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Invitro and *invivo* Evaluation of Fungicides against False Smut Disease of Rice in Hilly Zone of Karnataka, India

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A B S T R A C T

The efficacy of six different systemic fungicides and two combi-products were evaluated for inhibition of growth of pathogen at three different concentrations. Among systemic and combi-products fungicides were evaluated, the cent per cent inhibition was observed in Propiconazole 25 % EC, Tebuconazole 250 EC, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC and Tebuconazole 50 % + Trifloxystrobin 25 % WG at all the concentrations. Azoxystrobin 25 % SC resulted in 82.49, 100 and 100 per cent inhibition whereas difenconazole 25 EC recorded 75.08**,** 80.58, and 100 per cent inhibition of mycelia growth at 100, 200 and 500 ppm concentrations respectively. A field experiment was conducted at AHRS, Ponnampet during *Kharif* 2017 to find out the effective fungicide for the management of false smut of rice. It was inferred that the treatment with the fungicide Tebuconazole 50 % + Trifloxystrobin 25 % WP gave the lowest false smut disease severity of 2.80 per cent, which was followed by the Azoxystrobin 18.2 % + Difenconozole 11.4 % SC with 6.17 per cent, Propiconazole 25% EC with 10.50 per cent and Azoxystrobin 25 % SC with 15.87 per cent. On comparison with the grain yield obtained from each plot it was found that the treatment with Tebuconazole 50 % + Trifloxystrobin 25 % WP gave the highest grain yield of 55.42 q/ha which was followed by the Azoxystrobin 18.2 % + Difenconozole 11.4 % SC(53.72 q/ha), Propiconazole 25% EC(49.47 q/ha) and Azoxystrobin 25 % SC (48.36q/ha) as against grain yield of 38.68 q/ha in untreated control.

Introduction

Rice (*Oryza sativa* L.) is the most extensively cultivated food crop of the Asia and forms a major part of nourishment for half of the world's population. It is the primary source of energy and protein for 4.5 billion peoples in the most populous nations of Asia. Rice is a staple of in parts of Asia, Africa and South America to some extent of United States (Janick *et al*., 1981). More than 90 per cent of the world's rice is grown and consumed in Asia, where 60 per cent of the world's population lives. It accounts for 35-60 % of the caloric intake of three billion Asians (Guyer *et al*., 1998).

Worldwide, rice is cultivated in an area of about 161.4 million hectares, production of about 506.3 million tonnes and productivity of 3.14 tonnes per hectare. In India area under rice cultivation is 43.39 million hectare and production of about 104.32 million tonnes with 2.40 tonnes per hectare productivity. In Karnataka, it is grown in area of 1.06 million hectares with a production of 2.70 million tonnes and productivity of 2.67 tonnes per hectare (Annon, 2016). In India, rice crop is produced in almost all the zones including southern, northern and north-eastern zones. The major rice producing states of India are West Bengal, Andhra Pradesh, Tamil Nadu, Karnataka and Punjab. Rice not only a staple food but also a way of living in Asia, with more than 250 million farm households dependent on the crop for their livelihood.

The crop growth and production are affected by various biotic and abiotic factors. Biotic stresses include insect pests and diseases caused by fungi, bacteria, viruses, phytoplasmas and nematodes. abiotic stresses, drought, cold, heat, chemical injury, salinity and other non-parasitic disorders are also responsible for significant reduction in production and productivity. Fungal diseases like, blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*), brown spot (*Helminthosporium oryzae*), bakanae disease or foot rot (*Gibberella fujikuroi*), sheath rot (*Sarocladium oryzae*), leaf scald (*Microdochium oryzae*), narrow leaf spot (*Cercospora oryzae*), leaf smut (*Entyloma oryzae*) and udbatta disease (*Balansia oryzae*), bacterial diseases such as bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) are of economical importance. Viral disease such as rice tungro disease (*Rice tungro bacilliform virus* and *Rice tungro spherical virus*) is more prevalent and destructive under Indian condition. In addition to all these diseases, rice crop in recent past is prone to the false smut disease, which is one of the most emerging disease causing significant damage of rice yield and quality worldwide (Abbas *et al*., 2014).

