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*Full Length Research Paper*

# Pathological and rhizospherical studies on root-rot disease of squash in Saudi Arabia and its control

**Lobna Saleh Nawar**

Biology Department, Faculty of Science, King Abd El-Aziz University, Jeddah, Saudi Arabia. E-mail: lnawar@kau.edu.sa.

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**Isolations from diseased squash roots revealed the presence of *Alternaria tenuis, Aspergillus niger, Fusarium oxysporum, F. solani* and *Rhizoctonia solani.* The last two fungi were more frequent than any of the other fungi. Pathogenicity tests proved that squash plants were highly vulnerable to attack by *Fusarium solani* and *Rhizoctonia solani* in descending order, during the pre- and post-emergence stages. Isolates No.1 of *F. solani* and No.2 of *R. solani* were the most virulence pathogen and significantly retard the morphogenesis of survived squash plants. The in-vitro antagonistic effect of Trichoderma harzianum on root-rot pathogens revealed the presence of clear antagonistic action between them. The highest mean inhibition values were 78.86 and 41.94% RI against *F. solani* and *R. solani*, respectively. *T. harzianum* also exhibited a mycoparazitation associated with high level of growth reduction by its filtrate on the two pathogens. In vitro, benlate fungicide was found to be very toxic to the root-rot pathogens. Untreated squash seeds grown in the infested soil (positive control) with *F. solani* and *R. solani* showed higher percentage of infection. The plants grown under this treatment were significantly shorter with lesser weight than the corresponding figures of the other treatments. Soil or seed coated with *T. harzianum* and benlate had a significant lower percentage of infection (pre, post, dead plants and developed plants), significantly longer in height and better plant growth parameters. *T. harzianum* applied as soil or seed treatments enhancing the total microbial flora of squash rhizosphere at the first 30 days of plant growth and greatly increased the total fungal counts comparing with the corresponding figures of negative and positive controls. As the density of *T. harzianum* reached 99.3% and 100.0% in cultivated plants soil infested with *F. solani* and *R. solani* respectively, the other genera or species of fungi become greatly reduced or disappeared.**

**Key words:** squash, root-rot disease, Saudi Arabia

## INTRODUCTION

Squash *(Cucurbita pepo)* is one of the most important vegetable crops in Saudi Arabia that is consumed locally. Squash is subjected to attack by numerous pathogenic fungi, wherever the crop is grown. Root-rot caused by *Fusarium solani* and *Rhizoctonia solani* is considered among the most deleterious diseases, which cause great losses in many parts of the world including in Slovenia (Celar, 2000) in Italy (Fantino et al., 1989) and in Egypt (Madkour et al., 1983; Abdel-el-Rehim et al., 1987).

Biological control of plant diseases especially soilborne plant pathogens has been the subjected of much research in the last two decades. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan and Chet, 1994; Basim et al., 1999; Whipps and Lumsden, 2001; McLean et al., 2004). Although, biological control of root- disease fungi has been studied by many researches, few studies have been made on the control of squash root-rot disease by the use of *Trichoderma* spp. Hadar et al. (1979) and Elad et al. (1980) observed that the application of wheat bran colonized by *T. harzianum* to soil infested with *R. solani* and *Sclerotium rolfsii* reduced the incidence of disease caused by these pathogens in beans.

Considerable researches has been done to investigate antagonistic microbes for use in seed treatments as reported by Callan et al. (1990), Baird et al. (1994), Howell and Spepanovic (1995) and Mathre and Johnston (1995). Proliferation along the developing rhizosphere is one of the most important trails for antagonists applied to seed

as reported by Cook and Baker (1983). Benlate fungicide has been extensively used to enhance seed germination and seedling stand by reducing the severity of several diseases caused by various soilborne pathogens including *Pythium, Fusarium* and *Rhizoctonia* spp. (Baird et al., 1994; Mathre and Johnston, 1995).

The present study was conducted to specify the pathogens causing root-rot of squash in Saudi Arabia. Also the potency of an isolate of *Trichoderma harzianum* to protect squash plant by applying it to seed and soil was also tested. This study also examined the population dynamics of *Trichoderma* and *rhizospheric* microorganisms and its interactions with root-rot pathogens in squash rhizosphere.

## MATERIALS AND METHODS

### Isolation of pathogenic fungi

Squash seedlings and plants with symptoms of root-rot and wilt infections were collected from the growing areas at Saudi Arabia and brought to the laboratory. The infected samples were rinsed in tap water and the necrotic portions were excised and cut into 2-mm pieces, surface sterilized with 5% sodium hypochlorite for 30 s and rinsed in 4 successive changes of sterile distilled water. These were then plated on potato dextrose agar (PDA) and incubated at 28 ±2°C for up to 5 days under 12-h photoperiod.

