

## ROOT ROT DISEASE OF OLIVE TRANSPLANTS AND ITS BIOLOGICAL CONTROL\*

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### ABSTRACT

Several nurseries of olives in Fayoum and Giza were surveyed for root rot incidence during early summer of 2003. In Fayoum, root rot incidence reached 53% while in Giza, disease incidence was 44%. Disease symptoms consist of partial wilting, leaves browning and twig dieback, which was associated with severe root rot and basal stem cankers and followed, in most cases, by plant decline and death. The most frequently isolated fungi from rotted roots were *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Cylindrocarpon* sp. and *Alternaria alternata*. Isolation frequency of different fungi varied among olive cultivars. Generally, *Fusarium* spp. were the most frequently isolated pathogens and *Fusarium oxysporum* was the most frequent (35.5%) on all cultivars followed by *F. solani* (19.3%) *R. solani* (16.1%). Meanwhile, *S. rolfsii*, *F. moniliforme*, *Cylindrocarpon* sp. and *A. alternata* occurred at low frequencies. Pathogenicity tests showed that all tested isolates caused varied degrees of root rot symptoms on olive transplants, cvs. Manzanillo and Picual. *Fusarium oxysporum*, *F. solani* and *R. solani* caused the highest root rot incidence and severity on both cultivars. There was a positive correlation between disease severity on roots and severity of foliar symptoms. All evaluated olive cultivars were susceptible or extremely susceptible to fungal pathogens. All cultivars showed high disease severity with root rots, especially in response to infection by *F. solani*, *F. oxysporum* and *S. rolfsii*. However, the least foliar symptoms were recorded on cultivar Coratina. Application of two commercial biological control products (Rhizo-Plus and Trichoderma 2000) to soil, 24h before planting olive cuttings in the nursery, significantly reduced incidence of root rot on transplants of cultivars Manzanillo and Picual, up to 28 weeks after planting.

**Keywords:** Olive, Root rot, Fungal pathogens, Biological control, Rhizo-Plus, Trichoderma 2000.

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## INTRODUCTION

The olive oil and table olive industries play an important role in the agricultural and processing sectors of the major olive producing countries including Egypt and Syria. Olive plants are liable to attack by several soil borne pathogens, causing severe losses in yield and quality (Ghoneim *et al* 1996; Sánchez-Hernández *et al* 1998 & 2001; Agosteo *et al* 2001 & 2002 and Barreto *et al* 2002). Producers commonly suffer from losses due to death of transplants or mature plants. Root rot diseases of olive are primarily caused by the ubiquitous pathogens *Fusarium oxysporum*, *F solani*, *Rhizoctonia solani*, *Phytophthora* spp. and *Pythium* spp. (Teviotdale, 1994; Ghoneim *et al* 1996; Sánchez-Hernández *et al* 1998 & 2001 and Barreto *et al* 2002). These pathogens are capable of surviving in the soil in the absence of their host plants, and when weather conditions are not favorable for disease initiation and development (Bruehl, 1987). Such pathogens, under favorable conditions, might become destructive.

The main measure applied by growers to reduce losses due to these pathogens, especially at the early stages of plant development, are application of fungicides. However, lack of disease resistant varieties, high cost and inadequate protection by fungicides are the major obstacle in managing such pathogens (Teviotdale, 1994), and have prompted a search for alternatives for use in the control of soil borne pathogens. One of such alternatives is biological control using soil microorganisms that reduce the amount of inoculum or disease producing activity of pathogens (Cook, 1993). Successful bio-

logical control of several soil borne pathogens using various microbial antagonists including strains of *Trichoderma* species, fluorescent Pseudomonads and *Bacillus subtilis* were widely used worldwide (Weller, 1988; Tronsomo & Hjejord, 1998; Vannacci & Gullino, 2000; Zeidan & Farrag, 2002; Howell, 2004 and Jacobsen *et al* 2004).

The objective of this study was to investigate the nature of root rot diseases of olive transplants in Egypt and to evaluate the efficiency of certain biocontrol agents for controlling the disease.

## MATERIAL AND METHODS

### Isolation and identification of root rot pathogens

Different nurseries of olive in El-Fayoum and El-Giza districts were surveyed during early summer of 2003. Olive transplants, showing yellowing or dieback and death were used to isolate potential fungal pathogens from collar and roots as described by Sánchez-Hernández *et al* (1998). Purified isolates were maintained on potato dextrose agar (PDA) medium at 4°C till use.

