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USE OF SAPONIN-CONTAINING *Atriplex nummularia* TO SUPPRESS DAMPING-OFF OF COTTON SEEDLINGS

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ABSTRACT

Considerable variation was observed among four fungi, involved in damping-off of cotton seedling, as to their response to the application of *Atriplex nummularia* (AT) ground seeds to the growth medium. *Rhizoctonia solani* was resistant to the applied concentrations of AT. Contrary to the inhibitory effect on *Macrophomina phaseolina* and *Sclerotium rolfsii*, AT stimulated growth of *Fusarium oxysporum*. Analysis of variance for effects of different concentrations of AT on pathogenicity of the fungi indicated highly significant effects of concentrations, fungi, and their interaction. Due to the highly significant interaction between concentrations and fungi, an interaction least significant difference was calculated to compare between concentration means within each fungus. These comparisons showed that the fungi responded differently to the applied concentrations. The application of AT, as a seed treatment, at a rate as low as 3 g/kg seeds significantly decreased damping-off caused by any individual fungus, while the increase of application rate to 6 and 9 g/kg seeds did not significantly improve the efficiency of AT in controlling the disease. Moreover, in case of fungal mixture, the increase of application rate to 6 and 9 g/kg seeds resulted in significant reduction in efficiency of At compared to the rate of 3 g/kg seeds. Effect of AT on linear growth of the fungi under pure culture conditions was not related to its effect on pathogenicity of the fungi under greenhouse conditions. Twelve field trials were conducted in Sakha, El-Gemmeiza, Sirs El-Lian and Mallawy in 1999 (three planting dates in each location) to evaluate the effectiveness of treating seeds with AT in suppressing cotton seedling damping-off under field conditions. AT was effective in controlling damping-off only in Sirs El-Lian, where it significantly reduced it by 33.33, 33.76, and 39.55% in the first, second, and third planting dates, respectively. In this site, AT was as effective as the standard fungicide Monceren in controlling the disease. AT also significantly increased seedcotton yield in Sirs El-Lian by 35.65 and 38.42% in the second and third planting dates, respectively. In Sakha, AT significantly increased seedcotton yield by 15.38% in the third planting date. Seedcotton yield of all the other experiments was not affected by the application of AT.

INTRODUCTION

Cotton seedling damping-off is caused by a complex of seed-borne and soil-inhabiting organisms. These organisms are found in all cotton-producing areas of Egypt. Although the populations of inciting organisms differ from area to area, the pathogens most commonly involved in the disease complex are *Rhizoctonia solani* (El-Samawaty, 1999), *Fusarium* spp. (Aly *et al.*, 1996), *Macrophomina phaseolina* (Omar, 1999), *Pythium* spp. (Eisa, 1983), and *Sclerotium rolfsii* (Khashaba, 1972).

The disease occurs as pregermination decay of the seed, decay of the seedling on the way to the soil surface (Preemergence damping-off), partial or complete girdling of the emerged seedling at or near the soil surface (“Sore shin” or postemergence damping-off), and seedling root rot (Watkins, 1981).

The occurrence of major losses from cotton seedling damping-off is not uncommon in all cotton-producing areas in Egypt. These losses vary over years and locations but characteristically result in poor stands. Stands may be replanted if severely damaged and, even if damage is not severe enough for replanting, it may make weed control and other cultural practices difficult for the remainder of the season. Replanting, poor stands and seedling development, and weed competition ultimately affect plant maturity, fiber quality, and seed cotton yield (Kappelman, 1977). Thus, the widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian conditions. While effective fungicides are available (Aly *et al.*, 1992; Eisa *et al.*, 1992; Abdel Azizi *et al.*, 1996; and El-Samawaty, 1999), it is becoming increasingly evident that their widespread use is associated with some problems, such as the potential harmful effect on non-target organisms, the development of resistant races of the pathogens, and the possible carcinogenicity. Other problems include gradual elimination and phasing out of some compounds (Zaki *et al.*, 1998).

Recently, natural plant products have been widely used, as serious alternative to fungicides, for controlling plant pathogens because they are environmentally safe (Abdel-Rahman *et al.*, 1989 and Singh *et al.*, 1990).

