ROOT-ROT DISEASE OF GRAPEVINE IN EGYPT
Ziedan, E. H.
Plant Pathology Dept., National Research Centre, Dokki, Giza, Egypt.

ABSTRACT

Fusarium oxysporum Schlecht., Fusarium solani (Mart) Secco., Rhizoctonia solani Kuhn. and Macrophomina phaseolina (Tesia) God. isolated from grapevine plants grown in Beheira, Gharbia and Giza Governorates exhibited wilt and root-rot symptoms. Fusarium spp. were the most common isolated fungi (55.8%) followed by M. phaseolina (26.9%) and R. solani (17.3%). F. oxysporum was highly pathogenic fungus, showing root-rot and disease severity of grapevine cv. Thompson followed by R. solani and M. phaseolina. The isolated F. oxysporum and R. solani were non-pathogenic. Isolation trials from the rhizosphere yielded 17 isolates of bacteria, fungi and actinomycetes. Trichoderma harzianum (No. 8), Pencillium sp. (No. 4), Bacillus subtilis (No. 12) and Pseudomonas fluorescens (No. 10) showed their antagonistic effect against the isolated pathogenic fungi.

Keyword: Grapevine, Root-rot, Elltology and Antagonism

INTRODUCTION

Root-rot diseases continue to be a serious production constraint on grapevines in the Mediterranean basin. Root-rot syndrome caused by a number of pathogens viz: Rhizoctonia solani (Walker 1992 and 1994), Pythium ultimum (Ulkhede, 1992), Phytophthora cactorum (Latorre et al., 1994), Phymatotrichum omnivorum (Phymatotrichopsis omnivora) (Ramirez-Arredondo, 1994). Fusarium wilt caused by Fusarium oxysporum f.sp. herbicola (Andrade et al., 1995). In Egypt root-rot of grapevine caused by Fusarium solani, F. moniliforme, F. roseum, F. tricinctum, F. semitectum, F. equiseti, F. acuminatum, R. solani, M. phaseolina, Pythium ultimum and Botryodiplodia theobromae (Badawy 1973, Mourad, 1983 and Mahrous, 1994). The interaction between different pathogenic soilborne fungi was adequately reviewed by several authors. Zaher et al. (1979) found that different combination between pathogenic isolate of R. solani + F. oxysporum and F. solani increased the percentage of both pre and post emergency damping off of senna (Cassia aquifolia Delile.) than the single isolates. Biswas and Samajita (1991) noticed that F. moniliforme became activated in the presence of Colletotrichum falcatum of sugar cane. Ragab et al., 1997 found that the combination between M. phaseolina + F. semitectum followed by F. semitectum + R. solani significantly increased root-rot of Japanese persimmon ( Diospyros kaki L.). Ziedan (2000) indicated that root-rot of peanut significantly increased when a combination of Aspergillus niger and F. oxysporum was found.

The present work was conducted to study the role of soilborne fungi in the incidence of root-rot disease complex of grapevine and a preliminary evaluation of rhizospheric microorganism as a potential biocontrol agent(s).

MATERIALS AND METHODS

Isolation and identification of causal organisms

Twenty five samples of diseased grapevine roots (cv. Thompson seedless) were collected from Beheira, Gharbia and Giza Governorates. The diseased tissues were disinfected in 1% sodium hypochlorite solution for 2
min., rinsed in tap water three times and placed on sterilized tissue paper at room temperature until dry. The sterile tissues were then plated on potato dextrose agar (PDA), Czapek's and peptone glucose agar media for 3-5 days at 25±2°C. Fungal isolates were purified using hyphal tip and single spore culture techniques. Identification was carried in consultation with information from (Booth 1971), Barnett and Hunter 1972 and Nelson et al., 1983.

Pathogenicity test

Pathogenicity test was carried out in a greenhouse of Plant Pathology Dept. (NRC). Plastic pots (20 cm diameter) containing clay sandy soil were infested with different isolates of each fungal inoculum. The inoculum was prepared by growing each fungal isolates in sterilized corn meal and sand medium (75 g corn meal grain + 25 g clean pure sand + 100 water) at 25-28°C for two weeks. Soil was infested with the rate of 10% (w/w). Pots were watered every two days for a week before planting. One-year-old seedling of grapevine seedless cv. Thompson was planted in each pot. Five pots were used for each isolates as replicates. Four months later the percentage of root-rot and disease severity of shoot was determined while the scale of Weltz and Arthur, 1973 was followed viz: 0 = Healthy plant, 1 = Yellowish + 1/3 plant wilted, 2 = 2/3 plant wilted, 3 = Whole plant wilted, 4 = Plant dead.