The established fungal isolates were identified on the basis of morphological and microscopical characteristics of the vegetative and reproductive structures according to Barnett & Hunter (1987) for genera of imperfect fungi, Booth (1971) for *Fusarium* spp., Sneh *et al* (1991) for *Rhizoctonia* spp. and Ellis (1971) for *Alternaria* spp.

### Source of olive cuttings

Young rooted cuttings (six-months old) of five different olive cultivars, *i.e.*

Manzanillo, Picual, Koroniewski, Coratina and Ogizi, were obtained from nursery of Agricultural Research Center, Giza, Egypt and were used throughout the experiments.

### Pathogen's inoculum and inoculation

Inoculum of each tested fungal isolate was produced following the methods described by **Dhingra & Sinclair (1995)**. Spore suspension ( $1 \times 10^7$  spore/ml) of *Fusarium* spp., *Cylindrocarpon* sp. and *A. alternata* and mycelial fragments suspension ( $10^7$  colony forming units (cfu)/ml) of *R. solani* and *S. rolfsii* were prepared.

Young rooted cuttings were inoculated as described by **Sánchez-Hernández et al (1998)**. Roots were carefully cleaned under tap water and submerged for five minutes into the inoculum suspension. Meanwhile, autoclave-sterilized soil in each pot was infested with 30 ml conidial suspension of *Fusarium* spp., *Cylindrocarpon* sp. and *A. alternata* or 30 ml mycelial fragments suspension of *R. solani* and *S. rolfsii* per Kg soil. Inoculum of each pathogen was mixed separately with soil.

### Pathogenicity tests

Fungi consistently isolated from diseased tissues of olive roots were tested for potential pathogenicity in a greenhouse experiment. Young rooted cuttings (cvs. Manzanillo and Picual), inoculated as described above, were planted in black plastic bags (15cm diameter x 20cm height) containing pathogen-infested soil (1.6 Kg soil). One rooted olive cutting was planted in each pot and eight replicates were specified for each treatment.

Inoculated olive cuttings and control ones were placed in the greenhouse for up to 28 weeks. Plants were irrigated once a week. Meanwhile, root samples from inoculated and control plants were used to re-isolate each inoculated fungus and other fungi present in the root tissues.

### Cultivar reaction

Five olive cultivars (Manzanillo, Picual, Koroniewski, Coratina and Ogizi) were evaluated for their reactions to root rot pathogens. Virulent isolates of *F. oxysporum*, *F. solani*, *R. solani*, *S. rolfsii* and *A. alternata* were used throughout the study. Rooted cuttings of each cultivar were planted in plastic bags containing autoclave-sterilized sandy clay soil, infested with each pathogen, as previously mentioned. One rooted olive cutting was planted in each pot and eight replicates were specified for each treatment. The plants were grown under greenhouse conditions and were irrigated regularly. The incidence and severity of root rot was recorded after 28 weeks after transplanting.

### Biological control of root rot

Two commercial biological control products, kindly obtained from Modern Agricultural Company (PICO), Egypt, were examined for their capacity to suppress root-rot disease on olive transplants, cultivars Manzanillo and Picual. These bioagents are:

#### A. Rhizo-Plus

A biocontrol agent (*Bacillus subtilis*) FZB24 Manufacturer/Distributor: KFZB Biotechnik GmbH, Glienicke Weg 185, D-12489 Berlin, Germany.

## B. Trichoderma 2000

A biocontrol agent (*Trichoderma harzianum*) T166. Manufacturer/Distributor by Mycontrol, Ltd. USA.

Plastic bags, containing sterilized sandy-clay soil were treated with preparation of each bioagent (Rhizo-Plus or Trichoderma 2000) at the rate of 0.5% (w/w), 24h before planting of olive cuttings. At transplanting, soil was infested with each tested pathogen by adding 50 ml conidial or hyphal fragment suspension ( $10^7$  cfu/ml) to each bag. Each plastic bag was cultivated with one olive cutting and eight replicates were specified for each treatment. The plant bags were kept in the greenhouse and irrigated periodically till the end of the experiment. Growing plants were observed periodically and disease incidence and severity on shoots and roots was recorded, 28 weeks after planting.

### Disease assessment

Disease assessment for incidence of root rot on olive transplants was recorded, 28 weeks after planting. Severity of above ground symptoms and root rot symptoms was assessed, for each plant, using a 0-4 scale modified from **Sánchez-Hernández et al (2001)**. The disease severity was calculated using the following formula:

$$\text{Disease index} = \frac{\sum (\text{Class rating} \times \text{Class frequency})}{\text{Total numbers of plants} \times \text{highest rating}} \times 100$$

### Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the general linear model procedure of the Statistical

Analysis System (**SAS Institute, 1996**). Means were separated by least significant difference test (LSD) at 0.05.

