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Evaluation of Selective Fungicides and Biocontrol Agents for Suppression of Banded Leaf and Sheath Blight of Maize (*Zea mays*)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The investigation was carried out for managing leaf and sheath blight of maize (*Zea mays* L.) by using different fungicides and bio control agents. The bio control agents and fungicides exhibited inhibitory action against the test pathogen under laboratory condition. Field experiment conducted during kharif season 2016 revealed that two sprays of validamycin (0.2%), 30 and 40 days after sowing, gave maximum grain yield (30.0 q/ha) and 100-seed weight (208.0 g) with minimum percentage disease incidence (7%), (severity 1 on 1-5 scale), followed by difenoconazole @ 0.15% and hexaconazole (0.2%) for effective control of BSLB. Use of bio control agents *Trichoderma harzianum* and *Pseudomonas fluorescens* was found as best strategy for BSLB management.

Keywords: Bio control agents; foliar sprays; fungicides; Rhizoctonia solani f. sp. sasakii.

1. INTRODUCTION

The *Rhizoctonia solani* f. sp. *sasakii* causing banded leaf and sheath blight (BLSB) is one of the important fungi of corn worldwide. The fungus is commonly controlled by using fungicide because of non availability of resistant variety. Although the disease was observed in the western central Himalayan foothills region of India in early sixties, the importance was

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realized only in early 1970s when an epidemic occurred in warm and humid foothills areas in Mandi district of Himachal Pradesh [1]. In India, losses in grain yield have been recorded by 23.9-31.9% in ten cultivars [2]. The disease causes severe losses in several countries of Asia. Occurrence of disease has also been reported from other parts of the world [3]. Validamycin (0.1%) followed by difenoconazole (0.1%) as best in managing the disease [4]. BLSB can be effectively managed by seed treatment with the peat based formulation @ 16 g/Kg or soizolel application @ 2.5 Kg/ha [5]. carbendazim (0.2%) was most effective as seed treatment, showing 68.0% reduction in disease over the control and as a foliar spray (0.1%), it resulted least in disease severity (25.7%) and highest grain yield (31.5 q/ha) [6]. Since banded leaf and sheath blight pathogen is soil-borne and its occurrence has also been recorded) [7] field experiment was conducted to investigate the efficacy of fungicides and bio control agents against the disease as soil application and foliar sprays.

2. MATERIALS AND METHODS

The present investigation was carried out at Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India during year 2015-16.

2.1 Isolation of the Pathogen

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 Umerkote Odisha during the year 2015-16. Infected portion of collected leaves and leaf sheaths were cut into small pieces of 5 to 6 mm and the bits. Were surface sterilized with 1.0 per cent sodium hypochlorite solution for 1 minute. 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 preserved at 5°C. Further, these cultures were sub-cultured once in a month and used for future studies.

2.2 Isolation and Maintenance of Bio Control Agents

The fungal antagonist *Trichoderma. harzianum, T. viride* and *T. hamatum* used in present investigation were obtained from Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar and *Pseudomonas* spp., *Pseudomonas fluorescens* and *B. subtilis* were isolated from the native rhizosphere soils collected from healthy seedling of solanaceous family by following serial dilution plate count technique [9]. The rhizosphere soil from the roots (5-6 plants) was collected by vortexing. Ten gram of rhizosphere soil was placed in 100-ml sterile distilled water blank and thoroughly vortexed and diluted by serial dilution method. To get 10^{-1} dilution, 10 g of this soil was dissolved in 100 ml of sterile distilled water, from this 1 ml of soil suspension was taken and added to 9 ml of sterile distilled water to get 10^{-2} dilution. This was repeated until a dilution of 10^{-6} was obtained. From this 1 ml of the suspension was poured in agar plates and the plates were rotated for uniform spread of suspension. Plates were incubated at 28 ± 2^0C in BOD incubator in an inverted position. Three day old colonies of mycoflora were picked up and purified by single hyphal tip method whereas; one day old colonies of bacteria were picked up and purified by streak plate method. The fungal biological agents were maintained on PDA while bacterial cultures *Pseudomonas* on Nutrient Agar.

