Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 3244-3250

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 3244-3250**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.610.380

Morpho-Physiological Variability in *Alternaria carthami* Chowdhury Causing Safflower Leaf Spot

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Keywords

Alternaria carthami, Safflower, Variability, temperature, pH.

Article Info

Accepted:
26 September 2017
Available Online:
10 October 2017

A B S T R A C T

Twelve isolates of *Alternaria carthami* collected from major safflower growing parts of Northern Karnataka differed significantly with respect to Cultural, morphological and physiological variation. Among different tested media, host leaf extract recorded significantly higher (87.19 mm) radial growth followed by Czapeck's dox agar (81.92 mm) and Potato dextrose agar (81.25 mm). The conidia of different isolates shown variation with vertical (0-4) and horizontal (6-9) septum and also size varies from 29.6–84.4 x 9.1 –16.3 µm to 50.3-101.3 x 10.2-24.3 µm. The fungus under sturdy grew best at temperature of 25^0 C recorded 290.18 mg dry mycelial weight whereas, optimum range was between 25-30^0 C. Similarly the pH ranges from 6.0-7.0 was optimum for good growth and development of *A. carthami.* The highest dry mycelial weight (331.50 mg) of the fungus was recorded with of pH 7.0 followed by pH 6.0 (278.77 mg) and pH 5.0 (236.38 mg) and the least dry mycelial weight of 145.66 mg was recorded with pH 9.

Introduction

In India Safflower (*Carthamus tinctorius* L.) is known for its orange-red dye extracted from its brilliant florets besides high-quality edible oil rich in polyunsaturated fatty acids. Safflower oil is nutritionally similar to olive oil, as it contains high levels (78%) of linoleic or oleic acid. Leaves are rich in carotene, riboflavin and vitamin C owing to these seedlings and prunings are used as green leafy vegetable. The safflower production in India is about 1.89 lakh tonnes with bulk of the production concentrated in the states of Maharashtra and Karnataka. The leaf spot disease caused by *Alternaria carthami* Chowdhury is a major destructive disease of safflower in India. The disease occurred in epidemic form during 1997 in all safflower growing areas of Maharashtra, Andhra Pradesh and Karnataka states of India due to high humidity coupled with continuous rains during pre-flowering period. The disease caused severe losses in seed yield in the trials in most of the locations in Maharashtra and Karnataka. The disease has been reported to cause seed yield losses to the tune of 10 to 25 per cent (Indi *et al.*, 1988). Under severe conditions, it has been reported to cause 50 per cent loss in seed yield (Indi *et al.*, 1986 and Singh and Prasad, 2005). The disease assumes endemic proportion in most of the safflower growing areas of Karnataka, Maharashtra and Andhra Pradesh. The present

experiment on variability studies on cultural, morphological and physiological features of the pathogen are of immense utility in understanding the nature of the pathogen to arrive at pragmatic remedies to manage the disease.

Materials and Methods

The safflower leaves showing the typical leaf spot symptoms were collected from different northern parts of Karnataka *viz*., Dharwad *(Ac1)*, Hubli *(Ac2)*, Navalagund *(Ac3)*, Annigeri *(Ac4)*, Kundagol *(Ac5)*, Naragund *(Ac6)*, Rona *(Ac7)*, Hunagund *(Ac8)*, Badami *(Ac9)*, Bijapura *(Ac10)*, Bagevadi *(Ac11)* and Muddebihal *(Ac12)* and pathogen *A. carthami* was isolated by standard tissue isolation technique in the laboratory. Further, the pure culture of the fungus was obtained by single spore isolation method and maintained on Potato dextrose agar (PDA) slants for further use. Pathogenicity was proved by spraying spore and mycelial suspension on 30 days old manjira susceptible variety. The study was conducted in the Department of Plant Pathology, College of Agriculture, Dharwad, Karnataka, India.

