Journal of Advanced Zoology
ISSN: 0253-7214
Volume 44 Issue S-7 Year 2023 Page 1389: 1397

Compressive Study Of *Alternaria Solani* With Different Parameters Including Morphology, Physiology, Molecular And Pathogenicity Test

Khalaf Alhussaen*

**Department of Biology, College of Haqel, University of Tabuk, Saudi Arabia*

Article History

Received:
Revised:
Accepted:

Abstract

Alternaria spp. is an important pathogen include species are pathogenic, endophytic and saprobic which are caused a different plant diseases on seeds, plants, agricultural products. Isolates of Alternaria solani investigated in the present study were recovered from tomato plants have symptoms located in farms at Jordan Valley and collected from the market in city of Haqel, Tabuk area – Saudi Arabia. Population of the Alternaria solani were investigated based on morphology, physiology, molecular and pathogenicity characteristics. Alternaria solani isolates tested in the present study showed a variation between their populations based on morphology, physiology, molecular and pathogenicity characteristics. The optimum pH level of the Alternaria solani isolates tested in the present study were 6 to 7 and the optimum growth temperature were 25 to 30°C. This study were the first research in this area (Jordan and Saudi Arabia) to study Alternaria solani with different parameters including morphology, physiology, molecular and pathogenicity test.

Keywords: *Alternaria solani, morphology, physiology, molecular and pathogenicity test.*

Introduction:

Alternaria spp. is an important pathogen include species are pathogenic, endophytic and saprobic which are caused a different plant diseases on seeds, plants, agricultural products. *Alternaria solani* (*A. solani*) (Ellis and G. Martin) Sorauer species occupies a significant position among pathogenic species causing early blight disease and affects a range of crop plants. The disease of early blight on different economic plants include tomato and potato cause significant yield reductions (Olanya et al., 2009). The symptoms appear on tomato and potato when affected by *Alternaria solani* start on leaves that old, mature and lower, chlorotic and yellow brown spots then become large until one-half inch in diameter. The distinguish symptoms of early blight disease is a spot like target pattern (Agrious, 2005).

Alternaria solani is a pathogen that has a variable population based on molecular characterizations, physiological characteristics and morphological features isolated from various parts of the word (Pryor and Michailides, 2002; Alhussaen, 2012; Mallik et al., 2014; Alhussaen, 2019). Generally, pathogens have variable populations they were usually isolated from farms located in different areas. These variations affected the methods of disease managements, specifically when they tested for the fungicide usage.

Molecular, physiological and morphological characterizations are usually used to point the population variation of *A. solani*. It has large, long-beaked, and noncatenated spores, the septate mycelium is of dark brown colour which later become darker. The size of conidiophores varies from 50 to 90 µm having dark coloured conidia of 120-296 x 12-20 µm size (Simmons, 2000). These characteristics vary between isolates as Singh (1987) found *in vitro* conidia were grouped together as short chains with transverse (5-10) longitudinal septa (1-5).

Physiological characteristics for *A. solani* is vary when the optimum pH level were 6 to 7 and the optimum growing temperature were 25º C and 30º C when tested *in vitro* (Alhussaen, 2012). Other study found that *A. solani* has an optimum growth temperature of 25º C and pH level of 6.5 (Tatiana et al., 2010). Moreover, study in north Jordan found that *A. solani* has the optimum conditions were 25º C and pH 7 (Ibrahim et al., 2009).

Several molecular techniques were used successfully to study the variations between populations of *A. solani*. A study carried out in Jordan exhibited that population of *A. solani* isolates varied greatly under high temperature and humidity of different farms located in Jordan Valley (Alhussaen, 2019). Moreover, a dendrogram of plant pathogen papulation was prepared successfully using ITS sequencing of rDNA region (Onsekiz et al., 2018). Lourenco (et al., 2011), using different markers such as RAPD and AFLP, observed a significantly higher genetic variation in *A. solani* population in more than 150 isolates recovered from tomato and potato plants.

Investigation of pathogen populations based on genetic and pathogenic characteristics is lead to understand the epidemiology of the pathogen and will help in great successful management methods (Morris et al., 2000). Other study found that isolates of *A. solani* were variable in the population based on different parameters including morphology, pathogenic and genetic characteristics when all these isolates produced disease on the tested host (Varma et al., 2007).

