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ASSOCIATED FUNGI WITH SEEDS OF SOME EGYPTIAN COTTON CULTIVARS AND THEIR EFFECT ON THE PLANT MORTALITY, MYCOTOXIN PRODUCTION AND SEED OIL CONTENT

BY

Nawal A., Eisa*; El-Habbaa, G.M.*; Aboul-Ella, M.F. and Hassan, S.R.*****

* Agric. Botany Dept., Plant Pathology Branch, Fac. Agric., Benha University

** Central Lab. for Food and Feed, Agri. Res. Center, Giza

*** Field Crops Research Institute, Agric. Res. Center, Giza, Egypt.

ABSTRACT

Isolation trials from cotton seeds of cvs. Giza-86 and Giza-89 as well as damped-off seedlings resulted in several fungi belonging to 5 genera and 11 species. The isolated fungi were purified and identified as *Alternaria alternata* (Fr.) Keissler, *Aspergillus niger* Van Tieghem, *Fusarium dimerum* (Penz.) Arx, *Fusarium moniliforme* Sheld, *Fusarium nivale* (Fr.) Samuels & Hallett, *Fusarium roseum* Link emend., Snyder & Hansen, *Fusarium semitectum* Berk & *Rav, Fusarium tricinectum* (Corda) Sacc.,*Fusarium solani* (Mart.) Sacc. emend. Snyder & Hansen, *Penicillium spp* and *Rhizoctonia solani* Kuhn, *R. solani* isolates were mostly the dominant fungus isolated from cotton seeds of cvs. Giza-86 and Giza-89 before and after delinting as well as from the inner surface of testa and the rotten roots while, *Fusarium roseum* showed the highest frequency in the cotoyledons of both cvs. In general, the total number of isolated fungi from the cotoyledons was greatly low comparing with those isolated from seed testa for both cotton cvs. Among the 4 tested pathogens, *R. solani* was more aggressive as it caused the highest rates of pre- and post- emergence damping-off on both cotton cvs.. Also, increasing the inoculum level from 1 to 3% of soil weight increased gradually the percentage of infection. Also, means of survived cotton plants indicate that *R. solani* followed by *F. semitectum* were the highly pathogenic fungi at most the tested inoculum levels, whereas *F. roseum* was the least one. All the tested fungi were not able to produce aflatoxins (B1&B2), zearalenone, fumonisins and trichothesense *in vitro* on YES medium., Meanwhile, when cotton seed samples of both cvs. were infested with the same tested root rot pathogens, clear amounts of mycotoxins were detected in some cases. Moreover, infestation of cotton seeds with any of the tested root rot pathogens *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* affected negatively oil content of the seeds. Increasing the incubation period from 5 to 15 days decreased gradually oil contents for all treatments compared with the un-infested seeds. The highest decrease in oil content was recorded in the case of seed infestation with *R. solani* and *F. moniliforme* at any tested incubation period for the seeds of both cotton cvs.

Key words: cotton seeds, mycotoxin, oil content, and root rot fungi

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber and oil crops in Egypt and many other countries all over the world. It is attacked by several disorders, which resulted from insects, fungi, bacteria, nematodes and others at different stages of plant growth. Fungi are the widest pathogens but bacteria and viruses are sometimes involved. In this respect, Fulton and Bollenbacher (1959) isolated *Rhizoctonia solani, Pythium ultimum, Fusarium oxysporum, F. moniliforme, F. semitectum* and several other fungi from cotton seeds and seedlings and found that the most isolated fungi were pathogenic to cotton seedlings. Also, Alfred (1963) indicated that fungi belonging to *Alternaria, Aspergillus, Diplodia, Fusarium* and *Rhizoctonia* were associated with the seed hairs and the actual seeds during boll development. Kuch (1986) isolated *Fusarium equiseti* and *Fusarium semitectum* for more than 10% of the seeds at any sampling of delinted surface sterilized cotton seeds in the southern USA. *Rhizoctonia solani* was higher on roots with severe tissue damage than on roots exhibiting little or no damage (Huisman, 1988). On the other hand, Seneewong *et al.* (1991) found that *Fusarium* spp were the most prevalent fungal species isolated from inside the seed coat and from the embryo of 100 randomly selected seeds. The percentage of fungi occurring inside the seed coat was low. No fungi were found on the embryos during isolation from inside the seed coat and from the embryos of selected seeds from each lot. Wang *et al.* (1992) recorded high frequency of *Fusarium moniliforme* and *F. semitectum* from cotton seedlings and bolls while, *F. oxysporum, F. solani, F. equiseti* and *F. compactum* were less frequent. Mansoori and Hamdolahzadeh (1995) isolated *Alternaria alternata, Aspergillus niger, Fusarium acuminatum, Fusarium solani, Pythium ultimum, Rhizopus arrhizus* and *Rhizoctonia solani* from cotton seeds. Zhang *et al.* (1996) showed that *F. oxysporum, F. solani, F. equiseti* and *F. semitectum* were presented on the rhizoplane of cotton plants grown in pots containing cotton field soil. *F. oxysporum* and *F. solani* were the most dominant species. Palmateer *et al.* (2004) isolated fifty eight species of fungi belonging to 37 genera, including 9 species of *Fusarium. Fusarium oxysporum, F. solani* and *F. equiseti* were the most common members of this genus occurring at seedling stage.

As for mycotoxin production, Sankaranarayanan and Kumar (1985) isolated a toxin from the culture filtrate of a virulent strain of *F. oxysporum* f.sp. *vasinfectum* induced typical vein clearing symptoms in cotton shoots. Mazen *et al.* (1990) found that about 16 of the Egyptian cotton seeds and cotton seed products were naturally contaminated by aflatoxin B1 and B2. No citrinin, ochratoxin A, patulin, sterigmatocystin, diacetoxyscirpenol, T-2 toxin or zearalenone were detected in the samples assayed. Li *et al.* (1990) recorded that 4 strains of *F. moniliforme* [*Gibberella fujikuroi*] were isolated from cotton dust and when inoculated onto the growing cotton boll, inducing wilt in cotton.

