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Cultural and morphological characterization of *Alternaria sesami* (Kawa.) Mohanty and Behera causing leaf blight of sesame

Akash Bevanur, Jayalakshmi SK and Manthesha HD

Abstract

Sesame (*Sesamum indicum* L.) is one of the most ancient oilseed crop and rich source of oil, protein, calcium as well as phosphorus, which is widely naturalized in tropical regions around the world. Leaf blight of sesame caused by *Alternaria sesami* is an important disease inflicting heavy losses. Keeping in view, the present investigation was carried out to characterize the pathogen isolates of parts of Kalyana Karnataka based on cultural and morphological differences. Out of 20 isolates, AsR20 and AsK9 isolate had the highest number of horizontal and vertical septa, respectively. Whereas AsR20 and AsKp18 isolate had the highest conidial length and width, respectively. The maximum beak length was found in AsB3 isolate, while the maximum mycelial width was found in AsKp13 isolate. With respect to different media, the maximum mycelial growth of the test fungus was endorsed by potato dextrose agar medium (66.78 mm).

Keywords: Sesame, oilseed, *Alternaria*, mycelia, septa

Introduction

Sesame is mainly grown for its oil which has a pleasant taste and antioxidant properties (Bedigian *et al*., 1985) [4], accounted for the presence of secondary metabolites. Sesame seed is traditionally consumed directly as well as in confectionaries like cakes, cookies and many other baking products as well as seed cake which is nutritious feed for livestock (Laurenti, 2007) [12].

Sesame flourishes well in the tropical and sub-tropical regions, in plains as well as at an elevation of 4000 ft, but it is sensitive to low temperatures. In Northern India, it is mostly cultivated as *Kharif* crop, while in Andhra Pradesh two crops are taken up, one crop as rainfed crop (May-September) and other as *rabi* crop (November-January). The global sesame production scenario accounts for an area of 11.7 mha with the production of 6 mt and productivity of 512 kg/ha (Anon., 2020a). India is an important sesame growing country in the world, ranks second in acerage with an area of 1.72 mha (following Myanmar with 3.48 mha) and an annual production of 8.16 lakh tonnes and average productivity of 474 kg/ha. Among the states, Madhya Pradesh leads first in both acreage (4.24 lakh ha) and production (1.95 lakh tonnes) and Meghalaya ranks first in productivity (937 kg/ha). In Karnataka, the crop is grown in an area of 22 tha (0.022 m ha) with the production of 0.2 lakh tonnes and productivity of 921 kg/ha (Anon., 2020b) [2].

Among various fungal diseases, leaf spot/blight caused by *A. sesami* (Kawamura) has become one of the major constraints in the production and productivity of sesame all over the country in general and in the parts of Kalyana Karnataka, in particular. Around 73 per cent losses due to blight caused by *A. sesami* (Dolle, 1981) [5]. The typical symptoms of *Alternaria* leaf blight were recorded as small greyish spots on the lower surface of the leaves, on the upper surface concentric minute brown spots which later enlarge with characteristic concentric rings at the centre to produce a target board like pattern (Savitha *et al*., 2012) [18].

Asexual spores of fungi are one of the important means of dissemination particularly for an airborne fungal pathogens and used in identification and classification of the fungus. Savitha *et al.* (2013) [19] obtained six isolates of *A. sesami* (ALT1, ALT5, ALT8, ALT9, ALT10, ALT11) from Raichur, Gulbarga, Bidar, Dharwad, Hyderabad and Coimbatore and observed the growth and sporulation of *A. sesami* on synthetic and semi synthetic media. They found that PDA supported good growth (87.33 mm) and sporulation of *A. sesami* followed by PDA supplemented with $CaCO_3$ and host extract agar.

They also recorded morphological characters like conidial size, beak length and hyphal width and cultural characteristics like colony diameter, colony color, type of margin and sporulation *etc*. Variability among the isolates of *Alternaria* species will help us to know the variation which exist in the pathogen and also helps to know the severity of *Alternaria* leaf blight on sesame which differs among regions and between individual crops within a region. So henceforth, the present investigation was taken up with the objective to characterize twenty isolates of *A. sesami*.

