Short Communications

Microbial Bioagents: Economic multiplication and management of fungal-nematode complex on cumin

Nidhi Sharma* and P C Trivedi
Department of Botany, University of Rajasthan, Jaipur 302 004, India

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Mass-scale multiplication was done on cheaper substrates for application of bioagents in the management of plant-parasitic nematodes and fungal pathogens. The bioagents were isolated from the local field soils. Out of the 13 isolated fungi, most of the isolates of Trichoderma spp. that were found antagonistic to Fusarium oxysporum f. sp. cuminii in dual culture technique, were mass multiplied on cheaper agrowastes. Suitability of 6 substrates was screened and tea waste was found to be best followed by wheat bran and sorghum straw. Trichoderma harzianum (T5) had the maximum spore load per gram (SLPG) value on tea waste followed by T. hamatum (T16) on wheat bran. Three isolates of bacteria viz. Bacillus subtilis, Pseudomonas fluorescens and Rhizobium spp. were multiplied on nutrient broth, King’s B broth and yeast extract mannitol broth, respectively.

Keywords: biocontrol, Meloidogyne incognita, Fusarium oxysporum f. sp. cuminii, Trichoderma harzianum, T. viride, T. hamatum, Bacillus subtilis, Pseudomonas fluorescens

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A living, multiplying biocontrol agent potentially provides a continuous, non-chemical control of the pathogen. Most of the biocontrol agents are already present in the rhizosphere of plants but in minute quantities. Trichoderma spp. are among the most promising biocontrol agents. It is reported to be most widely distributed soil fungi. But it is usually necessary for biocontrol agents to proliferate and get established quickly. For this, their mass multiplication on economical substrates i.e. agrowastes is needed. In mass multiplication, the goal is a system that gives good spore yield, good shelf life at ambient temperature and a high percentage of viable spores.

Therefore, two approaches could be followed to transform a conducive soil into a suppressive soil: the first approach consists of enhancing the low levels of natural disease suppressiveness that exists in every soil and the second approach consists of the isolation of antagonistic microorganisms that can be mass produced and introduced to conductive soils to control Meloidogyne-Fusarium complex. The present study was undertaken, with the above objectives.

Rhizosphere soil was taken from the fields of cumin infected with Meloidogyne-Fusarium complex and different dilutions were prepared with distilled water. About 2 ml of suspension was poured (spread) over the surface of PDA in Petri-plates. Incubation was done at 25°C and examined daily. Different types of colonies were developed after 3-5 days. Each colony was sub cultured on test-tube slants and pure cultures of different fungi and bacteria were obtained. Different colonies were marked out with different colours. Slides were prepared for each of them and observed under microscope. The colony colour, morphological characters and their measurements were also taken into consideration. Identification was done using standard books and journals.

The fungal and bacterial cultures isolated from cumin fields infected with Meloidogyne-Fusarium complex were tested for their virulence in vitro by dual-culture technique. The effective microorganisms (fungi) were mass multiplied on one type of oil-cake (Gossypium arboreum L.); two types of straws viz. wheat and sorghum straw (Sorghum vulgare Pers.) and other waste materials like cotton seed (Gossypium spp.) tea waste (Camellia sinensis L.) and wheat bran.

About 150 g of agrowaste was washed and soaked in water for 6-10 h. The lumps of oil cakes were powdered and soaked in water; after the excess water was drained off, these were spread on the blotting paper. Moistened substrates were placed in 500-mL Erlenmeyer flasks and sterilized in an autoclave at 15 lbs. for 20 min. After autoclaving, the flasks were shaken vigorously for 2-3 min to separate the wet substrate. These autoclaved substrates were then inoculated with 1 mL of spore suspension of different fungi to be multiplied and were incubated at room temperature for 15 days. The flasks were shaken for the first two days to promote uniform growth of

*Author for correspondence:
Tel: 91-141-2711654; Fax: 91-141-2710880
E-mail: the-phantom81@rediffmail.com
fungus. After 15 days, about 5 g of substrate was taken out from each flask and dissolved in 30 mL water with 0.1 mL (2-3 drops) of tween 80. The sample was shaken for half an hour and the water solution was taken in another flask. From 30 mL, 25 mL of suspension was recovered and centrifuged. The supernatant was decanted and 2 mL of mounting fluid (1/3 glycerine + 2/3 95% alcohol) was added to the pellet. Spores were counted with the help of haemocytometer and spore load per gram was estimated by using following formula:

\[ \text{SLPG} = \frac{N \times V \times 10,000}{W} \]

Where,
- \( N \) = No. of spores in equal square of haemocytometer;
- \( V \) = Volume of mounting fluid added to the substrate;
- \( W \) = Weight of substrate

Different broths were prepared for the multiplication of bacteria, viz. nutrient broth for Bacillus, Kings B broth for Pseudomonas and yeast extract for Rhizobium.

Thirteen fungi isolated from the rhizosphere of cumin infected with Meloidogyne-Fusarium complex is given in Table 1. Out of them, most of the isolates of Trichoderma harzianum, T. viride, T. virens, T. hamatum were found antagonistic to Fusarium oxysporum f. sp. cumini in dual culture technique. Similarly, 3 isolates of bacteria viz. Pseudomonas fluorescens, Bacillus subtilis and Rhizobium spp. were found antagonistic. Therefore, all the above bioagents were selected for mass multiplication.

