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Cultural and Physiological studies on *Alternaria alternata* causing Blight of Marigold

Archana, A. M. [1]***, Lokesha, M. S.*** [2]***, Mahesh, Y. S.*** [1]***, A. M. Shirol*** [3] ***and Ashoka, N.*** [4]

[1]*Department of Plant Pathology, College of Horticulture, Bagalkot, India.*
[2]*Special Officer (Seeds) Seed Unit, University of Horticultural Sciences, Bagalkot, India.*
[3]*Department of Floriculture and Landscape Architecture, College of Horticulture, Bagalkot, India.*
[4]*Department of Agricultural Economics, College of Horticulture, Sirsi, India.*

(Corresponding author: Archana, A. M.)*
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ABSTRACT: Cultural and physiological studies on *Alternaria alternata* were studied at the Laboratory of Department of Plant Pathology, College of Horticulture, Bagalkot during 2018-2019. Marigold ranks first among the loose flowers in India. But, due to the increase in area, the crop is gradually becoming susceptible to many fungal and bacterial diseases mainly leaf blight which, affect the yield of the crop. The Potato Dextrose Agar (PDA) medium was used to maintain the pure culture isolated from infected marigold leaf and identified as *Alternaria alternata* (Fr.) Keissler. The cultural studies were conducted to know the effect of growth and sporulation in different media among them *A. Alternata* gave maximum mycelial growth (86.67) on the 8th day after incubation in a Potato dextrose agar (PDA) medium with better sporulation. Among liquid media, potato dextrose broth was observed most supportive for its growth. The various response of *A. Alternata* to different levels of temperature and pH showed that temperature of 30 °C and pH 6 was found congenial under *in vitro* conditions.

Keywords: Culture, morphology, physiology, *Alternaria alternata.*

INTRODUCTION

Marigold (*Tagetes erecta* L.) occupies a prominent place in ornamental horticulture. It is the most popular and commercial flower in India belongs to the Asteraceae family. The genus Tagetes has 33 species (Rydberg, 1945) in which two main species of marigold, *Tagetes erecta* L. and *Tagetes patula* L. are commercially grown for flower production and have their center of origin in Mexico and South Africa respectively.

Marigold procured popularity because of its adaptability to various types of soil, climate, longer blooming period, and more economic returns to farmers. The leaves and flowers possess medicinal value and it shows pharmacological properties *viz.*, anti-microbial, insecticidal, anti-bacterial, nematicidal, wound healing, anti-oxidant and larvicidal activity (Tripathy and Gupta, 1991). It has also been found effective to control the nematodes population when it is planted as intercrop and as organic manure (Polthance and Yamazaki, 1996). Mainly they are known to control root-knot nematodes, lesion nematodes, *Pratylenchus* spp., and *Meloidogyne* spp. infesting crop plants.

In India, among the loose flowers, the marigold ranks first. The total area under marigold cultivation in India is 66.13 thousand ha with an annual production of 603.18 thousand metric tonnes. It is mainly cultivated in the states of Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, and Haryana.

In Karnataka, Chamarajanagar, Haveri, Mysore, Bellary, and Belgaum are the major growing marigold districts (Anonymous, 2017). The area of marigold in Karnataka is 9830 ha and production are 87.34 thousand metric tonnes (Anonymous, 2016).

With the increase in area, the crop has become susceptible to soil, seed, and air-borne pathogens gradually. Marigold is infected by many fungal and bacterial diseases namely leaf spot, flower blight (*Alternaria* sp.), collar rot (*Phytophthora* sp.; *Pythium* sp.), wilt (*Fusarium oxysporum*), Cercospora leaf spot (*Cercospora melalopotamica*), damping-off (*Pythium* sp.), bacterial wilt (*Ralstonia solanacearum*), powdery mildew (*Oidium* spp.), Botrytis flower blight (*Botrytis* sp.) (Sohi, 1983; Pawar, 1971). Among these leaf spot and flower blight caused by *Alternaria* spp is one of the most destructive and economically important diseases and causes up to 50-60 % economic loss in flower yield (Cotty *et al.*, 1983) and induce 60 % disease severity in African marigold (Sen, 1996). Due to the destructive nature of the pathogen and the importance of the flower crop, this study was conducted to deeply understand the cultural as well as physiological characters of the pathogen. Similar kinds of studies were conducted in different crops infected by *Alternaria* spp. (Ramjegathesh and Ebenezar 2012; Nagrale *et al.*, 2013; Taware, 2014; Gholve *et al.*, 2015; Choudhary, 2017; Sinha and Alam, 2017; Gunda *et al.*, 2018 Reddy *et al.*, 2019).

