International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2023; 11(1): 149-155

Received: 09-12-2022
Accepted: 11-01-2023

Pavan D Bansod
M.Sc. Agriculture, Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Manisha J Gawai
M.Sc. Agriculture, Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Vitthal S Bodkhe
M.Sc. Agriculture, Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Corresponding Author:
Pavan D Bansod
M.Sc. Agriculture, Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Survey for incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha

Pavan D Bansod, Manisha J Gawai and Vitthal S Bodkhe

Abstract
A sclerotial fungus *Rhizoctonia bataticola* (Taub.) Butler a pycnidial stage known as *Macrophomina phaseolina* (Tassi) Goid. The fungus causes complex disease syndromes like charcoal root, root rot, seedling blight, foliage blight, tuber decay, dry rot, fruit rot, pod and seed rot in several economically important crops. Yield losses up to 77 per cent reported in soy bean due to *Rhizoctonia bataticola*. In the present investigation, various factors were studied on root rot of soybean caused by *Rhizoctonia bataticola*. Rapid roving survey in the major soybean growing areas of Vidarbha revealed the prevalence of *Rhizoctonia* root rot at all the locations where disease incidence ranged from 2.33 to 39.33 per cent. Thirteen different isolates of *Rhizoctonia bataticola* were isolated from different regions of the Vidarbha. Pathogenic abilities of different isolates of *R. Bataticola* on soybean cultivar TAMS-38 a susceptible variety, confirmed the isolates of *Rhizoctonia bataticola* to be pathogenic. The isolates of *R. Bataticola* were tentatively divided in five groups based on their pathogenic reaction as highly, strongly, moderately and weakly pathogenic and nonpathogenic. Potato Dextrose Agar media were the best media for the growth of *R. Bataticola*

Keywords: Root rot, *Rhizoctonia bataticola*, Vidharbha, Potato dextrose agar, C'zapek's Dox agar, Sabouraud's Agar, peptone agar media, sick soil, Pathogenic

Introduction
Rhizoctonia bataicola (Taub.) Butler (1925) = (*Sclerotium bataticola* Taub. (1913) (Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid. (1947) is a divers omnipresent ubiquitous soil-borne fungal pathogen, infecting more than 500 plant species. The pathogen causes different types of diseases viz. seed rot, seedling blight, root rot, charcoal rot, wilt, stalk rot, stem blight, fruit rot, seedling decay and leaf blight in crop plants. (Dhingra and Sinclair, 1978) [3] fungus produces either microsclerotia (primary source of inoculums) or pycnidial and infects plants from seedling to maturity. The disease is difficult to manage as pycnideospore and Sclerotia can survive in 2-15 years even in the absence of the host plant (Young *et al*., 1983; Baird *et al*., 2003). Soybean (*Glysine max* (L.) Merill) is the major oilseed crop in the world accounting for nearly 50% of total oilseed acreage as well production. It is unique crop of versatile nutritional attribute yielding both oil and protein. Soybean is called "Golden bean" of 21st century. Soybean rank second in vegetable oil production of India after groundnut. Over the past decade, productivity trend of soybean indicate that yields achieve are not attained due to profound adverse effect of biotic and abiotic factors. Soybean suffers from many diseases while root rot caused by *Rhizoctonia bataticola* is the most widespread disease of soybean.

Soybean cultivation has taken a big stride in the country during the past few years. The area under soybean is merely spread in latitudinal belt of about 15 to 25 ˚N comprising the state of Madhya Pradesh, Maharashtra, Chhattisgarh, Andhra Pradesh and Karnataka. These states together contribute to about 98% of the total soybean production in the country. The area under soybean in India during Kharif (monsoon) 2018 was 108.39 lakh hector with production of 114.83MT with average yield 1159 kg/ha. In Maharashtra, area sown under soybean is 36.39 lakh ha during Kharif (Mansoon) 2018. The estimated yield per ha and total production of soybean was 1054 kg/ha and 38.35 lakh MT, respectively (Soybean crop survey, SOPA, 2018).

Disease is more prevalent in some years. Although several measures can be taken to manage root rot pathogen under field condition efficient measures are not available. Host plant resistance has been considered as the only practicable way to manage this disease.

