INT. J. BIOL. BIOTECH., 16 (4): 1017-1025, 2019.

A PHYSIOLOGICAL STUDY OF NUTRITIONAL AND CULTURAL MEDIA ON GROWTH OF *ALTERNARIA SOLANI*, THE CAUSES OF EARLY BLIGHT DISEASE OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

Hina Zafar[1], S. Shahid Shaukat[2] and Syeda Azra Tariq[1]

[1]*Vertebrate Pest Control Institute, Southern-zone Agricultural Research Centre, Pakistan Agricultural Research Council, Old Blocks 9-10, University of Karachi, Karachi-75270, Pakistan.*
[2]*Institute of Environmental Studies, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Cultural studies were performed to evaluate nutritional requirement, particularly C and N for effective growth of *Alternaria. solani* in PDA media. Out of eight different carbon sources tested, glucose gave was found the most effective for the growth of *A. solani* whereas poor growth was found in the control (without any carbon source). Among the seven nitrogen sources tested threonine followed by urea gave optimal growth of *A. solani* while minimum growth occurred in the control. Media pH at nine different values was investigated for the growth of *A. solani.* The media pH of 5-7 gave maximal growth whilst pH 9 revealed minimum. Evidently, *A. solani* requires acidic pH for its growth. *A. solani* required a temperature range of 25 to 30°C for maximal growth while minimum was obtained at 5 °C . Furthermore, alternate light and dark (12 h each) was found to be fairly effective for highest growth of *A. solani*. Morphological characters and growth were examined in eight different media. Colony characters included were: margin of colony, colour of colony and substrate colour while colony diameter represented the growth of *A. solani*. When tested for media, PDA gave the highest growth, corn meal agar also supported noteworthy growth of *A. solani*, whilst lactic acid resulted in poor growth.

Key-words: *Alternaria solani*, early blight disease, tomato, growth media, fungal growth.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) crop is grown almost throughout the world particularly in tropical and temperate areas. Under greenhouse and field conditions tomato is attacked by many serious diseases. The yield of this crop is remarkably reduced by several important pathogenic fungi and oomycetes (Agrios, 2005). Tomato is used widely along with different meals usually in fresh form and is well known source of vitamins and minerals. According to Mathur and Shekhawat, (1986) *Alternaria solani* (Ellis and Martin) Jones and Grout, causes early blight disease in susceptible hybrids of tomato causing heavy losses (50 to 80%). Kallo and Banerjee (1993) stated that during early blight, when disease intensity is at peak it may eventually result in complete destruction of the crop. Mathur and Shekhawat (1986) stated that increase in the disease severity is usually due to high humidity, use of ineffective fungicides and often high rainfall. It has been noted that crops belonging to Solanaceae when cultivated in different regions, *Alternaria solani* usually parasitizes the crop (Singh, 1983). *Alternaria solani* infection results in the formation of toxins such as altersolanol, zinniol, alternaric acid, and macrosporin, with the result that these toxins disrupt various vital processes of the host plant, causing disease by adversely influencing the protoplast tissue (Agrios, 2005). Rotem (1966) observed that reduction occurs in certain key respiratory enzymes and toxins causing depletion of respiration rate while assimilation rate of the host is slightly enhanced. Furthermore, it was found that in the soil mixed with plant litter *Alternaria solani* may thrive at optimal temperature for a long duration.

To design control strategies it is necessary to understand the physiology and growth requirements of *Alternaia solani.* According to Pawar and Patel (1957) the growth of *A. ricini* requires sugars such as xylose, maltose and arabinose as carbon source. Chaturvedi (1966) observed that lactose, fructose, maltose and arabinose are effectively utilized by *A. alternata*. Gupta *et al*. (1970) demonstrated that increased sporulation and growth of *A.brassicae* occurs when media includes fructose among the eight monosaccharides tested. Further, they observed that maltose gave poor growth while mannitol gave excellent growth. Bhandari and Singh (1976) found that growth of *A. triticina* was maximum in fructose and mannose rather than glucose, ribose, xylose, arabinose and sucrose. Goyal (1977) recorded highest growth of *A. alternata* using maltose followed by other sugars tested. By contrast, mmannitol and galactose gave minimal growth. In accordance with Padmanabhan and Narayanaswamy (1977) the growth of *A. macrospora* was most luxuriant when fructose was used. For the optimal growth of *A. sesame* mannitol and to a lesser extent starch and lactose provided effective growth (Mohapatra *et al*., 1977). Mathur and

Sarboy (1977) registered highest growth of *A. alternata* on sucrose whereas the lowest growth resulted in media containing pentose sugars.