## RESULTS

### 1. Disease incidence

Several nurseries of olives in two districts in Egypt, *i.e.* Fayoum and Giza, were surveyed for root rot incidence during early summer of 2003. In all surveyed nurseries, root-rot disease was observed on all olive cultivars in moderate to high frequencies. In Fayoum, root rot incidence reached 53% while in Giza disease incidence was 44%.

### 2. Disease Symptoms

Disease symptoms observed on olive transplants grown in nursery are described. The symptoms appear on all parts of olive plants. Aerial symptoms consist of leaf chlorosis (Fig.1A), yellowing of leaves (Fig.1B), twig dieback (Fig.1C), leaves browning and defoliation (Fig.1D), followed, in most cases, by plant decline and death (Fig.1E). Although the above ground symptoms were unspecific, it was associated with severe root rot and basal stem cankers (Fig.1F).

### 3. Isolation and identification of the causal organism

A total of 62 fungal isolates were isolated from rotted roots of five olive cultivars collected from nurseries in two districts in Egypt. The most frequent isolates were identified according to their morphology and growth characters using specific keys for each fungal genus. These isolates were identified as: *Fusarium*

Fig. 1. Symptoms of root rot disease on olive transplants grown in different nurseries in Egypt.

(A): Leaf chlorosis, (B): Yellowing of foliage, (C): Die back of shoots, (D): Browning of the shoots, (E) Plant death, (F): Root-rot and collar cankers.

*oxysporum* Schlecht., *Fusarium solani* (Mart.), *Fusarium moniliforme* Sheldon, *Rhizoctonia solani* Kühn *Sclerotium rolfsii* Sacc., *Cylindrocarpon* sp. Wollen, and *Alternaria alternata* (Fr.) Kessiler (Table 1).

#### 4. Occurrence and frequency of the isolated fungi

Results in Table (1) show clearly that frequency of isolation of different fun-

gihas varied among olive cultivars and locations. Generally, *Fusarium* spp. were the most common pathogens in both districts and on all cultivars. *Fusarium oxysporum* was the most frequent (35.5%) on all cultivars followed by *F. solani* (19.3%) and *R. solani* (16.1%).

Meanwhile, *F. moniliforme*, *Cylindrocarpon* sp., *A. alternata* and *S. rolfsii* were recorded at low frequencies (9.7%, 9.7%, 6.5% and 3.2%, respectively).

Table 1. Frequency of occurrence of fungi isolated from woody cutting transplants, of five olive cultivars, obtained from two locations in Egypt during summer 2003.

| Fungi                       | Frequency of occurrence (%) |          |       |           |       | Mean |
|-----------------------------|-----------------------------|----------|-------|-----------|-------|------|
|                             | Giza                        |          |       | Fayoum    |       |      |
|                             | Manzanillo                  | Coratina | Pical | Koroneiki | Ogizi |      |
| <i>Alternaria alternata</i> | -                           | 22.2     | -     | 42.9      | 7.1   | 9.7  |
| <i>Cylindrocarpon sp.</i>   | 10                          | -        | 9.1   | -         | 7.1   | 6.5  |
| <i>Fusarium moniliforme</i> | 10                          | 22.2     | 4.5   | 14.2      | 7.1   | 9.7  |
| <i>Fusarium oxysporum</i>   | 40                          | 33.4     | 36.4  | 42.9      | 28.6  | 35.5 |
| <i>Fusarium solani</i>      | 20                          | -        | 27.3  | -         | 28.6  | 19.3 |
| <i>Rhizoctonia solani</i>   | 20                          | 11.1     | 18.2  | -         | 21.5  | 16.1 |
| <i>Sclerotium rolfsii</i>   | -                           | 11.1     | 4.5   | -         | -     | 3.2  |
| Total                       | 100                         | 100      | 100   | 100       | 100   | 100  |

