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PATHOGENICITY AND GROWTH HABIT *OF Rhizoctonia solani* ON DIFFERENT MEDIA

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ABSTRACT

Potato (*Solanum tuberosum* L.) is an important vegetable crop worldwide; however, its productivity is adversely affected by black scurf disease incited by *Rhizoctonia solani* Kühn. The present investigation aimed to establish the pathogenic role of *R. solani* and to assess its growth behaviour on different culture media. The pathogen was evaluated on nine culture media. Among them, Potato Dextrose Agar (PDA) consistently supported maximum growth (up to 90.00 mm), followed by Oatmeal Agar, Starch Agar (90.00 mm) and Potato Carrot Agar (89.3 mm) while Yeast Extract Agar (51.88 mm) and Czapek's Agar (60.53 mm) showed the least growth. The study concludes that *R. solani* is the causal agent of black scurf disease in potato, and PDA is the most suitable medium for its growth.
Keywords **:** Black scurf, PDA, culture media

Introduction

Potato (*Solanum tuberosum* L.), is an important vegetable crop that belongs to family Solanaceae, which is popularly known as "The King of Vegetables". Potato is a major vegetable crop that play a significant role in our daily diet. It serves as the primary source of starch and carbohydrates. the typical composition of a potato tuber per 100 g edible portion consists of 74.7 g of moisture, 1.6 g of protein, 0.1 g of fat, 22.6 g of carbohydrates, 97 kcal of energy, 10 mg of calcium, 40 mg of phosphorus, 0.70 mg of iron, 24 µg of carotene, 0.10 mg of thiamine, 0.01 mg of riboflavin, and 17 mg of vitamin C (Das, 2000). Potato is a nutritionally balanced food, offering relatively low energy while being a valuable source of high-quality protein, essential vitamins, minerals, and trace elements (Mehdi *et al*., 2008). The major potato producing Indian states are Uttar Pradesh (20.1 MT), followed by West Bengal (14.5 MT), Bihar (9.13 MT), Gujarat (4.02 MT), and Madhya Pradesh (3.95 MT) (DAC&W, GOI 2023). Black scurf, induced by the soil-borne fungus *Rhizoctonia solan*i Kuhn (Perfect stage is *Thanatephorus cucumeris*), is a globally distributed disease affecting quality and quantity of potato crops. It is widely distributed across India in varying amounts and poses a significant challenge in fields where potatoes are cultivated annually in the same field (Yanar, 2000). The fungus impedes plant growth by producing cankers on emerging sprouts, below-ground stems, and stolons, with tubers acquiring an unattractive appearance due to formation of black sclerotia on potato surface (Willersinn *et al*., 2015). Similar to other seed-borne pathogens (Arora, 2013), R. solani is transmitted through infected seed tubers, facilitating its long-distance dissemination. If once the pathogen becomes established in the soil, its sclerotia (resting stage) and mycelium can serve as additional sources of primary inoculum. Despite this, the extent of disease severity is not always indicative of yield

loss. However, the presence of black sclerotia on potato tubers affect the tuber quality by causing deformities and altering both the size and number of tubers. So that marketable value of tubers is significantly reduced. However, this disease can lead to substantial yield losses, reaching up to 40% in certain year (Woodhall *et al.*, 2008). Due to its broad host range and destructive nature, comprehensive studies on the biology of this fungus are necessary. Khan *et al.* (2016) assessed various culture media to determine their suitability for the growth of the potato black scurf pathogen, *Rhizoctonia solani.* If farmers are known optimal growth factors of disease development they can easily controlled through preventive management strategies. Due to insufficient information on the cultural characteristics of the potato black scurf fungus, this study aimed to identify suitable culture media for its growth.

Materials and Methods

Isolation, purification and identification of pathogen

Potato tubers showing characteristic black scurf symptoms, identified by small, crust-like dark or black sclerotia, were collected from farmers' fields for laboratory analysis. The infected tubers were thoroughly washed several times to remove adhering soil particles, after which the sclerotia along with a small portion of the surrounding potato tissue were aseptically excised using a sterilized scalpel. The excised samples were surface sterilized by immersion in 1.0% sodium hypochlorite solution for 30 seconds, followed by three rinses with sterile distilled water, and then dried on sterile tissue paper. Sterile Petri dishes containing 20 ml of Potato Dextrose Agar (PDA) medium were prepared under aseptic conditions, and the dried sclerotial pieces were placed onto the solidified medium inside a laminar airflow chamber. The inoculated petri dishes were sealed with parafilm and incubated at 25 ± 2°C, and fungal growth was monitored regularly.