Crop rotation, post-harvest removal of infected plant debris, manipulation of planting dates, adequate and timely fertigation, planting density and crop protection are the measures to manage the disease. Bioagent and fungicide application to seed and soil has been practiced in some cases, it is neither economical nor environmentally safe (Abavi and Pastoe-Carroles, 1990). Host plant resistance is the most economical and practical alternative for efficient management of root rot.

Material and Methods

Survey for incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha

An rapid roving field survey was conducted to know the incidence of *Rhizoctonia* root rot in major soybean growing area of Vidarbha, Maharashtra state viz. Amravati, Akola, Buldana, Washim, Nagpur, Wardha, Yavatmal during Kharif, 2018. In Amravati, the survey was conducted at Daryapur, Chandur bazaar, Amravati taluka. In Akola, survey was conducted in Akola, Murtizapur and Balapur taluka. From Buldana district, place like Shegaon, Chikhali taluka were selected. From Washim, mangrulpir, Malegaon and Washim talukas were selected for the survey. In Nagpur, survey was conducted in Hingna taluka whereas in Wardha district survey was conducted in Wardha and Seloo taluka and from Yavatmal district, root rot samples were collected from Pusad and Mahagaon taluka.

Root rot infected samples were collected from the fields of the above different places. Minimum samples were taken from one location. Each sample was taken in paper bag and labelled. The collected samples were further analyzed and used for isolation and detection of root rot pathogen.

Determination of *Rhizoctonia* root rot disease incidence

The diseased plants associated with root rot infecting fungi causing root rot was noted for the determination of disease incidence. In a field, one meter area mark at place randomly, and then number of diseased and total number of plants counted. Two fields selected from each village and the per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Collection of disease samples

Rhizoctonia root rot infected plant samples of soybean were collected from major soybean growing area of Vidarbha region of Maharashtra state during Kharif, 2018. (Table 1).

Surface sterilization of plant parts

Plant materials were surface sterilized using 0.1 per cent mercuric chloride solution for 30 second and then washing with sterile water thrice.

Culture media

Following media was used during laboratory studies

Potato Dextrose Agar (PDA) Potato (peeled and sliced)-200g, Dextrose-20g, Agar-20g, Distilled water-1000ml.

Isolation of plant pathogenic *Rhizoctonia bataticola* from diseased plant parts

For the purpose of isolation and obtaining pure culture of the pathogen, standard tissue isolation technique was followed. Disease root and stem portions were cut into small bits and washed well in tap water. These bits surface were sterilized with1:1000 sodium hypochloride solution for 30 second. Bits were washed thoroughly in sterilized distilled water three times to remove traces of sodium hypochloride and then aseptically transferred to sterile Petri plates containing potato dextrose agar media. The plates were incubated at room temperature (27 ± 2^0C) and as soon the emergence of mycelia from the infected portion, the hyphal tips were transferred to other plates for obtaining pure culture and isolates obtained were designated as R-1 to R-13. Purkayastha *et al*., (2006) [6] and Shekhar *et al*., (2006) [8].

Purification of fungal pathogenic cultures

Hyphal tip isolation

This method was used for maintaining pure culture of *R. Bataticola*. Hyphal tip isolation was done on water agar plates. Dilute mycelia suspension was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess was aseptically drained. Single mycelia bits were then marked under the microscopic field with ink on the glass surface of the plates and it was allowed to grow. Such plates were incubated at (27 ± 2^0C) and hyphae coming from each end cell of mycelia bit was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants under aseptic condition and incubated at temperature of (27 ± 2^0C) for 10 days.

Identification of pathogen

The identification of *Rhizoctonia bataticola* isolates was done on the basis of morphological characters described by Aghakhani and Dubey.

Maintenance of fungal culture

The receptive fungal pathogen cultures were maintained on PDA at room temperature by adopting subsequent sub culturing at periodically, regular intervals. Seven day old culture used for further studies.

Precaution to eliminate contamination

All isolation and inoculation work of microbial culture was carried out in laminar flow. The laminar air flow was sterilized by glowing ultraviolet lamp half an hour before use.

