Evaluation of Different Fungicides against *Rhizoctonia solani*, the cause of Sheath blight disease of Rice

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ABSTRACT

Sheath Blight disease of rice caused by *Rhizoctonia solani* is a destructive disease in rice growing areas of the world. It is distributed worldwide with the wide host range and causes substantial yield losses. Lack of durable sheath blight resistant rice varieties have led to develop sustainable control methods. The goal of the current investigation was to identify the most effective fungicides for the control of sheath blight. Nine fungicides were tested at two concentrations (recommended and half) in lab and field conditions against the rice sheath blight pathogen during the kharif season 2021. In vitro, Amistar Top (Azoxysrobin), Tilt (Propiconazole), Top Guard (Flutrifol+Tebuconazole), Nativo (Tebuconazole + Trifloxystrobin), Topsin-M (Thiophanate Methyl), Kachaloo (Difenoconazole) were found most effective, and completely (100%) inhibited *R. solani* mycelial growth. Field trial was conducted to evaluate the effect of these fungicides in controlling sheath blight disease. The results showed that fungicides differed significantly in respect of lesion height per infected plant. Application of Kachaloo (Difenoconazole) followed by Topsin-M (Thiophanate Methyl), Argyl Super (Clothaindine + Azoxysrobin), Nativo (Tebuconazole + Trifloxystrobin) was found most effective in suppressing the growth of pathogen and inhibited 81.31%, 80.96%, 74% and 73.22% disease respectively with half dose and 71.51%, 64.58%, 51.77% and 41.44 % with recommended dose as compare to control. Recommendation of the present studies regarding suitable fungicides for the control of sheath blight disease will help to the farmers to enhance their crop productivity by reducing the input cost.

Keywords: Rice, Sheath blight, Fungicides, Chemical control.

INTRODUCTION

Rice (*Oryza sativa* L.) is considered as one of the most significant cereal crops and a major contributor of food to the global food grain basket. It is a staple food for 2.7 billion people worldwide, approximately 90% world's rice grown in Asian continent (Al Salim *et al.*, 2016). Rice is the 2nd most significant staple food in Pakistan after wheat. Pakistan is one of the world's leading producers, exporters, and consumers of rice. It comes 10th in the

ranking of rice production (Khan *et al.*, 2022) and on 4th number among rice exporting countries. Maximum share of rice production 69% of the country is produced by Punjab province (Shahid *et al.*, 2014). Pakistan produces high-quality fine rice varieties, specifically basmati varieties with a distinctive aroma that are famous worldwide. These well-known basmati rice varieties are prone to a wide range of diseases. Major diseases including sheath blight, blast, bacterial leaf blight and brown spot are limiting the rice production in Pakistan (Shahbaz *et al*., 2021). Sheath blight disease is caused by fungus *Rhizoctonia solani*, accountable for 45% of yield losses (Margani and Widadi, 2018). The use of chemicals has great potential in disease reduction. Judicious use of chemicals is the most palatable and cost-effective choice for efficient disease management (Bhuvenishwari and Raju, 2012). The application of fungicides is widely used approach against ShB disease (Kandhari *et al.*, 2003). Chemical control ensures efficient and quick control of diseases, increase the crop yield with relatively better quality of produce. Fungicides possess spatial efficacy in controlling different rice diseases (Shahbaz *et al.*, 2021). The two most frequently used methods are foliar spray and seed treatment with fungicide to control ShB (Singh *et al.*, 2019). Fungicides helps to restrict the pathogen establishment on the sheaths and inhibit sclerotia formation by several means e.g. it damage the cell membrane of fungus, inhibit degrading enzymes (Kumar *et al.*, 2018), interfere in energy production key processes (Lal *et al.*, 2017), cell wall formation metabolic pathways accompanying with sterol and chitin biosynthesis (Singh *et al.*, 2019). It is necessary to advise the rice growers/ farming community to use a rotating schedule of fungicides in order to prevent infectious fungi from becoming resistant to these fungicides. The present study was aimed to examine the effect of various fungicides for inhibiting and controlling sheath blight disease. Nine fungicides were tested at recommended and half dose under lab and field conditions against the sheath blight pathogen during the kharif season 2021.