False smut occurs in most of the rice growing areas of the world including India, China, Japan, Southeast Asian countries, North and South America, Myanmar, Sri Lanka, Fiji, and Africa. Among the floral diseases of rice, false smut is gaining importance which is a post-flowering disease prevalent mostly during *Kharif* season. It is also known as Lakshmi disease and was believed to be a mark of a bumper harvest. Earlier it was regarded as sporadic but from the year 2000 onwards; it has been reported as an epidemic disease (Rush *et al.,* 2000; Singh and Pophaly, 2010).

False smut disease is caused by the pathogen *Ustilaginoidea virens* (Cooke) Takahashi, Whose teleomorph is *Claviceps Oryzasativa* (Hashioka), was first reported from Tirunelveli in Tami Nadu (Cooke, 1878) and most recently *Villosiclava virens* has been proposed as the new name for the teleomorph of the false smut fungus (Tanaka *et al.*, 2008). The fungus transforms individual grains of the panicle into greenish spore balls of velvety appearance. The spore balls are small at first and grow to a size two inches or more in diameter. They are smooth and are yellow covered by a membrane. Later, the membrane bursts and the colour of the ball becomes orange/yellow. When cut open, the ball is white in the center with three outer layers (Sciumbato and Street, 2000).

The fungus attack some of the weed species that commonly occur in rice fields and may also serve as sources of inoculum (Atia, 2004). The main reason for losses being incited is that the fungus attacks the panicles. About 15-20 percent losses have been reported by different workers from different provinces (Singh, 1998). It is an important devastating disease causing yield losses from 1 to 11 per cent (Atia, 2004).

Disease incidence of 10-20 per cent and 5-85 per cent respectively has been reported from Punjab and Tamil Nadu on different rice cultivars (Ladhalakshmi *et al.,* 2012).

Reports showed that rice false smut pathogen could produce two kinds of mycotoxins, namely Ustiloxins and Ustilaginoidins (Zhou *et al.,* 2012). This disease results in yield loss contaminated rice grains and. even more important, generating toxins poisoning to humans and domestic animals (Koiso *et al.*, 1994 and Zhou*et al.*, 2012).

Yield loss estimates due to *U. virens* were ranged from 0.2 to 49 per cent on different rice varieties in different regions of the country (Baruah *et al.,* 1992, Singh *et al.*, 1992 and Biswas, 2001). In Karnataka, rice hybrids cultivation is becoming very popular and farmers are adopting the technology easily and obtaining a good yield. Hybrid rice is mostly affected by the incidence of minor diseases like false smut incidence and the crop yield are badly affected by high fertility levels in an irrigated ecosystem. Looking to the expansion of hybrid rice area in Karnataka region, where the farming community almost depends on this important food crop, there isan urgent need to address the biotic stress likes false smut.

Very meager information is available about disease incidence, resistance cultivars and management aspect of false smut disease under field condition. Therefore the present research studies entitled "Investigation on false smut of rice incited by *Ustilaginoidea virens* (Cooke) in hilly and coastal zones of Karnataka" is undertaken with the following objectives:

Materials and Methods

In vitro evaluation of fungicides against *Ustilaginoidea virens*

The present investigation was carried out to evaluate systemic and combi-product fungicides for their efficacy on inhibition of the mycelial growth of *U. virens* by 'Poisoned food technique'.

All systemic fungicides and combi-products were tested at 100ppm, 200ppm and 500ppm concentrations by 'Poisoned food technique' against *U. virens*.

