Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2151
DOI URL: http://dx.doi.org/10.21474/IJAR01/2151

RESEARCH ARTICLE

MORPHOLOGICAL, PATHOGENIC, CULTURAL AND PHYSIOLOGICAL VARIABILITY OF THE ISOLATES OF *ALTERNARIA SOLANI* CAUSING EARLY BLIGHT OF TOMATO

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Manuscript Info

Manuscript History

Received: 25 September 2016
Final Accepted: 27 October 2016
Published: November 2016

Key words:-
Alternariasolani, isolates, variability, mycelial growth, tomato

Abstract

Alternariasolani is an important and widely distributed pathogen that caused early blight of tomato. Isolates of the *A. solani* were isolated from naturally diseased tomato fields in Hada Al Sham region, Jamoom. Therefore, the present investigation was undertaken to demonstrate morphological, cultural and physiological variation among the isolates of the pathogen.*A. solani* was isolated and pure culture for each isolate was obtained by Hyphal Tip isolation method. Morphological characteristics of *A. solani* including conidia size, length of beak, hypha and septa were studied under light microscope. Cultural characters such as colony colour, colony diameter, pigmentation, type of margin were studied using different culture media. PDA medium was used to study the effect of pH, temperature and light regime on the radial growth of *A. solani*

The results indicated that two isolates of the *A. solani* were identified and both of the test isolates showed a wide range of variability in respect of their mycelial and conidial dimentions and septation. On the culture media, maximum mycelial growth of AS1 was noted with Sabouraud's Agar medium (9 cm) followed by host agar medium (8.7cm) and PDA (7.9cm). While, the maximum linear growth of AS2 was recorded with Richard's Agar (9cm) followed by Czapeck's Agar (8.6cm) and Sabouraud's Agar (8.5cm).Isolates of *A. solani* showed highly variation in pigmentation, sporulation and feature of mycelial growth such as colony surface, growth margin and zonation.Temperature of 30°C was suitable for AS1 and AS2 and recorded the maximum mycelial growth of (7.05cm and 6.57cm, respectively). Optimum pH of PDA medium for the growth of AS1 and AS2 was 6.5. Alternative 12 h light and 12 h darkness recorded the maximum colony growth of AS1 (7.3cm), whereas, highest colony growth of 7.95cm was observed by AS2.

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Introduction:-

Tomato (*Lycopersiconesculentum* Mill.) belongs to the family Solanaceae. It is one of the most popular vegetables and a major horticultural crop grown all over the world and occupies a prominent position in the world's vegetable economy. It is the second most consumed vegetable after potato, ranking first among the processing crops, and unquestionably the most popular garden crop (FAO, 2007). About 152,956,115 tons of tomatoes were produced in the world in 2009 (FAO, 2009).The cultivated area in 2011, under tomato in Saudi Arabia was 14,175 hectares, which produced 483,588 tons, while the annual imports of tomato in Saudi Arabian was around 340,000 tons which cost of $20 million (FAOSTAT© FAO, 2013).

Farmers get lower yield mostly due to pests,diseases and sub-optimal fertilization. The most important factors responsible for the low productivity of tomato are diseases and insect pests. Among those diseases, early blight is one of foliar diseases of tomato caused by *Alternariasolani* is the most destructive and widespread in temperate,tropical and subtropical regions of the world, which causes a highest reduction in the quality andquantity of fruit yield (Hijmans*etal*., 2000). It can severely damage incurring a loss of 50 to 80% on tomato susceptible hybrids varieties. *A. solani* can infect all parts of the plant (causing leaf blight, fruit lesions andstem collar rot) and result in severe damage during all stages of plant development (Abada*et al.,* 2008).It can also cause disease on potato, pepper, and eggplant.

According to morphological characters and physiologic analysis of the pathogen, *A. solani* belongs to large, long beaked and concatenated spores (Simmons, 2000). The mycelium consisted of septate, branched, light brown hyphae, which turned darker with age. The conidiophores were short, 50 to 90 µm and dark coloured. Conidia were 120-296 x 12-20 µm in size, beaked, dark coloured and borne singly. However in culture they formed short chains. According to Singh (1987) the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

The organism is air-borne, soil inhabiting and the most common disease causing pathogen of the cultivated tomato in areas with heavy dew, frequent rainfall, and high humidity (Agrios, 2005). It may be seed borne and dispersed by water,wind, insects, workers and farm equipment. Spores can enter the leaf, steam or fruit. **(**Yonghao, 2013).