Pure cultures of the fungus were obtained by using the hyphal tip method as described (Malik *et al.,* 2014). The pure cultures of fungus were initially identified as *R. solani* through visual examination of their macro-morphological characteristics with the naked eye, and the identification was further confirmed microscopically according to the criteria outlined by Ogoshi (1987).

The identification of *Rhizoctonia solani* was based on their characteristic microscopic features, such as branching near the hyphal tips, right-angled mycelial branching and the formation of sclerotia with brown and black colours in 10 days old culture Fayyad *et al*. (2016).

Mass multiplication of *Rhizoctonia solani*

Millet grain (sorghum) were used for the mass multiplication of *Rhizoctonia solani*. The grains were soaked in water overnight, air-dried at room temperature, and autoclaved in polythene bag at 15 p.s.i. (121.6°C) for 20 minutes. Each bag was inoculated with a 5.0 mm mycelial bits obtained from a five-day-old culture of *R. solani* and incubated at 27 ± 1°C in BOD incubator for 15 days. To ensure uniform fungal colonization, the bags were shaken on alternate days. The inoculum thus produced was subsequently used for pathogenicity test (Kumar, 2018). The pots (30 cm diameter), filled with 5 kg of sterilized soil, were inoculated with 10 g of pathogen inoculum per kg of soil. The pots were watered and maintained for 48 hours to allow for effective pathogen colonization before potato seeds were sown.

Preparation of potato seed tubers

The Kufri Chipsona-1 potato seeds were surface sterilization by the soaking the tubers in a 1% sodium hypochlorite solution for three minutes, followed by a one-minute dip in 70% ethyl alcohol.

Experimental layout for Pathogenicity test

The soil mixture (clay and sand in a 1:1 ratio) was sterilized using 37% commercial formalin. A 1:9 dilution of formalin was prepared using water. The soil was sterilized with a formalin solution and then sealed with a polyethylene sheet for 48 hours. The soil was left exposed to air for 4–5 days to allow complete evaporation of formalin, as reported by El-Aziz *et al.* (2013). Pots (30 cm in diameter) were filled with sterilized soil and subsequently inoculated with *R. solani* at a rate of 10 g per kg of soil, followed by watering for 4 days prior to planting. The pots were arranged in CRD (complete random block design) at net house in Department of Plant Pathology, Chandra Shekhar University of Agriculture & Technology Kanpur, during the winter season under ambient temperature conditions. For each treatment, three replicates were used, with each replicate having three plants.

Assessment of different culture media on mycelial growth pattern of *R. solani*

The growth performance of *Rhizoctonia solani* was assessed using nine different culture media, including Potato Dextrose Agar, Oatmeal Agar, Potato Carrot Agar, Ray agar media Czapek's Agar, Starch Agar, Rose Bengal, yeast Agar and Corn Meal Agar media (The composition given media show in table 1)

were used to analyse the growth rate of *R. solani*. The culture media were prepared following the standardized procedure then autoclaved at 121.6 °C and 15 psi pressure for 20 minutes. The experiment was conducted using a completely randomized design with three replications. An equal volume (20 ml) of each medium was dispensed into Petri plates. A uniform mycelial bits of *Rhizoctonia solani* (5 mm in diameter) was inoculated at the centre of each plate and incubated at 25 ± 2°C. The diameter of pathogen growth was measured after 2, 4, and 6 days of incubation and recorded in millimetres (mm) using a scale (Arora, 2023).