### 5. Comparative pathogenicity of pathogens

All tested isolates were pathogenic, with varied degrees, to olive roots and showed also different levels of aerial symptoms (Table 2). Although, control non-inoculated plants showed a very low level of root necrosis, no foliar wilting was observed (Table 2). However, plants inoculated with the tested isolates showed less to severe root necrosis accompanied by less to severe crown and foliar symptoms. *Fusarium oxysporum*, *R. solani* and *F. solani* caused the highest root rot incidence and severity on both tested olive cultivars. Meanwhile, the infection percentage of root rot caused by *F. moniliforme* and *A. alternata* were moderate (37.5%). In all cases, no deep vascular discoloration was observed in root

or crowns of the diseased transplants. The isolate of *S. rolfsii* caused extensive necrosis on the roots and crowns with the appearance of white fungal mycelium growing around the collar of inoculated plants. Isolates of *F. oxysporum*, *F. solani* and *F. moniliforme* caused extensive root and crown necrosis on both cultivars. However, *A. alternata* and *Cylindrocarpon sp.* were also pathogenic and caused necrosis on the crown and too less extent on the roots.

The results showed also clearly that there is a positive correlation ( $r \geq 90$ ;  $P=0.05$ ) between disease severity on roots and severity of foliar symptoms. Foliar severity values were high in case of *F. solani*, *F. oxysporum* and *S. rolfsii* (Table 2). Meanwhile, all inoculated fungal isolates were also re-isolated successfully from roots of rotted plants.

Table 2. Pathogenicity of the most frequently isolated fungi from olive transplants to rooted woody cuttings of olive, cultivars Manzanillo and Picual<sup>x)</sup>.

| Pathogen                    | Cultivar                     |                                     |        |                              |                                     |      |
|-----------------------------|------------------------------|-------------------------------------|--------|------------------------------|-------------------------------------|------|
|                             | Manzanillo                   |                                     |        | Picual                       |                                     |      |
|                             | % of infection <sup>y)</sup> | Disease severity<br>% <sup>z)</sup> |        | % of infection <sup>y)</sup> | Disease severity<br>% <sup>z)</sup> |      |
| Shoots                      |                              | Roots                               | Shoots |                              | Roots                               |      |
| <i>Alternaria alternata</i> | 37.5                         | 41.5                                | 58.2   | 37.5                         | 50.0                                | 66.5 |
| <i>Cylindrocarpon sp.</i>   | 50.0                         | 33.2                                | 50.0   | 50.0                         | 41.5                                | 58.2 |
| <i>Fusarium moniliforme</i> | 37.5                         | 25.0                                | 50.0   | 37.5                         | 33.2                                | 50.0 |
| <i>Fusarium oxysporum</i>   | 87.5                         | 58.2                                | 75.0   | 87.5                         | 66.5                                | 83.2 |
| <i>Fusarium solani</i>      | 75.0                         | 66.5                                | 83.2   | 87.5                         | 75.0                                | 91.5 |
| <i>Rhizoctonia solani</i>   | 62.5                         | 41.5                                | 75.0   | 62.5                         | 58.2                                | 75.0 |
| <i>Sclerotium rolfsii</i>   | 67.5                         | 50.0                                | 75.0   | 75.0                         | 66.5                                | 83.2 |
| Non - infested              | 0.0                          | 0.0                                 | 16.5   | 0.0                          | 0.0                                 | 16.5 |
| LSD at P = 0.05             | 8.8                          | 6.6                                 | 10.2   | 10.0                         | 6.9                                 | 11.3 |

<sup>x)</sup> Data were recorded, 28 weeks after planting of rooted woody cuttings.

<sup>y)</sup> Figures are based on visible above ground symptoms.

<sup>z)</sup> Symptom severity was assessed on modified scales of **Sánchez-Hernández et al (2001)** where, 0= no symptoms to 4= plant dead.

## 6. Cultivar reaction

The results presented in Table (3) indicate disease severity values of root rot and foliar symptoms on five olive cultivars grown in artificially infested soil with five fungal pathogens. All evaluated cultivars were susceptible or extremely susceptible to such pathogens.

All cultivars showed high severity values of root rot, especially in response to infection with *F. solani*, *F. oxysporum* and *S. rolfsii*. Disease severity values on roots ranged from 91.5% on cv. Picual with *F. solani* to 58.2% on cv. Coratina with each of *F. oxysporum*, *R. solani* and

*S. rolfsii*. In case of *R. solani*, disease severity values on roots ranged from 75% on Manzanillo to 58.2% on Coratina. However, there were significant differences in foliar symptoms ratings on the tested cultivars. The least foliar symptoms were recorded on cultivar Coratina with all tested pathogens (Table 3). In case of *F. solani*, the severity values of foliar symptoms were 58.2% on cultivar Koroneiki and 75% on Ogizi, although root rot severity on both cultivars was 83.2%. It could be concluded that these cultivars are generally susceptible to all tested pathogens, although Coratina seem to be the least susceptible cultivar.