Hyphal tip transfer and the single spore technique were adopted whenever possible. Pure cultures of fungal isolates were identified using cultural and morphological feature with reference to Gilman (1957), Burnett and Hunter (1972) and Nelson et al. (1983).

### Pathogenicity test

Pathogenicity of the isolated fungi was tested using potting soil bioassay (Arafa, 1985). The soil used in the pathogenicity test was sterilized before being infested with fungal isolates at the rate of 1% by weight. The inoculums of the pathogen was prepared by growing each tested fungus on sand : barley : water medium ( 1:3:3, w:w:w) for two weeks at 28±2°C. Squash seeds were surface-sterilized with 3% sodium hypochlorite solution for 3 min. 15 seeds were sown in each pot and the pre and post emergence damping off were recorded 15 and 60 days after planting, respectively. Plant heights as well as their fresh and dry weights were estimated also at the end of the experiment.

Disease assessment

Percentage of pre-emergence damping off was determined after 15 days as:

$$\% \text{ pre- emergence} = \frac{\text{No. of ungerminated seeds / pot}}{\text{No. of sown seeds / pot}} \times 100$$

Percentage of post- emergence damping off was determined after 60 days:

$$\% \text{ post- emergence} = \frac{\text{No. of died seedlings /pot}}{\text{No. of survival plants / pot}} \times 100$$

Percentage of diseased plants was determined at the end of experiment as:

$$\% \text{ diseased plants} = \frac{\text{No. of infected plants with root-rot /pot}}{\text{No. of survival plants / pot}} \times 100$$

Reisolation of fungi from diseased plants were then done and compared with the original isolate.

### In-vitro test for antagonistic activity

Five millimeter discs of *T. harzianum* isolate was removed from the edge of colonies of 4 to 6 days-old PDA cultures and placed on one side of a 90-mm-petri dishes containing PDA medium. Similar discs of *F. solani* and *R. solani* isolates grown in the same manner were placed on the opposite side of petri-dishes. Each treatment was replicated four times. Cultures were observed daily and the degree of antagonism was calculated according to Kucuk and Kivanc (2003) as the percentage reduction (RI) in mycelial growth of the pathogen by:

$$RI = \frac{G1 - G2}{G1} X\ 100$$

Where RI = Percentage of mycelial growth reduction, G1 = Growth of pathogen fungus in control and G2 = Growth of pathogen fungus in treated plates.

### Determination of antifungal properties of culture filtrates of *T. harzianum*

*T. harzianum* isolate was inoculated into 100 ml PD broth medium and incubated at 25ºC in the dark, on a rotary shaker set at 50 rpm (Abd-el-Moity and shatla, 1981) for 14 days. The culture was filtered through 0.22 mm Millipore filters. The aliquot (2 ml) of this filtrate was placed in sterile petri-dishes and 15 ml of ¼ strength PDA at 45ºC was added. After the agar solidified, mycelial discs of the pathogens (5 mm) were placed gently in the center of the agar plates. The petri-dishes were sealed with polythene wrap, inverted and incubated at 25 ± 2ºC. Growth of the pathogens was recorded by measuring the diameter of the colonies in four directions along pre-drawn times, each day for 7 days or until the colony reached the plate edge (McLean et al., 2004). There were 4 replicates for each treatment and the degree of antagonism was calculated as the percentage reduction (RI) in mycelial growth of the pathogen as mentioned before

### Effect of benlate fungicide on the linear growth of pathogenic fungi

Benomyl {(methyl 1-(butylcarbamoyl) benzimidazol-2-yl carbamate) (as benlate 50% w.p)} was used at concentrations of 0, 6.25, 12.5, 25.0, 50.0 and 100.0 ppm based on the active ingredients. The fungicide is added to PDA medium after sterilization, while still warm. A 5 mm disc of *F. solani* and *R. solani*, obtained from the edge of the fungus colony, was transferred to the center of the plate. After the colonies of the control plates were reached about 80-90 mm in diameter, record were taken for the other treatments as measures of two vertical diameters and the average was calculated.

**Greenhouse experiment**

Experiments were carried out in artificially infested sandy loam soil. Soil was infested with the two pathogens as mentioned before in the pathogenicity test. Experiment was set up with plastic pot each containing 2.5 kg of soil sown with 15 squash seeds. *T. harzianum* was applied as seed coating or soil treatment in wheat bran preparation. A conidial suspension was prepared from cultures grown in petri-dishes containing 15 ml of PDA. Spores suspension was prepared and adjusted to 6 x 1010 conidia/ml. A seed coating was prepared from conidia collected and supplemented with CMC (v/v) as an adhesive. 5 ml of this suspension were used to coat 20 g of squash seed, which were immediately dried by warm ventilation.

Wheat bran at 10% moisture was amended with 40 ml of tap water per 100 g. The mixture was then autoclaved in 250 ml Erlenmeyer flasks for 1 h at 121ºC. Flasks were inoculated with spore suspension of *T. harzianum* and incubated for 14 days at 25 ± 2ºC. This preparation was mixed with soil at concentration of 5 g/kg of soil.

