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Morphological and Cultural Characterization of Isolates of *Alternaria sesami* Causing Sesame Leaf Blight

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A B S T R A C T

Diseased sesame samples were collected from the major growing regions of Andhra Pradesh for molecular and cultural characterization of *Alternaria sesami.* The collected isolates were characterized based on their conidial length, breadth and beak length and cultural characters (colour of the mycelium, growth on the PDA medium). Of the 12 isolates, most of the isolates were white in their colony colour which later turned into grayish brown, while a few isolates were brown with fluffy growth at the centre. After seven days of incubation highest mycelial growth was observed in NP 2 isolate (5.28 cm) isolate which was followed by EL 2, NAR 1 (5.27 cm). Average conidial size of the *A. sesami* is 24.88 - 34.64 µm X 9.61 - 12.13 µm with beak length of 5.12 - 8.32 µm and no. of horizontal and vertical septa varied from 2.76 - 3.82 and 1.31 - 1.76 respectively.

Introduction

Sesame (*Sesamum indicum* L.) is commonly known as "Till is one of the short duration crop grown throughout the year. Globally, in India sesame was cultivated in area (18.93 lakh ha) and production (413 kg ha^{-1}). India also has global market for white seeded types. In Andhra Pradesh, it occupies an area of 0.61 lakh ha with an annual production of 0.20lakhtonnes and with an average productivity of 321 kg ha^{-1} (Department of Agriculture, Cooperation & Farmers Welfare, GOI, 2016-17). Sesamum is attacked by several infectious plant pathogens which are responsible for major damaging factor to crop plants. Among important sesame diseases, *Alternaria* leaf spot is prevalent in all the sesame growing areas of the world and it is also reported in Kenya, Ethiopia, El-Salvador, Nigeria, India and USA (Verma *et al.*, 2005; Ojiambo *et al*., 2003; Kolte, 1985; Bhale *et al*., 1998).

Alternaria sesami is the incident of the sesame leaf blight disease is a highly variable pathogen which can cause seed rot, pre and post mergence losses, stem rot and leaf spots. It attacks seedlings, stems of young plants, leaves and pods of *Sesamum indicum* causing considerable damage to plants and fruits. The incidence of the disease in the major sesame growing areas ranged from 8 to 92 % (Fula, 2005) which was influenced by various environmental factors like temperature, light, humidity etc.

Therefore the present study was to study the morphological and cultural characters of the twelve isolates which were collected during the survey in the major sesame growing mandals of Visakhapatnam district during *kharif* 2016-2017.

Materials and Methods

Collection of diseased samples

Leaves of sesame showing typical blight symptoms were collected during the survey conducted and the fungus was isolated by the technique indicated below.

Sesame leaf were cut into small bits with the help of sterilized blade and were surface sterilized with sodium hypochlorite(1%) for 30 sec followed by three subsequent washings with sterilized distilled water and were blot dried using sterilized blotting paper. The cut samples were then placed on PDA medium in Petri plates. The plates were incubated at 25± 1^0C. Initial growth of the pathogen was sub-cultured in to Petri plates and well monitored for fungal growth. Pure cultures of all isolates were obtained by single spore method and maintained for further studies.

Morphological variability

The cultures of isolates were identified based on the characteristics of the colony, hyphae, conidiophore and conidia. Length, breadth and beak length were measured for 45 conidia for each isolate and compared with the dimensions for the *A. sesame* (Dolle, 1981; Shekarappa, 1999 and Ramegowda and Naik, 2008).

The identity of the fungus was confirmed by comparing the ranges of dimensions derived by the formula given by John (1970)

$\mu = \chi \pm t\ 0.05\ SE$

Where,

σ = Standard deviation
μ = Population mean
χ = Sample mean
η = Number of spores observed
t = Table t value (P=0.05)
SE = Standard error

Cultural variability

The isolates were cultured by inoculating 5 mm culture disc, cut from seven days old culture of *A. sesami* on to the center of the medium in each Petri dish and was incubated at room temperature in three replicates. Radial growth of each fungal isolate was calculated by taking the average of diameter measured on two axes. Other cultural characters like type of growth, colony colour were determined based on the works done by Raja and Reddy (2007), Verma *et al.*, (2007) and Tetarwal *et al.*, (2008).

