JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2016. Vol. **10**(4), p. 2697-2706

Cultural, Morphological and Pathogenic Variability in Different Isolates of *Alternaria carthami* of Safflower from Different Geographical Regions of Maharashtra

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http://dx.doi.org/10.22207/JPAM.10.4.28

(Received: 10 September 2016; accepted: 01 October 2016)

Cultural, morphological and pathogenic studies revealed a wide range of variability of among the 20 isolates of *A. carthami* of safflower which represented four geographic regions of the state of Maharashtra. All 20 test isolates exhibited a great cultural variability in mycelial growth, colony colour, growth speed, colony shape and sporulation. Significantly highest mycelial growth was found in isolate AcHl (90.00 mm), followed by AcBl (89.67 mm) and AcAn (88.83 mm). Among morphological variability in respect of mycelial width, conidial dimensions, beak length and number of vertical and horizontal septa eight isolates exhibited large mycelial width (7.05 to 9.80 μm), six isolates with medium sized with mycelium width (5.40 to 6.46 μm) and six with small sized mycelium width (3.25 to 4.86 μm). On the basis of average conidial dimensions (length X breadth), the test isolates were categorized into three groups *viz*., large, medium and small sized conidia were found in nine isolates of which average length ranged from 36.37 to 50.32 μm and width from 13.06 to 17.19 μm; seven isolates were with medium sized conidia (26.80-35.37 X 9.94-14.73 μm) and rest four isolates with small sized conidial (22.45-25.17 X 7.28-9.27 μm). About eight isolates exhibited long beak length (11.05 to 16.43 μm), seven isolates with medium beak length (8.13 to 11.27 μm) and rest five isolates were of short beak length (6.04 to 7.46 μm). Among the test isolates, horizontal septation on conidia was ranged from 1 to 12 and vertical septation from 0 to 3. All of the 20 isolates of *A. carthami* exhibited a wide range of pathogenic variability. However, the aggressive isolates (*viz*., AcHl, AcBl, AcAn, AcJg, etc.) showed least incubation period, highest leaf spot frequency with maximum sized leaf spots.

Keywords: *Alternaria carthami*, safflower, cultural, morphological, pathogenic variability.

Safflower is the most important *rabi* oilseed crops grown in India. In general, the crops have low average productivity due to the prevalence of various biotic and abiotic stresses. Diseases are among the major biotic stresses. *Alternaria* blight caused by *Alternaria carthami* is an important and a widespread destructive disease of safflower. Depending on the severity of the disease, yield losses as high as 25-60% have been reported in India (Singh and Prasad, 2005).

The *Alternaria* spp. are one of the widely distributed phytopathogens infecting a wide range of agronomical and horticulture crop plant species, cultivated worldwide. In *Alternaria* spp., the existence of a high level of variability *viz*., cultural, morphological and pathogenic etc. have earlier been reported by several workers (Rajender *et al*., 2013; Sharma *et al*., 2013; Giri *et al*., 2014; Nikam *et al*., 2015). The variants within population of the

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phytopathogen may certainly affect the rate of disease development and can induce infections even in disease tolerant or resistant host plant species. To develop an effective programme of breeding for disease resistance comprehensive understandings of pathogenic, morphological, cultural and pathogenic variability are essential. Therefore, in present studies, cultural, morphological and pathogenic variability of *A. carthami* isolates collected from various agro-climatic zones and geographical regions of state of Maharashtra was attempted.

MATERIALS AND METHODS

Cultural, morphological and pathogenic variability among *A. carthami* isolates

Cultural variability among *A. carthami* isolates

Twenty test isolates of *A. carthami* obtained one each from twenty districts under various geographical regions of the Maharashtra state were aseptically inoculated on PDA medium separately, maintaining three plates / isolate and incubated at 28±2 ^{0}C, in an BOD incubator. Observations on cultural characteristics *viz*., colony diameter, colony colour / pigmentation, appearance, growth rate, shape, margin and zonation were recorded after a week of incubation and sporulation was recorded at 15 days of incubation.

Morphological variability among *A. carthami* isolates

The morphological characteristics *viz*., hyphal width, size of conidia, length of beak of each test isolate (10 days old pure culture growth on PDA) were recorded by measuring with ocular micrometer, which was calibrated using stage micrometer, by applying standard procedure given by Aneja (2001) under the compound microscope (make: Labomed Vision 2000) at 400x magnification and under 10 random microscopic fields. Similarly, numbers of transverse and longitudinal septa / conidium were counted.

