



Characterization of Cultural and Morphological Variability in *Rhizoctonia solani* Isolates Associated with Root Rot of Ajwain (*Trachyspermum ammi* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author BLF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BLM and RKF managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i530431

Editor(s):

(1) Dr. Francisco Cruz-Sosa, Metropolitan Autonomous University Iztapalapa Campus, México.

Reviewers:

(1) Patel Gayatriben Bhagavandas, Kadi University, India.

(2) Ngho Doo Jules Patrice, University of Maroua, Cameroon.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67201>

Original Research Article

Received 04 February 2021

Accepted 08 April 2021

Published 13 April 2021

ABSTRACT

Rhizoctonia solani (teleomorph: Thanatephorus spp.) is a plant pathogenic fungus which had a wide host range. It is best known to cause many diseases in plants such as collar rot, root rot, damping-off, sheath blight, stem canker, web blight and wire stem throughout the world. *In vitro* study of the various isolates of *R. solani* for morphological and cultural characters and results revealed that this experiment the various isolates of *R. solani* differed in colony characters and showed black brown colored cultures with 90.0 mm colony diameter on 7th day of incubation under uniform environmental condition. The highest growth reported was isolates CHIRs-5 and sclerotial formation was recorded in all the isolates of *R. solani*. Morphology of sclerotia varied from 1.9×1.5 mm of DCHIRs-1 and 1.8×1.5 mm of RUDPRs-2.

Keywords: *R. solani*; isolates; cultural; morphological; rot and sclerotia.

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1. INTRODUCTION

Ajwain (*Trachyspermum ammi* (L.)) also known as carom seed or bishop weed and this is herbaceous herb belonging to the family *Apiaceae* and vastly grows in Egypt, Iran, Pakistan, Afghanistan and India as well as European region. It is more medical and pharmaceutical manuscript of medieval Persia, seeds of ajwain were highly administered by traditional healers and traditionally employed for different ailments. Due to its various chemical constituents, the herb seeds were also evaluated for its numerous pharmacological properties. Ajwain is erect, glabrous or minutely pubescent branched annual herb which grows up to 75-80 cm in height. Stem is striate, leaves are distant and 2-3 innately divided segments linear. Flowers are different in colour. The seeds are small yellowish brown in colour. It is a popular seed spice crop in India. In India, it is widely distributed and its production is concentrated mainly in Rajasthan followed by Gujarat, Madhya Pradesh, Bihar, Utter Pradesh, Punjab, Tamil Nadu, Andhra Pradesh, Haryana and West Bengal, respectively. Root rot (*Rhizoctonia solani* Kuhn) and Powdery mildew (*Erysiphe polygoni* D.C.) are two major diseases of ajwain [1,2]. Among these, the root rot disease is most destructive common disease of ajwain, caused by *R. solani*, resulted losses in yield as well as quality of the crop. [3] Studied the morphological and cultural characteristics of 53 *R. solani* this pathogen causes losses of % of yield as well as isolates collected from different host plants in Egypt.

2. MATERIALS AND METHODS

The diseased sample of ajwain crop infected by *R. solani* showing typical symptom were collected from different districts of ajwain growing areas of southern part of Rajasthan in air tight zip bag separately during survey. Collected sample were brought to the laboratory of plant pathology at Rajasthan college of Agriculture, Udaipur and pathogens were isolated on PDA media. All isolates of *R. solani* were studied for their morphological and cultural variability like: studies on growth rate, colony characters.

2.1 Studies on Cultural and Morphological Variability of Isolates of *Rhizoctonia solani*

All isolates of *R. solani* were grown on PDA medium. The melted medium was dispensed in

Petri plates and allowed to solidify. Five mm disc of the individual isolates of *R. solani* removed from the periphery of seven days old culture was aseptically placed in the centre of the PDA agar plate, maintained three replications for each isolate. The plates were incubated at $28 \pm 1^{\circ}\text{C}$. The variations in growth pattern and radial colony growth (diameter) of fungi in all isolates were recorded. Sclerotial production by isolates of *R. solani* was determined by removing bit (3 mm diameter) from three liner places across the centre of the colony, which were suspended in 10 ml sterile water in sterilized glass taste tube and agitated twice for about ten seconds, each time on a vortex shaker to dislodge conidia. The number of conidia and sclerotia in the resultant suspensions was determined using a haemocytometer, and expressed number of sclerotia per mm^2 of medium. For sclerotial size (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring 50 sclerotia of each isolates of *R. solani* using stage and ocular micrometer.

