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Effect of Environmental Conditions on Wilting and Root Rot Fungi Pathogenic to Solanaceous Plants

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Abstract: Twenty three isolates of *Fusarium oxysporum*, eight isolates of *Fusarium solani*, two isolates of *Verticillium dahliae* and four isolates of *Rhizoctonia solani* were isolated from tomato plants showing wilting and root rot symptoms at different localities in Dakahlia governorate, Egypt. These isolates varied in their aggressiveness against tomato plants. The influence of temperature, pH, light regime, sealing culture plates with Parafilm (1-10 layers) and type of media on the growth of two *F. oxysporum* f. sp. *lycopersici* (isolates 14 and 19), *F. solani*, *V. dahliae* and *R. solani* were evaluated under laboratory conditions in Petri dishes or in liquid culture. The incubation conditions of 25°C and improved aeration (obtained by not wrapping the culture plates) induced the optimal growth of all fungi tested. Among the culture media tested, potato dextrose agar (PDA) was the best medium for the growth of all fungi tested except for *F. oxysporum* f. sp. *lycopersici* (isolate 14) which grew best on lima bean agar. The continuous light induced the best growth for *F. oxysporum* f. sp. *lycopersici* (isolate 14) grew best under continuous darkness while diurnal light was the best for *V. dahliae* growth. In general, pH 8 (initial level) promoted the best growth of all fungi tested (isolate 19 of *F. oxysporum* f. sp. *lycopersici*, *F. solani*, *V. dahliae* and *R. solani*) except for *F. oxysporum* f. sp. *lycopersici* (isolate 19) which was best grown at pH 9 (initial level).

Key words: Physical factors, pH, temperature, light, aeration, culture medium, tomato, *Fusarium*, *Rhizoctonia*, *Verticillium*

INTRODUCTION

Solanaceous crops are economically important in both tropical and temperate regions. Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most economic vegetable crops in Egypt either for local consumption or exportation purposes. World losses in tomato yield can be referred to soil-born pathogens.

Fungal pathogens are considered as damaging agents causing a considerable reduction of its production. Several soilborne fungi attack tomato plant causing wilt diseases and root rot include *Fusarium* sp., *Verticillium dahliae* and *Rhizoctonia solani* (Sneh *et al.*, 1991; Awad, 1996; Iannou, 2000). These fungi are limiting factors for production of tomato fruits in good quality and high quantity (Larkin and Fravel, 2000; Iannou, 2000).

An understanding of the role of environmental conditions have on the infection and survival of these pathogens is necessary to develop cultural disease management practices. Therefore, the objectives of this study were to isolation, purification, identification of pathogenic fungi causing wilt and root rot diseases of

tomato and determine the optimum cultural conditions for mycelial growth by these fungi including pH, temperature, light, aeration and type of medium.

MATERIALS AND METHODS

Isolation, purification and identification of pathogenic fungi causing wilt and root rot diseases of tomato: Isolation trails were carried out from diseased samples collected from different localities at Dakahlia governorate. Isolation of the pathogens was conducted from roots of wilting and root rotted tomato plants at different stages of plant growth. Roots of diseased plants were washed carefully under tap water to remove the adhering soil particles. The washed roots were cut into small pieces and divided into two groups. The first one was surface sterilized by immersing the root pieces in 1% sodium hypochlorite solution for 5 min and then washed several times in sterilized distilled water to remove any residues of sodium hypochlorite. The second group was left without sterilization in order to isolate the surface organisms. The washed root pieces were dried between two sterilized filter

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paper, then transferred to potato dextrose agar (PDA) amended with rose Bengal (0.003%) and streptomycin sulfate (0.01%) in Petri dishes and incubated at 25±2°C for 4-7 days. The growing fungi were individually transferred to PDA medium. Pure cultures of fungi were obtained using single spore or hyphal tip technique. The fungal isolates were then identified according to Clements and Shear (1957) and Booth (1977). Pure cultures of the isolated fungi were transferred to PDA slants and kept in refrigerator at 4°C for further uses.

