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Effect of Environmental Conditions on Growth of *Alternaria alternata* Causing Leaf Blight of Noni

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Abstract: Effect of different pH levels, temperature, light intensity and media were tested against the growth of *A. alternata* under *in vitro* conditions. The results of experiment indicated that the growth of *A. alternata* was maximum in pH range of 6.00- 6.50 and temperature range of 25 - 30°C. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of *A. alternata* compared to continuous light and dark. Among the different media tested, host leaf extract medium supported significantly the maximum growth of all the fifteen isolates of *A. alternata* followed by potato dextrose agar (PDA). Further, the cultural charecters *viz*., colony and substrate colour, margin, topography, zonation, pigmentation, colony diameter (mm) and sporulation varied among the isolates.

Key words: *Alternaria alternata* · Leaf blight · Noni · Cultural characters

INTRODUCTION

Noni (*Morinda citrifolia* L.) is a small fruit-bearing, evergreen shrub, well known for its medicinal properties. The leaves, flowers, bark and roots on noni plants are being used for medicinal purposes [1, 2]. The extracts of noni fruits can be used to cure bacterial, viral, parasitic and fungal infections by stimulating the immune system. It also prevents malignant tumors [1]. The crop is being debilitated by an array of opportunistic pathogens. Among all, *A. alternata* which causes leaf blight disease is a major limiting factor in production, leading to heavy losses [3]. An understanding of the role of environmental conditions and its effect on infection and survival of the pathogen is necessary to develop cultural disease management practices. Therefore the objectives of this study included isolation, purification and identification of pathogenic fungus causing leaf blight disease of noni and determine optimal conditions for the mycelial growth of the fungus including pH, temperature, light and type of media.

MATERIALS AND METHODS

Isolation, Purification and Identification of Pathogen: The pathogen was isolated by tissue segment method [4] on PDA medium. Noni leaves showing characteristic of leaf blight symptom were cut into small pieces of 1.0 to 1.5 cm, surface sterilized with 0.1 per cent mercuric chloride for one min and washed in sterile distilled water thrice and blot dried with sterilized filter paper. The sterilized leaf bits were placed in Petri plates containing sterilized PDA medium. The plates were incubated at 28±2°C for four days and observed for the fungal growth. The fungus was purified by single spore isolation technique [5] and the purified isolates were maintained on PDA slants for further studies. Total of fifteen isolates were isolated from different growing areas.

The pathogen was identified up to species level based on their cultural and morphological characters. The identification was further confirmed from Indian Type Culture Collection and Identification, Culture Supply Services, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India.

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Pathogenicity Test: The pathogenicity of all fifteen purified isolates of *A. alternata* was confirmed by Koch's postulates. Two months old noni seedlings were maintained in glass house in mud pots at 28 ± 2°C and at 80 per cent Relative Humidity (RH). The potting mixture consist, two parts of laterite soil, one part of sand and one part of well decomposed farmyard manure. Three replications were maintained for each isolates as well as control treatment. The conidial suspension (5×10^5 spores ml^{-1}) was prepared in phosphate buffer (pH-7) from nine day old PDA culture of the different isolates. The spore suspensions were sprayed on to the noni seedlings while water was used for spraying the control treatment. The inoculation was done on cool evening hours. The severe symptoms were observed on inoculated plants. The symptoms were observed and compared with the original symptoms. The fungus was re-isolated from artificially inoculated noni leaves and compared with original pathogen.

Environmental Factors Affecting to the Growth of Isolates: The effect of pH, incubation temperature, light regime and type of culture media on the mycelial growth of *A. alternata in vitro* in agar plates was studied.

Effect of Hydrogen Ion Concentration (pH): The effect of pH on the growth of the pathogen was studied followed the method described by Kiryu [6] using PDA medium. Potato dextrose broth was prepared in 250 ml Erlenmeyer flask, each containing 100 ml broth. The pH of the broth was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 with a help of digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. The required quantity of agar was added to each flask and sterilized. The sterilized media of different pH levels were poured in the sterilized Petri plates in about 20 ml quantities and allowed to solidify. Nine mm discs from the actively growing ten day old cultures of different isolates were placed on the centre of the Petri plates. The plates were incubated at 28 ± 2°C for 10 days then the mycelial growth diameter was measured. Three replications were maintained for each treatment.

