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**D Prathyusha**
Ph.D. Scholar, Department of Plant Pathology, ANGRAU Agricultural College, Bapatla Andhra Pradesh, India

**AK Patibanda**
Professor, Department of Plant Pathology, ANGRAU, SMGR Agricultural College, Udayagiri, Nellore, Andhra Pradesh, India

**V Prasanna Kumari**
Professor and Head, Department of Plant Pathology, ANGRAU, Agricultural College, Bapatla, Andhra Pradesh, India

**P Madhusudhan**
Senior Scientist, Department of Plant Pathology, ANGRAU ARS, Nellore, Andhra Pradesh, India

**K Jayalalitha**
Principal Scientist, RRU (Crop Physiology), ANGRAU RARS, LAM, Guntur, Andhra Pradesh, India

**V Srinivasa Rao**
Professor and University Head Department of Statistics and Computer Applications, ANGRAU, Agricultural College Bapatla, Andhra Pradesh, India

**Corresponding Author:**
**D Prathyusha**
Ph.D. Scholar, Department of Plant Pathology, ANGRAU Agricultural College, Bapatla, Andhra Pradesh, India

# Evaluation of different single and combination fungicides against *in vitro* growth of *Pyricularia oryzae* causing rice blast disease

**D Prathyusha, AK Patibanda, V Prasanna Kumari, P Madhusudhan, K Jayalalitha and V Srinivasa Rao**

**Abstract**
Rice blast disease caused by *Pyricularia oryzae* is a major threat to the farmers as the disease severity may lead up to 80% crop loss in severe conditions. A laboratory experiment was conducted at Department of Plant Pathology, Acharya N.G. Ranga Agricultural University, Agricultural College, for testing the efficacy of eight different single and combination fungicides adopting poisoned food method against the pathogen *Pyricularia oryzae* causing rice blast disease. The experiment was carried out in a completely randomized design with eight treatments including control following 3 replications of each. Three concentrations (R – recommended dose; R/2 and 2R) each of different fungicides *viz*., Beam (Tricyclazole 75% WP @ 0.06%), Amistar (Azoxystrobin 25% SC @ 0.10%), Fujione (Isoprothiolane 5% EC @ 0.15%), Kasugamycin @ 0.25%, Folicur (Tebuconazole 25% EC @ 0.15%), Nativo 75% WG (Trifloxystrobin 50% + Tebuconazole 25% WG @ 0.08%), Custodia (Azoxystobin + Tebuconazole @ 0.15%) and Filia (Propiconazole + Tricyclazole @ 0.10%) were evaluated against the radial growth of pathogen. Results showed that except two fungicides *viz*., Kasugamycin and Azoxystrobin rest all the fungicides fully inhibited (100% inhibition) the mycelial growth of the pathogen at all the three different concentrations compared to control.

**Keywords:** Rice, *Pyricularia oryzae*, Fungicides, Poisoned Food Technique

## Introduction
Rice (*Oryza sativa* L.) is the second most important cereal cultivated globally and has been nourishing more than half of the world's population (Singh and Singh, 2019). India is the second largest producer (110.15 MT) of rice in the world while Andhra Pradesh is one among the leading states where huge production (7.49 MT) is achieved. Of all the biotic and abiotic factors that impact the yield, leaf blast disease caused by *Pyricularia oryzae* is of significant importance which can scale down the yield with 80% losses depending on the crop stage it's going to affect (Neupane & Kiran Bhusal, 2021) [9]. It is polycylic, hemibiotrophic foliar pathogen, prevalent globally in humid areas where the rice is being cultivated (Neelakanth *et al*., 2017) [7]. Seed borne nature and its capacity to infect all crop stages can impose severe devastation in crop. Cultural practices like use of resistant varieties, proper fertilizer application and weed management besides biological and chemical control measures offer the reliable disease control against leaf blast. The pathogen is highly variable and numerous pathotypes were reported so far. As the resistant cultivars are frequently being susceptible to the developing pathotypes, fungicide spraying is inevitable for efficient control of leaf blast where the resistance in crop is lacking (Groth, 2006; Morton and Staub, 2008; Pooja and Katoch, 2014) [2, 6, 10]. The present study was deployed to evaluate the efficacy of single and novel combinations of fungicide against rice blast.

## Material and Methods
A total of eight fungicides (Table 1) at three different concentrations (recommended (R), R/2 and 2R) were evaluated *in vitro* against *P. oryzae* by poisoned food technique (Nene and Thapliyal, 1993). Fungicide stock solution of 100000 ppm was prepared initially by dissolving 1 g of fungicide in 10 ml of sterile distilled water. Later, working standards of desired concentration of the poisoned media was made by using the formula $C_1V_1 = C_2V_2$

Where,
$C_1$ = concentration of the stock solution (ppm)
$V_1$ = volume of the stock solution to be added (ml)
$C_2$ = desired concentration (ppm)
$V_2$ = volume of PDA in which fungicide is to be amended (ml)

The poisoned medium (20ml) of the required concentration was poured in sterile petri plates under laminar air flow in triplicate and allowed to solidify. Culture discs of 5 mm diameter cut from the periphery of actively growing mycelium (seven days old) were inoculated in the centre of each petriplate under aseptic conditions and incubated at 28±1 °C in a BOD incubator. Unamended PDA plate inoculated with *P. oryzae* served as check. Radial growth of the test fungus was recorded 14 days after inoculation. Percent inhibition of growth over check was calculated using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = percent inhibition.
C = growth of *P. Oryzae* in unamended medium.
T = growth of *P. Oryzae* in amended medium.