Pathogenicity assay and pathogenic variability among of *A. carthami* isolates

In order to confirm identification of the disease and its causal agent, the pathogenicity test was attempted in pot culture under screen house conditions. Seeds of safflower cv. Manjira susceptible to Alternaria blight (*A. carthami*) were surface sterilized with 0.1 % HgCl2 and sown (@ 10 seeds/pot) in the earthen pots (30 cm dia.) filled with steam sterilized potting mixture of soil : sand : FYM (2 : 1 : 1). After two week, two healthy growing safflower seedlings per pot were maintained, watered regularly and kept in the screen house for further growth and development. The spore-cum-mycelial suspensions of *A. carthami* test isolates was prepared separately from 10 days pure culture in plates, by flooding with 5-10 ml sterile distilled water. This resultant suspension was suitably diluted with sterile distilled water to get inoculum concentration of 2 x 106 spores/ml. Thirty days old seedlings (2 / pot/ isolate / replication) of safflower cv. Manjira growing in earthen pots were artificially spray inoculated the spore-cum-mycelial suspension separately of the test isolates (Giri *et al*., 2013), the experiment was planned with CRD and all the test isolates replicated thrice. Safflower cv. Manjira seedlings grown in earthen pots and sprayed with sterile water (without inoculum) were maintained as uninoculated suitable control. These pots (both inoculated and uninoculated) were covered with polythene bags during evening hours and kept overnight, watered regularly to create optimum relative humidity and maintained in screen house for development of the disease symptoms.

From the artificially inoculated and Alternaria blight diseases safflower leaves the test isolates were reisolated separately on PDA medium and incubated at 28±2 0C. After a week of incubation the cultural and morphological characteristics developed of the test isolates were observed and compared with the characteristics (cultural and morphological) of the original test isolates obtained from naturally Alternaria blight diseased safflower foliage. To satisfy Koch's postulates, symptoms developed on artificial inoculated safflower leaves were compared with original symptoms on naturally diseased plants. Observations on incubation period (days to expression of initial symptoms), number of lesions / plant and diameter of the lesions were recorded.

RESULTS AND DISCUSSION

Cultural variability among *A. carthami* isolates

The results obtained on cultural characteristics *viz*., colony / mycelial growth,

growth rate, colony appearance, margin, sporulation and concentric zonation etc in respect of 20 test isolates of *A. carthami* grown on PDA are presented in Table 1 and depicted in Plate 1.

Mycelial growth

The results (Table 1) indicated that among the test isolates, mycelial growth was varied from 65.17 mm (AcDl) to 90.00 mm (AcHl). However, it was significantly highest in isolate AcHl (90.00 mm), followed by the isolates *viz*., AcBl (89.67 mm), AcAn (88.83 mm) all three were at par, AcJg (88.00 mm), AcPb (87.00 mm) both were at par, AcNd (86.50 mm), AcWs (85.50 mm) both were at par and AcOs (83.67 mm). In rest of the test isolates, mycelial growth was ranged from 65.17 mm to 79.33 mm; however, it was moderate in the isolate AcNs (71.17 mm), followed by AcSl (72.33 mm) and AcJl (73.00), all were at par and it was significantly minimum in AcDl (65.17 mm).

Colony colour

On the basis of colony colour, the test isolates were categorized into eight groups. Group I consisted four isolates with creamy white colony (AcPb, AcOs, AcSl and AcJg), group II contained of four isolates with creamy black colony (AcNd, AcAb, AcAn and AcDl), group III consisted two isolates with off white colony (AcHl and AcSt), group IV consisted two isolates with grayish black colony (AcBd and AcYt), group V consisted two isolates with light gray colony (AcJl and AcNs), group VI consisted three isolates with brown colony (AcWs, AcAk and AcBl), group VII consisted two isolates with olivaceous black colony (AcLt and AcNb) and group VIII consisted a single isolate with yellowish colony (AcAm).

Colony growth rate

On the basis colony growth rate, the test isolates were categorized as fast growing, moderate growing and slow growing. In fast growing category, the seven isolates included were AcPb, AcNd, AcHl, AcWs, AcAn, AcBl and AcJg; medium / moderate growing category the six isolates included were AcSt, AcOs, AbBd, AcAb, AcAk and AcLt and in slow growing category the seven isolates included were AcJl, AcYt, AcDl, AcAm, AcNb, AcNs and AcSl.

Colony shape and margin

On the basis of colony shape (circular or irregular) and colony margin (smooth or rough), the test isolates were categorized into two groups. The group I included about 11 isolates with circular colony and smooth margin, which were AcPb, AcNd, AcHl, AcBd, AcJl, AcYt, AcDl, AcBl, AcAm, AcNb and AcJg. The group II included the isolates with irregular colony and rough margin, which contained rest of the nine isolates *viz*., AcSt, AcOs, AcAb, AcWs, AcAk, AcLt, AcAn, AcNs and AcSl.

