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Morphological and Cultural Characterization of *Sclerotium rolfsii* Sacc on Chickpea and Its Management Using Combined Fungicide Molecules

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Collar rot stands as a significant affliction of chickpea, attributed to the pathogen Sclerotium rolfsii. The current research investigated the morphological and cultural diversity among 10 isolates of *S. rolfsii* collected from major chickpea cultivation regions of Karnataka by assessing their growth

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rate, colony color and appearance, and features of Sclerotium including color, arrangement, and maturity days of Sclerotia were recored on two different media. The isolates BSR 2, BSR 5, BRS 9 and BSR 10 exhibited notably rapid growth on both Potato Dextrose Agar (PDA) and Corn meal Agar (CMA). Overall, the growth of these isolates appeared denser, indicating more substantial mycelial formation on PDA compared to CMA. These findings underscore the existence of variability among the isolates. In vitro evaluation of fungicides for the management of collar rot was evaluated using new combi fungicides. Among those Carbendazim 12% + Mancozeb 63% (0.15%), Hexaconazole 4% WP + Zineb 68%, Carboxin 37.5% + Thiram 37.5 %, Tricyclazole18 % + Mancozeb 62% WP, Captan 70% + Hexaconazole 5% WP at 0.05 per cent concentration showed cent per cent inhibition. Hence, these combi fungicides have been proven effective in suppressing the pathogen, making them valuable tools for implementing successful disease management strategies during the initial stages of crop growth.

Keywords: Chickpea; Sclerotinia rolfsii; morphology; fungicides; variability.

1. INTRODUCTION

"Chickpea (*Cicer arietinum* L.) ranks as the world's third most significant food legume, trailing only dry bean and pea in importance. India holds the title of the largest producer of chickpeas, accounting for over 70% of global production. A significant challenge in agricultural production lies in effectively managing diseases caused by soil-borne pathogens. Among these diseases affecting chickpeas, collar rot stands out as a crucial issue, leading to seed rot and seedling mortality during the early stages of crop growth, up to 45 days after planting. Collar rot, caused by the fungus *Sclerotium rolfsii* Sacc., poses a significant threat to chickpea crops due to its rapid spread and destructive nature. Under favorable environmental conditions, this disease can lead to mortality rates ranging from 55% to 95% during the seedling stage" [1]. The *S. rolfsii* thrives in warm climates and is more prevalent in environments with high moisture and temperatures [2]. "The Control of this pathogen has proven challenging, likely due to its vigorous growth, broad host range and ability to produce numerous Sclerotia that can persist in the soil for several years" [3]. Efforts to manage collar rot in chickpea have been somewhat limited, as substantial levels of host plant resistance are lacking. However, the disease can be mitigated through the use of fungicides and appropriate crop rotation practices [4-5]. The present study aims to morphologically characterize the isolated fungus and evaluate the antifungal efficacy of novel fungicides against *S. rolfsii*, focusing on in vitro management of collar rot in chickpea.

2. MATERIALS AND METHODS

Isolation of pathogen *S. rolfsii* from diseased samples: Freshly diseased plant samples were collected from the research farm during the seedling and vegetative stages of the crop. The roots of the diseased plants, displaying symptoms, underwent thorough washing with water. Small segments of the infected roots were then carefully excised using a sterilized blade. These segments were surface sterilized by immersing them in a 1:1000 mercuric chloride (HgCl2) solution for one minute, followed by three rinses with sterilized distilled water to eliminate any residual HgCl2. Subsequently, the segments were aseptically transferred to Petri plates containing sterilized Potato Dextrose Agar (PDA) and incubated at a temperature of 25 ± 2°C for a period of three to five days. The plates were regularly monitored to observe the growth of fungal colonies originating from the different segments. Upon appearance of fungal colonies, they were transferred to PDA slants for the purpose of culture purification.

