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Cultural characteristics of *Alternaria alternata*

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Abstract
The present investigation revealed that all the eight culture media tested showed better growth and variable sporulation of *Alternaria alternata*. The mean colony diameter/mycelial growth recorded with all test media was ranged between 41.00mm (Soybean casein agar) to 76.70 mm (Sabouraud's aextrose agar). The radial growth of *Alternaria alternata* was maximum on Sabouraud's dextrose agar (76.70 mm) as compared to the rest media. The next best in order of merit was Oat meal agar (73.00 mm). These were followed by Potato dextrose agar (67.7mm), Malt extract agar (67.7 mm), Czapek's dox agar (66.00 mm), Yeast dextrose agar (64.7 mm) and Richard's agar (56.7 mm) were good in supporting the mycelial growth of the *Alternaria alternata*. The least mycelial growth noticed on Soybean casein agar medium (41.00 mm). The mycelium was creamy to ashy white in most of media except in case of Richard's agar, where mycelium showed dull white to green growth. Whereas, in case of Czapek's dox agar, the mycelium showed dull white to indigo growth. Sabouraud's dextrose agar, Potato dextrose agar, Malt extract agar and Oat meal agar recorded good sporulation (+++).

Keywords: Richard's agar, Sporulation, *Alternaria alternata*, growth characters

Introduction
Marigold (*Tagetes erecta*) is an important commercial flower crop, belonging to family Asteraceae. Marigold occupies an area of 66.13 thousand hectares with an annual production of 603.18 thousand metric tons in India (Horticultural Statistics at a Glance 2017). Marigold is used for various purposes i.e., cut flower, loose flower, pot plant and as bedding plant. It shows pharmacological properties *viz*., anti-microbial activity, insecticidal activity, anti-bacterial activity, nematicidal activity, wound healing activity, mosquitocidal activity, analgesic activity, antioxidant and larvicidal activity. Many diseases affect the crop, including leaf spots and blight (*Altenaria*, *Cercospora*, and *Septoria sp*.), powdery mildew (*Oidium sp.*, *Levelula taurica*), flower bud rot (*Alternaria dianthi)*, and damping off (*Pythium sp*.). *Alternania alternata* caused leaf spot and flower blight is a serious disease of marigolds, particularly African marigolds (*Tagetes erecta*). The disease is distinguished by circular dark-brown necrotic spots on the leaves, stem, and flowers. The petals and peduncle of the infected flower are discoloured in a distinctive brown, scorched, necrotic pattern. According to estimates, the disease caused a 50-60% reduction in flower yield (Cotty *et al*., 1983) [2]. These diseased flowers are unmarketable, and they cannot even be used to extract Xanthophylls, fatty acids, easters, and oils, which are used in the dye and perfume industries. Hence investigatigation was carried out *in vitro* evaluation of various culture media studies on morphological and cultural characteristics of *Alternaria alternata* infecting marigold.

Materials and Methods
Present investigations were carried out during 2020- 2021 in the Department of Plant Pathology and Agricultural microbiology, College of Agriculture, Pune-05.

1. Cultural Studies
The cultural characters of *Alternaria alternata* were studied on six non-synthetic/semi-synthetic and two synthetic solid media. The non-synthetic/semi-synthetic media *viz.* Potato dextrose agar, Oat meal agar, Malt extract agar, Sabouraud's dextrose agar, Yeast dextrose agar, Soybean casein agar and synthetic media *viz.* Richard's agar, Czapek's agar were used.
All media were sterilized at 1.054 Kg/cm^2 pressure at 121^{o}C for fifteen minutes. To carry out the study, 20 ml of each of medium was dispensed in 90 mm petri plates. Such petri plates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1 ^{o}C. Each treatment was replicated thrice. Observations were taken after seven days of

incubation. The colony diameter was recorded. The fungal colony colour, margin and sporulations were also recorded. The data on radial fungal growth was assessed statistically. The composition and preparation of the above mentioned synthetic and non-synthetic or semi-synthetic media was taken from 'Ainsworth and Bisby's Dictionary of the Fungi' by Ainsworth (1967) [1] and 'Plant pathological methods: fungi and bacteria' by Tuite (1969) [6].

Results and Discussion

1. Cultural Studies

1.1 Growth Characters on Different Solid Media

Cultural characteristics *viz*. mycelial growth, colony diameter and sporulation of *Alternaria alternata* were studied *in vitro* using eight culture media and the results obtained are presented in Table 1 and depicted in Plate 2 and Fig 1.

1.2 Mycelial Growth

The results presented in Table 1, Fig 1 and Plate 2 revealed that all the eight culture media tested showed better growth and variable sporulation of *Alternaria alternata*. The mean colony diameter /mycelial growth recorded with all the test media were ranged from mm 41.00 mm (Soybean casein agar) to 76.7 mm (Sabouraud's dextrose agar).

Maximum mycelial growth recorded on Sabouroud's dextrose agar (76.7 mm) as compared to the rest media. The next best in order of merit was Oat meal agar (73.00 mm). These were followed by Potato dextrose agar (67.7mm), Malt extract agar (67.7mm), Czapek's dox agar (66.00 mm), Yeast dextrose agar (64.7 mm) and Richard's agar (56.7 mm) were good in supporting the mycelial growth of the *Alternaria alternata*. The least mycelial growth noticed on Soybean casein agar medium (41.00 mm).