Oldman salt bush (*Atriplex nummularia*) was introduced into Egypt from Australia because it is well adapted for growth in saline alkaline soils, extreme arid conditions, and desert environment (Hussein, 1985). Currently, this plant is widely grown in the northwest coast (Matroh Governorate). It is used as forage for livestock due to its high nutritional value (Bickoff and Smith, 1972 and Draz, 1980).

A. nummularia (AT) is rich in saponins especially in wing bracts and inner seed coat (Hussein, 1985 and Assem, 1992).

Saponins are steroidal or triterpenoid glycosides found in many different plant species (Fenwick *et al.*, 1992 and Hostettmann and Marston, 1995). Many saponins have potent antifungal properties and, hence, it has been suggested that these compounds may play a role in protecting plants against attack by fungal pathogens (Osbourn, 1996). The major mechanism of toxicity of saponins to fungi is believed to be due to their membraneolytic action. Saponins complex with membrane sterols causing the formation of pores and, hence, the loss of membrane integrity (Osbourn, 1996). The antifungal activity of saponins against a variety of soilborne fungal pathogens has been demonstrated in previous studies (Aly *et al.*, 1996; Omar and Aly, 1996; Abdel-Momen *et al.*, 2000; and El-Sayed *et al.*, 2000).

The objective of this study was to determine effects of AT on linear growth and on pathogenicity of four soilborne fungi involved in damping-off of cotton seedlings and to evaluate the effect of AT, applied as seed treatment, on incidence of cotton seedling damping-off and on seedcotton yield under field conditions.

MATERIALS AND METHODS

Fungal isolates

Isolates of *Fusarium oxysporum* Schlech., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kuhn, and *Sclerotium rolfsii* Sacc. were isolated from roots of cotton seedlings infected with damping-off disease. Isolation, purification, and identification of these fungi were carried out at Cotton Pathology Lab., Plant Path. Res. Inst., Agric. Res. Center, Giza.

Seeds of AT as a source of saponin

Saponin-containing seeds of AT were dried at 70°C. The dried seeds were ground in Thompson Wiely mill. Saponin content in the ground seeds was determined by the modified method of Shany *et al.* (1970) and Khamis (1989). It was found that saponin content was 2% in the ground seeds.

Effect of AT on linear growth of the tested fungi

Ground seeds of AT was added to autoclaved PDA medium cooled to 50°C to obtain final concentrations of 0, 3, 6, 9 g/l. After thorough mixing, AT-amended PDA was dispensed into 9-cm Petri plates. Disks of mycelium, 6 mm in diameter, cut from the growing edge of cultures of the tested fungi, were placed in the center of plates amended with one of the four concentrations of AT. Each fungus-AT concentration was replicated five times. Linear growth at 25±3°C was recorded 4, 16, 5, and 8 days for *R. solani*, *F. oxysporum*, *M. phaseolina*, and *S. rolfsii*, respectively.

Effect of AT on incidence of cotton seedling damping-off under greenhouse conditions

Four batches of autoclaved soil were placed on greenhouse benches and separately infested with inoculum of each fungus at rates of 50, 50, 5, and 0.05 g/kg soil for *F. oxysporum*, *M. phaseolina*, *S. rolfsii*, and *R. solani*, respectively. In case of fungal mixture, autoclaved soil was placed on greenhouse bench and infested with mixture of the tested fungi to obtain final concentrations of 30, 30, 3, and 0.03 g/kg soil for *F. oxysporum*, *M. phaseolina*, *S. rolfsii*, and *R. solani*, respectively. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots and these were planted with 10 seeds per pot (cultivar Giza 86). Seeds were treated with different rates of AT ground seeds or the recommended rate of Monceren (Table 3). The greenhouse temperature during the experiment was ranging from 23.4°C to 38.5±3.5°C. Percentage of infected seedlings were recorded 45 days after planting.