Cultural and morphological variability: "Various isolates of *S. rolfsii* from the North Karnataka region were examined for their cultural and morphological traits, including growth rate and sclerotial formation, using two solid media: potato dextrose agar (PDA) and corn meal agar (CMA). Each isolate of *S. rolfsii* was cultured on PDA and CMA media. A 0.5 cm diameter mycelial disc of each isolate was placed at the center of the plate and replicated twice. The inoculated plates were then incubated at 26 ± 1°C for a period of 20 days. The radial growth of each colony in one direction was measured, and visual observations regarding sclerotial formation were recorded. Morphological characteristics based on mycelia (including mycelial growth, colony color, and appearance) and sclerotia (sclerotial color, shape, and arrangement on the surface of the media) were noted at 7 and 20 days of incubation, respectively, for each isolate" [4].

***In vitro* evaluation of fungicides against *S. rolfsii* by poison food technique:** "The effectiveness of seven combination fungicides Tubaconzole 50 % + Trifloxystrobin 25 % WG, Hexaconazole 4 % WP + Zineb 68 %, Captan 70 % + Hexaconazole 5 % WP, Propiconazole 13.9 % + Difenoconazole 13.9 %, Carboxin 37.5 % + Thiram 37.5 %, and Carbendazim 12 % + Mancozeb 63 % WP was assessed *in vitro* at various concentrations (20, 50, 100, 200, and 500 ppm) to determine their impact on the growth of *S. rolfsii* on Potato Dextrose Agar (PDA) medium using the poisoned food technique" [6].

Before the experiment, the pathogen *S. rolfsii* was inoculted on PDA medium for 7 days. The PDA medium was prepared and melted and the required amount of fungicide was added to achieve the desired concentrations. Twenty milli liters of poisoned medium was poured into each sterilized Petri plate, with a suitable control maintained without fungicide addition. To prevent bacterial contamination, a pinch of streptomycin sulfate was added to the medium during pouring. A five-millimeter mycelial disc was taken from the periphery of a 7-day-old colony of *S. rolfsii* and placed in the center of each Petri plate. The inoculated plates were then incubated at 25 ± 2°C, with four replications maintained for each treatment. The colony diameter was measured at the point of maximum growth of *S. rolfsii* in any of the treatments, and observations were recorded. Percent inhibition was calculated using the formula proposed by [7].

$$I\ (\%) = (C-T)/C \times 100$$

Where,

I = Per cent inhibition,
C = Growth in control
T = Growth in treatment

3. RESULTS

Cultural and morphological variability of pathogen: Significant variations were observed among the isolates regarding their total growth and growth rate on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) media (Fig. 1 & 2). Four isolates (BSR 1, BSR 2, BSR 5, BSR 7, BSR 9 and BSR 10) exhibited the maximum radial growth on PDA, reaching 96.10mm. Among these, BSR 2, BSR 5, and BSR 7 achieved 90.00mm growth within 3 days after inoculation (DAI), indicating their very fast growth. Another six isolates (BSR 1, BSR 2, BSR 5, BSR 7, BSR 9, and BSR 10) achieved 90.00mm diameter growth by 5 DAI, categorized as fast-growing. Three isolates (BSR 3, BSR 4, and BSR 8) exhibited growth of 81.00mm, 75.0mm, and 70.0mm, respectively, by 6 DAI, classifying them as moderate-growing. The isolate BSR 6 displayed the least growth (52.00mm) and was considered the slowest-growing isolate (Fig. 1).

Similarly, considerable variation in total growth and growth rate was observed among isolates on CMA. Six isolates (BSR 2, BSR 4, BSR 5, BSR 9 and BSR 10) achieved maximum radial growth (93.80mm) by the 4th DAI. Among them, BSR 3, BSR 6, and BSR 8 achieved maximum radial growth (90.00mm) by the 3rd DAI, while the remaining isolates reached this growth by the 4th DAI. The remaining isolates were categorized as slow-multiplying isolates. The least growth was observed in the isolate, BSR 1 (51mm) and considered as the slowest growing isolate among the 10 isolates (Fig. 2). The results of colony characters of *S. rolfsii* isolates on PDA and CMA (colony colour and appearance) are presented in Table 2.