Cultural variability of *A. carthami* on different solid medium

The cultural characters of 12 isolates of *A. carthami* were grown on various solid media *viz.*, Potato dextrose agar *(*PDA), host leaf extract (HLE), Czapeck's dox agar (CZ) and Richards' agar (RA). Five mm discs of actively growing *A. carthami* culture were cut using a cork borer and a single disc was placed in a Petri plate containing above media in aseptic condition. Each set of experiment was replicated thrice and the plates were incubated at 27 ± 1^0 C for 12 days. The radial growth of the pathogen from the centre to the periphery of Petri plate was measured for all the media and data was analyzed statistically.

Morphological variability of *A. carthami* on potato dextrose agar

The isolates of *A. carthami* were collected from Dharwad, Vijayapur, Gadag and Bagalkot districts (*Ac1* to *Ac12*) were used for the study. These isolates were grown on PDA and studied for morphological characters such as length and width of conidia, number of horizontal and vertical septa and beak length using Differential interference contrast microscope under 40X magnification.

Physiological variability of *A. carthami*

Effect of different temperature levels on the growth of *A. carthami*

All the twelve isolates of *A. carthami* were grown at 15, 20, 25, 30, 35 and 40^0 C on Potato dextrose broth (PDB). Thirty milliliters of PDB was prepared in 100 ml conical flask and then sterilized. Five mm diameter discs of ten days old mycelia of all the isolates of *A. carthami* were inoculated and incubated at different temperatures for 12 days, three replications were maintained. Cultures were filtered through Whatman No. 1 filter paper and dry mycelia weights were recorded.

Effect of different pH levels on the growth of *A. carthami*

All twelve isolates of *A. carthami* were grown on the PDB with pH levels of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by adjusting with 1N alkali (NaOH) or acid (HCl). Five mm mycelial discs of all the isolates were inoculated separately into 100 ml conical flasks containing 30 ml medium at different pH levels and three replications were maintained. After incubation at 27 ± 1^0 C for 12 days, the mycelial growth was harvested, washed, dried in hot air oven and the dry weights were recorded as described earlier.

Results and Discussion

Totally twelve isolates were collected from different northern parts of Karnataka. The isolation of pathogen was done using standard tissue isolation and the pure culture of the fungus was obtained by single spore isolation after eight days of inoculation. Based on cultural and spore morphology the fungus identified as *Alternaria carthami* and the isolates were coded as *Ac1* to *Ac12* with respect to their location names as Dharwad *(Ac1),* Hubli *(Ac2),* Navalagund *(Ac3),* Annigeri *(Ac4),* Kundagol *(Ac5),* Naragund *(Ac6),* Rona *(Ac7),* Hunagund *(Ac8),* Badami *(Ac9),* Bijapura *(Ac10),* Bagevadi *(Ac11)* and Muddebihal *(Ac12*). Further isolates were used for variability studies.

Cultural variability of *A. carthami* on different solid medium

The diversity in cultural characters of the isolates of *A. carthami* were subjected for their growth habit on different solid media. Among the four different media, host leaf extract agar has recorded significantly higher mean radial growth of 87.19 mm which was superior over other three media. Further, CZ (81.92 mm) and PDA (81.25 mm) both remained on par with each other while the lowest growth of the fungus was observed on RA (79.03 mm) respectively. Among different isolates, *Ac12* (88.25 mm) has recorded maximum mean radial growth, which was on par with *Ac5* (86.92 mm) followed by *Ac10* (84.50 mm), *Ac8* (84.42 mm) and *Ac11* (84.33 mm) while the least mean radial growth was recorded by *Ac4* (76.75 mm). With respect to isolates and media interaction, *Ac5* on RA (90.00 mm) showed maximum radial growth which was on par with *Ac12* on HLE (89.67mm). Result presented that the colour of colony varied from light grey to dark grey in HLE, PDA, CZ, media, whereas in RA it varied form creamy white to light pinkish. With respect to growth and margin, variations observed were from flat to rough raised and regular to smooth margin on all the four media. Further poor to good sporulation was recorded in HLE, whereas poor to moderate sporulation was observed on PDA and CZ while poor sporulation or no sporulation was seen in RA. Similar results were reported by Prasad *et al.*, (2012) who revealed that the maximum radial mycelia growth of *A. carthami* was more on skim milk agar medium followed by safflower leaf extract medium and Potato Carrot Agar medium and low growth was on Potato dextrose agar media. Sporulation was more on Potato carrot agar followed by safflower leaf extract agar media. The results are found in accordance with the reports of Somappa *et al.*, (2013) who observed the maximum radial growth on Czapeck's dox agar medium followed by PDA (Table 1).

