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EFFICACY OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST *SCLEROTIUM ROLFSII* CAUSING FOOT ROT DISEASE OF FINGER MILLET, UNDER *IN VITRO* CONDITIONS

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Abstract

Finger millet is one of the important millet crops widely cultivated across India, and more than 60 per cent of its area is concentrated in Karnataka. Although, it is found to be a hardy crop, it is also affected by many diseases, among them foot rot caused by *Sclerotium rolfsii* has been an increasing problem especially in irrigated and heavy rainfall areas. So a total of 14 fungicides and 5 bioagents were screened *in-vitro* against *Sclerotium rolfsii* causing foot of ragi. Out of which systemic fungicides, hexaconazole, propiconazole, difenconazole and combi products *viz*., Avatar, Nativo and Vitavax power were found effective, but the contact fungicide, mancozeb was found to be effective only at higher concentrations. Among the bioagents *Trichoderma harzianum* (GKVK) isolate was found to be effective than other biogents.

Key Words: *Sclerotium rolfsii*, fungicides, finger millet, foot rot, bioagents.

Introduction

Finger millet [*Eleusine coracana* (L.) Gaertn.,] is one of the important millet crops of India, commonly referred to as Ragi, Bird's foot, Nagli, Mandua in different regions of the country. It belongs to the family Poaceae. It is grown throughout the country extending from Tamil Nadu in the South to Uttarakhand in the North. This crop occupies more than 1.0 m. ha area in Karnataka and Karnataka alone contributes more than 60 per cent of its total production in the country. Finger millet is used as a staple food by many farming communities in south India because of its high nutritive value. Ragi is nutritionally rich with high quality protein; plenty of minerals, dietary fiber, phytochemicals and is having 8-10 times more calcium than rice and wheat and is recommended for diabetic patients. Although, ragi is known to be one of the hardiest crops, relatively free from pests and diseases, it is attacked by many diseases. Among these diseases, foot rot caused by *Sclerotium rolfsii* is one of the important emerging diseases of ragi and is on the increase in the recent past particularly under irrigated and high rainfall situations (Nagaraja and Reddy, 2009). The disease has been reported to cause more than 50 per cent yield loss (Batsa and Tamang, 1983).

Sclerotium rolfsii Sacc. is a well known and most destructive soil borne fungus initially described by Rolfs (1892) on tomato. The *Sclerotium rolfsii* is widely distributed and causes severe damage to more than 500 crops (Aycock, 1966).

Although there are several other *Sclerotium* producing fungi, the fungus characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus *Sclerotium* (Punja and Rahe 1992). *Sclerotium rolfsii* Sacc. is predominantly distributed throughout tropical and subtropical regions where, the temperature reaches higher levels during the rainy season. This pathogen causes a variety of symptoms on different hosts like collar rot in chickpea, southern blight of sugar beet, foot rot of finger millet, leaf spot in *Lotus meliloti*, bud rot of *Colocasia variagata and* fruit rot in *Citrullus vulgaris* etc. Consequently the diseases caused by this fungus are more serious in tropical and subtropical regions than in temperate regions and this pathogen is of major importance throughout the world.

Use of fungicides for the control of plant diseases is a common practice. As *Sclerotium* is a soil borne pathogen with a wide host range crop rotation may not be of much help, hence studies were undertaken to evaluate new fungicides and bioagents to know their efficacy against *Sclerotium rolfsii* for further utilization in field to manage the disease.

Material and Methods

Isolation of the Fungus

The part of foot region showing typical symptoms of foot rot disease of finger millet was used for isolating the causal fungus adopting the standard tissue isolation. Later, the bit of fungal growth was transferred to PDA slants for purification and maintenance of the culture.

Evaluation of Fungicides

The efficacy of four triazole systemic fungicides *viz.*, Hexaconazole, propiconazole, difenconazole, tricyclazole, and other two systemic fungicides carbendazim, and thiophanate methyl (at 25, 50, 100, 150, 200, 250 ppm), four non systemic fungicides *viz.*, captan, chlorothalonil, mancozeb and thiram (at 125, 250, 500, 750, 1000 ppm) and four combi products *viz.*, Avatar, Merger, Nativo and Vitavax power (at 125, 250, 500, 750, 1000 ppm) were assayed against *Sclerotium rolfsii.* These fungicides were evaluated under laboratory conditions by "Poison food technique".

Required quantity of individual fungicide was added separately into sterilized molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelium discs of 5 mm size from seven days old culture was cut by a sterile cork borer and one such disc was placed at the centre of each agar plate. The plate without any fungicide served as control. Three replications were maintained for each concentration. The plates were incubated at room temperature and the radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, calculated by using the formula given by Vincent (1947).