Poisoned food technique

The fungicides were added aseptically to sterilize PSA medium so as to get desired concentrations. Twenty ml of poisoned medium was poured to a Petri plates. Streptomycin sulphate at 1gm/l was added to the sterilized PSA medium before pouring into petri plates to avoid bacterial contaminations and 5 mm diameter of culture disc of the fungus was kept at the center of each Petri plate. Three replications were maintained for each treatment and the Petri plates were incubated at 25 ± 2^0C. The petridish without addition of fungicides and only with the PSA medium served as control. The radial growth of the fungus was recorded when the complete growth was obtained in control plate and per cent inhibition of growth of pathogen was calculated by using the formula given by Vincent's (1927).

$$I = (C-T)/ C \times 100$$

Where,
I = Per cent growth inhibition

C = Average diameter of mycelial growth in control set (mm)

T = Average diameter of mycelial growth in treatment set (mm)

Statistical analysis

Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) and ANOVA was done using factorial CRD for laboratory studies (Gomez and Gomez, 1984; Hosmand, 1988).

Chemical management of false smut under field condition

A field experiment was conducted at Agricultural and Horticultural Research Station, Ponnampet, during *kharif*, 2017-18 to find out the effective fungicide for the control of the false smut of rice.

Experiment was spread-out in Randomised Block Design (RBD) with nine treatments and three replications. Variety used was Tunga and the gross plot size was 10 sq. metres and all packages of practices were followed for conducting the experiment. Two sprays were given for each treatment at 50% flowering stage [80 days after transplanting (DAT)] and 100% flowering (90 DAT).

The observations on false smut disease incidence were recorded as per the methodology mentioned in previous section (3.2). Further, yield from all the treatments was weighed and recorded at the time harvest of the crop.

Statistical analysis

Statistical analysis was done as per the procedures given by Panse and Sukathme (1985). Actual data in percentage were converted to Arc sine values, before analysis according to the table given by Snedecor and Cochran (1967).

Results and Discussion

In vitro evaluation of fungicides against *Ustilaginoidea virens*

The efficacy of six different systemic fungicides and two combi-products were evaluated for inhibition of growth of pathogen at three different concentrations. The results indicated that, all the fungicides evaluated at different concentrations were effective on inhibition of growth of the *U. virens* (Table 1 and Plate 1). Among systemic fungicides evaluated, the cent per cent inhibition was observed in Propiconazole 25 % EC, Tebuconazole 250 EC, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC and Tebuconazole 50 % + Trifloxystrobin 25 % WG at all the concentrations. Azoxystrobin 25 % SC resulted in 82.49, 100 and 100 per cent inhibition whereas difenconazole 25 EC recorded 75.08**,** 80.58, and 100 per cent inhibition of mycelia growth at 100, 200 and 500 ppm concentrations respectively. However, Carbendazim 50 % wp and Kresoxim methyl 44.3 % were least effective with inhibition of85.47, 89.32 and 92.29 per cent and 40.25, 47.54 and 57.29 per cent at 100, 200 and 500 ppm concentrations respectively.

The false smut disease of paddy is caused by soil borne and air borne inoculums of *U. virens*, therefore, management under field condition is difficult. The peculiar infection process *i.e.* infection during flower opening stage make the management more complex than any other paddy diseases. Due to its soil borne and air borne nature the easiest disease management practices such as seed treatment, crop rotation *etc*., are ineffective. Moreover, it has been reported that the resistance breeding programme is slower due to limited resistance against this disease. Therefore, use of chemical fungicides is the only practical method suitable for management under field condition.

In the present study, eight novel fungicides (both single and combination) were tested against *U. virens* under *in vitro* condition. Among the different fungicides tested at different concentrations *in vitro*, Propiconazole 25 % EC, Tebuconazole 250 EC, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC and Tebuconazole 50 % + Trifloxystrobin 25 % WGshowed 100 per cent inhibition of the *U. virens* at all the three concentrations tested. Previous researchers have reported the effectiveness of many fungicides (both solo and combination) such as Carbendazim + Mancozeb (Hegde *et al.*, 2000b), Difenconazole 25 % EC, Propiconazole 25 % EC and Tebuconazole 250 EC (Chen *et al.*, 2013), Carbendazim, Propiconazole and tebuconazole (Saurabh *et al.*, 2014), Trifloxystrobin + Tebuconazole, Propiconazole (Ladhalakshmi *et al.*, 2014), Propiconazole, Tebuconazole, Trifloxystrobin + Tebuconazole (Shivamurthy, 2017) against false smut pathogen *in vitro* and our results are in complete consistence with their findings.