Effect of Temperature: Petri plates containing 20 ml of PDA medium were inoculated with nine mm mycelial disc from ten day old culture of different isolates. The inoculated plates were incubated at different temperature: 5, 10, 15, 20, 25, 30 and 35°C. The colony diameter was measured ten days after inoculation. Three replications were maintained for each treatment.

Effect of Light Regime: The pathogen cultures of different isolates on PDA was exposed to continuous light, dark and 12 h light and 12 h dark in an environment chamber maintained at 30°C. Mycelial disc of nine mm of each isolate was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelial growth was measured in each case ten days after inoculation.

Effect of Culture Media: Petri plates containing 20 ml of each of following media, PDA, oatmeal agar, Czapek Dox, Richards, Walks manns agar medium, water agar, Martins Rose Bengal Agar media and noni leaf extract agar were inoculated with nine mm diameter disc from ten day old cultures of different isolates. The inoculated plates were kept at 30°C. Three replications were maintained for each media. Colony diameter was measured ten day after inoculation. The different colony characters like pigmentation, sporulation zonation etc were recorded in PDA medium by visual observation.

Statistical Analysis: All the experiments were repeated once with similar results. The data were statistically analyzed [7] and the treatment means were compared by Dunkan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92-a developed by International Rice Research Institute Biometrics Units, The Philippines.

RESULTS AND DISCUSSION

Isolation, Purification and Identification of Pathogen: The process of isolation resulted in fifteen isolates of pathogen collected from different regions. All the isolates were confirmed by morphological and cultural characters as isolates of *A. alternata.*

Pathogenicity Test: Initially, eight days after inoculation dark circular brown spots appeared on the leaves which gradually coalesced to form large spots leading to blightening of leaves. Re-isolation trails revealed that the isolated fungi from diseased seedlings are found to be identical with those used for artificial inoculation. This is the first report on noni leaf blight caused by *A. alternata*.

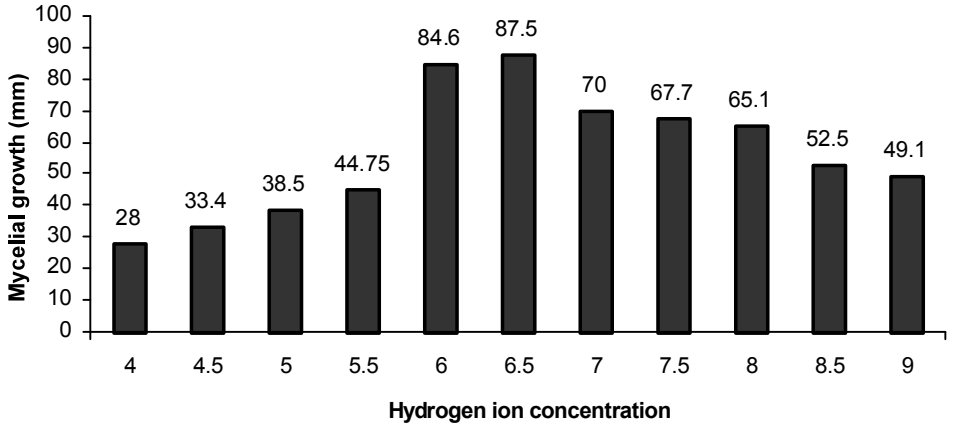

Fig. 1: Effect of hydrogen ion concentration (pH) on mycelial growth of *A. alternata*

Effect of Hydrogen Ion Concentration (pH) on the Mycelial Growth: Fungi generally utilize substrates in the form of solution only if the reaction of solution conducive to fungal growth and metabolism Kiryu [6]. This brings importance of hydrogen ion concentration for better fungal growth. Of all the eleven pH levels, pH 6.5 was found to be ideal and produced the maximum mean mycelial growth of 87.50 mm followed by pH 6.0 (84.60 mm) and pH 7.0 (70.00 mm). The mean mycelial growth was lowest at pH 4.0 which recorded 28.00 mm (Fig. 1). The pH below six and above seven was noticed to be inhibitory to the growth. The results of experiment indicated that *A. alternata* prefers pH range of 6.00- 6.50. This showed that the fungus prefers acidic pH for the growth. Cochrane [8] and Bilgrami and Verma [9] opined that in contrast to bacteria and actinomycetes, fungi are relatively tolerant to acidic ion than basic ions. The results obtained in the present study are in accordance with the results of Arunkumar (10). Gemawat and Ghosh [11] reported that pH 6.3 was best for the growth of *A. solani* and Samuel and Govind swami [12] observed that pH six was better for *A. carthami.* The inhibitory action of pH above 6.5 and below six was attributed to the unconducive reaction of the media.

