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RESEARCH ARTICLE

STUDY OF VARIABILITY AND SPORULATION BY ISOLATES OF *ALTERNARIA SOLANI* OF *LYCOPERSICON ESCULENTUM* (MILL.)

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ABSTRACT

Alternaria solani was studied on various parameters of cultural characters such as radial growth, pigmentation, pH, temperatures sporulation and differential host response. Among five isolates maximum radial growth was found in Sh isolates (75.2 mm) while minimum in Va isolates (56.5 mm). The maximum thickness of conidiogenous hyphae was recorded in Va isolate (9.56µ) and minimum (1.7µ) in Mi, Ba and Sh. *Alternaria solani* grew well on Czapek dox agar medium and Jenson medium. The maximum average radial growth was recorded 54.7 mm on PDA. The optimum temperature was recorded 25 ^{o}C with pH 7.5 for the pathogen. Pigmentation varied from brown to black color on these media, light yellow to black color at different temperatures and gray to brown color on different pH ranges. The sporulation was not found at any tested media, pH and temperatures except host decoction media. A method of inducing sporulation on host decoction media has been developed. Now it is possible to produce mature conidia in culture under aseptic condition in 20-25 days at 28 ^{o} C and pH 6.5. Septation of conidia were found to be greater in number from infected tomato leaves and fruits.

INTRODUCTION

The genus Alternaria is a large and important group of pathogenic fungi which cause significant number of important diseases on a wide range of agronomic and horticultural plants. *A. solani* (Ellis and Martin) Jones and Grout is most important pathogen causing severe early blight disease every year in tomato. It is one of the damaging as well as destructive disease of tomato. Due to this disease, about 80% yield loss was recorded in experimental field and severity varies from 15-90 per cent (Pandey *et al.,* 2003). Information on *in vitro* aspects pertaining to pathogenic variability, factors influencing the mycelial growth and sporulation are limited. The fungus is readily cultured on artificial media such as potato dextrose agar where it produces a deeply yellow pigmented gray/ black hairy colony. But among the different species of Alternaria, the *Alternaria solani* does not produce spore readily under laboratory conditions. It is easy to recognize *Alternaria* sp. by the morphology of their large conidia. They are catenate or solitary typically ovoid often beaked, pale brown to brown, multi-celled and muriform. (Ellis 1971). Several workers have attempted to increase sporulation by various methods. These methods have included exposure of fluorescent light, exposure of UV light, exposure of sun light, dehydration of medium and chemical treatment of culture (Douglas and Pavek, 1971). For Example Rath and Padhi (1973) reported that the sporulation in *Alternaria solani* in three days old culture on solid media exposing direct sunlight for 10 minutes and incubation at 20-25 ^{O}C temperature increased sporulation. Klimesova and Prasik (1989) reported that UV radiation had pronounced effect on five selected characters (length, Width, color, necrosity and length of the conidial beak) of these conidium. Distinct differences were found in 5 harbarium specimens of *Alternaria alternata* compared with corresponding isolates in cultures. These observations demonstrated the importance of studying the relation between natural material and their prospective cultures in order to assess correctly the general variability of the species. Pandey and vishwakarma (1999) have studied the morphological variability of the conidia of *Alternaria alternata* on culture media. He further reported the pathogenic isolates of *Alternaria alternata* reduced in its conidium and beak size from natural host to culture. A few workers viz. Rath and Padhi (1973), Stevenson and Pennypacker (1988), Sodlauskiene *et al.* (2003), Prasad *et al* (1973) and Rodriguez and Santana (1991) tested the isolates of *Alternaria* on particular temperatures for observing the

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cultural characters and sporulation of *A. solani*. The present study is completely different from previous workers. In this study cultural, morphological and an efficient method, proven consistently to induce sporulation in numerous isolates of *A. solani* has been developed. This makes it possible to produce spores under aseptic conditions in 20-25 days on host decoction media.