Morphological variability of *A. carthami* on potato dextrose agar

The morphology of all the isolates varied with respect to the conidia size, beak length and number of vertical and horizontal septa. The conidia of different isolates varied in septation (0-4 vertical and 6-9 horizontal). The isolates A*c1, Ac2, Ac4, Ac5, Ac6, Ac8, Ac10* and *Ac11* showed maximum horizontal septa up to 7-9 on the contrary minimum horizontal septa (6-7) was observed in the remaining isolates. The isolates Ac2 showed maximum of 0-4 vertical septa whereas minimum vertical septa (0-2) were observed in remaining isolates. Among the isolates, Ac10 and Ac7 showed maximum size of 50.3- 101.3 x 10.2-24.3 µm and 52.5-85.2 x 8.6-27.3 µm, respectively. The least size of the conidia was observed in isolate Ac2 (29.6-84.4 x 9.1-16.3). The presence of conidial beak was seen in all the isolates, but there was variation in terms of length of beak. The isolate Ac12 showed maximum beak length of 51.8-96.4 µm while the least beak length

was observed in isolate Ac9 (26.4-30.2 µm). Similar results were reported by Prasad *et al.*, (2012) where the variations amongst isolates of *A. carthami* conidia were owing to the production of conidia in either solitary or short chains besides the size of conidia varied from 28 x 80 µm to 40 x 104 µm. The present morphological description corroborated the earlier findings on *A. carthami* by Chowdhury (1944) where the observation was light brown conidia of variable shape, occurring in chains of at least two and consisting of 3-11 cells, with up to six longitudinal septa, measuring about 36-99 x 12-28 µm and beak length ranges from 15-84 x 3-5 µm. Moreover, Mahabaleswarappa (1981) opined that the shape of the conidia varied from roughly spherical to elongate, light brown, slightly constricted at the septa with long beak (Table 2).

Table.1 Morphological variation among different isolates of *Alternaria carthami*

Name of isolate	Septation		Conidia size Length (µm) x Width (µm)	Beak Length (µm)
	Vertical	Horizontal		
Ac1	0-3	7-9	34.1-101.1 x 9.2-27.2	27.5-61.3
Ac2	0-4	7-9	29.6-84.4 x 9.1-16.3	32.8-44.6
Ac3	0-2	6-7	42.0-93.6 x 18.7 -27.6	28.4-59.9
Ac4	0-2	7-9	46.9-97.9x 10.5-25.0	38.0-80.6
Ac5	0-2	7-9	36.2-84.4 x 8.5-13.1	26.7-43.9
Ac6	0-2	7-9	42.0-93.5 x 18.6-27.6	36.7-59.2
Ac7	0-2	6-7	52.5-85.2 x 8.6-27.3	34.7-61.2
Ac8	0-3	7-9	37.3-96.2 x 10.3-28.3	42.6-71.2
Ac9	0-2	6-7	30.3-96.2 x 8.6-20.1	26.3-30.3
Ac10	0-2	7-9	50.3-101.3 x 10.2-24.3	39.7-56.2
Ac11	0-2	7-9	39.0-100.4 x 12.3-26.9	44.7-84.23
Ac12	0-2	6-7	37.3-96.6 x 10.6-17.0	51.8-96.4

