Antagonistic Potential of Native Isolates of *Trichoderma viride* on Corm Rot Pathogen Complex of Saffron (*Crocus sativus*) in Kashmir

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Abstract: Investigation was undertaken to screen the potential native isolate of *Trichoderma viride* for bio suppression of corm rot pathogen complex, as *Trichoderma viride* are the most successful and widely used biocontrol agents. Taking the advantage and constraints of *Trichoderma viride* into consideration, efforts were made to encourage the native isolate against corm rot pathogens. Nine isolates of *Trichoderma viride* namely TKs, TKs1, TKs2, TKs3, TKs4, TKs5, TKs6, TKs7, and TKs8, were isolated from soils of different orchard plantations of Kashmir valley on modified *Trichoderma* Specific Medium (TSM). The isolates were studied for their cultural, morphometric characters and antagonistic potential against six newly recorded major fungal pathogens of saffron viz. sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Phytophthora* sp., *Fusarium oxysporum* f. sp. *gladioli*, *F. oxysporum* and *F. solani* individually on Potato Dextrose Agar, the culture morphology of all the isolates was found to be similar. The isolate TKs8, TKs9, TKs10, TKs11, TKs12, and TKs13, were found fully overgrown on all corm rot Pathogens of saffron, where as the isolates TKs1 failed to inhibit the *Phytophthora* sp. Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods.

Key words: Bio suppression, sterile basidiomycetes fungus, *Rhizoctonia solani*, *Phytophthora* sp., *Fusarium* spp., saffron

INTRODUCTION

During last fifteen years, saffron crop has been affected by severe rotting caused by sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium* f. sp. *gladioli*, *Fusarium oxysporum* and *Fusarium solani* (Mir and Devi, 2004) and reduction in yield has been reported. In 1980 the yield per hectare was 5.66 kg ha⁻¹ (Mir, 1992) and now its present productivity is 1.53 kg ha⁻¹ (Anonymous, 2009) which is the lowest in the world.

In recent years, attempts were also made to use a consortium of biocontrol agents to get persistent control of plant pathogens (Chauhe and Sharma, 2002). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Antagonistic fungi - 38 - especially *Trichoderma* spp. has been widely used against a number of phytopathogens (Rini and Sulochana, 2006) and parasitized hyphae of other fungi *in vitro* and brought about several morphological changes during destruction (Anatha and Murugesan, 2001). Screening of potential *Trichoderma* strains was done by Bandopadhyay et al. (2003) against major root pathogens and it was found that more or less all the strains checked the growth of the pathogen and stimulate plant defensive mechanisms (Hanson and Howell, 2004; Harman et al., 2004; Yadav et al., 2011).

*Trichoderma harzianum* is one efficient biocontrol that is commercially produced to prevent development of several soil pathogenic fungi (Jegathambigai et al., 2009). Biocontrol is an important approach for plant disease management under changing food habits and commercialization of agriculture (Manczinger et al., 2002).

Therefore, keeping in view medicinal importance and to remove the pesticidal residue of such valuable medicinal crop, the present study was undertaken for screening of several local antagonistic isolates of *T. viride*, obtained from different orchards of Kashmir valley, under *in vitro* conditions against few pathogens sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *gladioli*, *Fusarium gladioli*, *Fusarium solani*, *Phytophthora* sp. causing corm rot syndrome of saffron.

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MATERIALS AND METHODS

Collection of pathogen: Four pathogenic isolates namely sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium oxysporum* f. *sp. gladioli*, *Fusarium gladioli*, *Fusarium solani* and *Phytophthora* sp. were isolated, from infected corns from Kashmir valley from saffron growing area. The pathogens were maintained on PDA medium at 4°C.

Isolation of *Trichoderma* spp. (TK₁, TK₂, TK₃, TK₄, TK₅, TK₆, TK₇, TK₈, TK₉, and TK₁₀) was done from randomly collected soils from different vegetable fields and orchards of Kashmir valley by dilution plate technique using *Trichoderma* specific medium TSM (Elad et al., 1981) modified by Saha and Pan (1997).

