International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 1682-1685

Received: 14-05-2018
Accepted: 17-06-2018

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Combination of Azoxystrobin + Difenocanazole provides effective management of sheath blight of rice caused by *Rhizoctonia solani*

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Abstract
Sheath blight of rice, caused by the fungal pathogen *Rhizoctonia solani* kunh. (Sexual stage: *Thanetophorus cucumeris* (Frank) Donk.) is one of the major production constrains in rice growing countries of the world. Under conditions favoring disease, up to 50% grain yield may be lost. Use of short stature well tillering susceptible semi dwarf cultivars planted high densities, leading to dense canopies with moist microclimates, promote disease spread. Both seedlings and adult plants are equally affected but loss in much more when the disease appears in seedlings. The infection and spread of disease before the flag leaf stage revealed 20% grain loss. A trial was conducted to test the efficacy of different combinations of fungicide against sheath blight of rice. We report here in Azoxystrobin 18.2% w/w + difenoconazole 11.4 w/w SC (Amister) effectively reduced the sheath blight severity (11.11%) and also increased the grain yield as compare to other treatment and control. This study also demonstrated that rice producers can reduce sheath blight losses by planting moderately susceptible to moderately resistant cultivars and improve rice grain yield by applying Azoxystrobin 18.2% w/w + 11.4 w/w SC (Amister).

Keywords: Azoxystrobin + Difenocanazole, sheath blight, rice caused, *Thanetophorus cucumeris*

Introduction
Rice sheath blight, caused by the fungal pathogen *Rhizoctonia solani* kunh. (Sexual stage: *Thanetophorus cucumeris* (Frank) Donk) is a major constraint (second only to rice blast) to rice production, causing 5-10% yield losses in low land tropical Asia. The pathogen has a wide host range and can infect more than 32 plant families and 188 genera, often infecting legume crops grown in rotation with rice. The emergence of *Rhizoctonia solani* kunh. as economically important rice pathogen has been attributed to the intensification of rice cropping system with the development of new short stature, high tillering, high yielding varieties, high plant densities, and increase level of fertilizers and other inputs (Chahal *et al.* 2003, Siddiqi 1999) [2, 12] and these factors promote disease spread by providing favorable microclimate condition due to dense leaf canopy with and in increase leaf to leaf and leaf to sheath contact (Savary *et al.* 1995) [10]. Both seedlings and adults plants are equally affected but loss in much ore when the disease spread in seedlings. The older plants are attacked in flooded conditions and swampy rice fields (Dodman and Flentje 1970, Kannaiyan 1987, Shimamoto 1995) [5, 7, 11]. The infectionnad spread of disease before the flag leaf stage revealed 20% grain loss. Further, a strong relationship between the severity of symptoms and yield reduction was reported among cultivars (Marchetti and Bollchi 1991) [9]. Sheath blight can be effectively controlled with the application of systemic fungicides. In the present investigations we had evaluate 8 fungicide combinations for the management of sheath blight of rice.

Materials and Methods
Experimental Site
All field experiments were conducted during *Kharif* 2017-18 at the experimental field of Department of Plant Pathology situated in the Research Farm, campus of Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). Besides these field experiments, all laboratory work were carried out at Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

Materials

Glass wares and plastic wares

Whenever required, the glass wares of Borosil make plastic plates of Tarson make, blotter paper of standard grade, Micropipette and chemicals of standard grade (Merck, Qualigens, S.D. fine etc.) were used during the course of investigation. All the glass wares, polythene bags, ethyl alcohol, formalin, chemicals and other materials were procured from the Department of Plant Pathology, College of Agriculture, I.G.K.V, Raipur (C.G.).

Sterilization

All glasswares were sterilized in the oven at 180 ^{0}C for 60 min whereas the solid and liquid media were subjected to sterilization by autoclaving at (15 psi) 1.21 kg per cm^2 (121 ^{0}C) for 20 minutes.

