Efficacy of Antagonists on mycelium growth and carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* on Indian mustard

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**ABSTRACT**

*Sclerotinia sclerotiorum* (Lib.) de Bary is a soil borne pathogen capable of infecting more than 400 host plants worldwide. It is a major pathogen that plays a crucial role in reducing the yield in economically important crops. The capability of sclerotia to survive for more than 4 years becomes very difficult to manage the crop from the infection of *Sclerotinia* rot fungus. Stem rot of Indian mustard [*Brassica juncea* (L.) Czern & Cross] caused by *Sclerotinia sclerotiorum* is potentially a serious threat in many mustard growing areas in India. Treatments of seeds and foliar spray with of fungicides applied at regular intervals are effective in reducing infection, but uses of chemicals are hazardous, harmful for beneficial micro-organisms. Biological control of plant pathogens offers an exciting opportunity to manage plant diseases. In the present study, the efficacy of four bio-agents, viz., *Coniothyrium minitans*, *Aspergillus nidulans*, *Trichoderma harzianum*, and *Pseudomonas chlororaphis* were evaluated for the control of stem rot of Indian mustard. Results on bio-efficacy of different bioagents, when evaluated under glass house condition, the *Coniothyrium minitans* was the most effective agent and caused highest reduction (64.7 %) in carpogenic germination of sclerotia followed by *Aspergillus nidulans* (52.5 %) and *Trichoderma harzianum* (48.8 %), over control while *Pseudomonas chlororaphis* (48.3 %) was at par with *T. harzianum*. All the bioagents showed significant reduction effective in controlling the disease. Similar results were achieved when bioagents tested on dual inoculated plates.

**KEYWORDS**

Bio-management, antagonists, stem-rot, yield, rhizospheric population, carpogenic, sclerotia, *Sclerotiana*

**MATERIALS AND METHODS**

In the present studies, four bioagents viz. *Coniothyrium minitans*, *Aspergillus nidulans*, *Trichoderma harzianum*, and *Pseudomonas chlororaphis* were evaluated for their potential in controlling stem rot. Culture of antagonistic fungi obtained from IARI, New Delhi.
Mass Production of Antagonists

*Coniothyrium minitans*, *Aspergillus nidulans* and *Trichoderma harzianum* were multiplied on Potato Dextrose Agar in 250 ml conical flasks. Inoculated flasks were incubated in a biological oxygen demand (BOD) incubator at 25±2°C for 15 days. The flasks were flooded with sterilized distilled water. The fungi were harvested by scraping the mycelial mat from the flask with the help of a sterilized spatula. The collected mats were weighed and blended in a known volume of distilled water in an electric blender and a homogenous suspension was prepared. The strength of the bio-agent spores in the filtrate was adjusted to a desired level of 20 x 10^6 spores/ml using a haemocytometer.

*Pseudomonas chlororaphis* was mass multiplied on king’s B agar medium in 250 ml conical flasks. It is incubated in shaker incubator for 48 hours at 25±2°C. The bacterial colonies are harvested by scraping the bacterial colonies from the flasks.

Dual Cultures

*Sclerotinia sclerotiorum* and antagonists were inoculated singly and jointly on PDA in 5 Petri plates each at a distance of 1 cm apart. Two marks 1 cm apart was made on the back of lower Petri plates. One mark was inoculated with antagonists whereas the other with *S. sclerotiorum*, using sterilized inoculating needle on both occasions. The plates were incubated at 25 ± 2°C in a BOD incubator for 5 days. After incubation, plates were examined and radial growth of the antagonists and pathogenic fungi was measured on the basis of colony colour. Percent radial growth was calculated in relation to total area of the plate.

Testing of Efficacy of Bioagents on Carpogenic Germination of Sclerotia

Fifty-day-old laboratory produced and air-dried sclerotia (6-12 mm) were taken and buried in earthen pots (30cm dia.) containing steam sterilized sandy loam soil. Experiments were replicated 3 times. The pots were watered regularly to facilitate the carpogenic germination of sclerotia. The sclerotia buried at 2 cm depth in each pot. Each pot was saturated with spore suspension of antagonists. Observation on carpogenic germination was recorded up to 100 days of burial. Percent inhibition was recorded as per Vincent’s (1947) formula.