MATERIAL AND METHODS

A. Cultural studies

Solid media. The common semi-synthetic, synthetic, and natural media, in both solid and liquid form, are used to culture the fungus. In 12 different solid agar and liquid broth media (without agar) pure culture of the fungus was inoculated, namely Cornmeal (CMA/CMB), Czapek's Dox (CDA/CDB), Carrot extract (CEA/CEB), Glucose peptone (GPA/GPB), Rose Bengal (RBA/RBB), Host leaf extract (HLEA/HLEB), Malt Extract (MEA/MEB), Oatmeal (OMA/OMB), Potato dextrose (PDA/PDB), Richard's (RA/RB), Sabouraud's (SA/SB) and Waksman (WA/WB) were prepared and autoclaved at 121°C at 15 psi for 15-20 minutes. To each medium pH was adjusted to 6.0 before autoclaving. The equal quantity (20 ml) of every medium was poured into Petri plates (90 mm). The poured Petri plates were inoculated separately with uniform culture bits (5 mm) from 6-7 days good growing culture and incubated at 25±1 °C to each treatment three replication were kept and recorded its radial growth and days taken by mycelium to fill Petri plate were recorded.

Measurement of the radial growth. The growth of mycelium on various solid media was measured on the backside of each Petri plate by drawing two lines at right angles to each other through the center of the cultured plates. The diameters of the fungal mycelial growth on these lines were measured and the average of the two was indicated as the diameter of the colony in mm. therefore, rectilinear colony growth was measured into two directions. But in the case of irregular growth of the colony, it was measured by taking an average of the shortest and largest diameter of the fungal growth.

Liquid media. Twelve liquid media *i.e.*, Cornmeal (CMB), Czapek's Dox (CDB), Carrot (CB), Glucose peptone (GPB), Rose Bengal (RBB), Host leaf extract (HLEB), Malt Extract (MEB), Oatmeal (OMB), Potato dextrose (PDB), Richard's (RB), Sabouraud's (SB) and Waksman (WB). Before autoclaving, each medium pH was adjusted at 5.0. Each medium (60 ml) was poured separately in 100 ml Erlenmeyer conical flasks plugged with non-absorbent cotton and sterilized at 121 °C at 15 psi for 20 minutes in an autoclave. Then each flask was inoculated separately with equal sized (5 mm) culture bits from (6-7 days) vigorous growing culture and incubated at 25±1 °C for 14 days. Thereafter, already weighed Whatman filter papers No. 1 was used to filter out the mycelial contents, and the dry weight of the mycelial mat from each flask was recorded after keeping it in a hot air oven for 24 hours at 80 °C. For every treatment three replication was kept and data were recorded on the mycelium dry weight.

The weight of the dry mycelial mats of the fungus was calculated in mg as below shown:

DW = W2 – W1

Where,

DW = Mycelial dry weight,

W1= Weight of mycelial mat along with filter paper

W2= Weight of Whatman filter paper alone.

B. Physiological studies

The physiological studies of the identified pathogen were carried on by taking the Potato dextrose medium as basal medium.

Effect of temperature. The effect of different temperatures on the mycelial growth of the *A. alternata* was studied under *in vitro* conditions. Sixty ml of potato dextrose broth was taken in 100 ml Erlenmeyer flasks and sterilized at 121 °C at 15 psi for 20 minutes in an autoclave then, inoculated with a 5 mm disc of 7 days old uniform culture of the pathogen and incubated at 4 different levels of temperature *i.e.*, 15, 20, 25, 30, 35, and 40 °C. Each treatment was replicated thrice and data were recorded on the dry mycelial mat.

