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Variability in *Rhizoctonia solani* causing sheath blight of rice and its chemical management

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Abstract

Rhizoctonia solani is infamous fungus for its highly destructive pathogenic effect that can result in extensive damage of rice crop. To know the variability among the Indian isolates of *R. solani* inciting sheath blight in rice, four isolates were isolated from diseased samples of Uttar Pradesh, Uttrakhand, Gujarat and Karnataka states of India. Variability in isolates was observed with respect to the cultural, morphological and pathogenic level. Evaluation of toxicity of various fungicides against *R. solani* under *in vitro* condition showed the maximum sensitivity of *R. solani* by carbendazim even at 1 ppm concentration followed by Tebuconazole + trifoxystrobin and Hexaconazole. *R. solani* showed good tolerance to Kresoxim methyl even at 100 mg kg⁻¹ concentration. All tested fungicides namely Propiconazole, Hexaconazole, Kresoxim-methyl, Tebuconazole 50% + Trifloxystrobin 25%, Tricyclazole and Carbendazim caused complete inhibition of colony growth of *R.solani* at 1000 ppm. Foliar spray of Carbendazim @ 0.1% was found highly effective for the management of sheath blight in field condition with highest per cent reduction in disease (86.29%) followed by Tebuconazole + Trifloxystrobin (83.30%), Hexaconazole (74.20%) and Propiconazole (45.28%). Plots treated with Tricyclazole and Kresoxim Methyl had only 29.15 and 12.24 per cent reduction over control, respectively.

Keywords: Sheath blight, Rhizoctonia solani Kühn, fungicides, necrotropic, fungi

Introduction

Sheath blight incited by Rhizoctonia solani Kühn (Teleomorph: Thanatephorus cucumeris [A.B. Frank) Donk.]) is one of the most important fungal disease of rice, resulting in the significant yield loss in rice every year. This disease is alarming due to its intensive cultivation of modern high yielding varieties with high doses of nitrogenous fertilizers. Initial symptoms of sheath blight of rice appear in the form of circular, oblong or ellipsoid, greenish-grey watersoaked spots about 1cm long that occur on leaf sheath near the water level. These lesions enlarge and become oblong and irregular in outline, the center of which become grey white with brown margins. Lesions may coalesce to encompass the entire leaf sheath and stem (Rush and Lee, 1992)^[16]. Lesions may also appear on any part of sheath and several lesions may unite to encircle the whole culm. Under humid conditions, the infection may spread to upper leaf sheaths and leaf blades, which ultimately results in rotting of leaf sheath and drying up of the whole leaf. In severe cases, most of the leaves in a plant may be blighted. The lesion enlarge the centers of which become pale-green or grey and are surrounded by an irregular purple border (Webster and Gunnel, 1992)^[28]. Heavy infected plants produce poorly filled grains and may die immature panicle (Dasgupta, 1992)^[4] Singh et al. (2003)^[20] reported about the growth of mycelium on the affected parts of the plant under humid conditions and this aids in the spread of the disease to a considerable distance in the field through irrigation water. Lodging may occur in diseased plants, particularly in taller varieties (Rangaswamy and Mahadevan, 2005)^[15].

Rhizoctonia solani is a necrotropic Basidiomycetes fungus, known for its highly destructive lifestyle (Sinclair 1970; Anderson 1982; Gonzalez Garcia *et al.*, 2006) ^[19, 1, 6]. Based upon hyphal fusion affinity (known as anastomosis) isolates of *Rhizoctonia solani* has been classified into 14 anastomosis groups, namely AG-1 to AG-13 and AG-BI (Ogoshi 1987; Guillemaut *et al.*, 2003; Gonzalez Garcia *et al.*, 2006; Taheri *et al.*, 2007) ^[12, 7, 6, 24]. Based on characteristic features like colony morphology, biochemical genetic and pathogenicity,

