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CHARACTRIZATION OF ANASTOMOSIS GROUPS AND EVALUATION OF PATHOGENICTY OF *Rhizoctonia solani* ISOLATES INVOLVED IN FLAX SEEDLING BLIGHT.

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ABSTRACT

Forty eight isolates of *Rhizoctonia solani* were obtained from flax seedlings showed seedling blight. Anastmosis tests revealed that 12 isolates (25%) belonged to AG-2, while 36 isolates (75%) belonged to AG-4. Pathogenicity test on flax cultivar Shkha 1, under greenhouse conditions, showed that the pathogenic isolates in the pre-emergence stage represented 91.67 and 77.72% within AG-2 and AG-4, respectively. However, the pathogenic isolates of AG-4 representing 58.23% of the total isolates as well as the highest percentage of the pathogenic isolates (71.79%). This observations held true in the case of plant height and dry weight. These results indicate that both AGs 2 and 4 are important in the etiology of flax seedling blight. The importance of AG-2 is due to its high virulence, while the importance of AG-4 is due to its high prevalence .Cluster analysis suggested that, within each AG, isolates could be separated into subgroups with specific virulence patterns.

Keywords: *Rhizoctonia* solani, Anastomosis groups, Flax, Pathogenicity.

INTRODUCTION

Rhizoctonia solani Kuhn (teleomorph:*Thanatephorus cucumeris* (Fravk)Donk) is one of the more primitive basidiomycetes, *R. solani* exists in its vegetative form in nearly all agricultural soils. In this non-spore producing phase, the fungus lives saprophytically on dead plant remains but it can become vigorously parasitic when roots or other parts of a susceptible host penetrate the infested zone (Sneh *et al*., 1991). *R. solani* is a major cause of flax seedling blight throughout most of the flax producing countries (Anderson, 1977, Krylova, 1981, Kumud *et al*., 1997, and Andruszewska and Wysakowska, 1999). It attacks flax at early stages of development, destroying the roots and causing thinning or in severe infection, death of seedlings (Krylova, 1981). Under Egyptian conditions, *R. solani* is frequently isolated from blighted flax seedling (Personal communication); however, as far as we know, no attempts have been made to classify *R. solani* from flax into AGs. *Rhizoctonia solani* comprises a collection of noniterbreeding populations that are recognized through the anastomosis group concept (Ogoshi,1987). Isolates of *R.solani* are currently grouped into 18 AG and sub-groups on the basis of their anastomosis behaviour (Sneh *et al*., 1991). Since virulence and host range of these groups differ, Knowledge of anastomosis group affilation of an isolate involved in a particular disease has become very useful (Andderson, 1982; Sneh, *et al*., 1991). Therefore, in the present study, we identified AGs of *R. solani* involved in flax seedling blight and evaluated pathogenicty of 48 isolates under greenhouse conditions.

MATERALS AND METHODS

Isolation of *R. solani* from flax seedlings

Flax seedling showing seedling blight symptoms were collected from 48 fields in 6 governorates (Table 1) during the seasons of 2004 and 2005 .Ten-fifteen seedlings were obtained from each field . Segments with *Rhizoctonia* lesions were washed in running tap water, disinfested in 1% sodium hypochlorite for 5 min, and rinsed in sterilized water. Small pieces, 0.5cm of diseased tissues were plated on water agar and kept for 48h at 25 ± 1ºC. Hyphal tips were transferred to acidified PDA slants. Pure isolates were stored at 5 ºC and served as stock cultures. Identification the anastomosis groups (AGs) of isolated *Rhizoctonia solani*.

Microscopic characterization

Individual isolates were examined microscopically to verify branching near the distal septum of cells as well as the constriction of the branch near the point of origin(Sherwood, 1969; Anderson, 1982; Ogoshi, 1985).

Staining nuclei

Aliquots of acidified (HCL) 0.5% trypan blue in lactophonol (Burpe *et al*.,1978) was placed directly on young hyphae growing on agar media. Stained hyphae were covered with a slip and observed (400x) in a Petri dish, or a piece of agar infested with stained hyphae can be placed on microscope slide. Nuclei are stained dark blue to purple.

Hyphal fusion(Anastomosis)

Standard AG groups were kindly obtained from Cairo MERCIN, Faculty of Agriculture, Ain Shamis University. Isolates of *Rhizoctonia* are assigned to anastomosis group by pairing the isolates with 'tester' strains and observing the hyphae for fusion. Isolates paired on 2% water agar-coated slides or on agar in Petri dishes (Windels and Nabben, 1989). Mycelial transfers from the growing margins of young colony on PDA were plated 2-3cm apart in a slide in 9-cm Petri dish and incubated at 24 or room temperature until advancing hyphae made contact and slightly overlapped.

