

INFLUENCE OF GROWTH MEDIUM ON THE PATHOGENICITY OF SOME ISOLATES OF *RHIZOCTONIA* SPP. ON COTTON SEEDLINGS

EMAN A. OSMAN, SALWA A.F. EL-AKKAD AND
SEHAM E. ABD-EL-GHAFOUR

Plant Pathology Res. Institute, Agricultural Research Center, Giza, Egypt.

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Abstract

The nutritional status of seven multinucleate isolates of *Rhizoctonia solani* (AG4) and one binucleate isolate of *Rhizoctonia* was varied by growing the isolates on four growth media with variable nutritional values (water agar, potato dextrose agar, Czapeck's-dox agar, and sorghum extract agar). Effect of growth medium on pathogenicity of the isolates, on cotton cultivars Giza 80 and Giza 89, was evaluated under laboratory and greenhouse conditions. Percentage of seed germination and radicle length were used as criteria for judging pathogenicity of the isolates under laboratory conditions. Pre-emergence damping-off, post-emergence damping-off, survival, and dry weight were used for judging pathogenicity under greenhouse conditions. Growth medium was a highly significant ($p \leq 0.01$) or a significant ($p \leq 0.05$) source of variation in all the laboratory or greenhouse variables used for evaluating pathogenicity of the isolates. This result confirms the importance of the mycelium nutritional status in determining its pathogenicity. Pathogenicity showed differential responses to the growth media that is, a single isolate can be highly pathogenic when it is grown on a particular medium, but may show only low pathogenicity on another medium. Significant positive and negative correlations were observed between laboratory and greenhouse variables. Two regression models, derived from stepwise multiple regression analysis, were constructed for each cultivar to predict pathogenicity of the isolates under greenhouse conditions. The results indicate that laboratory tests by using sorghum extract agar as growth medium can be used as a rapid method for evaluating differences among *Rhizoctonia* isolates; however, this should not replace pathogenicity tests with seedlings under greenhouse conditions.

INTRODUCTION

The occurrence and severity of a plant disease is dependent upon the interaction of many factors in the physical and biological environment. Several factors, e.g., temperature, moisture and light, have been investigated extensively, whereas pathogen nutrition has received less attention. Most studies on pathogen nutrition have considered nutrient requirements for growth (Sims, 1960 and Williams, 1965), sporulation, and spore germination (Maier, 1968 and Phillips, 1995). The quantity and quality of nutrients needed for maximum virulence, and

the relative importance of the inoculum have not been adequately investigated (Weinhold *et al.*, 1969). However, the influence of the growth medium on the capacity of spores (Maire, 1968) and mycelium (Sims, 1960) to initiate disease, and the effect of supplying nutrients along with the inoculum (Kraft and Erwin, 1968; and Toussoun *et al.*, 1960) have been studied.

Rhizoctonia solani kuhn, [teleomorph *Thanatephorus cucumeris* (Frank) Donk] is a common Basidiomycota plant pathogen with a broad host range, causing a variety of plant diseases, including seed-rot, pre- and post-emergence damping-off, crown and root-rot, and foliage blights (Sinclair and Backman, 1989). Investigations with *Rhizoctonia*, showed that after rather short period in soil, the pathogen becomes less efficient in attacking cotton stems. This decrease in virulence may result from a loss of nutrients required to support pathogenic activity (Weinhold *et al.*, 1969).

The present study was initiated to evaluate the role of pathogen nutrition on virulence of *Rhizoctonia* spp. isolates to cotton seedlings under laboratory and greenhouse conditions and to study the effect of growth medium on the correlation between laboratory and greenhouse parameters used for evaluating pathogenicity of *Rhizoctonia* spp. isolates.

MATERIALS AND METHODS

Source of isolates

Cotton seedlings showing damping-off symptoms were collected from different cotton growing areas. Segments with *Rhizoctonia* lesions were separately washed in running tap water, disinfested in 1% sodium hypochlorite for 5 min. and rinsed once in sterilized water. Small pieces, 2-3mm, of diseased tissues were plated on water agar and kept for 48 h at $25 \pm 1^\circ\text{C}$. Hyphal tips were transferred to acidified PDA slants. Pure isolates were stored at 5°C and served as stock cultures. Identification of anastomosis group (AG) of isolates was carried out according to El-Akkad (1997).

Effect of growth medium on linear growth of *Rhizoctonia* spp.

Eight isolates of *Rhizoctonia* spp. were grown in water agar (WA), potato-dextrose agar (PDA), Czapeck's dox agar (CZ), and sorghum extract agar (SO) media (Booth, 1971). Five mm diameter disks, cut from the growing edges of cultures of the tested isolates on PDA, were placed on the four media in the center of 9-cm diameter Petri dishes and incubated at $25 \pm 1^\circ\text{C}$ in the dark. Colony growth was monitored and recorded after 3 days. Four replicates were used for

each isolate.

Effect of growth medium on pathogenicity of *Rhizoctonia* spp. in laboratory

Four substrates were used to evaluate the pathogenicity of *Rhizoctonia* spp. isolates under laboratory conditions. Pathogenicity was determined according to the method of Anderson (1977), Carling *et al.* (1986) and Muyolo *et al.* (1993) with some modifications. A 5-mm disk, cut from 3-day-old culture on WA, CZ, PDA and SO, was placed in the center of 9-cm Oetri dish containing 20 ml of the same substrate, incubated for 3 days at $25 \pm 1^\circ\text{C}$ in the dark, and then covered with sterilized wet soil. Five cotton seeds of cultivar Giza 89, or 80 were placed on soil of each plate. The seeds were previously surface-disinfested with 1% sodium hypochlorite, washed in sterilized water. Plates were incubated at $25 \pm 1^\circ\text{C}$ for 5 days in the dark, and then placed on the laboratory bench at the same temperature. Three replicates were used, and plates untreated with the pathogen served as a control. Pathogenicity was evaluated after 9 days of incubation on the basis of seed germination (%) and length of radicle (cm).