Regarding the effect of pathogenic fungi on seed oil content, Shadmanov and Alimukhamedov (1983) reported that infected seeds of cotton varieties and hybrids lost roughly half of their weight and half of their content of oil, nucleic acids and protein in comparison with healthy plants. Ataga and Akueshi (1986)

reported that *A. tenuis (A. alternata),. Curvularia lunata* [*Cochliobolus lunatus*], *Fusarium moniliforme* [*Gibberella fujikuroi*] and *Macrophomina phaseolina* infected sunflower seeds over 21 day increased the free fatty acid content, reduced the oil content and caused discoloration of the oil. Airede and Fsuruoso (1987) found that inoculating the autoclaved oil palm with *Aspergillus flavus, A. niger, Penicillium chrysogenum, P. janthinellum, Paecilomyces varioti, Syncephalastrum racemosum* or *Fusarium oxysporum.* for 0, 2, 4 and 8 weeks decreased the total oil content where, *A. flavus* caused the greatest change. Ahmed *et al.* (1994) found that infected sunflower seeds in Egypt with *F. oxysporum* had lower seed oil content, lower iodine values and higher acid numbers than the healthy seeds.

This work aimed to determine the associated fungi with some Egyptian cotton seeds used in cultivation and their effect on plant mortality, mycotoxin production and oil content.

MATERIALS AND METHODS

1- Isolation trials:

1a- Isolation from cotton seeds:

Seeds of two cotton cultivars *i.e.*, Giza-86 and Giza-89 were obtained from Cotton Research Institute, Agricultural Research Center, Giza, Egypt during 2000 and 2001. Samples were delinted by using 40% sulphuric acid for 3 minutes, then washed with sterilized distilled water (Helal *et al.* 1996). Hundred seeds were used for isolation before and after delinting by blotter test method as described by Paul *et al.* (1970). In this respect, the seeds were disinfested by immersing in 5% sodium hypochlorite for 3 minutes, then washed in sterilized distilled water and dried between two sterilized filter papers. Ten seeds representing each treatment were plated onto glass Petri dish (9 cm Φ) in two cycles over moistened filter paper where, the first cycle included 8 seeds while the other consisted of 2 seeds. The dishes were incubated at 25°C with a daylight regime of alternating cycles of near ultraviolet (UV) light for 12 hrs and 12 hrs darkness for 8 days. Observations of the resulted fungi were daily done using Stero-binocular microscope and the habit characters of different fungi were investigated after five or seven days post incubation. Also, preparations of the resulted and un-distinguished fungi were investigated under the microscope for complete identification.

1b- Isolation of fungi from the cotyledons and the inner surface of testa:

Hundred delinted seeds were inspected for the presence of any associated fungi in cotyledons and or those carried onto the inner surface of the testa. The seeds were soaked in tap water for 30 minutes, then surface sterilized in 3% sodium hypochlorite for 5 minutes,then washed several time in sterilized distilled water and dried between two sterilized filter papers The seed coats were then separated from the cotyledons containing embryos. Both testa and cotyledons were aseptically transferred to potato dextrose agar medium (PDA) containing 40 ppm streptomycin sulphate to avoid any bacterial growth. Plates were incubated at 25°C for 5–7 days and examined daily for the occurrence of

fungal growth. The emerged fungi were transferred individually to fresh (PDA) dishes. Purification of the isolated fungi was carried out as mentioned by Roncadori *et al.* (1971).

1c-Isolation of fungi from rotten roots of cotton seedlings:

Cotton roots and hypocotyls of seedlings showing damping-off symptoms were collected, then the infected parts were cut into small pieces, washed thoroughly with running tap water to remove any adhering soil particles. The pieces were surface sterilized as usual in 3% sodium hypochlorite for 3 minutes, then aseptically transferred to PDA plates. Plates were incubated at 25°C for 5–7 days and examined daily for the occurrence of fungal growth. The grown fungi were transferred individually to fresh (PDA) dishes. Purification of the isolated fungi was carried out as mentioned before.

All the isolated fungi from the different trials were identified according to their morphological and microscopical characters as described by Gilman (1957), Ram *et al.* (1970) Barnett and Hunter (1972), Sneh, *et al.* (1991) and Jens *et al.* (1991). Also, identification was Kindly confirmed at the Department of Fungal Taxonomy, Plant Pathology Institute, ARC. Giza, Egypt.

2- Pathogenicity tests:

Pathogenicity tests were carried out under greenhouse conditions at Agriculture Research Center, Giza. The fungal inocula were prepared using 500 ml conical flasks containing corn meal–sand medium. Each flask contained clean sand (25 g), corn meal (75g) and enough tap water to cover the prepared mixture and autoclaved for 30 minutes. The flasks were inoculated with each of the isolated fungi and incubated at 28°C for two weeks. Formalin sterilized clay pots (20 cm Φ) were filled with autoclaved loamy soil (5 kg soil/pot). The potted soils were infested with prepared fungal inocula for testing at rates of 1, 2 and 3% of soil weight. The added inocula were thoroughly mixed with the soil and watered regularly for 15 days before planting (Whitehead, 1957). Un- inoculated cornmeal–sand medium was added to the prepared soil as mentioned before to serve as check pots. Three pots were used as replicates for each particular treatment. Each pot was planted with 10 surface sterilized cotton seeds. Seeds of two cotton cultivars, *i. e.* Giza-86 and Giza-89 were used. Disease assessment was recorded as percentages of seeds showing pre–and post–emergence damping–off and survivals at 10 and 21 days after sowing, respectively.

3-Determination of mycotoxins produced by the tested pathogenic fungi:

3a- In cultures:

Mycotoxins *i.e.*, aflatoxins (B1' B2' G1' G2), fumonisins, zearalenone and trichothesense were determined by growing the tested pathogenic fungi in yeast extract sucrose medium (YES) consisting of 20g yeast extract and 200g sucrose and 1000 ml distilled water. Each 25 ml of the prepared YES medium were inoculated with 0.5 ml spore suspension of each isolate and then incubated for 15 days at 25°C (Park and Bullerman, 1981). Extraction and determination of mycotoxins were determined according to Anon. (1990).

