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Cultural and Physiological studies of *Alternaria brassicicola*(Schw.) Wiltshire of Cabbage (*Brassica oleracea* var. *capitata* L.)

DINH VIET TU, Y. M. SOMASEKHARA, AND M. K. SHIVAPRAKASH
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

ABSTRACT

A study was conducted to know the nutritional requirement of *Alternaria brassicicola*. Cultural studies of the pathogen revealed that, after ten days the growth of the fungus was maximum in yeast extract agar medium (90 mm) followed by potato dextrose agar (87 mm). The mean dry mycelial weight of the fungus was maximum on carrot extract broth (189 mg), followed by potato dextrose broth (187 mg) seven days after incubation. Both these media were also supported for good sporulation of the fungus. Maximum growth and sporulation of *A. brassicicola* was found at pH of 6 (dry mycelial weight was 154 mg) and the least growth was obtained at pH 4 (126 mg) and 10 (127 mg). The pH ranges from 5.5 to 7.5 is the best for the growth of *A. brassicicola* cause leaf spot of cabbage. The fungus made fairly good growth between temperature ranges of 20 to 35^{0}C. Maximum growth of the fungus was at 30^{0}C (86.08 mm) followed by 25^{0}C (75.83 mm). Optimum temperature for the growth and sporulation of the pathogen was found between 27 to 32^{0}C.

THE cabbage (*Brassica oleracea* var. *capitata*L.) crop is susceptible to a number of diseases caused by biotic and mesobiotic pathogens. Among various diseases, Alternaria leaf spot is the most destructive disease of cabbage in all the continents. This disease is known to be incited by *Alternaria brassicicola* infection. Alternaria leaf spot pathogens are necrotrophs and produces lesions surrounded by chlorotic areas on leaves, stems and siliquae causing reduction in the photosynthetic areas, defoliation, and early induction of senescence. Alternaria blight causes considerable reduction in quantity and quality of harvested cabbage product. The Alternaria leaf spot pathogen is seed borne, soil borne and air borne. The pathogens are greatly influenced by weather and nutrition with the highest disease incidence. This investigation included cultural and physiological factor for the growth of *Alternaria brassicicola* caused by leaf spot of cabbage. A suitable substrate is required for the growth of a pathogen. The morphological and cultural characteristics of the pathogen are essential for proper taxonomic status. The growth of *A. brassicicola* on different media has been reviewed. Swati Deep *et al*. (2014) reported that among seven types of media (PDA, Cauliflower leaf extract Agar, Carrot potato Agar, Oat meal agar, Czapek Dox agar, V8 juice agar and Corn meal agar), Potato dextrose agar and Cauliflower leaf extract agar were found optimum for the growth and sporulation of the *A. brassicicola*. Manika Sharma *et al*. (2013) reported that Potato Dextrose Agar, Cauliflower (Host) Agar medium and Carrot Potato Agar were good for all the cultures of *A. brassicae*. The nature of the activities of microorganism is such that the pH of the environment of a metabolizing culture will not remain constant for long (Munro, 1970). Lilly and Barnett (1951) reported that, the pH of the medium affected the rate and amount of growth and many other life processes. Kumar and Arya (1978) reported that, pH 6.0 was optimum for growth of *A. triticina*. Nishikado *et al*. (1941) reported that, pH 2, 5 and 10 are minimum, optimum and maximum, respectively for the growth of *A. macrospora*, while, Padmanabhan and Narayaswamy (1977) found pH 5 to 7 to be optimum for the growth of *A. macrospora.*

According to Mahabaleswarappa (1981) the optimum pH range for the germination of conidia of *A. carthami* was 5.0-6.0. Narasimha Rao and Rajagopalan (1978) found *A. helianthi* could grow and sporulation over a fairly wide range of pH 4.5 to 10.0 with maximum at neutral pH (7.0). The growth gradually increases up to neutral pH with a steep fall afterwards. The fungus grows poorly at 3.5 and 10.0 but fails to grow at pH 3.0, 10.5 and 11. Reddy and Gupta (1981) reported that the maximum growth of *A. helianthi* was at pH 6.0. Nallathambi and Thakore (2004) reported that pH 6.5 favoured maximum growth

of the *A. alternata*. The present investigation was carried to know the suitable media, pH and temperature for the growth of the pathogen.