3. RESULTS AND DISCUSSION

3.1 Morphological Characteristics of *R. solani*

All the isolates of *R. solani* differed in colony characters on 7th day of incubation under uniform environments and media. The first isolate UDPRs-1 had 90.0 mm growth diameter with 0.9×10^6 sclerotia/ mm^2 and had cottony, irregular margins, dully gray to black, very fast growing, suppressed at the centre. Second isolate RUDPRs-2 had growth diameter (68.0 mm) with sclerotial formation (0.5×10^6 sclerotia/ mm^2), and had velvety, aerial at centre, zonation present, slow growing, steel grey to black. Third isolate RAJRs-3 had growth diameter (70.0 mm) with sclerotial formation (0.6×10^6 sclerotia/ mm^2) and showed aerial, suppressed at the centre, irregular margins, dully grey to blackish medium fast growing. Fourth isolate KRAJRs-4 showed 75.0 mm growth diameter with woolly, entire margins, zonation absent, irregular margins, suppressed at the centre, very fast growing with 0.8×10^6 sclerotia / mm^2 . Fifth isolate CHIRs-5 showed 90.0 mm growth diameter with dull grey to black, zonation absent, irregular margins, dully gray to blackish, medium fast growing with 0.9×10^6 sclerotia / mm^2 sporulation. Sixth isolate DCHIRs-6 had growth diameter (55.0 mm) with cottony, zonation present, irregular margins, steel black, very slow growing and produced

Table 1. Radial growth, cultural characteristics and sclerotia formation of various isolates of *R. solani* incited root rot in ajwain on PDA after 7th day's incubation period at 28±1°C

S. No.	Isolates code	Colony characters			
		Growth diameter (mm)*	Growth rate (mm/day)*	Growth characters and colony colour	Sclerotia (1×10 ⁶ Sclerotia/mm ²)
1.	UDPRs-1	90.0	12.86	Irregular margins, cottony, dully grey to black, very fast growing, suppressed at centre	0.9
2.	RUDPRs-2	68.0	9.71	Velvety, aerial at centre, zonation present, slow growing, steel grey to black	0.5
3.	RAJRs-3	70.0	10.00	Aerial, suppressed at centre, irregular margins, dully grey to blackish, medium fast growing	0.6
4.	KRAJRs-4	75.0	10.71	Woolly, entire margins, zonation absent, medium fast growing	0.8
5.	CHIRs-5	90.0	12.86	Dull grey to black, Zonation absent, irregular margins, suppressed at centre, very fast growing	0.9
6.	DCHIRs-6	55.0	7.86	Cottony, zonation present, irregular margins, steel black, very slow growing	0.4
7.	PRARs-7	50.0	7.14	Velvety, aerial fealty growth with irregular margins, zonation absent, dull grey to black, very slow growing	0.3
8.	MPRARs-8	68.0	9.71	Fluppy, irregular margins, Steel black, zonation present, suppressed at centre, slow growing	0.5
SEm±		2.124	0.303		0.020
CD at 5%		6.443	0.920		0.060

* Mean of three replications; figures in parentheses are arcsine $\sqrt{\text{per cent angular transformed values}}$

Table 2. Growth measurement of sclerotia of different isolates of *Rhizoctonia solani* grown on PDA for 7thdays and incubated at 28±1°C

S. No.	Isolates code	Sclerotial morphology			
		Length (mm*)		Width (mm*)	
		Mean	Range	Mean	Range
1.	UDPRs-1	1.3 ±0.07	1.2-1.6	0.9±0.05	0.8-1.0
2.	RUDPRs-2	1.8 ±0.09	1.5-2.5	1.2±0.18	1.0-1.3
3.	RAJRs-3	1.6 ±0.07	1.5-1.9	1.1±0.06	0.9-1.2
4.	KRAJRs-4	1.4 ±0.06	1.2-1.8	1.0±0.05	0.8-1.1
5.	CHIRs-5	2.0 ±0.08	1.8-2.3	1.4±0.20	1.3-1.6
6.	DCHIRs-6	1.9 ±0.09	1.5-2.2	1.3±0.15	1.1-1.4
7.	PRARs-7	2.2 ±0.08	1.6-2.5	1.7±0.25	1.6-1.9
8.	MPRARs-8	1.5 ±0.07	1.2-2.0	1.1±0.10	0.9-1.3
SEm±			0.005		0.004
CD at 5%			0.015		0.001