According to Rane and Patel (1956) N requirement for the highest growth of *A. macrospora* are KNO_3 , NH_4NO_3 , peptone and sodium nitrate. *A. Alternata* and *A. citri* utilized the most common sources of N such as NO_3, organic form of N and NH_4NO_3 (Hasija, 1970). The study of Bhandari and Singh (1976) revealed that for good growth of *A. triticina* glutamine and asparagine are required. Goyal (1977) noticed that for most effective growth and sporulation of *A. alternata*, NO_3 nitrogen rather than NH_4 nitrogen was required. Padmanabhan and Narayanaswamy (1977) who tested various organic and inorganic nitrogen sources reported that for highest growth of *A. macrospora*, urea and $NaNO_3$ were required. The study of Mohapatra *et al*. (1977) revealed that ammonical form of N was better for vegetative growth than NO_3 of *A. sesame* (when parasitizing sesame crop).

It has been reported that for normal growth of *A. macrospora* a pH range of 4.8 to 5.2 and for *A. recini*grew pH range of 4.8 to 5.5 are required (Rane and Patel, 1956). In two studies highest growth of *A. alternata* was found to occur at pH 5.0 (Pawar and Patel, 1957). A study by Taber *et al*. (1968) regarding pH requirement of *A. raphani* disclosed that a pH range of 4.8 to 7.2. According to Hasija (1970) pH range of 4.4 to 6.4 was most suitable for growth of *A. citri* while high pH of 7.4 was not conducive to growth. Verma, (1970) recorded optimal growth of *A. alternata* at pH 5.4 to 7.4, whereas minimal expansion was found at acidic pH. Samuel and Govindaswamy (1972) working with *A. solani* recorded optimal mycelial growth at pH 5.0 while pH 7.0 was optimal for sporulation. A pH range of 5.0 to 7.0 was registered as optimal for the maximal growth of *A. macrospora* (Padmanabhan and Narayanaswamy, 1977). Working with *Alternaria sesame* the required pH ranged 3-10 with an optimum at 4.5 (Mohapatra *et al*., 1977). Mathur and Sarboy (1977) found maximum sporulation and growth of *A. alternata* at pH 5.5. According to Gemawat and Ghosh (1980) *A. solani* grew fairly well on a range of pH from 4.0 to 9.5, however, maximal growth and sporulation were registered at pH 6.3. Growth of *A. carthami* required pH within 5.3 to 8.1 of the media though the highest was obtained at pH 6.0 (Mahabaleswarappa , 1981).

Various studies on temperature requirement for *Alternaria solani* in culture disclosed that it can grow at varied temperature regimes from 5-35°C (Bonde, 1929; Verma, 1970; Gemawat and Ghosh, 1979). Kaul and Saxena (1988) in their study demonstrated that for effective growth of *A. solani* a temperature of 25°C was optimal while 35°C was unsuitable. Stevenson and Packer (1988) showed that a temperature of 25°C was most suitable for rapid germination of dark grown *A. solani.*

Lukens (1963) demonstrated that for the formation of condia of *A. solani* a prerequisite is that the cultures should be kept for six hours in dark subsequent to incubation. Sporulation and growth in Czapek Dox synthetic medium was meager in *A. solani* whilst in case of natural carrot leaf and semi-synthetic media sporulation was fairly high when media was exposed to radiance (Fencelli and Kimati, 1990).

Several workers have demonstrated that for the growth of *Alternaria solani* PDA is a good choice (Rotem 1906; Bonde 1929; Neergaard, 1945). Mohapatra *et al*. (1977) showed that PDA, Czapek Dox medium, Richard's medium, and oat-meal medium resulted in higher growth (*A. soani*) in that order. Study of Barksdale (1968) disclosed that for maximal sporulation and growth of *A. solani* PDA and lima bean agar were most conducive. Cheema *et al*. (1976) noticed that PDA > Czapek Dox agar > Yeast extract agar, in that order, gave rapid growth of *A. citri*. Joshi (1981) found that both Richard's agar and PDA could provide effective growth of *A. gomphrenae.* Mahabaleshwarappa (1981) stated that PDA and to a somewhat similar extent Richard's agar medium yielded highest and rapid growth of *A. carthami* while Kaul and Saxena (1988) while test in various media disclosed that PDA could result in cultural variation in *A. solani* regarding growth type, growth rate and colour of the colonies formed. According to Fencelli and Kimati (1990) recorded good sporulation and growth of *A. tenuis* in semi-synthetic media and sparse in Czapek Dox medium.