During the course of the biocontrol evaluation study, seeds were planted at depth of 2 cm in clay pot (25 cm). The untreated seeds grown in the soil free of the pathogen were also used (negative control). The experiment contained nine treatments that were arranged in randomized complete block design with 4 replicates. Control seeds were sacked in distilled water for 15 min. Disease was monitored to the flowering stage. The plants were lifted out and plant length was measured (cm). Disease incidence was assayed as the total percentage of infection. The percentage of reduction for the disease incidence due to the different seed treatments was calculated based on the percentage of infection relative to the positive control (untreated seeds sown in infested soil). The fresh weight (g/plant) was recorded and then the plants were dried in an electric oven at 70ºC for 24 h to calculate the dry weight.

Rhizosphere samples as adopted by Louw and Weblely (1959) were taken 15, 30, 45 and 60 days after sowing. The total microbial flora count was carried on modified soil extract agar medium according to Mahmoud et al. (1964). Martin's medium (Allen, 1961) was used for the counts of the total fungi and PDA rosebengal agar medium amended with choloramphenicol ( 80µ/ml) for Trichoderma spp. counts ( Kucuk and Kivanc, 2003).

The data obtained in the present study were subjected to statistical analysis according to Steel and Torrie (1960) whenever needed.

## RESULTS AND DISCUSSION

### Root-rot pathogens of squash

Isolation from root-rotted squash plants revealed the association of one or more of the following five fungal species; Alternaria tenuis, Aspergillus niger, Fusarium oxysporum, 3 isolates of Fusarium solani and 3 isolates of Rhizoctonia solani with diseased roots. *F. solani* and *R. solani* were more frequent than any of the other fungi (Table 1).These fungi were previously reported to be associated with squash root-rot disease in other countries (Martyn and Mclaughin, 1983; Pushpa et al., 1999). Upon testing the pathogenicity of these isolated fungi, all of them except Aspergillus niger, were found more or less able to attack squash at any stage of plant growth. The data also show that squash plants were highly vulnerable to attack by *F. solani* and *R. solani*, in descending order, during the pre- and post-emergence stages (Table 1 and Figure 1). As reported by several investigators, *F. solani* and *R. solani* have long been known to be the main organisms causing root-rot of squash (Madkour et al., 1983, Abdel-el-Rehim et al., 1987; Fantino et al., 1989; Celar, 2000).

Isolates No.2 of *F. solani* and *R. solani* showed highest pre-emergence (33.33%) compared to the control plants (0%). *F. solani* (No.1) and *R. solani* (No. 2) have the highest post-emergence of 93.18 and 67.35%, respectively. Isolate No.2 of *R. solani* and No.3 of *F. solani* caused a significant increase in the percentage of diseased plant (54.17 and 47.50%, respectively) compared to the control. Reisolations from diseased roots yielded only the same fungus used for the artificial infestation of the soil.

Squash plants which survived in soil never attained the normal growth either in height or fresh and dry weights (Table 1). The height of plants which survived in soil infested with the fungal isolates tested averaged only 65.42% of that grown in uninfested soil. Again *F. solani* isolate No.1 was the most aggressive fungus, which reduced the shoot length from 39.18 cm in the control to 7.05 cm in infested soil. A similar trend was also observed with plant weight. Average fresh and dry weights of plants that survived in infested soil were 67.25 and 64.69% of that the control treatment, respectively. The lowest fresh and dry weights were recorded when soil was infested with *F. solani* (isolate No.1) and *R. solani* (isolate No.2). Average fresh and dry weights of plants that survived in infested soil with *F. solani* were 3.73 and 1.22 g/plant, and were 10.93 and 2.36 g/plant in soil infested with *R. solani*, respectively.

*F. solani* and *R. solani* were not only the most aggressive pathogen but also the most frequently encountered fungus. For this reason, special attention was paid to *F. solani* and *R. solani*. The pathogenic properties of *R. solani* and *F. solani* in this study are in agreement with results obtained by Elad et al. (1980) who recorded that these fungi were capable of attacking a tremendous range of host plants causing seed decay, damping off, root rot and fruit decay.

