In-vitro assessment of biocontrol agents against Sclerotium rolfsii causing white rot of onion in Manipur

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Abstract
Antagonistic potential of five biocontrol agents were evaluated against Sclerotium rolfsii causing white rot of onion in Manipur. Trichoderma hamatum was most outstanding and effective against the test pathogen. The minimum mycelial growth of S. rolfsii was recorded in a dual culture with T. hamatum (2.0cm) followed successively by dual culture with T. harzianum and T. viride (3.7cm) respectively. Trichoderma hamatum, T. harzianum and T. viride showed 78.88%, 67.77% and 64.77% inhibition on mycelial growth of the pathogen respectively. T. harzianum formed a translucent inhibition of 0.1cm at the interaction site but eventually overgrew the test pathogen and completely inhibited sclerotia formation. Subsequently the test pathogen was also overgrown by T. hamatum and T. viride completely inhibiting sclerotia formation. Penicillium glabrum and P. citrinum were ineffective against S. rolfsii and both were overgrown by the pathogen.

Keywords: white rot, onion, Sclerotium rolfsii, biocontrol, sclerotia

Introduction
Onion (Allium cepa L.) is an important vegetable and spice crop in Manipur. It is mostly cultivated as a cool season crop. Onion suffers from many diseases which reduced crop yield considerably, alter the cropping pattern, lowers the quality of harvest produce, affects the local and export markets and cause significant economic losses (Mishra et al. 2014) [21]. White rot of onion is one of the prime diseases which reduced onion productivity. It is commonly observed in onion cultivating sites in the valley districts of Manipur and has traumatized onion cultivation in the state. Sclerotium rolfsii was first reported from Florida as a causal organism of tomato blight (Rolfs, 1892) [22]. The teleomorph of the fungus is Athelia rolfsii (Curzi) Tu and Kimbrough (Curzi, 1931; Tu and Kimbrough, 1978) [13, 39]. The symptoms commenced with the drying of leaves from the tips which extend downwards followed by blighting, drooping, wilting of leaves. The affected onion bulbs are watery, soft and rots accompanied by white cotton like mycelial growth of the fungus along with small white, brown to black colour sclerotia. The fungus attacks many hosts since the fungus has physiological, pathogenicity and also survives as mycelium in infected plants, plant debris and on dead organic materials (Mullen, 2001) [20]. Sclerotinia comprises of three layers namely, inner medulla, middle cortex and outer rind (Punja and Damiani, 1996) [28]. Moreover, the wall of sclerotia contains melanin pigment, elevated amount of lipid, ash and non-hydrolisable residue which are responsible for making sclerotia tolerance to biological and chemical degradation (Abbo Ellil, 1999; Chet et al., 1967) [3, 12]. The fungus sometimes developed hymenial layers for survival and also survives as mycelium in infected plants, plant debris and on dead organic materials (Mullen, 2001) [20]. The isolates of Sclerotium rolfsii from different hosts and locations varies in cultural, morphological, physiological, pathogenicity and genetic characters (Punja and Damiani, 1996; Punja and Sun, 2001; Shukla and Pandey, 2008) [28, 29, 37]. The substantial use of fungicides confers numerous harmful effects including residual issue, phytotoxic effect, health hazard, environmental degradation, climate change and non target effects on unrelated organisms (Ma et al., 2003; Boxall et al., 2009; Dias, 2012; Bollmann et al., 2014; Nettles et al., 2016) [19, 11, 15, 9, 24]. Although fungicides are included in effective and efficient management strategies against fungus usually developed resistance against fungicides. S. rolfsii isolates resistant to tebuconazole, flutolanil and pentachloronitrobenzene (PCNB) were reported from peanut.
et al., 1998) [36]. Hence, the potential and efficiency of fungicides are not constant since the effect varies from time to time. Thus, in recent years, biocontrol agents were tested for their effectiveness against several plant diseases. The utilization of biocontrol agents provides an alternative and appreciable strategy to manage the plant diseases as it does not degrade the environment and biodiversity. Therefore, the present investigation were carried out to evaluate five biocontrol agents that prevent both mycelial growth and sclerotia production of the fungus isolate causing white rot in onion under laboratory conditions.

Materials and Methods
Isolation, identification and maintenance of the pathogen
The diseased onion bulbs were collected. The cottony white mycelium from the infected bulb scales was inoculated on potato dextrose agar and was incubated at 28 ± 1°C for 4 days. The fungus was purified by hyphal tip cut method and re-isolated on PDA. The fungus was identified by comparison with relevant monographs. The culture of the fungus was maintained on PDA and was sub cultured periodically every week to freshly prepared PDA throughout the research period.

Collection and maintenance of biocontrol agents
Biocontrol agents namely, Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride, Penicillium glabrum and Penicillium citrinum were collected from the Department of Plant Pathology, College of Agriculture, Imphal. All the biocontrol agents were maintained on potato dextrose agar throughout the research period.