Field evaluation of the effectiveness of AT ground seeds in suppressing cotton seedling damping-off

Experiments were conducted at Sakha, El-Gemmeiza, Sirs El-Lian, and Mallawy Agricultural Research Stations in 1999. Each experiment was designed as a randomized complete block of five replicates; each replicate consisted of two 5-meter rows. Each row included 25 hills, each containing 10 seeds. Seeds of cotton (*Gossypium barbadense* L.) cultivars Giza 83, Giza 85, Giza 86, and Giza 89 were treated with AT ground seeds or Monceren, which were added to slightly moist seeds at a rate of 3g/kg seeds . The seeds were shaken thoroughly in plastic bags for 5 min. and allowed to dry before being planted. There were 3 planting dates in each site (Table 5). Percentage of infected seedlings were recorded 45 days from sowing. Seedcotton yield (cottonseed and lint before ginning) was picked on 15-30 October at each site.

Identification of fungi in soils of the experimental sites

Infected seedlings were removed from the field and washed under tap water to remove any adhering soil. Small pieces of necrotic root and hypocotyle tissue were surface sterilized with 10% Clorox solution for 2 minutes, and washed several times with sterilized water. The surface sterilized pieces were then dried on sterilized filter papers and plated on PDA medium amended with streptomycin sulfate and rose bengal to eliminate bacterial contamination. The plates were incubated at 26±3°C for 3-7 days. The developing colonies were identified according to Gilman (1966) or Barnett and Hunter (1979). Colonies of each fungus were expressed as percentage of the total developing colonies.

Statistical analysis of the data:

A randomized complete block design with five replications was used in all experiments. Least significant differences (LSD) was applied for comparing means. Percentage data were transformed into arc sine angles or square roots before carrying out analysis of variance (ANOVA) to produce approximately constant variance. ANOVA was performed with the MSTAT-C statistical package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State Univ., USA).

RESULTS and DISCUSSION

Considerable variation was observed among the tested fungi as to their response to the application of AT ground seeds to the growth medium (Table 1). *R. solani* was resistant to the applied concentrations of AT ground seeds. This resistance could be attributed to the ability of *R. solani* to detoxify the saponin of AT. This conclusion is in accordance with the previous studies, which indicated, that a number of fungal pathogens produce hydrolytic enzymes that detoxify saponin by the removal of sugar molecules (Wubben *et al.*, 1996). On the other hand, Omar and Aly (1996) showed that *R. solani* isolated from cotton seedlings was sensitive to pure synthetic saponin. Therefore, it seems reasonable to conclude that source of saponin plays an important role in determining the level of its antifungal activity even against the same fungus. Contrary to the inhibitory effect on *M. phaseolina* and *S. rolfsii*, AT stimulated growth of *F. oxysporum*. This stimulatory effect is in agreement with the findings of Leath *et al.* (1972) and Omar and Abd El-

Halim (1992). *M. phaseolina* and *S. rolfsii* were the only sensitive fungi to the application of AT to the growth medium. Evidently, *S. rolfsii* was the most sensitive fungus to AT. These two fungi were also sensitive to the application of pure synthetic saponin to PDA (Omar and Aly, 1996). It is noteworthy that AT showed the highest inhibitory effect on linear growth of *M. phaseolina* when it was applied to growth medium at a rate of 3 g/l. while the higher concentrations of 6 and 9 g/l were either ineffective or less effective in reducing *M. phaseolina* linear growth. This finding may be explained by the fact that low concentrations of saponin may exert more antifungal activity than higher ones. For example, Stuteville and Skinner (1987) found that alfalfa plants with low saponin content were more resistant to downy mildew than those with high saponin content.

Table 1: Effect of different concentrations (g/l) of *Atriplex nummularia*[a] on linear growth (cm) of four soilborne fungi involved in damping-off of cotton seedlings.

