J. Farm Sci., 36(4): (355-359) 2023

RESEARCH PAPER

In vitro sensitivity of *Rhizoctonia solani* f. sp. *sasakii* causing banded leaf and sheath blight of maize against different fungicides

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(Received: March, 2021 ; Accepted: December, 2023)

DOI: 10.61475/JFS.2023.v36i4.06

Abstract: The laboratory experiment was conducted to evaluate the new fungicide molecules against *Rhizoctonia solani* f. sp. *sasakii* by poisoned food technique under *in vitro* conditions. Out of eleven fungicides tested, the maximum (100%) mycelial inhibition was documented in eight treatments involving both systemic and combi fungicides *viz*., Carbendazim 50% WP, Propiconazole 25% EC, Tebuconazole 250 EC, Metiram 55% + Pyraclostrobin 5% WG, Fluopyram 17.7% + Tebuconazole 17.7% SC, Iprovalicarb 5.5% + Propineb 61.25%, Carboxin 37.5% + Thiram 37.5% WP and Tebuconazole 50% + Trifloxystrobin 25% WG at all the concentrations tested. Whereas, the least mycelial inhibition was recorded in Pencycuron 250 SC and Dimethomorph 50% WP at 0.05, 0.10 and 0.15 per cent concentrations, respectively.

Key words: Banded leaf, Fungicide, Maize, *Rhizoctonia solani,* Sheath blight

Introduction

Maize (*Zea mays* L) is grown in varied agro-climatic conditions throughout the world and it is one of the world's third most widely cultivated field crops after rice and wheat. It is called as 'Queen of Cereals' because of its high productive capacity among the cereal crops. Globally, more than 170 countries are growing almost 1147.7 million metric tons of maize from an area of 193.7 million hectares, with average productivity of 5.75 t/ha (Anon., 2020). Among the maize-producing nations, India ranks 4th in area and 7th in production, accounting for about 4 per cent of the global maize area and 2 per cent of total production. During 2021-22, the maize area was amounted to 10.04 million hectares with a production of 33.62 million MT and average productivity reached 3349 kg ha^{-1}. In Karnataka, the area under maize was 1.59 million ha which is of 15.87 per cent area of the country with the production of 5.22 million tons with the national share of 15.53 per cent and the productivity of 3279 kg/ha during the year 2021-22 (Anon., 2022). The major maize growing districts in Karnataka are Bagalkot, Belagavi, Bellary, Vijayapur, Chitradurga, Davanagere, Dharwad, Gadag, Haveri, Hassan, Koppal, Mandya, Mysore, Shimogaa and Uttara Kannada.

In contrast, the area under maize is increasing day by day due to its adaptability to varied climatic situations. The multiple utilities of maize as a 'food', 'fodder' and 'feed' make it more demand friendly and insulates it against low demand situations for enhancing farmer's income and livelihoods in almost all areas of its cultivation.

Although its area, production and productivity are increasing, but some of the biotic agents are limiting its growth and yield potential of the crop. Among the biotic agents, one of the major deterrents of maize is its sensitivity to a multitude of diseases that are detrimental to production and productivity. Maize is proned to several fungal diseases *viz.,* turcicum leaf blight, maydis leaf blight, downy mildews, rusts, and stalk rots, resulting in considerable loss in yield. Among them, banded leaf and sheath blight (BLSB) is responsible for a significant loss of grain yield from 11 to 40 per cent and even to 100 per cent in some cultivars in certain warm and humid areas where the conditions *viz.,* high relative humidity (90%), an optimum temperature of 28° C and rainfall during first week of infection congenial for pathogen (Madhavi *et al*., 2011, Izhar and Chakraborty 2013; Gao *et al.,* 2014).

In India, BLSB disease was first reported by Ullstrup in 1960 as banded leaf and sheath blight of maize caused by *Hypochonus sasakii* from the Tarai region of Uttar Pradesh (Payak and Renfro, 1966). The symptoms of the disease occur on all aerial parts of the plant except the tassel, but the symptoms on the leaves appear as irregularly globular to elongated and reflect as water-soaked areas. Later, these affected areas tend to be bleached and become straw coloured that leads to necrotic lesions (Ahuja and Payak, 1982). Lesions enlarge rapidly resulting in discoloured areas alternating with dark bands, which are often described as typical symptoms of BLSB (Rani *et al.,* 2013).