3b- In seeds:

100-g samples were homogenized in 200 ml methanol: water solution (8:2) in a blender for 3 min. The samples were filtered then, cleaned using 50 ml of clean up solution (150 g zinc sulphate +50 g phosphotungestic acid then dissolved in 1000 ml distilled water and filtered again using filter paper No. 4. About 75-ml of the collected filtrate were put in separating funnel containing 15-ml benzene, then shacked for 5 min. The upper layer was collected in a beaker and evaporated to dryness under steam of nitrogen.

Samples and standard aflatoxins (B1, B2, G1 and G2), zearalenone and fumonisins (Sigma, USA) were spotted on thin layer chromatography (TLC) plates at different concentrations: 2, 5, 7 and 10 µl, the spotted samples on TLC plates were eluted in eluting jar (contained, diethyl ether-methanol-water 96:3:1, v/v/v) for running. The running of samples was stopped when elution solvent reached the end line then TLC plates were dried and examined under ultraviolet detector at 365 nm.

Readings of mycotoxins were expressed as µg/kg sample = (S x Y x V) / (X x W)
Where: S = µl mycotoxins std. equal to unknown:

Y= Concentration of std. mycotoxins (aflatoxins, zearalenone and fumonisins) µg/ml.

V = µl of final dilution of sample.

X = µl sample extraction spotted giving fluorescent intensity equal to S.

W= weight of sample (100 g).

4- Determination of oil content:

Cotton seed samples (100-g, each) representing each cvs. Giza-86 and Giza-89 were inoculated with 0.5 ml spore suspension of each isolate then incubated for 15 days. Oil content was determined by extraction with petroleum ether (40-60°C b.p.) for 16 hrs using Soxhlet apparatus according to the method described by Anon. (1990) at different periods after inoculation.

RESULTS

I- Fungi associated with cotton seeds before and after delinting.

Isolation trials from cotton seeds (before and after delinting, testa and cotoyledons) and damped-off seedlings resulted in several fungi belonging to 5 genera and 11 species. Data in Table, (1 a) reveal that the total fungal isolates obtained from cotton seeds of cvs. Giza-86 and Giza-89 before delinting were 85 and 90 isolates, respectively. Out of them, *R. solani* produced the highest number of colonies with the highest frequency being 72.9 and 46.7% followed by *Fusarium moniliforme* and *F. roseum* from seeds of cvs Giza-86 and Giza-89, respectively. Meanwhile, *F. semitectum*, and *F. nivale* were more frequent in seeds of cv. Giza-89 than cv.Giza-86. Generally, all isolated fungi from cv Giza-86 except, *R. solani* were less frequent than on Giza-89 when isolation was carried out before delinting. Also, *Aspergillus niger, F. roseum* and *Penicillium spp* were not recorded on seeds of Giza-86 while *F. tricinectum* was not observed in seeds of Giza-89.

In delinted seeds, the total isolated fungi were 44 and 57 isolates from Giza-86 and Giza-89, respectively. Out of them, *F. moniliforme* and *R. solani* were more frequent within the seeds of cvs. Giza-86 where their frequency recorded 43.2 and 40.9%, respectively. Meanwhile, *R. solani* was the highest frequent in seeds of cv. Giza-89 (50.9%). On the other hand, *F. dimerum* was recorded only from seeds of cv. Giza-89 after delinting. However, it is pronounced from the results that many of the isolated fungi from seeds whether before or after delinting such as *Alternaria alternata, Aspergillus niger, Penicillium spp* and *F. semitectum* were isolated at low frequencies from seeds of both cvs. Also, it is clear that the total number of isolates obtained from the two cvs of cotton seeds after delinting were lesser than those isolated before delinting. In all cases, *R. solani* was mostly prevalent.

Table (1 a): Occurrence and frequency, of isolated fungi from cotton seeds (before and after delinting).

Isolated fungi	Frequency,% of the isolated fungi from cotton seeds,							
	Before delinting				After delinting			
	Giza-86		Giza-89		Giza-86		Giza-89	
	No.	F.	No.	F.	No.	F.	No.	F.
A.. alternata	2	2.4	3	3.3	-	-	2	3.5
A.. niger	-	-	2	2.2	-	-	-	-
F. dimerum	-	-	-	-	-	-	8	14.0
F.moniliforme	8	9.4	12	13.3	19	43.2	6	10.5
F. nivale	4	4.7	9	10.0	2	4.6	4	7.0
F. roseum	-	-	10	11.1	-	-	8	14.0
F.semitectum	5	5.9	9	10.0	5	11.4	-	-
F.tricinectum	4	4.7	-	-	-	-	-	-
Penicillium spp	-	-	3	3.3	-	-	-	-
R. solani	62	72.9	42	46.7	18	40.9	29	50.9
Total	85	100	90	100	44	100	57	100

No. = Number of isolated F. = Frequency % of the

Data in Table (1 b) show that the total fungal isolates obtained from the inner cotton seed testa of cvs. Giza-86 and Giza-89 were 69 and 43 isolates, respectively. Out of them, *R. solani* was the highest frequent fungus on both testa of cotton cvs. seeds where its frequency was 30.5 and 30.2%, respectively followed by *F. nivale* (30.2%) from seed testa of cv. Giza-89, *F. semitectum* (28.9 %) and *F. moniliforme* (24.6%) in the seed testa of cv.Giza-86. On the other hand, *A. alternata, F. solani, F. tricinectum, Penicillium spp* and *A. niger* were absent or isolated at low numbers from cotton seed testa of both cvs. Giza-86 or Giza-89.