MATERIALS AND METHODS

The media used for this purpose was Wakimoto.

Composition of Wakimoto Agar

Ingredient	For 1 Liter	For 500 ml

Agar	17g	8.5g
Peptone	5.0g	2.5g
Ca(NO3)2.4H2O	0.5g	0.25g
Sucrose	20.0g	10g
FeSO4	0.05g	0.025g

Procedure

Media was prepared in the required amount in the flask and autoclaved at 121ºC and 15lbs pressure for 20 minutes along with the petri plates. When culture media was cooled and near to solidify, poured into oven dried petri plates under sterilized conditions (in laminar flow chamber).

Plating of Samples

The diseased samples were cut into small pieces, this process done inside laminar flow. The 9 eppendrof tubes were arranged for washing of disease samples, 3 eppendrof tubes filled with 1 ml of 70% ethanol sol. for the surface sterilization and the other filled with 1ml autoclaved water. The diseased samples were dipped in to 70% ethanol sol. and then washed twice by dipping and shaking in autoclaved distilled water for 2 minutes. Four to five pieces of the sample were also placed on Wakimoto plates and left for overnight at 30ºC temperature.

Mycelial growth and sclerotia yield on agar

The mycelia of pathogen was isolated on Wakimoto and purified through hyphal tip / single sclerotial method (Rangaswami and Mahadevan, 2004). Pure culture maintained and stored in refrigerator at 5ºC for further studies.

In vitro evaluation of fungicide against *R. solani*

Poisoned food technique

In vitro study was conducted by taking nine chemicals viz. Amistar Top, Tilt, Cordate, Top Guard, Evito, Nativo, Argyl Super, Topsin-M, Kachaloo in recommended and half concentrations (Figure 1.1) using the 'poisoned food technique' (Nene & Thapliyal, 1982;

Schmitz, 1930) along with control (untreated) (Figure 1.2). These fungicides were poured into Wakimoto medium and mixed thoroughly before autoclaving (Table 1.1).When the media was autoclaved, aseptically poured into sterilized petri plates in the inoculation chamber and left to cool. Mycelial disc of 5 mm was placed in each Petri plate and incubated at 28 °C±. In control, no fungicide was used. Four replications were maintained of each treatment. The experiment was arranged in a completely randomized design.

Figure 1.1: Fungicides used against *Rhizoctonia solani* pathogen under lab and field conditions