The present study is an effort to address the problem of designing control strategies for *Alternaria solani*; it is imperative to understand the physiology and growth of *Alternaia solani* under various conditions. As a first step, an appropriate medium should be developed to examine *in vitro* control of the pathogen under study and later on strategies for control in the field can be developed equipped with the information of optimum cultural conditions.

MATERIALS AND METHODS

1. Nutritional influences on the growth of *Alternaria solani*

1.1. Effect of various carbon sources on the growth of *A. solani*

PDA broth was incorporated with seven different carbon sources that included sucrose, glucose, fructose, dextrose, maltose and lactic acid. Considering sucrose as the basis of C, the mass of each carbohydrate type was calculated to provide equal amounts of carbon.. Controls were kept without any carbon source in the medium. By using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide pH of the medium was adjusted to 7.0. Thirty ml of each

of the medium was poured into sterilized flasks (100 ml) which were subsequently sterilized and 5 mm discs procured from 9-days–old cultures that were inoculated with the fungus. Subsequently, these were subjected to incubation for six days at 27 ± 1°C. Triplicate samples for each treatment were maintained. Dry weights of mycelia were registered and the data set subjected to appropriate statistical tests in accordance with Zar (2008).

1.2. Effect of various nitrogen sources on the growth of *A. solani*

Various nitrogen sources including $(NH_4)_2SO_4$, NH_4Cl, KNO_3, Threonine, $NaNO_3$, Asparagine and NH_2CONH_2 (urea) were separately added to Richard's medium. For each of these cases on molecular weight basis, quantity of N required was calculated and as potassium nitrate present in the basal medium, equivalent amount of nitrogen incorporated. Controls did not receive any nitrogen source. The pH was maintained by using dilute acid or alkali to neutral. Subsequently, thirty ml of each of the medium was poured in a 100 ml sterile flask, sterilized and then 5 mm discs were obtained from *A. solani* inoculated 9-days-old culture. Later these were incubated at 27 ± 1°C for 6 days. Treatments and controls were replicated thrice. Dry mycelial weights were recorded and data analysed appropriately in accordance with Zar (2008).

2.2. Physiological influences on the growth of *Alternaria solani*

2.1. Influence of pH on growth of *Alternaria solani*

To examine the effect of pH on mycelial growth of *A. solani*, PDA broth served as a basal medium. With the aid of dilute acid or alkali the pH of the medium was regulated to various levels (*viz.* 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) using a Jenway (England) pH meter. With known pH, 30 mL of the medium was added to 100 ml flasks and then the medium was sterilized. Five mm discs of *A. solani* obtained from 8-day-old culture were inoculated and then incubated at 26 ±1°C for 10 days. For each treatment four replicates were kept and colony diameter was noted. Data were subjected to suitable statistical analysis.

2.2. Effect of temperature on the growth of *Alternaria solani*

To examine the effect of temperature on growth of *Alternaria solani* as a basal media P D (potato-dextrose) broth was employed. Thirty ml of the medium was poured into a 100 mL conical flasks and then sterilized. Five mm discs of 8-day-old culture of *Alternaria solani* were inoculated and subsequently incubated at different temperature regimes five, ten, fifteen, twenty, twenty five, thirty, thirty five and forty °C for eight days. For each treatment four replicates were maintained and colony diameters were determined. Data were subjected to appropriate statistical analysis.

2.3. Influence of light on the growth of *Alternaria solani*

It was examined if the growth of *Alternaria solani* was influenced by light using PDA as a basal medium. The cultures were given following dark and light regimes:

Dark h	Light h
24	0
16	8
12	12
8	16
4	20
0	24

Pure cultures were inoculated in Petri dishes containing PDA and incubated for eight days. Treatments were quadrupled and subjected to statistical inference.

2.4. Cultural media influence on the growth of *Alternaria solani*

Eight different solid cultural media were utilized to examine the growth of *Alternaria solani.*

- Potato Dextrose Agar
- Czapek Dox Agar
- Corn meal Agar
- Glucose Peptone Agar
- Malt extract Agar
- Oat meal Agar
- Richard's Agar
- Host leaf extract Agar

The details of constituents and the make-up process of different media appear in Tuite (1969).

