

Efficacy of different bio-agents, botanicals, fungicides and nano-fungicides against *Pyricularia oryzae*.

ABSTRACT

A study 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, during research period different bio-agent, botanicals, Azotrix (Azoxystrobin 16.7 % + Tricyclazole 33.3% SC) and nanofungicides are used and data were recorded regarding their efficacy in inhibition of mycelial growth of *Pyricularia oryzae* 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* (46.47%) were superior over *T. harzianum* (43.26 %) and *Pseudomonas fluorescense* (57.71 %) was better than *Bacillus subtilis* (48.54%), in botanicals bael leaf extract were found superior (86.32%) over neem leaf extract (74.43%), Fungicide (Azotrix @ 25, 50 and 100ppm) can reduce growth up to 85% over control and among nanofungicides Silver nanoparticles @ 10ppm was found best which can inhibit (61.87%) mycelial growth.

Keywords: Rice blast, *pyricularia oryzae*, botanicals, bioagents, Nanofungicides.

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. 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 Nations designated year 2004 as the "International Year of Rice". There are numbers of cultivars in rice crop but Basmati rice holds prime position for public demand due to its unique aroma, taste and features. The word 'Basmati' is 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).

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). The productivity of rice in Uttar Pradesh as comparing to West Bengal is quite low due to several biotic and abiotic stresses. Among the biotic stresses, diseases are the major constraints. The major diseases of rice crop are caused due to bacterial, fungal, viral and nematodes etc. The major fungal diseases of rice are blast (*Pyriculariaoryzae*), sheath rot (*Sarocladiumoryzae*), brown leaf spot (*Helminthosporiumoryzae*) and false smut (*Ustilaginoideavirens*). Among the diseases of rice, blast caused by *Pyriculariaoryzae* Cavara, is very devastating disease which can causes economically significant losses annually to the crop and yield loss as high as 70–80% when pre-disposing factors favour epidemic development (Piottiet *al.*, 2005).

MATERIALS AND METHODS

A study entitled “Efficacy of different bio-agents, botanicals, fungicides and nano-fungicides against *Pyriculariaoryzae*” was conducted during the period 2021-2023 at,

Department of Plant Pathology and Student Instructional Farm, Chandra Shekhar Azad University of agriculture and Technology. The suitable concentrations of botanical extract (@ 10 %, 20%, 40%, 60 %), bio-agents (*Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*), fungicides and nanofungicides were used against mycelial growth of *Magnaportha grisea* by the poison food technique (Shravelle, 1961) and dual culture technique (Dickinson and Skidmore, 1976) respectively. Per cent inhibition of growth were 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

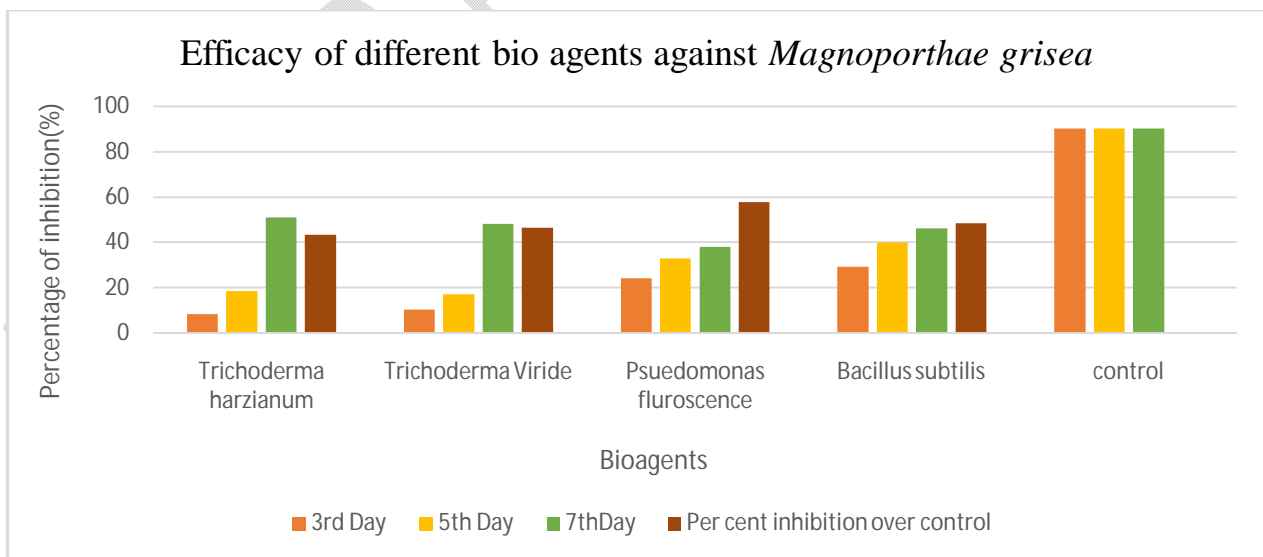
Dual culture technique was used for studying the mechanism of parasitism of the bioagent on *P. oryzae*. When fungal and bacterial isolates were paired with *P. oryzae*, among *Trichoderma* species, the percentage inhibition of fungal growth was recorded (Table 1). The percentage inhibition was significantly 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 fluorescens* respectively. Based on the data of Table, the