Sporulation and concentric zonation

The sporulation induced by the test isolates varied from poor (+) to excellent (++++). However, it was excellent (++++) in about eight isolates *viz*., AcNd, AcHl, AcAb, AcYt, AcWs, AcAn, AcBl and AcJg; good (+++) in seven isolates *viz*, AcPb, AcOs, AcBd, AcJl, AcAk, AcLt and AcNs and fair (++) in five isolates *viz*., AcSt, AcDl, AcAm, AcNb and AcSl. The zonation was present in about 11 test isolates *viz*., AcPb, AcNd, AcHl, AcBd, AcJl, AcYt, AcDl, AcAm, AcBl AcNb and AcJg; while, it was absent in rest of the 9 test isolates *viz*., AcSt, AcOs, AcAb, AcWs, AcAk, AcLt AcAn, AcNs and AcSl.

Morphological variability among *A. carthami* isolates

Results (Table 2 and Plate 2) revealed that all the 20 test isolates of *A. carthami* exhibited a wide range of variability in respect of mycelial width, conidial dimensions, beak length and number of vertical and horizontal septa.

Mycelial width

On the basis of mycelial width, the test isolates were categorized into three groups *viz*., large, medium and small sized mycelium. About eight isolates exhibited large mycelial width in the range of 7.05 to 9.80 µm. However, it was maximum in the isolate AcHl (9.80 µm), followed by AcBl (9.25 µm), AcAn (8.46 µm) and AcJg (8.15 µm); while, in rest of the four isolates (AcPb, AcWs, AcNd and AcOs) the mycelial width was ranged from 7.05 to 7.81µm.

In medium sized mycelium category, six isolates were grouped with mycelial width in the range 5.40 to 6.46 µm. However, maximum mycelial width was recorded in AcLt (6.46 µm) isolate, followed by the isolates *viz*., AcAk (6.30 µm), AcBd (6.10 µm), AcSt (5.89 µm), AcAb (5.70 µm) and AcYt (5.40 µm).

In small sized mycelium category, about six isolates were grouped with mycelial width in the range of 3.25 to 4.86 µm. However, it was maximum in the isolate AcJl (4.86 µm), followed by

Isolates of *A. carthami*, representing 20 districts of the Maharashtra state

Sr. No.	Districts	*A. carthami* Isolates	Agro-Climatic Zone	Av. Rainfall (mm)
Marathwada Region (08)				
1	Parbhani	AcPb	Assured Rainfall Zone (7)	700-900
2	Nanded	AcNd	Central Vidharbha Zone (8)	900-1150
3	Hingoli	AcHl	Central Vidharbha Zone (8)	900-1150
4	Latur	AcLt	Assured Rainfall Zone (7)	700-900
5	Osmanabad	AcOs	Assured Rainfall Zone (7)	700-900
6	Beed	AcBd	Scarcity Zone (6)	<700
7	Aurangabad	AcAb	Assured Rainfall Zone (7)	700-900
8	Jalna	AcJl	Assured Rainfall Zone (7)	700-900
Vidharbha Region (05)				
9	Buldana	AcBl	Assured Rainfall Zone (7)	700-900
10	Washim	AcWs	Central Vidharbha Zone (8)	900-1150
11	Akola	AcAk	Assured Rainfall Zone (7)	700-900
12	Amaravati	AcAm	Assured Rainfall Zone (7)	700-900
13	Yavatmal	AcYt	Central Vidharbha Zone (8)	900-1150
Khandesh Region (04)				
14	Jalgaon	AcJg	Assured Rainfall Zone (7)	700-900
15	Dhule	AcDl	Scarcity Zone (6)	<700
16	Nandurbar	AcNb	Western Maharashtra Plain Zone (5)	700-1200
17	Nasik	AcNs	Scarcity Zone (6)	<700
Western Maharashtra Region (03)				
18	Ahmadnagar	AcAn	Scarcity Zone (6)	<700
19	Satara	AcSt	Western Maharashtra Plain Zone (5)	700-1200
20	Solapur	AcSl	Scarcity Zone (6)	<700

AcSl (4.54 µm), AcNs (4.10 µm), AcAm (3.78 µm), AcNb (3.53 µm) and AcDl (3.25 µm).

Conidial size

The results revealed that the test isolates, exhibited a wide range of variability in respects of average conidial dimensions (length X breadth) and on the basis of which they were categorized into three groups *viz*., large, medium and small sized conidia.

