SHORT COMMUNICATION

Oryza Vol. 54 No. 4, 2017 (470-476)
DOI 10.5958/2249-5266.2017.00064.9

Evaluation of fungicides against sheath blight of rice caused by *Rhizoctonia solani* (Kuhn.)

KV Neha*, R Naveenkumar, P Balabaskar and P Manikandan

Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India

**Corresponding author e-mail: chuidruv91@gmail.com*

Received : 12 April 2016 Accepted : 5 October 2017 Published : 20 December 2017

ABSTRACT

Fixed plot survey for sheath blight disease conducted in some major rice growing areas of Cuddalore district, Tamil Nadu indicated that the Per cent disease index (PDI) varied with low to high level. The maximum PDI of 36.5% was recorded in Naduthittu followed by Vadakkumangudi (32.4%), Vallampadugai (26.5%), Muttlur (21.4%) and the least level of incidence was noticed in Ramapuram (10.5%). Four different inoculation methods were tested in the field. Among the four methods of artificial inoculation, sheath inoculum was found to be the best in plant infection. Out of different fungicides screened against the pathogen under in vitro condition, the new chemical ICF 310 at 1000 ppm concentration completely inhibited the growth of Rhizoctonia solani Kuhn, causing sheath blight disease. In pot culture different methods of treatment were applied by using ICF 310 against R. solani, where the fungicide ICF-310 at different levels showed significant influence on the incidence sheath blight of rice. Among the various treatments, combined application of ICF 310 as ST @ 2 g/kg of seeds + ICF- 310 @ 0.1% as seedling root dip + ICF- 310 @ 0.1% as foliar spray at 30 DAT and 45 DAT recorded the minimum sheath blight incidence (8.99%) at harvest. This was followed by combined application of ICF-310 as seed treatment @ 2g/kg of seeds + ICF- 310 @ 0.1% as foliar spray at 30 DAT+ 45 DAT which recorded the sheath blight incidence of 9.45% and the maximum sheath blight disease incidence was observed in untreated control.

Key words: *Pathogenicity, ICF 310, screening, in vitro, pot culture*

Rice (*Oryza sativa* L.) is an important cereal crop and staple food for a large part of world's population, especially in East and South Asia, Middle East, Latin America and West Indies. Rice, the most widely cultivated food crop in the world covers an area of 2.9 million acres worldwide, yielding an annual production of seven million tonnes with an average yield of 1080 kg/ acre (Anonymous, 2013).

Rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot and bacterial blight are the important ones (Ou,1985). Among these, sheath blight of rice is a destructive disease in all the rice growing areas of the world. The disease is caused by a soil-borne fungal pathogen, *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) anastomosis group 1 and sub-group 1A). Rice sheath blight, occurs throughout temperate and tropical production areas and is most prominent wherever rice is grown under intense production systems (Savary and Mew, 1996) and is second only to rice blast as the most economically important fungal disease of rice (Savary et al., 2006).

First reported in Japan in 1910, the disease became established in many oriental countries and as a result is often referred to as "Oriental leaf and sheath blight", 'sheath blight', '*Pellicularia* sheath blight', 'Sclerotial blight' and 'Banded blight of rice'. The widespread adoption of new, susceptible high yielding cultivars with large number of tillers and the changes in cultural practices associated with these cultivars favour the development of sheath blight and contribute greatly to the rapid increase in the incidence and severity of this disease in rice growing areas throughout the world (Srinivasachary et al., 2011). Apart from reducing from plant vigour and yield, the disease also causes grain discoloration at maturity, thus reducing the market value. Yield losses of 5-10% due to sheath blight has been estimated for tropical lowland rice in Asia (Savary and Willocquet, 2000). However, losses due to sheath blight disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas. When the disease spreads to upper parts of the plant and panicles, a total crop loss was observed (Srinivas et al., 2013).

The pathogen survives as mycelial or resistant structures known as sclerotia in plant debris and on weeds in rice growing areas. The ability of *R. solani* to produce sclerotia with a thick outer layer allows them to float and survive in water. *R.solani* survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Zachow et al., 2011). The sclerotia present in the soil or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of crops.

The natural infection of the sheath blight disease occurs at the seedling, tillering and booting stages of rice. Infection usually starts near the water line of rice plants in paddy fields. Lesions develop upward to the upper leaf sheaths and leaf blades. The centre of lesion become grayish white with brown margin, later several spots coalesce and show blight symptoms (Ou,1985). Thus entire plant often gets killed under severe cases (Rush and Lindberg, 1984).

