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Culture and physiological variability in *Rhizoctonia solani*, responsible for foliar and lesions on aerial part of soybean

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Abstract: Foliar blight of soybean is one of the major fungal diseases. *Rhizoctonia solani* isolated from soybean growing in tarai regions of Uttarakhand. Six isolates of *R. solani* has been characterized on the basis of cultural and physiological nature such as colony diameter, growth, colour and sclerotia formation were recorded. Potato Dextrose Agar (PDA) was found best for growth and development. Two isolates (Lakhimpur and Pantnagar) covered the whole plates (90 mm) in 48 hrs. However, maximum number of sclerotia and weight was recorded on Czapek Dox agar medium. Overall radial growth supporting is recorded Corn Meal Agar Medium. Varied range of temperatures *i.e.* 10, 15, 25, 30, 35 and 40^{0}C was tested and found better growth of different isolates of *R. solani* at 10 - 40^{0}C, with an optimum growth temperature at 30^{0}C. Isolates were grown on five broth media (Asthana & Hawkers, Potato Dextrose Agar, Czapek's Dox Agar, Corn Meal Agar and Richards Agar) for fresh, dry weight and oat meal broth culture filtrates of all isolates was used in phytotoxic effects. It recorded that maximum fresh and dry weight was observed on corn meal agar medium. The maximum reduction in radical and plumule length of germinating seeds were recorded in Haldichaur isolate.

Keywords: Cultural, Foliar blight, Physiological, Phytotoxic, *Rhizoctonia solani* and Soybean

INTRODUCTION

Foliar/Leaf blight of soybean caused by *Rhizoctonia solani* Kuhn is a severe disease and causes heavy loss in soybean production (Anwar *et al.*,1995). Soybean [*Glycine max* (L.) Merril] plants are infected by the pathogen at any stage of development, which causes very rapid defoliation and frequent crop failure (Wrather *et al.*, 2001). Fenille *et al.* (2002) have reported 31-60% yield losses due to foliar blight of soybean. The symptoms of aerial blight of soybean caused by *R. solani*, as leaf and pod spots, leaf blight, defoliation, stem and petiole lesions, cob web like mycelium and sclerotia developed over infected leaves were described by Atkins and Lewis (1954). The *R. solani* produces sclerotia as survival structure which is brown to black composed of clusters of melanin encrusted, thick walled cells, rich in nutrients, formed by repeated branching from short, thick, lateral hyphae, when produced on plant parts it is difficult to separate the sclerotia from their surrounding embeded sclerotia. Temperature is more considerable parameter for their growth and development along with sclerotia production. The phytotoxicity of their metabolites make spot on the foliage become greenish to reddish brown and later turn brown to black in colour. In severe form whole leaf may be blighted. It also inhibited root elongation causing seedling root rot, yellowing and shredding of cotyledons and leaves in soybean. Therefore, integrated management of plant disease based on the laboratory studies to identify the adverse climatic conditions or photoinsensitive varieties or rotational use of chemicals to control the plant disease is considerable as *R. solani* have been reported to shows variation in characteristics.

MATERIALS AND METHODS

Survey and collection of sample showing blight symptoms from soybean growing areas of tarai regions mainly in Uttarakhand were done. The isolates (Rudrapur, Sitarganj, Haldichaur, Lakhimpur, Durgapalpur and Pantnagar) of *R.solani*, were isolated on PDA and purified through hyphal tip/single sclerotial method (Rangaswami and Mahadevan, 2004). In cultural studies; mycelial discs of 5 mm diameter from 3 days old cultures of each isolates were transferred into the center of sterilized different culture media and plates were incubated for 5 days at 28±1^{0}C. The basic cultural characteristics such as colony diameter, colour and growth pattern were studied. The colony colour was determined with help of Munsell's