The growth rate of the fungus on each medium was calculated by the formula given by:

$$GR = \frac{S_{X+1}-S_X}{T_{X+1}-T_X}$$

Where, GR = Growth rate (mm hr^{-1}), S = Colony diameter (mm), T = Time (h)

Sporulation of each isolate was determined using Neubauer haemocytometer. Spores from each colony were harvested gently by scraping the colony with sterilized inoculation needle in 15 ml of sterile distilled water and the suspension was collected in a test tube. After thorough stirring, spore concentration was adjusted to 10^4 and was determined thrice and the average number of spores $ml^{-1}$was estimated for each isolate.

Based on spore concentration per unit area on culture medium isolates were categorized into

four types i.e., scanty sporulating (<4× 10^4 spores ml^{-1}, moderate sporulating (4-8 ×10^4 spores ml^{-1}), good sporulating (8.1- 12 ×10^4 spores ml^{-1}) and abundant sporulating (>12× 10^4 spores ml^{-1}) isolates.

Results and Discussion

Isolation and identification of the pathogen

The process of isolation resulted in the twelve isolates of the pathogen collected from different regions of Visakhapatnam district. All the isolates were confirmed as of *A. sesami.*

Morphological variability

In all the twelve isolates the conidia were muriform shape and light brown in color. The length of the conidia varies from 24.88 - 34.64 µm. Kokirapalli isolate, KP 2 produced longest (34.64 µm) conidia followed by AG 1 (32.50 µm) and VP 1 (31.55µm). The shortest spore producing isolate was AG 2 (24.88 µm) and was followed by EL 1 (25.10 µm).The width of the conidia varies from 9.61-12.13 µm. The spore produced by the isolate KP 2 (12.13 µm) was found to be the broadest followed by AG 1 (12.09 µm) and while it was least in NP 2 (9.61 µm). Generally all the isolates produced beaked conidia. The beak length of the conidia varied from 5.12 - 8.32 µm. KP1 had lengthiest beak (8.32 µm) followed by KP 2 (7.18µm) and VP 2 (7.17µm) while shortest beak was observed in AG 2 (5.12 µm). Number of horizontal and vertical septa varied from 2.76 - 3.82 and 1.31 - 1.76 respectively (Table 1). Mohanty and Behera (1958)descriptions were found as references to later works on *A. sesami* and Leppik and Sowell (1964)stated that his descriptions of *A. sesami* were in conformity with that of Mohanty and Behera. According to Leppik and Sowell (1964), pathogen was reported to have simple, erect, yellowish brown, 0-3 septate conidiophores, measuring 30-50 x 4.5-6.5µmand each bearing conidia singly or in chains at the apex. Conidia were reported to beobclavate, yellowish brown to dark brown that measured 30-100 X 10-28µm, with hyaline beak that was25-160 X 2-4µm in size. Dolle (1981) described the conidia of *A. sesami.* Sumathi (1997) reported that the isolates of *A. sesami* produced beaked and unbeaked conidia which were also observed in the present investigation. Shekarappa (1999), Ramegowda and Naik (2008) reported descriptions on the spore size of *A. sesami*. Savitha *et al.,* (2013) reported the spore dimensions of six isolates of *A. sesami* infecting sesame to range from 14.06-44.40 X 6.62-23.68 µm with maximum beak length of 3.50 - 17.02 µm and the present findings showed conformity with the work.

Cultural variability

The cultures were identified based on the colony characters and conidial dimensions. Cultures had light gray mycelium which later turned grayish white and developed concentric zonations. The centre of the colony was profuse to fluffy with whitish gray mycelium. Aged culture appeared completely black with no aerial mycelium. Most of the isolates were white in their colony colour that later turned in to grayish brown after seven days of incubation, while a few isolates remained brown since the day of incubation. The isolate KP 2, VP 2were darker than all isolates showing fluffy center with blackish brown flat margin. In majority of the isolates the colony was fluffy, raised at the center with entire margins, while uniform flat colony was noted in isolates NP 1 and EL2 (Table 2). Pigmentation of colonies of *Alternaria* spp was described to be variable as yellow, brown, black, brownish to greenish black on potato dextrose agar media (Ellis and Gibson, 1975 and Kumar *et al*., 2008).