Effect of different solid media on radial mycelial growth of *R. Bataticola* isolates

Four culture media were tested to understand cultural behaviour of thirteen isolates of *Rhizoctonia bataticola* and to identify the best growth supporting medium. The results are presented in Table 3 (Fig 5) Significance differences were

observed in respect different media among thirteen isolates of *R. Bataticola*.

Mass multiplication

Thirteen isolates of *Rhizoctonia bataticola* purified by hyphal tip method were multiplied by separately on sorghum grain medium in laboratory. Sorghum grains 200g + water 50ml was filled in 500ml conical flask and were autoclaved at 1.05 kg/cm2 for 15 minutes. The flasks were allowed to cool and inoculated with pure culture. The inoculated flask were inoculated at room temperature (27 ± 2^0C) for two weeks and shaken at every alternate day during incubation period. Sufficient quantity of inoculum was prepared and mixed in pots containing sterilized soil.

Preparation of sick soil for pots

The field soil + FYM (1:1) were sterilized for seven days by using formaldehyde chemical. Incubated flasks with full growth of fungus were then added in sterilized soil in 1:9 proportions (inoculum +soil). The plastic pots 12 cm diameter were filled the above mixture. The pots were watered and inoculated for 7 days to multiply pathogen in soil.

Pathogenicity test

The seeds of susceptible soybean cultivar TAMS-38 were used for studying the pathogenicity and infective capacity of isolates. The seed were sterilized with 0.1 per cent mercuric chloride for 1 minutes followed by three subsequent washing with sterile water to remove the traces of mercuric chloride. In each pot, 10 seeds were sown. One set of pots of sterilized soil, without inoculum was kept as control. The pots were watered as and when required and observations on the occurrence of root rot were recorded. On the basis of occurrence and symptoms, the isolates were identified as pathogenic. After proving the pathogenicity, re-isolation was made and compared with original culture.

Grouping of isolates

Thirteen isolates of *Rhizoctonia bataticola* were tested by sick soil method for their virulence against susceptible variety TAMS- 38. The per cent root rot was recorded on the basis of healthy and root rot infected plants. The isolates of *Rhizoctonia bataticola* were tentatively divided into five groups on the basis of virulence by reference Table 6. (Pawar, 2010).

Result and Discussion

Survey for incidence of *Rhizoctonia* root rot in major soybean growing area Vidarbha

In Amaravati district, survey was conducted in 3 village's viz. Kurha, Virulpurna, kathora of Chandurbazar taluka and some area of Amaravati. In Amaravati, maximum disease was observed in Virulpurna village (21.26%) followed by Kurha (13.67%) and least incidence of 9.31% was in Arjun nagar, Amaravati. In Akola district, highest disease incidence was observed in Vyala village (39.33%) of Balapur Taluka followed by Washimba (8.68%). In Washim district, survey was conducted in Mangrulpir Taluka, maximum disease observed in Bhapur village (10.40%) followed by Godhani (7.33%). Root rot incidence of 4.36 per cent and 7.68 per cent recorded in Nagpur and in Wardha district, disease incidence was observed 2.33 per cent and 18.26 per cent in Seloo Taluka. Disease incidence in Buldana and Yavatmal recorded 8.07 and 14.34 per cent, respectively.

The result of the present are in accordance to Sangeetha and Jahagirdar (2013) [7] who did roving survey during Kharif 2010 in major soybean growing areas of northern Karnataka to assess the distribution and incidence of root rot and reported that per cent disease incidence was range in 3.36 to 36.30 from different locations. The chance of maximum disease incidence depends upon favourable conditions, susceptible host and optimum inoculum in soil. The disease is favoured by drought and high soil temperature (Chand and Khirbat, 2009).

Symptoms of *Rhizoctonia* root rot

The infection started at the collar region of plants as water soaked areas and decaying of the root system takes place. The infection was found to spread to the roots of the plant and caused decay, which ultimately toppled and collapsed. These infected plants could be easily pulled out from the soil and exhibited brown discoloration of roots followed by rotting of roots. In addition, the extensive sloughing off of affected bark and shredding of roots (Fig 1) was also observed. In advanced stage, the aerial portion of the plants decayed completely (Smith and Cane 1997; Mengistu *et al*., 2011; Khan *et al*., 2012) [4].