The results obtained on PDA indicated that four isolates (BSR 1, BSR 2, BSR 5, BSR 7) displayed fluffy growth, while three isolates (BSR 3, BSR 4, BSR 6) exhibited cottony growth and the remaining three isolates (BSR 8, BSR 9 and BSR 10) demonstrated dense mat growth. Isolates BSR 1 also displayed as flower like pattern appearance of mycelium, while isolates BSR 4, BSR 7, and BSR 8 showed a wavy pattern. On CMA medium, four isolates (BSR 2, BSR 4, BSR 6, BSR 8) exhibited fluffy growth, two isolates (BSR 3 and BSR 7) showed cottony growth, two isolates (BSR 5, BSR 9) recorded dense mat growth, and the remaining two isolates (BSR 1 and BSR 10) displayed condensed growth. Overall, all *S. rolfsii* isolates demonstrated denser mycelial growth on PDA compared to CMA medium, indicating PDA as the preferred medium for the multiplication and maintenance of the pathogen. Regarding sclerotial characters such as time taken for production, colour and site of production, variations were observed among isolates on both PDA and CMA. Isolates BSR 2, BSR 3, BSR 5, and BSR 7 took the longest time for sclerotial production on PDA, while isolates BSR 9 and BSR 10 exhibited the shortest time. sclerotial colors ranged from brown to light orange, and the site of sclerotial production varied among isolates on both media. Similarly, variations in sclerotial characters were observed on CMA,

with some isolates showing faster sclerotial production compared to others. Isolates BSR 2, BSR 3, BSR 5, and BSR 7 required the maximum time for sclerotial production on CMA, while BSR 10 exhibited the shortest time (Table 2).

