

## To find out the efficacy of different bio-agents, botanicals, fungicides and nano-fungicides against *Pyriculariaoryzae*.

**Comment [AS1]:** The title should be "Efficacy of biological and chemical agents against *Pyriculariaoryzae*"

### ABSTRACT

A study entitled '~~To find out the efficacy of different bio-agents, botanicals, fungicides and nano-fungicides against *Pyricularia oryzae*~~' was conducted during the period 2021-2023 at Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur and the field experiments at Student Instructional Farm, Chandra Shekhar Azad University of agriculture and Technology. Objectives are to evaluate bio-agent, botanicals, Azotrix (Azoxystrobin 16.7% + Tricyclazole 33.3% SC) and nanofungicides alone to evaluate their efficacy in reduction of mycelial growth of *Pyriculariaoryzae* causing rice blast in Completely randomised design (CRD) under in-vitro condition by adopting principle of dual culture technique and food poisoning technique. All the selected treatments proved effective and reduce mycelial growth of pathogen among bioagents *T. viride* were superior over *T. harzianum*, and *Pseudomonas fluorescens* was better than *Bacillus subtilis*, in botanicals bael leaf extract superior over neem leaf extract, Azotrix alone can reduce growth upto 85% over control and among nanofungicides Silver nanoparticles was found best.

**Comment [AS2]:** Who write affiliation in abstract section?

**Keywords:** Rice blast, *pyriculariaoryzae*, botanicals, bioagents, Nanofungicides.

**Comment [AS3]:** Very bad writing and focus on research

### Introduction

Rice (*Oryza sativa* L.) belongs to the family *Poaceae* and sub-family *Oryzoideae*. It is an essential food crop grown worldwide ~~and gaining importance for more than half of world population~~. Rice is an annual crop which flourishes comfortably in hot and humid climates. It is a good nutritional source of carbohydrate which provides an instant energy. It is the major source of food for more than 2.7 billion people of the world and by the year of 2025, this number will grow to 3.9 billion people. Human consumption accounts for 85% of total production for rice. More than 90% of the world's rice is produced and consumed in the Asia-Pacific region. Rice has shaped the culture, diets and economic of thousands of millions of people. For more than half of the humanity "Rice is life". Considering its importance position, the United Nation designated year 2004 as the "International Year of Rice". There are numbers of cultivar in rice crop but Basmati rice hold prime position for public demand due to its unique aroma, taste and features. The word 'Basmati' is

**Comment [AS4]:** Write the name of country, where highest production of rice recorded with reference.

derived from two Sanskrit roots: (“vas” fragrance) and (may be entrenched or present from the beginning). Basmati, a unique fragrance rice, is a natural gift to the Indian subcontinent. It is the queen of rice. Epicureans praised its exquisite scent, taste, and texture, deeming it the most aromatic rice in the world. Aromatic rice emits a particular aroma in fields, during harvesting, storage, milling, cooking and eating. (Efferson, 1985).

Comment [AS5]: Any recent reference

The estimated total volume of milled rice produced worldwide reached over 502 million metric tons in the 2022/2023 crop year. India is the largest rice producing country accounting for about one third of the world acreage under the crop. The major rice growing states of India are West Bengal, Uttar Pradesh, Madhya Pradesh, Bihar, Odisha, Karnataka, Chhattisgarh, Andhra Pradesh and Gujarat. As per the Indian Agriculture Ministry, the area for rice production in 2023 is 411.52 lakh hectare, which is higher than 404.72 lakh hectare during 2022. India's estimated a production volume of rice was over 135 million metric tons having yield of 4.3 T/ha (Ministry of Agriculture & Farmers Welfare, 2023).

India is the largest exporter of rice globally. In 2022, the production of basmati rice was down 15 percent in 2021 due to heavy rainfalls during the harvesting seasons. However, overall rice exports increased to 46 percent as compared to the previous year because of an increase in the purchase of rice by neighbouring countries such as Bangladesh, Vietnam, and China. India is the leading exporter of Basmati Rice to the global market. The country has exported 4558972.23 MT of Basmati Rice to the world for the worth of Rs. 38524.11 Crores (4787.50 US\$ Mill.) during the year 2022-23 (APEDA).

Comment [AS6]: Introduction part is incomplete, because here author talk about only rice, but the title include fungus *Pyriculariaoryzae*.