High genetic resistance is not available for sheath blight and this disease is currently managed through use of fungicides (Savary et al., 2012). In the absence of suitable resistant donors, fungicides remain the main option to check this disease. Chemical control offers great potential and constitutes an important role in reducing crop losses caused by the rice diseases in the field. Most of the fungicides like benomyl, carbendazim, chloroneb, captafol, mancozeb, zineb, edifenphos, iprobenphos, thiophanate, carboxin etc. have been found effective for the control of disease under field conditions (Kannaiyan and Prasad, 1984; Roy, 1993; Singh and Sinha, 2004).

With this background the present study was formulated by the following objectives: 1. Survey, isolation and identification of the pathogen associated with sheath blight and establishing its pathogenicity. 2. To assess the in vitro efficacy of different fungicides against the test pathogen and 3. To assess the efficacy of selected fungicides *in vivo* (pot culture) condition for the management *Rhizoctonia solani*.

Survey on the occurrence of sheath blight of rice in Cuddalore District

A field survey (fixed plot survey) was conducted to assess the extent of sheath blight occurrence of rice in Cuddalore district. The villages where rice is traditionally grown are selected for assessing the prevalence of sheath blight disease caused by *Rhizoctonia solani*. Fifteen locations were selected for the survey. During the survey, the plants were found affected due to sheath blight disease and also the total number of plants observed were counted and recorded. For disease scoring, the typical assessment system for rice, developed by the International Rice Research Institute, Philippines (SES, 2002), was followed.

Disease Scale

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

Disease severity =

$$\frac{\text{Sum of disease grades} \times \text{No. of infected tillers/hill}}{\text{Total No. of Tillers} \times \text{Maximum disease grades} \times \text{No. of tillers assessed}} \times 100$$

Isolation, maintenance of the pathogen

The diseased rice plants showing the typical symptoms of sheath blight were collected from fifteen conventional rice growing areas of Cuddalore district. The pathogen was isolated in potato dextrose agar (PDA) medium from the diseased specimen showing the typical symptoms. The infected portion of the sheath was cut into small bits, surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds, washed in repeated changes of sterile distilled water and plated into PDA medium in sterilized Petri dishes. The plates were incubated at room temperature (28 ± 2^0C) for five days and were observed for the fungal growth. The fungus was subsequently purified and maintained in PDA slants and used for further studies.

Mass multiplication of *Rhizoctonia solani*

Rice leaf sheath were collected and cut it into small pieces of 4cm. Sheaths were transferred to open mouth bottles and closed with a cotton wool plug. The desired quantity of water was added and the bottles were sterilized at 15 psi for three successive days. The PDA medium was used to inoculate R. solani pathogen. From 10 days old culture of the pathogen grown in PDA, six discs (9 mm) were taken and inoculated into each bottle. The bottles were then incubated at room temperature (28 ± 2^0C) for 14 days and the inoculum thus prepared was used for subsequent studies.

Methods of inoculation of pathogen

Four methods were attempted on rice variety BPT 5204 under pot culture. Three replications were maintained for each treatment. These methods are as follows:

Grain inoculation method

The infected seeds were kept in between the leaf sheaths just above the water level.

Sheath inoculum method

The sheath inoculum prepared as described above was used to inoculate rice plants just above the water level at the maximum tillering stage of the rice crop (Gnanamanickam et al., 1992).

Sclerotia inoculum method

The leaf sheaths of R. solani were inoculated with 10 days old sclerotia. The sclerotial balls were kept in between the leaf sheaths with the help of a sterilized forcep. A small piece of wet cotton was kept above the sclerotial balls for maintaining the moisture condition (Singh et al., 2001)

Agar culture inoculation method

The grown up fungus on potato dextrose agar at room temperature was taken out in small bits with the help of a sterilized inoculation needle and inserted inside the leaf sheath of each tiller just above the water level. The inoculated plants were incubated and moved to the green house. The incidence was recorded after 60, 70 and 80 days after transplanting and the per cent disease incidence was calculated as described above. Four replications were maintained for each treatment (Singh et al., 2001).