Antagonistic potential of *Trichoderma viride* isolates on saffron pathogens: The antagonistic properties of fifteen isolates of *Trichoderma viride* were tested on PDA by dual culture plate technique. Paired cultures were observed for a total of 12 days before being discarded. All the ratings were done after contact between pathogen and the antagonist using a Bells scale (Bell et al., 1982) which is slightly modified (Class 1-7) as follows.

- **S₁**: The pathogen and the antagonist looked at the point of contact
- **S₂**: The antagonism starts overgrowth on pathogen.
- **S₃**: The pathogens starts overgrowth on mycoparasite
- **S₄**: The antagonist overgrew at least 15% of pathogen
- **S₅**: The antagonist overgrew at least 30% of pathogen
- **S₆**: The antagonist overgrew at least 60% of pathogen
- **S₇**: The antagonist completely overgrew the pathogen (100% overgrowth)

Identity of isolates of *Trichoderma* spp.: In general, colony morphology of all the isolates was more or less similar showing sparse to thin colony mycelial mass with whitish border in some cases. Sporulation started after 48h of incubation at 28±1°C for all the isolates (Table 2).

Micrometric measurements of *Trichoderma viride* (Table 1) showed that phialospore length ranged between 2.98-5.52 μm and breadth ranged from 2.71-4.6 μm and phialides length 9.22-12.56 and breadth 1.3-2.5. These characteristics, particularly the trifid phialophore with short phialides clearly resembled the identical characters of *Trichoderma viride* (Rifai, 1969).

Antagonistic potential of *Trichoderma viride* isolates against corn rot pathogens of saffron.

*Phytophthora* sp.: The results showed that isolate TK₁, TK₄, TK₅ and TK₁₀ were antagonistic to *Phytophthora* by totally overgrowing the pathogen within seven, nine and eleven day respectively. Isolate TK₁₀, TK₁ and TK₂ were antagonistic to *Phytophthora* overgrowing 75, 45 and 60%, respectively.

*F. oxysporum* f. *sp. gladioli*: The results showed that isolate TK₁, TK₂, TK₃, TK₄, TK₅ and TK₁₀ were antagonistic to *F. oxysporum* f. *sp. gladioli* by totally overgrowing the pathogen within 8 to 12 days. Isolates TK₆ and TK₁₀, were overgrowing the pathogen 90 and 45%, respectively.

**Sterile Basidiomycetes fungus:** The results against Basidiomycetes fungus showed that five isolates TK₁, TK₃, TK₄, and TK₁₀, totally overgrowing within nine, eight and 12 day, respectively. The remaining isolate TK₁₀, TK₉, TK₁ and TK₁₀, overgrew 90, 75, 45 and 15%, respectively.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Conidium (μm)</th>
<th>Phialide (μm)</th>
<th>Chlamydospore (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>B</td>
<td>L</td>
</tr>
<tr>
<td>TK₁</td>
<td>3.50-4.17</td>
<td>2.71-3.22</td>
<td>10.2-11.9</td>
</tr>
<tr>
<td>TK₂</td>
<td>4.21-4.87</td>
<td>3.20-3.62</td>
<td>9.41-9.51</td>
</tr>
<tr>
<td>TK₃</td>
<td>4.92-5.32</td>
<td>3.62-4.60</td>
<td>9.25-10.12</td>
</tr>
<tr>
<td>TK₄</td>
<td>3.79-4.22</td>
<td>3.10-4.52</td>
<td>9.82-10.45</td>
</tr>
<tr>
<td>TK₅</td>
<td>3.41-4.04</td>
<td>2.56-3.21</td>
<td>9.22-10.33</td>
</tr>
<tr>
<td>TK₆</td>
<td>3.62-3.97</td>
<td>3.11-3.96</td>
<td>9.99-12.22</td>
</tr>
<tr>
<td>TK₇</td>
<td>2.98-3.60</td>
<td>2.89-2.79</td>
<td>10.56-12.22</td>
</tr>
<tr>
<td>TK₈</td>
<td>4.27-4.62</td>
<td>3.56-3.88</td>
<td>9.66-9.22</td>
</tr>
<tr>
<td>TK₉</td>
<td>3.91-4.55</td>
<td>3.89-4.56</td>
<td>11.22-12.56</td>
</tr>
</tbody>
</table>
Table 2: Colony characters of *Trichoderma viride* isolates

