


Pathogenicity assay of some soil-borne fungi isolated from cotton seedlings



Taghreed Refai¹, Ibrahim N.M. Ali¹, Heba M.M. Abdel Nabi¹ Aly A. Aly², Mohamed I.I. Khalil¹, Kamel A. Abd-Elsalam^{2*} 

Address:

¹ Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

² Plant Pathology Research Institute, Agricultural Research Centre, 12619, Giza, Egypt

* Corresponding author's: e-mail: Kamel A. Abd-Elsalam, kamelabdelsalam@gmail.com

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ABSTRACT

Cotton is widely acknowledged as a major cash crop across the world, and its socioeconomic relevance, particularly in emerging nations, is well understood. Cotton seedling infections are among the most significant of the diseases that limit cotton lint and seed production. The goal of this study was to determine the pathogenicity of *Rhizoctonia solani*, *Fusarium moniliforme*, and *Machrophomina phaseolina* isolates taken from cotton roots to cotton seedlings in the Giza governorate. Seven fungus isolates were recovered from two cotton cultivars, including *Rhizoctonia solani*, *F. moniliforme*, and *M. phaseolina* (Giza 90 and Giza 94). It was discovered that the differences in pathogenicity between the isolates and controls vary by cultivar, indicating that the cultivars reacted differently to the isolates. All of the fungal isolates were harmful and reduced the survival rate on both cultivars. Pathogenic isolates had a negative impact on seed germination, root shoot length, and increased Cotton seedlings have a significant mortality rate. These problems might be overcome by growing cotton cultivars that are resistant to damping-off. In order to improve resistance, resistance genes must be introduced into cotton cultivars. Future cotton improvement techniques should incorporate damping-off management; however, the inclusion of numerous pathogenic fungi from various genera may confound efforts to create such approaches.

Keywords: Cotton seedlings complex, *Gossypium barbadense*, *Rhizoctonia solani*, *Machrophomina phaseolina*, *Fusarium moniliforme*

INTRODUCTION

Cotton (*Gossypium barbadense* L.) is a strategic field crop that is widely cultivated and traded around the world, as well as one of Egypt's most important export crops. Cotton seedling infections are a global issue caused by a symbiotic group of soil-borne microbes. *Rhizoctonia solani* and *Fusarium* spp. are among the organisms prevalent in Egypt's cotton-growing regions (Asran *et al.*, 2005). Cotton seedlings suffer from seedling blight, pre- or post-emergence damping-off, sore shin, and root rot caused by *R. solani* Kuhn, an anamorph of *Thanatephorus cucumeris* (Frank.) Donk (Fulton *et al.*, 1956). *R. solani* colonizes soft tissues and initiates infection. The fungus penetrates the epidermis and kills plant cells as a result of these precautions (Watkins, 1981).

Increased seed sowing depth during early chilly weather resulted in severe damping-off (Moubasher, 1958). *R. solani* is a common fungus found in the soil. *R. solani* is a species that affects a wide range of agricultural and horticultural crops (Ogoshi, 1987), and it is made up of genetically distinct groups (Adams, 1988). The behaviour of anastomosis is primarily used to identify and classify these groupings (Ogoshi, 1972). (Moustafa *et al.*, 1995) recovered many soilborne pathogens from infected cotton seedlings taken from several fields in Egypt, however *R. solani* AG-4 was the most often isolated cause pathogen of the illness, according to them. During pathogenicity experiments, some isolates of *R. solani* AG-4 inhibited emergence and induced root discolouration on cotton seedlings (Rush *et al.*, 1994).