This investigation has been performed to determine variation in population of *A. solani* isolated from various farms in Jordan Valley in 2012 as described in Alhussaen (2012 and 2019) and the population of *A. solani* from Tabuk region of Saudi Arabia. Nine isolates from tomato fruits, having early blight symptoms, were collected from the local market of Tabuk area, city of Haqel – Saudi Arabia based on morphological, pathogenic and genetic variation.

Materials and method:

Isolation:

In the spring of 2012, twelve isolates of *A. solani* recovered from infected tomato plants in Jordan Valley as described in Alhussaen (2012 and 2019). Nine isolates of tomato fruits, infested with early blight, were collected from the local market in Tabuk area, city of Haqel – Saudi Arabia (Table 1).

Table1: Isolates numbers, codes, country of isolates and place.

Isolate	Isolate code	Country of isolate	Place of isolate
1	JO1	Jordan	Farm 1
2	JO2	Jordan	Farm 1
3	JO3	Jordan	Farm 1
4	JO4	Jordan	Farm 2
5	JO5	Jordan	Farm 2
6	JO6	Jordan	Farm 2
7	JO7	Jordan	Farm 3
8	JO8	Jordan	Farm 3
9	JO9	Jordan	Farm 3
10	JO10	Jordan	Farm 4
11	JO11	Jordan	Farm 4
12	JO12	Jordan	Farm 4
13	SA1	Saudi Arabia	Market 1
14	SA2	Saudi Arabia	Market 1
15	SA3	Saudi Arabia	Market 1
16	SA4	Saudi Arabia	Market 2
17	SA5	Saudi Arabia	Market 2
18	SA6	Saudi Arabia	Market 2

19	SA7	Saudi Arabia	Market 3
20	SA8	Saudi Arabia	Market 3
21	SA9	Saudi Arabia	Market 3

Morphological characterization:
Different morphology features of *Alternaria solani* isolates were examined under power objective 40X using light microscope including hyphal color and size (length and width), also the number of septa in conidia and their size, length of beak. Seven days cultures of *Alternaria solani* were grown on PDA.

Physiological studies:
Effect of pH levels
Effect of pH on the growth of *Alternaria solani* was examined *in vitro* on PDA media. Various pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0) of the medium were adjusted by adding 0.1 N sodium hydroxide and 0.1 N hydrochloric. The discs of 5 mm size, taken from margin of culture, were inoculated and incubated in dark at 25±1°C up to 7 days. Three replications of each treatment were taken into consideration. After 7 days, the growth was recorded by measuring the diameter of the colony

Effect of temperature
In order to observe the optimum, lowest and the highest temperatures for fungal growth, PDA Petri dish cultures of the nine *A. solani* isolates were incubated under eight different temperatures from 5°C to 40°C (5°C intervals). All incubations were carried out at 25±1°C in the dark up to 7 days. For each isolate 3 replicates were used for each temperature. After 7 days, growth was measured using two diameter measurements perpendicular to each other.

Molecular Methods:
The DNA from *A. solani* mycelium was extracted and was amplified by PCR. The method of Paul (2000) was used to examine the Internal Transcribed Spacer (ITS1) region of the ribosomal nuclear DNA (rnDNA). Tools of the BLAST search http://blast.ncbi.nlm.nih.gov; it was used to analyze the sequences, and the sequences was amplified by ClustalW tools http://www.ebi.ac.uk; to examine the phylogram of the isolate and its relative.

Pathogenicity test:
The isolates of *A. solani* collected from various farms in Jordan Valley and from the local market of Tabuk area, city of Haqel – Saudi Arabia, were tested for pathogenicity (Table 1). In this method, healthy tomato fruits were inoculated with a pure culture of *A. solani* isolates tested in the present investigation.
A culture of 5 days old on PDA media incubated in the dark at 25°C ± 1°C of *A. solani* were used to cut a 5 mm disc from the margin and from a corresponding non-inoculated agar plate in the case of the control. The agar disc was placed on the surface of tomato fruits and near the disc an injury was made. Incubation of fruits was performed in the dark at 25°C ± 1°C for 7 days.

DATA ANALYSIS

The study was carried out using Completely Randomized Design (CRD). Rach treatment was replicated three times. For each treatment, growth area was measured with colony counter on Petri dish. Differences ($p \leq 0.05$) between treatment means were calculated with General Linear Model (GLM) ANOVA was used using SPSS software (VER 25).

