

## GLOBAL JOURNAL OF BIO-SCIENCE & BIOTECHNOLOGY

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## EXPLORATION OF VARIABILITY IN COLONY MORPHOLOGY AND VIRULENCE OF RHIZOCTONIA *BATATICOLA* ISOLATES CAUSING DRY ROOT ROT OF PULSES

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#### ABSTRACT

Variability in *Rhizoctonia bataticola* isolates from roots, leaf and seeds of pluses *viz.*, blackgram, greengram, cowpea, soyabean, and redgram were detected by virulence. Each and every isolate varied in its growth on different solid media. Irrespective of the media maximum mean mycelial growth was recorded by redgram root isolate (76.71mm). It was interesting to note that the eleven isolates varied in synthesized pink pigment and their intensity *viz.*, low (+), medium (++) and high (+++). As well as the temperature increased from 10° to 30°C the mycelial growth gradually increased and at 35°C there was gradual decrease respectively in blackgram root and seed, cowpea root, greengram shoot and root redgram shoot and root isolates. Irrespective of the isolates it was observed that the pH of 7 had supported the mycelial growth. There was drastic reduction of mycelial growth at pH 8 (34.3 mm). The pathogenic variability indicated that all the isolates were more aggressive on the original host from which it was isolated. Among the shoot, root and seed isolate the root isolate were more aggressive. Though the seed isolate was from blackgram host, it had recorded only 58 % root rot exhibiting its variability in pathogenicity. The experiment results indicated that the virulence of isolates from various plant parts deferred in their pathogenicity.

KEY WORDS: Rhizoctonia bataticola isolates, variability, pigment, temperature, pH, virulence

#### INTRODUCTION

Pulses are excellent source of protein, vitamins and minerals thus supplement with cereals to improve their nutritive value. In nature pulse crops are infected by many pest and diseases, particularly dry root rot which causes heavy damage compared to other diseases. Dry root rot caused by sclerotial form of *Rhizoctonia bataticola* (Pycnidial form of *Macrophomina phaseolina*) causes up to 60 % yield loss. It is a diverse omnipresent soil borne species with a wider host range involving more than 500 species in 75 families (Dhingra and Sinclair, 1978). It mainly produces micro sclerotia (primary source of inoculum) or pycnidia. It is both seed and soil borne and infects plants from seedling stage to maturity.

The ubiqutious, omnipresent sclerotial fungus Rhizoctonia *bataticola* (Taub.) Butl. in its pynidial state is known as Macrophomina phaseolina (Tassi.) Goid. from different host species differ in their morphological and cultural characters and even differences occur in the isolates from various parts of same host (Jain et al., 1973) Raut and Ingle (1989) in their investigation found that isolates from fifteen different cultivated crops had variation in vegetative (sclerotial) and reproductive (pycnidial) stages. The morphology and pathological variability so recorded has been restricted to one host species whereas comparative characteristic of isolates from different plant species have not been studied so far (Hooda and Grover, 1982). Based on morphological and cultural characters and pathogenic behaviour the six isolates of *R.bataticola* were classified into three groups, highly virulent (bean, bengal gram, and cowpea isolates), intermediate (sorghum and soyabean isolates) and mild (Gliricidia isolates) by Byadgi and Hedge (1985). Ghosh and Sen (1973) indicated that virulent isolates produced more sclerotia. Gupta and Kolte (1982) stated that leaf isolates produced abundant vegetative growth on PDA, it did not form sclerotia proportional to mycelial growth while the reverse way was true in the case of root isolates. The size of the sclerotia of the leaf isolates (98.10µm) was more than that of root isolates (88.28µm). He further reported that the two isolates differed from each other by their pycnidial size. Keeping in mind the investigation was carried out on pathogenic variability of R. bataticola from roots, leaf and seeds of blackgram, greengram, cowpea, soyabean, and redgram and to study the growth of isolates on different media, pH and temperature.

#### MATERIALS AND METHODS

#### Isolation and maintenance of the culture

Infected plant samples showing typical root rot symptoms on pulse crops (red gram, greengram, cowpea, soybean, blackgram) were collected and it was purified by single hyphal tip method (Riker and Riker, 1936).

#### Linear growth in different solid medium

The mycelial disc was punched from the individual pure culture isolates (11 isolates) using a sterile cork borer of 8 mm diameter and then placed in the middle of sterile Petri dishes containing different solid media *viz.*, PDA, oats meal agar, Czapek–Dox agar, Richard's and peptone

sucrose agar medium. The mycelial growth was recorded 3 DAI.