Biological control studies

Isolation of rhizospheric microflora

A number of bacterial, fungal and actinomycetes were isolated from rhizospheric soil samples collected from grapevine growing areas of Beheira, Giza and Qarbia using the method adopted by (Low and Webely, 1959). Dilutions were made up to 1×10⁻¹, 1×10⁻², 1×10⁻³ for isolating bacteria, actinomycetes and fungi respectively. Soil extract agar medium was used for bacterial isolation (Skinner et al., 1952), peptone dextrose agar medium for fungal isolation (Martin, 1950) and starch nitrate agar medium for actinomycetes (Waksman, 1957). One ml from the above dilutions was spreaded on the prepared media in a Petri dish and four replicate were used. The bacterial, fungal and actinomycetes colonies were checked 5 to 10 days after incubation in dark at 28°C. Antagonistic bacteria and actinomycetes isolates were identified according to the morphological and physiological characters recommended by (Harrigan and McCane, 1976 and Sneath, 1986).

Interaction between rhizospheric microflora and pathogenic fungi

The interaction between each of the grapevine pathogenic fungi i.e. F. oxysporum, M. phaseolina and R. solani and the rhizospheric microorganisms were assays in vitro on (PDA) according to (Ziedan 1993). Inhibition or reduction in the linear growth of the pathogenic fungi were recorded 5 days after incubation at 27±2°C.

Statistical analysis

Data were analyzed according to Snedecor and Cochran, (1980).
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Results
Survey of grapevine root-rot disease
The syndrome of grapevine root-rot disease complex, disease syndrome was commonly observed on the aerial plant parts showing yellowing, wilting, stunting, shortening of internode, while root showed necrotic lesions and discolorations (Fig. 1 and 2).

Isolation and identification of causal organisms
Conventional isolation technique from diseased grapevine roots yielded 52 isolates of fungi. Table 1: i.e., *Fusarium spp.* (55.0%), *M. phaseolina* (26.9%) and *R. solani* (17.3%). *Fusarium spp.* were the most prevailing fungus isolated from diseased grapevine plants collected from Gharbia (80%) followed by Giza (55.6%) and Beheira (51.7%). *M. phaseolina* was found at a percentage of 44.4% in Giza followed by Beheira (29.7%) but was not found in Gharbia. *Rhizoctonia solani* was found in Beheira at a rate of 27.8% followed by Gharbia (20%) Giza was free from the fungus.

<table>
<thead>
<tr>
<th>Fungus isolates</th>
<th>Governorate</th>
<th>% Fungal associated with grapevine root-rotted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beheira</td>
<td>Gharbia</td>
</tr>
<tr>
<td><em>Fusarium spp</em></td>
<td>29.8</td>
<td>7.7</td>
</tr>
<tr>
<td><em>M. phaseolina</em></td>
<td>15.4</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em></td>
<td>11.54</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Root rot infection types of grapevine in field
Routine isolation trails showed that different types of infection with fungal isolates i.e., (first model): single, double (second model) and (third model) combination between *Fusarium spp.*, *M. phaseolina* and *R. solani* were found (Fig. 3).

Data indicated that grapevine plants in Beheira, Gharbia, and Giza Governorates were infected with one or two and or three fungal infection models. Single and double infection types were observed at all Governorates. Third infection type was observed only in Beheira while, the higher percentage of single infection model was recorded in Gharbia followed by Giza. Meanwhile, the second model was observed only at Beheira (23.7%). Table 2 shows that different infection models with fungi were observed at Nubaria, the most cultivated province with grapevine in Egypt (Beheira Governorate). Single infection was due to *Fusarium spp.* (15.38%) and *R. solani* (15.38) while *M. phaseolina* as a single infection was not detected from infected root samples. The second infection model with double fungi viz: *Fusarium spp.* + *M. phaseolina* or *Fusarium spp* + *R. solani* were detected at (23.7%). Meanwhile, *R. solani* + *M. phaseolina* as a double infection model was not recorded. Finally, the third infection type viz: *Fusarium spp* + *R. solani* + *M. phaseolina* was detected at a rate (23.7%).
Fig (1): Root rot disease severity of grapevine shoot system
0 = Health plant, 1 = Yellowish+1/3 plant wilted, 2 = 2/3 plant wilted
3 = Whole plant wilted, 4 = Plant dead

Fig (2): Root rot disease severity of grapevine
0 = Normal colour (health), 1 = Slight brown discolouration
2 = Moderate brown discolouration, 3 = Dark brown discolouration

