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Research Article

EFFECT OF DIFFERENT VEGETABLE-GRAINS MEDIA ON VARIABILITY IN MYCELIAL GROWTH PATTERN AND SCLEROTIA FORMATION OF *RHIZOCTONIA SOLANI*

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ABSTRACT

Rhizoctonia solani is one of the most important soil-borne fungal pathogen causing significant yield losses in many agriculturally important crops globally. In the present investigation, several different vegetable-grains culture media *viz.*, Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal Dextrose Agar (BrDA) were evaluated to find out the appropriate media for growth and sclerotia formation of *R. solani*. Out of nine different culture media tested, maximum mycelial growth and sclerotia formation was recorded in MPDA while minimum mycelial growth was recorded in RDA. Maximum diameter of sclerotia was recorded in BDA.

Keywords: *Rhizoctonia solani, Vegetable-grains media, Sclerotia, Growth pattern of mycelia*

1. INTRODUCTION

Rhizoctonia solani, the most important species within the genus *Rhizoctonia*, is a soilborne plant pathogen with considerable diversity in cultural morphology, host range and aggressiveness. *Rhizoctonia solani* Kuhn is a soil-borne plant pathogen with considerable diversity in cultural morphology, host range and aggressiveness. The fungus is necrotroph in nature that infects economically important crops. *Rhizoctonia solani* (Teleomorph: *Thanatephorus cucumeris*) infected potato tubers [1]. Blight, rot, canker and damping off diseases in several crops by the infection of *Rhizoctonia* were earlier reported by researchers [2, 3]. *R. solani* with mature brown colored mycelia is able to form brown to black sclerotia that can persist in soil for long term [4]. Sclerotial development might be induced by application of suitable light intensities, pH and range of temperatures [5, 6]. In another research study conducted by Koley et al. [7], it was found that there was a correlation of sclerotia formation related to amount of nutrients in growth medium. On the basis of growth pattern of mycelia, development pattern of sclerotia on different growth media, isolates of *R. solani* were classified by the researcher Budiarti et al. [8]. Maximum numbers of sclerotia production were also correlated with composition of growth media [9, 10].

Keeping these earlier findings in mind, the present study has been undertaken to find out the appropriate composition of vegetable-grains culture media which will give comparatively better mycelial growth and more sclerotial formation in *Rhizoctonia solani* with a special emphasis on effect of different vegetable-grains media on cultural and morphological variability of that isolate.

2. MATERIAL AND METHODS

2.1. Isolation source of fungal isolate

Diseased mustard plant was spotted from the maize cultivating areas of Raiganj Block of Uttar Dinajpur, West Bengal. Infected parts of stem were collected in sterile polyethylene bags, labeled properly and brought to the Mycology and Plant Pathology Laboratory, Raiganj University. Collected infected part was cut into different segments. The segments were washed with distilled water to remove debris before applying the surface sterilization technique. Segments were treated with 0.1 % mercuric chloride for 2-3 min and rinsed with distilled water twice. Then it was treated with 70% alcohol for 1 min and washed 4-5 times with sterile distilled water. They were air dried aseptically under laminar air flow chamber and transferred centrally and aseptically in the petriplates containing freshly prepared Potato Dextrose Agar (PDA) medium and incubated at

28±2°C till the desired growth of fungus was obtained. In order to obtain the pure fungal culture, repeated subculture was practiced aseptically.

2.2. Maintenance, Preservation and preliminary identification of fungal isolate

The isolated culture was preserved on PDA slant at 4°C temperature. When required, they were allowed to grow on PDA media at 28±2°C. The seven day old culture showing good mycelial growth was used for further experimentation. The fungal isolate was also preliminary identified on the basis of microscopic and morphological observation which showed similar nature of mycelia and spore attachment with mycelia with the fungus- *Rhizoctonia*. After preliminary morphological identification, our desired isolated pure fungal isolate, isolated from rhizosphere of diseased mustard was coded as *Rhizoctonia solani*.