Material and Methods

The cultural characters of *A. brassicicola* were conducted on the following twelve different media (solid and liquid) *viz.,* Cabbage leaf medium, Oat meal medium,Carrot extract medium, Potato Dextrose medium, Czapek's medium, Richard's medium,Glucose Nitrate medium, Sabouraud's medium, Leonian's medium,Yeast extract medium, Malt extract medium and Starch's medium. Liquid medium was made in flask without agar. The composition and preparation of the mentioned synthetic and non- synthetic / semi-synthetic media was followed by Hawksworth *et al*. (1983). All the ingredients were dissolved in 400 ml distilled water and agar was dissolved separately in 500 ml of distilled water and mixed with the above solution and the volume was made up to 1000 ml. The medium was sterilized at 1.1kg / cm^2 pressure for 20 min. Each treatment with three replications was maintained. The plates were incubated for seven days with liquid media and ten days with solid media in incubators at 27±1^{0}C. After the incubation period, the mycelial growth (mm) was recorded and results were analysed statistically.

The growth of *A. brassicicola* was tested at different temperatures *viz.,* 10, 15, 20, 25, 30, 35 and 40^{0}C. Twenty ml of sterilized Potato dextrose agar (PDA) was poured into the sterile petriplates aseptically and allowed to solidify. Then the plates were inoculated with the 0.5 cm mycelial discs of *A. brassicicola.* Each treatment with three replications was maintained. The plates were incubated for ten days in incubators adjusted to required temperature levels mentioned above. After the incubation period, the mycelial growth (mm) was recorded and results were analysed statistically.

The growth of *A. brassicicola* was tested at different pH levels *viz.,* 4.0, 4.5, 5.0, 5.5, 6.0 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. Fifty ml of sterilized Potato dextrose broth (PDB) was poured into the sterile 100 ml flask. Then the flasks were inoculated with the 0.5 cm mycelial discs of *A. brassicicola*. Each treatment with three replications was maintained. The flasks were incubated for seven days at room temperature condition. After the incubation period, fungus was filtered with wahatsmen filter paper and kept in hot air oven at 60^{0}C. The mycelial weight was recorded and results were analysed statistically.

Results and Discussion

Among the twelve different solid media evaluated after ten days, the mean colony diameter of *A. brassicicola* was maximum in Yeast extract medium (90.00 mm) followed by Potato dextrose agar (87.00 mm), Carrot extract medium (84.58 mm), and minimum growth recorded in Sabouraud's dextrose agar (62.67 mm). The mycelium colour of fungus varied from white to light / dark grey and black. The growth varied from flat, raised fluffy to sparse. Pigmentation in the media also varied from off white, Greyish white to light grey, grey, light black and black. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation. The best sporulation of the fungus was recorded on Carrot extract dextrose agar followed by Potato dextrose agar and Cabbage leaf extract Media. Moderate sporulation was found in Oat meal agar, Czapek's agar, Yeast extract agar and Malt extract agar. Poor sporulation was recorded Sabouraud's agar, Richard's agar and Leonian's agar. While, mycelium of *A. brassicicola*is not sporulated in Starch's agar and Glucose nitrate agar after ten days cultured (Table I).

Among the twelve different liquid media evaluated, mean dry mycelial weight of *A. brassicicola* was maximum in Carrot extract medium (189.00 mg), followed by Potato dextrose broth (187.00 mg), Malt extract medium (181.00 mg).Whereas, mean dry mycelial weight was minimum in case of Richard's medium (134.00 mg) and Czapek's solution (140.00 mg). Colony colour of *A. brassicicola* changed from white to grey on Sabouraud's dextrose broth and Starch's broth; off white on Richard's broth, Glucose nitrate and Cabbage leaf extract broth; light grey on Carrot extract broth, Czapeck's broth and oat meal; grey on Potato dextrose broth and Lionian's broth. Mycelium of *A. brassicicola* grown on Malt extract broth has tight black colour. Excellent sporulation was recorded on Carrot extract broth, followed by potato dextrose broth, Malt extract