The primary symptom of early blight is the appearance of small dark brown spots on the lowest and oldest leaves. The tissue around the primary lesions may turns bright yellow, and if lesions are numerous, the entire leaves may become necrosis and chlorotic. The spotsget enlarged, they develop concentric rings which give them a bull's eye. In favorable weather conditions, disease develop, lesions can become numerous and plants defoliate, which damagethe quantity and quality of tomato fruits (Kouyoumjian, 2007).

Under natural epiphytotic condition, the *A.solani*have been found to exhibit a wide range of variability in disease symptoms. Morphological, cultural, pathogenic and molecular variability of the pathogen (*A. solani*) was also studies earlier by many workers (Babu*et al*. 2000, Ahmad, 2002, Naik*et al*, 2010). Analysis of pathogenic and genetic variation within pathogen populations is helpful in understanding host-pathogen coevolution, epidemiology and developing strategies for resistance management (Leung *et al*., 1993). Understanding the pathogen variability will help in developing the effective management strategies.

Materials and methods:-

Isolation and culture preparation:-

Tomato leaves showing typical early blight symptoms were collected in the early 2015 from growing tomato plants in different tomato growing fields of Hada al sham region of Jeddah, Saudi Arabia. The infected leaves were brought to laboratory and diseased leaves with *A. solani* would cut into small bits measuring about 5mm and surface sterilized in mercuric chloride solution for 1 min, rinsed twice with sterile distilled water. Pieces were then placed on Potato Dextrose Agar (PDA) medium and incubated under alternative 12 h light and 12 h dark at 28±2°C according to Naik *et al*., (2010). Pure culture of the fungus was obtained by Hyphal Tip Isolation Method.Pathogen was identified following the cultural and morphobiometric characteristics criteria (Ellis, 1971; Barnet and Hunter, 1972). Amongst the several geographical isolates collected from various fields, a representative isolate designation was picked up for detailed variability studies. These fungal isolates were grown on PDA slants, stored at 5°C in refrigerator and sub culturing was done subsequently at intervals of 30 days for further research studies. Laboratory experiments were conducted at the Plant Protection laboratory, Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid land agriculture, King Abdulaziz Univesity.

Morphological characterization:-
The morphological characteristics of representive isolate of the *A.solani* including hyphal width, length of beak, conidia size (length and width) and number of septa in conidia were recorded by slides under power objective 40X using light microscope. Ocular and stage micrometer were used to measure the size of conidia. The *A. solani* culture was 10 days old grown on PDA.

Pathogenicity test and pathogenic variability:-
In order to confirm the identification of early blight disease and its causal agent, the pathogenicity test was carried out under open filed condition. Four weeks seedlings of Supper Star tomato variety were transplanted into the field in the Agriculture Research Station of Hada Al Sham, department of arid land agriculture, King Abdul Aziz University in 2015. All the agricultural practices, fertilization and irrigation were performed in the proper manner. Three replications were maintained for each isolate and ten seedlings of tomato were growing in each replicate. Plant to plant and row to row space was 1m.

For each isolate of the *A.solani*, 10-day-old culture on PDA was added to 100 ml sterile distilled water and agitated using Waring blender at low speed for two minutes to release spores. The suspension was filtered using cheese cloth to remove PDA. The conidial suspension at 10^6 spores ml^{-1} (the inoculum density was measured by hemocytometer) of the isolates was prepared. The spore suspension was sprayed on tomato plants 20 days after transplatation of seedlings, and the inoculated tomato plants were covered by polythen bags for about 30 h to retain optimum humidity. The observed symptoms on plants for each isolate of the fungus were compared to the original symptoms of early blight. The pathogen was re-isolated from artificially inoculated tomato leaves and the morphological and cultural characteristics were compared with original pathogen. Forthermore, to study the virulence potential variability of each isolate, the disease insidence and severity were continuously measured. observation in each 14 days interval was recorded, using 0-5 disease scale (Pandey *et al.*, 2003) Disease incidence and percent disease index (PDI) for each isolate were calculated as follows:

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased Leaves} \times 100}{\text{Total Number of Leaves observed}}$$

$$\text{Percent disease index (PDI)} = \frac{\text{sum of all rating} \times 100}{\text{total number of observations} \times \text{maximum rating grade}}$$

Variability in cultural characteristics:-
The variability of representative isolates of *A.solani*was studied on various parameters of cultural characters such as colony colour, colony diameter, type of margin, sporulation and diversity in growth on different culture media *viz.*, potato dextrose agar (PDA), PDA with Caco3, host extract agar, Czapeck's agar, Sabouraud's and Richard's agar.

The required amount of agar was melted in 500 ml distilled water and the other ingredients for each medium were dissolved in separate conical flask containing 500 ml of distilled water. Then these two solutions were mixed thoroughly and by adding distilled water the volume become up to 1000 ml. In case of contamination by bacteria during culturing procedures, antibiotic *viz.*, ampicillin (50 mg L^{-1}) was added and then the media was autoclaved.

Then, an amount of 20 mL of each prepared medium was poured in a petri dish with 9 cm diameter. Four plates were used for each treatment. All plates were inoculated at the centre with a mycelial disc of 5 mm diameter with a 7 days old culture of the isolates. The whole procedure was performed under aseptic conditions in a laminar flow hood. The Petri dishes were incubated at 28±2 °C under a photoperiod of 12 hours. The culture parameters of each isolate on culture media were rocorded 10 days after incubation.

Physiological variability of the *A. solani*:-
a.Effect of pH levels:*In vitro* PDA medium is used to study the effect of pH on growth of *A.solani*. The pH of the medium was adjusted to different levels such as 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 by adding0.1 N hydrochloric acid and 0.1 N sodium hydroxide. About 5 mm discs were taken from 7 days old culture, then inoculated and incubated under 12 h light and 12 h dark at 28±2°C for 10 days. Four replications were used for each selected isolate of the pathogen. The diameter of colony growth was measured and recorded on 10^{th} day.

b.Effect of temperature: Eight temperatures from 5-40°C (5°C intervals) were used to incubate PDA Petri dish cultures of the selected *A. solani* isolates to find out the optimum temperature as well as the lowest and the highest temperatures at which fungal growth occurred. All incubation was carried out under 12 h light and 12 h dark for 10

days at 28±2°C. Four replicates were used for each of the selected isolate at each temperature and growth was measured after 10 days.

c.Effect of light regime: Isolates of *A.solani* on PDA were exposed to continuous dark,light and alternative 12 h dark and 12 h light in an aseptic chamber maintained at 28±2°C. Mycelial discs of 5 mm from each isolate were used to inoculate Petri plates. Four plateswere maintained for each treatment. For Inoculated plates, light intensity was adjusted to required level. The mycelial growth wasobsevered in each case ten days after inoculation.