Pathogenicity tests: All fungal isolates (37) obtained from isolation process were tested on tomato plants (cultivar: Super Marmand) in a greenhouse. Sterilized Erlenmeyer flasks, each containing 100 g of moistened ground corn:sand (3:1, w:w), were inoculated with cultures from each isolate using agar plugs from 7-day-old cultures. The inoculated medium was incubated at 25°C for 15 days. Plastic pots (15 cm in diameter) were filled with 500 g clay:sand (3:1, v/v) mixture. Soil was infested with each of the fungal isolates at a rate of 1% of soil weight (5 g infested corn/sand mixture per pot). Pots were irrigated to ensure the establishment of the tested isolates in the soil. Seeds of tomato cv. Super Marmand (susceptible to the wilt pathogens) were sown in the plant nursery and left to grow for 45 days. Tomato seedlings were transferred directly to the infested pots. Tomato seedlings transferred directly to non-infested pots served as a control. Three transplants were cultivated in each pot and three replicated pots were used for each treatment. Inoculated plants were weekly observed for development of disease symptoms (wilting and/or root rot). Forty days after inoculation, the number of survived plants was recorded and the percentages of plant mortality and survival plants were determined.

Environmental factors affecting growth of selected isolates: The effect of pH, incubation temperature, light regime, aeration and type of culture media on the growth of *F. oxysporum* f. sp. *lycopersici* (isolates 14 and 19), *F. solani*, *R. solani* and *V. dahliae* in agar plates was studied.

Effect of pH: Potato dextrose broth (PDB) medium was prepared in 250 mL Erlenmeyer flasks, each containing 100 mL of the medium. The pH of the medium was adjusted to 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10 with 1N NaOH and 1N HCl. For pH levels from 7.5 to 10, the medium was adjusted to these levels only after autoclaving. For the other pH levels, the medium was adjusted before autoclaving and then checked after autoclaving for any changes. Flasks were inoculated with 4-mm-diameter agar plugs of the test fungi and incubated

at 25°C. The mycelial mat was harvested 7 days after inoculation and dried in an oven at 70°C for 3 days. The dry weight of the mycelial mat was then recorded. Four replicates (flasks) were used for each treatment.

Effect of incubation temperature: Petri plates containing 20 mL of PDA medium were inoculated with 5 mm diameter discs from 10 day old cultures of each test fungus. The inoculated plates were incubated at five different temperatures: 15, 20, 25, 30 and 35°C. The colony diameters were measured 7, 4 and 21 days after inoculation for both *Fusarium* species, *R. solani* and *V. dahliae*, respectively. Four plates (replicates) were used for each treatment.

Effect of light: Petri plates containing 20 mL of PDA medium were inoculated with 5-mm-diameter discs from 10-day-old cultures of each test fungus. The inoculated plates were incubated at 25°C under three light photoperiods; Continuous Darkness (CD), Continuous Light (CL) and diurnal light (Dl, 12 h). Four plates (replicates) were used for each treatment. The colony diameters were measured 7, 4 and 21 days after inoculation for the two *Fusarium* species, *R. solani* and *V. dahliae*, respectively.

Effect of sealing of the culture plates: Cultures prepared as described above were incubated at 25°C and sealed with 0, 1, 2, 3, 5, 7 and 10 layers of Parafilm (American National Can, Greenwich, CT, USA). Four plates (replicates) were used for each treatment. The colony diameters were measured 7, 4 and 21 days after inoculation for the two *Fusarium* species, *R. solani* and *V. dahliae*, respectively.

Effect of culture media: Petri plates containing 20 mL of each of the following media: PDA, Lima Bean Agar (LBA), Potato Carrot Agar (PCA) and Tomato Dextrose Agar (TDA) were inoculated with 5 mm diameter discs from 10-day-old cultures of each test fungus. The inoculated plates were incubated at 25°C. Four plates (replicates) were used for each treatment. The colony diameters were measured 7, 4 and 21 days after inoculation for the two Fusarium species, R. solani and V. dahliae, respectively.

Statistical analysis: A complete randomized design was used in all experiments. Data collected from all experiments were statistically analyzed using the Statistical Analysis System package (SAS Institute, Cary, NC, USA). Differences between treatments were determined using Fishers Least Significant Difference (LSD) test and Duncans multiple range test (Duncun, 1955). All comparisons were performed at p = 0.05.

