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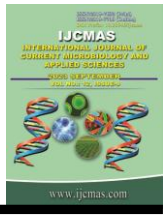

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Cultural and Physiological Characteristics of *Alternaria alternata*

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A B S T R A C T

Tobacco is one of the major important commercial crops grown throughout the country. Brown spot caused by *Alternaria alternata* is one of the major destructive disease causes heavy losses under field condition. Studies on cultural and physiological features of the pathogen are of immense use in understanding the nature of the pathogen. The radial growth of *A. alternata* was maximum on potato dextrose agar with colony diameter of 89.00 mm followed by Czapek's dox agar (87.00 mm) colour of the mycelium varied from whitish to dark grey. Physiological studies revealed that growth of the pathogen was maximum at temperature of 25°C (329.13 mg) and lowest at 5°C (67.16 mg), while the maximum dry weight was observed at pH 6.5 (586.53 mg).

Introduction

Tobacco (*Nicotiana tabacum* L.), belongs to solanaceae family is believed to have been introduced into India from its native Central America by Portuguese in 1603. In India tobacco is one of the important cash crops and it is cultivated in an area of about 4.93 lakh hectares (0.24 %) of total arable land in the country covering different types of tobacco *viz.*, cigarette tobacco, bidi tobacco, chewing tobacco, hookah tobacco, cheroot tobacco, cigar wrapper tobacco, cigar filler tobacco, oriental tobacco, dark fire cured tobacco, *etc.*, with a production of 800 M Kg. Tobacco is a source of medicine, edible protein oil, pesticide and organic acids. Tobacco crop is affected by many diseases among them brown spot disease incited by the fungus *Alternaria alternata (*Fries) Keissler is most destructive foliar diseases of flue cured tobacco (Anon., 1993). In this regard an experiment was conducted to know the Cultural and physiological characteristics of *Alternaria alternata*

Materials and Methods

The growth characters of the *A. alternata* were studied on ten different solid media 20 ml of each of the sterilized medium was poured into 90 mm diameter Petri dishes. Such plates were inoculated with 5 mm discs of 12 days old actively growing *A.*

alternata was placed at the centre of Petri dishes containing different media. Each treatment was replicated thrice. Colony diameter was recorded by averaging the linear growth of colony in two directions for each plate. Colony colour, substrate colour, margin of colony and topography of mycelium were also noted. The data on radial growth was analysed statistically.

The composition and preparation of the mentioned media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Hawksworth *et al.,* (1983). The cultural characters for the pathogen were studied on the following solid media *viz.,* Potato Dextrose Agar, Richard's Agar, Czapek's Dox Agar, Glucose Peptone Agar, Corn Meal Agar, Oat Meal Agar, Malt extract Agar, Sabouraud's Dextrose Agar, Host leaf extract Agar and Host leaf dextrose Agar.

Effect of temperature on growth of *A. alternata*

Potato dextrose broth was used as a basal medium to study the effect of different temperature on the growth of *A. alternata* 30 ml of the medium was added to 100 ml conical flasks and then the flasks were sterilized. Five mm discs taken from 10 days old culture of *A. alternata* were inoculated and incubated at different temperature *viz.*, 5, 10, 15, 20, 25, 30, 35 and 40°C for 12 days. Three replications were maintained for each treatment. The dry mycelial weight was recorded and analysed statistically.

Net dry weight of mycelium will be calculated as follows:

Net weight of mycelium = (Wt. of mycelium + filter paper) - (Wt. of filter paper)

Effect of pH on the growth of *A. alternata*

Potato dextrose broth was used as a basal medium to study the effect of pH on the growth of *A. alternata*. The pH of the medium was adjusted to various levels *viz.*, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 by adding 0.1 N sodium hydroxide and 0.1 N hydrochloric acid and it was determined by electronic pH meter. Thirty ml of the medium with known pH was added to 100 ml conical flasks and then the flasks were sterilized 5 mm discs taken from 12 days old culture of *A. alternata* were inoculated and incubated at 25 ± 1°C for 12 days. Three replications were maintained for each treatment. The dry mycelial weight was recorded and analyzed statistically.