Equipments Used

The following equipments or materials used in present investigation were-

1. Autoclave for media sterilization
2. BOD incubator for incubation
3. Binocular research microscope
4. Compound microscope
5. Hot air oven for glassware sterilization
6. Forceps, needles, blades, inoculation needle
7. Growth chamber
8. Laminar air flow for isolation and purification
9. Spirit lamp
10. Electronic weighing balance
11. Refrigerator
12. Centrifuger
13. Tissue lyser
14. Water bath
15. Vortex
16. PCR unit
17. Gel electrophoresis
18. Rotor
19. Microwave oven for melting the media

Chemicals Used

Fungicides such as Azoxystrobin + Difenocanazole is used against the test pathogen.

Medium used for fungal culture

4% Water Agar: For single spore isolation of *Colletotrichum* spp.

Potato Dextrose Agar: For culturing of *Colletotrichum* spp. pathogen

Oatmeal Agar Medium: For sporulation of *Colletotrichum* pathogen.

In vitro evaluation of fungicides against *C. truncatum* by Poison food technique

The fungus was grown on PDA medium for 8 days prior to setting up the experiment. The PDA medium was prepared and melted. The required quantity of fungicide was added to the melted medium to obtain the required concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates and suitable check was maintained without addition of fungicides. To avoid bacterial contamination, a pinch of streptomycin sulphate was added to the medium at the time of pouring. A5 mm mycelial disc was taken from the periphery of 8 days old colony of *C. truncatum* and placed in the centre of petriplate. The inoculated plates were incubated at 27 $\pm$ 1 ^{0}C and 3 replications were maintained for each treatment. Diameter of the colony was measured when maximum growth of the *C. truncatum* was reached in any of the treatments and the observations were recorded and per cent inhibition was calculated by using the formula of Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

Where,
I: Per cent inhibition
C: Mycelial growth in control
T: Mycelial growth in treatment

Analysis of Data

Analysis of data was done using CRD with additional treatment as control.

Result Discussion

The treatments i.e. mixture of two fungicide were assessed for curative effects.

A combination of Azoxystrobin 18.2% + difenoconazole 11.4% w/w SC (Amister) was effective in controlling sheath blight disease, followed by Zineb 68% + hexaconazole 4% WP (Avatar) and Fluxapyroxad 62.5 g/l + epoxiconazole 62.5 g/l EC (Adexar) (Table 1). In a rice ecosystem, in each season, more than one disease is observed and hence new fungicidal groups like oryzastrobin Qol are gaining importance as they are broadspectrum fungicides providing effective control against rice sheath blight and blast (Stammler *et al.* 2007) [13]. However, the broad spectrum fungicides may not give sufficient protection when the disease severity is very high. At present the ruling chemicals viz., Hexaconazole, Propiconazole, Validamycin, Carbendazim which are extensively used for the management of sheath blight disease (Chien and Chu 1973, Wakae and Matsura 1975, Viswanathan and Mariappan 1980a, b, Das and Mishra 1990, Van Eechout *et al.* 1991) [3, 17, 16, 4, 15]. Further, laboratory studies on two isolates of *R. solani* from rice and potato showed significant variation in response to different concentrations of fungicides (carbendazim, carboxin, pencycuron, Propiconazole and Validamycin) (Thind and Aggarwal 2005) [14]. Lore *et al.* (2005) [8], Biswas (2002) [1] evaluated and reported effectiveness of new fungicide Pencycuron (Moncern 250 EC) against rice sheath blight.

Fungicidal combination Azoxystrobin + difenoconazole was observed effective. Mixtures do not prevent resistant mutants from arising on a farm. They can, however, can slow the rate of spread of these mutants. A proper mixing partner is one that provides satisfactory disease control when used alone on the target disease. Azoxystrobin + difenoconazole have two different modes of action. Azoxystrobin is a Methoxy-acrylates and is a respiration inhibitor, i.e. it is a QoI affecting Complex III: cytochrome bc1 (ubiquinol oxidase) at Qosite (cyt b gene) where asdifenoconazole is a triazole and is a DMI/Demethylation inhibitor (G1: C14 demethylase in sterol biosynthesis (erg11/cyp15).

Difenoconazole is a broad spectrum fungicide that controls a wide variety of fungi. The mode of action of difenoconazole is that it is a sterol demethylation inhibitor which prevents the development of the fungus by inhibiting cell membrane

ergosterol biosynthesis in Aschomycetes, Basidomycetes and Deuteromycetes families.

