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Study on Cultural, Physiological and Nutritional Variability among Pathogen Isolates of *Alternaria helianthi*

Mahadevaswamy, G.[1*] Gangadharanaik G.[2] and Vijayalakshmi G.[3]

[1&2]Department of Plant Pathology, University of Agricultural and Horticultural Sciences, Shimoga, India

3Department of Plant Pathology, University of Agricultural Sciences, Bengaluru -560065, India

*Corresponding Author E-mail: msmahadevaswamy@gmail.com

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ABSTRACT

Alternaria leaf blight of sunflower is caused by Alternaria helianthi is an important disease of sunflower the four isolates of A. helianthi *designated as So, Ah1 Ah2 Ah3 and Ah4 were collected from different agroclimatic conditions and these isolates were characterized for cultural, nutritional and physiological studies. From the cultural studies it was observed that carrot agar medium supported good mycelial growth of all the isolates and the least was recorded with Czapek's dox agar medium both in solid as well as in liquid media. During the nutritional studies, among the carbon sources tested Maltose supported the maximum dry mycelial weight of 103.36 mg whereas, the least was recorded by Lactose (51.03 mg). Among the nitrogen sources, the maximum dry mycelial weight supported by Asperagine (60.22 mg) and the least dry mycelia weight was recorded by Ammonium chloride (34.16 mg). Physiological variability revealed that temperature has lot of influence on pathogen growth by influencing maximum dry mycelial weight at 30^{o} C (83.77 mg), whereas the least growth was found at 20^{0} C. The effect of pH on the growth of pathogen isolates revealed that pH 5 favoured good growth and development of all the isolates whereas least growth was observed at pH 4.*

Key words: *Alternaria helianthi, Cultural, Nutritional and Physiology studies.*

INTRODUCTION

Sunflower (*Helianthis annuus* L.) belongs to the family Asteraceae and it is one of the most important edible oilseed crop in India. It is a hardy and an herbaceous annual, which grows up to a height of 5 feet. The word *Helianthus* is derived from Greek word 'Helios' (sun) and 'anthos' (flower)[1]. Presently in India Sunflower crop is cultivated in an area of 0.487 million hectares with a production of 296 MT and a productivity of 608 kg/ha. The Alternaria leaf blight is a major disease of sunflower which causes an average yield and oil losses in India ranged from 28 to 80 per cent and 31 to 34 per cent, respectively[2]. In Karnataka, alternaria blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is one of the major diseases of sunflower. In northern Karnataka, Alternaria leaf blight is known to cause more than 80 per cent of yield loss under severe epiphytotic conditions[3].

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METERIAL AND METHODS

Collection and isolation of different isolates

The isolates collected from different districts of transitional zone of Karnataka and they have grouped into Ah1, Ah2, Ah3 and Ah4 based on the Morphological characters are utilized for further studies.

Cultural studies

Growth of *A. helianthi* isolates on solid media.

Four isolates of *A. helianthi* were grown on various solid media as well as liquid media *viz.,* Carrot agar, Czapek's agar, Oatmeal agar, Potato dextrose agar and Richard's agar. Five mm culture disc of ten days old actively growing *A. helianthi* was placed at the center of the Petri plate containing different media. The treatments were replicated three times. The radial growth from the center to the periphery of mycelia was measured and analyzed statistically.

Growth of *A. helianthi* isolates on Liquid media.

The study was carried out to see the variation among the isolates of *A. helianthi* with respect to their growth in different liquid broth media. All four isolates of *A. helianthi* were grown on carrot broth, Czapek's dox broth, Oatmeal broth, Potato dextrose broth and Richard's broth in three replications. Ten days old mycelial discs of 5 mm diameter were transferred aseptically into sterilized 100 ml flasks containing 30 ml of respective medium. They were incubated at 27 ± 1^0 C for 10 days. Each isolate was replicated thrice for a given medium. At the end of incubation period, the resulting growth of fungus was harvested and filtered through previously weighed Whatman No.1 filter paper and washed thoroughly with distilled water. It was dried at 40^{o}C for two days in hot air oven and weights were recorded.

Nutritional and Physiological variability

Effect of different carbon sources on the growth of *A. helianthi.*

Six carbon sources were taken by replacing them in Richard's broth solution. The carbon sources are viz., glucose, fructose, sucrose, maltose, dextrose and lactose. The pH of the medium is adjusted to 7.0 by using 0.1N sodium hydroxide or 0.1N hydrochloric acid.