TABLE I

Growth of A. brassicicola on different culture solid media

Media	Radial growth (mm) after 10 days	Colour	Type of growth	Pigmentation	Sporulation
Cabbage leaf medium	77.33	Dark grey	Flat growth with regular margin and zonation	Dark grey	+++
Carrot extract medium	84.58	Light black	Fluffy growth with irregular margin	Grey	++++
Czapek's solution agar	77.25	Light grey	Flat growth with regular margin	Light grey	++
Glucose nitrate agar	68.50	Light black	Flat growth with regular margin	Black	-
Leonian's agar	85.17	Greyish white	Fluffy raised with regular margin and zonation	Grey	+
Malt extract agar	77.58	Greyish white	Fluffy raised growth with regular margin	Off white	++
Oat meal agar	72.83	Off white	Fluffy raised with irregular margin	Off white	++
Potato dextrose agar	87.00	Grey	Flat growth with regular margin	Grey	+++
Richard's agar	62.17	Off white	Flat growth with regular margin	Off white	+
Sabouraud's agar	62.67	Greyish white	Fluffy raised with regular margin	Light grey	+
Starch's agar	67.83	Light grey	Fluffy raised growth with regular margin	Light grey	-
Yeast extract medium	90.00	Off white	Fluffy growth with irregular margin	Off white	++
Mean	76.08				
SEm±	1.37				
CD@5%	4.00				
Cv	3.93				

-No parasitization; + Weak parasitization;++ Medium parasitization; +++ High parasitizatio; ++++ Strong parasitization.

broth and moderate sporulation in Oat meal broth, Sabouraud's broth, Lionian's broth and Cabbage leaf extract broth. Poor sporulation was recorded Czapek's broth, Yeast extract broth, Richard's broth and whereas, in Glucose nitrate broth fungus was no sporulation after seven days cultured (Table II). On solid and liquid media, the morphological characters revealed that the fungus produces conidia and conidiophores, conidia were typically produced in chains on the conidiophore. The mycelium on twelve solid media was fluffy raised with regular margin and zonation. Hyphae were hyaline, septate and branched with off white to grey and black. The colour of the conidia was dark brown. The several media were tested for better growth of the *A. brassicicola* and found that Yeast extract agar, Potato dextrose agar, Carrot extract agar found good and sporulation of *A. brassicicola*. A similar result obtained on potato dextrose agar medium was reported by Pawar and Patel (1957). Barksdale (1968) also reported potato dextrose agar medium was the best medium for the growth and sporulation of *A. solani*. Cheema *et al*. (1976) reported that *A. citri* grows rapidly on PDA followed by Yeast extract agar. Manika Sharma *et al*. (2013) reported that potato dextrose agar and carrot extract agar were good for better growth of *A. brassicae*. Swati Deep *et al*. (2014) also reported that Potato dextrose agar and Carrot extract

TABLE II

Growth of A. brassicicola on different liquid media

Culture media	Dry mycelial weight (mg)	Colour of mycelium	Sporulation
Cabbage leaf extract broth	164	Off white	++
Carrot extract broth	189	Light grey	++++
Czapek's broth	140	light gey	+
Glucose nitrate broth	141	Off white	-
Leonian's broth	166	Grey	++
Malt extract broth	181	Light black	+++
Oat meal broth	179	light grey	++
Potato dextrose broth	187	Grey	+++
Richard's broth	134	Off white	+
Sabouraud's dextrose broth	143	White to grey	++
Starch's broth	158	White to grey	+
Yeast extract broth	153	Off white	+
Mean	160		
SEm±	0.03		
CD@5%	0.08		
Cv%	3.13		

- No parasitization; + Weak parasitization ++ Medium; parasitization; +++ High parasitization; ++++ Strong parasitization.

agar were found better for the growth and sporulation of *A. brassicicola*. Roy (1969) found good growth of *A. dauci* causing leaf blight of carrot in potato dextrose medium.

