



Research Article

IN VITRO STUDY OF NEW GENERATION CHEMICALS AGAINST *Rhizoctonia solani* Kuhn CAUSING SHEATH BLIGHT OF RICE

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Abstract- Rice (*Oryza sativa* L.) is the world's second most important cereal crop and is becoming a model plant for cereals. It is the staple food crop for Asian countries, supplying nearly 23% of the per capita energy for six billion people worldwide. Sheath blight of rice caused by *Rhizoctonia solani* Kuhn is a serious threat in all rice growing countries of the world. This disease is distributed in almost all the rice growing states of India. The disease is alarming due to its intensive cultivation of modern high yielding varieties of Rice with high doses of nitrogenous fertilizers. Though the resistant varieties are the best method of management, as the major gene (s) for resistance not yet found and resistance is majorly governed by polygenes, still management of this disease is depended upon the chemicals. This study includes the *In-vitro* evaluation of seven new generation chemical compounds which includes mainly strobilurins and triazols for testing their efficacy against Sheath blight disease. In *In-vitro* study 4 fungicides screened at 5 different concentrations (10,20,40,80,160ppm) and 2 were at 0.5,1,2,4,8ppm concentrations by using poisoned food technique. Among them Tebuconazole 50% + Trifloxystrobin25%WG gave the lowest ED₅₀ value (0.0054g/L) followed by the Hexaconazole5%SC (0.005ml/L) and Propiconazole25%EC (0.011ml/L).

Keywords- Fungicides, *In vitro*, Sheath blight, *Rhizoctonia*, Management

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Introduction

Rice is the staple food crop for Asian countries. Despite advances in rice production technology, diseases remain a major cause of yield loss and lower profits on world rice farms. Use of high-yielding cultivars with less overall disease resistance and greater nitrogen (N) fertilizer requirements has increased rice yields but has also increased diseases. Rice is grown in shorter rotations or no rotations, on increasingly less fertile soils and with decreasing irrigation capacity all will contribute to severe disease condition [1]. Sheath blight disease of rice caused by *Rhizoctonia solani* Kuhn is a serious threat in all rice growing areas of the world. The disease was first recorded from Japan [2]. In India, the disease was first reported from Gurudaspur, Punjab [3]. The market share of fungicide used on rice in India during 2010-11 is Rs 380 crores, of which blast and sheath blight fungicides alone constitute 280 crores and the share of fungicides used against brown spot, BLB, grain discoloration, stem rot and false smut is 100 crores [4]. Globally 8.4 % of fungicides market share is for rice [5]. Since systemic fungicide introduction in the 1960s, they generally provide better disease management than non-systemic products. Development of resistance against fungicides in pathogens poses a threat to the sustainable crop production. To mitigate this problem new generation chemicals with novel mode of action have been introduced in the market in the past few years. Optimisation of doses and selection of effective fungicide are the two major challenges for effective management of sheath blight disease.

Dutta and Kalha (2011) reported all the test fungicides, botanicals/plant leaf extracts and bio agents tested against *Rhizoctonia solani* causing sheath blight of

rice were showed to be static and significantly inhibited mycelial growth of the test pathogen over untreated control [6]. Hong Zhou *et al.* (2013) showed the EC₅₀ values of Boscalid, Jinggangmycin and Hexaconazole were 1.0692, 96.2859 and 0.0240 µg/mL, respectively [7]. Moni *et al.* (2010) conducted *in-vitro* and *in-vivo* evaluation of 5 new fungicides namely Nativo, Hexa, Follicur, Propi and Evaeilt Super against rice sheath blight disease [8]. The highest yield of 4.83 t/ha and 4.89 t/ha and the lowest DSI (disease severity index) 0.25% and 14.69% were observed in Nativo treated plots during 2008 and 2009, respectively. In present study attempt was made to find better chemical with good efficacy to manage this devastating disease which is very important in high rainfall areas.

Materials and Methods

Pathogen Isolation: The test pathogen viz., *Rhizoctonia solani* Kuhn was isolated from infected rice sheath and leaf, by using Modified Ko and Hora medium [5,9,10]. The inoculated culture plates were incubated at 27±1°C. The hyphal tip culture was done to get the pure culture of the pathogen. *Rhizoctonia* pure culture was maintained on PDA [11] at 4°C for further use.