The status of banded leaf and sheath blight of maize in northern Karnataka was reported by Rajput and Harlapur (2014) and it is one of the destructive emerging diseases of maize in the northern Karnataka in the recent past. In the present scenario, management of BLSB is considered to be very essential because the disease caused by *Rhizoctonia solani* f. sp. *sasakii* AG1-IA is the most predominant and serious limiting factor for the efficient cultivation of maize in the world. So, the management of BLSB disease by the fungicides is extremely important to reduce crop destruction and to prevent the yield loss.

Although the continuous use of fungicides is dangerous for the ecosystem, there is no other way to combat the pathogen, and many times the situation demands the farmer to rely on

these synthetic pesticides. Thus, the thrust for formulating the management strategy is a key to avoid its widespread occurrence and spreading to new areas through fungicidal approach as a preventive measure. Hence, a study on *in vitro* evaluation of new fungicidal molecules against *Rhizoctonia solani* Kuhn f. sp. *sasakii* (Exner) causing banded leaf and sheath blight of maize have been undertaken to provide first-hand information in confirming fungal sensitivity against specific fungicide which serves as a reliable basis for field testing.

Material and methods

In vitro studies

The study on evaluation of fungicides *in vitro* was conducted in the Department of Plant Pathology, College of Agriculture, UAS, Dharwad during 2018-19.

The efficacy of new fungicide molecules was tested at three different concentrations *viz.,* (0.05%, 0.1% and 0.15%) against the pathogen by following 'poisoned food technique'. The required concentrations of fungicides was prepared and incorporated into sterilized, cooled potato dextrose agar. Twenty ml of poisoned medium was poured into 80 mm sterilized Petri plates and all plates were inoculated with actively growing culture of the pathogen with five mm mycelial disc from the periphery of the culture plate. Three replications were maintained for each treatment. These plates were incubated at 27 ± 1 °C until full growth of the pathogen in control treatment and colony diameter was recorded. Per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) as follows:

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

Where,

I = Per cent inhibition of mycelial growth

C = Growth of mycelium in control

T = Growth of mycelium in treatment

The fungicide molecules evaluated against *Rhizoctonia solani* f. sp. *sasakii* under *in vitro* was listed in Table 1. The data was statistically analysed using completely randomized design (CRD) through two way anova from online software OP Stat, developed by Hissar Agricultural University, Haryana. The treatments were compared with the untreated control for better understanding and for getting conclusive evidences. The critical difference used was at 1 per cent. The results are presented in Table 2.

Results and discussion

In vitro efficacy of fungicides against *Rhizoctonia solani* f. sp. *sasakii*

In the present experiment, totally eleven fungicides were evaluated against *Rhizoctonia solani* f. sp. *sasakii* under *in vitro* by the poisoned food technique and data is presented in the Table 2, Plate 1 and Fig. 1.

Table 1. List of fungicide molecules evaluated against *Rhizoctonia solani* f. sp. *sasakii* under *in vitro*

Fungicide name	Trade name
Metiram complex 55% + Pyraclostrobin 5% WG	Cabrio top
Carbendazim 50% WP	Bavistin
Dimethomorph 50% WP	Acrobat
Fluopyram 17.7% + Tebuconazole 17.7% SC	Luna Experience
Iprovalicarb 5.5% + Propineb 61.25% WP	Melody Duo
Pencycuron 250 SC	Monceren
Propiconazole 25% EC	Tilt
Fenamidon 10% + Mancozeb 50%	Sectin
Tebuconazole 250 EC	Folicure
Carboxin 37.5% + Thiram 37.5% WP	Vitavax power
Tebuconazole 50% + Trifloxystrobin 25% WG	Nativo
Control (untreated)	

All the fungicides evaluated were significantly inhibited the mycelial growth of the test pathogen except Pencycuron 250 SC (ineffective) and Dimethomorph 50% WP (least effective) over control for per cent mycelial inhibition. Among the 11