Chemical management of false smut under field condition

A field experiment was conducted at AHRS, Ponnampet during *Kharif* 2017 to find out the effective fungicide for the management of false smut of rice and the results obtained are presented below (Table 2 and Plate 2).

Among the different treatments, two sprays of Tebuconazole (50 %) + Trifloxystrobin (25 %) WP was highly effective in the management of false smut disease with least infected tillers (3.51 %), which was followed by Azoxystrobin 18.2 % + Difenconozole 11.4 % SC, Propiconazole 25% EC and Azoxystrobin 25 % SC with 4.77, 5.67, and 6.80 per cent infected tillers respectively. The highest per cent infected tillers were observed in untreated control (11.56 %).

In terms of per cent infected grains, the treatment with Tebuconazole 50 %+ Trifloxystrobin 25 % WP recorded the lowest percentage of infected grains (0.80 %), which was followed by the treatment of Azoxystrobin 18.2 % + Difenconozole 11.4 % SC with 1.29 % and Propiconazole 25% EC with 1.85%. The highest per cent of infected grains was observed in untreated control (6.23%).

List of chemicals evaluated for their efficacy against *U. virens*

Sl. No.	Fungicide	Trade name
1	Azoxystrobin25% SC	Amistar 25% SC
2	Difenconazole25% EC	Score 25% EC
3	Kresoxim methyl44.3 % SC	Ergon 44.3 % SC
4	Propiconazole25% EC	Tilt 25% EC
5	Carbendazim50 % WP	Bavistin 50 % WP
6	Tebuconazole250 EC	Folicur 250 EC
7	Azoxystrobin 18.2%+ Difenconazole 11.4% SC	Amistar top 325 SC
8	Tebuconazole 50% + Trifloxystrobin 25% WG	Nativo 75 % WG
9	Control	

List of fungicides evaluated under field condition against *U.virens*

Sl. No.	Treatment	Concentration
1	Azoxystrobin 25 % SC	1.0 ml/l
2	Difenconazole 25 % EC	1.0 ml/l
3	Kresoxim methyl 44.3 % SC	1.0 ml/l
4	Propiconazole 25 % EC	1.0 ml/l
5	Carbendazim 50 % WP	1.0 g/l
6	Tebuconazole 250 EC	1.0 ml/l
7	Azoxystrobin 18.2%+ Difenconazole 11.4% SC	1.0 ml/l
8	Tebuconazole 50% + Trifloxystrobin 25% WG	0.4 g/l
9	Untreated Control	……….

Table.1 *In vitro* evaluation of fungicides against *Ustilaginoidea virens*

Sl. No.	Fungicide	Inhibition (%)			
		Concentration (ppm)			
		100	200	500	Mean
1	Azoxystrobin 25 % SC	82.49 *(65.30)	100.00 (90.05)	100.00 (90.05)	94.16 (81.80)
2	Difenconazole 25 % EC	75.08 (60.08)	80.58 (63.90)	100.00 (90.05)	85.22 (71.34)
3	Kresoxim methyl 44.3 % SC	40.25 (39.40)	47.54 (43.61)	57.29 (49.22)	48.36 (44.08)
4	Propiconazole 25 % EC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
5	Carbendazim 50 % WP	85.47 (67.63)	89.32 (70.96)	92.29 (73.92)	89.03 (70.83)
6	Tebuconazole 250 EC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100 (90.05)
7	Azoxystrobin 18.2% + Difenconazole 11.4% SC	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100 (90.05)
8	Tebuconazole 50% + Trifloxystrobin 25% WG	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100 (90.05)
9	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean		75.92 (65.84)	79.72 (69.85)	83.29 (73.71)	79.64 (69.80)
		S.Em±		**C.D at 1%**	
F		0.46		1.73	
C		0.28		1.06	
F×C		0.26		1.00	