Effect of Different Temperature on the Mycelial Growth: Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. All the fifteen isolates grew well at temperature of 30°C (89.45 mm) followed by 25°C (86.11 mm) and 35°C (70.27 mm). The least growth was observed at 5°C (9.70 mm) (Table1). From the study, it is clear that temperature ranging from 25 - 30°C is better for the growth *of A. alternata*. The results are supported by Martin and Fernandez [13] and Garibaldi *et al*. [14] who reported that 27°C was the optimum temperature for the growth of *A. alternata*.

Effect of Light Intensity on the Mycelial Growth: Light has profound effect on the mycelial growth of *A. alternata*. The exposure of the fungus to alternate cycles of 12 h light and 12 h darkness for 10 days resulted in the maximum mycelial growth of *A. alternata* (87.73 mm) which was significantly superior over other two treatments tested (Table 2). The mycelial growth of fungus exposed to continuous light resulted in 37.10 mm and continuous darkness resulted in 22.72 mm growth. The study agreed well with results of Arunkumar [10] that alternate cycles of 12 h light and 12 h darkness resulted in maximum growth of *A. solani*.

Effect of Culture Media: Every living being requires food for its growth and reproduction and fungi are not an exception to it [6]. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life process. Neither all media are equally good for all fungi nor there can a universal substrates or artificial medium on which all fungi grow well. So different media including synthetic and non synthetic were tried for the growth of *A. alternata*.

Among the different medium tested, host leaf extract medium supported significantly the maximum growth of all the fifteen isolates of *A. alternata* with mean mycelial

Table 1: Effect of different temperatures on the mycelial growth of *A. alternata* isolates

	Colony diameter (mm) at different temperature *						
Isolate	5˚C	10˚C	15˚C	20˚C	25˚C	30˚C	35˚C
AA1	10.11^{g}	18.00^{f}	42.00^{e}	62.50^{d}	85.15^{b}	90.00^{a}	68.53^{c}
AA2	08.30^{f}	19.50^{e}	38.20^{d}	54.50^{c}	88.25^{a}	90.00^{a}	64.34^{b}
AA3	11.00^{f}	23.45^{e}	46.00^{d}	61.00^{c}	88.90^{a}	90.00^{a}	67.48^{b}
AA4	09.20^{f}	18.30^{e}	43.50^{d}	60.00^{c}	90.21^{a}	89.50^{a}	72.41^{b}
AA5	11.85^{f}	19.65^{e}	44.00^{d}	60.25^{c}	88.00^{a}	89.00^{a}	69.40^{b}
AA6	10.00^{f}	20.65^{e}	48.50^{d}	62.00^{c}	88.37^{a}	88.00^{a}	72.54^{b}
AA7	8.30^{f}	18.60^{e}	48.00^{d}	76.30^{b}	83.00^{a}	90.00^{a}	68.00^{c}
AA8	10.50^{e}	19.85^{d}	49.00^{c}	68.30^{b}	88.50^{a}	90.00^{a}	69.00^{b}
AA9	09.25^{g}	19.00^{f}	48.00^{e}	75.80^{d}	80.40^{b}	90.00^{a}	72.10^{c}
AA10	10.25^{g}	18.75^{f}	47.90^{e}	70.00^{c}	81.00^{b}	89.00^{a}	63.14^{d}
AA11	11.00^{g}	21.00^{f}	46.75^{e}	64.41^{d}	80.25^{b}	89.75^{a}	76.41^{c}
AA12	08.75^{f}	17.50^{e}	48.90^{d}	61.73^{c}	88.37^{a}	90.00^{a}	72.67^{b}
AA13	09.25^{g}	18.24^{f}	47.50^{e}	70.50^{c}	83.00^{b}	88.75^{a}	68.50^{d}
AA14	08.75^{f}	18.50^{e}	49.00^{d}	63.25^{c}	89.25^{a}	87.75^{a}	71.30^{b}
AA15	09.00^{f}	17.00^{e}	48.00^{d}	68.20^{c}	89.00^{a}	90.00^{a}	78.30^{b}
Mean	9.70	19.20	46.35	65.25	86.11	89.45	70.27