**Table 1:** Details of Treatments for *in vitro* evaluation of fungicides

| Treatments | Fungicides | Trade Name | Recommended Conc (R) | R/2 | 2R |
|---|---|---|---|---|---|
| $T_1$ | Tricyclazole | Beam | 0.06%(600 ppm) | 0.03%(300 ppm) | 0.12%(1200 ppm) |
| $T_2$ | Azoxystrobin | Amistar | 0.10%(1000 ppm) | 0.05%(500 ppm) | 0.20%(2000 ppm) |
| $T_3$ | Kasugamycin | Kasu B | 0.25(2500 ppm) | 0.125(1250 ppm) | 0.5(5000 ppm) |
| $T_4$ | Isoprothiolane | Fuji-one | 0.15%(1500 ppm) | 0.075%(750 ppm) | 0.30%(3000 ppm) |
| $T_5$ | Tebuconazole | Folicur | 0.15%(1500 ppm) | 0.075%(750 ppm) | 0.30%(3000 ppm) |
| $T_6$ | Trifloxystrobin + Tebuconazole | Nativo | 0.08(800 ppm) | 0.04(400 ppm) | 0.16(1600 ppm) |
| $T_7$ | Azoxystrobin 11% + tebuconazole 18.3 % SC (0.15%) | Custodia | 0.15%(1500 ppm) | 0.075%(750 ppm) | 0.30%(3000 ppm) |
| $T_8$ | Propiconazole + Tricyclazole | Filia | 0.10%(1000 ppm) | 0.05%(500 ppm) | 0.20%(2000 ppm) |
| $T_9$ | Control | | | | |

## Result and Discussions

Upon evaluation of fungicides mentioned in Table 2, a significant reduction in mean radial growth of the pathogen was noticed with the increasing concentration. All the treatments except for Kasugamycin and Azoxystrobin, tested at three concentrations were found effective over the control with cent % inhibition of the pathogen.

Decrease in mycelial growth with the increased concentration was noticed in both Kasugamycin and Azoxystrobin fungicide treatments. At half of the recommended concentration (R/2), least percent inhibition of 67.44% was noticed in Azoxystrobin where the maximum radial growth of 2.93 cm was observed, followed by Kasugamycin with 84.11% inhibition (1.43 cm).

**Table 2:** *In vitro* efficacy of fungicides against *Pyricularia oryzae*

| S. No. | Treatments | Dose R (%) | Colony diameter (cm)❖ and Percent inhibition over control | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | R/2 | | R | | 2R | |
| | | | Radial growth (cm) | Percent inhibition over control | Radial growth (cm) | Percent inhibition over control | Radial growth (cm) | Percent inhibition over control |
| 1 | Tricyclazole 75% WP | 0.06 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| 2 | Azoxystrobin 25% SC | 0.10 | 2.93(1.98) | 67.44 | 2.60(1.89) | 71.11 | 2.50(1.87) | 72.22 |
| 3 | Isoprothiolane 5% EC | 0.15 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| 4 | Kasugamycin 3% SL | 0.25 | 1.43(1.56) | 84.11 | 1.30(1.51) | 85.55 | 0.80(1.34) | 91.11 |
| 5 | Tebuconazole 25% EC | 0.15 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| 6 | Trifloxystrobin 50% + Tebuconazole 25% WG | 0.08 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| 7 | Azoxystobin 11% + Tebuconazole 18.3% SC | 0.15 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| 8 | Propiconazole 10.7% + Tricyclazole 34.2% SE | 0.10 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 | 0.00(1.00) | 100.00 |
| | Mean | - | 0.48 | | 0.47 | | 0.46 | |
| 9 | Control | | 9.00 (3.16) | | | | | |
| | SEM ± | | 0.01 | | 0.01 | | 0.01 | |
| | C.D. ($p \leq 0.05$) | | 0.02 | | 0.03 | | 0.02 | |
| | CV% | | 1.40 | | 1.27 | | 1.13 | |

❖Means of three replications
*Values in parenthesis are square root transformed values