As for the isolated fungi from cotoyledons,(Table, 1b) the total fungal isolates *i.e.*, 14 and 19 were isolated from the cotoyledons of cvs. Giza-86 and Giza-89 seeds, respectively. Out of them, *F. roseum* showed the highest frequency (28.5 and 31.5%) from cotoyledons of both cotton cvs. seeds followed by *F. moniliforme* from cvs Giza-86 and Giza-89 seeds (21.3 and 15.8%) respectively. Meanwhile, *F. solani* was not recorded in cotoyledons of both cotton cvs. However, *Penicillium spp., A. alternata* and *A. niger* as well as, some other fungi were isolated in low frequency. In general, the total number of isolated fungi from the cotoyledons was greatly lower than those isolated from seed testa for both cotton cvs tested.

Table (1 b): Occurrence and % of isolated fungi from testa and cotoyledons of cotton seeds.

Isolated fungi	Frequency,% of isolated fungi from cotton seeds,							
	Testa				Cotyledons			
	Giza-86		Giza-89		Giza-86		Giza-89	
	No.	F.	No.	F.	No.	F.	No.	F.
A.alternata	-	-	2	4.7	-	-	2	10.5
A. niger	1	1.5	-	-	2	14.3	1	5.3
F.moniliforme	17	24.6	4	9.3	3	21.3	3	15.8
F. nivale	10	14.5	13	30.2	2	14.3	1	5.3
F. roseum	-	-	8	18.6	4	28.5	6	31.5
F. semitectum	20	28.9	2	4.7	1	7.2	1	5.3
F. solani	-	-	1	2.3	-	-	-	-
F.tricinectum	-	-	-	-	1	7.2	3	15.8
Penicillium spp.	-	-	-	-	1	7.2	-	-
R. solani	21	30.5	13	30.2	-	-	-	-
Unknown	-	-	-	-	-	-	2	10.5
Total	69	100	43	100	14	100	19	100

No. = Number of F. = Frequency % of the

Regarding the isolated fungi from damped-off seedlings of cotton, data in Table (1 c) show that 6 fungal isolates were obtained from the rotten roots of cv. Giza-86, two of them were *R. solani*. Meanwhile, 14 isolates were obtained from the rotten roots of cv. Giza-89, out of them 5 isolates were *R. solani*. It is clear that *R. solani* was the most frequent fungus followed by *F. moniliforme* and *A. niger*. However, *Penicillium spp.* and *F. tricinectum* recorded the lowest frequency.

Table (1 c): Occurrence frequency % of isolated fungi from cotton damped-off seedlings

Isolated fungi	Frequency, % of isolated fungi from cotton rotten roots				
	cv. Giza-86		cv. Giza-89		Mean of frequency %
	No.	F.	No.	F.	
A. niger	1	16.67	2	14.29	15.48
F. moniliforme	1	16.67	3	21.43	19.05
F. tricinectum	1	16.67	1	7.14	10.42
Penicillium spp.	-	-	2	14.29	7.14
R. solani	2	33.32	5	35.71	34.52
Unknown	1	16.67	1	7.14	11.90
Total	6	100	14	100	

No. = Number of isolated F. = Frequency, % of the isolated

2- Pathogenicity tests:

Data in Table (2) indicate that *R. solani* was the highest pathogenic fungus among all of the tested fungi where it caused the highest pre-emergence

damping-off when the first three inoculum levels *i.e.*, 1, 2, 3% were used on both cotton cvs. Giza-86 and Giza-89. In this respect, *R. solani* caused the highest pre-emergence damping–off percentage followed by *F. semitectum, F. moniliforme* and *F. roseum*. Also, increasing the inoculum level from 1 to 3% increased gradually the percentage of pre–emergence damping–off, where the highest pre-emergence was recorded at 3% inoculum level for all the tested pathogens.

Regarding the post emergence damping-off, it is clear that the infection values ranged from 3.3 to 12.2 % in the case of cotton cv. Giza-86 and 3.3-15.5 % in the case of cv. Giza-89. *R.solani* and *F. semitectum* were the most virulent pathogens at this disease stage while; *F. roseum* was the least virulent one. However, increasing inoculum level of each pathogen from 1 to 3% increased gradually to reach its maximum at 3% inoculum level.

As for the plant survival, the results indicate that increasing inoculum level from 1 to 3 % gradually decreased the percentages of survived cotton plants. In this respect, the least plant survival was at 3% inoculum level in case of cv. Giza-86 infection with *R. solani,* while the highest survival was at 1% inoculum level in case of cv. Giza-89 infection with *F. roseum*. Also, the means of survived cotton plants indicate that *R. solani* followed by *F. semitectum* were the highly pathogenic fungi at most tested inoculum levels whereas *F. roseum* was the least one in this respect onto both the tested cotton cvs.

Table (2): Effect of different inoculum levels on the incidence of pre- and post emergence damping-off.

Disease parameters	Tested fungi	Survived and damped-off seedlings % at different inoculum levels							
		cv.Giza-86			Mean	cv.Giza-89			Mean
		1	2	3		1	2	3	
Pre-emergence %	*R. solani*	23.4	26.7	36.7	21.7	10.1	16.7	43.4	17.5
	F. semitectum	13.4	20.1	30.1	15.9	6.7	10.1	20.1	9.2
	F. moniliforme	16.7	23.4	23.4	15.9	6.7	13.4	13.4	8.3
	F. roseum	10.1	16.7	23.4	12.6	3.4	13.4	13.4	7.5
Mean		15.9	21.7	28.4	16.5	6.7	13.4	22.6	10.7
Post-emergence %	*R. solani*	3.3	10.0	12.2	6.4	3.3	10.0	15.5	7.2
	F. semitectum	3.3	8.9	12.2	6.1	3.3	8.9	11.1	5.8
	F. moniliforme	3.3	7.7	10.0	5.3	3.3	6.6	11.1	5.3
	F. roseum	3.3	6.6	7.7	4.4	3.3	5.5	7.7	4.1
Mean		3.3	8.3	10.5	5.5	3.3	7.8	11.4	5.6
Survival-%	*R. solani*	73.3	63.3	51.1	71.9	86.6	73.3	41.1	75.3
	F. semitectum	83.3	71.1	57.7	78.0	90.0	81.1	68.8	85.0
	F. moniliforme	80.0	68.6	66.6	78.8	90.0	80.0	75.5	86.4
	F. roseum	86.6	76.7	68.9	83.1	93.3	81.1	78.9	88.3
Mean		80.8	69.9	61.1	78.0	90.0	78.9	66.1	83.8

Where: Natural of un–germinated seeds in Pre–emergence damping–off stage (control) = *(16.7%)
Natural of dead seedling in Post-emergence damping–off stage (control) =** (23.3%)

3- Effect of the isolated fungi on mycotoxin production:

Data in Table (3) reveal that all the tested fungi were not able to produce any kind of mycotoxins *i.e.*, aflatoxins (B1 & B2), zearalenone, fumonisins and trichothesense when the tested fungi were grown in cultures of YES medium.

