In-vitro evaluation of fungicides and biocontrol agents against \textit{R. solani} causing root rot of cotton in Rajasthan

Rammniwas Yadav, Sushila Choudhary, Kalpana Yadav, Rajendra Prasad Jat and Rajpal Yadav

Abstract

Root rot pathogens (\textit{R. solani}) were isolated in majority from diseased cotton plants showing root rot symptoms collected from farmer’s field during survey. The pathogenicity of recovered isolates of \textit{R. solani} were separately confirmed by Koch’s postulates by growing susceptible cotton cultivar “Jai BG-II” in pathogen inoculated pots also observed incidence and typical symptoms by respective isolates. In view of increasing disease incidence in cotton growing areas, attempts were made to evaluate six (systemic and non-systemic) fungicides viz., Carbendazim, Vitavax power, Azoxystrobin, Rhizolex, Tebuconazole and Thiram at 50, 100, 250 and 500 ppm concentrations against \textit{R. solani} in vitro. Sensitivity of Carbendazim, Vitavax power and Tebuconazole was found more pronounced against \textit{R. solani}, whereas Carbendazim was highly effective against \textit{R. solani} at all the concentrations with 100 per cent inhibition of mycelial growth. In \textit{vivo} studies for evaluating comparative efficacy of four \textit{Trichoderma} isolates. The efficacy of the \textit{T. viride} isolates (T-5 and T-3) found to be highly effective against \textit{R. solani} pathogens in vitro. However, \textit{T. aureoviride} was found to be less effective than \textit{T. viride}.

Keywords: \textit{Rhizoctonia solani}, management, fungicides, biocontrol agents, disease incidence etc.

I. Introduction

Cotton (\textit{Gossypium} spp.) is one of the most important fibers and cash crop belongs to genus \textit{Gossypium} of the family \textit{Malvaceae}. It is originated as a tropical and subtropical perennial plant, but is produced as an annual crop in many temperate regions around the world. Cotton is cultivated in about 80 countries in the world in which top five producers are India, China, Pakistan, USA and Brazil (Anonymous, 2016-17) [2]. Cotton is popularly known as “white gold” and it is the crop with multiple uses that supplies five basic products viz. - lint, oil, seed, hulls and linters. Among them the lint is the most important product of cotton plant and provides much of the high quality fiber for the textile industry. India is the first largest cotton producer, consumer and exporter in the world; it is cultivated in an area about 11.87 million hectares with the 30.15 million tons production and 4.32 q ha\(^{-1}\) productivity respectively. Agriculture statistics at a glance-2016 (2017-a). In Rajasthan total area under cotton cultivation is around 4.4 lac hectares with production of 13.2 lac tones and productivity of 5.01 q ha\(^{-1}\). Agriculture statistics at a glance-2016 (2017-b).

Most common fungal diseases in cotton are leaf spot and leaf blight caused by (\textit{Alternaria macrospora}, \textit{Alternaria alternata}, \textit{Cercospora gossypina}, \textit{Cochliobolus spicifera}, \textit{Myrothecium oridum} (Kamal and Moghal, 1968; Jagirdar and jagirdar, 1980; Jiskani 1992 and jaskani 2001) [13, 9, 10, 11]. Anthracnose (\textit{Colletotrichum gossypii}), Areolate mildew (\textit{Cercospora gossypii}), Ascochyta blight (\textit{Ascochyta gossypii}) and black root rot (\textit{Thielaviopsis basicola}), boll rot is caused by several pathogen including (\textit{Ascochyta gossypii}, \textit{Colletotrichum gossypii}, \textit{Fusarium spp.}, \textit{Lasiodiplodia theobromae}, \textit{Rhizoctonia solani}). Under favorable environmental condition Charcoal rot (\textit{Macrophomina phaseolina}), Fusarium wilt (\textit{Fusarium oxysporum f. sp. vasinfectum}), powdery mildew (\textit{Leveillula taurica}), cotton rust (\textit{Puccinia schedonardii}, \textit{Puccinia caccabata}), Sclerotium stem rot (\textit{Sclerotium rolfsii}) and Damping-off and root rot (\textit{Rhizoctonia solani} (Silva et al. 1995 and Belot and Zambiasi, 2007) [18, 4]. Seed treatment with fungicides is the appropriate method for control the seed/soil borne diseases but, the fungus \textit{R. solani} is non-spore forming and soil borne, it’s propogules and sclerotia are evenly distributed in the soil and fungicides are not able to reach at the target point for manage the disease up to longer period. On other hand overzealous and indiscriminate use of most of the synthetic fungicides has created different type of environmental and toxicological
problems. Attention has been paid towards exploitation of non-hazardous bio control agents or botanical amendment in plant protection. Therefore, the ultimate aim of research has been the development of integrated control strategies, as such; use of alternative methods like eco-friendly botanicals and biological control with integration of fungicides to manage this disease.