MATERIALS AND METHODS

A. solani was studied on various parameters of cultural and morphological variability such as radial growth, pigmentation, pH, temperatures, sporulation and pathogenic variability along with differential host response of pathogen on tomato. These all sets of experiments have been discussed as follows- Cultural and morphological variability of different isolates of *A. solani* on PDA. Five selective isolates of *A. solani* were taken for representing Varanasi (Va), Mirzapur (Mi), Robertsganj (Ro), Shillong (Sh) and Bangalore (Ba) of different zones of the country. The cultures were already maintained in laboratory. All these isolates were tested for their cultural and morphological variations on Potato dextrose agar (PDA) medium only. For each isolates 5 Petri plates were poured with potato dextrose agar medium. After solidification of the medium 5 mm culture bits of each isolates were inoculated onto the PDA Petri plates by maintaining the aseptic conditions. These inoculated Petri plates were kept in BOD at 25±1°C for growth. The data was recorded after 3 days of inoculation and radial growth was measured per day upto 9^{th} day.

The thickness of conidiogenous hyphae of different isolates were measured by calibrations through ocular and stage micrometer. The growth rate, cultural, and morphological characters were studied. In another experiment three types of nutrient media viz. ASM (*Alternaria* sporulation medium), freshly prepared V-8 juice agar, and readymade available V-8 juice agar medium (Hi-media) containing asparagine was taken for above mentioned five isolates for the cultural and morphological study. Pathogen was incubated at 25±1°C for 11 days. The growths of different isolates of *A. solani* were measured on these medium and the variability were recorded. For cultural and morphological variability of *A. solani* on different nutrient media, pH and temperatures, pure culture of one pathogenic isolate of *A. solani* was grown on fourteen different synthetic, semi-synthetic and natural media i.e. V-8 juice medium (V-8 JM), Malt extract agar (MEA), Corn meal agar (CMA), Potato dextrose rose bengal agar (PDRBA), Casein hydrolysate medium (CHM), Richard synthetic agar (RSA), Oat meal agar (OMA), Rose bengal chloramphenical agar (RBCA) Potato dextrose agar (PDA), Czapek dox agar (CDA), Jenson media (JM), Asthana and Hawker medium (AHM), Malt extract potato dextrose agar (MEPDA) and *Alternaria* sporulation medium (ASM) for the study of cultural and morphological variability.

These media were prepared as per standard composition (Dhingra and Sinclair 1995). Twelve pH ranges i.e. 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5 were adjusted through pH meter (Thermo Orion) by adding few drops of either HCl or NaOH solution in PDA medium. Five different temperatures i.e. 15^0C, 20^0C, 25^0C, 30^0C and 35^0C were maintained in BOD incubator separately. PDA was selected as standard basal medium for both temperature and pH study. Ten days old culture of *A. solani* was inoculated on PDA with 5 mm culture disc and incubated at 25 ^{o}C for the study of variability on various pH ranges and the same size of culture bits were taken for various temperatures range. Five replication of each set of experiment maintained for each test media, pH and temperatures. The data of radial growth rate, pigmentation and colony character were recorded after 3 days of inoculation and continued up to 9^{th} day. The experimental data was subjected to statistically analysis using factorial CRD design. Sporulation of *A. solani* on host decoction media:- The following *three* combination of host decoction medium were prepared and tested for the sporulation of *A. solani.*

Preparation of host decoction media: The tomato leaf extract and fruit juice were prepared by grinding in a mixer and it was filtered with a muslin cloth. Then the juice was centrifuged at 8000 rpm for 10 minutes at room temperature. The supernatant was taken as medium preparation at each step.

Ist combination of host decoction media*:* 20 ml of 1 % autoclaved agar was poured in a Petri plates and after solidification, the freshly prepared tomato leaf extract was passed through bacterial proof filter in each agar plates under aseptic condition. Then 7 days of old culture bits (5 mm size) of *A. solani* was inoculated on this Petri plates and incubated at 25±1°C and data were recorded after every 7 days intervals upto 21^{th} day.

IInd combination of host decoction media: 200 ml ripe tomato fruit juice as well as tomato leaf extract were taken in flask separately which contains 5 g of celite powder in both and it was centrifuged at 8000 rpm for 10 minutes at room temperature. The supernatant was taken as medium. 2 g. of Agar and 2 g. of Sucrose were added in this supernatant and pH is adjusted to 6.5. Then it was autoclaved at 15 lbs for 15 minute, after sterilization of 0.1% $CaCO_3$ was mixed in this medium under aseptic condition. It was poured in Petri plates and again 2 ml of freshly prepared tomato fruit juice and leaf extract added separately onto the Petri plates through bacterial proof filter. Then 7 days of old culture bits of *A. solani* (5 mm size) were inoculated on this Petri plates and incubated at 25±1°C. The observations were recorded after every 7 days intervals upto 21^{th} day.