In large sized conidial group, about nine isolates were included with their average conidial length in the range of 36.37 to 50.32 µm and their average width in the range of 13.06 to 17.19 µm. Among these isolates, highest average conidial size (length X breadth) was recorded in the isolate AcAk (50.32 X 15.02 µm), followed by the isolates *viz*., AcLt (45.80 X 14.62 µm), AcBd (41.45 X 16.06 µm), AcBl (40.35 X 13.06 µm), AcAb (39.40 X 15.31 µm), AcAn (39.11 X 13.56 µm) and AcPb (38.30 X 15.62 µm). Rest of three isolates in this group recorded conidial size in the range of 36.37-37.55 X 16.35-17.19 µm.

In the medium sized conidial group, seven isolates were included, of which average conidial size was ranged from 26.80-35.37 X 9.94-14.73 µm. Among these isolates, maximum average conidial size was recorded in the isolate AcAm (35.37 X 9.94 µm), followed by the isolates *viz*., AcJl (32.27 X 12.76 µm), AcYt (30.00 X 12.27 µm) and AcNd (29.38 X 11.24 µm). Rest of the three isolates in this group recorded average conidial size in the rage of 26.80-28.20 X 10.78-14.30 µm.

In small sized conidial group, only four isolates were grouped, of which average conidial size was ranged from 22.45-25.17 X 7.28-9.27 µm. Among these isolates, maximum conidial size was recorded in the isolate AcSt (25.17 X 7.93 µm), followed by the isolates *viz*., AcSl (23.92 X 9.27 µm) and AcNs (23.57 X 8.44 µm); while it was least in the isolate AcDl (22.45 X 7.28 µm).

Table 1. Cultural variability among the isolates of *A. carthami*

Characters	Isolates AcPb (Parbhani) 1	AcNd (Nanded) 2	AcHl (Hingoli) 3	AcSt (Satara) 4	AcOs (Osmanabad) 5	AcBd (Beed) 6	AcAb (Aurangabad) 7	AcJl (Jalna) 8	AcYt (Yavatmal) 9	AcWs (Washim) 10
Colony Dia. (mm)	87.00	86.50	90.00	77.83	83.67	79.33	75.67	73.00	74.50	85.50
Colour	Creamy white	Black	Off white	Off white	Creamy white	Grayish / black	Black	Light gray	Grayish black	Brown
Appearance	Cottony	Cottony	Cottony	Cottony	Fluffy	Feathery	Feathery	Feathery	Fluffy	Fluffy
Growth speed	Fast	Fast	Fast	Medium	Medium	Medium	Medium	Slow	Slow	Fast
Shape	Circular	Circular	Circular	Irregular	Irregular	Circular	Irregular	Circular	Circular	Irregular
Margin	smooth	smooth	Smooth	rough	rough	smooth	rough	smooth	smooth	rough
Sporulation	+++	++++	++++	++	+++	+++	++++	+++	++++	++++
Zonation	Present	Present	Present	Absent	Absent	Present	Absent	Present	Present	Absent
Characters	AcAk (Akola) 11	AcLt (Latur) 12	AcAn (Ahmadnagar) 13	AcDl (Dhule) 14	AcBl (Buldana) 15	AcAm (Amravati) 16	AcNb (Nandurbar) 17	AcNs (Nasik) 18	AcSl (Solapur) 19	AcJg (Jalgaon) 20
Colony Dia. (mm)	80.17	81.83	88.83	65.17	89.67	68.83	67.00	71.17	72.33	88.00
Colour	Brown	Olivaceous black	Black	Black	Brown	Yellowish	Olivaceous black	Light gray	Creamy white	Creamy white
Appearance	Feathery	Fluffy	Feathery	Feathery	Fluffy	Cottony	Feathery	Fluffy	Cottony	Cottony
Growth	Medium	Medium	Fast	Slow	Fast	Slow	Slow	Slow	Slow	Fast
Shape	Irregular	Irregular	Irregular	Circular	Circular	Circular	Circular	Irregular	Irregular	Circular
Margin	Rough	Rough	Rough	Smooth	Smooth	Smooth	Smooth	Rough	Rough	Smooth
Sporulation	+++	+++	++++	++	++++	++	++	+++	++	++++
Zonation	Absent	Absent	Absent	Present	Present	Present	Present	Absent	Absent	Present

Sporulation : ++++ = Excellent, +++ = Good, ++ = Fair, + = Poor, Dia : Diameter
Colony / mycelial growth : SE± : 0.46, CD (P = 0.01) : 1.29