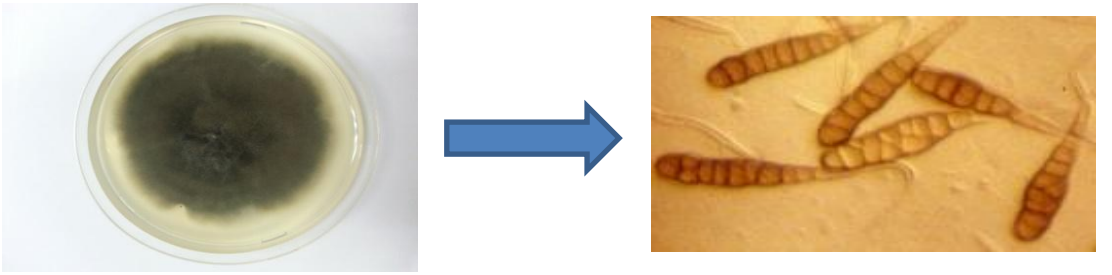
Results:-

Morphological characterization of the *A. solani* isolates:-

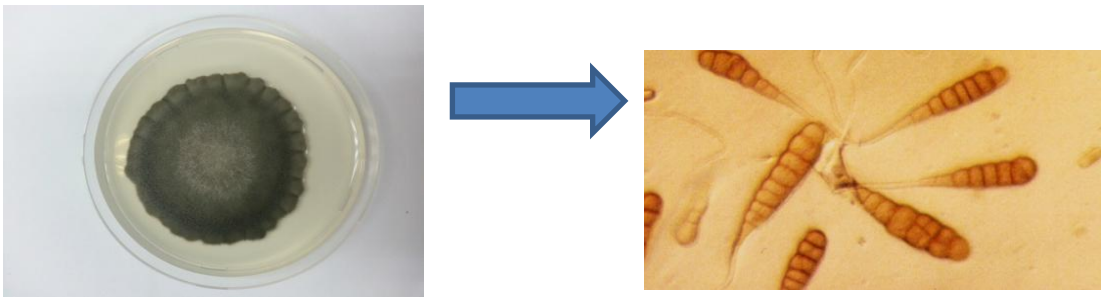
Investigations on morphological characteristics of the test isolates of *A. solani*showed highly variability based on their mycelial width, conidial size, beak length and septations (Table 1). Ten slides from edge of active pure culture of each isolate were prepared using of light microscope, all the observed fungal feature of *A.solani* were recorded. The fungus at first produced cottony growth which is dark, ranging from grey to black with tints of brown orolive. Colonies are spreading hairy and grey brown to black, the whole texture of the fungus was similar to cotton.The conidiophores of the fungus were formed singly or in groups, strait or flexuous brown to olivaceous and solitary, muriform and ellipsoidal tapering to a beak and pale or olivaceous brown (Fig. 1). The average conidial size (L × W) was higher in the isolate AS1 (25-44 × 7-15µm). Whereas, it was comparatively lower in isolate AS2 (20-30 × 5-13µm). The conidial septations (No. of vertical septa andhorizontal) were also found to be varied in the test isolates of the fungus.

However, the number of septation was maximum in the isolate AS1 (2-11 and 1-4) while it was (2-8 and 1-3) in the isolate AS2. The maximum length of beak was also recorded in the isolate AS1 (6-10 µm). Whereas, it was 4-8 µm in the isolate AS2. The mycelial width was found to be higher in AS1 (0.8=25 µm) while, the lower mycelial width was noted with isolate AS2 (0.7-2 µm).

Fig. 1:-



Pure culture of isolate (AS1) Conidia of AS1



Pure culture of isolate (AS2) Conidia of AS2

Table 1:- Morphological characteristics of *A. solani* isolates causing early blight on tomato

No	Isolates	Size of conidia (µm)		Septa in conidia		Length of beak (µm)	Mycelial width (µm)
		Length	Width	Horizontal septa	Vertical septa		
1	**AS1**	25-44	7-15	2-11	1-4	6-10	0.8-2.5
2	**AS2**	20-30	5-13	2-8	1-3	4-8	0.7-2

The conidial features of *A. solani* test isolates investigated in the present study are in accordance with *A. solani* conidia characteristics which were basically described by Ellis and Ellis (1985). According to Singh (1987) who reported that, the conidia of *A.solani* contained 5-10 transverse septa and 1-5 londitudinal septa. Ahmad (2002) studied the variability in condial morphology of *A. solani* causing early blight of tomato and reported comparatively large size (175× 12.5µm) condia and it's larger beak length (47-65µm). Nikam*et al*. (2015) also reported that, amongst test isolate of *A. solani*, the highest mycelial width was recorded in AsJI (64.2µm). The average conidial size (L×B) and their beak length were highest in the isolate AsBd (42.18×15.18 and 13.1µm) respectively. Conidial septation of horizontal and vertical septa was also found to be varied among the test isolates of *A. solani*. Naik*et al*. (2010) found that, the conidia were solitary, straight or slightly flexuous, 19.5 -70 µm in length and 6-22 µm width with 2 -7 transverse septa and 1-4 longitudinal septa.