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soil colour chart (Munsell, 1954). Based on mycelial pigmentation, the cultures were assigned in different groups as dark brownish, dark white and dirty brown. Colony growth pattern was recorded by visual observation according to growth of hyphae. Colony diameter growth rate was recorded on the five different media i.e. three synthetic media (Asthana and Hawkers, Czapek's agar and Richard's agar), one semi synthetic medium (PDA) and one natural medium (Corn Meal Agar medium) after 48 hrs of inoculation at 28±2^{0}C with the help of scale. The isolates were classified into slow; splash, fast, thin and fluffy growth. Growth was measured of the each isolate with three replications. The number, weight, colour, texture (smooth and rough) and patterns of sclerotia formed were recorded. In another physiological experiment; a total of six different temperature viz. 10, 15, 25, 30, 35 and 40^{0}C were evaluated to find out the suitable temperature level for radial growth and sclerotia formation, were incubated and observed after at 72 and 120 hrs, respectively. Each treatment was replicated thrice. A total of five broth medium viz. (Asthana & Hawkers, Potato Dextrose Agar, Czapek's Dox Agar, Corn Meal Agar and Richards Agar) were tested for biomass (fresh and dry weight) production. 125 ml medium was taken in flask and inoculated with 5 mm disc of the each isolates. Inoculated flasks in triplicates were incubated for 15 days at 28±1^{0}C. After incubation period mycelial mat and sclerotia were filtered through Whatman No.41 filter paper. The weight of dried mycelium along with sclerotia was recorded by subtracting the weight of filter paper from the total weight. Although, the result of biomass production was recorded after 15th day and observed that oat meal broth medium supports biomass for all the isolates. Therefore, culture filtrate on oat meal broth medium was used phytotoxic effect on soybean variety NRC-64, for seed germination of soybean. The healthy looking seeds of soybean Cv. NRC-64 were surface sterilized with two per cent chlorex solution for one minute and kept for soaking in culture filtrate for 12 hrs. The 25 soaked seeds were taken out and placed in sterilized wetted blotter paper and kept for 3 days for seed germination. Along with sterilized distilled water soaked seeds were kept as check. Observations were recorded after 70 hrs of incubation at 28± 1^{0}C. Percent inhibition over control was calculated by applying the following formula (Mc kinney, 1923).

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition, C = radical/plumule length in control, T = radical/plumule length in treatment

RESULTS AND DISCUSSION

Colony diameter, growth pattern and colour showed great diversity among the all isolates. On the basis of growth pattern, the isolates were categorized into five groups: slow, splash, fast thin and fluffy. Only two isolates (Rudrapur and Sitargang) showed slow growth. Burpee *et al.* (1980*)* had grouped the growth pattern in three groups. Colony colour of all the isolates were grouped into 4 groups: dark brownish, dirty white, milky white and dirty brownish. Three isolates (Sitargang, Haldichaur and Pantnagar) showed the dirty white mycelial pigmentation. Sunder *et al.* (2003*)* had reported varied brownish pigmentation of mycelial structure. Colony diameter and growth rate were recorded after 48 hrs. Isolates were grown on different media. PDA was found best for growth and development in comparison to others. Two isolates (Lakhimpur and Pantnagar), covered the whole plates (90 mm) in 48 hrs (Table 1). Singh *et al.* (1974) reported that PDA was found best for radial growth. The maximum number of sclerotia and weight was recorded on Czapek Dox agar medium. Tiwari and Khare (2002) reported that Czapek's Dox Agar was best for sclerotial production. Sclerotial colour grouped into two: dark brown and light brown and sclerotial formation pattern grouped in excellent, good and fair (Table 2). Sinha and Ghufran (1988) reported that more variations in the number weight and colour. The data

Table 1. Effect of culture medium on radial growth different isolates of *R. solani* at 28±1^{0}C after 48 hrs of incubation period.