In vitro analysis: The response of *Rhizoctonia solani* Kuhn towards different fungicide molecules at different concentrations was performed following the Poisoned Food Technique [12]. Potato dextrose agar used as base medium for evaluation of *R. solani*. Inoculated Petri plates were incubated at 28±1°C for 3-4 days and the growth (diameter) of the pathogens was measured. Per cent inhibition in growth for different concentrations of individual test fungicide as

compared to fungicide non-amended one was calculated and the ED₅₀ value for the individual fungicide was computed by using the log- probit analysis. Percent inhibition was calculated by using the given formula:

$$\text{Mycelial growth inhibition (\%)} = [(dc-dt)/dc] \times 100$$

Where dc = average diameter of fungal colony in control

dt= average diameter of fungal colony in treatment group

Fungicides used for bioassay and field evaluation

S.No	Fungicide	Concentrations
1	F1.	Azoxystrobin 23%SC (Amister) 10, 20,40,80 and160 ppm
2	F2.	Difenconazole 25% (Score) 10, 20,40,80 and160 ppm
3	F3.	Validamycin 3% (Sheathmar) 10, 20,40,80 and160 ppm
4	F4.	Propiconazole 25% (Tilt) 10, 20,40,80 and160 ppm
5	F5.	Tebuconazole25% EC (Folicure) 10, 20,40,80 and160 ppm
6	F6.	Tebuconazole 50%+Trifloxystrobin 25% WG (Nativo) 0.5, 1.0,2.0,4.0 and8 ppm
7	F7.	Hexaconazole 5%SC (Contaf) 0.5,1.0,2.0,4.0 and 8 ppm

Data was analyzed using statistical package Indo-stat software. The comparisons were done at 0.05 probability level.

Results and Discussion

Fungal growth at different chemical concentrations

All the test fungicides showed decreased growth of *R. solani* with the increase in concentrations which is depicted in [Table-1]. Among the fungicides tested at higher concentrations, Propiconazole 25% EC showed no growth at 40 ppm concentration but others showed at least little growth at the same concentration. Whereas, Tebuconazole 25% EC ceased the growth of test fungus at 80 ppm concentration. Among the test fungicides which were tested at lesser concentrations, Hexaconazole 5%SC showed very less growth i.e., only 9.67 mm. at 8 ppm concentration followed by Tebuconazole 50%+Trifloxystrobin 25% WG showing 15.17 mm growth. The fungicides tested at lower concentrations i.e. .5, 1, 2, 4,8ppm were initially tested at higher concentrations (10, 20, 40, 80, 160) but even at 10 ppm 100% inhibition was observed. So these molecules (F6, F7) are considered as highly toxic against the test pathogen.

Table-1 Mean values of growth (mm) of *Rhizoctonia solani* in *In-vitro* bioassay

Concentration	Growth of <i>R.solani</i> in-vitro					Concentration	F ₆	F ₇
	F ₁	F ₂	F ₃	F ₄	F ₅			
10ppm	42.33	29.17	81.50	15.17	20.67	0.5ppm	49.83	11.67
20ppm	36.50	25.50	67.83	14.50	8.00	1ppm	37.33	10.33
40ppm	36.00	19.83	56.33	0.00	6.50	2ppm	28.33	10.50
80ppm	30.33	17.67	40.00	0.00	0	4ppm	16.17	10.67
160ppm	27.67	13.17	27.50	0.00	0	8ppm	15.17	9.67
C.V%	14.44	5.47	12.49	5.09	22.72	C.V%	11.65	4.72
C.D.(5%)	11.25	3.17	13.45	2.09	8.43	C.D.(5%)	8.18	2.00
SEM	3.65	1.03	4.37	0.68	2.7	SEM	2.66	0.65

Analysis of percent inhibition effect of test chemicals

In case of percent inhibition, [Table-2] the maximum was observed for Hexaconazole 5% SC and Tebuconazole 50%+Trifloxystrobin 25% WG showing 89.26% and 83.15% at just 8 ppm concentration with the lowest ED₅₀ values 0.005 ml/L and 0.0054g/L respectively. At higher concentration lowest ED₅₀ was found in

case of propiconazole i.e. 0.011ml/L. Dutta and kalka (2011), Moni *et al.* (2010) considered similar type of compounds for *in-vitro* studies against *R. solani* they found significant inhibition of mycelial growth of fungus [6,8]. Hexaconazole 5% SC was found to give lowest ED₅₀ value than the others in present investigation. This finding is corroborated with findings of Hong Zhou *et al.* (2013) [7].