#### Liquid medium - mycelial dry weight

Mycelial disc (9 mm) was taken from 3-4 days old culture and inoculated individually into each conical flask containing PD, Oats meal, Czapek– Dox, Richard's and peptone sucrose broths, incubated at room temperature (28  $\pm$  2°C) for 7 days. After 7 days the mycelial mat was filtered through (Whatman No. 42) filter paper and oven dried at 60<sup>o</sup>C for 48 hours (Singh and Kaiser, 1994). The dry weight of mycelium of each isolate was recorded using electronic weighing balance.

### Pigment production of isolates

The pigment synthesis of pathogen was observed at regular intervals in peptone sucrose broth and the intensity (+ low intensity, ++ medium intensity and +++ high intensity) was recorded (Suriachandraselvan and Seetharaman, 2003).

### Temperature

The eleven isolates of *R. bataticola* were inoculated on to PDA medium in sterile Petri plates and kept in different temperature *viz.*,  $10^{\circ}$ ,  $15^{\circ}$ ,  $20^{\circ}$ ,  $25^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$ C. The diameter of the mycelial growth was recorded after 3 DAI. Three replications were maintained.

### pН

Mycelial disc (9 mm) placed in the middle of sterilized Petri dishes containing PDA medium with different pH levels *viz.*, 6.5, 7.0, 7.5 and 8.0. The diameter of the mycelial growth was measured after 3DAI. Three replications were maintained.

# Mass multiplication of *R. bataticola* inoculum for virulence studies

The isolates of the fungus were multiplied in sand maize medium (Riker and Riker, 1936).

# Assessing the pathogenic variability of *R. bataticola* isolates

The potting mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The inoculum of each isolate of *R. bataticola* collected from different parts of pulse crops were separately mixed at 100g/

kg (w/w) with the unsterilized soil, filled in 30 cm earthen pots ten days before sowing (Sundravadana, 2002). Surface sterilized (using 0.1% MgCl<sub>2</sub> solution for 30 sec. followed by two washings in sterile water) blackgram, greengram, cowpea, soybean, redgram seeds were sown @ 10 seeds per pot. Three replications were maintained in a completely randomized design. The pots were maintained in glass house with regular, judicious and uniform watering. The root rot incidence was recorded at 15 (seedling mortality) and 60 DAS and the per cent disease incidence (PDI) was calculated as below.

### **RESULT AND DISCUSSION**

The ubiqutious, omnipresent sclerotial fungus *Rhizoctonia* bataticola (Taub.) Butl. in its pynidial state is known as *Macrophomina phaseolina* (Tassi.) Goid. This fungal pathogen causes dry root rot in mostly all pulse crops. It is reported to attack root, leaf, stem, pod and seeds of pulse crops. Research was undertaken to study the pathological variability of this dry root rot pathogen.

It was observed that the shoot, root and seed isolate of *R. bataticola* from blackgram differed in their virulence (Table 1). The shoot isolate had recorded maximum seedling mortality on blackgram (16.10 %) and was significantly superior followed by greengram (15.29 %) and minimum seedling mortality was recorded on redgram (7.88 %) soybean (5.3 %) and cowpea (9.33 %), the same trend was observed after 60 DAS.

The blackgram shoot and root isolate was highly virulent and significantly superior against blackgram respectively recording 90.60 % and 88.58 % of root rot incidence. The next best susceptible host was greengram. It was interesting to note that the seed isolate was less virulent on its original host. The seed isolate has recorded only 58.00 per cent root rot incidence on blackgram and it was followed by greengram (56.28 %).

**TABLE 1.** Pathogenic Variability of R. bataticola blackgram isolates on pulse crops

Host	Seedli	ng mortality (%)	15 DAS	Root rot percentage (%) 60 DAS			
	Shoot Isolates	Root Isolates	Seed Isolates	Shoot Isolates	Root Isolates	Seed Isolates	
Blackgram	16.10 <sup>a</sup>	20.33 <sup>a</sup>	13.28 <sup>a</sup>	90.60 <sup>a</sup>	88.58 <sup>a</sup>	58.00 <sup>a</sup>	
	(23.66)	(26.8)	(21.37)	(72.17)	(70.27)	(49.60)	
Cowpea	9.33°	13.34 <sup>c</sup>	8.12 <sup>c</sup>	65.82 <sup>c</sup>	63.51 <sup>d</sup>	51.94 <sup>c</sup>	
	(17.85)	(21.42)	(16.56)	(54.22)	(52.83)	(46.11)	
Greengram	15.29 <sup>b</sup>	19.20 <sup>b</sup>	12.29 <sup>b</sup>	87.00 <sup>b</sup>	73.29 <sup>b</sup>	56.28 <sup>b</sup>	
	(23.02)	(25.98)	(20.52)	(68.88)	(58.88)	(48.61)	
Redgram	7.88 <sup>d</sup>	10.59 <sup>d</sup>	6.59 <sup>d</sup>	61.50 <sup>d</sup>	69.28 <sup>c</sup>	51.51 <sup>d</sup>	
	(16.3)	(18.98)	(14.87)	(51.69)	(56.34)	(45.86)	
Soyabean	5.3 <sup>e</sup>	6.60 <sup>e</sup>	3.20 <sup>e</sup>	$48.00^{e}$	53.10 <sup>e</sup>	38.00 <sup>e</sup>	
	(13.3)	(14.88)	(10.3)	(43.85)	(46.77)	(38.05)	

