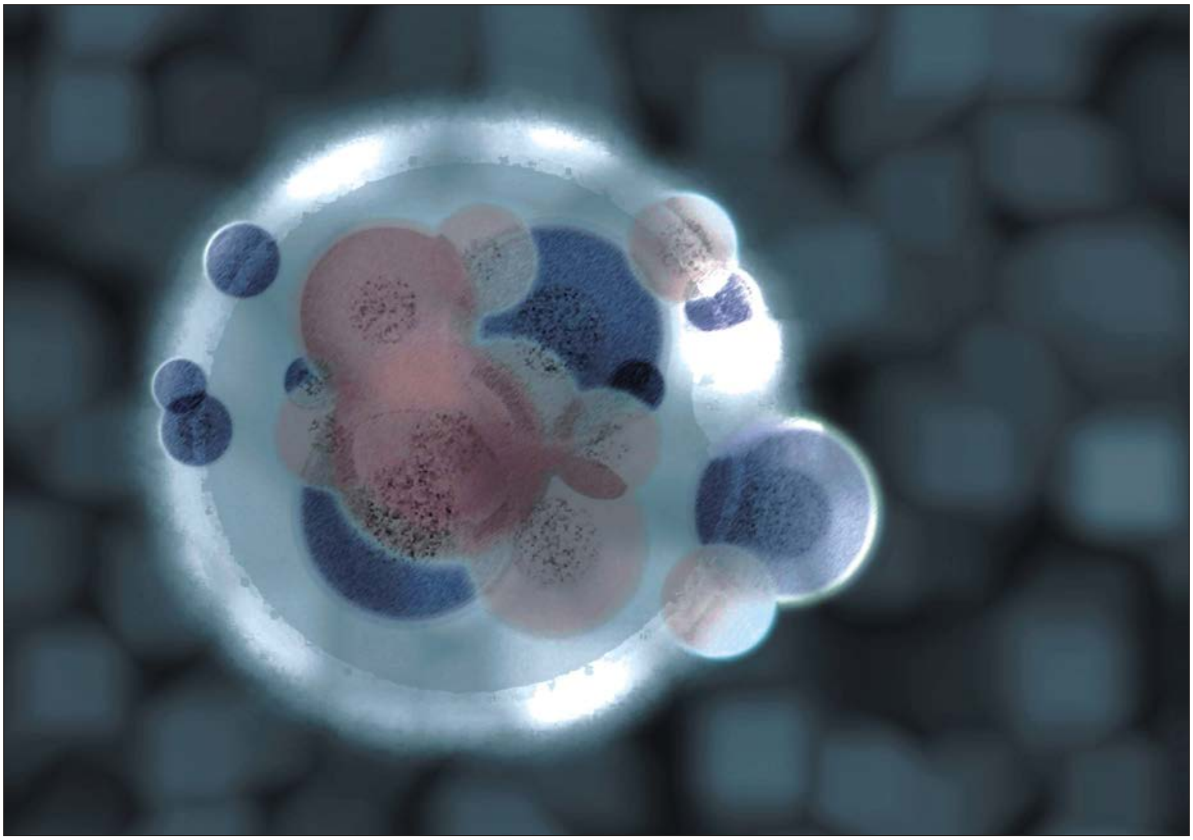

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Effect of Soil Acidity on Diseases Caused by *Pythium ultimum* and *Fusarium oxysporum* on Tomato Plants

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Abstract: *Pythium ultimum* and *Fusarium oxysporum* are important pathogens in agriculture crops and cause serious diseases that resulted in reducing the yield of many crops. The effect of soil acidity on the occurrence and severity of diseases caused by *Pythium ultimum* and *Fusarium oxysporum* were examined. Pathogens were grew at pH levels of 2-10 *in vitro*. *Pythium ultimum* and *Fusarium oxysporum* isolates have an optimum growth pH level of 6 and 7 and grew well at pH level of 5 and slightly at pH level of 4 and 8 but no growth at pH levels of 2, 3, 9 and 10. Six treatments (4, 5, 6, 7, 8 and 9) were tested *in vivo* to examine the effect of soil pH on the occurrence and severity of disease. The pH level of 5 was the best for plant growth but not to both pathogens. However, more disease severity by the two pathogens were found at the pH levels of 6 and 7. The pH levels could be used to reduce the plant disease reduction by knowing the optimum levels for plants and pathogens.

