PATHOLOGICAL STUDIES ON COTTON SEEDS USED IN AGRICULTURE

BY

EL-Sayed Abd-ELReheim Hassan
B.Sc., Agric. Sc., Moshtohor, Zagazig University, Benha Branch 1991
M. Sc., Agric. Sc., (Agronomy), AL-Azhar University,1998

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CONTENTS

INTRODUCTION

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 occupies a prominent position in Egyptian agriculture. It is still the main cash crop for a sizeable section of Egyptian farmers where they named it the white gold. As well as, it is the main raw material for the largest national industry where it is the main source of seed oil and the textile industry at locally and world scale. In this respect, there are continuous efforts toward improving its productivity and quality. In Egypt, the cultivated area with cotton reached about 696,000 feddan (290.000 hectare) yielded approximately about 5,075,000 kantar (kantar = 157.5 Kg) with an average of 7.3 kantar/feddan, according to FAO Production Yearbook (2003). It is attacked by several disorders, which are usually from insects, fungi, bacteria, nematodes and others at the different stages of growth. Fungi are the most wide pathogens but bacteria and viruses are also sometimes involved. Parasitic soil–inhabiting nematodes may also be associated with these fungi and seedling damage is often increased when the crop is attacked by both groups of organisms **(Brodie and Cooper, 1964** and **Cauquil and Shepherd, 1970)**. Cotton seedling diseases whether pre- or post- emergences are worldwide problem, often causing serious stand losses. A number of soil and seed-borne pathogens can infect cotton seedlings individually or in association as a disease complex. In Egypt, every increase in cotton seedling disease about 1% can cause losses in the total yield estimated by 0.05-0.09 kantars/feddan **(Aly *et al.*1994** a and b). Cotton plants are

subjected to attack by various pathogenic fungi causing several diseases during different stages of growth Among these diseases seed rot and seedling damping–off. A number of pathogenic fungi including seed–borne and soil–borne pathogens such as *Alternaria* spp, *Fusarium* spp., *Rhizopus* spp. and *Aspergillus* spp are the most frequently identified seed borne pathogens in cotton. They are often associated separately or in combination with seed rot pre–emergence damping–off **(Minton and Garber 1983)**. The seedling disease syndrome encompassed any host–pathogen interactions with debilitate the plant between planting and about one month after emergence. The symptoms may be divided into four groups depending on the stage of development when damage occurs and which part of the plant is affected;

The first stage where damage can occur immediately after planting, resulting in seed decay. Under sub–optimal conditions for plant growth, the newly emerging roots and shoots are vulnerable to attack, resulting in pre- emerging damping–off. As well as, the seedling remains vulnerable to attack, by soil–borne fungi after emerging, especially when growth is slow and infection of the hypocotyls at this stage is referred to as post–emerging damping off. Finally, symptoms may become apparent on the cotyledons and first leaves, as a distinct spotting or a more generalized blight. At mature stage, the unopened cotton bolls yielded cultures belonging to genera *Alternaria, Aspergillus, Diplodia, Fusarium* and *Rhizopus* and these fungi are associated with the seed hairs and the actual seed during boll development **Wiles (1963)**. External infection of cotton seeds has an effect on seedlings growth, and severe boll rot are usually followed by an

increase in seedling diseases. Cotton seedling disease caused by *Rhizoctonia solani* reduces the number of stand plants and usually causes considerable yield losses. The behavior of plant pathogens and assurance of root diseases are related distinctly to certain rhizosphere phenomena **(Curl. 1982)**.

This work aimed to investigate the effect of acid delinted seeds on the fungi ability which associated with seeds. Isolation and identification of fungi associated with seed rot after and before acid delinted seeds. Testing pathogenicity of the isolated fungi at different levels of soil infestation. Evaluating effect of some fungicides and commercial bioagents on disease incidence through determining variation in some physiological, biochemical and growth characters of cotton plants under artificial infection. Studying reaction of different cotton cultivars (Varietal reaction) against root-rot infection.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1- The causal microorganisms:

Arndt (1935 and 1943); Fulton and Bollenbacher (1958); Moubashr (1958) and Ranney and Bird (1958) reported that *Rhizoctonia solani, Pythium ultimum ,Fusarium oxysporum, F.moniliforme, F. semitectum* and several other fungi were pathogenic to cotton seedlings as well as the infection was generally greater under cool and rainy weather.

Ranney (1962) found that *Rhizoctonia solani, Pythium* spp. *Fusarium* spp. and *Glomerella gossypii* were involved in cotton damping –off disease complex.

Alfred (1963) indicated that fungi belonging to *Alternaria, Aspergillus, Diploidia, Fusarium* and *Rhizoctonia* were associated with the seed hairs and the actual seed during boll development. External infection of cotton seeds affected seedlings growth.

Lumsden *et al.* (1983) found in greenhouse experiments that root-rot of cotton was attributed to *Rhizoctonia solani* and *Fusarium* spp.

Kuch (1986) recorded that *Fusarium equiseti* and *Fusarium semitectum* were isolated for more than 10% of the seed at any sampling of delinted surface sterilized cotton seeds in the southern USA.

Huisman (1988) found that *Rhizoctonia solani* was higher on roots with severe tissue damage than on roots

exhibiting little or no damage, for the other fungi, colonies were equally abundant at roots without damaged tissues

Seneewong *et al.* (1991) reported that *Fusarium* spp were the most prevalent fungi 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 also low. Only 2.7 % of the seed coats were infected. The number of embryos containing fungi was negatively when were isolated from inside the seed coat and from the embryos of selected seeds from each lot.

Wang *et al.* (1992) recorded that among 200 isolates from 194 cotton seedlings and bolls during 1978–1990; 61% were *Fusarium moniliforme* [*Gibberella fujikuroi*] var. *intermedium*, 28% *Fusarium semitectum*, [*Fusarium pallidoroseum*], 4.0 % *Fusarium oxysporum* , 3.5 % *Fusarium solani*, 3% *Fusarium equiseti* and 0.5 % *Fusarium compactum*. Inoculation tests demonstrated that *G. fujikuroi* var. *intermedium* was the predominant pathogen causing seedling and boll red rot of cotton and had a wide host range.

Monga *et al.* (1994) obtained 13 isolates of *Rhizoctonia solani*, from cotton seedlings, which were categorized into 4 distinct groups on the basis of cultural characteristics.

Mansoori and Hamdolahzadeh (1995) isolated *Alternaria alternata, Aspergillus niger, Fusarium accuminatum, 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 present on the rhizoplane of cotton plants grown in pots containing cotton field soil. *F. oxysporum* and *F. solani* were the most dominant species

Wrather *et al.* (2002) used samples consisted of 10 cotton seedlings, 2 to 3 weeks after emergence, with symptoms of seedling diseases. (*Fusarium, Pythium, Rhizoctonia* and *Thielaviopsis*) and Three species of cultured fungi from the roots were identified viz., *Rhizoctonia solani*, *Thielaviopsis basicola* and *Pythium ultimum*. Repeated tests of pathogenicity confirmed that *R. solani* AG-4, *T. basicola* and *P. ultimum* were the major causal agents of post-emergence cotton seedling disease in Missouri.

Palmateer *et al.* (2004) studied the mycoflora of upland cotton in Alabama. Plants were sampled at seedling, first bloom, full bloom, and maturity stages of development. Fifty eight species of fungi belonging to 37 genera were isolated, including 9 species of *Fusarium. Fusarium oxysporum, F. solani* and *F. equiseti* were the most common members of this genus occurring at all four sampling stages. Eight species accounted for 67% of the total fungi isolated during the two-years study. *Alternaria alternata* was the most common fungus which encountered accounting for 19 and 10% of the total fungi isolated in 2000 and 2001 respectively. Twenty species of fungi were reported for the first time colonizing upland cotton.

Wang *et al.* (2004) isolated *Fusarium* spp. from stems and rhizosphere soils of 79 populations of four *Gossypium*

species native to Australia in 2001. *F. solani* was more common in the soil from *G. sturtianum* populations than from *G. bickii* populations, and that *F. crookwellense* was more common in South Australian soils than in Queensland and Northern Territory soils. *Fusarium oxysporum* had a relatively greater relative density in the rhizosphere soils of *G. australe* plants than in that of sympatrically growing *G. sturtialnum* plants. Fifteen of the *F. oxysporum* isolates produced typical fusarium wilt symptoms on cultivated cotton (*G. hirsutum* cv. 'Siokra 1-4), therefore they were classified as wild *Fusarium oxysporum* f.sp *vasinfectum.* Soil samples collected from *G. sturtianum* populations in South Australia had the highest incidence of wild *Fov* (24%). Two wild *Fov* isolates were similar in virulence to a cotton field *Fov* isolate in the glasshouse experiments, indicating that they could incite Fusarium wilt disease in cotton fields.

2- Varietal reaction:

Naim (1964) reported that there were differences between the two Egyptian cotton varieties Ashmoni and Karnak and they were varied in their susceptibility to damping–off disease caused by *Rhizoctonia solani.* The Karnak variety was found to be the less susceptible than the Ashmoni variety.

Salem (1969) demonstrated that both Egyptian and American cotton varieties were susceptible at different degrees to *Rhizoctonia solani.*

Eissa (1983) found that American cotton varieties were more resistant to infection with *Rhizoctonia solani* and *Pythium ultimum* than most of the Egyptian cotton varieties.

Panhwar *et al.* (1993) compared four Pakistanian cotton varieties, which were with 4 multi–adversity (i.e., multi–disease) resistant varieties introduced from Texas, USA, in trials at Sakrand during 1988–1990. The obtained data from the tested 3 years showed significant varietals differences for seedling rot and root rot diseases.

Heping and Michael (1997) evaluated the susceptibility of 12 Upland cotton cultivars to three soil borne fungi, *Pythium ultimum, Rhizoctonia solani* and *Thielaviopsis basicola* in greenhouse experiments. Based on symptoms development and seedling survival, cultivars highly resistant to *P.ultimum* included Data Pina (DP) 6166, Prema, DP 6100, and Maxxa. A relatively low incidence of pre-emergence damping–off caused by *R. solani* was occurred in Chembreed 7, DP 6100 and Rayale, although all cultivars were subsequently suffered significantly from post–emergence damping–off. Seed treatment with carboxin and pentachloronitobenzene for the control of *R. solani* induced damping–off resulted in stand increases in all 12 cultivars in greenhouse tests and in 3 of 6 cultivars in field trials.

Zhang *et al.* (2003) stated that Wanmian 21 is a derivative of the cross combination Huaibei 2 X Huaibei 8. They found that the variety was resistant to Fusarium wilt and Verticillium wilt,

Liu *et al* (2003) recorded that Liaomian 18 is a derivative of the cross combination H7-7109 x 1036-6. Fusarium wilt severity index reached 0.38 and the Verticillium wilt severity index recorded 7.5. It is also highly resistant to seedling disease

and boll blight.

3 - Effect of fungicides:

David and Sinclair (1968) reported that protection against pre- and post-emergence damping-off was occurred when seedlings were grown in soil treated with 250 ppm Vitavax. Vitavax used as seed treatment (80Z /1001b. of seeds) gave significantly greater protection than did a standard. Results from *in vitro* studies showed that Vitavax was fungistatic to *R. solani* at 1 ppm.

Papavizas *et al.* (1980) detected that the new systemic fungicide N.\cyclohexyl–N-methoxy–2.5dimethyl–3–furancarbozamide(BAS 389) was combined in acetone with one of the two other new systemic fungicides N-(2,6–dimethylphenyl).N-(mthoxyacetyl) alanine methylester (CGA–48988) and propyl [3dimthylamino) propyl] corbamate Hcl (SN 66752) and infused into Stoneville 213 and Acala Sj-2 acid-delinted seed of cotton (*Gossypium hirsutum*). The treatments appreciably reduced seedling disease in soil artificially infested with *Rhizoctonia solani* or *Thielaviopsis basicola* alone, in soil infested with *R. solani* and *Fusarium* spp. and in soil infested with a mixture of *Pythium spp., R. solani* and *T.basicola*. None of the fungicides used alone consistently reduced seedling disease in soil artificially infested with *Rhizoctonia solani*.

Barakat and Osman (1981) found that dressing of cotton seeds with any of the fungicides Captan, Quinolate 15 and Euparen has significantly improved the emergence of seedlings,

but thiabendazole (TBZ) was ineffective. All applied fungicides did not affect root or shoot lengths comparing with the control plants. Dry weight of shoots was reduced especially with Euparen and Quinolate 15 treatments.

Eissa *et al.* (1987) reported that damping–off disease caused by *Rhizoctonia solani, Sclerotium rolfsii, Pythium ultimum* and *Macrophamina phaseolina* is the most important disease of cotton in Egypt. Different fungicides were screened–out against the damping–off fungi under greenhouse conditions. All compounds used satisfactory controlled the pathogens, however, to different extent. Fourteen fungicides were screened for control of *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium ultimum* and M. *Phaseolina* of cotton seedlings damping-off under greenhouse conditions. All of the fungicides controlled the pathogens.

Hillocks and Gumer (1988) recorded that seven fungicides, together with the standard compounds quintozene and benodanil, were tested against. *Rhizoctonia solani* in the laboratory and were then evaluated for the control of seedling disease in cotton grown in field plots, inoculated with *R. solani.* Both seed dressing and in–furrow applications gave some measure of control with all fungicides tested, but in–furrow treatments were the more effective, especially against post-emergence damping–off. Compounds giving the best control of seedling loss were tolclofosmethyl as a seed dressing and pencycuron plus captan in–furrow, reflecting results from the laboratory test. However, other compounds were also as

effective as the standard compound quintozene, in controlling seedling loss.

Abdel–Shahaid *et al.* (1989) tested the fungicide Benlate (benomyl) 50% as a soil treatment in the greenhouse to control damping–off of cotton seedlings caused by each of *Rhizoctonia solani* and *Fusarium solani* . The results indicated that, this compound has fungistatic and systemic action. It effectively protected cotton seedlings against *R. solani* and *F. solani* at concentrations as low as 20 ppm. *In vitro* bioassay studies showed that *R. solani* was more sensitive than *F. solani* to benomyl.

Alagarsamy and Jeyaraian (1989) found that out of 5 fungicides tested against growth of *R. solani* in culture, tolclofos- methyl was the most inhibitory followed by carbendazim.

Schultels and Nu-Flou (1989) used Apron/Chloroneb fungicide as seed treatment to control the fungal disease complex of cotton where co-formulation of metalaxyl and chlorobeb was controlled *Pythium ultimum* and *Rhizoctonia solani,* respectively.

Osman *et al.* (1990) reported that tolclofos- methyl at 4 ppm followed by benomyl at 8 ppm were more effective against *R. solani in vitro* than dichlofuamid, copper oxyquinalate + carboxin or Pencycuron.Growth inhibitions of *R. solani* by benomyl or tolclofos methyl was reduced by presence of ammonium sulfate in PDA. Ammonium sulfate alone had no effect on fungal growth.

Sawant and Mukhopadhyay (1990) mentioned that metalaxyl was highly inhibitory to *Pythium aphanidermatum* at 2.9 ppm combination products (Ridomil MZ [metalaxyl + mancozeb] and metalaxyl + Zineb) and significantly less inhibitory than metalaxyl alone. Seed treatment with 1000 ppm metalaxyl gave complex protection against damping–off for the entire 28 day susceptible period. Metalaxyl was detected in sugarbeet seedling roots and stems, and leaves up to 24 d and 44 d, respectively after seed treatment.

Youssef (1990) found that the fungicides Homi, Monceren, Rhizolex and Vitavax / Captan were effective against lentil seedling damping–off caused by *R. solani, Macrophamina phaseolina* and *Fusarium solani.*

Henriquez and Mantealegre (1992) tested 11 fungicides *in vitro* for controlling *S. rolfsii* and found that Vitavax-T, Basitac, Vitavax, Rhizolex, Bayleton and Baytan, were the most effective.

Ilyas *et al.* (1992) tested 10 fungicides against *Fusarium oxysporum F. sp.* cicri, in soil drench on infected potted chickpea plants in greenhouse. They found that Benomyl was the most effective, followed by Rhizolex and Topsin–M.

Stachewicz and Burth (1994) tested the sensitivity of 118 isolates of *R. solani* collected from 12 different areas in Germany to thiabendazole, carbendazim, pencycuron, and tolclofos–methyl and fenpicloni *in vitro*. They found differences among the isolates in their sensitivity to each of the tested fungicides.

Youssef *et al.* (1995) concluded that delinting the seeds of Egyptian cotton cvs. Giza 45 and 75 by the brush machine for one and two times before coating with the Monceren fungicide resulted in improving seed germination and seedling growth characteristics as compared to the acid delinted seeds or the non–delinted seed (Fuzz seeds).

Helal *et al.* (1996) found that the delinted cotton seeds which were dressed with Rhizolex before planting in infested and non-infested soil in the greenhouse gave the highest seedling emergence and the highest percentage of survived seedlings as will as, delinted seeds with 50 % diluted sulfuric acid gave the lowest rots diseased seedlings. Seed dressing improved seedling vigor at the two stages of seedling growth in the greenhouse. Rhizosphere microflora were not affected with different treatments in the greenhouse.

Davis *et al.* (1997) reported that twenty-five field trials conducted over a 3–year period in five San Jooguin Valley countries included the following treatments: nontreated cotton seed, seed treated with myclobtanil for the control of *Rhizoctonia solani* induced damping–off, seed treated with metalaxyl for the control of *Pythium.* Seed treated with a combination of the two fungicides resulted in improved stands in 15 of 18 fields. Metalaxyl did not increase stands in any field in 1993 to 1994. In 1995, the combination of fungicides increased stands relative to the nontreated seeds and was more effective in increasing stands than myclobutanil or metalaxyl alone. Apparently, seeds protected with a fungicide active against *R. solani* were more susceptible to infection by *Pythium spp*. In general, the fungicide

seed treatment active against *R. solani* increased stands of the cultivar Maxxa regardless of soil type and pathogen population. Increased stands from the metalaxyl treatment was occurred in 1of the 3 year of the study.

El–Safety *et al.* (2001) reported that the cotton seed dresser fungicides Monceren, Monceren–combi, Monceren–euparen, Rhizolex-T and Vitavax 300 with the fertilizer superphosphate and herbicides cotoran, goal and stomp in a soil infested with *Rhizoctonia solani* Kuhn under suitable conditions for infection in the greenhouse, significantly reduced the infection.

El–Deeb *et al.* (2002) recorded that the effect of three fungicides on the incidence of root and pod rots in peanut was evaluated and compared under greenhouse and field conditions. The fungicides were Vitavax–Thiram, Rhizolex–T and Topsin M70. All treatments reduced the percentage of root and pod rots in both the greenhouse and the field.

El–Habbaa *et al.* (2002) mentioned that evalution of different fungicides revealed that, Maxim was the most effective as it prevented *in vitro* the growth of *F. solani, M. phaseolina* , *B.theobromae* , *S .rolfsii* and *R solani*, at 1-5ppm, followed by Benlate at (10–800ppm). Vitavax–T (25–200 ppm) and Rhizolex–T (200–800 ppm). Meanwhile, Apron and Monceren had no or little effect and failed to produce a considerable reduction in growth of all tested fungi even at (800 ppm).

Goulart (2002) conducted an experiment in greenhouse to evaluate the efficiency of several fungicides, applied as seed

dressing to control damping–off caused by *R. solani* using the cotton cv Delta Opal. The effect of the fungicide treatment on initial and final seedling emergence was observed with distinction to triadimenol + pencycuron + tolylfluanid and triadimenol + tolylfluanid followed by carboxin + thiram, triadimenol and carboxin + thiram + carbendazim. The most efficient treatments in the control of cotton seedling post–emergence damping–off were triadimenol + pencycuron + tolylfluanid followed by triadimenol, triadimenol + tolylfluanid and carboxin + thiram. No phytotoxic effects were observed on cotton.

Sultanov *et al.* (2002) reported that sodium humate was identified as an effective seed treatment for increasing the resistance of cotton to plant pathogens in Uzbekistan. The fungicide Darman–4 [of unstated composition] was also found to be an effective seed treatment against number of diseases in terms of both, biological effectiveness and yield increase.

Chavan *et al.* (2003) found that the efficacy of mancozeb, copper oxychloride, carbendazim captan and captafol in inhibiting the growth of *F. oxysporum* f. *carthami* was evaluated *in vitro*. Each fungicide at rates of 0.1, 0.2 and 0.3 % a. I, respectively (except carbendazim normal rates of 0.025, 0.05 and 0.1 % a. I) was incorporated into a sterilized medium. Carbendazim applied at all rates was superior in the inhibition of fungal growth, resulting in a mean colony diameter of 5.0 mm.(38.3 mm in the untreated control) followed by captafol (13.8-16 mm), captan, (14.5–16.8 mm) and mancozeb (15.00–

17.8mm). Copper oxychloride was effective only when applied at normal and above normal rates (28.0–32.6 mm).

4- Biological control:

Howell (1982) found that a strain of *Gliocladium virens* isolated from the parasitized hyphae of *Rhizoctonia solani*, significantly suppressed damping-off incited in cotton seedlings by this pathogen and by *Pythium ultimum* when the antagonist was placed with cotton seeds planted in infested soil treatment. *Gliocladium virens* parasitized on *Rhizoctonia solani* by coiling around and penetrating the hyphae. *Pythium ultimum* was not parasitized by *Gliocladium. virens* but was strongly inhibited by antibiosis.

Beagle and Papavizas (1985) recorded that the efficacy of several fungal antagonists for control of *Rhizoctonia solani* on potato was evaluated in greenhouse and field tests. Fermentor biomass (FB) preparations of *Trichoderma viride* (T–1–R9) and *Gliocladium virens* (GI – 21) were applied as dusts to seed potatoes infested with sclerotia of *R. solani* before planting , reduced disease incidence in the field by 50 and 55%, respectively. In the greenhouse, up to 88 % reduction in germination of sclerotia was obtained by treating sclerotial–infested tubers with FB of T-1-R9 before planting. FB preparations of *T. viride* (T–1R 9), *T. harzianum* (WT–6), *T. hamatum* (-4). and *G. virens* (G1–21) added to the soil in a mixture of pulverired pyrophyllite (anhydrous aluminum silicate), significantly reduced numbers of propagules of *R. solani.* Results suggested that both soilborne and tuberborne

propagules of *R. solani* can be effectively reduced by biological means.

Lewis and Papavizas (1985) found that mycelial preparations of eight of 14 isolates of *Trichoderma* spp. and *Gliocladium virens* reduced survival of *Rhizoctonia solani* at least 50% in pathogen-infested beet seed in soil and in soil infested with sand / cornmeal inoculum of the pathogen. All antagonistic isolates reduced saprophytic growth of *R. solani* from infested beet seed into soil. Isolates of *T. hamatum* and *G. virens* were more effective than those of *T. harzianum* and T. *viride*. Population densities of all antagonistic isolates were increased 10^4-10^6-fold during 3 weeks of incubation after adding the mycelial preparations to soil. Conidial preparations of isolates, added to the soil in amounts equal to propagules in mycelial preparations did not reduce survival of *R. solani* or its growth through the soil. Mycelial preparations, but not conidia, of most isolates of *Trichoderma* spp., and *G. virens* prevented damping-off of cotton, sugar beet and radish seedlings in the greenhouse.

Lumsden and Locke (1989) reported that 50 isolates of bacteria and fungi, including species of *Pseudomonas, Bacillus, Trichoderma* and *Penicillium.* and twenty isolates of *G. virens* showed clear variation in their efficacy in controlling *P. ultimum* and *R. solani* for damping-off of zinna, cotton and cabbage. Some isolates controlled *P. ultimum* but not *R. solani* and control of *P. ultimum* was effective when sporangial inoculum of the pathogen was introduced at the time of planting

the host seed, however, control of *R. solani* required prior contact of *G. virens* with inoculum of *R. solani.*

Abed–El Moiety *et al.* (1990) mentioned that *Trichoderma harzianum*, *Gliocladium virticilloides*, *G. roseum*, *Cheatomium cochliodes* and *C. globosum,* varied in their antagonistic effect against four pathogenic soilborne fungi. The pathogens, i.e. *Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii* and *Macrophamina phaseoli* are involved in the damping–off and root rot syndromes of cotton seedlings. *T. harzianum* and *G. virticilloides* were more effective in controlling the disease than other tested antagonists.

Howell (1991) recorded that air dried and ground seed-coating preparations of *Gliocladium virens* were variable in their efficacy for biocontrol of cotton seedling disease induced by *Pythium ultimum*. Seed coat preparations of *G. virens* may be used to control Pythium damping–off of cotton seedlings, and in combination with fungicide, the amount of fungicide necessary to effect control can be reduced.

Truner and Bachman (1991) found that treatment of peanut seeds with *Bacillus subtilis* improved seed germination and emergence, increased and reduced levels of root cankers caused by *R. solani* AG4, and increased root growth.

Mukheriee *et al.* (1995) reported that an isolate of *Trichoderma harzianum* was less effective than *G. virens* in suppressing *S. rolfsii* and *R. solani* was compared with *G .virens* for various mechanisms of antagonism *in vitro* viz., antagonism in dual culture / hyphal parasitism. Parasitism of sclerotia and

antibiosis of *G. virens* and *T. harzianum* were equally effective in parasitizing the hyphae of *R. solani*. Only *T. harzianum* parasitized the hyphae of *S. rolfsii.*

Zhang *et al.* (1995) mentioned that isolates of *Fusarium* species from the rhizoplane of cotton can be grouped into pathogenic and non pathogenic strains. The most pathogenic species was *F. solani*. Experiments on the interactions between *Fusarium* spp. and the biocontrol agents, *B. subtilis* and *G. virens,* indicated that *Fusarium* spp. on the cotton rhizoplane were significantly reduced by *B. subtilis* stains GBo3 and GB07 and *G. virens* strain G- 4 and G – 6 which were applied as seed treatments.

Ellis and Johan (1996) reported that plant growth promoting rhizobacterium (PGPR), *Pseudomonas fluorescens* strain WCS417 has been shown to induce systemic resistance against *Fusarium oxysporum* in several plant species without inducing synthesis of pathogensis–related proteins (PR).

Aqil and Batson (1999) found that the potential of a radical assay for selecting biocontrol agents was investigated using 2 rhizobacteria with known biocontrol activity, *Pseudomonas fluorescens* (Dagger G isolate) and *Bacillus subtilis* (GBo3), against *Pythium ultimum*, *Fusarium oxysporum* and *Thielaviopsis basicola*, the causal pathogens of seedling disease of cotton. Both rhizobacteria significantly reduced symptoms development induced by all pathogens.

Howell *et al.* (2000) recorded that research on the mechanisms employed by the biocontrol agent *Trichoderma*

virens to suppress cotton (*Gossypium hirsutum*) seedling disease incited by *Rhizoctonia solani* has shown that mycoparasitism and antibiotic production are not the major contributors to successful biological control. They examined the possibility that seed treatment with *T, virens* stimulates defense responses, as indicated by the synthesis of terpenoids in cotton roots and found that the analysis of extracts of cotton roots and hypocotyls grown from *T, virens* treated seeds showed that terpenoids synthesis and peroxides activity were increased in the roots of treated plants, but not in the hypocotyls of these plants or in the untreated controls.

Gopalakrishnan *et al.* (2003) found that out of twelve *Trichoderma,* isolates tested [belonging to the species *T. hamatum, T. viride*, *T. virens* (*Gliocladium virens)* and *T. harzianum]*, cotton isolate *T. viride* (97) exhibited the fastest growth rate and strong antagonism against *Rhizoctonia solani in vitro* recording 56.2 % inhibition in dual culture. All the isolates tested enhanced cotton cv. RCH2 seed germination when treated with mycelial suspensions of biological control agents *in vivo*. The disease incidence in biological control agent treatments ranged between 14.8 -32.4% while pathogen treatment recorded 82 %.

5- Biochemical changes:

Alberto and Diamond (1963) found that mycelium of *Fusarium oxysporum f. lycopersici* was appeared in the stems of tomato plants 6 days after sowing. Polyphenol oxidase activity in diseased stems was 3–10 times higher than in healthy stems. In

diseased stems, the polyphenol oxidase activities associated with infection was raised gradually until the ninth day then increased sharply thereafter. Poplyphenol oxidase activity was well correlated with the severity of foliar symptoms and quantity of mycelium.

Bell (1967) found that introduction of conidia of *Verticillium albo–atrum* into boll cavities or xylem vessels of excised stems of *Gossypium hirsutum* or *G. barbadense* induced a marked accumulation of either–soluble phenolic compounds after 24–72 hr. The predominant compound was identified as gossypol on the bases of Rf values ultraviolet absorption spectra and derivatives formed with aniline 2,4 dinitrophenyl–hydrazine and phloroglucinol. Inoculated bolls or stem sections of glanded and glandless Acala 4–42 produced equivalent amounts of gossypol. Gossypol synthesis also was induced by sporanigospores of *Rhizopus nigricans*, cupric and mercuric ions and various metabolic inhibitors. Purified gossypol had LD50 values of 20–100 ppm (50–250 uM) against spore germination of various fungi. The behavior of gossypol in cotton is similar to that of described phytoalexin.

Mellon and Lee (1985) mentioned that the distribution of peroxidase (s) in non–inoculated cotton bolls and flowers was qualitatively determined. The soluble and bound forms of cotton peroxidase (s) were investigated for change elicited by fungal infection. Inoculation of cotton bolls, 30 d post pathogenicity with *Aspergillus. flavus, Fusarium equiseti, F. moniliforme* (*Gibberella fujikuroi*), *F. semitectum* or *Rhizoctonia solani*

followed by a 6–d incubation period stimulated soluble peroxidase activity 2 to 6 folds.

Nuritdinova *et al.*(1986) studied of the cell walls in healthy and infected plants of the resistant *Gossypium hirsutum*, variety Tashkent and the susceptible S 4727, using race 1 of *V. dahliae.* The cell walls of the susceptible S 4727 contained more carbohydrates than those of Tashkent 1 before and after infection. After infection, content of most sugars was lower and of most amino acids were higher in Tashkent than S 4727.

Richard and Alain (1986) found 10 additional pathogenesis–related proteins (b6a–b6b–b10a–b10b–b11a–b11b–b12a–b13–b14–b15) in intercellular fluid extracts of stressed "Xanthi–ne" tobacco leaf tissue using two dimensional polyacrylamide gel electrophoresis. Four Proteins (b12–b13–b14–b15) were only resolved by using native polyacrylamide gel electrophoresis for basic proteins in the first dimension gel and they were best extracted in 0.05 M tri –HCl (pH 7.5) and 0.05 M CaCl 2 as the infiltration buffer. These findings indicated that at least 23 proteins were accumulated extracellularly after various types of stress in "Xanthi–ne" tobacco green tissue.These proteins probably represent several groups or families of plant stress proteins.