### *In-vitro* antagonistic action

Agar plates inoculated with the pathogenic fungal isolates of *F. solani* and *R. solani* and the isolates of *T. harzianum* (Table 2) revealed the presence of clear antagonistic action between them. Marked inhibition in the linear growth of the two fungi was occurred. The highest mean inhibition values, 78.86 and 41.94% RI, were obtained against *F. solani* and *R. solani*, respectively, with *T. harzianum*. Table 2 also showed that *T. harzianum* exhibited a mycoparazitism associated with high level of growth reduction induced by its filtrate on the two pathogens, 80.95 and 52.78% RI, respectively. R. solani

**Table 1.** Pathogenicity of isolates representing fungal species associated with root-rot of squash and their effect on Morphogenesis of squash.

| Tested fungal isolates | Root-rot diseased plant (%) | | | ****Morphogenesis data of survival plants | | |
|---|---|---|---|---|---|---|
| | *Pre-emergence | **Post-emergence | ***Diseased plant | Shoot length/ mm | Fresh weight/g | Dry weight/g |
| *Alternaria tenuis* | 5.00 | 8.06 | 1.92 | 37.38 | 22.95 | 6.34 |
| *Aspergillus niger* | 0.0 | 0.00 | 0.00 | 36.28 | 24.60 | 6.68 |
| *Fusarium oxysporum* | 14.17 | 11.42 | 2.50 | 22.78 | 21.43 | 6.15 |
| *Fusarium solani -1* | 30.00 | 93.18 | 16.67 | 7.05 | 3.73 | 1.22 |
| *Fusarium solani -2* | 33.33 | 60.02 | 38.33 | 29.50 | 17.68 | 5.59 |
| *Fusarium solani -3* | 30.00 | 40.24 | 47.50 | 17.85 | 17.88 | 5.15 |
| *Rhizoctonia solani-1* | 20.00 | 43.71 | 33.66 | 27.33 | 14.25 | 4.44 |
| *Rhizoctonia solani-2* | 33.33 | 67.35 | 54.17 | 23.00 | 10.93 | 2.36 |
| *Rhizoctonia solani-3* | 10.00 | 34.93 | 23.06 | 29.48 | 14.83 | 4.51 |
| Uninfested controls | 0.00 | 0.00 | 0.00 | 39.18 | 24.50 | 7.29 |
| L.S.D. at 5% | 7.948 | 10.840 | 18.711 | 6.321 | 10.460 | 2.492 |

*Pre-emergence at 15 days after sowing.
** Post- emergence at 30 days after sowing.
*** Diseased plants at 60 days after sowing.
**** Average per plant.

**Figure 1.** Plants raised from the a- control treatment

b- heavily infected soil with *R. solani*

**Table 2.** *In-vitro* assessment of growth reduction percentage of *Fusarium solani* and *Rhizoctonia solani* root-rot aggressive pathogens to squash on media containing culture of *Trichoderma harzianum.*

| Pathogen | Growth reduction (%) on medium containing *T.harzianum* | |
|---|---|---|
| | Culture filtrate | mycelium |
| *Fusarium solani* | 80.95 | 78.86 |
| *Rhizoctonia solani* | 52.78 | 41.94 |

showed more resistance to *T. harzianum* than *F. solani* in the two tests. A number of species within the genus Trichoderma are well known for their biological control capabilities against a wide range of commercially important plant pathogens (Whipps and Lumsden, 2001; McLean et al. 2004). They are known to produce a number of antibiotics, such as trichodermin, trichodermol A and harzianolide (Elad et al., 1980; Claydon et al.,

**Table 3.** Effect of benlate fungicide incorporated into PDA medium on linear growth (mm) of F. solani and *R. solani* root-rot aggressive pathogens to squash.

| Pathogen | Benlate concentration (ppm) | | | | | | L.S.D at 5 % |
|---|---|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25.0 | 50.0 | 100.0 | |
| *Fusarium solani* | 83.0 | 47.75 | 27.50 | 15.15 | 0.00 | 0.00 | 6.442 |
| *Rhizoctonia solani* | 90.0 | 16.75 | 0.00 | 0.00 | 0.00 | 0.00 | 12.064 |

**Table 4a.** Effect of *T. harzianum* and benlate fungicide on root-rot incidence caused by *F. solani* or *R. solani* and plant morphogeneses of squash after 60-days old.

| Infestation with | ***Fusarium solani*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Treatment | Disease expression % | | | Plant morphogenesis | | |
| | | Pre-em. | Post-em. | Diseased plants | Shoot length (cm) | Fresh weight (g) | Dry weight (g) |
| Control | Uninfested | 5.00 | 10.56 | 0.00 | 35.50 | 27.92 | 7.59 |
| | Infested | 35.00 | 53.57 | 66.67 | 16.48 | 11.88 | 8.29 |
| Benlate | Seed dresser | 5.00 | 7.78 | 8.68 | 39.83 | 33.84 | 8.29 |
| *T, harzianum* | Soil | 12.50 | 20.40 | 20.69 | 41.45 | 42.19 | 10.36 |
| *T, harzianum* | Seed | 20.00 | 25.00 | 42.16 | 36.20 | 28.84 | 6.69 |
| L.S.D. at 5% | | 9.866 | 9.603 | 20.579 | 8.599 | 23.338 | 1.591 |
| ***Rhizoctonia solani*** | | | | | | | |
| Control | Uninfested | 5.00 | 10.56 | 0.00 | 35.50 | 27.92 | 7.59 |
| | Infested | 25.00 | 36.61 | 95.00 | 15.20 | 8.73 | 3.06 |
| Benlate | Seed dresser | 0.00 | 0.00 | 2.50 | 37.22 | 37.23 | 8.37 |
| *T, harzianum* | Soil | 10.00 | 5.56 | 17.71 | 39.13 | 33.77 | 10.50 |
| *T, harzianum* | Seed | 10.00 | 19.44 | 18.16 | 33.83 | 36.45 | 7.13 |
| L.S.D. at 5% | | 3.888 | 9.279 | 11.192 | 7.135 | 13.198 | 4.26 |

1991). These compounds were responsible for most of the inhibition of fungal phytopathogens.