Psuedomonasfluroscence 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. & Fig 1.).

Table:1. Efficacy of different bio agents against *Magnoporthae grisea*

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

Fig:1. Efficacy of different bio agents against *Magnoporthae grisea*



2. In vitro evaluation of botanicals on the inhibition of mycelial growth of *Magnoporthae grisea*

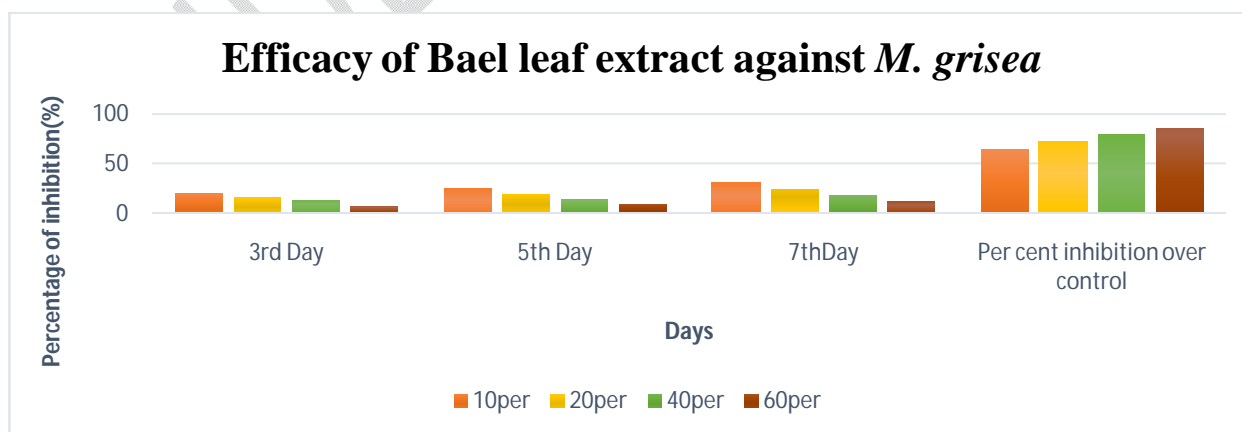
2.1. Evaluation of Bael leaf extract against *Magnoporthae grisea* in vitro

The efficacy of Bael leaf extract against *M. grisea*, at 10%, 20%, 40% and 60% concentrations is shown in table (Table 2 & Fig 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 *Magnoporthae 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
Control		90	90	90	
C.D. @ 5%		1.753	2.133	2.644	
SE(m)±		0.497	0.605	0.75	