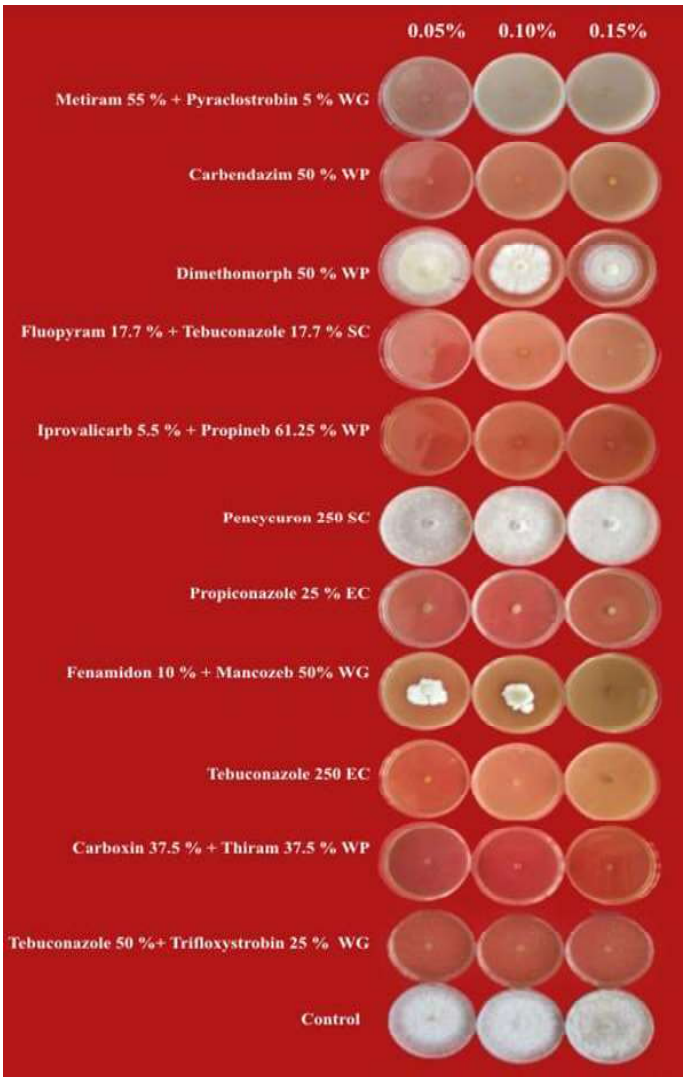

Plate 1. *In vitro* efficacy of fungicides against *Rhizoctonia solani* f. sp. *sasakii*

Table 2. *In vitro* efficacy of the fungicides against *Rhizoctonia solani* f. sp. *sasakii*

Treatment No.	Treatments	Per cent Inhibition of Mycelial Growth			
		Concentration (%)			Mean
		0.05	0.10	0.15	
T_1	Metiram 55% + Pyraclostrobin 5% WG	100.00(10.05)*	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_2	Carbendazim 50% WP	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_3	Dimethomorph 50% WP	1.53(1.46)	22.37(4.83)	23.75(4.97)	15.88(3.75)
T_4	Fluopyram 17.7% + Tebuconazole 17.7% SC	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_5	Iprovalicarb 5.5% + Propineb 61.25% WP	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_6	Pencycuron 250 SC	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)
T_7	Propiconazole 25% EC	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_8	Fenamidon 10% + Mancozeb 50% WG	46.95(6.91)	56.95(7.61)	100.00(10.05)	67.97(8.19)
T_9	Tebuconazole 250 EC	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_{10}	Carboxin 37.5% + Thiram 37.5% WP	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_{11}	Tebuconazole 50% + Trifloxystrobin 25% WG	100.00(10.05)	100.00(10.05)	100.00(10.05)	100.00(10.05)
T_{12}	Control (untreated)	0.00(10.05)	0.00(10.05)	0.00(10.05)	0.00(10.05)
	Mean	77.13(8.16)	79.94(8.53)	83.98(8.77)	80.35(8.39)
				S.Em±	C.D. at 1%
	Fungicides (F)			0.05	0.16
	Concentration (C)			0.03	0.08
	F×C			0.10	0.30

*Mean of three replications, Figures in the parenthesis are square root transformed values