IIIrd combination of host decoction media: The tomato juice was extracted from ripe and semi-ripe fruits separately. The whole juice was transferred in a conical flask which contains 5 g of celite powder and centrifuged at 8000 rpm for 10 minutes at room temperature. The supernatant was taken as medium. The total volume of the medium maintained up to 250 ml after adding 1 % agar and 1% dextrose in it. Before pouring of this medium 0.5 % $CaCO_3$ powder was added in this medium and inoculation was done as mentioned in Ist and IInd combinations of host decoction media. These all combinations of host decoction medium were tested more than three times for the sporulation study of *A. solani.*

Comparative study between conidial morphology of *A. solani* from host decoction media and infected host tissues

Conidial morphology of *A. solani* was studied from different parts of infected plant such as leaves, stem, and fruits.

Temporary slides were prepared separately from these parts of plant tissue. The horizontal septa, vertical septa, and septa in beak of different conidia were counted from infected host tissue for the study in morphological variability of conidia. The same tissues were incubated in BOD for 12 hours at 25±1°C in moisture box and again data of conidial morphology was taken after incubation. The temporary slides were prepared in lacto phenol by scrapping of the infected tissue. The comparative study of conidial morphology had done in both cases i.e. from culture spore and host spores of *A. solani.*

RESULTS

Variability of *A. solani* on PDA

The five isolates of *A. solani* exhibited significant variation for their cultural character, pigmentation and per day growth rate. Colonies of different isolates of the pathogen varied from white to dark black, circular to irregular, smooth to rough, with or without concentric zonation. The radial growth varied from 56.5 to 75.2 mm after 9 days of incubation. The pigmentation varied from brown to brownish black (Table 1). Maximum radial growth was 75.2 mm of Sh isolate while lowest growth was 56.5 mm in Va isolate. Radial growth was almost similar to Ro, Mi, Sh isolates. The variability in radial growth among these isolates has been presented in Fig. 1 from 4th day to 9th day. Periodical radial growth of these five isolate was recorded every day from 4th to 9th day of incubation which revealed that fastest growth was between 5th to 7th days in Ro, and Mi isolate. Sh isolate reflected maximum growth at initial stage between 4-5 days, and later on between 8-9 days. (Table 2)

media i.e. ASM, V-8 juice agar, and V-8 juice agar (synthetic) which containing asparagine. Pathogen was incubated at 25±1°C for 11 days. The growth patterns observed significantly different for the test isolates. Radial growth of *A. solani* varied from 24.33 mm to 52.83 mm on a ASM, 25.50 mm to 53.33 mm on V-8 juice agar medium and 26.50 mm to 48.51 mm on V-8 juice agar synthetic medium (Table 3). All the isolates were significantly different from each other. The maximum radial growth of Mi, and Ba isolate were recorded on V-8 juice agar medium while Va, and Sh on V-8 juice agar synthetic. Although all five isolates of *A. solani* were pathogenic culture but great variability in radial growth were recorded within the isolate as well as among these three media. ASM did not supported formation of conidia under the present test condition (Plate 1a).

Plate 1a. Variability of Sh Isolates on three selective media

Table 1. Cultural, morphological variability and sporulation of different isolates of *A. solani* on PDA

Isolates	Pigmentation	Avegarge radial growth (mm) days after inoculation						Width of Conidiogenous hyphae (µ)	Mycelial growth/ colony characters				Sporulation
		4th	5th	6th	7th	8th	9th		Circular/irregular	Smooth/ rough	Gwoth rate	Zonation	
Va	Brown	29.9	39.8	46.5	48.5	50.2	56.5	9.56	Irregular	Smooth	4.4	Concentric zonztion	No
Ro	Black brown	31.0	40.5	51.0	62.7	66.5	72.8	1.7	Irregular	Rough	6.9	Without zonation	No
Mi	Brownish black	30.5	38.7	51.6	58.3	66.7	72.9	1.7	Circular	Smooth	7.0	Without zonation	No
Ba	Brownish black	25.4	32.7	41.5	49.6	59.7	64.5	1.17	Circular	Smooth	6.5	Concentric zonation	No
Sh	Black brown	30.1	41.0	49.9	56.3	63.7	75.2	1.17	Irregular	Smooth	7.5	Concentric zonation	No