Rhizoctonia solani AG-1 has been further divided into six intraspecific groups i.e IA,IB,IC,ID,IE and IF(Ogoshi 1987; Yang and Li 2012; Wibberg et al., 2013) [12, 31, 30]. AG-1 IA causes sheath blight disease in rice. Several workers have reported that sclerotia are first grey white, later brown to black in color, sub-globose slightly flattened in shape and vary from 0.5-5.0 mm in size (Matsumoto and Yamato, 1935; Ryker, 1939; and Palo, 1926) [10, 17, 13]. Sclerotia of Rhizoctonia solani are quite variable. Studied by (Exner and Chilton, 1943; Whitney and Parameter, 1964) ^[5, 29] on variation among single spore isolates showed that sclerotia of sibling isolates varied widely in size, shape, surface texture and distribution on culture medium. Agricultural chemicals have played an important role for management of sheath blight and other diseases of rice. Prophylactic and therapeutic sprays of Carbendizim, Carboxim and Kitazin using as soil drenching with Carbendazim, Carboxin and PCNB effectively controlled sheath blight disease (Viswanathan and Mariappan, 1980) [27]. Das and Mishra (1990) [3] obtained best disease control with Topsin-M (Thiophanate methyl) seed treatments followed by foliar sprays of same fungicide. Since new combination of fungicides are available in the market and the new races of the pathogens is continuously evolving due to gene and protein expansion because of various genetic mechanisms. Therefore, it is most important to know the toxic response of old and new combination of fungicides on currently evolving strains and races of the pathogens. The main aim of the research was to study the variability among Indian isolates of Rhizoctonia solani Kühn inciting sheath blight of rice, In vitro evaluation of toxicity of fungicides against the Rhizoctonia solani infecting rice and field evaluation of fungicides against the Rhizoctonia solani inciting sheath blight of rice.

Material and Methods

Isolation, characterization and pathogenicity test of *Rhizoctonia solani*

During the isolation of different isolates of R. solani from different geographical locations of India for studies on variability in this fungus, four isolates were collected from diseased plants of rice. Isolates were named as R. solani FA (Faizabad), R. solani PA (Panthnagar), R. solani GU (Gujarat) and R. solani KA (Karnataka). Based on the characteristics and morphology of hyphae, hyphal branches, size and septation pattern of hyphal development, sclerotia production and ability to cause the sheath blight in rice, the collected isolates were identified as R. solani AG-1. Variability studies were done for variation in 4 isolates of R. solani collected from different locations of India. Each isolates were grown on the PDA medium and radial growth, cultural characters like colony morphology, colour, growth morphology, growth rate, presence of constriction in the origin of the branch, septum formation near the branch origin, right/acute angle branching type and sclerotial colour, number, shape and production pattern and morphological characteristics like size of mycelium and sclerotia were studied. The pathogenicity test of each isolate was also done with standard protocols to identify the pathogenic ability of each isolates.

In-vitro evaluation of fungicides against R. solani

For *In vitro* evaluation of fungicides, poisoned food technique (Nene and Thapliyal, 1993)^[11] was adopted and isolate of *R. solani* collected from Kumarganj, Faizabad was used as test fungus for this study. Six fungicides *viz* Propiconazole, Hexaconazole, Kresoxim-methyl, Tebuconazole 50% +

Trifloxystrobin 25%, Tricyclazole and Carbendazim were added in the slightly cooled PDA medium at the rate of 1mg/ liter, 5mg/ liter, 10mg/ liter, 100mg/ liter and 1000mg/ liter and poured into 90 mm diameter Petri plates. PDA medium without fungicides were served as control. After solidification, 5 mm discs of Rhizoctonia solani were cut from the margin of actively growing colony and inoculated on to the center of the Petri dish containing the poisoned PDA medium. Petri plates were incubated at 27 ± 2 o C temperature. Five Petri plates were used as replicate for each treatment. The experiment was laid out in a Complete Randomized Design with five replications. After a specific period of incubation *i.e.*, after control plate reached approximately 90 mm diameter, radial growth of test fungus in the entire poisoned medium was observed. The colony diameter of the pathogen in control plates was also recorded and per cent inhibition over control was worked out according to the equation given by Vincent (1947)^[26].