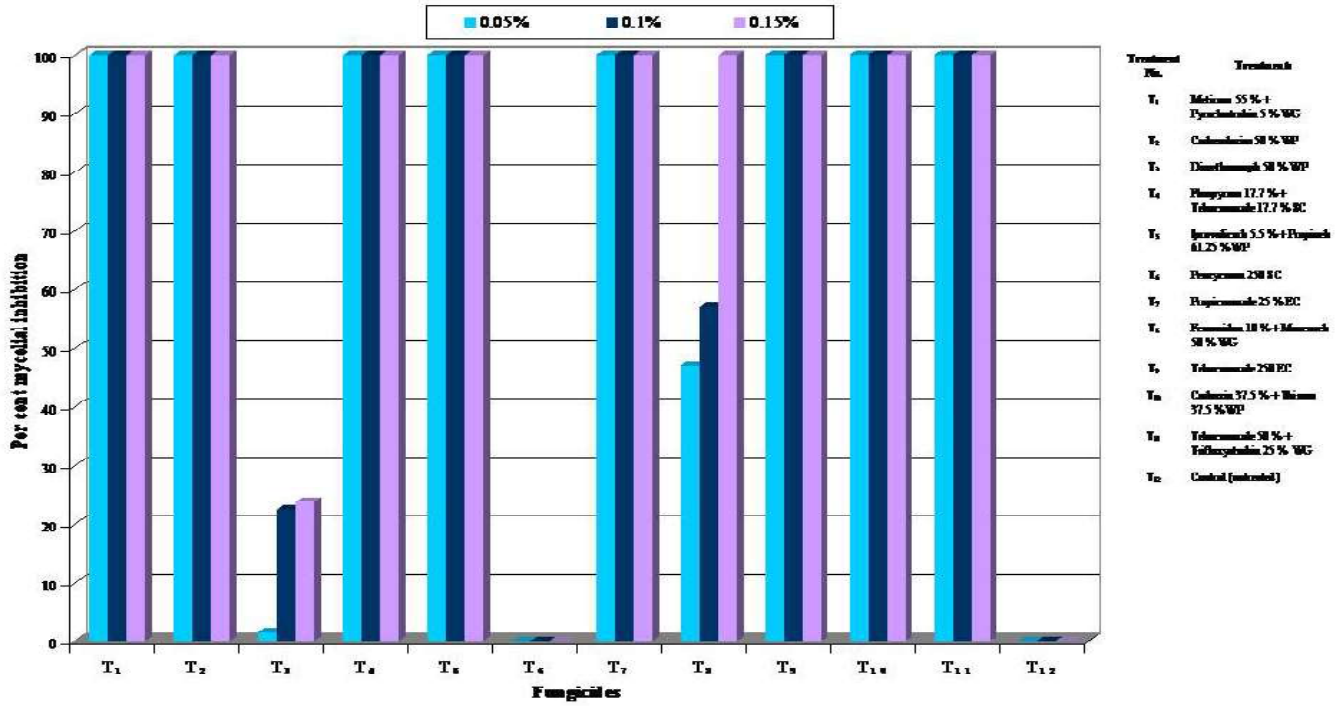

Fig 1. Sensitivity of *Rhizoctonia solani* f. sp. *sasakii* against different fungicides under *in vitro*

fungicides tested at three different concentrations (each @ 0.05, 0.10 and 0.15%), the fungicides *viz.,* Carbendazim 50% WP, Propiconazole 25% EC, Tebuconazole 250 EC, Metiram 55% + Pyraclostrobin 5% WG, Fluopyram 17.7% + Tebuconazole 17.7 % SC, Iprovalicarb 5.5% + Propineb 61.25%, Carboxin 37.5% + Thiram 37.5% WP and Tebuconazole 50% + Trifloxystrobin 25% WG resulted with cent per cent (100%) mycelial growth inhibition followed by Fenamidon 10% + Mancozeb 50% WG, with 46.95, 56.95 and 100 per cent mycelial growth inhibition @ 0.05, 0.10 and 0.15%, respectively (Plate.1). However, these treatments were statistically superior over control in limiting the growth of the pathogen. Hence, the above said fungicides were worked well in limiting the growth of the pathogen. This may be due to the presence of active ingredient in correct dose and action of fungicides *viz.,* Metiram 55% + Pyraclostrobin 5% WG, Metiram belongs to dithiocarbamates having multisite mode of action and pyraclostrobin belongs to methoxy carbamates having mode of action on complex 11 of fungal respiration; ubiquinol oxidase, quinone site, Carbendazim 50% WP belongs to methyl benzimidazole carbamates targets on mitosis: â-tubuline assembly, Fluopyram 17.7% + Tebuconazole 17.7%