Key words: *Pythium ultimum*, *Fusarium oxysporum*, tomato plants, severity, pathogens

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is the most important fresh vegetable crop in Jordan and many other countries in the world. Present world production, which exceeded 133 million metric tons in 2007, occupied approximately 4.7 million ha (FAO, 2007). In Jordan, tomatoes are the most widely cultivated vegetable crop with a total area of about 98800 dunums, with a total production of 435100 metric tons (Ministry of Agriculture in Jordan, 2010).

Pythium species and *Fusarium* species are important pathogens in agriculture crops. They cause serious diseases that resulted in reducing the yield of many crops. These diseases including damping-off of seeds and seedlings, root rots and crown rots of older plants. *Fusarium* species also cause a vascular wilt of tomato plants. These two pathogens affect a wide variety of hosts of any age including tomato plants. The environmental conditions that are favored to tomato (especially in greenhouse which always has high humidity and moderate to high temperature) are the favorite for soil-borne pathogens including *Pythium* and *Fusarium* (Agrios, 2006).

The genus *Pythium* was created by Pringsheim in 1858 with the description of *Pythium monospermum* Pringsh as the type species (Martin, 1992). At the present time, more than 200 species have been described worldwide (Mathew *et al*., 2003; Abdelghani *et al*., 2004), but only 120 species have been given valid names (Dick, 1990) and the rest were placed in five groups (F, T, G, P and HS) (Van der Plaats-Niterink, 1981; Dick, 1990). Members of the genus *Pythium* are now classified as fungal-like organisms in the phylum Oomycota in the kingdom Chromista and not in the kingdom Fungi (Deacon, 2006).

Fusarium is a large genus of filamentous fungi widely spread in soil and effecting plants. *Fusarium oxysporum* is the most important species and it's a common soil-borne plant pathogen with a worldwide distribution. As a species, it probably causes more economic damage to agricultural crops than any other plant pathogen (Correll, 1991).

Different factors are very important in the occurrence and severity of plant diseases including environmental conditions and soil acidity (pH). Plants and pathogens have an optimum pH level for their growth and reproduction. When these optimum pH levels are known for plants and pathogens, diseases could be reduced by changing pH levels to be an optimum to plants not to pathogens. Various studies reported that pH level could be used to reduce plant disease severity and reproduction (Blank and Murray, 1998; Narisawa *et al*., 2005; Pane *et al*., 2011; Sakuma *et al*., 2011). For example, Waterer (2002) reported that common scab disease on potato plants caused by *Streptomyces scabies* could be controlled by decreasing soil pH level below 5.2 and he found this level not affecting the yield. Moreover, pH level had a significant positive linear relationship with species diversity and disease incidence of *Pythium* (Broders *et al*., 2009).

The present study was designed to examine the effect of soil acidity (pH) on the occurrence and severity of diseases caused by *Pythium ultimum* and *Fusarium oxysporum* on tomato plants. Moreover, this study will point the pH level that may reduce the damage on tomato plants caused by *Pythium ultimum* and *Fusarium oxysporum in vivo* and *in vitro*.

MATERIALS AND METHODS

Isolations: *Pythium* and *Fusarium* species were isolated from roots of diseased tomato seedlings grown in Jordan Valley on March, 2011. Samples were placed separately in labeled plastic bags and kept at 4°C until isolations. Root systems were washed under tap water and root pieces were plated on Potato Dextrose Agar (PDA) media. Cultures were incubated at 25±1°C for 2-3 days and identified according to (Van der Plaats-Niterink, 1981) for *Pythium* spp. and (Burgess and Summerell, 1992) for *Fusarium* spp.