Fungus	Linear growth[b] at a concentration of				LSD	
	0	3	6	9	P < 0.05	P< 0.01
Rhizoctonia solani	8.80	9.00	8.85	9.00	NS	NS
Fusarium oxysporum	6.50	7.7	7.50	7.43	0.11	0.16
Macrophomina phaseolina	8.65	7.03	8.85	8.32	0.27	0.37
Sclerotium rolfsii	9.00	5.15	4.60	4.50	0.43	0.60

[a] ***A. nummularia* was added to PDA as ground seeds.**
[b] **Linear growth at 25±3°C after 4, 16, 5, and 8 days for *R. solani*, *F. oxysporum*, *M. phaseolina*, and *S. rolfsii*, respectively.**

ANOVA (Table 2) for effects of different concentrations of AT on pathogenicity of four soilborne fungi, involved in damping-off of cotton seedlings, indicated highly significant effects of treatments, fungi, and their interaction. Due to the highly significant interaction between treatments and fungi, an interaction least significant difference (LSD) was calculated to compare treatment means within each fungus (Table 3). These comparisons showed that percentage of infected seedlings in the infested control (control 2) was much higher than that in the sterile soil (control 1) for all the tested fungi and their mixture- that is, all treatments were tested, for effectiveness in controlling the disease, under high disease pressure. This high disease pressure is considered as a prerequisite condition for any meaningful evaluation of AT as a fungicide. The comparisons also showed that the differences in disease incidence between treatments and infested control were not the same for each fungus-that is, fungi responded differently to the application of treatments. For example, application of AT, as seed treatment, at a rate of 3 g/kg seeds significantly reduced pathogenicity of *R. solani* by 62.16%, while the same treatment reduced pathogenicity of *M. phaseolina* only by 28.26%. Pathogenicity of *R. solani* was reduced by 70.27% when cotton seeds were treated with AT at a rate of 6 g/kg seeds, while this treatment reduced pathogenicity of *S. rolfsii* only by 20.41%. The application of AT, as a seed treatment, at a rate as low as 3 g/kg seeds significantly decreased damping-off caused by any individual fungus, while the increase of application rate to 6 and 9 g/kg seeds did not significantly improve the efficiency of AT in controlling the disease. Moreover, in case of fungal mixture, the increase of application rate to 6 and 9 g/kg seeds resulted in significant reduction in efficiency of AT compared to the rate of 3 g/kg seeds. These findings may be explained by the fact that saponin stimulated seed germination at low concentrations, while it reduced it at higher concentrations (Nord and Van Atta, 1960 and Omar and Aly, 1996). AT, applied at a rate of 3 g/kg seeds, was as effective as Monceren in controlling damping-off caused by *R. solani*, *F. oxysporum*, *S. rolfsii*, and fungal mixture. This result is in concert with the findings of other workers who found that saponin was as effective as the fungicides Vitavax/Captan, Benlate, Sumosclex, and Rizolex T (Omar and Abd El-Halim, 1992; Omar and Aly, 1996 and Omar *et al.*, 1996). On the other hand, *M. phaseolina* was a notable exception because the application of AT at a rate of 3 g/kg seeds was significantly less effective than Monceren in controlling damping-off caused by this fungus.

Table 2: Analysis of variance of effects of different concentrations (g/l) of *Atriplex nummularia* on pathogenicity of four soilborne fungi involved in damping-off of cotton seedlings.

Source of variation [a]	d.f.	M.S.	F-value
Replication	4	398.3307[b]	2.7107*
Treatment (T)	5	7498.5039	51.0286**
Fungus (F)	4	4036.4954	27.4691**
T x F	20	380.5995	2.5900**
Error	116	146.9470	

[a] **Replications were random, while each of treatments and fungi were fixed.**
[b] **Arc sin-transformed data.**
[c] **Significant at P $\leq$ 0.05 (*) or P $\leq$ 0.01 (**).**

Table 3: Effects of different concentrations (g/kg seeds) of *Atriplex nummularia* (AT) and the fungicide Monceren on pathogenicity of for soilborne fungi involved in damping-off of cotton seedlings.