Effect of pH. The effect of different pH levels on the mycelial growth of the pathogen was studied. Sixty ml of potato dextrose broth was pipetted in 100 ml conical flasks and with the help of a pH meter seven different pH levels *i.e.* 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were adjusted by using N/10 HCl or N/10 NaOH solutions. Inoculation was done same procedure in the case of liquid media. Data on dry weight were recorded.

RESULTS AND DISCUSSION

A. Cultural studies

On solid media. The data revealed that potato dextrose agar medium had significantly the highest growth (86.67 mm) of the pathogen at eight days of the incubation followed by oatmeal agar medium (82.33 mm) which are on par with each other. The minimum growth of the pathogen was recorded on the rose bengal agar (55.67 mm) (Table 1). The present finding conforms with Devappa and The jakumar (2016). It is concluded that PDA has a simple formulation and more nutrient contents, supporting the best mycelial growth of the fungus. Besides variation in growth rate, *A. alternata* exhibited little variation in color also where white color was observed in the case of oatmeal agar and Richard agar exhibited as grey at the center with white at the margin and dark brown to black in carrot agar whereas rest of the media colony color varied from grey to dark grey. The colony margin of the pathogen varied from uniform to wavy. Black pigmentation was found in Potato dextrose agar, Rose bengal agar, Host leaf extract agar, Malt extract agar, Waksman agar, Carrot agar, whereas Hazel pigmentation was found in oatmeal agar, Richard Agar, Sabouraud's agar, and dark brown pigmentation was seen in Czapek's dox agar and glucose peptone agar, but there was no pigmentation in case of cornmeal agar. The better sporulation (++++) was observed in Potato dextrose agar. The next best media was found to be oatmeal agar and Sabouraud's agar and Host leaf extract agar (Table 2). These results were in contradiction to the findings of Reddy *et al.* (2019), who found maximum radial mycelial growth of the *A. alternata* (90 mm) in PDA with excellent sporulation (++++) and Czapek's doxes agar showed poor sporulation.

On liquid media. The data presented in Table 3 showed that the Potato dextrose broth medium produced significantly the highest dry weight (359.67 mg) growth of the fungus after incubation at 25±1°C The observations are contradictory with the results of Kantwa *et al.* (2006), who observed the maximum growth and sporulation on potato dextrose broth. This variation in the fungi growth in all the media is mainly for 14 days followed by Carrot broth (304.00 mg) and Potato dextrose broth is significantly superior overall other media. Cornmeal broth supported the least growth (47.00 mg) of the fungus.

because of the media composition and carbon source used. For the better growth of *A. alternata*, dextrose, maltose, and xylose were carbon sources significant (Patil and Suryawamshi 2015).

Table 1: Growth of *A. alternate* on different solid media.

Sr. No.	Treatments	Mean Radial Growth (mm)
1.	Corn Meal Agar	82.00
2.	Czapek`S Dox Agar	56.67
3.	Carrot Agar	76.66
4.	Glucose Peptone Agar	73.00
5.	Host Leaf Extract Agar	72.33
6.	Malt Extract Agar	74.33
7.	Oat Meal Agar	82.33
8.	Potato Dextrose Agar	86.67
9.	Rose Bengal Agar	55.67
10.	Richard`S Agar	71.33
11.	Sabourauds`S Agar	63.33
12.	Waksman Agar	83.00
	S. Em±	**1.47**
	CD @ 1 %	**4.32**