*Mean of three replications

In a row, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 2: Effect of light intensities on mycelial growth of *A. alternata* isolates

	Mycelial growth (mm)*		
Isolate	Light	Dark	12 h alternate dark and light
AA1	35.40^{c}	24.00^{b}	89.80^{a}
AA2	36.25^{c}	23.00^{b}	88.00^{a}
AA3	35.00^{c}	24.99^{b}	84.20^{a}
AA4	38.21^{c}	23.41^{b}	86.02^{a}
AA5	37.21^{c}	23.00^{b}	88.50^{a}
AA6	38.64^{c}	28.00^{b}	88.75^{a}
AA7	37.25^{c}	23.82^{b}	88.12^{a}
AA8	34.21^{c}	22.00^{b}	84.50^{a}
AA9	35.68^{c}	21.50^{b}	89.54^{a}
AA10	38.26^{c}	19.85^{b}	87.24^{a}
AA11	37.98^{c}	19.23^{b}	88.31^{a}
AA12	38.17^{c}	21.56^{b}	88.46^{a}
AA13	36.74^{c}	20.47^{b}	88.79^{a}
AA14	39.45^{c}	23.00^{b}	86.34^{a}
AA15	38.00^{c}	23.00^{b}	89.41^{a}
Mean	37.10	22.72	87.73

*Mean of three replications

In a row, means followed by a common letter are not significantly different at the 5% level by DMRT

growth of 89.80 mm followed by PDA (89.34 mm) and oatmeal agar (83.72 mm) and Walksman's medium (80.23 mm) (Table 3). Least mean mycelial growth was observed in water agar (9.84 mm). The results are on par with the result obtained in an experiment conducted by Ramjagathesh [15] where in among ten media tested for the growth of *A. alternata* causing leaf blight of onion, host leaf extract agar medium (9.0 cm) followed by PDA (8.24 cm) and the lowest growth was recorded in water agar medium (0.92 cm). PDA was the best medium for

Table 3: Effect of different media on mycelial growth of *A. alternata* isolates

	Mycelial diameter (mm)							
Isolate	Water agar	Richards	Oatmeal Agar	PDA	MRB	Czapek Dox	Host leaf extract	Walksman's
AA1	08.20^{f}	79.00^{b}	90.00^{a}	90.00^{a}	21.00^{de}	20.00^{de}	90.00^{a}	84.05^{a}
AA2	08.80^{def}	78.88^{b}	88.00^{b}	89.50^{b}	19.85^{ef}	21.00^{bcde}	89.50^{a}	80.05^{b}
AA3	09.20^{def}	76.20^{c}	84.20^{de}	90.00^{a}	20.00^{ef}	23.00^{a}	90.00^{a}	79.50^{bc}
AA4	11.70^{ab}	81.20^{a}	86.02^{cd}	90.00^{a}	23.00	21.50^{abcd}	90.00^{a}	80.00^{b}
AA5	10.00^{cde}	81.50^{acd}	81.00^{hij}	88.95^{c}	21.00^{de}	21.00^{bcde}	89.00^{a}	78.05^{c}
AA6	08.80^{def}	76.02^{cd}	84.00^{efg}	89.00^{c}	23.00^{bc}	21.00^{bcde}	90.00^{a}	80.01^{b}
AA7	10.23^{bcd}	75.00^{e}	86.00^{c}	90.00^{a}	23.80^{bc}	21.65^{abcd}	90.00^{a}	84.50^{a}
AA8	08.50^{ef}	74.65^{cd}	79.00^{ij}	90.00^{a}	19.65^{f}	22.65^{ab}	90.00^{a}	79.54^{a}
AA9	11.20^{abc}	72.35^{e}	80.05^{j}	88.50^{e}	19.00^{f}	22.60^{ab}	90.00^{a}	78.02^{bc}
AA10	08.80^{def}	73.65^{de}	82.00^{ghi}	88.75^{d}	23.02^{abc}	19.85^{cde}	90.00^{a}	80.10^{c}
AA11	09.20^{def}	69.85^{f}	83.85^{def}	89.55^{b}	21.00^{de}	21.00^{bcde}	89.00^{a}	84.36^{ab}
AA12	11.70^{ab}	78.88^{b}	84.20^{de}	88.67^{d}	22.0^{bcd}	21.00^{bcde}	90.00^{a}	78.02^{c}
AA13	09.36^{def}	76.20^{cd}	81.20^{fhj}	87.61^{f}	23.50^{ab}	19.85^{e}	90.00^{a}	79.25^{bc}
AA14	10.00^{cde}	81.20^{a}	84.00^{de}	89.50^{b}	21.00^{e}	20.00^{de}	90.00^{a}	80.00^{b}
AA15	11.90^{a}	81.50^{a}	82.35^{fgh}	90.00^{a}	21.45^{cde}	22.00^{abc}	89.50^{a}	78.05^{c}
Mean	09.84	77.11	83.72	90.00	21.48	21.21	89.80	80.23