***In vitro* experiments:** To examine the effect of pH on the growth of *Pythium ultimum* and *Fusarium oxysporum* in vitro. PDA media was made and adjusted the pH level to 2, 3, 4, 5, 6, 7, 8, 9 and 10 with a help of digital pH meter using 0.1 N Hydrochloric acid and 0.1 N sodium hydroxide. *Pythium ultimum* and *Fusarium oxysporum* were inoculated centrally on a Petri dish (90 mm diameter) containing 20 mL PDA with a 5 mm diameter plug taken from the edge of actively growing 3-5 days old cultures on PDA incubated at 25±1°C in the dark. All cultures were incubated for 2-5 days, to allow sufficient time for the different fungi and oomycetes to reach the edge of the Petri dishes. Three replicates were used for each fungus or oomycete at each pH level. Growth was measured after 5 days, using two diameter measurements perpendicular to each other. Colony radius was calculated for each of these times.

***In vivo* experiments:** The effect of soil pH on disease severity of *Pythium ultimum* and *Fusarium oxysporum* on tomato plants was examined. Tomato seedlings were planted in pots contain 1:1 peat moss and perlite. The pH of the mixture was 5.6-6. pH was adjusted to 4, 5, 6, 7, 8 and 9 using an irrigation solution of 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. Three pots were used for each pH level for *Pythium ultimum* and three pots were used for each pH level for *Fusarium oxysporum*. Three pots were used for each pH level as control for both pathogens. Plants were kept for four weeks from the time of inoculation to harvest in glasshouse and irrigated three times a week with the right solution to keep the pH level in the pot at the same. Plants were assessed every three days (visual observation) and at the end of the experiments, wet weight and dry weight of shoots and roots were obtained individually for all plants. Re-isolation of the pathogens was attempted.

Inoculum preparation: Macerated agar culture of two PDA plate cultures of *Pythium ultimum* were blended in 500 mL sterile distilled water with three pulses each of two seconds, for inoculating plants. Corresponding non-inoculated two PDA agar plates were blended in 500 mL sterile distilled water as above for use with control plants. The macerated agar was added at the rate of 20 mL per plant. The same method was used to *F. oxysporum*.

Data analysis: General Linear Model (GLM) ANOVA (SPSS VER 14) was used to find the differences ($p \leq 0.05$) of the growth on the plates between the pH levels tested. GLM ANOVA (SPSS VER 14) was used to find the differences ($p \leq 0.05$) between the mean wet weight and dry weight for shoots and roots for inoculated and non-inoculated (control) plants from the *in vivo* experiments. Heteroscedastic data were transformed as required using log or square root transformations.

RESULTS

Pythium ultimum isolates tested have an optimum growth pH level of 6 and 7 (Table 1). Isolates tested also grew well at pH level of 5. *Pythium ultimum* isolates grew slightly at pH level of 4 and 8. There were no growth of *Pythium ultimum* isolates at pH levels of 2, 3, 9 and 10. There were significant differences in colony growth between *Pythium ultimum* isolates tested at all pH levels tested ($p \leq 0.00$). However, no significant differences in colony growth between *Pythium ultimum* isolates tested

Table 1: Mean colony growth (CM^2) of *Pythium ultimum* isolates from tomato roots grown on PDA media with different pH levels at 5 days after inoculation and incubation in the dark at 25±1°C

pH level	Growth (CM^2)
2	0.00^a
3	0.00^a
4	9.70^b
5	50.70^c
6	56.72^d
7	56.72^d
8	12.70^b
9	0.00^a
10	0.00^a

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$

at pH levels of 6 and 7 ($p \leq 1.00$). Moreover, no significant differences in colony growth between *Pythium ultimum* isolates tested at pH levels of 4 and 8 ($p \leq 0.25$).

The optimum pH level of colony growth of *Fusarium oxysporum* isolates found in this study are 6 and 7 (Table 2). Moreover, *Fusarium oxysporum* isolates were grew well at pH level of 5. At pH levels of 4 and 8, *Fusarium oxysporum* isolates were grew slightly. However, no growth was found at pH levels of 2, 3, 9 and 10 of all *Fusarium oxysporum* isolates tested. There were significant differences in colony growth between *Fusarium oxysporum* isolates tested at all pH levels tested ($p \leq 0.00$). Moreover, the colony growth of *Fusarium oxysporum* isolates tested at pH level of 5 found to be significant different from all pH levels tested ($p \leq 0.00$). However, no significant differences in colony growth between *Fusarium oxysporum* isolates tested at pH levels of 6 and 7 ($p \leq 1.00$).