Effect of growth medium on pathogenicity of *Rhizoctonia* spp. In greenhouse

A modified inoculum layer technique (Windels and Nabben, 1989) was used to examine the pathogenicity of isolates under greenhouse conditions. Eight isolates of *Rhizoctonia* spp. were cultured on WA, PDA, CZ, and SO and were incubated for 7 days at $25 \pm 1^\circ\text{C}$. The mixture of each medium and fungal mycelium was used to separately infest an autoclaved clay soil at a rate of 0.5 g/Kg soil. Seeds of cotton (*Gossypium barbadense* L.) cultivars Giza 89 or Giza 80 were sown in 10-cm-diameter sterilized clay pots at a rate of 10 seed per pot. Four replicates were used for each treatment. The temperature in the greenhouse was $26 \pm 4^\circ\text{C}$ throughout the experiment. The results were recorded as preemergence damping-off at 15 days after sowing, while post-emergence damping-off, surviving seedlings, and dry weight were recorded after 45 days.

Statistical Analysis

A completely randomized block design with four replications (greenhouse experiments) or 3 replicates (laboratory experiments) was used in the present study. Percentage data were transformed into arcsine angles (at the replicate level) before carrying out the analysis of variance (ANOVA) to produce an approximately constant variance. Least significant difference (LSD) was applied to compare treatment means. ANOVA of the data and correlation and regression analysis were performed with appropriate computerized programs (M STAT-C).

RESULTS

Effect of growth medium on linear growth of *Rhizoctonia* spp.

ANOVA (Table 1) showed that linear growth of isolates of *Rhizoctonia* spp. was significantly affected by the isolate, the growth medium, and the interaction between the isolate and the medium. Regarding the means in Table (2), it was clear that the isolates of *Rhizoctonia* spp. showed variation in their linear growth on different media. While PDA medium was the best medium for growth of isolate no. 1, SO medium was the best medium for growth of isolate no. 3. The growth of isolate no. 2 and no. 8 was similar on PDA, CZ and SO media; their growth also reached its maximum on these media. SO medium was the most suitable media for growth of isolates no. 5 and no. 7, while PDA the best for the growth of isolate no. 6. The maximum growth of isolate no. 4 was observed on SO and PDA media. It is noteworthy that SO medium was the most suitable medium for growth of most of the isolates, while WA medium was the least. In general, the variability in the linear growth of the isolates was evident on PDA and CZ media.

Table 1. Analysis of variance of the effect of growth medium on linear growth of *Rhizoctonia* spp. isolates.

Sources of variation	df	MS	F ^a
Isolate (s)	7	28.194	359.440**
Media (M)	3	123.274	1571.609**
S X M	21	4.361	55.599**
Error	96	0.078	

^aF. value is significant at $p \leq 0.01$ (**)

Table 2. Effect of growth medium on linear growth of *Rhizoctonia* spp. isolates

Isolate	Media				Mean
	Water Agar (WA)	Potato Dextrose Afir (PDA)	Czapek's Dox (CZ)	Sorghum (SO)	
S1	1.47	6.57	4.75	6.13	4.73
S2	6.82	9.00	9.00	9.00	8.46
S3	4.10	5.72	6.63	8.00	6.11
S4	2.38	9.00	8.13	9.00	7.13
S5	3.47	8.65	8.55	9.00	7.42
S6	2.63	8.52	5.38	8.38	6.22
S7	3.47	5.88	5.97	8.05	5.84
S8	7.25	9.00	9.00	9.00	8.56
Mean	3.95	7.79	7.17	8.32	

^aLinear growth in cm after three days at $25 \pm 1^\circ\text{C}$ in the dark

LSD for interaction between isolates and media = 0.392 ($p \leq 0.05$) or = 0.518 ($p \leq 0.01$)

Effect of growth medium on pathogenicity of *Rhizcottonia* spp. isolates on cultivar Giza 89 in laboratory:

ANOVA (Table 3) shows that the percentage of seed germination and the radicle length of cotton cultivar Giza 89 were significantly affected by the isolate used in soil infestation, the growth medium and the interaction between the isolate and the medium. Percentage of seed germination of cultivar Giza 89 on the different media significantly decreased by some isolates, while the percentage of seed germination was not affected by some others (Table 4). Isolate no. 1 caused 26.3 % reduction in seed germination on PDA medium, [(Control germination% - Treatment germination%)/Control germination %], while the same isolate reduced the seed germination by 65% on CZ medium, and it did not affect seed germination if grown on WA medium. Isolate no. 8 significantly reduced the seed germination on all media; however, its inhibitory effect ranged from 26.3% on WA medium to 57.9% on PDA medium. Isolate no. 5 caused complete inhibition of the seed germination on CZ and SO media. The same isolate (no. 5) caused 63.2% reduction on PDA medium, whereas no significant reduction in the seed germination was experienced on WA medium. Isolate no. 6 caused 100% reduction in seed germination on SO, 70% reduction on CZ medium, and had no significant effect on seed germination on WA and PDA media. On the contrary, isolates no. 3 and no. 7 had no significant effects on seed germination on any of the media tested. It can be seen that the effect of the fungal isolate on the seed germination of cultivar Giza 89 depends on the medium on which the isolate was grown.

It is noteworthy that all the tested isolates significantly reduced the radicle length on SO medium isolates especially no. 5 and no. 6, which completely inhibited the radicle growth (no germination) (Table 4). Isolate no. 4 reduced the radicle length by 60.3 % on PDA, by 73.1% on CZ, and by 73.9% on SO medium, while it increased the radicle length by 65.2% on WA medium. This stimulatory effect was also observed in case of isolates no. 2, 3 and 7. The increase in the radicle length by some isolates only on WA may indicate that these isolates were able to synthesize a stimulatory substance on this medium or that reduced amounts of metabolites inhibiting plant growth are formed in such a nutritionally poor medium.

Table 3. Analysis of variance of effect of *Rhizoctonia* spp. isolates on seed germination and radicle length of cotton (Cultivar Giza 89) under effect of different growth media.

Sources of variation	df	Seed germination		Radicle Length	
		MS	F ^a	MS	F
Isolate (s)	8	5036.046	24.086**	67.691	119.724**
Media (M)	3	4106.618	19.641**	184.201	325.792**
S X M	24	1034.024	4.946**	9.069	16.041**
Error	108	209.085		0.565	

^aF. value is significant at $p \leq 0.01$ (**)

Table 4. Effect of *Rhizoctonia* spp. isolates on seed germination and radicle length of cotton (Cultivar Giza 89) under effect of different growth media.