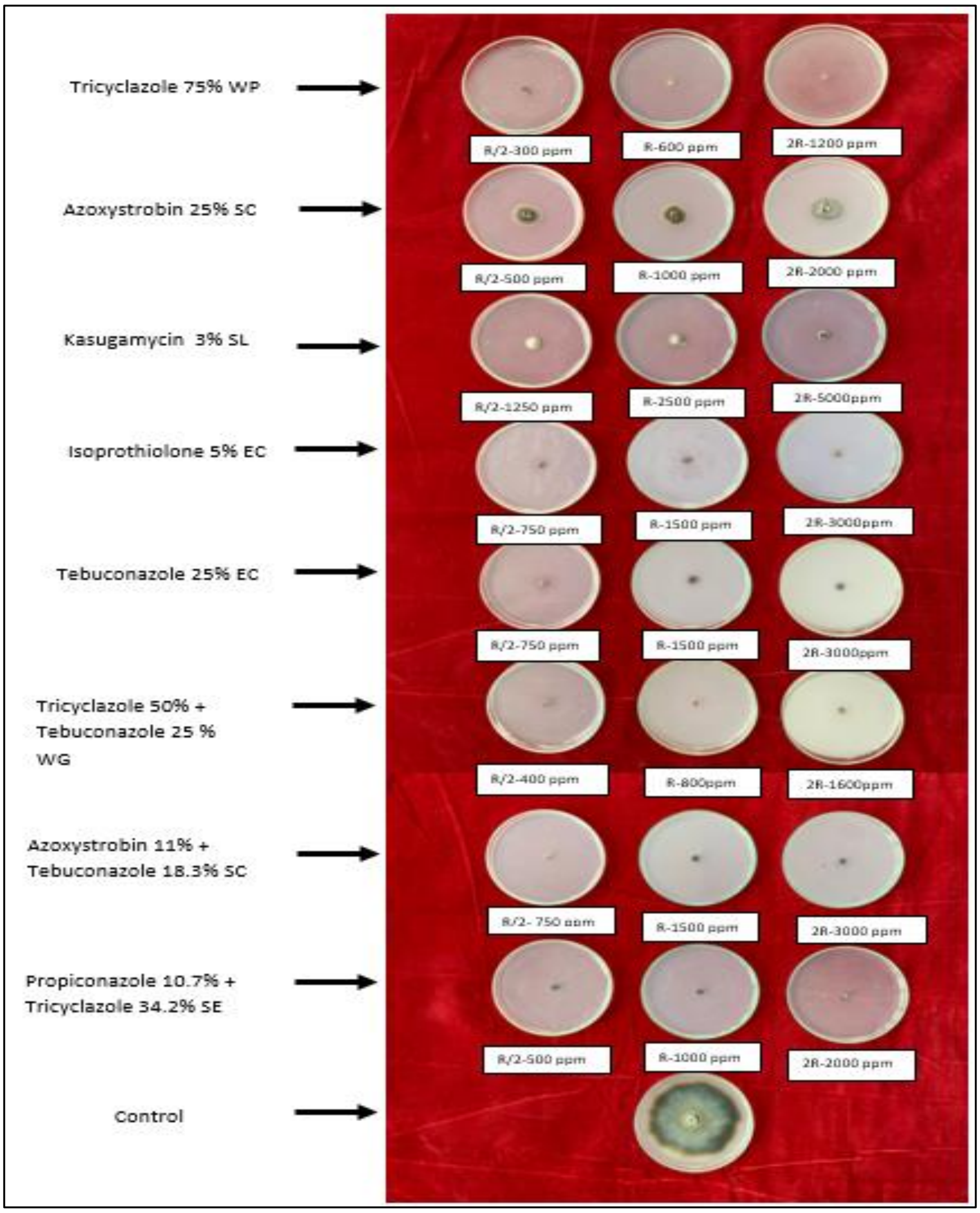


**Fig 1:** Efficacy of chemicals on radial growth of *P. oryzae in vitro*

Though, at the recommended concentration (R) complete inhibition of the pathogen was not noticed intreatments of Kasugamycin and Azoxystrobin. The maximum inhibition of 85.55% was observed in Kasugamycin (1.30 cm), while Azoxystrobin found to be the least effective with 71.11% inhibition over the control. However at twice the dose of recommendation (2R), Azoxystrobin and Kasugamycin was continued to be the least performing among all with 72.22% and 91.11% inhibition respectively, compared to control (Table 2; Fig 1).

The present findings are in agreement with the results of Kavanashree *et al*., 2019; Kulmitra *et al*., 2017 [5]; Neelakanth *et al*., 2017 [7]; Kafle *et al*., 2021; Singh *et al*., 2019 [11]; Vidhyashankar *et al*., 2020 [12]. Ergosterol, being the essential for fungal cell wall structure can cause deleterious effect on cell wall integrity if its biosynthesis was inhibited (Dupont *et al*., 2012) [1]. Majority of the fungicides which showed complete inhibition of mycelia growth were of triazole group members. They proved to specifically inhibit sterol biosynthesis which is essential for maintaining cell wall integrity.

## Conclusion

In the present study, the efficacy of recent fungicides is explored for their potential against the rice blast pathogen. Novel fungicide combinations which can control the pathogen efficiently with least dosages can offer multiple sites of action, exhibiting least chances of resistance development. Fungicide formulations of such *viz*. Trifloxystrobin + Tebuconazole (Nativo), Azoxystobin + Tebuconazole (Custodia) and Propiconazole + Tricyclazole (Filia) can be exploited for the rice blast control even at half of the recommended doses.

**Conflict of Interest:** None declared

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