The Pharma Innovation Journal 2022; 11(4): 1544-1548

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(4): 1544-1548

www.thepharmajournal.com
Received: 13-01-2022
Accepted: 26-03-2022

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Effect of different media, pH and temperature on the growth of *Rhizoctonia solani causing web blight of urd bean* under *in vitro* Conditions

Babli, SP Tiwari and Rani Chodari

Abstract
Rhizoctonia solani is a multiparous widely distributed plant pathogen. Web blight caused by *Rhizoctonia solani* causes huge yield loses in urdbean (*Vigna mungo*). All the commercially grown varieties are susceptible. Being a typical soil borne fungus,. It was carried out to know the efficacy of different among the four tested media for colony growth of *R. solani* from urd bean, Richard's Agar medium was found most suitable and supported the growth of *R. solani*. However, Czapek's Dox Agar medium least supported the growth of *R. solani*. Further, it was observed that *R. solani* showed maximum radial growth at 25 ^{0}C. The maximum radial growth of *R. solani* was recorded a pH 7.0 after different incubation period. Further, it was observed that neutral pH 7.0 and slightly alkaline pH favours the colony growth of *R. solani* in comparision to acidic pH. However, neutral pH 7.0 found most suitable for growth of *R. solani*.

Keywords: *Rhizoctonia solani,* cultural and morphological characters, mycelial growth

Introduction
Urd bean (*Vigna mungo* (L.) Hepper) is an important pulse crop of India. This crop is a major source of dietary proteins, minerals and vitamins for the vegetarian population of India. It is also rich in phosphoric acid. Urd bean is also cultivated as a mixed crop with finger millet or barnyard millet in the hills of Uttaranchal during the *kharif* season. In North India, it is grown in *kharif* and summer season. In India, urd bean is cultivated in 2.89 million ha area with production of 1.28 million tones and productivity of 440 kg/ha (Anonymous, 2003). It is cultivated in Kharif, spring and summer seasons in India and covers 3.77 million hectares area and produces 1.52 million tonnes. (Purushottam and Singh, 2015) [11]. Despite being an important pulse crop its productivity has been quite low probably due to various biotic and abiotic constraints. Urdbean is vulnerable to a variety of diseases *viz.*, anthracnose (*Glomerella lindemuthianum*), dry root rot (*Macrophomina phaseolina*), leaf spot (*Cercospora canescens*), powdery mildew (*Erysiphe polygoni*), rust (*Uromyces phaseoli*), web blight (*Rhizoctonia solani*), Mosaic and leaf crinkle (Bara, 2007) [1]. Among the biotic constraints, web blight disease of urdbean caused by *Rhizoctonia solani* Kuhn [Teleomorph: *Thanatephorous cucumeris* (Frank) Donk] is considered as an important constraint accountable for losses in production as well as productivity in India up to 20-30% (Kumar *et al.* 2018) [7]. The disease had been reported in other countries like Pakistan, Sri Lanka, West Indies, Japan, Philippines, Myanmar, North America, South America, Argentina, Brazil, and Mexico too beside India. The disease has been reported from various urdbean growing areas of India including; Punjab, Haryana, Bihar, Rajasthan, Uttarakhand, Madhya Pradesh, Uttar Pradesh, West Bengal, Himachal Pradesh and Jammu and Kashmir (Shailbala and Tripathi, 2007) [15]. The disease appears about 21-25 days after sowing depending on cultivars, environmental conditions, crop stages and cultivation practices (Dubey and Patel, 2001; Shailbala and Tripathi, 2007) [15]. Seed quality and grain yield are heavily affected in this disease. Web blight of urd bean is a seed and soil borne disease (Saksena and Dwivedi, 1973; Dwivedi and Saksena, 1974) [16] and managed by chemical seed treatment (Dubey and Dwivedi, 1988) [3]. The chemicals not only disturb the ecology of soil but also develop hazardous impacts on surroundings including *Rhizobium* spp. Biological seed treatment with fungal antagonist has significant promise against such devastating pathogens (Mukhopadhyay, 1994) [9] but suitable methods of seed treatment and optimum doses are ingredients for successful management. The first report of occurrence of web blight on urdbean caused by *Rhizoctonia solani* Kuhn [Teleomorph:

Thanatephorous cucumeris (Frank) Donk] in India was reported by Saksena and Dwivedi in 1973 [16]. This disease is known to occur in other leguminous crops like mungbean (Dwivedi and Saksena, 1975) [5], pigeonpea (Dwivedi and Saksena, 1975) [5], cowpea (Lakshman *et al.* 1979) [8], soybean (Verma and Thapliyal, 1976) [17], groundnut (Dwivedi and Dubey, 1986) [4] and rice bean (Jalali, 1989) [6].