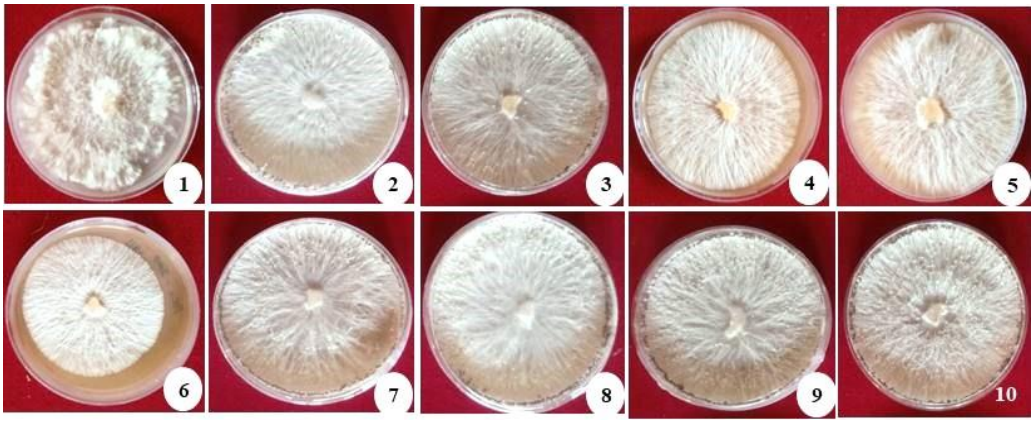

Fig. 1 Mycelial growth of *S. rolfsii* isolates on PDA media after 7 days

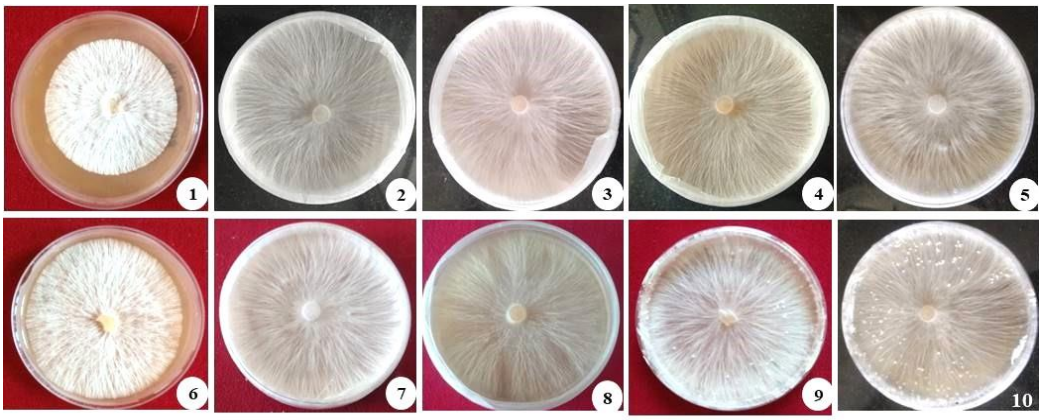

Fig. 2 Mycelial growth of *S. rolfsii* isolates on CMA media after 7 days

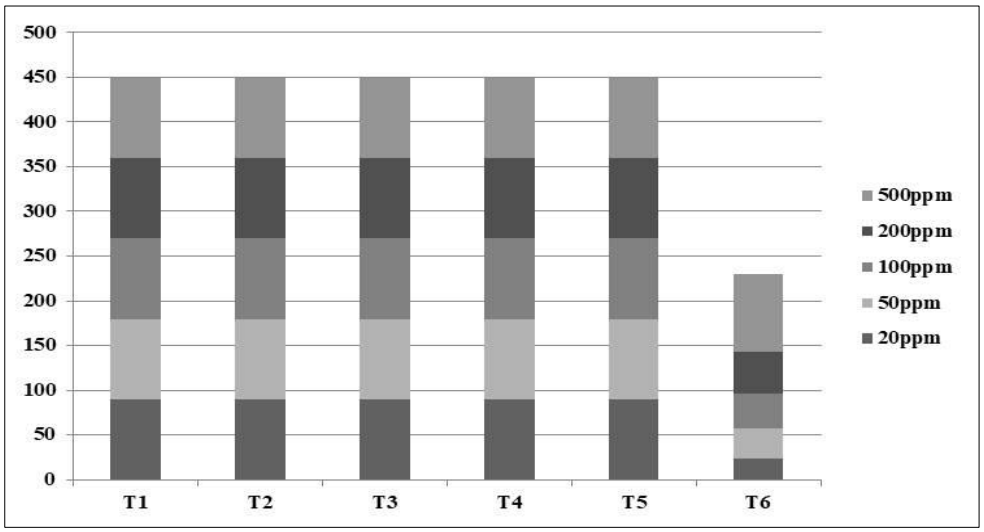

Fig 3. Effect of new combi fungicide molecules on the inhibition of radial growth of *S. rolfsii* Sacc

Table 1. A Comparative Analysis of Morphological Characteristics of *S. rolfsii* Sacc. on both PDA and CMA

Sl. No	Isolate	PDA				CMA			
		Colour	Shape	Arrangement	Maturity (days)	Colour	Shape	Arrangement	Maturity (days)
1	BSR-1	Dark Brown	Spherical	Central and peripheral	11	Dark brown	Irregular	Peripheral	10
2	BSR-2	Light Brown	Irregular	Formed on upper petriplate	17	Dark brown	Spherical	Peripheral	14
3	BSR-3	Dark brown	Spherical	Peripheral	18	Dark brown	Irregular	Peripheral	22
4	BSR-4	Brown	Spherical	Scattered all over plate	22	Brown	Irregular	Centralised	26
5	BSR-5	Dark Brown	Spherical	Peripheral	22	Dark brown	Spherical	Peripheral	14
6	BSR-6	Dark Brown	Irregular	Scattered all over plate	22	Brown	Irregular	Peripheral	19
7	BSR-7	Light Brown	Irregular	Scattered all over plate	15	Dark brown	Spherical	Scattered	14
8	BSR-8	Light orange	Irregular	Scattered all over plate	11	Dark brown	Irregular	Scattered	22
9	BSR-9	Brown	Spherical	Formed at edges on upper petriplate	6	Dark brown	Spherical	Peripheral	10
10	BSR-10	Brown	Spherical	Scattered all over plate	15	Brown	Spherical	Peripheral and Scattered	10

