Evaluating the efficacy of different bio control agents against *Magnaporthe grisea* under *in vitro* condition

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Abstract

Rice blast causes yield losses to Basmati rice farmers in worldwide. Although this problem is currently being addressed through the use of resistant rice varieties, fungicide and crop rotation farming, these methods alone do not form a durable, long lasting solution in mitigating disease. Here Antagonist activity of different bio-agent such as *Chaetomium globosum*, *Trichoderma viride*, *Bacillus subtilis*, *Trichoderma harzianium* and *Pseudomonas fluorescens* tested their efficacy as bio-control agents against to *Magnaporthe grisea*. *Trichoderma viride*, *Trichoderma harzianium* and *Chaetomium globosum* is known for its mycoparasitic properties, whereas *Bacillus subtilis* and *Pseudomonas fluorescens* have antagonistic mechanism for the control of fungal disease. The results showed that the dual inoculation of bio-control agents caused significant (p≤0.05) inhibition of *M. grisea* as compared to a single agent. The 100% inhibition of the fungal (*M. grisea*) radial growth was recorded *C. globosum* at different time intervals 72, 96 and 120hrs.

Keywords: *In vitro* management, *Magnaporthe grisea*, bio-control, dual plate culture

Introduction

Basmati rice is an important staple food grain crop in the world. Basmati rice is an important export commodity among the food grains in India. The unique feature of “Basmati Rice” such as extra long slender grain, length wise excessive elongation on cooking, soft and fluffy texture of cooked rice, and pleasant aroma which together determine uniqueness of “Basmati Rice”. Aside their cooking qualities, “Basmati Rice” also reported to have low glycemic index and are micro nutrient rich epically for iron and zinc (Dwivedi, 1997) [1]. Basmati has attained “Heritage rice” status as it is considered as “Farmers Cultivar” being maintained and grown by farmers of Punjab regions of India and Pakistan. In India its different varieties are mostly cultivated in the districts of karnal, Panipat, Kurukshetra, Kaithal, Amritsar, Fatehgarh, Gurudaspur, Hoshairpor, Jalandhar, Patiyala and Sangruoor in Punjab, Kangra, Solan, Una and Mandi in Himachal Pradesh mundi in Rajasthan and in several districts of Uttar Pradesh. Basmati is grown in limited areas of extent in Jammu and Kashmir. The Basmati rice has special demand in diet and international market. The total area of Basmati rice in India about 2.10 million hectares and production 8.70 million tonnes (Anonymous, 2014-2015) [2]. The area of Basmati rice in Uttar Pradesh is 354.39 thousand hectare with a production of 1260.69 thousand tons (Anonymous, 2014-2015) [2]. The average productivity of Pusa Basmati-1121 has 35.0 Q/ha this variety is very demand (Anonymous, 2014-15) [2]. Basmati rice farming now a days is facing many environmental problems caused by input of chemical fertilizers and pesticides. The use of chemical fertilizers and pesticides has led to soil decline with fertility, ecosystem damage, elimination of soil and emergence of resistant pathogens. Therefore the use of eco-friendly bio-fertilizers should be encouraged. Beneficial microorganisms have been reported to be involved in maintaining agricultural production, protecting the ecosystem and decreasing the use of chemical fertilizers (Adesemoye and Kloepper, 2009) [3].

*Magnaporthe grisea* (Anamor Pyricularia grisea Sacc. synonym Pyricularia oryzae Cav.) causes Basmati rice blast disease in rice cultivation areas worldwide (Chin, 1975, Kato, 2001) [4, 5]. Disease severity has increased recently due to the use of intensive agronomic practices that favour disease development. Blast disease severity is triggered by excess of N fertilization (Faria et al., 1982, Correa-Victoria et al., 2004) [6, 7] as well as rainfall and high humidity.
Cultural practice, cultivating resistant varieties and the use of synthetic fungicides are the three strategies used to control Basmati rice blast (Ghazanfar et al., 2009, IRRI, 2010) [8, 9]. Among them disease caused by fungus Pyricularia grisea is a most serious disease as compared to other diseases of Basmati rice. It cause stem and neck blast of paddy. This disease generally causes yield loss of 10-20% but in severe cases yield loss may reach up to 80% (Chaudhary, 1999 and Koutroubas et al., 2009) [10, 11]. However it may be vary according to adopted plant protection measures and growing cultivars. The excess use of chemicals resulted in environmental pollution and ill health to biotic community as a whole. Therefore, the biological method of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. Furthermore, obvious pollution of the environment and the toxic effects of synthetic chemicals on non-target organisms including humans have prompted investigations on pesticides of plant origin (Amadioha, 2000) [12].

