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A comparative Study of Onion Purple Blotch (Caused by *Alternaria porri*) and Tomato Early Blight (Caused by *A. solani*) Diseases in Southern Ghors of Jordan

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Abstract

Two species of *Alternaria* were collected from southern Ghors of Jordan, isolated and characterized: *A. porii*, the causal agent of purple blotch of onion, and *A. solani*, the causal agent of early blight of tomato. Among planted onion cultivars, Beit Alpha was found to be highly susceptible to purple blotch, and Tebal was highly resistant under field conditions. Morphologically, there were distinct differences in conidia and mycelial growth rates between *A. porri* and *A. solani*. *Alternaria porii* was able to infect tomato and cause severe symptoms on its leaves; however, *A. solani* was not able to infect onion *in vitro*. Copper sulfate was the most effective salt in reducing the growth of both species *in vitro* and completely inhibited their fungal growth.

Keywords: Disease management, fungal diseases, plant pathogens, vegetable crops.

1. Introduction

Alternaria is a fungal genus that belongs to class Ascomycetes (formerly, classified in domain Eukaryota, kingdom Fungi, phylum Deuteromycota, class Hyphomycetes, order Hyphales, series *Porosporae* (Ellis & Gibson, 1975) and includes 299 species (saprophytic and pathogenic species). They are environmentally ubiquitous and are a part of common fungal flora that act as natural agents of decay and decomposition. About 20% of agricultural spoilage and decay are caused by *Alternaria* species. Crop losses caused by *Alternaria* species may reach up to 80% of the yield (Kirk *et al.,* 2008; Nowicki *et al.,* 2012). Variability is a well-known phenomenon in genus *Alternaria* and was noticed as changes in spore shape and size, mycelial growth, sporulation and pathogenicity (Mohsin *et al.,* 2016). Based on phylogenetic and morphological studies, *Alternaria* is currently divided into 26 sections (Woudenberg *et al.,* 2015). *Alternaria* sect. *porri* is the largest section containing most *Alternaria* species that have medium to large conidia and long beaks, some of which are important plant pathogens. The *porri* clade includes *A. porri* (Ellis) Cif. which causes purple blotch disease of onion as well as *A. solani* which causes early blight disease of tomato (Simmons, 1995).

Tomato (*Solanum lycopersicum* L.) is an economic solanaceous vegetable crop of high importance worldwide. Tomato is grown in an area of more than 5 million hectares around the world (WPTC, 2018) and of about 24500 hectares in Jordan with a local production of about 1.7 million tons (Anonymous, 2016). Onion (*Allium cepa* L.) is an important bulbous vegetable crop of a high global importance grown in an area of 3.7 million hectares (FAO, 2010). In Jordan, onion occupies an area of 5700 hectares that produce about 12200 tones of yield (Anonymous, 2016).

Early blight (target leaf spot) disease of tomato caused by *A. solani* is one of the world's most destructive diseases which affect tomato crops. The causal organism is air-borne and soil inhabiting. It is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). Symptoms of the disease usually appear on leaves, stems, petioles, twigs and fruits under favorable conditions resulting in defoliation, drying off of twigs and premature fruit drop; thus, the disease causes 50-86% loss in fruit yield (Mathur and Shekhawat, 1986).

Purple blotch of onion is a major disease throughout the world including Jordan, which is caused by *A. porri* (Islam *et al.,* 2001). This disease can cause 30-50% yield reduction (Pascua *et al.,* 1997). It causes extensive damage to bulb- as well as seed-crops. It is also a major limiting factor in the cultivation of onion (Savitha *et al.,* 2014; Priya *et al.,* 2015, Ramesh *et al.,* 2017).

Conidia, chlamydospores mycelia of *A. solani* and *A. porri* survive on plant debris and in soil. Both species have typical dry-dispersed conidia that are produced away from the host surface on aerial conidiophores (Fitt *et al.,* 1989; Everts and Lacy, 1996). Atmospheric temperature, humidity, wind speed and conidial spore concentration are the factors that are closely correlated with *A. solani* and *A.*

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porri disease occurrence. Wind, rain and insects are the primary source of disseminating inoculum of the pathogen (Rotem, 1994).