Pathogenicity test

Substrate for growth of each *Rhizoctonia solani* isolate was prepared in 500-ml glass bottle, each bottle contained 50 g of malt grains and 40 ml of tap water. Contents of bottle were autoclaved for 30 minutes. Inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize malt for 2 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infestsd separately with inoculum of each isolate at a rate of 1g/kg of soil. Infested soil was dispended in 10-cm-diameter clay pots and these were planted with 20 seeds per pot (cultivar Sakha1). In the control treatment, no fungal inoculum was added to the autoclaved soil. Pots were randomly distributed on a greenhouse bench under a temperature regime 24± 6 oC. Pre-emergence damping-off was recorded 15 day after planting, while, survival plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

Table (1) : Isolates of *Rhizoctonia solani* from flax seedling used in this study.

Isolats no.	Geographic origin	AG group	Previous crop
1	Kafer-EL-sheikh	AG-4	Rice
2	Kafer-EL-sheikh	AG-4	Cotton
3	Kafer-EL-sheikh	AG-2	Corm
4	Kafer-EL-sheikh	AG-4	Rice
5	Kafer-EL-sheikh	AG-4	Cotton
6	Kafer-EL-sheikh	AG-4	Rice
7	Kafer-EL-sheikh	AG-4	Rice
8	Kafer-EL-sheikh	AG-2	Cotton
9	Kafer-EL-sheikh	AG-4	Corn
10	Damiatta	AG-4	Corn
11	Damiatta	AG-2	Corn
12	Damiatta	AG-4	Cotton
13	Damiatta	AG-4	Corn
14	Damiatta	AG-4	Rice
15	Damiatta	AG-4	Cotton
16	Damiatta	AG-4	Corn
17	Damiatta	AG-2	Corn
18	Damiatta	AG-2	Corn
19	Gharbiya	AG-4	Rice
20	Gharbiya	AG-4	Corn
21	Gharbiya	AG-4	Rice
22	Gharbiya	AG-2	Rice
23	Gharbiya	AG-4	Coton
24	Gharbiya	AG-2	Corn
25	Gharbiya	AG-4	Coton
26	Gharbiya	AG-4	Coton
27	Gharbiya	AG-4	Coton
28	Beheira	AG-4	Rice
29	Beheira	AG-2	Corn
30	Beheira	AG-2	Coton
31	Beheira	AG-4	Corn
32	Beheira	AG-4	Rice
33	Beheira	AG-4	Coton
34	Beheira	AG-4	Corn
35	Sharqiya	AG-2	Rice
36	Sharqiya	AG-4	Rice
37	Sharqiya	AG-4	Corn
38	Sharqiya	AG-4	Coton
39	Sharqiya	AG-4	Coton
40	Sharqiya	AG-4	Rice
41	Sharqiya	AG-4	Rice
42	Sharqiya	AG-2	Rice
43	Sharqiya	AG-4	Corn
44	Daqahlyia	AG-4	Corn
45	Daqahlyia	AG-4	Coton
46	Daqahlyia	AG-2	Corn
47	Daqahlyia	AG-4	Corn
48	Daqahlyia	AG-4	Rice

Statistical analysis of data

Pathogenicity test was carried out in a randomized complete block design of three replicates. Percentage data were transformed into $\sqrt{x+0.5}$ or arc sine angles to produce approximately constant variance before carrying out the analysis of variance (ANOVA). Duncan's multiple range test was used to compare between isolate means. ANOVA and correlation analyses were carried out by MSTAT statistical package. Cluster analysis of *R.solani* isolates wase performed with the softweare package spss 6.0.

RESULTS AND DISCUSSION

Number of nuclei

Determination of the number of nuclei in vegetative hyphal cell is an important process in the identification of *Rhizoctonia solani* (Parmeter and Whitney 1970). Colonies of *Rhizoctonia solani* were stained with trypan blue and observed to determine numbers of nuclei in individual cells. All the isolates were multinucleate (Fig. 1),contain from 3 to 8 nuclei per cell.

Hyphal anastomsis

Microscopic examination showed that the isolates were divided into two anastomosis groups. 36 isolates (75%) belonged to AG-4, while 12 isolates (25%)belonged to AG-2 (Table 1). Perfect fusion between tester isolate and tested isolate is shown in Figs.2and3.