3. Statistical analysis

For the data sets derived from of each the above experiment, analysis of variance (ANOVA) by completely randomized design (CRD) was performed. As a post-hoc test Duncan's multiple range test and least significant difference (LSD) at p=0.05 were employed. In addition, Scheffe's multiple contrast test was also performed since it is a flexible test that permits comparison of groups of means (or one mean against all) (Zar, 2008).

RESULTS

1. Results of Nutritional studies

1.1. The outcome of the use of various sources of C on *in vitro* growth of *Alternaria solani*

Carbon is an essential element for the growth of a fungus. The use of eight different carbon sources was examined for the role in the growth of *A. solani* while the basal medium free from added carbon was kept as control. Various sources of carbon gave a significant difference ($F=83.72$; $p<0.001$) as shown by a one-way ANOVA. Among all carbon sources, glucose and sucrose gave maximum growth while minimum growth was observed in control (Table 1). Scheffe's multiple contrast test was performed with sucrose against all other C-sources that yielded a significant difference against between the two groups ($p <0.010$). Scheffe's test for lactic acid against all other C-sources was also found significant ($p<0.01$).

Table 1. Mean and standard error of colony diameter recorded when Carbon was provided by various carbohydrates to record the effect on growth of *A. solani.*

S.N	Carbon sources	Mean and S.E
1	Glucose	5.93 ± 0.03 a
2	Sucrose	5.96 ± 0.03 a
3	Fructose	5.2 ± 0.15 b
4	Lactose	4.96 ± 0.15 b
5	Dextrose	4.33 ± 0.08 b
6	Maltose	3.83 ± 0.03 c
7	Manitol	5.23 ± 0.12 d
8	Lactic acid	2.8 ± 0.30 e
9	Control	1.7 ± 0.15 f

$LSD_{0.05}$ = 0.468

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different ($p<0.05$).

Table 2. Mean and standard error of colony diameter recorded in when various N-sources were added on the growth of *A. solani.*

S.N	Nitrogen sources	Mean and S.E
1	Ammonium sulphate	3.5 ± 0.05 a
2	Ammonium chloride	3.5 ± 0.23 b
3	Asparagine	5.5 ± 0.23 b
4	Sodium nitrate	3.5 ± 0.23 b
5	Potassium nitrate	4.76 ± 0.08 c
6	Threonine	6.03 ± 0.03 c
7	Urea	4.8 ± 0.15 c
8	Control	2.46 ± 0.08 d

$LSD_{0.05}$ = 0.693

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different ($p<0.05$).

1.2. The effect of different Nitrogen containing salts on the *in vitro* growth of *Alternaria solani*

To examine the influence of different N- sources on the growth of *A. solani* we tested 7 nitrogen containing . Controls were not incorporated with any N-source. A one-way ANOVA yielded a significant difference between N-

sources (F=25.49; p<0.001). Maximum growth was found in urea and Threorine while minimum growth occurred in the controls (Table 2). Comparison of threonine with the rest of N-sources using Scheffe's multiple contrast test gave a significant difference (P<0.01).

2. Results of physiological studies

2.1. Effect of pH-regimes on the *in vitro* fungal (*A.solani*) growth

In this experiment the influence of various pH-regimes on growth of the fungus *Alternaria. solani* was examined. Basal medium was PD broth and pH was regulated using 0.1 N NaOH and 0.1 N HCl to achieve the required pH of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. Growth of *A. solani* at various pH-regimes differed highly significantly (F=39.52; p<0.001) when the appropriate ANOVA was performed. Fungal growth was highest at pH 6, while pH of 5.0 and 7.0 gave slightly lesser expansion (Table 3). A pH of 9.0 gave the least. When a pH of 6 was compared against all other pH-regimes used, Scheffe's multiple contrast test showed a remarkable difference between the two groups (p<0.05). However, when lowest colony diameter (at pH=8) was tested against the rest surprisingly a non-significant difference resulted.

Table 3. Mean and standard error of colony diameter recorded in different pH on the colony diameter expansion of *A. solani*.

S.N	pH	Mean and S.E
1	4	4.36 ± 0.31 a
2	5	5.66 ± 0.17 ab
3	6	6.03 ± 0.06 b
4	7	5.33 ± 0.08 c
5	8	3.03 ± 0.29 d
6	9	3.26 ± 0.12 d

$LSD_{0.05}$ = 0.620

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different (p<0.05).

