

Original Research Article

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Cultural and Morphological Variability among the Isolates of *Sclerotium rolfsii* Sacc. Causing Wilt Complex Disease of Betelvine (*Piper betle* L.)

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ABSTRACT

Keywords

Betelvine, Wilt complex, *Sclerotium rolfsii*, isolates, Cultural variability, Morphological variability

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Wilt complex is one of the important diseases of betelvine causing severe losses in betel leaf production. In the present study Cultural characters of *Sclerotium rolfsii* was studied on six different solid media. Among the solid media tested, maximum radial growth was observed on potato dextrose agar and oat meal agar. Ten isolates of *S. rolfsii* collected from different locations of northern Karnataka were studied on potato dextrose agar for their cultural and morphological variability. Colony diameter ranged from 75.33 to 90.00 mm. Colony colour varied from buff white to pure white, with smooth to coarse texture and regular to irregular margin. Production of sclerotial bodies started from 4th day. Sclerotial bodies are ellipsoidal to spherical in shape with light brown to dark brown colour.

Introduction

Betelvine (*Piper betle* L.) is one of the important perennial horticulture crops of commercial and medicinal value. It is a perennial, dioecious creeper belonging to the family *Piperaceae* and originated central and eastern Malaysia. Successful cultivation of betelvine suffers from root and aerial diseases among these wilt/ root rot caused by many fungal pathogens like *Phytophthora* spp., *Rhizoctonia solani*, *R. bataticola*, *Fusarium* spp., *Pythium* spp. and *Sclerotium rolfsii* along with root knot nematode *Meloidogyne incognita* results in significant yield losses (Brahmankar *et al.*, 2011).

Wilt or foot rot caused by *Sclerotium rolfsii* has become a major limiting factor for successful cultivation of betelvine. Chowdary (1945) reported some diseases of betelvine in Sylhet, Assam with special emphasis on sclerotium wilt. *S. rolfsii* is a polyphagous and most destructive soil borne fungus with wide host range and was first reported by Rolfs (1892) as a cause of tomato blight in Florida.

Materials and Methods

Isolation and identification of fungus

Betelvine plants showing typical wilting symptoms were collected from orchards in

different parts of northern Karnataka. The isolation of the fungus was made by following standard tissue isolation technique on Potato dextrose agar medium. Microscopic examination of the fungus culture was done to record morphological and cultural characters. The identification of *Sclerotium rolfsii* Sacc. was done based on white aerial mycelium and production sclerotial bodies in the culture plate by following Barnett and Hunter (1972).

Cultural characters of *Sclerotium rolfsii* Sacc. on different solid media

Cultural characters of *S. rolfsii* was studied on six different solid media viz., Potato dextrose agar, Oat meal agar, Rrichard's agar, Czapek's dox agar, Host extract agar and Host extract dextrose agar so as to identify the best medium for further studies on variability.

Twenty ml of each medium was poured aseptically into 90 mm diameter Petriplates. After solidification, five mm discs of the fungus was selected from actively growing culture using a cork borer and a single disc placed at the centre of each Petridish.

Each treatment was replicated five times and they were incubated at 27 ± 1 °C for 12 days. Cultural characters such as the colony diameter, colony colour, and production of sclerotial bodies were recorded when the maximum growth was attained on any one of the tested media.

Cultural and morphological study of *Sclerotium rolfsii* isolates on PDA

Ten different isolates of *S. rolfsii* collected from different parts of northern Karnataka were grown on potato dextrose agar medium for the cultural and morphological studies. The cultural and morphological characters (radial growth, colony colour, type of margin, type of growth, texture and sporulation) were recorded after 10 days of incubation, three

replications were maintained for each isolate.

Results and Discussion

The effect of different culture media on the growth of *S. rolfsii* differed significantly. The maximum growth of *S. rolfsii* was observed on Potato dextrose agar (90.00 mm) and Oat meal agar (90.00 mm).

The least growth was recorded in Host extract dextrose agar (33.17 mm). In all the six media, the mycelial growth varied from poor to excellent. Colour of the mycelium varied from buff white to white with both regular to irregular margin and smooth to coarse texture.

Production of sclerotial bodies varied from 4th day of inoculation to 8th day in different solid media. The excellent growth of mycelium, raised colony, smooth margin and early initiation of sclerotial bodies was observed in case of Potato dextrose agar (Table 1) (Plate 1). Basamma *et al.*, (2012) and Ronakkumar and Sumanbhai (2014) observed Potato dextrose agar and Oat meal agar found to be best for growth and sclerotial production of *S. rolfsii*.

The pathogen showed minimum colony diameter on Host extract agar medium this may be due to presence of antifungal properties of essential oils in betelvine leaf that possessed inhibition of mycelial growth (Pradhan *et al.*, 2013).

The data presented in Table 2 and Plate 2 revealed that, variation was observed among 10 isolates of *S. rolfsii* on Potato dextrose agar, Maximum radial growth of 90.00 mm was observed in CHG Sr2, HRH Sr4, KST Sr7, SHG Sr9 and YDL Sr10 isolates. Least colony diameter was recorded in isolate SNR Sr8 (75.33 mm); colony colour varied from buff white to pure white, with smooth to coarse texture and regular to irregular margin.