Results:

Morphological characterization:
The isolates of *A. solani* recovered from Jordan Valley farms and local market of Saudi Arabia, show a significant variation in the morphological characterization. Regarding the shape, colour and arrangement, the shape of conidia was straight, slightly flexuous, muriform or ellipsoidal tapering to beak, pale and sometimes branched. The size of conidia varied from 25-75 µm in length and 10-21 µm in width (Figure 1, Table 2). The morphological study of mycelium shows that it was septate having 2-7 transverse and 1-5 longitudinal septa.

The width of mycelium was 0.8-1.5 µm. The morphology of conidiophores exhibits that they were brown to olivaceous brown in colour and were arranged in groups or singly.

Table 2: Morphological features for isolates of *A solani* recovered from Jordan Valley farms and local market from Saudi Arabia.

Isolate Code	Mycelial width (µm)	Conidia size (µm) Length	Width	Length of beak (µm)	Septa in conidia Horizontal	Vertical
JO1	0.8-1.2	25-50	10-15	8-10	3-5	1-4
JO4	1.1-1.5	35-65	10-20	10-14	2-5	1-3
JO7	0.9-1.3	45-75	15-20	12-17	4-7	1-3
JO10	1.0-1.4	40-65	10-15	11-15	3-6	2-4
SA1	1-1.7	37-70	11-19	9-13	2-4	1-3
SA4	0.9-1.6	35-71	12-21	10-16	3-7	2-3
SA7	1.2-1.7	38-69	11-17	11-16	3-5	2-5

Figure 1: Mean of morphological features for isolates of *A. solani* recovered from Jordan Valley farms and local market from Saudi Arabia.

Effect of pH levels:

The optimum pH level of the *A. solani* isolates tested was 6 to 7. Isolates grew well at pH 4.5, 5, 5.5 and 7.5. On the other hand, lower growth was observed at pH 4 and 8 for all isolates tested (Figure 2, Table 3).

No significant differences were found in mycelium growth between *A. solani* isolates tested at each pH levels (P=0.56). However, significant differences were noticed in the colony growth at different pH levels for each isolate (P=0.00). Nevertheless, no significant difference in mycelium growth was observed for each isolate at pH 6, 6.5 and 7 (Table 3).

Table 3: Mean growth (mm) of 7 isolates of *A. solani* incubated on PDA with nine pH levels (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8) in the dark. Growth was measured after 7 days. Within a column, means with the same letter are not significantly different from each other at $P \leq 0.01$. n = 3 for each isolate at each pH level.

	Mycelial diameter growth (mm) of different isolates						
pH level	Jo1	Jo4	Jo7	Jo10	SA1	SA4	SA7
4	3.9 b	3.3 b	3.5 b	3.3 b	3.4 b	3.7 b	3.6 b
4.5	5.3 c	5.1 c	5.4 c	5.0 c	5.2 c	5.5 c	5.1 c
5	6.8 d	7.0 d	6.9 d	6.6 d	7.0 d	6.9 d	6.6 d
5.5	6.7 d	6.6 d	6.8 d	6.8 d	6.6 d	6.8 d	6.8 d
6	8.1 e	7.9 d	8.3 e	8.1 e	7.9 d	8.3 e	8.1 e
6.5	8.6 e	8.7 e	8.5 e	8.5 e	8.7 e	8.5 e	8.5 e
7	8.8 e	8.9 e	8.6 e	8.7 e	8.9 e	8.6 e	8.7 e
7.5	5.4 c	5.0 c	5.2 c	5.3 c	5.0 c	5.2 c	5.3 c
8	2.1 a	2.0 a	2.1 a	2.0 a	2.0 a	2.1 a	2.0 a