RESULTS AND DISCUSSION

Isolation and identification of tomato wilt and root rot pathogens: The process of isolation from both surface-sterilized and non sterilized roots resulted in 37 fungal isolates belonging to three fungal genera, i.e., *F. oxysporum*, *F. solani*, *V. dahliae* and *R. solani*. Twenty three isolates of *F. oxysporum* f. sp. *lycopersici*, eight isolates of *F. solani*, two isolates of *V. dahliae* and four isolates of *R. solani* were obtained.

Pathogenicity test: The first sign of wilting and root rot on tomato appeared around 40 days after inoculation and gradually intensified. Lower leaves developed the wilting first, then extended to the upper leaves. Vascular discoloration was evident from the early stages of infection, extending upward throughout the plant.

The most virulent isolates against tomato plants were *F. oxysporum* f. sp. *lycopersici*-14 (isolate 14) and *F. oxysporum* f. sp. *lycopersici*-19 (isolate 19), *F. solani* (isolate 1), *V. dahliae* (isolates 1 and 2) as well as *R. solani* (isolate 3) (Table 1).

Effect of environmental factors on the growth of pathogenic fungi

Effect of pH: In general, pH 8 (initial level; just when prepared and before autoclaving) promoted the best growth of all fungi tested except for *F. oxysporum* f. sp. *lycopersici* (isolate 14) which was best grown at pH 9 (initial level). Data in Table 2 show that initial pH levels of 9 and 10 gave the highest growth of *F. oxysporum* f. sp. *lycopersici* (Isolate 14). However, isolate 19 of the same fungus was best grown at initial pH levels from 7.5 to 10. In this regard, Mousa (2004) reported that pH 7 gave the

Table 1: Disease data resulted from inoculation of tomato plants with different pathogenic fungi, 40 days after inoculation

| Pathogens | Isolate No. | Treatment ^a | No. of survival plants | Plant mortality (%) | Survival plants (%) |
|---------------------------------|-------------|------------------------|------------------------|---------------------|---------------------|
| F. oxysporum f. sp. lycopersici | - | - (Control) | 3.00a | 0.00e | 100.00a |
| | 1 | + | 2.33abc | 22.33cde | 77.67abc |
| | 2 | + | 2.67ab | 11.00de | 89.00ab |
| | 3 | + | 2.67ab | 11.00de | 89.00ab |
| | 4 | + | 1.33cde | 55.67abc | 44.33cde |
| | 5 | + | 2.00abcd | 33.33bcde | 66.67abcd |
| | 6 | + | 1.33cde | 55.67abc | 44.33cde |
| | 7 | + | 2.00abcd | 33.33bcde | 66.67abcd |
| | 8 | + | 1.67bcde | 44.33abcd | 55.67bcde |
| | 9 | + | 1.33cde | 55.67abc | 44.33cde |
| | 10 | + | 2.00abcd | 33.33bcde | 66.67abcd |
| | 11 | + | 1.33cde | 55.67abc | 44.33cde |
| | 12 | + | 1.33cde | 55.67abc | 44.33cde |
| | 13 | + | 2.00abcd | 33.33bcde | 66.67abcd |
| | 14 | + | 0.67e | 77.67a | 22.33e |
| | 15 | + | 2.33abc | 22.33cde | 77.67abc |
| | 16 | + | 2.67ab | 11.00 de | 89.00ab |
| | 17 | + | 1.00de | 66.67ab | 33.33de |
| | 18 | + | 2.33abc | 22.33cde | 77.67abc |
| | 19 | + | 0.67e | 77.67a | 22.33e |
| | 20 | + | 2.33abc | 22.33cde | 77.67abc |
| | 21 | + | 2.00abcd | 33.33bcde | 66.67abcd |
| | 22 | + | 1.67bcde | 44.33abcd | 55.67bcde |
| | 23 | + | 1.67bcde | 44.33abcd | 55.67bcde |
| F. solani | - | - (Control) | 3.00a | 0.00 d | 100.00a |
| | 1 | +` | 0.33 d | 89.00a | 11.00d |
| | 2 | + | 3.00a | 0.00d | 100.00a |
| | 3 | + | 1.67bc | 44.33bc | 55.67bc |
| | 4 | + | 1.33cd | 55.67ab | 44.33cd |
| | 5 | + | 3.00a | 0.00d | 100.00a |
| | 6 | + | 2.00abc | 33.33bcd | 66.67abc |
| | 7 | + | 2.67ab | 11.00cd | 89.00ab |
| | 8 | + | 3.00a | 0.00d | 100.00a |
| V. dahliae | - | - (Control) | 3.00a | 0.00b | 100.00a |
| | 1 | +` ′ | 1.67b | 44.33a | 55.67b |
| | 2 | + | 1.67b | 44.33a | 55.67b |
| R. solani | - | - (Control) | 3.00a | 0.00c | 100.00a |
| | 1 | + | 0.67bc | 77.67ab | 22.33bc |
| | 2 | + | 1.33b | 55.67b | 44.33b |
| | 3 | + | 0.00c | 100.00a | 0.00c |
| | 4 | + | 1.33b | 55.67b | 44.33b |