Materials and Methods

Collection and isolation of the pathogenic isolates from various geographical regions

During the field survey carried out in *Kharif* 2021, a large number of blight infected sesame leaf samples were collected from different places *viz*., Raichur, Kalaburgi, Bidar and Koppal. Sesame leaves infected with *A. sesam*i were collected with prominent symptoms such as concentric rings, dark brown, round to irregular patches.

Isolation and identification of *A. sesami*

The pathogen, *A. sesami* was isolated from the infected parts of sesame by standard tissue isolation procedure. The infected leaf bits were washed thoroughly with sterilized distilled water and small pieces of diseased leaf bits adjoined to the healthy tissue were cut with the help of a scapel and sterilized by immersing in mercuric chloride solution (0.1%) for 30 seconds in order to eliminate surface contaminants and repeatedly washed with sterilized distilled water to remove the traces of mercuric chloride before transferring them to Petri plates containing sterilized potato dextrose agar (PDA) under aseptic condition. The Petri plates were incubated at room temperature (25± 2 °C) for 10 days. The mycelial growth emerging from the diseased leaf bits was transferred directly into fresh PDA plates. The culture was purified by single spore isolation technique. The pure culture thus obtained was transferred into culture medium with the help of sterilized needle.

Cultural and morphological characterisation of *A. sesami*

The studies were carried out to know the variation in the pathogen collected from different parts of Kalyana Karnataka causing blight in sesame. Cultural and morphological variations were assessed among the different isolates of the pathogen.

Morphological characterization among the different isolates of *Alternaria* spp.

Morphological characters of the fungus such as mycelial width, size of the conidia, number of transverse and longitudinal septa, length of beak were measured under high power objective (40X) using ocular and stage micrometer through a compound microscope.

Effect of different solid media on growth of *A. sesami*

A five mm disc cut from seven days old culture of *A. sesami* was inoculated on various solid media to find out the suitable medium *viz*., synthetic and non synthetic media to record variations in cultural characteristics, colony diameter and sporulation. Colony diameter on each media was calculated by taking the average of diameter measured at 3rd, 6th and 9th days.

The media used were
1. Potato dextrose Agar
2. Richard’s Agar
3. Czapak’s Dox Agar
4. Saubard’s Agar
5. V8 juice Agar
6. Asthana’s and Hawker’s
7. Potato carrot Agar
8. Oat meal Agar
9. Malt extract Agar
10. Corn meal Agar

The composition and preparation of the above mentioned synthetic and non-synthetic media were obtained from Ainsworth and Bisby’s "Dictionary of the fungi" (Hawksworth *et al*., 1983) [8].

Results and Discussion

Effect of different solid culture media on mycelial growth and sporulation of *A. sesami*

As described, the diversity of cultural characteristics of *A. sesami* was studied in 10 different cultural media, which included both synthetic and non-synthetic media. The data pertaining to the effect of different culture media on growth and sporulation of *A. sesami* has been presented (Table 2, Plate 1 and fig. 1). The mycelial growth of *A. sesami* was influenced by different media compositions on its growth and sporulation. Among the different media tested, PDA had the highest mean mycelial growth of 66.78 mm, followed by potato carrot dextrose agar medium (62.15 mm), and the lowest was noticed on Asthana and Hawker’s medium (25.17 mm) followed by malt extract agar medium (44.34 mm). PDA was frequently reported to be the optimum medium for *Alternaria* spp. fungus (Gholve *et al*., 2017; Savitha *et al*., 2013) [6, 19].

Sporulation of *A. sesami* was shown to be excellent on oatmeal agar, but moderate on PDA and potato carrot dextrose agar medium. Czapeck's Dox agar, Sabouraud's agar, and malt extract agar medium showed good sporulation, while V-8 juice agar, corn meal agar, Asthana and Hawkers' agar medium, and Richard's agar medium showed poor sporulation.

In the current study, PDA exhibited good pathogen growth because of its simple formulation, which allows for the best mycelial development of the fungus (Saha *et al*., 2008) [15], but it includes too many nutrients, which leads to sporulation loss (UKNCC, 1998) [21]. According to Waggoner and Horsfall (1969) [23], *A. solani* requires a carbon supply (sugar) for increased sporulation, while excessive sugar availability restricts conidia generation. However, oatmeal agar has less sugar than PDA, which promotes sporulation. Similarly, pathogen growth and sporulation were improved in potato carrot dextrose agar medium, as carrot provides a low amount of carbohydrates, which aids sporulation in many imperfect fungi. (Kirk *et al*., 2008) [10].