2.2. Effect of various temperature regimes on *in vitro* radial expansion of *A. solani.*

The object of this study was to examine the influence of various temperature regimes on the colony diameter of *Alternaria solan.* The medium was PD-broth and temperatures regimes were 5, 10, 15, 20, 25, 30, 35 and 40°C for 5 days. The appropriate analysis of variance yielded a significant difference among the radial growth for varied temperature regimes (F=128.61; p<0.001). The maximum radial growth was supported by 25 °C followed by 30 °C whilst the least occurred at 5°C (Table 4). Thus it is apparent that a temperature regime of 25°C to 30°C is most suitable. Scheffe's multiple contrast test for diameter expansion at 25°C and that at rest of the temperatures regimes, showed remarkable difference at a probability level of <0.05.

Table 4. Mean and standard error of colony diameter recorded at varied temperature regimes for *A. solani* diameter expansion.

S.N	Temperature	Mean and S.E
1	5°C	2.2 ± 0.57 a
2	10°C	2.8 ± 0.05 a
3	15°C	3.26 ± 0.12 b
4	20°C	5.2 ± 0.05 b
5	25°C	6.03 ± 0.06 b
6	30°C	5.86 ± 0.14 c
7	35°C	5.23 ± 0.13 d
8	40°C	5.06 ± 0.08 e

$LSD_{0.05}$ = 0.390

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different (p<0.05).

2.3. Results of different light / dark regimes on radial expansion of *Alternaria solani in vitro*

In this experiment the influence of light/dark regimes on the expansion of *A. solani* was examined. *A. solani* was exposed to continuous darkness and continuous light and alternate cycles of dark and light for different periods

of time for 6 days (Table 2.5).One-way ANOVA showed significant difference in light/dark regimes tested ($F=19.47$; $p<0.001$). The radial expansion was highest when the cultures received alternate exposures (12h day light/ darkness for 12h) (Table 5). This alternate exposure regime when tested against the rest of the light/dark regimes using Scheffe's test showed a significant effect compared to the rest of light/dark treatments ($p<0.05$).

Table 5. Mean and standard error of colony diameter recorded in different light interval on the growth of *A. solani.*

S.N	Exposed intervals (hours)	Mean and S.E
1	4 h dark 20 h light	5.36 ± 0.14 a
2	12h dark 12 h light	6.03 ± 0.06 a
3	8 h dark 16 h light	5.23 ± 0.18 b
4	8 h light 16 h dark	4.76 ± 0.08 b
5	24 h light 24 h dark	5.96 ± 0.06 c

$LSD_{0.05}$ = 0.390

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different ($p<0.05$).

Table 6. Diameter expansion and colony characteristics s of *Alternaria. Solan i*on various types of media

S.N	Media	Colony characters		
		Colour of colony	Substrate colour	Margin of colony
1	Potato Dextrose agar	Dark brown	Light brown	Irregular
2	CzapekDox agar	Whitish	Light colour	Thin flat, smooth
3	Corn meal agar	Dark brown	Dark greyish	Irregular
4	Glucose peptone agar	Greyish	Light greyish	smooth wavy
5	Malt extract agar	Light brown	Greyish	regular margin
6	Oat meal agar	Grey	Greyish	Smooth
7	Richard's agar	Light brown	Light greyish	Smooth
8	Host leaf extract agar	Dull white	Greyish	Smooth, irregular

Table 7. Mean and standard error of colony diameter recorded in different media on the growth of *A. solani*

S.N	Media	Mean and S.E
1	Potato Dextrose agar	5.93 ± 0.12 a
2	CzapekDox agar	4.7 ± 0.25 ab
3	Corn meal agar	6.03 ± 0.08 ab
4	Glucose peptone agar	5.4 ± 0.15 b
5	Malt extract agar	5.7 ± 0.15 b
6	Oat meal agar	5.33 ± 0.12 b
7	Richard's agar	5.83 ± 0.12 c
8	Host leaf extract agar	3.46 ± 0.20 d

$LSD_{0.05}$ = 0.549

The pair of means having common letter against them do not differ significantly while those that have all letters different against them from the other are significantly different ($p<0.05$).

3. Result of Cultural studies using various media

3.1. Results of different media on growth of *Alternaria solaniin vitro*

In this experiment morphological characters and growth of *A. solani* was observed on eight different media. Variations in morphological characters including margin of colony, colony colour and colour of substrate were noticed (Table 6). In other experiment eight different media were evaluated for radial growth of *A. solani* and on eight different solid media it showed highly significant difference in its growth. Analysis of variance (ANOVA) showed significant differences in fungal growth in different media tested ($F=24.23$; $p<0.001$). Corn meal agar supported maximum growth followed by potato dextrose agar (PDA). Richard's agar, malt extract agar, glucose

peptone agar, oat meal agar and minimum was observed in host leaf extract agar (Table 7). When growth on corn meal medium was checked against that on other media a significant difference was found. However, a comparison of PDA against growth in the rest of the media showed a non-significant difference.