Pathogenicity test and pathogenic variability:-

In order to confirm the identification of the early blight and its causal agent, the pathogenicity test was conducted under field conditions using susceptible tomato variety of Supper Star revealed that, after two weeks from inoculation of tomato seedlings, typical symptoms of early blight was appeared on the leaves, which was similar to those observed on naturally infected tomato plants and the morphological and cultural characteristics of the *A.solani* were compared with original pathogen. The isolates of the *A. solani* collected from two geographical regions expressed differences in their virulence. Both of *A. solani* isolates were successfully able to infect tomato foliage appearing infection symptoms of early blight, but the degree of infection was varied. Among the isolates of *A. solani*, AS1 isolate was mostly virulent.However, recorded maximum level of disease incidence (53.5%) and disease severity (32%), whereas, the AS2 isolate noted the least early blight incidence of 27% and disease severity of 18%. AS1 isolate produced properly clear early blight symptoms on leaves,stem and even fruits and was found to be virulent causing sever disease in tomato plants (Fig 2). Meanwhile, AS2 was rated as less virulent.

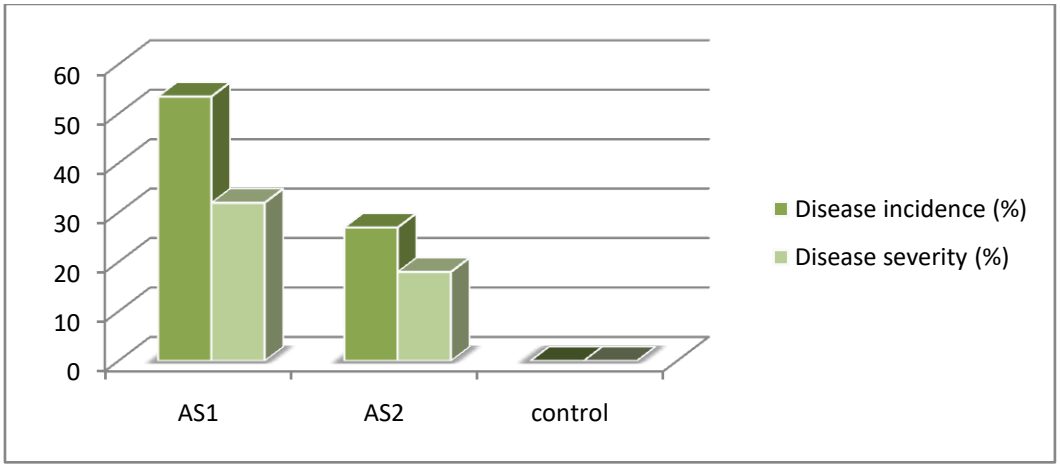


Fig 2:- Pathogenic variability of the *A.solani* Isolates

A.solani was basically identified based on symptom. Currently, symptomatology is not the only reliable method for detection of plant disease but it is a primary step in disease diagnosis (Batool*et al*., 2011), however, the modern identification techniques *viz.,* PCR were found to be more reliable for diagnosis of plant disease. Pathogenicity test was carried out by spraying conidial suspension of the pathogen on tomato seedlings for confirmation of pathogen association with host. Similar approach was followed by different scientists (Castro *et al*., 1999; Vloutoglou and Kalogerakis, 2000) conducted the pathogenicity test by spraying conidial suspensions. Conidial suspensions of *A. solani* were more effective for preparation of inoculum load and pathogenicity test against tomato and potato early blight (Rotem,1994).

Cultural variability of *A. solani*isolates:-

The indicated results from*A.solani*isolates showed variations in their colony growth on various solid culture media. The mycelial growth was recorded after ten days of inoculation. Two test isolates of *A.solani* (AS1 and AS2) produced various size of radial growth of mycelium and exhibited differences in pigmentation, sporulation, growth margin, zonation and growth surface on the culture media.

Radial growth:-
Amongst test media, the maximum average radial growth by isolate AS1 was observed in sabouraud's agar culture media (9.0 cm) followed by host extract agar (8.7 cm), PDA (7.9 cm), Czapeck's agar (7.2 cm), and PDA +CaCo3 (6.0 cm). While, the lowest rate of fungal growth was recorded in richard's agar (4.7 cm). but the maximum mean mycelial growth by isolate AS2 was observed in richard's agar(9.0 cm) followed by Czapeck's agar (8.6 cm), sabouraud's agar (8.5 cm), PDA + CaCo3 (6.8 cm), PDA (6.1 cm) and rose bengal (5.4 cm). Whereas, the minimum average mycelial growth was recorded in host extract agar (3.3 cm) (Table 2).