Presently, the two diseases are mainly managed by chemical fungicides, although the international trend is shifting towards more environmentally safe measures of plant disease control (Sallam, 2011; Savitha *et al.*, 2014).

The objectives of this study are to isolate and characterize the species of *Alternaria*, which cause diseases of onion and tomato grown in southern Ghors' open fields and to investigate the effects of different treatments on the suppression of the characterized pathogenic *Alternaria* species *in vitro*.

2. Materials and Methods

2.1. Field work

Several field trips were made to southern Ghors of Jordan, which is a common agricultural area under drip irrigation planted with tomato and onion crops during the pre-harvest stage of 2017/2018 fall. Visual disease observations of tomato and onion were carried out, particularly for diseases caused by *Alternaria* under open field conditions. Many diseased-plant samples were collected for laboratory pathogen isolation and identification. Disease incidence was assessed as the proportion % of diseased plants. Disease severity was scored by following 0-5 scale (Sharma, 1986) and further as a per cent disease index that was worked out by using a formula proposed by Wheeler (1969). Loss in crop yield was visually estimated in the field as the percentage of reduction in bulb size due to purple blotch disease.

2.2. Laboratory work

2.2.1. Pathogen isolation and characterization

Pathogen isolation was done by taking some superficial fungal growths of *A. solani* and *A. porri* from leaf lesions showing the typical symptoms of early blight of tomato and purple blotch of onion, respectively. They were cultured on potato dextrose agar (PDA) medium at 25°C in darkness for two weeks. The culture was then visually examined after slide preparation under a stereoscopic microscope at 100 and 400X for conidial morphology and characterization.

2.2.2. Pathogenicity test

Pathogenicity of the two *Alternaria* species was tested on tomato and onion leaves *in vitro*. A 5 cm-in-diameter piece of tomato (GS12, a susceptible cultivar) compound leaf or mature onion (Beit Alpha, a susceptible cultivar) leaf was rinsed in sterile distilled water, plated on Petri-dish and sprayed with a conidial suspension (at 10^6 conidia per ml) of each pathogen. Three plates were assigned for each treatment as replicates and three plates were sprayed with distilled water as non-treated control. All inoculated plates were incubated at 25°C and 16/8 light/dark inside a plant growth chamber for one week. Disease-symptoms development was visually monitored and photographed at the end of the incubation period.

2.2.3. In vitro fungicide bioassay

To investigate the effects of different chemical treatments on the suppression of the two pathogenic species *in vitro*, homogenized solutions of copper sulfate (at 2.5 g/l), sulfur (80% of a commercial product (c.p.) at 7 g/l), carbendazim (50% of c.p. at 2.5 g/l) and thiophenate methyl (70% c.p. at 1 g/l) were prepared under laboratory conditions. A 0.5 cm-in-diameter disc was taken from a pure culture of *A. porri* or *A. solani* and placed on the center of a freshly prepared PDA plate after treating it uniformly with 0.5 ml of each of the previous solutions. Five treated and cultured plates were assigned as replicates for each treatment and five untreated cultured plates were kept as a control. All plates were incubated at 25°C in darkness for one week. After one week, the diameter of the fungal growth on the plate of each species per treatment was measured using a 20 cm-in-length ruler and compared with that of the untreated control.

2.3. Statistical Analysis

Data was analyzed statistically using regression models where general linear model (GLM) procedure was implemented (SPSS software version 11.5; SPSS Inc., Chicago, USA). Least significance difference (LSD) test and t-test were applied for mean separation at the 0.05 probability level (Steel *et al.*, 1997).

3. Results

3.1. Field work

All planted tomato cultivars were found highly susceptible to early blight caused by *A. solani* as indicated by high disease incidence (about 85%) and severity (about 62%). No tomato cultivars resistant or tolerant were noticed during the trips in southern Ghors. Onion cultivar Tebal was found highly resistant to purple blotch (the disease incidence and severity were less than 15 %) compared to another cultivar (Beit Alpha) that was highly susceptible with a disease incidence and severity higher than 85% (Table 1). Purple blotch of onion was a highly destructive disease that had caused high economic losses especially in the susceptible cultivar Beit Alpha with approximately 39.3 % compared to 4.2 % bulb reduction in the resistant one, Tebal.