Abuo–Taleb *et al.* (1987) mentioned that *F. oxysporum f. sp. vasinfectum* caused vascular wilt in the susceptible cotton cvs. Giza 74 and Bahtim 110 (glandless), but not in the resistant Acala 502, Giza 70 and Menoufi. Resistance was associated with marked accumulation of total phenolic compounds and free

gossypol. A gradual increase in the level of total phenols was observed in both resistant and susceptible cultivars up to 72 h after inoculation and in gossypol up to 144 h.

Borkar and Verma (1990) studied the resistant cotton cvs. 101–1028 which its intercellular fluid (IF) contained higher conc. of sugar than the susceptible Acala–44. Inoculation of the IF *in vitro* increased the total sugar content only in the IF of the susceptible cultivar, indicating production of exopolysaccharide. The total phenol content was higher in the IF of the susceptible cultivar, although total and dihydroxyphenol contents in the leaves were low compared with the levels in resistant plants. During host – pathogen interaction total and dihydroxyphenol contents increased in 101 – 1028 and its IF but decreased in Acola 44. The resistance of cotton to *X. compestris* was related to the higher total sugar contents in the IF which was responsible of the inhibition of growth and multiplication of the pathogen.

Hiremath and Savanur (1990) found that in collections of cotton leaves from healthy and infected plants there were increase in total sugars, reducing sugars, total phenols and amino acids in disease bottom leaves and lesser increase in disease to cotton leaves, indicating that *Alternaria macrospora* had greater effect on the metabolism of the lower leaves.

Vlassova (1993) recorded that detached roots of the highly susceptible cotton variety S-4727 and the relatively resistant variety Tashkent–1 were inoculated with a virulent strain of *Verticillium dahliae* biotype Kh1–288-race 2. Peroxidase and phenoloxidase activities were determined on the

4 th day after fungal penetration into and on the 7 th day during intensive colonization of the root. An increase in peroxidase and phenoloxidase activities was observed during the early stage of infection in the susceptible and resistant varieties. At the later stage, oxidase activity became similar in inoculated and healthy roots. An increase in total phenol content was only detected in inoculated roots of the resistant variety. It was suggested that detached roots can be used as a model to study wilt resistance in cotton varieties.

Vlassova *et al.* (1994) suggested that phenols and their oxidation production products play a role in both specific and non specific defence reactions for detached cotton roots of resistant and susceptible cultivars, inoculated with a virulent strain of *Verticillium dahliae.*

Zhang *et al.* (1995) cultured *F. oxysporum* f.sp. *vasinfectum* in modified liquid Richard's medium. Resistant cotton cell lines were selected using culture filtrates of the pathogen. It was shown that levels of reducing sugars, total soluble sugars, soluble proteins, tannins, gossypol and some amino acids were increased after a 2 days treatment with 6% filtrates, but all the analysed substances in sensitive cell lines were decreased. They suggested that the resistance of cell lines to toxic filtrates may depend on the ability of cell lines to synthesize protective substances when invaded by toxins. The results may be useful as a reference for the study of mechanisms of resistance to wilt in cotton.

Franchoan and Lan (1997) found that cotton *(Gossypium hirsutum L.)* hypocotyls tissue responded with increased lignification following treatment a protein–lipopolysaccharide elicitor from *Verticillium dahliae,* the causative agent of vascular wilt disease in cotton. The induction of defense reaction was investigated over a period of 0–35 hr. Following exposure to the elicitor, increased synthesis and deposition of lignin and lignin–linked phenolic polymers occurred. The defense responses in two cultivars of *G. hirsutum* (cvs. 19 and Acala, 151–70 resistant and susceptible to *V. dahliae,* respectively) were compared. The resistant cultivar exhibited higher and earlier induced levels of enzyme activity and lignin–like polymers compared to the susceptible cultivar. This indicates that the effectiveness of induced defense responses depends on their rapid initiation, development and accumulation and suggests a possible correlation between the timing and intensity of lignin–linked polymer accumulation and resistance / tolerance of *G. hirsutum* seedling against *V. dahliae.*

Gurdeep *et al.* (1998) mentioned that biochemical changes in the apical leaves of cotton varieties suggested that cotton leaf curl bigeminivirus (CLCV) infection results in the malfunctioning of polyphenol metabolism in susceptible varieties. Healthy leaves of resistant cultivar LD–327 had higher total phenol, ortho-dihydrakyl-phenol, and flavonol contents as well as higher peroxidase activity. Poplyphenol oxidase (Catechol oxidase) phenylalanine ammonialyase and tyrosine ammonialyase activities were similar to those of susceptible cultivar F-846.The intensity of infection varied in the apical

leaves of F–846. Peroxidase activity was increased in moderately infected leaves compared with in healthy leaves, but decreased in the severely infected leaves.Catechol oxidase was increased in all leaves with increasing disease severity.

Howell *et al.* (1999) reported that on the mechanisms employed by the biocontrol agent *Trichoderma virens* to suppress cotton (*Gossypium hirsutum*) seedling disease incited by *Rhizoctonia solani* has shown that mycoparasitism and antibiotic production are not major contributors to successful biological control. They examined the possibility that seed treatment with *T. virens* stimulates defense responses, as indicated by the synthesis of terpenoids in cotton roots. Analysis of extracts of cotton roots and hypacotyls grown from *T. virens* treated seeds showed that terpenoids synthesis and peroxidase activity were increased in the roots of treated plants.

Sherif and El–Habbaa (2000) found that the higher decrease in gibberellins was associated with yellow leaf curl infection. Peroxidase and Polyphenoloxidase activities were found to be considerably higher in infected tomato leaves with *Alternaria solani, Pseudomonas syringae* and viruses than in healthy leaves. Viral induction with yellow leaf curl and leaf roll of tomato leaves exhibited higher activity of peroxidase followed by *P. syringae* infection while polyphenoloxidase was higher with *A. solani* infection than *P. syringae* and viral infection. The alteration of protein fractions from healthy and infected tomato leaves exhibited 5 polypeptides relative to infection with *P. syringae* and *A. solani* and 3 polypeptides relative to viral infection. They added that chlorophyll (a & b) and carotenoids

were highly reduced in infected leaves with *Pseudomonas syringae* and *Alternaria solani* than viral infection compared with healthy plants.

Chakrabarty *et al.* (2002) mentioned that six cotton lines possessing different degrees of resistance to grey–mildew (*Ramularia areola*) were used to study the biochemical basis of resistance. Induced rather than constitutive levels of phenylalanine amino lyase (PAL), total phenol, gossypol and flavonols played crucial role in governing resistance. The phenolic proline and total sugar upon infection appeared important for resistance. Resistant plants also possessed higher levels of constitutive as well as induced tannins and constitutive proline. Amino acid and carbohydrate metabolism of healthy and diseased plants did not directly reveal the significance of these constituents in resistance except that they were remarkably reduced as a result of infection.

Yuan *et al.* (2002) found that the root exudates for resistant seven cotton cultivars inhibited spore germination and mycelium growth of *V. dahliae,* but the root exudates of susceptible ones had an opposite effect to pathogen. The kinds and amount of amino acids in the root exudates of the susceptible cultivars were much higher than the resistant ones. Phenylalanine and protein were found only in the root exudates of the susceptible cultivars. The carbohydrate content in the root exudates of the susceptible cultivars was distinctly higher than the resistant ones.

6-Effect of fungicides on seedling parameters in presence of infection:

Murumkar and Chavan (1985) found that the infection of *Cicer arietinum* by *F. oxysporum* f.sp. *ciceri* resulted in a reduction in chlorophyll and increase in organic acids, polyphenols and carbohydrates.

Arinze and Sokirko (1986) mentioned that in lab. experiments respiration intensity, chlorophyll and carotenoids content and the total and working surface were reduced in maize seedlings grown from seeds inoculated with *Penicillium glaucum, Fusarium moniliforme* [*Gibberella fujikuroi*], *Aspergillus glaucus* and *A. niger*. Peroxidase activity in seedling leaves was varied with the pathogen species.

Alagrsamy *et al.* (1989) reported that seed treatment trials with the fungicide, carbendazim gave the best germination followed by tolclofos-methyl. Post-emergence mortality was the least with carboxin. In a soil drenching experiment carbendazim was superior to the other fungicides in improving germination and controlling post-emergence mortality. Yield of seed cotton was improved by carbendazim and tolclofos-methyl soil treatments.

Gowily and Abdel-Kader (1995) studied the effect of salinity on the infection rate of tomato plants with *F. oxysporum. f.sp. lycopersici*. Salinity stress increased the susceptibility of tomato plants to Fusarium wilt, especially at high salt concentrations. Increasing salinity with infection decreased plant height, fresh and dry weight and chlorophyll content. Protein

content and chlorosis were generally increased with salt induced stress and infection.

Youssef *et al.* (1995) demonstrated that the delinting seed of Egyptian cotton cvs. Giza 45 and 75 by the brush machine one or two times before coated with the Monceren fungicide resulted in improving seed germination and seedling growth characteristics.

Nwachukwu and Umechuruba (1997) investigated that the effects of *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Colletotrichum dematium* and *Fusarium moniliforme* [*Gibberella fujikuroi*] on the nutrient values of African yam bean, *S. stenocarpa*, seeds. All the fungi caused significant reduction in carbohydrate, oil and dry matter contents of African yam bean seeds, while protein, ash, fibre and moisture contents of the seeds were significantly increased when compared with uninoculated seeds.

Ahmed *et al.* (2000) found that cotton seeds dressed with Monceren 25% exhibited the highest speed of germination in non-infested soil, while the lowest speed of germination was obtained from acid delinted seeds without seed dressing in infested soil. Seeds dressed with Monceren 25% gave the largest values of plant height when they were mechanically delinted and grown in non-infested soil, while they gave the lowest plant height when acid delinted and grown in infested soil.

Felaifel *et al.* (2000) tested 12 fungicides on peanut seed germination, pod rot, plant dry weight, bacterial nodulation, protein and oil content under artificial inoculation with *R. solani*

and *F. solani*. They found that the different fungicides acted on the given parameters depending on the pathogen species, the fungicide itself and the studied traits. The effect on the plant dry weight, nodule counts, protein and oil content were varied greatly according the inoculated fungi and the fungicidal compounds but no clear relation had been detected among the fungicidal activity of the compound and any of these traits.

EL-Deeb *et al.* (2002) demonstrated that the fungicides, Vitavax-Thiram, Rizolex-T and Topsin-M70 at all treatments increased pod yield of peanut compared to the non-treated. The fungicidal treatments gave a higher yield than that obtained in the alternative compound treatments.

7- Effect of pathogenic fungi on seed oil content:

Shadmanov and Alimukhamedov (1983) reported that infected seeds of cotton (*G. hirsutum*) 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, whereas this was not true for *G. barbadense*.

Ataga and Akueshi (1986) studied the bio-deterioration in sunflower seeds inoculated with *A. tenuis (A. alternata) Curvularia lunata* [*Cochliobolus lunatus*], *Fusarium moniliforme* [*Gibberella fujikuroi*] and *Macrophomina phaseolina* over 21 day. It was clearly that they increased the free fatty acid content, reduced the oil content and caused discoloration of the oil.

Airede and Fsuruoso (1987) found that autoclaved oil palm kernels were inoculated with spores of seedborne isolates

of *Aspergillus flavus, A. niger, Penicillium chrysogenum, P. janthinellum, Paecilomyces varioti, Syncephalastrum racemosum or Fusarium oxysporum.* At 0, 2, 4 and 8 weeks after inoculation, the moisture content, oil, free fatty acids (FFA), sugars and protein nitrogen were estimated. The principal biochemical changes induced by those fungi were increases in moisture content and FFA, decreases in total oil and total sugars and a degradation of protein nitrogen. *A. flavus* caused the greatest change, and *P. varioti* caused the least change in the moisture conditions of this experiment.

Shivpuri *et al.* (1990) recorded that eighty-two Indian mustard seed samples were collected from 9 agro-climatic zones of Rajasthan, India, and 16 fungal species were isolated. The effects of these fungi were studied on the quantity and quality of Indian mustard oil. *Fusarium oxysporum, Phoma lingam* [*Leptosphaeria maculans*] and *P. nebulosa* reduced, 6 isolates increased and 7isolates did not effect oil content. All the fungi caused an unpleasant odour in oil and changed the oil colour significantly.

Ahmed *et al.* (1994) found that *Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii* [*Corticium rolfsii*] and *Fusarium oxysporum* from the diseased sunflower plants collected from different governorates in Egypt. Infected seeds from plants with *F. oxysporum* had lower seed oil content, lower iodine values and higher acid numbers than the healthy seeds. Diseased seeds showed a change in the normal colour of oil except in the case of cultivar Miak.

Ataga and Umechuruba (1998) mentioned that stored seeds of African yam bean (*Sphenostylis stenocarpa*) which were inoculated with seedborne *Botryodiplodia theobromae, Fusarium pallidoroseum* and *Penicillium oxalicum* resulted increasing in moisture content, free fatty acids, crude protein, fibre and ash content. As well as, decreases in dry matter, oil and carbohydrates during the incubation period of 21 days.

Srivastava and Pandey (2000) reported that infested Kusum (*Schleichera oleosa*) seeds with several fungi during one year of storage *i.e., Fusarium solani, Aspergillus fumigatus, A. flavus, A. niger* and *Paecilomyces variotii* resulted in changes in the biochemical properties of oil, which became more pronounced commensurate to increase in duration of seed-fungus association. At the end of one, year the total oil content diminished to less than half, saponification value was (SV) increased by 2.89% and fat acidity value was enhanced by 77%. The changes in these values were gradual over the intervening months evidently because of fungal activity indicating breakdown of kusum oil into fatty acids, and loss of short chain glyceride fatty acids.

Adekunle and Badejo (2002) studied the effect of the 5 tested fungi, *A. wentii* and *Fusarium solani* [*Nectria haematococca*] on the biochemical properties of artificially-infected oil in the corm of *C. esculentus* (collected between November 1999 and July 2000 in Lagos, Nigeria) after 14 days of incubation. The moisture content of the corm was 27.30+or-0.41% and the oil yield was 15.44+or-0.38%. The essential oil

was edible and non-rancid with free fatty acid value of 3.25+or-0.27%.

8-Mycotoxins:

Sankaranarayanan and Kumar (1985) isolated a toxin from the culture filtrate of a virulent str. (I 5) of *F. oxysporum* f.sp. *vasinfectum* from wilt sick soils near Coimbatore, induced typical vein clearing symptoms in cotton shoots. Characteristics of the endotoxin, which is host specific, are described.

Mazen *et al.* (1990) studied thirty-nine species belonging to 16 fungal genera isolated from Egyptian cotton seeds, cotton seed meal and cotton seed cake on 1% glucose-Czapek's agar medium incubated at 28 °C *Aspergillus* was the most frequent genus and it emerged in 87-100% of the samples contributing 70-98% of total fungi in the 3 substrates tested. The most common species were *A. niger, A. flavus, A. fumigatus, A. terreus* and *Rhizopus stolonifer; A. niger, A. fumigatus* and *Penicillium corylophilum* and *A. niger, A. flavus, A. terreus, A. nidulans* and *R. stolonifer*, respectively. Cotton seeds and cotton seed products were naturally contaminated by aflatoxin B1 and B2. Approx. 16% of the different substrates tested were positive for aflatoxin contamination. No citrinin, ochratoxin A, patulin, sterigmatocystin, diacetoxyscirpenol, T-2 toxin or zearalenone were detected in the samples assayed.

Li *et al.* (1990) recorded that the 4 strains of *F. moniliforme* [*Gibberella fujikuroi*] were isolated from cotton dust and inoculated onto the growing cotton boll, inducing wilt in cotton. This strain was cultured in rice medium and extracted

by Mirocha's method. The crude extract was tested on rabbit skin and pea seed germination. A toxin purified and identified by TLC, HPLC and H-NMR, was confirmed to be T-2 toxin.

Pieckova and Jesenska (1996) mentioned that the mycobiota of 24 samples of flax and 45 samples of cotton processed in Slovak textile factories during a 3-year period were studied. Mean colony forming units (cfu) of 12.2 x 10^6 of microfungi per g of flax samples and 3.3 x 10^5 cfu of micromycetes per g of cotton samples isolated. Species isolated included *Aspergillus flavus, A. fumigatus, A. glaucus, A. restrictus, A. terreus, A. versicolor, Alternaria sp., Cladosporium sp., Fusarium sp.* and *Penicillium sp.* Among of 55 *A. flavus* isolates tested, 17 (30.9%) produced aflatoxin B1.

Vidhyasekaran *et al.* (1997) isolated a toxin from the rice sheath blight pathogen, *R. solani*, which induced characteristic symptoms of the disease on rice. The toxin was partially purified and was identified as a carbohydrate containing glucose, mannose, N-acetylgalactosamine and N-acetylglucosamine. The toxin was also detected in infected rice leaves. Highly virulent isolates produced more toxin than less virulent isolates. Several *R. solani* isolates from rice and one each from cotton and tomatoes produced a similar toxin. All rice cultivars tested were susceptible to the pathogen and sensitive to the toxin. Host specificity of the toxin was demonstrated using hosts and nonhosts of the pathogen.

Hasan (2001) reported that the effect of pencycuron and pencycuron-c on cotton (*Gossypium barbadense*) seed mycoflora, aflatoxin production and seed viability was studied.

At 8% seed moisture content (mc), pencycuron and pencycuron-c promoted *Aspergillus niger, A. flavus* and *Penicillium corylophilum* growth count. The *A. niger* utilized pencycuron as nitrogen source more than pencycuron-c. Seeds with 15% mc lost their viability faster than those with 8% mc, and it was more evident as storage time was increased. Such loss was occurred faster when seeds were treated with pencycuron, whereas pencycuron-c exerted significant activation in the viability compared to the control. The fungal species had high biodegradation activity and produced aflatoxin in different parts of cotton boll (fibre, valves, and seeds). Pencycuron and pencycuron-c inhibited aflatoxin B1 and B2 production in seeds, but did not affect aflatoxin G1 and G2.

Wei *et al.* (2004) recorded that trichothecenes are biologically active mycotoxins produced by several fungi. Trichothecin was extracted and purified from *Trichothecium roseum* S-24 isolated from a cotton field and cultured in the presence or absence of chitin. The mycotoxin was purified using filter paper, Kieselgel 60 silica gel chromatography, reverse-HPLC, and silica gel thin layer chromatography. Chitin had a significant effect on the amount of purified trichothecin. The results of TLC-BIOASSAY showed that trichothecin (1, 5, 10 and 50 micro g) inhibited the growth and development of *Verticillium dahliae, Magnaporthe grisea, Alternaria alternata* and *Fusarium moniliforme [Gibberella fujikuroi]*. Complete inhibition was observed in *V. dahliae* at 10 and 50 micro g trichothecin, and in *M. grisea* and *A. alternata* at 50 micro g trichothecin.

MATERIALS AND METHODS

MATERIALS AND METHODS

I- Isolation trials:

1- 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 seasons. Samples were delinted by using concentrated sulphuric acid (40%) for 3 minutes then washed with sterilized distilled water several times (**Helal *et al.* 1996).** Hundred seeds were used for isolation before delinted and after delinted by blotter test method as described by **Paul et *al.* (1970)**. These seeds were disinfected by immersing in 5% sodium hypochlorite solution 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 moisten 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 compound microscope to complete identification.

2- Isolation from cotyledons and 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. In this respect, the seeds were soaked in tap water for 30 minutes, then surface sterilized in 3% sodium hypochlorite for 5 minutes, followed by treatment in 70 % ethanol for two minutes then washed several times in sterilized distilled water and dried between two sterilized filter papers. The seed coats were then separated far 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 (**Christensen, 1957**). The emerged fungi were transferred individually to fresh (PDA) dishes. Purification of the isolated fungi was carried out as mentioned before **Roncadori *et al.* (1971).**

3-Isolation from rotten roots of cotton seedlings:

Cotton roots and hypocotyls of damping-off seedlings 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 by immersing in 5% sodium hypochlorite solution for 3 minutes followed by 70 % ethanol for two minutes then washed several times in sterilized distilled water and dried between two sterilized filter papers. Surface sterilized samples were aseptically transferred to PDA plates as mentioned before. 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 before.

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

II- 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 then tightly sealed and autoclaved for 30 minutes. The flasks were inoculated by any of the isolated fungi and incubated at 28°C for two weeks. Clay pots (20 cm Φ) were sterilized by immersing in 5% formalin solution for 15 minutes and left for 15 days to get rid of formalin odour, then filled with autoclaved loamy soil (2 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 to ensure the distribution growth of the fungi **(Whitehead, 1957).** Un-inoculated cornmeal–sand medium was added to the prepared

soil as mentioned before to serve as control pots. Three pots were used as replicates for each particular treatment. Each pot was planted with 10 surface sterilized cotton seeds. Two cotton cultivars, i. e. Giza-86 and Giza-89 seeds obtained from Cotton Research Institute, ARC, were used. Disease assessment was recorded as percentages of pre–and post–emergence damping–off and survivals were recorded at 10 and 21 days after sowing, respectively as following:

$$\text{Pre–emergence damping–off \%} = \frac{\text{No.of un–germinated seeds}}{\text{No.of planted seeds}} \times 100$$

$$\text{Post-emergence damping–off \%} = \frac{\text{No.of dead seedlings}}{\text{No.of planted seeds}} \times 100$$

Survived plants %= 100–(% pre–emergence damping- off + % Post emergence damping–off)

III – Laboratory studies:

1-Evaluation of some fungicides on the growth of the pathogenic fungi:

This experiment was conducted to study the effect of some fungicides i.e., Maxim, Premis, Topsin–M70 – Rizolex–T, Vitavax–T70 and Vitavax–T40 at different concentrations on the linear growth of pathogenic fungi which were selected according to their pathogenic capabilities after the pathogenicity tests. The tested fungicides **(Table 1)** were tested at different concentrations, i.e. 0, 1, 5, 10, 25, 100, 200 and 400 ppm based on their active ingredient. In this respect, sterilized PDA medium was mixed with any of the tested concentration immediately

before solidification and poured into plates (7 cm Φ). Plates with poisoned or un–poisoned PDA medium were inoculated, at the center with equal discs (4 mm) taken from the margin of 7 days–old cultures of the above mentioned pathogenic fungi and incubated at 28°C. Four plates were used for each treatment as replicates. Linear growth of each tested fungus was measured daily until its mycelial growth was completely covered the surface of the medium in the control treatment (un–poisoned medium) and the averages of the two perpendicular diameters of the fungal growth in mm were calculated as described by **Mahrous (1974)**.

2-Evaluation of some commercial bio-agents on growth of the tested pathogenic fungi:

In this experiment, the commercial bio-agents, i.e. Plantguard (each ml contains about $30x10^6$ spore of *Trichoderma harzianum*) and Rizo-N (each gram contains about $30x10^6$ cfu of *Bacillus subtilis*) were used to test their effect on the linear growth of the tested pathogenic fungi under study. Two equal amounts, i.e., 100 µl Plantguard or 100 µg of Rizo-N were spotted (for the first) or streaked (for the second) at two opposite sides on the surface of (PDA) medium and apart equal distances from the peripheral side of the plates.

Treated as well as un–treated plates (9 cm Φ) were inoculated simultaneously at their center (between the two spots or streaks of the tested bio-agent) each with a disc of any other tested pathogenic fungus. Four plates were used as replicates for

each treatment and all plates were incubated at 25° C for about 5–7 days until plates of control treatment (without bio-agent) were covered by the mycelial growth of any of the tested fungi. Then, the average linear growth of tested fungi was recorded in mm.

Table (1): List of the tested fungicides, their active ingredients, recommended dose and manufactures

Trade name & Producing Company	Common name & Active ingredient	Chemical formula	Dose/ kg seeds
Maxim (Syngenta,Swizerland)	Fludioxonil-metalaxyl (3.5%)	4,2,2difluoro1,3benzodioxol4y1)-1H–pyrrole carbonitrite(1UPAC).	3 ml
Premis (France)	Triticonazole (BSI,PaE-150)	5–[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1H1,2,4–triazol=1–methyl) cyclopentanal	5 ml
Topsin-M-70 (Nippon-soda, Japan)	Thiophonate–methyl (70%)	Dimethyl(1.2phenylene)bis (iminocarbono–thioye)biscarbonate	3 g
Rizolex–T (Sumitomo, Japan)	Tolclofos–methyl+ Thiram (50%)	O-(2,6-dichloro-p-tolyl) O,O-dimethyl phosphorothioate (IUPAC). 30%-Tetramethylthiuram disulfide (IUPAC).	3 g
Vitavax–T70 (Mniroyal, England)	Carboxim + Thiram (75%)	5,6–dihydro-2-methyl–N-phenyl–1.4–oxathiin-3-carboxyamide–Bis(dimethylthio–carbamayl disulfide	3g
Vitavax– T40 (Mniroyal, England)	Carboxin+Thiram (40%)	5,6–dihydro-2-methyl–N-phenyl–1.4–oxathiin-3-carboxyamide–Bis(dimethylthio–carbamayl disulfide	3 g

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

A- ***In vitro*:**

Mycotoxins i.e., aflatoxins (B1' B2' G1' G2), fumonisins, zearalenane and trichothense were determined by growing the tested pathogenic fungi in yeast extract sucrose medium (YES) which consists of 20g yeast extract and 200g sucrose and 1000 ml distilled water. Each 25 ml of 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 prospected mycotoxins were determined according to **Anon. (1990).**

B- ***In vivo:***

4-Determination of aflatoxins, fumonisins and zearalenone:

Mycotoxins (aflatoxins, fumonisins and zearalenone) were determined according to **Anon. (1990)**. In this respect, 100-grams samples were homogenized in 200 ml methanol: water solution (8:2) in a blender at high speed for 3 min. The samples were filtered using filter paper No. 1 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 collected filtrate were put in separating funnel containing 15-ml benzene, then shaked for 5 min. The upper layer was collected in a glass beaker and evaporated till 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, respectively) 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 (UV) wavelength 365 nm.

Mycotoxins µg / kg samples = (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 (mycotoxins such as aflatoxins, zearalenone and fumonisins.
W= weight of sample (100 g).

VI- Greenhouse experiments:

1-Effect of some fungicides and commercial bio-agents on disease incidence of cotton under greenhouse conditions:

The effect of some fungicides and commercial bio-agents on root–rot incidence expressed as cotton dead plants under greenhouse conditions was investigated. In this study, loamy soil was infested with 3% inoculum level of any of the tested fungal inoculum and distributed in sterilized pots (25 cm Φ) as previously mentioned.

A-Effect of different fungicides on the incidence of root– rot:

In this study, fungicides namely Premis and Maxim were used at the rate of 5 ml/kg seeds while, Topsin–M70, Rizolex–T, Vitavax–T70 and Vitavax-T40 were used at the rate of 3g /kg seeds to determine their efficiency against cotton root–rot infection (dead plants). Cotton seeds cvs Giza-86 and Giza-89 were mixed with the tested fungicides (each separately) plus glue suspension as adhesive material (10 ml/kg seed) in a closed glass container and then shaken vigorously for 10 minutes. After seed dryness, the treated seeds were sown in pots (25 cm Φ) at the rate of 10 seeds/pot. In control treatments, cotton seeds were sown in potted soil infested with any of the pathogenic fungi (without treatment).

B- Effect of Plantguard and Rizo-N bio-agents on root-rot incidence:

Effect of two commercial bio-agents i.e., Plantguard (*Trichoderma harzianum*) and Rizo–N (*Bacillus subtilis)* against pathogenic fungi causing root–rot of cotton cultivars was studied. In this respect, seeds of cotton cvs (Giza-86 and Giza-89) were mixed with Rizo–N and Plantguard at the rate of 3g and 3 ml/kg seeds of the two treatments, respectively and sown at the rate of 10 seeds/pot . Three replicates (pots) for each treatment were used.

The incidence of root–rot disease (dead plants) of cotton caused by the tested pathogens was determined 21 days after sowing for all the aforementioned greenhouse studies. Also,

values of shoot length, root length and dry weight of the entire grown plants in the pots were determined.

V- Field experiments:

Effect of some fungicides, and commercial bio-agents on root–rot incidence under field conditions was carried out during 2 successive seasons i.e., 2000 and 2001. Seeds were treated as mentioned before in the greenhouse experiments and planted in naturally infested field at Sakha Agricultural Research Station, Kafr EL–Sheikh, Egypt. Experimental plot was 3 x 5.5 m^2 containing 3 rows and 20 cm apart between each two hills. Three randomized replicates were used for each treatment. The normal field practices of cotton cultivation were performed. Each plot was planted with 375 cotton seeds at the rate of 125 seed/row (five seeds/hill). Percentages of damping–off (pre– and post–emergence damping–off) were recorded after 21 days from sowing, and the percentages of survived plants were determined at 45 days after sowing. Plant height, number of fruiting branch, number of bolls/plant, cotton weight/ boll, cotton yield/plant, cotton lint/plant, cotton seeds/plant, cotton yield/feddan, cotton lint/feddan, cotton seeds/feddan, fiber length and fiber strength for each treatment as affected by the different treatments were also determined.

IV- Chemical determinations:

1. Chemical analysis of treated cotton plants:

Cotton plants cvs. Giza-86 and Giza-89 were grown in inoculated pots (25 cmϕ) with tested root-rot pathogens. Then,

cotton leaf samples were taken 21 days post inoculation for further chemical determinations.

Extraction:

Cotton leaf samples (2 g) of each treatment were cut into small portions. These portions were immediately placed in 50 ml of 95% ethanol in brown bottles and kept in darkness at room temperature till dryness then homogenized in sterile mortar as recommended by **Bozarth** and **Diener (1963).** The resultant homogenate was filtered through filter paper. The residue was thoroughly washed with 80% ethanol. The ethanolic extracts were air dried at room temperature till near dryness and then were quantitatively transferred to 10 ml 50% isopropanol, and used for chemical analysis of sugars and phenols.

A- Determination of sugar content:

Sugars (Total, reducing and non-reducing) were determined spectrophotometrically with picric acid as described by **Thomas and Dutcher (1924).** The sugar content was calculated as mg glucose from standard curve prepared for glucose. The following two solutions were used for the determination of the total soluble and reducing sugars.

Picrate-picric solution:

Thirty six grams of picric acid were added to 500 ml of a 1% solution of sodium hydroxide in one liter flask, 400 ml of hot water were added and the mixture was shaken occasionally until the picric acid was dissolved, and afterwards, it was cooled and diluted to one liter.

Sodium carbonate solution:

Twenty grams of sodium carbonate were dissolved in 100 ml of distilled water. For determination of total soluble sugars, 0.5 ml of a given sample was placed in 70 ml test tube, containing 5 ml of distilled water plus 4 ml picrate-picric solution and then the mixture was boiled for 10 minutes, on a water bath. After cooling, one ml of sodium carbonate was added and the mixture was boiled again for 10 minutes, then cooled and completed to 50 ml with distilled water. The optical density of the developed color was measured by using spectrophotometer (SPECTRONIC 20-D) in the presence of a blank at 540 nm.