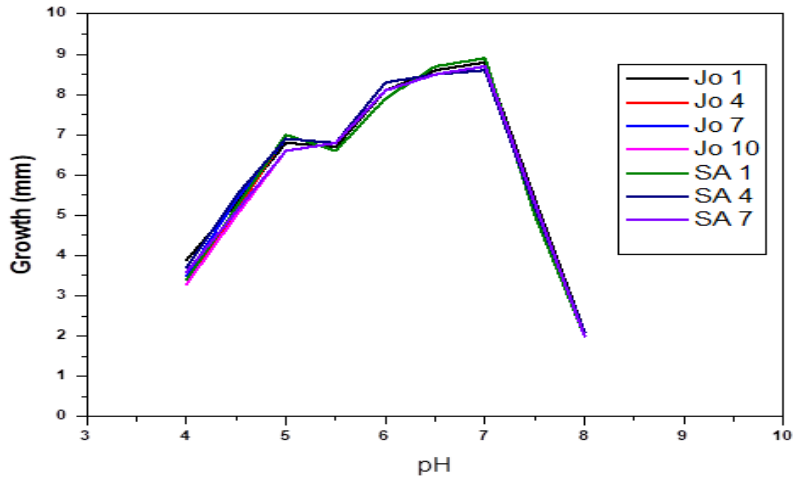

Figure 2: Mean growth (mm) of 7 isolates of *A. solani* incubated on PDA with nine pH levels (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8) in the dark.

Effect of temperature:

Isolates tested of *A. solani* have an optimum growth temperature of 25°C to 30°C. At temperature, 20°C *A. solani* isolates grew slightly 2.6 cm. Moreover, Limited growth was occurred at temperatures of 10°C and 40°C, but no growth was appeared at temperature of 5°C (Figure 3, Table 4).

Significant differences were appeared in colony growth among the different isolates of *A. solani* at each temperature tested (P=0.01). Moreover, variation in temperature significantly affected the growth of colony for each isolate (P=0.00). Nevertheless, a non-significant effect of temperature on mycelial growth was observed at 25 °C and 30 °C (Table 4).

Table 4: Mean growth (mm) of 7 *A. solani* isolates incubated at eight different temperatures from 5°C to 40°C on PDA in the dark. Growth was measured after 7 days. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.01$. n = 3 for each isolate at each temperature.

Temperature	Mycelial diameter growth (mm) of different isolates						
(C°)	Jo1	Jo4	Jo7	Jo10	SA1	SA4	SA7
5	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
10	0.1 b	0.2 b	0.2 b	0.1 b	0.2 b	0.3 b	0.1 b
15	1.1 c	1.9 c	1.2 c	1.0 c	1.3 c	1.7 c	1.5 c
20	2.1 d	2.8 d	2.4 d	2.8 d	2.6 d	2.7 d	2.6 d
25	8.6 e	8.8 e	8.8 e	8.5 e	8.6 e	8.8 e	8.6 e
30	8.8 e	8.7 e	8.9 e	8.3 e	8.9 e	8.7 e	8.7 e
35	2.9 d	3.5 d	3.1 d	3.5 d	3.4 d	3.6 d	3.2 d
40	0.2 b	0.1 b	0.1 b	0.1 b	0.1 b	0.1 b	0.1 b