Pathogenicity test

Pathogenicity of 48 isolates of *Rhizoctonia solani* was evaluated on flax cultivar Sakha1 under greenhouse condition (Table 2). Twelve isolates (25%) belonged to AG-2, while 36 isolates (75%) belonged to AG-4 (Table 3). The pathogenic isolates in the pre-emergence stage represented 91.67 and 77.78% within AG-2 and AG-4, respectively. However, the pathogenic isolates of AG-4 represented 58.23% of the total isolates as well as the highest percentage of the pathogenic isolates (71.79%) . This observations held true in the casa of plant height and dry weight . These results are in agreement with the results of Anderson (1977) who found that *Rhizoctonia solani* isolates belonging to AGs 2 and 4 caused seed decay of flax. These findings may indicate that the prevailing environmental conditions during flax growing season are more favorable for the prevalence of AG-4. The results also indicate that both AGs 2 and 4 are important in the etiology of flax seedling blight. The importance of AG-2 is due to its high virulence , while the importance of AG-4 is due to its high prevalence . There were no clear differences between AGs 2 and 4 regarding their isolation frequencies from the different regions in the Nile Delta (Table 4). Thus, the isolates frequencies of AGs 2 and 4 were 50 and 47% , respectively from East Delta region (Damiatta, Sharqiya, and Daqahylia). As to West Delta region(Gharbiya and Behira) , the isolation frequency was 33.33% for each group. AGs 2 and 4 were isolated from Kafr EL-Sheikh (North Delta region) at isolation frequencies of 16.67 and 16.44%, respectively. Within each governorate, the isolation frequency of AG4 was much greater than that of AG-2. Thus , while the isolation frequency of AG-4 ranged from 66.67 to 80% it ranged from 33 to 33.20% for AG-2 .