Table.1 Morphological characters of conidia of *A. sesami* isolates

S. No.	Isolates	Spore dimensions (µm)						Number of septa	
		Length	**Population mean (µm)**	**Breadth**	**Population mean (µm)**	**Beak length**	**Population mean (µm)**	**Horizontal**	**Vertical**
1	AG 1	32.50	32.50±2.01(1.16)	12.09	12.09±2.01(0.49)	5.55	5.55±2.01(0.36)	3.48	1.55
2	AG 2	24.88	24.88±2.01(1.16)	11.50	11.50±2.01(0.38)	5.12	5.12±2.01(0.43)	2.76	1.36
3	KP 1	31.10	31.1±2.01(0.57)	9.62	9.62±2.01 (0.33)	8.32	8.32±2.01(0.97)	3.06	1.44
4	KP 2	34.64	34.64±2.01(1.24)	12.13	12.13±2.01(0.32)	7.18	7.18±2.01(0.73)	3.40	1.73
5	VP 1	31.55	31.55±2.01(1.13)	10.13	10.13±2.01(0.31)	5.92	5.92±2.01(0.34)	3.57	1.48
6	VP 2	29.16	29.16±2.01(0.99)	10.49	10.49±2.01(0.27)	7.17	7.17±2.01(0.66)	3.13	1.42
4	NP 1	32.10	32.10±2.01(0.90)	11.9	11.90±2.01(0.29)	5.95	5.95±2.01(0.42)	3.02	1.64
8	NP 2	25.20	25.20±2.01(0.68)	9.61	9.62±2.01(0.21)	5.14	5.14±2.01(0.32)	3.02	1.31
9	EL 1	25.10	29.2±2.01(0.84)	11.60	11.60±2.01(0.41)	5.97	7.97±2.01(0.46)	2.90	1.36
10	EL 2	30.31	30.1±2.01(0.90)	11.77	11.77±2.01(0.46)	6.30	6.30±2.01(0.47)	3.40	1.57
11	NEL 2	25.44	30.61±2.01(0.92)	10.33	10.33±2.01(0.38)	5.68	5.68±2.01(0.23)	3.82	1.44
12	NAR 1	28.89	28.89±2.01(0.74)	11.14	11.14±2.01(0.39)	5.37	6.37±2.01(0.24)	3.22	1.76
	SEm±	0.64		0.23		0.17		0.08	0.04
	CD (P≤0.05)	2.16		0.79		0.59		0.27	0.16
	CV %	4.32		4.33		5.54		5.06	6.45

Table.2 Cultural characteristics of *A. sesami* isolates after seven days of incubation

S. No.	Isolates	Colony colour	Type of growth
1	**AG 1**	White turned to light grayish brown	Fluffy raised center with regular outer margin
2	**AG 2**	White turned to grayish brown	Cottony fluffy centre with raised light coloured margin
3	**KP 1**	White turned to grayish brown	Grayish fluffy raised centre with raised whitish margin
4	**KP 2**	White turned to dark grayish brown	Grayish Fluffy centre with brown flat Margin margin
5	**VP 1**	White turned to grayish brown	Fluffy centre with circular growth grayish white raised margin
6	**VP 2**	Dark brown	Fluffy centre with distinct flat margin
7	**NP 1**	White turned to light brown	Uniform cottony growth with brown regular margin
8	**NP 2**	Light grayish turned to blackish brown	Fluffy centre with blackish brown flat margin
9	**EL 1**	Light grayish	Fluffy raised centre with grayish raised margin
10	**EL 2**	Light grayish	Cottony growth at centre with whitish raised margin
11	**NEL 2**	White turned to dark brown	Fluffy centre with grayish brown flat margin
12	**NAR 1**	Light grayish turned to dark brown	Fluffy raised centre with circular growth having flat margin