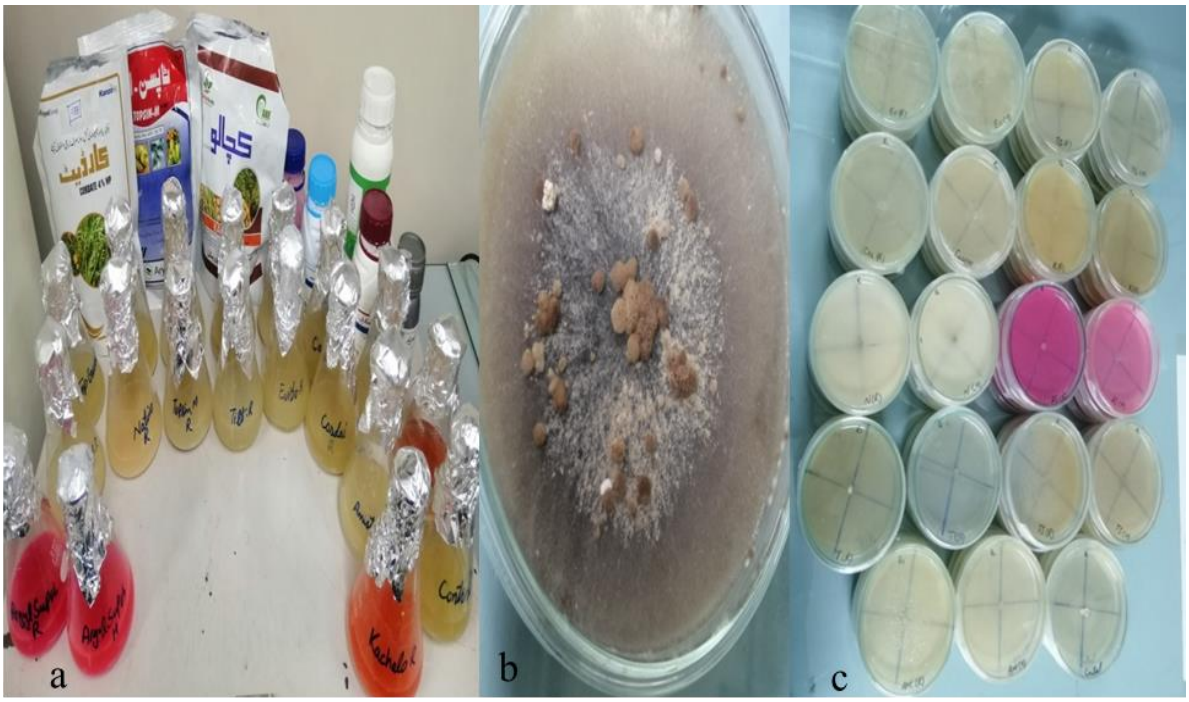

Figure 1.2: a) Preparation of poison food using fungicides in media, b) Inoculum of *Rhizoctonia solani*, c) Disc inoculation in the center of media plates

Table 1.1: Fungicides concentrations to obtain desired doses

S. No.	Recommended Doses of all the fungicides			Water in liter	ml or gm/liter	uL/liter	uL/100 ml	uL/50 ml
1	Amistar Top	Azoxysrobin	200 ml/acre	100	2	2000	200	100
2	Tilt	Propiconazole	80 ml/acre	100	0.8	800	80	40
3	Cordate	Kasugamycin	300 gm/acre	100	3	3000	300	150
4	Top Guard	Flutrifol+Tebuconazole	200 ml/acre	100	2	2000	200	100
5	Evito	Fluoxystrobin	125 ml/acre	100	1.25	1250	125	62.5
6	Nativo	Tebuconazole+Trifloxystrobin	65 gm/acre	100	6.5	6500	650	325
7	Argyl Super	Clothaindine + Azoxysrobin	100 gm/acre	100	1	1000	100	50
8	Topsin-M	Thiophanate Methyl	250 gm/acre	100	2.5	2500	250	125
9	Kachaloo	Difenoconazole	250 gm/acre	100	2.5	2500	250	125
10	Control							

The growth of *R. solani* was recorded at 48, 72 and 96 post hour inoculation and mycelial diameters were recorded when control had reached the margins of the plates by using formula (Vincent, 1947).

$$\mathbf{I = C\text{-}T/C \times 100}$$

Where,

I = Percent inhibition of fungal growth. C = Control. T = Treated Plates.

In vivo evaluation of fungicides against *Rhizoctonia solani*

A field experiment was conducted at the Agriculture Farm of Nuclear Institute for Agriculture and Biology (NIAB), during kharif season of 2021. Super basmati nursery was transplanted in puddled field after thirty days. NPK fertilizers were applied according to departmental recommendations. Artificial inoculation was made by placing the tooth pick immersed inoculum in sheath (Figure 1.3). Three replications of each treatment were maintained. Each replication contains 5 plants The field trials were laid out in a Randomized Block Design to assess the efficacy of ten treatments (9 chemicals viz. Amistar Top, Tilt, Cordate, Top Guard, Evito, Nativo, Argyl Super, Topsin-M, Kachaloo in recommended and half concentrations and one control) against rice sheath blight disease (Figure 1.4). These chemicals were sprayed twice at fifteen days interval to the respective plots after the appearance of the disease symptoms in field and water sprayed plots were kept as control. Statistix 8.1 software was used to perform statistical analysis on the recorded data.