2.3. Composition of Different vegetable-grains media

Growth and sclerotia formation of *Rhizoctonia solani* were studied on nine different vegetable-grains media viz. Nutrient Dextrose Agar (Pentose 5g; $NaCl_2$ 5g; Beef extract 1.5 g; Yeast extract 1.5 g; Agar 20g; 20g Dextrose; Distilled water 1L), Carrot Dextrose Agar (Carrot 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Czapek's Dox Dextrose Agar ($NaNo_3$ 3g; K_2HPO_4 1g; $MgSO_4$ 0.5g; KCl 0.5g; $FeSO_4$ 0.01g; sucrose 30g; Agar 15g; Dextrose 15g; Distilled water 1L), Brinjal Dextrose Agar (Brinjal 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Beet Dextrose Agar (Beet 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Rice Dextrose Agar (Rice grain 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Banana Dextrose Agar (Banana 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Maize Dextrose Agar (Maize grain 200g; Dextrose 20g; Agar 20g; Distilled water 1L), Maize Potato Dextrose Agar (Maize grain 100g; Potato 100g; Dextrose 20g; Agar 20g; Distilled water 1L) and Potato Dextrose Agar (Potato 200g; Dextrose 20g; Agar 20g; Distilled water 1L).

2.4. Preparation of Inoculum of Rhizoctonia solani to evaluate mycelial growth and sclerotia formation on different vegetable grains based media

The seven days old culture with good mycelial growth was used by taking 5mm diameter disc and single sclerotium from the culture were also taken separately for growing on ten different sterile culture media. Each medium (20ml) were used for pouring plates (100mm) and plates were inoculated with 4 mm mycelial discs taken from advancing zones of mycelia of *R. solani* of 5 days old cultured of PDA. The plates were incubated at 28±1°C for study of growth, colony pattern, sclerotia formation, characters of sclerotia etc. Each treatment was replicated thrice. The whole set up was arranged to study the morphological characters in triplicates. The petriplates were then incubated for seven days at 28±2°C in a BOD incubator and colony characters of fungus was recorded along with some important parameters.

2.5. Study of sclerotial characteristics

Sclerotial characteristics of the isolate on ten different media were studied by comparing with the key given by Burpee et al. [11].

Aerial: Sclerotia formed with in aerial mycelium

Embedded: Sclerotia formed with in substrate

Size of sclerotia: (a) Large, (b) Small

Colour of sclerotia: (a) Light brown, (b) Brown, (c) Dark brown, (d) Deep dark brown

Location and Pattern of sclerotial formation: Sclerotia either produced on the surface of agar (aerial) or submerged in the medium. The production of sclerotia by the isolate on different media was recorded as circular manner concentrated towards periphery; irregularly scattered but more towards the centre of the colony; irregular very sparsely scattered and scattered irregularly all over the colony surface.

Sclerotia either with rough border or with smooth border.

3. RESULTS AND DISCUSSION

3.1. Microscopic observation of fungal isolate

Rhizoctonia solani showed brown colouration of mature hyphae, right-angled hyphal branching, constriction at the point of branching (Fig. 1). The isolate also produced blackish to whitish sclerotia when allowed to grow on different media at different stages of their growth.

3.2. Variability in mycelial colour and growth pattern of R. solani on different vegetable-grains media

The colour of the mycelia was varied from white [during growth of mycelia on Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal

Dextrose Agar (BrDA)] to white with light gold [On Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA) and Carrot Dextrose Agar (CDA)] in appearance. Nature of mycelial growth of the isolate varied as per media composition as noticed as abundant (When growing on BtDA, NDA, PDA, MPDA and BrDA media), moderate (in RDA, MDA and BDA media) as well as slight growth (in CDA medium) in nature (Table 1; Fig. 2). The differences in the intensity of the colour might also correspond to the amount of pigments released by respective isolate in the media. The colour production may also be due to release of secondary metabolites like toxins. *R. solani* produced abundant mycelial growth which may be either due to the inherent nature of the isolate to go for quick or produced abundant growth in early stages before setting the sclerotia. Similar observations were observed in a study where about the brown colony colour of *R. solani* maize isolates Hc whereas the isolates Bc, Jr and Rf had white colour [12]. Srinivas [13] also categorized the *Rhizoctonia solani* f. sp. *sasakii* isolates from maize based on texture, abundance of their mycelia growth and colony appearance.