Table.2 Growth of *A. carthami* isolates on different solid media

Name of isolate	Redial growth (mm)				Mean redial growth (mm)
	HLE	PDA	CZ	RA	
Ac1	88.00	75.00	82.67	68.00	**78.42**
Ac2	86.67	81.00	81.33	70.33	**79.83**
Ac3	81.00	88.00	70.00	77.33	**79.08**
Ac4	83.67	72.33	79.00	72.00	**76.75**
Ac5	88.67	84.67	84.33	90.00	**86.92**
Ac6	89.33	83.33	71.33	78.67	**80.67**
Ac7	88.33	76.00	85.67	74.67	**81.17**
Ac8	88.33	81.00	87.00	81.33	**84.42**
Ac9	86.33	81.33	81.00	86.67	**83.83**
Ac10	89.00	79.00	84.67	85.33	**84.50**
Ac11	87.33	85.67	89.33	75.00	**84.33**
Ac12	89.67	87.67	86.67	89.00	**88.25**
Mean	**87.19**	**81.25**	**81.92**	**79.03**	**82.35**
	Isolate (I)		**Media (M)**		**I x M**
S.Em. ±	0.54		0.31		1.09
CD at 1%	2.18		1.26		4.36

Note: HLE- Host leaf extract, PDA-Potato dextrose agar, CZ- Czapeck's Dox agar, RA- Richards' agar

Table.3 Effect of different temperature levels on the growth of *A. carthami* isolates

Name of Isolate	Temperature levels ^{0}C (Dry mycelial weight in mg)						Mean
	15	20	25	30	35	40	
Ac1	66.00	212.50	343.00	249.75	188.75	68.00	**188.00**
Ac2	58.75	156.00	291.50	231.50	122.50	71.25	**155.16**
Ac3	62.75	166.00	300.25	272.75	126.25	84.00	**168.66**
Ac4	66.25	150.50	283.25	240.25	87.50	79.50	**151.20**
Ac5	72.00	185.75	290.25	263.00	144.50	79.50	**172.50**
Ac6	69.75	208.25	284.50	249.00	138.00	66.75	**169.37**
Ac7	59.00	195.50	331.00	244.25	134.75	65.75	**171.87**
Ac8	66.25	174.75	241.00	284.75	130.50	85.75	**163.83**
Ac9	74.25	178.00	228.75	254.25	128.75	68.75	**155.45**
Ac10	67.25	208.75	231.25	309.25	172.50	69.75	**176.50**
Ac11	67.00	219.25	256.50	221.25	112.25	75.50	**158.62**
Ac12	60.50	143.50	322.75	235.70	92.75	72.25	**154.54**
Mean	**65.81**	**183.22**	**290.18**	**248.20**	**131.54**	**73.89**	**165.47**
	Isolate (I)		**Temperature (T)**		**I x T**		
S.Em. ±	1.00		0.70		2.45		
CD at 1%	4.01		2.83		9.83		

Table.4 Effect of different pH levels on the growth of *A. carthami* isolates

Name of Isolate	Different pH levels (Dry mycelial weight in mg)						Mean
	4	5	6	7	8	9	
Ac1	236.00	286.00	315.00	384.67	215.00	189.00	**270.94**
Ac2	130.67	233.67	289.33	385.00	235.00	131.33	**234.17**
Ac3	101.00	214.00	230.67	285.67	210.00	102.67	**190.67**
Ac4	88.00	239.33	284.67	365.67	216.00	131.00	**220.78**
Ac5	184.33	232.67	274.67	325.00	188.67	144.33	**224.94**
Ac6	146.00	267.33	288.00	395.33	247.67	190.00	**255.72**
Ac7	87.33	153.00	198.33	259.33	147.33	103.67	**158.17**
Ac8	187.67	235.67	276.33	261.33	178.67	95.33	**205.83**
Ac9	155.67	237.67	325.33	273.67	152.00	120.00	**210.72**
Ac10	247.67	289.33	315.00	425.33	255.00	203.67	**289.33**
Ac11	184.67	244.00	265.00	290.33	215.00	159.33	**226.39**
Ac12	179.67	204.00	283.00	326.67	220.67	171.67	**230.94**
Mean	**160.72**	**236.38**	**278.77**	**331.50**	**206.75**	**145.16**	**226.55**
	Isolate (A)			**pH (B)**			**A X B**
S.Em. ±	1.01			0.71			2.49
CD at 1%	4.05			2.86			9.92

