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Research Article

IN -VITRO STUDY OF FUNGICIDES AND AN ANTIBIOTIC AGAINST *Rhizoctonia solani* f.Sp. *Sasakii* CAUSING BANDED LEAF AND SHEATH BLIGHT OF MAIZE

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Abstract- Sixteen fungicides and one antibiotic namely: Propiconazole, Hexaconazole, Difenconazole, Thiophenate-Methyl, Carbendazim, Tebuconazole, Mancozeb, Copper oxychloride, Zineb, Chlorothalonil, Carbendazim+Mancozeb, Carboxin+Thiram, Tricyclazole+Mancozeb, Hexaconazole+Zineb, Carbendazim+Iprodione, Trifloxystrobin+Tebuconazole and Validamycin were screened *in-vitro* by using poisoned food techniquefor their effect on inhibition of mycelium growth of *Rhizoctonia solani f .sp. sasakii* ,the causal agent of banded leaf and sheath blight of maize. Among systemic fungicides screened, maximum inhibition of myceli um growth was observed in Propiconazole and Carbendazim, while maximum inhibition of mycelium growth was observed in Carbendazim+ Mancozeb and Carbendazim + Iprodione among combi-product fungicides tested. Maximum inhibition of mycelium growth was observed in Mancozeb and Validamycin among the tested non-systemic fungicides.

Keywords- *Rhizoctonia solani* f.sp. *sasakii,* Fungicides, Antibiotic and *in-vitro* growth

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Introduction

Maize (*Zea mays* L.) fetch most significant role in cereal production in the world. Maize is grown throughout the world under a wide range of climatic conditions. Maize was introduced to India from USA during the beginning of 17th century [3].Maize is one of the important crops in India occupying fifth place in area and third place in production. Maize occupied an area of nearly of 8.26 mha, having production 19.73 mt and productivity 2295 kg/ ha, in India [1]. It is one of the major crops of North Karnataka and is being cultivated under rainfed and irrigated conditions. Around 112 diseases of maize have been reported so far from different parts of the world. Of these, 65 are known to occur in India leading to about 15-20% yield losses annually [8]. Among them Banded leaf and sheath blight (BLSB) of maize caused by *Rhizoctonia solani* f. sp. *sasakii* Exner is gaining economic importance in North Karnataka. Several fungicides like carbendazim, thiophanatemethyl, iprobenfos, captan, mancozeb + thiophanatemethyl, copper oxychloride and thiram are found to be effective in inhibiting the growth of pathogen in *in-vitro* condition [9]. Some chemicals like carbendazim and thiophanatemethyl also proved most effective as they caused the maximum inhibition of mycelial growth [7]. Yet, limited information is available on its sustainable management. Therefore, the present investigation was aimed to study inhibition of mycelia growth of *R. solani* f. sp. *sasakii*at different concentrations of several fungicides. In view of the increasing importance of BLSB disease, the present study was carried out. The purpose of the study was to evaluate different fungicides under laboratory conditions to find out the most effective one for final use. The results of this study will be helpful to the maize growers to adopt the most appropriate control strategy.

Material and methods

The present investigation was carried out at Department of Plant Pathology, Agricultural College, University of Agricultural Sciences, Dharwad, Karnataka, India during the year, 2013.

Isolation of the pathogen

The diseased leaves and leaf sheaths of maize plants showing characteristic symptoms of banded leaf and sheath blight were collected from Northern Karnataka during the year 2012. Infected portion of collected leaves and leaf sheaths were cut into small pieces of 5 to 6 mm and the bits. For 1 minute, they were surface sterilized with 1.0 per cent sodium hypochlorite solution. Consequently, they were washed three times with double distilled sterilized water, and then aseptically transferred to sterilized Petri plates containing sterilized PDA medium in aseptic condition. These plates were incubated at 26 ± 1 °C for four days to obtain luxuriant growth of the fungus. Purification of isolated fungi was done using hyphal tip technique. The principle growth characters like morphological, cultural and sclerotia formation were considered for identification of pure cultures of causal organism. These characters were compared and identified as *R. solani* f. sp. *sasakii* based on the observed traits [8]. The pure culture of the fungus was sub cultured on PDA slants and kept in laboratory at 28 ± 2°C for 15 days. In refrigerator, at 5°C such mother culture slants were preserved. Further, these cultures were sub-cultured once in a month and used for future studies.