2.3 Dual Culture Method

In vitro antagonistic activity of *T. harzianum, T. viride* and *T. hamatum* against *Rhizoctonia solani* was studied in dual culture technique. Petri dishes (90 mm) containing 20 ml of sterile PDA were inoculated with a 5 mm diameter plug of 7-day- old pure culture of pathogenic fungi and different strains of *Trichoderma* at antagonistic poles of PDA plates and incubated at 25°C. Radial growth of pathogen was measured at 24 h intervals. In case of *Pseudomonas fluorescens, B. subtilis* and *Pseudomonas* spp parallel streaking was done on one side of the agar plate and incubated at 27°C for 24 h. After the incubation period a 5 mm diameter mycelial plug of actively growing *R. solani* was placed on the opposite pole and incubated at 25°C for 7 days. Control petri dishes were inoculated with pathogens and a sterile agar plug. Three replications were maintained for each treatment.

Percentage inhibition of pathogen was calculated by the following formula.

I = (R1- R2 / R1) x 100

Where R2 denotes the radial growth of the pathogen towards the opponent antagonist and R1 denotes the radial growth of the pathogen towards opposite side.

The experiment was repeated thrice.

2.4 Slide Culture Method

For each pathogen - *Trichoderma* interaction, a clean slide was placed in 9 cm diameter plates and sterilized. Then a small amount of autoclaved melted PDA was spread over the slide to make a thin PDA film on the slide. A 5 mm diameter mycelial plug of one week old *R. solani* was placed at one end of the slide containing PDA media while on the opposing end different strains of *Trichoderma* were placed. Then 1 ml of double distilled water was added to the plate to prevent drying and then incubated at 25±1°C for 7 days. After incubation period the meeting area of pathogen-*Trichoderma* hyphae was observed under a light microscope.

Effect of volatile substance produced by *T. harzianum*, *T. viride* and *T. hamatum* on growth of the pathogen. Petri plates containing 20 ml of PDA were inoculated separately with 5 mm disc of antagonists and incubated for 5 hours. After this lid of each plate was replaced by a bottom containing PDA previously inoculated with the disc of the pathogen and sealed together with paraffin film. The control sets did not contain the antagonist. The cultures were incubated at 25°C.The studies were conducted in three replicates. Radial growth was measured at 24 hours intervals and percent inhibition was determined using the formula:

I (Percent inhibition) = (C2 - C1 / C2) x 100

Where, C2 means growth of *R. solani* in control and C1 means growth of *R. solani* in treatment.

2.5 Efficacy of Fungicides in the Management of BLSB

The efficacy of eight fungicides *viz.,* thiophenate-M 70 WP, validamycin 3 SL, probineb 70 WP, difenconazole 25 EC, carbendazim 12% + mancozeb 63%, carbendazim 50 WP, mancozeb 75 WP and hexaconazole 5 EC were evaluated *in vitro* at 0.05, 0.10, 0.15 and 0.2 per cent concentration using poison food technique.

The pathogen *Rhizoctonia 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 quantities of fungicides to obtain the desired concentration on the basis of active ingredient of the chemical. Poisoned medium was poured in each of the sterilized Petri plates. Mycelial disc of 5 mm was taken from the periphery of the 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. The colony diameter was measured in two directions and average was worked out. The percent inhibition of growth was calculated by using the formula [10].

I (Percent inhibition) = (100(C-T)/C) x 100

Where,

I = Per cent inhibition of mycelium growth
C = Growth of mycelium in control
T = Growth of mycelium in treatment
Treatment details

2.6 Evaluation of Fungicides and Bio Control Agents under Field Condition

Field experiment was conducted during kharif season 2016 at Central Agricultural Research Farm, Bhubaneswar farm, Bhubaneswar in a Randomized block design with three replications to assess the possibility of managing disease by different eight fungicides and six bio agents. Seeds of moderate susceptible maize hybrid Vivek Hybrid- 43 were treated with bio control agents, viz. *T. viride T. hamatum, Pseudomonas fluorescens, B. subtilis Pseudomonas* spp and *Trichoderma harzianum* (10 g/Kg) using gum as sticker. The treated seeds were spread over a clean paper and dried in cool and shaded place sown immediately after drying. Foliar spray with eight fungicides, viz. Thiophenate - M 70 WP, validamycin 3 SL, probineb 70 WP, difenconazole 25 EC, carbendazim 12% + mancozeb 63%, carbendazim 50 WP, mancozeb 75 WP and hexaconazole 5 EC 50% (1 g/l) and six bio control agents, viz. *P. fluorescens* (10 g/Kg) and *T. harzianum* (10 g/Kg) were given 30 and 40 days after sowing. The field trail was conducted under irrigated, sandy red loam soil conditions. Hybrid Vivek Hybrid - 43 was shown

on 10 June 2016 in a plot size 3 m × 3 m with spacing of 60 cm × 20 cm in Randomized Block Design with 3 three replications.