Table 3. Reaction of different olive cultivars to infection by various fungal pathogens, under greenhouse conditions <sup>Y)</sup>

| Pathogen                    | Disease Severity (%) <sup>Z)</sup> |          |       |           |       |            |          |       |           |       |
|-----------------------------|------------------------------------|----------|-------|-----------|-------|------------|----------|-------|-----------|-------|
|                             | Shoots                             |          |       |           |       | Roots      |          |       |           |       |
|                             | Manzanillo                         | Coratina | Pical | Koroneiki | Ogizi | Manzanillo | Coratina | Pical | Koroneiki | Ogizi |
| <i>Alternaria alternata</i> | 41.5                               | 25.0     | 50.0  | 33.2      | 50.0  | 58.2       | 50.0     | 66.5  | 66.5      | 66.5  |
| <i>Fusarium oxysporum</i>   | 58.2                               | 41.5     | 66.5  | 58.2      | 58.2  | 75.0       | 58.2     | 83.2  | 75.0      | 75.0  |
| <i>Fusarium solani</i>      | 66.5                               | 50.0     | 75.0  | 58.2      | 75.0  | 83.2       | 75.0     | 91.5  | 83.2      | 83.2  |
| <i>Rhizoctonia solani</i>   | 41.5                               | 33.2     | 58.2  | 50.0      | 58.2  | 75.0       | 58.2     | 75.0  | 75.0      | 75.0  |
| <i>Sclerotium rolfsii</i>   | 50.0                               | 41.5     | 66.5  | 58.2      | 66.5  | 75.0       | 58.2     | 83.2  | 75.0      | 83.2  |
| Non – infested              | 0.0                                | 0.0      | 0.0   | 0.0       | 0.0   | 16.5       | 8.3      | 16.5  | 7.5       | 15.5  |
| LSD at P = 0.05             | 4.3                                | 4.2      | 4.4   | 4.8       | 4.1   | 11.2       | 10.3     | 12.4  | 12.8      | 11.7  |

<sup>Y)</sup> Data were recorded, 28 weeks after planting of rooted woody cuttings.

<sup>Z)</sup> Symptom severity was assessed on modified scales of Sánchez-Hernández *et al.* (2001), where 0= no symptoms to 4= plant dead

## 7. Biological control of root rot

Results in Table (4) indicate that, treatment of rooted olive cuttings (cv. Manzanillo) with the bioagents, Rhizo-plus and Trichoderma 2000 have significantly reduced root-rot disease on olive transplants, after 28 weeks from planting. Trichoderma 2000 reduced disease severity on olive roots by 33.3% in cases of *F. oxysporum*, *F. solani* and *R. solani*, and by 43 % and 66.7% for *A. alternata* and *S. rolfsii*, respectively. Meanwhile, foliar wilt ratings were also reduced in plants treated by Trichoderma 2000. However, Rhizo-Plus was more effective than Trichoderma 2000 in reducing severity of root rot or foliar symptoms, as it reduced

root rot severity by 78% and 55.7% with *S. rolfsii* and *F. oxysporum*, respectively.

Results in Table (4) indicate also that, both tested bioagents significantly reduced root-rot disease on olive transplants (cv. Pical). In most cases, Rhizo-Plus was more effective than Trichoderma 2000 in reducing severity of root rot, although they showed similar effect in reducing foliar symptoms on shoots due to *F. oxysporum*, *S. rolfsii* and *A. alternata*, up to 28 weeks after treatment.

## DISCUSSION

This study revealed the nature of root rot disease of olive in Egypt. Survey conducted during early summer of 2003 revealed that the disease is widespread and

Table 4. Effect of two biocontrol products, Rhizo-Plus and Trichoderma 2000, on the incidence of root rot on olive transplants, cvs. Manzanillo and Picual, grown in sandy clay soil infested by different fungal pathogens, under greenhouse conditions<sup>x)</sup>.