Azoxystrobin is also a broad spectrum fungicide of the class of synthetic compounds called β-methoxyacrylates. These chemicals are derived from naturally occurring strobilurins, highly effective phytotoxic compounds produced by two species of mushrooms found naturally in Czech forests. It acts as a systemic fungicide which has curative, translaminar and preventative action.

The mode of action of azoxystrobin is to prevent the respiration of fungi due to the disruption of electron transport chain, preventing ATP synthesis (this occurs as the azoxystrobin binds to the Qo site of Complex III within the mitochondrion). Azoxystrobin has the broadest spectrum of any antifungal treatment, and it is effective against all 4 major groups of fungi like Ascomycota, Deuteromycota, Basidiomycota and Oomycota.

The fungicide azoxystrobin moves translaminarly as well as systemically (in the plant's vascular system, or "plumbing"). In terms of practical significance, systemic movement (when it occurs) and translaminar movement help to compensate for incomplete spray coverage.

Conclusion

A combination of Azoxystrobin 18.2% + difenoconazole 11.4% w/w SC (Amister) was effective in controlling sheath blight disease, followed by Zineb 68% + hexaconazole 4% WP (Avatar) and Fluxapyroxad 62.5 g/l + epoxiconazole 62.5 g/l EC (Adexar)

Table 1: Evaluation of new Fungicide against sheath blight of rice (*R. Solani*)

Treatments	Technical and Trade name	Dose per littre of water	Percent Disease Index	Percent decrease over control
T1	Flusilazole 12.5% + carbendazim 25% SC (Luster)	1.0 ml	14.81 (22.57)	57.44
T2	Azoxystrobin 18.2% w/w + difenoconazole 11.4 w/w SC (Amister)	1.0 ml	11.11 (19.46)	68.07
T3	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC (Custodia)	1.5ml	13.33 (21.40)	61.69
T4	Tricyclazole 18% + mancozeb 62% WP (Merger)	2.5gm	16.29 (23.78)	53.18
T5	Zineb 68% + hexaconazole 4% WP (Avatar)	2.5gm	11.85 (20.11)	65.94
T6	Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo)	0.4gm	14.07 (22.00)	59.56
T7	Mancozeb 50% + carbendazim 25% WS (Sprint)	2.5gm	15.55 (23.18)	55.31
T8	Fluxapyroxad 62.5 g/l + epoxiconazole 62.5 g/l EC (Adexar)	1.5m	12.59 (20.75)	63.82
T9	Control	-	34.8 (36.12)	-
SE(m) ±			0.718	
CD at 5%			2.172	

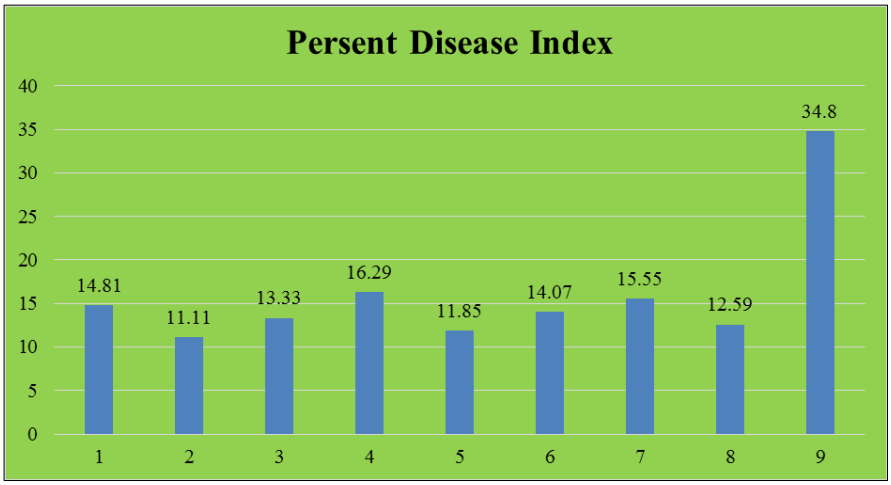

Fig 1: Evaluation of new Fungicide against sheath blight of rice (*R. Solani*)

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