Isolate	Media/ Radial growth (mm)					
	Asthana and Hawkers	Potato dextrose agar	Czapek's dox agar	Corn meal agar	Richards agar	Mean
Rudrapur	37.00	88.00	81.20	72.00	55.20	66.68
Sitargang	32.40	88.20	68.00	74.80	64.00	65.48
Haldichaur	38.00	88.80	76.20	79.40	72.60	71.00
Lakhimpur	38.40	90.00	83.00	74.40	72.80	71.72
Durgapalpur	29.80	85.00	83.20	78.20	63.80	67.88
Pantnagar	42.60	90.00	72.00	68.00	74.60	69.88
Mean	36.36	88.33	77.26	74.46	67.06	
LSD (P=0.05)	a = 3.61 b = 3.29	a × b= 8.07 CV (%) = 9.38				

a = isolate, b = media, a x b = interaction, *mean of three replications

Table 2. Effect of culture medium on sclerotia number and weight of different isolates of R. solani

Isolates	Number of sclerotia*						Weight of sclerotia (mg)*					
	Asthana and Hawkers	Potato dextrose agar	Czapek's dox agar	Corn meal agar	Richards agar	Mean	Asthana and Hawkers	Potato dextrose agar	Czapek's dox agar	Corn meal agar	Richards agar	Mean
Rudrapur	57.66	77.33	56.00	63.66	26.66	56.26	6.33	5.40	7.30	4.60	2.60	5.24
Sitargang	50.33	48.33	119.33	73.00	5.33	59.33	4.66	5.66	6.00	5.60	0.66	4.53
Haldichaur	33.66	65.66	72.00	68.00	13.66	50.60	5.33	6.33	3.66	7.33	1.00	4.73
Lakhimpur	52.00	61.00	46.33	63.33	0.00	44.43	6.66	5.33	7.33	5.66	0.00	5.00
Durgapalpur	37.33	39.33	26.00	53.00	5.00	32.12	4.00	4.60	5.33	5.00	0.33	3.85
Pantnagar	65.66	50.00	76.00	67.00	0.00	51.33	6.00	5.33	5.66	6.33	0.00	4.66
Mean	59.50	56.94	66.00	64.66	8.44		5.50	5.44	5.88	5.27	0.77	
LSD (P = 0.05), a =1.170; b =1.068; a × b =2.61; CV (%) =36.50												

a = isolate, b = media, a x b = interaction, * mean of three replications

Table 3. Effect of temperature range on the radial growth of different isolates of *R. solani*.

Isolate	Radial growth (mm)*																			
	10^0C				15^0C				25^0C				30^0C				35^0C			
	24	48	72	Mean	24	48	72	Mean	24	48	72	Mean	24	48	72	Mean	24	48	72	Mean
Rudrapur	6.4	12.2	37.4	18.6	7.0	13.0	42.0	20.0	12.6	35.8	75.2	41.2	14.0	42.2	84.6	46.9	12.4	30.4	59.6	34.0
Sitarganj	6.2	12.0	36.0	18.0	6.0	13.2	40.2	19.8	9.6	24.2	48.0	27.2	14.6	39.4	75.6	43.2	11.6	32.2	61.2	35.0
Haldichaur	0.0	6.0	24.2	10.1	7.6	15.6	53.2	25.5	11.2	33.8	68.0	37.6	10.2	30.6	62.4	34.4	11.2	32.6	62.5	36.3
Lakhimpur	6.2	12.8	38.8	19.3	7.2	13.6	41.8	20.8	11.0	27.2	55.0	31.1	12.2	42.8	86.4	47.1	10.4	24.0	47.8	27.4
Durgapalpur	6.0	13.0	34.8	19.6	6.2	15.6	46.8	22.9	12.0	31.7	57.8	33.8	10.2	29.0	60.0	33.6	13.2	20.8	43.4	25.8
Pantnagar	0.0	6.2	25.8	10.7	6.6	17.0	53.8	25.8	11.0	32.8	70.2	38.0	14.8	41.6	81.4	45.9	14.0	32.4	70.4	40.6
Mean	4.13	10.4	33.7		6.8	14.8	46.3		11.2	30.9	62.4		12.7	37.6	75.0		12.1	29.5	57.9	
LSD (P=0.05); a = 0.898; b =0.635; a × b =1.55 CV (%) =7.68																				

a = isolate, b = temperature, a x b = interaction, * mean of three replications

Table 4. Effect of broth culture medium on biomass (fresh weight) production.