On the other hand, when cotton seed samples of both cvs. Giza-86 and Giza-89 were infested with the tested root rot pathogens, clear amounts of mycotoxins (ppb) were detected in some cases. In this respect, *F.semitectum* produced 600 and 200 ppb of zearlenone mycotoxin into the infected seeds of cvs.Giza-86 and Giza-89, respectively. Also, *F. roseum* produced 250 and 200 ppb into the infected seeds of cvs. Giza-89 and Giza-86, respectively. On the other hand, *R. solani* and *F. moniliforme* were not able to produce zearlenone mycotoxin into the cotton seeds of both tested cvs. As for fumonisins mycotoxins, only *F. moniliforme* produced 200 and 300 ppb into the infected seeds of cvs. Giza-86 and Giza-89, respectively. In addition, none of the four tested isolates was able to produce aflatoxins into the infested cotton seeds, meanwhile aflatoxins were detected only in naturally contaminated cotton seeds of both tested cvs.

Table (3): Mycotoxins produced by the isolated fungi in the infested cotton seeds with some pathogenic fungi

Tested fungi	Produced mycotoxins (ppb)					
	Zearlenone (ppb)		Fumonisins (ppb)		Aflatoxins (ppb)	
	cv.Giza-86.	cv.Giza-89.	cv.Giza-86.	cv.Giza-89.	cv.Giza-86.	cv.Giza-89.
R.solani	0.0	0.0	0.0	0.0	0.0	0.0
F.moniliforme	0.0	0.0	200.0	300.0	0.0	0.0
F.roseum	200.0	250.0	0.0	0.0	0.0	0.0
F.semitectum	600.0	200.0	0.0	0.0	0.0	0.0
Control	0.0	0.0	0.0	0.0	250.0	650.0

4- Changes in the oil content of infested cotton seeds:

Data in Table (4) reveal that infestation of cotton seeds with any of the tested root rot pathogens *i.e.*, *R. solani, F. moniliforme, F. semitectum,* and *F. roseum* affected negatively oil content of the seeds. In this respect, all the tested pathogens decreased the percentages of oil content of the tested cotton seeds of cvs Giza-86 and Giza-89 compared with the uninfested seeds (control) at any incubation period *i.e.*, 5,10 and 15 days. It is clear also that increasing of the incubation period from 5 to 15 days has decreased gradually the percentages of oil content in all treatments compared with the un-infested seeds (check). The highest decrease in oil content was recorded in the case of seed infestation with *R. solani* and *F. moniliforme* at any tested incubation period for the seeds of both cotton cvs.

Table (4): Effect of treating the seed of cotton seeds cvs. Giza-86 and Giza-89 with the root-rot pathogens on oil content (%) incubation at 25 °C.

Treatment	% of oil content					
	5 days		10 days		15 days	
	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89
R. solani	20.5	22.5	21.5	23.5	22.5	24.5
F. moniliforme	20.5	22.5	21.5	23.5	22.5	24.5
F. roseum	21.5	23.5	22.5	24.5	23.0	25.0
F. semitectum	21.5	23.5	22.5	24.5	23.0	25.0
Control	24.5	26.5	24.5	26.5	24.5	26.5

DISCUSSION

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber and oil crops in Egypt and in many other countries allover the world. It is liable to attack by several disorders, which resulted from insects, fungi, bacteria, nematodes and others at different stages of plant growth (Cauquil and Shepherd 1970). A number of soil and seed borne pathogens can infect cotton seedlings individually or in association as a disease complex. A number of pathogenic fungi including seed–borne and soil–borne pathogens such as *Alternaria spp, Fusarium spp., Rhizopus spp.* and *Aspergillus spp.* are frequently identified seed borne pathogens in cotton (Minton and Garber 1983).

Isolation trials from different parts of cotton seeds of cvs Giza-86 and Giza-89 (before and after delinting, testa and cotyledons) resulted several fungi belong to 5 genera and 11 species. The isolated fungi were identified as *Alternaria alternata, Aspergillus niger, Fusarium dimerum, Fusarium moniliforme, Fusarium nivale, Fusarium roseum, Fusarium semitectum, Fusarium tricinectum, Fusarium solani, Penicillium spp* and *Rhizoctonia solani.* The isolated fungi from different parts of cotton seeds as well as rotten roots of Giza-86 and Giza-89 cvs. were differed in their frequency from one part to another. Generally, *R. solani* was the most frequent followed by *F. moniliforme* and *F. roseum* and the other *Fusarium* spp from most of different cotton seed parts. However, many of the isolated fungi, whether before or after delinting, like *A. alternata, A. niger, Penicillium spp* and *F. semitectum* were infrequently from seeds of cvs. Giza-86 and Giza-89. Also, it is clear that the number of total fungi isolated from the seeds of the two cotton cvs. after delinting were lesser than those isolated before delinting. In general, the total number of the isolated fungi from cotyledons was greatly lower comparing with those isolated from the inner surface of seed testa for both cotton cvs. Regarding the isolated fungi from damped-off seedlings, six isolates were isolated from the damped-off seedlings of Giza-86, two of them are *R. solani.* Meanwhile, 14 isolates were isolated from the rotten roots of Giza-89, 5 isolates of them are *R. solani.* Also, *R. solani* was the most frequent fungus followed by *F. moniliforme* and *A. niger.* However, *Penicillium spp.* and *F. tricinectum* were the lowest frequent fungi isolated from the rotten roots of cotton seedling. These results are in agreement with those obtained by Fulton and Bollenbacher (1959) who isolated *F. oxysporum, F. moniliforme, F. semitectum* and *R. solani* from cotton seedlings. Alfred (1963) isolated species belonging to