Cotton Seed Parameter	Isolate	Growth Medium				Mean
		WA	PDA	CZ	SO	
Seed Germination (%)	S1	95(83.36) ^a	70(57.10)	35(35.78)	35(35.78)	58.75(53.01)
	S2	85(60.27)	90(76.72)	80(66.91)	80(66.91)	81.25(67.70)
	S3	75(63.74)	85(73.55)	85(73.55)	80(70.38)	81.25(70.31)
	S4	90(76.72)	85(70.08)	50(45.00)	70(60.86)	73.75(63.16)
	S5	80(70.67)	35(35.78)	0	0	28.75(26.61)
	S6	80(63.43)	80(66.91)	30(32.90)	0	47.50(40.81)
	S7	95(83.36)	80(66.91)	85(70.08)	75(64.03)	83.75(71.09)
	S8	70(60.86)	40(38.95)	50(45.00)	70(60.58)	57.50(51.35)
	Control	95(83.36)	95(83.36)	100(90.00)	95(83.36)	96.25(85.02)
	Mean	83.9(71.75)	73.3(63.26)	57.2(51.02)	56.1(49.10)	

^aPercentage data were transformed into arc sine angles before carrying out analysis of variance to produce approximately constant variance. Transformed data are shown in parentheses.

LSD (transformed data) for the interaction between isolates and media = 20.25 ($p \leq 0.05$) or = 26.76 ($p \leq 0.01$).

Radicle Length (cm)	S1	6.63	0.50	0.50	0.50	2.03
	S2	8.88	5.50	2.50	3.25	5.03
	S3	8.75	3.13	6.25	0.50	4.91
	S4	9.50	2.38	1.75	1.50	3.78
	S5	1.50	0.75	0	0	0.56
	S6	5.63	0.50	0.50	0	1.41
	S7	10.50	3.75	5.25	3.13	5.66
	S8	5.00	0.63	0.50	0.50	1.66
	Control	5.75	6.00	6.50	5.57	6.00
	Mean	6.79	2.57	2.64	1.79	

LSD for the interaction between isolates and media = 1.05 ($p \leq 0.05$) or = 1.39 ($p \leq 0.01$).

Effect of growth medium on pathogenicity of *Rhizoctonia* spp. Isolates on cultivar Giza 80 in laboratory:

ANOVA (Table 5) show that both seed germination and radicle length of cotton cultivar Giza 80 were affected by the isolate and the growth medium, while the interaction between the isolate and the medium significantly affected the radicle length only. There was significant difference between the general mean of seed germination of cultivar Giza 80 on WA medium and that on both PDA medium and SO medium (Table 6), while the difference was nonsignificant between the general mean of seed germination on WA medium and that on CZ medium. It is noteworthy that there were no significant differences among the means of seed germination on the three media SO, CZ, and PDA. When the general means of seed germination on different media were compared, it was found that WA was the most suitable media for seed germination, while SO was the least suitable regardless of the isolate. When the general means of seed germination of cultivar Giza 80, under the effects of different isolates of *Rhizoctonia* spp., were compared, it was found that isolates nos. 1, 2, 5, 6, and 8 significantly reduced the percentage of seed germination regardless of the growth medium. Isolate no. 8 was the most pathogenic isolate as it inhibited the seed germination by 35.1% compared with the control, followed by isolate no. 6, which reduced the seed germination by 32.5%. On the other hand, isolate no. 2 was the least pathogenic, as it caused only 11.7% reduction in germination. Isolates nos. 3, 4, and 7 did not significantly affect seed germination of this cultivar.

Since radicle length of cultivar Giza 80 was affected by isolate x growth medium interaction, LSD was used to compare between isolate means within growth media. These comparisons revealed that isolate no. 1 caused significant reduction in the radicle length by 88.5% compared with the control on PDA medium, while the same isolate had no significant effect on the radicle length on WA medium (Table 6). Isolate no. 2 caused significant increase in radicle length (20.8%) on WA medium, while it reduced the radicle length significantly on other media. The maximum reduction of 52% was on SO medium compared with the control. It is worth noting that isolate no. 8 reduced the radicle length significantly on all the growth media and this reduction reached its maximum level on SO medium (92%). It can also be noticed that all isolates reduced the radicle length on PDA and SO media, while the variability among the isolates was clear on WA and CZ media.

Table 5. Analysis of variance of the effect of *Rhizoctonia* spp. isolates on seed germination and radicle length of cotton (Cultivar Giza 80) under effect of different growth media.

Sources of variation	df	Seed germination		Radicle Length	
		MS	F ^a	MS	F
Isolate (s)	8	1476.196	5.245**	56.630	86.981**
Media (M)	3	1540.701	5.474**	53.668	100.694**
S X M	24	193.458	0.687	2.355	4.418**
Error	108	281.437		0.533	

^aF. value is significant at $p \leq 0.01$ (**)

Table 6. Effect of *Rhizoctonia* spp. isolates on seed germination and radicle length of cotton (Cultivar Giza 80) under effect of different growth media.

Cotton Seed Parameter	Isolate	Growth Medium				Mean
		WA	PDA	CZ	SO	
Seed Germination (%)	S1	85(70.08) ^a	70(60.86)	70(60.86)	75(60.27)	75.00(63.02)
	S2	90(76.72)	80(66.91)	90(76.72)	80(66.91)	85.00(71.81)
	S3	100(90.00)	90(76.72)	85(70.08)	80(66.91)	88.75(75.93)
	S4	95(83.36)	80(70.67)	95(83.36)	95(83.36)	91.25(80.19)
	S5	95(83.36)	70(64.62)	80(70.38)	75(60.27)	80.00(69.66)
	S6	70(73.55)	55(48.17)	80(66.91)	55(47.88)	65.00(59.13)
	S7	90(76.72)	85(73.55)	90(76.72)	80(70.67)	86.25(74.41)
	S8	90(76.72)	60(54.81)	65(57.97)	35(35.78)	62.50(56.32)
	Control	95(83.36)	95(83.36)	95(83.36)	100(90.00)	96.25(85.02)
	Mean	90.0(79.32)	76.1(66.63)	83.3(71.82)	75.0(64.67)	

^aPercentage data were transformed into arc sine angles before carrying out analysis of variance to produce approximately constant variance. Transformed data are shown in parentheses.

LSD (transformed data) for the interaction between isolates = 11.74 ($p \leq 0.05$) or = 15.52 ($p \leq 0.01$).