Effect of different nitrogen sources on the growth of *A. helianthi*

Various nitrogen sources were taken by replacing them in Richard's broth solution. The nitrogen sources viz., ammonium nitrate, ammonium sulphate, ammonium chloride, asparagine and urea. The pH of the medium is adjusted to 7.0 by using 0.1N sodium hydroxide or 0.1N hydrochloric acid.

Physiological studies

Effect of different temperature levels on the growth of isolates of *A. helianthi*

Growth of each isolate was tested at 20, 25, 30, 35 and 40 ^{0}C. During the investigation 30 ml of potato dextrose broth was poured into 100 ml conical flask and sterilized. Five mm diameter of ten days old mycelial discs of all the isolates of *A. helianthi* were incubated at different temperatures. Cultures were filtered through Whatman No.1 filter paper and washed thoroughly with distilled water. It was dried at 40^{o}C for two days in hot air oven and dry mycelia weight was recorded.

Effect of different pH levels on the growth of isolates of *A. helianthi*

Isolates of *A. helianthi* were grown on the potato dextrose broth at different pH levels of viz., 4.0, 5.0, 6.0, 7.0, and 8.0. The pH levels were adjusted by adding 1 N alkali (NaOH) or acid (HCl). Ten days old, five mm mycelial discs of all the isolates were inoculated separately into conical flasks containing 30 ml medium at different pH levels. For each treatment three replications were maintained. The flasks were after incubated at 27 ± 1^0 C for 10 days, the mycelial growth was harvested, washed, dried in hot air oven and the dry weight was recorded as described earlier

RESULTS AND DISCUSSIONS

Cultural studies

Growth of A. helianthi on different solid media:

The four isolates of *A. helianthi viz.,* Ah1, Ah2, Ah3 and Ah4 were studied for their growth habit on different solid media *viz.,*

carrot agar, Czapek's dox agar, Oat meal agar, Potato dextrose agar and Richard's agar (Table 1). The results obtained from data revealed that, Among them isolate Ah1 showed highest radial growth on carrot agar (71.83 mm) and found significantly superior over other medium tested. However it is found on par with PDA (67.57 mm). Isolate Ah2 showed a radial growth of 80.00 mm and differed significantly compare to oatmeal agar (74.77 mm), PDA (68.80 mm), Czapek's dox agar (66.37 mm) and Richard's agar (59.83 mm) medium. The growth studies of isolate Ah3 revealed that Carrot agar supported maximum radial growth (81.00 mm) and found significantly superior over other medium tested *viz.,* PDA and Oatmeal agar at 76.97 mm and 76.70 mm respectively, however the least growth was recorded on Richard's agar (66.08 mm). Isolate Ah4 recorded the maximum radial growth of 78.67 mm on Carrot agar followed by PDA and Oatmeal agar at 77.57 mm and 76.53 mm respectively and were on par with each other and significantly differed from other medium. However Czapek's dox agar recorded least growth of 60.70 mm.

Growth of A. helianthi on different liquid broth media:

Effect of different broth culture medium *viz.,* Carrot broth, Czapek's dox broth, oat meal broth, Potato Dextrose Broth and Richard's broth on growth of different isolates of *A. helianthi* was studied and the data is presented in Table 2. The growth studies revealed that among four isolates tested isolate Ah1 recorded highest dry mycelial weight of 68.40 mg on PDB, which is on par with carrot broth with a dry mycelial weight of 66.37 mg and differed significantly from Richard's broth (55.87 mg) and Oatmeal broth (49.20 mg). However, least dry mycelial weight was recorded on Czapek's dox broth (42.03 mg). The carrot broth supported the maximum dry mycelial weight of isolate Ah2 (75.70 mg) and significantly differed with other culture medium tested. Followed by Oat meal broth, PDB and Richard's broth at 66.43 mg, 63.47 mg and 57.77 mg respectively. However the least dry mycelial weight was found in Czapek's dox broth (51.60 mg). Isolate Ah3 recorded the maximum dry mycelial weight (88.90 mg) on carrot broth whereas; the least dry mycelial weight was recorded on Richard's broth (69.17 mg) medium. The highest dry mycelial weight of 80.70 mg was recorded with Ah4 on Carrot broth which was on par with PDB (80.53 mg) and found significantly superior over all other media tested *viz.,* Czapek's dox broth (69.87 mg), Richard's broth (67.00 mg), and Oatmeal broth with 60.91 mg.