## MATERIALS AND METHODS

A study entitled To find out the efficacy of different bio-agents, botanicals, fungicides and nano-fungicides against *Pyriculariaoryzae* was conducted during the period 2021-2023 at, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur and the field experiments at Student Instructional Farm, Chandra Shekhar Azad University of agriculture and Technology, Kanpur. The suitable concentrations of botanical extract (@ 10 %, 20%, 40%, and 60 %), bio-agents (*Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*), fungicides and nanofungicides will be used against mycelial growth of *Magnoporthaegrisea* by the poison food technique (Shravelle, 1961) and dual culture

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Comment [AS10]: 1. *Magnoporthaegrisea* cause different disease from *Pyriculariaoryzae*  
2. Why author use *Magnoporthaegrisea* fungus while in title have *Pyriculariaoryzae*.

technique (Dickinson and Skidmore, 1976) respectively. Per cent inhibition of growth will be calculated by following formula as given by (Horsfall, 1956) and data obtained on per cent inhibition were subjected to statistical analysis-

$$X = \frac{Y-Z}{Y} \times 100$$

Where, X=Percent growth inhibition

Y=Growth of fungus in control (mm)

Z=Growth of fungus in treatment (mm)

### Recording observations:

The present investigation was conducted under the laboratory conditions to study the “Development of Bio-intensive Integrated Disease Management (BIDM) practices against blast of basmati rice caused by *Magnaportha grisea*” The findings are presented to focus the research of other scientists' that has a direct or indirect relationship with the current topic.

### Results And Discussion

#### 1. In vitro screening of the bioagent antagonists

Dual culture technique was used for studying the mechanism of parasitism of the bioagent on *P. oryzae*. When fungal and bacterial isolates were paired with *P. oryzae*, among *Trichoderma* species, the percentage inhibition of fungal growth was recorded (Table 1). The percentage inhibition was significantly superior for *Trichoderma Viride* (46.47 per cent), compared to *Trichoderma harzianum* (43.26 per cent) and hence *Trichoderma Viride* was selected for further studies (Table 1). The effectiveness of the potential bio control agent, This observation is in line with findings of (Singh *et al.*, (2005) and (Nayar (1996) where it could reduce the leaf blast by treatment with *Trichoderma* sp. and *Pseudomonas fluorescense* respectively. Based on the data of Table, the *Pseudomonas fluorescense* which had higher percentage of inhibition on *P. oryzae* (57.71 per cent) than *Bacillus subtilis* (48.54 per cent) was selected as the bacterial antagonist for further studies (Table 1).

Comment [AS11]: Author have no focused in his research.  
This is just a joke.

**Table 1. Efficacy of different bio agents against *M. grisea***

Radial mycelial growth(mm)					
Name of Bioagents	3rd Day	5th Day	7thDay	Percent inhibition over control	
<i>Trichodermaharzianum</i>	8.56	18.61	51.06	43.26	
<i>TrichodermaViride</i>	10.51	17.31	48.17	46.47	
<i>Psuedomonasfluroscence</i>	24.31	32.96	38.06	57.71	
<i>Bacillus subtilis</i>	29.21	40.13	46.31	48.54	
control	90	90	90		
C.D.	2.317	3.298	5.288		
SE(m)	0.657	0.935	1.499		

## 1.2 In vitro evaluation of botanicals on the inhibition of mycelial growth of *P. oryzae*

### 1.2.1 Evaluation of Bael leaf extract against *M. grisea* in vitro

The efficacy of Bael leaf extract against *M. grisea*, at 10%, 20%, 40% and 60% concentrations is shown in table (Table 2). The results of in vitro study evinced that, at 10% concentration the mycelium growth inhibition was 65.28% of *M. grisea* while, at 20% concentration it was 72.37% inhibition of *M. grisea* and at 40% concentration mycelium inhibition was 79.62% however, at 60% concentration maximum mycelium growth inhibition was recorded 86.32% of *M. grisea*. The evaluated data are in favour of findings of previous researcher work done like Dutta and Kalha (2011); Routa *et al.*, (2012)

**Table 2 Efficacy of Bael leaf extract against *M. grisea***

			Radial mycelial growth (mm)		
Name of Botanicals	Cocentration	3rd Day	5th Day	7thDay	Percent inhibition over control
Bael leaf extract	10%	20.12	26.12	31.06	65.28
	20%	16.43	19.56	24.86	72.37
	40%	12.89	14.21	18.34	79.62

	60%	7.53	8.91	12.31	86.32
<b>Control</b>		90	90	90	
<b>C.D.</b>		<b>1.753</b>	<b>2.133</b>	<b>2.644</b>	
<b>SE(m)</b>		<b>0.497</b>	<b>0.605</b>	<b>0.75</b>	

### 1.2.2 Evaluation of Neem leaf extract against *M. grisea* in vitro

Another botanical i.e., Neem leaf extract also used against *M. grisea* at 10%, 20%, 40% and 60% concentrations is shown in table (Table 3). The results of in vitro study revealed out that, at 10% concentration the mycelium growth inhibition was 62.82% of *M. grisea* while, at 20% concentration it was 67.95% inhibition of *M. grisea* and at 40% concentration mycelium inhibition was 74.43% however, at 60% concentration maximum mycelium growth inhibition was recorded 80.92% of *M. grisea* and result are in line of Amadioha (2000) and Kishore *et al.*, (2007).