LSD for media = 7.83 ($p \leq 0.05$) or = 10.35 ($p \leq 0.01$)

Radicle Length (cm)	S1	5.00	0.63	1.50	1.00	2.03
	S2	7.25	3.13	4.13	3.00	1.38
	S3	6.63	4.13	6.13	3.50	5.09
	S4	6.13	2.50	4.38	3.50	4.13
	S5	2.00	0.75	1.50	1.75	1.50
	S6	3.50	0.50	1.88	0.75	0.66
	S7	5.50	2.00	4.00	2.75	3.56
	S8	2.38	0.50	1.63	0.50	1.25
	Control	6.00	5.50	5.50	3.25	5.81
	Mean	4.93	2.18	3.40	2.56	

LSD for the interaction between isolates and media = 1.02 ($p \leq 0.05$) or = 1.35 ($p \leq 0.01$).

Greenhouse Tests

Regarding the cotton cultivar Giza 80, ANOVA (Table 7) show that the isolate of *Rhizoctonia* spp. was a source of highly significant variation in all the parameters under study. The growth medium was not a source of significant variation in the percentage of the post emergence damping-off; however, a highly significant or significant variation were reflected in the other parameters (pre-emergence damping-off, survival, and dry weight). The interaction between the isolate and the medium was significant or highly significant in all the parameters under study. Therefore, and interaction LSD was calculated to compare between isolates means with growth medium for all the parameters tested. These comparisons showed that isolates nos. 1, 5, and 6 caused significant increases in the percentages of pre-emergence damping-off when the growth media were PDA, CZ, or SO, while the same isolates had no significant effect on the percentage of pre-emergence damping-off when grown on WA (Table 8). Isolate no. 7 caused significant increase in the percentage of the pre-emergence damping-off only when grown on CZ. It can be noticed that all the isolates tested had no significant effect on the percentage of the seedling mortality in the pre-emergence stage when the growth medium was WA.

With respect to post-emergence damping-off, isolate no. 2 caused higher level of infection when grown on CZ or SO media; however, it had no significant effect during this stage when grown on WA or PDA. Isolate no. 1 caused significant increase in infection, only when grown on CZ medium, while isolate no. 8 caused significant increase in infection only when grown on SO medium. All the isolates tested showed no significant effects on post emergence damping-off when the growth medium was WA or PDA. It is noteworthy that most of the isolates tested showed higher levels of pathogenicity at the pre-emergence stage compared to the post-emergence stage.

Isolates nos. 1, 5, and 6 caused highly significant reduction in the surviving seedlings when the growth media were SO, CZ, and PDA, while the same isolates did not cause significant reduction in the surviving seedlings when propagated on WA.

Isolate no. 7 caused significant reduction in the surviving seedlings when grown on CZ. Isolates nos. 7 and 8 showed opposite effects on the percentage of the surviving seedlings grown on CZ. Inoculum of isolate no. 7 taken from CZ was highly pathogenic, while that of the isolate no. 8 taken from the same medium was non-pathogenic.

Table 7. Analysis of variance of the effect of *Rhizoctonia* spp. isolates, growth medium, and their interaction on damping-off of cotton seedlings (Cultivar Glza 89) under greenhouse conditions.

Sources of variation	df	Pre-emergence damping-off		Post-emergence damping-off		Survival		Dry Weight	
		MS	F ^a	MS	F	MS	F	MS	F
Replicates	3	416.37	1.04	1.69	0.46	367.81	0.94	27463.33	0.57
Isolate (s)	8	5417.08	16.55**	16.04	4.32**	6671.69	16.98**	644102.25	13.46**
Media (M)	3	4208.61	10.52**	4.23	1.14	5597.56	14.24**	179060.52	3.74*
S X M	24	1178.73	2.95**	6.75	1.82*	1285.42	3.27**	119441.76	2.50**
Error	105	399.87		3.72		392.97		47862.45	

^aF value is significant at $p \leq 0.05$ (*) or at $p \leq 0.01$ (**)

Table 8. Effect of growth media on pre-emergence damping-off, post-emergence damping-off, survival, and dry weight of cotton seedlings (Cultivar Giza 89) induced by *Rhizoctonia* spp. isolates under greenhouse conditions.

Parameter	Isolate	Growth Medium				Mean
		WA	PDA	CZ	SO	
Pre-emergence damping-off	S 1	15(16.45) ^a	100(90.0)	65(57.69)	95(83.36)	68.75(61.88)
	S 2	35(35.78)	30(32.62)	50(45.0)	40(35.19)	38.75(37.15)
	S 3	20(19.33)	15(16.45)	25(29.73)	10(13.28)	17.50(19.70)
	S 4	20(23.09)	45(41.83)	35(32.31)	10(13.28)	27.50(27.63)
	S 5	20(26.57)	95(83.36)	100(90.0)	75(67.50)	72.50(66.86)
	S 6	30(29.42)	80(66.91)	95(83.36)	90(80.19)	73.75(64.97)
	S 7	25(29.73)	30(29.14)	75(67.50)	15(19.92)	36.25(36.57)
	S 8	15(16.45)	25(26.26)	15(16.45)	50(45.00)	26.25(26.04)
	Control	35(32.31)	20(23.09)	30(29.14)	35(32.31)	30.00(29.21)
	Mean	23.9(25.46)	48.9(45.52)	54.4(50.13)	46.7(43.34)	

^aPercentage data were transformed into arc sine angles before carrying out analysis of variance to produce approximately constant variance. Transformed data are shown in parentheses.

LSD (transformed data) for the interaction between isolates and media = 28.0 ($p \leq 0.05$) or = 37.0 ($p \leq 0.01$).

Post-emergence damping-off	S 1	10(2.12)	0(0.71)	30(4.84)	5(1.66)	11.25(2.33)
	S 2	15(2.47)	10(2.62)	35(5.80)	40(6.56)	25.00(4.36)
	S 3	10(2.12)	5(1.66)	0(0.71)	0(0.71)	3.75(1.30)
	S 4	0(0.71)	0(0.71)	5(1.66)	0(0.71)	1.25(0.95)
	S 5	15(2.47)	5(1.66)	0(0.71)	10(2.12)	7.50(1.74)
	S 6	0(0.71)	20(4.03)	5(1.66)	5(1.66)	7.50(2.02)
	S 7	0(0.71)	10(2.62)	0(0.71)	5(1.66)	3.75(1.42)
	S 8	0(0.71)	5(1.66)	5(1.66)	25(4.39)	8.75(2.10)
	Control	5(1.66)	5(1.66)	0(0.71)	5(1.66)	3.75(1.42)
	Mean	3.11(1.52)	6.7(1.93)	8.9(2.05)	10.6(2.35)	

LSD (transformed data) for the interaction between isolates and media = 2.7 ($p \leq 0.05$) or = 3.57 ($p \leq 0.01$).