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

Sr. No.	Treatments	Colony character				Sporulation	Spore size (µm) (L x B)
		Colony color	Surface	Margin of the colony	Pigmentation		
1.	Corn Meal Agar	Grey	Smooth	Uniform	No	+	35.12 – 39.16 × 7.80 – 9.45
2.	Czapek`s Dox Agar	Grey /whitish with dark border	Smooth	Irregular	Dark brown	+	34.6-38.5 ×8.20 – 12.02
3.	Carrot Agar	Dark brown to black	Rough	Uniform	Black	++	24.10 – 29.69 × 7.22–9.09
4.	Glucose Peptone Agar	Grey with dark at the center	smooth	Uniform	Dark brown	++	31.60 –35.38 × 7.00–09.22
5.	Rose Bengal agar	Dark grey	Rough	Irregular	Black	+	29.72 – 38.00 × 6.84 – 7.89
6.	Host Leaf Extract Agar	Dark grey	Smooth	Uniform	Black	+++	37.67 – 45.12 × 10.02–12.22
7.	Malt Extract Agar	Light brown	Rough	Irregular	Black	++	26.15 –35.10 × 6.80–8.45
8.	Oat Meal Agar	white	smooth	Irregular	Hazel	+++	36.60-37.24 × 6.47-9.8
9.	Potato Dextrose Agar	Dark Grey	Smooth	Uniform	Black	++++	35.58-40.8 × 7.69-10.04
10.	Richard`s Agar	Whitish grey	Smooth	Uniform	Hazel	+	34.5-37.10 × 7.12-8.80
11.	Sabouraud`s Agar	Grey	Smooth	Wavy	Hazel	+++	33.15-38.71 × 6.15-7.90
12.	Waksman Agar	Grey	Smooth	Regular	Black	++	34.84-36.25 × 7.80-9.75

- No suporulation, + Poor, ++ Moderate, +++ good, +++

Table 3: Growth of *A. alternate* on different liquid media.

Sr. No.	Treatments	Mean Dry Weight (mg)
1.	Corn Meal Broth	47.00
2.	Czapek`s Dox Broth	156.00
3.	Carrot Broth	304.00
4.	Glucose Peptone Broth	65.00
5.	Host Leaf Extract Broth	102.33
6.	Malt Extract Broth	185.67
7.	Oat Meal Broth	212.00
8.	Potato Dextrose Broth	359.67
9.	Rose Bengal Broth	152.67
10.	Richard`s Broth	121.33
11.	Sabourauds`s Broth	221.33
12.	Waksman Broth	173.33
	S. Em±	**1.05**
	CD @ 1 %	**3.10**

B. Physiological studies

Effect of pH on growth of *Alternaria alternata.* The results in the Table 4 depict that pH ranging from 4.0 to 9.0 supports the growth of the pathogen. Maximum growth of the *A. alternata* was recorded at pH 6.0 (329.30 mg) which was significantly superior to all other pH followed by pH 7.0 (278.00 mg). The least growth was observed at pH 4.00 (150.67 mg). The results obtained in the present study are under the results of Taware (2014), who reported that pH 6.5 was best for the growth of *Alternaria carthami* and Odenapur (2011) observed that pH 6.0 was better for *A. alternata*. The inhibitory action of pH above 7.0 and below 6.0 was attributed to the unconducive reaction of the media.

Effect of temperature on growth of *Alternaria alternata*. The *A. alternata* growth varied significantly with different temperature levels. Results indicate that *A. alternata* grew at a wide range of temperatures ranging from 15 to 40 °C. But the maximum mean dry mycelial weight (296.67 mg) was observed at 30 °C which is considered to be optimum for the better growth of the pathogen followed by 25 °C (219.00 mg) after fourteen days of incubation. The least growth of the pathogen was observed at 40 °C (42.33 mg) (Table 5). The results are supported by Hubballi (2010), who reported that 30 °C was the optimum temperature for the growth of *A. alternata*.

Table 4: Effect of pH levels on mycelial growth of the *A. alternata.*

Sr. No.	pH levels	Mean dry mycelial weight (mg)
1	4	150.67
2	5	224.33
3	6	329.3
4	7	278.00
5	8	201.67
6	9	157.33
S. Em ±		**1.32**
CD @ 1 %		**3.56**

Table 5: Effect of temperature on mycelial growth of the *A. alternata.*

Sr. No.	Temperature (^{o}C)	Mean dry mycelial weight (mg)
1.	15	99.33
2.	20	191.67
3.	25	219.00
4.	30	296.67
5.	35	127.33
6.	40	42.33
S. Em ±		**1.25**
CD @ 1%		**3.89**

CONCLUSION

In the present study, *Alternaria alternata* was isolated from the infected marigold crop. Twelve different media were used to observe the growth and sporulation. Potato dextrose media was found best for growth and sporulation. Maximum growth was found at 6.0 pH of media followed by 7.0 and temperature of 30 °C was found congenial for the pathogen growth. The present study will be helpful in the research being carried out by different workers studying this pathogen to understand its congenial condition for host-pathogen interaction.

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