The above technique was applied also for determination of reducing sugars except that picrate-picric acid and sodium carbonate were added together at the same time and boiled only for 10 minutes.

Total and reducing sugars concentrations were calculated as milligrams of glucose per one gram fresh weight according to a standard curve of glucose. However, the non-reducing sugars were determined as the difference between the total and the reducing sugars.

B- Determination of phenolic compounds:

Phenolic compounds were determined using the colorimetric method of analysis described by **Bary and Thorpe (1954)**. Phenol reagent (Folin-Ciocalteu reagent) was prepared by boiling a mixture of 100 g of sodium tungestate, 25 g of sodium molybdate, 700 ml of distilled water, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid

under reflex for 10 hours in a water bath. Then 150 g of lithium sulphate, 50 ml of distilled water and a few drops of bromine were added to the mixture and boiled again for 15 minutes without a reflex condenser to remove excess bromine, then cooled, diluted to 1 liter with distilled water and filtered.

The free phenols were determined as follows, one ml of the phenol reagent and 5 ml of a 20% solution of sodium carbonate were added to the isopropanol sample (0.2 ml) and diluted to 10 ml with warm water, (30-35°C). The mixture was let to stand for 20 minutes and read using spectrophotometer (SPECTRONIC 20-D) at 520 nm against a reagent blank.

For total phenols determination, 10 drops of concentrated hydrochloric acid were added to the isopropanol sample (0.2 ml) in a test tube, heated rapidly to boiling over a free flame, with provision for condensation. Then the tubes were placed in a boiling water bath for 10 minutes. After cooling 1ml of the reagent and 2.5 ml of 20% Na_2CO_3 were added to each tube. The mixture was diluted to 50 ml with distilled water, and after 20 minutes was determined using spectrophotometer (SPECTRONIC 20-D) at 520 nm against a reagent blank.

The total and free phenol contents were calculated for each treatment as milligrams of catechol per one-gram fresh weight according to standard curve of catechol. The conjugated phenols were determined by subtracting the free phenols from the total phenols.

C- Determination of photosynthetic pigments:

Chlorophyll (A), Chlorophyll (B) and Carotenoids were

determined according to the method suggested by **Wettestien. (1957)**

A fixed fresh weight (0.2 g) sample from fourth leaf (from the top of the plant) were ground in mortar with 5 ml 85% acetone for extraction of pigments in the presence of 0.5 g of purified sand to facilitate the grinding and 0.5 g $CaCo_3$ salt to neutralize the acidity of the sap, for preventing transformation of chlorophyll to pheaphytin. The homogenate was transferred to be centrifuged for 15 min. at 4000 rpm, then repeated another time with small volume of acetone in order to get the pigments free. The supernatant was diluted to 25 ml with 85% acetone and its optical density was measured at wave length of 662, 644 and 440.5, nm respectively.

The concentration of photosynthetic pigments was calculated as follows:-

Chlorophyll (A) = (9.784 X E 662 – 0.99 x E 664)

Chlorophyll (B) = (21.426 X E 644 – 4.65 X E 662)

Carotenoids = {4.695 X E 440.5 – 0.263 (Chl. (A) + Chl. (B)}

Where E = Reading recorded at the selective wave length.

2- Determination of oil content:

Cotton seed samples (100-grams for each sample) of both 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 0 time, 5, 10 and 15 days post inoculation.

Physiological response of resistance.

3-SDS – Protein electrophoresis:

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was preformed according to the method of **Laemmli (1970)** which was modified by **Studier (1973)**

Preparation of plant material:

Cotton plant leaves were taken at 21 days old and kept under –20°C till use. Then one gram of each plant sample was treated with liquid nitrogen and ground with 2 ml Lan's buffer (2x) using mortar and pestle. Samples were transferred to Eppendorf tubes, and kept at 4°C over night, then centrifuged for 20 min at 12000 rpm at 4 °C. Supernatants containing water soluble protein fractions were subjected for further analysis by SDS electrophoresis. **(Okuna *et al.*, 1991).** The stock solutions used for protein electrophoresis were as follows:

Lan's buffer (2X)

SDS	10ml
Glycerol	20ml
1m tris (pH 8.8)	12ml
0.25 M ETDA	1.6µl
H_2O (dd.)	26.4 ml

Acrylamide stock (Kept in dark, 4 C°)

A. Resolving gel

Acrylamide	30 g
Bis–Acrylamide	0.8 g
H_2O (dd.)	Up to 100 ml

B. Stacking gel:

Acrylamide	30 g
Bis–Acrylamide	1 g
H_2O (dd.)	Up to 100 ml

Buffers:

A. Resolving gel buffer (4X tris, pH 8.4 at 4°C

Tris	18.15 g
HCl (conc.)	3.50 ml
H_2O (dd.)	Up to 100 ml

B . Stacking gel buffer (1m tris – HCl, pH 6.8 at 4°C)

Tris	12.11 g
H_2O (dd.)	Up to 100 ml

Adjust pH to 6.8 by HCl.

C. Run buffer:

Tris	15.14 g
Glycine	72.07 g
10 %SDS	5000 ml
H_2O (dd.)	Up to 5 liters

Gel preparation:

Vertical slab (18x16) gel electrophoresis apparatus was used as marketed by Hoefer SE 600 series (His). All glass plates were washed thoroughly with distilled water, then surface sterilized with ethanol. Spacers of 1.5 mm were used.

A. Resolving gel (15% Acrylamide)

Acrylamide stock (for resolving gel.)	31.66 ml
Tris (4x pH 8.4)	16.25 ml
H_2O (dd.)	16.10 ml

This solution was filtered then the following ingredients were added:

SDS (10 %)	750 µl
Ammonium persulfate (10%) freshly prepared	500 µl
TEMED	100 µl

The two gels were poured simultaneously to a height of 1:5 below the bottom of the comb. Gels were overlaid with filtered isopropanol and left to polymerize for at least 1h. The following ingredients were added.

B. Stacking gel.

Acrylamide stock (for resolving gel.)	2.66 ml
Tris (4x pH 6.8)	2.50 ml
H_2O (dd.)	14.70 ml

This solution was filtered then the following ingredients were added

SDS (10 %)	250 µl
Ammonium persulfate (10%) freshly prepared	100 µl
TEMED	40 µl

These staking gel solutions were quickly poured over the two resolving gels and 15 well – combs were used. Gels were left to polymerize for 45 min before gels were run.

Application of samples:

A volume of 75 µl protein extract for each sample were added to 10 µl mercaptoethanol (10 % v/v) to each sample. Samples were boiled for 3 min and added to 5 µl bromophenol blue and glycerol 100 µl of each sample were loaded on the gel .

Gel running and visualization .

Four liters of the run buffer were poured into the running tank to be precooked by flooding cold water (4°C) through cooling tubes. The run buffer (800 ml) was added to upper tank just before running gels where run at 100 volt for quarter of hour then the voltage was raised to 250 volt till the samples reached one inch from the bottom of the gel. Gels were removed from the apparatus and placed in plastic tanks, then covered with visualized solution. Gels were shaked gently overnight.

The composition of the visualized solution was as following:

Methanol	445 ml
Coomassie brilliant blue – R 250	1 g
Acetic acid (conc.)	90 ml
H_2O (dd.)	445 ml

After removing the visualization solution, gels were covered with a dis-visualization solution (Freshly prepared) of the following composition:

Methanol	700 ml
Acetic acid (conc.)	200ml
H_2O (dd.)	Up to 3500ml

Gel was agitated gently for one hour. After removing the solution, a new one was added. This step was repeated several times until gel background is clear, then they were photographed.

Preparation of plant materials:

Five grams of cotton leaves of cvs Giza-86 and Giza-89 at 21 days old were taken and kept under – 20°C till use. Then, one g of each plant sample was treated by liquid nitrogen and ground with 2 ml phosphate buffer (pH 6.5) 0.1 M using mortar and pestle. Samples were transferred to Eppendorf tubes and then

centrifuged for 20 min at 12000 rpm at 4°C. Supernatants containing peroxidase and esterase enzymes were stored at –80°C till use. Three replicates were prepared for each treatment as described by **Biles and Mertyn (1973).**

4-Changes in some electrophoretic isozyme related to resistance:

Polyacrylamide gel electrophoresis (PAGE) was performed exclusively in vertical slab 18x16 cm (Bio-Rad) according to the method described by **Stegemann *et al.* (1985).**

Stock solution buffers.

Acrylamide stoke (30 %)

Acrylamide	29.29g
N.N. methyle bis – acrylamide	0.12g
H2O (dd.)	100 ml

Gel Buffer: -

Tris	5.451 g
Boric acid	1.546 g
EDTA, Na2 salt	0.292g
H2O (dd.) up to	100 ml

These stocks were kept at 4°C

Gel preparation:

Polyacrylamide standard gel at pH 8.6 (8%) was prepared as follows:

30% Acrylamide	25 ml
Gel buffer	75ml
Sodium sulfite	30mg
Ammonium peroxidase phosphate (2%)	4 ml.
TEMED	100 µl

The gel was poured into the plate and 15 well comb were placed immediately. Gel polymerization took about 30 minutes.

Samples application:

A volume of 50 µl of each sample was mixed with 10-µl bromophenol blue, then 60 µl of this mixture were loaded on the prepared gel.

Enzyme visualization:

A-Esterase activity:

Gels were incubated in 0.1 M phosphate buffer pH7 for 15 min and then transferred into reaction mixture containing 30 mg fast blue RR – salt (stabilizer diazonium) as the coupler, 0.5 ml of 1% (W/V) -naphthyl acetate in a 50% acetone as the substrate and 24.5 ml distilled water. Incubation was carried out at 25°C in the dark for about 20 min., after which, the reaction was halted by a 7 % acetic acid solution

B- Peroxidase activity:

The isozymes patterns of peroxidase were visualized by the method of **Novaky** and **Hampton (1968)** which was applied by incubating the gels in the staining solution contained 7% acetic acid and 1% sodium acetate and was saturated with benzidine dihydrochloride. Hydrogen peroxide was added to this solution to give a concentration of 0.1 % or 0.01 % just prior to use.

Statistical analysis:

All the aforementioned experiments which carried out under Lab., greenhouse and field conditions were performed in a complete randomized block and split design. All data were analyzed according to **Snedecor and Cochran (1989).**

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

I-Isolation, purification and identification of fungi associated with cotton seeds before and after delinting.

Isolation trials from cotton seeds (before and after delinting, testa and cotoyledons) and rotten roots 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.) v. Arx, *Fusarium moniliforme,* J. 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* Kuehn in addition to some unidentified fungi.

The obtained data **(Table, 2-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 (62 and 42 isolates) with the highest frequency being 72.9 and 46.7% followed by *Fusarium moniliforme* and *Fusarium roseum* from seeds of cvs Giza-86 and Giza-89, respectively. Meanwhile, *Fusarium semitectum,* and *Fusarium nivale* were more frequent from seeds of cv. Giza-89 than on cv.Giza-86. Generally, all isolated fungi from cv Giza-86 except, *R. solani* were lesser in their frequency from than on Giza-89 when isolation was carried out before delinting. Also, *Aspergillus niger, Fusarium roseum* and *Penicillium spp* were not recorded on seeds of Giza-86 while *Fusarium tricinectum* was not recorded from seeds of Giza-89.

Whereas, *F. dimerum* was not isolated from both undelinted cotton cvs seeds.

As for the isolated fungi after delinting, the total isolated fungi were 44 and 57 isolates from Giza-86 and Giza-89, respectively. Out of them, *Fusarium moniliforme* and *R. solani* were the highest frequent fungi within the seeds of cvs. Giza-86 where their frequency recorded 43.2 and 40.9%, respectively. Meanwhile, *R. solani* was only the highest frequent one from seeds of cv. Giza-89 after delinting where its frequency was 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,*

Table (2-a): Occurrence and, % of the isolated fungi from cotton seeds (before and after delinting).

Isolated fungi	Occurrence and, % of the isolated fungi from cotton seeds									
	Before delinting				Mean of Freq.%	After delinting				Mean of Freq.%
	Giza-86		Giza-89			Giza-86		Giza-89		
	No.	F.	No.	F.		No.	F.	No.	F.	
A.. alternata	2	2.4	3	3.3	2.8	-	-	2	3.5	1.8
A.. niger	-	-	2	2.2	1.1	-	-	-	-	-
F. dimerum	-	-	-	-	-	-	-	8	14.0	7.0
F.moniliforme	8	9.4	12	13.3	11.4	19	43.2	6	10.5	26.9
F. nivale	4	4.7	9	10.0	7.4	2	4.6	4	7.0	5.8
F. roseum	-	-	10	11.1	5.6	-	-	8	14.0	7.0
F.semitectum	5	5.9	9	10.0	7.9	5	11.4	-	-	5.7
F.tricinectum	4	4.7	-	-	2.4	-	-	-	-	-
Penicillium spp	-	-	3	3.3	1.7	-	-	-	-	-
R. solani	62	72.9	42	46.7	59.8	18	40.9	29	50.9	45.9
Unknown	-	-	-	-	-	-	-	-	-	-
Total	85	100	90	100		44	100	57	100	

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

Aspergillus niger, Penicillium spp and *Fusarium semitectum* were isolated in low frequencies from seeds of cvs.Giza-86 and Giza-89. 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 the dominant.

Data in **Table (2-b)** show the associated fungi obtained from the inner surface of cotton seed testa and cotoyledons. In this respect, 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 *Fusarium nivale* (30.2%) from seed testa of cv. Giza-89, *Fusarium semitectum* (28.9 %) and *Fusarium moniliforme* (24.6%) in respect to the seed testa of cv.Giza-86. On the other hand, *Alternaria alternata, Fusarium solani, Fusarium tricinectum, Penicillium spp* and *Aspergillus niger* were not isolated or isolated in low numbers from cotton seed testa of both cvs. Giza-86 or Giza-89. As for the isolated fungi from cotoyledons, the total fungal isolates i.e., 14 and 19 were isolated from the cotoyledons of cvs. Giza-86 and Giza-89 respectively. Out of them, *Fusarium roseum* showed the highest frequency fungus (28.5 and 31.5%) from cotoyledons of both cotton cvs. seeds followed by *Fusarium moniliforme* from cvs Giza-86 and

Giza-89 (21.3 and 15.8%) respectively. Meanwhile, *Fusarium solani* was not recorded from cotoyledons of seeds of both cotton cvs. However, *Penicillium spp., Alternaria alternata* and *A. niger* as well as, some others fungi were isolated at low frequencies. 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 tested.

Regarding the isolated fungi from rotten roots of cotton seedlings, data in **Table (2-c)** show that 6 fungal isolates were obtained from the rotten roots of cv. Giza-86, two of them were o *R. solani*. Meanwhile, 14 isolates were obtained from the rotten roots of cv. Giza-89, 5 isolates of them were belonging to *R. solani*. It is clear that *R. solani* was the most frequent fungus followed by *Fusarium moniliforme* and *Aspergillus niger*. However *Penicillium spp.* and *Fusarium tricinectum* recorded the lowest frequency.

Table (2-b): Occurrence and, % of the isolated fungi from testa and cotoyledons of cotton seeds.

Isolated fungi	Frequency and percentages of isolated fungi									
	Testa				Mean of F..%	Cotyledons				Mean of F.%
	Giza-86		Giza-89			Giza-86		Giza-89		
	No.	F.	No.	F.		No.	F.	No.	F.	
A.alternata	-	-	2	4.7	2.3	-	-	2	10.5	5.3
A. niger	1	1.5	-	-	0.7	2	14.3	1	5.3	9.8
F.moniliforme	17	24.6	4	9.3	16.9	3	21.3	3	15.8	18.6
F. nivale	10	14.5	13	30.2	22.5	2	14.3	1	5.3	9.8
F. roseum	-	-	8	18.6	9.3	4	28.5	6	31.5	30.0
F.semitectum	20	28.9	2	4.7	16.8	1	7.2	1	5.3	6.3
F. solani	-	-	1	2.3	1.2	-	-	-	-	-
F.tricinectum	-	-	-	-	-	1	7.2	3	15.8	11.5
Penicillium spp.	-	-	-	-	-	1	7.2	-	-	3.6
R. solani	21	30.5	13	30.2	30.3	-	-	-	-	-
Unknown	-	-	-	-	-	-	-	2	10.5	5.3
Total	69	100	43	100		14	100	19	100	

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

Table (2-c): Occurrence and, % of the isolated fungi from cotton rotten roots

Isolated fungi	Frequency and percentages of isolated fungi from cotton rotten roots				
	cv. Giza-86		cv. Giza-89		Mean of F.%
	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 isolate fungi F.= Frequency % of the isolated fungi

II- Pathogenicity tests:

Data in **Table (3)** indicate that *R. solani* was the highest pathogenic fungus among all of the tested fungi where it caused the highest infection of 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 levels from 1-3% increased gradually the percentage of pre–emergence damping–off where the highest pre-emergence was recorded at 3% inoculum for all tested pathogens.

Regarding, the post emergence damping-off, it is clear that the post infection ranged from 3.3 to 12.2 % in case of cotton cv. Giza-86 and 3.3-15.5 % in case of cv. Giza-89. *R.solani* and *F. semitectum* were the most virulent pathogens at this disease stage meanwhile, *F. roseum* was the least virulent one. However, increasing inoculum level of each pathogen from 1 -3% increased gradually post infection 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 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, it is clear from the results that 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 (3): Pathogenicity test of some isolated fungi from cotton seeds at different levels on two cotton cvs.Giza-86 and Giza-89.

Disease parameters	Tested fungi	Disease index at different inoculum levels									
		cv.Giza-86				M.	cv.Giza-89				M.
		0	1	2	3		0	1	2	3	
Pre-%	*R. solani*	00.0	23.4	26.7	36.7	21.7	00.0	10.1	16.7	43.4	17.5
	F. semitectum	00.0	13.4	20.1	30.1	15.9	00.0	6.7	10.1	20.1	9.2
	F.moniliforme	00.0	16.7	23.4	23.4	15.9	00.0	6.7	13.4	13.4	8.3
	F .roseum	00.0	10.1	16.7	23.4	12.6	00.0	3.4	13.4	13.4	7.5
Mean		00.0	15.9	21.7	28.4	16.5	00.0	6.7	13.4	22.6	10.7
Post-%	*R. solani*	00.0	3.3	10.0	12.2	6.4	00.0	3.3	10.0	15.5	7.2
	F. semitectum	00.0	3.3	8.9	12.2	6.1	00.0	3.3	8.9	11.1	5.8
	F.moniliforme	00.0	3.3	7.7	10.0	5.3	00.0	3.3	6.6	11.1	5.3
	F .roseum	00.0	3.3	6.6	7.7	4.4	00.0	3.3	5.5	7.7	4.1
Mean		00.0	3.3	8.3	10.5	5.5	00.0	3.3	7.8	11.4	5.6
Survival %	*R. solani*	100.0	73.3	63.3	51.1	71.9	100.0	86.6	73.3	41.1	75.3
	F. semitectum	100.0	83.3	71.1	57.7	78.0	100.0	90.0	81.1	68.8	85.0
	F.moniliforme	100.0	80.0	68.6	66.6	78.8	100.0	90.0	80.0	75.5	86.4
	F .roseum	100.0	86.6	76.7	68.9	83.1	100.0	93.3	81.1	78.9	88.3
Mean		100.0	80.8	69.9	61.1	78.0	100.0	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%)

III-Chemical changes in some plant constituents of cotton detached leaves due to infection with root rot pathogens.

1- Sugars and phenols changes:

Data in **Table (4-a)** indicate that infestation the soil with root rot pathogens increased the reducing, non-reducing and total sugars in leaves of cotton plants of both cvs (Giza-86 and Giza-89). The highest increase in reducing and non-reducing sugars in cotton leaves of cvs Giza-86 and Giza-89 were recorded in case of infestation the soil with *F. moniliforme* followed by each of *R .solani* and *F. semitectum,* respectively, while the lowest amount of reducing sugars was recorded when the soil was infested with *F. roseum* comparing to un-infested soil (control). On the other hand, total sugars were also high in cotton leaves of both cvs. (Giza-86 and Giza-89) in case of infestation the soil with *F. moniliforme* followed by *R.solani* and *F. semitectum.* While the least increase was in case of infestation the soil with *F .roseum.*

Regarding phenols, it is clear from data **(Table, 4-b)** that infestation the soil with root rot pathogens affected positively the content of total, free and conjugated phenols in leaves of cotton plants cvs, Giza-86 and Giza-89. In this respect, the lowest amount of phenols as mg/g fresh weight (total, free and conjugated phenols) of both cvs.(Giza-86 and Giza-89) was recorded in case of infestation the soil with *F .roseum* comparing with the other tested soil borne pathogens. Meanwhile, the highest increase in the amount of determined phenols as mg/g fresh weight (total, free and conjugated phenols) of both cvs. (Giza-86 and Giza-89) was recorded in the case of infestation the soil with *R. solani* followed by *F. moniliforme* and *F.*

semitectum, respectively. It is clear also that all the determined phenols (total, free and conjugated phenols) were higher in cv. Giza-89 than those determined in cv. Giza-86.

Table (4-a): Chemical constituents in cotton detached leaves of cotton cvs. Giza-86 and Giza-89, 21 days post sowing in soil infested with root-rot pathogens.

Tested fungi	Sugars (mg/g fresh weight)					
	Reducing		Non-reducing		Total	
	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89
R.solani	6.77	6.77	13.47	14.25	20.24	21.02
F.moniliforme	7.55	7.16	14.24	15.41	21.79	22.57
F.roseum	5.84	6.07	11.28	11.83	17.12	17.90
F.semitectum	6.77	6.15	12.69	14.09	19.46	20.24
Control	5.37	5.60	10.20	11.75	15.57	16.35

Table (4-b): Chemical constituents in cotton detached leaves of cotton cvs. Giza-86 and Giza-89, 21 days post sowing in soil infested with root-rot pathogens.

Tested fungi	Phenols (mg/g fresh weight)					
	Free		Conjugated		Total	
	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89	cv.Giza-86	cv.Giza-89
R.solani	9.27	13.53	3.23	4.71	12.50	18.24
F.moniliforme	8.53	12.06	3.09	4.18	11.62	16.24
F.roseum	7.65	8.53	2.79	3.09	10.44	11.62
F.semitectum	8.53	10.15	2.80	3.68	11.33	13.83
Control	5.59	6.62	2.06	2.35	7.65	8.97

2- Changes of chlorophyll (A), (B) and carotenaids.

Data in **Table (5)** reveal that infestation the soil with the tested root rot pathogens *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* before sowing cotton seeds affected

negatively the content of chlorophyll when determined in leaves of cotton seedlings as mg/g fresh weight of both the tested cvs after 21 days post sowing. In this respect, all the tested root rot pathogens decreased the content of chlorophyll A and B and the total chlorophyll in cotton leaves comparing with uninfested soil (control) of both the two cvs Giza-86 and Giza-89. The highest decrease in chlorophyll A and B as well as total chlorophyll was recorded in case of infestation the soil with *F. moniliforme, F. semitectum, F. roseum* and *R. solani* respectively comparing with uninfested soil (control) of both the two cultivars.

The results also indicate that infestation the soil before sowing with any of the root rot pathogens do not affect clearly the carotenoids content in the leaves of grown cotton plants of both tested cotton cvs. comparing with un-infested soil (control) where all the determined carotenoids contents were ranged from 0.91 to 0.93 mg/g fresh weight.

Table (5): Changes of chlorophyll and carotenoids content in the leaves of cotton cvs. Giza-86 and Giza-89, 21 days post sowing in infested soil with root-rot pathogens.

Tested fungi	Chlorophylls (mg/g fresh weight)						Carotenoids (mg/g fresh weight)	
	A		B		Total			
	Giza-86	Giza-89	Giza-86	Giza-89	Giza-86	Giza-89	Giza-86	Giza-89
R.solani	2.801	3.000	1.476	1.554	4.291	4.554	0.93	0.92
F.moniliforme	1.946	2.033	1.380	1.548	3.326	3.581	0.91	0.94
F.roseum	2.613	2.611	1.440	1.483	4.053	4.094	0.91	0.92
F.semitectum	2.425	2.523	1.362	1.316	3.787	3.839	0.93	0.92
Control	4.254	4.468	2.380	2.695	6.634	7.163	0.93	0.93

3 – Changes in electrophoretic protein patterns:

Data in **Table (6) and Fig. (1)** show the variation in fractionated protein patterns in leaves of two cotton cultivars (Giza-86 and Giza-89) as a result of infection with *Rhizoctonia solani* and *Fusarium* spp. The results of exhibited protein bands ranged from 208.6 to 10.5 KDa. The obtained results suggest that the infection with pathogens increased the number of fractionated protein bands comparing with control treatment (un-infected).

In this respect, the infected cotton plants (cv. Giza-86) with *R. solani* revealed 15 protein bands comparing with control plants (11 band), some of them are similar in their molecular weight to as in control while some others were newly formed corresponding to infection with the pathogen like, 100.0, 82.9, 40.1, 29.8, 17.5, 14.6 and 10.5 KDa. Meanwhile, infection with *F .moniliforme* revealed 12 protein bands; some of them are new formed like 100.0, 47.7, 40.1 and 16.1 KDa. Also, the inoculated plants cv. (Giza-86) with *F. roseum* revealed 12 bands, among them the new protein bands were 192.6, 148.4, 93.1 and 16.1 KDa. On the other hand, the inoculated cotton plants cv. (Giza-86) with *F. semitectum* revealed 16 protein bands comparing with the un-inoculated ones (11 band), among them, the new formed bands were 192.6, 148.4, 116.2, 93.1, 47.7, 40.1, 28.9 and 16.1 KDa. The results indicate also that the inoculated cotton plants cv. (Giza-86) with *F. roseum* and *F. semitectum* revealed typical protein bands at 192.6, 148.4, 93.1 and 16.1 KDa, as well as they were differed completely from protein bands of *R. solani* infection, meanwhile they were partially similar to those

produced when plants were inoculated with *F. moniliforme* at 16.1 KDa. Moreover, The inoculated cotton plants cv. (Giza-86) with each of *R. solani* and *F. moniliforme* produced typical bands in response to infection at 100.0, 40.1 and 17.5 KDa. These appeared protein bands differed in their condense, where some of them were clear faint and some others appeared intensive although, they have the same molecular weight.

As for cotton cv.Giza-89, results show clear variation in the fractionated protein patterns which resulted in the leaves as a result of infection with each of *Rhizoctonia solani* and *Fusarium* spp. These exhibited protein bands ranged from 208.6 to 11.9 KDa. The obtained results clearly indicate that the infection with *R. solani* and *F. roseum* revealed protein bands lesser in their number comparing with control treatment (un-infected) meanwhile, only the infected plants with *F. semitectum* revealed protein bands more than in control treatment. On the other hand, all infected cotton plants (cv. Giza-89) produced few of new bands in response to infection with root rot pathogens such as 17.5 KDa in case of *R. solani*; 20.1 KDa with *F. moniliforme*; 82.9 and 21.6 KDa with *F. roseum* as well as, 130.9, 99.5, 96.3, 46.4, 36.3, 30.4 ,16.1 and 13.8 KDa in case of *F. semitectum*. On the other hand, the inoculated cotton plants (cv. Giza-89) with *F. roseum* and *F. semitectum* revealed typical protein band at 21.6 KDa.

Table (6): Molecular weight of formed protein patterns in leaves of cotton plants sown in soil previously inoculated with root rot pathogens.