Results and Discussion

Growth of *A. alternata* on different solid media

Every living being requires food for its growth and reproduction and fungi are not an exception. Fungi derive the food from the substrate upon which they grow. To culture fungi artificially, the essential nutrients needed for the growth and development are necessary to supplement in the medium. So, in order to find out the best sources of nutrients for the fungus in study, different synthetic, semi-synthetic and non-synthetic media were tested. The radial growth of the fungus was used to determine growth on solid media.

A. alternata isolated from tobacco leaves was grown on ten different media. Among those maximum radial growth of *A. alternata* was observed on potato dextrose agar media (89.00 mm) followed by Czapek's dox agar (87.00 mm), Host leaf extract with dextrose (86.00 mm) and Host leaf extract agar (84.00 mm). Similar results were obtained by Hubballi *et al.,* (2010) in which host leaf extract medium supported significantly more and maximum growth of all the 15 isolates of *A. alternata* followed by potato dextrose agar (PDA). Ramjegathesh and Ebenzer (2012) found maximum growth of *A. alternata* on host leaf extract agar media followed by Czapek's dox agar and potato dextrose agar media. Mesta (2006) and Manjunath *et al.,* (2010) reported that 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).

Table.1 Cultural and morphological characteristics of *A. alternata* on different solid media

Sl. No	Media	Radial growth (mm)	Colony characters			
			Colour of colony	Substrate colour	Margin of colony	Topography of mycelium
1	Richard's Agar	83.00	Light grey	Light greyish	Regular margin	Raised fluffy growth
2	Potato Dextrose Agar	89.00	Whitish grey	Light brownish	Regular margin	Raised fluffy growth
3	Czapek'sDox Agar	87.00	Whitish grey	Light colour	Irregular margin	Medium raised
4	Glucose Peptone Agar	59.00	Dark grey	Light greyish	Regular margin	Raised fluffy growth
5	Malt extract Agar	81.00	Black colour	Greyish	Regular margin	Medium raised
6	Sabourad's agar	77.28	Cream to grey	Yellowish	Regular margin	Flat mycelial
7	Host leaf extract Agar	84.00	Dark grey	Dark greyish	Regular margin	Raised fluffy growth
8	Host leaf extract with dextrose	86.00	Dark grey	Dark greyish	Regular margin	Raised fluffy growth
9	Oat meal Agar	66.00	Greyish white	Light greyish	Regular margin	Flat mycelial
10	Corn meal Agar	79.00	Whitish	Light brown	Regular margin	Medium Flat
	SEm±	0.57				
	C D at 1%	2.32				

Table.2 Impact of different temperature ranges on growth of *A. alternata* in potato dextrose broth

Sl. No.	Temperature (°C)	Dry mycelial weight (mg)
1	5	67.16
2	10	88.30
3	15	125.26
4	20	198.16
5	25	329.13
6	30	222.56
7	35	131.36
8	40	94.80
SEm±		**0.80**
C D at 1%		**3.32**

Table.3 Effect of different pH ranges on growth of *A. alternata* in potato dextrose broth

Sl. No.	pH	Dry mycelial weight (mg)
1	3.0	110.80
2	3.5	182.50
3	4.0	210.17
4	4.5	256.37
5	5.0	329.08
6	5.5	419.93
7	6.0	505.03
8	6.5	586.53
9	7.0	437.90
10	7.5	336.31
11	8.0	296.72
12	8.5	208.88
SEm±		**0.92**
C D at 1%		**3.63**

