Bioefficacy of Bioagents against Pathogenic Mycoflora of Soybean Seeds

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ABSTRACT
Soybean (Glycine max L. Merrill.) crop is affected by number of pathogenic fungi, which are mostly seed borne, causing significant qualitative and quantitative losses. Though, majority of seed borne fungi are being managed with fungicidal seed treatments, but due to their harmful effects, their use needs to be reduced. Therefore, present in vitro study was conducted to assess bioefficacy of eight bioagents against three major pathogenic seed borne fungi viz., Fusarium, Verticillium, Macrophomina phaseolina and Alternaria alternata, by applying Dual Culture Technique. Three separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. The results revealed that all of the eight test bioagents significantly inhibited mycelial growth of Fusarium, Verticillium, Macrophomina phaseolina and Alternaria alternata, over untreated control. However, in case of F. verticillioioides significantly highest mycelial growth inhibition was achieved with T.virens (85.93%), followed by, A. niger (84.63%), T. hamatum (66.85%), T. asperellum (63.52%), T. harzianum (62.77%), and T. koningii (52.96%). P. fluorescens (45.00%) was found least effective. Whereas, in M. phaseolina, significantly highest mycelial growth inhibition was resulted with T.virens (83.71%), followed by, T. vires (83.33%), T. harzianum (82.96%), A. niger (70.37%), T. asperellum (39.26%), P. fluorescens (30.55%) and T. koningii (24.07%). While for A. alternata, significantly highest mycelial growth inhibition was with T. hamatum (84.91%), followed by, T. vires (82.04%), A. niger (77.41%), T. asperellum (75.93%), T. harzianum (71.85%), T. koningii (55.37%) and P. fluorescens (43.15%).

Keywords: Pathogenic mycoflora of soybean seeds, bioagent

INTRODUCTION
Soybean (Glycine max L. Merrill.) is a worldwide economic crop and the most important cultivated legume with hundreds of food, feed and industrial uses [15]. Soybean is a protein rich oilseed, which is presently number one edible oil source globally. Soybean seed is highly nutritious containing about 40% protein, 30% carbohydrates, 5% fibre, 0.5% lecithins, 4% saponins and 18-20% vegetable oil [6]. In India during 2016-17, soybean was cultivated on an area of 10.60 M ha with a production of 7.13 million MT. The productivity was 0.61 MT/ha which is popularly grown in the state of Madhya Pradesh, Maharashtra and Rajasthan [2]. Several phytopathogenic and saprophytic fungal species have been reported on soybean seeds. The most important fungal seedborne diseases of soybean are Fusarium collar rot (Fusarium semitectum) [10], Downy mildew (Perenospora manshurica), Aspergillus flavus, Aspergillus niger, Curvularia lunata, Penicillium digitatum, Rhizopus stolonifer, Anthracnose/pod blight (Colletotrichum truncatum), Alternaria leaf spot (Alternaria alternata), Cercospora leaf spot (Cercospora sojina), Purple seed stain (Cercospora kikuchii) [3], Charcoal rot (Macrophomina phaseolina), Stem rot (Sclerotinia sclerotiorum) [6, 8]etc. These phytopathogenic fungi associated with soybean seeds cause qualitative losses by reducing oil quality due to increased free fatty acids as well as quantitative losses by seed deterioration and seedling mortality, leading to accountable seed yield losses [1, 14]. Therefore, present study on in vitro bioefficacy of bioagents against pathogenic mycoflora of soybean seeds was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during 2017-18.

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MATERIALS AND METHODS
Isolation, identification and pathogenicity of seed borne fungi
Previous season stored seeds of soybean cultivars MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 were collected from Oilseeds Research Station, Latur and Seed Processing Unit (National Seed Project) VNMKV, Parbhani. These seeds were plated aseptically onto autoclaved and cooled Potato Dextrose Agar medium, in separate sterile glass petri plates and incubated at room temperature. After a week of incubation, various fungal colonies developed on PDA plates were observed under stereomicroscope, distinguished on the basis of colony colour and growth habit, further re-isolated on fresh PDA plates and incubated at room temperature. Based on morpho-cultural characteristics and microscopic observations, the most predominant fungi identified were Fusarium verticillioides, Macrophomina phaseolina and Alternaria alternata. The pathogenicity of these three fungi was proved by seed inoculation and standard blotter paper techniques, by using the surface sterilized (2% Sodium hypochlorite solution) seeds of soybean cultivars MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335.

In vitro evaluation of bioagents
A total of eight biocontrol agents (Table. 1) were evaluated in vitro against Fusarium verticillioides, Macrophomina phaseolina and Alternaria alternata separately, by applying Dual Culture Technique [5]. Three separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. Observations on linear mycelial growth of the test fungi and test bioagents were recorded separately at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungi. Per cent mycelial growth inhibition of the test fungi with the test bioagents are presented in Table 1 and Fig. 1.

RESULT AND DISCUSSION
The results obtained on per cent of mycelial growth inhibition of two test fungi viz., F. verticillioides, M. phaseolina and A. alternata with the test bioagents are presented in Table 1 and Fig. 1.

F. verticillioides inhibition
The results (Table.1 and Fig.1A) revealed that all of the eight test bioagents significantly inhibited mycelial growth of F. verticillioides, over untreated control, which was ranged from 45.00– 85.93 per cent. However, significantly highest mycelial growth inhibition was achieved with T.virens (85.93%), followed by, A. niger (84.63%), T. hamatum (66.85%), T. asperellum (63.52%), T. harzianum (62.77%), and T. koningii (52.96%). P. fluorescens was found least effective (45.00%).