Carrot agar medium not only supported maximum radial growth of all the isolates tested, even supported increased dry mycelial weight of these isolates when grown in liquid media. Whereas least growth was recorded on Czepek's dox agar. This may be attributed to effective utilization of inherent complex nature of natural media by *A. helianthi* isolates. This is in agreement with the observation made by the author[4] who observed carrot broth supporting good fungal growth owing to provision of some additional nutrients.

Nutritional study

Effect of different carbon sources

The four isolates of *A. helianthi* were studied for their growth response to different carbon sources by replacing them in Richard's broth (Table 3). The quantity of each carbon compound tried was determined on the basis of their molecular weight so as to provide equivalent amount of carbon as that of sucrose present in the basal medium. The carbon compounds used were Glucose, Fructose, Lactose, Maltose, Dextrose and Mannitol.

The data obtained thus revealed that among different carbon sources tested dextrose supported the highest dry mycelial weight of isolate Ah4 (90.93 mg) which differed significantly compared to Ah3 (81.87 mg) and Ah1 (79.72 mg) whereas, isolate Ah2 (70.96 mg) recorded the lowest dry mycelial weight. Glucose as a carbon source, the isolate Ah4 showed highest dry mycelial weight of 96.52 mg which is significantly superior over other isolates *viz.,* Ah3, Ah2, with a mean dry mycelial weight of 91.15 mg, 75.86 mg

respectively However the mean minimum dry mycelial weight was recorded by Ah1 (72.33 mg). Isolate Ah4 effectively utilized Mannitol as a carbon source and recorded the highest dry mycelial weight of 98.40 mg and found significantly superior over other isolates tested *viz.,* Ah3 (73.09 mg), Ah2 (66.18 mg) and Ah1 (65.41 mg). In Maltose as a carbon source the maximum dry mycelial weight was recorded with isolate Ah4 (103.36 mg) which differed significantly compared to other isolates. However the isolate Ah2 recorded least dry mycelial weight of 56.58mg. Fructose as a carbon source supported the maximum dry mycelial weight of isolate Ah3 (87.00 mg) which is significantly superior over other isolates tested followed by Ah4 (84.37 mg) and Ah1 (64.19 mg) whereas, the lowest dry mycelial weight was recorded by isolate Ah2 (57.24 mg). The maximum dry mycelia weight of 75.07 mg was recorded by isolate Ah2 when Lactose was used as a carbon source which is significantly superior over other isolates tested followed by Ah4 (72.08 mg), Ah3 (60.15 mg) and Ah1 (51.03 mg) respectively.

These findings are well supported by the different authors[5,6] who found that growth of *A. alternata* was maximum on maltose followed by sucrose, starch, glucose and least dry weight was obtained in lactic acid because of poor utilization of lactic acid as it is less rapidly available to the fungus.

Effect of different nitrogen sources:

The data obtained thus revealed that ammonium nitrate, was efficiently utilized by the isolate Ah3 showing highest dry mycelial weight of 49.51 mg followed by Ah4 (47.15 mg) and were on par with each other and significantly superior over Ah1 (43.41 mg) and Ah2 (45.36 mg).Ah3 utilizes maximum quantity of ammonium sulphate as a nitrogen source and recorded maximum dry mycelial weight of 53.22 mg which is significantly superior over other isolates followed by Ah4 (45.97 mg). However Ah1 recorded the least dry mycelial weight of 37.23 mg followed by Ah2 (38.18 mg) Asperagine as a nitrogen source favoured the growth of isolate Ah3 (60.22 mg) and is on par with Ah1 (58.23 mg) and found significantly superior over other isolates Ammonium chloride as a source of nitrogen was efficiently utilized by isolate Ah4 and recorded highest dry mycelial weight of 51.47 mg and found significantly superior over other isolates *viz.,* Ah2, Ah3 which recorded the dry mycelial weight of 43.77 mg and 43.70 mg respectively. However Ah1 recorded the least dry mycelial weight of 34.16 mg. On urea the isolate Ah2 recorded the highest dry mycelial weight of 51.20mg and was significantly superior, over others. However isolate Ah3 recorded lowest dry mycelial weight of 40.63 mg and was on par with Ah4 (42.87 mg).