Screening of fungicides against *R. solani* under *in vitro*

Six fungicides belonging to different combination's *viz.,* Avtar (Hexaconazole 4% + Zineb 68% WP), ICF 310 (Mancozeb 68% + Hexaconazole 4%), Indofil- M45 (Mancozeb), Antracol (Propineb), Indofil- Z 78 (Zineb 75%), Merger (Tricyclazole 18% + Mancozeb 62% WP) and Carbendazim 50% WP at 0.1% conc. were screened against the pathogen under in vitro condition to find out their relative efficacy in inhibiting the growth of *R. solani* by using poisoned food technique. On the basis of the performance of the fungicides *in vitro*, the selected fungicide was taken for further studies.

Effect of selected fungicides on the disease severity of sheath blight in pot culture

A pot culture experiment was conducted with rice variety BPT 5204. All the agronomic practices were followed according to the recommendations of the State Agricultural Department. Standard blanket fertilizer recommendation of 150:50:50kg NPK/ha was followed. The causal organism R. solani was artificially inoculated at maximum tillering stage by using the standard sheath inoculum technique just above the water line at 30 DAT. The chemical fungicides were sprayed as per the treatment schedule (Table 5). Three replications per treatment, a suitable control and carbendazim at 1000 ppm were also maintained. The pots were maintained in glass house and all the standard agronomic procedures were followed. All the observations *viz.,* the sheath blight disease incidence (PDI) at different intervals, plant height (cm), number of tillers and yield (grain and straw) in g/pot) were assessed and recorded at harvest following standard procedures. The disease incidence was recorded at 60 DAT, 90 DAT and at harvest stage. The disease severity was assessed using 0-9 scale, the typical assessment system for rice developed by the International Rice Research Institute (SES, 2002).

Survey on the incidence of sheath blight of rice in Cuddalore district of Tamil Nadu

Fixed plot survey conducted in some major rice growing areas of cuddalore district, indicated that the per cent Disease Index (PDI) varied with low to high level. The maximum PDI of n36.5% was recorded in Naduthittu followed by Vadakkumangudi (32.4%), Vallampadugai (26.5%), Muttlur (21.4%) and moderate incidence was noticed in Shivapuri (19.5%), Periya kannadi (19.1%), Vandurayanpattu (18.5%), Keerapalayam (18.0%), Vadalore (17.2%), Kantha kumaran (16.0%), Orathur (15.2%), Permapattu (14.1%), Thiththampalayam (13.9%) and Bhuvanagiri (13.5%) with the least level of incidence noticed in Ramapuram (10.5%) (Table 1). Generally, the crop grown in clay soils and at panicle initiation stage of the crop recorded fairly higher disease incidence. Present findings are in agreement with Singh (2002) who reported all cultivars of rice being susceptible to sheath blight, but the degree of susceptibility varies and sheath blight is usually severe on cultivars that are short, high tillering and responsive to high fertilizer in comparison to tall cultivars with fewer tillers (Dath, 1990). Available literature also revealed that the amount of crop yield loss of 5-60 per cent by the disease varied from place to place (Jia et al., 2012).

Cultural characters of *R. solani* isolates

All the fifteen isolates of sheath blight pathogen *R. solani* produced light brown to brown colour sclerotia. The maximum sclerotial population was observed in Naduthittu isolate of 1 to 3 mm. The diameter of sclerotia ranged from 1.4-2.5 mm in Table 2.

Table 2. Cultural characters of different isolates of *R. solani*

Sl. No	Locality	Colour	Topography	Sclerotial size (µm)
1.	Shivapuri	Whitish brown	Aerial	M
2.	Permapattu	Light brown	Aerial	M
3.	Periyakannadi	Dark brown	Aerial	S
4.	Kanthakumaran	Whitish brown	Aerial	L
5.	Naduthittu	Yellowish brown	Aerial	L
6.	Orathur	Very pale brown	Aerial	L
7.	Vandurayanpattu	Dark brown	Aerial	L
8.	Keerapalayam	Light brown	Aerial	L
9.	Muttlur	Very pale brown	Aerial	S
10	Vallampadugai	Yellowish brown	Aerial	S
11	Ramapuram	Very pale brown	Aerial	M
12	Bhuvanagiri	Dark brown	Aerial	M
13	Thiththampalayam	Light brown	Aerial	L
14	Vadalore	Very pale brown	Aerial	L
15	Vadakkumangudi	Yellowish brown	Aerial	L

S- Small (< 1.5 mm), M- Medium (1.5 mm) and L- Large (1.5 to 3mm) (Basu et al., 2004).