Growth of the isolate AS1 were mostly different significantly among the five different selective culture media such as PDA, sabouraud's agar, czapeck's agar, richard's agar and host extract agar. While, it was not significantly different between PDA+CaCo3 and rose bengal. Meanwhile, the means of mycelial growth of isolate AS2 was different significantly among five tested media *viz*., richard's agar, PDA +CaCo3, PDA and host extract agar. But the average of radial growth was not different significantly in sabouraud's agar, rosebengal and czapeck's agar.

Table 2:- Effect of different culture media on the radial growth diameter (cm) of *A. solani*isolates

No.	Medium	Colony diameter (cm) of the isolates		Mean (M**)**
		AS1	AS2	
1	PDA	7.9 b*	6.1 d	7.01
2	PDA+CaCo3	6.0 de	6.8 c	6.4
3	Sabouraud's Agar	9.0 a	8.5 ab	8.75
4	Czapeck's Agar	7.2 c	8.6 a	7.9
5	Richard's Agar	4.7 f	9.0 a	6.9
6	Rose Bengal	5.5 de	5.4 de	5.45
7	Host Extract Agar	8.7 a	3.3 g	6.01
Mean (I)		7.0	6.8	---
Source		LSD at 5%		
Media(M)		0.42		
Isolate(I)		ns		
M×I		0.60		
CV (%) = 6.0				

(*)Mean values followed by the same letter are not significantly different at $p < 0.05$

Pigmentation:-
Isolate of *A.solani* exhibited great variability in pigement production on different test culture media (Table 3a and b). The isolate of AS1, produced brownish black pigment on rose bengal, host extract agar and richard's agar while redish black pigement produced on PDA, czapeck's agar, PDA+CaCo3 and sabouraud's agar. Whereas, isolate AS2 produced brownish black pigement on PDA, czapeck's agar, PDA+CaCo3 and sabouraud's agar. But whitish black pigment was produced on rose Bengal and host extract agar. Whereas, on richard's agar culture media, grey pigment was produced.

Sporulation:
Condia production was observed in both of the *A. solani* isolates but sporulation was varied from poor to good rate (Table 3a and b). However, the isolate AS1 showed poor sporulation in rose bengal, czapeck'sagar, good rate sporulation was recorded in PDA, host extract agar and PDA+CaCo3. Poor rate of conidial production by the isolate AS2 was occurred on the culture media of rose bengal, host extract agar and sabouraud agar while fair spore production was noted on czapeck's agar. The PDA and PDA+ CaCo3 were the best media for isolate AS2 that produced good rate of sporulation. Hence, the Richard's agar culture media however did not showed any rate of sporulation

Mycelial growth patterns:-
Mycelial growth patterns were observed on different culture media (Table 3a and b).Isolate AS1 grew with circular margin on czapeck's agar, rose bengal, PDA and host extract agar. While irregular growth margin was showed on sabouraud's agar and czapeck's agar, PDA+CaCo3 and richard's agar. Smooth surface colony was observed on rose bengal, PDA, czapeck's agar and host extract agar. Meanwhile, PDA+CaCo3, richard's agar and sabouraud's agar media were recorded with rough surface colony. Among culture media, rose bengal, PDA+CaCo3 and sabouraud's

agar showed clear zonation of fungal colony. Growth margin of isolate AS2 was irregular shape on rose bengal and sabouraud's agar. Where, it was circular on other five tested culture media. Rough surface colony of AS2 was achieved on rose Bengal, PDA, host extract agar, PDA+CaCo3 and sabouraud's agar while on richar's agar and czapeck's agar, surface of the fungal colony was smooth. Zonation of colony was not seen on czapeck's agar, PDA+CaCo3 and richard's agar.