Fungus	Infected seedlings (%) [a] — Treatment: Control [b]	Control [c]	Monceren (3 g)	AT (3 g)	AT (6 g)	AT (9 g)
Rhizoctonia solani	14 (21.69)	74 (59.57)	24 (26.27)	28 (28.33)	22 (26.70)	32 (33.17)
Fusarium oxysporum	8 (12.69)	60 (50.82)	16 (23.31)	22 (27.18)	28 (31.33)	30 (32.91)
Macrophomina phaseolina	5 (14.31)	92 (79.67)	30 (32.66)	66 (54.73)	70 (57.69)	70 (57.04)
Sclerotium rolfsii	5 (14.31)	92 (75.25)	70 (57.51)	64 (53.58)	72 (58.89)	70 (57.69)
Fungal mixture	22 (27.60)	98 (86.31)	38 (37.33)	38 (37.68)	78 (67.67)	76 (66.69)

LSD (Transformed data) for fungus x treatment = 15.18 (P $\leq$ 0.05) or 20.06 (P $\leq$ 0.01).

[a] **Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produced approximately constant variance. Transformed data are shown in parentheses.**
[b] **Sterile soil.**
[c] **Sterile soil infested with the designated fungus.**

Effect of AT on linear growth of the tested fungi under pure culture conditions was not related to its effect on the pathogenicity of these fungi under greenhouse conditions. Thus, while AT had no effect on linear growth of *R. solani* in the pure culture, it significantly reduced its pathogenicity by 56.76-70.27% in the greenhouse test. AT stimulated growth of *F. oxysporum* on PDA, while it significantly suppressed damping-off caused by this fungus by 50.00-63.33% in the greenhouse test. *S. rolfsii* was the most sensitive fungus to AT in pure culture; however, AT showed modest ability to suppress damping-off caused by this fungus in the greenhouse test. Taken together, these results suggest that toxicity of AT to the pathogens was unlikely to be the mechanism by which AT suppressed damping-off caused by the pathogens. Evidently, another mechanisms should account for such a suppression.

It is now well established that resistance can be induced in plants which lack genes for resistance by pretreating with a number of abiotic agents, which alters disease reaction to subsequent challenge inoculation (Misaghi, 1982).

Accordingly, it seems reasonable to conclude that AT suppressed damping-off of cotton seedlings by inducing resistance to the pathogens involved in the disease.

Twelve field trials were conducted in Sakha, El-Gemmeiza, Sirs El-Lian, and Mallawy in 1999 to evaluate the effectiveness of AT in suppressing cotton seedling damping-off under field conditions. Typical symptoms of seedling damping-off were observed on infected seedlings in each site. Frequency of fungi isolated from the infected seedlings is shown in Table 4. Of the isolated fungi, *R. solani* and *Fusarium* spp. are considered as major causes of cotton damping-off, while the other fungi like *Aspergillus* spp. and *Rhizopus* spp. are unimportant disease agents except when the cotton seedlings are weakened (Watkins, 1981). The lowest disease level was observed in Sirs El-Lian, where the percentages of infected seedlings in the control treatments were ranging from 31.10-33.90% (Table 5).

Table 4: Isolation frequency of fungi from cotton seedlings infected with postemergence damping-off in the experimental sites.

Fungus	Isolation frequency (%)[a]			
	Sakha	El-Gemmeiza	Sirs El-Lian	Mallawy
Fusarium spp.	31.8	0.0	13.0	55.6
Rhizoctonia solani	0.0	46.7	44.2	11.6
Alternaria spp.	2.9	0.0	0.0	0.0
Chaetomium spp.	0.0	0.0	4.0	0.0
Aspergillus spp.	11.9	0.0	30.9	4.0
Penicillium spp.	4.0	0.0	0.0	0.0
Rhizopus spp.	27.5	53.3	7.9	25.6
Cladosporium spp.	2.9	0.0	0.0	3.2
Nigrospora spp.	4.0	0.0	0.0	0.0
Unidentified (Sporulating)	15.0	0.0	0.0	0.0

[a] Colonies of each fungus were expressed as percentage of the total developing colonies. Each value was the mean of 5 replications (plates).

AT was effective in controlling damping-off only in Sirs El-Lian, where it significantly reduced it by 33.33, 33.76, and 39.55% in the first, second, and third planting dates, respectively. In this site, AT was as effective as the standard fungicide Monceren in controlling the disease. AT also significantly increased seedcotton yield in Sirs El-Lian by 35.65 and 38.42% in the second and third planting dates, respectively.

