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Dasharathe BG
M.Sc., Student, College of Agriculture, MPKV, Rahuri, Maharashtra, India

Deore PB
Assistant Professor, College of Agriculture, MPKV, Rahuri, Maharashtra, India

MN Agam
M.Sc., Student, College of Agriculture, MPKV, Rahuri, Maharashtra, India

Corresponding Author:
Dasharathe BG
M.Sc., Student, College of Agriculture, MPKV, Rahuri, Maharashtra, India

Effect of fungicides on different isolates of *Sclerotium rolfsii* from Khandesh region

Dasharathe BG, Deore PB and MN Agam

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Abstract
The present investigation was carried out at Plant Pathology Section, College of Agriculture, Dhule. The material and methods used, and procedures followed to investigate the "Effect of fungicides on *Sclerotium rolfsii* isolates of groundnut from Khandesh region" *Sclerotium rolfsii* Sac is a destructive soil borne fungal pathogen with wide host range that includes groundnut, an important oil seed crop in India. The fungicides Propiconazole and Tebuconazole recorded cent percent mycelial inhibition followed by SAAF, Carbendazim, Benomyl, Captain, Rodomel and Thiophanate methyl. The results also indicated that all the six isolates recorded no effect of fungicides on colony color, shape of sclerotia bodies, color of sclerotia bodies, position of sclerotia bodies and numbers of sclerotia bodies present in one position. It was also pointed out that the increase in the concentration of fungicides doesn't have any effect on colony color, shape of sclerotia bodies, color of sclerotia bodies, position of sclerotia bodies and numbers of sclerotia bodies present in one position. The variation in size of sclerotia bodies, number of sclerotia bodies per cm2 and test weight of sclerotia bodies among all the six isolates due to effect of eight fungicides were found statistically significant. The fungicide SAFF was most effective in reducing size of sclerotia bodies, number of sclerotia bodies per cm^2 and test weight of sclerotia bodies followed by Carbendazim and Benomyl. While the fungicide Thiophanate methyl was less effective in reducing size sclerotia bodies, number of sclerotia bodies per cm^2 and test weight of sclerotia bodies followed by Rodomel. It was also pointed out that the increase in the concentration of fungicides there was reduction in size of sclerotia bodies, number of sclerotia bodies per cm^2 and test weight of sclerotia bodies among all the six isolates.

Keywords: *Sclerotium rolfsii*, propiconazole, tebuconazole

Introduction
Groundnut is believed to be the native of Brazil. The plant was introduced by Portuguese into Africa from where it was introduced into North America. It was introduced into India during the first half of the sixteenth century from one of the Pacific Islands of China, where it was introduced earlier from either Central America or South America (Jaisani, 2009) [1].

The major groundnut producing countries of the world are India, China, Nigeria, Senegal, *Sudan*, Burma and USA which occupied 18.9 million ha area with 17.8 million tons production of groundnut in the world, these countries account for 69% of the area and 70% of the production. The China is the highest groundnut producer in world followed by India. (Anonymous, 2011) [3].

Sclerotium rolfsii Sac is a soil inhabitant, non-target, polyphagous and a ubiquitous facultative parasite. It has a wide range infecting cultivated crops viz., potato, groundnut, soybean, sunflower, chili, tomato, cotton, lucerne, wheat and onion etc. It is documented that, fungus infects more than 500 plant species (Rupe, 1999) [4].

Among the soil borne diseases, stem rot caused by *Sclerotium rolfsii* is gaining a serious status. This disease is also referred as Sclerotium blight, Sclerotium wilt, Southern blight, Southern stem rot and white mold. This fungus is distributed throughout the world and is particularly prevalent in warmer climate and significant yield losses can be seen in monoculture or short rotation with other crops which are susceptible to this pathogen (Aken and Dashiell, 1991) [5].

Among the crops viz., soybean, peanut, sugar beet, pepper, tomato and potato suffer maximum losses whereas sorghum, wheat, rice, lentil, betel vine, alfalfa, cotton, sugarcane, tobacco, sunhemp, sunflower, chrysanthemum, gladiolus and other ornamental species suffer minor damage (Ansari, 2005) [6].

Garren (1959) [7] has estimated the losses due to *Sclerotium rolfsii* is to the extent of 10 to 20 m dollars annually in Southern USA.