Bands No.	Cotton cvs.									
	cv. Giza-86					cv. Giza-89				
	C1	1	2	3	4	C2	1	2	3	4
1	208.6	208.6	208.6	208.6	–	208.6	–	–	–	–
2	–	-	–	192.6	192.6	192.6	192.6	192.6	192.6	192.6
3	–	–	–	148.4	148.4	148.4	148.4	148.4	148.4	–
4	130.9	130.9	–	–	–	–	–	–	–	130.9
5	–	–	–	–	116.2	116.2	116.2	116.2	116.2	–
6	–	100.0	100.0	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	99.5
8	–	–	–	–	–	–	–	–	–	96.3
9	–	–	–	93.1	93.1	93.1	93.1	93.1	93.1	93.1
10	–	–	–	–	–	–	–	–	91.9	–
11	88.1	–	–	88.1	88.1	88.1	88.1	88.1	88.1	88.1
12	–	82.9	–	–	–	–	–	–	82.9	–
13	77.2	77.2	77.2	77.2	77.2	77.2	77.2	77.2	77.2	77.2
14	60.4	60.4	60.4	60.4	60.4	60.4	60.4	60.4	60.4	60.4
15	51.2	51.2	51.2	51.2	51.2	51.2	51.2	51.2	51.2	51.2
16	–	–	47.7	–	47.7	47.7	–	47.7	47.7	–
17	–	–	–	–	–	–	–	–	–	46.4
18	45.4	45.4	–	–	45.4	45.4	–	45.4	–	–
19	41.5	–	–	–	–	–	–	–	–	–
20	–	40.1	40.1	-	40.1	40.1	40.1	40.1	40.1	–
21	–	–	–	–	–	–	–	–	–	36.3
22	–	–	–	–	–	35.5	35.5	35.5	35.5	–
23	35.0	–	35.0	35.0	35.0	–	–	–	–	–
24	–	–	–	–	–	–	–	–	–	30.4
25	–	29.8	–	–	–	–	–	–	–	–
26	–	–	–	–	28.9	–	–	–	–	–
27	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6
28	–	–	–	–	–	22.1	22.1	22.1	–	–
29	–	–	–	–	–	–	–	–	21.6	21.6
30	–	–	–	–	–	–	–	20.1	–	–
31	–	17.5	17.5	–	–	–	17.5	–	–	–
32	–	–	16.1	16.1	16.1	–	–	–	–	16.1
33	–	–	–	–	–	15.1	15.1	15.1	–	15.1
34	–	14.6	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	13.8
36	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9
37	–	10.5	–	–	–	–	–	–	–	–
Total	**11**	**15**	**12**	**12**	**16**	**17**	**15**	**17**	**16**	**18**

C1= Control cv.Giza-86 C2= Control cv.Giza-89 1= infested soil with *R. solani* 2 = infested soil with *F. moniliforme* 3 = infested soil with *F. roseum* 4 = infested soil with *F. semitectum*

EXPERIMENTAL RESULTS

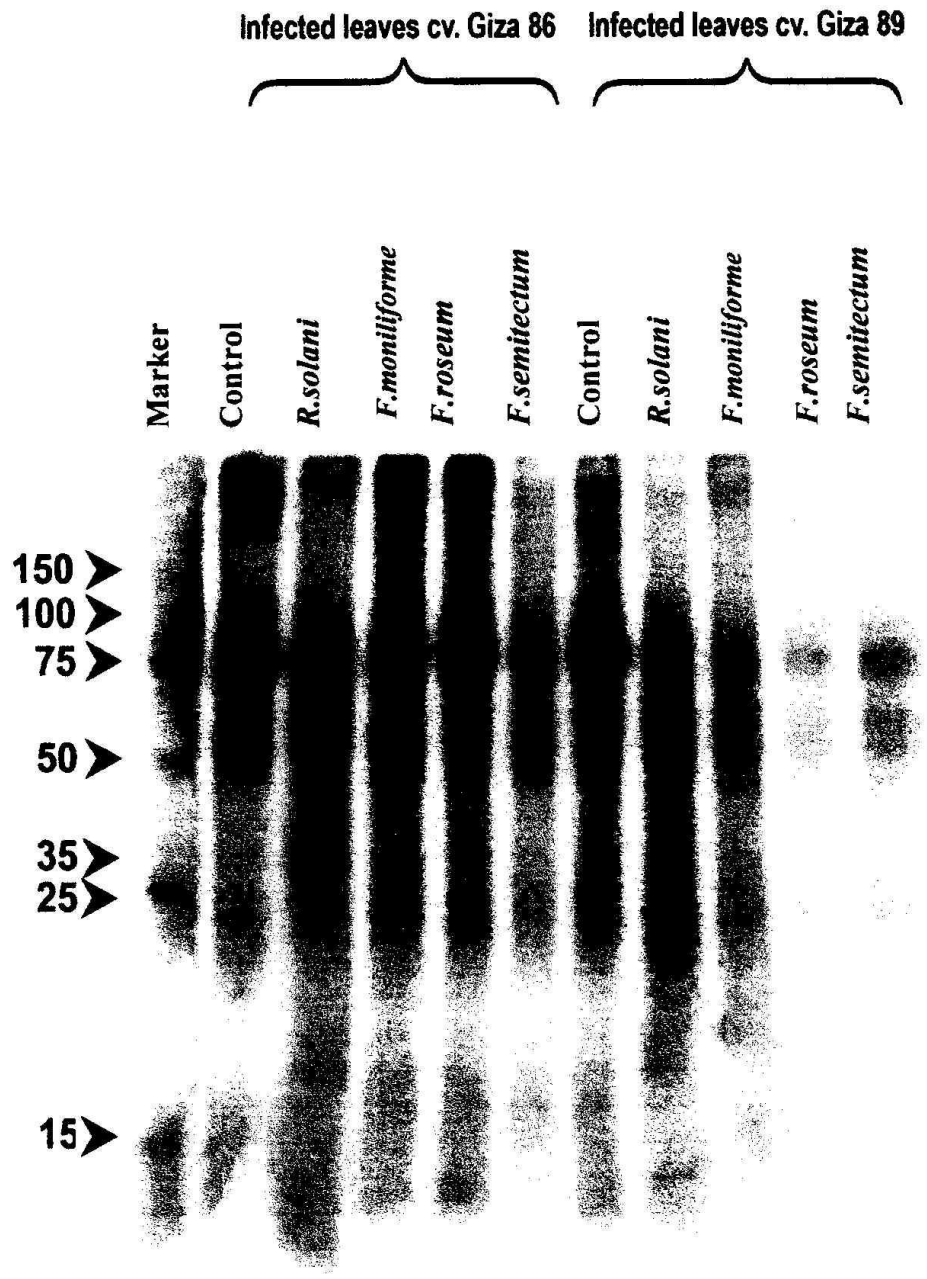

Figure (1): Electrophoretic protein patterns on SDS-PAGE in leaves of cotton cvs Giza-86 and Giza-89 previously planted in soil infested with root rot pathogens.

4-Changes in some electrophoretic isozyme related to resistance:

A- Esterase isozyme:

Data in **Table (7) and Fig. (2)** indicate clearly that infestation the soil with any of the tested root rot pathogens *i.e., R. solani, F. moniliforme, F. roseum* and *F. semitectum,* before sowing cotton seeds in this soil do not affect esterase isozyme content in cotton leaves of growing seedlings at 21 days post sowing in case of cv Giza-86 comparing to un-infested control. In this respect, all the protein patterns of esterase isozyme resulted due to any infestation treatment were equal to those appeared with the control treatment where they showed the same values of Rf except with *R. solani* which resulted a new protein band at 0.36.

As for cv. Giza-89, the same trend was also true except that the infestation treatment with *R. solani* where, five new protein bands of esterase isozyme were formed at Rf values 0.21, 0.36, 0.62, 0.71 and 0.79.

Table (7): The Rf values of esterase isozyme patterns in cotton leaves of cvs Giza-86 and Giza-89.

No.of band	cv.Giza-86.					cv.Giza-89.				
	Control	*R.solani*	*F.moniliforme*	*F.roseum*	*F.semitectum*	Control	*R.solani*	*F.moniliforme*	*F.roseum*	*F.semitectum*
1	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034
2	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093
3	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
4	-	-	-	-	-	-	0.21	-	-	-
5	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
6	-	0.36	-	-	-	-	0.36	-	-	-
7	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
8	-	-	-	-	-	-	0.62	-	-	-
9	-	-	-	-	-	-	0.71	-	-	-
10	-	-	-	-	-	-	0.79	-	-	-
Total	**5**	**6**	**5**	**5**	**5**	**5**	**10**	**5**	**5**	**5**

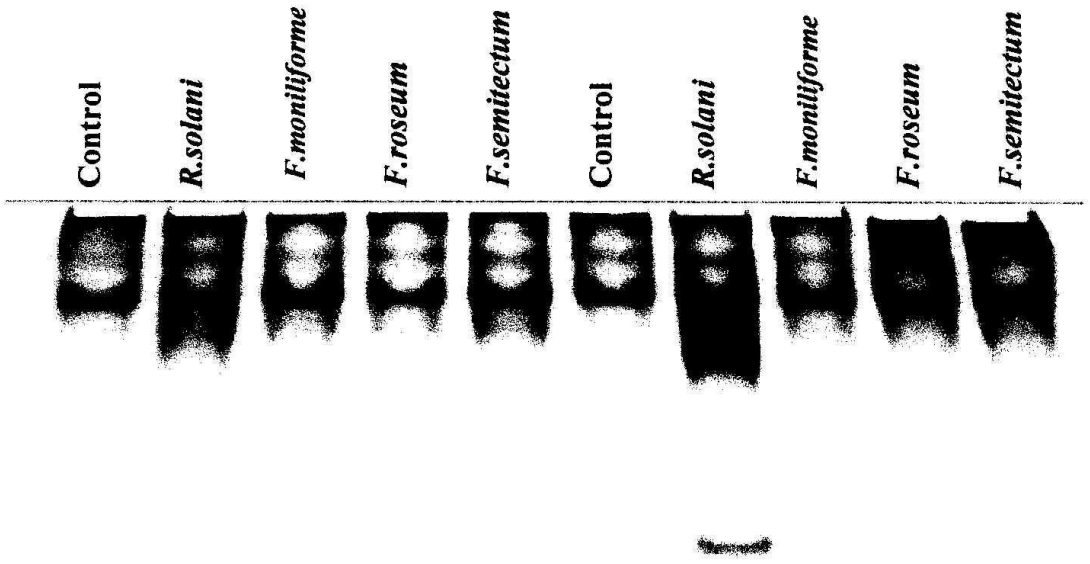

Fig. (2): Electrophoretic protein patterns of esterase isozyme in the leaves of cotton cvs Giza-86 and Giza-89.

B-Peroxidase isozyme:

Data presented in Table **(8)** and illustrated in **Fig. (3)** reveal that soil infestation with the tested root rot pathogens *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* before sowing cotton seeds showed clear variations in peroxidase isozyme patterns of the two tested cotton cvs.

As for cv. Giza-86, the obtained results indicate that two new protein bands at Rf 0.10 and 0.12 were developed due to soil infestation with *R. solani* comparing with the control treatment (un-infested). Meanwhile, infestation with *F. moniliforme* did not reveal any new bands comparing with the control treatment (un-infested). Whereas, soil infestation with *F.*

semitectum, and *F. roseum* revealed only one new band of peroxides isozyme at Rf. 0.10.

Concerning cv. Giza-89, soil infestation with the tested root rot pathogens resulted in clear changes in formation of peroxidase isozyme where many protein bands were disappeared comparing to those of un-infested one (control), meanwhile, no new protein bands were formed with all infestation treatments.

Table (8): The Rf values of peroxidase isozyme patterns in cotton leaves of cvs.Giza-86 and Giza-89.

No.of band	cv.Giza-86					cv.Giza-89				
	Control	*R.solani*	*F.moniliforme*	*F.roseum*	*F.semitectum*	Control	*R.solani*	*F.moniliforme*	*F.roseum*	*F.semitectum*
1	-	0.10	-	0.10	0.10	0.10	0.10	-	-	0.10
2	-	0.12	-	-	-	0.12	-	-	-	-
3	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
4	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	-	-
5	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
6	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Total	**4**	**6**	**4**	**5**	**5**	**6**	**5**	**4**	**3**	**4**

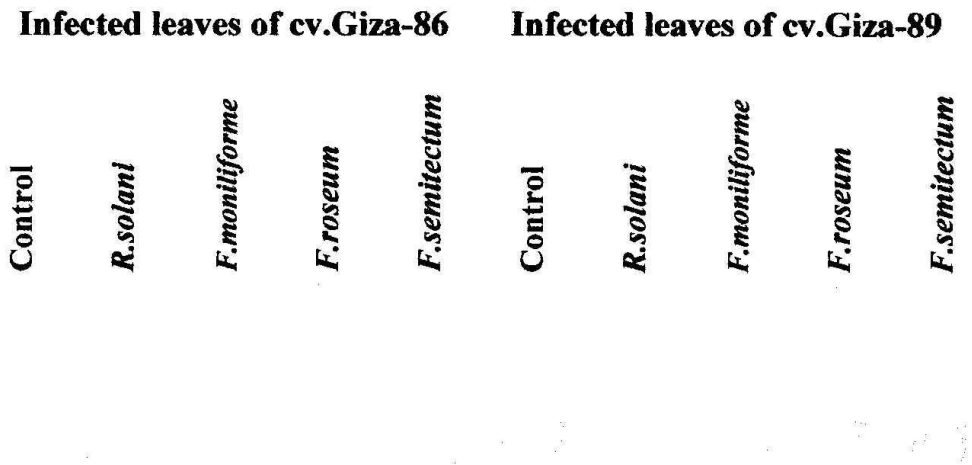

Fig. (3) Electrophoretic protein patterns of peroxidase isozyme in the leaves of cvs Giza-86 and Giza-89.

5- Changes in oil content of infested cotton seeds:

Data in **Table (9)** 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 root rot pathogens decreased the percentages of oil content into the tested cotton seeds of cvs Giza-86 and Giza-89 comparing with the uninfested seeds (control) at any incubation period *i.e.,* 5, 10 and 15 days. It is clear also that increasing incubation period from 5 to 15 days decreased gradually the determined percentages of oil contents for all treatments compared with the un-infested seeds (control). The highest decrease in percentages of oil contents 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 (9): 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.

Treatments	Days after inoculation)					
	15 days		10 days		5 days	
	Giza-86.	Giza-89.	Giza-86.	Giza-89.	Giza-86.	Giza-89.
R.solani	22.5	24.5	21.5	23.5	20.5	22.5
F.moniliforme	22.5	24.5	21.5	23.5	20.5	22.5
F.roseum	23.0	25.0	22.5	24.5	21.5	23.5
F.semitectum	23.0	25.0	22.5	24.5	21.5	23.5
Control	24.5	26.5	24.5	26.5	24.5	26.5

IV- Effect of the isolated fungi on mycotoxin production:

Results in **Table (10)** reveal that all the tested fungi were not able to produce any kind of mycotoxins *i.e.*, aflatoxins (B1 & B2), zearalenane, fumonisins and trichothense when the fungi were grown *in vitro* on specific YES medium, no one of the abovementioned mycotoxins was detected.

On the other hand, when the 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 of 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 in to the infested cotton seeds, meanwhile aflatoxins were appeared only in naturally contaminated cotton seeds of both tested cvs.

Table (10): Mycotoxins produced by the isolated fungi in the infested cotton seeds with some pathogenic fungi

Tested fungi	Produced mycotoxins (ppb)					
	Zearlenone		Fumonisins		Aflatoxins	
	Giza-86.	Giza-89.	Giza-86.	Giza-89.	Giza-86.	Giza89.
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

V- Laboratory studies

A- Effect of some fungicides on the growth of the tested fungi:

This experiment was conducted to study the effect of different concentrations of some fungicides on the linear growth of *R. solani, F. moniliforme, F. roseum* and *F. semitectum.*

Data in **Table (11-a)** indicate that all the tested fungicides affected the linear growth of *R. solani* to values ranged from 4 to 68 mm comparing with the un-treated one (control. In this respect, Premis, Maxim and Topsin-M were the best effective fungicides on growth of *R. solani.* It is clear also, that Topsin-M was the highest effective one at the concentrations 5-400ppm

where the resulted linear growth was only 9 mm followed by Premis at concentrations of 5-400ppm (13 to 9 mm) and Maxim at 10-400 ppm (10 mm).

Table (11-a): Effect of some fungicides on the linear growth (mm) of *R. solani*, 5 days post inoculation at 28°C.

Fungicides	The linear growth of *R. solani* (mm) at different concentrations (ppm)									Mean
	Co.	1	5	10	25	50	100	200	400	
Maxim	70	12	11	10	10	10	10	10	10	17.1
Premis	70	14	13	9	9	9	9	9	9	16.8
TopsinM70	70	54	9	9	9	9	9	9	9	20.7
RizolexT50	70	66	52	44	42	36	25	19	16	41.1
VitavaxT70	70	68	68	68	64	61	42	41	9	54.5
VitavaxT40	70	46	46	42	39	27	16	15	4	33.8
Mean	70.0	43.3	33.2	30.3	28.8	25.3	18.5	17.2	9.5	30.7

L.S.D. at 5 % for Concentration (C) 0.11 Fungicides (F) 0.09 C x F 0.27

On the other hand, Maxim and Premis were more effective than Topsin-M at concentration 1 ppm. Moreover, the least effective fungicide on the linear growth of *R. solani* was Vitavax-T70 especially at the tested concentrations ranged from 1 to 200 ppm where the average of the linear growth was 54.5 mm.. It is clear that increasing the concentration from 1 to 400 ppm increased gradually the effect of the tested fungicides in reducing the growth of *R. solani.*

Data in **Table (11-b)** indicate that Maxim and Premis were the best effective fungicides on linear growth of *Fusarium moniliforme* where the resulted linear growth was only 9 mm at all the tested concentrations which ranged between 1-400ppm Meanwhile, Topsin-M was also effective at the tested concentrations ranged from 5-400 ppm where its linear growth

was only 9 mm. On the other hand, Rizolex-T, Vitavax-T70 and Vitavax-T40 were less effective than the fist three fungicides especially at the tested concentrations ranged from 1-50 ppm. It is clear also that increasing the concentrations of the tested fungicides from 1 to 400ppm increased gradually the effect on the average of resulted linear growth. It is clear also that Vitavax-T40, Rizolex-T and Vitavax-T70 were the least effective fungicides on linear growth of tested *Fusarium moniliforme* respectively.

Table (11-b): Effect of some fungicides on the linear growth (mm) of *Fusarium moniliforme*, 5 days post inoculation at 28°C.

Fungicides	The linear growth of *Fusarium moniliforme* at different concentrations (ppm).									Mean
	Co.	1	5	10	25	50	100	200	400	
Maxim	70	9	9	9	9	9	9	9	9	15.8
Premis	70	9	9	9	9	9	9	9	9	15.8
Topsin-M70	70	31	9	9	9	9	9	9	9	18.2
RizolexT50	70	44	44	44	18	9	9	9	9	28.4
VitavaxT70	70	52	34	26	25	9	9	9	9	27.0
VitavaxT40	70	42	41	33	30	28	9	9	9	30.1
Mean	70	31.2	24.3	21.6	16.6	12.2	9	9	9	22.5

L.S.D. at 5 % for Concentration (C) 0.13 Fungicides (F) 1.1 C x F 0.32

Results in **Table (11-c)** indicate that Premis followed by Topsin-M were the highest effective fungicides on linear growth of *Fusarium roseum* where they gave the least average of linear growth (15.8 and 18.6 mm) respectively. Premis fungicide gave the least average of the linear growth (9 mm) at all the tested concentrations, *i.e.,* 1 to 400 ppm. Meanwhile, Topsin-M gave the same average of linear growth (9 mm) at the tested concentrations ranged from 5-400ppm. On the other hand,

Vitavax-T70 followed by Rizolex-T were the least effective fungicides on the linear growth of *Fusarium roseum*. Also, increasing the concentrations from 1-400 increased gradually the effect of tested fungicides.

Table (11-c): Effect of some fungicides on the linear growth (mm) of *Fusarium roseum*, 5 days post inoculation at 28°C.

Fungicides	The linear growth of *Fusarium roseum* at different concentration(ppm).									Mean
	Co.	1	5	10	25	50	100	200	400	
Maxim	70	53	52	47	42	40	35	31	9	42.1
Premis	70	9	9	9	9	9	9	9	9	15.8
Topsin-M70	70	35	9	9	9	9	9	9	9	18.6
Rizolex–T50	70	58	53	52	49	47	39	28	24	46.6
Vitavax–T70	70	64	64	62	53	52	32	18	16	47.8
Vitavax–T40	70	65	64	54	45	33	18	11	9	41.0
Mean	70	47.3	41.8	38.8	34.5	31.6	23.6	17.6	12.6	35.3

L.S.D. at 5 % for Concentration (C) 0.06 Fungicides (F) 0.05 C x F 0.15

Results in **Table (11-d)** reveal clearly that Premis followed by Topsin-M were the best effective fungicides on growth of *Fusarium semitectum in vitro* where they gave the least average of growth (20.7 and 22.0 mm) respectively. Meanwhile, Rizolex-T was the least effective one on the growth of *Fusarium semitectum*. Also, increasing the concentration from 1 to 400 ppm increased gradually the effect of the tested fungicides.

Table (11-d): Effect of some fungicides on the linear growth (mm) of *Fusarium semitectum,5* days post inoculation at 28°C.

Fungicides	The linear growth of *Fusarium semitectum* at different concentrations (ppm).									Mean
	Co.	1	5	10	25	50	100	200	400	
Maxim	70	29	29	29	29	28	28	25	9	30.6
Premis	70	25	24	22	10	9	9	9	9	20.7
Topsin-M70	70	46	28	9	9	9	9	9	9	22.0
Rizolex–T50	70	61	49	48	42	38	37	24	15	42.6
Vitavax–T70	70	45	46	46	43	33	9	9	9	34.4
Vitavax–T40	70	51	46	45	43	33	9	9	9	35.0
Mean	70	42.8	37.0	33.0	29.3	25.0	16.8	14.1	10.0	30.8

L.S.D. at 5 % for Concentration (C) 0.1 Fungicides (F) .08 C x F 0.24

B- Effect of some antagonists on the growth (mm) of the tested fungi:

Data in **Table (12)** show the effect of the tested bioagents in this study on the linear growth of all the tested root-rot pathogens. In this respect, Rizo-N was better than Plant Guard in its effect on growth of *R. solani*, *F. moniliforme* and *F. semitectum* as well as comparing to control treatment Meanwhile, Plant Guard was better only on its effect on growth of *F. roseum.*

Table (12): Effect of two antagonists on the linear growth (mm) of the tested root rot fungi, 5 days after incubation at 28° C

Bioagents	The linear growth of the tested pathogens.				
	R.solani	*F.moniliforme*	*F.roseum*	*F.semitectum*	Mean
Control	70	70	70	70	70.0
Rizo-N	43	45	39	47	43.5
Plant Guard	50	53	34	52	47.3
Mean	54.3	56.0	47.6	56.3	53.6

VI–Greenhouse studies

A- Effect of treating cotton seeds of Giza-86 and Giza-89 cvs. with some fungicides on the percentage of dead plants:

Data in **Table (13-a)** reveal that sowing cotton seeds cv Giza-86 in soil infested with any of the pathogenic fungi whether individually or in combination in the absence of any fungicidal treatment (control-2) resulted in high percentage of dead plants. In this respect, the highest percentage of dead plants was occurred in the case of soil infestation the with the combined inoculum **viz.,** *R. solani* x *F. moniliforme x F. roseum* followed by *R. solani* x *F. moniliforme* x *F. semitectum* and *F. moniliforme* x *F. semitectum*. The analogous percentages of dead plants were 80.0, 76.7 and 76.7%, respectively. Also, it is clear that infested soil with fungi whether individually or in combination gave over 50% dead plants with significant differences among most of the infestation treatments, whereas it was 40% in the un-infested soil planted with un-treated seeds with any of the tested fungicides.

On the other hand, treating the cotton seeds with fungicides before sowing in infested soil with fungi whether individually or in combination resulted in a clear reduction in percentage of dead plants where the percentage of dead plants ranged from 16.7 to 43.3%. Moreover, the highest effective fungicides in reducing the percentage of dead plants were respectively, Topsin-M, Premis and Maxim while, the least effective fungicide was Rizolex-T. Also, Topsin-M, Premis and Maxim fungicides were more effective in decreasing the percentage of dead plants than other tested fungicides in all soil

infestation treatments with clear significant difference between the tested fungicides in this respect. Also, treating cotton seeds with fungicides before sowing in the un-infested soil (control–1) resulted in the least percentage of dead plants with superiority of Premis and Maxim over Topsin-M, Vitavax-T70, Vitavax-T40 and Rizolex-T.

Table (13-a): Effect of treating cotton seeds cv.Giza-86 with some fungicides on the percentage of dead plants, 21 days post sowing.

Tested fungi at 3% inoculum	% of dead plants of cotton seedlings (Giza-86)							Mean
	Maxim	Premis	Topsin-M	Rizolex-T	Vitavax-T70	Vitavax-T40	**Co. (2)	
R. solani (1)	20.0	20.0	23.3	43.3	26.7	33.3	56.7	31.9
F. moniliforme (2)	23.3	23.3	20.0	36.7	26.7	30.0	56.7	30.9
F. roseum (3)	26.7	20.0	20.0	30.0	23.3	30.0	56.7	29.5
F. semitectum (4)	26.7	33.3	20.0	40.0	23.3	30.3	56.7	32.9
R. solani x (2)	23.3	23.3	23.3	36.7	36.7	36.7	67.7	35.4
R.solani x (3)	23.3	23.3	26.7	30.0	30.0	40.0	66.7	34.2
R. solani x (4)	16.7	16.7	26.7	40.0	30.0	33.3	73.3	33.8
F. moniliforme x (3)	26.7	26.7	20.0	33.3	36.7	30.0	73.3	35.2
F. moniliforme x (4)	26.7	23.3	23.3	33.3	36.7	30.0	76.7	35.7
F . roseum x (4)	26.7	23.3	20.0	33.3	30.0	30.0	66.7	32.8
R. solani x (2) x (3)	36.7	26.7	23.3	43.3	33.3	30.0	80.0	39.0
R. solani x (2) x (4)	36.7	26.7	23.3	43.3	33.3	30.0	76.7	38.5
R. solani x (3) x (4)	23.3	20.0	23.3	36.7	36.7	33.3	70.0	34.7
F.moniliforme(3)x (4)	23.3	26.7	20.0	30.0	30.0	33.3	70.0	33.3
R.solani x (2)x(3)x(4)	30.0	23.3	30.0	36.7	40.0	36.7	73.3	38.5
*Control (1)	16.7	16.7	20.0	26.7	20.0	23.3	16.7	20.0
Mean	25.4	23.3	22.7	35.8	30.8	31.8	64.8	33.5

*Control-1= Seeds treated with fungicides only without infestation
**Control – 2 = Infested soil with fungi without seed dressing.

L.S.D. at 5% for	Treatment = T	Fungi =F	T x F
	2.80	2.87	7.59

Data in **Table (13-b)** indicate that sowing cotton seeds cv. Giza-89 in soil infested with any of the pathogenic fungi whether individually or in combination in the absence of any fungicidal treatment (control-2) resulted in high percentage of dead plants ranged from 53.3 to 73.3%. In this respect, the high percentage of dead plants was occurred in the case of infestation the soil with the inoculum consisted of *R. solani* x *F. moniliforme x F. roseum x F. semitectum* (73.3%) followed by *R. solani* x *F. moniliforme, R. solani* x *F. roseum, R. solani* x *F. semitectum* and *R. solani* x *F. moniliforme x F. roseum* (66.7%). Also, it is clear that there were a clear significant differences between most of the infestation treatments.

Table (13-b): Effect of treating cotton seeds cv. Giza-89 with fungicides on the percentage of dead plants 21 days post sowing.

Tested fungi at 3% inoculum	% of dead plants of cotton seedlings (Giza-89)							Mean
	Maxim	Premis	Topsin-M	Rizolex-T	Vitavax-T70	Vitavax-T40	**Control (2)	
R. solani (1)	20.0	16.7	16.7	36.7	30.0	26.7	66.7	30.5
F. moniliforme (2)	30.0	20.0	23.3	36.7	30.0	30.0	53.3	31.1
F. roseum (3)	23.3	16.7	20.0	26.7	23.3	30.0	53.3	27.6
F. semitectum (4)	26.7	20.0	20.0	26.7	30.0	30.0	53.3	29.5
R. solani x (2)	40.0	23.3	20.0	33.3	30.0	33.3	66.7	32.3
R. solani x (3)	26.7	23.3	20.0	30.0	30.0	30.0	66.7	32.3
R. solani x (4)	26.7	20.0	20.0	30.0	26.7	33.3	66.7	31.8
F. moniliforme x (3)	20.0	20.0	23.3	30.0	30.0	30.0	63.3	30.8
F.moniliforme x (4)	23.3	20.0	23.3	30.0	30.0	30.0	53.3	29.8
F .roseum x (4)	23.3	23.3	23.3	30.0	33.3	26.7	56.7	29.5
R .solani x (2) x (3)	23.3	20.0	20.0	36.7	30.0	33.3	66.7	31.4
R.solani x (2) x (4)	33.3	20.0	20.0	36.7	30.0	33.3	60.0	33.3
R solani x (3) x (4)	26.7	23.3	23.3	30.0	30.0	36.7	60.0	31.4
F.moniliforme(3)x(4)	23.3	26.7	26.7	26.7	30.0	30.0	60.0	31.8
R.solani x (2) x (3) x(4)	26.7	26.7	26.7	30.0	40.0	30.0	73.3	36.2
*Control (1)	16.7	16.7	16.7	23.3	16.7	23.3	13.4	18.6
Mean	25.6	21.0	21.4	29.5	28.7	29.1	58.3	30.4

*Control-1= Seeds treated with fungicides only without infestation
**Control – 2 = Infested soil with fungi without seed dressing.

L.S.D. at 5% for	Treatment = T	Fungi =F	T x F
	2.12	2.89	7.64

On the other hand, treating cotton seeds cv. Giza-89 with fungicides before sowing in infested soil with fungi whether individual or in combination resulted in a clear reduction in the percentage of dead plants which ranged from 16.7 to 40.0%. Moreover, Premis and Topsin-M were the highest effective fungicides in reducing the percentage of dead plants where they

gave the lowest percentage of dead plants, being 21.0 and 21.4% followed by Maxim treatment (25.6%). Meanwhile, the least effective fungicides were in case of treating the seeds with Rizolex-T and Vitavax-T40. Also, Topsin-M, Premis and Maxim fungicides were more effective in decreasing the percentage of dead plants than the other tested fungicides in all cases of soil infestation with clear significant difference between the tested fungicides in this respect. Also, treating cotton seeds with fungicides before sowing in un-infested soil (control–1) resulted in the least percentage of dead plants with superiority of Maxim, Premis, Topsin-M, and Vitavax-T70 over Rizolex-T and Vitavax-T40 in this field.

B– Effect of treating cotton seeds cvs. Giza-86 and Giza-89 with antagonists on percentage of dead plants, 21 days after sowing.

Data in **Table (14)** show that treating cotton seeds of Giza-86 and Giza-89 cvs.with commercial antagonists (Rizo-N or Plant Guard) before sowing in infested soil with the pathogenic fungi whether individually or in combination decreased significantly the dead plants comparing with the highest percentages of dead plants in the soil infested only with the pathogenic fungi (control–2).

As for Giza-86, the dead cotton seedlings percentages were high in the infested soil with the pathogenic fungi whether used individually or in combination where they ranged from 56.7 to 80.0%. In this respect, the highest percentage of dead plants was due to infestation the soil with *R. solani* x *F. moniliforme* x *F. roseum* (80.0%) followed by *F. moniliforme* x *F. semitectum*

and *R. solani* x *F. moniliforme* (76.7%). Meanwhile, soil infestation with any of the pathogenic fungi alon resulted in 56.7% dead plants. On the other hand, treating cotton seeds cv Giza-86 with Rizo-N or Plant Guard antagonists reduced the infection of cotton seedlings 13.3 – 23.3% in the case of Rizo-N while it was 20.0 – 30.0% with Plant Guard treatment. Also, it is clear from the obtained results that treating cotton seeds with Rizo-N was better than Plant Guard in reducing infection of cotton seedlings where the average of dead plants were 19.0 and 23.0%, respectively.

Regarding Giza-89, the same trend was true where the infection of cotton seedlings were high in soil infested with any of the pathogenic fungi whether individually or in combination, being 53.3 – 73.3%. As well as, treating the seeds of cotton cv. Giza-89 with Rizo-N or Plant Guard antagonists gave similar results to those of Giza-86. In this respect, treating cotton seeds cv Giza-89 with Rizo-N was better than Plant Guard treatment in reducing the percentages of dead plants, being 23.7 and 27.5, respectively. It is clear also, that the percentage of dead plants was high in the un-infested soil (control-1) where it reached 40.0% in the case of Giza-86 and 36.7% in case of Giza-89. Meanwhile, using antagonists reduced these percentages to 13.3 and 16.7% for Giza-86 and 20.0 and 23.3% for Giza-89 in case of using Rizo-N and Plant Guard, respectively.

Table (14): Effect of treating cotton seeds cvs.Giza-86 and Giza-89 with antagonists on the percentage of dead plants, 21 days after sowing.