Sakha experiment in the third planting date was heavily infested with late season insects, which severely reduced seedcotton yield. In spite of the unfavorable conditions, AT significantly increased seedcotton yield by 15.38% (P < 0.10).

Table 5: Effect of treating cotton seeds with ground seeds of *Atriplex nummularuia* on incidence of cotton seedling damping-off and on seedcotton yield under field conditions in 1999.

Location and cultivar	Treatment	Planting date[a]								
		First			Second			Third		
		Infection			Infection			Infection		
		%	Trans.	Yield[b]	%	Trans.	Yield	%	Trans.	Yield
Sakha (Giza 86)	*A. nummularia*	95.92	9.79[c]	3.97	72.20	8.49[c]	1.15	53.80	...	0.60[x]
	Monceren	93.80	9.68	4.00	68.10	8.23	1.13	51.20	...	0.55
	Control	92.80	9.63	3.67	74.50	8.62	1.15	39.10	...	0.52
	LSD (P ≤ 0.05)		**NS**	**NS**		**NS**	**NS**	**NS**		**NS**
	LSD (P ≤ 0.01)		**NS**	**NS**		**NS**	**NS**	**NS**		**NS**
El-Gemmeiza (Giza 89)	*A. nummularia*	49.00	44.42[d]	7.70	47.00	43.28[d]	4.63	49.50	44.71d	2.92
	Monceren	20.00	26.13	8.57	19.00	25.74	5.53	24.50	29.61	3.75
	Control	51.00	45.57	7.40	50.00	45.00	4.50	50.00	44.71	2.80
	LSD (P ≤ 0.05)		**7.89**	**NS**		**4.57**	**NS**		**7.37**	**NS**
	LSD (P ≤ 0.01)		**11.95**	**NS**		**6.92**	**NS**		**11.16**	**NS**
Sirs El-Lian (Giza 85)	*A. nummularia*	22.60	4.75[c]	4.00	20.60	4.53[c]	4.30	18.80	4.31[c]	7.17
	Monceren	22.40	4.73	5.02	19.40	4.40	6.50	18.80	4.32	7.18
	Control	33.90	5.81	5.50	31.10	5.58	3.17	31.10	5.58	5.18
	LSD (P ≤ 0.05)		**0.57**	**NS**		**0.35**	**1.93**		**0.63**	**0.96**
	LSD (P ≤ 0.01)		**0.87**	**NS**		**0.53**	**NS**		**0.95**	**1.45**
Mallawy (Giza 83)	*A. nummularia*	63.40	...	7.63	68.50	...	9.07	78.60	8.86[c]	12.50
	Monceren	66.40	...	10.20	69.90	...	7.43	78.10	8.83	10.47
	Control	67.50	...	10.20	67.30	...	8.67	85.40	9.24	12.70
	LSD (P ≤ 0.05)	**NS**		**NS**	**NS**		**NS**		**NS**	**NS**
	LSD (P ≤ 0.01)	**NS**		**NS**	**NS**		**NS**		**NS**	**NS**

[a] In Sakha, planting dates were 18/4, 12/5, and 28/5. In El-Gemmeiza, planting dates were 29/3, 15/4, and 3/5. In Sirs El-lian, planting dates were 25/3, 8/4, and 22/4. In Mallawy, planting dates were 26/3, 13/4 and 3/5.

[b] Seedcotton yield (Kentar/feddan)

[c] Percentage data were transformed into $\sqrt{X}$ before carrying out the analysis of variance to produce approximately constant variance.

[d] Percentage data were transformed into arc sine angles before carrying out the analysis of variance.

[x] Significant increase compared to the control (P ≤ 0.10, LSD = 0.06).

Seedcotton yield of all the other experiments was not affected by the application of AT.

It is worthy of mention that the standard fungicide Monceren was effective in controlling the disease only in El-Gemmeiza and Sirs El-lian.

The failure of AT to suppress the disease in Sakha, El-Gemmeiza, and Mallawy could be related to the relatively high disease pressure, which obscured any potential resistance induced by AT. On the other hand, AT was effective in suppressing the disease in Sirs El-lian because the disease pressure reached its lowest level in this site. Another possibility for such a failure is the degradation of AT saponin by indigenous microorganisms in soil of the experimental sites.