Table 2. Morphological variability among the isolates of *A. carthami*

Sr. No.	Isolates /Districts	Av. Mycelial width (µm)	Av. Size of conidia (µm) Length	Breadth	Av. Beak length (µm)	No. of Septa(Range) H	V
1	AcPb(Parbhani)	7.81	38.30	15.62	11.27	1 - 5	1 – 2
2	AcNd(Nanded)	7.50	29.38	11.24	8.10	1 - 4	0 – 2
3	AcHl(Hingoli)	9.80	36.37	16.35	16.43	1 - 4	1 – 3
4	AcSt(Satara)	5.89	25.17	7.93	06.86	1 - 4	0 – 1
5	AcOs(Osmanabad)	7.05	27.87	11.70	07.46	1 - 5	0 - 1
6	AcBd(Beed)	6.10	41.45	16.06	13.03	1 - 6	1 - 2
7	AcAb(Aurangabad)	5.70	39.40	15.31	12.04	1 - 5	1 - 2
8	AcJl(Jalna)	4.86	32.27	12.76	10.59	3 - 5	0 - 1
9	AcYt(Yavatmal)	5.40	30.00	12.27	10.24	2 - 4	0 - 1
10	AcWs(Washim)	7.50	28.20	14.13	09.01	4 - 8	0 - 2
11	AcAk(Akola)	6.30	50.32	15.02	14.65	3 - 8	0 - 1
12	AcLt(Latur)	6.46	45.80	14.62	15.24	4 - 8	1 - 2
13	AcAn(Ahmadnagar)	8.46	39.11	13.56	13.45	2 - 5	1 - 2
14	AcDl(Dhule)	3.25	22.45	7.28	06.04	1 - 4	0 - 1
15	AcBl(Buldana)	9.25	40.35	13.06	14.34	4-12	1 - 3
16	AcAm(Amravati)	3.78	35.37	9.94	11.05	1 - 3	0 - 1
17	AcNb(Nandurbar)	3.53	26.80	10.78	09.47	1 - 3	0 - 1
18	AcNs(Nasik)	4.10	23.57	8.44	07.10	2 - 5	1 - 2
19	AcSl(Solapur)	4.54	23.92	9.27	06.50	2 - 4	1 - 2
20	AcJg(Jalgaon)	8.15	37.55	17.19	12.96	2 - 6	0 - 2

H : Horizontal, V : Vertical

Table 3. Pathogenic variability among *A. carthami* isolates

Sr. No.	Isolates / Districts	Av. incubation period (days)*	Av. no. of spots*	Av. size of spot(mm^2)*
1	AcPb (Parbhani)	7	16.33	14.28
2	AcNd (Nanded)	7	15.67	13.88
3	AcHl (Hingoli)	5	22.00	17.14
4	AcSt (Satara)	7	10.50	09.07
5	AcOs (Osmanabad)	7	10.67	09.52
6	AcBd (Beed)	8	09.67	08.64
7	AcAb (Aurangabad)	7	13.33	11.90
8	AcJl (Jalna)	8	08.50	07.16
9	AcYt (Yavatmal)	8	09.00	07.79
10	AcWs (Washim)	7	14.00	12.96
11	AcAk (Akola)	7	11.00	10.43
12	AcLt (Latur)	7	12.50	11.10
13	AcAn (Ahmadnagar)	5	19.67	15.82
14	AcDl (Dhule)	9	05.00	02.80
15	AcBl (Buldana)	5	21.33	16.54
16	AcAm (Amravati)	9	06.33	04.77
17	AcNb (Nandurbar)	9	05.50	03.18
18	AcNs (Nasik)	9	07.00	05.37
19	AcSl (Solapur)	8	07.67	06.72
20	AcJg (Jalgaon)	6	18.50	15.11
	SE±	0.28	0.15	0.13
	CD (P = 0.01)	0.82	0.41	0.34

* : Mean of three replications, Av. : Average, No. : Number

Beak length

On the basis of conidial average beak length, the test isolates were also categorized into three groups *viz*., long, medium and short beaked.

In long beaked group, about eight isolates were included with average beak length in the range of 11.05 to 16.43 µm. However, it was highest in the isolate AcHl (16.43 µm), followed by the isolates *viz*., AcLt (15.24 µm), AcAk (14.65 µm), AcBl (14.34 µm), AcAn (13.45 µm), AcBd (13.03 µm), AcJg (12.96 µm) and AcAb (12.04 µm).