Assessment of biocontrol agents against Sclerotium rolfsii
Five biocontrol agents were evaluated under laboratory conditions by dual culture method on the mycelial growth and sclerotia production of the fungus. Potato dextrose agar (PDA) was prepared and sterilized at 121°C at 15lbs for 20 minutes. PDA was poured into sterilized petriplates and allowed to solidify. 5mm disc of all the biocontrol agents and the test pathogen were inoculated in opposite direction at 3 cm apart from each other. 5mm mycelial disc of the test pathogen alone were inoculated on the centre. This serves as a control. Three replications were maintained for each treatment. All the inoculated petriplates were incubated at 28 ± 1°C in inverted position until the control plates were fully grown by the test fungus. Bell’s scale was used to compare the antagonistic activities of five biocontrol agents
Bell’s scale (Bell et al., 1982) [7] with slight modification (Class I-VI):
Class I = The antagonist completely overgrew the pathogen (100% or overgrowth).
Class II = The antagonist overgrew at least ⅓ rd (75% overgrowth) of the pathogen surface.
Class III= The antagonist colonizes on half of the growth of the pathogen (50% overgrowth). Class IV = The pathogen and the antagonist locked at the point of contact.
Class V = The pathogen overgrew the mycoparasite.
Class VI = Formation of inhibition zone between pathogen and antagonist.

The per cent inhibition of mycelial growth of test fungus over control was calculated by using the formula recommended by Dennis and Webster (1971) [14]

\[
PI = \frac{C-T}{C} \times 100
\]

Where PI= Percent inhibition
C = linear growth of the fungus in control
T = linear growth of the fungus in treatment

Per cent inhibition of sclerotia production was calculated after 35 days of incubation by adopting the formula described by Vincent (1927) [40] as

\[
PI = \frac{C-T}{C} \times 100
\]

Where PI= Percent inhibition
C = number of sclerotia produced by the fungus in control
T = number of sclerotia produced by the fungus in treatment

Results and Discussion
The fungus was identified as Sclerotium rolfsii Saccardo based on morphological characteristics and taxonomic keys available in the literatures (Saccardo, 1913; Mordue, 1974; Punja, 1985) [22, 26, 33].

Bell’s scale
All the evaluated biocontrol agents showed different antagonistic potential against Sclerotium rolfsii. Two Trichoderma species namely, T. viride and T. hamatum and two Penicillium species namely, P. glabrum and P. citrinum came in contact with S. rolfsii after 2 days of incubation. However, T. harzianum form a translucent inhibition zone of 0.1cm at interaction site. However, on the fourth day of incubation T. harzianum came in contact with S. rolfsii and subsequently overgrew the pathogen completely. The test pathogen S. rolfsii was completely overgrown by T. harzianum, T. hamatum and T. viride after 5 days of incubation while P. glabrum and P. citrinum were completely overgrown by S. rolfsii after 5 days of incubation. Bell’s scale disclosed Class I by T. hamatum and T. viride, Class VI and subsequently Class I by T. harzianum and Class V by P. glabrum and P. citrinum. The present results are in corroboration with the findings of Amin et al. (2010) [2] who reported that Bell’s scale revealed Class I category by two isolates (Th-1 and Th-2) of T. harzianum and three isolates of T. viride (Tv-1, Tv-2 and Tv-3) in dual culture with S. rolfsii. Similarly, Biswas and Sen (2000) [8] reported that T8 and T10 isolates of T. harzianum in a dual culture with S. rolfsii showed Class I of Bell’s scale. Yaqub and Shahzad (2005) [41] reported that T. harzianum inhibited the growth of S. rolfsii and exhibits coiling around the mycelium of S. rolfsii on fourth day of incubation and showed B type of interaction in which the biocontrol agent and pathogen colony does not meet, however, the biocontrol agent produced coils around the hyphae of S. rolfsii which corroborates to Class VI of Bell’s scale. The present results are also in agreement with the findings of Basumatry et al. (2015) [6] who reported that Penicillium species tested against S. rolfsii was ultimately overgrown by the pathogen which confirms Class V category of Bell’s scale. Senthilkumarana et al. (2010) [34] also reported that T. harzianum and T. viride in dual culture with S. rolfsii showed Class I category of Bell’s scale. Appearance of translucent inhibition zone in dual culture of Trichoderma species could be attributed to antibiosis caused by antibiotics, volatile and non volatile metabolites, hydrolytic enzymes and

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Efficacy of biocontrol agents on the mycelial growth of Sclerotium rolfsii