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>36 h</th>
<th>After 60 h</th>
<th>After 90 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK_3</td>
<td>3.8</td>
<td>White growth appears inoculum, sparse very thin mycelium hardly seen</td>
<td>Sparse 4 cm mycelium growth, media become yellow around inoculum, after light green sporulation</td>
</tr>
<tr>
<td>TK_5</td>
<td>3.5</td>
<td>White mycelial growth on the inoculum, very thin mycelium around the inoculum</td>
<td>Compact white mycelium on inoculum, light green sporulation around the inoculum 2.5 cm after raised cotton growth 8.6 cm</td>
</tr>
<tr>
<td>TK_4</td>
<td>6.3</td>
<td>Sparse whitish thick growth</td>
<td>Compact fluffy light green sporulation on older regions</td>
</tr>
<tr>
<td>TK_6</td>
<td>5</td>
<td>White growth on the inoculum, encircled sparse mycelium</td>
<td>On inoculum white growth, dense lightly sparse fluffly green sporulation 4.5 cm</td>
</tr>
<tr>
<td>TK_7</td>
<td>6</td>
<td>Yellow growth on inoculum, surround sparse white growth</td>
<td>Yellow growth on inoculum, around 2 cm sparse light green sporulation</td>
</tr>
<tr>
<td>TK_8</td>
<td>3.9</td>
<td>Thin sparse growth 3.9 cm around the inoculum</td>
<td>Yellow growth appears on inoculum sporulate light green sporulation appears white dense growth at periphery</td>
</tr>
<tr>
<td>TK_10</td>
<td>4</td>
<td>Very thin mycelial growth around the inoculum</td>
<td>Around inoculum 5.5 cm dia. White mycelial growth. Surrounded by sparse whitish green mycelium 1.5 cm dia, encircled by dark green 0.5 cm dia.</td>
</tr>
<tr>
<td>TK_11</td>
<td>7</td>
<td>Thick raised white mycelium</td>
<td>Around the inoculum very light green fluffly mycelium encircled by 1 cm dense green sporulation</td>
</tr>
<tr>
<td>TK_12</td>
<td>6.3</td>
<td>Around inoculum 3 cm white mycelium</td>
<td>Fluffy mycelium like balls, surrounded dark green sporulation</td>
</tr>
</tbody>
</table>

Table 3: Hyperparasitic potential of *T. viride* wild isolates on fungal pathogens of saffron

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Basidiomycetes fungus</th>
<th>Rhizoctonia solani</th>
<th>Phyllosticta sp.</th>
<th>F. oxysporum f. sp.declini</th>
<th>F. oxysporum</th>
<th>F. solani</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK_3</td>
<td>D: 5</td>
<td>R: 5</td>
<td>D: 5</td>
<td>R: 5</td>
<td>R: 5</td>
<td>R: 5</td>
</tr>
<tr>
<td>TK_4</td>
<td>D: 7</td>
<td>R: 7</td>
<td>D: 7</td>
<td>R: 7</td>
<td>R: 7</td>
<td>R: 7</td>
</tr>
<tr>
<td>TK_7</td>
<td>D: 8</td>
<td>R: 8</td>
<td>D: 8</td>
<td>R: 8</td>
<td>R: 8</td>
<td>R: 8</td>
</tr>
<tr>
<td>TK_8</td>
<td>D: 9</td>
<td>R: 9</td>
<td>D: 9</td>
<td>R: 9</td>
<td>R: 9</td>
<td>R: 9</td>
</tr>
<tr>
<td>TK_10</td>
<td>D: 10</td>
<td>R: 10</td>
<td>D: 10</td>
<td>R: 10</td>
<td>R: 10</td>
<td>R: 10</td>
</tr>
<tr>
<td>TK_11</td>
<td>D: 11</td>
<td>R: 11</td>
<td>D: 11</td>
<td>R: 11</td>
<td>R: 11</td>
<td>R: 11</td>
</tr>
<tr>
<td>TK_12</td>
<td>D: 12</td>
<td>R: 12</td>
<td>D: 12</td>
<td>R: 12</td>
<td>R: 12</td>
<td>R: 12</td>
</tr>
</tbody>
</table>

D: Days before contact. R: Rating. **: An average of five individual observation. *: The numerical value represents the days required for attaining S to S; stage of modified Bell’s scale.