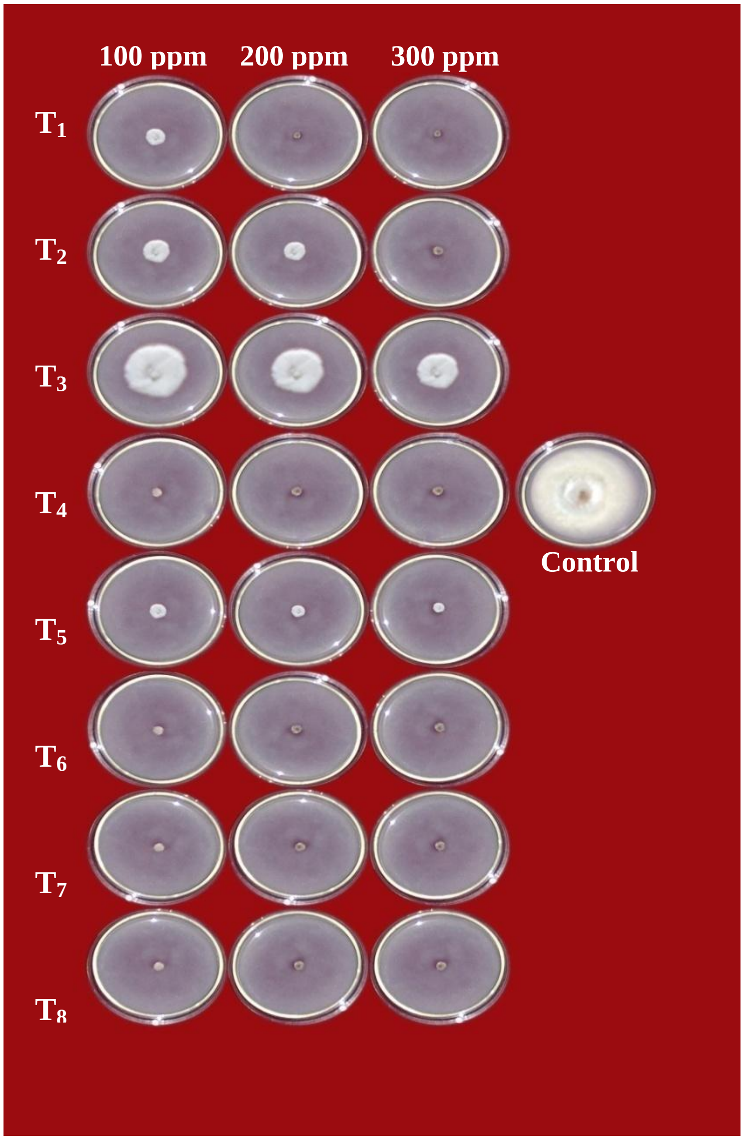
*Figures in parenthesis are arc sin transformed values

Table.2 Chemical management of false smut of rice under field condition during *Kharif* -2017-18