Alternaria, Aspergillus, Diplodia, Fusarium and *Rhizoctonia* from cotton seed hairs and the actual seed during boll development. Mazen *et al.* (1990) isolated thirty-nine species belonging to 16 fungal genera from Egyptian cotton seeds. The most common species were *A. niger, A. flavus, A. fumigatus, A. terreus, Rhizopus stolonifer, Penicillium corylophilum A. terreus, A. Nidulans,* respectively. Also, Seneewong *et al.* (1991) isolated *Fusarium* spp from inside the cotton seed coat and from the embryo of 100 randomly selected samples to be the most prevalent fungal species. Moreover, Palmateer *et al.* (2004) found that *Fusarium oxysporum, F. solani* and *F. equiseti* were the most common fungi at the seedling stage of the upland cotton in Alabama.

Concerning the pathogenicity tests, *R. solani* caused the highest infection by pre-emergence damping –off followed by *F. semitectum, F. moniliforme* and *F. roseum.* Regarding post emergence damping-off, *R.solani* and *F. semitectum* were the most virulent pathogens at this disease stage, meanwhile *F. roseum* was the least virulent one. Also, increasing the inoculum levels from 1 to 3% increased gradually the percentage of infection by % pre– and post emergence damping–off caused by any of the tested pathogens. Meanwhile, increasing inoculum level from 1 to 3 % gradually decreased the percentages of the survived cotton plants. These results could be interpreting in light of the findings obtained by Fulton and Bollenbacher (1959) who demonstrated that *F. oxysporum, F. moniliforme, F. semitectum* and *R. solani* isolated from cotton seedlings, were the most pathogenic fungi among twenty-two fungi tested for their pathogenicity to cotton seedlings. Also, Ranney and Bird (1958) verified that the most important disease attacking cotton seedlings is damping–off caused by *Rhizoctonia solani, Fusarium* spp and *Pythium* spp., Salem (1969) mentioned that both Egyptian and American cotton varieties were susceptible at different degrees to infected with *R. solani.* Wang *et al.* (1992) isolated *F. moniliforme, F. semitectum, F. oxysporum, F. solani, F. equiseti* and *F. compactum* from cotton seedlings and bolls during 1978–1990. They added that inoculation tests revealed that *Fusarium moniliforme* was the predominant pathogen causing seedling and boll red rot of cotton and had a wide host range. Also, Heping and Michael (1997) verified the ability of *Rhizoctonia solani* and some other soil fungi infecting cotton plants. Moreover, Wang *et al.* (2004) isolated many Fusarium isolates from stems and rhizosphere soils of 79 populations of four *Gossypium* species cultivated in Australia during 2001.

Regarding mycotoxin production, no one of the tested isolates was able to produce any of aflatoxins (B1 & B2), zearalenone, fumonsins and trichothense when grown *in vitro* on specific YES medium. On the other hand, infestation the cotton seed samples of both cvs Giza-86 and Giza-89 with the tested damping-off pathogens produced considerable amounts of mycotoxins in some cases. In this respect, *F. semitectum* and *F. roseum* produced zearalenone into infected seeds of cv.Giza-86 and cv.Giza-89 while, *R. solani* and *F. moniliforme* were not able to produce zearalenone into cotton seeds of both tested cvs. As for fumonisins, only *F. moniliforme* produced them into infected seeds of Giza-86 and cv.Giza-89. In addition, no of the four tested isolates was able to produce aflatoxins into infested cotton seeds, meanwhile, aflatoxins were found only in naturally contaminated cotton seeds of the two tested cvs. The obtained results are in line with the findings of Li, *et*

al. (1990) who detected T-2 toxin in rice medium cultures of 4 strains of *F. moniliforme* which were isolated from cotton dust. While, Mazen *et al.* (1990) reported that cotton seeds and cotton seed products were naturally contaminated by aflatoxin B1 and B2. No citrinin, ochratoxin, patulin, sterigmatocystin, diacetoxyscirpenol, T-2 toxin or zearalenone were detected in the samples assayed. On the other hand, Vidhyasekaran *et al.* (1997) reported that several *R. solani* isolates from rice and one from each cotton and tomatoes produced a N-acetylgalactosamine and N-acetylglucosamine toxins.

Infestation of cotton seeds with the tested damping-off pathogens, *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* decreased the percentages of oil content in the tested cotton seeds of cvs Giza-86 and Giza-89 comparing with uninfested seeds (control) incubated for 5-15 days. It is clear also that increasing the incubation period from 5-15 days decreased gradually the determined percentages of oil contents due to the activity of the tested pathogens comparing with un-infested seeds. The highest decrease in percentages of determined oil contents was recorded in case of infestation seeds with each of *R. solani* and *F. Moniliforme*. These results are in agreement with the findings of Ataga and Akueshi (1986) who found that *A. tenuis [A. alternata], Curvularia lunata, Fusarium moniliforme* and *Macrophomina phaseolina* growing well on sunflower seeds and caused biodeterioration over 21 days as well as reducing the oil content and causing discoloration of oil. Also, the results obtained by Airede and Fsuruoso (1987) concerning the inoculated oil palm kernels with spores of some seed-borne fungi verify the results of the present work. Also, Ataga and Umechuruba. (1998) found that inoculating seeds of African yam bean with *Fusarium pallidoroseum* decreased oil and carbohydrates during the incubation period of 21 days.

REFERENCES

Ahmed, K.G.M.; El-Said, S.I.A,; Fawzy, R.N.; Badr, A.E. and Abdallah, M.A. (1994): Pathological study on sunflower plant, chemical, biological control, and seeds oil content. Ann. of Agric. Sci., Moshtohor, 32: 1529-1543.