Means followed by a common letter are not significantly different at the 5% level by DMRT.

	Seedling morta	lity (%) 15 DAS	Root rot (%	6) 60 DAS
Host	Shoot Isolates	Root Isolates	Shoot Isolates	Root Isolates
-		Cowpea isolates		
Blackgram	11.68 <sup>b</sup>	13.89 <sup>c<sup>1</sup></sup>	51.94 <sup>c</sup>	76.51 <sup>b</sup>
U	(19.98)	(21.88)	(46.11)	(61.01)
Cowpea	13.34 <sup>a</sup>	19.26 <sup>a</sup>	78.86 <sup>a</sup>	$80.87^{a}$
· · F - · ·	(21.42)	(26.03)	(62.63)	(64.07)
Greengram	11.29°	14.30 <sup>b</sup>	58.00 <sup>b</sup>	70.00 <sup>c</sup>
e	(19.64)	(22.21)	(49.60)	(56.7)
Redgram	8.28 <sup>d</sup>	10.59 <sup>d</sup>	44.22 <sup>d</sup>	41.23 <sup>d</sup>
e	(16.73)	(19.00)	(41.68)	(39.95)
Sovabean	$4.00^{e}$	6.30 <sup>e</sup>	23.30 <sup>e</sup>	20.28 <sup>e</sup>
	(11.54)	(14.53)	(28.86)	(26.78)
Greengram isol	ates	(	()	(
Blackgram	17.42 <sup>a</sup>	19.43 <sup>a</sup>	67.45 <sup>b</sup>	72.79 <sup>b</sup>
	(24.67)	(26.15)	(55.21)	(58.56)
Cowpea	$16.35^{b}$	18 36 <sup>b</sup>	66 82 <sup>b</sup>	$74.25^{b}$
compeu	(23.85)	(25.37)	(54.83)	(59.51)
Greengram	$15.28^{\circ}$	$19.26^{a}$	(5 1.05) 75 60 <sup>a</sup>	(59.51) 76.00 <sup>a</sup>
Greengrun	(23, 02)	(26.03)	(60.4)	(60.67)
Redgram	(25:02) 7 59 <sup>d</sup>	$10.59^{d}$	46 82°	(00.07) 45 73°
iteugruin	(15.99)	(18.99)	(43.18)	(42.55)
Sovahean	(19.99) 4 80 <sup>e</sup>	$6.30^{e}$	29.80 <sup>d</sup>	28 00 <sup>d</sup>
Soyubean	(12.66)	(14, 53)	(33.09)	(32.00)
Redoram isolate	(12:00)	(11.55)	(55.07)	(52:07)
Blackgram	7 35 <sup>b</sup>	10 37 <sup>b</sup>	40.87 <sup>b</sup>	48 32 <sup>b</sup>
Didekgruin	(15.73)	(18 79)	(39.74)	(44.04)
Cownea	(10.73) 5 072°	9 33 <sup>d</sup>	31.23°	38.13°
compea	(13.84)	(17.79)	(33.96)	(38,13)
Greengram	7 20 <sup>b</sup>	(17.77) 9.80°	30.28 <sup>b</sup>	(90.15) 49.30 <sup>b</sup>
Oreengram	(15.56)	(18.24)	(38.82)	(11.6)
Redoram	(15.50) 17.23 <sup>a</sup>	(10.24) 21.26 <sup>a</sup>	(30.02) 78 17 <sup>a</sup>	(44.0) 82 $47^{a}$
Keugrann	(24.55)	(27.46)	(62.15)	(65, 26)
Sovahean	(24.33) 2.60 <sup>d</sup>	(27.40) (27.40)	(02.13) 23 30 <sup>d</sup>	(05.20) 29.30 <sup>d</sup>
Soyabean	(9.28)	(11.96)	(28.86)	(32,77)
Sova bean isola	(9.20) tes	(11.90)	(28.80)	(32.77)
Blackgram	5 3 3 <sup>°</sup>	10.67 <sup>c</sup>	28 80 <sup>b</sup>	33 12 <sup>b</sup>
Diackgrain	(12.26)	(10.07)	(22.45)	(25, 22)
Common	(15.50) e 22 <sup>b</sup>	(19.03) 12.14 <sup>b</sup>	(32.43)	(33.32)
Cowpea	(16, 77)	12.14	20.07	28.09
Crooncrom	(10.77)	(20.39)	(2/.10)	(32.00)
Greengram	5.50	10.00	20.90	33.28 (25.24)
Dadama	(13.30)	(19.00)	(31.24)	(33.24)
Keagram	2.50	3.8U	23.10	29.95
C1 -	(9.09)	(11.23)	(30.1)	(33.1/)
Soyabean	$1/.30^{\circ}$	22.60	/0.80	81.60
	(24.30)	(28.37)	(01.21)	(04.01)