Infection of rice plants by different methods of inoculation with *R. solani*

With a view to find out the best inoculation methods, four methods were tested in the field *viz.,* Grain inoculation method, sclerotial inoculation method, sheath inoculum method and Agar culture method. Among the four methods of artificial inoculation, sheath inoculum was found to be the best in plant infection. It recorded a mean per cent infection of 64.94 per cent (Table 3). The next best method of inoculation was sclerotial inoculation method which recorded 20.09 per cent

Table 1. Survey on the incidence of sheath blight of rice in Cuddalore district.

Sl. No.	Locality	Crop stage	Soil type	Sheath blight (%)
1.	Shivapuri	Panicle initiation	Clay loam	19.5
2.	Permapattu	Panicle initiation	Clay loam	14.1
3.	Periyakannadi	Panicle initiation	Clay loam	19.1
4.	Kanthakumaran	Panicle initiation	Clay	16.0
5.	Naduthittu	Panicle initiation	Clay	36.5
6.	Orathur	Panicle initiation	Clay loam	15.2
7.	Vandurayanpattu	Grain filling	Clay loam	18.5
8.	Keerapalayam	Grain filling	Clay loam	18.0
9.	Muttlur	Panicle initiation	Sandy loam	21.4
10.	Vallampadugai	Panicle initiation	Clay	26.5
11.	Ramapuram	Panicle initiation	Clay loam	10.5
12.	Bhuvanagiri	Panicle initiation	Clay loam	13.5
13.	Thiththampalayam	Grain filling	Sandy loam	13.9
14.	Vadalore	Grain filling	Clay loam	17.2
15.	Vadakkumangudi	Grain filling	Clay	32.4

Table 3. Effect of different methods of inoculation with R. solani on the per cent sheath blight incidence.

Sl. No.	Methods	Days after inoculation (per cent infected leaf sheaths)					
		5	7	9	12	15	Mean
1	Grain inoculation method	0.00	20.00	44.00	66.0	82.0	42.4
2	Sclerotial inoculum method	3.46	5.00	8.00	28.00	56.00	20.09
3	Sheath inoculum method	26.72	28.00	70.00	100.00	100.00	64.94
4	Agar method	4.66	6.00	8.00	18.00	18.00	10.93
	SEd	0.705	0.803	1.777	1.725	1.379	1.668
	CD (p=0.05)	1.759	2.004	4.434	4.303	3.439	4.168

infection. In line with the present study, several authors have successfully used sheath inoculum method for the artificial inoculation of the pathogen (Park et al., 2008: Lore et al., 2012). Xing et al. (2013) reported that when injury was made, the rice sheaths exhibited maximum infection by *R. solani*, whereas in the present study the sheath inoculums produced maximum incidence of sheath blight even without any injury.

Screening for fungicides against on the mycelial growth of *R. solani*

Out of six different fungicides tested against the test pathogen, the new chemical ICF 310 at 1000 ppm concentration completely inhibited the growth of the *R. solani*. This was followed by Avtar with 90.84 mm, Merger (86.37%), Carbendazim 50% WP (74.85), Indofil Z-78 (67.74%), Indofil M-45 (59.76%) and Antracol (59.25%) at 1000 ppm conc. in the decreasing order of merit. The maximum growth of 90 mm was observed in control (Table 4). Several chemical fungicides have been tested and reported effective by the earlier workers. Ali and Archer (2003) reported that fungicides *viz.,* carbendaim, cycproconazole SC, fenpropimorph EC, fludoxanil 2.5SC, hexaconazole 250 SC, iprodione 50WP, pencycuron 250Flo, propiconazole 250EC and tolclofos-methyl500 Flo were found effective against sheath blight under in vitro condition. Sudhakar et al. (2005) have also found fungicides *viz.,* carbendazim, hexaconazole and propiconazole to be effective against *R. solani* (sheath blight) *in vitro*. Lore et al. (2012) reported about kresoxim methyl 40% + hexaconazole 8%, hexaconazole and propiconazole being most effective against *R. solani* under in vitro. All these earlier reports corroborated with the present findings.

