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Evaluation of culture media for the growth of *Rhizoctonia solani* causing black scurf of potato

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Abstract

Rhizoctonia solani Kuhn [*Thanatephorus cucumeris* (Frank) Donk] is most important pathogen of potato causing black scurf. In the present investigation, seven culture media *viz.*, Potato dextrose agar, Corn meal agar, Water agar, Czapek's agar, Malt extract agar, Potato leaf agar and Potato stem agar were evaluated to find out the appropriate growth of *R. solani*. Out of seven culture media tested, maximum mycelial growth and sclerotia per plate was recorded in potato dextrose agar while minimum mycelial growth was recorded in Water agar. Maximum sclerotia diameter was recorded in Malt extract agar.

Keywords: Water agar, Czapek's agar, Malt extract agar

Introduction

Potato (Solanum tuberosum L) is a tuber crop belongs to the family Solanaceae. Potato is an important crop among all vegetables and has an important role in our daily diet. It is the main source of starch and carbohydrates. The average composition of potato tuber per 100 g edible portion is moisture (74.7 g), protein (1.6 g), fat (0.1 g), carbohydrates (22.6 g), energy (97 kcal), calcium (10 mg), phosphorus (40 mg), iron (0.70 mg), carotene (24 µg), thiamine (0.10 mg), riboflavin (0.01 mg), vitamin-C (17 mg) (Das, 2000)^[2]. Potato is a balanced food containing less energy but nutritional high quality of protein, essential vitamins and minerals including trace elements (Mehdi et al., 2008) [7]. Black scurf caused by Rhizoctonia solani Kuhn (Thanatephorus cucumeris) is a soil born fungus disease of potato worldwide. It is distributed in India in varied proportions and is a major problem in field wherever potato is grown year after year in the same field. In plants, carbohydrates are available in effortless as well as in complex form. Before utilization, fungi convert the complex forms into simple water soluble sugars of low molecular weight. It has been shown that various fungi respond differently with a particular compound and the fungi exhibit marked variation in the utilization of different carbohydrate sources. A vital and extensive knowledge of nutritional patterns and factors influencing the growth of fungi is a prerequisite for any study leading to the understanding relationship between host and pathogen. Not much consideration has been given on the culture and growth media parameters of the R. solani. Therefore, extensive knowledge on the influence of various culture media on growth of the R. solani as well as characteristics of sclerotia of the fungus from black scurf infected potato tubers is needed to be developed for suitable management strategies of the disease and may help in physiological and taxonomical study of the fungus.

Materials and Methods

Evaluation of culture media for growth of Rhizoctonia solani

Seven culture media *viz.*, Potato dextrose agar (Peeled and sliced potato 200 g, Dextrose 20 g, Agar 20 g and Distilled water 1000 ml), Corn meal agar (Cornmeal, 20.0 g, Glucose 20.0 g, Agar 20.0 g and Distilled Water 1000 ml), Water agar (Agar 20 g and Distilled Water 1000 ml), Czapek's agar (Sucrose 20 g, Sodium nitrate 2 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g, Agar 20 g and Distilled Water 1000 ml), Malt extract agar Malt Extract 20.0 g, Peptone 10.0 g, Agar-agar 20.0 g and Distilled water 1000 ml), Potato leaf agar (Potato leaf 200 g, Dextrose 20 g, Agar

20 g and Distilled water 1000 ml) and Potato stem agar (Potato stem 200 g, Dextrose 20 g, Agar 20 g and Distilled water 1000 ml) were used to compare the growth rate of *R. solani*. The culture media were prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for 20 minutes. Mycelial discs of the seven days old culture of *R. solani* (5 mm diameter) were placed on the middle of each pre poured medium and incubated at 25 ± 1 °C (Dela Paz *et al.*, 2006) ^[3]. The experiment laid out in complete randomized design and replicated four times. The diameter of the growth of the fungus was measured after 2, 4 and 6 days of the incubation and growth was taken in mm with the help of a scale. Number of sclerotia and diameter of sclerotia were recorded after 6 days of the incubation.

Results and Discussions

In the study, seven culture media viz Potato dextrose agar, Corn meal agar, Water agar, Czapek's agar medium, Malt extract agar, Potato leaf agar medium and Potato stem agar were evaluated to find out the appropriate growth of *R*. *solani*. Mycelial growth was recorded at 2, 4 and 6 days after inoculation. The data on mycelial growth, number of sclerotia and diameter of sclerotia are presented in Table-1 and Fig-1, 2 and 3 respectively.

Mycelial growth of R. solani on seven culture media

At 2 days after inoculation, significantly maximum mycelial growth was observed in Potato dextrose agar (28.25 mm) which was found at par with Potato stem agar medium (26.00 mm) followed by Potato leaf agar medium (24.25 mm) while minimum mycelial growth of *R. solani* was recorded in Water agar (8.25 mm) followed by Malt extract agar (9.75 mm), Czapek's agar medium (15.25 mm) and Corn meal agar (18.25 mm). Statistically Water agar and Malt extract agar were found at par with each other at 2 days after inoculation.

Significantly maximum mycelial growth of *R. solani* was recorded in Potato dextrose agar (56.33 mm) at 4 days after inoculation followed by Potato stem agar medium (48.75 mm), Potato leaf agar medium (36.75 mm) and Corn meal agar (28.25 mm), however minimum mycelium growth was observed in Water agar (18.75 mm) followed by Malt extract agar (24.67 mm), Czapek's agar medium (26.00 mm). Statistically, Water agar and Malt extract agar found similar at 4 days after inoculation.