Material and Methods

The experiment was laid out in a complete randomized design (CRD) Cultural characters of *R. solani* were studied on 20 ml sterilized Potato Dextrose Agar medium. Five mm mycelium discs of *R. solani* isolates were transferred aseptically at the center of each Petri plate in three replications and incubated at 24±2ºC for 15 days. Colony characters were recorded as follows: a) growth of the fungus, b) ability to produce sclerotia, c) appearance:- submerged/cottony/fluffy d) formation of sclerotia:- scattered/smooth/clumped; shape of sclerotia.

Cultural characteristics and Radial growth

Cultural characteristics of *Rhizoconia solani* was studied on four culture media namely Potato Dextrose Agar (PDA), Czapek's Dox Agar (CZDA), Richard's medium and Corn Meal Agar (CMA)

Effect of temperature on growth of *R. solani* isolates

Effect of four different temperatures *viz.* 15 °C, 20 °C, 25 °C and 30 °C, was studied on growth of *Rhizoctonia solani* on potato dextrose agar. Mycelial discs of 5mm diameter were cut from the edge of a 3 days old culture of five isolates grown at 25 ºC and were transferred to the center of the 90mm Petri dish and incubated at different temperatures. Each treatment was replicated three times in a completely randomized design. The average diameter of the fungal colony was recorded at 24hrs, 48hrs, 72hrs, 96hrs and 120hrs after incubation.

Effect of pH on growth of *R. solani* isolates

Effect of six different pH *viz.* 4, 5,6,7,8 and 9 was studied on growth of *Rhizoctonia solani* on potato dextrose agar. The pH of the medium was adjusted to desired level by using N/10HCl or N/10NaOH.Mycelial discs of 5mm diameter were cut from the edge of 3 days old culture of five isolates grown at 25 ºC and were transferred to the center of 90mm Petri dish and incubated at different temperatures. Each treatment was replicated three times in a completely randomized design. The average diameter of the fungal colony was recorded at 24hrs, 48hrs, 72hrs, 96hrs and 120hrs after incubation.

Results

Cultural Characterization Isolated pathogen *R. solani* causing web blight of urd bean was subjected to cultural characterization based on colony morphology, type, radial growth on different media, pH and temperature.

Colony morphology and radial growth

Five days old culture of *R. solani* on potato dextrose agar medium produced light brown colouredhyphal growth. Microscopic study clearly indicated constriction at the point of branching and right angle branching in matured hyphae. The isolate shared typical characteristics of *R. solani* like a) branching at right angle near the distal septum of the cell in young vegetative hyphae, b) formation of a septum in the branch near the point of origin, c) constriction of the branch at origin d) undifferentiated sclerotia and g) absence of rhizomorphs. Sclerotia were produced after 10 days of growth of culture. The sclerotia were irregular in shape and brown to black in colour. Ten days old culture of *R. solani* showing sclerotia production has been shown in Plate Colony radial growth was recorded at regular interval on PDA medium and it was observed that after 48 hours of incubation period 23.50 mm radial growth was attained by the culture. However, complete petri plate growth was recorded after 7 days of incubation period. Production of sclerotia started after prolonged incubation of the culture and it started after 10 days of incubation. Thereafter, massive irregular shaped, brown to black colouredsclerotia were produced. Colony radial growth of *R. solani* after different incubation periods has been shown in table.