Suitability of 6 types of agrowastes was tried under laboratory conditions for growth and proliferation of Trichoderma isolates (Table 2). Fungus could be observed on the surface of agrowastes within 2 days. After 15 days of inoculation, there was sufficient

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fungi/Bacteria</th>
<th>S. No.</th>
<th>Name of the fungus (Strains)</th>
<th>Spore load per gm on different substrates (SLPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fusarium oxysporum</td>
<td>1.</td>
<td>Trichoderma harzianum (T₁)</td>
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</tr>
<tr>
<td>2.</td>
<td>T. harzianum (T₂)</td>
<td>2.</td>
<td>T. harzianum (T₁)</td>
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<tr>
<td>3.</td>
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<td>T. harzianum (T₃)</td>
<td>2.46×10⁹</td>
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<td>4.</td>
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<td>4.</td>
<td>T. harzianum (T₄)</td>
<td>3.54×10⁹</td>
</tr>
<tr>
<td>5.</td>
<td>T. harzianum (T₅)</td>
<td>5.</td>
<td>T. harzianum (T₅)</td>
<td>6.92×10⁹</td>
</tr>
<tr>
<td>6.</td>
<td>T. harzianum (T₆)</td>
<td>6.</td>
<td>T. harzianum (T₆)</td>
<td>3.54×10⁹</td>
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<tr>
<td>7.</td>
<td>T. harzianum (T₇)</td>
<td>7.</td>
<td>T. harzianum (T₇)</td>
<td>3.69×10⁹</td>
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<tr>
<td>8.</td>
<td>T. harzianum (T₈)</td>
<td>8.</td>
<td>T. harzianum (T₈)</td>
<td>4.94×10⁹</td>
</tr>
<tr>
<td>9.</td>
<td>T. viride (T₉)</td>
<td>9.</td>
<td>T. viride (T₉)</td>
<td>5.96×10⁹</td>
</tr>
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<td>10.</td>
<td>T. viride (T₁₀)</td>
<td>10.</td>
<td>T. viride (T₁₀)</td>
<td>8.12×10⁹</td>
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<td>11.</td>
<td>T. viride (T₁₁)</td>
<td>11.</td>
<td>T. viride (T₁₁)</td>
<td>2.02×10⁹</td>
</tr>
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<td>12.</td>
<td>T. viride (T₁₂)</td>
<td>12.</td>
<td>T. viride (T₁₂)</td>
<td>7.08×10⁹</td>
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<td>13.</td>
<td>T. viride (T₁₃)</td>
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<td>T. viride (T₁₃)</td>
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</tr>
<tr>
<td>14.</td>
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<td>T. viride (T₁₄)</td>
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<td>15.</td>
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<td>T. viride (T₁₅)</td>
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<td>16.</td>
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<td>17.</td>
<td>Paecilomyces lilacinus (T₁₇)</td>
<td>17.</td>
<td>Paecilomyces lilacinus (T₁₇)</td>
<td>4.23×10⁹</td>
</tr>
</tbody>
</table>

T₁, T₉, T₁₇=from Jaipur, T₂ & T₁₃=from Chittorgarh, T₃ & T₁₄=from Hardwar, T₆, T₈ & T₁₁=from Aligarh, T₇, T₁₂ & T₁₅=from Ag. Co., T₅=from J.S, T₁₀ & T₁₆=from Udaipur
fungal growth. Tea waste was found to be best substrate for all the isolates followed by wheat bran and sorghum straw. Cotton cake was least favoured by the bioagent fungi. T3, T6, T8, T14 and T16 isolates were best multiplied on wheat bran; T4 and T12 on sorghum straw and T9 on cottonseed. Rest of the isolates had maximum SLPG on tea waste. T. harzianum (T5) had the maximum SLPG value, viz. 6.02 × 10^7 on tea waste followed by T. hamatum (T16) on wheat bran as 4.93 × 10^7. In case of bacteria, two of them showed characteristic change in colour of broths; in case of Bacillus, it turned yellow and in Pseudomonas, green to reddish brown.

The amendment of soil with decomposable organic matter is recognized as an efficient method of changing soil and rhizosphere environments, thereby adversely affecting the life cycle of pathogens and enabling the plant to resist the attack of pathogens through better vigour and/or altered root physiology.

There are many reports where addition of a variety of organic materials to soil resulted in a definite reduction of the nematode population^5-8. Thus, the combination of biocontrol agents and organic matter is a great boon for the plant to overcome the disease.

Using oil cakes as a substrate for multiplication of the fungus could serve dual functions, firstly as a nutrient base for the growth of fungus and secondly oil cakes have nematicidal properties^9. It is well known that application of oil cakes to soil inhibits the population of plant parasitic nematodes^9,10. Root-knot control through amendments is preferred over chemical control on the ground that the former is easy to apply, material is locally available and repeated application in every crop is not needed. Oil cakes are also cheaper than fumigants.

Tribe and Ahmed^11 made use of barley autoclaved in plastic bags as the solid support and nutrient for biocontrol agents. The grain created a large surface area under the right humidity condition. Jatala^12 has reported rice as a good substrate for multiplication of Paecilomyces lilacinus. Sharma^13 found maize, bajra and jowar grains as good substrates for growth and proliferation of the fungus and noted effective control of root-knot nematode, M. incognita using these grains. It was also noted that root-knot index and final nematode population was less in grains + nematode+ fungus treatment as compared to grains+ nematode treatment. Maximum control was obtained with 20 g of fungus infested. Mass multiplication of T. harzianum and T. virens was tried on coffee pulp, tea waste, neem cake and FYM and of these, tea waste supported maximum growth and sporulation^14.

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References