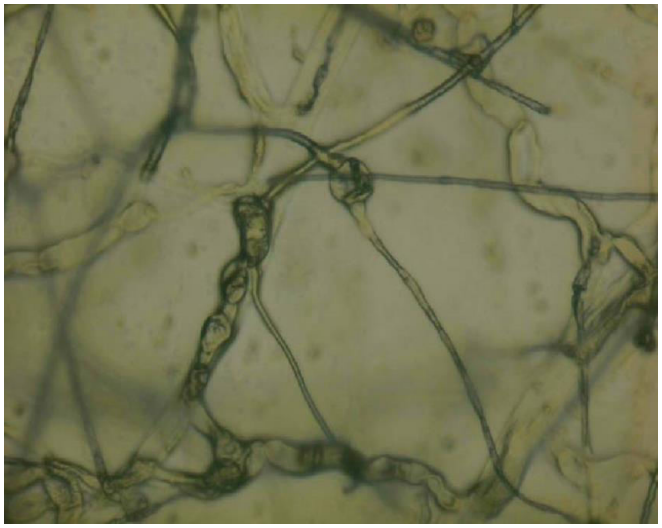


Fig. 1: Microscopic observation of *Rhizoctonia solani* showing hyphal branching with spore

3.3. Effect of vegetable-grains media on sclerotial variability of *R. solani*

3.3.1. Growth pattern, number and color of sclerotia

Maximum sclerotia formation was recorded in MPDA medium. Pattern of sclerotia formation of the isolate on NDA, PDA and BDA media was in circular manner concentrated towards periphery whereas peripheral with few centralized on BtDA, CDA and BrDA media. Sclerotia were also categorized as sub-peripheral on RDA, sub-centralized on MDA and were formed in irregular manner scattered all over the colony surface on MPDA medium. Coloration of sclerotia also varied from prominent black to white with or without exudates over the surface, when the isolate was grown on different media. Highest numbers (41) of sclerotia of the isolate were produced on MPDA medium, followed by 34 in NDA, 33 in CDA, 32 in BtDA, 28 in PDA, 21 in BrDA and 18 in BDA media. Lowest numbers (4) of sclerotia were noticed in RDA medium (Table 1; Fig. 2). In support of our research findings, Srinivas [13] assigned the sclerotia of *R. solani* isolates causing BLSB in maize into three different colour groups based on pigmentation. Anderson [14] reported that the isolates of *Rhizoctonia* were characterized on the basis of dominant pigmentation of the sclerotia. According to the observation by Anderson, the only character that clearly distinguished the sclerotia was the formation of colour.