Airede, C.E. and Fsuruoso, O.F. (1987): Deterioration of shelled oil palm kernels caused by seedborne fungi. J. Sci. of Food and Agric., 40: 293-304.

Alfred, B. W. (1963): Relation of seed–borne fungi to boll rots of cotton. Phytopathology, 53: 984.

Anonymous (1990): Official Methods of Chemical Analysis, 15th ed, Kenneth Helrich edit, Published by the Association of Official Analytical Chemists.(A.O.A.C.) Inc., Virginia, USA.

Ataga, A.E. and Akueshi, C.O. (1986): Changes in oil and free fatty acid contents of sunflower seeds inoculated with *Alternaria tenuis* Auct, *Curvularia lunata* (Waiker) Boedijn, *Fusarium moniliforme* Sheld. and *Macrophomina phaseolina* (Tassi) Gild. Phytopathologia-Medit., 25: 44-46.

Ataga, A.E. and Umechuruba, C.I. (1998): Biochemical changes in African yam bean, *Sphenostylis stenocarpa*, (Hochst ex. A. Rich) Harms seeds caused by *Botryodiplodia theobromae* Pat, *Fusarium pallidoroseum* (Cooke) Sacc. and *Penicillium oxalicum* Currie and Thom. Global-J.of-Pure and Applied. Sci., 4: 381-384.

Barnett, H.L. and B.B. Hunter (1972): Illustrated Genera of Imperfect Fungi. 3rd ed. Burgess Publishing Co. Minneapolis, Minn. 241 pp.

Cauquil, J. and Shepherd, R.L. (1970): Effect of root-knot nematode-fungi Comminations on cotton seedling disease. Phytopathology, 60: 448–51.

Fulton, N.D. and Bollenbacher, K. (1959): Pathogenicity of fungi isolated from diseased cotton seedling in Arkansas. Phytopathology, 49: 684–689.

Gilman, J.C. (1957): A Manual of Soil Fungi. Cambridge Univ. Press, Ames, Iowa, U.S.A., 450 p.

Helal, S.E.; Mohamed, H.Z. and Salem, F.H. (1997): The effect of cotton seed delinting method on certain seed characters, rhizosphere microflora and control of *Rhizoctonia solani*. Bull. Fac. Agric. Cairo Univ., 48: 413- 434.

Heping, W. and Michael, R.D. (1997): Susceptibility of selected cotton cultivars to seedling disease pathogens and benefits of chemical seed treatments. Pl. Dis., 81: 1085–1088.

Huisman, O.C. (1988): Colonization of field grown cotton roots pathogenic and saprophytic soilborne fungi. Phytopathology, 78: 716–721.

Jens, C. F.; Thrane, V. and Mathur, S.B. (1991): An -Illustrated manual on Identification of Some Seed-borne *Aspergillus, Fusarium, Penicillium* and their Mycotoxins. Danish Government Institute of Seed Pathology for Developing Countries. Denmark.

Kuch, M.A. (1986): Mycoflora of cotton seed from the Southern United States: a Three year study of distribution and frequency. Mycology, 78: 796–712

Li, S.; Chen, M.; Liu, B.; Liao, T.; Wang, G.; Zheng, S.; Chen, W.; Yuan, H.; Chen, R. and Huang, J. (1990): T-2 toxin produced from a mould-*Fusarium moniliforme*. J. of Toxicol., (1): 104

Mansoori, B. and Hamdolahzadeh, A. (1995): Seed test and seedling disease of cotton in Gorgon and Gonbad. Applied Entomology and Phytopathology, 62: 1-2 17 (en), 80–83.

Mazen, M.B.; El-Kady, I.A. and Saber, S.M. (1990): Survey of the mycoflora and mycotoxins of cotton seeds and cotton seed products in Egypt. Mycopathologia, 110: 133-138.

Minton, E.B. and Garber, (1983): Controlling the seedling disease complex of cotton. Pl. Dis., 67: 115 – 118.

Palmateer, A.; McLean, K.; Morgan-Jones, G. and Santen, E. (2004): Frequency and diversity of fungi colonizing tissues of upland cotton. Mycopathologia, 157: 303-316. (c. f. Rev .Pl. Pathol., 83(10): p1234).

Park, K.Y. and Bullerman, R. (1981): Increased aflatoxin production by *Aspergillus parasiticus* under conditions of cycling temperature. J. of Food Science, 46:1147-1151.

Paul, N.A.; Ambat, K.L and Mathur, S.B. (1970): Seed health testing of rice III, Reprint of Int. Seed Test. Ass., 35:157-163.

Ram N.; S.M. Mathur and P. Neergaard (1970): Seed-borne fungi of mung bean (*Phaseolus aureus* Roxb.) from India their significance. Proc. Mt. Seed Test Assoc., 25: 225-241.

Ranney, C.D. and Bird, L.S. (1958): Survey of the primary fungi involved in the seedling disease of cotton. Texas Agric. Exp. Sta. Prog. Report, 2020: 1-3.

Roncadori, R.W.; Mccarter, S.M. and Crauford, J.L (1971): Influence of fungi on cotton seed deterioration prior to harvest. Phytopathology, 61: 1326–1328.

Salem, F.H. (1969): Cultural, pathogenic and physiological studies on *Rhizoctonia solani* Kuhn, the Casual Agent of Sore Shin Disease in the U.A.R. MSc. Thesis, Plant Pathology, Fac. of Agric., Cairo University.

Sankaranarayanan, V. and Kumar, S.U. (1985): A new toxin responsible for the early symptom in Fusarium wilts disease of cotton. Current Science India, 54: 196-197.

Seneewong, A.; Bashin, C.C. and Baston, W.E. (1991): The relationship between internal disease organisms and germination of gin run cotton seed (*Gossypium hirsutum* L.). J. Seed Technology, 15: 91-

Shadmanov, R.K. and Alimukhamedov, K.A. (1983): Characteristics of seed quality in interspecific hybrids and initial forms of cotton in relation to wilt attack. Uzbekiston-Biologija-Zurnali, 1: 24-27.