Recommended dose of fertilizers and insect control measures were followed as per the package and cultural practice of ACRIP, Maize, OUAT, Bhubaneswar [11]. Culture of *R. solani* f. sp. *sasakii* was multiplied on autoclaved barley grains and artificially inoculated on untreated plots. Inoculations were made by inserting 2-3 maize grains covered with fungal growth of pathogen isolates gently between the rind and the leaf sheath of plants. High humidity was maintained during disease development by frequent watering and irrigation by sprinkler [12] and [8].

The observations on disease severity were recorded at silk drying stage using 1-5 scale [13], The per cent disease index (PDI) and per cent efficacy of disease control (PEDC) over the control were calculated by using the following formula [14].

On the basis of 100-seed weight, grain and stover yield and per cent increase in grain and stover yield was calculated. The percentage data were angularly transformed prior to statistical analysis and all data subjected to analysis of variance using Randomized Block Design [15].

3. RESULTS AND DISCUSSION

There were statistically significant differences among all the treatments for percent Inhibition. The percent Inhibition was significantly differed by different bio control agents and chemicals *in vitro* (Table 1).

Among all the fungal bio agents *Trichoderma harzianum* recorded the highest per cent inhibition (68.8%). *T. viride* and *T. hamatum* were recorded the 62.2 and 59.4 percent inhibition *in vitro* conditions respectively.

Among all the bacterial bio agents while *Pseudomonas* spp. recorded the highest per cent inhibition (48.0%). while *Pseudomonas fluroscens* and *Bacillus subtilis* recorded 47.1 and 43.2 percent inhibition under *in vitro* conditions respectively.

Among the eight fungicides tested, validamycin at 0.2%, difenoconazole 0.1% and

Table 1. Effect of bio agents against *R. solani* caused by BLSB on maize

S. no	Bio agent	Radial growth in (mm)	Inhibition (%)
1	*Trichoderma harzianum*	28.1	68.8
2	*T. viride*	34.0	62.2
3	*T .hamatum*	36.5	59.4
4	*Pseudomonas* sp.	46.8	48.0
5	*Pseudomonas fluroscens*	47.6	47.1
6	*Bacillus subtilis*	51.1	43.2
7	Control (No bio agent)	90.0	-
	SE(m)+	2.14	-
	CD (0.05)	6.47	-

Table 2. Effect of fungicides against *R. solani* caused by BLSB on maize

S. No	Fungicide	Radial growth in (mm) at different hrs					Inhibition (%)
		24	48	72	96	120	
1	thiophenate-M 70 WP @ 0.1%	0.0	5.0	10.0	12.0	15.0	85.0
2	validamycin 3 SL @ 0.2%	0.0	0.0	0.0	0.0	0.0	100.0
3	probineb 70 WP @ 0.2%	0.0	5.0	10.0	15.0	20.0	80.0
4	difenoconazole 25 EC @ 0.1%	0.0	0.0	0.0	0.0	0.0	100.0
5	carbendazim 12%+mancozeb 63% @ 0.3%	5.0	6.0	7.0	8.0	12.0	88.0
6	carbendazim 50 WP @0.1	3.0	5.0	6.0	7.0	9.0	91.0
7	mancozeb 75 WP 0.3%	0.0	5.0	10.0	15.0	20.0	80.0
8	hexaconazole 5 EC @ 0.2%	0.0	0.0	0.0	0.0	-	100.0
9	Control (No fungicide)	15	30	45	60	90.0	-

Table 3. Effect of bio agents against BLSB of maize (*R. solani*)

S. No	Bio agent	Dosage	Mean PDI	Inhibition (%)	Yield (q/ha)
1	*Trichoderma harzianum*	0.2	26.2 (30.79)	46.0	27.93
2	*T. viride*	0.2	28.3 (32.14)	42.3	26.45
3	*T .hamatum*	0.2	30.8(33.77)	37.2	21.45
4	*Pseudomonas* sp.	1.0	35.8(36.75)	27.0	26.75
5	*Pseudomonas fluroscens*	1.0	26.5(30.95)	51.9	26.95
6	*Bacillus subtilis*	0.1	26.0	47.0	24.75
7	Control (No bio agent)	-	49.1	-	17.85
	SE(m)+		2.14	-	1.55
	CD (0.05)		6.47	-	4.60