Table-2 Percent growth inhibition and ED₅₀ values for test fungicides

Fungicide	Percent reduction in growth at different concentrations					R ²	ED ₅₀ (ml/L)
	10ppm	20ppm	40ppm	80ppm	160ppm		
Azoxystrobin 23% SC	52.96	59.44	60.00	66.30	69.26	0.89	0.038
Difenconazole 25% EC	67.59	71.67	77.96	80.37	85.37	0.98	0.028
Validamycin 3% L	9.44	24.63	37.41	55.56	69.44	0.94	0.060
Propiconazole 25% EC	83.15	83.89	100.00	100.00	100.00	0.82	0.011
Tebuconazole 25% EC	77.04	91.11	92.78	100.00	100.00	0.86	0.013
Fungicide	0.5ppm	1ppm	2ppm	4ppm	8ppm	R ²	ED ₅₀
Tebuconazole50%+ Trifloxystrobin25% WG	44.63	58.52	68.52	82.04	83.15	0.96	0.0054g/L
Hexaconazole 5%SC	87.04	88.52	88.33	88.15	89.26	0.60	0.005

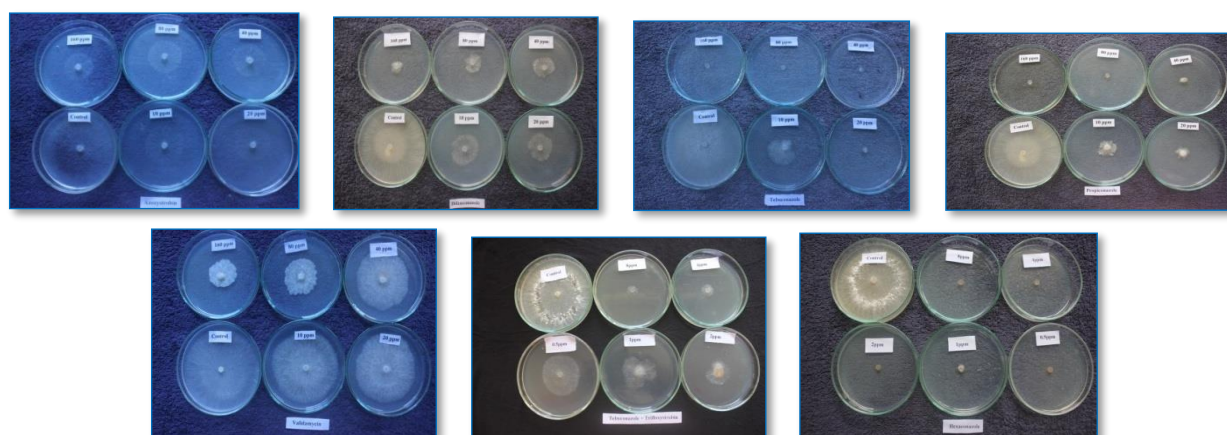


Fig-1 Growth of *Rhizoctonia* under *In vitro* bio assay experiment

Conclusion

Sheath blight disease of rice caused by a soil borne pathogen, *Rhizoctonia solani* is having high economic importance in Terai region of West Bengal. In present study among seven chemicals Hexaconazole 5% SC has shown the highest antifungal activity with lowest ED₅₀ (0.005) value followed by Tebuconazole 50%+ Trifloxystrobin 25% WG. So these chemicals can be recommended to the farmers for control of sheath blight. As there is no absolute resistance found for breeding resistant varieties still chemical control is dependable for managing this destructive disease in many areas.

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Abbreviations:

EC: Emulsifiable concentrate

ED₅₀: Effective Dose for 50% of population

SC: Suspension concentrate

WG: Wettable granules

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

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