SC contains two fungicide molecules, Fluopyram belongs to benzamides that targets on succinate dehydrogenase (quinone) inhibitor; Tebuconazole belongs to triazoles (Demethylation inhibitors) sterol biosynthesis inhibitors Class-I that targets on C-14 demethylation in sterol biosynthesis;, Iprovalicarb 5.5% + Propineb 61.25% contains two fungicide products Iprovalicarb and Propineb, the fungicide molecule Iprovalicarb belongs to carbamates/ carboxylic acid amides that targets on phospholipid biosynthesis and cell wall deposition and Propineb belongs to dithiocarbamates group that targets on multisite contact activity; Propiconazole fungicide have the same function as that of Tebuconazole as mentioned above. The fungicide Carboxin 37.5% + Thiram 37.5% WP contains two fungicide molecules, first one *ie.,* Carboxin belongs to carboxamides group that targets on succinate dehydrogenase complex II in fungal respiration, Thiram belongs to dithiocarbamates M3 group having multi-site contact activity; and the Tebuconazole 50% + Trifloxystrobin contains two fungicide molecules, Tebuconazole function was same as explained above and fungicide Trifloxystrobin belongs to oximino acetates (Quinone outside inhibitors) that targets on complex III of fungal respiration ubiquinol oxidase quinone site. All the tested effective fungicides and their respective concentrations were shown positive results because they were hitted on the target site of *Rhizoctonia solani* f. sp. *sasakii* as explained in the earlier statements. The present findings are in accordance with several workers results *viz*., Akhtar *et al.* (2010), Bastide *et al*, (2016), Devi and Thakur, (2016), Raji *et al.* (2016), Rajput *et al*. (2016), Sharma *et al.* (2017), Malik *et al*. (2018) indicating the fungicides that works well because of the active ingredients were targeting the site of action in the pathogen.

The fungicides Pencycuron 250 SC and Dimethomorph 50% WP showed the least mycelial inhibition in all the three (0.05, 0.10 and 0.15%) concentrations tested, respectively. The fungicide Pencycuron belongs to phenylureas group that targets on cell division and the fungicide Dimethomorph belongs to belongs to carbamates/ carboxylic acid amides that targets on phospholipid biosynthesis and cell wall deposition. The ineffectiveness of these fungicides on the pathogen *Rhizoctonia solani* f. sp. *sasakii* may be due to the usage of less concentration or due to presence of less active ingredient in the fungicides Pencycuron and Dimethomorph.

Conclusions

In vitro efficacy of fungicides serves as a preliminary step in confirming fungal sensitivity against specific fungicide and, it serves as a reliable basis for field testing and therefore, an attempt was made to check the sensitivity of the pathogen in laboratory conditions through poisoned food technique. The results revealed that Carbendazim 50% WP, Propiconazole 25% EC, Tebuconazole 250 EC, Metiram 55% + Pyraclostrobin 5% WG, Fluopyram 17.7% + Tebuconazole 17.7% SC, Iprovalicarb 5.5 % + Propineb 61.25%, Carboxin 37.5% + Thiram 37.5% WP and Tebuconazole 50% + Trifloxystrobin 25% WG at 0.05, 0.10 and 0.15 per cent concentration showed 100 per cent mycelial inhibition of *Rhizoctonia solani* f. sp. *sasakii.* Hence, the above said fungicides can be used for testing *in vivo* to limit the growth of the pathogen.

Acknowledgement

Authors are thankful to Department of Plant Pathology, College of Agriculture, Dharwad for providing the laboratory facilities during the study and I am honoured to work in the University of Agricultural Sciences, Dharwad.

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