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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 Antagonistics 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 ($p \leq 0.05$) inhibition of *M. grisea* as compared to a single agent. The100% 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, Fathegarh, Gurdaspur, 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-1121has 35.0 Q/ha this variety is very demand (Anonymous, 2014-15) [2]. Basmati rice farming now a day 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 (Anamorf *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 ^{0}C in BOD incubator plate no 1. After 4-5 days, fine radiating myceial 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 lifted 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. fluoresces* 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 ^{0}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].

$$[I\% = (C-T)/C] \times 100$$

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 Gouraman is 1995 [21] who observed that antagonistic such as *T. harzianium* and *Chetomium 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*

S. No.	Antagonistic	Radial growth after 72 h.	Inhibition %	Radial growth after 96 h.	Inhibition %	Radial growth after 120 h.	Inhibition %
1	*Trichoma harzianium*	26.16	34.28	30.16	53.49	37.20	55.93
2	*Chaetomium globosum*	0.00	100	0.00	100	0.00	100
3	*Pseudomonas fluorescens*	24.50	40.72	31.66	53.49	35.16	58.35
4	*Trichoderma viride*	12.70	69.27	20.13	68.96	25.33	69.99
5	*Bacillus subtilis*	20.16	51.22	29.16	55.04	34.16	59.54
6	Control	41.33		64.86		84.43	
	Sem±	0.72		0.82		0.54	
	CD at 5%	2.26		2.58		1.69	
	C.V @ 5 %	5.98		4.74		2.32	

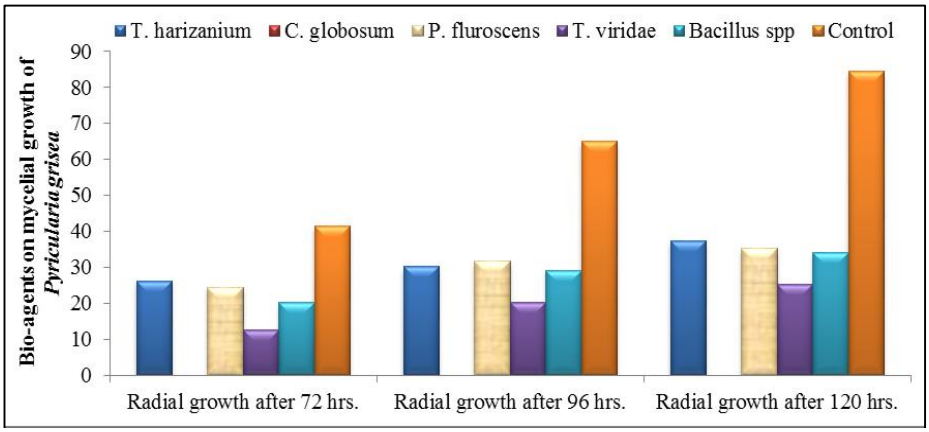

Fig 2: Effect of different Bio-agents on mycelial growth of *Pyricularia grisea in vitro*

Plate 1: Pure culture of *P. grise*

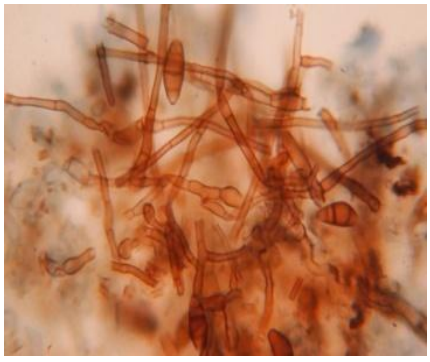

Plate 2: Mycelum and conidia of *P. grisea*

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 'Basmati 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 'Basmati rice' farmers. Biological control is an innovative, cost effective and eco-friendly approach for managing diseases of 'Basmati rice'.