Isolation

The standard tissue isolation technique was followed to obtain *Rhizoctonia* culture from infected plant parts showing root rot symptoms. Potato dextrose agar was used as basal medium for isolation of the fungus. Thus thirteen different isolates of the fungus were isolated from soybean roots. Isolation of *Rhizoctonia bataticola* from infected samples of different host plants was also reported by Sinclair and Shurlleff (1975) [9], Su, *et al*., (2001), Purkayastha *et al*., (2006) [6] and Shekhar *et al*., (2006) [8], Isolation and designation details of *Rhizoctonia bataticola* isolates collected from different locations are furnished in Table 2.

Effect of different solid media on radial mycelial growth of *R. Bataticola* isolates

Four culture media were tested to understand cultural behaviour of thirteen isolates of *Rhizoctonia bataticola* and to identify the best growth supporting medium. The results are presented in Table 3 (Fig 5) Significance differences were observed in respect different media among thirteen isolates of *R. Bataticola*. Among media, Potato dextrose agar media found to support maximum growth mean (75.75 mm) followed by C'zapek's Dox agar (71.57 mm) while minimum growth was noted in peptone agar media.

Pathogenicity

The pathogenicity test of 13 isolates of *Rhizoctonia bataticola* isolated from the different parts of the Vidarbha region was tested by using cultivar TSMS 38. The results are presented in Table 4 and Fig 6.The result indicates that amongst all the thirteen isolates of *Rhizoctonia bataticola* all proved to be pathogenic to cultivar TAMS 38. On the basis of per cent

mortality these thirteen isolates were grouped as highly pathogenic (1), strongly pathogenic (2), moderately pathogenic (7) and weakly pathogenic (3).

Grouping of *Rhizoctonia bataticola* isolates based on their pathogenic ability

The data based on pathogenic variability in different isolates of *Rhizoctonia bataticola* are given in Table 5. The isolates of *Rhizoctonia bataticola* were tentatively divided in five groups based on their pathogenic ability on cultivar TAMS 38 (Fig 6).