Table 4. Effect of fungicides on per cent disease index of banded leaf and sheath blight

S. No	Fungicide	Dosage	PDI (%)	Inhibition (%)
1	thiophenate-M 70 WP	0.1	15.0	83.1
2	validamycin 3 SL	0.2	7.0	92.1
3	probineb 70 WP	0.2	45.0	49.4
4	difenoconazole 25 EC	0.1	8.0	91.0
5	carbendazim 12%+ mancozeb 63%	0.3	29.0	67.4
6	carbendazim 50 WP	0.1	39.0	56.1
7	mancozeb 75 WP	0.3	41.0	53.9
8	hexaconazole 5 EC	0.2	8.9	89.8
9	Control (No fungicide)	-	89.0	-

Table 5. Bio efficacy of fungicides against (*R. solani*) on vivek maize hybrid - 43

S. No	Fungicide	DosTage	Severity (1-5)	Yield (q/ha)	1000 grain wt (g)	Cobs /plant
1	thiophenate-M 70 WP	0.1	2.0	21.6	178.0	0.9
2	validamycin 3 SL	0.2	1.0	30.0	188.0	1.0
3	probineb 70 WP	0.2	4.0	18.6	179.0	0.9
4	difenoconazole 25 EC	0.1	1.0	25.2	208.0	1.1
5	carbendazim 12%+ mancozeb 63%	0.3	3.0	21.4	183.0	0,9
6	carbendazim 50 WP	0.1	4.0	18.5	183.0	0.9
7	mancozeb 75 WP	0.3	4.0	22.0	183.0	0.9
8	hexaconazole 5 EC	0.2	2.0	22.3	185.0	1.0
9	Control (No fungicide)	-	5.0	17.5	174.0	0.9
	SE(m)+			0.23	0.08	0.12
	CD (0.05)			0.69	0.26	0.34

hexaconazole 0.2% recorded maximum mean mycelia inhibition which were significantly superior to all other treatments followed by carbendazim+ mancozeb 0.3%, thiophenate methyl 0.1%, probineb 0.2% and mancozeb 0.3% under laboratory condition (Table 2).

There were statistically significant differences among all the treatments of biological control agents for PDI under field condition. Among six bio agents 0.2% *Trichoderma harzianum* recorded highest grain yield (2793 Kg/ha) compared to other treatments and 46.0 percent inhibition over control. Among the bacterial bio

agents tested *Pseudomonas fluroscens* recorded the highest percent inhibition (51.9) compared to all other treatments which also, recorded the grain yield 2695 Kg/ha (Table 3).

There were statistically significant differences among all the treatments for PDI. The PDI significantly differed by different chemicals tested *in vitro* and foliar application of chemicals (Tables 4 and 5). Validamycin was recorded the highest per cent inhibition over control plot (92.1%). Similar trend was observed the difenoconazole (91%), hexaconazole (89.8%), thiophenate methyl (83.1%) [16] (Table 4).

Foliar spray differed significantly in grain yield in all fungicides tested. Maximum grain yield was observed in two sprays of validamycin @ 0.1% at 30 and 40 DAS (30.0 q/ha) over the control (17.5 q/ha) and was statistically at par with probineb (18.6 q/ha), carbendazim 18.5 q/ha.

In vitro screening of fungicides reveal the efficacy of various fungicides and provide first hand information confirming fungi toxicity against specific pathogen and therefore it serves as a reliable basis for field testing validamycin, difenoconazole and hexaconazole are found to be effective in inhibiting the growth of pathogen in *in vitro* condition. Under field condition also, all the fungicides except mancozeb were effective in reduce severity [13,4,17,8].

Management of BLSB disease of maize by treating seed with carbendazim and *T. harzianum* as also reported by [18]. The present investigation also substantiates that BLSB disease can be managed by two sprays of validamycin 3 SL @ 0.2% 30 and 40 DAS.

4. CONCLUSION

From the study, it can be concluded that bio control agents *Trichoderma harzianum* and *Pseudomonas fluroscens* could effectively inhibit the *Rhizoctonia* growth under *in vitro* condition and can manage the BLSB disease under *in vivo* condition and can manage the BLSB disease under *in vivo* condition also. Additionally, two sprays of validamycin @ 0.2%, difenoconazole @ 0.1% and hexaconazole 0.2% can also be recommended to reduce the disease incidence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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