Seedling diseases are caused by organisms that can be found in most soils. They stay put eternally once they've established themselves. They build structures that allow them to survive year after year in the earth (Hillocks, 1992). The fungus survives saprophytically on dead plant remains in its vegetative form in practically all agriculture soils in the non-spore producing phase, but it can become actively parasitic when roots or other elements of a susceptible host penetrate the infected zone (Watkins, 1981). *R. solani* was a major source of cotton damping-off throughout much of Egypt's cotton-growing areas, according to (Moustafa *et al.*, 1995; Asran-Amal *et al.*, 2005). Several studies have shown that *R. solani* isolates belonging to AG4 are pathogenic (Baird and Carling, 1997; Baird *et al.*, 2004; Colyer and Vernon, 2005). The behaviour of the anastomosis is used to identify and classify these groupings (Anderson 1982 and Ogoshi 1976). Cotton seedling damping-off was caused by *R. solani*, which was linked to severe necrosis on the root and/or hypocotyl (Malero-Vara and Jimenez-Diaz 1990).

Colonization occurs when *M. phaseolina* infects cotton roots or stems of internal tissue progresses quickly and plants die due to the destruction of vascular tissues. Examination of parasitized tissue reveals the disintegration of parenchyma with many small, black sclerotia attached to the vascular bundles (Watkins, 1981).

M. phaseolina is widely distributed on Egyptian ground, and it is easily and frequently Late in the growth season, cotton roots were isolated. Thus, when (Aly et al., 1996) collected and assayed *M. phaseolina* was recovered from 37.5 percent of the samples analysed from 88 samples of infected cotton roots from 12 Egyptian governorates. However, it was not clear whether this variation represented usable resistance in a breeding program. In Egypt, commercial cotton (*Gossypium barbadense* L.) exhibits no resistance to *M. phaseolina* (Aly et al., 2006); consequently, more research is needed to recognize charcoal rot- genotypes that are resistant A comprehensive knowledge of the degree to which there is diversity in virulence among *M. phaseolina* isolates would help devolve Cotton cultivars that are resistant to a wide range of pests and diseases. Therefore, this investigation The goal of this study was to see how pathogenicity differed across isolates of *M. phaseolina* originated in various Egyptian locales and their interactions with cotton cultivars. *M. phaseolina*. The pathogen that causes charcoal rot (ashy stem) in cotton is a seed- and soilborne disease with a wide host range. (Dhingra and Sinclair, 1978). *M. phaseolina* is also a plurivorous More than 500 host species have been infected by a fungus (Sinclair, 1982). When *M. phaseolina* infects cotton roots or stems, internal tissue colonisation occurs quickly, and the plant dies. A dry rot is visible on the damaged portions, with many microscopic black sclerotia dispersed throughout the wood and softer tissues (Watkins, 1981). Such information could improve soil management practices for predicting and reducing the incidence of charcoal rot. Differential interactions among cotton genotypes and isolates.

Fusarium species have been linked to a number of cotton illnesses, including the seedling disease complex, wilt, and boll rot (Abd-Elsalam et al., 2006). Although the extent of losses from Fusarium root rot is unknown, the high percentage of pathogenic isolates from cotton shows that *F. solani* may be a significant pathogen (Nelson and Windels, 1992; Hwang et al., 1995) suggest that Fusarium root rot can seriously reduce yields of field. *F. solani* is one Among the organisms involved in the *Gossypium* spp. seedling disease complex (Davis et al., 1981; Johnson, 1981). Seed rot, pre- and post-emergence damping-off, and seedling root rot are some of the disease signs that cause stand reductions and reduced seedling vigour, which delays growth and maturity. Fusarium solani caused significant reductions in emergence of cotton and increased root discoloration of surviving seedlings (Batson and Trevathan, 1988). *F. oxysporum* was the most prevalent Fusarium species isolated from roots, according to (Nelson and Windels, 1992), however isolates were either slightly pathogenic or nonpathogenic. *F. solani* isolates, on the other hand, were nearly all extremely pathogenic (Nelson et al., 1997). Fusarium was commonly isolated from damping-off-infected cotton seedlings in cotton (Aly et al., 1996; El Samawaty, 1999). *F. solani* isolates from cotton were found to be pathogenic in 83 percent of cases (Abd-Elsalam et al., 2006). According to (Batson and Borazjani, 1984), the percentage of seedlings emerging from fumigated soil experimentally infected with *F. solani* was much lower than the percentage of seedlings emerging from non-infected soil. Devolving cotton cultivars with stable resistance would benefit from a comprehensive understanding of the level of virulence variation. The main aim of the current study to assess pathogenicity assay for cotton seedlings pathogens in two commercial cotton cultivars now available for planting and breeding in cotton-growing regions in Egypt.