Fig:2 Efficacy of Bael leaf extract against *M. grisea*



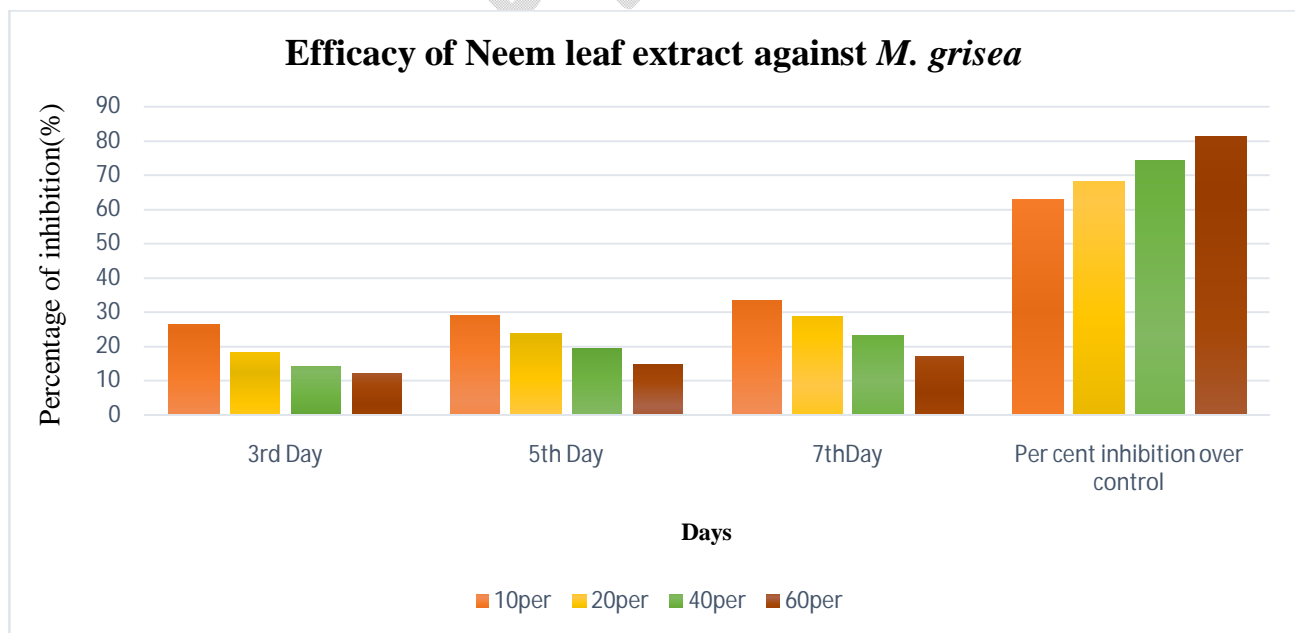
2.2. Evaluation of Neem leaf extract against *M. grisea* in vitro

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 & Fig 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	Cocentration	3rd Day	5th Day	7thDay	Percent inhibition over control
Neem leaf extract	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
Control		90	90	90	
C.D.		2.116	2.601	3.069	
SE(m)		0.6	0.737	0.87	

Fig: 3.Efficacy of Neem leaf extract against *Magnoporthaegrisea*



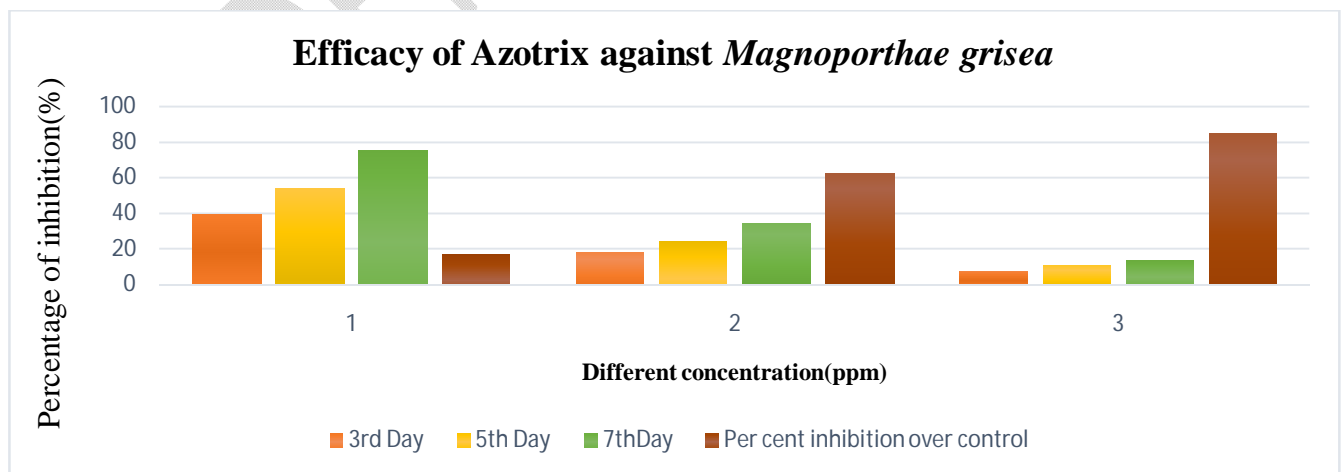
3. Evaluation of Fungicide against *Magnoporthaegrisea* in vitro