Efficacy of fungicides and antibiotic assay

In-vitro evaluation of various fungicides and an antibiotic to check the mycelial growth was done through poisoned food technique on PDA medium

[2]. The experiment was conducted in Completely Randomized Design (CRD) with 17 treatments and 3 replications. Six systemic fungicides *viz.,* propiconazole 25 EC, hexaconazole 5 EC, difenoconazole 25 EC, thiophanate methyl 60 WP, carbendazim 50 WP and tebuconazole250 EC were evaluated *in-vitro* at 0.025, 0.05 and 0.1% concentration and four non systemic fungicides *viz*., mancozeb 75 WP, copper oxychloride 50 WP, zineb 68 WP and chlorothalonil 65 WP were evaluated *in-vitro* at 0.1, 0.15 and 0.2 % concentration. In addition, six combi-product fungicides *viz*., carbendazim 12%+ mancozeb 63%, carboxin 36.5% + thiram 36.5%, tricyclazole 18% + mancozeb 62%, hexaconazole 4% + zineb 68%, carbendazim 25% + iprodione 25% and trifloxystrobin 25%+ tebuconazole 50% were evaluated *in-vitro* at 0.1, 0.15 and 0.2% concentration. Antibiotic validamycin was screened at 0.03, 0.04 and 0.05% concentrations. *R. solani* f. sp. *sasakii* was grown on PDA medium in petri plates for four days prior to experiment. Fungicide suspension was prepared in PDA by adding requisite quantity of fungicides to obtain the desired concentration on the basis of active ingredient of the chemical. Sterilized Petri plates were filled with such kind of Poisoned medium. Mycelial disc of 4 mm was taken from the periphery of four days old culture and placed in the centre and incubated at 28 ± 2°C till growth of the fungus reaches the periphery in the control plate. Three replications were maintained for each treatment. Average of colony diameter was measured in two directions and inhibition percentage was calculated with formula [11].

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

Where, I = Per cent inhibition of mycelium growth; C = Growth of mycelium in control; T = Growth of mycelium in treatment.

Results and Discussion

Results were presented in [Tables-1, 2 and 3]. Results revealed that all the tested fungicides significantly inhibited mycelial growth of the fungus. Among the six systemic fungicides tested, propiconazole 25% EC (100%) and carbendazim50% WP (100%) gave maximum mean mycelial inhibition which were significantly superior to all other treatments followed by hexaconazole 5% EC (98.0%) and tebuconazole 250% EC (95.28%). At 0.025% concentration, propiconazole 25% EC and carbendazim 50% WP (100% and 100% mycelial inhibition respectively) were statistically on a par with each other as well as significantly superior over remaining fungicides followed by hexaconazole 5% EC (94.1%). At 0.05% concentration, significantly maximum mean per cent inhibition of mycelial growth was recorded in propiconazole 25% EC and found to be effective (100% mycelial inhibition) which was statistically on par with hexaconazole 5% EC (100%) and carbendazim 50%WP (100%) and was significantly different with all others. All systemic fungicides showed 100% inhibition at 0.01%. Among six systemic fungicides tested propiconazole 25% EC, carbendazim 50%WP and hexaconazole 5% EC were found to be most effective at all concentrations whereas tebuconazole 250% EC, difenoconazole 25% EC and thiophanate methyl 70 WP were found least effective at lower concentrations. Among four non-systemic fungicides and one antibiotic tested, mancozeb 75 WP (94.0%) and validamycin (88.3%) gave maximum mean mycelial inhibitions, which were significantly superior to all other treatments followed by chlorothalonil 75 WP (83.8%) and zineb 78 WP (82.4%). At 0.1% concentration, mancozeb 75 WP gave maximum mean mycelial inhibition of 88.8%, which was significantly superior over other fungicides followed by chlorothalonil 75 WP (80.5%). At 0.15% concentration mancozeb 75 WP gave maximum mean mycelial inhibition of 95.5%, which was significantly superior over other non-systemic fungicides, tested, followed by validamycin (88.8%) and chlorothalonil 75 WP (82.2%). At 0.2% concentration, mancozeb 75 WP gave maximum mean mycelial inhibition of 97.70 %, which was on par with validamycin (95.5%). Among the combi-product fungicides tested, carbendazim 12%+ mancozeb 63% and carbendazim 25% + iprodione 25% were found to be most effective at all concentrations. Carbendazim 12%+ mancozeb 63% and carbendazim 25% + iprodione 25% at 0.1% gave 100.0% inhibition and were found to be the most effective fungicides. Similar trend was observed in the treatment with carbendazim 12% + mancozeb 63% and carbendazim 25% + iprodione 25% at 0.1, 0.15 and 0.2% (100%). The carbendazim 12%+ mancozeb 63%, carbendazim 25% + iprodione 25%, carboxin 37.5% + thiram 37.5% and trifloxystrobin 25% + tebuconazole 50% @ 0.2% concentration were found to be most effective and gave 100.0 % mycelial inhibition of fungus which is statistically on par with each other. Among the six systemic, four non-systemic, six combi-product fungicides and one antibiotic tested, propiconazole 25% EC, carbendazim 50% WP, carbendazim 12 %+ mancozeb 63 % and carbendazim 25 % + iprodione 25 % gave 100.0% mean mycelial inhibition at all their tested concentration. Propiconazole @5.2 X 10^{-2} µg a.i. ml^{-1} was found very effective against *R. solani* f. sp. *sasakii* under *in-vitro* conditions [6]. Carbendazim also inhibited 95-100% of fungal growth of *R. solani* [4,5,10].