Table 2. Average radial growth rates of different isolates of *A. solani*

Isolates	Average radial growth (mm) per day after inoculation				
	4-5 days	5-6 days	6-7 days	7-8 days	8-9 days
Va	9.9	9.2	7.2	6.0	9.7
Ro	9.5	10.5	11.7	3.8	6.3
Mi	8.2	12.9	6.7	8.4	6.2
Ba	7.3	8.8	8.1	10.1	4.8
Sh	10.9	8.9	6.4	7.4	11.5

Variability of five isolates of *A. solani* on three selective nutrient media

It was observed that the five different isolates of *A. solani* were significantly different in each other on three different

Sporulation was not recorded on these selective media. The thickness of conidiogenous hyphae varied from 1.17 to 9.56 µ. Minimum thickness of conidiogenous hyphae observed in Sh, Ro, Mi, and Ba isolate i.e. 1.17 µ. The maximum thickness of

conidiogenous hyphae was observed in Va isolate. (Table 1) The fastest growth rate after 9 days of incubation was recorded 7.5 mm in Sh isolate and slowest growth rate was recorded 4.4 mm in Va isolate. Irregular margin, dark brown, smooth, velvety, with zonation was recorded in Va isolate; smooth, Irregular margin, black brown without zonation colony in Ro isolate; circular, smooth margin, fluffy, slight velvety, brownish black, without zonation in Mi isolate; circular margin, fluffy, depressed center with concentric zonation, brownish black colony in Ba isolate, and irregular margin, gray periphery, black brown with concentric zonation colony character was recorded in Sh isolate. (Table 1)

Table 3. Variability in radial growth of *A. solani* isolates on selected nutrient media

Isolates	Radial growth (mm)		
	A.S.M.	V-8 uice Agar	V-8 uice Agar (Synthetic)
Va	24.33	25.50	26.50
Ro	52.83	47.03	48.51
Mi	44.50	52.50	42.50
Ba	46.50	53.33	41.00
Sh	42.93	43.36	45.70
C.D. at	3.24	2.72	2.48

Variability of *A. solani* on different nutrient media

The variability in radial growth was closely observed on fourteen different nutrient media. The maximum average radial growth (54.74 mm) was recorded on CDA medium followed by JM (50.14 mm). Both media were significantly different to each other. However, JM and PDA (48.5 mm) were significantly at par to each other. CHM (46.5 mm), AHM (45.8mm) and RSA medium (45.3 mm) were significantly at par to each other and statistically belong to third group in supporting maximum mean radial growth of *A. solani*. (Fig. 1 and Plate 1b) Maximum radial growth of *A. solani* on 9th d of incubation was recorded 77.0 mm on CHM. The radial growth varied between 74.6 to 77.0 mm on CDA, JM, RSA, AHM and CHM on 9th day. PDA also supported very good radial growth of 70.7 mm. Lowest growth was recorded on Malt extract agar medium followed by V-8 JM. The slow growth on synthetic V-8 JM may be due to asparagine) Sporulation was not recorded on different nutrient media.