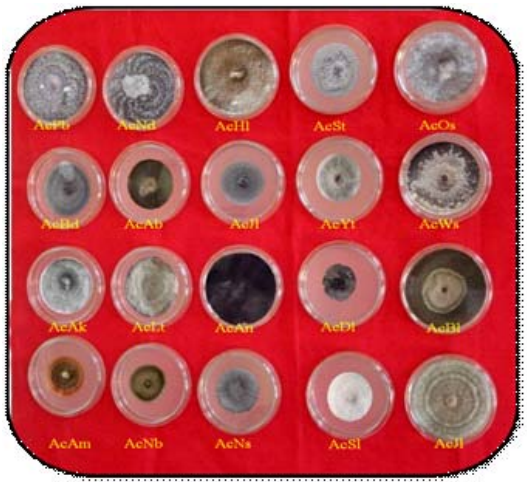

Plate 1 : Cultural variability among the test isolates of *A. carthami*

In medium beaked group, about seven isolates were included with average beak length in the range of 8.13 to 11.27 µm. Among these isolates, maximum average beak length was recorded in AcPb (11.27 µm), followed by the isolate *viz*., AcAm (11.05 µm), AcJl (10.59 µm), AcYt (10.24 µm), AcNb (9.47 µm), AcWs (9.01 µm) and AcNd (8.10 µm),

In small beaked group, about five isolates were included with average beak length in the range of 6.04 to 7.46 µm. Among these isolates, maximum average beak length was recorded with the isolate AcOs (7.46 µm), followed by AcNs (7.10 µm), AcSt (6.86 µm), AcSl (6.50 µm) and AcDl (6.04 µm).

Septation

Results revealed marked variability among the test isolates in respect of their horizontal and vertical septa on the conidia. Among the test isolates, horizontal septation was ranged from 1 to 12 and vertical septation from 0 to 3. Of the test isolates, AcBl recorded maximum horizontal (4-12) and vertical (1-3) septations, followed by the isolate *viz*., AcWs and AcLt (each H : 4-8 and V : 0-2), AcAk (H : 3-8 and V : 0-1), AcJl (H : 3-5 and V : 0-1), AcJg (H : 2-6 and V : 0-2), AcAn and AcNs (each H : 2-5 and V : 1-2) and AcYt (H : 2-4 and V : 0-1). Rest of the isolates recorded horizontal and vertical septation in the range of 1-3 to 1-6 and 0-1 to 0-3.

Thus, from this foregoing results it has been inferred that all 20 test isolates of *A. carthami* representing four geographic regions of the Maharashtra state exhibited a varied range morphological characteristics. The mycelial width, conidium size and their beak length were varied from small to large, as well as septations (H and V) were ranged from 1-12 (horizontal) to 0-3 (vertical).

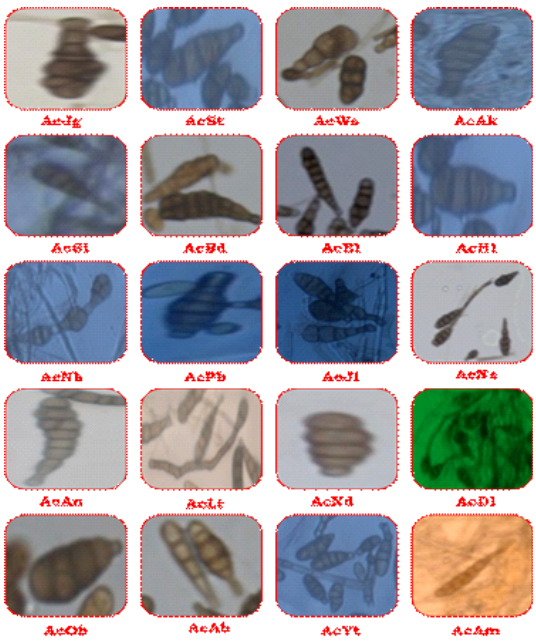

Plate 2 : Close-up view of conidia of *A. carthami* test isolates

Plate 3 : Pathogenicity test and pathogenic variability of *A. carthami* isolates on safflower Cv. Manjira

Cultural and morphological variability studies in *A. carthami, A. brassicae, A. sesame, A. solani, A. lini,* isolates was attempted earlier by several workers. Mortensen (1983) reported mycelial growth of the *A. carthami* isolates ranged from 81 to 32 mm on PDA within 8 days, conidial amount from 0.1 X 10^5 to 19.3 X 10^5 per plate. Mycelial growth in isolate 4 and 43-1 was rapid mycelial growth (81 and 79 mm) and it was slow isolates S-a, 22 and 49-1 (65, 64 and 60 mm, respectively). Vishwanath *et al.* (2002) reported cultural variability among three isolates of *A. brassicae* in respect of growth and colony chararacters. Savitha and Naik (2004) that the isolates of *Alternaria* spp. infecting sesamum produced fluffy to raised dark brown or light grey mycelium (72-88 mm) on PDA. Cultural variability among the isolates of various *Alternaria* spp. reported earlier were *A. brassicae* (Prasad *et al*., 2012; Singh and Singh, 2014), *A. helianthi* (Rajender *et al*., 2013).