Tested fungi at 3% inoculum	% dead plants of cotton seedlings cvs.							
	Giza-86				Giza-89			
	Rizo-N	Plant Guard	Control (2)**	Mean	Rizo-N	Plant Guard	**Contro l (2)	Mean
R. solani (1)	16.7	20.0	56.7	31.1	23.3	33.3	66.7	41.1
F. moniliforme (2)	20.0	20.0	56.7	32.3	26.7	26.7	53.3	35.6
F. roseum (3)	13.3	20.0	56.7	30.0	20.0	26.7	53.3	33.3
F. semitectum (4)	16.7	20.0	56.7	31.1	20.0	26.7	53.3	33.3
R. solani x (2)	20.0	23.3	76.7	40.0	23.3	26.7	67.7	39.2
R. solani x (3)	20.0	20.0	66.7	35.6	26.7	26.7	66.7	40.0
R. solani x (4)	20.0	20.0	73.3	37.8	26.7	30.0	66.7	41.1
F. moniliforme x (3)	20.0	23.3	73.3	38.8	20.0	23.3	63.3	35.5
F.moniliforme x (4)	20.0	20.0	76.7	38.9	20.0	23.3	53.3	32.2
F .roseum x (4)	16.7	23.3	66.7	35.6	20.0	23.3	56.7	33.3
R .solani x (2) x (3)	23.3	26.7	80.0	43.3	26.7	30.0	66.7	41.1
R.solani x (2) x (4)	23.3	26.7	76.7	42.2	26.7	30.0	60.0	38.9
R solani x (3) x (4)	20.0	30.0	70.0	40.0	26.7	30.0	60.0	38.9
F.moniliforme x (3)x (4)	20.0	30.0	70.0	40.0	26.7	30.0	60.0	38.9
R.solani(2)x(3)x(4)	20.0	26.7	73.3	40.0	26.7	30.0	73.3	43.3
*Control (1)	13.3	16.7	16.7	15.6	20.0	23.3	13.4	18.9
Mean	19.0	23.0	65.5	35.7	23.7	27.5	58.4	36.5

*Control-1= Seeds treated with fungicides only without infestation

**Control – 2 = Infested soil with fungal without seed dressing.

L.S.D. at 5% for:	Bioagents (b)	Fungi (F)	b x F
cv. Giza-86	6.28	3.31	5.74
cv. Giza-89	3.82	4.24	7.35

C-Effect of treating cotton seeds cvs. Giza-86 and Giza-89 with some fungicides and commercial antagonists on some growth characters of cotton plants at 21 days old.

Data in **Table (15-a)** indicate clearly that treating cotton seeds (cv. Giza-86) with certain fungicides or antagonists before sowing increased significantly the shoot length of the most resulted seedlings at 21 days old. In this respect, the highest increase in the shoot length of the resulted seedlings was in case of treating seeds with Rizolex-T and Rizo-N and planted in soil infested with *F.moniliforme* where the resulted shoot lengths measured 21.7 and 21.2 cm, respectively. Also, treating the seeds with Topsin-M and Vitavax-T40 increased the shoot length in the same infested soil with *F.moniliforme*. Meanwhile, the least shoot length was recorded in case of planting seeds treated with Rizolex-T and Rizo-N in soil infested with *F. moniliforme* where the average shoot length reached 21.7 and 21.2 cm, respectively. Also, planting seeds treated with Topsin-M and Vitavax-T40 increased the shoot length. In case of treating the cotton seeds before sowing with Premis fungicide and Plant Guard bioagent in soil infested with *F.semitectum* and *R.solani*, the estimated shoot length of seedlings recorded 12.7 and 13.0 cm in case of Premis and 12.9 cm in case of Plant Guard in soil infested with *R. solani*. On the other hand, treating the cotton seeds with fungicides or antagonists before sowing in normal soil without infestation (control-2) with any of tested root rot fungi improved the shoot length of the resulted seedlings in case of Maxim, Vitavax-T40, Rizo-N and Plant Guard treatments more than other treatments and control.

Table (15-a): Effect of treating cotton seeds cv Giza-86 with some fungicides and commercial antagonists before sowing in infested soil with root rot fungi on some growth characters of cotton plants at 21 days old.

Seed treatments	Infestation treatment					Mea
	R.sola	*F.monilifor*	*F.semitect*	*F.roseu*	**Co	
Shoot length (cm)						
Maxim	19.8	19.3	18.4	19.3	20.6	19.5
Premis	13.0	18.0	12.7	17.6	16.4	15.5
Topsin-M	16.3	20.2	18.0	19.6	17.4	18.3
Rizolex-T	19.6	21.7	18.4	19.4	19.6	19.7
Vitavax-T70	17.8	16.9	18.8	19.5	19.3	18.4
Vitavax-T40	15.1	20.0	18.3	20.0	20.3	18.7
Rizo-N	17.2	21.2	15.3	20.7	20.1	18.9
Plant Guard	12.9	16.8	15.7	15.0	21.0	16.3
*Control-1	17.6	19.5	14.0	18.3	19.4	17.8
Mean	16.8	19.3	16.6	18.9	19.3	18.2
Root length (cm)						
Maxim	5.4	5.4	6.9	3.4	4.3	5.1
Premis	7.6	6.3	8.2	4.4	4.3	6.2
Topsin-M	4.6	5.3	3.4	5.1	4.6	4.6
Rizolex-T	6.9	3.3	4.5	4.7	3.7	4.6
Vitavax-T70	6.4	5.2	6.5	5.8	4.1	5.6
Vitavax-T40	7.0	6.0	4.4	5.0	4.2	5.3
Rizo-N	5.7	6.3	3.5	5.4	4.7	5.1
Plant Guard	5.4	4.8	5.3	4.0	3.3	4.6
*Control-1	7.1	7.6	5.8	4.7	3.0	5.6
Mean	6.3	5.6	5.4	4.7	4.0	5.2
Dry weight (g) of plantlets						
Maxim	1.1	0.63	0.42	0.62	0.71	0.69
Premis	1.1	0.51	0.50	0.70	0.71	0.70
Topsin-M	0.84	0.65	0.47	0.66	0.66	0.65
Rizolex-T	0.63	0.64	0.46	0.74	0.73	0.64
Vitavax-T70	0.47	0.50	0.62	0.67	0.73	0.59
Vitavax-T40	0.44	0.70	0.48	0.70	0.75	0.61
Rizo-N	0.47	0.50	0.47	0.77	0.67	0.58
Plant Guard	0.42	0.62	0.42	0.82	0.66	0.59
*Control-1	0.84	0.67	0.97	0.69	0.75	0.78
Mean	0.70	0.60	0.53	0.71	0.71	0.65

*Control – 1 = Infested soil with fungi without seed dressing.
**Control-2= Seeds treated with fungicides only and planted in uninfested soil

L.S.D.at 5% for:	Fungi (F)	Treatment(T)	(FxT)
Shoot length	0.0809	0.108	0.242
Root length	0.025	0.0.0081	0.077
Dry weight	0.006	0.0.008	0.0182

In general, the obtained results showed that Rizolex-T and Maxim were the best tested Fungicides in increasing the shoot lengths of the seedlings while, Premis and Plant Guard negatively affected the shoot lengths of cotton seedlings.

On the other hand, treating cotton seeds with fungicides or antagonists before sowing in soil infested with the tested pathogenic fungi reduced significantly the root length in most treatments. Meanwhile, some fungicides treatments increased the seedlings root length like Premis seed treatment in soil infested with *R.solani* (7.6 cm) and in soil infested with *F. semitectum.* As well as, there was a clear increase in root length in case of seed treatment Maxim, Vitavax-T70 and Plant Guard seed treatments before sowing in soil infested with *F.semitectum.* Also, treating cotton seeds with fungicides or antagonists before sowing in uninfested soil with any of the pathogenic fungi (control-2) improved the root length of the seedlings more than planting un-treated seeds in normal soil (control-1).

Concerning the dry weight, treating cotton seeds with selected fungicides or antagonists before sowing in infested soil with the tested pathogenic fungi reduced significantly the dry weight of the resulted seedlings in most treatments comparing with un-treated seeds planted in infested soil with the tested fungi (control-1) except, Maxim and Premis in soil infested with *R.solani* (1.1g) as well as, Plant Guard, Rizo-N and Rizolex-T in soil infested with *F. roseum.*

Results in **Table (15-b)** reveal also that treating cotton seeds cv Giza-89 with fungicides or antagonists before sowing in infested soil with the tested root rot fungi increased significantly

the shoot length of the seedlings in some cases and decreased it in some others comparing with the un-treated seeds in normal soil (control-1). In this respect, Rizo-N followed by Topsin-M were the best seed treatments for increasing the shoot length of cotton seedlings in growing soil infested with *R. solani.* While, Topsin-M and Vitavax-T70 treatments were the best in increasing the shoot lengths of seedlings growing in soil infested with *F.moniliforme*. Meanwhile, all other treatments reduced the shoot lengths which became lesser than those of control-1 treatment. Also, planting seeds treated whether with fungicides or antagonists in soil infested with *F.semitectum* reduced the shoot lengths of the seedlings in comparison with those of control-1 treatment. On the other hand, treating the seeds with Topsin-M, Vitavax-T70 or Rizo-N before sowing in soil infested with *F.roseum* was the best in increasing the shoot length of the seedlings more than other treatments and control-1, meanwhile, Plant Guard treatment was the least in this respect where it reduced the shoot length. In addition, treating the seeds with Rizo-N, Maxim and Vitavax-T70 before sowing in uninfested soil with the tested fungi (control-2) was the best in improving the shoot lengths of resulted seedlings more than those of other treatments and control-1. In general, Rizo-N, Topsin-M and Vitavax-T70 were the best seed treatments in this respect.

As for root length, treating the cotton seeds cv Giza-89 with Topsin-M fungicide before sowing in infested soil with *R.solani* or *F.moniliforme* only increased the root length of the resulted seedlings comparing with other fungicides or antagonists treatments which decreased the root length in most

cases compared with control-1 treatment. Meanwhile, all seed treatments with fungicides or antagonists before sowing in infested soil with *F.semitectum* decreased the root length of the resulted seedlings to values lesser than those of control-1 treatment. On the other hand, seed treatment with Rizolex-T was the best treatment in case of infested soil with *F. roseum* in increasing significantly the root length of the resulted seedlings comparing with other treatments and control. In the same time, treating the seeds of Giza-89 with fungicides or antagonists before sowing in uninfested soil (control-2) reduced the root lengths compared to control-1 treatment in this respect.

Regarding dry weight of resulted seedlings at 21 days old, treating the cotton seeds cv Giza-89 with any of the fungicides or antagonists before sowing in soil infested with *R.solani* decreased the dry weight of resulted seedlings comparing with control treatment. While seed treatment with any of the tested fungicides and antagonists before sowing in infested soil with *F. moniliforme* increased significantly the dry weight of the resulted seedlings comparing with control-1 treatment, where Vitavax-T40, Maxim, Premis and Topsin-M were the best seed treatments in this respect. Also, Rizo-N and Premis were the best seed treatments before sowing in soil infested with *F.semitectum* in increasing the dry weight of the resulted seedlings comparing with control-1 treatment whereas other seed treatments reduced significantly the dry weight of the resulted seedlings.

Table (15-b): Effect of treating cotton seeds cv. Giza-89 with some fungicides and commercial antagonists before sowing in infested soil with root rot fungi on some growth characters of cotton plants at 21 days old.

Seed treatments	Infestation treatment					Me
	R.sol	*F.monilif*	*F.semitec*	*F.rose*	**Co	
Shoot length (cm)						
Maxim	16.3	18.5	15.3	18.3	20.	17.7
Premis	14.3	18.7	14.2	18.0	16.	16.4
Topsin-M	17.2	21.2	18.7	20.2	17.	18.9
Rizolex-T	15.3	19.4	17.5	18.6	18.	17.8
Vitavax-T70	16.4	20.6	17.1	19.7	19.	18.7
Vitavax-T40	16.1	17.8	19.1	18.4	18.	17.9
Rizo-N	17.6	18.7	19.5	19.2	21.	19.3
Plant Guard	15.6	15.2	15.3	14.7	13.	14.9
*Control-1	15.4	20.0	20.0	17.7	18.	18.4
Mean	16.0	18.9	17.4	18.4	18.	17.8
Root length (cm)						
Maxim	6.3	6.2	3.6	7.2	4.5	5.6
Premis	6.2	5.5	4.5	6.5	4.3	5.4
Topsin-M	7.7	7.0	4.2	6.4	3.9	5.8
Rizolex-T	6.2	6.3	6.4	7.4	4.0	6.1
Vitavax-T70	4.4	3.7	5.0	7.1	3.1	4.7
Vitavax-T40	6.0	5.3	5.4	5.2	3.0	5.0
Rizo-N	6.0	5.0	5.1	3.6	3.6	4.7
Plant Guard	3.3	4.6	4.6	4.7	3.0	4.0
*Control-1	7.0	6.6	6.5	7.0	6.6	6.7
Mean	5.9	5.6	5.0	6.1	4.0	5.3
Dry weight (g) of plantlets						
Maxim	0.43	0.76	0.39	0.59	0.6	0.57
Premis	0.56	0.75	0.58	0.62	0.6	0.64
Topsin-M	0.47	0.73	0.47	0.65	0.6	0.59
Rizolex-T	0.42	0.60	0.47	0.75	0.6	0.57
Vitavax-T70	0.43	0.80	0.44	0.62	0.6	0.58
Vitavax-T40	0.41	0.62	0.50	0.67	0.7	0.59
Rizo-N	0.44	0.65	0.58	0.66	0.8	0.64
Plant Guard	0.66	0.61	0.44	0.47	0.4	0.53
*Control-1	0.86	0.51	0.54	0.76	0.6	0.66
Mean	0.52	0.67	0.49	0.64	0.6	0.59

*Control- 1 =Infested soil with fungi without seed dressing.
**Control-2= Seeds treated with fungicides only and planted in uninfested soil

L.S.D.at 5% for:	Fungi (F)	Treatment(T)	(FxT)
Shoot length.	0.156	0.209	0.469
Root length.	0..019	0.025	0.057
Dry weight.	0..0192	0.0258	0.0577

Meanwhile, seed treatments with the selected fungicides or antagonists before sowing in soil infested with *F. roseum* decreased the dry weight of the resulted seedlings at 21 days old comparing with control-1. On the other hand, Rizo-N and Vitavax- T40 were the best seed treatments before sowing in the uninfested soil (control-2) in increasing the dry weight of the resulted seedlings in un-infested soil comparing with control-1 (un-treated seeds).

VII– Field trials:

Effect of some fungicides and commercial bioagents:

1- Effect on disease incidence:

Data in **Table (16)** indicate that, all the tested fungicides and commercial bioagents affected positively disease incidence under field conditions. In this respect, Maxim was the most effective fungicide on reducing disease incidence of cotton plants where it gave the highest percentages of survived plants of cotton cvs. Giza-86 and Giza-89 during the both growing seasons of 2000 and 2001 being 76.7 & 78.0% and 80.7 & 82.0%, respectively.

Meanwhile, Topsin-M70 followed Maxim in this respect without significant differences. Slight difference was noticed between Vitavax-T70 and Maxim or Topsin-M7. Rizolex-T was the least effective fungicide, where the survived plants were 64.7 and 71.4% during 2000 growing season and 72.0 and 69.6% during season 2001 for both cvs. Giza-86 and Giza-89, respectively.

Table (16): Effect of some fungicides and commercial bioagents on damping off incidence of the two cotton cvs. Giza-86 and Giza-89, under field conditions during 2000- 2001 growing seasons.

Treatments	% Disease incidence and survived plants.																	
	cv.Giza-86									cv.Giza-89								
	Season2000			Season2001			Mean			Season2000			Season2001			Mean		
	Pre	Post	Sur.	Pre	Post	Sur.	Pre	Post	Sur.	Pre	Post	Sur.	Pre	Post	Sur.	Pre	Post	Sur.
Maxim	16.7	6.7	76.6	16.0	3.3	80.7	16.4	5.0	78.7	18.0	4.0	78.0	15.3	2.7	82.0	16.7	3.4	80.0
Topsin-M70	22.7	4.0	73.3	22.0	3.0	75.0	22.4	3.5	74.2	20.7	2.7	76.7	19.3	1.3	79.4	20.0	2.0	78.1
Rizolex-T	29.3	6.0	64.7	26.0	2.0	72.0	27.6	4.0	68.4	22.0	6.7	71.4	26.7	3.7	69.6	24.3	5.2	70.5
Vitavax-70	22.7	4.0	73.3	21.3	4.7	74.0	22.0	4.4	73.7	22.0	4.0	74.0	20.7	4.0	75.3	21.4	4.0	74.7
Vitavax-40	23.3	7.3	69.4	22.7	3.3	74.0	23.0	5.3	71.7	25.3	1.3	73.4	22.0	3.3	74.7	23.7	2.3	74.1
Rizo-N	28.0	5.0	67.0	26.7	2.0	71.3	27.4	3.5	69.2	24.7	4.7	70.6	23.3	3.3	73.4	24.0	4.0	72.0
Plantguard	27.3	8.0	64.7	26.0	5.3	68.7	26.6	6.7	66.7	26.7	5.3	68.0	25.3	4.0	70.7	26.0	4.7	69.4
Control	52.7	13.3	34.0	54.7	13.3	32.0	53.7	13.3	33.0	52.7	11.3	36.0	54.7	12.7	32.6	53.7	12.0	34.3
Mean	27.8	6.9	65.4	26.9	4.6	68.5	27.4	5.7	67.0	26.5	5.0	68.5	25.9	4.4	69.7	26.2	4.7	69.1

L. S .D .at 5% for:	cv.Giza-86.			cv.Giza-89.		
	Pre.	Post.	Survival.	Pre.	Post.	Survival.
Season2000	4.22	3.97	2.99	3.15	2.29	3.59
Season2001	2.54	2.39	3.08	5.43	2.64	6.11

Regarding the effect of some commercial bioagents under field conditions, data in **Table (16)** indicate that, the bioagent Rizo- N was the best for its effect on reducing disease incidence and increasing survived plants of cvs. Giza-86 and Giza-89 in both 2000 and 2001 growing seasons, being 67.0 & 70.6% and 71.3 & 73.4 % on the average, respectively.

2- Effect on plant height (cm):

Data in **Table (17)** indicate that treating cotton seeds with fungicides or commercial bioagents before sowing improved the plant height of cotton plants cvs Giza-86 and Giza-89 more than untreated ones (control). In this respect, Vitavax–T70 followed by Vitavax–T40 and Rizolex–T50 were the best effective fungicides in increasing cotton plant height during the two growing seasons (2000-2001) where they resulted in a remarkable increase over other treatments. On the other hand, the other tested fungicides were also more effective than the un-treated control in increasing the plant height of tested cotton plants. Moreover, Rizo-N and Plant Guard bioagents improved also the plant height of the tested cotton plants more than those of the un-treated control and sometimes better than some tested fungicides. Plant height in the case of Plant Guard treatment was higher than of those treated with Rizo-N treatment.

Data indicate also that the plant height of cotton plants cv. Giza-86 was higher than those of Giza-89 cultivar in both seasons under the effect of all tested treatments.

Table (17): Effect of some fungicides and commercial bioagents on plant height (cm) of cotton plants cvs.Giza-86 and Giza-89 during 2000 and 2001 growing seasons

Treatments	Plant height (cm)					
	Season 2000		Mean	Season 2000		Mean
	cv.Giza-86	cv.Giza-89		cv.Giza-86	cv.Giza-89	
Maxim	126.3	124.7	125.5	124.7	123.7	124.2
Topsin-M	128.7	117.7	123.2	128.0	117.7	122.8
Rizolex-T	1297	124.0	126.8	1287	123.3	126.0
Vitavax-T70	135.3	133.0	134.1	128.7	129.0	128.8
Vitavax-T40	133.7	129.3	131.5	128.7	127.7	128.2
Rizo-N	126.3	123.3	124.8	125.7	123.3	124.5
Plant Guard	127.3	125.0	126.2	127.0	123.3	125.2
Control	124.7	117.0	120.8	121.0	115.0	118.0
Mean	129. 0	124.3	126.6	126.6	122.8	124.7

L.S.D.at 5% for	Season 2000	Season 2001
Cotton cultivars = (C)	0.843	0.933
Treatments = (T)	1.690	1.87
C x T	2.386	2.64

3- Effect on fruiting branches of cotton plants:

Data in **Table (18)** show that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also the number of fruiting branches on growing plants. In this respect, Vitavax-T70 followed by Vitavax-40 were the best effective fungicides in increasing the number of fruiting branches onto both cvs. Giza-86 and Giza-89 during the growing seasons 2000 and 2001. Also, treating the seeds before sowing was effective in increasing the number of fruiting branches of both cvs. Giza-86 and Giza-89 during the growing seasons 2000 and 2001 comparing with some other tested fungicides and untreated

control. Plant Guard and Rizo-N were more effective than many of the tested fungicides and control in this respect.

Table (18): Effect of some fungicides and commercial bioagents on fruiting branches of cotton plants cvs.Giza-86 and Giza-89 during 2000 and 2001 growing seasons

Treatments	Number of fruiting branches / plant					
	Season 2000		Mean	Season 2000		Mean
	cv.Giza-86	cv.Giza-89		cv.Giza-86	cv.Giza-89	
Maxim	12.0	12.3	12.2	11.3	12.0	11.7
Topsin-M	12.0	11.0	11.5	10.7	10.7	10.7
Rizolex-T	12.3	12.0	12.2	12.3	12.0	12.1
Vitavax-T70	15.3	14.3	14.8	12.7	14.0	13.4
Vitavax-T40	15.0	13.7	14.4	13.0	12.0	12.5
Rizo-N	12.7	11.7	12.2	12.7	11.0	11.5
Plant Guard	13.3	12.3	12.7	12.0	11.7	11.5
Control	11.3	10.3	10.8	10.7	9..0	9.8
Mean	13.0	12.2	12.6	11.9	11.6	11.7

L.S.D.at 5% for	Season 2000	Season 2001
Cotton cultivars = (C)	0.464	N.S.
Treatments = (T)	1.690	1.01
C x T	n.s.	n.s.

4- Effect on bolls number of cotton plants:

Data in **Table (19)** indicate that treating cotton seeds cvs.Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens led to increasing the number of opened bolls/plant compared with control treatment (untreated seeds). Meanwhile, the only exception in this respect was Topsin-M70 treatment where it was less effective especially onto cv. Giza-86 during season 2000. Also, it is clear that the highest increases for both cotton cvs was produced by using Vitavax-T70 followed by Vitavax-T40 and Rizo-N, respectively. during

season 2000. Whereas, Vitavax-T70, Vitavax-T40, Plant Guard and Rizo-N were the most effective for treatment the seeds of both cotton cvs. Giza-86 and Giza-89 during the second season 2001.

Table (19): Effect of some fungicides and commercial bioagents on mature opened bolls of cotton plants cvs.Giza-86 and Giza-89 during 2000 and 2001 growing seasons

Treatments	Number of opened bolls/plant					
	Season 2000		Mean	Season 2000		Mean
	cv.Giza-86	cv.Giza-89		cv.Giza-86	cv.Giza-89	
Maxim	18.0	20.3	19.2	16.0	19.3	17.6
Topsin-M	14.3	20.0	17.2	14.3	19.3	16.8
Rizolex-T	17.3	19.0	18.2	15.3	17.3	16.3
Vitavax-T70	21.3	24.3	22.8	17.0	22.7	19.8
Vitavax-T40	21.3	23.0	22.2	21.3	21.7	21.5
Rizo-N	19.3	23.0	21.2	17.3	21.0	19.2
Plant Guard	18.3	21.0	19.6	18.3	20.0	19.2
Control	14.7	18.7	16.7	12.7	17.0	14.8
Mean	18.1	21.2	19.6	16.5	19.8	18.2

L.S.D.at 5% for	Season 2000	Season 2001
Cotton cultivars = (C)	0. 68	0.577
Treatments = (T)	1.36	1.154
C x T	n.s,	1.63

5- Effect on the weight of cotton yield:

Data in **Table (20)** indicate that treating cotton seeds cvs.Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens also led to increasing the weight of yielded cotton for each boll. In this respect, Vitavax-T70 and Vitavax-T40 were the best for seed treatment in increasing the weight of yielded cotton for each boll during the two growing seasons for both tested cotton cvs. Giza-86 and Giza-89. Also, Rizo-N and Plant Guard were more effective than many

fungicides and the to un-treated control in increasing the weight of yielded cotton for each boll at the first and the second season specially for cv. Giza-89. Maxim and Rizolex fungicides followed by Vitavax-T70 and Vitavax-T40 in their effect during seasons 2000 and 2001 specially for cv. Giza-89. Also, treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also cotton yield (g)/plant of growing plants. In this respect, Vitavax-T70 followed by Vitavax-40 were the most effective fungicides in increasing the cotton yield (g)/plant for both cvs. Giza-86 and Giza-89 during 2000 growing season. Meanwhile, Vitavax-T40 followed by Rizo-N were the most effective treatments in increasing the cotton yield (g)/plant onto both cvs. Giza-86 and Giza-89 during season 2001. It is clear also, that Plant Guard and Rizo-N were more effective than many of the tested fungicides, i.e., Maxim, Topsin-M and Rizolex-T as well as the un-treated control in this respect.

It is clear also, that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also the yield of cotton (kantar/feddan).

Table (20): Effect of some fungicides and commercial bioagents on cotton weight (g)/boll , yield of cotton (gram) per plant and cotton yield (kantar/feddan) of cotton plants cvs.Giza-86 and Giza-89 during 2000 and 2001 growing seasons.

Treatments	Season 2000		Mean	Season 2001		Mean
	cv.Giza-	cv.Giza-		cv.Giza-	cv.Giza-	
Cotton weight (g)/boll						
Maxim	2.2	2.3	2.3	2.2	2.3	2.3
Topsin-M	2.3	2.2	2.7	2.2	2.2	2.2
Rizolex-T	2.2	2.3	2.7	2.2	2.3	2.2
Vitavax-T70	2.4	2.6	2.5	2.2	2.5	2.4
Vitavax-T40	2.4	2.6	2.5	2.3	2.4	2.4
Rizo-N	2.2	2.4	2.3	2.2	2.4	2.3
Plant Guard	2.2	2.4	2.3	2.2	2.3	2.3
Control	2.2	2.2	2.2	2.1	2.1	2.1
Mean	2.3	2.4	2.4	2.2	2.3	2.3
Yield of cotton (gram) per plant						
Maxim	39.0	46.1	42.1	34.5	44.0	39.3
Topsin-M	32.6	44.7	38.7	31.8	42.7	37.3
Rizolex-T	38.3	43.7	41.0	33.3	39.5	36.4
Vitavax-T70	51.8	64.2	58.0	37.9	57.1	47.5
Vitavax-T40	50.2	58.9	54.5	48.6	52.1	50.4
Rizo-N	42.9	55.8	49.4	38.1	49.9	44.0
Plant Guard	41.1	49.5	45.3	40.5	45.0	42.7
Control	31.5	41.1	36.3	26.7	36.0	31.4
Mean	40.9	50.5	45.7	36.4	45.8	41.1
Cotton yield (kantar/feddan)						
Maxim	5.7	7.8	6.7	4.9	7.4	6.2
Topsin-M	4.5	7.4	5.9	4.5	6.5	5.5
Rizolex-T	6.4	9.4	7.9	4.8	7.4	6.1
Vitavax-T70	10.4	13.7	12.1	6.6	10.6	8.6
Vitavax-T40	9.5	12.3	10.9	8.5	9.9	9.2
Rizo-N	6.1	14.2	10.2	5.2	9.1	7.2
Plant Guard	6.2	9.2	7.7	5.9	7.0	6.5
Control	4.0	5.7	4.8	3.0	4.9	3.9
Mean	6.6	10.0	8.3	5.4	7.8	6.6

LSD at 5% for	Cotton cultivar = (C)		Treatments = T		Cx T	
	2000	2001	2000	2001	2000	2001
Cotton weight (g)/boll	0.051	0.023	1..36	0.046	n. s.	0.066
Yield of cotton (gram) / plant	5.534	4.974	1.374	2.689	n. s.	3.803
Cotton yield(kantar/feddan)	0.675	0.796	1.35	0.798	1.9	1.125

In this respect, Vitavax-T70 followed by Vitavax-T40 were more effective for seed treatment in increasing cotton yield (kantar/feddan) for cv. Giza-86 during season 2000 while,

Vitavax-T70 followed by Rizo-N and Vitavax-T40 were the most effective for treatment seeds of cv. Giza-89 during the same season. On the other hand, Vitavax-T40 followed by Vitavax-T70 were the best during season 2001 for Giza-86, meanwhile, the reverse was true for Vitavax-T70 followed by Vitavax-T40 which were the most effective fungicides in increasing the cotton yield of cv. Giza-89 during the same season.

6- Effect on the yield of cotton lint:

Data in **Table (21)** indicate that treating cotton seeds cvs.Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens led to increasing the average yield of cotton lint (g)/plant compared with control treatment (untreated seeds). The only exception in this respect was Topsin-M treatment which was less effective for both cvs.Giza-86 and Giza-89 during the two growing seasons 2000 and 2001 comparing with the other tested fungicides. Also, it is clear that he highest increase for both cotton cvs. was produced by using Vitavax-T70 followed by Vitavax-T40 and Rizo-N, respectively during season 2000. Whereas, Vitavax-T40 and Plant Guard were the most effective treatments of Giza-86 while, Vitavax-T70 followed by Vitavax-T40 and Rizo-N were the best during season 2001.

Data in **Table (21)** show also, that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also the yield of cotton lint (kantar/feddan). In this respect, seed treatment with each of Vitavax-T70 followed by Vitavax-T40 were more effective in

increasing the yield of cotton lint (kantar/feddan) for cv. Giza-86 during season 2000 while, Rizo-N followed by Vitavax-T70 and Vitavax-T40 were the most effective seed treatments for cv. Giza-89 during the same season. On the other hand, Topsin-M and Vitavax-70 showed the best effect in increasing the yield of cotton lint of cv.Giza-86 during season 2001.

Table (21): Effect of some fungicides and commercial bioagents on the yield of cotton lint (g)/plant and yield of cotton lint (kantar/feddan) for cotton plants cvs.Giza-86 and Giza-89. during 2000 and 2001 growing seasons.