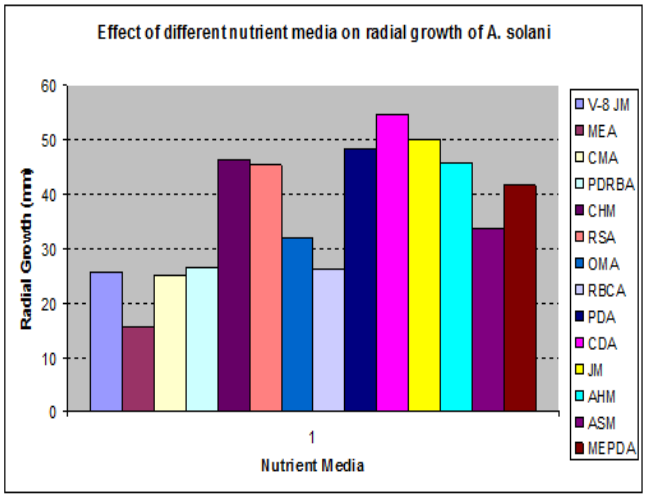

Fig 1. Effect of different nutrient media radial growth of *A. solani*

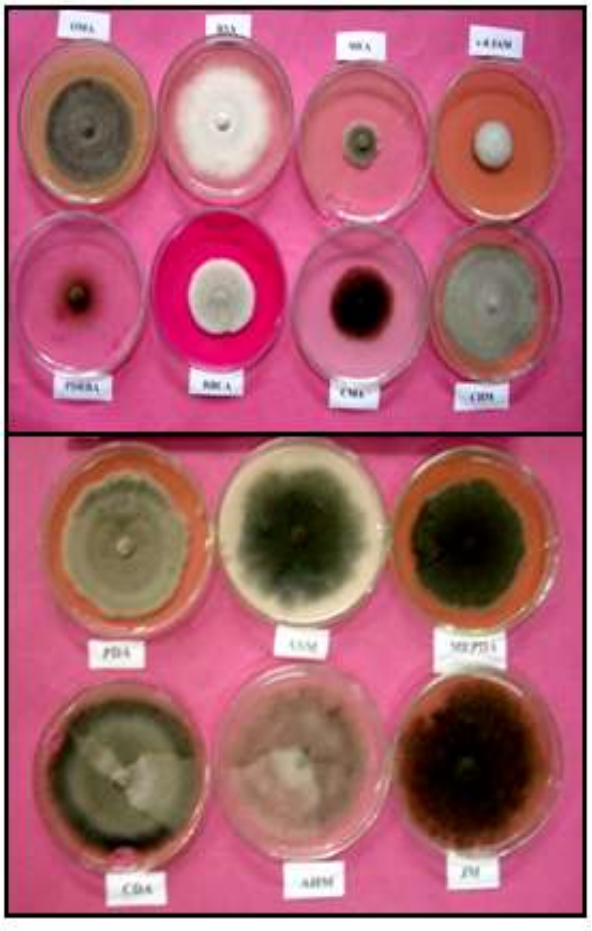

Plate 1b. Growth and Sporulation of *A. solani* on different nutrient media

Variability *A. solani* on different temperatures

Radial growth was found significantly different to each other at different temperatures. The most favorable temperature for growth of the pathogen was 25°C. Maximum average radial (48.65 mm) growth was recorded at 25°C temperatures while minimum average radial growth (25.97 mm) was at 35°C temperatures. (Fig. 2) The radial growth of *A.*

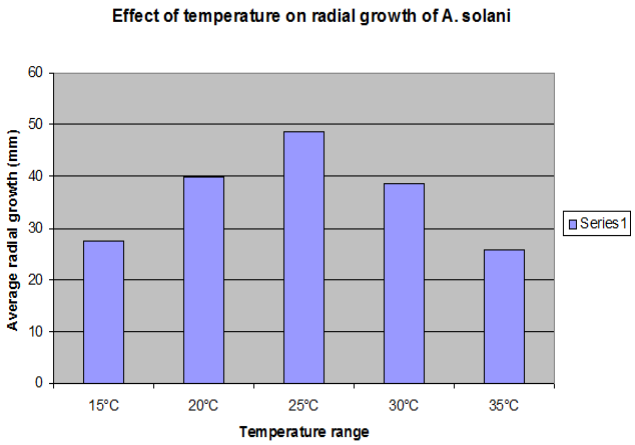

Fig 2. Effect of temperature on radial growth of *A. solani*

solani was maximum (70.0 mm) on 9th d of incubation. As the temperature decreased or increased from 25°C, the drastic

growth reduction was observed. This trend of observation was continued from first observation (3rd day) to seventh observation (9th day). Inability of conidia production at any stage indicating that variable temperature range (15-35°C) cannot induce spore formation. (Fig 2)

Variability of *A. solani* on different pH

Behaviour of growth and colony character of *A. solani* was studied on different pH ranges between pH 4.0-9.5 on PDA at 25±1°C up to 9th days of incubation. Maximum radial growth 83.9 mm was recorded at pH 7.5 on 9th days. Maximum average radial growth was observed at pH 7.5 (52.54 mm) and minimum average radial growth was observed at pH 4.0 (18.67mm). Light acidic and slightly alkaline pH was suitable for the growth of *A. solani*. Apparent acidic (4.0) and alkaline pH (9.5) was reducing radial growth drastically and not Suitable for the growth of *A. solani*. Minimum radial growth was recorded 29.2 mm on 9th day at pH 4.0.

highly significant on the growth of *A. solani*. The best suited pH was 7.5 for the growth of *A. so* where light brown pigment with zonation observed as colony characters. Colony characters varied from whitish gray to dark gray, dark brown with zonations at different pH. Sporulation did not observe under any pH ranges, which indicates that also variation in pH between 4.0 - 9.5 could not induce the sporulation in *A. solani* on PDA. Differences in radial growth were found significantly at different pH. This trend of observation was continued from first observation (3rd day) to seventh observation (9th day).