**TABLE 2.** Pathogenic Variability of *R. bataticola* isolates on other pulse crops

Means followed by a common letter are not significantly different at the 5% level by DMRT.

The *R. bataticola* pathogen isolated from the shoot and root of cowpea differed in its virulence on the other pulse hosts (Table 2). The results revealed that the cowpea shoot isolate was virulent on cowpea causing 13.34 per cent seedling mortality and was significantly superior and was followed by blackgram (11.68 %) and greengram (11.29 %) and they were on par with one another in their infection whereas seedling mortality was very low in redgram (8.28 %) and soybean (4.0 %). The root isolates were more virulent compared to the shoot isolate and had

recorded 19.26 % seedling mortality on cowpea. The same trend was observed after 60 DAS. The root isolate of cowpea recorded the maximum of 80.87 % root rot incidence on cowpea and it was significantly superior. This was followed by blackgram (76.51 %) and greengram (70.0 %). Soybean recorded the least incidence of 20.28 % root rot incidence and the results confirmed that the pathogen are more aggressive on the original host in which it has been isolated and the root isolate are more virulent in inducing root rot compared to the shoot isolates.

Isolates	PDA	Richard	Oats	Czapek Dox	PSA	Mean	
Blackgram							
i) Shoot	72.48 <sup>e</sup>	51.24 <sup>ef</sup>	60.40 <sup>e</sup>	62.81 <sup>g</sup>	47.01 <sup>h</sup>	58.78	
ii) Root	76.85 <sup>d</sup>	58.79cd	63.21d	$65.82^{f}$	54.48 <sup>e</sup>	63.83	
iii) Seed	87.29 <sup>b</sup>	64.60 <sup>b</sup>	70.29 <sup>ab</sup>	66.80 <sup>c</sup>	67.90 <sup>b</sup>	71.77	
Cowpea							
i) Shoot	76.87 <sup>d</sup>	57.50 <sup>d</sup>	62.89 <sup>d</sup>	64.39 <sup>fg</sup>	61.99 <sup>d</sup>	64.72	
ii) Root	81.60 <sup>c</sup>	59.60 <sup>c</sup>	$68.60^{bc}$	73.80 <sup>d</sup>	$66.70^{b}$	70.76	
Greengram							
i) Shoot	72.48 <sup>e</sup>	49.93 <sup>f</sup>	54.36 <sup>g</sup>	64.93 <sup>fg</sup>	52.04 <sup>f</sup>	58.74	
ii) Root	76.25 <sup>d</sup>	51.77e	58.19 <sup>f</sup>	70.03 <sup>e</sup>	55.98 <sup>e</sup>	62.44	
Redgram							
i) Shoot	87.29 <sup>b</sup>	69.80 <sup>a</sup>	71.29 <sup>a</sup>	79.29 <sup>b</sup>	64.70 <sup>c</sup>	74.44	
ii) Root	89.85 <sup>a</sup>	60.40 <sup>c</sup>	61.89 <sup>c</sup>	84.46 <sup>a</sup>	71.58 <sup>a</sup>	76.71	
Soyabean							
i) Shoot	$68.29^{f}$	$44.60^{h}$	51.60 <sup>h</sup>	59.39 <sup>h</sup>	49.29 <sup>g</sup>	54.63	
ii) Root	72.20 <sup>e</sup>	48.20 <sup>g</sup>	54.29 <sup>g</sup>	$63.70^{fg}$	$52.60^{f}$	58.19	
Mean	78.31	56.03	66.48	68.64	58.54		

<b>IABLE 3.</b> Growth of <i>R. bataticola</i> isolates in different solid mediur	TABLE 3.	Growth	of R.	bataticola	isolates	in	different solid medium
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Means followed by a common letter are not significantly different at the 5% level by DMRT.