Morphological variability in respect of conidial size, beak length and conidial septation observed in present study in respect of *A. carthami* coincides with several earlier workers. Deokar and Raghuwanshi (2002) studied six isolates of *A. carthami* and reported variability in conidial size (42-69 X 11-17 µm length and 41-58 X 10-14 µm breadth), horizontal septation (0-7), vertical septation (0-3), small to large beak length and light brown to dark brown conidia. Ramegowda and Naik (2008) reported variability in mycelial width (2.87-6.95 µm), large sized conidia with medium to long beak length in the isolates of *A. macrospora* causing leaf blight of cotton. Prasad *et al.* (2012) reported conidial length (96-120 X 24-32 µm) and breadth (24-40 µm), with transverse septa (7-9) and longitudinal septa (2-4) in *A. carthami*. Sharma *et al.* (2013) reported variability in conidial length (37.88 to 57.65 µm) and width (6.0 to 9.5 µm), transverse septa (2.33 to 6.0) and minimum number of longitudinal septa in *A. brassicae* isolates. Singh *et al.* (2014) studied morphological variability among *A. brassicae* isolates and reported their hyphal width (3.0-5.9 µm), muriform or ovate conidia (86.40-240.5 X 15.5-30 µm), transverse septa (5-16), longitudinal septa (0-8) and beak length (10-130 µm).

Pathogenicity test and pathogenic variability

Pathogenicity test (Plate 3) of *A. carthami* (20) was conducted in screen house using Alternaria blight susceptible safflower cv. Manjira and results obtained on pathogenic traits *viz*., incubation period, number of spots and size of the spots are presented in Table 3.

Symptoms

All 20 test isolates of *A. carthami* were found pathogenic to safflower. The symptoms induced under pathogenicity test were identical to those symptoms observed on naturally diseased safflower crop foliage during survey.

Incubation period

Results revealed that among various pathogenic traits of *A. carthami*, the incubation period was varied in the susceptible safflower cv. Manjira. On the basis of incubation period (day to expression of first symptom), the test isolates were categorized in three groups *viz*., A (highly aggressive with minimum of 5-6 days incubation period), B (moderately aggressive with moderate of 7-8 days incubation period) and C (less aggressive with 9 or more days incubation period). Accordingly, in group A the isolates (04) included were AcHl, AcAn, AcBl and AcJg; in group B the isolates (12) included were AcPb, AcNd, AcSt, AcOs, AcAb, AcWs, AcAk, AcLt, AcBd, AcJl, AcYt and AcSl and in group C the isolates (04) included were *viz*., AcAm, AcNb, AcDl and AcNs.

Frequency of leaf spots

There were significant variations in the frequency of leaf spots (average number of spots / plant) on safflower cv. Manjira (Table 3) and it was ranged from 5.00 (AcDl) to 22.00 (AcHl). On the basis of frequency of the leaf spots, the test isolates were categorized in to three groups *viz*., A (highly virulent = leaf spot frequency in the range of 22.00 to 15.67 / plant), B (moderately virulent = leaf spot frequently in the range of 10.50 to 14.00 / plant) and C (less virulent = leaf spot frequency in the range of 5.00 to 13.33 / plant). In group A of highly virulent isolates the leaf spot frequency was ranged from 15.67 (AcNd) to 22.00 (AcHl) per plant; however, it was significantly highest with the isolate AcHl (22.00), followed by AcBl (21.33), AcAn (19.67), AcJg (18.50), AcPb (16.33) and AcNd (15.67). In group B of moderately virulent isolates, the leaf spot frequency was ranged from 10.50 (AcSt) to 14.00 (AcWs); however, it was significantly maximum with the isolate AcWs (14.00), followed by AcAb (13.33), AcLt (12.50), AcAk (11.00), AcOs

(10.67) and AcSt (10.50), later two isolates were at par. In group C of less virulent isolates, the leaf spot frequency was ranged from 5.00 (AcDl) to 13.33 (AcAb) per plant; however, it was significantly maximum with the isolate AcLt (12.50), followed by AcAk (11.00), AcOs (10.67), AcSt (10.50), AcBd (9.67) and AcYt (9.00).

Size of leaf spots

Size / diameter of the spots induced by *A. carthami* isolates on foliage of safflower cv. Manjira was also found to be varied significantly among the test isolates and it was ranged from 2.80 to 17.14 mm^2.