**Rhizoctonia solani:** The results showed that all isolates were antagonistic to *Rhizoctonia solani* by totally overgrowing the pathogen with six to nine day except isolate TK_13, it overgrew only 75% even after day.

**F. oxysporum:** The results showed that isolate TK_3, TK_5, TK_7, TK_8, TK_10, and TK_11 were antagonistic to *F. oxysporum* by totally overgrowing the pathogen within 6 to 9 days. Isolates TK_3, TK_6, and TK_11 did not progress beyond 30% over after day. The remaining isolate TK_4 totally fails to overgrow the host pathogen even up to 12 days of inoculation spive of attaining the point of contact of the third day.

**F. solani:** The result shows that five isolates TK_1, TK_2, TK_4, TK_5, and TK_13 were highly antagonistic to *Fusarium solani*, totally overgrowing the pathogen within 6 to 11 days. Isolates TK_4 and TK_11 were overgrew the pathogen 30% whereas TK_10 15% and TK_8 failed to overgrow the host pathogen even after 12 days of inoculation, in spite of attaining the point of contact on the 4th day of inoculation.

The overview of the results (Table 3) showed that the isolates TK_1, TK_2, TK_4, TK_5, TK_6, TK_7, TK_10, TK_11, and TK_15 were found fully overgrown on all corn rot Pathogens of saffron, where as the isolates TK_13 failed to inhibit the *Phytophthora* sp. To identify these, isolates of
Trichoderma spp. have been listed in the tables that reached class-I (S.) stage within 6-11 days of inoculation. However, based on this information the antagonistic Trichoderma viride did not allow an early selection of isolates, as the variability in the antagonistic characteristic within the isolate and isolate-pathogen interaction was very high.

The above observations established the fact that Trichoderma isolates existing in their natural conditions in natural ecosystem do differ with respect to their growth and antagonistic potential. Similarly Li et al. (2001) studied eighteen isolates of Trichoderma spp. of these isolates, TR13 showed greatest antagonists effects against Rhizoctonia solani. Several research papers that have appeared in the literature do reveal the fact that various species and isolates of fungal antagonist Trichoderma suppress mycelial growth, reduce root rots, increase plant growth and induce resistance in various crops with which Sclerotium rolfsii (Tian et al., 2001; Das and Dutta, 2002; Palomar et al., 2002), Rhizoctonia solani (Li et al., 2001; Burgess and Hepworth, 1996, Zapata et al., 2001; Ziedan and Mahmoud, 2002; Gaikwad and Nimbalkar, 2003; Yossen et al., 2003, Fravel and Lewis, 2004, Hajlaoui et al., 2001; Singh et al., 2003; Huang and Erickson, 2004; Salehpour et al., 2005) are associated. It is clear that the success of bioagents introduces in soil does not guarantee the control the target pathogen(s) because plants, physicochemical and biological factors of soil affect establishment, proliferation and antagonistic activities of the introduced bioagents. It is necessary that, identified antagonist efficiency against foot, root rot and damping off should be investigated and examined in vivo conditions also, the results of such survey would be reported by the authors in near future (Shaigan et al., 2008).

It is in this context that to ensure success of introduced bioagents, they should be isolated for the local areas where they exist. Since, they have already faced various processes of evaluation, their application would be feasible and result oriented. We reviewed the literature to find out that have others worked on these aspects. Literature analysis revealed that comparative studies have been done with various species (Kucuk and Kivanen, 2003; Chang et al., 2006) studied Trichoderma isolates from different soil sampled and grouped them according to their antagonistic potential and chitin utilization.

CONCLUSION

The overgrowth by the antagonist under in vitro conditions may be good criteria of selecting an isolate shows good performance under in vitro conditions. The trend of the results also indicated that there was not only variability amongst the isolates of Trichoderma viride with differential degree of a stagnation towards a single pathogen but also towards different pathogens.

The results of the study are the pointer to the fact that the antagonists should be isolated from different systems and locations to create a huge genetic pool and tested for their antagonistic potential against variety of the targeted plant pathogens and recommended specifically for different locations and systems. The present study clearly indicates the high potential of biocontrol agent, Trichoderma viride isolates for different plant pathogens. Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods.

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