MATERIAL AND METHODS

Fungal isolates:

Random samples collected from numerous places in cotton-producing districts of Egypt were used to isolate the fungus. A total of 10 to 15 seedlings with various damping-off signs were included in each sample. Infected seedlings were taken out of the field and thoroughly cleaned under running tap water to eliminate any soil that had adhered to them. Small pieces of necrotic tissue (about 0.5 - cm/long) were freshly prepared with a 10% chlorox solution for two minutes before being rinsed numerous times with sterile water. To eliminate bacterial contamination, the surface-sterilized pieces were bottled dry between sterilized filter sheets and plated (five pieces per plate) on potato dextrose agar medium (PDA) modified with streptomycin sulphate or penicillin G and Rose Bengal. For 3-7 days, the plates were incubated at 20 \pm 3 °C. Single spore or hyphal tip procedures were used to purify the emerging colonies. At the cotton pathology lab, Plant Pathology Research Institute, Agric. Res. Center, Giza, pure cultures of the isolated fungus were identified to genus level according to (Gilman, 1966) or (Barnett and Hunter, 1979). Assiut University Mycology Center (AUMC), Assuit, Egypt, graciously identified pure isolates to species level from a random sample of 15 isolates.

Pathogenicity assay:

50 g of sorghum grains and 40 ml of tap water were combined in a 500-ml glass container to provide a substrate for the development of fungal isolates. The contents of the bottle were autoclaved for 30 minutes. After being collected from a one-week-old culture on PDA, isolate inocula was aseptically placed in the bottle and allowed to colonize sorghum for three weeks. The current experiment utilized autoclaved clay loam soil. Different batches of soil were inoculated with an inoculum of each isolate at rates of 50, 50, and 1 g/kg of soil for *Fusarium*, *Machrophomina*, and *Rhizoctonia*, respectively. Infested dirt was placed into 15-cm clay pots before being loaded with 10 seedlings each (cultivars Giza 90 or Giza94). In the

control treatment, no fungal inoculum was introduced to the autoclaved soil. Temperatures ranging from 24°C to 30°C were used to randomly put the pots on a greenhouse bench. Preemergence damping-off, survival, plant height (cm/plant), and dry weight (mg/plant) were assessed 15 days after planting, followed by postemergence damping-off, survival, plant height (cm/plant), and dry weight (mg/plant) 45 days after planting. **Figure 1** depicts the pathogenicity test stages for pathogenic soil-borne fungus isolated from cotton seedlings.