Treatments	Season 2000		Mean	Season 2001		Mean
	Giza-86	Giza-89		Giza-86	Giza-89	
Yield of cotton lint (g)/plant						
Maxim	12.9	15.4	14.2	11.8	14.7	13.3
Topsin-M	10.8	14.8	12.8	10.6	14.2	12.4
Rizolex-T	12.8	14.6	13.7	11.1	13.2	12.2
Vitavax-T70	17.2	21.4	19.3	12.6	19.0	15.8
Vitavax-T40	16.7	19.6	18.2	16.0	17.3	16.6
Rizo-N	14.3	18.6	16.5	12.7	16.6	14.6
Plant Guard	13.7	16.5	15.1	13.5	15.0	14.3
Control	10.5	13.7	12.1	8.9	12.0	10.5
Mean	13.6	16.8	15.2	12.2	15.3	13.7
Yield of cotton lint (kantar/feddan)						
Maxim	5.7	7.8	6.8	5.4	7.9	6.7
Topsin-M	4.5	7.9	6.2	9.2	7.0	8.1
Rizolex-T	6.4	9.4	7.9	5.3	8.0	6.6
Vitavax-T70	10.4	13.7	12.0	7.0	11.3	9.2
Vitavax-T40	9.5	12.3	10.9	5.0	10.6	7.5
Rizo-N	6.1	14.2	10.2	5.9	9.8	7.8
Plant Guard	6.2	9.2	7.7	6.3	7.8	7.0
Control	4.0	5.7	4.8	3.3	5.3	4.3
Mean	6.6	10.0	8.3	5.9	8.5	7.2

LSD at 5% for	Cotton cultivar = (C)		Treatments =		Cx T	
	Season 2000	Seaso	Seaso	Seaso	Seaso	Seaso
Yield of cotton	1.8	1.682	N.S.	0.877	N.S.	1.241
Yield of cotton	1.9	1.096	0.675	0.387	1.35	0.776

Meanwhile, Vitavax-T70 followed by Vitavax-T40 and Rizo-N were more effective fungicides in increasing the yield of cotton lint of cv. Giza-89 during the same season.

7- Effect on the yield of cotton seeds (g)/plant and yield of cotton seeds (kg/feddan)

Data in **Table (22)** show that treating cotton seeds cvs.Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also the yield of cotton seeds (g)/plant. In this respect, Vitavax-T70 followed by Vitavax-T40 and Rizo-N were the best treatments during season 2000 on both tested cotton cvs.Giza-86 and Giza-89.While, Vitavax-T40 and Rizo-N were the best seed treatments during season 2001 in increasing the yield of cotton seeds of cv. Giza-86. Vitavax-T70 followed by Vitavax-T40 and Rizo-N were the best seed treatments for cv Giza-89 during the same season. Rizo-N was more effective than Plant Guard in increasing the yield of cotton seeds (g)/plant of both cotton cvs.Giza-86 and Giza-89 during the two growing seasons of 2000 and 2001.

Data indicate also, that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens increased also the yield of cotton seeds (kg/feddan). In this respect, using the tested fungicides and bioagents resulted in noticeable increase in yield of cotton seeds for both cotton cvs.Giza-86 and Giza-89 during the two growing seasons comparing with the un-treated control.

Table (22): Effect of some fungicides and commercial bioagents on the yield of cotton seeds (g) / plant and yield of cotton seeds (kg/feddan) of cotton cvs.Giza-86 and Giza-89 during 2000 and 2001 growing seasons.

Treatments	Season 2000		Mean	Season 2001		Mean
	Giza-86	Giza-89		Giza-86	Giza-89	
Yield of cotton seeds (g) / plant						
Maxim	26.0	30.7	28.4	22.7	29.3	26.0
Topsin-M	21.7	29.6	25.7	21.2	28.5	24.8
Rizolex-T	25.5	29.1	27.3	22.2	26.2	24.2
Vitavax-T70	34.6	42.8	38.7	25.3	38.0	31.7
Vitavax-T40	33.5	39.3	36.4	31.9	34.7	33.3
Rizo-N	28.6	37.3	33.0	27.0	33.2	30.1
Plant Guard	27.4	32.9	30.2	25.4	30.0	27.7
Control	20.9	27.4	24.2	17.8	24.0	20.9
Mean	27.3	33.6	30.4	24.2	30.5	27.3
Yield of cotton seeds (kg/feddan)						
Maxim	574.1	805.2	689.6	513.2	777.4	645.3
Topsin-M	470.3	803.9	637.1	447.1	673.9	560.5
Rizolex-T	647.1	936.8	791.9	493.2	759.5	626.4
Vitavax-T70	1065.5	1404.3	1234.9	685.8	1102.5	894.2
Vitavax-T40	966.1	1258.9	1112.3	881.0	1034.4	957.7
Rizo-N	614.1	1172.7	893.4	545.3	741.5	643.4
Plant Guard	630.9	925.1	778.0	601.9	772.1	686.9
Control	404.4	616.5	510.5	315.4	506.4	410.9
Mean	671.6	990.4	831.0	560.4	796.0	678.2

LSD at 5% for	Cotton cultivar = (C)		Treatments = T		Cx T	
	Season 2000	Season 2001	Season 2000	Season 2001	Season 2000	Season 2001
Yield of cotton seeds (g) /	3.735	3.241	2.703	1.835	N.S.	2.59
Yield of cotton seeds	48.0	63.35	69.0	126.69	135.8	n.s

Moreover, Vitavax-T70 and Vitavax-T40 were the most effective seed treatments for both tested cotton cvs during season 2000. On the other hand, Vitavax-T40 followed by Vitavax-T70 showed the best effect for cv.Giza-86 during season 2001 while,

Vitavax-T70 and Vitavax-T40 were the most effective seed treatments for cv. Giza-89 during the same season.

8- Effect on the fiber length:

Data in **Table (23)** reveal that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens improved also the fiber length of yielded cotton. In this respect, The tested fungicides and bioagents improved clearly the fiber length of yielded cotton for both cotton cvs. Giza-86 and Giza-89 during the two growing seasons comparing to un-treated seeds (control). Therefore, Vitavax-T70 followed by Vitavax-T40 were the most effective seed treatments in increasing the fiber length of the yielded cotton of the two cotton cvs during seasons 2000 and 2001.

Table (23): Effect of some fungicides and commercial bioagents on the fiber length (mm) of cotton cvs.Giza-86 and Giza-89.during 2000 and 2001 growing seasons.

Treatments	Fiber length (mm)					
	Season 2000		Mean	Season 2000		Mean
	cv.Giza-86	cv.Giza-89		cv.Giza-86	cv.Giza-89	
Maxim	31.7	32.1	31.9	31.6	32.2	31.9
Topsin-M	31.9	31.9	31.9	31.9	31.8	31.8
Rizolex-T	33.0	32.1	32.6	33.0	32.4	32.7
Vitavax-T70	33.9	33.2	33.6	33.7	33.1	33.4
Vitavax-T40	33.4	32.8	33.1	33.3	32.8	33.0
Rizo-N	32.9	32.2	32.6	32.8	32.1	32.5
Plant Guard	33.3	31.9	32.6	32.9	31.8	32.4
Control	31.2	31.5	31.4	31.2	31.4	31.3
Mean	32.7	32.2	32.5	32.6	32.2	32.4

L.S.D.at 5% for	Season 2000	Season 2001
Cotton cultivar = (C)	0.304	0.294
Treatments = T	0.61	0.588
Cx T	n. s.	n. s.

9- Effect on the fiber strength:

Data in **Table (24)** reveal that treating cotton seeds cvs. Giza-86 and Giza-89 with fungicides and/or bioagents for controlling root rot pathogens improved also the fiber strength of the yielded cotton. In this respect, most the determined values of fiber strength (micron) were clearly different from the those of the un-treated one (control). Vitavax-T70, Vitavax-T40 and Plant Guard gave the best effect in improving the fiber strength of the yielded cotton over the other tested fungicides and bioagents during the two growing, i.e., seasons 2000 and 2001.

Table (24): Effect of some fungicides and commercial bioagents on the fiber strength of cotton cvs. Giza-86 and Giza-89. during 2000 and 2001 growing seasons.

Treatments	Fiber strength (micron)					
	Season 2000		Mean	Season 2000		Mean
	cv.Giza-	cv.Giza-		cv.Giza-	cv.Giza-	
Maxim	17.9	17.9	17.9	17.8	17.9	17.8
Topsin-M	17.9	18.1	18.0	17.8	18.1	17.9
Rizolex-T	18.2	17.9	18.0	18.2	17.8	18.0
Vitavax-T70	18.8	18.3	18.6	18.6	18.2	18.4
Vitavax-T40	18.7	18.2	18.5	18.4	18.2	18.3
Rizo-N	18.1	18.2	18.2	18.1	18.1	18.1
Plant Guard	18.5	18.0	18.3	18.4	17.9	18.1
Control	17.6	17.6	17.6	17.5	17.7	17.6
Mean	18.2	18.0	18.1	18.1	17.9	18.0

L.S.D.at 5% for	Season 2000	Season 2001
Cotton cultivar = (C)	0.110	0.108
Treatments = T	0.213	0.216
Cx T	0.302	0.306

DISCUSSION

DISCUSSION

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber and oil crops in Egypt and in many other countries all over the world. It is attacked by several disorders, which are resulted from insects, fungi, bacteria, nematodes and others at the different stages of growth **(Brodie and Cooper 1964, Cauquil and Shepherd 1970)**. Cotton seedling diseases whether pre or post emergence are world wide problem, often causing serious stand losses. A number of soil and seed borne pathogens can infect cotton seedlings individually or in association as a disease complex. Cotton plants are subjected to attack by various pathogenic fungi causing several diseases during different stages of growth. Among these diseases are the seed rot and seedling damping – off. A number of pathogenic fungi including seed–borne and soil–borne pathogens such as *Alternaria spp*, *Fusarium spp*., *Rhizopus spp*. and *Aspergillus spp*. are the most 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 in several fungi which belong to 5 genera and 11 species. The isolated fungi were purified and 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*. As well as, some of the isolated fungi were stayed unknown (without identification). The

isolated fungi from different parts of cotton seeds as well as rotten roots of Giza-86 and Giza-89 were differed in their frequencies from part to another. Generally, *R. solani* was the highest frequent fungus followed by *Fusarium moniliforme* and *Fusarium roseum* and then the other *Fusarium* spp from most of the different cotton seed parts. However, many of the isolated fungi, whether before or after delinting, like *Alternaria alternata, Aspergillus niger, Penicillium spp* and *Fusarium semitectum* were isolated at low frequencies from seeds of cvs. Giza-86 and Giza-89. Also, it is clear that the total isolated number from two cvs of cotton seeds of the two cvs. after delinting were lesser than those before delinting. In general, the total number of isolated fungi from cotyledons was greatly lower comparing with those of inner surface of seed testa for both cotton cvs tested. Regarding the isolated fungi from rotten roots of cotton seedlings, six isolates were isolated from the rotten roots of Giza-86, two of them are belonging to *R. solani*. Meanwhile, 14 isolates were isolated from rotten roots of Giza-89, 5 isolates of them are belonging to *R. solani*. Also, *R. solani* was the most frequent fungus followed by *Fusarium moniliforme* and *Aspergillus niger*. However, *Penicillium spp.* and *Fusarium tricinectum* were the lowest frequent fungi that isolated from rotten roots of cotton seedlings. These results are in agreement with the obtained findings of **Fulton and Bollenbacher (1958)** who isolated *Fusarium oxysporum, F.moniliforme, F. semitectum* and *Rhizoctonia solani* from cotton seedlings. While, **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* and *Rhizopus stolonifer;* and *Penicillium corylophilum;* and *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, the results of **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 throughout the 2000 and 2001 growing seasons.

Concerning the pathogenicity, tests *R. solani* caused the highest % 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 % pre– and post emergence damping–off with all the tested pathogens. As for survival %, it was found that 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 (1958)** who demonstrated that *Fusarium oxysporum, F. moniliforme, F. semitectum* and *Rhizoctonia solani,* which were 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 which is caused by *Rhizoctonia solani, Fusarium* spp and *Pythium* spp. While, **Salem (1969)** mentioned that both Egyptian and American cotton varieties were susceptible at different degrees to *Rhizoctonia solani.* **Wang *et al.* (1992)** isolated *Fusarium moniliforme, Fusarium semitectum, Fusarium oxysporum, Fusarium solani, Fusarium equiseti* and *Fusarium compactum* from cotton seedlings and bolls during 1978–1990. They added also 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.

Infestation the soil with root rot pathogens increased the reducing, non-reducing and total sugars in leaves of cotton plants of both cvs (Giza-86 and Giza-89). The highest increases in reducing and non-reducing sugars in cotton leaves of cvs Giza-86 and Giza-89 was recorded in case of infestation the soil with each of *F. moniliforme* followed by *R. solani* and *F. semitectum,* respectively while, the lowest amount of reducing sugars was obtained when the soil was infested with *F. roseum* comparing to un-infested soil (control). Regarding phenols, it is clear that infestation the soil with root rot pathogens affected positively the content of total, free and conjugated phenols in the leaves of

cotton plants cvs, Giza-86 and Giza-89. In this respect, the highest increase in the amount of determined phenols as mg/g fresh weight (total, free and conjugated phenols) of both cvs.(Giza-86 and Giza-89) was recorded in case of infestation the soil with *R. solani* followed by *F. moniliforme* and *F. semitectum,* respectively while the lowest ones were recorded in case using *F. roseum* . It is clear also, that all the determined phenols (total, free and conjugated phenols) were higher in general in cv. Giza-89 than those determined in cv. Giza-86. The obtained results are in agreement with those of **Nuritdinova *et al.*(1986)** who studied the cell walls in healthy and infected plants of the resistant *Gossypium hirsutum* variety Tashkent-1 and the susceptible S 4727 , using race 1 of *V. dahliae*, and found that the cell walls of S 4727 contained more carbohydrates than those of Tashkent-1 before and after infection. While, **Abuo-Taleb *et al.* (1987)** found a gradual increase in the level of total phenols in both resistant and susceptible cotton cultivars up to 72 h after inoculation and in gossypol up to 144 h. Also, **Borkar and Verma (1990)** reported that cotton infection with *X. campestris pv, malvacearum* increased the total sugar content and the total phenol content only in the leaves of the susceptible cultivar than in the leaves of the resistant one. Similar results were obtained also by **Hiremath and Savanur (1990)** who found that cotton leaves collected in from healthy and infected plants there was increase in total sugars , reducing sugars, total phenols and amino acids in disease bottom leaves and lesser increase in diseased to cotton leaves , indicating that *Alternaria macrospora* had greater effect on the metabolism of the lower leaves. **Vlassova (1993)** mentioned that an increase in the total phenol

content was only detected in the inoculated roots of the resistant cotton variety (Tashkent–1) with a virulent strain of *Verticillium dahliae* biotype Kh1–288 race 2. Also, **Chakrabarty *et al.* (2002)** reported that induced rather than constitutive levels of phenylalanine amino lyase (PAL), total phenol, gossypol and flavonols played crucial role in governing resistance to grey–mildew (*Ramularia areola*). The effect of phenolic proline and total sugar upon infection appeared important for resistance.

Infestation the soil with the tested root rot pathogens *i.e.*, *R. solani, F. moniliforme, F. semitectum,* and *F. roseum* before sowing cotton seeds in this soil affected negatively the content of chlorophyll when was determined in the leaves of cotton seedlings as mg/g fresh weight of both tested cvs after 21 days post sowing. In this respect, infection with any of the tested root rot pathogens decreased the content of chlorophyll A and B and also total chlorophyll in cotton leaves comparing with those grown in the uninfested soil (control) of the two cotton cvs. (Giza-86 and Giza-89). The highest decrease in chlorophyll A and B as well as total chlorophyll was recorded in case of infestation the soil with *F. moniliforme, F. semitectum, F. roseum* and *R. solani,* respectively comparing with those grown in the uninfested soil (control) of the two cultivars. The results indicate also that growing cotton plants in the infested soil before sowing with root rot pathogens do not affect clearly on carotenoids content in the leaves of the grown cotton plants of the tested cvs comparing with those grown in the un-infested soil (control). These results are in harmony with those of **Murumkar and Chavan (1985)** who found that infection of *Cicer arietinum*

with *F. oxysporum f.sp. ciceri* resulted in a reduction in chlorophyll and increase in organic acids, polyphenols and carbohydrates. Similar results also were obtained on maize by **Arinze and Sokirko (1986)** who found that inoculating maize seeds with *Fusarium moniliforme* [*Gibberella fujikuroi*] reduced chlorophyll and carotenoids content and the total chlorophyll in grown seedlings. Also, the results of **Sherif and El–Habbaa (2000)** support the obtained results. They found that **infected** tomato leaves with *Pseudomonas syringae, Alternaria solani,* and yellow leaf curl virus exhibited high reduction in chlorophyll (a & b) and carotenoids compared with healthy leaves.

As for the variation in fractionated protein patterns of the two cotton cultivars (Giza-86 and Giza-89), as a result of infection with *Rhizoctonia solani* and *Fusarium spp,* it was found that the infection with root rot pathogens increased the number of fractionated protein bands comparing with control treatment (un-infected). In this respect, the infected cotton plants (Giza-86) with *R. solani* revealed 15 protein bands comparing with control plants (11 band), some of them are similar in their molecular weight to as in control while some others are newly formed corresponding to infection with the pathogen. Meanwhile, infection with *F .moniliforme* revealed 12 protein bands; some of them are new formed. Also, The inoculated plants (Giza-86) with *F. roseum* revealed 12 bands with some new protein bands. On the other hand, the inoculated cotton plants (Giza-86) with *F. semitectum* revealed 16 protein bands comparing with the un-inoculated ones (11 bands) where the new formed bands were 192.6, 148.4, 116.2, 93.1, 47.7, 40.1,

28.9 and 16.1 KDa. The results indicate also that the inoculated cotton plants (Giza-86) with *F. roseum* and *F. semitectum* revealed typical protein bands at 192.6, 148.4, 93.1 and 16.1 KDa as well as they are differed completely with the protein bands of *R. solani* infection, meanwhile they were similar partially with the inoculated plants with *F. moniliforme* at 16.1 KDa. Moreover, The inoculated cotton plants (Giza-86) with *R. solani* and *F. moniliforme* produced typical bands in response to infection at 100.0, 40.1 and 17.5 KDa. These appeared protein bands are differed in their condense where some of them are clear faint and some others appeared intensive although they have the same molecular weight.

As for cotton cv. Giza-89, there were clear variations in fractionated protein patterns in the leaves of Giza-89 as a result of infection with *Rhizoctonia solani* and *Fusarium spp.* These exhibited protein bands ranged from 208.6 to 11.9 KDa. Infection with each of *R. solani* and *F. roseum* caused lower number of protein bands comparing with control treatment (un-infected). Meanwhile, only infected plants with *F. semitectum* revealed protein bands more than in those of the control treatment. On the other hand, all infected cotton plants (Giza-89) produced few of new bands in response to infection with root rot pathogens viz., 17.5 KDa in case of *R. solani*, 20.1 KDa with *F. moniliforme*, 82.9 and 21.6 KDa with *F. roseum* as well as, 130.9, 99.5, 96.3, 46.4, 36.3, 30.4, 16.1 and 13.8 KDa in case of *F. semitectum*. On the other hand, inoculated cotton plants (Giza-89) with *F. roseum* and *F. semitectum* revealed typical protein band at 21.6 KDa. The obtained results *i.e.*, fractionated protein

bands of cotton cvs Giza-86 and Giza-89 in relation to infection with *R. solani* and *Fusarium* spp. could be interpreting in light that infections stimulate formation of pathogenesis–related proteins as a normal result of reaction in the plant to soil-borne infection. Also, the resulted pathogenesis–related proteins may be differed according to the plant age, plant type and the kind of infection. The results obtained by **Richard and Alain (1986), Liu *et al.* (1995), Sherif and El–Habbaa (2000)** and **Yuan *et al.* (2002)** support greatly this interpreting.

Infestation the soil with any of the tested root rot pathogens, *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* before sowing cotton seeds did not affect esterase isozyme content in cotton leaves of growing seedlings at 21 days old in case of cvs Giza-86 and Giza-89 comparing with un-infested control. In this respect, the resulted protein patterns of esterase isozyme due to infestation treatments were equal to those appeared with those of the control treatment where they showed the same values of Rf. The only exception was for *R. solani*, which formed one new protein band at 0.36 with cv Giza-86 and five new protein bands of esterase isozyme at Rf values 0.21, 0.36, 0.62, 0.71 and 0.79 with cv Giza-89. On the other hand, Infestation the soil with the same pathogens under the same conditions caused clear variations in peroxidase isozyme patterns of the two tested cotton cvs Giza-86 and Giza-89. As for Giza-86, the obtained results showed that soil infestation with *R.solani* caused the formation of two new protein bands at Rf 0.10 and 0.12 comparing with control treatment (un-infected). Meanwhile, infestations with *F. moniliforme* do not reveal any

new bands comparing with control treatment (un-infected).Whereas, infestation with *F. semitectum,* and *F. roseum* revealed only one new band of peroxides isozyme at Rf. 0.10. Concerning cv Giza-89, infestation the soil the with tested root rot pathogens resulted in clear changes in formation of peroxidase isozyme, where many protein bands were disappeared comparing to those of un-infested control, meanwhile, no new protein bands were formed with under the effect of any infestation treatment. These results are in harmony the those obtained by **Mellon and Lee (1985)** who found that inoculated cotton bolls, 30 days post infection with *A. flavus, Fusarium equiseti, F. moniliforme, F. semitectum* or *Rhizoctonia solani* followed by a 6 – d incubation period stimulated soluble peroxidase activity 2 to 6– fold. **Vlasova (1993)** observed an increase in peroxidase and phenol oxidase activities during the early stage of infection of the highly susceptible cotton variety S-4727 and the relatively resistant variety Tashkent–1 with a virulent strain of *Verticillium dahliae* biotype Kh1–288 race 2. Similar results also were obtained by **Sherif** and **El–Habbaa (2000)** who found that peroxidase and polyphenoloxidase activities were considerably higher in infected tomato leaves in relation to infection with *Alternaria solani, Pseudomonas syringae* and virus than in healthy leaves.

Infestation of cotton seeds with the tested root rot pathogens, *i.e., R. solani, F. moniliforme, F. semitectum,* and *F. roseum* decreased the percentages of oil content tested cotton seeds of cvs Giza-86 and Giza-89 comparing with uninfested seeds (control) at all incubation period which ranged between 5-

15 days. It is clear also that increasing 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 (control). The highest decrease in percentages of determined oil contents was recorded in case of infestation the seeds with each of *R. solani* and *F. moniliforme* at all tested incubation period for seeds of both cotton cvs. These results are in agreement with the findings of **Ataga and Akueshi (1986)** who found *A. tenuis [A. alternata]*, *Curvularia lunata*, *Fusarium moniliforme* and *Macrophomina phaseolina* grew well on sunflower seeds and caused biodeterioration over 21 days as well as reduced the oil content and caused discoloration of the oil. Also, the results of **Airede and Fsuruoso (1987)** concerning the inoculated oil palm kernels with spores of some seed-borne fungi verified the obtained results. 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. Moreover, the results of **Srivastava and Pandey (2000) and Adekunle and Badejo (2002)** interpret the obtained results in this respect.

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 those tested root rot pathogens produced clear amounts of mycotoxins (ppb) in some cases. In this respect, *F.semitectum* and *F. roseum*

produced Zearalenone mycotoxin into infected seeds of cv.Giza-86 and cv.Giza-89 while, *R. solani* and *F. moniliforme* were not able to produce Zearalenone mycotoxin into cotton seeds of both tested cvs. As for fumonisins mycotoxins, 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. These 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. Approx. 16% of the different substrates tested were positive for aflatoxin contamination. 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 each from cotton and tomatoes produced a N-acetylgalactosamine and N-acetylglucosamine toxins.

All of the tested fungicides reduced the growth of all the tested pathogens. In this respect, they reduced the linear growth of *R. solani* where Premis, Maxim and Topsin-M were the most effective fungicides. It is clear, also that Topsin-M was the highest effective one at the concentrations 5-400ppm followed by Premis at concentrations of 10-400ppm and Maxim at 10-400ppm. On the other hand, Maxim and Primes were more

effective than Topsin-M at concentration 1ppm. Moreover, Vitavax-T70 was also effective fungicide in reducing the growth of *R. solani*. Also, increasing the concentration from 1 to 400ppm increased gradually the effect of the tested fungicides in reducing the growth of *R. solani*. Concentrations 200 and 400 ppm were more effective than others. Also, Maxim and Premis as well as Topsin-M were the most effective fungicides in reducing the growth of *Fusarium moniliforme*. On the other hand, Rizolex-T, Vitavax-T70 and Vitavax-T40 were less effective than the fist three fungicides. On the other hand, Premis followed by Topsin-M were the highest effective fungicides in reducing the growth of *Fusarium roseum* and *Fusarium semitectum* more than other tested fungicides. Premis fungicide and Topsin-M gave the highest reduction at all tested concentrations except that of 1 ppm Topsin-M. The tested fungicides may be affect many biochemical processes in the cells of the pathogens, *i.e.*, enzymes, cell walls, ergestrol, DNA synthesis, cell division and others. Thus, these fungicides had a great potentiality to control many root rot pathogens although they may differ in their effect. In this respect, **Alagarsamy and Jeyaraian (1989)** and **Osman *et al.* (1990)** found that tolclofos-methyl was the most inhibitory fungicide against the growth of *R. solani* in culture. While, **Anwar *et al.* (1991)** reported that benomyl gave 100 % effect even at 20 ppm against growth of *Fusarium solani*. Also, **El–Habbaa *et al.* (2002)** revealed that Maxim was the most effective as it prevented *in vitro* the growth of *F. solani, M. phaseolina, B.theobromae, S. rolfsii* and *R solani* at 1-5ppm, followed by Benlate at (10–800ppm),Vitavax–T (25–200 ppm) and Rhizolex–T (200–800 ppm).

Concerning the effect of the bioagents, Rizo-N and Plantguard bioagents reduced the linear growth of all the tested root-rot pathogens comparing to un-treated one control. Rizo-N and Plantguard succeeded in reducing the linear growth of *R. solani, F. roseum, F. moniliforme* and *F. semitectum* to remarkable values. Moreover, Rizo-N was better than Plantguard in its effect in reducing the linear growth of all the tested fungi. The obtained results are in agreement with those obtained by **Dennis and Webster (1971)** who reported that *Trichoderma* spp. produced the antibiotic tricodermol which can inhibit the growth of several fungi. While, **Pusey and Wilson (1984)** reported that *Bacillus subtilis* exerted a heat stable antibiotic affect spore germination or developing the germ tube of some root pathogens. Also, **Abed–El Moiety *et al.* (1990)**, **Mukheriee *et al.* (1995)**, **Podile and Prakash (1996)** verified the antagonistic potentialities of *Trichoderma* spp., *B. subtilis* and some other bio-agents in controlling many root rot pathogens. **El-Habbaa (1997)** mentioned that *T. harzianum*-127 produced volatile substances with clear inhibitory effect, which proved to be static for fungi. As well as, he confirmed the antagonistic potentialities of *Trichoderma* spp using scanning electron microscope where they formed the coiled hyphae around the host hyphae as well as photographed the successful parasitism of *Trichoderma sp.* on the tested host hyphae. **El-Habbaa and Mohamed (2001)** confirmed the efficacy of released Trichoderma-volatile substances in controlling *Botrytis cinerea* in vitro. In addition, **Gopalakrishnan *et al.* (2003)** reported that many isolates of *T. hamatum, T. viride, T. virens* (*Gliocladium virens)* and *T. harzianum* controlled effectively the pathogens

Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii and *Macrophamina phaseolina* which cause the damping–off and root rot syndromes of cotton seedlings.

As for the pathogenic potentialities of the tested fungi whether individually or in combination in the presence or absence of any fungicidal treatment, it is clear that sowing cotton seeds of cvs Giza-86 and Giza-89 in soil infested with any of the pathogenic fungi whether individually or in the combination in absence of any fungicidal treatment resulted in high percentage of dead plants where, especially with the combined inoculum of the four tested fungi *i.e., R.solani, F. moniliforme, F. roseum and F. semitectum*. Meanwhile, treating cotton seeds with fungicides before sowing in infested soil with the tested fungi resulted in a clear reduction in the percentage of dead plants. Topsin-M, Premis and Maxim were the most effective fungicides while, Rizolex-T was the least effective one. Also, treating cotton seeds with fungicides before sowing in un-infested soil resulted in the least percentage of dead plants with superiority of Premis and Maxim over Topsin-M, Vitavax-T70, Vitavax-T40 and Rizolex-T. Also, it could be concluded that the fungicides Premis, Topsin-M70, and Maxim were the best in controlling cotton root–rot caused by any of *R. solani, F. moniliforme, F. roseum* or *F. semitectum* and in case using of the combined inoculum. Disease incidence on Giza-89 cv, in most treatments was lower than the corresponding values on Giza-86. These results are confirmed by those obtained by **David** and **Sinclair (1968)** who found that treating the soil or seeds with Vitavax gave significantly greater protection against pre- and post-emergence

damping-off. **Abdel–Shahaid *et al.* (1989)** reported that the fungicide Benlate (benomyl) 50% as a soil treatment in the greenhouse protected effectively cotton seedlings against *R. solani* and *F. solani* at concentrations as low as 20 ppm. *In vitro*, bioassay studies showed that *R. solani* was more sensitive than *F. solani* to benomyl therefore, **Youssef *et al.* (1995) Helal *et al.* (1996)** and **El–Safety *et al.* (2001)** confirmed that treating cotton seeds with fungicides, *i.e.*, Monceren, Rizolex, Vitavax 300 and others before planting in infested and non infested soil in the greenhouse gave the highest seedling emergence and the highest percentage of survived seedlings as well as improved seedling vigor at the two stages of seedling growth in the greenhouse. As well as, **El–Habbaa *et al.* (2002)** reported that Maxim was the most effective as it prevented *in vitro* the growth of *F. solani, M. phaseolina* , *B.theobromae* , *S. rolfsii* and *R solani*, at 1-5ppm, followed by Benlate at (10–800ppm). Vitavax–T (25–200 ppm) and Rizolex–T (200–800 ppm). Also, **Goulart (2002)** found *in vitro*, and greenhouse studies, that dressing seeds of cotton seeds with fungicides controlled damping–off caused by *R. solani.*

On the other hand, treating cotton seeds of Giza-86 or Giza-89 cvs with commercial antagonists (Rizo-N or Plantguard) before sowing in infested soil with the pathogenic fungi whether individually or in combination decreased significantly the dead plants comparing with the highest percentages of dead plants growing from untreated seeds and planted in soil infested with the pathogenic fungi. It was clear that treating cotton seeds cv Giza-86 with Rizo-N or Plantguard antagonists reduced the infection of cotton seedlings to low values. Meanwhile, treating

the cotton seeds with Rizo-N was better than Plantguard in this respect. Generally, it could be concluded that the commercial antagonists whether Rizo-N or Plantguard might be useful in controlling cotton infection caused by *R. solani*, *F. moniliforme*, *F. roseum* and *F. semitectum* on cotton seedlings of both cvs. Giza-86 and Giza-89 These results are in harmony with those of **Lumsden** and **Locke (1989) Howell (1991),** and **Mishra** and **Singh (2000).**

Treating cotton seeds (cvs Giza-86 and Giza-89) with certain fungicides or antagonists before sowing increased significantly the shoot length of most resulted seedlings at 21 days old. In this respect, the highest increase in the shoot length of the resulted seedlings (cv Giza-86) was in case of treating seeds with Rizolex-T and Rizo-N in soil infested with *F. moniliforme*. Meanwhile, Rizo-N followed by Topsin-M were the best seed treatments in increasing the shoot lengths of cotton seedlings (cv Giza-89) in soil infested with *R. solani*. Also, all seed treatments of cv Giza-89 seeds whether with fungicides or antagonists and planted in soil infested with *F. semitectum* reduced the shoot lengths of resulted seedlings to lengths lesser than in control-1 treatment. On the other hand, treating cotton seeds with fungicides or antagonists before sowing in normal soil without infestation (control-2) improved the shoot length of the resulted seedlings in case of Maxim. Vitavax-T40, Rizo-N and Plantguard treatments more than in other treatments and control. In general, Rizolex-T and Maxim were the best tested fungicides in increasing the shoot lengths of the resulted

seedlings of cv Giza-86 while, Rizo-N, Topsin-M and Vitavax-T70 were the best in case of cv Giza-89.