Survival	S 1	75(67.50)	0(0.0)	5(6.64)	0(0.0)	20.00(18.54)
	S 2	45(41.53)	60(51.05)	15(16.45)	15(16.45)	33.75(31.37)
	S 3	70(60.86)	80(66.91)	75(60.72)	90(76.72)	78.75(66.19)
	S 4	80(66.91)	55(48.17)	60(54.81)	90(76.72)	71.25(61.65)
	S 5	65(54.22)	0(0.0)	0(0.0)	15(16.45)	20.00(17.67)
	S 6	70(60.58)	0(0.0)	0(0.0)	5(6.64)	18.75(16.80)
	S 7	75(60.27)	60(51.05)	25(22.50)	80(66.91)	60.00(50.78)
	S 8	85(73.55)	70(57.10)	80(66.91)	25(25.97)	65.00(55.88)
	Control	60(54.53)	75(60.27)	70(60.86)	60(51.05)	66.25(56.68)
	Mean	69.4(59.99)	44.4(37.17)	36.7(32.05)	42.2(37.43)	

LSD (transformed data) for the interaction between isolates and media = 2.7 ($p \leq 0.05$) or = 3.57 ($p \leq 0.01$).

Dry Weight/Plant (mg)	S 1	613.00	0	308.75	0	230.44
	S 2	409.00	491.50	336.25	446.25	420.75
	S 3	398.75	606.50	566.75	483.25	513.81
	S 4	426.00	748.75	500.00	729.00	600.94
	S 5	520.25	0	0	320.00	211.31
	S 6	323.25	0	0	0	50.81
	S 7	748.50	392.75	305.75	649.00	524.00
	S 8	661.00	833.00	515.25	770.00	694.81
	Control	446.25	400.75	507.50	379.25	433.44
	Mean	505.11	385.92	337.81	420.19	

LSD for the interaction between isolates and media = 2.7 ($p \leq 0.05$) or = 3.57 ($p \leq 0.01$).

All the tested isolates did not significantly affect the dry weight per plant when grown on WA. On the other media, isolates exhibited variations in their effects on the dry weight. For example, isolates nos. 1, 2 and 7 on CZ medium did not affect the dry weight. Isolate no. 4 caused the highest increase in dry weight (85.8%) when the growth medium was PDA. Isolate no. 8 also increased dry weight (107.9%) if grown on PDA. It can be noticed that isolate no. 8 caused an increase in the dry weight per plant compared with the control with all the growth media; however, this increase was nonsignificant in case of WA and CZ media, while it was significant in case of SO medium and highly significant with PDA. The most likely explanation for such increases in dry weight is that less competition for available nutrients occurred in the infested pots due to the less number of surviving seedlings. On the contrary, more competition occurred in the control pots due to the higher number of surviving seedlings. Thus, the less competition in the infested pots (less survivals) improved the performance of the single seedlings in terms of dry weight up to a certain time.

Concerning the cotton cultivar Giza 80, ANOVA (Table 9) show that the pathogen (*Rhizoctonia* spp.), the growth medium, and their interaction were sources of significant or highly significant variation in all variables under study. *Rhizoctonia* isolates nos. 1 and 2 caused significant or highly significant increases in pre-emergence damping-off when the inoculum was grown on any growth medium except SO medium (Table 10), while isolates nos. 5 and 6 caused highly significant increases in the disease incidence at the same stage when the inoculum was raised on any growth medium except WA.

Isolate no. 4 exhibited a significant increase in disease incidence in this stage only when grown on PDA. Isolate no. 5 grown on PDA or CZ media and isolate no. 1 on PDA caused the highest mortality in the pre-emergence stage (100%). It can be noticed that 75% of *Rhizoctonia* isolates were highly pathogenic on cultivar Giza 80 when inocula were derived from PDA cultures.

In general, the isolates were less pathogenic in the post emergence stage. Isolates nos. 2 and 6 caused significant or highly significant increases in post emergence damping-off when grown on CZ and SO media, while isolate no. 5 behaved similarly only when the inoculum was grown on SO medium. It is noteworthy that all the tested isolates had no significant effect on post-emergence damping-off when they were grown on PDA medium. Only one isolate (isolate no. 1) caused significant increase the percentage of infected seedlings in this stage when WA was used as a growth medium.

Table 9. Analysis of variance of the effect of *Rhizoctonia* spp. isolates, growth medium, and their interaction on damping-off of cotton seedlings (Cultivar Glza 80) under greenhouse conditions.

Sources of variation	df	Pre-emergence damping-off		Post-emergence damping-off		Survival		Dry Weight	
		MS	F ^a	MS	F	MS	F	MS	F
Replicates	3	195.85	0.66	4.6	1.29	211.71	0.58	19001.41	0.54
Isolate (s)	8	5632.93	18.99**	21.72	6.10**	9694.22	26.53**	646284.94	18.20**
Media (M)	3	1176.70	15.09**	12.37	3.47*	6641.25	18.17**	156896.08	4.42**
S X M	24	885.51	2.99**	6.97	1.96**	983.61	2.69**	73567.62	2.07**
Error	105	296.61		3.56		365.44		35501.54	

^aF value is significant at $p \leq 0.05$ (*) or at $p \leq 0.01$ (**)

Table 10. Effect of growth media on pre-emergence damping-off, post-emergence damping-off, survival, and dry weight of cotton seedlings (Cultivar Giza 80) induced by *Rhizoctonia* spp. isolates under greenhouse conditions.