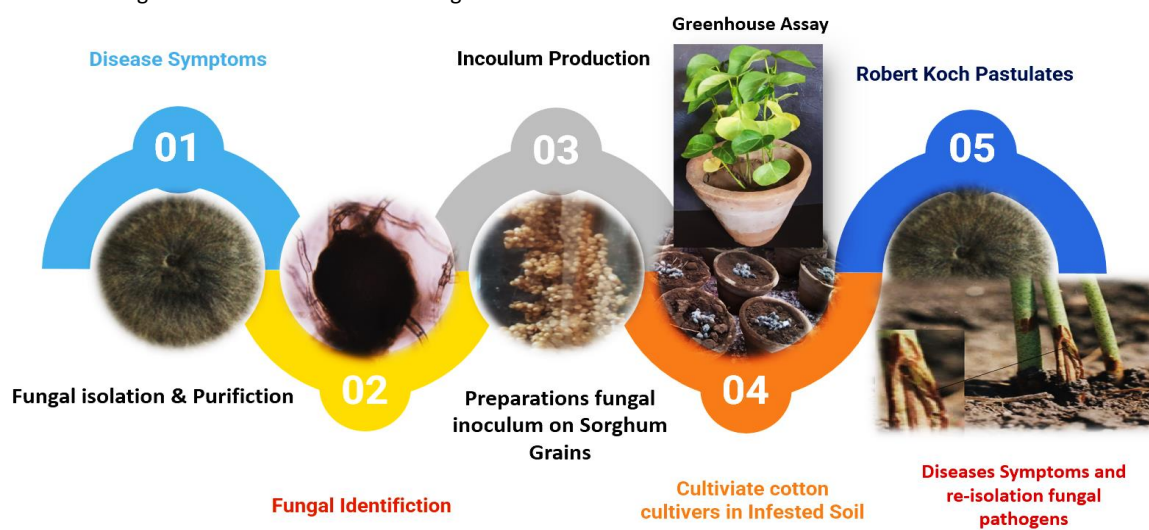


Fig. 1. Pathogenicity test steps of pathogenic fungi isolated from cotton-diseases seedlings

Statistical Analysis:

The current study employed a completely randomized complete block design with five replicates. The data was subjected to an analysis of variance (ANOVA) using the MSTAT-C statistical programme. To create essentially constant variance, percentage data were translated into arc sine angle before being subjected to ANOVA. The SPSS 22.0 software suite was used to conduct correlation and regression analysis (Gomez and Gomez, 1984).

RESULTS

Pathogenicity of nine isolates on two commercial cultivars in greenhouse settings, as well as control. When cotton seedlings were grown in contaminated soil, they developed root rot signs on the hypocotyl and main root, as well as substantial decreases in plant weight, dry weight, and stem length when compared to the control. Pathogenicity tests indicated the pathogen's direct involvement in root rot symptoms, which may result in cotton seedling damping-off. Control seedlings exhibited no symptoms (See Fig. 2).

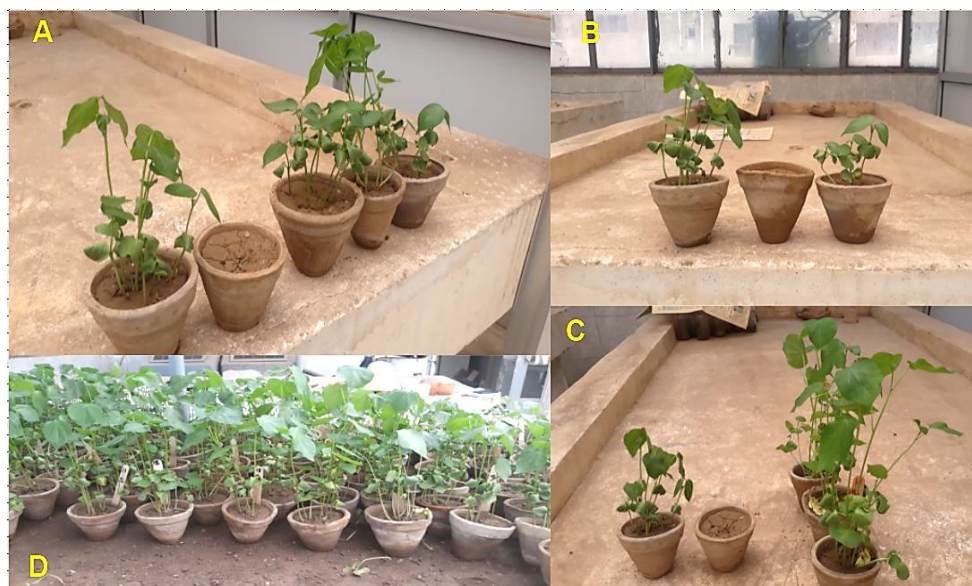


Fig. 1. Pathogenicity test of three soil-borne fungi against cotton seedling after 45 days under greenhouse conditions. (A) soil infested with *R. solani*, (B) soil infested with *M. phaseolina*: (C) soil infested with *F. moniliforme*, and (D) infested with three pathogenic fungi.