	Mycelial dry weight (mg)					PSA	
Isolates	PDA	Richard	Oats	Czapek Dox	Mycelial dry weight	Pigment synthesis	Mean
Black gram							
i) Shoot	681.1 <sup>d</sup>	449.5 <sup>d</sup>	597.0 <sup>c</sup>	591.1 <sup>d</sup>	499.6 <sup>d</sup>	+	563.6
ii) Root	693.6 <sup>bcd</sup>	484.6 <sup>c</sup>	612.2 <sup>bc</sup>	637.4 <sup>c</sup>	502.8 <sup>d</sup>	+++	586.1
iii) Seed	702.6 <sup>bc</sup>	491.6 <sup>c</sup>	631.2 <sup>a</sup>	$688.0^{a}$	541.8 <sup>c</sup>	+++	611.0
Cow pea							
i) Shoot	630.9 <sup>e</sup>	406.6 <sup>e</sup>	551.1 <sup>d</sup>	596.0 <sup>d</sup>	560.2 <sup>d</sup>	++	543.5
ii) Root	706.2 <sup>b</sup>	522.6 <sup>b</sup>	624.2 <sup>ab</sup>	685.0 <sup>a</sup>	643.1 <sup>a</sup>	++	636.2
Green gram							
i) Shoot	613.3 <sup>e</sup>	448.9 <sup>d</sup>	541.6 <sup>d</sup>	579.8 <sup>d</sup>	645.5 <sup>a</sup>	++	565.8
ii) Root	695.3 <sup>bcd</sup>	573.9 <sup>a</sup>	625.1 <sup>ab</sup>	686.2 <sup>a</sup>	649.2 <sup>a</sup>	++	645.9
Red gram							
i) Shoot	685.6 <sup>cd</sup>	513.0 <sup>b</sup>	621 <sup>ab</sup>	655.0 <sup>b</sup>	$407.8^{\mathrm{f}}$	+++	576.4
ii) Root	751.3 <sup>a</sup>	565.0 <sup>a</sup>	638.0 <sup>a</sup>	694.8 <sup>a</sup>	652.4 <sup>a</sup>	++	660.3
Soya bean							
i) Shoot	491.6 <sup>g</sup>	307.0 <sup>g</sup>	408.2 <sup>e</sup>	$435.0^{f}$	$411.2^{f}$	++	404.6
ii) Root	$528.0^{\mathrm{f}}$	$338.0^{\mathrm{f}}$	399.1 <sup>e</sup>	462.0 <sup>e</sup>	431.1 <sup>e</sup>	++	431.6
Mean	652.6	463.7	568.0	610.0	540.4		
						-	-

TABLE 4. Growth of R. bataticola isolates in different liquid media

+ : low intensity

++ : medium intensity

+++ : high intensity

Means followed by a common letter are not significantly different at the 5% level by DMRT.

The root rot causative agent *R. bataticola* was isolated from shoot and root of greengram and this two isolates behaved differently on the different pulse crops. Compared to the shoot isolate the root isolate was more virulent in causing seedling mortality and root rot (Table 2) .The root isolate of *R. bataticola* was highly pathogenic on greengram and blackgram causing respectively 19.26 % and 19.43 % seedling mortality and they were on par with one another.