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![Graph showing percentage of grapevine root-rot infection](image)

Fig(3) Percentage of grapevine root-rot infection

<table>
<thead>
<tr>
<th>Infection types</th>
<th>Fungi</th>
<th>Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singly</td>
<td>Fusarium spp.</td>
<td>15.38</td>
</tr>
<tr>
<td></td>
<td>Rhizoctonia solani</td>
<td>15.68</td>
</tr>
<tr>
<td></td>
<td>M. phaseolina</td>
<td>0.00</td>
</tr>
<tr>
<td>Double</td>
<td>Fusarium spp. + R. solani</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>Fusarium spp. + M. phaseolina</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>R. solani + M. phaseolina</td>
<td>0.00</td>
</tr>
<tr>
<td>Third</td>
<td>Fusarium spp+ R. solani M. phaseolina</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Table (2): Percentage of fungal infection types of grapevine at Beheira Governorate

**Pathogenicity test**

A number of fungal isolates from diseased grapevine with root-rot, yellowing, wilting, stunting and discoloration of root system and phloem as shown in Fig (1 and 2) were prepared in pure cultures of *R. solani*. 2 isolates of *F. oxysporum* from Beheira, two isolates of *F. solani* and *M. phaseolina* from Giza and two isolates of *F. oxysporum* from Gharbia were tested against grapevine seedlings (Cv. Thompson seedless, one-year-old). Data in Table (3) show that *F. oxysporum* isolates from Beheira and Gharbia were the most and significantly pathogenic isolates induced 100% root-rot of grapevine plants and high disease severity. Three isolates of *R. solani*, *F. solani* and *M. phaseolina* showed moderately root-rot syndrome (50%) and disease severity. On the other hand, *R. solani* and *F. oxysporum* isolated from Beheira and Gharbia respectively were recorded as non-pathogenic isolates.
Table (3): Pathogenicity test of fungal isolates

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Governorate</th>
<th>Root-rot of grapevine Infection</th>
<th>O. severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoctonia solani</td>
<td>Beheira</td>
<td>00.0 A</td>
<td>0.0 C</td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>Beheira</td>
<td>50.0 B</td>
<td>2.0 B</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>Beheira</td>
<td>100.0 C</td>
<td>4.0 C</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>Giza</td>
<td>50.0 B</td>
<td>2.0 B</td>
</tr>
<tr>
<td>M. phaseolina</td>
<td>Giza</td>
<td>50.0 B</td>
<td>2.0 B</td>
</tr>
<tr>
<td>P. oxysporum</td>
<td>Gharbia</td>
<td>00.0 C</td>
<td>00.0</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>Gharbia</td>
<td>100.0 A</td>
<td>4.0 A</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>00.0 C</td>
<td>0.0 C</td>
</tr>
</tbody>
</table>

Values in a column followed by the same letter are not significantly different (P≤0.05) according to Duncans multiple range tests.

Symptomatology of grapevine root-rot disease

The expression of symptoms in root-rot disease of grapevine caused by Fusarium spp., M. phaseolina and R. solani showed to be similar to those of root-rot on other plants. Root-rot symptoms on shoot system appeared as chlorosis, yellowing wilting and withering of leaves which were pulled up easily. Fig (1) i.e. 0= healthy, 1 = yellowish +1/3 plant wilted, 2= 2/3 plant wilted, 3= whole plant wilted, 4= plant dead showed sever wilt and brownish root-rot. Severity in the root system started and appeared on root and stem basal portion as slight brown to dark black discoloration of phelom. They recorded as follows in Fig (2) 0= Healthy root (Normal colour), 1 = Slight brown discoloration, 2 = Moderate brown discoloration, 3 = Brown brown discoloration.

No differences were observed due to different pathogenic fungal isolate of shoot system and root systems.

Rhizospheric studies

Data in Table (4) show the isolated fungi and bacteria from grapevine rhizosphere i.e bacterial isolates (B. subtilis, 3 P. fluorescens), 2 Streptomyces isolates and 8 fungal isolates, 2 T. harzianum, 2 A niger, 2 Pencillium spp. and two unidentified fungi. Four isolates i.e Pencillium spp., T. harzianum (No.8), P. fluorescens (No 10), B. subtilis (No 12) showed a highly antagonistic effect against the three causal organisms i.e F. oxysporum, M. phaseolina and R. solani. A high reduction in pathogenic fungal growth by T. harzianum was observed. The fungal growth of the tested fungi was severely inhibited in the presence P. fluorescens.