Table 1. Disease incidence and severity of purple blotch disease on two onion cultivars at fully mature bulb stage under field conditions of southern Ghors of Jordan:

Cultivar	Disease incidence %	Disease severity %
Beit Alpha	87.3 [1] a[2]	91.7 a
Tibal	12.8 b	13.6 b

[1] Average of 10 plants/ treatment

[2] Means within columns followed by the same letters are not significantly different at 0.05 probability level using t-test.

3.2. Laboratory work

3.2.1. Pathogen isolation and characterization

Morphologically, there were distinct differences in conidia morphology and mycelial growth rates between *A. porri* and *A. solani* (Figure 1). The conidia of *A. solani* are relatively larger in size and their cells are more swollen than that of *A. porri*. Furthermore, the growth rate of *A. solani* was faster than *A. porri*. The mycelium of *A. solani* was able to cover the whole plate within a week (see the control plates, Figure 3).

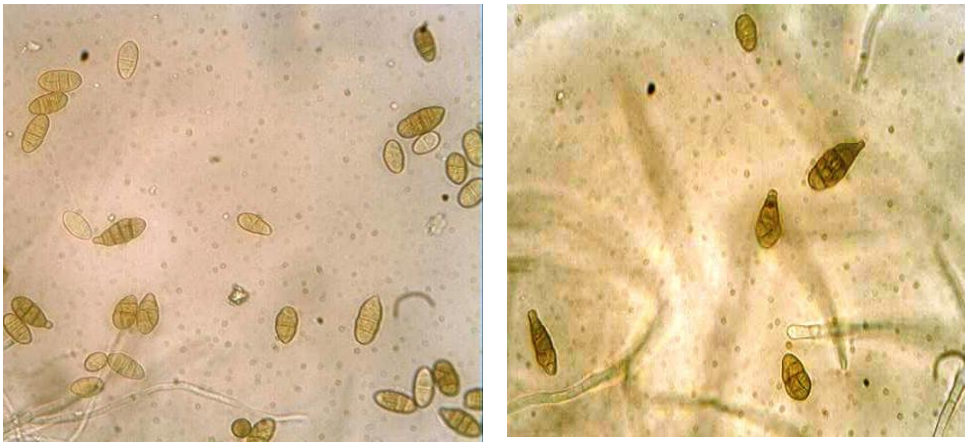

Figure 1. Conidia of *Alternaria porii* (left) and conidia of *A. solani* (right) under 400X total magnification.

3.2.2. Pathogenicity test

Alternaria porii was able to infect tomato and cause severe symptoms on its leaves, but *A. solani* was not able to infect onion *in vitro* (Figure 2). The pathogenicity of *A. porri* on tomato was confirmed by the positive re-isolation of the pathogen from symptomatic tomato leaves showing wide light brown lesions surrounded by wide yellow halo at the end of incubation period (Figure 2).