| Pathogen                    | Treatment        | Disease severity <sup>y)</sup> |                          |       |                          |        |                          |       |                          |
|-----------------------------|------------------|--------------------------------|--------------------------|-------|--------------------------|--------|--------------------------|-------|--------------------------|
|                             |                  | Manzanillo                     |                          |       |                          | Picual |                          |       |                          |
|                             |                  | Shoots                         |                          | Roots |                          | Shoots |                          | Roots |                          |
|                             |                  | Mean                           | Efficacy % <sup>z)</sup> | Mean  | Efficacy % <sup>z)</sup> | Mean   | Efficacy % <sup>z)</sup> | Mean  | Efficacy % <sup>z)</sup> |
| <i>Alternaria alternata</i> | Non-treated      | 41.5                           |                          | 58.2  |                          | 50.0   |                          | 66.5  |                          |
|                             | Rhizo-Plus       | 8.2                            | 80.2                     | 33.2  | 43.0                     | 8.2    | 83.6                     | 33.2  | 50.0                     |
|                             | Trichoderma 2000 | 8.2                            | 80.2                     | 33.2  | 43.0                     | 8.2    | 83.6                     | 33.2  | 50.0                     |
| <i>Fusarium oxysporum</i>   | Non-treated      | 58.2                           |                          | 75.0  |                          | 66.5   |                          | 83.2  |                          |
|                             | Rhizo-Plus       | 16.5                           | 71.7                     | 33.2  | 55.7                     | 8.2    | 87.7                     | 33.2  | 60.1                     |
|                             | Trichoderma 2000 | 16.5                           | 71.7                     | 50.0  | 33.3                     | 8.2    | 87.7                     | 41.5  | 50.1                     |
| <i>Fusarium solani</i>      | Non-treated      | 41.5                           |                          | 75.0  |                          | 75.0   |                          | 91.5  |                          |
|                             | Rhizo-Plus       | 16.5                           | 60.2                     | 41.5  | 44.7                     | 16.5   | 78.0                     | 25.0  | 72.7                     |
|                             | Trichoderma 2000 | 16.5                           | 60.2                     | 50.0  | 33.3                     | 8.2    | 89.1                     | 41.5  | 54.6                     |
| <i>Rhizoctonia solani</i>   | Non-treated      | 41.5                           |                          | 75.0  |                          | 58.2   |                          | 75.0  |                          |
|                             | Rhizo-Plus       | 16.5                           | 60.2                     | 41.5  | 44.7                     | 16.5   | 71.7                     | 33.2  | 55.7                     |
|                             | Trichoderma 2000 | 16.5                           | 60.2                     | 50.0  | 33.3                     | 8.2    | 85.9                     | 41.5  | 44.7                     |
| <i>Sclerotium rolfsii</i>   | Non-treated      | 50.0                           |                          | 75.0  |                          | 66.5   |                          | 83.2  |                          |
|                             | Rhizo-Plus       | 8.2                            | 83.6                     | 16.5  | 78.0                     | 8.2    | 87.7                     | 25.0  | 70.0                     |
|                             | Trichoderma 2000 | 8.2                            | 83.6                     | 25.0  | 66.7                     | 8.2    | 87.7                     | 41.5  | 50.1                     |
| Non-infested                |                  | 0.0                            |                          | 16.5  |                          | 0.0    |                          | 16.5  |                          |
| LSD at P=0.05               |                  | 8.5                            |                          | 9.7   |                          | 12.5   |                          | 12.5  |                          |

<sup>x)</sup> Data were recorded, 28 weeks after planting of rooted woody cuttings.

<sup>y)</sup> Symptom severity was assessed on modified scales of Sánchez- Hernández *et al.* (2001) where 0= no symptoms to 4= plant dead.

<sup>z)</sup> Efficacy of treatment = (control-treatment) / control %.

causes serious losses in surveyed nurseries at Fayoum and Giza districts. The results indicated that, although the above ground symptoms were unspecific, it was associated with severe root rot and basal stem cankers. Several fungal pathogens, *i.e.* *F. oxysporum*, *F. solani*, *F. moniliforme*, *R. solani*, *S. rolfsii*, *Cylindrocarpon sp.* and *A. alternata*, were isolated from rotted roots of different olive cultivars. These results are in agreement with other studies which indicated that soil borne fungi are mainly responsible for root-rot diseases of olive transplants and trees and cause severe damage and reduction in yield (Teviotdale, 1994; Ghoneim *et al* 1996; Sánchez-Hernández *et al* 1998 & 2001 and Barreto *et al* 2002). Generally, the results indicate clearly that *Fusarium* spp. were the most common pathogens in both districts and all cultivars. *Fusarium oxysporum* was the most frequent on all cultivars followed by *F. solani* and *R. solani*. It has been also reported that *Fusarium* species have commonly been associated with root rot of olive transplants (Boullila *et al* 1993; Ghoneim *et al* 1996; Sánchez-Hernández *et al* 1998 and Barreto *et al* 2001 & 2002). Meanwhile, *S. rolfsii*, *A. alternata*, *F. moniliforme* and *Cylindrocarpon sp.* occurred at low frequencies. However, most of these fungal species are very frequent in the field soils of the area surveyed (Ghoneim *et al* 1996). Such pathogens, under favorable conditions, might become destructive (Sánchez-Hernández *et al* 1998). Variation in pathogens and disease incidence in different sites might be attributed to one or more of factors including soil types, soil moisture content, inoculum density of the pathogens, other agricultural practices, cultivars, and interaction