Other culture media that have been reported to support good growth and sporulation of *Alternaria* spp. include malt extract agar (Reddy *et al*., 2019) [14], Sabouraud's agar (Tele *et al*., 2021) [20], V-8 juice agar medium (Parvin *et al*., 2021) [13], Richards’s agar and oatmeal agar (Valvi *et al*., 2019) [22]. The results obtained in this study were confirmed with findings of several workers (Krishna *et al*. (2018) [11]; Gholve *et al*. (2017) [6]; Savitha *et al*. (2013) [19].

Cultural characteristics of *A. sesami* on different solid media

By the ninth day of incubation, *A. sesami* exhibited a diversified behaviours on several solid media (Table 3; Plate 2). The colony colour varied as light grey (Asthana and Hawkers' agar, corn meal agar, oat meal agar, potato dextrose agar, Sabouraud's agar medium), grey (V-8 juice agar medium), dark grey (potato dextrose carrot agar), whitish to greyish (Czapek's Dox agar, Richard's agar medium) and light grey to brown (malt extract agar medium) on the different media studied.

On most of the media tested, *A. sesami* colony margin displayed a smooth, regular margin; however, on Czapek's Dox agar medium and Richard's agar medium, it generated a slightly irregular margin. *A. sesami* developed rough, irregular margins on Asthana and Hawkers' agar and Sabouraud's agar mediums. On majority of the media, the fungus produced sparse mycelium, but the media like Ashthana and hawker's, PDA and malt extract agar medium showed dense mycelium, whereas it produced fluffy mycelium on Czapek's Dox agar and potato dextrose carrot agar, Asthana and Hawkers' agar medium. Among the ten media evaluated, most of them showed indistinct zonations, whereas distinct zonations were noticed in case of Ashthana and hawker's, Czapek's Dox agar, Richard's agar and V-8 juice agar medium. These cultural characteristics of *A. sesami* were recorded earlier also by Sangeetha *et al.* (2016) [16]; Parvin *et al*. (2021) [13]; Ginoya and Gohel (2015) [7]; Gholve *et al*. (2017) [6].

Morphological characterisation among the isolates of *A. sesami*

Twnety isolates of *A. sesami* were isolated from blight infected leaves collected from various sesame growing districts of Kalyana Karnataka during the survey (Table 1; Plate 3). and these isolates were selected for microscopic observations with one from each taluk chosen.

The morphological variation of the conidia generated was also investigated under 40X magnification (Table 4). The conidia from various isolates varied in the number of horizontal septa they possessed. Isolates AsB5, AsKp15 had 2 and AsK9 had 3 horizontal septa, while isolates AsB4, AsK10, AsKp16, AsKp17 and AsK10 ranged from 2 to 3. Majority of the isolates *viz.*, AsB3, AsB6, AsK7, AsK8, AsKp13, AsR18, and AsR19 ranged from 3 to 4 horizontal septa, while AsB2 and AsK11 isolates ranged from 3 to 5. The isolate AsB1 and AsK12 ranged 4 to 5 horizontal septa and the maximum number of horizontal septa was observed in AsR20 isolate which showed 4 to 6 septa in its conidia.

Most of the isolates showed variation in the number of vertical septa. As3, As4, AsB5, AsB6, AsK8, AsK10, AsK11, AsKp13, AsKp14, AsKp15 and AsR18 isolates ranged between of 0 to 1 vertical septa whereas AsK7, AsK12, and AsR20 isolates showed only 1 vertical septa. AsK9 identified had the highest number of vertical septa (1 to 2) were recorded while in the remaining isolates no vertical septa were observed.

The mean conidial length of various isolates of *A. sesami* ranged from 21.71 µm (AsKp16) to 40.18 µm (AsR20). The maximum average conidial length was observed in AsR20 (40.18 µm) followed by AsK12 (37.28 µm) and AsK7 (34.43 µm), whereas AsKp16 found to possess shortest length of conidium (21.71 µm) followed by AsB4 (21.77 µm) and AsKp17 (22.94 µm). The average width of conidia ranged from 6.98 µm (AsKp16) to 15.04 µm (AsR20). However AsR18 recorded maximum conidial width (16.01 µm), followed by AsK7 (15.56 µm), and AsR20 (15.34 m), while AsKp16 had the least conidial width (6.98 µm), followed by AsB2 (8.64 µm) and AsK12 (8.78 µm).