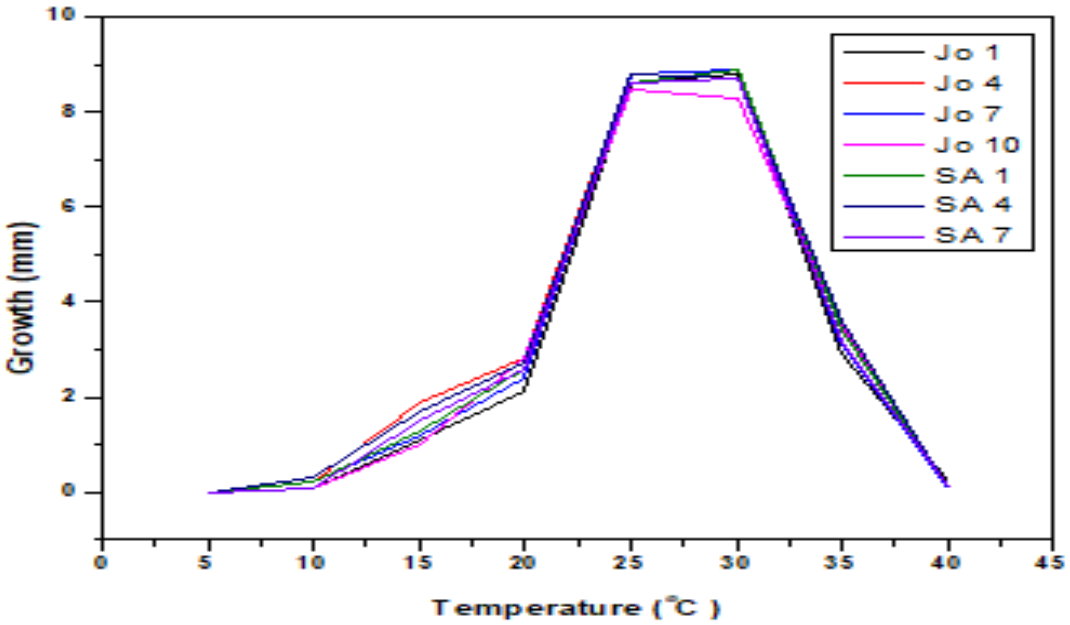

Figure 3: Mean growth (mm) of 7 *A. solani* isolates incubated at eight different temperatures from 5°C to 40°C on PDA in the dark. Growth was measured after 7 days.

DNA Sequences:

The sequences of *A. solani* isolates from different farms in the Jordan Valley corresponding to *A. solani* from Gen-Bank (Table 5) with code (LT714700). The length of sequence for isolate (JO1) was 777 bp, and was 100% similarity to the sequence of *A. solani* (LT714700). However, the length of (JO4) isolates sequence was 546 bp, and (JO7) isolates sequence length was 470 bp, and (JO10) isolates sequence was 550 bp, in length were 99% similarity to the sequence of *A. solani* (LT714700).

Isolates recovery from local market from Saudi Arabia corresponding to *A. solani* (DQ084021) from Gen-Bank (Table 5). The sequence length of isolate of SA1 was 786 bp, and was 100% similarity to the sequence of *A. solani* (DQ084021). However, the sequence length of isolates (SA4) was 751 bp, and the length sequence of (SA7) was 768 bp, and were 99% similarity to the sequence of *A. solani* (DQ084021).

Table 5: Sequences and the corresponding sequences from GenBank of *A. solani* isolated from tomato fruits and plants with early blight symptoms from farms in the Jordan Valley and local market from Saudi Arabia.

Isolate code	Sequence length (bp)	Match from GenBank (Location)^	GenBank accession number	Identities (%)	Gaps
JO1	777	*Alternaria solani* SEH-As 1999 (PAKISTAN)	LT714700	100	0
JO4	546	*Alternaria solani* SEH-As 1999 (PAKISTAN)	LT714700	99	0
JO7	470	*Alternaria solani* SEH-As 1999 (PAKISTAN)	LT714700	99	0
JO10	550	*Alternaria solani* SEH-As 1999 (PAKISTAN)	LT714700	99	0
SA1	786	*Alternaria solani* 2005 (USA)	DQ084021	100	0
SA4	751	*Alternaria solani* 2005 (USA)	DQ084021	99	1
SA7	768	*Alternaria solani* 2005 (USA)	DQ084021	99	0

Variation observed with the ITS region of rDNA sequence:

UPGMA dendrogram (Figure 1) were described by the length (bp) of the sequence for ITS region of rDNA. Isolates were divided into six division at similarity coefficient 0.1. The isolates collected from tomato fruits from the market in Saudi Arabia into two groups at similarity coefficient 0.1. Nevertheless, isolates come from various farms in Jordan Valley were divided into four division at similarity coefficient 0.1. In these six divisions isolates recovery from same area and have similar morphology and physiology features and matched the same isolates from the GenBank were come together in the dendrogram (Figure 1).

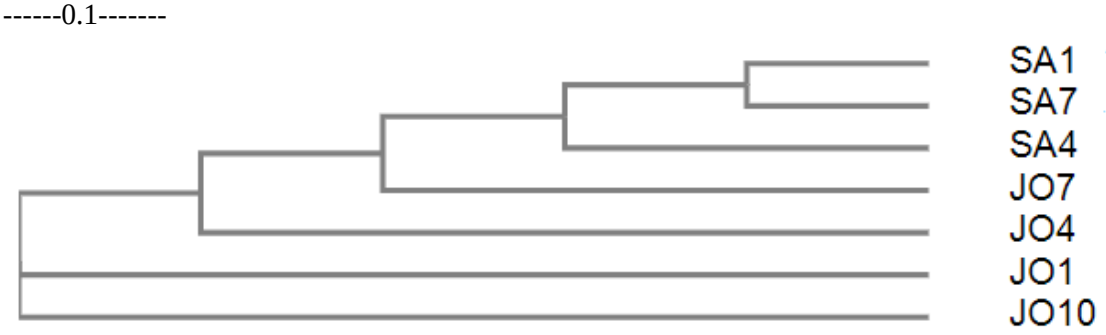

Figure 1: Dendrogram of *Alternaria solani* by DNA sequences.