Table 1 : Composition of different media

Sr. No	Culture media	Ingredients	As per litter (1000 ml)
1.	Potato Dextrose Agar (PDA)	Peeled sliced potato, Dextrose, Agar, Distilled Water	200g, 20g, 15-20g
2.	Oatmeal Agar (OMA)	Oatmeal, Agar, Distilled Water	60g, 15-20g
3.	Potato Carrot Agar (PCA)	Potato, Carrot, Agar, distilled water	20g, 20g, 15-20g
4.	Ray Agar Media (RCA)	Rye Grains (Infusion), Agar, distilled water	60g, 15-20g
5.	Czapek's Agar Media (CAM)	Sucrose, Sodium nitrate, Dipotassium phosphate, Magnesium sulphate, Potassium chloride, Ferrous sulphate, Agar Distilled Water	20g, 2g, 1g, 0.5g, 0.5g, 0.01g, 15-20g
6.	Starch Agar	Soluble Starch, Beef Extract, Agar, distilled water	20g, 3g, 15-20g
7.	Rose Bengal Agar Media (RBAM)	Soy Peptone, Dextrose, Potassium Dihydrogen, Phosphate Magnesium Sulphate, Rose Bengal, Agar, Distilled Water	5g, 10g, 1g, 0.5g, 0.05g, 15-20g
8.	Corn Meal Agar (CMA)	Corn Meal (Infusion), Agar, Distilled Water	50.0g , 15.0g
9.	Yeast Agar (YA)	Yeast Extract, Peptone, Dextrose, Agar, Distilled Water	10g, 20g, 20g, 15-20g

Result and Discussion

Isolation, purification and identification of pathogen

Isolation and identification of *R. solani* cultures were carried out using morphological and microscopic characteristics. During early growth, the mycelia of isolated fungi appeared light brown, and abundant aerial hyphae were produced throughout the growth period. As the cultures matured, their colour deepened, becoming very dark brown after 21 days. At three days old culture formed concentric rings but they usually disappeared as the cultures matured and their colour deepened. On the 10 day, sclerotia appeared around the edges of the petri dishes. After 21 days of growth, sclerotia were randomly distributed on the agar surface and within the agar. Young sclerotia were tan in colour, turning dark brown as they matured, and their size varied from 1.5 mm to 5 mm in diameter. The microscopic visual observation shows the hyphae exhibit branching at both right and acute angles, with a septum frequently present near the point of branch formation (Plates 1). The study also revealed the presence of hyphal constrictions at branching points, a distinctive morphological trait of *R. solani* in accordance with standard taxonomic descriptions (Khedri *et al.,* 2014). The pathogen under study was identified as *R. solani* Kühn on the basis of cultural and morphological characters as described by Parameter and Whitney (1970) on PDA medium.

Plate 1 : Identification of *R. solani* sowing typical character a. Pure culture b. Right angel branching c. Healthy Potato d. Infected Potato tuber (Black sclerotia on tuber surface)

Pathogenicity test

The pathogenicity assay indicated that the isolate fungi was pathogenic to the susceptible potato variety, producing typical symptoms such as black sclerotia on the tuber surface, while the control plants remained symptom-free. These results support the conclusion that *Rhizoctonia solani* is the agent responsible for the black scurf disease. (Plate 1)

Assessment of different Culture Media on mycelial growth pattern of *R. solani*.

The study assessed nine culture media including Potato Dextrose Agar, Oatmeal Agar, Potato Carrot

Agar, Ray Agar media, Capek's Agar, Starch Agar, Rose Bengal, yeast Agar and Corn Meal Agar media to determine the most suitable medium for the growth of *R. solani.* The growth of the mycelium was observed at 2, 4 and 6 days following inoculation. The result regarding mycelial growth and sclerotia count is shown in Table 2

At 2 days' post-inoculation, the maximum mycelial growth was recorded on Potato Dextrose Agar (37.77 mm), followed by oatmeal Agar (36.39 mm), Starch Agar (35.73 mm) and Carrot Agar (29. 93mm). The lowest mycelial growth was observed on Yeast Agar (21.79 mm), with Czapek's Agar (23.24 mm), Corn Agar (27.7 mm) and Rose bangal Agar (28.56 mm) showing progressively higher growth (Plate 2)

At 4 days' post-inoculation, the maximum mycelial growth of *R. solani* was recorded on Potato Dextrose Agar (83.36 mm), followed by Oatmeal Agar (83.05 mm), Starch Agar (83.01 mm), and Potato Carrot Agar (82.14 mm). The lowest growth was observed on Yeast Extract Agar (35.79 mm), with Czapek's Agar (43.11 mm) Cornmeal Agar (74.9 mm) and Ray Agar (77.72 mm) showing marginally higher growth. (Plate 2)