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استعمال نبات الأتربلكس نيوميولاريا المحتوى على مادة السابونين لمقاومة مرض موت بادرات القطن

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درس تأثير مطحون بذرة نبات الأتربلكس نيوميولاريا على النمو الطولى لأربعة من فطريات التربة ، المسببة لمرض موت بادرات القطن ، وذلك تحت ظروف المعمل. تباينت الفطريات موضع الدراسة فيما بينها بشكل ملحوظ من حيث الإستجابة التى أظهرتها بعد إضافة مطحون البذرة إلى بيئة النمو ، لم يؤثر مطحون البذرة على النمو الطولى لفطر ريزوكتونيا سولانى ، فى حين ثبط النمو الطولى لكل من فطرى ماكروفومينا فسيولينا وسكليروشيم رولفسياى ، اما فطر فيوزاريوم أوكسيسبورم فقد حدث تنشيط لنموه. درس تأثير المطحون على القدرة المرضية للفطريات ، عند استعماله لمعاملة البذرة بتركيزات مختلفة ، وذلك تحت ظروف الصوبة. أظهر تحليل التباين أن الفطريات والتركيزات وتفاعل الفطريات × التركيزات كانت كلها مصادر معنوية للتباين فى القدرة المرضية ، نظراً لمعنوية تفاعل الفطريات × التركيزات فإن أقل فرق معنوى إستعمل للمقارنة بين تأثير التركيزات على القدرة المرضية لكل فطر على حدة ، أظهرت هذه المقارنات أن الفطريات تباينت فيما بينها من حيث درجة الاستجابة لتركيزات المطحون. إن إستعمال المطحون لمعاملة البذرة بمعدل ٣ جم/كجم بذرة أدى إلى حدوث إنخفاض معنوى فى القدرة المرضية لجميع الفطريات ، إلا أن زيادة هذا المعدل إلى ٦ و٩ جم/كجم بذرة لم تؤدِّ إلى حدوث زيادة معنوية فى كفاءة المطحون فى مقاومة المرض. فى حالة خليط الفطريات ، أدى إستعمال المطحون بمعدل ٦ و ٩ جم/كجم بذرة إلى نقص معنوى فى الكفاءة مقارنة بكفاءة المطحون عند إستعماله بمعدل ٣ جم/كجم بذرة. أظهرت الدراسة عدم وجود علاقة بين تأثير المطحون على النمو الطولى للفطريات تحت ظروف المعمل ، وتأثيره على القدرة المرضية تحت ظروف الصوبة. أجريت ١٢ تجربة حقلية فى سخا والجميزة وسرس الليان وملوى خلال موسم ١٩٩٩ (٣ مواعيد زراعة فى كل موقع) وذلك لتقييم فعالية المطحون فى مقاومة مرض موت بادرات القطن تحت ظروف الحقل. أدى إستعمال المطحون لمعاملة البذرة قبل الزراعة إلى إنخفاض مستوى الاصابة بالمرض فى سرس الليان بنسب ٣٣,٣٣ و ٣٣,٧٦ و ٣٩,٥٥% فى مواعيد الزراعة الأول والثانى والثالث على التوالى ، بينما لم يؤثر إستعمال المطحون على مستوى الإصابة بالمرض فى باقى التجارب ، الجدير بالذكر أن فعالية المطحون فى مقاومة المرض فى سرس الليان كانت تعادل فعالية المبيد الفطرى الجهازى مونسرين. ترتب على إستعمال المطحون فى سرس الليان حدوث زيادة معنوية فى محصول القطن الزهر بنسب ٣٥,٦٥ و ٣٨,٤٢% فى مواعيد الزراعة الثانى والثالث ، على التوالى ، أدى إستعمال المطحون فى سخا إلى حدوث زيادة معنوية فى محصول القطن الزهر فى ميعاد الزراعة الثالث بنسبة ١٥,٣٨% ، أما محصول القطن الزهر لباقى التجارب فإنه لم يتأثر بإستعمال المطحون.