Further, the conidia had transverse septa (up to 11) and longitudinal septa (up to 6), the total length ranged from 22.8-119.4 µm including of beak length with beak length being 8.8-52.5 µm and width of conidia 9.65-20.65 µm. Observations on morphological characteristics of *A. carthami*, dark grey colonies, conidiophores simple erect, septate, 40-80 µm length, conidia solitary, straight, size of conidia without beak 40-100 µm long, 10-15 µm thick, number of transverse septa 4-10 and longitudinal septa 4-7, beak with 2-4 septa and 30-65 µm length (Park and Lee, 2003) remained valid testimonials for the current results. The variations among the isolates were also observed by earlier workers (Mortensen and Bergman, 1983; Deokar and Raghuwanshi, 2002).

Physiological variability of *A. carthami*

Effect of different temperature levels on growth of *A. carthami* isolates

The growth of the isolates gradually increased from 15^0C to 25^0C and then showed reduction in dry mycelial weight from 30^0C onwards. Among different incubated temperatures, the highest mean dry mycelial weight was recorded at 25^0C (290.18 mg) followed by 30^0C (248.20 mg) while the lowest was observed at 15^0C (65.81 mg). Isolates *Ac8*, *Ac9* and *Ac10* showed good growth at 30^0C on the contrary rest of all the isolates showed good growth at 25^0C. The significant interaction difference was also observed between isolates and temperature. The highest dry mycelial weight recorded in *Ac1* (343.00 mg) at 25^0C which was significantly superior over other isolates and temperature combination and least dry mycelial weight was recorded at 15^0 C in isolate *Ac2* (58.75 mg).

The experimental results on different temperatures revealed that, all the isolates grew well at 25^0 C and also at 30^0 C. Therefore 25 to 30^0 C temperature was optimum for growth and development of the fungus. These results were in confirmation with the reports of Chowdhury (1944); Mahabaleswarappa (1981); Arumkumar (2006); Ramegowda and Naik (2008); Naik *et al.*, (2010); they also revealed that 25-30^0C was optimum for the growth of the fungus (Table 3).

Effect of different pH levels on growth of *A. carthami* isolates

The isolates, reactions and their interaction offered significant differences with respect to the dry mycelial weight of *A. carthami* at different pH level of the media. Growth of all the isolates gradually increased from acidic to neutral pH and then showed the drastic reduction at alkaline pH.

Among different pH levels, mean dry mycelial weight remained higher at pH 7 (331.5 mg), followed by pH 6 (278.8 mg) and the least dry mycelia weight was recorded at pH 9 (145.2 mg). The interaction between isolates and pH also resulted in significant variations with respect to dry mycelial weight. The growth of *Ac10* (425.3 mg) at pH 7 which was significantly superior over growth of other isolates and pH levels, followed by *Ac6* (395.3 mg), *Ac2* (385.0 mg) *Ac1* (384.7 mg) which were on par with each other. The least dry mycelia weight was recorded at pH 4 in *Ac7* (87.3 mg) which was on par with Ac4 (88.0 mg) at same pH as well as with *Ac8* (95.3 mg) at pH 9 (Table 4).

The present investigation revealed that the pathogen grows well in the pH range of 4-9, but optimum pH range for better growth and development of *A. carthami* was pH 6 to 7. The results were found similar with studies of Chowdhury (1944) who reported the maximum dry mycelial weight at pH 6, with

an optimum pH of 6-7 growth of *A. carthami* and also by Mahabaleswarappa (1981) who reported the maximum dry mycelial weight at pH 6. The earlier workers also observed significant growth was pH range of 6 to 7 (Arunkumar, 2006; Mesta, 2006).

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How to cite this article:

Madhu, M.R., M.P. Basavarajappa, I.D. Anand and Chidanand Lokapur. 2017. Morpho-Physiological Variability in *Alternaria carthami* Chowdhury Causing Safflower Leaf Spot. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 3244-3250. doi: https://doi.org/10.20546/ijcmas.2017.610.380