Fig 1: Symptoms of *Rhizoctonia* root rot

Fig 2: Survey of Vidarbha region of Maharashtra

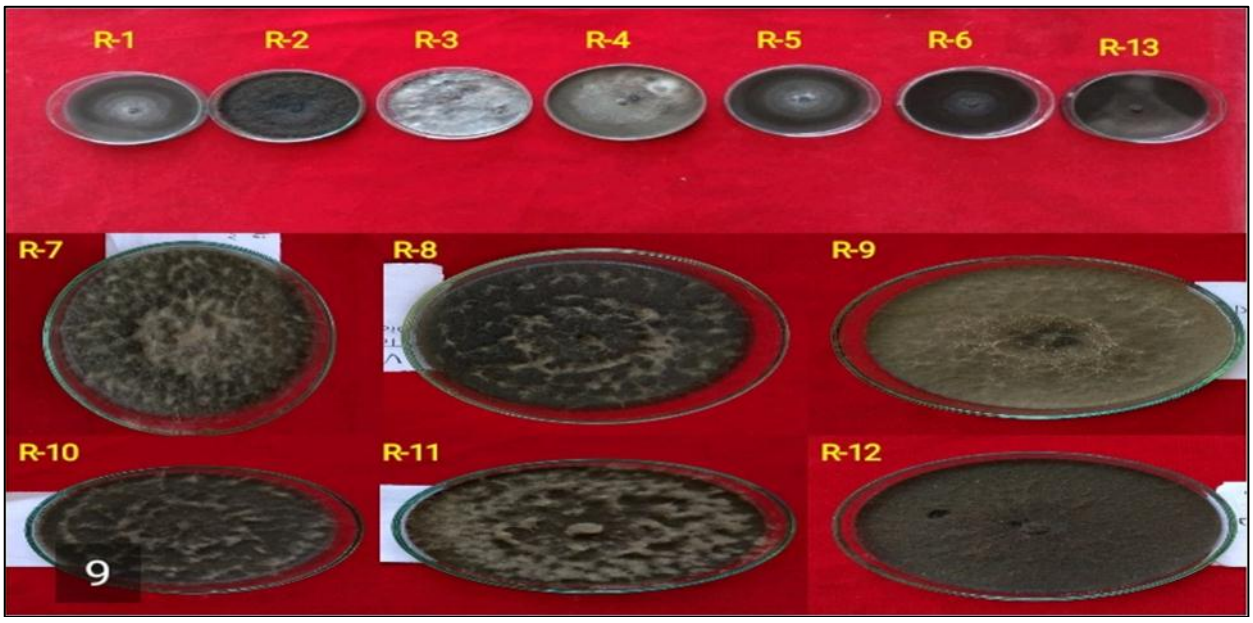

Fig 3: Isolates of *R. Bataticola* collected from Vidarbha region

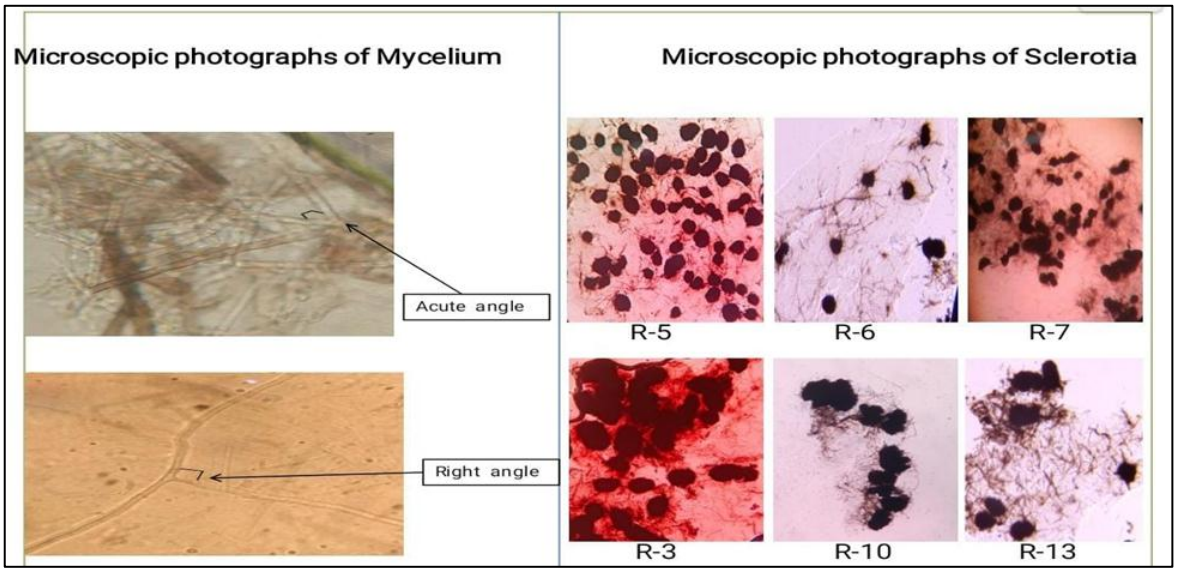

Fig 4: Microscopic view

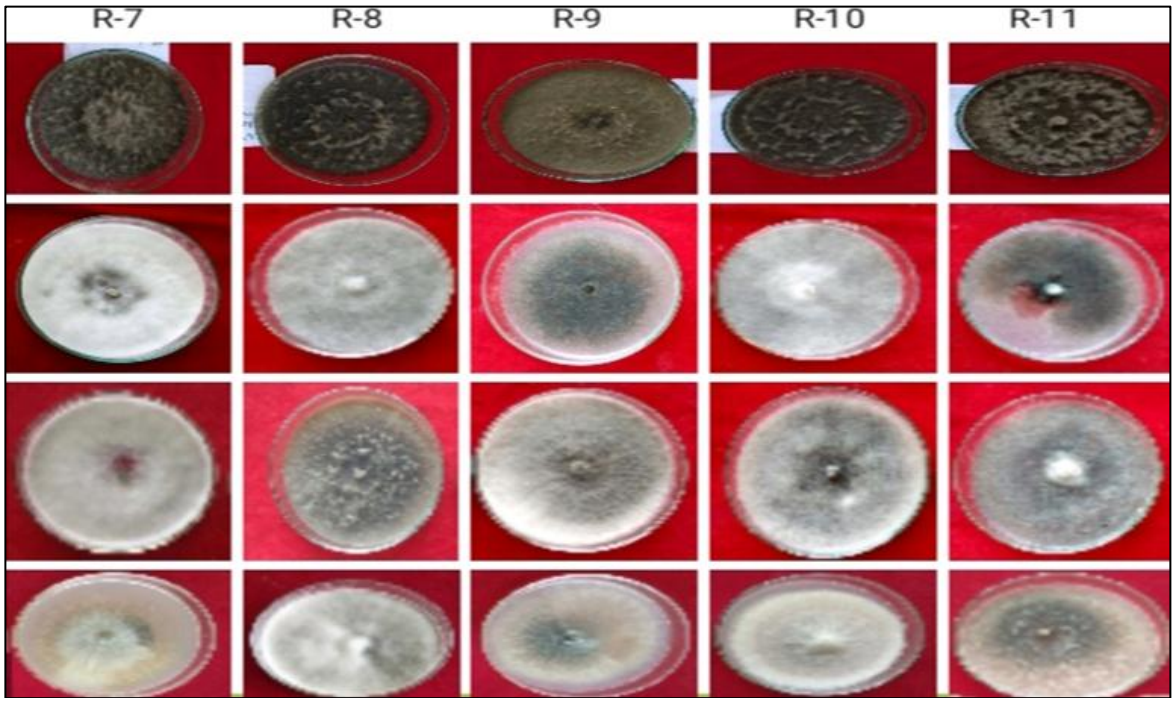

Fig 5: Growth of *R. Bataticola* on different media

Fig 6: Pathogenicity test of *R. Bataticola* against TAMS 38

Table 1: Survey for incidence of *Rhizoctonia* root rot in Vidarbha region

State	District	Taluka	Village	Variety	Per cent of incidence of Rhizoctonia root rot
Maharashtra	Washim	Mangrulpir	Godhani	JS 335	7.33
		Mangrulpir	Bhapur	JS 335	10.40
	Nagpur	Hingna	Hingna	JS 335	4.36
		Hingna	Waddhamana	JS 335	7.68
	Buldhana	Chikhali	Girola	JS 335	8.07
	Yavatmal	Mahagaon	Bori Izara	JS 335	14.34
	Wardha	Shelu	Hamdapur	JS 335	2.33
		Shelu	Dahegaon	JS 335	18.26
	Amravati	Chandurbazar	Kurha	JS 335	13.67
		Chandurbazar	Virul Purna	JS 335	21.26
		Amravati	Amravati (RRC)	JS 335	9.31
	Akola	Balapur	Vyalla	JS 335	39.33
		Akola	Washimba	JS 335	8.66