Sporulation of *A. solani* on host decoction media: Out of three combinations of the host decoction medium, third combination was found very effective for the good sporulation of *A. solani*. However sporulation were found in all set of experiments but in third combination after 20-25 days of inoculation a typical conidia of *A. solani* were observed (Plate 2).

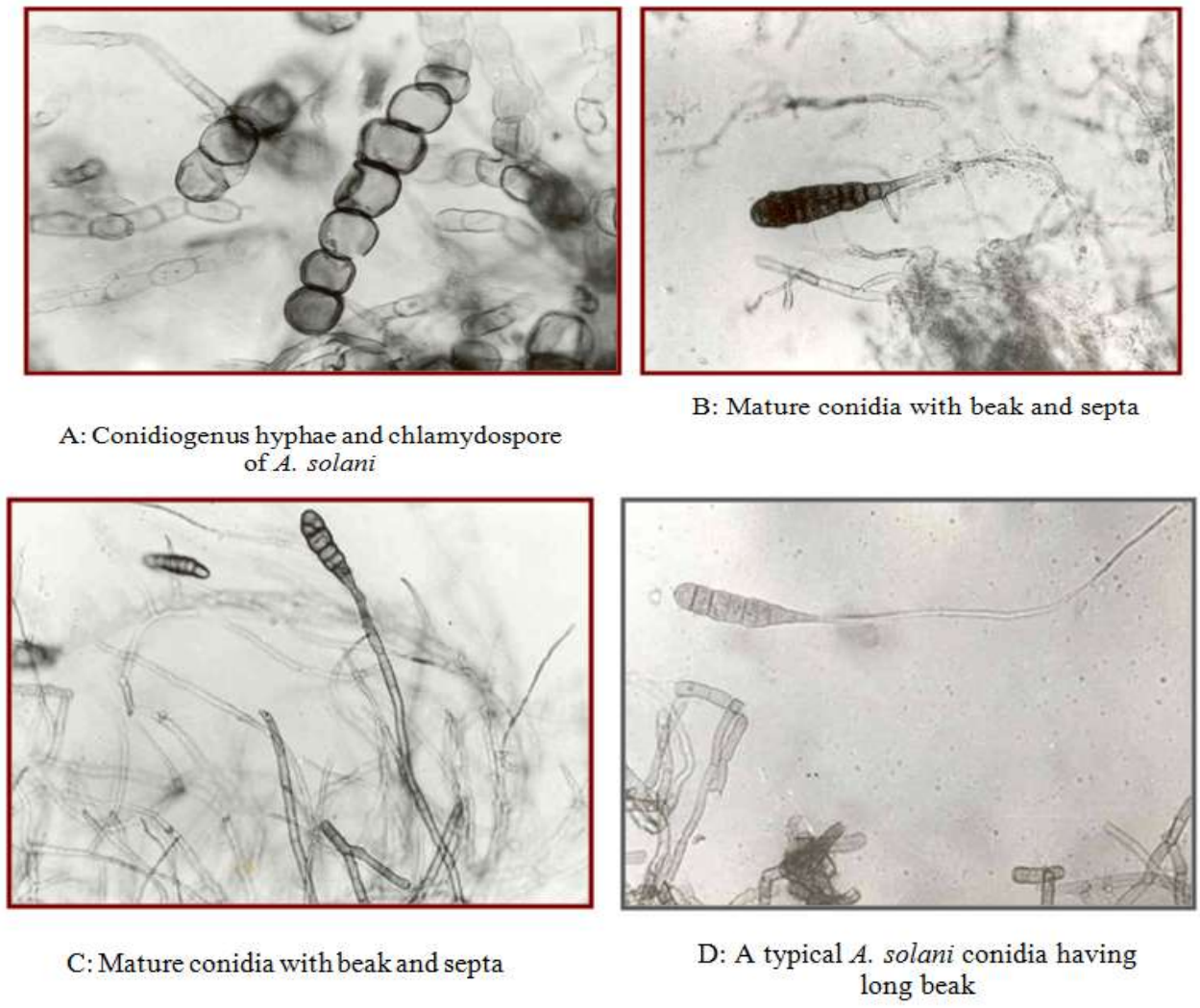

Plate 2. Sporulation in *A. solani* on Host decoction media

Neutral pH was also not promoting growth highly in comparison to pH 6.5 and 7.5. (Fig. 3) It is because of the complete balance of cationic and anionic charged nutrient particle, which does not readily made available to the growing fungi. The gradient charge may be zero at neutral pH hence there may be no movement of any nutrient material in the medium. Effect of period (days) and different pH ranges were

Few conidiogenous hyphae were also produce in this media. (Plate 2A) The total host extracts either tomato juice or leaf extract were pass through bacterial filter under aseptic condition to prevent contamination and maintain heat sensitive vitamin and growth hormone and celite powder was added just for the removing of the colouring matter of tomato juice.

Table 4. Septation of *A. solani* from different parts of host tissue and from host decoction media

	Infected host*			Incubated host*			From culture*		
	Leaf	Stem	Fruit	Leaf	Stem	Fruit	Ist combination	IInd combination	IIIrd combination
No.of horizontal septa	8.8	8.8	10.1	9.1	9.9	11.2	8.0	7.5	8.2
No. of vertical septa	1.0	1.3	2.1	2.4	1.9	2.0	1.3	1.8	1.6