Isolates	Colony diameter(mm)							
isolates	10 <sup>o</sup> C	15 <sup>o</sup> C	20 <sup>0</sup> C	25 <sup>o</sup> C	30 <sup>o</sup> C	35 <sup>0</sup> C	wiean	
Blackgram								
i) Shoot	-	19.33 <sup>g</sup>	29.30 <sup>h</sup>	35025 <sup>g</sup>	71.78 <sup>d</sup>	74.49 <sup>c</sup>	46.03	
ii) Root	-	22.07 <sup>f</sup>	31.30 <sup>g</sup>	44.12 <sup>e</sup>	76.45 <sup>c</sup>	75.86 <sup>c</sup>	49.86	
iii) Seed	-	30.00 <sup>cd</sup>	$36.20^{\circ}$	51.00 <sup>c</sup>	$87.60^{a}$	81.60 <sup>b</sup>	57.28	
Cowpea								
i) Shoot	-	29.50 <sup>d</sup>	33.14 <sup>e</sup>	53.91 <sup>b</sup>	70.08 <sup>d</sup>	71.48 <sup>d</sup>	51.60	
ii)Root	-	$30.20^{\circ}$	34.20 <sup>d</sup>	$58.00^{a}$	77.10 <sup>c</sup>	75.60 <sup>c</sup>	55.02	
Greengram								
i) Shoot	-	$21.44^{\rm f}$	29.11 <sup>h</sup>	47.31 <sup>b</sup>	62.71 <sup>e</sup>	62.01 <sup>e</sup>	44.51	
ii) Root	-	26.18 <sup>e</sup>	31.01 <sup>g</sup>	48.16 <sup>d</sup>	71.74 <sup>d</sup>	71.23 <sup>d</sup>	49.66	
Redgram								
i) Shoot	-	39.30 <sup>b</sup>	$50.10^{b}$	54.00 <sup>b</sup>	85.10 <sup>b</sup>	84.60 <sup>a</sup>	62.62	
ii) Root	-	42.73 <sup>a</sup>	$56.70^{a}$	57.90 <sup>a</sup>	85.86 <sup>ab</sup>	85.85 <sup>a</sup>	65.80	
Soyabean								
i) Shoot	-	$16.20^{i}$	20.39 <sup>j</sup>	$41.00^{f}$	63.00 <sup>e</sup>	63.29 <sup>e</sup>	40.77	
ii) Root	-	18.20 <sup>h</sup>	25.30 <sup>i</sup>	44.00 <sup>e</sup>	71.00 <sup>d</sup>	70.29 <sup>d</sup>	45.75	
Mean		28.83	34.23	48.51	74.76	74.20		

TABLE 5. Effect of temperature on the growth of *R. bataticola* isolates

TABLE 6. Effect of pH on growth of different isolates of R. bataticola

<b>.</b>						
Isolates	Ph6.5	Ph6.5 Ph 7 I		Ph 7.5 Ph 8		
Black gram						
i) Shoot	70.46 <sup>d</sup>	71.67 <sup>d</sup>	$67.04^{\text{ f}}$	$30.50^{\text{ f}}$	59.91	
ii) Root	76.55 °	77.45 °	69.83 <sup>de</sup>	33.71 <sup>e</sup>	64.38	
iii) Seed	85.10 <sup>a</sup>	84.29 <sup>a</sup>	79.90 <sup>b</sup>	38.20 <sup>d</sup>	71.80	
Cow pea						
i) Shoot	72.17 <sup>d</sup>	75.97 °	67.88 ef	30.55 <sup>f</sup>	61.56	
ii) Root	80.29 <sup>b</sup>	82.60 <sup>b</sup>	$74.10^{\circ}$	41.60 <sup>b</sup>	61.64	
Green gram						
i) Shoot	70.56 <sup>b</sup>	72.07 <sup>d</sup>	$66.54^{\text{ f}}$	$30.50^{\text{ f}}$	59.91	
ii) Root	74.44 <sup>c</sup>	77.86 <sup>c</sup>	70.43 <sup>d</sup>	32.81 <sup>e</sup>	63.68	
Red gram						
i) Shoot	82.10 <sup>b</sup>	88.60 <sup>a</sup>	79.10 <sup>dc</sup>	39.70 °	72.37	
ii) Root	84.46 <sup>a</sup>	89.15 <sup>a</sup>	83.06 <sup>a</sup>	44.22 <sup>a</sup>	75.22	
Soya bean						
i) Shoot	58.20 <sup>f</sup>	61.10 <sup>f</sup>	57.29 <sup>h</sup>	22.29 <sup>h</sup>	49.72	
ii) Root	61.10 <sup>e</sup>	65.21 e	60.80 <sup>g</sup>	26.29 <sup>g</sup>	53.35	
Mean	71.13	76.90	70.50	33.67		

Mean followed by a common letter are not significantly different at the 5% level by DMRT.

The root isolate of greengram had recorded 76 % root rot incidence after 60 DAS and it was significantly superior and was followed by cowpea which recorded 74.25 % root rot incidence. The isolates of greengram were not aggressive on soybean. The root isolate recorded only 28 % root rot incidence on soybean after 60 DAS proving its less virulent nature on soybean.