^{*-:} Fungus-free control, +: Inoculated. Soil was infested with each of the fungal isolates at a rate of 1% of soil weight (5 g infested corn/sand mixture per pot). Pots were irrigated to ensure the establishment of the tested isolates in the soil. Seeds of tomato cv. Super Marmand (susceptible to the wilt pathogens) were sown in the plant nursery and left to grow for 45 days, b: Values within a column for each fungus followed by the same letter(s) are not significantly different according to LSD test (p = 0.05)

Table 2: Effect of pH levels on the growth of F. oxysporum f. sp. lycopersici (isolates 14 and 19) and F. solani after 7 days of incubation at 25°C

| pН | | lyc opersi | rum f. sp. ci - 14 | lycopers | <i>orum</i> f. sp. ici - 19 | F. solani | : | V. dahlid | se | R. solan | i |
|-----------------------|----------------------|------------------|-----------------------|------------------|--------------------------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|
| Before autoclaving | After autoclaving | pH at harvest | Dry weight (g) | pH at harvest | Dry weight (g) | pH at harvest | Dry weight (g) | pH at harvest | Dry weight (g) | pH at harvest | Dry weight (g) |
| 2.00 | 2.15 | 2.00 | 0.3055bca | 1.77 | 0.2791cde | 2.03 | 0.3276gh | 2.25 | 0.2588h | 2.01 | 0.4597f |
| 2.50 | 2.67 | 2.31 | 0.2988bc | 2.12 | 0.2757ed | 2.12 | 0.3293gh | 2.68 | 0.3524g | 2.52 | 0.3087g |
| 3.00 | 3.18 | 2.54 | 0.1915f | 2.61 | 0.1502i | 2.44 | 0.2585h | 3.16 | 0.1653i | 3.24 | 0.3574fg |
| 3.50 | 3.62 | 2.80 | 0.1917f | 3.26 | 0.1685hi | 3.03 | 0.2930h | 3.23 | 0.2588h | 5.04 | 0.8860d |
| 4.00 | 4.13 | 3.43 | 0.1920f | 3.61 | 0.1868ghi | 5.98 | 0.3275gh | 3.99 | 0.2595h | 6.88 | 0.6253e |
| 4.50 | 4.61 | 3.48 | 0.2182ef | 3.91 | 0.1920gh | 6.58 | 0.3925efg | 4.53 | 0.2521h | 7.04 | 0.6967e |
| 5.00 | 5.16 | 3.51 | 0.2427def | 2.75 | 0.2161fg | 5.15 | 0.4362cdef | 4.40 | 0.3584fg | 7.04 | 1.1552a |
| 5.50 | 5.61 | 3.92 | 0.2816bcd | 2.85 | 0.2357f | 6.42 | 0.4146def | 4.68 | 0.4285defg | 7.15 | 1.0932abc |
| 6.00 | 6.01 | 4.30 | 0.2581ede | 6.11 | 0.2419ef | 7.49 | 0.4418cdef | 4.46 | 0.4442cdef | 7.13 | 1.1304ab |
| 6.50 | 6.27 | 4.47 | 0.2773bcd | 6.40 | 0.2352f | 7.49 | 0.3769fg | 5.64 | 0.5070cd | 6.80 | 1.1013abc |
| 7.00 | 6.46 | 4.61 | 0.3001bc | 6.76 | 0.2502ef | 7.42 | 0.5067bc | 5.06 | 0.4747cde | 6.39 | 1.0716abc |
| _b | 7.50 | 4.84 | 0.2990bc | 6.62 | 0.3185abc | 7.32 | 0.5062bc | 4.48 | 0.4383def | 6.56 | 0.9881cd |
| _ | 8.00 | 4.68 | 0.3274b | 6.94 | 0.3555a | 7.40 | 0.6426a | 4.46 | 0.6772a | 7.24 | 1.1822a |
| - | 8.50 | 4.84 | 0.2732cd | 6.58 | 0.3064bcd | 7.35 | 0.4661cde | 4.44 | 0.3852fg | 7.42 | 0.9243d |
| - | 9.00 | 4.92 | 0.3995a | 7.23 | 0.3320ab | 7.80 | 0.5822ab | 4.64 | 0.5295bc | 6.32 | 1.1387ab |
| _ | 9.50 | 5.26 | 0.2748cd | 6.87 | 0.3270ab | 7.24 | 0.4915cd | 4.84 | 0.3951efg | 7.66 | 1.0025bcd |
| _ | 10.00 | 5.16 | 0.4361a | 7.21 | 0.3550a | 7.88 | 0.5980a | 5.35 | 0.6090ab | 5.22 | 0.9658cd |
| LSD 0.05 | | - | 0.05 | - | 0.04 | - | 0.08 | - | 0.09 | - | 0.14 |