between the host and the pathogenic fungi (Ghoneim *et al* 1996; Sánchez-Hernández *et al* 1998 & 2001 and Barreto *et al* 2001 & 2002).

The pathogenicity tests demonstrated that all tested isolates were clearly pathogenic to olive and reproduced typical symptoms of root rot in rooted cuttings of cvs. Manzanillo and Picual. *Fusarium oxysporum* and *F. solani* caused the highest root rot incidence and severity on transplants of both tested olive cultivars. Isolate of *F. oxysporum*, *F. solani* and *F. moniliforme* showed extensive root and crown necrosis on both cultivars. Variation in pathogenicity of different isolates of *Fusarium* spp. from olive trees have also been reported (Ghoneim *et al.* 1995; Sánchez-Hernández *et al.* 1998 and Barreto *et al.* 2001&2002). Meanwhile, the results showed also that there is a positive correlation between disease severity on roots and severity of foliar symptoms.

Several factors may interact with incidence of diseases on olive trees (Martelli *et al* 2002). The plant material and rooting conditions may affect the infection by certain fungal pathogens (Teviotdale, 1994). Latent infections may spread during rotting phase (Martelli *et al* 2002). High humidity conditions accomplished by mist treatment may favor certain fungal pathogens. In this study, plant material used for the pathogenicity tests came from a commercial nursery that could be the reason why it was not possible to have plants totally free of root rot fungi. This fact could determine the appearance of some level of root rot in control plants and could interfere with the experimental evaluations, since fungi present in plant roots were similar to some isolates tested such as *F. solani* or

*F. oxysporum* (Sánchez-Hernández *et al* 1998).

The results of the present study demonstrate that five olive cultivars, *i.e.* Manzanillo, Coratina, Picual, Koroneiki and Ogizi were generally susceptible to all tested pathogens. All cultivars showed higher disease severity with root rots, especially in response to the infection with *F. solani*, *F. oxysporum* and *S. rolfsii*. However, there were significant differences in foliar wilt ratings on the tested cultivars. However, Ghoneim *et al* (1996) found that olive cultivars, *i.e.* Ogizi, Dolci and Manzanillo were susceptible to different soil borne fungi, whereas cultivars Krygula and Picual were less susceptible. Resistant cultivars can be the key in managing diseases as Verticillium wilt of olive, and to this regard some olive accessions with promising resistant traits have been selected (Ciccarese *et al* 2002 and López-Escudero *et al* 2004).

Control of various soil borne diseases with biocontrol formulation have been popular with grower all over the world (Vannacci and Gullino, 2000). The results of the present study revealed the effectiveness of two commercial biological control products (Rhizo-Plus and Trichoderma 2000), for suppression of root-rot on transplants of olive cultivars, Manzanillo and Picual. Both bioagents effectively reduced disease incidence and severity in artificially-infested soil; and also stimulated plant growth in sterilized-non infested soil (Unpublished data). Successful biological control of several soil borne pathogens on different horticultural crops has been reported (Utkhede and Li, 1989; Harris *et al* 1994, Nemeč *et al* 1996; Vannacci & Gullino, 2000; Kexiang *et al* 2002 and

Howell, 2004). Production of vigorous olive transplants which are more resistant to soil borne plant pathogenic fungi is advantageous to the producer as well as to the farmer. Application of beneficial microorganisms (e.g. *Bacillus subtilis* and *Trichoderma harzianum*) to the propagative mixture during production of transplants in the nursery makes the use of such microorganisms for both biological control and plant growth enhancement more feasible (Baker, 1989; Harris *et al* 1994; Inbar *et al* 1994 and Harman, 2004).