Also, treating cotton seeds cv Giza-86 with fungicides or antagonists before sowing in infested soil with the tested pathogenic fungi reduced significantly the root length, dry weight of the resulted seedlings most cases of treatments comparing with un-treated seeds in infested soil with the tested fungi (control-1). However, some fungicide treatments increased the seedlings root length like Premis seed treatment with soil infested with *R. solani* and soil infested with *F. semitectum*. As well as, there was a clear increase in root length in case of treatment the seeds with Maxim and Vitavax-T70 and Plantguard seed treatments before sowing in soil infested with *F. semitectum*. Also, treating cotton seeds with fungicides or antagonists before sowing in noninfested soil without infestation with any of the pathogenic fungi (control-2) improved the root length of the resulted seedlings more than un-treated seeds planted in soil (control-1). On the other hand, treating cotton seeds of cv. Giza-89 with Topsin-M fungicide before sowing in infested soil with *R. solani* or *F. moniliforme* only increased the root length of the resulted seedlings comparing with other fungicides or antagonists treatments which decreased the root lengths in most cases compared with control-1 treatment. Meanwhile, all seed treatments with fungicides or antagonists before sowing in infested soil with *F. semitectum* decreased the root lengths of the resulted seedlings to lengths lesser than that of control-1 treatment. On the other hand, seed treatment with Rizolex-T was the best treatment in case of infested soil with *F.*

roseum in increasing significantly the root length of the resulted seedlings comparing with other treatments and control. In the same time, treating the seeds of Giza-89 with fungicides or antagonists before sowing in uninfested soil (control-2) reduced the root lengths compared to control-1 treatment in this respect. Also, treating cotton seeds cv Giza-89 with any of the fungicides or antagonists before sowing in soil infested with *R. solani* reduced the dry weight of the resulted seedlings at 21 days old. Meanwhile, all seed treatments with any of the tested fungicides and antagonists before sowing in infested soil with *F. moniliforme* increased significantly the dry weight of the resulted seedlings comparing with control-1 treatment. Vitavax-T70, Maxim, Premis and Topsin-M were the best seed treatments in this respect. Also, Rizo-N and Premis were the best seed treatments before sowing in soil infested with *F. semitectum* in increasing the dry weight of the resulted seedlings comparing with control-1 treatment, whereas other seed treatments reduced significantly the dry weight of the resulted seedlings. On the other hand, Rizo-N and Vitavax-T40 were the best seed treatments before sowing in the uninfested soil (control-2) in increasing the dry weight of the resulted seedlings in un-infested soil comparing with control-1 (un-treated seeds). These results are in harmony of those collected by **Alagrsamy** and **Jeyaraian (1989)** who found that treating cotton seeds with carbendazim and tolclofos-methyl fungicides, gave the best germination, respectively while carboxin treatment gave the least post-emergence mortality. **Youssef *et al.* (1995)** reported that treating the delinted seeds of Egyptian cotton cv Giza 45 with monceren fungicide improved seed germination and seedling growth

characteristics. Also, **Ahmed *et al.* (2000)** found that dressing cotton seeds with Monceren 25% improved germination and plant height in non-infested soil. Similar results were obtained also by **Felaifel *et al.* (2000)** and **EL-Deeb *et al.*(2002)** whom demonstrated that the fungicides, Vitavax-T, Rizolex-T and Topsin-M70 increased many of growth characters and pod yield of peanut compared to the non-treated.

On the other hand, treating cotton seeds with fungicides and commercial bioagents reduced root rot incidence under field conditions. Maxim and Topsin-M followed by Vitavax-T70 were the most effective fungicides on reducing disease incidence of cotton plants (cvs Giza-86 and Giza-89) during growing seasons 2000 and 2001. Also, Rizo- N was the most effective bioagent on reducing disease incidence and increasing the survived plants of both cotton cvs during the two growing seasons. Meanwhile, treating cotton seeds with fungicides or commercial bioagents before sowing for controlling root rot pathogens increased also plant height, number of fruiting branches on growing plants, number of the opened bolls/plant, weight of yielded cotton for each boll, cotton yield (g)/plant, average yield of cotton lint (g)/plant, yield of cotton seeds (g)/plant, yield of cotton (kantar/feddan), yield of cotton lint (kantar/feddan), yield of cotton seeds (kg/feddan) and fiber length and strength of yielded cotton of both cotton cvs (Giza-86 and Giza-89) during the two growing seasons comparing to un-treated seeds (control). Generally, Vitavax-T70, Vitavax-T40 and Rizo-N were the best seed treatments in this respect. It is pronounced also that Plantguard and Rizo-N were more effective than many of the

tested fungicides, *i.e.*, Maxim, Topsin-M and Rizolex-T as well as un-treated control. The obtained results are in harmony with those obtained by **Abdel–Shahaid *et al.*(1989), Youssef *et al.* (1995), Helal *et al.* (1996), Ahmed *et al.* (2000), Felaifel *et al.* (2000), El–Safety *et al.* (2001) EL-Deeb *et al.*(2002)** and **Goulart (2002)**.

SUMMARY

SUMMARY

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 the different stages of growth. Cotton seedling diseases whether pre or post emergences are world wide problem, often causing serious stand losses. A number of soil and seed borne pathogens can infect cotton seedlings individually or in association as a disease complex. Cotton plants are subjected to attack by various pathogenic fungi causing several diseases during different stages of growth.

The obtained results of the present study could be summarized as follows:

1- Isolation trials from different parts of cotton seeds of cvs Giza-86 and Giza-89 (before and after delinting, testa and cotyledons) resulted in several fungi which belonging to 5 genus and 11 species. The isolated fungi were purified and identified as *Alternaria alternata, Aspergillus niger, Fusarium dimerum, Fusarium moniliforme, Fusarium nivale, Fusarium roseum, Fusarium semitectum, Fusarium tricinectum, Fusarium solani, Penicillin spp* and *Rhizoctonia solani.* As well as, some isolated fungi stayed unknown without identification.

2- The isolated fungi from different parts of cotton seeds as well as rotten roots of Giza-86 and Giza-89 were differed in their frequencies from part to another. Generally, *R. solani* was the highest frequent fungus followed by *Fusarium*

moniliforme and *Fusarium roseum* and then the others *Fusarium* spp.

3- It is pronounced that many of isolated fungi whether before or after delinting like *Alternaria alternata, Aspergillus niger, Penicillium spp* and *Fusarium semitectum* were isolated at low frequencies from seeds of Giza-86 and Giza-89. Also, it is clear that the total isolated number from two cvs of cotton seeds after delinting were lesser than those before delinting. The total number of isolated fungi from cotyledons was greatly low comparing with those of inner surface of seed testa for both cotton cvs tested.

4- Regarding the isolated fungi from rotten roots of cotton seedlings, six isolates were isolated from rotten roots of Giza-86, two of them were belonging to *R.. solani*. Meanwhile, 14 isolates were isolated from rotten roots of Giza-89, 5 isolates of them were belonging to *R.. solani*. Also, *R.. solani* was the most frequent fungus followed by *Fusarium moniliforme* and *Aspergillus niger*. However, *Penicillium spp.* and *Fusarium tricinectum* recorded the lowest frequent fungi that isolated from rotten roots of cotton seedlings.

5- Concerning pathogenicity, *R. solani* caused the highest % pre-emergence damping –off followed by *F. semitectum, F. moniliforme* and *F. roseum*. Meanwhile post emergence damping-off, *R.solani* and *F. semitectum* were the most virulent pathogens at this disease stage, while *F. roseum* was the least virulent one. Also, increasing the inoculum levels from 1 to 3% increased gradually % pre– and post emergence damping–off with all tested pathogens. As for survival %, it

is pronounced that raising inoculum level from 1 to 3 % gradually decreased percentages of survived cotton plants.

6- Infestation the soil with root rot pathogens increased the reducing, non-reducing and total sugars in leaves of cotton plants of both cvs (Giza-86 and Giza-89). The highest increases in reducing sugars in cotton leaves of cvs Giza-86 and Giza-89 were recorded in case of infestation the soil with *F. moniliforme* followed by *R. solani* and *F. semitectum*, respectively while the lowest amount of reducing sugars in case of soil infested with *F. roseum* comparing to un-infested soil (control).

7- As for non – reducing sugars in cotton leaves of both cvs (Giza-86 and Giza-89), the same trend was true where, the highest increase was recorded also in case of infestation the soil with *F. moniliforme* followed by *R. solani* and *F. semitectum* respectively while, the least increase was in case of infested soil with *F. roseum* comparing with un-infested soil.

8- Regarding phenols, it is clear that infestation the soil with root rot pathogens affected positively the content of total, free and conjugated phenols in leaves of cotton plants cvs, Giza-86 and Giza-89. In this respect, the highest increase in amount of determined phenols (total, free and conjugated phenols) of both cvs (Giza-86 and Giza-89) were recorded in case of infestation the soil with *R.solani* followed by *F. moniliforme* and *F. semitectum* respectively while the lowest ones were recorded in case *F. roseum* . It is clear also that all

determined phenols content were higher in general in cv. Giza-89 than those determined in case of Giza-86.

9- Infestation the soil with tested root rot pathogens i.e. *R. solani*, *F. moniliforme*, *F. semitectum*, and *F. roseum* before sowing cotton seeds in were affected negatively the content of chlorophyll when determined in leaves of cotton seedlings of both tested cvs after 21 days post sowing. In this respect, all the tested pathogens decreased the content of chlorophyll A and B and then total chlorophylls in cotton leaves comparing with uninfested soil (control) of both two cvs (Giza-86 and Giza-89). The highest decrease in chlorophyll A and B as well as total chlorophyll was recorded in case of infestation the soil with *F. moniliforme*, *F. semitectum*, *F. roseum* and *R. solani* respectively comparing with uninfested soil (control) of both two cultivars. The results indicated also that infestation the soil before sowing with root rot pathogens do not affect clearly on carotenoids content in leaves of both tested cvs comparing with un-infested soil (control).

10- As for the variation in fractionated protein patterns of two cotton cultivars (Giza-86 and Giza-89) due to infection with *Rhizoctonia solani* and *Fusarium* spp, it is pronounced that the infection with those pathogens increased the number of fractionated protein bands comparing with control treatment (un-infected). In this respect, the infected cotton plants (Giza-86) with *R. solani* revealed 15 protein bands comparing with control plants (11 band), some of them are similar in their molecular weight to as in control while some

others are newly formed corresponding to infection with the pathogen.

11- Meanwhile, infection with *F .moniliforme* and *F. roseum* on cv. Giza 86 revealed 12 protein bands. some of them are new formed. On the other hand, the inoculated cotton plants (Giza-86) with *F. semitectum* revealed 16 protein bands comparing with the un-inoculated ones (11 band) where the new formed bands were 192.6, 148.4, 116.2, 93.1, 47.7, 40.1, 28.9 and 16.1 KDa. The results indicate also that the inoculated cotton plants (Giza-86) with *F. roseum* and *F. semitectum* revealed typical protein bands at 192.6, 148.4, 93.1 and 16.1 KDa as well as they differed completely with those protein bands of *R. solani* infection, meanwhile they were similar partially with the inoculated with *F. moniliforme* at 16.1 KDa.

12. Moreover, The inoculated cotton plants (Giza-86) with *R. solani* and *F. moniliforme* produced typical bands in response to infection at 100.0, 40.1 and 17.5 KDa. These appeared protein bands are differed in their condense where some of them are clear faint and some others appeared intensive although they have the same molecular weight.

13- As for cotton cv Giza-89, there were clear variations in fractionated protein patterns in leaves due to infection with *Rhizoctonia solani* and *Fusarium spp.* These exhibited protein bands were ranged between 208.6-11.9 KDa. It is pronounced that the infection with *R. solani* and *F. roseum* revealed protein bands lesser in their number comparing with control treatment (un-infected)

meanwhile, only the infected plants with *F. semitectum* revealed protein bands more than in control treatment.

14- On the other hand, all infected cotton plants (Giza-89) produced few of new bands as in response to infection with root rot pathogens like 17.5 KDa in case of *R. solani*, 20.1 KDa with *F. moniliforme*, 82.9 and 21.6 KDa with *F. roseum* as well as, 130.9, 99.5, 96.3, 46.4, 36.3, 30.4 ,16.1 and 13.8 KDa in case of *F. semitectum*. On the other hand, the inoculated cotton plants (Giza-89) with *F. roseum* and *F. semitectum* revealed typical protein band at 21.6 KDa.

15- Infestation the soil with tested root rot pathogens i.e. *R. solani*, *F. moniliforme*, *F. semitectum*, and *F. roseum* before sowing cotton seeds in this soil do not affect esterase isozyme content in cotton leaves of growing seedlings at 21 days old in case of cvs Giza-86 and Giza-89 comparing to un-infested one (control). In this respect, all resulted protein patterns of esterase isozyme of all infestation cases were equal to those appeared with control treatment where they taken the same values of Rf. The only exception was for *R. solani* which formed one new protein band at 0.36 with cv Giza-86 and five new protein bands of esterase isozyme at Rf values 0.21, 0.36, 0.62, 0.71 and 0.79 with cv Giza-89.

16- Infestation the soil with tested root rot pathogens *i.e.*, *R. solani*, *F. moniliforme*, *F. semitectum*, and *F. roseum* before sowing cotton seeds in this soil incited clear

variations in peroxidase isozyme patterns of two tested cotton cvs Giza-86 and Giza-89.

17- As for Giza-86, it is pronounced from the obtained results that the infestation with *R.solani* formed two new protein bands at Rf 0.10 and 0.12 comparing with control treatment (un-infected), meanwhile, the infestation with *F. moniliforme* do not reveal any new bands comparing with control treatment (un-infected). Whereas, the infestation with *F. semitectum,* and *F. roseum* revealed only one new band of peroxides isozyme at Rf. 0.10.

18- Concerning cv Giza-89, infestation the soil with tested root rot pathogens resulted in clear changes in formation of peroxidase isozyme where many protein bands were disappeared comparing to those of un-infested one (control), meanwhile, no one of new protein bands were formed with all infestation treatments.

19- Infestation of cotton seeds with tested root rot pathogens i.e. *R. solani, F. moniliforme, F. semitectum,* and *F. roseum* decreased the percentages of oil content into both tested cotton seeds of cvs Giza-86 and Giza-89 comparing with uninfested seeds (control) at all incubation days which ranged between 5-15 days. It is clear also that increasing incubation days from 5-15 days decreased gradually the determined percentages of oil contents for all tested pathogens comparing with un-infested seeds (control). The highest decrease in percentages of determined oil contents were recorded in case of infestation the seeds with *R. solani*

and *F. moniliforme* at all tested incubation days for seeds of both cotton cvs.

20- Regarding mycotoxin production, of the tested fungi that isolated from cotton seeds were not able to produce any of aflatoxins (B1 & B2), zearalenane, fumonsins and trichothecenes when grown *in vitro* on specific YES medium.

21- On the other hand, infestation the cotton seed samples of both cvs (Giza-86 and Giza-89) with those tested root rot pathogens produced clear amounts of mycotoxins (ppb) in some cases. In this respect, *F.semitectum* and *F. roseum* produced zearlenone mycotoxin onto infected seeds of cv.Giza-86 and cv.Giza-89 while, *R. solani* and *F. moniliforme* were not able to produce Zearlenone mycotoxin onto cotton seeds of both tested cvs. As for fumonisins mycotoxins, only *F. moniliforme* produced onto infected seeds of Giza-86 and cv.Giza-89. In addition, no one of the four tested isolates was able to produce aflatoxins onto infested cotton seeds, meanwhile it is pronounced that aflatoxins were appeared only on naturally contaminated cotton seeds of both tested cvs.

22- All of tested fungicides reduced the growth of all the tested pathogens. In this respect, they reduced the linear growth of *R. solani* where Premis, Maxim and Topsin-M were the best effective fungicides. It is clear also that Topsin-M was the highest effective one at the concentrations 5-400ppm followed by Premis and Maxim at concentrations of 10-400ppm. On the other hand, Maxim

and Primes were more effective than Topsin-M at concentration 1ppm. Moreover, the effective fungicide in reducing the growth of *R. solani* was Vitavax-T70. Also increasing the concentration from 1 to 400ppm increased gradually the effect of tested fungicides in reducing the growth of *R. solani* where the concentration like 200 and 400 ppm were more effective than others.

23- Also, Maxim and Premis as well as Topsin-M were the most effective fungicides in reducing the growth of *Fusarium moniliforme*. On the other hand, Rizolex-T , Vitavax-T70 and Vitavax-T40 were less effective than the frist three fungicides although they reduced the growth of *F. moniliforme*. On the other hand, Premis followed by Topsin-M were the highest effective fungicides in reducing the growth of *Fusarium roseum* and *Fusarium semitectum* more than other tested fungicides. Premis fungicide and Topsin-M gave the highest reduction at all tested concentrations except 1 ppm for the second.

24- Concerning the effect of bioagents, Rizo-N and Plantguard bioagents reduced the linear growth of all tested root-rot pathogens comparing to un-treated one (control) where, Rizo-N and Plantguard succeeded in reducing the linear growth of *R. solani*, *F. roseum*, *F. moniliforme* and *F. semitectum* to remarkable values. Moreover, Rizo-N was better than Plantguard in its effect in reducing the linear growth of all tested fungi.

25- As for the pathogenic potentialities of tested fungi whether individual or in combination in presence or absence of any

fungicidal treatment. It is clear that sowing cotton seeds of cvs Giza-86 and Giza-89 in soil infested with any of the pathogenic fungi whether individual or in combination in absence of any fungicidal treatment resulted in high percentage of dead plants. The high percentages of dead plants were occurred with the combined inoculum of the for tested fungi i.e., *R.. solani*, *F. moniliforme*, *F. roseum* and *F. semitectum*. Meanwhile, treating cotton seeds with fungicides before sowing in infested soil with fungi whether individual or in combination resulted in a clear reduction in percentage of dead plants. Fungicides Topsin-M, Premis and Maxim were the best effective while, Rizolex-T was the least effective one for reduction in pathogens.

26- Also, treating cotton seeds with fungicides before sowing in un-infested soil resulted in the least percentage of dead plants with superiority of Premis and Maxim over Topsin-M, Vitavax-T70, Vitavax-T40 and Rizolex-T.

27- Moreover, it could be concluded that the fungicides Premis , Topsin-M70, and Maxim might be the best in controlling cotton root–rot which caused by any of *R. solani*, *F. moniliforme*, *F. roseum* or *F. semitectum* and in case of the combined infection of them. Disease incidence on Giza-89 cv, in most treatments was lower than its corresponding values on Giza-86 .

28- On the other hand, treating cotton seeds of Giza-86 or Giza-89 cvs with commercial antagonists (Rizo-N or Plantguard) before sowing in infested soil with pathogenic

fungi whether individual or in combination decreased significantly the dead plants comparing with the control. It was clear that treating cotton seeds cv Giza-86 with Rizo-N or Plantguard antagonists reduced the infection of cotton seedlings to low values. Meanwhile, treating the cotton seeds with Rizo-N was better than Plantguard in reducing the root rot infection of cotton seedlings.

39- Generally, it could be concluded that the commercial antagonists whether Rizo-N or Plantguard might be useful in controlling cotton root–rot infection caused by *R. solani*, *F. moniliforme*, *F. roseum* and *F. semitectum* on cotton seedlings of both cvs. Giza-86 or Giza-89

30- Treating cotton seeds (cvs Giza-86 and Giza-89) with certain fungicides or antagonists before sowing increased significantly the shoot length of most resulted seedlings at 21 days old. In this respect, the highest increase in the shoot length of resulted seedlings (cv Giza-86) was in case of treating seeds with Rizolex-T and Rizo-N in soil infested with *F. moniliforme*. Meanwhile, Rizo-N followed by Topsin-M were the best seed treatments in increasing the shoot lengths of cotton seedlings (cv Giza-89) in soil infested with *R. solani*.

31- Also, all seed treatments of cv Giza-89 whether with fungicides or antagonists in soil infested with *F. semitectum* reduced the shoot lengths of resulted seedling to lengths lesser than in control-1 treatment. On the other hand, treating the cotton seeds with fungicides or

antagonists before sowing in normal soil without infestation (control-2) with any of tested root rot fungi improved the shoot length of resulted seedlings in case of Maxim, Vitavax-T40, Rizo-N and Plantguard treatments more than other treatments and control. In general, Rizolex-T and Maxim were the best tested fungicides in increasing the shoot lengths of resulted seedlings of cv Giza-86 while, Rizo-N, Topsin-M and Vitavax-T70 were the best in case of cv Giza-89.

32- Also, treating cotton seeds cv Giza-86 with fungicides or antagonists before sowing in infested soil with tested pathogenic fungi reduced significantly the root length, and dry weight of resulted seedlings in most cases of treatments comparing with un-treated seeds in infested soil with tested fungi (control-1). However, some fungicides treatments increased the seedlings root length like Premis seed treatment with soil infested with *R. solani* and soil infested with *F. semitectum*. As well as, there was a clear increase in root length in case of Maxim and Vitavax-T70 and Plantguard seed treatments before sowing in soil infested with *F. semitectum*.

33- Also, treating cotton seeds with fungicides or antagonists before sowing in normal soil without infestation with any of pathogenic fungi (control-2) improved the root length of resulted seedlings more than un-treated seeds in normal soil (control-1). On the other hand, treating the cotton seeds cv Giza-89 with Topsin-M fungicide before sowing in infested soil with *R. solani* or *F. moniliforme* only increased the

root length of resulted seedlings comparing with other fungicides or antagonists treatments. Meanwhile, all seed treatments with fungicides or antagonists before sowing in infested soil with *F. semitectum* decreased the root lengths of resulted seedlings to lengths lesser than that of control-1 treatment. On the other hand, seed treatment with Rizolex-T was the best treatment in case of infested soil *F. roseum* in increasing significantly the root length of resulted seedlings comparing with other treatments and control.

34- In the same time, treating the seeds of Giza-89 with fungicides or antagonists before sowing in normal soil (control-2) reduced the root lengths compared to control-1 treatment. Also, treating the cotton seeds cv Giza-89 with any of fungicides or antagonists before sowing in soil infested with *R. solani* reduced the dry weight of resulted seedlings at 21 days old. While all seed treatments with any of tested fungicides and antagonists before sowing in infested soil with *F. moniliforme* increased significantly the dry weight of resulted seedlings comparing with control-1 treatment where Vitavax-T70, Maxim, Premis and Topsin-M were the best seed treatments in this respect. Also, Rizo-N and Premis were the best seed treatments before sowing in soil infested with *F. semitectum* in increasing the dry weight of resulted seedlings comparing with control-1 treatment whereas other seed treatments reduced significantly the dry weight of resulted seedlings. On the other side, Rizo-N and Vitavax-T40 were the best seed treatments before sowing in the normal soil (control-2) in

increasing the dry weight of resulted seedlings in un-infested soil comparing with control-1 (un-treated seeds)

.35- On the other hand, treating cotton seeds with fungicides and commercial bioagents reduced root rot incidence under field conditions. Maxim and Topsin-M70 followed by Vitavax-T70 were the best effective fungicides on reducing root–rot incidence of tested cotton plants (cvs Giza-86 and Giza-89) during growing seasons 2000 and 2001. Also, Rizo- N was the best effective bioagent on reducing root-rot incidence and increasing the survived plants of both cotton cvs during the two growing seasons.

36- Meanwhile, treating cotton seeds with fungicides or commercial bioagents before sowing for controlling root rot pathogens increased also plant height, number of fruiting branches on growing plants, number of the opened bolls/plant, weight of yielded cotton for each boll, cotton yield (g)/plant, average yield of cotton lint (g)/plant, yield of cotton seeds (g)/plant, yield of cotton (kantar/feddan), yield of cotton lint (kantar/feddan), yield of cotton seeds (kg/feddan) and fiber length of yielded cotton of both cotton cvs (Giza-86 and Giza-89) during the two growing seasons comparing to un-treated seeds (control). Generally, Vitavax-T70, Vitavax-T40 and Rizo-N were the best seed treatments in this respect. It is pronounced also that Plantguard and Rizo-N were more effective than many of tested fungicides like Maxim, Topsin-M and Rizolex-T as well as un-treated ones (control).

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ARABIC SUMMARY

الملخص العربي

يعتبر القطن واحداً من أهم محاصيل الألياف والزيت فى مصر والكثير من دول العالم. يتعرض محصول القطن للعديد من الآفات الحشرية والأمراض الفطرية والبكتيرية والنيماتودية وغيرها من الآفات طوال مراحل نموه المختلفة. ومن أكثر الأمراض الخطيرة تلك التى تصيب بادرات القطن سواء قبل أو بعد الإنبات مسببة خسائر كبيرة. يتعرض القطن أيضاً لبعض الأمراض نتيجة الإصابة بمسببات الأمراض الكامنة فى البذور أو التربة سواء منفردة أو مجتمعة. ومن ثم لا تمر مرحلة من مراحل نمو القطن إلا وتعرض للإصابة بالكثير من الأمراض المتسببة عن الفطريات بصفة خاصة.

وقد أجريت هذه الدراسة بغرض التعرف على أهم الفطريات الممرضة المحمولة على البذرة والمسببة لأمراض بادرات القطن وتأثير إزالة زغب بذرة القطن بحامض الكبريتيك على تواجد تلك الفطريات المحمولة على البذرة. كما تناولت الدراسة أيضآ تقييما لتأثير بعض المبيدات الفطرية و المستحضرات الحيوية على مسببات أمراض بادرات القطن وعلى بعض خصائص النمو لنباتات القطن تحت ظروف المعمل والصوبة والحقل. كما شملت الدراسة أيضآ دراسة التغيرات البيوكميائية فى النبات كرد فعل على العدوى.

ويمكن تلخيص النتائج المتحصل عليها فى هذه الدراسة فيما يلي:

١- أجريت تجربة لعزل الفطريات المحمولة فى مواضع مختلفة من بذور صنفين من القطن هما جيزة – ٨٦ وجيزة –٨٩ (قبل وبعد إزالة الزغب، القصرة، الفلقات) وتم عزل العديد من الفطريات، تم التعرف على ١١ نوع تنتمي إلى ٥ أجناس بعد تنقيتها وتعريفها وهى ألترناريا ألترناتا، أسبرجلس نيجر، فيوزاريوم دايميرم، فيوزاريوم مونيليفورم، فيوزاريوم نيڤال، فيوزاريوم روزيم، فيوزاريوم سيميتكتم، فيوزاريوم تيرايسينكتم، فيوزاريوم سولانى، أنواع أخرى. بنيسليوم، ريزوكتونيا سولانى، إلى جانب بعض العزلات التي لم يتم التعرف عليها.

٢- اختلفت نسبة تكرار الفطريات المعزولة من بذور القطن والتي تسبب أعفان الجذور لصنفي القطن جيزة-٨٦ وجيزة-٨٩ من نفس الأماكن. عموماً كان ريزوكتونيا سولانى أكثر الفطريات تكرارا وتبعة فطر فيوزاريوم مونيليفورم و فطر فيوزاريوم روزيم وعزلات أخرى أغلبها تتبع جنس فيوزاريوم.

٣- كانت النسب التكرارية الفطريات المعزولة من البذور قبل أو بعد إزالة الزغب مثل ألترناريا ألترناتا، أسبرجلس نيجر، بنيسليوم، فيوزاريوم سيميتكتم منخفضة فى بذور الصنفين جيزة-٨٦، جيزة -٨٩. عموما كانت أعداد الفطريات المعزولة من البذور بعد نزع الزغب أقل من تلك المعزولة قبل إزالة الزغب. كذلك كانت أعداد الفطريات المعزولة من الفلقات قليلة جداً إذا ما قورنت بتلك المعزولة من الأسطح الداخلية لقصرة بذور صنفى القطن موضع الاختبار.

٤- تم عزل ٦ عزلات فطرية من جذور بادرات القطن صنف جيزة-٨٦ المتعفنة اثنتان منها تتبع فطر ريزوكتونيا سولانى. فى حين تم عزل ١٤ عزلة فطرية من جذور بادرات الصنف جيزة-٨٩ المتعفنة كانت خمسة منها تتبع الفطر ريزوكتونيا سولانى. كان الفطر ريزوكتونيا سولانى الأكثر تكراراً يليه فيوزاريوم مونيليفورم وأسبرجلس نيجر. أما أقل العزلات الفطرية المعزولة من جذور القطن المتعفنة تكراراً فكانت أنواع بنيسليوم وفيوزاريوم تيرايسينكتم.

٥- أثبتت اختبارات القدرة المرضية أن الفطر ريزوكتونيا سولانى أكثر الفطريات المعزولة قدرة على إحداث المرض، حيث سبب أعلى نسبة إصابة بموت البادرات قبل ظهورها فوق سطح التربة تلاه فى ذلك فطريات فيوزاريوم سيميتكتم، فيوزاريوم مونيليفورم وفيوزاريوم روزيم. بالنسبة لموت البادرات بعد ظهورها فوق سطح التربة كانت فطريات ريزوكتونيا سولانى وفيوزاريم سيميتكتم أكثرها إحداثاً للمرض فى تلك المرحلة. بينما كان أقلهم خطورة فطر فيوزاريوم روزيم. لوحظ أن خطورة الإصابة تزداد بزيادة مستوى اللقاح من ١ إلى ٣% لكل

الفطريات المختبرة. كما لوحظ أن نسبة نباتات القطن الحية المتبقية تتناقص تدريجياً بزيادة معدلات اللقاح من ١ إلى ٣%.

٦- أسفر تقدير السكريات المختزلة والغير مختزلة والكلية فى أوراق صنفي القطن (جيزة-٨٦ وجيزة-٨٩) المنزرعة فى تربة معداة بالفطريات المسببة لأعفان الجذور عن وجود زيادة ملحوظة مقارنة بتلك المنزرعة فى تربة غير معداة. وسجلت أعلى معدلات الزيادة فى السكريات المختزلة فى أوراق نباتات صنفى القطن السابقين المنزرعة في تربة معداة بفيوزاريوم مونيليفورم يليها تلك المعداة بريزوكتونيا سولانى و فيوزاريوم سيميتكتم على التوالى. بينما سجلت أقل كمية من السكريات المختزلة فى أوراق النباتات المنزرعة فى تربة معداة بفيوزاريوم روزيم مقارنة بالتربة الغير معداة (الكنترول).