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

Where

I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

Evaluation of fungicides for management of sheath blight of rice in field conditions

Evaluation of fungicides for management of sheath blight of rice was done at Student's Instructional Farm of NDUAT, Kumarganj, Faizabad. Rice variety Pusa Basmati-1 was transplanted in the several plots of 4x3 m size for spray of various fungicides. The fungicidal treatment of rice for management of sheath blight is were foliar spray of Propiconazole @ 0.1% after symptom appearance (T1), foliar spray of Hexaconazole @ 0.1% after symptom appearance (T2), foliar spray of Kresoxim-methyl @ 0.1% after symptom appearance (T3), foliar spray of Tebuconazole 50% + Trifloxystrobin 25%@ 0.1% after symptom appearance (T4), foliar spray of Tricyclazole@ 0.1% after symptom appearance (T5), foliar spray of Carbendazim @ 0.1% after symptom appearance (T6) and Unsprayed Control (T7)

A simple, rapid and mass inoculation technique developed by Bhaktavatsalam (1978) to induce sheath blight disease in rice was applied to develop artificial sheath blight disease for evaluation of fungicides in fields condition. *R. solani* isolate FA multiplied on autoclaved stem pieces (2-3 inches in length) of water sedge soaked in 1 percent peptone solution for 8-10 days were inoculated. Four to five stem bits colonized with fungal mycelia and sclerotia of *R. solani* isolate FA were placed in between the tillers in the central region of the hill, 5-10cm above the waterline and then tied with a rubber band to maintain high humidity in the microclimate. After appearance of sheath blight symptoms in fields, fungicides were sprayed once and the disease scoring was done based on the 0-9 scale given below.

Scale description for scoring sheath blight disease of rice (0-9 scale)

Relative lesion height

Grade

- 0 No infection
- 1 Vertical spread of the lesion up to 20% of plant height
- 3 Vertical spread of the lesion up to 21-30% of plant height
- 5 Vertical spread of the lesion up to 31-45% of plant height
- 7 Vertical spread of the lesion up to 46-65% of plant height
- 9 Vertical spread of the lesion up to 66-100% of plant height

Observations on disease index will be recorded from 10 randomly selected plants from each plot and will be graded based on Standard Evaluation System (SES) scale (0-9) (Anonymous, 2002)^[2]. The yield will be recorded from each plot and later converted to kg/ha⁻¹. The cost economics will be worked out to calculate B: C ratio in the field management trail.

Results

Variability in different isolates of R. solani

Studies on cultural and morphological variability of four isolates were studied and variability in relation to mycelium, branching type, radial growth, growth pattern, pattern of sclerotial production, sclerotial colour and shape, mycelial and sclerotial diameter, sclerotial production, constriction and septum and in the ability to incite symptoms (Table-1, 2, 3 and 4). In general, the colony colour of R. solani isolates varied from white to light brown. During early stage of growth, colony of R. solani nearly white but all older colonies had shades of brown. Isolate FA, GU, PA appeared as light brown colour in old cultures whereas isolates KA had more shade brown colour in comparison to other isolates. All four isolates were differing in their growth pattern, having flat velvety (R. solani KA), raised thread (R. solani FD), flat and raised thread (R. solani GU) and raised thread (R. solani PA). Pattern of sclerotial production varied between the isolates. R. solani KA isolate produced sclerotia in the central portion, peripheral distribution was observed in FA and PA isolate and scattered pattern was observed in GU isolate. Colour of sclerotia varied between the isolates. Blackish brown sclerotia were produced in KA and PA isolate, brow sclerotia in FA isolate and black colour in GU isolate. There was also variation in the shape of sclerotia. Round irregular was found in KA & PA, irregular shape in FA and Globular in GU isolate. KA isolate had right angle branching and isolate FA, GU and PA had acute angle. The largest mycelia width was recorded in GU isolate with mean mycelia width of 6.4µm and diameter was the smallest with 1.38 mm and other isolates KA, FA and PA had 6.06, 6.16,6.34µm width and 2.28, 2.2, 2.46mm diameter respectively Three isolates expressed low level sclerotial production (KA, FA and PA) moderate level sclerotial production was found in GU isolate. Constriction at the point of origin and septum was found near the branching of mycelium in all the four isolates. Variation in radial growth was observed in four isolates of R. solani. The radial growth ranged from 84.4 mm to 89.8mm. Highest growth was observed in GU isolate. Comparatively moderate growth was observed in PA, FA and KA isolates. Inoculation of different isolates of R. solani on the rice variety Pusa basmati-1 resulted in the development of symptom of sheath blight of rice within 3-5 days after inoculation. Based on the minimum time required for incitation of symptom and lesion size, isolate FA and PA was found more virulent on the rice variety Pusa basmati-1.