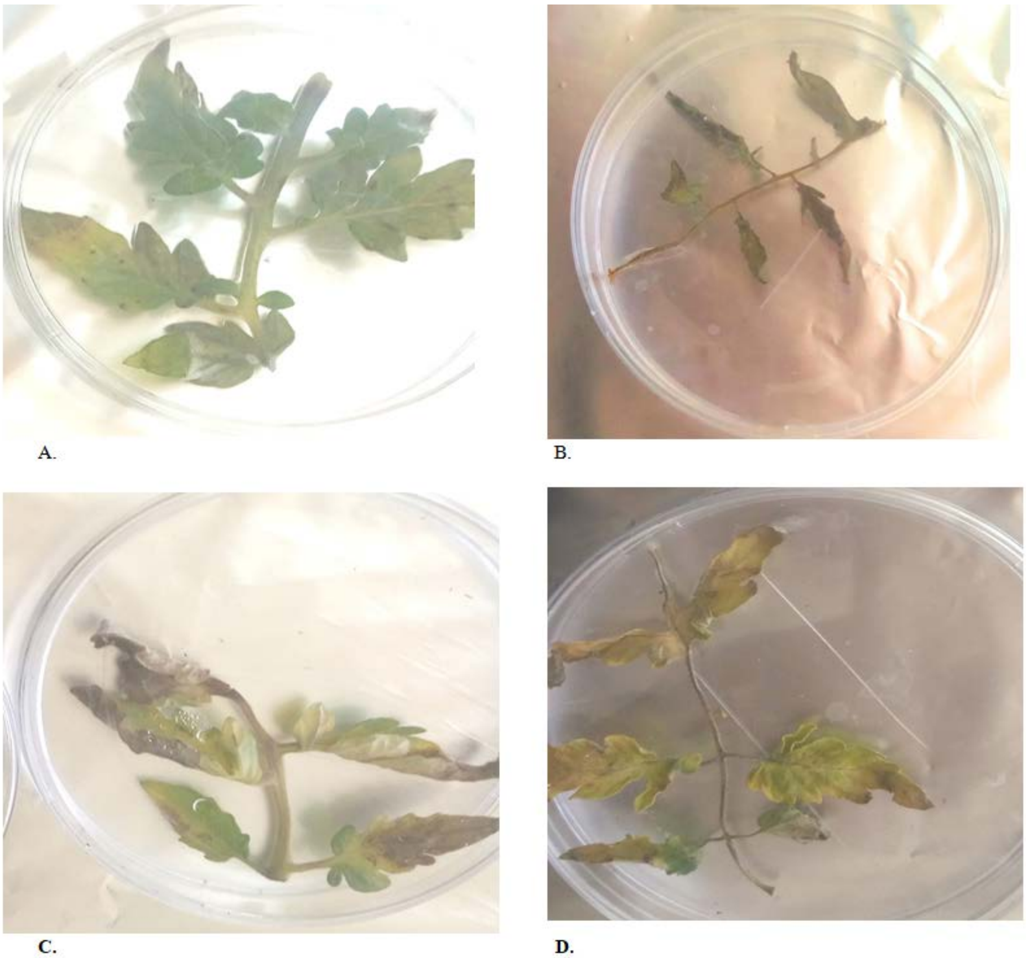

Figure 2 Symptoms resulted from artificial inoculation and infection of tomato with *Alternaria porii* (in A: after one week and in B: after two weeks) or with *A. solani* (in A: after one week and in B: after two weeks).

3.2.3. In vitro fungicide bioassay

Copper sulfate was the most effective treatment. It was more effective than sulfur and even than the two systemic fungicides; carbendazim and theophenate methyl in reducing the growth of *A. porri* and *A. solani* in the bioassay test. Copper sulfate treatment completely inhibited the fungal growth of both *Alternaria* species *in vitro* (Figures 3 & 4).