Generally, the results of this study demonstrated that root rot is a serious additional threat to olive production in Egypt. It affects olive plants in the nursery, commercial orchards and landscape plantings. The disease is expanding in olive-growing nurseries, probably due to both the use of infected propagative material and planting in contaminated soil. There are no available resistant cultivars and many registered fungicides to control root-rot and wilt diseases in horticulture crops are ineffective against wide array of soil borne pathogens. Such diseases are notifiable and efforts should be made to eliminate it before it becomes established in the olive orchards especially in new plantations.

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## مرض عفن الجذور في شتلات الزيتون ومكافحته حيويًا

[٢٦]

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المصابة هي *Fusarium solani*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Cylindrocarpon* sp., *Alternaria alternata*, *Sclerotium rolfisii*. تفاوتت نسب عزل تلك الفطريات من جذور الشتلات المصابة تبعاً للصنف ومنطقة الزراعة، وبصفة عامة كانت أنواع *Fusarium* هي أكثر الفطريات المعزولة من منطقتي الحصر فبلغت نسبة عزل فطر *Fusarium oxysporum* (٣٥,٥%) يليه *Fusarium solani* (١٩,٣%) ثم الفطر *Rhizoctonia solani* (١٦,١%) بينما عزلت فطريات *Alternaria alternata*, *Fusarium solani*, *Cylindrocarpon* sp. و *moniliforme* *Sclerotium rolfisii* بنسب أقل. أظهرت اختبارات القدرة المرضية أن كل الفطريات المختبرة كانت قادرة علي إحداث عفن للجذور مع ظهور درجات تأثير مختلفة علي المجموع الخضري علي صنفى الزيتون (منزانييلو وبيكوال). أعطت العدوى بفطريات *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfisii* أعلى شدة إصابة، وكان هناك علاقة

يصاب الزيتون بأمراض أعفان الجذور والذبول والتي تسبب خسائر شديدة تحت نظم الزراعة المختلفة، ومن ثم فقد استهدفت هذه الدراسة حصراً لإصابة بأمراض عفن الجذور في بعض مناطق إكثار الشتلات بجمهورية مصر العربية وتحديد مسبباتها المرضية، ومحاولة مكافحتها باستخدام عوامل المكافحة الحيوية

أوضحت نتائج حصر المرض بمشائل الزيتون بمحافظة الفيوم والجيزة خلال الصيف المبكر لعام ٢٠٠٣ أن أمراض عفن الجذور على الشتلات كانت أكثر وجوداً في الفيوم (٥٣%) مقارنة بالجيزة (٤٤%). تفاوتت أعراض الإصابة على المجموع الخضري فشملت ظهوراً اصفراراً على الأوراق، ذبول جزئي للمجموع الخضري أحياناً، تلون الأوراق باللون البني، وموت أطراف الفروع من القمة متجهاً نحو الداخل والذي كان مصاحباً لوجود عفن على الجذور و تقرحات في منطقة التاج بالقرب من سطح التربة، وفي معظم حالات الإصابة الشديدة كان يحدث تدهور و موت للنبات. كانت أكثر الأنواع الفطرية المعزولة من الشتلات

الناشئ عن الإصابة بمختلف الفطريات المختبرة على شتلات الزيتون صنفى منزائيللو، بيكوال حيث تفاوتت فاعلية المركبين وفقاً لنوع الممرض والصنف المستخدم، وتراوحت الكفاءة عامةً بين ٣٣,٣% إلى ٨٥,٨%.

توضح الدراسة أن أمراض عفن الجذور تمثل مشكلة في بعض مناطق إكثار الشتلات بجمهورية مصر العربية ونظراً لتعدد الفطريات المسببة للمرض وعدم وجود أصناف مقاومة فإنه يجب إجراء مزيد من الدراسة لوضع استراتيجية متكاملة لمكافحة هذا المرض ضمن برامج مكافحة أمراض الزيتون تحت نظم الزراعة المختلفة في مصر.

إرتباط موجب بين شدة الإصابة على المجموع الجذري وشدة الأعراض على المجموع الخضري.

كانت كل أصناف الزيتون المختبرة قابلة للإصابة أو شديدة القابلية للإصابة وفقاً لنوع الممرض وسجلت أعلى شدة إصابة في حالة فطريات *Fusarium solani* ، *Fusarium* ، بينما *Sclerotium rolfsii* ، *oxysporum* ، كانت أعراض الإصابة على المجموع الخضري أقل ما يمكن في حالة الصنف كوراتينا. أدى استخدام كلا من المركبين الحيويين Rhizo-Plus ، Trichoderma 2000 كعاملات للتربة قبل الزراعة، إلى إختزال معنوي في شدة الإصابة بعفن الجذور

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