Plate.1 Effect of different solid media on growth of *A. alternata*

Plate.2 Muriform conidia

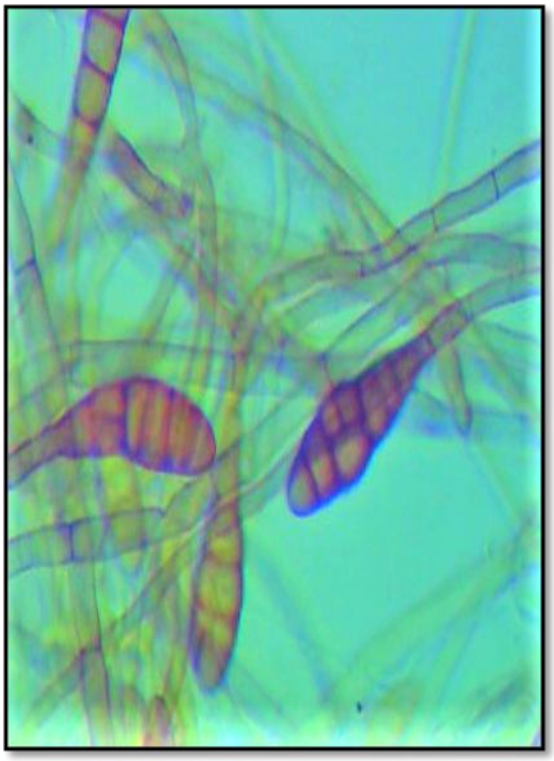

Plate.3 Conidia of *A. alternata* in chain

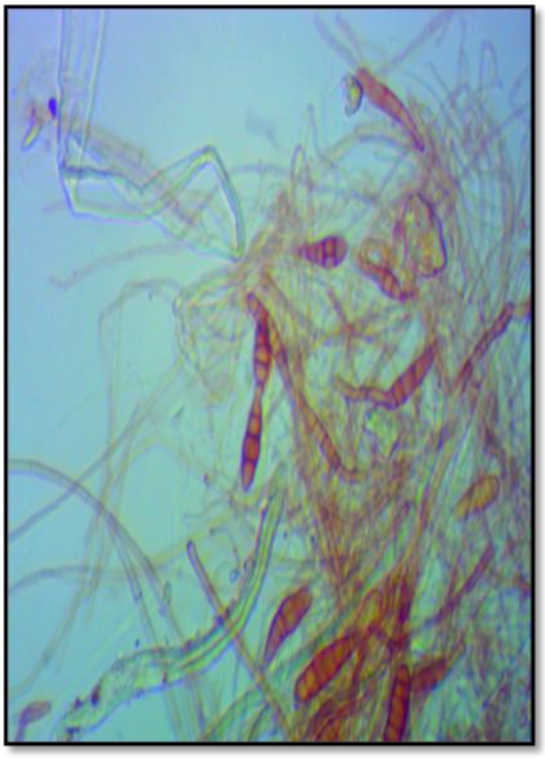

Plate.4 Effect of temperature on growth of *A. alternata* in potato dextrose broth