Table.3 Variation in cultural characteristics of *A. sesame* isolates

S. No.	Isolates	Diameter of the colony (cm) DAI							Mean	Spore conc X 10^4
		1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**		
1	AG 1	0.60	0.83	1.47	2.22	2.78	3.42	4.08	2.2	5.33
2	AG 2	0.53	0.97	1.22	2.33	2.97	3.58	4.13	2.24	11.33
3	KP 1	0.72	1.08	1.53	2.53	3.10	3.77	4.48	2.45	16.67
4	KP 2	0.63	1.13	1.43	2.33	2.98	3.98	5.07	2.50	11.67
5	VP 1	0.80	1.18	1.62	2.30	3.10	4.12	5.12	2.60	28.33
6	VP 2	0.83	1.17	1.52	2.58	3.08	4.18	5.08	2.63	44.67
7	NP 1	0.87	1.23	1.63	2.67	3.12	4.18	5.25	2.70	71.67
8	NP 2	0.87	1.18	1.72	2.83	3.23	4.15	5.28	2.75	41.00
9	EL 1	0.80	1.08	1.53	2.50	2.98	4.12	5.12	2.59	17.67
10	EL 2	0.62	1.03	1.58	2.62	3.18	4.02	5.27	2.61	20.67
11	NEL2	0.83	1.28	1.75	2.82	3.22	4.12	5.12	2.73	39.00
12	NAR 1	0.90	1.27	1.78	2.87	3.08	4.08	5.27	2.75	52.67
	SEm±	0.014	0.021	0.027	0.016	0.020	0.020	0.027		1.11
	CD (P≤0.05)	0.04	0.06	0.07	0.04	0.05	0.05	0.08		2.92
	CV %	5.66	5.75	5.18	1.90	1.95	1.52	1.67		11.04

Table.4 Growth rate of different *A. sesami* isolates

S. No.	Isolates	GROWTH RATE (per day)						
		1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**
1	AG 1	0.01	0.01	0.03	0.03	0.02	0.03	0.03
2	AG 2	0.01	0.02	0.01	0.05	0.03	0.03	0.02
3	KP 1	0.02	0.02	0.02	0.04	0.02	0.03	0.03
4	KP 2	0.01	0.02	0.01	0.04	0.03	0.04	0.05
5	VP 1	0.02	0.02	0.02	0.03	0.03	0.04	0.04
6	VP 2	0.02	0.01	0.01	0.04	0.02	0.05	0.04
7	NP 1	0.02	0.02	0.02	0.04	0.02	0.04	0.04
8	NP 2	0.02	0.01	0.02	0.05	0.02	0.04	0.05
9	EL 1	0.02	0.01	0.02	0.04	0.02	0.05	0.04
10	EL 2	0.01	0.02	0.02	0.04	0.02	0.03	0.05
11	NEL 2	0.02	0.02	0.02	0.04	0.02	0.04	0.04
12	NAR 1	0.03	0.02	0.02	0.05	0.01	0.04	0.05

Table.5 Correlation between radial growth, growth rate, spore concentration and PDI

	RADIAL GROWTH	GROWTH RATE	SPORE CONC	PDI
RADIAL GROWTH	1.000			
GROWTH RATE	0.935	1.000		
SPORE CONC	0.641	0.496	1.000	
PDI	0.366	0.368	0.370	1.000

r value= 1.79

Fig.1 Radial growth of different *A. sesami* isolates on different days of incubation