Pathogenicity test:

The isolates of *A. solani* studied, were found to be pathogenic to tomato fruits tested. Symptoms start of chlorotic and abscise prematurely spots. These spots have concentric rings as target-like pattern and surrounded by a yellow halo. However, no symptoms appeared in the control (non-inoculated) plants (Table 6).

Inoculated and the control (non-inoculated) fruits exhibited significant differences (P=0.00) in disease severity. In addition, significant differences were found between inoculated isolates recovery from Jordan valley and the isolates recovery from local market of Saudi Arabia (P=0.00) (Table 6). However, non-significant difference in disease severity was found in the isolates recovered from Jordan valley (P=0.08). Moreover, a non-significant difference in disease severity was found in the isolates recovered from the local market of Saudi Arabia (P=0.06).

Table 6: Mean disease severity for *A. solani* isolates from farms located in Jordan Valley and from local market from Saudi Arabia. Means followed by the same letter are not significantly different from each other.

Isolate code	Mean disease severity (cm)
JO1	1.2 a
JO4	1.1 a
JO7	1.2 a
JO10	1.3 a
SA1	1.8 b
SA4	1.8 b
SA7	2.0 b
Control	0.0 c

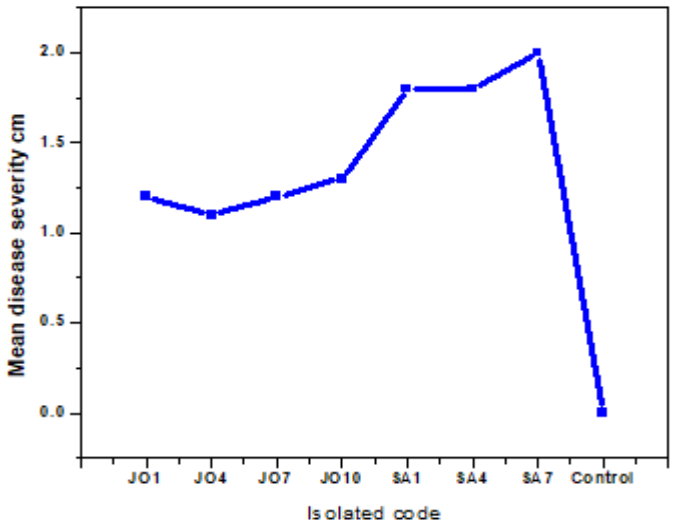

Figure: Mean disease severity for *A. solani* isolates from farms located in Jordan Valley and from local market from Saudi Arabia.

Discussion:

Alternaria solani isolates tested in the present study recovered from farms in Jordan Valley and the local market from Saudi Arabia showed a variation between their populations based on morphology, physiology, molecular and pathogenicity characteristics. This is the first research in this area (Jordan and Saudi Arabia) to study *A. solani* with different parameters including morphology, physiology, molecular and pathogenicity test. In Jordan, fungicides sensitivity and disease management studies were done on *A. solani* (Al-Mughrabi, 2004; Abu-El Samen and Al Shudifat, 2012; Abu-El Samen et al., 2016). In Jordan, the first study on the physiological, morphological and molecular characterization of *A. solani* was perfomed by Alhussaen (2012 and 2019). Whereas, in Saudi Arabia few researchers investigated the *A. solani* management techniques (Rahmatzai et al., 2017; Baka, 2010).

The results of this study pointed that the optimum growing temperature of the isolates recovered in this study was 25º C and 30º C. Moreover, the pH level of 6 to 7 were found to be the optimum for *A. solani* when they tested *in vitro*. These results were published by Alhussaen (2012 and 2019) contestant with Ibrahim et al., (2009) who found that the optimum conditions for *A. solani* isolated from north of Jordan were 25º C and pH 7 when they study the ability of *A. solani* to utilize the polyester-polyurethane, and with Naik (*et al.* 2010) who reported that temperatures 25º C and 30º C were the optimum of growth and the maximum sporulation of *A. solani*.