٧- أظهر إخبار السكريات الغير مختزلة فى أوراق كلا صنفى القطن (جيزة-٨٦، جيزة-٨٩) أنها أخذت نفس المنحى السابق، حيث بلغت أقصى زيادة أيضاً فى التربة المعداة بفيوزاريوم مونيليفورم يليه التربة المعداة بريزوكتونيا سولانى ثم فيوزاريوم سيميتكتم على التوالي، بينما سجلت أقل زيادة فى أوراق القطن المنزرعة فى تربة معداة بفيوزاريوم روزيم مقارنة بالتربة الغير معداة.

٨- فيما يخص الفينولات: تبين أن زراعة نباتات القطن صنفى جيزة-٨٦ وجيزة-٨٩ فى تربة معداة بمسببات أعفان الجذور تزيد محتوى الأوراق من الفينولات الكلية، الفينولات الحرة و الفينولات المرتبطة. وقد سجلت أعلى نسبة زيادة فى كمية الفينولات السابقة (ملجم/جم) من الوزن الغض لأوراق كلا القطن صنفى عندما زرعت البذور فى تربة معداة بريزوكتونيا سولانى يليها تلك المعداة بفيوزاريوم مونيليفورم ثم فيوزاريم سيميتكتم على التوالى. بينما سجلت أقل نسبة فينولات فى أوراق النباتات المنزرعة فى تربة معداة بفيوزاريوم روزيم. وعموماً لوحظ أن محتوى الفينولات المقدرة كان أعلى دائما فى أوراق الصنف جيزة-٨٩ عن مثيلتها المقدرة فى الصنف جيزة-٨٦.

٩- أثرت عدوى التربة بمسببات أعفان الجذور مثل ريزوكتونيا سولانى، فيوزاريوم مونيليفورم، فيوزاريوم سيميتكتم وفيوزاريوم روزيم قبل زراعة بذور القطن فيها سلبياً على محتوى الكلوروفيل فى أوراق بادرات كلا صنفى القطن (ملليجم/جم من الوزن الغض) بعد ٢١ يوم من الزراعة. فقد لوحظ انخفاض فى الكلوروفيل (أ، ب) والكلوروفيل الكلي مقارنة بمحتوى أوراق صنفى القطن(جيزة-٨٦، جيزة-٨٩) المنزرعة فى تربة غير معادة (الكنترول). سجل أعلى انخفاض فى محتوى الكلوروفيل (أ، ب) والكلوروفيل الكلى فى العينات المأخوذة من نباتات زرعت فى تربة معداة بفيوزاريوم مونيليفورم، فيوزاريوم سيميتكتم، فيوزاريوم روزيم و ريزوكتونيا سولانى على التوالى مقارنة بتلك المنزرعة فى تربة غير معداة (الكنترول) بكلا صنفى القطن. وبتقدير محتوى أوراق صنفى القطن (سواء المنزرعة فى تربة معداة أو غير معداة) من الكاروتينات لم يلاحظ أى تغير فيه.

١٠- بتفريد البروتينات المستخلصة من صنفى القطن (جيزة-٨٦، جيزة-٨٩) المصابة بريزوكتونيا سولانى، فيوزاريوم تبين أن عدد حزم البروتين المتكونة نتيجة الإصابة أكثر منها فى الكنترول (الغير مصاب). وبمطابقة الحزم البروتينية فى نباتات القطن (جيزة-٨٦) المصابة بريزوكتونيا سولانى أظهرت ١٥ حزمة بروتينية مقارنة بالكنترول (١١ حزمة بروتينية) بعضها متشابهة فى الوزن الجزيئى مع الكنترول بينما البعض الأخر ظهر نتيجة العدوى بمسببات الأمراض.

١١- ثبت أن عدوى صنف القطن (جيزة-٨٦) بفيوزاريوم مونيليفورم أظهر ١٢ حزمة بروتينية بعضها تكون نتيجة الإصابة. فى حين تسببت عدوى نفس الصنف بفيوزاريوم سيميتكتم فى تكوين ١٦ حزمة بروتينية مقارنة بالكنترول (١١حزمة بروتينية) وكانت الحزم البروتينية الجديدة ذات أوزان ١٩٢,٦ ، ١٤٨,٤ ، ١١٦,٢ ، ٩٣,١ ، ٤٧,٧ ، ٤٠,١ ، ٢٨,٩ ، ١٦,١ كيلودالتون. لوحظ أن هناك تشابه بين الحزم البروتينية المتكونة نتيجة إصابة صنف جيزة-٨٦ بفيوزاريوم روزيم، فيوزاريم سيميتكتم عند أوزان ١٩٢,٦ ، ١٤٨,٤ ، ٩٣,١ ، ١٦,١

كيلودالتون. ولكنها تختلف تماماً عن الحزم المتكونة نتيجة الإصابة بريزوكتونيا سولانى، وتتشابه إلى حد ما البروتينات المتكونة نتيجة الإصابة بفيوزاريوم مونيليفورم عند ١٦,١ كيلودالتون.

١٢- وجد أن عدوى نباتات القطن (جيزة-٨٦) بريزوكتونيا سولانى وفيوزاريوم مونيليفورم تسببت فى إنتاج حزم متطابقة إستجابة للعدوى عند ١٠٠، ٤٠,١ ، ١٧,٥ كيلودالتون. إلا أن الحزم المتكونة تتباين فى درجة كثافتها بين الحاد والشاحب رغم أن لها نفس الوزن الجزيئي.

١٣- بالنسبة لصنف القطن (جيزة- ٨٩) ظهرت اختلافات واضحة بين حزم البروتين المستخلص من أوراق النباتات المصابة صنف (جيزة- ٨٩) نتيجة الإصابة بريزوكتونيا سولانى وبفيوزاريوم. تراوحت أوزان تلك الحزم بين ١١,٩ و ٢٠٨,٦ كيلودالتون. من الواضح أن الإصابة بريزوكتونيا سولانى وفيوزاريوم روزيم أظهرت حزم بروتينية أقل عدداً مقارنة بالكنترول، بينما أظهرت النباتات المصابة بفيوزاريوم سيميتكتم حزم بروتينية أكثر من الكنترول.

١٤- من ناحية أخرى أنتجت نباتات الصنف جيزة -٨٩ قليلاً من الحزم الروتينية الجديدة كاستجابة للإصابة بمسببات أمراض أعفان الجذور مثل إنتاج حزمة وزنها ١٧,٥ كيلودالتون عند الإصابة بريزوكتونيا سولانى ، ٢٠,١ كيلودالتون عند الإصابة بفيوزاريوم مونيليفورم و ٨٢,٩، ٢١,٦ كيلودالتون عند الإصابة بفيوزاريوم روزيم، علاوة على ذلك تكونت حزم أوزانها ١٣٠,٩ ، ٩٩,٥ ، ٩٦,٣ ، ٦٤,٤ ، ٣٦,٣ ، ٣٠,٤ ، ١٦,٤-١ ، ١٣,٨ كيلودالتون عند الإصابة بفيوزاريوم سيميتكتم. من ناحية أخرى، ظهرت حزمة متطابقة وزنها ١٢,٦ كيلودالتون فى نباتات القطن (جيزة -٨٩) المصابة بفيوزاريوم روزيم، وفيوزاريوم سيميتكتم.

١٥- وجد أن عدوى التربة بمسببات أعفان الجذور مثل رايزوكتونيا سولانى وفيوزاريوم سيميتكتم وفيوزاريوم روزيم قبل زراعة بذور القطن لا تؤثر على

محتوى أوراق بادرات القطن (جيزة -٨٦ ، جيزة -٨٩) عمرها ٢١ يوم من المشابه الإنزيمي إستيريز مقارنة بالكنترول. كما وجد أن البروتينات الناتجة عن مشابه إنزيم إستيريز في جميع حالات العدوى متساوية مع مثيلتها في الكنترول حيث كان لهما نفس قيم الـ Rf. فيما عدا استثناء وحيد يتمثل فى تكوين حزمة بروتينية لها قيمة Rf تعادل ٠,٣٦نتيجة إصابة صنف جيزة ٨٦ برايزوكتونيا سولانى، كما ظهرت خمسة حزم جديدة لها قيم Rf هى : ٠,٢١ ، ٠,٣٦ ، ٠,٦٢ ، ٠,٧١ ، ٠,٧٩ عند إصابة الصنف جيزة ٨٩ برايزوكتونيا سولاني.

١٦- أيضاً لوحظ أن عدوى التربة بمسببات أعفان الجذور مثل رايزوكتونيا سولانى وفيوزاريوم سيميتكتم وفيوزاريوم روزيم قبل زراعة بذور القطن تؤثر بوضوح على محتوى أوراق بادرات القطن (جيزة -٨٦ ، جيزة -٨٩) من نماذج إنزيم بيروكسيديز أيزوزايم.

١٧- بالنسبة للصنف جيزة -٨٦ أظهرت النتائج أن عدوى التربة برايزوكتونيا سولانى نتج عنه حزمتين جديدتين من البروتين لهما قيم Rf هما ٠,١٠ ، ٠,١٢ إذا ما قورنت بالكنترول (التربة الغير معداة). بينما لم تتسبب عدوى التربة بفيوزاريوم مونيليفورم فى ظهور أى حزم جديدة مقارنة بالكنترول. أما عدوى التربة بفطريات فيوزاريم سيميتكتم ، وفيوزاريوم روزيم فأظهرت حزمة بروتينية جديدة فقط من أنزيم البيروكسيديز لها قيمة Rf تساوي ٠,١٠.

١٨- وجد أن زراعة الصنف جيزة -٨٩ فى تربة معداة بمسببات أعفان الجذور تسبب فى حدوث تغير واضح فى تكوين أنزيم البيروكسيديز يستدل عليه باختفاء العديد من حزم البروتين مقارنة بالكنترول (الغير معداة)، فى حين لم تتكون حزم بروتينية جديدة نتيجة كل معاملات العدوى.

١٩- عدوى بذور صنفي القطن (جيزة-٨٦، جيزة-٨٩) بمسببات أعفان الجذور وهى ريزوكتونيا سولانى، فيوزاريوم مونيليفورم فيوزاريوم سيميتكتم وفيوزاريوم روزبم قللت من النسبة المئوية لمحتوى البذور من الزيت مقارنة

(الكنترول) فى كل فترات التحضين التى تراوحت بين ٥ و ١٥ يوماً. وقد لـوحظ أن محتوى البذور من الزيت يتناقص تدريجياً بزيادة فترة التحضين من ٥ إلى ١٥ يوماً نتيجة العدوى بكل مسببات المرض السابقة مقارنة بالكنترول. وبلغت أعلـى نسبة انخفاض لمحتوى الزيت فى البذور المعداة بريزوكتونيا سولانى، فيوزاريـوم مونيليفورم عند كل فترات تحصين البذور المعداة لكلا الصنفين.

٢٠- بالنسبة لإنتاج الميكوتوكسين(السموم الفطرية) وجد أن جميـع الفطريـات المعزولة من بذور القطن لا تستطيع إنتاج افلاتوكسينات (ب١، ب٢)، زيرالينـان، فيومونسين و ترايكوثينات عندما تمت تنميتها فى المعمل على بيئة متخصصة مثل بيئة مستخلص الخميرة.

٢١- من ناحية أخرى وجد أن عدوى بذور القطن (جيـزة-٨٦، جيـزة -٨٩) بمسببات أعفان الجذور جعلتها تنتج كميات واضحة من الميكوتوكسين (ppb) فـى بعض الحالات. حيث وجد أن فطري فيوزاريوم سـيميتكتم وفيوزاريـوم روزيـم ينتجان ميكوتوكسين الزيرالينين فى بذور صنفى جيزة- ٨٦، جيزة- ٨٩ المصابة، بينما فطرى ريزوكتونيا سولانى وفيوزاريوم مونيليفورم فلم ينتجـا ميكوتوكسـين الزيرالينين فى بذور كلا صنفي القطن. بالنسبة لميكوتوكسين الفيومـونزنس، فقـد انتجه فطر فيوزاريوم مونيليفورم فقط فى بذور جيزة- ٨٦، جيزة-٨٩ المصـابة. علاوة على ذلك لم يثبـت أن أى مـن الأربعـة فطريـات المختبـرة قـد انـتج افلاتوكسينات فى بذور القطن المصابة إلا أنه يبدو أن الأفلاتوكسينات قد تنتج فقط على بذور كلا صنفي القطن المختبرة المصابة طبيعياً.

٢٢- أثرت كل المبيدات الفطرية المختبـرة علـى نمـو مسـببات الأمـراض المختبرة. تبين ذلك من خلال خفض أو تثبيط مبيدات بريمس و ماكسيم و توبسين-م بفعالية للنمو الخطى لفطر ريزوكتونيا سولانى، وكـان أكثـرهم فعاليـة مبيـد توبسين-م بتركيزاته من ٥ - ٤٠٠ جزء فى المليون يليه في ذلك مبيدات بـريمس وماكسيم بتركيزات من ١٠ - إلى ٤٠٠ جزء فى المليون. وعلى النقـيض أظهـر

مبيدى بريمس وماكسيم تفوقاً على التوبسين-م عند تركيز ١ جزء فـى المليـون. علاوة على ذلك فان أكثر المبيدات فعالية فى خفض نمو فطر رايزوكتونيا سولانى كان الڤيتافاكس ت٧٠. لوحظ أيضاً أن زيادة التركيز مـن ١ - ٤٠٠ جـزء فـى المليون زادت تدريجياً عمل على خفض أو تثبيط نمو رايزوكتونيا سولاني ومـن بينها كانت تركيزات مثل ٢٠٠ ، ٤٠٠ جزء فى المليون أكثرها فاعلية.

٢٣- كانت مبيدات ماكسيم و بريمس علاوة على توبسين- ام أكثـر المبيـدات الفطرية فعالية في تقليل نمو فيوزاريوم مونيليفورم. أما مبيدات رايـزوليكس-ت و الڤيتافاكس ت٧٠ و الڤيتافاكس ت٤٠ فكانت أقل المبيدات المختبـرة فعاليـة عـن السابقة فى خفض نمو فيوزاريوم مونيليفورم. ومن ناحية أخرى أظهر مبيد بريمس تلاه توبسين-م فعالية فى خفض نمو فيوزاريوم روزيم عن المبيدات الأخرى، أكثر من المبيدات الفطرية الأخرى المختبرة . أظهر مبيدي بريمس وتوبسين-م أعلـى معدلات خفض النمو بجميع التركيزات المختبرة فيما عدا تركيـز ١ جـزء فـى المليون بالنسبة للتو بسين. كان مبيدى بريمس و توبسين-م أكثر المبيدات فعاليـة فى خفض نمو فيوزاريوم سيميتكتم تحت ظروف المعمل.

٢٤- المركبات الحيوية التجارية المختبرة قللت نمو مسـببات أعفـان الجـذور المختبرة ومن أفضلها ريزو-ن الذى كان أكثر فعالية من البلانت جارد فى تخفيض النمو الخطي لجميع الفطريات المختبرة.

٢٥- درست القدرة المرضية للفطريات المختبرة سواء منفردة أو فى توليفة فى وجود أو غياب أي مبيد فطرى، وقد تبين أن زراعة بذور صنفى القطـن جيـزة-٨٦، جيزة -٨٩ فى تربة معداة بأى من الفطريات الممرضة سواء منفردة أو فـى توليفة فى غياب المبيدات الفطرية أدت إلى حدوث نسبة موت عالية بين بـادرات القطن، وكانت أكثر نسبة لموت البادرات عندما تمت عـدوى التربـة بفطريـات رايزوكتونيا سولانى وفيوزاريوم مونيليفورم وفيوزاريـوم روزيـم وفيوزاريـوم سيميتكتم. بينما وجد أن معاملة بذور القطن بالمبيدات الفطرية قبل زراعتهـا فـى

التربة المعداة بفطر أو مجموعة فطريات نتج عنه نقصاً واضحاً فى نسبة النباتات الميتة، وفى هذا الصدد أظهرت مبيدات توبسين-م، بريمس و ماكسيم فعالية أكثر من غيرها من المبيدات المختبرة وكان أقلهم فعالية ريزولكس-ت.

٢٦- لوحظ أيضاً أن معاملة بذور القطن بالمبيدات الفطرية قبل زراعتها فى تربة غير معداة أعطت أقل نسبة موت للبادرات، وتفوق فى ذلك مبيدات بريمس وماكسيم عن مبيدات توبسين-م والڤيتافاكس ت٧٠ و الڤيتافاكس ت٤٠ وريزوليكس-ت.

٢٧- يستنتج مما سبق أن المبيدات الفطرية بريمس وتوبسين-م وماكسيم كانت أفضل المبيدات المختبرة فى مقاومة مسببات أعفان الجذور التى تسببها فطريات رايزوكتونيا سولانى وفيوزاريوم روزيم وفيوزاريوم مونيليفورم وفيوزاريوم سيميتكتم، وحتى فى حالة الإصابة بتوليفة من تلك الفطريات. و فى حالة كل التراكيب التوافقية الناتجة عن هذه الفطريات. عموماً كانت نسبة حدوث المرض على الصنف جيزة ٨٩ فى معظم المعاملات أقل من القيم المماثلة فى حالة الصنف جيزة-٨٦.

٢٨- وجد أيضاً أن معاملة بذور القطن صنفى جيزة-٨٦ وجيزة-٨٩ بالمركبات الحيوية التجارية (الريزو-ن أو البلانت جارد) قبل زراعتها فى التربة المعداة بواحد أو أكثر من الفطريات الممرضة تقلل نسبة موت البادرات بصورة معنوية مقارنة مع نسبة الموت المرتفعة التى تحدث فى التربة المعداة بالفطريات الممرضة. معاملة بذور القطن صنفى جيزة-٨٦ وجيزة-٨٩ بالمركبات الحيوية التجارية الريزو-ن أوبلانت جارد قللت نسبة إصابة البادرات إلى أقل قيمة. علاوة على ذلك فان معاملة بذور القطن بالمركب الحيوى ريزو-ن كانت أفضل المعاملة بالبلانت جارد في خفض نسبة إصابة البادرات بأعفان الجذور.

٢٩- وجد أن المعاملة بالمركبات الحيوية التجارية سواء الريزو-ن أو البلانت جارد قد تكون مفيدة فى مقاومة تلوث بذور صنفى القطن جيزة-٨٩، جيزة-٨٦

بمسببات أعفان الجذور المتسببة عن فطريات رايزوكتونيا سولاني وفيوزاريوم مونيليفورم وفيوزاريوم روزيم وفيوزاريوم سيميتكتم.

٣٠- وجد أن معاملة بذور صنفي القطن جيزة-٨٦ وجيزة-٨٩ بمبيدات فطرية معينة والمركبات الحيوية قبل الزراعة يزيد طول الريشة زيادة معنوية فى معظم بادرات الناتجة بعد ٢١ يوم. وفى هذا الصدد سجلت أعلى زيادة فى طول الريشة فى البادرات الصنف جيزة-٨٦ الناتجة بعد معاملة البذور بالمبيد الفطرى الريزوليكس-ت والمركب الحيوى ريزو-ن فى التربة الملوثة بفطر فيوزاريوم مونيليفورم. وكانت المعاملة بالريزو-ن ثم توبسين-م أفضل معاملات البذرة المسببة لزيادة طول الريشة فى بادرات صنف القطن جيزة-٨٩ فى التربة المعداة بفطر رايزوكتونيا سولانى.

٣١- كل معاملات بذور صنف جيزة-٨٩ سواء بالمبيدات الفطرية أو المركبات الحيوية فى التربة المعداة بفيوزاريوم سيميتكتم تقلل طول ريشة البادرات الناتجة بكثير عن الكنترول-١. أما معاملة بذور القطن بالمبيدات الفطرية أو المركبات الحيوية قبل الزراعة فى التربة العادية (دون عدوى كنترول-٢) فقد أدت إلى زيادة طول ريشة البادرات الناتجة عن معاملة البذور بمبيدات ماكسيم وڤيتافاكس- ت٤٠ والمركبات الحيوية ريزو-ن أو البلانتا جارد أو الناتجة عن المعاملات الأخرى أو الكنترول. عموماً أثبتت النتائج أن مبيدات ريزوليكس-ت وماكسيم كانت أفضل المبيدات الفطرية المختبرة فى زيادة طول ريشة بادرات صنف جيزة-٨٦، بينما المركب الحيوى ر يزو-ن والمبيدات توبسين-م وڤيتافاكس- ت٧٠ كانت الأفضل فى حالة الصنف جيزة-٨٩.

٣٢- وجد أيضاً أن معاملة بذور القطن صنف جيزة-٨٦ بالمبيدات الفطرية أو المركبات الحيوية قبل زراعتها فى التربة المعداة بالفطريات الممرضة لها تأثير ملحوظ فى نقص طول الجذور، والوزن الجاف فى المعاملة إذا ما قورنت بالبذور الغير معاملة والمنزرعة فى التربة المعداة بالفطريات المختبرة (كنترول-١). ومن

المبيدات التى عوملت بها البذور وزادت من طول الجذور المعاملة بمبيد بريمس ثم زراعتها فى تربة معادة برايزوكتونيا سولاني أو فيوزاريم سيميتكتم. علاوة علـى ذلك أدت معاملة البذور بمبيدات ماكسيم وڤيتافاكس-ت٧٠ أو بالمركـب الحيـوى البلانتا جارد إلى زيادة طول جذور البادرات المعاملة حتى إذا زرعت فـى تربـة معداة بفطر فيوزاريوم سيميتكتم .

٣٣- وجد أن معاملة بذور القطن بالمبيدات الفطرية أو المركبات الحيوية قبـل زراعتها فى التربة العادية الغير معداة بأى من الفطريات الممرضة (كنتـرول-٢) تزيد من طول جذور البادرات الناتجة أكثر من تلك الناتجة من بذور غير معاملـة بالمبيدات الفطرية ومنزرعة فى تربة غير ملوثة (كنترول-١). من ناحية أخـرى، وجد أن معاملة بذور القطن صنف جيزة-٨٩ بالمبيد الفطـرى توبسـين-م قبـل الزراعة فى التربة معداة بفطر رايزوكتونيا سولانى أو فيوزاريوم مونيليفورم أدت إلى زيادة فى طول الجذور إذا ما قورنت ببقية المبيـدات أو المركبـات الحيويـة الأخرى التى أدت إلى تناقص طول الجذور فى أغلب الأحيان مقارنة بـالكنترول-١. بينما أدت كل معاملات البذور سواء بالمبيدات الفطرية أو المركبـات الحيويـة قبل الزراعة فى تربة معداة بفطر فيوزاريوم سيميتكتم إلى تقليـل طـول جـذور البادرات الناتجة مقارنة حتى بأقل الأطوال المسجلة فى معاملة (كنترول-١). ومن ناحية أخرى ، فإن معاملة البذور بالمبيد الفطرى رايزوليكس-ت كانـت أفضـل المعاملات فى حالة التربة المعداة بفطر فيوزاريوم روزبم حيث أدت لزيادة معنوية فى طول جذور البادرات الناتجة مقارنة بالبذور المعاملـة بالمبيـدات الفطريـة الأخرى أو معاملة الكنترول.

٣٤- فى نفس الوقت، وجد أن معاملة بذور القطن صنف جيزة -٨٩ بالمبيدات الفطرية أو المركبات الحيوية قبل زراعتها فى تربـة غيـر معـداة بالفطريـات (كنترول-٢) يقلل طول الجذور مقارنة بمعاملة الكنترول-١. أيضاً وجد أن معاملة بذور القطن صنف جيزة -٨٩ بأى من المبيدات الفطرية أو المركبات الحيوية قبل

زراعتها فى تربة معداة بفطر رايزوكتونيا سولانى تقلل الوزن الجاف للبادرات الناتجة بعد ٢١ يوم من الزراعة. بينما أدت معاملة البذور بأى من المبيدات الفطرية و المركبات الحيوية قبل الزراعة فى تربة معداة بفطر فيوزاريوم مونيليفورم إلى حدوث زيادة ملحوظة فى الوزن الجاف للبادرات الناتجة مقارنة بمعاملة الكنترول-١ وفى هذا الصدد كانت المعاملة بمبيدات ثيتافاكس-ت٧٠، وماكسيم و بريمس وتوبسين-م أفضل المعاملات. أيضاً وجد أن المركب الحيوى ر يزو-ن والمبيد الفطرى بر يمس كانت أفضل معاملات البذور قبل الزراعة فى التربة المعداة بفطر فيوزاريوم سيميتكتم حيث زادت من الوزن الجاف للبادرات الناتجة مقارنة بمعاملة الكنترول-١، فى حين تسببت بقية المعاملات الأخرى فى حدوث خفض معنوى فى الوزن الجاف للبادرات الناتجة. من ناحية أخرى ، وجد أن معاملة البذور بالمركب الحيوى ر يزو-ن والمبيد الفطرى ثيتافاكس-ت٤٠ كانت أفضل المعاملات قبل الزراعة فى تربة غير معداة (الكنترول-٢) واستدل عليها من زيادة الوزن الجاف للبادرات الناتجة مقارنة الكنترول-١ (البذور الغير معاملة).

٣٥- من ناحية أخرى، قللت معاملة بذور القطن بالمبيدات الفطرية أو المركبات الحيوية من التأثير المرضى لمسببات أعفان الجذور تحت ظروف الحقل. وكانت المعاملة بالمبيدات الفطرية ماكسيم و توبسين-م ٧٠ يليهما الثيتافاكس-ت٧٠ أفضل المعاملات فعالية فى خفض التأثير المرضي لمسببات أعفان الجذور لصنفى القطن المختبرين (جيزة-٨٦، جيزة-٨٩) خلال موسمى الزراعة ٢٠٠٠ ، ٢٠٠١ . ومن بين المركبات الحيوية كان ريزو-ن أفضلها في خفض التأثير المرضى لمسببات أعفان الجذور حيث زاد من نسبة النباتات الحية فى كلا صنفى القطن خلال ذات الموسمين الزراعيين.

٣٦- أدت معاملة بذور القطن بالمبيدات الفطرية أو المركبات الحيوية التجارية قبل زراعتها لمقاومة مسببات أعفان الجذور إلى زيادة من طول الساق، عدد

الأفرع المثمرة على النباتات، عدد اللوز المتفتح/نبات ، وزن اللوزة (جم)/نبـــات، محصول القطن (جم)/نبات، متوسط محصول القطن الشعر (جم)/نبات، محصـــول بذرة القطن (جم)/نبات، محصول القطن الزهر (قنطار/ فدان)، محصـــول القطـــن الشعر (قنطار/ فدان)، محصول بذرة القطن (كجم/ فدان) وطول شعرة القطن فى محصول كلا صنفى القطن (جيزة-٨٦ ، جيزة-٨٩) خلال الموسمين الـــزراعيين مقارنة بالبذور الغير معاملة (الكنترول). عموما، كانت معاملة البـــذور بمبيـــدات ڤيتافاكس-ت٧٠ وڤيتافاكس -ت٤٠ والمركب الحيوى ر يزو-ن أفضل المعاملات في هذا الصدد. كما تبين أن المركبات الحيوية بلانتا جارد و ريزو-ن لهما تـــأثير واضح مقارنة بالمبيدات الفطرية المختبرة مثل ماكسيم أو ريزوليكس-ت عن الغير معاملة (الكنترول).

دراســـات مرضيـــــة علي بـــذور القطـــن المستخدمـــة فى الزراعـــة

رسالة مقدمة من

السيـــد عبد الرحيـم حســـن

بكالوريوس علوم زراعية- كلية الزراعة بمشتهر- جامعة الزقازيق-فرع بنها ١٩٩١

ماجستير علوم زراعية (محاصيل)-كلية الزراعة- جامعة الأزهر ١٩٩٨

للحصول على

درجة دكتوراه الفلسفة فى العلوم الزراعية (أمراض نبات)

وقد تمت مناقشة الرسالة والموافقة عليها من قبل اللجنة :

الأستـــاذ الدكتـــور / محمـــد فاروق عطيـــة .....

أستـــاذ أمراض النبات - كلية الزراعـــة- جامعـــة القاهرة.

الأستـــاذ الدكتـــور / نـــوال عبد المنعـــم عيســـى .....

أستـــاذ أمراض النبات - كلية الزراعة بمشتهـــر- جامعـــة بنها.

الأستـــاذ الدكتـــور / جهاد محمد دسوقي الهبـــاء .....

أستـــاذ أمراض النبات - كلية الزراعة بمشتهـــر- جامعـــة بنها.

دكتـــور/ فتحى جـــاد محمـــد .....

أستـــاذ أمراض النبات المساعد - كلية الزراعة بمشتهـــر— جامعـــة بنها.

تاريخ الإمتحان : ١٦/ ١٢ /٢٠٠٦

دراســـات مرضيـــة علي بـــذور القطـــن المستخدمـــة فى الزراعـــة

رسالة مقدمة من

السيـــد عبد الـرحيـم حســـن

بكالوريوس علوم زراعية كلية- الزراعة بمشتهر- جامعة الزقازيق -فرع بنها١٩٩١

ماجستير علوم زراعية (محاصيل)-كلية الزراعة- جامعة الأزهر ١٩٩٨

لجنة الإشراف:

الأستـاذ الدكتـور / نـوال عبدا لمنعم عيسى .....

أستـــاذ أمراض النبات - كلية الزراعـــة بمشتهـــر— جامعـــة بنهـــا.

الأستـاذ الدكتـور / جهـاد محمـد دسوقى الهبـاء .....

أستـــاذ أمراض النبات - كلية الزراعـــة بمشتهـــر— جامعـــة بنهـــا.

الأستـاذ الدكتـور / محمـود إبراهيـم العميـرى .....

مديـــر معهـــد بحـــوث المحاصيـــل الحقلية - مركـــز البحـــوث الزراعيـــة.

دراســـا ت مرضيـــــة علي بـــذور القطـــن المستخدمـــــة فى الزراعـــة

رسالة مقدمة من

السيـــد عبد الـرحيـم حســـن

بكالوريوس علوم زراعية كلية- الزراعة بمشتهر- جامعة الزقازيق -فرع بنها ١٩٩١

ماجستير علوم زراعية (محاصيل)-كلية الزراعة -جامعة الأزهر ١٩٩٨

للحصـــول على

درجـة دكتـوراه الفلسفـة فى العلوم الزراعية

(أمراض نبات)

قسـم النـبات الزراعى

فـرع الفطـر وأمراض النبـات

كلية الزراعـة بمشتهـر

جامعـة بنهـا

٢٠٠٦