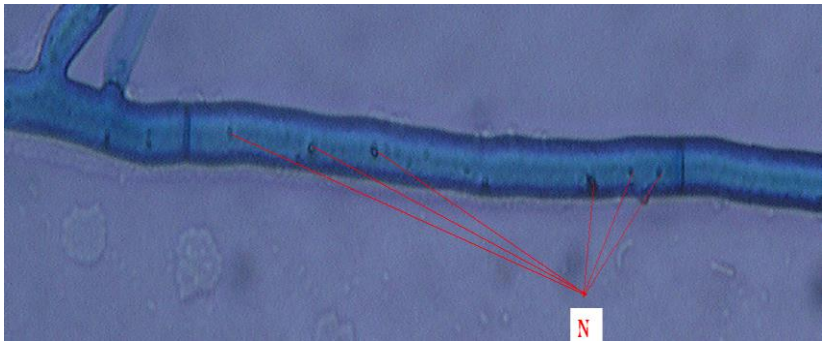

Fig.(1): Staining Nuclei (N) in vegetative hyphae of *Rhizoctonia Solani*

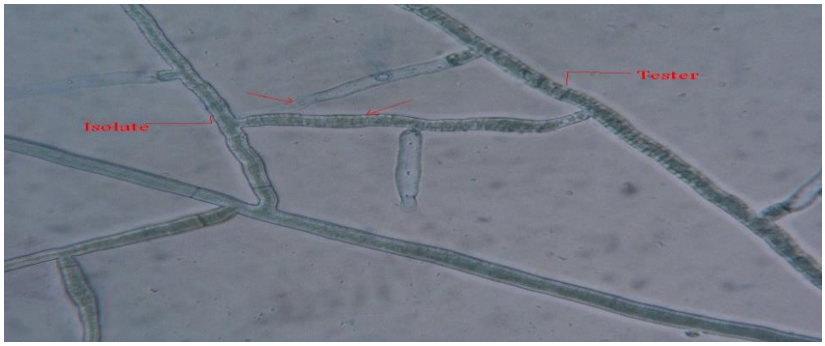

Fig.(2): Perfect fusion between hyphae of *Rhizoctonia solani*

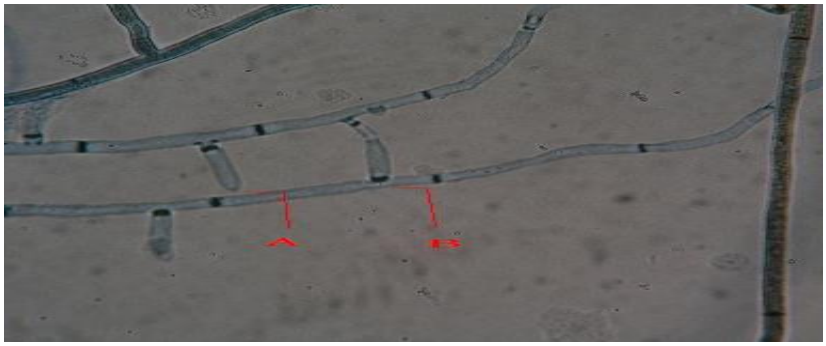

Fig.(3): Perfect fusion between hyphae of *Rhizoctonia solani*

- **contact of hyphae.**
- **fusion of cells.**
- **Note lack of plasmolysis of fused cells.**

Table (3): Classification of pathogenic *Rhizoctonia solani* from flax based on AG group .

Seedling growth variable	AG Group	Total number of tested isolates	Total number of pathogenic isolates	Percentage of isolates within AG group	Percentage of total isolates[a]	Percentage of pathogenic isolates
Pre-emergence damping-off	AG2-2	12	11	91.67	22.92	28.21
	AG-4	36	28	77.78	58.33	71.79
Survival	AG2-2	12	12	100	25	25
	AG-4	36	36	100	75	75
Plant height	AG2-2	12	12	100	25	25
	AG-4	36	36	100	75	75
Dry weight	AG2-2	12	11	91.67	22.92	26.19
	AG-4	36	31	86.11	64.58	73.81

[a]**Total of 48 isolates of Rhizoctonia solani were tested for pathogenicity on seedlings of flax cultivar sakha1 under greenhouse conditions .**

Table (4) : Distribution of Rhizoctonia solani AGs based on their geographic origin

AG group	No of Isolates	Governorate					
		Kafr-EL-sheikh	Damiatta	Gharbiya	Behira	Sharqiya	Daqahlyia
AG2-2	12	2	3	2	2	2	1
AG-4	36	7	6	7	5	7	4

AG-2 showed the highest isolation frequency after corn, while AG-4 showed its highest isolation frequency after cotton (Table 5). This result indicates that previous crop exerted a differential effect on the isolation frequency of the two groups. Within each previous crop, AG-4 was always the predominant group . Our results are in agreement with the other reports, which indicate that preceding crop plays an important role in enhancing or suppressing the different AGs of *R.solani*. For example, maize as a preceding crop in rotation may keep up the inoculum potential in the soil and therefore play an important role in the epidemiology of *Rhizoctonia* root rot of sugar beet (Ithurrant *et al*., 2004). Schillinger and paulitz (2006) reported that *Rhizoctonia* bare patch in wheat was reduced with barely rotations.

Correlation among variables used for evaluating pathogenicity of *R.solani* isolates on flax seedlings are shown in Table 6 significant ($p<0.01$) negative correlation was observed between pre-emergence damping–off and survival. Pre-emergence damping-off was correlated with plant height for isolates of AG-4 and for all isolates, while it was nonsignificant for isolates of AG-2. Correlation between pre-emergence damping-off and dry weight was nonsignificant. Post-emergence damping-off and plant height were negatively correlated for AG-4 isolates and for all isolates. Survival was negatively correlated with pre-emergence damping-off. The least number of significant

correlations was observed in the case of AG-2, this could be attributed to its small sample size (n=12).

Eight groups of similar isolates were identified by cluster analysis (Fig. 4). These groups were isolates 7,26,11,38,46,41; isolates of 6,12,44,33,24,1,8; isolates of 20,36,5,13,16,30,43; isolates 28,40,23; isolates 18,35,3,21,47,19; isolates of 9,25,2,14,37,42; isolates of 4,32,10,15,45,29, and isolates of 22,34,17,39,27,48,31. Neither AG nor geographic origin was associated with virulence pattern of the isolates because, most of the groups included isolates belonging to AG-4 and AG-2 and isolated from North, West, and East Delta regions. Similarly, previous crop was not associated with virulence pattern. Cluster analysis suggested that within each AG, isolates could be separated into subgroups with specific virulence patterns.

Table (5) : Distribution of AGs of *Rhizoctonia solani* based on previous crop.

AGs	No.of Isolats	Previous crop		
		Cotton	Corn	Rice
AG2-2	12	2	7	3
AG-4	36	13	10	13

Table (6) : Correlation among variables used for evaluating pathogenicity of *R.solani* isolates on flax seedlings (cultivar Sakha 1) under greenhouse conditions.