Table (4): Antagonistic effect of rhizospheric microorganism to causal root-rot of grapevine

<table>
<thead>
<tr>
<th>Antagonistic microorganism</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F. oxysporum</td>
</tr>
<tr>
<td></td>
<td>reduction Lg%</td>
</tr>
<tr>
<td>Pencillium sp (4)</td>
<td>10.0</td>
</tr>
<tr>
<td>T. harzianum (5)</td>
<td>0.0</td>
</tr>
<tr>
<td>P. fluorescens (10)</td>
<td>13.0</td>
</tr>
<tr>
<td>B. subtilis (12)</td>
<td>6.0</td>
</tr>
</tbody>
</table>

L.g = Linear growth
* zone inhibition
DISCUSSION

Root-rot disease of grapevine was found to be caused by *F. oxysporum* Schlecht. *F. solani* (Martiussi Sacc., *R. solani* Kuhn and *M. phaseolina* (Tass.) Goid. (Badawy, 1973; Walker, 1992 and 1964; Mourad, 1993; Mahrous, 1994 and Andrade et al., 1995).

The observed root-rot symptoms of grapevine were found similar to those of other plants *i.e.* chlorosis, external and internal pheol discoulouration. These observation were similar to what obtained by Wettz and Arthur (1973) of chrysanthemum, Ziedan (1993) in sesame Carver et al., 1996 of pink, El-Mohamedy (1998) in citrus, and Ziedan (2000) in peanut.

The fungal isolation from diseased root showed three types of infection. These type might be due to the pathosystem in different governorates (Zaheer et al., 1979, Biswas and Samajjti 1991, Ragab et al., 1997 and Ziedan, 2000). These infection models of grapevine may be attributed to relationships between saprophytic ability of the fungus, the host and the environmental condition under natural field conditions.

*Fusarium* spp. followed by *M. phaseolina* and *R. solani* were the most isolated fungi from Behera, Garribi and Giza Governorates. Pathogenicity test indicated that *F. oxysporum* isolated from Behera and Garribi were the most pathogenic fungal isolates causing high percentage of root-rot incidence (100%) in cv. Thompson, the famous variety in Egypt. *Fusarium solani, M. phaseolina* and *R. solani* isolates showed moderately effect as root-rot on grapevine plant. On the other hand, some isolates of *F. oxysporum* and *R. solani* were recorded as non pathogenic fungi (Badawy, 1973 and Mourad, 1993, Mahrous, 1994). Two isolates of grapevine rhizosphere *i.e.* *Trichoderma harzianum* (No.8) and *P. fluorescens* (No.10) showed highly antagonistic effects against the pathogenic fungi causing a high reduction in the linear growth and restricted inhibition zones presenting antibiosis activities.

Further studies on the possibility of using these isolates as biocontrol agents are needed.

REFERENCES


مرض عفن جذور النعناع في مصر

السيد حسن زيدان

قسم امراض النباتات المركز القومي للبحوث - الدقيق - جيرة - مصر

تهدف البحث된 لتعريف على أهم مسببات مرض عفن جذور نباتات النعناع في محافظات البحرية والبحيرة والEPHIR، وبعض مضيفات الفيروسات المسببة للأمراض في النباتات، باستعمالها في برامج الفحص والبحثية.

وتقدم في المقالة المذكورة:

1. تم الحصول على مسببات

Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani, Fusarium oxysporum.

العثورا من نباتات النعناع المصابة بمرض عفن جذور النعناع والمحصول عليها اعراض الجذور وعفن

الجذور من محافظات البحرية والبحيرة والجريدة.

2. وافق الأنواع الموجودة في النبات Fusarium

هي أكثر الطرق المعزولة تكراراً بنسبة 65.8% (نسبة) تحت الفصل Fusarium phaseolina

(17.3%) (و أن عزلات الفطر Fusarium oxysporum

له أكثر عزلات القدرة في ارتفاع النعناع في نباتات النعناع. (صنف طومسون.)

Macrophomina phaseolina، Rhizoctonia solani

النوع

وحينما جرت في بناء عزلات الفطر

المحمولة عليها من محافظات الرديرية والجريدة على الترتيب ليس لها

الجذور على ارتفاع النعناع. (8)

- Pen. T. harzianum

النعناع حيث تم إن عزلة من الفطر (8) من نوع Pen. fluorescens

(4) وعزلة من كلا من الفطر B. subtilis (12)

(10) ذات قدرة جذابة عالية ضد الطرق المعزولة، والتي تسبب مرض عفن جذور

النعناع.