Research Article
CODEN (USA): IJPLCP

ISSN: 0976-7126
Kumar et al., 13(6):11-14, 2022

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Evaluation of Culture Medium for the Growth and sporulation of *Rhizoctonia bataticola* Causing Dry Root Rot of Chickpea (*Cicer arietinum*)

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Article info

Received: 18/04/2022

Revised: 11/05/2022

Accepted: 18/06/2022

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Abstract

Rhizoctonia bataticola (Taub.) is the cause of dry root rot of chickpea. This Pycnidia phase is found in *Macrophomina phaseolina* (Tassi) Goidis. It is a fungal pathogen that lives in the soil. This research was conducted at Department of Plant Pathology, SVPUA&T Meerut. In this research, dry root rot affected samples were collected from Chickpea fields. Ten different culture media were used in this study for evaluation for the best growth of *Rhizoctonia bataticola.* The fungus *R. bataticola* gave maximum mycelial growth on potato dextrose agar (87.93 mm) followed by cornmeal agar 80.16 mm and oatmeal agar 75.26 mm, while potato dextrose Bengal agar showed the least growth on solidmedium at 30.21 mm. Of the ten liquid broths tested, potato dextroe broth had the highest mean mycelial dry weight (154.20 mg), followed by cornmeal broth (125.80 mg), oatmeal broth (123.30 mg), and V-8 juice broth (117.40 mg). . Potato dextrose (53.66 mg) gives the least mycelial dry mass in Bengal broth. Sclerotia production was noticed highest in PDA and lowest in SDA.

Key words: Chickpea, *Rhizoctonia bataticola*, Media, Growth

Introduction

Chickpea (*Cicer arietinum* L.) is a legume commonly known as "Chana or Bengal gram". Chickpea belongs to the legume family (Leguminaceae), which is cultivated in many countries. It has high nutritional value in Indian vegetarian diet as it contains 21.1 percent protein, 61.5 percent carbohydrates and 4.5 percent fat, 6 percent crude fiber and 6 percent soluble sugar. (Ahlawat *et al.*, 1996). The causal organism of dry root rot of chickpea is *Rhizoctonia bataticola.* It is a soil inhabiting fungi. It is a necrotrophic fungal pathogen which is seed and soil borne in nature (Sharma and Pande, 2013). t is one of the most important diseases that occurs mostly in dry conditions and dominates the seedling, flowering and balsa stages of the crop. It is widespread and infects both monocots and dicots in more than 284 plant species (Farr *et al.*, 1995). The growth of *R. bataticola* is enhanced by high soil temperature i.e. more than 30^0 C) and low soil moisture condition which favours its growth.

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Significant reduction of 25- 70 per cent in chickpea production due to this disease in India has been reported (Pande and Sharma, 2010). The viability and growth of *Rhizoctonia bataticola* is also affected by environmental conditions, such as rising temperatures due to global warming, low soil moisture due to low rainfall, and high soil pH, i.e. soil alkalinity (Khare *et al*., 1970).

Material and Method [4-7]

Isolation and purification of the pathogen

Chickpea roots with typical symptoms of dry root rot were isolated. These roots were washed with tap water to remove soil and other dirt. 4-5 mm pieces or root pieces were taken for isolation. During isolation, these pieces were sterilized with 1% sodium hypochlorite (NaOCl) solution, then washed twice in sterile distilled water, dry these pieces by placing them on sterile absorbent paper. Now these pieces were transferred to sterilized PDA medium which was poured earlier. with forceps and placed in a BOD incubator for incubation at 27 ± 10 C (Anurag and Ved, 2019). The incubation period of 4 days was characterized by the examination of fungal colonies on bicycles, with the underside of each mycelium growth marked and visualized using a light microscope.

Growth of *Rhizoctonia bataticola* on solid & liquid media

The growth of *R. bataticola* on different types of solid media such as corn flour agar, rose bengal agar, pikovskaya agar, sabouraud dextrose agar, potato dextrose bengal agar, V-8 juice agar, oatmeal agar and potato dextrose agar was compared using liquid and solid media separately. In a 250 ml conical flask, 100 ml of liquid medium was prepared, i.e. medium without agar, a pinch of streptomycin was added to each flask as an antibiotic. For solid medium, 100 ml of each medium was prepared and 15-20 ml of each medium was transferred to Petri dishes immediately before pouring, a drop of streptomycin was added to each flask as an antibiotic. The fungal inoculum was added to an Erlenmeyer flask by slightly stirring the filamentous mat using a cork borer taken from a seven-day culture. Plates were incubated at 27 ± 10°C for two days and repeated three times. Potato dextrose agar plates were used as control. Radial mycelial growth was measured five days after incubation. To inoculate *R. bataticola* into an Erlenmeyer flask, a mycelial plate (5 mm) was taken from a 5-day culture and inoculated separately into each 250 ml Erlenmeyer flask containing sterilized corn broth, rose bengal broth, pikovskaya broth, Sabouraud dextrose agar, potato dextrose rose Bengal broth, V-8 juice, oat agar, and potato dextrose broth and kept in a BOD incubator at (27 ± 1 °C) for ten days for incubation. After 10 days, the mycelial mat was separated from the remaining broth using Whatman No.1 filter paper, dried in the sun for 4 days and immediately weighed on an electronic scale (Sharma and Pande, 2013). A conical flask containing potato dextrose broth was used as a control. The mycelial dry weight of each broth was recorded.