• Mean of three replications

• In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 4: Cultural characters of *A. alternata* isolates in different media

	Colony characters							
Isolate	Water agar	Richards Agar	Oatmeal Agar	PDA	MRB	Czapek Dox	Host leaf extract	Walksman's
AA1	Black	White	Dark brown	Dark brown	White	Black	Brown	White
AA2	Black	White	Whitish brown	Blackish white	Dark white	Black	Brown	Dark white
AA3	Black	White	Brownish white	Dark brown	Reddish White	Black	Brownish white	White
AA4	Black	White	Brownish white	Dark brown	Reddish White	Black	Brown	Greyish white
AA5	Black	White	Brownish white	Brownish black	White	Black	Brown	White
AA6	Black	White	Brownish black	Dark brown	White	Black	Brown	White
AA7	Black	White	Dark brown	Dark brown	White	Black	Brown	White
AA8	Black	White	Brown	Blackish white	Reddish White	Black	Brown	Dark white
AA9	Black	White	Dark brown	Dark brown	Reddish White	Black	Brownish white	White
AA10	Black	White	Whitish brown	Dark brown	Dark white	Black	Brown	Greyish white
AA11	Black	White	Brownish white	Brownish black	Reddish White	Black	Brown	White
AA12	Black	White	Brownish black	Dark brown	Reddish White	Black	Brown	White
AA13	Black	White	Brownish white	Dark brown	White	Black	Brown	Greyish white
AA14	Black	White	Brownish white	Brownish black	White	Black	Brown	White
AA15	Black	White	Whitish brown	Dark brown	White	Black	Brownish white	White

the growth of *A. carthami* inciting leaf blight of safflower [16], *A. solani* causing leaf blight of tomato [17], *A. alternata* causing leaf blight of tobacco and jasmine [18], *A. palandui* causing leaf blight of onion [19]. Prasad [20] reported that out of four non-synthetic media and three synthetic media tested for the growth of *A. solani*, PDA supplemented with the $CaCO_3$ and Sabourd's agar was found to be best media.

Besides variation in growth rate, the isolates of *A. alternata* exhibited variation in respect of colony colour also. The isolate AA1 produced black coloured colonies on water agar and Czapek Dox agar, white coloured colonies on Richard's agar, Martins Rose Bengal agar (MRB) and Walksman's agar. In case of PDA and oat meal agar, dark brown colonies and brown coloured colonies on host leaf extract agar. The other isolates also showed variations in colony colour on different media and the details are furnished in Table 4. The results obtained in the present study agreed very well with the findings of Anand *et al.* [21] where, he has noticed variations in the colony colours of *A. alternata* in different media.