According to ANOVA Table (1), cultivar was a non-significant cause of variance in plant height and dry weight. Fungal isolates were a very significant source of variance in both examined variables ($P=0.00$). Interactions between cultivars and isolates were shown to be a non-significant source of variance in both evaluated variables. Table (1), revealed that cultivar was a very highly significant source of variation in plant height ($p=0.00$), a substantial source of variation in survival ($p=0.05$), and a non-significant source of variation in dry weight ($p=0.08$). Treatment was a very significant cause of variance in all of the variables investigated ($p=0.00$). The interaction between cultivar and treatment was a substantial source of variance in all of the variables studied.

Table 1. The influence of certain fungus (treatments), cultivars, and their interactions on some growth factors of cotton seedlings grown in greenhouse conditions was studied using analysis of variance.

Growth variables and source of variation	D.F	Mean square	F. value	P > F
Survival				
Replicates	4	304.398	1.296	0.28
Cultivars (V)	1	914.020	3.893	0.05
Treatments (T)	7	4799.219	20.440	0.00
V x T	7	534.292	2.276	0.04
Error	60	234.792		
Plant height				
Replicates	4	14.181	0.395	0.81
Cultivars (V)	1	572.450	15.952	0.00
Treatments (T)	7	235.287	6.556	0.00
V x T	7	301.249	8.394	0.00
Error	60	35.887		
Dry weight				
Replicates	4	0.285	1.131	0.35
Cultivars (V)	1	0.801	3.174	0.08
Treatments (T)	7	1.435	5.683	0.00
V x T	7	0.544	2.154	0.05
Error	60	0.252		

ANOVA was used to achieve an almost constant variance by transforming percentage data into arc sine angle. For the fungi x cultivars interaction, the LSD (transformed data) was 19.19 ($p=0.04$). Because the fungal x cultivars interaction was significant ($p=0.04$), an interaction LSD was used to compare fungi means within each cultivar. All fungi were harmful on both cultivars, according to these comparisons. On both cultivars, F3, Rs37, and M4 (18.00%) were the most effective isolates in decreasing survival Table (2). On Giza 90, isolate M4 was the most pathogenic isolate, whereas isolate F3 was the most pathogenic isolate on Giza 94. On Giza 90, isolates F1 and F3 were similarly pathogenic, but isolate F3 was more harmful. These interactions imply that the difference between isolates may vary depending on cultivar. It was found that the variations in pathogenicity between the isolates and controls differed by cultivar, indicating that the cultivars reacted differentially to the isolates. All fungal isolates were pathogenic and decreased the survival percentage on both cultivars.

Table 2. Effect of some fungi, and cultivars interaction on survival of cotton seedlings cultivated in infested soil growing in a greenhouse

Isolates	Survival (%)					
	Giza 90 %	Transformed ^a	Giza94 %	Transformed ^a	Mean %	Transformed ^a
F1	50	45.000	40	35.950	45	40.475
F3	42	39.688	0	0.000	21	19.844
Rs3	44	44.266	58	46.154	51	45.210
Rs37	40	41.538	34	35.266	37	38.402
M4	18	19.380	14	16.846	16	18.113
M6	66	57.688	60	50.312	63	54.000
M12	62	52.200	68	55.838	65	54.019
Control	96	84.688	100	90.000	98	87.334
mean	52.250	48.056	46.750	41.296	49	44.676

Table (3) displays the effects of the most pathogenic fungal isolates, cultivars, and their interactions on the dry weight and plant height of cotton seedlings produced in a greenhouse. M4 isolate was the only isolation that resulted in a substantial reduction in Giza 90 plant height, whereas F3 isolate was the only isolate that resulted in a significant reduction in Giza 94 plant height. Giza 90 plant height increased significantly as a result of F3 and Rs3. On Giza 94, none of the isolates enhanced

plant height. Giza 90's dry weight was significantly reduced by M4, F1 and Rs 37, while Giza 94's dry weight was significantly reduced by M4, F3. The pathogenicity of *R. solani* isolates was tested on two cotton cultivars in a greenhouse setting. Regardless of isolate, the difference in overall means between the two cultivars was not significant.