There were no disease symptoms evident on plants inoculated with *Pythium ultimum* or the control plants (Table 3) at pH level of 5. Disease symptoms appeared to be more severe on the plants inoculated with *Pythium ultimum* compare with the control at pH levels of 6 and 7. At pH levels of 4 and 8, plants showed limited growth on both inoculated and control plants. *Pythium ultimum* were successfully re-isolated from all inoculated plants and not from the control plants.

Significant differences in wet weight and dry weight of shoots and roots were found at pH levels of 6 and 7 between inoculated plants and the control plants (Table 3). Moreover, Significant differences in wet weight and dry weight of roots and dry weigh of shoots were found at pH level of 8 between inoculated plants and the control plants, but not in wet weight of shoots. However, no significant differences in wet weight and dry weight of shoots and roots were found at pH levels of 4 and 5 between inoculated plants and the control plants.

Tomato plants grew well at pH levels of 5 and 7 on both treatments, inoculated with *Fusarium oxysporum* and control plants and showed no disease symptoms (Table 4). However, disease symptoms were evident on plants grew at pH level of 6 compare with the control. Plants grew at pH levels of 4 and 8 showed limited growth on both inoculated and control plants. *Fusarium oxysporum* were successfully re-isolated from all inoculated plants and not from the control plants.

Significant differences were found in wet weight and dry weight of shoots and roots at pH levels of 5, 6 and 7 between inoculated plants and the control plants (Table 4). Moreover, significant differences in wet weight and dry weight of roots and dry shoots were found at pH level of 8 between inoculated plants and the control but not in wet shoots. However, no significant differences in wet weight and dry weight of shoots and roots were found at pH level of 4 between inoculated plants and the control plants.

Table 2: Mean colony growth (CM^2) of *Fusarium oxysporum* isolates from tomato roots grown on PDA media with different pH levels at 5 days after inoculation and incubation in the dark at 25±1°C

pH level	Growth (CM^2)
2	0.00^a
3	0.00^a
4	2.07^a
5	44.16^b
6	56.72^d
7	56.72^d
8	3.96^a
9	0.00^a
10	0.00^a

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$

Table 3: Mean wet and dry weights of shoots and roots of tomato plants inoculated with *Pythium ultimum* after four weeks with five treatments

	Mean weight (g)			
Treatments	Wet shoot	Dry shoot	Wet root	Dry root
pH 4				
Infected	48.33^a	2.37^a	3.01^a	0.74^a
Control	49.59^a	2.57^a	3.03^a	0.75^a
pH 5				
Infected	149.76^b	4.59^b	3.94^b	0.91^b
Control	157.33^b	5.67^b	4.52^b	1.02^b
pH 6				
Infected	88.64^d	3.75^b	2.75^b	0.68^a
Control	190.67^b	7.02^d	4.99^d	1.03^b
pH 7				
Infected	97.45^d	5.98^c	2.68^b	0.67^a
Control	200.71^b	6.89^d	5.62^d	1.06^b
pH 8				
Infected	79.18^d	2.97^a	3.98^c	0.85^c
Control	92.81^d	3.67^b	4.57^d	0.99^d

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$

Table 4: Mean wet and dry weights (g) of shoots and roots inoculated with *Fusarium oxysporum* after four weeks with five treatments

	Mean weight (g) of growth parameter			
Treatments	Wet shoot	Dry shoot	Wet root	Dry root
pH 4				
Infected	51.26^a	2.94^a	2.86^a	0.81^a
Control	60.07^a	2.99^a	2.91^a	0.89^a
pH 5				
Infected	100.27^b	3.87^b	3.01^b	0.77^a
Control	142.79^c	5.13^c	4.76^d	1.12^b
pH 6				
Infected	73.89^d	3.27^b	2.94^b	0.72^a
Control	197.72^c	7.82^d	5.42^d	1.14^b
pH 7				
Infected	100.95^d	6.12^c	2.79^b	0.72^a
Control	197.59^c	7.01^d	5.37^d	1.23^b
pH 8				
Infected	86.41^d	2.53^a	4.18^c	0.94^c
Control	90.85^d	3.95^b	4.49^d	1.17^d