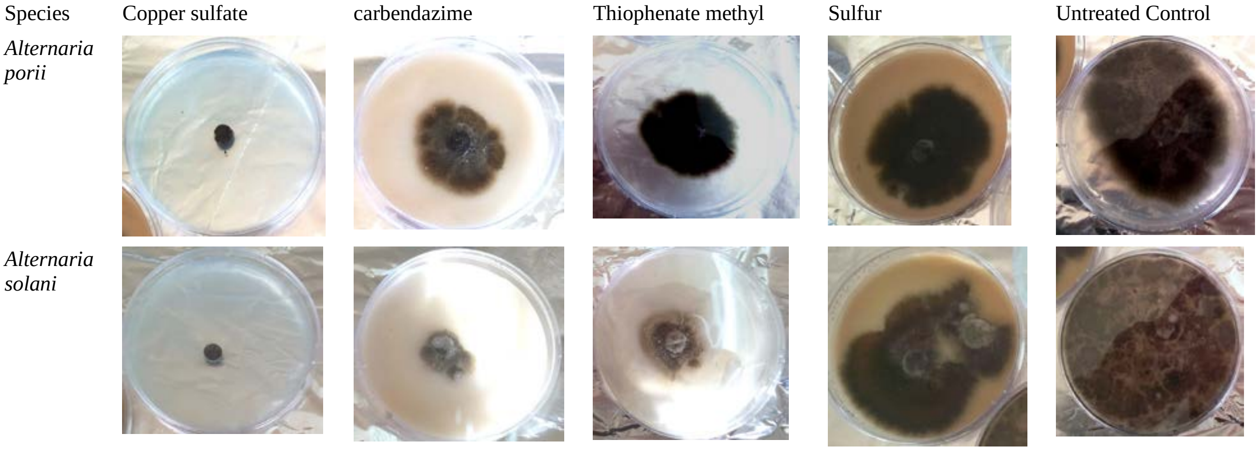

Figure 3: Fungal growth of *Alternaria porii* and *A. solani* on PDA treated with different chemical treatments.

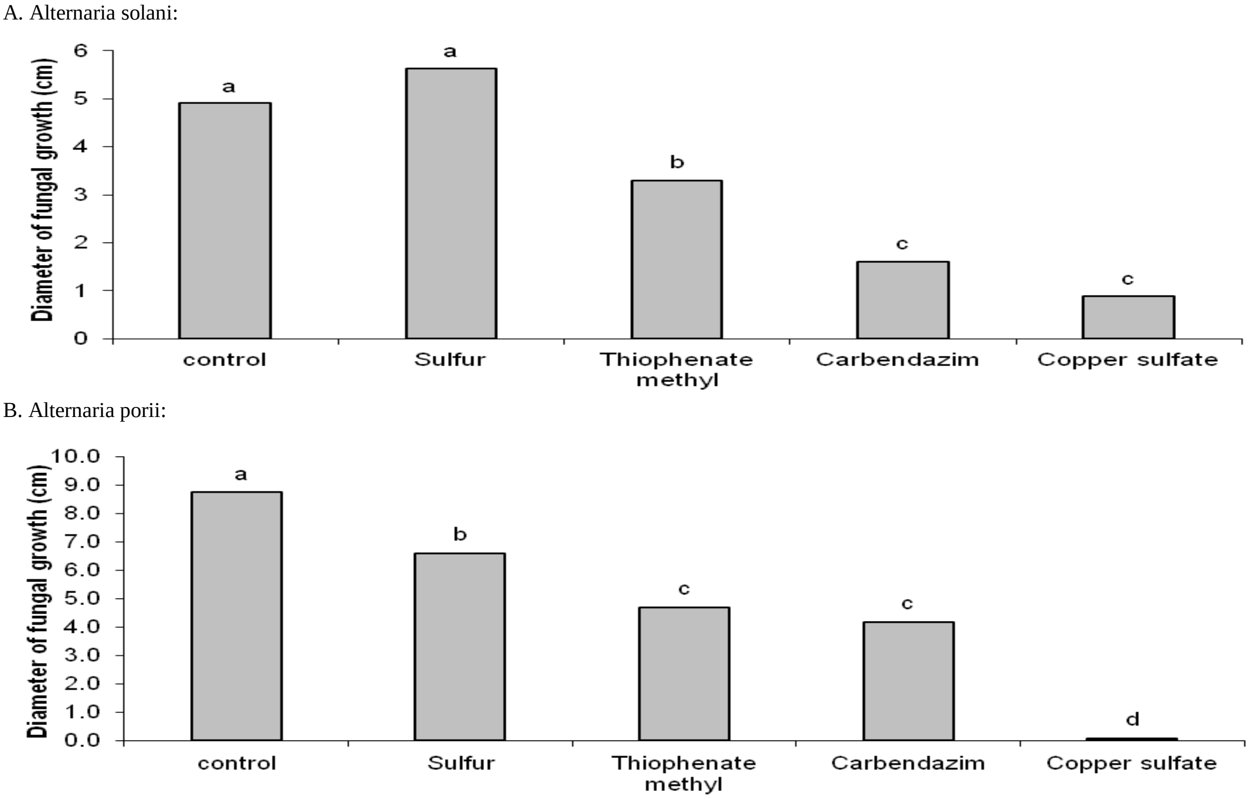

Figure 4: Fungal growth (in cm) of *Alternaria solani* and *A. porii* on PDA treated with different treatments.