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

Where,

I = per cent inhibition
C = growth in control
T = growth in treatment

Evaluation of Bio-Agents

In vitro evaluation was carried out with five biogents *viz.*, *Trichoderma harzianum* Rifai (Th-55 isolate), *Trichoderma harzianum* Rifai (biocontrol lab UAS, GKVK isolate), *Trichoderma viride* Pers. Ex. S. F. Gray (Tv-27 isolate), *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn. by dual culture technique for their antagonistic effect against *Sclerotium rolfsii* under *in-vitro* conditions against ragi Mandya isolate of *Sclerotium rolfsii* through dual culture technique.For this study both bioagents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus.

Dual culture technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the Petri plate to the middle of the Petri plate and the test fungus placed at the other end. The plates were incubated at 27±1℃ and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = per cent inhibition
C = growth in control
T = growth in treatment

Results and Discussion

Evaluation of Fungicides

Among the six systemic fungicides tested three triazole compounds *viz*., hexaconazole, propiconazole and difenconazole were found to be highly effective at all concentrations and were significantly superior over control as these inhibited cent per cent growth of *Sclerotium rolfsii* (Table 1). The other fungicides *viz.*, carbendazim, tricyclazole and thiophanate methyl did not show any inhibition of the mycelial growth of *Sclerotium rolfsii* at any of the six concentrations tested and was only comparable to control. These results were supported by many previous workers *viz*., Chowdhury *et al*. (1998); Virupaksha Prabhu and Hiremath (2003); and Arunasri *et al*. (2011); who reported that the Triazoles (Hexaconazole, Propiconazole, Difenconazole) were highly inhibitive to the growth of *Sclerotium rolfsii*. whereas, Johnson and Subramanyam (2000) found carbendazim as least effective on *Sclerotium rolfsii*.

Table 1: Effect of systemic fungicides on *Sclerotium rolfsii*

Treatments	Per cent inhibition of mycelial growth						
	25ppm	**50ppm**	**100ppm**	**150ppm**	**200ppm**	**250ppm**	**Mean**
T1: Tricyclazole (Baan 70% WP)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T2: Propiconazole (Tilt 25% EC)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
T3: Difenconazole (Score 25% EC)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
T4: Hexaconazole (Contaf 5% EC)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
T5: Carbendazim (Bavistin 50 % WP)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T6: Thiophanate methyl (Roko 70% WP)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	50.00	50.00	50.00	50.00	50.00	50.00	
		Fungicide		**Concentration**		**F X C**	
SEm±		**3.20**		**3.20**		**7.85**	
C.D at 1%		**6.37**		**6.39**		**15.67**	
CV %		**19.60**					

Figures in parenthesis are arcsine transformed values

Among the non systemic fungicides, mancozeb was highly effective at higher concentration as it inhibited the *S. rolfsii* by 54.88, 73.33 and 83.00 per cent at 500, 750 and 1000ppm respectively. Captan also inhibited the mycelial growth to 44.44 per cent only at 1000ppm concentration while thiram inhibited the pathogen growth up to 38.55 per cent at 1000ppm concentration. Chlorothalonil was found to be least effective in inhibiting the growth of *Sclerotium rolfsii* as it inhibited only 11.18 per cent at 1000ppm concentration (Table 2). These results were in accordance with the results of Sujatha (1991) and Johnson and Subramanyam (2000).

Table 2: Effect of Non Systemic fungicides on *Sclerotium rolfsii*

Treatments	Per cent inhibition of mycelial growth					
	125ppm	**250ppm**	**500ppm**	**750ppm**	**1000ppm**	**Mean**
T1: Captan (Captaf 50% WP)	0.00	11.88 (20.20)	28.22 (32.10)	43.44 (41.20)	44.44 (41.80)	25.59
T2: Chlorothalonil (Kavach 75% WP)	0.00	2.66 (9.38)	3.33 (10.51)	11.11 (19.47)	11.88 (20.16)	5.65
T3: Mancozeb (Indofil M-45 75% WP)	16.10 (23.70)	29.66 (32.99)	54.88 (47.80)	73.33 (58.90)	83.00 (65.64)	51.39
T4: Thiram (Thiram 75% WP)	0.00	0.00	16.10(23.70)	32.22 (34.60)	38.55 (38.40)	17.37
Control	0.00	0.00	0.00	0.00	0.00	
Mean	4.02	11.05	25.63	40.02	44.29	
		Fungicide	**Concentration**	**F X C**		
	SEm±	**7.63**	**6.83**	**3.41**		
	C.D at 1%	**29.19**	**26.11**	**13.06**		
	CV %	**23.65**				

Figures in parenthesis are arcsine transformed values

Out of four combi products *viz.*, vitavax power (Thiram 37.5% + Carboxin 37.5%), avatar (Hexaconazole 4% + Zineb 68%), merger (Tricyclazole 18% + Mancozeb 62%) and nativo (Tebuconazole + Trifloxystrobin) tested except merger (Tricyclazole 18% + Mancozeb 62%) all other combi products showed cent per cent inhibition at all the five

concentrations tested (Table 3). Merger is also an effective fungicide as it showed complete inhibition of the pathogenic growth at 1000ppm concentration, but the inhibition was only up to 57.44, 73.00, 84.88 and 87.11 per cent at 125, 250, 500 and 750ppm concentration respectively. These results are in agreement with Virupaksha Prabhu and Hiremath (2003); and Arunasri *et al*. (2011); who reported that the combi products containing triazoles *viz.*, Avatar, Merger and Nativo were highly inhibitive to the growth of *Sclerotium rolfsii*. Vyas and Joshi (1977) and Sujatha (1991) reported that carboxin was highly effective on *Sclerotium rolfsii*. In our studies also carboxin and triazole containing fungicides showed the complete inhibition of mycelial growth of *Sclerotium rolfsii*.