Treatments

T1= Amistar Top	(Azoxysrobin)
T2= Tilt	(Propiconazole)
T3= Cordate	(Kasugamycin)
T4= Top Guard	(Flutrifol+Tebuconazole)
T5= Evito	(Fluoxystrobin)
T6= Nativo	(Tebuconazole+Trifloxystrobin)
T7= Argyl Super	(Clothaindine + Azoxysrobin)
T8= Topsin-M	(Thiophanate Methyl)
T9= Kachaloo	(Difenoconazole)
T10= Control	(Distilled water)

Figure 1.3: Mass multiplication of *Rhizoctonia solani* with immersed toothpicks in media for field

Figure 1.4: Evaluation of nine fungicides under field conditions through foliar spray

RESULTS

In vitro efficacy of various fungicides on *R. solani* mycelial growth

Amistar Top (Azoxysrobin), Tilt (Propiconazole), Top Guard (Flutrifol+ Tebuconazole), Nativo (Tebuconazole+Trifloxystrobin), Topsin-M (Thiophanate Methyl) and Kachaloo (Difenoconazole) showed effectiveness as compared to control. These fungicides were individually effective against the pathogen even at the recommended and half dose by completely (100%) inhibiting the mycelial growth and sclerotia formation. At recommended dose Argyl Super (Clothaindine + Azoxysrobin) fungicide was effective and inhibited the 69.2 % mycelial growth of *R. solani* and comparatively less effective in half dose 52.2%. Evito (Fluoxystrobin) inhibited 43.1% mycelial growth at half dose and 11.9 % at recommended dose (Table 1.2). The least effective fungicide was Cordate (Kasugamycin) having 33.3% inhibition on recommended dose and 8.1% respectively on half dose.

Table 1.2: Effect of Fungicides on mycelial growth of *R. solani* under lab conditions

Sr.No	Fungicides	Disease Inhibition on recommended dose (%)	Disease Inhibition on half dose (%)
1	Amistar	100.0 a	100.0 a
2	Tilt	100.0 a	100.0 a
3	Top Guard	100.0 a	100.0 a
4	Evito	11.9 f	43.1 d
5	Cordate	33.3 e	8.1 g
6	Nativo	100.0 a	100.0 a
7	Argyl Super	69.2 b	52.2 c
8	Topsin M	100.0 a	100.0 a
9	Kachaloo	100.0 a	100.0 a
10	Control	0.0 h	0 h
			alpha = 0.050

In vivo efficacy of fungicides against *R. solani*

The effect of fungicides was significantly different in respect of lesion height of the infected plant (Table 1.3). Spraying of Kachaloo (Difenoconazole) followed by the Topsin-M (Thiophanate Methyl), Argyl Super (Clothaindine + Azoxysrobin), Nativo (Tebuconazole + Trifloxystrobin) was found most effective in suppressing sheath blight and inhibited 81.31%, 80.96%, 74% and 73.22% disease respectively with half dose and 71.51%, 64.58%, 51.77% and 41.44 % with recommended dose as compare to control. Cordate (Kasugamycin) inhibits 46.91% disease in half dose, Top Guard (Flutrifol+Tebuconazole) and Tilt (Propiconazole) suppressed 41.54% and 41.25% at half dose, 37.24% and 42.29% at recommended dose. Evito (Fluoxystrobin) were found least effective with 35.78 at half dose and 13.29 at recommended dose. Amistar Top (Azoxysrobin) was not effective with 21.59% disease inhibition in half dose and 20.64% disease inhibition in recommended dose (Table 1.3).