M. phaseolina inhibition
The results (Table.1 and Fig.1B) revealed that all of the eight test bioagents significantly inhibited mycelial growth of M. phaseolina, over untreated control and it was ranged from 24.07– 83.71 per cent. However, significantly highest mycelial growth inhibition was resulted with T. hamatum (83.71%), followed by, T. virens (83.33%), T. harzianum (82.96%), A. niger (70.37%), T. asperellum (39.26%), P. fluorescens (30.55%) and T. koningii (24.07%).

A. alternata inhibition
The results (Table.1 and Fig.1C) revealed that all of the eight test bioagents significantly inhibited mycelial growth of A. alternata, over untreated control and it was ranged from 43.15– 84.91 per cent. However, significantly highest mycelial growth inhibition was resulted with T. hamatum (84.91%), followed by, T. virens (82.04%), A. niger (77.41%), T. asperellum (75.93%), T. harzianum (71.85%), T. koningii (57.37%) and P. fluorescens (43.15%).

Various species of Trichoderma, Aspergillus niger and P. fluorescens are most commonly and commercially exploited bioagents/ antagonists to combat several seed borne and/or soil borne plant pathogens. Fungicidal / fungistatic effects of these bioagents have been attributed to various mechanisms exerted such as antibiotic, lysis, mycoparasitism, competition, production of volatile/nonvolatile compounds etc. In present study also, various species of Trichoderma, Aspergillus niger and P. fluorescens were found as efficient antagonists against three major seed borne pathogenic fungi viz., F. verticillioides, M. phaseolina and A. alternata of soybean.
Fig. 1 *In vitro* efficacy of various bioagents against *F. verticillioides*, *M. phaseolina* and *A. alternata* associated with soybean seeds

A. *Fusarium verticillioides*  
B. *Macrophomina phaseolina*  
C. *Alternaria alternata*
Table.1 In vitro efficacy of various bioagents against F. verticillioides, M. phaseolina and A. alternata associated with soybean seeds

<table>
<thead>
<tr>
<th>Tr. No.</th>
<th>Treatments</th>
<th>Inhibition* (%)</th>
<th>F. verticillioides</th>
<th>M. phaseolina</th>
<th>A. alternata</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Trichoderma asperellum</td>
<td>63.52 (52.84)</td>
<td>39.26 (38.79)</td>
<td>75.93 (60.61)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trichoderma harzianum</td>
<td>62.77 (52.39)</td>
<td>82.96 (65.61)</td>
<td>71.85 (57.95)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Trichoderma hamatum</td>
<td>66.85 (54.84)</td>
<td>83.71 (66.19)</td>
<td>84.91 (67.14)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Trichoderma koningii</td>
<td>52.96 (46.69)</td>
<td>24.07 (29.38)</td>
<td>55.37 (48.08)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Trichoderma virens</td>
<td>85.93 (67.96)</td>
<td>83.33 (65.90)</td>
<td>82.04 (64.92)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Aspergillus niger</td>
<td>84.63 (66.91)</td>
<td>70.37 (57.02)</td>
<td>77.41 (61.62)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Pseudomonas fluorescens</td>
<td>45.00 (42.13)</td>
<td>30.55 (33.55)</td>
<td>43.15 (41.06)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>Control (Untreated)</td>
<td>00.00 (00)</td>
<td>00.00 (00)</td>
<td>00.00 (00)</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.91</td>
<td>0.70</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>CD (P=0.01%)</td>
<td></td>
<td>2.66</td>
<td>2.06</td>
<td>2.23</td>
<td></td>
</tr>
</tbody>
</table>

*: Mean of three replications. Figures in parentheses are Arcsine values

These results of the present study are in agreement with previous findings of several workers [8, 12, 14, 15]. Gupta and Ansari [8] revealed that seedling mortality was reduced maximum with T. viride and P. fluorescens individually or in combination with fungicide and bioagent seed treatments, rather it enhanced seed germination over untreated control. Rahman et al. [12] reported that all the three species of Trichoderma viz., T. harizanum, T. hamatum and T. viride showed excellent control of seedborne M. phaseolina and also increased germination significantly and thereby production of healthy seedlings. Rajeswari et al. [13] reported that seed treatments with T. viride (6g/kg), mancozeb (2.5g/kg) and neem oil (10 ml/kg) not only enhanced the seed quality but also were effective in reducing total seed mycoflora and seedling mortality. Seeds of safflower cultivars Nira and Manjeera treated with T. viride @ 6g kg-1 also 32 recorded higher seed germination (90% and 87%) and seedling vigour (1529,1383) with less number of total fungal colonies (7% and 12%).

Hence, from ongoing results and discussion, it is concluded that soybean pathogenic three major seedborne fungi viz., F. verticillioides, M. phaseolina and A. alternata could be managed with the bioagents viz., A. niger, various species of Trichoderma and P. fluorescens.

**CONCLUSION**

Agroforestry can support food production, increase the total efficiency and stability of the system in North Eastern region. It holds promise to satisfy all human needs (food, fuel, fodder, timber etc.) and it also can act as an insurance against drought, flood and natural calamities those are familiar to north-east region of India. With inclusion of agroforestry with different crop components and livestock one can be benefitted in terms of monetary as well as ecological sustainability. Thus, area based effective research strategies in North Eastern region is required for meeting the diversified needs of the people as well as for increasing the food production

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