Table 2: *Rhizoctonia* root rot samples collected from major soybean growing area of Vidarbha, Maharashtra

State	District	Taluka	Village	Isolated designation
Maharashtra	Washim	Mangrulpir	Godhani	R-1
		Mangrulpir	Bhapur	R-13
	Nagpur	Hingna	Hingna	R-2
		Hingna	Waddhamana	R-12
	Buldhana	Chikhali	Girola	R-3
	Yavatmal	Mahagaon	Bori Izara	R-4
	Wardha	Shelu	Hamdapur	R-5
		Shelu	Dahegaon	R-6
	Amravati	Chandurbazar	Kurha	R-7
		Chandurbazar	Virul Purna	R-8
		Amravati	Amravati (RRC)	R-9
	Akola	Balapur	Vyalla	R-10
		Akola	Washimba	R-11

Table 3: Radial growth of *R. Bataticola* on different media

Sr. No.	Media	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10	R-11	R-12	R-13	Mean
1.	Potato Dextrose Agar (mm)	83.1	80.4	75.1	61.0	88.6	73.9	90.0	90.0	81.4	81.0	81.0	90.0	90.0	75.75
2.	C'zapek's Dox Agar (mm)	77.3	73.9	65.6	63.9	80.1	78.3	68.0	76.5	66.4	75.6	68.8	62.4	73.1	71.57
3.	Sabouraud's Agar (mm)	63.0	60.6	59.2	60.8	63.9	64.8	65.5	69.7	72.2	60.8	58.5	71.3	69.7	64.66
4.	Peptone Agar (mm)	54.8	54.0	69.7	48.3	57.0	60.8	54.7	80.1	60.0	66.4	53.2	56.2	60.0	59.65