The control of this disease through fungicide application however has adverse effects on the environment and negatively affects the soil microbiota. Therefore, the main objective of the present study was to assess the effect of bio agent on mycelia growth of M. grisea in vitro condition.

Materials and Methods

Fungal isolation

The fungus Pyricularia grisea was isolated from diseased samples collected from experimental site of the university as per method mentioned by Tuite, 1969 [13]. The typical symptoms of rice blast cut with the help of sterilized blade. These pieces were washed thoroughly water and placed for 30 second in 0.1 per cent mercuric chloride solution.Followed by washing thrice with sterilized water thoroughly. Excess water was removed by placing on the folds of sterilized blotting paper. Then after, dry pieces were placed on poured sterilized PDA medium in sterilized Petri plates. Petri dishes were incubated at 26±1 °C in BOD incubator plate no 1. After 4-5 days, fine radiating mycelial growth was observed and subsequently it was transferred a new petri-dish containing sterilized PDA medium plate no 2. Pathogenecity test was also carried out at boot leaf stage and periodical observations were made for the development of symptoms on panicle. Re-isolations were made and the identity of the fungus was confirmed as per the original description to prove Koch’s postulates.

In vitro Antagonistic activity

Antagonistic studies of different bio agents such as Trichoderma harzianum, Pseudomonas fluorescens, Chaetomium globosum, Trichoderma viride and Bacillus subtilis bio agents were conducted using dual culture technique. Autoclaved PDA was poured in sterilized petridish and allowed to solidify. Four petridish for each bio agents were used. Six mm disc of seven days old test fungus and bio agent were cut with the help of Cork borer and transferred in petridishes. Four discs of bio agents were inoculated at four peripheral points of the plates and test fungi were placed in centre of petridishes. Three days old culture of P. fluorescens and B. Subtilis streaked around the disc of test fungus of two sites. Control plates were kept where culture disc of test fungus was grown in same condition on potato dextrose agar without bio agents. The plates were incubated in BOD incubator at 26±1 °C for seven days. All in vitro tests of antagonism were performed three times. After incubation period the mycelia inhibition was calculated at different time intervals (72hrs, 96hrs and 120hrs). Dual culture method (Rao, 2003) [14] was employed to evaluate the antagonistic activities of T. harzianum and T. viride. Similarly, Pseudomonas fluorescens isolate was assessed for potential antagonistic activity against P. grisea isolates on PDA using dual culture technique (Rangajaran et al., 2003) [15].

Four mm diameter mycelial disc was cut from an actively growing P. grisea culture and placed on the surface of fresh PDA medium at the center of the Petri plates. A loopfull of actively growing Pseudomonas fluorescens isolate was placed opposite to the P. grisea disc and streaking the Pseudomonas fluorescens isolate on the plate at four locations, approximately 3 cm from the centre. Plates inoculated with P. grisea isolates and without Pseudomonas fluorescens and Trichoderma species alone were used as control. Degree of antagonism was determined by measuring the radial growth of pathogen (radial mycelia growth reduction) of P. grisea isolates by five bio-agent species of in dual culture in relation to growth of the control and percentage of inhibition was calculated by using the following equation (Riungu et al., 2008) [16].

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

Where,

I = percentage inhibition of pathogen by antagonists
C= radial growth measurement of the pathogen in the control plates and
T= radial growth of the pathogen in the experimental plates

Results and Discussion

Effect of different bio-agents on Pyricularia grisea

Antagonistic activities of five bio-agents against Pyricularia grisea in vitro by dual culture methods evaluated.Colony diameter of both bio control agent and the test fungus were recorded after each72, 96 and 120 hours by giving straight line in the centre of both colonies with permanent marker. The results recorded mentioned in Table 1 and Fig 2. After 72hrs maximum radial growth 41.33 mm was recorded in control. The maximum inhibition of the fungal radial growth was recorded in Chaetomium globosum (100 per cent inhibition) followed by Trichoderma viride (69.27 per cent) and Bacillus subtilis (51.22 per cent). Minimum inhibition radial growth was found in Trichoderma harzianum (34.28 per cent) followed by Pseudomonas fluorescens (40.72 per cent).