Azotrix(Combination of Azoxystrobin 16.7 % + Tricyclazole 33.3% SC) fungicides were evaluated at 25, 50 and 100ppm, the percentage of inhibition of all of them were significantly different from each other (Table 3.& Fig 3.). The highest percent inhibition 85.03% over control was recorded at 100ppm, after that 61.87% at 50ppmand 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 byNaiket *al.*, 2014; Kunovaet *al.*, 2013 and Surapuet *al.*,2017 against *P. oryzae*.Ravikumaret *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 Azotrixagainst *M. grisea*

Radial mycelial growth (mm)					
Name of Fungicide	Cocentration(ppm)	3rd Day	5th Day	7thDay	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
Control		90	90	90	
C.D.		3.417	4.708	6.49	
SE(m)		0.848	1.168	1.61	

Fig: 4.Efficacy of Azotrixagainst *Magnoporthae grisea*



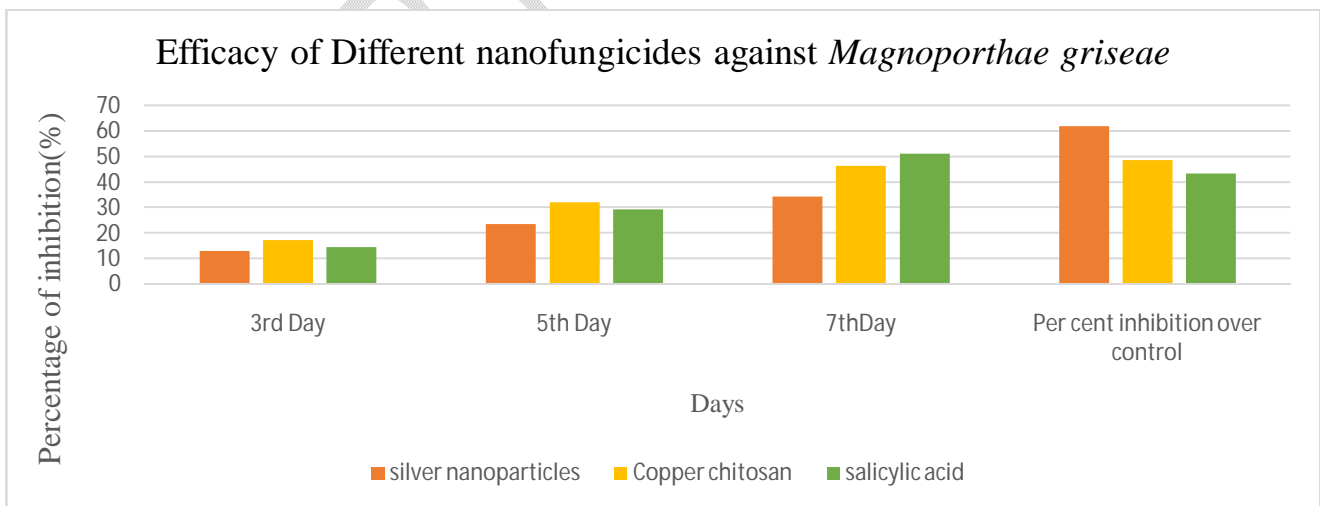
4. Evaluation of Nanofungicide against *Magnoporthae grisea* in vitro

Among three synthetic nanofungicide namely silver nanoparticles, copper chitosan and salicylic acid nanoparticles having concentration 10, 20 and 100 respectively (Table 4). 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 100ppm with respect to other nanoparticles (Table 4. & Fig 4.). The above finding are in favour of Elamawiet *et al.*, 2013; Silva *et al.*, 2018 and Gadereret *et al.*, 2014.