Cultural characteristics of *Rhizoctonia solani* on different media

All media evaluated against *R. solani* were able to cover the full petri plate growth on the 6th day after incubation, but the rate of growth varied determining the preference for a substrate for growth. It was observed that after 48hrs of incubation period maximum radial growth of 26.16 mm on Richard's Agar medium. However, minimum radial growth of 17.6 mm was recorded on Czapek's Dox agar medium. The colony radial growth increases with increased incubation period until full petri plate growth. After 144hrs of incubation period maximum radial growth of 90.00 mm was recorded on Richard's Agar medium. However, Czapek's Dox Agar medium least supported the growth of *R. solani* from urd bean and minimum radial growth of 71.83 mm (table 5). The colony morphology of *R. solani* on different media did not show any significant variation and light brown-submerged mycelial growth was recorded on all the four media. The graphical representation of colony radial growth of *R. solani* from urd bean after different incubation periods on different media is given in fig.1. Pictorial representation of different isolates of *R. solani* from urd bean on different media is given

Effect of temperature on growth of *R. solani*

The *R. solani* colony radial growth was maximum at 25 C. However, at 30 C colony radial growth was better than at 15 C and 20 C. The maximum colony radial growth of 24.50 mm was recorded at 25 C after 48hrs of incubation period. Similar pattern of radial growth was observed after a prolonged incubation period of 72hrs, 96hrs, 120hrs and 144hrs. Maximum radial growth of 90.00 mm was recorded at 25 after 144hrs of incubation period. (table.6, plate.3).

Effect of pH on growth of *R. solani*

It was observed that *R. solani* showed differential behaviour at different pH. However, maximum radial growth of *R. solani* was recorded at a pH 7.0 after different incubation periods. Further, it was observed that neutral pH 7.0 and slightly alkaline pH favours the colony growth of *R. solani* in comparison to acidic pH. Maximum colony radial growth of 25.33 mm was recorded at 7.0 pH after 48 hrs of incubation period. Slightly alkaline conditions of the medium favoured better for colony radial growth of *R. solani* in comparison to acidic conditions and it was observed that colony radial growth of 22.00 mm was recorded at pH 8.0 after 48 hrs of

incubation period. Minimum colony radial growth of 6.66 mm was recorded at pH 4.0 after 48 hrs of incubation period. Maximum radial growth of 89.83 mm was recorded at pH 7.0 after 144 hrs of incubation period. In this way neutral pH 7.0 found most suitable for growth of *R. solani*. Detailed data for *R. solani* after different incubation periods at six different pH are presented in table 7, plate 4 and figure 3

Table 1: Effect of different media on mycelial growth of *Rhizoctonia solani* after different incubation period

Treatment		Medium			Colony Radial growth (mm) after*	
48(Hrs.)	72(Hrs.)	96(Hrs)		120(Hrs.)	144(Hrs.)	
T1	PDA	23.50	34.16	49.50	70.16	89.00
T2	Czapek's Dox Agar	17.16	28.50	38.50	59.50	71.83
T3	Richard's Agar	26.16	37.83	54.50	75.83	90.00
T4	Corn meal Agar	19.50	31.50	43.33	66.50	87.58
S.Em±	A= 0.241, B= 0.269, A x B= 0.539					
CD (5%)	A= 0.691, B= 0.773, A x B= 1.546					

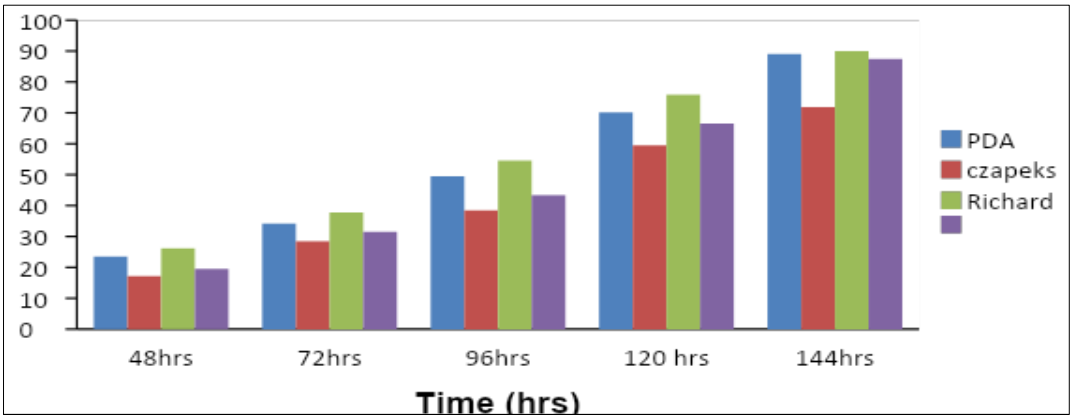

Fig 1: Graphical representation of effect of different media on mycelial growth of *Rhizoctonia solani* after different incubation period