The yield loss up to 75-80 percent has been reported in New Mexico (Aycock, 1966) [8]. The recent literature revealed that many workers tested different fungicides viz., Carbendazim, Captain, Furmecyclox, 2,6-dichloro 4-nitroaniline, Cycloheximide, Carvoxime, PCNB, Benomyl, Mancozeb, Thieved, Dithiane M-45, Topspin-M, Propiconazole, Propineb, Rodomel, Tebuconazole, Thiophanate methyl to manage the fungi. While previous reports indicated use of Mercuric chloride, formaldehyde and formaldehyde to control *Sclerotium rolfsii* causing stem rot (Addison and Chona, 1971) [9].

Materials and Methods

The effect of eight fungicides on six isolates of *Sclerotium rolfsii* were studied *in vitro* at three different concentrations of fungicides *viz,* 100 ppm, 250 ppm and 500 ppm using poison food technique on potato dextrose agar (PDA) medium. CRD design used for this experiment Nines treatments and three replications.

Based on active ingredient, requisite quantity of each fungicide was calculated. The PDA medium was prepared and sterilized at 1.1 kg/cm² pressure for 15 min in conical flasks (500 ml/cap). When the sterilized media was cold up to 40 ^{0}C, the desired quantity of each fungicide was mixed thoroughly in separate conical flask containing sterilized PDA. The Fungicide amended PDA medium was then poured aseptically in glass Petri plates (90 mm diameter) and allowed to solidify at room temperature. Each fungicide treatment at three concentrations and isolates were replicated thrice. After solidification of the medium, the plates were inoculated aseptically with a 5 mm culture disc obtained from a week old actively growing pure culture of *Sclerotium rolfsii* isolates. The disc was placed on PDA in an inverted position in the center of the petri plate and plates were incubated at 28$\pm$2 ^{0}C. Petri plates filled with plain PDA (without any fungicide) and inoculated with culture disc of *Sclerotium rolfsii* isolate was maintained as untreated control.

Observations to be recorded

Changes in the mycelial growth and morphological characteristics of individual isolates of *Sclerotium rolfsii* of groundnut on PDA will be studied by poison food technique and observations were recorded as follows:

a) Colour of sclerotia bodies

After incubation up to thirty days, the color of ten sclerotia bodies per replication was recorded by comparing with standard color chart.

b) Size of sclerotia bodies

After incubation up to thirty days, diameter of ten sclerotia bodies per replication of each isolate was recorded with the help of Dogmatic caliper and observations were statistically analyzed.

c) Position of sclerotia bodies

For individual isolate, the position of sclerotia bodies in petri plate was recorded. Whether the sclerotia bodies are formed at edge, near edges, at the top of Petri plates, near concentric circle or at center of petri plate and whether they are distributed uniformly or irregular in a petri plate.

Results and Discussion

Effect of fungicides on colony color of *Sclerotium rolfsii* isolates

The effects of all eight fungicides on the colony color of each isolate of *Sclerotium rolfsii* were recorded at 72 hrs by comparing with standard color chart. The fungicides Propiconazole and Tebuconazole reported cent percent inhibition of all six isolates at all three concentrations tested. The results presented in Table 1, recorded variation in colony color among six isolates but at different concentrations of fungicides no variation in colony color was recorded in each isolate. Also, the different fungicides tested against individual isolate doesn't have any effect on colony color of an individual isolate tested.

At three concentrations of eight fungicides, the colonies of isolate Sr1 and Sr6 were cottony white, while colonies of Sr3 and Sr5 were white in color. The colonies of isolated Sr2 and Sr4 were pure white and dim white respectively.

At three concentrations of eight fungicides, the colonies of isolate Sr1 and Sr6 were cottony white, while colonies of Sr3 and Sr5 were white in color. The colonies of isolated Sr2 and Sr4 were pure white and dim white respectively. The fungicides Propiconazole and Tebuconazole reported cent percent inhibition of all six isolates at all the three concentrations tested. The variations in colony color among six isolates of *Sclerotium rolfsii* were recorded but different fungicides and their different concentrations recorded no variation in colony color in an isolate and among the six isolates.

Effect of fungicides on appearance of growth of *Sclerotium rolfsii*

The results presented in Table 2 showed variation in appearance of growth among all the six isolates of *Sclerotium rolfsii*. The fungicides Propiconazole and Tebuconazole reported cent percent inhibition of all six isolates at all the three concentrations tested. The isolate Sr1 and Sr5 recorded clear mycelial growth while isolate Sr2, Sr3, Sr4 and Sr6 recorded thin mycelial growth, full growth of mycelium, bread like mycelial growth and tuft of mycelium, respectively. It was observed that individual isolation does not show any variation in appearance of growth when tested against eight different fungicides at three different concentrations. From this it is concluded that different fungicides and their different concentrations tested doesn't have any effect on appearance of growth of *Sclerotium rolfsii* isolates.