Table 3. Under greenhouse circumstances, the interaction of fungi and cultivars on plant height and dry weight of cotton seedlings cultivated in infested soil).

Isolates	Plant vigor parameters					
	Plant height (cm/ plant)			Dry weight (g/plant)		
	Giza90	Giza94	mean	Giza90	Giza94	mean
F1	16.880	12.160	14.520	0.772	0.764	0.768
F3	31.720	0.000	15.860	1.294	0.000	0.647
Rs3	28.580	25.660	27.120	1.152	0.938	1.045
Rs37	20.940	16.340	18.640	0.636	0.681	0.658
M4	9.460	12.040	10.750	0.210	0.204	0.217
M6	18.380	19.900	19.140	1.038	1.176	1.107
M12	21.400	20.660	21.030	1.146	1.198	1.172
Control	20.580	18.380	19.480	1.558	1.244	1.401
mean	20.992	15.643	18.318	0.976	0.776	0.876

(F=*F. moniliforme*) (M=*Machrophomina phaseolina*) (R=*Rhizoctonia solani*)

LSD ($p \leq 0.05$) for isolates x cultivars for plant height=7.50 and for dry weight=0.63

DISCUSSION

Cotton seedling damping-off is a worldwide issue that frequently results in significant stand loss if it is not handled. This disease complex is caused by seed-borne and soil-borne organisms, both flora and fauna, acting separately or in combination (Mohamed and Akladiou, 2017; Mansour et al., 2020). *R. solani* is a virulent fungal pathogen with a diverse host range. It was discovered in all cotton-producing districts of Egypt (Asran-Amal et al., 2005). *M. phaseolina* was discovered to be pathogenic to 11 commercial cotton cultivars, with significant diversity in their response to infection (Abd-Elsalam, 2010). Zaki and his colleagues recently screened soil pathogenic fungi implicated in cotton seedling damping-off and discovered that *Fusarium* spp. (44%), *R. solani* (44%), and *M. phaseolina* (12%) were the most prevalent fungus engaged in the disease. *R. solani*, *F. moniliforme*, and *M. phaseolina* isolates obtained from cotton roots to cotton seedlings in the Giza governorate were tested for pathogenicity. Giza 90 and Giza 94 were selected for this study because Giza 90 is the most common cotton cultivar in upper Egypt and Giza 94 is the most common cotton cultivar in lower Egypt (Nile Delta). According to Zaki et al., Giza 94 was somewhat more vulnerable to infection than Giza 90 (2022).

The goal of this study was to assess the pathogenicity of several soil-borne pathogenic fungus isolated from Giza governorate on two marketed cotton cultivars. Here, we identified the most significant commercial cotton cultivars belonging to G. barbadense; among these cultivars, Giza 90 and Giza 94 were chosen for the current study because Giza 90 is Upper Egypt's major cotton cultivar, whilst Giza 94 is Lower Egypt's dominating cotton cultivar (Nile Delta). The results of this study increase the number of *R. solani*, *F. moniliforme*, and *M. phaseolina* species known to be harmful to cotton seedlings in Egypt. As indicated in this study, Giza 94 was marginally more vulnerable to infection than Giza 90. The Koch's postulate, which established a "cause and effect link" between three pathogenic fungus and the reported symptoms, has been confirmed.