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References

1. Dwivedi DK. Response of scented rice genotypes to nitrogen under mid up land situation. Indian Journal of Agronomy. 1997; 42(1):74-76.
2. Anonymous. State-wise-Basmati-rice-Production, w.w.w. airea and w.w.w.apeda.com, 2015.
3. Adesemoye AO, Torbert HA, Kloepper JW. Plant growth-promoting Rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol, 2009; 58:921-929.
4. Chin KM. Fungicidal control of the rice blast disease. Mardi Research Bulletin. 1975; 2(2):82-84.
5. Kato H. Rice blast disease. Pesticide Outlook February, 2001, 23-25.
6. Faria JCD, Prabhu AS, Zimmermann FJP. Effect of Nitrogen fertilization and fungicidal sprays on blast and yield of upland rice. Pesq Agropec Bras. 1982; 17(6):847-852.
7. Correa-Victoria FJ, Tharreau D, Martinez C, Vales M, Escobar F, Prado G. Studies on the rice blast pathogen, resistance genes, and implications for breeding for durable blast resistance in Colombia. In: Kawasaki S, editor. Rice blast: interaction with rice and control. Proceedings of the third international rice blast conference. Dordrecht, the Netherlands. Kluwer Academic Publishers, 2004, 214-27.
8. Ghazanfar MU, Wakil W, Sahi ST, Saleem Y. Influence of various fungicides on the management of rice blast disease. Mycopath. 2009; **7**(1):29-34.
9. IRRI. Rice blast International Rice Research Institute. www.knowledgebank.irri.org/factsheetsPDFs/.RiceFactSheets, Mar 2010.
10. Chaudhary B. Effect of blast disease on rice yield. Nepal Agriculture Research Journal. 1999; 3:8-13.
11. Koutroubas SD, Katsantonis D, Ntanos DA, Lupotto E. Blast disease influence on agronomic and quality traits of rice varieties under Mediterranean conditions. Turk. J. Agric. 2009; 33:487-494
12. Amadioha AC. Controlling rice blast *in vitro* and *in vivo* with extracts of Azadirachta indica. Crop Protection. 2000; 19:287-290.
13. Tuite J. Plant Pathological Methods, Fungi and Bacteria. Burges Publishing Company, New York, 1969, 239.
14. Rao SN, Kulkarni S. Effect of Trichoderma spp. on growth of Sclerotium rolfsii Sacc. Journal of Biological Control. 2003; 17:181-184.
15. Rangajaran S, Saleena LM, Vasudevan P, Nair S. Biological suppression of rice diseases by Pseudomonas spp. Under saline soil conditions. Plant Soil. 2003; 251:73-82
16. Riungu GM, Muthorni JW, Narla RD, Wagacha JM, Gathumbi JK. Management of Fusarium head blight of wheat and deoxynivalenol accumulation using antagonistic microorganisms. Plant Pathol J. 2008; 7:13-19.
17. Whipps JM. Microbial interaction and biocontrol in the rhizosphere. J Experi Bot. 2001; 52(1):487-511.
18. Larena I, Melgarejo P, Cal ADe. Production, survival and evaluation of solid substrate inoculums of Penicillium oxalicum, a biocontrol agent against Fusarium wilt of tomato. Phytopathology. 2002; 92:863-869.
19. Srinon W, Chuncheen K, Jirattiwarutkul K, Soytong K, Kanokmedhakul S. Efficacies of antagonistic fungi against Fusarium wilt disease of cucumber and tomato and the assay of its enzyme activity. J Agri Technol. 2006; 2(2):191-201.
20. Yaqub F, Shahzad S. *In vitro* evaluation of microbial antagonists against Sclerotium rolfsii. Pak J Bot. 2005; 37(4):1033-1036.
21. Gouramanis GD. Biological and chemical control of rice blast disease (Pyricularia oryzae) in North Greece. Cahiers Options Méditerranéennes. 1995; 15:61-68.
22. Watanabe N. Antagonism by various kinds of Trichoderna fungi to airborne plant pathogens. Bulletin of the Faculty of Agriculture-Meiji University. 1985; 68:1-9.