2. Materials and Methods

2.1 In vitro efficacy of fungicides (Poison food technique)
relative efficacy of different systemic and non-systemic fungicides was evaluated by using poisoned food technique (Schmitz, 1930) [19]. In vitro efficacy of fungicides was tested against the most virulent/aggressive isolate of R. solani (SGN Rs-03) with six fungicides viz., Thiram 75% WP (Gupta Chemicals (p.) Ltd., Mumbai), Vitavax power 75% WP [carboxin] (Pesticide India Ltd., Udaipur), Bavistan 50% WP [carbendazim] (BASF India Ltd., Mumbai), Tebuconazole 25.9 w/w [Folicular 250 EC] (Bayer Crop Science, India Ltd., Mumbai), Rhizolex 50%WP [telcloros-methyl] (Sumitomo chemicals ltd.) and Azoxytribin 23% SC [Amistar] (Syngenta ltd.) were tested at four concentrations i.e., 50, 100, 250 and 500 ppm. Desired quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized Petri dishes and allowed to solidify. Each plate was inoculated with three mm disc of fungal culture and incubated at 28±1 °C for seven days. In each treatment three replications were allowed for.

In each treatment three replications were allowed for measuring the growth of the test pathogen in dual culture and in control plates. The per cent mycelial inhibition zone of pathogen was calculated using following formula:

\[ I = \frac{(C - T)}{C} \times 100 \]

Where,
I = Per cent mycelial inhibition zone
C = Growth of fungal plant pathogen in control (mm)
T = Growth of fungal plant pathogen in dual culture plate (mm)

3. Results and Discussion

3.1 Isolation of the pathogen, Purification and identification of the pathogen
The disease samples of cotton root rot were also collected from different villages of six districts during the surveys to isolate the pathogen. The most of root rot samples yielded Rhizoctonia, Fusarium spp. and Sclerotinia spp., whereas, in majority of R. solani colonies were recovered.

To purify R. solani single sclerotia was picked under a stereo-binocular microscope and using single hyphal tip culturing on PDA plates. The cultures of different isolates were identified on the basis of morphological characters of the fungus and compared with the standard description (Holiday, 1981 and Mordue, 1988) [7, 16].

3.2 In vitro evaluation of fungicides (poisoned food technique)
Six fungicides Carbendazim, Vitavax power, Azoxytribin, Rhizolex, Tebuconazole, and Thiram were evaluated at four concentrations viz., 50, 100, 250 and 500 ppm by poisoned food technique for their effectiveness against R. solani. All the test fungicides significantly inhibited the mycelial growth of R. solani at all concentrations. The Carbendazim was found the most effective that completely 100 per cent inhibited the mycelial growth of R. solani at all concentrations (50, 100, 250 and 500 ppm) followed by Vitavax power was found effective with inhibition of 95.6, 95.9, 96.1 and 96.9 per cent mycelial growth of R. solani at 50, 100, 250 and 500 ppm respectively. Third most effective fungicide was Tebuconazole which showed 91.4, 94.4, 96.1 and 96.7 per cent growth inhibition at 50, 100, 250 and 500 ppm respectively. It was also observed that Tebuconazole found at par at 250 and 500 ppm with Vitavax power, followed by Rhizolex and Azoxytribin showed 81.7, 84.4, 88.4, 91.7 and 70.2, 73.3, 76.1, 79.2 per cent inhibition at 50, 100, 250 and 500 ppm, respectively. Thiram was found least effective at all concentrations against R. solani with 19.4, 22.3, 25.2 and 30.8 per cent growth inhibition of R. solani at 50, 100, 250 and 500 ppm concentration respectively (Table-1, Fig.-1). Similar results have been observed by several workers, where fungicides like Carbendazim, Vitavax and Tebuconazole have been reported to be effective for control of root rot of cotton (Yadav et al. 2003) [21] and on other crop (Vadhera et al. 1997) [20]; (Meen and Chatopadhyay 2002) [15]; (Dutta and Kalha 2011) [16]; (Rajendraprasad et al. 2017) [17].
Table 1: Comparative efficacy of different fungicides on the growth of *R. solani* at various concentrations (ppm) *in vitro*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fungicides</th>
<th>Mycelial growth (mm)*</th>
<th>Per cent growth inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>Carbendazim</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Vitavax power</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>Azoxistrobin</td>
<td>26.8</td>
<td>24.0</td>
</tr>
<tr>
<td>4</td>
<td>Rhizolex</td>
<td>16.5</td>
<td>14.0</td>
</tr>
<tr>
<td>5</td>
<td>Tebuconazole</td>
<td>7.7</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>Thiram</td>
<td>72.5</td>
<td>69.9</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>90.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