Table 3.Effect of Combi products against *Sclerotium rolfsii*

Treatments	**Per cent inhibition of mycelial growth**					
	125ppm	**250ppm**	**500ppm**	**750ppm**	**1000ppm**	**Mean**
T1: Hexaconazole 4% + Zineb 68% (Avtar)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
T2: Tricyclazole 18% + Mancozeb 62% (Merger)	57.44 (49.27)	73.00 (58.69)	84.88 (67.10)	87.11 (69.0)	100.00 (90)	80.48
T3: Tebuconazole 50% + Trifloxystrobin 25% (Nativo)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
T4: Carboxin 37.5%+ Thiram 37.5% (Vitavax power)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00 (90)	100.00
Control	0.00	0.00	0.00	0.00	0.00	
Mean	89.36	93.25	96.22	96.77	100.00	
			Fungicide	**Concentration**	**F X C**	
	SEm±		**2.65**	**2.37**	**1.19**	
	C.D at 1%		**10.14**	**9.07**	**4.54**	
	CV%		**2.30**			

Figures in parenthesis are arcsine transformed values

Evaluation of Bioagents

The antagonistic microorganism's *viz.*, *Trichoderma harzianum* Rifai (Th-55 isolate), *Trichoderma harzianum* Rifai (biocontrol lab UAS, GKVK isolate), *Trichoderma viride* Pers. Ex. S. F. Gray (Tv-27 isolate), *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn. were evaluated by dual culture technique for their antagonistic effect against *Sclerotium rolfsii* under *in-vitro* conditions. Inhibition zone in mm was recorded and the per cent inhibition was calculated.

At 7 days after inoculation maximum inhibition of mycelial growth (61.88%) was noticed in *Trichoderma harzianum* Rifai (biocontrol lab UAS, GKVK isolate), which was followed by *Trichoderma viride* Pers. Ex. S. F. Gray (Tv-27 isolate) (57.77%) and *Trichoderma harzianum* Rifai (Th-55 isolate) (56.33%). *Pseudomonas fluorescens* and *Bacillus subtilis* did not show any inhibition of mycelial growth of *Sclerotium rolfsii* as the pathogen grew over the bioagents (Table 4).

At 14 days after inoculation all the isolates does not show any variation in increasing the inhibition percentage except *Trichoderma harzianum* Rifai (biocontrol lab UAS, GKVK isolate) which continued its growth on the pathogen and inhibited up to 81.11% growth. However, the bacterial antagonist doesn't show any inhibition of the mycelial growth of *Sclerotium rolfsii* even after 14 days of inoculation. These results were in accordance with the results of Bari *et al.* (2000); Kulkarni (2007) and Basamma (2008).

Table 4: Antagonistic effect of different bioagents against *Sclerotium rolfsii*.

Sl. No.	**Bioagents**	**Per cent inhibition of mycelial growth**	
		7 days	**14 days**
1	*Trichoderma harzianum* (GKVK)	61.88 (51.90)*	81.11 (64.20)
2	*Trichoderma harzianum* (Th-55)	56.33 (48.63)	56.33 (48.63)
3	*Trichoderma viride* (Tv-27)	57.77 (49.47)	57.77 (49.47)
4	*Pseudomonas fluorescens* (GKVK)	0.00	0.00
5	*Bacillus subtilis* (GKVK)	0.00	0.00
6	Control	0.00	0.00
	SEm±	**1.81**	**1.57**
	C.D at 1%	**4.03**	**3.15**
	CV %	**7.60**	**3.90**

Figures in parenthesis are arcsine transformed values

Conclusions

Systemic fungicides like hexaconazole, propiconazole, difenconazole and combi products, Avatar (Hexaconazole 4% + Zineb 68%), Nativo (Tebuconazole 50% + Trifloxystrobin 25%) and Vitavax power (Thiram 37.5% + Carboxin

37.5%) showed complete inhibition of the pathogen at all the concentrations tested. Whereas, the contact fungicide mancozeb was found inhibitive only at higher concentrations. By this study it was observed that the triazoles and triazole containing combi products were found to be effective even at low concentrations under lab conditions against *Sclerotium rolfsii*. Among the bioagents, *Trichoderma harzianum* (GKVK) isolate showed maximum inhibition of *Sclerotium rolfsii.* However these fungicides and bioagents may be tested under field conditions for confirming the efficacy.

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