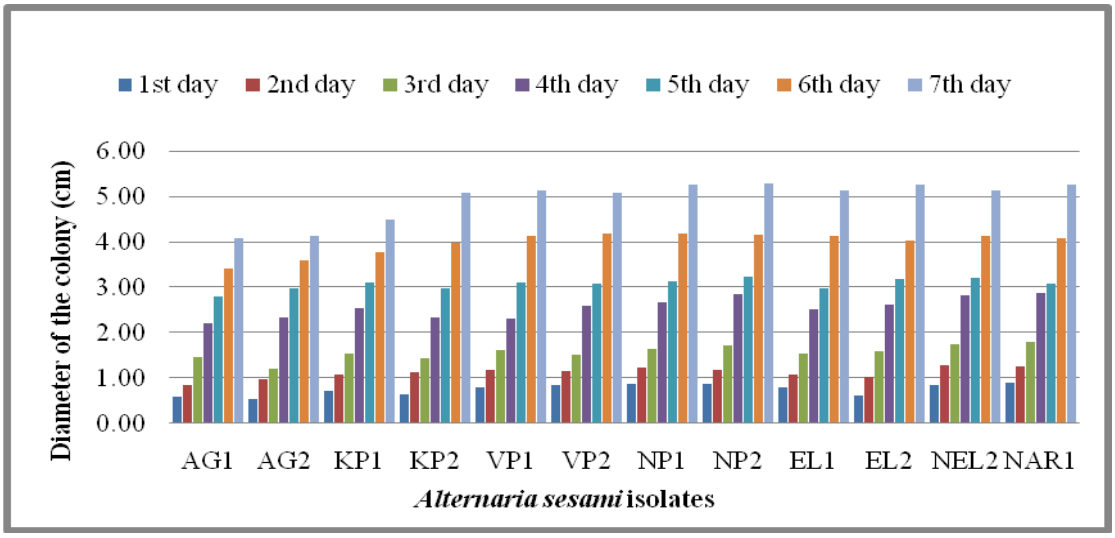
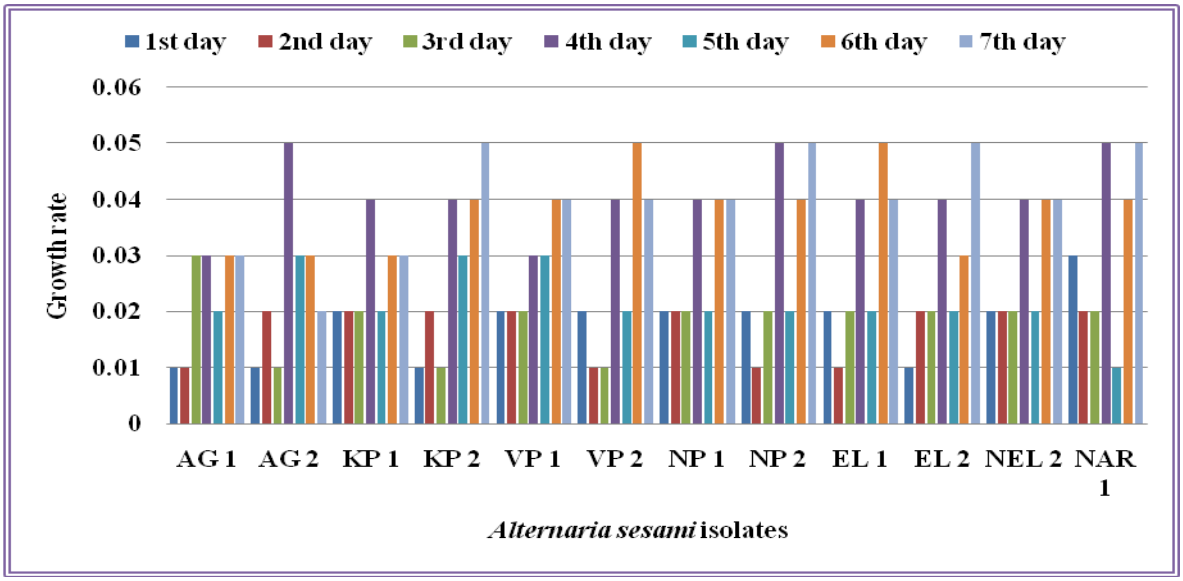


Fig.2 Growth rate of different isolates of *A. sesami* on different days of incubation



Rotem (1994) stated that variation in the cultural characteristics (colour, growth and sporulation) makes it possible to find as many races as possible in all isolates Variation in colony margin was described as serrated, wavy and entire (Nagrale *et al*., 2013).