At 6 days after inoculation, maximum mycelial growth was recorded on Potato dextrose agar (90. 00 mm) and statically the similar result also recorded in Oatmeal Agar, Starch Agar followed by Potato Carrot Agar (89.3 mm), Rose Bangal Agar (88.6 mm), and Ray Agar (87.33 mm). The minimum growth was observed on Yeast Extract Agar (51.8 mm), followed by Czapek's agar medium (60.53 mm), Cornmeal Agar (86.36mm) (Plate 2)

The study revealed that *R. solani* exhibited maximum growth on Potato Dextrose Agar (PDA) at 2, 4, and 6 days after inoculation compared with other media. Similar findings were recorded by Lalan *et al.* (2013), Singh and Singh (2007), Khan *et al.* (2016), Gupta (2017), Sharma *et al.* (2013) and Kumar *et al.* (2014) who reported that R. solani exhibited the most rapid growth on Potato Dextrose Agar (PDA). Because PDA is considered a highly nutritive medium for the growth of *R. solani,* primarily due to its carbon-rich composition that supports its nutritional requirements. Additionally, it has been noted that PDA is commonly used as a medium due to its straightforward formulation and its ability to support the growth of various plant pathogenic fungi. Several studies have shown that PDA is optimal for the colony growth of various fungal species (Meera *et al.*, 2012)