Table 2. Sclerotial characters of different isolates of *S. rolfsii* Sacc. on PDA & CMA

		PDA			CMA		
S. No	Isolates	Colour	Shape	Arrangement	Colour	Shape	Arrangement
1	BSR-1	Fast	Extra white	Thick strands, upward growth and cottony at centre	Dark brown	Spherical	Dense cottony, aggregated
2	BSR-2	Fast	White	Thin strands, suppressed, wavy like pattern	Dark brown	Irregular	Thick strands, sparse growth, aggregate at centre, upright growth
3	BSR-3	Fast	White	Thin strands, suppressed, wavy like pattern	Brown	Irregular	Flower like, cottony, Fluffy, branches like clear at centre
4	BSR-4	Fast	Cottony white	Fluffy at margins, wavy appearance	Dark brown	Spherical	Flower like, cottony, Fluffy, branches like clear
5	BSR-5	Fast	Extra white	Thick strands, sparse, upright growth	Brown	Irregular	Sparse, suppressed thin strands
6	BSR-6	Very fast	Light Orange	Thin strands, thick strands at centre	Dark brown	Spherical	Thick strands, upright growth (distance between strands is more than others)
7	BSR-7	Moderate	Light white	Suppressed, dense cottony at centre, upright	Dark brown	Irregular	Suppressed, Fluffy at margins
8	BSR-8	Very fast	Dirty white	Dense mat like appearance	Dark brown	Spherical	Cottony, dense mat like appearance
9	BSR-9	Moderate	Light white	Fluffy, wavy margins towards at edges, dense at margins	Brown	Spherical	Dense cottony, condensed at centre
10	BSR-10	Very fast	Dirty white and light brownish	Suppressed strands and dense mat like appearance	Dark brown	Irregular	Sparse, suppressed growth

Table 3. Effect of new combi fungicides on per cent inhibition of radial growth of *S. rolfsii* Sacc. at different concentrations (ppm).

Sl. no	Fungicide/ Treatments	Per cent inhibition of mycelial growth of *S. rolfsii* *					Mean
		20	**50**	**100**	**200**	**500**	
1	T1:Tebuconzole 50%+Trifloxystrobin 25% WG	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
2	T2:Captan 70% + Hexaconazole 5%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
3	T3:Propiconazole13.9%+Difenoconazole13.9%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
4	T4:Carboxin 37.5%+Thiram 37.5%	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
5	T5: Hexaconazole 4% WP + Zineb 68%,	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0 (86.93)	100.0
6	T6:Carbendazim 12% + Mancozeb 63%	15.69 (23.27)	31.10 (33.87)	39.16 (38.72)	52.77 (46.57)	100.0 (86.93)	47.74
		0.00	0.00	0.00	0.00	0.00	
	Mean	77.42	80.82	89.86	92.12	100.0	
			Fungicide	Concentration	F × C		
	SEm±		0.315	0.274	0.638		
	C.D at 5%		0.831	0.788	1.916		

*Average of four replication
Figures in parentheses are angular transformation

In vitro* evaluation of new fungicide molecules against *S. rolfsii: The efficacy of six combi products *viz*., The effectiveness of seven combination fungicides Tubaconzole 50% + Trifloxystrobin 25% WG, Hexaconazole 4% WP + Zineb 68%, Captan 70% + Hexaconazole 5% WP, Propiconazole 13. % + Difenoconazole 13.9%, Carboxin 37.5% + Thiram 37.5%, and Carbendazim 12% + Mancozeb 63% WP were evaluated against *S. rolfsii* at five different concentrations (20, 50, 100, 200, and 500 ppm) under *in vitro* by the poisoned food technique revealed the mean mycelial growth suppression ranging from 23.27 to 100 per cent in total.