Isolate	Biomass (fresh weight) in gm*					
	Asthana and Hawkers	Potato dextrose agar	Czapek's dox agar	Corn meal agar	Richards agar	Mean
Rudrapur	8.26	11.33	9.19	13.85	10.91	10.70
Sitarganj	6.53	12.36	8.60	9.22	10.66	9.47
Haldichaur	8.30	11.60	8.67	12.84	7.48	9.78
Lakhimpur	12.52	17.57	8.36	18.14	11.54	13.62
Durgapalpur	8.29	13.72	8.30	23.24	11.45	13.00
Pantnagar	6.81	15.87	7.39	17.60	9.68	11.40
Mean	8.45	13.74	8.41	15.81	10.28	
LSD (P=0.05) a = 0.282 b = 0.257 a × b= 0.630 CV (%) = 3.40						

a = isolate, b = medium, a x b = interaction, *mean of three replication

Table 5. Effect of broth culture medium on biomass (dry weight) production.

Isolate	Biomass (dry weight) in gm*					
	Asthana and Hawkers	Potato dextrose agar	Czapek's dox	Corn meal agar	Richards agar	Mean
Rudrapur	1.68	2.36	2.35	2.26	2.70	2.27
Sitarganj	1.68	2.52	2.29	1.91	2.26	2.13
Haldichaur	1.50	2.33	2.32	2.09	2.20	2.08
Lalkhimpur	1.87	2.43	1.82	2.57	2.68	2.27
Durgapalpur	1.72	1.66	2.25	5.87	2.68	2.23
Pantnagar	1.63	2.19	1.90	2.65	2.56	2.18
Mean	1.68	2.24	2.15	3.39	2.51	
LSD (P=0.05), a = 0.142; b = 0.130; a × b= 0.319; CV (%) = 8.88						

a=isolate, b=medium, a x b = interaction, *mean of three replications

presented in (Table 3) revealed that radial growth of different isolates of *R. solani* were significantly varied when recorded after 72 hrs of the inoculation among themselves at different temperature levels except 40^{0}C, where no, radial growth was recorded. However, maximum radial growth was recorded in isolate Lakhimpur and Pantnagar, at 30^{0}C, 86.60 and 81.40 mm, respectively. Radial growth and sclerotia formation varied with culture media but it was observed that oat meal supports all the isolates of *R. solani*. Singh *et al.* (1974) and Dubey and Dewivedi (1992) also observed optimum growth of *R.solani* at 28^{0}C. The minimum radial growth was recorded after 72 hrs in isolate of Haldichaur (24.20 mm) followed by Pantnagar (25.80 mm) and Sitarganj (36.00 mm) at 10^{0}C. The optimum radial growth was observed at 15^{0}C and 25^{0}C for all the isolates of *R. solani*. Further, it was observed that all the isolate of *R. solani* had excellent sclerotia formation at 30^{0}C. Optimum sclerotia formation was occurred at 25 and 35^{0}C while minimum sclerotia were occurred at 10 - 15^{0}C. Tiwari and Khare (2002) and Grosch and Kofoet (2003) also reported that the optimum hyphal growth was measured over temperature range of 20-30^{0}C with optimum at 25^{0}C and sclerotic production were observed at 30 – 35^{0}C on different media. The results are similar to those reported by Lim *et al.* (1987) who reported poor fungal growth at 35 and 15^{0}C. The results obtained in the present investigation suggest that 30^{0}C temperature is the optimum for the growth and sclerotia production. The data (Tables 4 & 5) revealed that isolates Lakhimpur and Durgapalpur gave maximum growth (fresh weight i.e. more than 13 gm). It was closely followed by Pantnagar isolate (11.40 gm). Among the medium, maximum biomass (Fresh weight) was recorded on the Corn meal medium (15.81 gm) followed by potato dextrose medium (13.74 gm) and Richard's medium (10.28 gm). However, maximum biomass (fresh weight) was recorded in the isolate of Durgapalpur (23.24gm) and Pantnagar (17.60 gm). The minimum biomass (fresh weight) was recorded in the medium Asthana and Hawker's medium. In this Corn meal broth medium also supports the growth *Rhizoctonia solani* isolates. Maximum biomass (dry weight) was recorded in isolate of Rudrapur and Lakhimpur (2.27 g) followed by Durgapalpur (2.23 g), the reason is due to fluffy mycelium having more water holding capacity and during drying water evaporated and reduced dry weight. In general, among the medium, maximum biomass (dry weight) was recorded on Corn Meal (3.39 g) closely followed by Richard's medium (2.51 g) and potato dextrose medium

