Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 85-87
Received: 21-01-2019
Accepted: 25-02-2019

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Studies on morpho-cultural characters of *Alternaria alternata* infecting groundnut crop by using various culture media

Reddy VV, Ghante PH and Kanase KM

Abstract
Alternaria alternate is one of the most important foliar fungus causing leaf blight of Groundnut. *A. alternata*'s cultural characters were studied on different culture media *viz.,* Corn meal agar, Conn's agar, Czapek's dox agar, Potato dextrose agar, Malt extract agar, Richard's agar, V-8 juice, Host leaf extract agar (20%), Host stem extract (20%) and Host root extract (20%) as well as morphological characters such as mycelial growth, colony colour, colony diameter and sporulation *etc*. were observed after specific period. The various culture media tested revealed preference of *A. alternata* to PDA exhibit black to brownish colour colony, with excellent sporulation (++++), with large sized conidia (42.15 - 47.16 µm) and significantly highest mycelial growth (90 mm), was produced followed by Malt extract agar (85 mm), Host leaf extract agar (84 mm), Host stem extract agar (82 mm), Richard's agar (78 mm), Conn's agar (65 mm), Host root extract agar (52 mm), Czapek's dox agar (40 mm) and V-8 juice (38 mm), respectively.

Keywords: Groundnut, *A. alternata in vitro,* morpho-cultural characters and culture media

Introduction
Groundnut (*Arachis hypogea* L.) is one of the most important leguminous oilseed crops belonging to family Fabaceae and sub-family Papilionaceae, which comprise important edible oilseed crops in the world. It improves soil fertility by fixing atmospheric nitrogen and also used as a fodder for cattle. Groundnut is as source of high quality edible oil (44-56%), easily digestible protein (22-30%), carbohydrates (10-25%), vitamins (E, K and B complex), minerals (Ca, P, Mg, Zn andFe) and fiber. Groundnut shell can be used as fuel, animal feed, cattle litter, and filler in feed and fertilizer industry and in making particle boards and alcohol and acetone after fermentation. Haulm (above ground vegetative parts) used as animal fodder or in manuring, being groundnut roots add nitrogen (100-152 kg/ha) to the soil (Nigam, 2014) [11]. The area, production and productivity of groundnut in the country during the year 2016-17 were 47.68 lakh ha, 74.01 lakh MT and 15.52 q/ha, respectively. The area, production and productivity of groundnut in Maharashtra during the year 2016-17 were 2.11 lakh ha, 2.62 lakh MT and 12.39 q/ha, respectively (Anonymous, 2016) [1].
The leaf blight of groundnut caused by different species of *Alternaria* has been minor disease. The leaf blight disease of groundnut caused by *Alternaria alternata* was reported by Balasubramanian (1979) [2], Subrahmanyam *et al.,* (1981) [16], Vyas *et al.,* (1985) [17] and Narain *et al.,* (1987) [10]. Patil and Hiremath (1996) [12] reported *Alternaria tenuissima* and *Alternaria arachnidis.* Among the various diseases leaf blight incited by *Alternaria* spp. *(Alternaria alternata)* affecting groundnut has been reported as one of the important diseases causing potential yield losses in groundnut crop (Kumar *et al.,* 2012 and Kantawa *et al.,* 2014) [6, 5]. Hence, investigation was carried out with *in vitro* evaluation of various culture media studies on morpho-cultural characters of *Alternaria alternata* infecting groundnut.

Materials and Methods
The experiment was conducted at Department of Plant Pathology, College of Agriculture Latur, VNMKV, Parbhani (M.S.). The pathogen was isolated from diseased leaves of Groundnut on PDA incubated at 27±1ºC. For the purpose, a total of ten culture media *viz.,* Corn meal agar, Conn's Agar, Czapek's dox Agar, Potato dextrose agar, Malt extract agar, Richard's agar, V-8 juice, Host leaf extract Agar (20%), Host stem extract (20%) and Host root extract (20%) were used. Autoclaved (@ 15 lbs / inch^2, for 15 min) and cooled (45 °C) culture media were poured (20 ml /plate) separately in sterile glass petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the media, these petri plates

(three plates / treatment / replication) were separately inoculated by placing in a 5 mm culture disc obtained from actively growing 7 days old pure culture of *Alternaria alternata* and incubated at temperature of 27±1 ºC. The mycelial growth, colony diameter, colony colour, sporulation *etc*. was recorded. For morphological characterization of the test isolates of *Alternaria alternata* temporary mount of pure culture of the test strains were prepared in 0.1% Lacto-phenol cotton blue stain, on clean glass slide and observed under research microscope. An observation on morphological characters *viz*., size of conidia was recorded. The size of conidia was measured using ocular micrometer (calibrated using stage micrometer) under the microscope at 100 X magnification.