3.3.1.1. Different media used for growth of Rhizoctonia solani

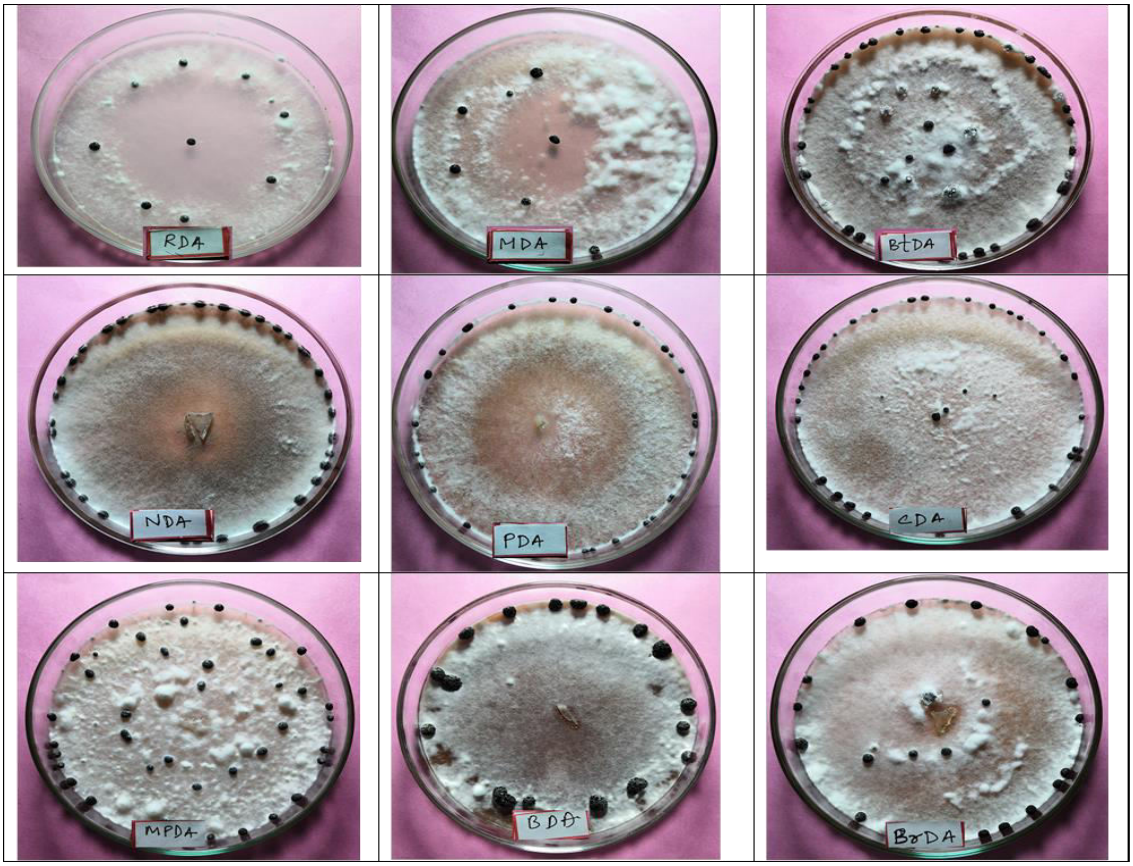
Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal Dextrose Agar (BrDA).

3.3.2. Shape and size of sclerotia

Sclerotia of the isolate showed a wide range of variation in shape as per the changes of the composition of the different media. Ranges were from spherical (in BrDA, RDA, CDA media) to oval (in BDA) and elliptical (in NDA medium). Size of sclerotia was categorized into large and small based on diameter of sclerotia. Maximum diameter of sclerotia (32.5 mm^2) was recorded in BDA medium whereas minimum diameter of sclerotia (3.94 mm^2) was found in CDA (Table 2; Fig. 3). In a research work carried out by Madhavi et al. [15], the rice isolate *R. solani* (RS28) recorded highest (19.00) sclerotial count compared to maize isolates. Sclerotial colour in all isolates except for RS 24 was yellowish brown to dark reddish brown whereas the rice isolate was dark yellowish brown.