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$

DISCUSSION

This study demonstrated that root diseases caused by *Pythium ultimum* and *Fusarium oxysporum* could be reduced by growing tomato plants in soil or media with pH level of 5. Based on the experiments using five pH levels, plants grew in media with pH level of 5 inoculated with *Pythium ultimum* showed no disease symptoms compare with control plants. Plants grew in media with pH levels of 6 and 7 and inoculated with *Pythium ultimum* showed wilted plants compare with the control plants. On the other hand, plants inoculated with *F. oxysporum* at pH level of 5 showed a few disease symptoms compare with the control plants. Moreover, plants grew at pH levels of 6 and 7 and inoculated with *F. oxysporum* showed more welted plants compare with the control plants. All plants grew at pH levels of 4 and 8 inoculated with *Pythium ultimum* or *Fusarium oxysporum* showed limited growth on both inoculated and control plants.

Significant differences in wet weight and dry weight of shoots and roots were found at pH levels of 6 and 7 between inoculated plants with *Pythium ultimum* and the control plants. Other researchers have found that root disease on coriander plants caused by *Pythium ultimum* is most destructive at pH levels of 5.5 to 6 (Lumsden and Ayers, 1975; Garibaldi *et al*., 2010). Moreover, significant differences were found in wet weight and dry weight of shoots and roots at pH levels of 5, 6 and 7 between inoculated plants with *Fusarium oxysporum* and the control plants. Caracuel *et al*. (2003) found that pH level of 6 was the best for *Fusarium oxysporum* growth and virulence.

The relation between root diseases and pH levels were investigated in this study. pH level of 5 was the best for growing tomatoes without symptoms. This mean tomato plants grow well and strong at pH level of 5. The optimum pH level for tomato in soil was found to be 5-6 (Preedy and Watson, 2008). Moreover, the optimum pH level for the two pathogens used in this study was 6 and 7 (Table 2). When pH level is optimum for the tomato plants and not for the pathogens, it could make tomato plants strong enough to resist the pathogens. This method of control diseases called creating conditions favorable for plants and unfavorable to the pathogen (Agrios, 2006). The agriculture methods for controlling plant diseases include the previous method were used to reduce various plant diseases (Kerruish, 2001).

On the other hand, tomato plants grow at pH levels of 6 and 7 and inoculated with *Pythium ultimum* or *Fusarium oxysporum* showed disease symptoms. The reason for this could be the pathogens at these pH levels were very pathogenic. The optimum pH level for the two pathogens used in this study was found to be 6 and 7 (Table 1 and 2). Garibaldi *et al*. (2010) found the high level of symptoms and growth reduction on coriander plants by *Pythium ultimum* was at pH level of 6. Other *Pythium* species such as *Pythium irregular* have also an optimum pH level of 6-6.5 (Athalye *et al*., 2009). Moreover, Vaz *et al*. (2010) found the best growth of *Fusarium oxysporum* occur was on media with pH level of 7. Furthermore, Merhej *et al*. (2010) report that the fungal of *Fusarium graminearum* development stopped when dropped the pH level in the media to 4.7-5.9.

Tomato plants grew at pH levels of 4 and 8 showed limited growth on infected and control treatments. This could be due to high in acidity or alkaline for tomato plants and both pathogens for the growth and reproduction. The optimum growth pH levels of pathogens on agar culture was found to be 6-7 and grow well at level 5, but only a very small amount of growth could occur at pH levels of 4 and 8 (Table 1, 2). Several studies have reported that the optimum growth pH level of *Pythium ultimum* and *Fusarium oxysporum* are 6-7 (Qian and Johnson, 1987; Vaz *et al*., 2010). Buckingham and Whittingham, 2008 record that most vegetables include tomato grow at pH level of 5-7.

CONCLUSION

The present study shows that pH level can control root diseases and reduce the yield losses caused by *Pythium ultimum* and *Fusarium oxysporum*. This method is easy to apply and not expensive in monitoring and changing pH levels in media or even soil. Moreover, this results will help farmers growing vegetables in hydroponic systems including Nutrient Film Technique (NFT) system in reducing their crops damage. Farther study is needed to assess this method in commercial.

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