DISCUSSION

According to Bilgrami and Verma (1978) among the essential elements, carbon occupies the unique position because living organisms require carbon as a functional and structural component. Its percentage is about fifty percent in terms dry weight. Eight carbon compounds, were tested for the measurement of *in vitro* growth of *A.solani*.. Optimal colony diameter increase of *A. solani* was given by Sucrose and Glucose, whereas, Lactic acid and in control (without any carbon source) gave minimal growth. The results are in accordance with those resported by Gupta *et al*., (1970) in which glucose gave maximum growth of *Alternaria brassicae* among the eight monosaccharides tested. It can be readily explained with respect to molecular structure of glucose that is the basic monosaccharide (6-carbon compound) highly soluble in water and readily mobilized into fungal mycelia.

Nitrogen is an essential ingredient for the biosynthesis of proteins. However, various N-sources do not contribute towards protein synthesis in a similar way and consequently to the growth and development of fungal tissue. In the present study, seven nitrogen contributing sources were evaluated for growth of *A. solani* in which asparagine and threonine (which are amino acids) proved to be the most effective towards the growth of *A. solani.* This could be due to the fact that these amino acids contain the amino groups (α-amino-) that are readily incorporated into the protein structure. Thus these N-sources are most effective for the development of both reproductive and vegetative tissue. Not unexpectedly, compared to control (without N-source), all other N-sources gave better results compared to controls. Likewise, Misra and Mahmood (1960)) reported that 3 amino acids (valine, arginine, alanine) supported good growth of *Colletotrichum capsici.* According to Lilly and Barnett (1951) the amino acids alanine and glycine are directly used in protein synthesis. Highest mycelial growth of *G. cingulata* was recorded when amino acids l-leucine and dl- methionine acted as the N-source while $NaNO_3$, NH_4NO_3 and urea gave low growth (Sing and Shankar, 1971). According to Cochrane (1958) it might be because of the release of NH_3 in high concentration during autoclaving, urea could have broken down to ammonia resulting in toxicity to the fungus.

This investigation disclosed that a pH 6.0 was most suitable for maximal growth of *A. solani* while minimal occurred at pH 8. According to Lilly and Barnett (1951) rate and growth of the fungus is to a great extent affected by hydrogen ion concentration of the medium. This indicates that *A. solani* is acid tolerant and prefers acidic pH over the alkaline pH. In general, fungi thrive better in acidic media (acidic pH) compared to basic ions (alkaline pH) which contrasts with the bacteria and actinomycetes (Cochrane, 1958; Bilgrami and Verma, 1978). The results concord with those given by Verma (1970) and Gemawat and Ghosh (1980) according to whom a pH of 6.0 was the most suitable pH for the growth of *Alternaria* spp..

Temperature is regarded as a predominating factor with regard to vegetative and reproductive growth of fungi. Present investigation demonstrated that maximal growth occurred at 25 °C while minimum was obtained at 5°C. Similar experiment was conducted by Kaul and Saxena (1988) which disclosed that the most suitable temperature for vegetative growth of *A. solani* was 25°C. Furthermore, Chowdhury (1944) also reported that for highest growth of *A. carthami* temperatures of 30°C and 25°C were most suitable.. According to Bonde (1929) *A. solani* can grow within a wide temperature range (15 to 40°C), it was further noted that a temperature of 25°C was optimal for the growth.

One of the overriding factor for growth and spore formation is light (Timmick *et al*., 1951; Drayton, 1937; Leonian, 1924). The present investigation showed that *A. solani* gave highest growth, when *A. solani* cultures were given alternate dark and light conditions (12 h dark then with 12 h light). Luckens (1963) reported that conidia formation of *A. solani* was initiated when the culture were given six hours of darkness. Furthermore, abundant sporulation occurred at 18°C for 8 h , Zhu *et al*., (1985) demonstrated that *A. solani* sporulated profusely at 18°C (8 h) when cultures (corn meal agar) were exposed to fluorescent light. Fencelli and Kimati (1990) investigated that after exposure for 8 h of light *A. dauci* gave maximum sporulation and growth.