At 6 days after inoculation, significantly maximum mycelial growth was observed in Potato dextrose agar (85.00 mm) followed by by Potato stem agar medium (28.33 mm), Potato leaf agar medium (27.33 mm) and Corn meal agar (26.33 mm), however minimum mycelium growth was observed in Water agar (20.67 mm) followed by Malt extract agar (24.67 mm), Czapek's agar medium (26.00 mm). Statistically, Water agar and Malt extract agar found similar at 6 days after inoculation.

This study showed that growth of *R. solani* was best on PDA at 2, 4 and 6 after inoculation as compared to other media. Present finding agreed with Parmeter (1965) ^[8] who observed that the *R. solani* grew most rapidly on PDA, because PDA is supposed to be the most nutritive media for

growth of R. solani as it use carbon principally for its nutrition purpose. Singh and Singh (2007)^[11] reported that maximum fungal colony diameter of *R. solani* (89.7 mm) was recorded on potato dextrose agar, which at on par with PDA + rice leaf extract agar. Csondes (2007)^[1] reported that the malt extract, Sabouraud-glucose and potato-dextroseagar media was most advantageous media for the growth of Macrophomina phaseolina. Gupta (2017)^[4] reported that maximum growth of the R. solani at seven days after inoculation was recorded on potato dextrose agar, followed by corn meal agar, Richards agar, malt extract agar, and water agar while its minimum growth was recorded on oat meal agar. Kumar et al. (2014) ^[5] reported that PDA medium supported maximum mycelial growth (60.1 mm) followed by Coon's (57.5 mm). However, the minimum mycelial growth was recorded on Czapek's Dox agar and oat meal medium (7.2 mm). According to Meena and Chattopadhyay (2002) [6] Czapek's agar was the best in supporting mycelial growth of Rhizoctonia solani Kuhn. Sharma et al. (2013)^[10] reported that maximum mycelia growth of R. solani was recorded on Potato dextrose agar (88.33 mm) Followed by Czapek's dox agar (77.26 mm) and Corn meal agar (74.46 mm).

Number of sclerotia

Data presented on table-1 showed that significantly maximum number of sclerotia was recorded in Potato dextrose agar (57.75 sclerotia/plate) followed by Potato stem agar medium (44.75 sclerotia/plate), Potato leaf agar medium (29.25 sclerotia/plate), Corn meal agar (15.75 sclerotia/plate), Malt extract agar (14.75 sclerotia/plate) and Czapek's agar medium (12.50 sclerotia/plate). No sclerotia was produced by R. solani in Water agar medium. According to Kumar et al. (2014) ^[5] more number of sclerotia produced on yeast potato peptone dextrose agar (YPPDA) medium (89.0) followed by oat meal medium (66.0). Sclerotial production was minimal on Czapek's Dox agar (11.0). Sclerotium yields were obtained maximum on Malt Yeast extract medium for AG 3 and PDA for AG 2-1 of R. solani (Ritchie et al., 2009)^[9]. Singh et al. (1974)^[12] reported the maximum number of sclerotia and weight was recorded on Czapek Dox agar medium. Tiwari and Khare (2002) ^[13] reported that Czapek's Dox Agar was best for sclerotial production. Sharma et al. (2013)^[10] reported that maximum sclerotia of *R. solani* produced by Czapek's Dox agar followed by Corn meal agar and Potato dextrose agar.

Diameter of sclerotia

Data presented on table-1 showed that maximum diameter of sclerotia was recorded in Malt extract agar (1.68 mm) followed by Corn meal agar (1.61 mm), Czapek's agar medium (1.55 mm), Potato dextrose agar (1.42 mm), Potato leaf agar medium (1.35 mm) and Potato stem agar medium (1.23 mm). Kumar *et al.* (2014) ^[5] observed bigger sclerotia in Richard's Agar (4.95) followed by Yeast Peptone Potato Dextrose agar (4.25), Asthana & Hawkers (3.95), Yeast Extract Dextrose agar (3.65), Malt extract agar (2.45) and Brown's agar (2.40).

Table 1: Mycelial growth, sclerotial number and sclerotial diameter of R. solani on culture medium

Medium	Mycelial growth (mm) at DAI*			Number of	Diameter of
	2	4	6	sclerotia/plate	sclerotia (mm)
Potato dextrose agar	28.25	54.75	90.00	57.75	1.42
Corn meal agar	18.50	28.25	43.50	15.75	1.61
Water agar	8.25	18.50	36.00	0.00	0.00
Czapek's agar	15.25	27.00	42.50	12.50	1.55
Malt extract agar	9.75	21.25	30.25	14.75	1.68
Potato leaf agar	24.25	36.75	59.25	29.25	1.35
Potato stem agar	26.00	48.75	77.50	44.75	1.23
SE.m ±	0.96	1.31	1.04	1.61	-
C.D. at 5%	2.81	3.86	3.05	4.73	-

*Days after inoculation



Fig 1: Mycelial growth of R. solani on culture media



Fig 2: Number of sclerotia of R. solani on culture media



Fig 3: Diameter of sclerotia of R. solani on culture media

Conclusion

In article, the study evaluated seven culture media to assess the growth of *R. solani*, a significant pathogen affecting various crops. The results indicated that Potato Dextrose Agar (PDA) consistently supported the highest mycelial growth, number of sclerotia, and diameter of sclerotia compared to other media at different time intervals after inoculation. This finding aligns with previous research suggesting PDA as the most nutritive medium for *R. solani* growth. Moreover, observations on sclerotial production and

size across different media further emphasized the superiority of PDA in supporting the pathogen's development. These findings provide valuable insights for researchers and practitioners aiming to understand and manage *R. solani* infections effectively, contributing to improved disease control strategies in agricultural settings.

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