The data presented in Table 3 revealed that out of the six combo fungicides, four viz., Tubaconzole 50% + Trifloxystrobin 25% WG, Captan 70% + Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5 % + Thiram 37.5% were found to be highly effective at all the concentrations with 100 per cent inhibition in mycelial growth of *S. rolfsii*. The Carbendazim 12% + Mancozeb 63% was found to be least effective in lower concentrations but a higher concentration (500 ppm), showed 100 per cent inhibition of mycelial growth of *S. rolfsii* (Fig.3).

4. DISCUSSION

Studying morphological and cultural variability is crucial for understanding the behavioral patterns of *S. rolfsii* growth across diverse media. The majority of our isolates exhibit distinct morphological and cultural patterns when grown on PDA compared to CMA. Several researchers [8- 20] reported that “the most suitable medium for better growth of *S. rolfsii* was Potato dextrose agar medium”. Similar results were also observed in studies by [21] where they evaluated “effects of different culture media on *S. rolfsii* mycelial growth, sclerotial production”. [22] examined “total of seven media were used for studying the growth of *S. rolfsii* in Lentil” and [23, 24] also proved “the efficiency of PDA in enhancing mycelial growth of fungus out of nine different cultural media. Their results reported that potato-dextrose medium was most suitable for mycelial growth and sclerotia production of *S. rolfsii*. Thus, the present observation confirmed earlier reports of PDA media suitability for the growth of *S. rolfsii* isolates. The efficacy of 6 combo fungicides was tested at different concentrations of 20, 50, 100, 200 and 500 ppm against *S. rolfsii* on PDA by poisoned food technique *in vitro* condition with new combo fungicide. It revealed that fungicides, Hexaconazole 5% EC, Propiconazole 25% EC, Tubaconzole 50% + Trifloxystrobin 25% WG, Captan 70% + Hexaconazole 5% WP, Propiconazole 13 % + Difenoconazol and Carboxin 37.5% + Thiram 37.5% showed complete inhibition of the pathogen at all the concentrations tested”. Whereas, Carbendazim 12% + Mancozeb 63% was found inhibitive only at higher concentrations (100 ppm). The *in vitro* testing of fungicides gives valuable data on their ability to effectively combat a pathogen in the shortest period of time, and as a result, These results are in agreement with the findings of other studies [25-27] who reported that “the combo products containing triazoles *viz*., Avatar, Merger and Nativo were highly inhibitive to the growth of *S. rolfsii”*. Similarly the studies by [28-31] who observed that “100 per cent inhibition of mycelial growth by these combi fungicides against *S. rolfsii* Sacc. from different crop system” [32]. also reported “the effectiveness of combi fungicides tebuconazole + trifloxystrobin against foot rot pathogen of tomato (*S. rolfsii*)”. Similarly, [33] in their “studies on in vitro evaluation of fungicides against *S. rolfsii*, the causal agent of collar rot of gerbera noticed 100 per cent inhibition of the growth of the pathogen by the fungicides”.

5. CONCLUSION

The present study examined the morphological and cultural variability of ten *S. rolfsii* isolates from major Karnataka chickpea-growing areas. The isolates were analyzed using two solid media, namely potato dextrose agar and corn meal agar, and their growth rates, colony colors and appearances, and sclerotium colors, arrangements, and maturity days were noted. The results showed that the isolates, BSR 2, BSR 5, BSR 9, and BSR 10, grew extremely quickly on both PDA and CMA. In comparison to CMA, PDA showed higher overall isolation growth (dense mycelial production and sclerotial bodies). Thus, the current study demonstrated the existence of variability among the isolates, effect of various culture media on cultural characteristics and sclerotial development. In the present studies the fungicides, Hexaconazole 5% EC, Propiconazole 25% EC, Tubaconzole 50% + Trifloxystrobin 25% WG, Captan 70% + Hexaconazole 5% WP, Propiconazole 13% + Difenoconazol and Carboxin 37.5% + Thiram 37.5% were highly effective even at least concentration of 25 ppm under in vitro conditions. The least per cent inhibition was observed in

Carbendazim 12% + Mancozeb 63% which was found effective only at 500 ppm tested. Therefore, it would be beneficial for selecting suitable disease management strategies by utilizing new generation fungicide molecules under field condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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