## Effect of benlate fungicide on the linear growth of the pathogens

Data in Table 3 showed that benlate completely inhibited the growth of *F. solani* and *R. solani* at 50.0 ppm and 12.5 ppm, respectively. These results are totally agreement with those of Baird et al. (1994) and Mathre and Johston (1995). Benomyle was found by other workers (Geypens, 1976; Cole and Cole, 1978) to be very effective against *R. solani* at 3.2µg/ml. Differences in effective doses could be explained in views of the observations of Geypens (1976) that highly virulent isolates of *R. solani* were more sensitive to thiophanate methyl a fungicide related to benlate than the less virulent isolates.

## *In vivo* effect of *T. harzianum* and benlate

Squash plant cultivated in untreated soil (negative control) suffered very little from damping off (pre and post emergence) without root-rot symptoms on the survival plants (Table 4 and Figure 1a). On the other hand, seed grown in the infested soil (positive control) with *F. solani* or R¬¬. solani significantly increased the percentage of pre and post-emergence damping off and dead plants ( Figures 2b,c,d). Compared with the positive control, *T. harzianum* treatments and benlate fungicide significantly produced a lower percentage of infection. Benlate reduced the disease severity to the maximum values of 5.00 and 7.78% for pre and post-emergence respectively in soil infested with *F. solani*, and 0% in soil infested with *R. solani*. The obtained results could be explained on the basis that benomyl might act directly on the pathogen to reduce its infectivity or on the host in some way which increases its resistance to infection (Baird et al., 1994; Mathre et al., 1995).

Adding *T. harzianum* antagonistic fungi to the soil or applying as seed coating significantly reduced the root-rot infection at both pre, post-emergence and dead plants (Tables 4 and Figure 2a). In this respect *T. harzianum* as soil treatment proved to be more effective than seed coating in soil treated with the both pathogens. The finding are consistent with the results of several investi-

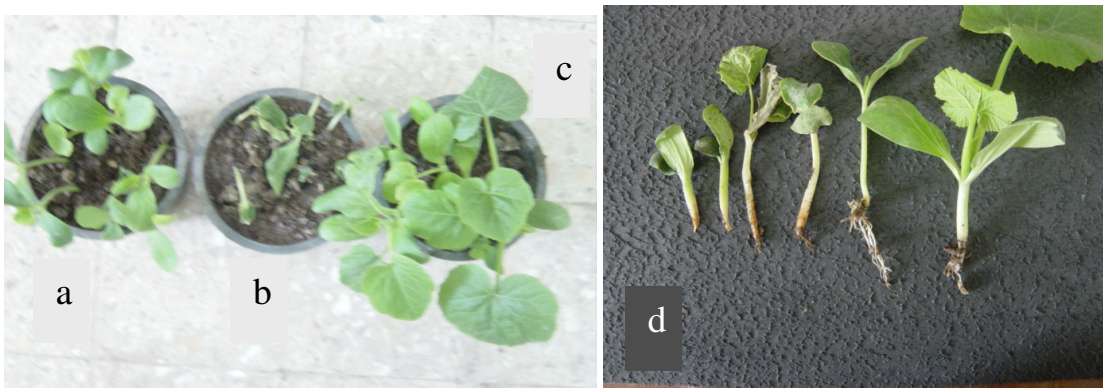


**Figure 2.** Plants raised from soil treated with: a) -*T. harzianum;* b) -*R. solani,* c) -*F. solani,* d) - Young seedling of squash showing post emergence damping-off.

**Table 4b.** Rhizospheric microflora of squash plants in infested soil with *F. solani* and *R. solani* and applied with *T. harzianum* and benlate during different period of plant growth.