SEm± 0.531 0.614 0.134 0.106 0.082 0.078 0.074 0.089

CD (P= 0.05) 1.57 1.82 0.39 0.31 0.24 0.23 0.22 0.26

* Average of four replications; Figures given in parentheses are arcsine √ per cent angular transformed values.

### Plate 1: Inhibition of mycelial growth of *R. solani* by different fungicides

#### 3.3 *In vitro* evaluation of biocontrol agents (Dual culture technique)

Efficacy of biocontrol agents *T. viride* (T-5), *T. harzianum* (Th.J. 89-2), *T. viride* (T-3) and *T. aureoviride* (Ta-D 91-5) was studied *in vitro* against *R. solani*.

Results indicate that all the biocontrol agents viz., *T. viride*, *T. harzianum*, *T. viride* (T-3) and *T. aureoviride* were have antagonistic activity to the growth of *R. solani* *in vitro*. Maximum and significant high per cent inhibition of *R. solani* growth (84.5 per cent) was recorded by *T. viride* (T-5) in dual culture method, followed by *T. viride* (T-3) 80.0 per cent while the *T. harzianum* (Th.J. 89-2) was showed 77.7 per cent mycelial growth inhibition. Minimum growth inhibition was recorded by *T. aureoviride* (Ta-D 91-5) which was 72.3 per cent (Table-2, Fig.-2). Similar results have been observed by several workers, where biological control agents like *T. viride*, *T. harzianum* and *T. aureoviride* have been reported to be effective for control of root rot caused by *R. solani*. (Bunker and Mathur 2001) [5]; Mathur and Gurjar 2002) [14]; (Arya et al. 2017) [3]; (Hassanein 2012) [8].
Table 2: Per cent inhibition zone of mycelial growth of *R. solani* with four isolates of *Trichoderma* spp. by dual culture technique

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biocontrol agents</th>
<th>Growth of <em>R. solani</em></th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Growth (mm)*</td>
<td></td>
</tr>
<tr>
<td><em>T1</em></td>
<td><em>Trichoderma viride</em> (T-5)</td>
<td>13.9</td>
<td>84.5 (66.9)</td>
</tr>
<tr>
<td><em>T2</em></td>
<td><em>Trichoderma harzianum</em> (Th.J, 89-2)</td>
<td>20.1</td>
<td>77.7 (61.8)</td>
</tr>
<tr>
<td><em>T3</em></td>
<td><em>Trichoderma viride</em> (T-3)</td>
<td>18.0</td>
<td>80.0 (63.4)</td>
</tr>
<tr>
<td><em>T4</em></td>
<td><em>Trichoderma aureoviride</em> (Ta-D 91-5)</td>
<td>24.95</td>
<td>72.3 (58.3)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>90.0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>SE±m</td>
<td>0.699</td>
<td>0.573</td>
</tr>
<tr>
<td></td>
<td>CD (P= 0.05)</td>
<td>2.10</td>
<td>1.73</td>
</tr>
</tbody>
</table>

* Average of four replications; Figures given in parentheses are arcsine √ per cent angular transformed values.

Plate 2: Evaluation of fungal biocontrol agent against *R. solani* (SNG Rs-03) by dual culture technique *in vitro*

4. References