Parameter	Isolate	Growth Medium				Mean
		WA	PDA	CZ	SO	
Pre-emergence damping-off	S1	25(29.73) ^a	100(90.0)	60(58.28)	40(35.19)	56.25(53.30)
	S2	25(29.73)	45(42.12)	55(48.17)	45(41.83)	42.50(40.46)
	S3	15(19.92)	20(26.57)	35(36.06)	35(36.06)	30.00(29.65)
	S4	15(19.92)	30(35.78)	10(13.28)	15(19.92)	17.50(22.23)
	S5	10(13.28)	100(90.0)	100(90.0)	70(60.86)	70.00(63.54)
	S6	25(22.50)	85(70.08)	70(60.86)	65(57.69)	61.25(52.78)
	S7	15(16.45)	10(13.28)	20(26.57)	20(19.33)	16.25(18.91)
	S8	15(16.45)	15(19.92)	30(29.14)	35(32.31)	23.75(24.46)
	Control	0	0	10(9.81)	15(19.92)	6.25(7.43)
	Mean	16.1(18.67)	45.0(43.08)	43.3(41.35)	37.8(35.90)	

^aPercentage data were transformed into arc sine angles before carrying out analysis of variance to produce approximately constant variance. Transformed data are shown in parentheses.

LSD (transformed data) for the interaction between isolates and media = 24.11 ($p \leq 0.05$) or = 31.87 ($p \leq 0.01$).

Post-emergence damping-off	S1	25(3.73)	0.0(0.71)	40(4.84)	20(3.54)	21.25(3.20)
	S2	0.0(0.71)	15(3.57)	25(4.99)	25(4.99)	16.25(3.56)
	S3	0.0(0.71)	5(1.66)	0.0(0.71)	0.0(0.71)	1.25(0.95)
	S4	10(2.62)	10(2.62)	0.0(0.71)	0.0(0.71)	5.00(1.66)
	S5	0.0(0.71)	0.0(0.71)	0.0(0.71)	25(4.39)	6.25(1.63)
	S6	5(1.66)	15(3.57)	30(4.68)	35(5.30)	21.25(3.81)
	S7	0.0(0.71)	0.0(0.71)	0.0(0.71)	0.0(0.71)	0.0(0.71)
	S8	5(1.66)	15(3.08)	5(1.66)	20(4.03)	11.25(2.61)
	Control	0.0(0.71)	5(1.66)	0.0(0.71)	5(1.66)	2.50(1.18)
	Mean	5.0(1.47)	7.2(2.03)	11.1(2.19)	14.4(2.89)	

LSD (transformed data) for the interaction between isolates and media = 2.64 ($p \leq 0.05$) or = 3.49 ($p \leq 0.01$).

Survival	S1	50(41.53)	0	0	40(35.19)	22.50(19.18)
	S2	75(60.27)	40(39.23)	20(19.62)	30(25.38)	41.25(36.12)
	S3	85(70.08)	75(60.27)	65(53.94)	65(53.94)	72.50(59.55)
	S4	75(63.74)	55(47.88)	90(76.72)	85(70.08)	76.25(64.61)
	S5	90(76.72)	0	0	5(6.64)	23.75(20.84)
	S6	70(60.86)	0	0	0	17.5(15.21)
	S7	85(73.55)	90(76.72)	80(63.43)	80(70.67)	83.75(71.09)
	S8	80(70.67)	70(60.86)	65(57.69)	45(41.83)	65(57.76)
	Control	100(90.00)	95(83.38)	90(80.19)	80(33.91)	91.25(80.12)
	Mean	78.9(67.49)	47.2(40.92)	45.6(39.07)	47.8(41.18)	

LSD (transformed data) for the interaction between isolates and media = 26.76 ($p \leq 0.05$) or = 35.37 ($p \leq 0.01$).

Dry Weight/Plant (mg)	S1	409.00	0	0	326.25	183.81
	S2	587.25	650.00	313.75	344.25	473.81
	S3	495.75	528.50	544.25	357.25	481.44
	S4	602.50	661.50	588.75	553.25	601.50
	S5	587.50	0	0	81.75	167.31
	S6	146.75	0	132.50	75.00	88.56
	S7	651.50	510.75	554.75	584.75	575.44
	S8	590.00	664.50	544.25	615.00	603.44
	Control	413.75	292.75	557.25	418.50	420.56
	Mean	498.22	367.56	359.50	372.89	

LSD for the interaction between isolates and media = 236.80 ($p \leq 0.05$) or = 348.67 ($p \leq 0.01$).

Isolates nos. 1, 2, and 6 caused significant or highly significant reductions in the percentage of surviving seedlings, while isolates nos. 3, 7, and 8 had no significant effect regardless of the growth medium. Isolate no. 5 caused 100% seedlings mortality when grown on PDA and CZ media, while it had no effect on the survival when grown on WA. Isolate no. 6 was pathogenic regardless of the medium; however, it showed the lowest pathogenicity when grown on WA.

Regarding the dry weight per plant of cotton cultivar Giza 80, it was found that isolate no. 6 caused significant reduction in the dry weight of surviving seedlings regardless of the growth medium. The maximum reduction in the dry weight per plant of the surviving seedlings (82.1 %) was observed when the inoculum was taken from SO – grown culture. Isolate no. 5 caused highly significant reduction in the dry weight of the surviving seedlings (80.5%) when the inoculum was grown on SO medium. Isolates nos. 2,4 and 8 significantly increased the dry weight per plant when their inocula were grown on PDA medium. Of these isolates, isolate no. 8 caused the highest increase (127%).

Relationship between laboratory and greenhouse parameters

Effects of growth media on the correlation between laboratory and greenhouse parameters for evaluating pathogenicity of *Rhizoctonia* spp. isolates on cultivar Giza 89 and Giza 80 are shown in Tables (11) and (12), respectively. Linear growth, seed germination, and radicle length were measured under laboratory conditions, while the other parameters were measured under greenhouse conditions.

A significant positive correlation ($r=0.67$, $p\leq 0.10$) was observed between linear growth and post emergence damping-off of Giza 80 when the growth medium was PDA. On the other hand, the linear growth showed no correlation with any other greenhouse parameter regardless of the growth medium and cultivar (Tables 11 and 12). Significant correlations were observed between seed germination of Giza 89 on SO medium and all the greenhouse parameters except post emergence damping-off (Table 11). Seed germination was also correlated with the plant dry weight when the growth medium was CZ (Table 11). In case of Giza 80, seed germination was correlated only with the pre-emergence damping-off and dry weight when the growth medium was WA (Table 12). Radicle length of Giza 89 was positively correlated with the pre-emergence damping-off and survival when the growth medium was PDA and with pre-emergence damping-off when the growth medium was SO (Table 11). The radicle length of Giza 80 on CZ medium was correlated with survival and dry weight, while the radicle length on SO medium was correlated with post-emergence damping-off and survival (Table 12).