Cultural characteristics are often noted when fungi grow on different solid media. In the present study for investigating the cultural characteristic and variation in growth, *A. solani* isolated from infected tomato plants was grown on eight solid media. Among eight solid media corn meal agar and potato dextrose agar supported maximum growth of *A. solani. A. solani*, for its growth preferred non synthetic media to synthetic media. These results correspond well with those of Gemawat and Ghosh (1920), who tested the growth of *A. solani* in seven solid media and observed that PDA gave good sporulation with excellent growth after 10 days of incubation. Furthermore, many researchers including Bonde (1929), Neergaard (1945), Rotem (1966) and Mazzonetto *et al*. (1996) observed best

growth of *Alternaria* spp. on potato dextrose agar. Similar results have been reported by Mahadevaiah (1993), Joshi (1981) and Mahabaleswarappa (1981). Variation in morphological characters including margin of colony, colony colour and substrate colour were noticed on *A. solani*. Several workers Bonde (1929), Rotem (1966), Henning and Alexander (1959) and Kaul and Saxena (1988) observed differences in cultural characters like type of growth, growth rate, sporulation on different media, colony colour and colour of the substrate. Our, results, in general, seems to correspond with their findings.

REFERENCE

Agrios, G. (2005). *Plant pathology*, 5th ed., New York, Elsevier.

Barksdale, T.H. (1968). Resistance of tomato seedling to early bright. *Phytopathology*, 58: 443-446.

Bhandari, J.K.S. and R.S. Singh (1976). Effect of carbon and nitrogen sources on the growth of *Alternaria triticina*. *Indian Phytopathology*, 29: 88-89.

Bilgrami, K.S. and R.N. Verma (1978). *Physiology of Fungi*. Vikas Publishing House, New Delhi, P.498.

Bonde, R. (1929). Physiological strains of *Alternaria solani*. *Phytopathology*, 19: 533-548.

Chaturvedi, C. (1966). Utilization of oligo saccharides by three imperfect fungi. *Mycopathologia et mycologia applicata* 29: 323-330.

Cheema, S.S., S.P. Kanpur, J.S. Chohah and R. Jayarajan (1976). Cultural studies on *Alternariacitri*the casual organism of stylar rot of citrus. *Indian Phytopathology*, 29: 328-329.

Chowdhury, S. (1944). An *Alternaria* disease of saflower. *Journal of Indian Botanical Society*, 23: 59-65.

Cochrane, V.W. (1958). *Physiology of fungi*, John Wiley and Sons, Inc.New York, 524pp.

Drayton, F.L. (1937). The effect stage of *Botrytis convoluta*.*Mycologia*, 29: 305-318.

Fencelli, M.I. and H. Kimati (1990). Influence of culture media and fluorescent light on the sporulation of *Alternaria dauci*. *Phytopathologica*, 16: 248-252

Gemawat, P.D. and S.K. Ghosh (1980). Studies on the physiology of growth of *Alternariasolani*. *Indian Journal of Mycology Plant Pathology*, 9: 138-139.

Goyal. K.N. (1977). Effect of pH, carbon and nitrogen nutrition on the growth of *Alternaria tenuis*. *Indian Journal of Mycology and Plant Pathology*, 7: 155-157.

Gupta, R.B.C., B.G. Desai and V.N. Pathak (1970). Carbon requirements of *Alternaria brassicae, Nova Hedwigia*, 19: 349-350.

Hasija, S.K. (1970). Physiological studies of *Alternaria citri* and *Alternaria tenuis*. *Mycologia*, 63: 289-295.

Henning, R.G. and W.J. Alexander (1959). Evidence of existence of physiological races of *A. solani*. *Plant Disease Reporter*, 47: 659-643.

Joshi, P.V. (1981). *Studies on Alternaria gomphrenae causing leaf and stem blight of Gomphrena globosa Linn*. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, P.103

Kallo.G. and M.K. Banerjee (1993). Early blight resistance in *Lycopersicon esculentum* Mill. Transferred from *L. pimpinellifolium* (L.) Mill. and *L. hirsutum* f. *glabratum* Mull. *Gartenbauwiss*, 58: 238-240

Kaul, A.K. and H.K. Saxena (1988). Physiologic specialization in *Alternaria solani* causing early blight of tomato. *Indian Journal of Mycology Plant Pathology*, 18: 128-132.

Leonian, L.H. (1924). A study of factors promoting pycnidium formation in some Sphaeropsdales. *American Journal of Botany*, 11: 19-50.

Lilly, V.G. and H.L. Barnett (1951). *Physiology of the fungi*. McGraw Hill Book Company Inc., New York 439pp.