Among the nitrogen sources tested maximum dry mycelial weight was supported by Asperagine (60.22 mg) with isolate Ah3 and the least dry mycelial weight was recorded with Ammonium chloride (34.16 mg) by isolate Ah1. The same was also in agreement with the findings obtained by authors[8] and Threonine supported maximum dry mycelial weight of *A. solani* followed by Asparagine[7].

Physiological studies

Effect of different temperature:

All the four isolates were studied for their growth potential at different temperature levels on Richard's broth medium and the results are presented in Table 5.

From the result it was observed that at 20^0 C the isolate Ah3 recorded the maximum dry mycelial weight of 54.58 mg which was on par with Ah4 (53.15 mg) however it was found significantly superior over Ah2 (46.76 mg) and Ah1with dry mycelial weight of 38.63 mg. Isolate Ah3 recorded highest dry mycelial weight of 67.00 mg at 25^0 C and found significantly superior over other isolates. However isolates Ah4, Ah2 and Ah1 recorded the dry mycelial weight of 60.80 mg, 56.74 mg, and 45.62 mg respectively. At 30^0 C isolate Ah4 responded well and recorded the highest dry mycelial weight of 83.77 mg and was on par with Ah3 (82.21 mg) whereas, Ah2 recorded dry mycelial weight of 72.30 mg followed by isolate Ah1 with the least dry mycelial weight of 66.41 mg. At 35^0 C isolate Ah3 responded well with the highest dry mycelial weight of 71.41 mg which was on par

with Ah2 (71.26 mg) and differed significantly with other isolates. Among the isolates tested isolate Ah3 recorded the maximum dry mycelial weight of 42.09 mg at 40^0 C and was on par with Ah4 (40.73 mg), and differed significantly over other.

Among the isolate tested at different temperatures levels, the highest mean dry mycelial weight was recorded at temperature 30^0 C (76.17 mg) showed significantly superior over 35^0 C (66.84 mg),25^0 C (57.54 mg) and 20^0 C (48.28 mg), whereas the least dry mycelial weight of 37.03 mg recorded at 40^0 C.

These findings were confirmation with the results obtained by the author[6] recorded highest dry mycelial weight at temperature 30^0 C followed by 25^0C and 35^0 C, while at 20^0 C least dry mycelial weight was recorded. Similar findings the fungus grew well from 18-30^0C, but the growth was more rapid at 28^0C and 30^0C[9].

Effect of different pH level:

Effect of different pH level on the growth of four isolates in terms of dry mycelial weight at different pH range tested *viz.*, 4, 5, 6, 7 and 8 the data are presented in Table 6. The data obtained from the observation revealed that there was a significant difference among the isolates with respect to pH requirement.

Among the different pH level tested, isolate Ah3 responded well to pH 4.00 and recorded the maximum dry mycelial weight of 58.41mg and differed significantly other isolate *viz.*, Ah4 (55.22 mg), Ah2 (50.11 mg) and Ah1 at 42.18 mg. The isolate Ah4 was very well responded to the change in H^+ ion concentration with the maximum dry mycelial weight of 86.61 mg at pH 5.00 and found significantly superior over other isolates. However isolate Ah1 showed least response with the lowest dry mycelial weight of 54.13 mg. At pH 6.00 the maximum dry mycelial weight was recorded by isolate Ah3 (76.96 mg) and was significantly superior over other isolates followed by Ah4 (71.02 mg), which is on par with Ah1 (70.97 mg). Whereas isolate Ah2 (67.75 mg) recorded the least dry mycelial weight. The maximum dry mycelial weight was recorded at pH 7.00 by the isolate Ah3 (66.15 mg) which differed significantly from Ah4 (59.43 mg) and Ah2 (54.99 mg). However, the least dry mycelial weight was recorded by Ah1 (50.37 mg). At pH 8.00 the isolate Ah3 (56.50 mg) recorded the maximum dry mycelial weight which was superior over other isolates followed by Ah4, Ah2 which recorded the dry mycelial weight of 53.18 mg and 49.40 mg respectively. However Ah1 recorded the least dry mycelial weight of 45.60 mg.

Among the different pH levels tested, the highest mean dry mycelial weight was recorded at pH 5 (72.51 mg) and was on par with pH 6 (71.68 mg) but significantly superior over all other pH levels. Least dry mycelial weight was recorded at pH 8 (51.17 mg) and was on par with pH 4 (51.48 mg). These results indicating wider adoptability of different isolates to different pH level and their virulence. This result was confirmed by authors[10,7,8] found that maximum dry mycelial weight was observed at pH 6.0 followed by pH 5.0 and the minimum dry mycelial weight was recorded at pH 8.0 by isolates of *A. alternata*.