Table 4: Pathogenicity test of *Rhizoctonia bataticola* against variety TAMS 38

Sr.No	Isolates	Total no. of seeds sown	Total no. of seeds germinated	No. of plants infected	Days to initiate symptoms	Percet mortaly due to pathogen (45 DAS)	Place
1	R-1	10	6	2	41	33.33	Godhani
2	R-2	10	7	3	45	42.85	Hingna
3	R-3	10	5	2	49	40.00	Girola
4	R-4	10	6	3	44	50.00	Bori Ijara
5	R-5	10	5	2	48	40.00	Hamdapur
6	R-6	10	6	4	43	66.66	Dahegaon
7	R-7	10	7	4	44	57.14	Kurha
8	R-8	10	6	1	49	16.66	Virulpurna
9	R-9	10	7	2	47	28.57	Amaravati
10	R-10	10	6	1	42	16.66	Vyala
11	R-11	10	5	2	48	40.00	Washimba
12	R-12	10	6	3	50	50.00	Waddhamna
13	R-13	10	6	1	48	16.66	Bhapur
14.	Control	10	9	0	0	0	

Table 5: Grouping of *R. Bataticola* isolates on the basis of pathogenicity

Sr. No.	Category	Per cent of mortality	Isolates
1	Non pathogenic	0	Nil
2	Weakly pathogenic	1-20	R-8, R-10, R-13
3	Moderately pathogenic	21-50	R-1, R-2, R-3, R-4,R-5, R-9,R-11,R-12
4	Strongly pathogenic	50-70	R-6, R-7

References

1. Abavi GS, MA Pastor Corrales. Root rots of beans in Latin America and Africa: Diagnosis, Research, Methodologies and Management strategies Cali. Colombia. CIAT; 1990.
2. Aghakhani M, SC Dubey. Morphological and pathogenic variation among isolates of Rhizoctonia bataticola causing dry root rot of chickpea. Indian Phytopath. 2009;62(2):183-189.
3. Dhingra OD, JB Sinclair. Biology and pathology of *Macrophomina phaseolina.* Impresna Universitaria, Universidade Federal De Vicosa, Vicosa- 36. 570 Minas Gerais-Brazil; c1978. p. 166.
4. Khan Riyaj Ahmad, Towseef Ahmad Bhat, Krishan Kumar. Management of chickpea (Cicer arietinum L.) dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler. International Journal of Research in Pharma-ceutical and Biomedical Science. 2012;3(4):1539-1548.
5. Maglekar RK, JG Raut. Survey of soybean diseases in Vidarbha and influence of few diseases on plant and yield parameter. PKV Res. J. 1997;21:103-104.
6. Purkayastha, B Kaur, N Dilbaghi, A Chaudhury. Characterization of *Macrophomina phaseolina*. The charcoal rots pathogen of cluster bean, using conventional techniques and PCR-based molecular markers. Plant Patho. 2006;55:106-116.
7. Sangheetha TV, Shamrao Jahagirdar. Survey for assessment of disease incident of root rot of soybean Karnataka J. Agric. Sci. 2013;26(4):563-564.
8. Shekhar M, RC Sharma, Lokendra Singh, Ram Dutta. Morphological and pathogenic variability of *Macrophomina phaseolina* (Tassi) Goid incitent of charcoal rot of maize in India Indian Phyto Path. 2006;59(4): 453-459.
9. Sinclair JB, MC Shurlletf. Compendium of soybean diseases Amer. Phytopath Soc. Inc Minnesota. USA; c1975. p. 8-10.
10. Smith GS, ON Carvil. Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina*. Plant Dis. 1997;81:363-368.
11. SU G, SO Suh, S Schneiderand, JS Russin. Host specialization in the charcoal rot fungus. *Macrophomina Phaseolina* Phytoparh. 2001;91:120-126.
12. Young PA. Charcoal rot of Plant in East Texas, Texas Agriculture Experiment Station Bulletin. 1949;712:1-33.