1.3 Growth Characteristics

Alternaria alternata growth characteristics in different solid media revealed that Sabouraud's Dextrose Agar, Oat meal agar, Potato dextrose agar, and Malt Extract agar supported the most growth of the fungal colony. Except for Malt extract agar, Yeast Dextrose Agar, and Potato Dextrose Agar, which had wavy margins, and Czapek's Dox Agar and Richard's Agar, which had serrated margins. Except for Richard's Agar, where fungus showed dull white to green development, the mycelium was creamy to ashy white in most media. The mycelium on Czapek's Dox Agar, on the other hand, grew in a dull white to indigo colour. (Table 1 and Plate II)

1.4 Sporulation

All eight culture medium tested showed sporulation ranging from poor (+) to outstanding (++++). However, sporulation was outstanding (++++) on Sabouraud's dextrose agar, Oat meal agar, Potato dextrose agar, and Malt extract agar. On Yeast dextrose agar, good (+++) sporulation was observed. Media Czapek's dox agar and Richard's agar showed moderate (++) sporulation, whereas Soybean casein agar showed low (+) sporulation (Table 1).

Results of present study on the effect of various culture media on mycelial growth and sporulation are in conformity with several scientists *viz*. Koley and Mahapatra (2015) [4], Ginoya and Gohel (2015) [3], Nagrale *et al*. (2013) [5] who reported maximum growth of *Alternaria* spp. on Sabouraud's dextrose agar.

Table 1: *In vitro* effect of various culture media on mycelial growth, cultural characteristic and sporulation of *Alternaria alternata*

Sr. No	Name of cultural media	Av. Colony dia.* (mm)	Sporulation	Colony characters
1	Potato Dextrose Agar	67.7	++++	Creamy to white, wavy margin, circular, mycelium with concentric ring
2	Oat Meal Agar	73	++++	Ashy white to light green, entire margin circular
3	Soybean Casein Agar	41	+	Ashy white to light green, entire margin circular
4	Czapek's Dox Agar	66	++	Dull white to indigo, serrated margin, circular.
5	Richard's Agar	56.7	++	Dull white to light green, serrated margin, circular.
6	Yeast Dextrose Agar	64.7	+++	Ashy white, wavy margin, roughly circular, mycelium with concentric ring.
7	Sabouraud's Dextrose Agar	76.7	++++	Greyish to ashy white, entire margin, circular.
8	Malt Extract Agar	67.7	++++	Ashy white to greyish, wavy margin, circular, mycelium with concentric ring.
	S.E (m) ±	0.909		
	C.D 0.01	2.782		

*Mean of three replications.

Sporulation (No. of conidia)

+: Poor (Below 5) +++: Good (16-30)

++: Moderate (6-15) ++++: Excellent (above 30)

Plate 2: Cultural variability of *Alternaria alternata*
PDA: Potato Dextrose Agar
OMA: Oat Meal Agar
SCA: Soybean Casein Agar
CzDA: Czapek's Dox Agar
RA: Richard's Agar
YDA: Yeast Dextrose Agar
SDA: Sabouraud's Dexrrose Agar
MEA: Malt Extract Agar

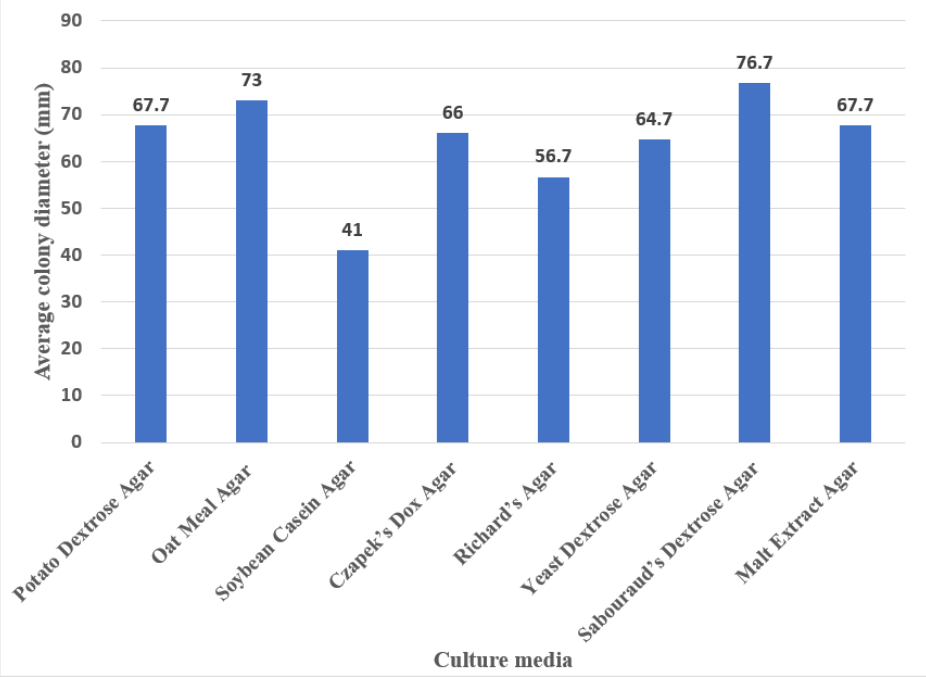

Fig 1: *In vitro* effect of different culture media on mycelial growth of *A. alternata*

Conclusion
SDA, OMA, PDA, and MEA were found the excellent media for mycelial growth and sporulation of *Alternaria alternata.*

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