Based on average size (mm^2) of the leaf spots, the test isolates were categorized into three groups *viz.*, A (with maximum average leaf spot size in the range 12.96 to 17.14 mm^2), B (with medium average leaf spot size in the range of 7.16 to 11.90 mm^2) and C (with small average leaf spot size in the range of 2.80 to 6.72 mm^2). In group A, those isolates which induced large sized spots (12.96 to 17.14 mm^2) were included and significantly large sized leaf spots were induced by the isolates AcHl (17.14 mm^2), followed by the isolates AcBl (16.54 mm^2), AcAn (15.82 mm^2), AcJg (15.11 mm^2), AcPb (14.28 mm^2), AcNd (13.88 mm^2) and AcWs (12.96 mm^2). In group B of medium sized leaf spots, maximum sized spots were induced with the isolate AcAb (11.90 mm^2), followed by the isolates AcLt (11.10 mm^2), AcAk (10.43 mm^2), AcOs (9.52 mm^2), AcSt (9.07 mm^2), AcBd (8.64 mm^2), AcYt (7.79 mm^2) and AcJl (7.16 mm^2). In group C of small sized leaf spot, maximum sized spots were induced with the isolate AcSl (6.72 mm^2), followed by AcNs (5.37 mm^2) and AcAm (4.77 mm^2); whereas significantly smallest sized leaf spots were induced by the isolates AcDl (2.80 mm^2) and AcNb (3.18 mm^2).

Thus, from this ongoing results it was concluded that all 20 isolates of *A. carthami* representing four geographic regions of the Maharashtra state exhibited a wide range of pathogenic variability. However, the aggressive isolates (*viz.*, AcHl, AcBl, AcAn, AcJg, AvNd, etc.) showed highly virulent least incubation period, highest leaf spot frequency and maximum sized leaf spots. Whereas, moderately virulent / aggressive isolates exhibited each moderate incubation period, leaf spot frequency and their size. In less virulent isolates *viz.*, AcAm, AcDl, AcNb and AcNs through incubation period was maximum (> 9 days), but leaf spot frequency and their size were of lower degree.

Pathogenic association of *A. carthami* with safflower causing Alternaria leaf spot / blight was reported earlier by several workers under controlled conditions of screen house by inoculating spore cum mycelial suspension (*A. carthami*) on susceptible safflower cultivars (Singh and Prasad, 2005; Ranaware *et al.*, 2010; Gholve *et al*., 2015).

The cultural and morphological characteristics of *A. carthami* found in present study are in consonance with the earlier reported (Deokar and Raghuwanshi, 2002; Taware *et al.*, 2014). Gholve *et al.* (2015) reported that *A. carthami* produced initially white, cottony profused aerial mycelium, which gradually turned greenish grey in colour. Aged culture completely black with no aerial mycelium on Potato dextrose agar Microscopic characteristics such as brownish black septate mycelium and dark brown beaked conidia with transverse and longitudinal septation of *A. carthami* observed during present study were also reported earlier by several workers (Ranaware *et al.*, 2010; Prasad *et al.*, 2012; and Gholve *et al.*, 2015).

Pathogenic diversity among isolates of the *Alternaria* spp. infecting various oilseeds and vegetable crops was reported in past. Meena *et al*. (2011) reported pathogenic variability in *A. brassicae* isolates in respect of disease severity on host differentials and lesion size. Virulent isolates produced large sized lesions with higher per cent disease severity. Sigh *et al.* (2013), Sharma *et al*. (2013) and Giri *et al.* (2014) were of similar opinion in respect of pathogenic variability in *A. brassicae* causing leaf blight of rapeseed-mustard. Kolte *et al*. (2005) reported pathogenic variability in leaf spots produced. Virulent isolates produced large spots with dark margins. Jadhav *et al*. (2011) reported existence of pathogenic variability in *A. macrospora* isolates from cotton. They found that in virulent isolates the incubation period very short (8-9 days), large lesion size and maximum number of lesions per unit leaf area; whereas, the less virulent isolates required maximum incubation period (8-13 days), small sized lesion and their minimum frequency.

Thus, in present study pathological, cultural, morphological and pathogenic variability

observed among the isolates of *A. carthami* may be attributed to their geographic distribution in the Maharashtra state, long tern influence of weather parameters at a particular location, ability of the pathogen to adopt the safflower varieties grown etc.

ACKNOWLEDGEMENT

I greatly acknowledge to my Dr. K. T. Apet, Head, Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani for their support during research work for the completion of my Ph.D. degree programme.

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