Lukens, R.J. (1963). Photo-inhibition of sporulation in *Alternariasolani*. *American Journal of Botany*, 50: 720-724.

Mahabaleshwarappa, K.B. (1981). *Studies on leaf spot of safflower (Carthamus tinctorius Linn) caused by Alternaria carthami.* Choudhary M.Sc. (Agri) Thesis.University of Agricultural Sciences, Bangalore, P.54.

Mahadevaiah, P.H. (1993). *Studies on variation survival and management of Alternaria solani sorauer, the incitant of early blight of tomato.* M.Sc. (Agri) Thesis, University of Agricultural Sciences, Bangalore, 106 pp.

Mathur, K. and K.S. Shekhawat (1986). Chemical control of early blight in Kharif sown tomato *Indian Journal of Mycology Plant Pathology*, 16: 235-238.

Mathur, S.B. and A.K. Sarboy (1977). Physiological studies on *Alternaria alternata* from sugar beet. *Indian Journal of Phytopathology*, 30: 432-434.

Mazzonetto, F., J.O. Menten, A.L. Paradela and M.A. Galli (1996). Effect of culture media on mycelial growth and conidial production of *Alternaria* spp. in beans. *Ecossistema*, 21: 59-61.

Misra, A.P. and M. Mahmood (1960). Effect of carbon and nitrogen nutrition on growth and sporulation of *C. capsici*. *Journal of the Indian Botanical of Society*, 39: 314-321.

Mohapatra, A., A.K. Mohanty and N.N. Mohanty (1977). Studies on physiology of the sesame leaf blight pathogen, *Alternaria sesami*. *Indian Phytotopathology*, 30: 332-334.

Neergaard, P. (1945). Danish species of *Alternaria* and *Stemphylium*, Hamphry Millfor. Oxford University Press, London, P.566.

Padmanabhan, P. and P. Narayanaswamy (1977). Growth studies of *Alternaria macrosporazimm incitant* of leaf spot disease of cotton. *Madras Agricultural Journal*, 64: 258-261.

Pawar, V.H. and M.K. Patel (1957). *Alternaria* leaf spot of *Ricinus cumunis* L. *Indian Phytopathology*, 10: 110-114.

Rane, M.S. and M.K. Patel (1956). Disease of cotton in Bombay-I, *Alternaria* leaf spot. *Indian Phytopathology*, 9: 106-113.

Rotem, J. (1966). Variability in *Alternaria porri f. solani*. *Israel Journal of Botany*, 15: 48-57.

Samuel, G.S. and C.V. Govindaswamy (1972). Effect of vitamins and levels of pH on the growth and sporualtion of *Alternaria solani*, the causal agent of the leaf blight disease of seasame (*Sesamum indicum*). *Indian Journal of Mycological Plant Pathology*, 2: 185-186.

Singh, R.S. (1983). *Diseases of Vegetable Crops*. Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, Bombay, Culcutta, P.419.

Singh, R.A. and G. Shankar (1971). Effect of physical factors and nutrition on growth and perithecial development of *Glomerella cingulatain vitro*. *Indian Phytopathology*, 24: 728-734.

Stevenson, R.E. and S.P. Packer (1988). Effect of radiation temperature and moisture on conidial germination of *Alternaria solani*. *Phytopathology*, 78: 926-930.

Taber, R.A., T.C. Venterpoor and W.A.S. Tabr (1968). A comparative nutritional study of *Alternaria raphani, A. Brassicae* and *A. brassicola* with special reference to *A. raphani. Phytopathology*, 58: 609-616.

Timmick, M.B., V.G. Lilly and H.L. Barnett (1951). The influence of light and other factors upon sporulation of *Diaporthe phaseolorum* var. *Botatis* from soybean. *Phytopathology*, 41: 327-336.

Tuite, J. (1969). *Plant pathological methods*. Minneapolis, Minnesota: Burgess Publishing Company.

Verma, V. (1970). Effect of temperature and hydrogen-ion concentration of three pathogenic fungi. *Sydowia*, 23: 164-168.

Zar, J.H. (2008.) *Biostatistical Analysis*.5th ed. Prentice Hall, Englewood Cliffs, New Jersey.

Zhu, Z.Y., Y.M. Huang and Y.H. Li (1985). An efficient technique for inducing profuse sporulation of *Alternaria solani* in pure culture. *Acta Mycologica Sinica*, 4: 180-184.

(Accepted for publication October, 2019)