Sl. No.	Treatments	Dosage (g or ml/l)	Per cent infected tillers	Per cent infected grains	Disease severity (%)	Reduction in disease severity over control (%)	Yield (q/ha)
1	Azoxystrobin 25% SC	1.0	6.80 *(15.01)	2.33 (8.73)	15.87 (23.56)	77.98	48.36
2	Difenconazole 25% EC	1.0	7.65 (16.03)	2.96 (9.86)	22.65 (28.56)	68.57	46.48
3	Kresoxim methyl 44.3 SC	1.0	10.48 (18.87)	5.37 (13.37)	56.31 (49.25)	21.87	41.48
4	Propiconazole 25% EC	1.0	5.67 (13.71)	1.85 (7.63)	10.50 (18.85)	85.43	49.47
5	Carbendazim 50 WP	1.0	9.68 (18.09)	3.75 (11.08)	36.26 (36.33)	49.69	43.12
6	Tebuconazole 250 EC	1.0	8.73 (17.17)	3.31 (10.43)	28.94 (32.12)	59.85	45.09
7	Azoxystrobin 18.2% + difenconazole 11.4% SC	1.0	4.77 (12.56)	1.29 (6.40)	6.17 (14.37)	91.44	53.72
8	Tebuconazole 50% + Trifloxystrobin 25% WG	0.4	3.51 (10.64)	0.80 (4.75)	2.80 (8.47)	96.11	55.42
9	Control	-	11.56 (19.86)	6.23 (14.45)	72.08 (60.04)	-	38.68
S. Em ±			**0.82**	**0.80**	**2.97**	**-**	**1.54**
CD at 5%			**2.48**	**2.41**	**8.91**	**-**	**4.63**
CV (%)			**9.08**	**14.47**	**17.06**	**-**	**5.71**

*Figures in parenthesis are arc sin transformed values

Plate.1 Effect of fungicides on inhibition of mycelial growth of *Ustilaginoidea virens*



T_1: Azoxystrobin 25 % SC, T_2: Difenconazole 25 % EC, T_3: Kresoxim methyl 44.3 % SC, T_4: Propiconazole, 25 % EC, T_5: Carbendazim 50 % WP, T_6: Tebuconazole 250 EC, T_7: Azoxystrobin 18.2 %+ Difenconazole 11.4 % SC, T_8: Tebuconazole 50 % + Trifloxystrobin 25 % WG

Plate.2 General Field view of an experimental plot

Among all the treatments two sprays of Tebuconazole 50 % + Trifloxystrobin 25 % WP was highly effective in reducing the false smut disease severity with least disease severity of 2.80 per cent, followed by Azoxystrobin 18.2 % + Difenconozole 11.4 % SC with 6.17 per cent, Propiconazole 25% EC (10.50%) and Azoxystrobin 25 % SC (15.87%). The highest percentage of disease severity was observed in untreated control (72.08 %).

On comparison with the grain yield obtained from each plot it was found that the treatment with Tebuconazole 50 % + Trifloxystrobin 25 % WP gave the highest grain yield of 55.42 q/ha which was followed by the Azoxystrobin 18.2 % + Difenconozole 11.4 % SC (53.72 q/ha), Propiconazole 25% EC (49.47 q/ha) and Azoxystrobin 25 % SC (48.36q/ha) as against grain yield of 38.68 q/ha in untreated control.

It was inferred that the treatment with the fungicide Tebuconazole 50 % + Trifloxystrobin 25 % WP gave the lowest false smut disease severity of 2.80 per cent, which was followed by the Azoxystrobin 18.2 % + Difenconozole 11.4 % SCwith 6.17 per cent, Propiconazole 25% EC with 10.50 per cent and Azoxystrobin 25 % SC with 15.87 per cent. Kresoxim methyl 44.3 % SC was recorded least effective with disease severity of 56.31 per cent, however found significantly different with 72.08 per cent in untreated control.

In case of rice, many researchers have reported the increased grain yield after application of fungicides due to reduction in biotic stress on plant during critical growth stages (Sood and Kapoor, 1997, Tirmali *et al.*, 2001, Prabhu *et al.*, 2003, Usman *et al.*, 2009, Naik *et al.*, 2012, Bhuvaneshwari and Raju, 2012, Bag *et al.*, 2016 and Pramesh *et al.*, 2016a&b). For management of false smut disease, efficacy of many fungicides has been reported previously (Chen *et al.*, 2013, Kumar, 2015, Raji *et al.*, 2016 and Hosagoudar, 2018).

In the present study, in addition to the previously reported fungicides, we are reporting the efficacy of new combination of fungicides such as Tebuconazole 50 % + Trifloxystrobin 25 % WG and Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, for the management of false smut disease under field condition.

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