Table 3a:- Evaluation of culture media for growth characteristics of isolate AS1

No.	Medium	Pigmentation	Sporulation	Growth margin	Growth surface	Zonation
1	Rose Bengal	Brownish black	Poor	Circular	Smooth	Yes
2	Potato Dextrose Agar	Redish black	Good	Circular	Smooth	Not
3	Czapeck's Agar	Redish black	Poor	Irregular	Smooth	Not
4	Host Extract Agar	Brownish black	Good	Circular	Smooth	Not
5	PDA+CaCo3	Redish black	Good	Irregular	Rough	Yes
6	Richard's Agar	Brownish black	Poor	Irregular	Rough	Not
7	Sabouraud's Agar	Redish black	Poor	Irregular	Rough	Yes

Table 3b:- Evaluation of culture media for growth characteristics of isolate AS2

No.	Medium	Pigmentation	Sporulation	Growth margin	Growth surface	Zonation
1	Rose Bengal	Whitish black	Poor	Irregular	Rough	Yes
2	Potato Dextrose Agar	Brownish black	Good	Circular	Rough	Yes
3	Czapeck's Agar	Brownish black	Fair	Circular	Smooth	Not
4	Host Extract Agar	Whitish black	Poor	Circular	Rough	Yes
5	PDA+CaCo3	Brownish black	Good	Circular	Rough	Not
6	Richard's Agar	Grey	No	Circular	Smooth	Not
7	Sabouraud's Agar	Brownish black	Poor	Irregular	Rough	Yes

In the study of colony characteristics of *A. solani* isolates, the mycelial growth patterns, sporulation, radial growth and pigmentation on different culture media were found to be significantly different. The results of the present research work on cultural variability of *A.solani* isolates observed are similar to the findings of earlier workers. Nikman*et al*., 2015 reported that all of the isolates of *A.solani* exhibited a great variability in respect to their mycelial growth, colony color, colony diameter, colony zonation and sporulation. But Singh *et al*., 2014 observed the cultural variability amongst isolates *A.solani*, some of them were significantly different in radial growth, pigmentation, culture color, growth margin and sporulation.

Physiological variability of *A.solani* isolates:-

a.Effect of pH levels on the growth of *A. solani* isolates:-

Behavior of growth and colony character of *A. solani* isolates were studied on different pH ranged between pH 4.0-8.0 on PDA at10th day of incubation. Highly significant difference in the colony growth of *A. solani*isolates was observed at different pH levels (Table 4). Light acidic and slightly alkaline pH was suitable for the growth of *A. solani* isolates. The optimum pH level for both of tested isolate of *A.solani* (AS1 and AS2) was 6.5 and maximum radial growth was obtained (7.3 cm and 6.1 cm, respectively). However, the tested isolates of *A. solani* grew well at pH levels of 6 and 7. Thus, recorded the highest colony growth (6.9 cm) and 7.1 cm) by AS1 and (5.9 cm and 5.9 cm) by AS2, respectively. Moderated mycelial growth of AS1 was noted at 5.5, 7.5, 5.0, 8.0, and 4.5. Whereas, in AS2 it was seen at pH levels of 5.5, 5.0, 4.5, 4.0.Apparent acidic (4.0) and alkaline pH (8.0) was reducing radial growth drastically and not suitable for the growth of *A. solani* isolates. The minimum growth in AS1 was obtained (3.5 cm) at pH 4.0. While in AS2 the least mycelial growth was observed (4.0 cm) at pH 8.0.Differences in radial growth were found significantly at different pH levels for both of the *A. solani* isolates.

Table 4:- Effect of pH levels on the growth of *A. solani* isolates

No.	pH	Radial growth (cm) of the isolates		Mean (p)
		ASH1	ASH2	
1	4.0	3.5 i*	4.3 hj	3.90
2	4.5	5.5 f	4.5 h	4.98
3	5.0	6.3 d	4.9 g	5.60
4	5.5	6.7 bc	5.5 f	6.10
5	6.0	6.9 bc	5.9 e	6.41
6	6.5	7.3 a	6.1 de	6.71
7	7.0	7.1 ab	5.7 ef	6.43
8	7.5	6.3 d	4.2 hj	5.25
9	8.0	5.5 f	4.0 hj	4.75
Mean (I)		6.12	5.01	---
Source		LSD at 5%		
pH (p)		0.23		
Isolates (I)		0.15		
P×I		0.33		
CV (%) = 4.2				

(*)Mean values followed by the same letter are not significantly different at $p < 0.05$