RESULTS AND DISCUSSION

Radial Growth

All the antagonists or *S. sclerotiorum* grew luxuriantly and covered the whole surface of PDA in Petri Plates (Table 1). Dual inoculated plates showed a discernible competition between the antagonists and the pathogen. *C. minitans* proved strongest antagonists as it greatly restricted the radial growth of *S. sclerotiorum* followed by *A. nidulans*, *T. harzianum* and *P. chlororaphis*. (Table 1).

Effect of Fungal Mycoparasites

Amongst the tested biological control agents, *C. minitans* was the most effective agent and caused highest reduction (64.7 %) in carpogenic germination of sclerotia followed by *A. nidulans* (52.5 %) and *T. harzianum* (48.8%) over control while *Pseudomonas chlororaphis* (48.3 %) was at par with *T. harzianum* (CD _0.05_ = 3.30; Table 2)Earlier, Whipps and Budge (1990) reported that spores (4x10^6 spores/ml) of *Gliocladium virens* and *Coniothyrium minitans* has more infectivity and decreased viability of sclerotia of *Sclerotinia sclerotiorum* compared with solid substrate inoculum. This probably arose as a result of the massive direct contact of spores with sclerotia. Huang and Erickson (2000) found that The carpogenic germination of sclerotial bodies of *S. sclerotiorum* was reduced by the mycoparasites *C. minitans* and *Talaromyces flavus. C. minitans* into potting mixture at the rate of 10^6 spores/g of potting medium or soil was effective (60-85% control) in controlling Sclerotinia rot of lettuce, cabbage and beans (Stewart _et al._, 2001). Huang and Kozub (1991) investigated that continuous monoculture of sunflower increased the natural population of *C.*
Table 1. Effect of antagonists on the radial growth of *Sclerotinia sclerotiorum* on PDA in Petri plates

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Radial growth %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. sclerotiorum</em> + <em>A. nidulans</em></td>
<td>23.5 76.5</td>
</tr>
<tr>
<td><em>S. sclerotiorum</em> + <em>C. minitans</em></td>
<td>15.2 84.8</td>
</tr>
<tr>
<td><em>S. sclerotiorum</em> + <em>T. harzianum</em></td>
<td>28.0 72.0</td>
</tr>
<tr>
<td><em>S. sclerotiorum</em> + <em>P. chlororaphis</em></td>
<td>44.4 65.6</td>
</tr>
</tbody>
</table>

Table 2. Effect of antagonists on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Apothecia-producing sclerotia (%)</th>
<th>Inhibition over check (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>96.5</td>
<td>0</td>
</tr>
<tr>
<td><em>C. minitans</em></td>
<td>43.6</td>
<td>64.7</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>51.8</td>
<td>48.8</td>
</tr>
<tr>
<td><em>A. nidulans</em></td>
<td>49.5</td>
<td>52.5</td>
</tr>
<tr>
<td><em>P. chlororaphis</em></td>
<td>50.9</td>
<td>48.3</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>1.99</td>
<td>3.30</td>
</tr>
</tbody>
</table>

*minitans* and *Trichoderma* spp., which in turn reduced the severity of sunflower wilt under field conditions. Rodriguez and Godeas (2001) demonstrated that soil-borne strains of *Gliocladium virens*, *Trichoderma harzianum* and *Aspergillus* sp. caused inhibition mediated by antibiosis against *S. sclerotiorum*. Singh and Kaur reported the mycoparasitism of *Trichoderma harzianum* (Th38) and *E. purpureascens* against *S. sclerotiorum* in 2001. The disease suppression was due to the effective saprophytic colonization of petals by the antagonistic fungi. Savchuk and Fernando (2004) reported that *Pseudomonas* spp. (DF41) and *P. chlororaphis* (PA23) are effective biocontrol agents against *S. sclerotiorum* of canola and inhibited the germination of ascospores. The findings of the present study correlate with the study of other workers viz., Whipps and Budge (1990); Huang and Erickson, 2000; Stewart et al., (2001); Huang and Kozub (1991); Rodriguez and Godeas (2001); Singh and Kaur (2001); Savchuk and Fernando (2004). The difference in the efficacy of bio-agents may be due to differences in strain, dosage and agro-ecological environment that affect the population dynamics of pathogen and its biological control agents. These investigations suggest that losses caused by *S. sclerotiorum* could be checked by interrupting the infection cycle *i.e.* reducing ascosporic inoculum load by deep ploughing of *Sclerotinia* infested fields along with the incorporation of biological control agents at the time of sowing.

REFERENCES


Mehta, N., Hieu, N. T. and Sangwan, M. S. (2010) Efficacy of botanicals against *S. sclerotiorum*