4. Discussion

The two diseases caused by *A. solani* and *A. porii*, common in southern Ghors of Jordan, resulted in considerable plant-yield losses as indicated from our field observations. The influence of purple blotch disease was more severe in the susceptible onion cv. Beit Alpha than cv. Tebal which appeared to be resistant. Therefore, the use of resistant cultivars of onion could be a useful, easy-to-use and effective tool to reduce crop losses due to the disease. The use of resistant cultivars of tomato could be useful also in tomato since no cultivars resistant or tolerant to early blight disease were noticed during the trips in southern Ghors. It might be due to the loss of high genetic variability. Low genetic resistance to pest and diseases in commercial tomato cultivars induces farmers, under conventional crop systems, to use high levels of pesticides that do not meet consumer requirements (Modolon *et al.*, 2012). Epidemics do not generally occur until late in the season, when the plants are most susceptible. However, disease-progress curves differ depending on location and prevailing weather conditions.

Early blight can be controlled by effective use of cultural practices such as a 3-5 year crop rotation with non-host crops or resistant cultivars, site selection, sanitation of fields, providing proper plant nutrition, avoiding water stress and planting disease-free seed (Madden *et al.,* 1978). Generally, the best crops for rotation are forage crops and grains, including maize. Successive or consecutive cropping of tomato or potato in one field promotes an earlier appearance of early blight (Shtienberg and Fry, 1990).

As it is revealed by the morphological observations, the two *Alternaria* species (*A. solani* and *porii*) have variations in the shape and size of conidia. In addition, there are differences in the growth rate between them on PDA medium. These results agree with the previous studies (Ellis & Gibson, 1975; Pryor and Gilbertson, 2000; Hussein, 2019).

Alternaria porii was able to infect tomato and causes severe symptoms on its leaves but *A. solani* was not able to infect onion *in vitro*. The pathogenicity of *A. porri* on tomato was confirmed by the positive re-isolation from symptomatic tomato leaves showing wide light brown lesions surrounded by wide yellow halo. In a study using ITS and mitochondrial SSU sequences of *Alternaria* species,) showed a distinct number of species-clades. Although the porri clade includes both *A. porri* and *A. solani* as related species, there are differences in their pathogenicity to different hosts that could be due to phylogenetic and enzymatic variability (Pryor and Gilbertson, 2000; Hussein, 2019).

Our study revealed that both *Alternaria* species (*A. porri* and *A. solani*) were destructive plant pathogens. This result has an agreement with the previous studies (Pelletier, 1988; Shuman, 1995). *Alternaria solani* is a polycyclic pathogen as many cycles of infection are possible during a season (Shuman, 1995). Primary infections on new plantings of potatoes or tomatoes are caused by over-wintering inoculum (Pscheidt, 1985). The pathogen over-winters as mycelium or conidia in plant debris, soil, infected tubers and fruits or other host plants (Pelletier, 1988; Shuman, 1995; Al-Ameiri *et al.,* 2015).

Among tested salts and fungicides, copper containing fungicides (e.g. copper sulfate) could be effective in protecting plants from both diseases. A number of foliar fungicides can be used to manage early blight of tomato as they were effective. Copper-oxychloride, mancozeb and chlorothalonil are the most frequently used protectant fungicides for early blight management but they provide insufficient control under high disease pressure (Holm *et al.,* 2003; Pasche and Gudmestad, 2008).

5. Conclusion

Both *Alternaria* species (*A. solani* and *A. porii*) were found naturally occurring in southern Ghors of Jordan and causing purple blotch of onion and early blight of tomato that resulted in considerable yield losses. Onion cv. Tebal was found to be resistant to purple blotch under field conditions. *Alternaria porii* was able to infect both crops *in vitro*. Copper sulfate was effective in the suppression of fungal growth of both *Alternaria* species *in vitro*.

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