The highest inhibition of the fungal radial growth was recorded in Chaetomium globosum (100 per cent inhibition) followed by Trichoderma viride (68.96 per cent) and Bacillus subtilis (55.04 per cent) respectively. After 96 hrs maximum radial growth 64.86 mm was recorded in control. The highest inhibition of the fungal radial growth was recorded in Chaetomium globosum (100 percent) followed by Trichoderma viride (59.33 per cent) and Bacillus subtilis (53.61 per cent) respectively. The maximum radial growth (84.43 mm) was recorded in control each after 120 hrs. All the five bio agents tested, reduce the radial growth invariably in comparison to control. All the bio agents taken in this investigation were effective against P. grisea and significantly inhibited the radial growth in vitro. Most effective performance among the bio agent was exhibited by Chaetomium globosum.

The use of antagonistic fungi to control the destructive plant pathogens is getting more importance since few decades (Whipps, 2001) [17]. Fungal bio-control agents like Trichoderma spp., Gliocladium spp., Penicillium spp., etc.
have proved to be effective for controlling numerous destructive plant pathogens (Larena et al., 2002, Srinon et al., 2006, Yaqub & Shahzad, 2005) [18-20]. Our results are in accordance to those reported by Gouramanis 1995 [21] who observed that antagonistic such as T. harzianium and Chaetomium globosum gave 70-88% mycelial and conidial inhibition of P. grisea. Similarly, Watanabe 1985[22] evaluated different species of Trichoderma against 24 airborne plant pathogens including M. oryzae and found that T. harzianum, T. koningii, T. Pseudo koningii and T. Viride had strong antagonistic potential. He also found that selective isolates of T. harzianum and T. Viride showed severe antagonism against M. grisea, while T. Polysporum was weaker antagonist. However, this bio-control agent demonstrates a powerful antagonistic behaviour in the control of rice disease. It can therefore be concluded that Chaetomium globosum is an effective biological control agent.

### Table 1: Effect of different bio-agents on mycelial growth of *Pyricularia grisea*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antagonistic</th>
<th>Radial growth after 72 h.</th>
<th>Inhibition %</th>
<th>Radial growth after 96 h.</th>
<th>Inhibition %</th>
<th>Radial growth after 120 h.</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trichoma harzianium</em></td>
<td>26.16</td>
<td>34.28</td>
<td>30.16</td>
<td>53.49</td>
<td>37.20</td>
<td>55.93</td>
</tr>
<tr>
<td>2</td>
<td><em>Chaetomium globosum</em></td>
<td>0.00</td>
<td>100</td>
<td>0.00</td>
<td>100</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>24.50</td>
<td>40.72</td>
<td>31.66</td>
<td>53.49</td>
<td>25.33</td>
<td>69.99</td>
</tr>
<tr>
<td>4</td>
<td><em>Trichoderma viride</em></td>
<td>12.70</td>
<td>69.27</td>
<td>20.13</td>
<td>68.96</td>
<td>25.33</td>
<td>69.99</td>
</tr>
<tr>
<td>5</td>
<td><em>Bacillus subtilis</em></td>
<td>20.16</td>
<td>51.22</td>
<td>29.16</td>
<td>55.04</td>
<td>34.16</td>
<td>59.34</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>41.33</td>
<td>64.86</td>
<td>41.33</td>
<td>64.86</td>
<td>41.33</td>
<td>64.86</td>
</tr>
<tr>
<td></td>
<td>SEM±</td>
<td>0.72</td>
<td>0.82</td>
<td>0.82</td>
<td>0.54</td>
<td>0.54</td>
<td>0.54</td>
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<tr>
<td></td>
<td>CD at 5%</td>
<td>2.26</td>
<td>2.58</td>
<td>2.58</td>
<td>1.69</td>
<td>1.69</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>C.V @ 5%</td>
<td>5.98</td>
<td>4.74</td>
<td>4.74</td>
<td>2.32</td>
<td>2.32</td>
<td>2.32</td>
</tr>
</tbody>
</table>

Fig 2: Effect of different Bio-agents on mycelial growth of *Pyricularia grisea in vitro*

**Plate 1:** Pure culture of *P. grisea*

**Plate 2:** Mycelium and conidia of *P. grisea*

### Conclusion

Studies were also conducted on disease management with bio agents against blast disease. All the five bio agents i.e., such as *Trichoderma harzianium, Chaetomium globosum, Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* inhibited the growth of fungus significantly but *Chaetomium globosum* completely inhibited the radial growth *in vitro*. One of the most serious rice diseases in the ‘Basmoti rice’ sector is fungal disease infection. Plant diseases need to be controlled to maintain the quality and abundance of food quality. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic practices, growers often rely heavily on chemical fertilizers and fungicides. A more balanced, cost effective and eco-friendly approach can be adopted by ‘Basmoti rice’ farmers. Biological control is an innovative, cost effective and eco-friendly approach for managing diseases of ‘Basmoti rice’.

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