Effect of various concentrations of fungicides radial growth of *R. solani* AG-1A

The observations on the toxic effect of various fungicides used in present study showed higher sensitivity of *R. solani* isolate FA to Carbendazim which caused 82.67 per cent inhibition at 1mg kg⁻¹ and completely checked the mycelial growth at 5mg kg⁻¹ or at greater concentrations followed by Hexaconazole and Tebuconazole + trifoxystrobin which inhibited 33.78 and 40.44% per cent mycelial growth at 1 mg kg⁻¹ concentration respectively. Tebuconazole + trifoxystrobin

and Hexaconazole caused more than 80% inhibition in mycelial growth at 10 mg ai kg⁻¹ and completely ceased the mycelial growth at 100 mg ai kg⁻¹or greater concentrations. Propiconazole and Tricyclazole caused little inhibition in mycelial growth at1mg kg-1but increased the toxicity at 5-10 mg kg⁻¹and completely ceased the mycelial growth at 100 and 1000mg kg⁻¹. R. solani was found completely tolerant to Kresoxim methyl up to 10 mg kg⁻¹concentration but Kresoxim methyl caused 47.78 per cent and 100 per cent inhibition in mycelial growth at 100mg kg⁻¹and1000 mg kg⁻¹respectively (Figure-1, Table- 5). Observation of toxic effect of various fungicides on R. solani indicates that Carbendazim, Tebuconazole + trifoxystrobin and Hexaconazole are most toxic to the R. solani even at 1 mg a.i. kg⁻¹ concentration and application of these fungicides could be used as best substitute of other fungicides.

Evaluation of fungicides for management of sheath blight in field conditions

Application of all fungicides was found significant with respect to the reduction of disease severity and yield of rice. Spray of Carbendazim (0.1%) was found highly effective for the management of disease with least PDI of 8.99 followed by Tebuconazole + Trifloxystrobin (0.1%) having 10.95 PDI. The spray of Hexaconazole (0.1%) and Propiconazole (0.1%)was also effective with 16.92 and 35.89 PDI. The untreated control treatment recorded highest PDI of 65.60. The highest per cent reduction in disease was recorded in Carbendazim (0.1%) treated plants (86.29%) followed by Tebuconazole + Trifloxystrobin (0.1%) treatment (83.30%). Hexaconazole (0.1%) and Propiconazole (0.1%) showed 74.20% and 45.28% reduction in disease respectively. Plot treated with Tricyclazole (0.1%) and Kresoxim Methyl (0.1%) showed lowest in controlling the disease by recording PDI of 46.47 and 57.56 with 29.15 and 12.24 per cent reduction over control, respectively.