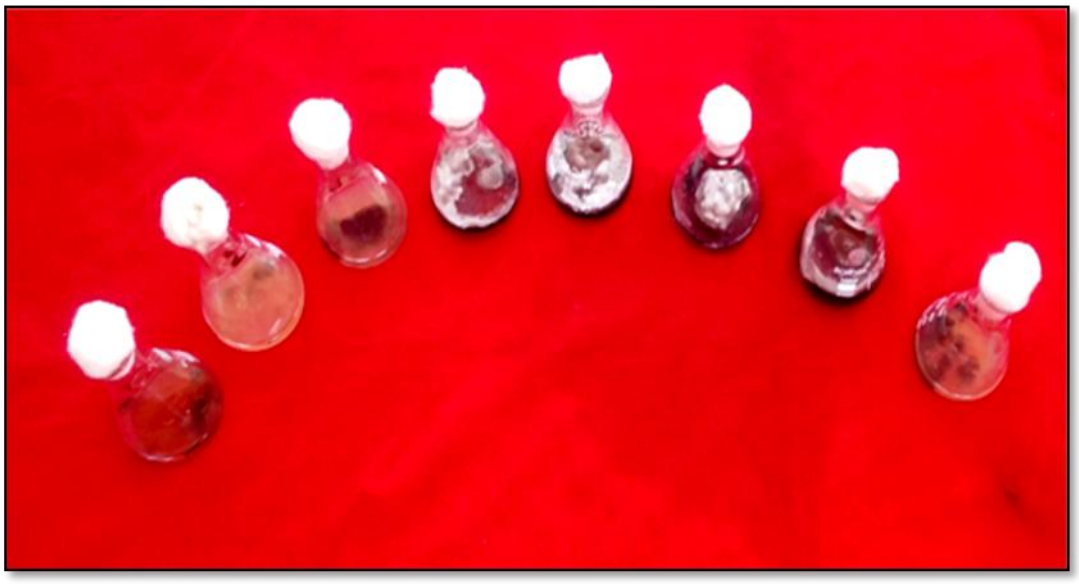

Plate.5 Effect of pH on growth of *A. alternata* in potato dextrose broth

Effect of temperature on growth of *A. alternata* in liquid media

The maximum growth of the test fungus was recorded at 25°C (329.13 mg) followed by 30°C (222.56 mg) while least growth was observed at 5°C. The findings of the present study are in close conformity with observations of Hanumanthaiah (1943) who reported that the fungus *Alternaria* spp. grow between 20°C and 30°C and the optimum being 25°C. Kumar and Arya (1978) reported that the best growth of *A. triticina* was obtained at 25°C. Choudhary *et al.*, (2017) found maximum mycelial growth at 25 ºC. Thus from the present study it was obvious that the optimum temperature for the growth of *A. alternata* was between 20°C to 30°C.

Effect of pH on *A. alternata* in liquid media

The hydrogen ion concentration (pH) of the medium and the growth of the fungus are interrelated. Every organism has minimum, maximum and optimum pH for the growth. Among the twelve different pH levels used for study, the maximum dry mycelial weight was observed at pH 6.5 (586.53 mg) followed by pH 7.0 (437.90 mg) and the minimum dry mycelial weight was recorded at pH 3.0 (110.80 mg). Verma (1970) reported optimum pH for the growth of *A. alternata* was 6.6 moderate growth was observed at pH of 4.4 and poor growth was observed at pH of 2.7, 3.4 and 8.0.

Similarly, Padmanabhan and Narayanswamy (1977) reported that a pH range of 5.0 to 7.0 was optimum for the growth of *A. macrospora*. Mahabaleswarappa (1981) observed that *A. carthami* made fairly good growth between pH range of 5.3 to 8.1 and maximum growth of the fungus was at pH 6.0. Choudhary *et al.*, (2017) showed among the eight pH levels, pH 6.5 was found to be ideal and produced the maximum dry mycelial weight of 845 mg.

Ten different solid media were used to study the cultural and morphological characteristics of *A. alternata*. The radial growth of *A. alternata* was maximum on Potato dextrose agar with colony diameter of 89.00 mm followed by Czapek's dox agar (87.00 mm) colony characters of *A. alternata viz.*, colour of the mycelium varied from whitish to dark grey. The growth varied from flat to raised and margin varied from regular to irregular.

Physiological studies revealed that growth of the pathogen was maximum at temperature of 25°C (329.13 mg) and lowest at 5°C (67.16 mg). The maximum dry weight was observed at pH 6.5 (586.53 mg) followed by pH 7.0 (437.90 mg) and the minimum dry mycelial weight was recorded at pH 3.0 (110.80 mg).

Future scope

Cultural, morphological and physiological characterisation of different isolates of *A. alternata.*

Molecular characterisation of different isolates of *A. alternata.*

Studies on effects of different levels of relative humidity on growth and sporulation of *A. alternata.*

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