^a: Values within a column followed by the same letter(s) are not significantly different according to LSD test (p = 0.05), ^b: For pH levels from 7.5 to 10, the medium was adjusted to these levels only after autoclaving

highest growth of F. oxysporum f. sp. lycopersici. However, his finding is slightly different from our findings, it was apparent from our results (Table 2) that different isolates can best grow at slightly different range of pH. This conclusion is supported by our finding that within 7 days of incubation, both isolates (14 and 19) of F. oxysporum f. sp. lycopersici have differently changed the pH of the culture broth medium. Both isolates have changed the initial alkali pH levels of the broth medium to acidic pH with being the final pH with isolate 14 became more acidic than with isolate 19 (Table 2). This might indicate that the metabolites of isolate 14 either are more acidic or more in quantity than those of isolate 19. The highest growth of F. solani was obtained when grown on broth medium with initial pH levels of 8, 9 and 10 (Table 2). It seems that the metabolites of F. solani are either less acidic or less in quantity than both isolates of F. oxysporum f. sp. lycopersici (Table 2). For V. dahliae, our results showed that its best growth was obtained at pH of 8 (initial level) (Table 2). This finding is unlike those reported by Domsch et al. (1980) who found that the optimum pH for the growth of V. dahliae was between 5.3 and 7.2 and Abada (1994) who reported that pH 6.0-7.2 favoured V. dahliae. On the other hand, the best growth of R. solani was obtained at a wider range of pH levels namely, from pH 5 to pH 9 (Table 2). This range is wider than the range reported by Abada (1994) i.e., pH 7.2-8.0.

Effect of temperature: The pathogenic fungi tested were able to grow at a wide range of temperature. Data in Table 3 indicate that 25°C was the common optimum temperature for all tested fungi. At 35°C all fungi except