Table 6. Effect of corn meal broth culture filtrate isolates of *R. solani* on soybean (variety NRC-64) seed germination.

Isolates	Germination percent*		Seedlings	
	Germinated	Non-germinated	Radical length (cm)*	Plumule length (cm)*
Rudrapur	68.00	32.00	3.95	5.18
Sitarganj	72.00	28.00	2.00	6.20
Haldichaur	10.00	90.00	1.32	3.60
Lalkhimpur	55.32	54.68	1.99	4.15
Durgapalpur	65.32	34.68	2.12	6.12
Pantnagar	24.00	76.00	1.36	4.34
Check	100.00	00.00	11.32	17.72
LSD (P=0.05)			1.58	1.50
CV (%)			63.48	33.61

*Mean of three replications

(2.24 g). However, the maximum biomass (dry weight, 5.87 g) was recorded in the isolate of Durgapalpur grown on Corn meal medium. The minimum biomass (dry weight) was recorded on the Asthana and Hawker's medium. The data revealed (Table 6) that the fifteen days old culture filtrate on corn meal broth of different isolate of *R. solani* significantly reduced the radical length and plumule length of the germinated seeds as compared to check. Verma (1973) also reported that phytotoxic metabolites produced by soybean isolate of *R.solani*, inhibited radical elongation, showed seedling root rot yellowing the shredding of Cotyledons and leaves. The maximum reduction in radical length was recorded in Haldichaur isolate by giving 1.32 cm radical length. This was closely followed by Pantnagar (1.36cm). Jain and Thapliyal (1980) observed that *R.solani* isolated from soybean leaf produced non-host specific toxic metabolites, capable of producing leaf symptoms, supporting the present investigation. The organism produced more phytotoxic metabolites in the medium on which it grow, which causes inhibition on seed germination and inhibition of root elongation. The next best in radical length reduction was recorded in the isolate Lakhimpur (1.99 cm). The maximum reduction in plumule length was recorded in Haldichaur isolate (3.60 cm) followed by Lakhimpur (4.15 cm) and Pantnagar (4.34 cm). Similarly, Sherwood and Lindburg (1962) reported that pectinolytic and cellulytic enzymes produced by the isolate of *R.solani*, to be phytotoxic for seed germination

Conclusion

The genus *Rhizoctonia* is considered as a complex mixture of filamentous fungi, having in common the possession of a non-spored imperfect state, usually referred to as the *Rhizoctonia* anamorph. The *Rhizoctonia* anamorph is characterized by several common features present among members of the entire *Rhizoctonia* species complex. The phytopathological studies in the complex have represented the major contingent of contributions in the group, especially in the case of *R. solani.* Further, molecular characterization is needed for understanding of biology, physiology and systemic study.

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