Table 2: Effect of different temperatures on mycelial growth of *Rhizoctonia solani* after different incubation periods Treatment

Treatment		Temperature			Colony Radial growth (mm) after*	
48(Hrs.)	72(Hrs.)	96(Hrs)	120(Hrs.)		144(Hrs.)	
T1	150C	7.16	16.66	27.50	35.00	54.33
T2	200C	9.50	22.16	33.50	46.33	71.50
T3	250C	24.50	41.16	61.00	79.16	90.00
T4	300C	22.50	35.33	57.50	76.50	88.16
S.Em±	A= 0.263, B= 0.294, A x B= 0.588					
CD (5%)	A= 0.754, B= 0.844, A x B= 1.687					

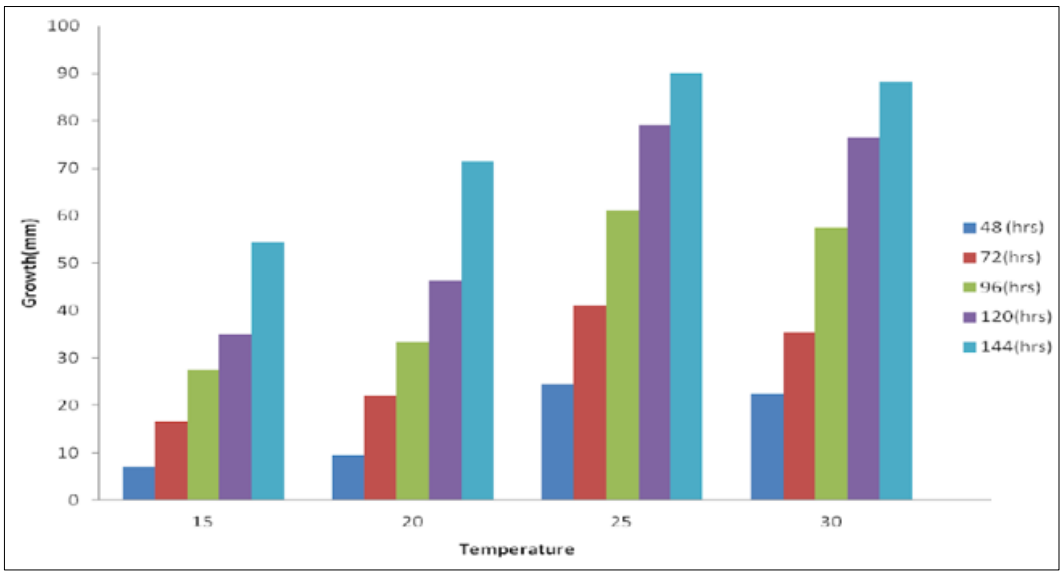

Fig 2: Graphical representation of effect of different temperatures on mycelial growth of *Rhizoctonia solani* after different incubation periods

Table 3: Effect of different pH on mycelial growth of *Rhizoctonia solani* after different incubation periods

Treatment		pH			Colony Radial growth (mm) after*	
48(Hrs.)		72(Hrs.)	96(Hrs)		120(Hrs.)	144(Hrs.)
T1	4.0	6.66	17.16	25.50	37.53	55.50
T2	5.0	11.00	23.16	41.83	54.66	75.50
T3	6.0	15.83	31.50	52.33	65.83	80.50
T4	7.0	25.33	39.50	67.50	82.83	89.83
T5	8.0	22.00	43.66	56.50	70.50	88.50
T6	9.0	17.50	37.83	37.83	57.33	85.33
S.Em±		A= 0.240, B= 0.219, A x B= 0.536				
CD (5%)		A= 0.680, B= 0.621, A x B= 1.521				