Table 1: Effect of nine different vegetable-grains media on mycelial colour, growth pattern, number, color of sclerotia of *Rhizoctonia solani*

Media	Mycelia Colour	Mycelial Growth	Sclerotial Growth Pattern	Number of Sclerotia/plate	Appearance and Colour of Sclerotia
RDA	White	Moderate	Sub- Peripheral	4	Prominent and black with exudates over the surface
MDA	White	Moderate	Sub-Centralised	6	Appeared white with exudates with over the surface
BtDA,	White and light gold	Abundant	Peripheral and few centralised	32	Prominent and black with exudates over the surface
NDA	White and light gold	Abundant	Peripheral	34	Prominent black with exudates
PDA	White and light gold	Abundant	Peripheral	28	Prominent black with exudates
CDA	White and light gold	Slight	Peripheral and few centralised	33	Prominent and black with exudates over the surface
MPDA	White	Abundant	Scattered	41	Prominent black with exudates
BDA	White	Moderate	Peripheral	18	Prominent and black
BrDA	White	Abundant	Peripheral and few centralised	21	Prominent and black with exudates over the surface



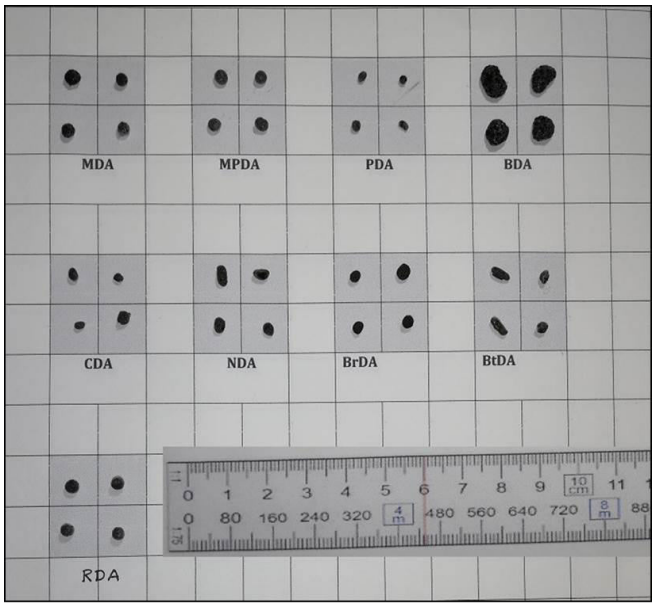
Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal Dextrose Agar (BrDA)

Fig. 2: Variability in mycelial colour, growth pattern of *Rhizoctonia solani* along with variation in colour and growth of sclerotia on nine different vegetable-grains culture media.

Table 2: Influence of different vegetable-grains media on shape and size of sclerotia of *Rhizoctonia solani*

Media	Length (mm)	Breath (mm)	Diameter (mm^2)	Shape
BDA	6.5±0.09	5.0±0.09	32.5±0.06	Oval
BrDA	3.0±0.01	2.25±0.08	6.75±0.018	Sphere
RDA	3.0±0.01	3.0±0.01	9.0±0.015	Sphere
CDA	2.25±0.08	1.75±0.07	3.94±0.33	Sphere
MDA	2.75±0.55	2.75±0.55	7.56±0.02	Round, Sphere
MPDA	3.75±0.06	3.0±0.01	11.25±0.07	Oval and Sphere
NDA	4.0±0.57	2.0±0.05	8.0±0.11	Ellipse

±- *SE; mm- mili meter; Different media used: Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal Dextrose Agar (BrDA).*



Rice Dextrose Agar (RDA), Maize Dextrose Agar (MDA), Beet Dextrose Agar (BtDA), Nutrient Dextrose Agar (NDA), Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA), Maize Potato Dextrose Agar (MPDA), Banana Dextrose Agar (BDA) and Brinjal Dextrose Agar (BrDA).

Fig. 3: Study of variation in size of sclerotia of *Rhizoctonia solani* on different composition of media.

4. CONCLUSION

The overall results of the present study will pave the way to search appropriate media for growth and sclerotia formation of *Rhizoctonia solani*. Maize Potato Dextrose Agar (MPDA) could be used as suitable vegetable-grains based media for production of maximum mycelial growth whereas Banana Dextrose Agar (BDA) could be utilized to induce production of large sized sclerotia.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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