Because the fungi that cause cotton damping-off are non-specialized and have a broad host range, rotating cotton with other crops is a risky strategy for treating the disease. Develop new cotton cultivars in Egypt are prone to damping-off is urgently needed, as this study has shown, because damping-off resistance has not been prioritized in the development of these cultivars. As a result, the sole commercially accessible management practice for managing the disease is seed-dressing fungicides (Mohamed and Akladiou, 2017; Mohamed et al. 2018; Zaki et al., 2022). It is becoming increasingly clear that their widespread use is linked to a number of issues, including harm to non-target organisms, the development of pathogen-resistant races, and the possibility of carcinogenicity. Other difficulties include the gradual phase-out and removal of specific fungicides (Zaki et al., 1998).

CONCLUSION

Analysis of variance indicated that isolates and interactions between isolates' cultivars were significantly major sources of variation in all of the examined characteristics. Isolates, as well as the interaction between cotton cultivars and isolates, are statistically significant, indicating that *R. solani* isolates pathogenic to cotton show physiologic specialisation. Cotton cultivars that are resistant to damping-off might help to alleviate these problems. To increase resistance, resistance genes must be introduced into cotton cultivars. Thus, future cotton improvement efforts should incorporate resistance breeding and biological control for cotton disease damping-off. In addition, due to climatic change, future research should look into the pathogenicity of pathogenic fungi isolated from cotton plants on a low frequency.



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فحص القدرة المرضية لبعض الفطريات القاطنة في التربة والمعزولة من بادرات القطن

تغريد رفاي¹ ، إبراهيم ن.م علي¹ ، هبة محمد محمد عبد النبي¹ علي عبد الله علي² ، محمد إبراهيم محمد¹
كامل ا. عبد السلام^{2*}

¹ قسم النبات الزراعي ، كلية الزراعة ، جامعة قناة السويس ، الإسماعيلية ، مصر.
² معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، 12619 ، الجيزة ، مصر

* المؤلف المراسل: kamelabdsalam@gmail.com

الملخص

يُعرف القطن على نطاق واسع بأنه محصول نقدي رئيسي في جميع أنحاء العالم ، كما أن أهميته الاجتماعية والاقتصادية ، لا سيما في الدول الناشئة ، مفهومة جيداً. تعد عدوى بادرات القطن من أهم الأمراض التي تحد من إنتاج القطن الياف وبذور المحصول. الهدف من هذه الدراسة هو تحديد القدرة المرضية لعزلات *Rhizoctonia solani* و *Fusarium moniliforme* و *Machrophomina phaseolina* المأخوذة من جذور القطن إلى شتلات القطن في محافظة الجيزة. تم استخلاص سبع عزلات من الفطريات من صنفين من القطن هما *R. solani* و *F. moniliforme* و *M. phaseolina* (جيزة 90 وجيزة 94). تم اكتشاف أن الفروق في الأمراض بين العزلات والضوابط تختلف باختلاف الصنف ، مما يشير إلى أن الأصناف تتفاعل بشكل مختلف مع العزلات. جميع العزلات الفطرية كانت ضارة وتقلل من معدل البقاء على قيد الحياة في كلا الصنفين. كان للعزلات الممرضة تأثير سلبي على إنبات البذور ، وطول الفسائل الجذرية ، كما أن زيادة شتلات القطن لها معدل نفوق معنوي. يمكن التغلب على هذه المشاكل من خلال زراعة أصناف قطن مقاومة للتخميد. من أجل تحسين المقاومة ، يجب إدخال جينات المقاومة في أصناف القطن. يجب أن تتضمن تقنيات تحسين القطن المستقبلية إدارة التخميد ؛ ومع ذلك ، فإن إدراج العديد من الفطريات المسببة للأمراض من مختلف الأجناس قد يربك الجهود المبذولة لخلق مثل هذه الأساليب.

الكلمات المفتاحية: بادرات القطن، فيوزاريوم، ماكروفومينا، موت البادرات، ريزوكتونيا سولاني