The results of the experiments indicated that *A. solani*isolates prefer a pH of 6.5 for its colony growth. Hydrogen ion concentration of the PDA culture medium has a profound effect on the amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). The results of the present investigation indicated that optimum pH level for the growth of *A. solani*was 6.5. However, optimal and good colony growth of the fungus was also noted at pH 5.5, 6.0 and 7.0. This shows that *A. solani* isolates prefer acidic pH rather than alkaline pH indicating its acid tolerance and interesting. Arunakumara*et al*., 2015, also reported that, the optimum pH for the growth of *A. solani* was in the range of 6.5 to 7.0.

b.Effect of temperature on the linear growth of *A. solani*:-

There was significant difference in the linear growth of *A. solani* isolates at different levels of temperature (Table 5). Maximum colony growth (7.05 cm) in isolate AS1 was recorded at 30 °C followed by 25 °C, 35 °C, 20 °C, 15 °C and least colony growth was obtained (2.2 cm) at 10 °C. The optimum temperature for isolate AS2 was also 30 °C, which recorded the highest colony growth (6.5 cm) followed by 25 °C, 35 °C, 20 °C, 15 °C and least growth was observed (2.1 cm) at 10 °C.

Table 5:- Effect of temperature on the radial growth (cm) of *A.solani*

Temperature ranges (°C)	Isolate ASH1	Isolate ASH2	Mean (T)
10	2.22 i*	2.15 i	2.187
15	2.97 j	3.52 g	3.25
20	4.87 f	4.9 f	4.88
25	6.15 c	5.97 c	6.06
30	7.05 a	6.57 b	6.81
35	5.90 d	5.42 e	5.66
40	3.55 g	3.37 h	3.46
Mean (I)	4.67	4.56	--
Source	LSD at 5%		
Temperature (T)	0.088		
Isolates (I)	0.047		
T × I	0.124		
CV (%) = 1.88			

(*)Mean values followed by the same letter are not significantly different at $p < 0.05$

Temperature is the most important physical environment factor for influencing vegetative and reproductive activity of the fungi. In the present study on the influence of temperature *A. solani* showed maximum growth at 30°C. Kaul

and Saxena (1988) also reported the temperature of 25°C being good for recording the maximum colony growth of *A. solani*.Alhussaen 2012 pointed that the optimum growing temperature for the isolate of *A.solani* was 25-30 °C.

c.Effect of light regimes on the diameter of radial growth (cm) of *A. solani:-*

The mycelial growth of *A. solani* isolates was indicated significant differences at different light conditions. Interaction between average radialgrowth of the isolates were significantly different (Table 6). Moreover, the maximum average mycelial growth (7.3 cm) of isolate AS1 was recorded, when the incubated plates were exposed to alternative 12 h light and 12 h darkness, followed by continuous light (6.72 cm) and the least average radial growth of the fungus (5.75 cm) was noted with continuous dark condition. Whereas, in isolate AS2, maximum mean radial growth (7.95 cm) was found when plates were incubated under continuous light followed by alternative 12 h dark and 12 h light and minimum colony growth (5.92 cm) of the fungus was recorded in continuous darkness.

Table 6:- Effect of light regimes on the diameter of radial growth (cm) and sporulation of *A. solani*

Light condition	**Isolate ASH1**	**Isolate ASH2**	**Mean (L)**
Darkness	5.75 d	5.92 d	5.8 c
Continuous light	6.72 c	7.95 a	7.3 b
12 h light 12 h dark	7.30 b	6.47 c	8.6 a
Mean (I)	6.58 a	6.78 a	---
Source	LSD at 5%		
Isolates (I)	0.21		
Light (L)	0.26		
I× L	0.37		
CV (%) =3.75			

(*)Mean values followed by the same letter are not significantly different at $p < 0.05$

Conclusion:-

Investigation ofmorphological, cultural and physiological variability within pathogen population is the most helpful in understanding host pathogen coevolution, epidemiology and developing strategies for disease management.

Acknowledgment:-

The authors are thankful to department of arid land agriculture, faculty of meteorology, environment and arid land agriculture, king Abdul Aziz University for providing the laboratory and field facilities to carried out this research work.

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