The ITS region of rDNA sequences of *A. solani* recovery from Jordan Valley farms and from local market of Saudi Arabia, were used to confirm the identification to species level and to study the population distinction. The isolates of *A. solani* obtained from Jordan Valley farms were found to be 100% matching to the isolates (LT714700) in the Gen-Bank. Moreover, the *A. solani* isolates obtained from local market of Saudi Arabia also showed 100% similarity to the isolates (DQ084021) in the Gen-Bank. Identification by sequences region of the ITS of rDNA for fungi is giving a correct identification and saving time and effort (Miguel et al., 2010; Edin, 2012; Sudheer et al., 2013; Ozkilinc et al., 2018).

Perusal of the results show significant variations in morphological, physiological and molecular characterization in the of *A. solani* isolates recovered from Jordan Valley farms as well as from local market of Saudi Arabia. In Jordan there was no study investigated the population of *A. solani* based on morphology, physiology, molecular and pathogenicity characterization. Alhussaen (2019) performed the first and the only study in Jordan on *A. solani* population variation isolated from plants of Tomato from diverse farms in Jordan Valley using the sequences of ITS region of rDNA. Moreover, in Jordan there were a few studies examined the variation of *A. solani* of sensitivity of fungicides (Al-Mughrabi, 2004; Abu-el Samen and Al Shudifat, 2012).

In the world, various studies look into the population of *Alternaria solani* based on both morphology and molecular characteristics (Petrunak and Chrits, 1992; Weir *et al.* 1998; Van der Waals *et al.* 2004; Lourenço *et al.* 2009). Several other studies showed that population of *Alternaria solani* exhibit significant variation in terms of pathogenicity (Kumar et al., 2008; Naik et al., 2010).

Perusal of the data of the present study showed a strong pathogenicity of the isolates of *Alternaria solani* to tomato fruits. The isolates recovery from local market of Saudi Arabia were more pathogenic from the isolates recovery from Jordan valley. The results also showed that inoculated and the control (non-inoculated) fruits differ significantly (P=0.00) in disease severity. In addition, significant differences were found between inoculated isolates recovery from Jordan valley and the isolates recovery from local market of Saudi Arabia (P=0.00) (Table 6). These variations could be the climate changes between Jordan Valley and Saudi Arabia. These results are consist with Pugliese (et al., 2012) who found some climate parameters effect the *Alternaria japonica* disease incidents and severity when increased the combination of CO2 and temperature.

References:

1. Abu-El Samen, F.M. and Al Shudifat, A, (2012). Sensitivity of tomato early blight isolates (*Alternaria solani*) from Jordan to Mancozeb, Chlorothalonil and Azoxystrobin fungicides. Phytopathology 101: S2 (Abstract).
2. Agrios. GN, (2005). 'Plant pathology.' (Academic Press: San Diego, USA)
3. Alhussaen, K. (2012). Morphological and physiological characterization of *Alternaria solani* isolated from tomato in Jordan valley. Res. J. of Biolog. Sci. 7: 316-319.
4. Alhussaen, K. (2019). Variation in the population of *Alternaria solani* by using sequencing of ITS1 isolated from tomato plants from Jordan valley. J. of Biolog. Sci. 19: 46-50.