*Each value is a mean of three replications.
A = Treatment
B = Replication
C = Interaction (Treatment x Replication)

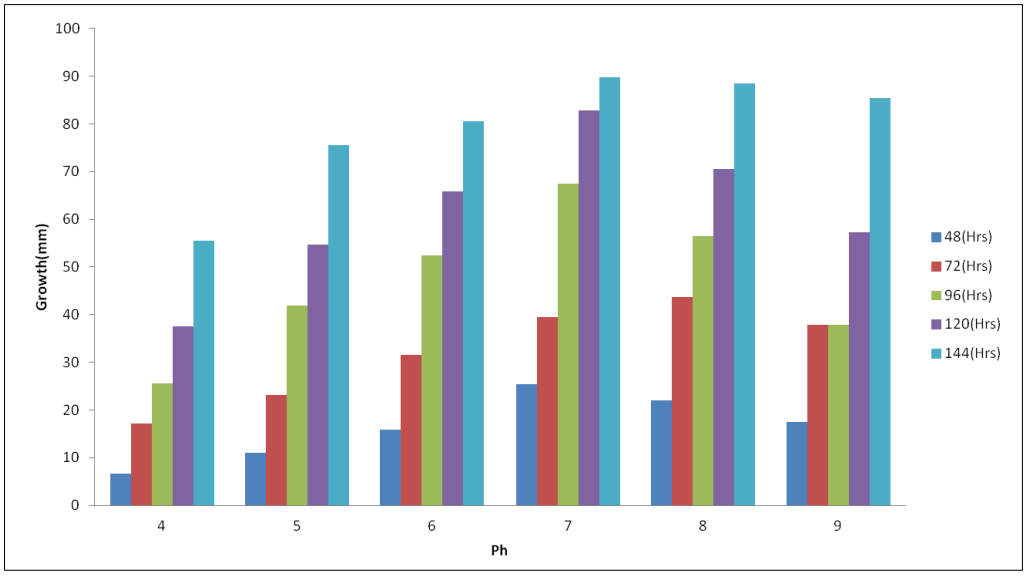

Fig 3: Graphical representation of effect of different pH on mycelial growth of *Rhizoctonia solani* after different incubation periods

Discussion and Conclusion

Among the four tested media for colony growth of *R. solani* from urd bean, Richard's Agar medium was found most suitable and supported the growth of *R. solani*. However, Czapek's Dox Agar medium least supported the growth of *R. solani* and minimum radial growth of 71.83 mm was recorded. To identify the optimum temperature for growth of R. solani, four different temperatures were tested for its colony growth under in vitro conditions and it was observed that *R. solani* showed maximum radial growth at 25 C. However, it was observed that *R. solani* was not able to grow well at low temperatures (15 C and 20 C). Further, at 30 C colony radial growth was better than at 15 C and 20 C. To identify the suitable pH for maximum growth of *R. solani*, it was grown on six different pH (4, 5, 6, 7, 8 and 9) under laboratory conditions on potato dextrose agar medium and colony radial growth was recorded. The maximum radial growth of *R. solani* was recorded at a pH 7.0 after different incubation periods. Further, it was observed that neutral pH 7.0 and slightly alkaline pH favours the colony growth of *R. solani* in comparison to acidic pH and maximum colony radial growth of 89.83 mm was recorded at pH 7.0 after 144 hrs of incubation period. However, minimum radial growth of 55.50 mm was recorded at pH 4.0 after 144 hrs of incubation period. In this way neutral pH 7.0 found most suitable for growth of *R. solani*. The results with respect to morphological, cultural and sclerotial characters of *R. solani* observed in the present investigations have also been recorded and described by several workers (Singh *et al.*, 2002; Srinivas, 2002 [12], Sharma *et al.*, 2004 and Akhtar *et al.*, 2009; Dubey *et al.*, 2011; 2012) in urd bean and other crops where findings are matching with the present investigation. Richard's Agar medium was found most suitable and supported the growth of *R. solani*. *R. solani* showed maximum radial growth at 250C temperature and pH 7.0.

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