*Rhizoctonia bataticola* was isolated from the shoot and root of redgram and the same was utilized to find out the pathogenicity on other pulse crops (Table 2).As in the other host, it was observed that compared to shoot and root isolate the latter was more virulent causing increased root rot incidence on its original host. The root isolate of redgram had respectively recorded 10.37, 9.8 and 9.33 % seedling mortality on blackgram, greengram and cowpea since they were on par with one another .The maximum seedling mortality of 21.26 % was recorded on the redgram and it was significantly superior. The same trend was observed after 60 DAS. The root isolate recorded the maximum of 82.47 % root rot incidence in redgram and it was significantly superior. The soybean crop recorded the least incidence of 29.3 % root rot against root isolate of redgram. The dry root rot pathogen isolated from the shoot and root of soybean was cross inoculated to study the virulence of the isolates on other pulse crops . It was interesting to note that soybean isolates of root and shoot was not that much pathogenic on other pulse crops. As that of the other crops the root isolate was more virulent and it had caused seedling mortality to tune of 22.6 % on soybean and it was significantly superior .The reading

taken after 60 DAS revealed that the root isolate was virulent only on its original host from which it was isolated. The root isolate of soybean recorded 81.6 % root rot on soybean and it was individually significantly superior revealing the compatibility of the host and its pathogen (Table 2). Several workers have worked on this line. Isolates of *R. bataticola* from different host or same host have been reported to show wide variation in virulence and pathogenicity (Hooda and Grover, 1988, Raut and Ingle, 1989; Bansal, *et al.*, 1990 Sobti and Sharma, 1992).

The growth of the eleven isolates in solid and liquid media was tested to study the variability. Redgram root isolate recorded the maximum growth of 89.85 mm in PDA and it was individually significantly superior. It was followed by blackgram seed and redgram shoot isolate which respectively recorded 87.29 and 87.29 mm and they were on par with one another. Redgram shoot isolate and blackgram seed isolate respectively recorded 71.29 and 70.29 mm mycelial growth and they were on par with one another on oats medium. This was followed by cowpea root isolate which recorded 68.6 mm mycelial growth. The maximum mycelial growth was recorded by redgram shoot isolates in Richard's medium (69.8 mm), by redgram root isolates in Czapek-Dox Agar medium (84.46 mm) and Peptone sucrose Agar medium (71.58 mm) and they were significantly superior. In general it was observed that PDA medium had recorded maximum mycelial growth followed by Czapex-Dox medium, Oats media supported the growth of redgram shoot isolate and blackgram seed isolate (Table 3). The same trend was observed in liquid both. Among the eleven isolates the mycelial dry weight of redgram root isolate was individually significantly superior in all the five media tested viz., PD broth (751.3 mg), Richards broth (565 mg) oats broth (638 mg), Czapek - Dox broth (694.8 mg) and peptone sucrose broth (652.41 mg). This was followed by greengram root isolate. The mycelial dry weight of cowpea root isolate was individually significantly superior in oats (624.2 mg), Czapek - Dox broth (685 mg) and peptone sucrose (643.1 mg) broth (Table 4). The growth of different isolates in the different liquid culture broth varied considerably. In general the growth of soybean shoot and root isolate of R. bataticola was considerably less when compared with the other isolates. It was interesting to note that the eleven isolates inoculated in the peptone sucrose liquid broth synthesized pink pigment and their intensity varied among the isolates *viz.*, low (+), medium (++) and high (+++). The blackgram shoot isolate had recorded low pink pigment while intensity was moderate in cowpea, greengram, soybean shoot and root isolates and in redgram root isolate. High pigmentation was observed in blackgram root, seed and redgram shoot isolate. There was no pigment formation in potato dextrose, Richards, oats and Czapek - Dox broths (Table 4).

#### REFERENCES

Albach, R.F. and Bruton, B.D. (1983) Pigment analysis of honeydew melon infected with *Macrophomina phaseolina*. Phytochemical Society of North America Newsletter. 2, 23-24. Ghosh and Sen (1973) reported that all the isolates under study secreted a red pigment in Sabourauds' medium. Polypeptide chains in peptone in Sabourauds medium probably might have stimulated pigment in these isolates. Relative virulence in pathogens has been associated with the synthesis of chromogenic toxins. Phytotoxic red pigments have been reported in *Alternaria eichorniae* (Charodattan and Rao, 1982) and *Cercospora beticola* (Lynch and Geoghegan, 1979). A red pigment absorbing at 520 nm was produced *in vitro* by a canta loup fruit isolate of *M. phaseolina* (Albach and Bruton, 1983). Ulukas (1984) reported that *M. phaseolina* isolates from onion, carnation, olive and chickpea produced a dense red colour and the pigment production was the best on potato yeast extract agar.