These results are in confirmation with several workers (Alam *et al.*, 2004; Gour and Sharma, 1994; Prashant Kumar *et al.*, 2011) [10, 11, 12].

Effect of fungicides in color of sclerotia bodies of *Sclerotium rolfsii* isolates

After incubation up to thirty days, the colors of ten sclerotia bodies were recorded by comparing with standard color chart (Table 3). The fungicides Propiconazole and Tebuconazole reported cent percent inhibition of all six isolates at all the three concentrations tested. All the six isolates recorded variation in color of sclerotia bodies. The sclerotia bodies of Sr1 and Sr6 were chocolate brown while Sr3 and Sr5 were yellowish brown. The sclerotia bodies of Sr2 and Sr4 were light brown and brown respectively.

From the results presented in Table 3, it was pointed out that sclerotia bodies color of an individual isolate doesn't have

any effect when tested against eight different fungicides at three different concentrations. From this it is concluded that different fungicides and their different concentrations tested doesn't have any effect on color of sclerotia bodies of *Sclerotium rolfsii* isolates.

Table 1: Effect of fungicides on colony color of *Sclerotium rolfsii* isolates.

Sr. No	Treatment	Colony Color of *Sclerotium rolfsii* isolates																	
		Sr-1			Sr-2			Sr-3			Sr-4			Sr-5			Sr-6		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
1	Benomyl	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
2	Captain	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
3	Carbendazim	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
4	Propiconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Rodomel	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
6	SAAF	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
7	Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Thiophanate methyl	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW
9	Control	CW	CW	CW	PW	PW	PW	W	W	W	DW	DW	DW	W	W	W	CW	CW	CW

CW: Cottony White PW: Pure White
W: White DW: Dim White
CW: Cottony White No growth

Table 2: Effect of fungicides on appearance of growth of *Sclerotium rolfsii* isolates.

Sr. No	Treatment	Appearance of growth of *Sclerotium rolfsii* isolates																	
		Sr-1			Sr-2			Sr-3			Sr-4			Sr-5			Sr-6		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
1	Benomyl	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
2	Captain	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
3	Carbendazim	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
4	Propiconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Rodomel	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
6	SAAF	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
7	Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Thiophanate methyl	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM
9	Control	CMG	CMG	CMG	TnMG	TnMG	TnMG	FGM	FGM	FGM	BLMG	BLMG	BLMG	CMG	CMG	CMG	TM	TM	TM

Short Form **Meaning**
CMG : Clear mycelial growth
TnMG : Thin mycelial growth
FGM : Full growth of mycelium
BLMG : Bread like mycelial growth
TM : Tuft of mycelium
- : No growth

Effect of fungicides on the size of sclerotia bodies of *Sclerotium rolfsii* isolates

The results presented in Table 4 showed that all the eight fungicides tested at three concentrations resulted in a statistically significant effect on size of sclerotia bodies. The results also indicated that increase in the concentration of all the eight fungicides tested reported reduction in the size of sclerotia bodies of all six *Sclerotium rolfsii* isolates. The fungicides Propiconazole and Tebuconazole were recorded cent percent inhibition in all six isolates at 100 ppm, 250 ppm and 500 ppm concentration. All the eight fungicides tested at three concentrations *viz.*, 100ppm, 250ppm and 500ppm significant effect on size of sclerotia bodies of all the isolates of test pathogen over untreated control. The results indicated that increase in the concentration of all the eight fungicides tested reported reduction in the size of sclerotia bodies among all six isolates of *Sclerotium rolfsii*. Among the fungicides tested, Propiconazole and Tebuconazole were recorded cent percent inhibition of mycelial growth at all three concentrations tested.

Table 3: Effect of fungicides on color of sclerotia bodies of *Sclerotium rolfsii* isolates.

Sr. No	Treatment	Color of sclerotia bodies of *Sclerotium rolfsii* isolates																	
		Sr1			Sr2			Sr3			Sr4			Sr5			Sr6		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
1	Benomyl	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
2	Captain	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
3	Carbendazim	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
4	Propiconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Rodomel	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
6	SAAF	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
7	Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Thiophanate methyl	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB
9	Control	CB	CB	CB	LB	LB	LB	YB	YB	YB	B	B	B	YB	YB	YB	CB	CB	CB

Short Form		Meaning
CB	:	Chocolate brown
LB	:	Light brown
YB	:	Yellowish brown
B	:	Brown
-	:	No growth

Table 4: Effect of fungicides on the size of sclerotia bodies of *Sclerotium rolfsii* isolates.