5. Al-Mughrabi, K. (2004). Sensitivity of Jordanian isolates of *Alternaria solani* to mancothane. *Phytopathol. Med.* 43: 14–19.
6. Edin, V. (2012). Identification by using specific primers for *ALTERNARIA SOLANI*, with using F129L analysis associates with loss sensitivity. Crop Protection. 38: 72-73.
7. Harvey, PR. Butterworth, PJ. Hawke, BG. and Pankhurst, CE. (2000). Genetic variation among populations of *Pythium irregulare* in southern Australia. *P. Path. (Oxford)* 49: 619-627.
8. Ibrahim, N. Anwar, M. Khalid, M. Ismail, M. Hamzah, M. and Toshiaki, N. (2009). Polyester-polyurethane Biodegradation by Alternaria Solani, Isolated from Northern Jordan, Adv. Environ. Biol., 3(2): 162-170.
9. Kumar, V. Haldar, S. Pandey, K. Singh, R. Singh, A. and Singh, P. (2008). Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. W. J. of Micro. and Bio. 24 (7) 1003-1009.
10. Lourenço, Jr. Rodrigues, T. Campos, A. Bragança, C. Scheuermann, K. Reis, A. Brommonschenkel, S. Maffia, L. and Mizubuti, E. (2011). Genetic Structure of the Population of *Alternaria solani* in Brazil. J. of Phyt. 159: 233–240.
11. Lourenço, V. Moya, A. González-Candelas, F. Carbone, I. Maffia, L. and Mizubuti, E. (2009). Molecular diversity and evolutionary processes of Alternaria solani in Brazil inferred using genealogical and coalescent approaches. Phyto. 99:765-774.
12. Malik, P. Shankar, R. Malik, V. Sharma, N. and Mukherjee, TK. (2014). Green chemistry based benign routes for nanoparticle synthesis. J. Nano. 24:1–14.
13. Miguel, A. Isabel, G. Nicolette, P. Rosario, M. and Teresa, G. (2010). PCR detection and identification of Alternaria species-groups in processed foods based on the genetic marker Alt a 1. F. Cont. 22 (12): 1745-1756.
14. Morris, F. Connolly, M. and Clair, D. (2000). Genetic diversity of Alternaria alternata, isolated from tomato in California assessed using RAPDs. Myc. Res. 104: 286–292.
15. Naik, M. Prasady, Y. Bhat, K. and Devika, R. (2010). Morphological, Physiological, Pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. In. Phyt. 63 (2): 168-173.
16. Olanya, M. Honeycutt, C. Larkin, R. and Griffin, T (2009). The effect of cropping systems and irrigation management on development of potato early blight. J. of Gen. P. Pathol. 75 (4): 267-275.
17. Ozkilinc, H. Rotondom, F. Pryor, B. and Peever, T. (2018). Contrasting species boundaries between sections Alternaria and Porriof the genus *Alternaria*. P. Pathol. 67: 303-314.
18. Paul, B. (2000). ITS1 region of the rDNA of *Pythium megacarpum* sp. nov., its taxonomy and its comparison with related species. FEMS Microbiol. Lett. 186: 229-233.
19. Petrunak, DM. and Chrits, BJ. (1992). Isozyme variability in *Alternaria solani* and *A. alternate*. Phyto. 82:1343-1347.
20. Pongpisutta, R. (2005). Variability of *Phytophthora cinnamomi* in New South Wales. The University of Sydney. PhD thesis.
21. Pryor. BM. and Michailides, TJ. (2002). Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with Alternaria late blight of pistachio. Phyto. 92:406-416.
22. Simmons, E. (2000). *Alternaria* themes and variations (244-286) species on Solanaceae. Myco. 75:1-115.
23. Singh, SN. (1987). Response of chilli cultivars to Alternaria alternata and losses under field conditions. Farm Sci. J. 2(1): 96-97.
24. Sudheer, K. Ruchi, S. Prem, L. and Alok, K. (2013). Rapid detection and quantification of Alternaria solani in tomato. Scientia Hort. 151 (28): 184-189.
25. Tatiana, T. Rodrigues, L. Maffia, O. and Eduardo, S. (2010). *In vitro* production of conidia of *Alternaria solani*. Trop. P. Pathol. 35(4): 203-212.
26. Van der, W. Korsten, L. and Slippers, B. (2004). Genetic Diversity among *Alternaria solani* Isolates from Potatoes in South Africa. P. Dis. 88: 959-964.
27. Verma, M. Brar, S. Tyagi, R. Sahai, V. Prévost, D. Valéro, J. and Surampalli, R. (2007). Bench-scale fermentation of Trichoderma viride on wastewater sludge: rheology, lytic enzymes and biocontrol activity. Enz. Micro. Tech. 41: 764-771.
28. Verma, P. Singh, S. and Gandhi, S. (2007). Variability among Alternaria solani isolates causing early blight of tomato. In. Phyto. 60(2): 180-186.
29. Weir, T. Huff, D. Christ, B. and Romaine, C. (1998). RAPD-PCR analysis of genetic variation among isolates of *Alternaria solani* and *Alternaria alternata* from potato and tomato. Mycologia 90(5):813-821