Table:5.Efficacy of Different nanofungicides against *Magnoporthae grisea*

Radial mycelial growth (mm)					
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.		2.175	4.147	6.435	
SE(m)		0.539	1.029	1.596	

Table: 6.Efficacy of Different nanofungicides against *Magnoporthae grisea*



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*.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

References

Anonymous: https://agriwelfare.gov.in/en/Agricultural_Statistics_at_a_Glance

Dutta, U., & Kalha, C. S. (2011). In vitro evaluation of fungicides, botanicals and bioagents against *Rhizoctonia solani* causing sheath blight of rice and their integration for effective management of the disease under field conditions. *Plant Disease Research*, 26(1), 14-19.

Dutta, U., & Kalha, C. S. (2011). In vitro evaluation of fungicides, botanicals and bioagents against *Rhizoctonia solani* causing sheath blight of rice and their integration for effective management of the disease under field conditions. *Plant Disease Research*, 26(1), 14-19.

- Kunova, A., Pizzatti, C. and Cortesi, P. (2013). Impact of Tricyclazole and Azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus (*Pyriculariaoryzae*). *Pest Manage. Sci.*, 69(2):278-284.
- Naik, V.K.B. and Jamadar, M.M. (2014). In- vitro bioassay of different fungicides against blast of pearl millet caused by *Pyriculariagrisea* (Cooke.) Sacc. *Karnataka J. Agril. Sci.*, 27(1): 88-90.
- Nayar, K. 1996. Development and evaluation of a biopesticide formulation for control of foliar pathogens of rice. Ph.D. Thesis, Tamilnadu Agricultural University, Coimbatore, 186p.
- Piotti, E., Rigano, M. M., Rodino, D., Rodolfi, M., Castiglione, S., Picco, A. M., & Sala, F. (2005). Genetic structure of *Pyriculariagrisea* (Cooke) Sacc. isolates from Italian paddy fields. *Journal of Phytopathology*, 153(2), 80-86.
- Ravikumar MR, Ganapathi T. Pre-mix evaluation of azoxystrobin 16.7% + tricyclazole 33.3% SC in controlling Sheeth blight, blast and brown spot diseases of rice (*Oryzasativa* L.). *Pharma Innovation* 2022;11(3):189-194.
- Routa, S., Thatoib, H.N. and Tewaria, S.N. (2012). Performance of aqueous extract of *Aegle marmelos* based Amaext-a, an antifungal product against *Pyriculariagrisea* causing blast disease of rice. *Archives of Phytopathology and Plant Protection*, 45(20): 2507–2518.
- Routa, S., Thatoib, H.N. and Tewaria, S.N. (2012). Performance of aqueous extract of *Aegle marmelos* based Amaext-a, an antifungal product against *Pyriculariagrisea* causing blast disease of rice. *Archives of Phytopathology and Plant Protection*, 45(20): 2507–2518.
- Singh, R.P., Kumar, M.S., Madhavilatha, L., Singh, S.P., Singh, R.B. and Singh, H.B. 2005. Reduced disease incidence and increased rice production in upland irrigated and rainfed ecosystem through application of *Trichoderma harzianum* NBRI-1055 and *Azospirillum*. *Indian J. Plant Pathol.* 23(1&2):37-41
- Surapu, R. B., Rani, C. D., Aruna, J., Vijay, S., Reddy, P. N., & Prasad, M. S. (2017). In Vitro Evaluation of Fungicides against Rice Blast Isolates to Assess Development of Fungicidal Resistance. *International Journal of Current Microbiology and Applied Sciences*, 53-60.