Results and Discussion

Cultural characters

The growth *A. alternata* was studied based on the cultural and morphological characters. The observations were recorded for mycelial growth, colony colour, colony diameter and sporulation *etc*. after specific period.

Radial mycelial growth / colony diameter

The results revealed that, all ten test culture media resulted with moderate to maximum mycelial growth of *A. alternata.* However, mycelial growth rate was maximum on Potato Dextrose Agar (90 mm) followed by Malt extract agar (85 mm), Host leaf extract Agar (84 mm), Host stem extract Agar (82 mm), Richard's Agar (78 mm), Conn's Agar (65 mm), Host root extract Agar (52 mm), Czapek's dox Agar(40 mm) and V-8 juice (38 mm), respectively.

Colony characteristics

Colony colour

The colour of the colony was observed from reverse side of petri-dish. The observations on colony pigmentation were taken at 8 days after incubation. The data showed that, the cultures were initially black in colour, later on; it became dark black in colour. On PDA, it produced black and brownish colony, while on Host Root extract agar, Czapek's Dox agar, Corn agar, Richard's agar, and Host stem extract agar, Light black to white colour colony was observed on Malt extract and Host leaf extract agar.

Mycelial growth

The *Alternaria alternata* pathogen of groundnut was also observed for their colony appearance. Slight difference was observed in appearance of colony. It had good growth and smooth margin to irregular margin on Potato Dextrose Agar (PDA), Malt extract agar, Host leaf extract agar and Host stem extract agar was observed. Moderate growth was observed in Richard's agar and Conn's agar media and slow growth was observed in Root extract agar, Corn meal Agar, Czapek's dox agar and V-8 juice.

Table 1: Growth and characteristics of *A. alternata* on different solid media

Sr. No.	Media	Colony diameter(mm)	Colony colour	Mycelial growth	Sporulation
1	Corn meal agar	45	Dark black colony	Slow growth	+
2	Conn's agar	65	Black colour growth is observed	Good growth with irregular margin	++
3	Czapek's dox Agar	40	Brownish colour growth is observed	Slow growth with smooth margin	+
4	Potato dextrose agar	90	Black colour mycelium	Good growth with irregular margin	++++
5	Malt extract agar	85	Whitish mycelium	Good growth with irregular margin	+++
6	Richard's agar	78	Black colour growth is observed	Moderate growth with smooth margin	++
7	V-8 juice	38	brownish mycelium	slow growth with smooth margin	+
8	Host leaf extract agar (20%)	84	Whitish and brownish mycelium	good growth with irregular margin	+++
9	Host stem extract (20%)	82	Brownish white Buffy growth is observed	Good growth with smooth margin	+++
10	Host root extract (20%)	52	Brown in colour	Slow growth is observed	+
	SE ±	0. 148	-	-	-
	CD at 1%	0.434	-	-	-

*Avg. of three replications, Figures in parenthesis are Arc sine transformation values. +: Poor (1-50 conidia/microscopic field 100x); ++: Fair (51-100); +++: Good (101-150); ++++: Excellent (>150).