Efficacy of fungicide (ICF-310) on the incidence of *R. solani* causing sheath blight of rice (pot culture)

Out of different types of fungicides application, the fungicide ICF-310 at different levels showed significant influence on the incidence of sheath blight of rice. Among the various treatments, combined application of ICF 310 as ST @ 2 g/kg of seeds + ICF- 310 @ 0.1% as seedling root dip + ICF- 310 @ 0.1% as foliar spray at 30 DAT and 45 DAT (T8) recorded the minimum sheath blight incidence (8.99%) at harvest (Table 5). This was followed by combined application of ICF-310 as seed treatment @ 2g/kg of seeds + ICF-310 @ 0.1% as foliar spray at 30 DAT+ 45 DAT) (T7) which recorded the sheath blight incidence of 9.45%

Table 4. Screening of fungicides against the mycelial growth of R. solani by poisoned food technique.

Tr. No.	Fungicides @ 0.1%	Mycelial growth (mm) 1000 ppm	Per cent inhibition 1000 ppm
1	Avtar (Hexaconazole 4% + Zineb 68% WP)	8.24	90.84
2	ICF 310 (Mancozeb 68% + Hexaconazole 4%)	0.00	100
3	Indofil- M45 (Mancozeb)	36.21	59.76
4	Antracol (Propineb)	36.67	59.25
5	Indofil- Z 78 (Zineb 75%)	29.03	67.74
6	Merger (Tricyclazole 18%+ Mancozeb 62% WP)	12.26	86.37
7	Carbendazim 50 % WP (0.1%)	22.63	74.85
8	Control	90.00	-
	SEd	4.059	1.210
	CD (P=0.05)	8.790	0.559

Table 5. Efficacy of fungicide (ICF-310) on the incidence of *R. solani* causing sheath blight (pot culture).

Tr. No.	Treatments	Sheath blight incidence (%)			Per cent decrease over control		
		60 DAT	90 DAT	At harvest	60 DAT	90 DAT	At harvest
1.	ICF- 310 as ST @ 2 g /kg of seeds	20.55(26.95)	22.11(28.04)	27.98(31.93)	27.69	33.50	46.56
2.	ICF-310 @ 0.1% as Seedling root dip	21.32(27.49)	25.99(30.65)	29.45(32.86)	24.98	21.83	43.75
3.	ICF-310 @ 0.1% as Foliar spray at 30 DAT	18.21(25.26)	15.01(22.79)	31.37(34.06)	(35.92)	(54.85)	40.08
4.	ICF-310 @ 0.1% as foliar spray at 30 DAT + 45 DAT	16.55(24.00)	18.86(25.73)	19.43(26.15)	41.76	43.27	62.89
5.	$T_1 + T_2$	11.98(20.25)	12.79(20.95)	13.83(21.83)	57.84	61.53	73.58
6	$T_1 + T_3$	10.21(18.63)	10.15(18.57)	11.49(19.81)	64.07	69.47	78.05
7	$T_1 + T_4$	8.88(17.33)	9.12(17.57)	9.45(17.90)	68.75	72.57	81.95
8	$T_1 + T_2 + T_4$	8.32(16.76)	8.67(17.12)	8.99(17.44)	70.72	73.92	82.83
9	Carbendazim (0.1% as soil drench) + ST @ 2.0 g /kg of seeds	10.98(19.35)	12.63(20.81)	14.69(22.53)	61.36	62.01	71.94
10	Control	28.42(32.21)	33.25(35.21)	52.36(46.35)	-	-	-
	SEdCD (p=0.05)	0.1000.221	0.1190.262	0.1190.251	0.2230.472	0.1620.343	0.1760.372

and the maximum sheath blight disease incidence was observed in untreated control. The new fungicide was proved better than the chemical carbendazim 50 WP in reducing the sheath blight incidence. Chemical control of the sheath blight disease is successful at field level in majority of the cases (Kandhari et al., 2003). Most of the fungicides like benomyl, carbendazim, chloroneb, captafol, mancozeb, zineb, edifenphos, iprobenphos, thiophanate, carboxin etc. have been found effective for the control of the sheath blight disease under field conditions (Kannaiyan and Prasad, 1984; Singh and Sinha, 2004). Out of these, benomyl, carbendazim, edifenphos and iprobenphos were the most effective chemicals (Roy, 1993).

During the recent days, many chemical fungicides are being used for the management of sheath blight disease of rice. However, the new chemical fungicide ICF 310 WP is to be used for the management of sheath blight of paddy when used singly. Further, the present study indicates that the various growth promoting substances produced by P. fluorescens and suppression of *R. solani* by the action of their combination would have contributed significantly to the growth promotion and yield enhancement of rice crop.

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