R. solani had the least growth. The maximum growth of F. oxysporum f. sp. lycopersici (isolate 14) was obtained at 25°C followed by 20°C, while at 35°C, the lowest growth was obtained. However, F. oxysporum f. sp. lycopersici (isolate 19) had a wider range of temperature for its best growth which was obtained at 20 and 25 followed by 30°C. These findings are supported by the finding of Mousa (2004) who reported that the maximum growth of F. oxysporum f. sp. lycopersici was obtained at 25°C followed by 30°C. The maximum mycelial growth of F. solani was obtained at 25 and 30°C followed by 20 and 30°C. These results are partially in concurrence with other studies i.e., Christine (1986), Gracia-Garza and Fravel (1998) and Rekah et al. (2000) who indicated that fungal growth of Fusarium spp. was best between 20-25°C, then declined sharply at 35°C and totally inhibited at 40°C. The best growth of V. dahliae was obtained at 25°C followed by 20°C. The maximum mycelial growth of R. solani was obtained at 25 and 30°C while at 15°C, the least growth was occurred. Our results are supported by Windels and Brantner (2000), Okada and Ferris (2001) and Grosch et al. (2004) who found that R. solani prefers temperature range of 25-28°C while Verticillium sp. prefer 24°C (Schnathorst, 1981) and by Schnathorst (1981) who reported that the best growth of Verticillium sp. was obtained at 24°C.

Effect of light regime: The light regime had a significant effect on culture growth of the test pathogens. Data in Table 4 show that maximum mycelial growth of *F. oxysporum* f. sp. *lycopersici* (isolate 14), *F. solani* and *R. solani* occurred under continuous darkness or

Table 3: Effect of different temperature on the growth of pathogenic fungi

| Temperature (°C) | Growth diameter (cm) | | | | |
|------------------|---|---|-----------------|----------------------|--------------------|
| | Fusarium oxysporum f. sp. lycopersici - 14 | Fusarium oxysporum f. sp. lycopersici - 19 | Fusarium solani | Verticillium dahliae | Rhizoctonia solani |
| 15 | 3.01ca | 3.83c | 2.45c | 2.28d | 1.78c |
| 20 | 5.36b | 6.36a | 5.39b | 5.18b | 5.19b |
| 25 | 6.25a | 6.88a | 7.71a | 6.39a | 7.60a |
| 30 | 2.95c | 5.29b | 5.81b | 3.78c | 8.11a |
| 35 | 1.11d | 1.18d | 2.61c | 1.78d | 4.75b |
| LSD 0.05 | 0.55 | 1.04 | 0.69 | 0.73 | 0.50 |

^a: Values within a column followed by the same letter(s) are not significantly different according to LSD test (p = 0.05)

Table 4: Effect of different light on the growth of pathogenic fungi

| | Growth diameter (cm) | | | | | | | |
|---------------------|---|---|-----------------|----------------------|--------------------|--|--|--|
| Light regime | Fusarium oxysporum f. sp. lycopersici - 14 | Fusarium oxysporum f. sp. lycopersici - 19 | Fuscrium soloni | Verticillium dahliae | Rhizoctonia solani | | | |
| Continuous light | 7.94ab ^a | 9.00a | 8.40a | 5.35b | 9.00a | | | |
| Diurnal light | 7.06b | 8.48b | 7.44b | 6.48a | 8.31b | | | |
| Continuous darkness | 8.01a | 8.44b | 8.34a | 5.88ab | 8.59ab | | | |
| LSD 0.05 | 0.95 | 0.11 | 0.14 | 0.87 | 0.44 | | | |

e: Values within a column followed by the same letter(s) are not significantly different according to LSD test (p = 0.05)

Table 5: Effect of sealing of the culture plates on the growth of pathogenic fungi

| | Growth diameter (cm) | | | | | | |
|------------------|---|---|-----------------|----------------------|--------------------|--|--|
| NLP ^a | Fusarium oxysporum f. sp. lycopersici - 14 | Fusarium oxysporum f. sp. lycopersici - 19 | Fusarium solani | Verticillium dahliae | Rhizoctonia solani | | |
| 0 | 9.00a ^b | 9.00a | 9.00a | 6.04a | 9.00a | | |
| 1 | 8.50a | 8.31b | 8.16ab | 6.04a | 8.25b | | |
| 2 | 7.09b | 7.65bc | 7.10bc | 3.13c | 7.71c | | |
| 3 | 7.49b | 7.91cd | 6.55c | 5.29ab | 8.15abc | | |
| 5 | 7.20b | 6.83e | 6.94bc | 4.63b | 9.00a | | |
| 7 | 7.45b | 7.18de | 7.63bc | 3.16c | 9.00a | | |
| 10 | 7.16b | 7.21de | 6.94bc | 2.41c | 9.00a | | |
| LSD 0.05 | 0.81 | 0.62 | 1.31 | 1.21 | 0.46 | | |