Septa in beak	2.0	2.7	3.1	2.1	2.2	2.2	1.1	1.2	1.2

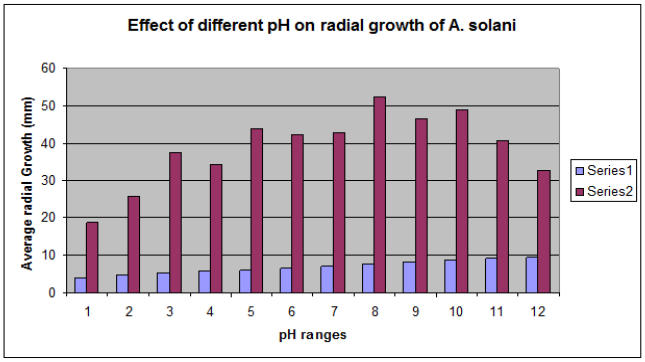

Fig 3. Effect of different pH ranges on radial growth of *A. solani*

Present findings clearly indicate that ripe tomato juice and $CaCO_3$ play very significant role in spore induction of *A. solani* as compared to leaf extract and unripe fruit juice. Shahin and Shepard (1979) developed an efficient technique for inducing profuse sporulation of *A. solani*. They had also used $CaCO_3$ with agar and were found good sporulation of 18-28 hours of inoculation and incubation of Petri plates at 28 ^{O}C.

DISCUSSION

The present findings clearly indicate significant variability in the cultural characters of five isolates of *A. solani*. Sporulation was not recorded on PDA. Similar type of observation was taken by Klimesova and Prasik (1989) in case of *A. alternata*. Even after 9th d of incubation, differences in radial growth rate was found significantly on different nutrient media. CHM, RSA and AHM were almost similar for the periodical radial growth of the pathogen. PDA and JM were also similar in growth rate but the longer period of incubation, increases the intensity of conidiogenous hyphae formation. Variations in colony characters of the pathogen were also recorded on same fourteen nutrient media on 9th d. Colony of *A. solani* was variable on these media. Blackish brown colony was recorded on V-8 JM, ASM and MEPDA. Whitish brown colony was on RSA, RBCA, AHM while light gray colony was observed on rest of the media. The velvety black colony was found in CMA, PDRBA, OMA.