Plate.1 Growth of *Sclerotium rolfsii* on different solid media



Legend:

1. Czapek dox agar
2. Host extract agar
3. Oat meal agar
4. Richard's agar
5. Host extract dextrose agar
6. Potato dextrose agar

Plate.2 Cultural and morphological diversity among the isolates of *Sclerotium rolfsii*



Legend :

- | | | |
|--------------------|-----------------|--------------|
| 1. Cholachagudda-2 | 2. Kesti | 3. Harihara |
| 4. Guttal | 5. Savanur | 6. Shiggaon |
| 7. Banashankari | 8. Kappalaguddi | 9. Yadihalli |
| 10. Hediya | | |

Table.1 Effect of different solid media on the growth of *Sclerotium rolfsii*

| Name of the media | Colony diameter in mm | Colony colour | Type of margin | Texture | Growth characters |
|----------------------------|-----------------------|---------------|----------------|---------|--|
| Potato dextrose agar | 90.00 | Cottony white | Regular | Smooth | Growth is excellent, raised colony, sclerotial initiation on the 4 th day, sclerotia ellipsoidal and brown |
| Czapek dox agar | 87.33 | White | Regular | Smooth | Growth is good, flat colony, sclerotial imitation on the 7 th day, sclerotia ellipsoidal and brown |
| Richards' agar | 83.33 | Buff white | Irregular | Smooth | Growth is good, flat colony, sclerotial formation on the 8 th day, sclerotia ellipsoidal and dark brown |
| Oat meal agar | 90.00 | Cottony white | Regular | Smooth | Growth is excellent, margin smooth, raised colony, sclerotial initiation on the 5 th day, sclerotia ellipsoidal and brown |
| Host extract agar | 33.17 | White | Irregular | Coarse | Growth is poor, raised colony, sclerotial initiation on the 6 th day, sclerotia ellipsoidal and dark brown |
| Host extract dextrose agar | 42.17 | White | Irregular | Coarse | Growth is poor, raised colony, sclerotial initiation on the 6 th day, sclerotia ellipsoidal and dark brown |
| S. Em.± | 0.3 | | | | |
| C.D. @ 1% | 1.3 | | | | |

Table.2 Morphological and cultural variability among the isolates of *Sclerotium rolfsii* on Potato dextrose agar

| Name of the isolate | Source | colony diameter in mm | colony colour | Type of margin | Texture | Growth characters |
|---------------------|--------------------|-----------------------|---------------|----------------|---------|---|
| BSK <i>Sr1</i> | Banashankari | 78.67 | Buff white | Regular | Smooth | Growth is excellent, flat colony, sclerotial initiation on the 5 th day, sclerotia distribution all over the plate, ellipsoidal and dark brown |
| CHG <i>Sr2</i> | Cholachagudda 2 | 90.00 | Buff white | Regular | Smooth | Growth is excellent, flat colony, sclerotial imitation on the 5 th day, sclerotia distribution all over the plate, spherical and light brown |
| GTL <i>Sr3</i> | Guttal | 89.67 | White | Irregular | Coarse | Growth is excelent, flat colony, sclerotial formation on the 6 th day, sclerotia distribution at the periphery, ellipsoidal and dark brown |
| HRH <i>Sr4</i> | Harihar | 90.00 | Buff white | Regular | Smooth | Growth is excellent, flat colony, sclerotial initiation on the 6 th day, sclerotia distribution at the periphery, ellipsoidal and light brown |
| HDY <i>Sr5</i> | Hediyala | 81.67 | White | Regular | Smooth | Growth is good, flat colony, sclerotial initiation on the 4 th day, sclerotia distribution at the centre, spherical and dark brown |
| KPD <i>Sr6</i> | Kappalguddi | 81.33 | Buff white | Irregular | Smooth | Growth is good, flat colony, sclerotial initiation on the 6 th day, sclerotia distribution at the centre, spherical and dark brown |
| KST <i>Sr7</i> | Kesti | 90.00 | Pure white | Irregular | Coarse | Growth is excellent, raised colony, sclerotial initiation on the 5 th day, sclerotia distribution at the periphery, spherical and dark brown |
| SNR <i>Sr8</i> | Savanur | 75.33 | Buff white | Irregular | Coarse | Growth is moderate, raised colony, sclerotial initiation on the 7 th day, sclerotia distribution at the periphery, ellipsoid and light brown |
| SHG <i>Sr9</i> | Shiggaon | 90.00 | Pure white | Regular | Smooth | Growth is excellent, raised colony, sclerotial initiation on the 5 th day, sclerotia distribution at the periphery, ellipsoidal and dark brown |
| YDL <i>Sr10</i> | Yadihalli | 90.00 | Buff white | Irregular | Coarse | Growth is excellent, raised colony, sclerotial initiation on the 6 th day, sclerotia all over the plate, spherical and light brown |
| | S. Em.± | 0.88 | | | | |
| | C.D. @ 1% | 3.57 | | | | |

There was variation in sclerotia production, Sclerotial initiation was seen on 4th day to 7th day, regarding distribution of sclerotial bodies maximum isolates produced sclerotia at the periphery of Petrisplate and other isolates produced at the centre and distributed all over the plate. Sclerotial bodies are ellipsoidal to spherical in shape with light brown to dark brown colour. These results are in agreement with the findings of Kakade *et al.*, (2017) who observed the variability among 13 isolates of *S. rolfsii* on Potato dextrose agar, where colony diameter ranged from 51.70 to 80.30 mm. Colour of the mycelium varied from cottony white to buff white either smooth or wavy margin. The colour of sclerotial bodies varied from brown to dark brown with spherical to sub spherical shape. Similar observations were also made by Manu (2012) and Savita *et al.*, (2016).

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