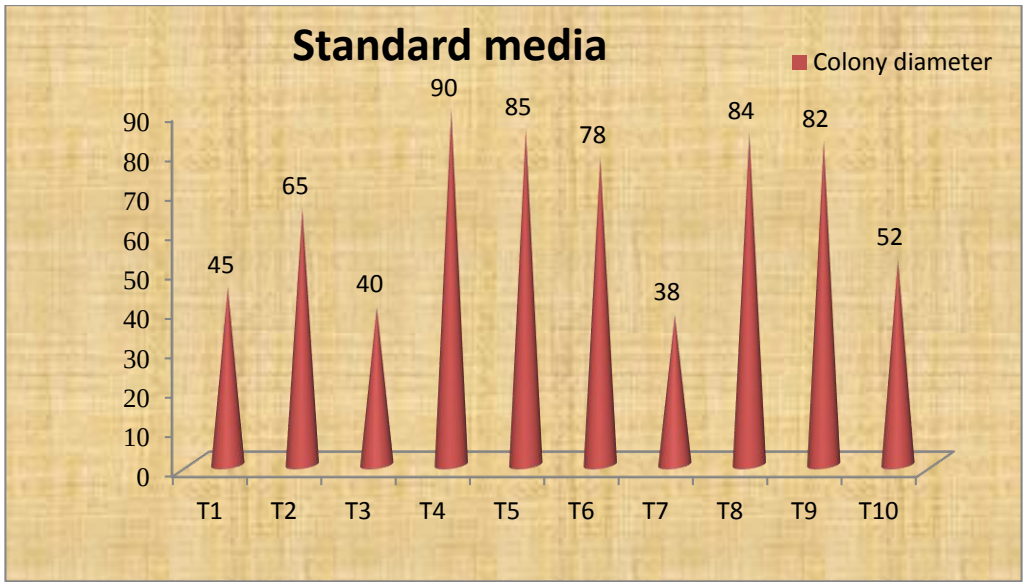

Fig 1: Per cent growth of *A. alternata* over standard media

T_1 Corn meal agar
T_2 Conn's Agar
T_3 Czapek'sdox Agar
T_4 Potato dextrose agar
T_5 Malt extract agar
T_6 Richard's agar
T_7 V-8 juice
T_8 Host leaf extract agar (20%)
T_9 Host stem extract (20%)
T_{10} Host root extract (20%)

Sporulation

All the ten culture media tested exhibited a wide range of sporulation of test pathogen. However, excellent sporulation (++++) was induced on Potato Dextrose Agar; whereas, good sporulation (+++) was recorded on Malt extract, Host leaf extract, host stem extract and Richard's agar. While, fair sporulation was observed in Conn's agar, Corn meal agar and Root extract agar and poor sporulation was observed in Czapek's dox agar and V-8 juice.

These results are in conformity to the findings of several earlier workers. They reported the radial growth of *A. alternata* was highest on Potato dextrose agar as compared to other media, who reported high level of variation in cultural characteristics in isolates obtained from different solid media (Gohel *et al.*, 2007, Hubbali *et al.*, 2010, Singh *et al.*, 2012, Manika *et al.*, 2013, Nagrale *et al.*, 2013 and Selvamani *et al.*, 2013) [3, 4, 15,8, 14].

Table 2: Morphological characteristics of *A. alternata* on different solid media

Sr. No.	Media	Size of Conidia	
		Length (µm)*	Width (µm)*
1.	Corn meal agar	38.12 – 42.16	07.80 – 09.45
2.	Conn's agar	29.82 – 38.10	06.64 – 07.80
3.	Czapek'sdoxagar	38.40 – 42.08	08.20 – 12.02
4.	Potato Dextrose agar	42.15 – 47.16	08.26 – 12.14
5.	Malt extract agar	26.45 –35.00	06.80–08.40
6.	Richard's agar	33.40 – 37.28	07.80–08.16
7.	V-8 juice	24.00 – 29.59	07.12–09.19
8.	Host leaf extract agar (20%)	38.47 – 45.12	10.12–14.22
9.	Host stem extract agar	32.60 –36.38	07.10–09.22
10.	Host root extract agar	22.44 – 28.37	08.16– 10.12

*Mean of ten observations
Morphological characters

Morphological characteristics of *A. alternata* on different media

Results depicted in table 2 that, *A. alternata* exhibited a wide range of variability in respect of length and width of conidia and septation in mycelial dimensions.

Mycelial characters

Light microscopy studies revealed that, mycelium of test pathogen was observed septate on all tested media. The mycelium was brown initially, but developed dark black colour in advanced stage and grow rapidly.

Conidial dimensions

From the data presented in table 2, it is observed that, conidial size of *Alternaria alternata* ranged from 24 to 47.16 µm. However, large sized conidia were produced on the Potato dextrose agar (42.15 - 47.16 µm) followed by host leaf extract agar (38.47 - 45.12 µm), Czapek's dox agar (38.40 - 42.08 µm), Corn meal agar (38.12 - 42.16 µm), Richards agar (33.40 - 37.28 µm), Host stem extract agar (32.60 -36.38 µm), Conns agar (29.82 - 38.10 µm), Malt extract agar (26.45 - 35.00 µm), V-8 juice (24.00 - 29.59 µm), and small conidial size were found Host root extract agar (22.44 - 28.37µm), respectively. These results are in conformity to the finding of several earlier workers (Raja and Ramana, 2007; Kumar *et al.*, 2012 and Nagrale *et al.*2013) [13, 6, 8, 9].

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