^aNLP = Number of layers of Parafilm, ^b: Values within a column followed by the same letter(s) are not significantly different according to LSD test (p = 0.05)

continuous light, however isolate 19 of *F. oxysporum* f. sp. *lycopersici* grew best under the continuous light. The maximum mycelial growth of *V. dahliae* was obtained under diurnal light or continuous darkness. Although there are many well-documented studies on the effects of light on fungi, most of these deal with the promotion or inhibition of sporulation. Griffin (1981) has pointed out that light modulation of *in vitro* fungal growth must be interpreted cautiously because pre-exposure of media to light can result in growth inhibition. Evidence was obtained indicating that the inhibition may be due to the formation of peroxidase in the medium. In our study, the culture media were inoculated immediately after their preparation.

Effect of sealing of the culture plates: In general, all fungitested produced the highest level of growth when the culture plates were not wrapped with Parafilm (improved aeration) (Table 5). In other words, the mycelial growth of pathogenic fungitested was inhibited by increasing the Parafilm wrappings around the culture plates. This effect

may be due to a reduction of air exchange in wrapped cultures and an increase in the concentration of CO_2 as suggested by Cotty (1987). This finding is similar to the one reported by Shabana *et al.* (2001) who stated that the best mycelial growth of *Alternaria eichhorniae* (a biocontrol agent for waterhyacinth) was obtained in the unwrapped cultures or those sealed with one layer. The only pathogen that generally was not affected with the level of aeration (the number of Parafilm layers) was *R. solani* which covered the whole plate regardless the number of Parafilm wrapping around the culture plates.

Effect of culture media: The growth of the tested fungi varied depending on the type of medium. In general, PCA was the best common medium for the growth of all fungi tested (Table 6). However, for each individual isolate, there are also other medium/media that is/are as good as PCA. For instance, LBA also promoted the best growth of isolate 14 of *F. oxysporum* f. sp. *lycopersici* while PDA also promoted the best growth of all other fungi tested (Table 6). There was no significant difference among all

Table 6: Effect of different media on the growth of pathogenic fungi

| | Growt. | h diamete | er (cm) |
|--|--------|-----------|---------|
|--|--------|-----------|---------|

| | Fusarium oxysporum f. sp. | Fusarium oxysporum f. sp. | | | |
|----------------------|---------------------------|---------------------------|-----------------|----------------------|--------------------|
| Medium | lyc opersici - 14 | lycopersici - 19 | Fusarium solani | Verticillium dahliae | Rhizoctonia solani |
| Potato dextrose agar | 8.04b ^a | 8.76a | 8.34a | 9.00a | 9.00a |
| Lima bean agar | 9.00a | 8.49b | 8.05b | 8.31b | 9.00a |
| Potato carrot agar | 9.00a | 8.60ab | 8.38a | 9.00a | 9.00a |
| Tomato dextrose agar | 5.55c | 5.26c | 4.93c | 8.46b | 9.00a |
| LSD 0.05 | 0.51 | 0.24 | 0.28 | 0.17 | 0 |

a: Values within a column followed by the same letter(s) are not significantly different according to LSD test (p = 0.05)

media used in promoting the mycelial growth of R. solani, which filled the whole plate on all media within 4 days after inoculation (Table 6). However, PDA is considered a general medium for growth due to its nutritional factors, which are essential for growth, this medium is considered as a limiting factor for growth of Fusarium sp. (Marcia et al., 1983; Angela and Carlos, 2000). On the other hand, PDA was shown to favour growth of F. oxysporum f. sp. lycopersici (Mousa, 2004) and F. oxysporum f. sp. niveum (El-Shami, 1984). Present results do not support the statement of Marcia et al. (1983) and Angela and Carlos (2000) but agree with the findings of Mousa (2004) and El-Shami (1984) since PDA induced the best linear growth for F. oxysporum f. sp. lycopersici (isolate 19) and F. solani in the present study.

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