AG	Variable	Variable 1	2	3	4	5
2-2[a]	1-Pre-emergence damping-off		0.167	-0.993**	-0.521	0.005
	2-Post emergence damping-off	0.167		-0.280	-0.011	-0.064
	3-Survival	-0.993**	-0.280		0.508	0.001
	4-Plant height	-0.521	-0.011	0.508		0.052
	5-Dry weight	0.005	-0.064	0.001	0.052	
4[b]	1-Pre-emergence damping-off		0.101	-0.980**	-0.564**	-0.080
	2-Post emergence damping-off	0.101		-0.288	-0.476**	0.231
	3-Survival	-0.980**	-0.288		0.623**	0.029
	4-Plant height	-0.564**	-0.476**	0.623**		0.005
	5-Dry weight	-0.080	0.231	0.029	0.005	
All[c] AGs	1-Pre-emergence damping-off		0.101	-0.983**	-0.544**	-0.057
	2-Post emergence damping-off	0.101		-0.274	-0.398**	0.190
	3-Survival	-0.983**	-0.274		0.588**	0.018
	4-Plant height	-0.544**	-0.398**	0.0588		0.015
	5-Dry weight	-0.057	0.190	0.018	0.015	

****Correlation is significant at (P<0.01).**
[a]No = 12
[b]No = 36
[c]No = 4

Crop[a]	Originc[b]	AG[c]
R	N	4
Cot	W	4
Cor	E	2
Cot	E	4
Cor	E	2
R	E	4
R	N	4
Cot	E	4
Cor	E	4
Cot	W	4
Cor	W	2
R	N	4
Cot	N	2
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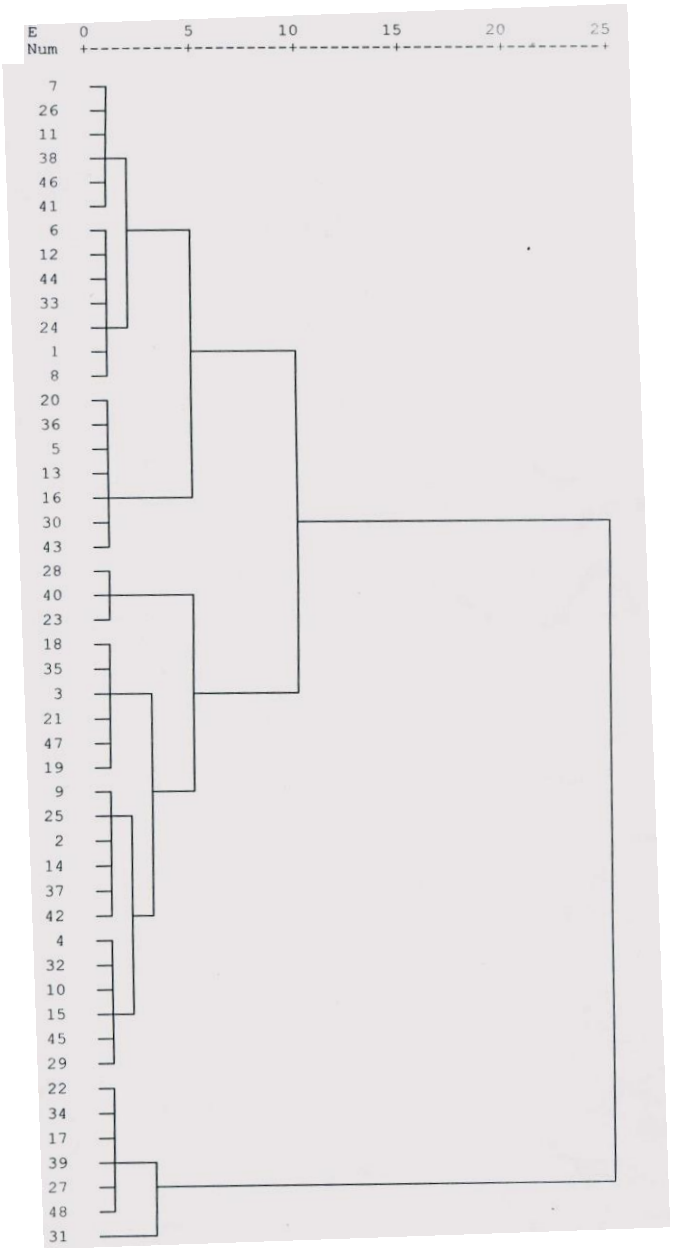