Stepwise multiple regression was used to construct four regression models to predict pathogenicity of *Rhizoctonia* spp. isolates under greenhouse conditions as a function of seed germination and radicle length. R^2 of the models ranged from 38.50 to 39.80% (Table 13).

Table 11. Effect of growth media on correlation between laboratory parameters and greenhouse parameters that evaluate pathogenicity of *Rhizoctonia* spp. On cotton cultivar Gixa 89.

Growth Media	Parameters	Parameters					
		7	6	5	4	3	2
Water Agar (WA)	1- Linear growth	0.0907 ^a	-0.2877	0.1254	0.2282	0.0033	-0.7751*
	2- Seed germination	0.3772	0.2607	-0.2135	-0.1572	0.3661	
	3- Radicle length	0.0913	-0.0320	-0.2480	0.2336		
	4- Pre-emergence damping-off	-0.4767	-0.7734*	0.1455			
	5- Post-emergence damping-off	-0.2168	-0.7343*				
	6- Survival	0.4447					
	7- Dry weight						
Potato-Dextrose Agar (PDA)	1- Linear growth	0.1501	-0.1613	0.1115	0.1426	-0.1555	-0.3059
	2- Seed germination	0.1355	0.2962	0.2222	-0.3438	0.6430 ^x	
	3- Radicle length	0.3873	0.6271 ^x	0.0858	-0.6541 ^x		
	4- Pre-emergence damping-off	-0.8609**	0.9820**	-0.0081			
	5- Post-emergence damping-off	-0.3262	-0.1809				
	6- Survival	0.9083**					
	7- Dry weight						
Czapek's Dox Agar (CZ)	1- Linear growth	0.2303	0.34641	-0.0240	-0.3797	-0.1666	-0.083
	2- Seed germination	0.6706 ^x	0.4702	0.1165	-0.5564	0.8475**	
	3- Radicle length	0.4974	0.3979	-0.2392	-0.3198		
	4- Pre-emergence damping-off	-0.9562**	-0.9092**	-0.0559			
	5- Post-emergence damping-off	0.0359	-0.3649				
	6- Survival	0.8767**					
	7- Dry weight						
Sorghum Extract Agar (SO)	1- Linear growth	0.5802	0.1778	0.4008	-0.3720	0.1281	0.1129
	2- Seed germination	0.7340*	0.6309 ^x	0.2594	-0.8243*	0.7590*	
	3- Radicle length	0.4625	0.4619	0.3426	-0.6824 ^x		
	4- Pre-emergence damping-off	-0.8207*	-0.9192**	0.0771			
	5- Post-emergence damping-off	0.1789	-0.4629				
	6- Survival	0.6701 ^x					
	7- Dry weight						

^aLinear correlation coefficient (r) is significant at $p \leq 0.01$ (**), $p \leq 0.05$ (*) or $p \leq 0.10$ (x)

Table 11. Effect of growth media on correlation between laboratory parameters and greenhouse parameters that evaluate pathogenicity of *Rhizoctonia* spp. On cotton cultivar Gixa 89.

Growth Media	Parameters	Parameters					
		7	6	5	4	3	2
Water Agar (WA)	1- Linear growth	0.4044 ^a	0.4133	-0.5417	-0.0799	0.1086	0.2295
	2- Seed germination	0.8142*	0.5151	-0.2689	-0.6903 ^x	0.2239	
	3- Radicle length	0.277	-0.0071	-0.1851	0.2840		
	4- Pre-emergence damping-off	-0.6183	-0.7840*	0.444			
	5- Post-emergence damping-off	-0.2980	-0.9043**				
	6- Survival	0.501					
	7- Dry weight						
Potato-Dextrose Agar (PDA)	1- Linear growth	0.0962	-0.3365	0.6723 ^x	0.1857	-0.3308	-0.5513
	2- Seed germination	0.4951	0.5890	-0.4149	-0.5011	0.8713**	
	3- Radicle length	0.6136	0.5659	0.0229	-0.5595		
	4- Pre-emergence damping-off	-0.9108**	-0.9808**	-0.2427			
	5- Post-emergence damping-off	0.4048	0.0544				
	6- Survival	0.8505**					
	7- Dry weight						
Czapek's Dox Agar (CZ)	1- Linear growth	0.2996	0.2351	-0.4898	-0.0359	0.0734	0.1190
	2- Seed germination	0.3779	0.3919	-0.3463	-0.3221	0.7198*	
	3- Radicle length	0.6782 ^x	0.6210 ^x	-0.4293	-0.5764		
	4- Pre-emergence damping-off	-0.9063**	-0.9193	0.3745			
	5- Post-emergence damping-off	-0.6363	-0.7092				
	6- Survival	0.9592**					
	7- Dry weight						
Sorghum Extract Agar (SO)	1- Linear growth	0.0698	-0.0809	0.0621	0.0890	0.2256	-0.1122
	2- Seed germination	0.0210	0.4534	-0.5530	-0.3528	0.8278*	
	3- Radicle length	0.2769	0.6249 ^x	-0.7235	-0.5137		
	4- Pre-emergence damping-off	-0.9144**	-0.9729**	0.8443**			
	5- Post-emergence damping-off	-0.6789 ^x	-0.9454**				
	6- Survival	0.8494**					
	7- Dry weight						

^aLinear correlation coefficient (r) is significant at $p \leq 0.01$ (**), $p \leq 0.05$ (*) or $p \leq 0.10$ (x)

Table 13. Regression models that describe pathogenicity (Y) of *Rhizoctonia* spp. isolates on cotton seedlings under greenhouse conditions as a function of seed germination (X_2) or radicle length (X_3) under laboratory conditions.

Cultivar	Growth Medium	Regression model	Coefficient of determination (R^2)	F. value
Giza 80	WA	 ^a		
	PDA			
	CZ	$Y^b = 103.2299 - 13.77844 X_3$	38.5	$3.76x^c$
	SO	$Y = 89.66571 - 15.95974 X_3$	39.05	$3.84x$
Giza 89	WA			
	PDA	$Y = 84.08528 - 11.55361 X_3$	39.33	$3.89(x)$
	CZ			
	SO	$Y = 96.74815 - 0.7170371 X_2$	39.80	$3.97(x)$

^a No regression model could be constructed.

^b Pathogenicity was evaluated as the percentage of dead seedlings after 45 days from planting date.