The effect of temperature on the mycelial growth of R. bataticola was recorded after 3 DAI. It was observed that 15°, 20° and 25°C had recorded respectively a mean mycelial growth of 26.83, 34.23 and 48.51 mm. The maximum mycelial growth was recorded at 30° and 35°C respectively recording 74.76 and 74.20 mm. As the temperature increased from 10° to 30°C the mycelial growth gradually increased and at 35°C there was gradual decrease of 0.59, 6, 1.5, 0.7, 0.51 0.5, 0.001 and 0.71 mm, respectively in blackgram root and seed, cowpea root, greengram shoot and root redgram shoot and root and soybean root isolates. There was an increase of 2.71, 1.4, 0.29 mm mycelial growth respectively in shoot isolates of blackgram, cowpea and soybean (Table 5). Temperature is the specific limitation for the growth of the fungal pathogen and for its pathogenicity. Though it is very well known that dry root rot pathogen is high temperature loving, this experiment was carried out to observe if there was variation among the isolates of pulse crops.

It has been well established that hydrogen ion concentration in the growing media influenced growth and metabolism of fungal pathogen. It is universally known that pH 7 supports the mycelial growth of most of the fungi. This experiment was carried out in order to find out the pH preference of the eleven isolates (Table 6). The effect of pH on the mycelial growth of R. bataticola was assessed after 72 hrs after inoculation. It was observed that the pH 7 had supported 78.3 mm mean mycelial growth. There was drastic reduction of mycelial growth at pH 8 (34.3 mm). At pH 7 all the isolates had good mycelial growth and it ranged from 61.10 mm (soybean shoot isolate) to 89.15mm (redgram root isolate). It was observed that among the eleven isolates, the redgram root isolate was individually significantly superior in all the pH tested viz., pH 6.5 recorded 84.46 mm, pH 7 recorded 89.15 mm, pH 7.5 recorded 83.06 mm and pH 8 recorded 44.22 mm mycelial growth. The pathogenecity of isolates on different pulse host revealed that the isolates were virulent on the original host in which it has been isolated.

Bansal, R,K., Sobti, A.K. and Meth,a S.M. (1990) Cultural variability among seven isolates of *Rhizoctonia*. Indian Phytopathology. 43, 448 - 450.

Byadgi, A.S. and Hedge, R. (1985) Variation among the isolates of *Rhizoctonia* from different host plants. Indian Phytopathology. 38, 297 - 301.

Charodattan, R. and Rao, K. V. (1982) Bostrycin and 4deoxybostrycin: two nonspecific phytotoxins produced by *Alternaria eichorniae*. Applied Environment and Microbiology. 43, 846 - 849.

Ghosh, S.K. and Sen, S. (1973) Comparative physiological studies on four isolates of *Macrophomina phaseolina*. Indian Phytopathology. 26, 615 - 621.

Gupta, S.C. and Kolte, S.J. (1982) A comparative study of two isolates of *Macrophomina phaseolina* from leaf and root rot groundnut. Indian Phytopathology. 35, 222 - 225.

Hooda, I. and Grover, R,K. (1982). Studies on different isolates, age and quantity of inoculum of *Rhizoctonia bataticola* in relation to disease development in mungbean. Indian Phytopathology. 35, 619 - 623.

Hooda, I. and Grover, R.K. (1988) Effect of age, quantity of inoculum and isolates of *Macrophomina phaseolina* on the pathogens of mungbean and its control by chemicals. Indian Phytopathology. 41, 107 - 117.

Raut, J.C. and Ingle, R.W. (1989) Variation in isolates of *Rhizoctonia bataticola*. Indian Phytopathology. 42, 506 - 508.

Sharma, Y.K., Gaur, R.B. and Bisnoi, H.R. (2004) Cultural, morphological and physiological variability in *Macrophomina phaseolina*. Journal of Mycology and Plant Pathology. 34, 532-534.

Singh, R.D.N. and Kaiser, S.A.K.M. (1994) Effect of different media and pH levels on growth and cultural characteristics of charcoal rot pathogen (*Macrophomina phaseolina*) infecting maize. Crop Science. 7, 282 - 287.

Sobti, A.K. and Sharma, L.C. (1992) Cultural variability among three isolates of *Rhizoctonia bataticola* from groundnut. Indian Phytopathology. 41, 149 - 151.

Sundravadana, S. (2002) Management of blackgram (*Vigna mungo* (L). Hepper) root rot (*Macrophomina phaseolina* (Tassi.) Goid with bioagents and nutrient. M.Sc (Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Ulukus, I. (1984) A suitable medium for production of soluble red pigments by some strains of *Macrophomina phaseolina*. Journal of Turkish Phytopathology. 13, 53 - 61.