With respect to yield, treatments varied significantly with Carbendazim (0.1%) recorded highest yield of 5083 kg/ha with 90.66% increase over control. The next best fungicide was Tebuconazole + Trifloxystrobin (0.1%) with the yield of 4916 kg/ha with 84.39 per cent increase over control. Hexaconazole (0.1%) and Propiconazole (0.1%) resulted in comparatively higher yield 4750 and 4583 kg/ha when compared to least yield of Kresoxim Methyl (0.1%) with 3833 kg/ha. Untreated control had the yield of 2666 kg/ha (Table6).

Discussion

Fungi isolated from the sheath blight infected rice host were appeared as white colony with mycelial branching, sclerotia formation and formation of monilified cell similar to *Rhizoctonia solani* infecting rice. Hence the isolated fungi isolated from rice sheath blight symptoms were identified as *Rhizoctonia solani* AG-1. The incitation of symptom by inoculation of each isolates of *R. solani* on rice variety Pusa Basmati confirmed the authenticity of *R. solani* AG-1isolates (Table-3). The variability in the isolates of *R. solani* in relation of their colony growth, morphological characters, number and pattern of sclerotia formation, size of sclerotia, branching type etc. may be attributed to the origin of each isolate from different geographical locations. Variation in the pathogenicity of each isolate exists which is nature norms.

The observations of *in vitro* sensitivity and tolerance of R. solani AG-1 with common fungicides indicated that carbendazim, Tebuconazole + trifoxystrobin and Hexaconazole were most toxic to R. solani AG-1. This fungus showed good tolerance to Kresoxim methyl even at 100 mg kg⁻¹ concentration (Table- 5). The variable toxicity of tested fungicides may be attributed to the chemical nature and toxic nature of fungicides against R. solani AG-1. Prasad (2005) also reported the complete mycelial growth inhibition of R.solani by Hexaconazole, Carbendazim, Propiconazole, Benomyl, Thiphanate methyl and Carbendazim + Mancozeb. Higher efficacy of Carbendazim and good potential of Tebuconazole + trifoxystrobin, Hexaconazole and Propiconazole @ 0.1% in reduction of disease incidence of sheath blight and highest yield in comparison to other fungicides may be attributed to the toxic principles present in these fungicides and their unique mode of action against pathogen. Carbendazim, Hexaconazole and Propiconazole was also found effective by other workers (Thangasamy and Rangasawamy, 1989; Syryadai and Kadir, 1989; Surulirajan and Kandhari, 2003; Sudhakar et al., 2005; Prasad 2005; Kandhari, 2007; Johnson et al., 2013; Santhakumari and Rehumathniza, 2004)^[25, 22, 23, 21, 14, 18].

 Table 1: Radial growth and sclerotial production of different isolates

 of *Rhizoctonia solani*

Isolate	Mean radial growth (mm)	Sclerotial production
R. solani (KA)	87.0	Low
R. solani (FA)	89.4	Low
R. solani (GU)	89.8	Moderate
R. solani (PA)	84.4	Low
S.Em.±	1.57	
CD (P=0.01)	6.51	

*Low level (<15/sq inch) **Moderate level (15-50/sq inch)

 Table 2: Morphological variation in different isolates of *Rhizoctonia* solani

Isolate	Mean mycelia width(µm)	Mean sclerotial diameter(mm)		
R. solani (KA)	6.06	2.28		
R. solani (FA)	6.16	2.20		
R. solani(GU)	6.40	1.38		
R. solani (PA)	6.34	2.46		

Table 3: Pathogenicity test of R. solani on Pusa basmati-1 in pot experiment

Isolates	Date of appearance of necrotic lesion after inoculation	Lesion length (in mm)
R. solani FA	3 rd day	5mm
R. solani PA	4 th day	4mm
R. solani KA	5 th day	3mm
R. solani GU	5 th day	3mm

Table 4: Cultural variability in the different isolate of Rhizoctonia solani AG-1