Pigmentation on these media varied from brown yellowish to black. However, the concentric zonation was observed in some of the media from upper and lower sides. No spore production was observed in any of the above medium. However, only cultural variability was recorded among the different nutrient media. It is clear from the present findings that sporulation could not induced on the above tested media upto 9th d of incubation at constant temperature of 25±°1C. Our findings corroborate the observation of Rath and Padhi (1973) where they reported that best growth was at 20-25°C temperature. Gupta and Nikhraj (1972) reported that best temperature for sporulation of *A. solani* was 22.5°C at pH 6 in light conditions whereas in dark condition the sporulation was reported at pH 7.0 on the same temperature. In present experiment, differences in radial growth, colony characters and pigmentation of *A. solani* were recorded at different temperatures on PDA after nine days of incubation. The colony character appeared as light gray and pigmentation was recorded as light yellow at 35°C. At 25°C colonies color was gray with light brown pigment. Colony character varied from dark gray to light gray and pigmentation on reverse side of culture plate was brown, black, oily yellow and greenish brown. Gupta and Nikhraj (1972) reported that best pH for the growth and sporulation of early blight pathogen of potato was 7.0.

The pathogen of their study may be different in nature from our pathogen because they had mentioned about sporulation and spore germination, which could not prove after several efforts in *A. solani*. Septation of a conidium was found to be greater in the incubated host as compared to natural host and culture. Maximum number of septation was found in infected tomato fruit tissue in both conditions viz. incubated, infected host and followed by spore from culture plates. The numbers of horizontal septa were recorded 4-11, vertical septa 1-4 and septa in beak recorded 1-5 in a conidium while from culture plates the number of horizontal septa 5-8, vertical septa1-2 and septa in beak 0-1 in a conidium and along beak in a conidium were also observed. (Table 4) It means septation of a conidium is reducing in culture medium as compare to natural host. Pandey and Vishwakarma (1999) also studied the pathogenic isolates of *A. alternata* reduced in its conidium and beak size from natural host of culture medium; therefore it is proving the results.

In present findings a well developed and maximum no. of septa recorded in tomato fruit which was incubated as 25±1°C because host nutrition is very important for size of spore. Our findings are similar to the observations of Ahmed (2002) he had reported that the no. of horizontal septa was 5-11 in the tomato leaf. In tomato fruit largest conidia observed in size and shape, because it has sufficient nutritional requirement for growth and development of the pathogen. Several workers Prasad *et al* (1973), Lukens and Horsfall (1973) Madan and Thind (1979), Stevenson and Pennypacker (1988), Ghosh and Gemawat (1979), Zhu *et al* (1985), Kvasnyuk (1985), Vakalounakis (1982) and Bernal *et al* (2002) have reported for sporulation by using different artificial media and other treatment. Prasad *et al* (1973) reported that sporulation was induced when fully grown *A. solani* cultures was dipped in or sprayed with sterilized distilled water, and kept partially covered at different temperatures. Cold water dips (4°C, 4 Min) or sprays at 4°C or 28°C followed by incubation at room temperature (13-26°C) in diffuse sunlight produced the spores within 0 hour. The cultures yielded a number of subsequent spore crops when scraped, and dipped after each conidial harvest.

Madan, and Thind (1979) had seen the role of trace elements on growth and sporulation of *A. solani*. Out of 20 trace elements tested Ca was determined for good sporulation in *A. solani*. Rodriguez and Santana (1991) reported sporulation on Yucca Glucose Agar, Malanga Glucose Agar and Sweet Potato Glucose Agar. Some researchers such as Kumagai and Oda (1969), Kaoru and Mitsuo(1970), Prasad and Dutt (1974), Singh (1967), Fourtouni *et al* (1998), Lukens (1962), Douglas and Pavek (1971) and Cotty (1987) have done work on effect of light for sporulation of *A. solani* and also its other species as *A. tegetica*, *A. alternate*, and *A. kikuchiana.* Kumagai and Oda (1969) found sporulation in *A. solani* only on exposure to irradiation by U. V. light followed by a period of darkness while Kaoru amd Mitsuo (1970) reported that effect of continuos white fluorescent light (340 mμ and 365m μ) exposed with short distance on culture plates of *A. solani* increased the spore production on three selective nutrient media i.e. V-8 juice medium, pear leaf juice medium and dry apricot V-8 juice medium. We have also studied sporulation on V-8 juice medium but only conidiogenous hyphae were found on both PDA and V-8 juice medium. Therefore the present work is completely different from earlier workers. Our finding shows that sporulation was not found on PDA, Alternaria sporulation medium, V-8 juice agar and v-8 juice agar (synthetic) without any light treatments. But successfully sporulation is induced in host decoction media. These host decoction combinations for conidial formation of *A. solani* was observed suitable and further standardization of temperature and pH and different carbon and calcium sources required getting

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