Sr. No	Treatment	Size of sclerotia bodies (mm)																	
		Sr1			Sr2			Sr3			Sr4			Sr5			Sr6		
		100 PPM	250 PPM	500 PPM	100 PPM	250 PPM	500 PPM	100 PPM	250 PPM	500 PPM	100 PPM	250 PPM	500 PPM	100 PPM	250 PPM	500 PPM	100 PPM	250 PPM	500 PPM
1	Benomyl	1.61	1.52	1.39	1.33	1.11	0.96	1.14	0.97	0.82	0.99	0.86	0.69	0.92	0.74	0.55	0.85	0.68	0.50
2	Captain	1.63	1.55	1.44	1.38	1.17	1.04	1.17	1.04	0.92	1.00	0.89	0.73	0.93	0.82	0.68	0.86	0.72	0.55
3	Carbendazim	1.54	1.36	1.23	1.27	1.06	0.87	1.05	0.83	0.60	0.98	0.78	0.58	0.90	0.67	0.43	0.83	0.57	0.29
4	Propiconazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	Rodomel	1.64	1.60	1.52	1.41	1.19	1.07	1.23	1.13	1.03	1.13	1.07	1.00	0.94	0.86	0.77	0.88	0.81	0.70
6	SAAF	1.27	1.07	0.85	1.03	0.76	0.57	0.92	0.67	0.41	0.92	0.68	0.39	0.84	0.56	0.29	0.76	0.43	0.13
7	Tebuconazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	Thiophanate methyl	1.65	1.62	1.55	1.43	1.25	1.12	1.28	1.20	1.13	1.17	1.13	1.09	0.95	0.90	0.84	0.89	0.82	0.74
9	Control	1.66	1.66	1.66	1.75	1.75	1.75	1.30	1.30	1.30	1.20	1.20	1.20	0.97	0.97	0.97	0.89	0.89	0.89
	S.E. ±	0.37	0.41	0.43	0.32	0.26	0.29	0.39	0.42	0.22	0.45	0.21	0.24	0.26	0.18	0.32	0.19	0.15	0.12
	C.D.	1.11	1.22	1.28	0.95	0.79	0.87	1.16	1.25	0.66	1.34	0.63	0.71	0.78	0.54	0.95	0.58	0.46	0.37

* : No growth

At 100 ppm, 250 ppm and 500 ppm concentration of fungicides smallest size of sclerotia bodies were recorded in treatment with SAAF in isolate Sr6 (0.76mm), Sr6 (0.43mm) and Sr6 (0.13mm) respectively. In all the six isolate the smallest size of sclerotia was recorded in treatment with SAAF. While highest sclerotia size was recorded in control followed by Thiophanate methyl.

These results are in confirmation with Singh *et al.*, (2010). They studied *in vitro* effect of ten fungicides *viz.*, Mancozeb, Quintozene, Vitara, Thiram, Carbendazim, Companion, Captive, Aureofungin, Thiophanate methyl and Ziram on sclerotia production *Sclerotium rolfsii*, causing collar rot of chickpea. They reported no sclerotia formation with fungicides Mancozeb and Quintozene. The next best fungicides were Vitara (Size: 2.00 mm), Thiram (Size: 1.80 mm) and Companion (Size: 1.10 mm). While Thiophanate methyl was found least effective (size: 1.20 mm). The similar results were reported by Rao *et al.*, (2004) [13] in their studies on efficacy of bioagents in controlling potato wilt caused by *Sclerotium rolfsii* [*Vorticism Rolfson*].

Summery and Conclusion

The mycelium inhibition among all the six isolates increased with increased concentration of fungicides. The variations were recorded among six isolates of *Sclerotium rolfsii*, but different fungicides and their different concentrations tested doesn't have any effect on colony color, appearance of growth, color of sclerotia bodies, position of sclerotia bodies and number of sclerotia bodies present in one position. Increase concentration of all the eight fungicides tested reported reduction in the size of sclerotia bodies, number of sclerotia bodies per cm^2 and test weight of sclerotia bodies among all six isolates of *Sclerotium rolfsii*.

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Conflict of Interest

None.

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