**a-** number of total microbial flora (x $10^5$ / g dry soil)

| Sampling (days) after sowing | Untreated soil (negative control) | Soil infested with *F. solani* | | | | Soil infested with *R. solani* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz* Seed tr. | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz.* Soil tr. |
| 15 | 100.0 | 133.7 | 1151.6 | 454.6 | 828.9 | 216.7 | 3024.6 | 1940.0 | 2181.8 |
| 30 | 83.5 | 37.5 | 430.1 | 140.5 | 241.9 | 35.5 | 432.3 | 309.4 | 594.2 |
| 45 | 42.7 | 60.7 | 158.8 | 8.4 | 20.2 | 96.5 | 230.2 | 3.4 | 13.5 |
| 60 | 18.1 | 12.5 | 22.6 | 0.8 | 11.9 | 32.3 | 124.7 | 2.3 | 5.3 |

**b-** number of total fungal count (x $10^4$ / g dry soil)

| Sampling (days) after sowing | Untreated soil (negative control) | Soil infested with *F. solani* | | | | Soil infested with *R. solani* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz* Seed tr. | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz.* Soil tr. |
| 15 | 8.2 | 82.2 | 43.1 | 259.9 | 122.9 | 77.1 | 12.0 | 194.0 | 49.3 |
| 30 | 10.3 | 128.3 | 22.7 | 3210.2 | 323.7 | 79.6 | 13.9 | 5447.4 | 41.5 |
| 45 | 37.9 | 282.9 | 26.0 | 2100.8 | 117.5 | 100.9 | 27.8 | 2288.3 | 4.8 |
| 60 | 42.2 | 720.6 | 5.6 | 3466.0 | 166.4 | 124.5 | 3.0 | 233.2 | 7.9 |

**c-** number of *Trichoderma* spp. count (x $10^3$ / g dry soil)

| Sampling (days) after sowing | Untreated soil (negative control) | Soil infested with *F. solani* | | | | Soil infested with *R. solani* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz* Seed tr. | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz.* Soil tr. |
| 15 | 14.5 | 0.0 | 14.5 | 155.9 | 94.03 | 0.0 | 35.9 | 13.25.1 | 164.3 |
| 30 | 5.2 | 0.0 | 29.8 | 291.6 | 98.9 | 0.0 | 20.4 | 23369.5 | 628.6 |
| 45 | 0.0 | 0.0 | 47.4 | 10504.2 | 89.3 | 17.5 | 27.8 | 15255.5 | 227.3 |
| 60 | 0.0 | 2.8 | 86.7 | 28162.9 | 541.8 | 17.5 | 30.5 | 2478.1 | 116.7 |

gators (Hadar et al., 1979; Elad et al., 1980; Hussain et al., 1990). Other authors have speculated that antagonistic activity of some microorganisms against the plant pathogens may be due to the ability of these agents to grow and sporulate on seed and thereafter to become

**Table 5.** The frequency occurrence of fungi(%) in the rhizosphere of squash plants of soil infested with *F. solani* and *R. solani* and applied with *T. harzianum* and benlate after 60-days-old of plant growth.

| Isolated fungal genera and species | Negative control | Soil infested with *F. solani* | | | | Soil infested with *R. solani* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz* Seed tr. | Positive control | Benlate Seed tr. | *T. harz.* Soil tr. | *T. harz.* Soil tr. |
| *Alternaria tenius* | 8.8 | _ | _ | _ | _ | 4.5 | _ | _ | _ |
| *Aspergillus flavus* | 17.6 | _ | _ | _ | _ | 15.2 | _ | _ | _ |
| *Aspergillus niger* | 29.4 | 6.2 | 20.0 | _ | _ | _ | _ | _ | 36.4 |
| *Aspergillus* spp. | - | - | - | - | - | 18.2 | - | - | - |
| *Fusarium solani* | - | 80.8 | 20.0 | - | 1.8 | - | - | - | - |
| *Fusarium* spp. | 2.9 | - | - | - | 8.9 | - | 25.0 | - | - |
| *Mucor* spp. | 5.9 | 9.2 | 20.0 | - | - | 3.0 | 75.0 | - | - |
| *Penicillium* spp. | 29.4 | 3.1 | 20.0 | - | - | 24.2 | - | - | 18.2 |
| *Rhizoctonia solani* | - | - | - | - | - | 34.8 | - | - | 9.1 |
| *Rhizopus nigricans* | 5.9 | - | - | 0.7 | - | - | - | - | 9.1 |
| *T. harzianum* | - | - | - | 99.3 | 89.3 | - | - | 100.0 | 27.3 |
| *Trichoderma* spp. | - | 0.8 | 20.0 | - | - | - | - | - | - |

established in large numbers in soil (Whipps and Lumsden, 2001; McLean et al., 2004).

Comparing with uninfected soil (negative control), squash plants grown in infested soil with F. and *R. solani* (positive control) were significantly shorter. Pre-sowing of squash seeds with *T. harzianum* as soil or seed treatments and with benlate as seed coating produced plants that are significantly longer in height. Trichoderma spp. have been found to show potential as biological agents against seed and root rotting pathogens (Okigbo and Ikediugwu, 2000). Also, *T. harzianum* is known to produce extracellular cell wall degrading enzymes such as chitinases and cellulases which are important features of mycoparasite for the colonization of their host fungi (Di Pietro, 1995).