Table 5: Cultural characters of *A. alternata* isolates in PDA medium

Isolate	Colony colour	Substrate colour	Margin	Topography	Zonation	Pigmentation	*Colony diameter (mm)	Sporulation
AA1	Dark brown	Light brown	Irregular	Raised fluffy growth	Without Zonation	Black	90.00 [a]	+++
AA2	Blackish white	Greyish	Smooth	Flat mycelial growth	Without Zonation	Yellow	89.50 [b]	++
AA3	Dark brown	Brown	Smooth	Medium flat growth	Without Zonation	Brownish black	90.00 [a]	++
AA4	Dark brown	Light greyish	Smooth	Medium raised	Concentric Zonation	Black	90.00 [a]	+
AA5	Brownish black	Light grayish	Irregular	Raised fluffy growth	Concentric Zonation	Brown	88.95 [c]	+++
AA6	Dark brown	Greyish	Smooth	Flat mycelial growth	Concentric Zonation	Greenish black	89.00 [c]	+
AA7	Dark brown	Light brown	Smooth	Medium fluffy growth	Concentric Zonation	Black	90.00 [a]	++
AA8	Blackish white	Greyish	Smooth	Flat mycelial growth	Concentric Zonation	Black	90.00 [a]	++
AA9	Dark brown	Light brown	Smooth	Medium flat growth	Concentric Zonation	Black	88.50 [e]	+++
AA10	Dark brown	Light greyish	Smooth	Medium raised	Concentric Zonation	Yellow	88.75 [d]	+
AA11	Brownish black	Brown	Irregular	Raised fluffy growth	Concentric Zonation	Brownish black	89.55 [b]	++
AA12	Dark brown	Light greyish	Smooth	Flat mycelial growth	Without Zonation	Black	88.67 [d]	++
AA13	Dark brown	Light greyish	Smooth	Raised fluffy growth	Without Zonation	Brown	87.61 [f]	++
AA14	Brownish black	Greyish	Smooth	Flat mycelial growth	Without Zonation	Greenish black	89.50 [b]	+++
AA15	Dark brown	Brown	Irregular	Medium flat growth	Without Zonation	Black	90.00 [a]	+++

+ Poor sporulation: 1-10 spore / microscopic field (100X)

++ Medium sporulation: 11-50 spores/ microscopic field (100X)

+++ Good sporulation: More than 100 spores/ microscopic field (100X)

*Mean of three replications, In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Colony Characters on PDA: The results revealed that there is a considerable variation among the colony characters of the isolates collected from different locations (Table 5). Most of the isolates (AA1, AA3, AA4, AA6, AA7, AA9, AA10, AA12, AA13 and AA15) had dark brown coloured colonies whereas two isolate (AA2 and AA8) produced blackish white coloured colonies. Isolate AA5, AA11 and AA14 produced brownish black coloured colonies. Many of the isolates produced smooth margin whereas, isolates AA1, AA5, AA11and AA15 produced irregular margin.

There was variation with respect to topography of the colony. Four isolates (AA1, AA5, AA11 and AA13) had raised fluffy growth while, two isolates (AA4 and AA10) had medium raised growth. Five isolates (AA2, AA6, AA8, AA12 and AA14) produced flat mycelial growth whereas, three isolates (AA3, AA9 and AA15) grown with medium flat mycelium. One isolate AA7 had medium fluffy growth.

Seven isolates (AA1, AA2, AA3, AA12, AA13, AA14 and AA15) were without concentric rings in the colony. On the contrary eight isolates (AA4, AA5, AA6, AA7, AA8, AA9, AA10 and AA11) had concentric rings in the colony growth.

There was a considerable diversity with respect to pigmentation of isolates. Isolate AA1, AA4, AA7, AA8, AA9, AA12 and AA15 produced black pigment while two isolates (AA2 and AA10) had yellow pigment. Isolates AA5 and AA13 produced brown coloured pigment whereas two isolates (AA3 and AA11) occurred with brownish black pigmentation. Isolates AA6, AA14 and AA5 were having greenish black and brown pigmentation, respectively.

Highest colony diameter of 90.00 mm was observed in isolates AA1, AA3, AA4, AA7, AA8 and AA15 while the lowest colony growth of 87.61mm was recorded in AA13.

All the isolates had sporulation on PDA. Good sporulation was recorded in five isolates (AA1, AA5, AA9, AA14 and AA15); while moderate sporulation was observed in seven isolates (AA2, AA3, AA7, AA8, AA11, AA12 and AA13) and poor sporulation was recorded in three isolates (AA4, AA6 and AA10).

The results were on par with the findings of Arunkumar [10] where in variation in colony characters of *A. solani* on PDA was reported. The variation in the colony characters of the isolates was attributed to the geographical variation from where isolates were collected and also virulence of the isolates. Several workers notably

Kaul and Saxena [22] observed differences in cultural characters like growth rate, type of growth, colony colour of the substrate and sporulation of the isolates.

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