Amidst the biocontrol agents evaluated against the antagonist, three Trichoderma species were effective in reducing the mycelial growth of S. rolfsii. The minimum mycelial growth of S. rolfsii was recorded in a dual culture with T. hamatum (2.0cm) followed successively by dual culture with T. harzianum (2.90cm) and T. viride (3.17cm) respectively. T. hamatum gave maximum inhibition of 78.88% on mycelial growth of S. rolfsii followed by T. harzianum giving 67.77% and T. viride giving 64.77% inhibition on mycelial growth of the test pathogen. Both Penicillium glabrum and P. citrinum were ineffective against S. rolfsii giving only 3.0% and 1.45% inhibition on mycelial growth of the test pathogen. The mycelial growth of S. rolfsii was maximum in a dual culture with P. citrinum (8.87cm) followed by dual culture with P. glabrum (8.73cm). Singh and Singh (1994) reported that three isolates of T. harzianum (Th-1, Th-2 and Th-3) and two isolates of T. viride (Tv-1 and Tv-2) showed maximum inhibition of mycelial growth of S. rolfsii with Th-2 giving 73.40%, Th-1 giving 67.22%, Th-3 giving 57.23%, Tv-2 giving 59.62% and Tv-1 giving 53.53% inhibition of mycelial growth of the test pathogen after 96 hours of incubation. Barakat et al. (2006) also reported that T. harzianum (J14) and T. hamatum (T36) were the most effective isolates against S. rolfsii at 25°C in dual culture showing 79% inhibition on mycelial growth of S. rolfsii and both Trichoderma species grew over the test pathogen and formed branches that coiled around the pathogen. He also stated that T. harzianum (J14), T. hamatum (T36) and T. viride (J8) grew over and parasitized the hyphae of S. rolfsii. Basumatry et al. (2015) reported that T. harzianum and T. viride inhibited the mycelial growth of S. rolfsii producing a clear inhibition zone with T. harzianum giving 77.39% and T. viride giving 76.54% mycelial growth inhibition of the pathogen. He also stated that Penicillium species was ineffective and gave 29.05% mycelial growth inhibition of the test pathogen and was ultimately overgrown by the pathogen after 7 days of incubation. Similarly, Karikeyyan et al. (2006) reported that Tv1 isolate of T. viride gave 69.40% inhibition on mycelial growth of S. rolfsii in dual culture while Tv2 and Tv3 isolates gave 56.35% and 56.90% mycelial growth inhibition after 5 days of incubation. Bosah et al. (2010) reported that Penicillium species failed to produce obvious significant inhibitory effect against S. rolfsii and the pathogen even outgrew the growth of Penicillium on the fourth day of incubation.

Efficacy of biocontrol agents on sclerotia production by Sclerotium rolfsii

All the three Trichoderma species completely inhibited sclerotia production of S. rolfsii giving 100% inhibition as the pathogen was completely overgrown and parasitized by the three Trichoderma species. However, the test pathogen could produce sclerotia in a dual culture with both the Penicillium species. Penicillium glabrum gave 66.02% inhibition on sclerotia production and P. citrinum gave 62.83% inhibition on sclerotia production of S. rolfsii over control. The present results corroborates to the findings of Shaigan et al. (2008) who reported that T. viride, T. harzianum and T. hamatum parasitizes the hyphae of S. rolfsii and also destructs and lyses already produced sclerotia at 98.5, 86.5 and 85% respectively.
Plate III: Dual culture of *T. viride* and *S. rolfsii*

Plate IV: Dual culture of *P. glabrum* and *S. rolfsii*

Plate V: Dual culture of *P. citrinum* and *S. rolfsii*

Plate VI: Culture of *S. rolfsii* (Control)

**Table 1:** Comparision of antagonistic effect of biocontrol agents by Bell’s scale

<table>
<thead>
<tr>
<th>Biocontrol agent</th>
<th>Duration of point of contact between biocontrol agent and pathogen</th>
<th>Bell’s scale</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>No contact (Inhibition zone) up to 3rd day and contact on 4th day</td>
<td>VI &amp; I</td>
</tr>
<tr>
<td><em>Trichoderma hamatum</em></td>
<td>2nd day</td>
<td>I</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>2nd day</td>
<td>I</td>
</tr>
<tr>
<td><em>Penicillium glabrum</em></td>
<td>2nd day</td>
<td>V</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>2nd day</td>
<td>V</td>
</tr>
</tbody>
</table>

**Table 2:** Efficacy of biocontrol agents against *Sclerotium rolfsii*

<table>
<thead>
<tr>
<th>Biocontrol agent</th>
<th>Mycelial growth (cm)*</th>
<th>Inhibition (%) of mycelial growth</th>
<th>Sclerotia production*</th>
<th>Inhibition (%) on sclerotia production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>2.90 (1.84)</td>
<td>67.77</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><em>Trichoderma hamatum</em></td>
<td>2.00 (1.58)</td>
<td>78.88</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>3.17 (1.91)</td>
<td>64.77</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><em>Penicillium glabrum</em></td>
<td>8.73 (3.04)</td>
<td>3.00</td>
<td>331</td>
<td>66.02</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>8.87 (3.06)</td>
<td>1.45</td>
<td>362</td>
<td>62.83</td>
</tr>
<tr>
<td>Control</td>
<td>9.00 (3.08)</td>
<td>-</td>
<td>-</td>
<td>974</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SE(d) ±</th>
<th>CD₉₀,₯₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.04)</td>
<td>0.1 (0.03)</td>
</tr>
</tbody>
</table>

*Mean of three replications, Figures in parenthesis are (√x+0.5) transformed values

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Fig 1: Growth rate of biocontrol agents and S. rolfsii in dual culture

Fig 2: Antagonistic effect of biocontrol agents against S. rolfsii in dual culture

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