*Mean no. of fifty sclerotia and ± S.D. of mean value

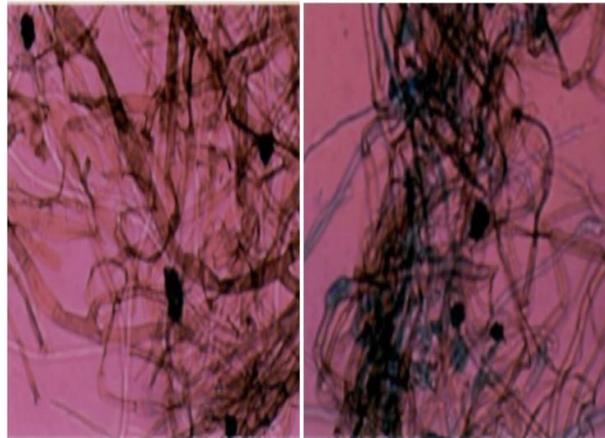


Fig. 1. Mycelium and Sclerotia formation of *R. solani*

0.4×10^6 mm²/sclerotia. Seven isolate PRARs-7 had growth diameter (50.0 mm) with velvety, aerial fealty growth with irregular margins, zonation absents, dull grey to black, very slow growing and produced 0.3×10^6 sclerotia/mm² and eight isolate MPRARs-8 had growth diameter (68.0 mm) with fluppy, irregular margins, steel black, zonation present, suppressed at the centre, slow growing and produced 0.5×10^6 sclerotia/mm² (Table 1 & Fig. 1). In another study, 112 isolates of *R. solani* were collected from soil; root and collar rot or foliage blight infected various plants from several locations in Haryana, India. Of these, 43% belonged to anastomosis group-1 (AG-1 IA, AG-1 IC), 37% to AG-4, 9% to AG-3 and 11% to AG-7. Studies on 20 selected isolates of different AGs revealed considerable variability in their cultural and morphological characters, growth and virulence. Results of pathogenicity tests using mung-bean cv. K 851, chickpea cv. C 235, rice cv. Jaya and sugarcane cv. COJ 64 showed that isolates of AG-1, AG-4 and AG-7 were moderate to highly virulent on hypocotyls of chickpea and mung-bean [4]. Similar results also have been reported that five isolates (I₁ to I₅) of *R. solani* from fenugreek plants collected from different locations in Chhattisgarh varied in the rate and type of growth, colony colour, hyphal width and sclerotial production. Isolate I₃ showed the highest growth on PDA medium and highest pathogenicity [5] and also these findings supported various scientists in the while studying variability of *R. solani* isolate found six isolates as light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown reported by Lal and Kandhari [6].

3.2 Variability in Sclerotial Morphology

Sclerotial formation was recorded in all the eight isolates of *R. solani* studied. Morphology of sclerotia especially varies in terms of length and width among different isolates collected from various locations.

Among the isolates of *R. solani*, sclerotia of DCHIRs-6 were the highest in size measuring $1.9(1.5-2.2) \times 1.3(1.1-1.4)$ mm, followed by sclerotia of isolate RUDPRs-2 which measured $1.8(1.5-2.5) \times 1.2(1.0-1.3)$ mm. Isolate RAJRs-3 recorded sclerotia of size $1.6(1.5-1.9) \times 1.1(0.9-1.2)$ mm and those of MPRARs-8 measured $1.5(1.2-2.0) \times 1.7(0.9-1.3)$ mm, the sclerotia of isolates KRAJRs-4 measuring $1.4(1.2-1.8) \times 1.0$

$(0.8-1.1)$ mm, the sclerotia of isolates CHIRs-5 measuring $2.0(1.8-2.3) \times 1.4(1.3-1.6)$ mm, the sclerotia of isolates PRARs-7 measuring $2.2(1.6-2.5) \times 1.7(1.6-1.9)$ mm and the sclerotia of isolates UDPRs-1, the smallest were of measuring $1.3(1.2-1.6) \times 0.9(0.8-1.0)$ mm (Table 2). All the eight isolates of *R. solani* differed in colony characters and showed black coloured cultures with 90.0 mm colony diameter on 7th day of incubation under uniform environments and medium. Sclerotial formation was recorded in all the six isolates of *R. solani*. Morphology of sclerotia varied from 1.9×1.5 mm of DCHIRs-1 and 1.8×1.5 mm of RUDPRs-2. The results showed variations in growth and sclerotial formation among *R. solani* isolates [7,8]. The similar experiment was conducted an experiment in which a total of 25 isolates of *R. solani* were collected from rice plants from different regions of Uttar Pradesh viz., Azamgarh, Basti and Faizabad, and reported for variability using morphological and virulence characteristics. Virulence diversity was analyzed by inoculation on 10 rice cultivars under greenhouse conditions at 28°C. In most of the isolates, the disease symptoms appeared at 48 h after inoculation but in some of them the disease appeared at 96 h after inoculation and this study supported by Debbarma and Dutta [9] and reported morphological variability was studied in 6 isolates of *R. solani* having different hosts from Assam. Colony size, colony growth, colour and sclerotia formation (ring at periphery, peripheral or scattered), location (surface) and texture (smooth or rough) varied in these isolates. The disease severity of *R. solani* isolates was analyzed by AUDPC (area under disease progress curve) value on the basis of lesion length recorded at days 4, 8, 12 and 16 after inoculation. A comparative analyses of *R. solani* isolates indicated that fast growing isolates having macro-sized sclerotia were highly virulent compared to slow growers with micro-sized sclerotia.

4. CONCLUSION

This study was observed that the various isolates have great diversity in cultural or morphological characters. *Rhizoctonia solani* represents a diverse group of fungi that differs in many significant features. Identify of *Rhizoctonia solani* isolates to some taxonomic level is superior importance for further studying their epidemiology and control of this pathogen in different crops.

ACKNOWLEDGEMENTS

The Authors are thankful to Professors & Head, Department of Plant Pathology, Rajasthan College of Agriculture under the Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan (India) and Director of General, Indian Council of Agricultural Research, New Delhi, India for financial support to carry out the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

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