Data presented in Table 5 indicated that total microbial flora count in the rhizosphere of squash plants cultivated in the uninfested soil and soil infested with *F. solani* and *R. solani* of different treatments decreased as the plant grows. The total microbial flora in soil artificially infested with the two pathogenic fungi (positive control) is generally higher than that of the uninfested soil (negative control) during most stages of plant growth. Benlate fungicide as seed dressing also stimulates the total microflora, increasing their numbers in the rhizosphere. Generally, application of *T. harzianum* as soil or seed treatments to soil infested with *F. solani* and *R. solani* increased the total microbial flora count during the first 30 days, while after that date the opposite trend was observed. Increase in fungal population be attributed to root exudates and sloughs which supplied the fungi with nutrients to grow and proliferate. Similar results were also obtained in Egypt by Ibrahim et al. (1996) on tomato and Sahab et al. (2001) on sesame. Benlate treatment greatly reduced the number of fungi in the rhizosphere of soil infested with *F. solani* and *R. solani* as the plant grew.

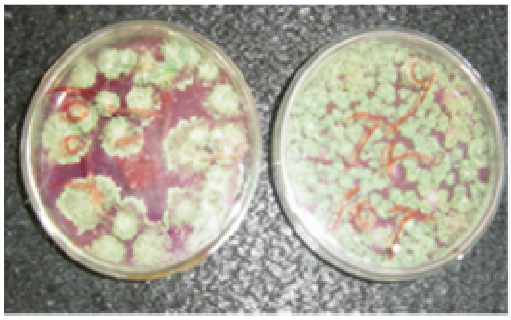

**Figure 3.** Colonies of *T. harzianum* on plate of specific medium isolated from the rhizosphere of squash plants.

Trichoderma spp. Treatment greatly increased the number of the antagonistic fungus in the rhizosphere of squash plants (Figure 3). Counts of *T. harzianum* in the rhizosphere of plants grown in soil infested with the two pathogens were always greater than those comparable ones of seed treatment.

## Conclusion

General, quantitative and qualitative differences in fungi were observed in rhizosphere soil of noninfested and *F. solani* and *R. solani* infested soil. More thane 8 genera were isolated from the infested and non-infested soil. As expected, the occurrence of *Fusarium spp.* (including *F. solani*) and *R. solani* showed higher frequency in soil infested with *F. solani* and *R. solani* (positive control) respectively than the negative control. Application of benlate as seed dreesing in soil inoculated with pathogenic fungi greatly reduced the occurrence of *Fusarium* and *Rhi*

*-zoctonia* spp. Inoculation with *T. harzianum* as soil treatment greatly reduced or eliminated most of fungal species. The same result was also obtained by Aziz et al. (1997) who found that germination of conidia of root-rot fungi in bean rhizosphere soil was inhibited after soil or seed application with Trichoderma. The great reduction of the pathogen population densities in the rhizosphere soil could be a result of lower proliferation rate of the pathogen in the rhizosphere already colonized by the antagonist (Muhammad and Amusa, 2003).

**REFERENCES**


Abd-El-Moity TH, Shatla MN (1981). Biological control of white rot disease of onion (Sclerotium cepivorum) by Trichoderma harzianum. Phytopathol. Z. 100: 107-114.

Abdel-Rehim MA, Abou-Taleb EM, Al-Mounofe OA, Raffat FM, A Tohamy (1987). the efficacy of seed treatment with calcium compounds in controlling damping-off disease of certain vegetable crops. Alex. J. Agric. Res. 32(1): 333-344.

Allen ON (1961). Experiments on soil bacteriology. Burgess Publishing Co. Minneapolis, Minnesota, USA. pp: 147.

Arafa MK (1985). Studies on Fusarium wilt of cumin. M.Sc. Thesis, Assiut University, Egypt.

Aziz NH, El-Fouly MZ, El-Essawy AA, MA Khalaf (1997). Influence of bean seedling root exudates on the rhizosphere colonization by Trichoderma lignorum for the control of *R. solani*. Bot. Bull. Acad. Sin. 38: 33-39.

Baird RE, Nankam C, Moghaddam PF, J Pataky (1994): Evaluation of seed treatments on Shrunken-2 sweet corn. Plant Dis. 78: 817-821.

Basim, H.; Ozturk SB. and O. Yegen (1999): Efficacy of a biological fungicide Trichoderma harzianum Rifai, T-22 against seedling root-rot pathogens (*R. solani*, Fusarium sp.) of cotton. GAP-Environmental Symposium , Fianbufa, Turkey. pp: 137-144.

Burnett HL, BB Hunter (1972). Illustrated genera of imperfect fungi. Burgess Pub. Co. Minneapolis, Minnesota, USA. pp: 241.

Callan NW, Mathre DE, JB Miller (1990). Bio-priming seed treatment for biological control of Pythium ultimum pre-emergence damping-off in sh2 sweet corn. Plant Dis., 74: 368-372.