	Mycelial characters				olony characters	Sclerotia characters		
Isolates	Colour	Branching type	Formation of septa in the base	Growth	rowth Morphology		Colour	Shape
R.solani KA	Initially white later dark brown	Right angle	Present	Fast	Flat velvety	Central	Blackish brown	Round irregular
R.solani FA	Initially white to light brown	Acute angle	Present	Fast	Raised thread growth	Peripheral	Brown	Irregular
R.solani GU	Initially white to light brown	Acute angle	Present	Fast	Flat and raised thread growth	Scattered	Black	Globular
R.solani PA	Light brown	Acute angle	Present	Fast	Raised thread growth	Peripheral	Blackish brown	Round irregular

Table 5: Effect of different concentration of six fungicides on radial growth of Rhizoctonia solani AG-1 Isolated from Rice

	Radial growth (mm) and per cent inhibition of <i>R. solani</i> at different concentration of fungicides									
	Fungicidal concentrations (mg ^{-kg})									
Fungicides	1mg ^{-kg}		5mg ^{-kg}		10mg ^{-kg}		100mg ^{-kg}		1000mg ^{-kg}	
	Growth	%	Growth	%	Growth	%	Growth	%	Growth	%
	(mm)	Inhibition	(mm)	Inhibition	(mm)	Inhibition	(mm)	Inhibition	(mm)	Inhibition
Propiconazole	82.4	8.44 ^{a A}	56.8	36.89 ^{a B}	49.2	45.33 ^{a C}	0.0	100 ^{a D}	0.0	100 ^{a D}
Hexacconazole	59.6	33.78 ^{bA}	25.6	71.56 ^{b B}	17.8	80.22 ^{b C}	0.0	100 ^{a D}	0.0	100 ^{a D}
Kresoxim methyl	90.0	0.00 ^{c A}	90.0	0.00 ^{c A}	90.0	0.00 ^{c A}	47.0	47.78 ^{b B}	0.0	100 ^{a C}
Tebuconazole + Trifoxystrobin	53.6	40.44 ^{d A}	11.0	87.78 ^{d B}	8.0	91.11 ^{d C}	0.0	100 ^{a D}	0.0	100 ^{a D}
Tricyclazole	86.8	3.56 ^{e A}	77.8	13.56 ^{e B}	65.6	27.11 ^{e C}	0.0	100 ^{a D}	0.0	100 ^{a D}
Carbendazim	15.6	82.67 ^{f A}	0.0	100.00 ^{f B}	0	100.00 ^{f B}	0.0	100 ^{a B}	0.0	100 ^{a B}
Control	90.0	NA	90.0	NA	90.0	NA	90.0	NA	90.0	NA

Percent inhibition (%) data (in comparison to growth in control) in Colum superscript with different lowercase letters are the significantly different at p=0.05.

Percent inhibition (%) data (in comparison to growth in control) in rows superscript with different uppercase letters are the significantly different at p=0.05



Fig 1: Effect of different concentration of six fungicides on radial growth of *Rhizoctonia solani* AG-1 Isolated from Rice on Potato Dextrose Agar medium

Table 6: Effect of various fungicides for management of sheath blight of rice in field conditions

Treatments	Treatment details	Percent disease index	Percent reduction over control	Mean yield (kg/ha)	Percent increase over control	B:C
T1	Propiconazole@ 0.1%	35.89	45.28	4583	71.90	1.67
T_2	Hexaconazole@ 0.1%	16.92	74.20	4750	78.16	1.82
T3	Kresoxim Methyl@ 0.1%	57.56	12.24	3833	43.77	1.22
T_4	Tebuconazole+Trifloxystrobin@ 0.1%	10.95	83.30	4916	84.39	1.84
T5	Tricyclazole@ 0.1%	46.47	29.15	4375	64.10	1.60
T ₆	Carbendazim@ 0.1%	8.99	86.29	5083	90.66	2.00
T ₇	Control	65.60		2666		0.60
	S.Em±	0.26		14.67		
	CD at 5%	0.80		22.60		

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