Radial growth of *A. sesame* isolates on PDA significantly differed among the twelve isolates tested (4.08 - 5.28 cm) (Table 3 and Fig. 1). Savitha *et al.,* (2013) reported that radial growth of *A. sesami* isolates to range between 3.5 and 8.8 cm when cultured on different media like PDA, host extract agar, oat meal agar, Czapeck's agar, Sabourd's agar and Richard's agar and the present results ascertain the previous report.

Growth rate among the isolates varied from 0.01 to 0.05 in seven days of incubation (Table 4 and Fig. 2). Sporulation in *A. sesame* isolates after seven days of incubation varied from 5.33 $x10^4$ (AG 1) to 71.67 $x10^4$ ml^{-1} (NP 1). NP1 isolate was actively sporulating among all the isolates. AG 1 isolate was found to have moderate sporulation, two isolates AG 2 and KP 2 had good sporulation while most of the other isolates were found to produce abundant sporulation (Table 3). Nagrale *et al*., (2013) found varied spore concentration in Gerbera *A. alternata* isolates when tested on 20 different media ranged from 0.02 x 10^4 cm^{-2}(Tap water) to 0.61 x 10^4 cm^{-2}(PDA) which were exactly corresponding with the results of Kapoor and Hingorani (1958); Lonnaidis and Main (1973) Variation in sporulation of *A. solani* isolates on PDA was reported by Singh *et al.,* (2014) who observed sporulation in only two of 10 isolates to range from 0.5 X 10^3 to 2.0x$10^3$$ml^{-1}$ when the inoculated petri plates were kept in BOD at 25±2 ^{0}C for growth.

Significant positive correlation was found between radial growth and growth rate (0.935) while non-significant but positive correlation was observed between radial growth and PDI (0.366) and growth rate and PDI (0.368) (Table 5).

The present findings clearly indicate significant variability in the cultural characters of twelve isolates of *A. sesami.* Sporulation was abundant on the PDA media. Similar type of observation was taken by Rajender *et al.,* (2013) in *A. helianthi.* The

radial growth of the pathogen increased from the day of incubation on the PDA media. Variation in growth of *A. solani* on PDA was reported (Tong *et al*., 1994; Babu *et al*., 2000; Kumar *et al*., 2008; Naik *et al*., 2010; Singh *et al*., 2014 and Nikam *et al*., 2015). Pachori *et al.,* (2016) reported that average mycelial growth rate of *A. solani* isolates after seven DAI to range between 29.5 mm to 35.5 mm.

The colour of the colonies were light gray in the beginning and turned to grayish white with concentric zonations and the aged culture was completely black with no aerial mycelium which was in corroboration with Rotem (1994) stated that variation in the cultural characteristics (colour, growth and sporulation) makes it possible to find as many races as possible in all isolates.

Pigmentation of the colonies of the isolates varied from yellow, brown, brownish to greenish black on PDA. Spore production was abundant for most of the isolates. The present findings were in corroboration with variation in sporulation of *A. solani* isolates on PDA was reported by Singh *et al.,* (2014) who observed sporulation in only two of 10 isolates when the inoculated petri plates were kept in BOD at 25±2 ^{0}C for growth. In contrast to the above findings in the present study all isolates sporulated when incubated at room temperature with alternate cycles of light and darkness. It was reported that light to have a profound influence on growth and sporulation of fungi (Padhi and Rath, 1974; Naik *et al*., 2010).

The measurements of the conidia varied for different isolates when grown on the PDA medium. The conidial dimensions in the present study are in agreement with the earlier descriptions of Leppik and Sowell (1964). Dolle (1981) described the conidia of *A. sesami.* Sumathi (1997) reported that the isolates of *A. sesami* produced beaked and unbeaked conidia which were also observed in the present investigation. Shekarappa (1999), Ramegowda and Naik (2008) reported descriptions on the spore size of *A. sesami*. Savitha *et al.,* (2013) reported the spore dimensions of six isolates of *A. sesami* infecting sesame to range from 14.06-44.40 X 6.62 - 23.68 µm with maximum beak length of 3.5-17.02 µm.

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