Table-1 *In-vitro evaluation of systemic fungicides against Rhizoctonia solani f. sp. sasakii*

Fungicide	Per cent inhibition of mycelial growth Concentration (%)			Mean
	0.025	0.05	0.1	
Hexaconazole 5 EC	94.1 (75.9)	100.0 (90.0)	100.0 (90.0)	98.0 (81.9)*
Propiconazole 25 EC	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Difenoconazole 25 EC	91.7 (73.3)	94.1 (75.9)	100.0 (90.0)	95.2 (77.4)
Thiophanate methyl 70 WP	91.7 (73.3)	94.1 (75.9)	100.0 (90.0)	95.2 (77.4)
Carbendazim 50 WP	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Tebuconazole 250 EC	91.7 (73.3)	94.1 (75.9)	100.0 (90.0)	95.2 (77.4)
Mean	94.3 (76.9)	97.0 (80.1)	100.0 (90.0)	
	Fungicide (F)	Concentration (C)	F X C	
S Em ±	0.7	0.6	1.4	
C D (1%)	3.0	2.6	5.6	

*Figures in the parentheses indicate arc sine transformed values

Table-2 *In-vitro evaluation of contact fungicides and antibiotic against Rhizoctoniasolanif. sp. sasakii*

Fungicide	Per cent inhibition of mycelial growth Concentration (%)			Mean
	0.1	0.15	0.2	
Chlorothalonil 75 WP	80.5 (63.8)	82.2 (65.1)	88.8 (70.5)	83.8 (66.3)*
Zineb 78 WP	77.7 (61.8)	80.5 (63.8)	88.8 (70.5)	82.4 (65.2)
Copper oxychloride 50 WP	57.7 (49.5)	74.4 (59.6)	82.2 (65.8)	73.7 (59.1)
Mancozeb 75 WP	88.8 (70.5)	95.5 (77.8)	97.7 (81.4)	94.0 (75.9)
Mean	76.2 (60.8)	83.19 (66.4)	89.4 (71.6)	
	Fungicide (F)	Concentration (C)	F X C	
S Em ±	0.8	0.6	1.5	
C D (1%)	3.4	2.7	6.0	
Antibiotics				
	0.03	0.04	0.05	
Validamycin	80.5 (63.8)	88.8 (70.5)	95.5 (77.8)	88.3 (70.0)

Table-3 *In-vitro evaluation of combi product fungicides against Rhizoctonia solani f. sp. sasakii*

Fungicide	Per cent inhibition of mycelial growth Concentration (%)			Mean
	0.1	0.15	0.2	
Carbendazim 12 % + Mancozeb 63 %	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)*
Carboxin 37.5 % + Thiram 37.5 %	99.3 (85.3)	100.0 (90.0)	100.0 (90.0)	99.7 (88.7)
Tricyclazole 18 % + Mancozeb 62 %	28.1 (31.8)	45.1 (41.8)	45.1 (41.8)	39.4 (38.4)
Hexaconazole 4 % +Zineb 68 %	55.0 (47.5)	68.8 (56.0)	75.6 (60.4)	66.4 (54.6)

Carbendazim 25 % + Iprodione 25 %	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Trifloxystrobin 25 % + Tebuconazole 50%	99.6 (86.7)	100.0 (90.0)	100.00 (90.0)	99.8 (88.9)
Mean	80.3 (71.9)	85.5 (76.3)	86.6 (77.0)	84.1 (75.1)
	Fungicide(F)	Concentration(C)	F X C	
S Em ±	0.9	0.7	1.7	
C D (1%)	3.6	2.9	6.9	

Conflict of Interest: None declared

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