Sneh, B.L.; Burpee and A. Ogoshi (1991): Identification of *Rhizoctonia* species. Am. Phytopathology, Soc., St. Paul, MN. 133 pp.

Vidhyasekaran, P.; Ponmalar, T.; Samiyappan, R.; Velazhahan, R.; Vimala,R.; Ramanathan,A.; Paranidharan,V. and Muthukrishnan,S. (1997): Host-specific toxin production by *Rhizoctonia solani*, the rice sheath blight pathogen. Phytopathology, 87: 1258-1263.

Wang, B.; Brubaker, C.L. and Burdon, J.J. (2004): *Fusarium* species and Fusarium wilt pathogens associated with native *Gossypium* populations in Australia. Mycol. Res., 108: 35-44. (c. f. Rev. Pl. Pathol, 83 (7): p 840).

Wang, G.C.; Gu, Z.F. and Lou, X. (1992): Studies on the pathogens of Fusarium root rot of cotton. Acta Phytopathological Sinica, 22: 211–215.

Whitehead, M.D. (1957): Sorghum medium suitable for the increase of inoculum for studies of soil–borne and certain other fungi. Phytopathology, 47: 450.

Zhang, J.; Howell, C.; Starr, J. and Wheeler, M. (1996): Frequency of isolation and the pathogenicity of *Fusarium* species associated with roots of healthy cotton seedlings. Mycol. Res., 100: 747-752.

الفطريات المصاحبة لبذور بعض أصناف القطن المصرية وتأثيراتها على موت النباتات وإنتاج التوكسينات ومحتوى الزيت فى البذور

نوال عبد المنعم عيسى*، جهاد محمد الهباء*، محمد فتحى أبو العلا**، ا لسيد عبد الرحيم حسن***

* قسم النبات الزراعى – كلية الزراعة بمشتهر – جامعة بنها

** المعمل المركزى للأغذية والأعلاف – مركز البحوث الزراعية – جيزة

*** معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية – جيزة

أظهرت تجارب العزل من بذور القطن صنفى جيزة-٨٦ وجيزة-٨٩ قبل وبعد إزالة الزغب ومن السطح الداخلى للقصرة والفلقات بالإضافة إلى الجذور المصابة بالعفن إلى وجود العديد من الفطريات التى تتبع خمس أجناس وأحد عشر نوعا ، وقد عرفت الفطريات المنقاه على أنها ألترناريا ألترناتا، أسبرجلس نيجر، فيوزاريوم ديمارم، فيوزاريوم مونيليفورم، فيوزاريوم نيفالى، فيوزاريوم روزم ، فيوزاريوم سميتكتم، فيوزاريوم ترايسنيكتم، فيوزاريوم سولانى، أنواع من البنسيليوم

بالإضافة إلى الريزوكتونيا سولانى وبعض الفطريات التى لم يتم تعرفها. وقد كان فطر الريزوكتونيا سولانى هو أكثر الفطريات المعزولة سيادة من بذور القطن صنفى جيزة-٨٦ وجيزة-٨٩ قبل وبعد إزالة الزغب ومن السطح الداخلى للقصرة ، بالإضافة إلى الجذور المصابة بالعفن بينما كان فطر فيوزاريوم روزم هو الأكثر تكرارا على فلقات كلا الصنفين، وبشكل عام كان العدد الكلى للفطريات المعزولة من الفلقات منخفضا بدرجة كبيرة مقارنة بالفطريات المعزولة من القصرة لكلا الصنفين المختبرين. ولقد كان فطر الريزوكتونيا سولانى هو أعلى الفطريات مرضية من بين أربع فطريات مختبرة حيث سبب أعلى نسبة إصابة بموت البادرات قبل وبعد خروجها فوق سطح التربة لكلا الصنفين المختبرين جيزة-٨٦ وجيزة-٨٩. وقد إتضح أيضا أن زيادة مستوى اللقاح من ١-٣ % سبب أرتفاعاً تدريجيا فى نسبة الإصابة بموت البادرات قبل وبعد خروجها فوق سطح التربة. فضلا عن ذلك فقد أشارت متوسطات نباتات القطن المتبقية أن فطر الريزوكتونيا سولانى متبوعا بفطر فيوزاريوم سميتكتم هما الأكثر مرضية عند معظم مستويات اللقاح المختبرة فى حين كان الفطر فيوزاريوم روزم هو أقلها مرضية على كلا الصنفين المختبرين. لم تكن كل الفطريات المختبرة قادرة على إنتاج أى نوع من توكسينات الأفلاتوكسين (ب١، ب٢) أو الزيرالينون أو الفيومنسنز أو الترايكوسيسنز عندما نميت الفطريات معمليا على بيئة السواى إى إس المتخصصة، فى حين أن تلويث عينات بذور القطن (صنفى جيزة-٨٦ وجيزة-٨٩) بنفس فطريات أعفان الجذور المختبرة كشف عن وجود كميات واضحة من الميكوتوكسينات مقدرة بجزء فى البليون فى بعض الحالات الملوثة. وعلى الجانب الآخر، فقد أثر سلبيا تلويث بذور القطن بأى من فطريات عفن الجذور المختبرة (ريزوكتونيا سولانى ، فيوزاريوم مونيليفورم ، فيوزاريوم سميتكتم ، فيوزاريوم روزم) على محتوى هذه البذور من الزيت. كما كان واضحا أن زيادة فترة التحضين من ٥-١٥ يوم قد خفضت تدريجيا نسبة الزيت المقدرة فى كل المعاملات مقارنة بالبذور الغير معاملة، كما سجل أعلى إنخفاض فى محتوى الزيت فى حالة تلويث البذور بفطرى ريزوكتونيا سولانى ، فيوزاريوم مونيليفورم (لكل منها على حدة)عند أى فترة تحضين مختبرة لبذور كلا الصنفين.