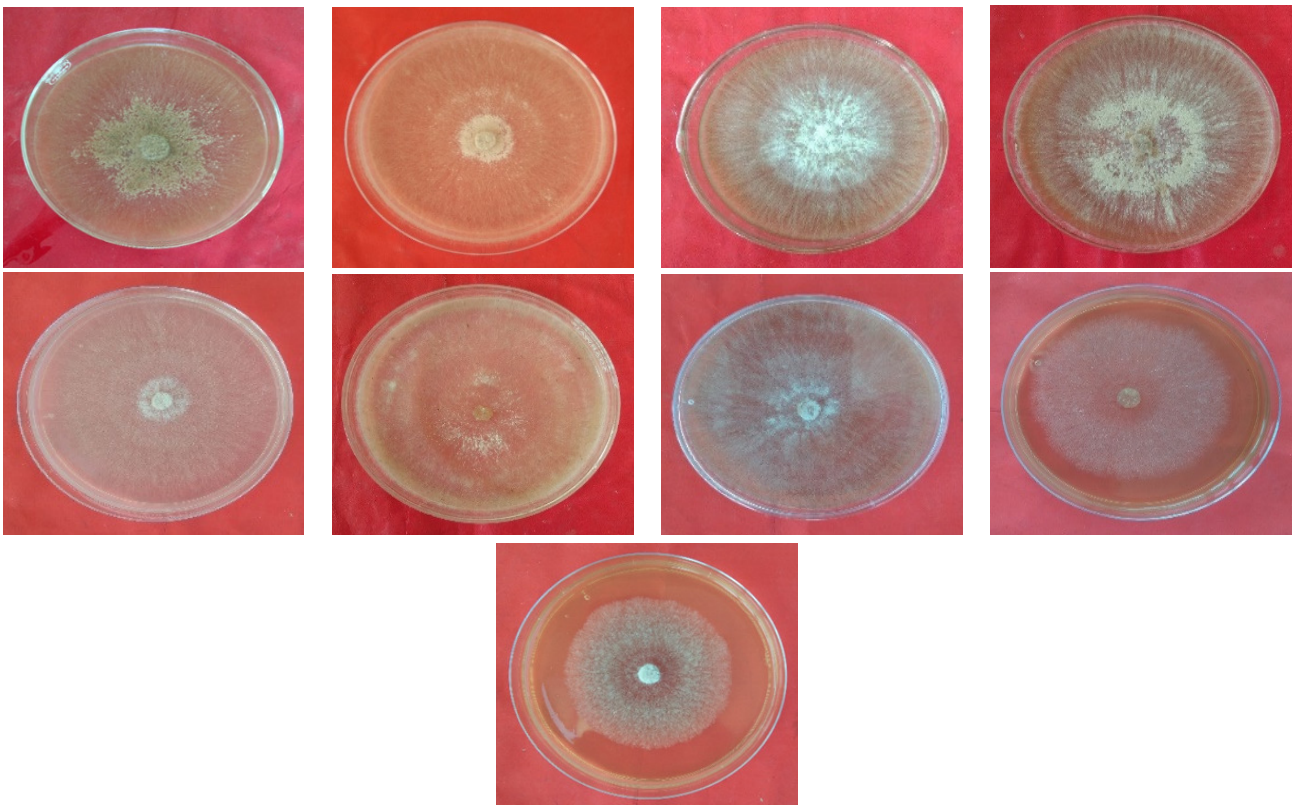

Plate 2 : Mycelial growth of R. solani on different culture media
1.Potato Dextrose Agar (PDA) 2. Oatmeal Agar (OMA)) 3. Starch Agar (SA) 4. Potato Carrot Agar (PCA 5 Rose Bengal Agar (RBA) 6. Corn Meal Agar (CMA) 7. Ray Agar Media (RGM) 8. Czapek's Agar (CZA) 9. Yeast extract Agar media

Table 2 : Assessment of different Culture Media on mycelial growth pattern of *R. solani.*

	Day After Inoculation (DAI)				
Sr.No	**Culture Media**	**2 DAY**	**4 DAY**	**6 DAY**	**Pigmentation**
1	Potato Dextrose Agar (PDA)	36.77	83.36	90	Dark Brown
2	Oatmeal Agar (OMA)	36.39	83.05	90	Brownish white
3	Potato Carrot Agar (PCA)	29.93	82.14	89.3	Light brown
4	Rose Bengal Agar (RBA)	28.56	80.57	88.6	Pinkish white
5	Corn Meal Agar (CMA)	27.7	74.9	86.36	Yellowish white
6	Starch Agar (SA)	35.73	83.01	90	Pale Brown
7	RAY AGAR (RA)	29.04	77.72	87.333	Light Brown
8	Czapek's Agar (CZA)	23.24	43.11	60.53	hyaline
9	Yeast Extract Agar (YEA)	21.79	35.79	51.88	Pale brown
	C.D. at 5%	1.39	1.85	1.63	
	C.V.	2.69	1.50	1.15	

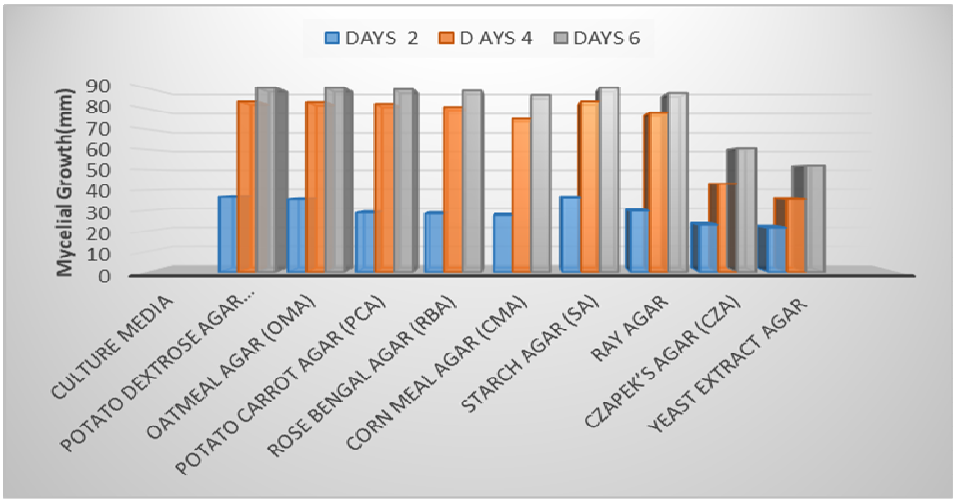

Fig. 2 : Assessment of different Culture Media on mycelial growth pattern of *R. solani*

Conclusion

The study successfully isolated and identified *Rhizoctonia solani* as the causal agent of black scurf disease in potatoes based on its unique morphological characteristics, such as hyphal branching at acute and right angle. Pathogenicity tests confirmed the ability of the fungus to produce typical symptoms of black sclerotia on potato tubers and this study also revealed that Potato Dextrose Agar (PDA) facilitated the highest mycelial growth of *R. solani*. This observation supports earlier findings that PDA is a highly nutritive medium for the growth of a wide range of plant pathogenic fungi, owing to its high carbon content. While other media also supported growth, PDA consistently yielded the highest mycelial development. These findings offer important insights for researchers and practitioners seeking to better understand *R. solani* infections, thereby enhancing disease control strategies in agricultural practices.

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