**Table 3** Efficacy of Neem leaf extract against *M. grisea*

Radial mycelial growth (mm)					
Name of Botanicals	Concentration	3rd Day	5th Day	7th Day	Percent inhibition over control
<b>Neem leaf extract</b>	10%	26.21	29.12	33.46	62.82
	20%	18.31	23.56	28.84	67.95
	40%	14.29	19.54	23.01	74.43
	60%	11.98	14.61	17.17	80.92
<b>Control</b>		90	90	90	
<b>C.D.</b>		<b>2.116</b>	<b>2.601</b>	<b>3.069</b>	
<b>SE(m)</b>		<b>0.6</b>	<b>0.737</b>	<b>0.87</b>	

### 1.2.3 Evaluation of Fungicide against *M. grisea* in vitro

Azotrix (Combination of Azoxystrobin 16.7 % + Tricyclazole 33.3% SC) fungicides were evaluated at 25ppm, 50 ppm and 100ppm, the percentage of inhibition of all of them were significantly different from each other (Table 4). The highest percent inhibition 85.03% over control was recorded at 100ppm, after that 61.87% at 50ppm and least inhibition percentage observed at 25ppm was 17%. The increase in per cent of inhibition was significantly increases with increase in

concentration of fungicide. The similar results were also reported by Naik *et al.*, 2014; Kunova *et al.*, 2013 and Surapuet *et al.*, 2017 against *P. oryzae*. Ravikumar *et al.*, 2022 in his research concluded that Azoxystrobin 16.7% + Tricyclazole 33.3% SC @ 750 ml/ha was best in controlling the sheath blight, blast and brown spot disease of paddy.

**Table 4** Efficacy of Azotrix against *M. grisea*

Radial mycelial growth (mm)					
Name of Fungicide	Concentration (ppm)	3rd Day	5th Day	7th Day	Percent inhibition over control
Azotrix (Azoxystrobin 16.7% + Tricyclazole 33.3% SC)	25	39.21	53.96	74.7	17
	50	18.12	24.13	34.31	61.87
	100	7.23	11.12	13.47	85.03
<b>Control</b>		90	90	90	
<b>C.D.</b>		<b>3.417</b>	<b>4.708</b>	<b>6.49</b>	
<b>SE(m)</b>		<b>0.848</b>	<b>1.168</b>	<b>1.61</b>	

#### 1.2.4 Evaluation of Nanofungicide against *M. grisea* in vitro

Among three synthetic nanofungicide namely silver nanoparticles, copper chitosan and salicylic acid nanoparticles having concentration 10, 20 and 100 respectively (Table 5). Silver nanoparticles show the maximum percent inhibition of mycelium over control i.e., 61.87% at 10 ppm while copper chitosan inhibit upto 48.55% percent of mycelium growth at 20 ppm. However salicylic acid significantly inhibit 43.21% mycelium growth at 100 ppm with respect to other nanoparticles (Table 5). The above findings are in favour of Elamawiet *et al.*, 2013; Silva *et al.*, 2018 and Gadereret *et al.*, 2014.

**Table 5** Efficacy of Different nanofungicides against *M. grisea*

Radial mycelial growth (mm)
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Name of Nanofungicide	Cocentration(ppm)	3rd Day	5th Day	7thDay	Percent inhibition over control
silver nanoparticles	10	12.96	23.56	34.31	61.87
Copper chitosan	20	17.13	32.12	46.3	48.55
salicylic acid	100	14.54	29.36	51.12	43.21
Control		90	90	90	
C.D.		<b>2.175</b>	<b>4.147</b>	<b>6.435</b>	
SE(m)		<b>0.539</b>	<b>1.029</b>	<b>1.596</b>	

### Conclusion

Our findings showed that radial growth of fungal mycelium were inhibited as influenced by different treatments. Among the bio-agents and botanicals *Pseudomonas fluorescens*, *Trichoderma viride*, bael leaf extract and neem leaf extract suppressed the growth of *P. oryzae* and proved its potential as bio-control. More studies are therefore needed to confirm the current findings and to determine the most effective formulation against *P. oryzae*.

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