Table 1.3: Effect of Fungicides on sheath blight disease under field conditions

Sr. No	**Fungicides**	**Disease Inhibition on recommended dose (%)**	**Disease Inhibition on half dose (%)**
1	Amistar	20.64 h	21.59 h
2	Tilt	42.29 f	41.25 f
3	Top Guard	37.24fg	41.54 f
4	Evito	13.29 i	35.78 g
5	Cordate	18.59 h	46.91e
6	Nativo	41.44 f	74 b
7	Argyl Super	51.77 d	73.22 b
8	Topsin M	64.58 c	80.96 a
9	Kachaloo	71.51 b	81.31 a
10	Control	0.00 j	0.00 j
			alpha = 0.050

DISCUSSIONS

Sheath blight has been successfully controlled with fungicidal sprays, which are best at preventing disease spread worldwide. The control of the disease depends on the timely administration of potent fungicides. Various fungicides are available and use commercially against different rice diseases. But no fungicide was claimed and marketed specifically for sheath blight disease in Pakistan. In this view, the current study was conducted to evaluate commercially available fungicide e.g Amistar Top, Tilt, Cordate, Top Guard, Evito, Nativo, Argyl Super, Topsin-M, Kachaloo against sheath blight disease in laboratory and field conditions. All the fungicides were effective with varying degrees of significance in reducing sheath blight disease as compared to control. Under the present studies, Amistar Top (Azoxysrobin), Tilt (Propiconazole), Top Guard (Flutrifol+Tebuconazole), Nativo (Tebuconazole+Trifloxystrobin), Topsin-M (Thiophanate Methyl) and Kachaloo (Difenoconazole) showed effectiveness as compared to control. These fungicides were individually effective against the pathogen even at the recommended and half dose by completely (100%) inhibiting the mycelial growth and sclerotia formation in laboratory. In field condtions Kachaloo (Difenoconazole) was found most effective in suppressing sheath blight and inhibited 81.31% disease with half dose and 71.51% with recommended dose as compare to control followed by the Topsin-M (Thiophanate Methyl) 80.96% and 64.58%, Nativo (Tebuconazole + Trifloxystrobin) 74% and 41.44 % and Argyl Super (Clothaindine + Azoxysrobin), 73.22% and 51.77% disease reduction with half dose and with recommended dose as compare to control respectively. Different scientific studies provide strong support for the current study. According to Hunjan *et al.* (2012), fungicides such as trifloxystrobin + tebuconazole, tebuconazole, and pro-piconazole shown better levels of effectiveness against the rice pathogen *R. solani* in lab conditions. Bag, 2009 reported the effectiveness of trifloxystrobin 25% + tebuconazole 50% in field conditions against sheath blight disease from West Bengal. Propiconazole and hexaconazole totally block the radial development of *Rhizoctonia solani* at 1000 ppm concentration (Tiwarie *et al.*, 2002). Hexaconazole has been proven to be a successful fungicide against rice sheath blight (Johnson *et al.*, 2013). Nativo and Bavistin, two of the novel formulations, were found effective in

reducing the pathogen's ability to develop mycelia and produce sclerotia at lower concentrations (Sriraj *et al.*, 2014). Kumar *et al*., (2017) carried out experiment to evaluate the efficacy of different fungicides copper Oxichloride, Mancozeb, Carbendazim, Hexaconazole and Propiconazole through poisoned food technique.

The conclusion of the present study is that Kachaloo (Difenoconazole) was found most effective in controlling the sheath blight disease and can be recommended to the farmers. Moreover Topsin-M (Thiophanate Methyl), Nativo (Tebuconazole + Trifloxystrobin) and Argyl Super (Clothaindine + Azoxysrobin) which have also been proved effective and can be recommended for sheath blight disease control. These research findings will contribute to enhance rice productivity ultimately its export.

Conflict of interest: There is no conflict of interest.

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