Celar F (2000). Cucurbit diseases. Sodobno Kmetijstvo, 33(4): 162-165. (C.F. Rev. Plant Path. 79: 1037).

Claydon N, Hanson JR, Truneh A, AG Avent (1991). Harzianolide, a butenolide metabolite from cultures of Trichoderma harzianum. Phytochem. 30: 3802-3803.

Cole DL, JS Cole (1978). Field control of soreshin (*R. solani*) of tobacco with benomyl and benodanil, Ann. App. Biol. 90: 187.

Cook RJ, KF Baker (1989). The nature practice of biological control of plant pathogens, APS Press, St. Paul, pp: 539.

Elad T, Chet J, J Katan (1980). Trichoderma harzianum a biocontrol effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology 70: 119-121.

Fantino MG, Fantuz F, S Gennari (1989). Study of squash rot caused by *F. solani* f. sp. cucurbitae. Difesa delle piante, 12(1-2): 147-153.

Geypens M (1976): Evaluation of some fungicides for control of damping- off in beans and tomatoes. Med. Fac. Landbouww. Rijks Univ. Gent. 41: 669.

Gilman CJ (1957). A manual of soil fungi. 2nd ed. Iowa State College Press. USA pp. 450.

Hadar Y, Chet I, Y Henis (1979). Biological control of *R. solani* damping –off with wheat bran culture of *Trichoderma harzianum*. Phytopathology. 69: 64-68.

Howell CR, RD Stepanovic (1995). Michanism in the biocontrol of Rhizoctonia solani – induced cotton seedling disease by Gliocladium virens: Antibiosis. Phytopathology. 85: 469-472.

Hussain S, Ghaffar A, M Aslam (1990). Biological control of Macrophomina phaseolina charcoal rot of sunflower and mung bean. Egypt J. Phytopathol. 130: 157-160.

Ibrahim LA, Ragab MM, Sahab AF, Nehal Elmugy (1996): Biocontrol of tomato root-rot and wilt. Egypt J. Phytopathol. 24(1-2): 45-56.

Kucuk C, M Kivanc (2003). Isolation of Trichoderma spp. and determination of their antifungal, biochemical and physiological features. Turk Biol. 22: 247-253.

Louw HA, Webely DW (1959). The bacteriology of root region of cat plant grown under controlled pot culture conditions. J. Appl. Bacteriol. 22: 216-226.

Madkour MA, Abou-Taleb EM, AM Okasha (1983). Aceton inhibition of *R. solani* growth. Phytopathol. Z. 107: 111-116.

Mahmoud SA, Abou El-Fadle M, M El-mofty (1964). Studies on the rhizosphere microflora of a desert plant. Folia Microbiol. 9: 1.

Martyn RD, RJ Mclaughin (1983). Susceptibility Of summer squash to the watermelon wilt pathogen, F. oxysporum f .sp. niveum. Plant Dis. 67(3): 263-266.

Mathre DE, Johnston RH, Cllan NW, Mohan SK, Martin JM, JB Miller (1995). Combined biological and chemical seed treatments for control of two seedling diseases of sh2 sweet corn, Plant Dis 79: 1145-1148.

McLean, K.L.; Dodd, S.L.; Sleight, B.E.; Hill, R.A. and A. Stewart (2004): Comparison of the behavior of a transformed hygromycin resistant strain of Trichoderma atoviride with the wild-type strain. New Zealand Plant Protection, 57: 72-76.

Muhammad S, NA Amusa (2003). In-vitro inhibition of some seedling blight inducing pathogens by compost-inhabiting microbes. Afr. J. Biotechnol. 2(6): 161-164.

Nelson PE, Toussoum TA, WF Marasas (1983). Fusarium spp. An illustrated manual for identification . The Pennsylyvania University, Parl, USA. pp: 198.

Okigbo RN, FEO Ikediugwu (2000). Studies in biological control , Post-harvest rot in yams using Trichoderma viride. Phytopathology 148: 235-240.

Pushpa K, Borkar GM, DV Patil (1999). Study on seed borne of pumpkin, cucumber, watermelon and muskmelon. J. Soil Crops 9(2): 234-238.

Sahab AF, Elewa IS, Mostafa MH, EH Ziedan (2001): Integrated control of wilt and root-rot diseases of sesame crop in Egypt. Egypt. J. Appl. Sci. 16(7): 448-462.

Sivan A, I Chet (1994). Integrated control of Medica on growth and interaction between a range of soilborne glasshouse pathogens and antagonistic fungi. Phytopathology 10: 127-142.

Steel PG, JH Torri (1960). Principles and procedures of statistics. Mc-Grow-Hill Book Company Inc. New York.

Whipps JM, RD Lumsden (2001). Commercial use of fungi as plant disease biological control agents: status and prospects. CABI Publishing, Wallingford, United Kingdom. pp: 9-22.