^c F. value is significant at $p < 0.10$

DISCUSSION

For infection by *Rhizoctonia solani* to occur, the propagules of the fungus must germinate or resume growth. Hyphae must then grow to the host, form infection structures, penetrate the epidermis, and establish themselves in the host. These processes require energy and the synthesis of new structural materials. Only three sources are available to provide the required nutrients: (i) propagules of the fungus; (ii) soil solution and organic matter in soil; and (iii) exudates from the host plant (Weinhold *et al.*, 1969). Early studies (Weinhold *et al.*, 1969) showed that when the mycelium of *Rhizoctonia solani* was deficient in either carbon or nitrogen, it could grow vegetatively, but its ability to attack the stem of cotton seedlings was markedly reduced. In the present study, the nutritional status of *Rhizoctonia* mycelium was varied by growing the pathogen on media with varying nutritional values. Pathogenicity of this mycelium on cotton seedlings was evaluated under laboratory and greenhouse conditions. Growth medium was a source of highly significant ($p \leq 0.01$) or significant ($p \leq 0.05$) variation in all the laboratory and greenhouse variables used to evaluate pathogenicity of the fungus. This result confirms the importance of the mycelium nutritional status in determining its pathogenicity. Pathogenicity showed differential responses to the growth media *i.e.* a single isolate can be highly path-

ogenic when grown on a particular medium, but may show only low pathogenicity on another medium. The effect of growth medium on the pathogenicity of plant pathogenic fungi, as have been demonstrated herein., was also demonstrated by Phillips (1965) with *Fusarium roseum f. cerealis* and Maier (1986) with *F. solani f. phaseoli*, who found that the medium on which spores were produced influenced their pathogenic capability. From the practical point of view, our results imply the following: (i) when evaluating pathogenicity of *Rhizoctonia* spp. the nutritional status of the inoculum must be taken into account. Criteria such as dry weight or volume of inoculum are not necessarily indicative, because the fungus can make satisfactory vegetative growth on a medium, yet the mycelium will exhibit a degree of virulence far below the potential of the fungus (Weinhold *et al.*, 1969), (ii) Because crop rotation may play any important role in managing soil-borne diseases, it is desirable to rotate cotton with crops whose residues are not nutritionally adequate for increasing pathogenicity of *Rhizoctonia* spp. on cotton. However, more research is needed to determine such crops.

Two regression models, derived from stepwise multiple regression analysis, were constructed for each cultivar to predict pathogenicity of *Rhizoctonia* isolates under greenhouse conditions. The results indicate that the appropriate model for prediction was affected by cotton cultivar, growth medium, and the laboratory variable used for prediction. WA was not an appropriate growth medium to predict pathogenicity *Rhizoctonia* isolates on any cultivar. PDA was not appropriate in the case of Giza 80, while it was in the case of Giza 89. On the contrary, CZ was appropriate in the case of Giza 80 while it was not in the case of Giza 89. SO was appropriate for predicting the pathogenicity of isolates on both cultivars provided the use of radicle length and seed germination as predictors in the case of Giza 80 and Giza 89, respectively. It is worthnoting that linear growth was not included in any model. This may be due to the fact that linear growth is a function of saprophytic capability of the fungus, which may not be related to its pathogenic capability. Our results, which are in agreement with those reported by Muyolo *et al.* (1993), suggest that laboratory tests by using SO can be used as rapid methods for evaluating pathogenicity differences among *Rhizoctonia* isolates; however, they should not replace pathogenicity tests with seedlings in greenhouse which are confirmative.

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تأثير بيئة النمو على قدرة عزلات فطر الريزوكتونيا على إصابة بادرات القطن

إيمان أمين عثمان، سلوى أحمد العقاد، سهام عزت عبد الغفور

معهد بحوث أمراض النبات - مركز البحوث الزراعية - الجيزة - مصر

أمكن التأثير علي الحالة الغذائية لسبع عزلات من فطر الريزوكتونيا سولاني وعزلة من فطر الريزوكتونيا سولاني وعزلة من فطر الريزوكتونيا ثنائي الأنوية، وذلك بتنميتها علي أربع بيئات تتفاوت فيما بينها من حيث القيمة الغذائية (بيئة الآجار المائي وبيئة آجار البصل والديكستروز وبيئة تشابكس آجار مستخلص الذرة الرفيعة). قيم تأثير بيئة النمو على القدرة المرضية للعزلات تحت ظروف المعمل والصوبة باستعمال صنفى القطن جيزة ٨٩ وجيزة ٨٩، أستعملت النسبة المئوية للإنبات وطول الجذير كمعايير للحكم على القدرة المرضية للعزلات تحت ظروف المعمل، فى حين أستعملت الإصابة فى مرحلتى ما قبل ومل بعد ظهور البادرات فوق سطح التربة والبادرات السليمة فى نهاية التجربة وكذلك الوزن الجاف للبادرات كمعايير للحكم على القدرة المرضية للعزلات وتأثيرها تحت ظروف تاصوبة. كانت بيئة النمو مصدرا عالى المعنوية أو مصدرا معنويا للتباين فى جميع المؤشرات المستعملة لتقييم القدرة المرضية تحت ظروف تامعمل الصوبة، مما يؤكد أهمية الحالة الغذائية للميسليوم فى تحديد قدرته المرضية. أظهرت القدرة المرضية للعزلات استجابة متباينة لبيئة النمو المستعملة بحيث يمكن القول بأن العزلة ذات القدرة المرضية العالية عند تنميتها على بيئة نمو معينة قد تهر قدرة مرضية منخفضة عند تنميتها على بيئة أخرى. أمكن الكشف عن العديد من الارتباطات المعنوية الموجبة والسالبة بين مؤشرات المعمل والصوبة المستعملة لتقييم القدرة المرضية للعزلات. أمكن لكل صنف قطن - عن طريق تحليل البيانات باستخدام الانحدار المتعدد المرحلى - التوصل إلى معادلتين للتنبؤ بالقدرة المرضية للعزلات تحت ظروف الصوبة. أظهرت النتائج أن الاختبارات المعملية باستخدام بيئة مستخلص الذرة الرفيعة يمكن استعمالها كطريقة سريعة لتقييم الاختلافات فى القدرة المرضية للعزلات، وإن كان ذلك لا يعنى الاستغناء عن إجراء اختبارات القدرة المرضية تحت ظروف الصوبة.