Temperature is one of the important factors for the growth of an organism. The results indicated that the maximum colony diameter of *A.brassicicola* was observed at 30^{0}C (86.08 mm) followed by 25^{0}C (75.83 mm) and at 35^{0}C (72.50) mm and there was significant difference between the these treatments. The minimum colony diameter was recorded at 40^{0}C (11.00 mm) and 5^{0}C (12.42 mm) and this was followed by 10^{0}C (15.43mm) and 15^{0}C (17.10 mm). The maximum sporulation was found at 30^{0}C and significantly superior to other temperature levels, followed by 25^{0}C and 35^{0}C and moderate sporulation at 20^{0}C. There was no sporulation at 15^{0}C, 5^{0}C and 40^{0}C (Table III). The findings of the present study were in close conformity with the observations of Rotem (1994) who reported that the conidia of *A. brassicicola* germinate over a wide range of temperatures, with the optimum at 28 to 31^{0}C. Sporulation can occur over a wide range of temperatures and is optimal at 20 to 30^{0}C. Ronald and Diana (2011) reported that sporulation occurred at a temperature range of *A. brassicicola* from 8-24^{0}C, where mature spore occur after 14-24 hours. Optimum temperatures were between 16 and 24^{0}C and sporulation time range from 12 to 14 hours.

Hydrogen ion concentration is an important factor promoting the growth and development of fungi. The present study was carried out to know the best pH level for the growth of *A. brassicicola*. The results indicated that pH range of 5.5 to 7.5 was optimum for the growth of *A. brassicicola*. Maximum dry mycelia

TABLE III

Effect of temperature on the growth of A.brassicicola on PDA

Temperature (^{0}C)	Mycelium growth (mm)		Sporulation
	7 days	10 days	
5	11.25	12.42	-
10	14.13	15.43	-
15	16.67	17.10	-
20	34.45	48.92	+
25	38.00	75.83	+++
30	74.58	86.08	++++
35	55.92	72.75	++
40	10.17	11.00	-
Mean	31.89	42.44	
S.Em±	0.43	0.41	
CD@5%	1.30	1.23	
Cv%	2.28	1.77	

- No parasitization; + Weak parasitization ++ Medium; parasitization; +++ High parasitization ++++ Strong parasitization.

weight was observed at pH 6.0 with 154.00 mg, followed by pH 6.5 (148.00 mg) and pH 5.5 (145.00 mg). Minimum dry mycelial weight was recorded at pH 4.0 (126.00 mg), pH 10 (127.00 mg) and pH 9.5 (130 mg).

Among 13 pH levels, pH 6 was found best in sporulation of *A. brassicicola* and found significantly superior over all the other pH levels and followed by pH 6.5. At pH 5.5, 7.0 and 7.5 were found medium sporulation of fungus. Poor sporulation was found at pH 4.5, 5.0, 8.0 and 8.5. Colony of *A. brassicicola* was completely no sporulation at pH 9.0, 9.5, 10 and 4.0. (Table IV). The hydrogen ion concentration (pH) of the medium and the growth of the fungus were interrelated. Every organism has minimum, maximum and optimum pH for the growth. The results indicated that the maximum growth and sporulation of *A. brassicicola* was observed at pH 6.0, followed by pH 6.5 and the minimum was recorded at pH 4.0 and at pH 10. Similar findings have been reported by Padmanabhan and Narayaswamy (1977) who found pH 5 to 7 to be 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. These findings correlates with the results obtained in the present study where pH 6.0 supported the maximum growth of *A. brassiccicola* and pH 5.5 and pH 6.5 were optimum for the growth of the pathogen. The present investigation found that, Carrot extract medium, potato dextrose broth and malt extract medium were found to good in growing Alternaria leaf spot fungi with the temperature range from 25 to 30^{0}C. The pH ranges from 5.5 to 7.5 is the best for the growth of *A. brassicicola* cause leaf spot of cabbage.

TABLE IV

Effect of pH on growth of A.brassicicolain Potato dextrose broth

pH levels	Dry mycelial weight (mg)	Sporulation
4.0	126	-
4.5	132	+
5.0	140	+
5.5	145	++
6.0	154	++++
6.5	148	+++
7.0	141	++
7.5	140	++
8.0	137	+
8.5	133	+
9.0	132	-
9.5	130	-
10.0	127	-
Mean	137	
S.Em±	0.06	
CD @ 5%	0.19	
CV%	1.64	

- No parasitization; + Weak parasitization ++ Medium parasitization; +++ High parasitization and ++++ Strong parasitization.

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