Results and Discussion

Radial growth and mycelial dry weight of *R. bataticola* isolates on different solid and liquid media. There was a significant difference in radial growth between different media. The average radial mycelial growth of the isolates on different solid media was studied and it varied from 28.50 mm to 88.63 mm. Among the Ten solid media, potato dextrose agar medium was significantly superior and recorded the highes t mycelial growth of 87.93 mm and this was followed by corn meal agar (80.16 mm), oat meal agar (75.26 mm), V-8 juice agar (69.18 mm), rose bengal agar (62.50 mm), pikovskaya agar (54.10 mm) and sabouraud dextrose agar (48.65 mm), Carrot agar (35.28), Tomato juice agar (40.05) and potato dextrose rose bengal agar media (30.21 mm) (Table 1). Maximum growth in PDA may be due to presence of some additional nutrients in this media (Devi and Singh, 1998). A similar observation on the effect of different media on the growth of *R. bataticola* was made by (Sundravadana *et al.*, 2012). The same trend was observed in liquid medium, *R. bataticola* mycelial dry weight was highest in potato dextrose broth and was significantly better in all eight media tested, potato dextrose-rose broth gave the lowest mycelial dry weight of 53.66 mg (Suriachandra *et al.*, 2003) studied on growth different media and reported *R. bataticola* grew best on PDA, corn meal agar and oat meal agar. Sclerotia production was excellent on PDA.

Table.1 Growth of *R. bataticola* culture on different Solid and liquid media

S.No.	Media	Mycelial growth in mm	Mycelial dry weight (mg)	Sporulation	Colony Colour
1	Pikovskaya agar	54.10	94.60	++	Grey
2	Oat meal agar	75.26	123.30	+++	GreyishBlack
3	Sabouraud dextrose agar	48.65	88.50	+	Dark Grey
4	Potato dextrose agar	87.93	154.20	++++	DarkBlack
5	V-8 Juice agar	69.18	117.40	+++	Dark Gray
6	Rose Bengal agar	62.50	102.10	+++	Dark Gray
7	Corn meal agar	80.16	125.80	++++	GreyishBlack
8	Potato dextrose rose bengal agar	30.21	53.66	+	WhitishGrey
9	Carrot agar	35.28	65.10	++	Grey
10	Tomato juice agar	40.05	68.26	++	Balck
SEm±		0.61	0.78		
CDat 5%		1.10	1.21		

Average of three replication

++++High Sporulation, +++&++Medium Sporulation and +Low Sporulation

Dry mycelial weight was found to be maximum on day 14 of the incubation period (154.20 mg) and remained significantly better than the other nine treatments. Dry mycelial weight increased up to 14 days followed by weight loss. The decrease in mycelial mass is due to the autolysis of the mycelium and the lack of nutrients in the environment during incubation after the optimal number of days. The present studies confirmed the variability in mycelial growth and mycelial dry weight of *R. bataticola* cultured in various culture media.

Colony color and sporulation of *R. bataticola* were studied on different agar media (Table 1). The highest sporulation, or sclerotia production, occurred on potato dextrose agar and cornmeal agar, followed by oatmeal agar, V-8 juice agar, and rose bengal agar. Sabouraud dextrose agar and potato dextrose Bengal agar gave the least sporulation. The color of the colony was also different in PDA medium in different environments, its culture gives a dark black color, similarly in cornmeal agar medium it gives a dark gray black color and in potato dextrose agar, in bengal agar it gives a whitish gray color (Aboshosha *et al*., 2007; Fernandez *et al.*, 2006).

Conclusion

In the present investigation out of ten different solid media, Potato dextrose agar media was noticed to the best medium for highest mycelia growth and abundant sporulation of the *Rhizoctonia bataticola*, Followed by Corn meal agar medium (CMA), which was significantly at par with Oat meal agar medium. The color of the mycelia colony was also varied in different solid medium.

Acknowledgement

This research was supported by Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India.

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