Fig.(4): Phenogrom based on average linkage cluster analysis of virulence of 48 isolates of R.solani on flax cultivar Sakha 1

a Pervious crop Rice (R), Cotton (Cot), and Corn (Cor).
b Geographic origins of isolates were East Delta (E), North Delta (E), North Delta (N), and Wast Delta (W).
c Anastomosis groups

REFERENCES

Andruszewska, A., and I. Wysakowska .1999. Pathogenicity of some *Rhizoctonia solani* Kuhn isolates on flax. Progress in Plant Protection, 39: 914-916.

Anderson, N.A.1982. The genetics and pathogenicity of *Rhizoctonia solani*. Annual Review of Phytopathology, 20: 329-374.

Anderson, N. A.1977. Evaluation of the *Rhizoctonia* complex in relation to seedling blight of flax. Plant Disease Reporter, 61: 140-142.

Burpee, L.L., P.L. Sanders, H. Cole, Jr., and S.H. Kim. 1978. A staining technique for nuclei of *Rhizoctonia solani* and related fungi. Mycologia, 70: 1281-1283.

Kumud, K., S. Jitendra, and M.D. Yadav. 1997. Fungi associated with linseed seeds, their effect and chemical control. Annals of Plant Protection Sciences, 5: 179-183.

Krylova, T.V. 1981. *Rhizoctoniosis* of fibre flax. Mikologiya-i-Fitopatologiya, 15: 511-513.

Ogoshi, A.1987. Ecology and Pathogenicity of Anastomosis groups of *Rhizoctonia solani* Kuhn. Annual Review of Phytopathology,25:125-143.

Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Fitopatologia Brasileira, 10: 371-390.

Parmeter, J. R. JR. and H.S.Whitney.1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: J. R. Parmeter Jr., ed. Biology and pathology of *Rhizoctonia solani*. University of California Press, Berkeley. 255 pp.

Schillinger, WF. and T.C. Paulitz. 2006. Reduction of *Rhizoctonia* bare patch in wheat with barley rotations. Plant Disease, 90: 302-306.

Sneh, B.; L. Burpee and A. Ogoshi. 1991. Identification of *Rhizoctonia* species. APS Press, USA. 133pp.

Ithurrart, M. EF., G. Buttner. and J.Petersen. 2004. *Rhizoctonia* root rot in sugar beet (*Beta vulgaris* sp*. altissima*) - epidemiological aspects in relation to maize (*Zea mays*) as a host plant. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz,111: 302-312.

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توصيف المجموعات الالتحامية وتقييم القدرة المرضية لعزلات فطر الريزكتونيا سولانى المسببة لمرض لفحة بادرات الكتان .

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أمكن الحصول على ٤٨ عزلة لفطر الريزوكتونيا سولانى من بادرات كتان مصابة بمرض لفحة الباردات . أظهر الفحص الميكروسكوبي إن ١٢ عزلة (٢٥%) كانت تتبع المجموعة الالتحامية AG2 فى حين أن ٣٦ عزلة (٧٥%) كانت تتبع المجموعة الالتحامية AG4 . أظهر اختبار القدرة المرضية لهذه العزلات على صنف سخا ١ تحت ظروف الصوبة ان ٩١٫٦٧ % من عزلات المجموعة ٢ وكذلك٧٢٫٧٢ % من عزلات المجموعة ٤ كانت ممرضة فى مرحلة ما قبل ظهور البادرات فوق سطح التربة . العزلات الممرضة التابعة للمجموعة ٤ مثلت ٥٨٫٢٣ % من اجمالى العزلات المختبرة كما مثلت ٧١٫٧٩ % من إجمالى العزلات الممرضة . وكانت تلك النتائج متوافقة مع تأثير العزلات على طول البادرات و وزنها الجاف . أكدت النتائج أن العزلات التابعة لكل من المجموعتين ٢ و ٤ تلعب دورا هاما في إحداث لفحة بادرات الكتان حيث أتضح أن عزلات المجموعة ٢ كانت ذات قدرة مرضية عالية فى حين أن عزلات المجموعة ٤ كانت أكثر أنتشارا . أظهر التحليل العنقودي إن كل من المجموعة الالتحامية ٢ و ٤ تنقسم إلى ٨ مجموعات فرعية من العزلات التي تتميز كل منها بنمط محدد من حيث القدرة المرضية.

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