Table 1 Growth of *A. helianthi* isolates on different solid media

Sl. no	Isolates	Media				
		Richard's agar	Oatmeal agar	Carrot Agar	PDA	Czapek's dox agar
		Radial growth(mm)				
1	Ah1	66.25	66.87	71.83	67.57	64.37
2	Ah2	59.83	74.77	80	68.8	66.37
3	Ah3	66.08	76.7	81	76.97	68.9
4	Ah4	72	77.57	78.67	76.53	60.7
Mean		66.04	73.98	77.88	72.47	65.08
		S.Em±		CD at 1%		
Isolates (I)		0.77		2.97		
Media (M)		0.69		2.66		
I x M		1.55		5.95		

Table 2. Growth of *A. helianthi* isolates on different liquid media

Sl. no	Isolates	Media				
		Richard's broth	Oat meal broth	Carrot broth	PDA	Czapek's dox broth
		Dry mycelial weight (mg)				
1	Ah1	55.87	49.2	66.37	68.4	42.03
2	Ah2	57.77	66.43	75.7	63.47	51.6
3	Ah3	69.17	72.7	88.9	83.97	73.63
4	Ah4	67	60.91	80.7	80.53	69.87
Mean		62.45	62.31	77.92	74.09	59.28

	S.Em±	CD at 1%
Isolates (I)	0.71	2.71
Media (M)	0.63	2.42
I x M	1.42	5.43

Table 3. Effect of different Carbon sources on the growth of *A. helianthi* isolates

Sl. no	Isolates	Carbon sources					
		Dextrose	Glucose	Mannitol	Maltose	Fructose	Lactose
		Dry mycelial weight (mg)					
1	Ah1	79.72	72.33	65.41	70.24	64.19	51.03
2	Ah2	70.96	75.86	66.18	56.58	57.24	75.07
3	Ah3	81.87	91.15	73.09	99.52	87	60.15
4	Ah4	90.93	96.52	98.4	103.36	84.37	72.08
	Mean	80.87	83.96	75.77	82.43	73.2	64.25

	S.Em±	CD at 1%
Isolates (I)	0.61	2.32
Media (M)	0.5	1.89
I x M	1.22	4.63

Table 4. Effect of different Nitrogen sources on the growth of *A. helianthi* isolates

Sl. no	Isolates	Nitrogen sources				
		Ammonium nitrate	Ammonium sulphate	Asperagine	Ammonium chloride	Urea
		Dry mycelial weight (mg)				
1	Ah1	43.41	37.23	58.23	34.16	46.2
2	Ah2	45.36	38.18	53.04	43.77	51.2
3	Ah3	49.51	53.22	60.22	43.7	40.63
4	Ah4	47.15	45.97	51.43	51.47	42.87
Mean		46.36	43.65	55.73	43.28	45.23

	S.Em±	CD at 1%
Isolates (I)	0.67	2.56
Media (M)	0.55	2.09
I x M	1.35	5.13

Table 5. Effect of different temperature levels on the growth of *A. helianthi* isolates

Sl. no	Isolates	Temperature (^{0}C)				
		20	25	30	35	40
		Dry mycelial weight (mg)				
1	Ah1	38.63	45.62	66.41	55.51	30.6
2	Ah2	46.76	56.74	72.3	71.26	34.7
3	Ah3	54.58	67	82.21	71.41	42.09
4	Ah4	53.15	60.8	83.77	69.16	40.73
Mean		48.28	57.54	76.17	66.84	37.03
		S.Em±		CD at 1%		
Isolates (I)		0.7		2.69		
Media (M)		0.63		2.4		
I x M		1.41		5.38		

Table 6. Effect of different pH levels on the growth of *A. helianthi* isolates.

Sl. no	Isolates	pH				
		4	5	6	7	8
		Dry mycelial weight (mg)				
1	Ah1	42.18	54.13	70.97	50.37	45.6
2	Ah2	50.11	66.58	67.75	54.99	49.4
3	Ah3	58.41	82.72	76.96	66.15	56.5
4	Ah4	55.22	86.61	71.02	59.43	53.18
Mean		51.48	72.51	71.68	57.73	51.17
		S.Em±		CD at 1%		
Isolates (I)		0.67		2.57		
Media (M)		0.6		2.3		
I x M		1.34		5.14		

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