The beak length of conidium of various isolates varied from isolate to isolate and ranged from 3.93 µm (AsKp16) to 8.69 µm (AsB3). The maximum beak length was noticed in As3 (8.69 µm), followed by AsK12 (7.37 µm), and AsB5 (7.31 µm), while AsKp16 had recorded shortest beak (3.93 µm), followed by AsK9 (4.01 µm), and AsK10 (4.02 µm). Similarly the isolates varied in their mycelium width, with isolate AsKp13 having the maximum mycelium width (4.83 µm), followed by AsKp17 (4.69 µm), and AsK12 (3.02 µm) had the least mycelial width (Plate 3).

The results obtained in the current study were confirmed with the findings of several workers. Savitha *et al*. (2004) [17] studied the morphological variations of *A. sesami* where conidial length varied from 14.01 µm to 44.40 µm and the width ranging from 4.7 to 11.1 µm with 2 to 10 horizontal and 0 to 2 vertical septa. In the study conducted by Banne *et al*. (2021) [3], the ten isolates of *A. alternata* showed varied morphological variability. Among the ten, four isolates *i.e*., Ac7, Ac8, Ac9 and Ac10 showed large sized mycelial width which ranged from 7.15-9.48 µm. Two isolates *i.e.*, Ac-9 and Ac-10 isolates were showed large sized conidia (42.17-60.29 X 9.40-11.77). Three isolates Ac-5, Ac-9 and Ac-8 showed large sized beak length, ranging from 13.52-15.29 µm, whereas horizontal and vertical septation ranged from 1-9 and 1-5 respectively.

According to conidial length, conidial breadth, and the number of septa, Jyothsna *et al*. (2018) [9] noted the morphological heterogeneity of *A. sesami*. The highest conidial size was 24.88-34.64 µm x 9.61-12.13 µm, while the range of the average number of horizontal and vertical septa varied from 2.76-3.82 and 1.31-1.76 respectively.

Table 1: Designation of *Alternaria* spp. isolates collected from different sesame growing areas of Kalyana Karnataka region

District	Village	Isolate code
Bidar	Bhalki	AsB1
	Kohinoor	AsB2
	Kodambal	AsB3
	Khanapur	AsB4
	Hudagi	AsB5
	Chitaguppa	AsB6
Kalaburgi	Alapur	AsK7
	Pattan	AsK8
	Bableshwar	AsK9
	Keribosgha	AsK10
	Kamalapur	AsK11
	ZARS, Kalaburgi	AsK12
Koppal	Tawargere	AsKp13
	Hagedal	AsKp14
	Kukunpalli	AsKp15
	Vankalkunta	AsKp16
	Budhgumpa	AsKp17
Raichur	Huligudda	AsR18
	MARS, UAS, Raichur	AsR19
	Karadkal	AsR20

Table 2: Effect of solid culture media on mycelial growth and sporulation of *A. sesame*

Sl. No.	Media	Colony diameter (mm)*	Sporulation
1	Asthana and Hawkers's agar	40.18	+
2	Corn meal agar	77.33	+
3	Czapek's Dox agar	86.44	++
4	Malt extract agar	68.40	++
5	Oat meal agar	87.11	++++
6	Potato dextrose agar	90.00	+++
7	Potato dextrose carrot agar	88.22	+++
8	Richard's agar	81.77	+
9	Sabouraud's agar	87.11	++
10	V-8 juice agar	74.89	+
S. Em. ±		0.57	
C.D ($p \leq 0.01$)		1.7	

* Mean of three replications, DAI-Days After Incubation

Note:

++++ Excellent sporulation

+++ Moderate sporulation

++ Good sporulation

+ Poor sporulation

Table 3: Cultural characteristics of *A. sesami* on different solid media

Sl. No.	Media	Colony colour	Type of margin	Mycelial character
1	Asthana and Hawkers's agar	Light grey colour	Irregular with rough whitish margin	Dense mycelium with distinct zonations
2	Corn meal agar	Light grey colour	Regular with smooth whitish margin	Sparse mycelium with indistinct zonations
3	Czapek's Dox agar	Whitish to greyish colour	Slighlty irregular with smooth whitish margin	Fluffy mycelium with distinct zonations
4	Malt extract agar	Light grey to brown colour	Regular with rough whitish margin	Dense mycelium with indistinct zonations
5	Oat meal agar	Light grey colour	Regular with rough whitish margin	Sparse mycelium with indistinct zonations
6	Potato dextrose agar	Light grey colour	Regular with smooth greyish margin	Dense mycelium with indistinct zonations
7	Potato dextrose carrot agar	Dark grey colour	Regular with smooth grayish margin	Fluffy mycelium with indistinct zonations
8	Richard's agar	Whitish to grey colour	Slighlty irregular colony with rough and whitish margin	Sparse mycelium with distinct zonations
9	Sabouraud's agar	Light grey colour	Irregular with rough greyish margin	Sparse mycelium with indistinct zonations
10	V-8 juice agar	Grey colour	Regular with smooth dark grey margin	Sparse mycelium with distinct zonations

Table 4: Morphological characterisation of different isolates of *Alternaria* spp.

Isolate code	Size of conidia (µm)*				Septa in conidia		Length of the beak (µm)*		Mycelial width (µm)*	
	Length		Width		HS	VS				
	Range	Mean	Range	Mean			Range	Mean	Range	Mean
AsB1	24.72-31.80	29.08*	10.23-13.22	11.66*	4-5	0	4.86-6.86	5.62	3.50-4.66	3.89*
AsB2	29.62-34.42	32.25	7.96-9.85	8.64	3-5	0	5.93-8.49	6.91	2.88-4.43	3.87
AsB3	29.31-31.78	30.38	11.09-13.11	12.36	3-4	0-1	8.10-9.23	8.69	3.57-4.13	3.89
AsB4	19.84-24.40	21.77	11.69-12.33	11.38	2-3	0-1	6.86-7.39	7.11	2.47-4.29	3.44
AsB5	23.32-25.42	24.37	11.48-13.98	12.94	2	0-1	6.15-8.79	7.31	3.57-4.09	3.85
AsB6	23.65-29.57	25.83	10.13-12.94	11.48	3-4	0-1	5.50-6.07	5.85	2.65-4.63	3.61
AsK7	31.07-37.63	34.43	10.54-15.56	13.56	3-4	1	4.78-6.29	5.40	2.46-3.86	3.25
AsK8	27.53-35.07	31.23	9.76-13.26	11.45	3-4	0-1	5.07-7.93	7.19	3.62-4.13	3.80
AsK9	28.61-32.68	30.30	10.53-16.32	12.76	3	1-2	3.56-4.28	4.01	2.58-4.02	3.80
AsK10	27.53-30.10	28.55	11.43-13.51	12.51	2-3	0-1	3.83-4.27	4.02	3.35-4.72	4.28
AsK11	28.67-35.70	31.20	3.13-11.64	10.01	3-5	0-1	4.08-4.26	4.16	3.07-4.80	3.77
AsK12	35.94-38.86	37.28	8.15-9.30	8.78	4-5	1	6.15-8.83	7.37	2.78-3.24	3.02
AsKp13	29.71-32.69	31.36	9.73-12.99	11.20	3-4	0-1	6.02-7.13	6.65	4.05-5.54	4.83
AsKp14	24.33-27.07	25.89	11.39-12.09	11.67	2-4	0-1	6.98-7.15	7.05	3.28-4.34	3.68
AsKp15	20.72-27.98	23.73	9.35-11.64	10.25	2	0-1	5.50-6.07	6.05	3.07-5.09	3.91
AsKp16	20.99-24.08	21.71	6.04-7.62	6.98	2-3	0	3.67-4.16	3.93	3.54-4.32	3.92
AsKp17	21.34-24.34	22.94	8.75-9.78	9.33	2-3	0	3.98-4.23	4.10	4.12-5.71	4.69
AsKp18	25.08-31.54	28.56	14.02-16.01	15.04	3-4	0-1	6.73-7.31	6.97	3.36-3.76	3.57
AsR19	30.93-33.27	32.05	9.55-13.93	11.54	3-4	0	6.25-7.89	6.82	3.96-4.21	4.09
AsR20	37.51-43.33	40.18	11.14-15.34	13.34	4-6	1	6.16-7.96	6.88	2.48-4.60	3.77

*Mean of three observations; HS-Horizonal septa, VS-Vertical septa.

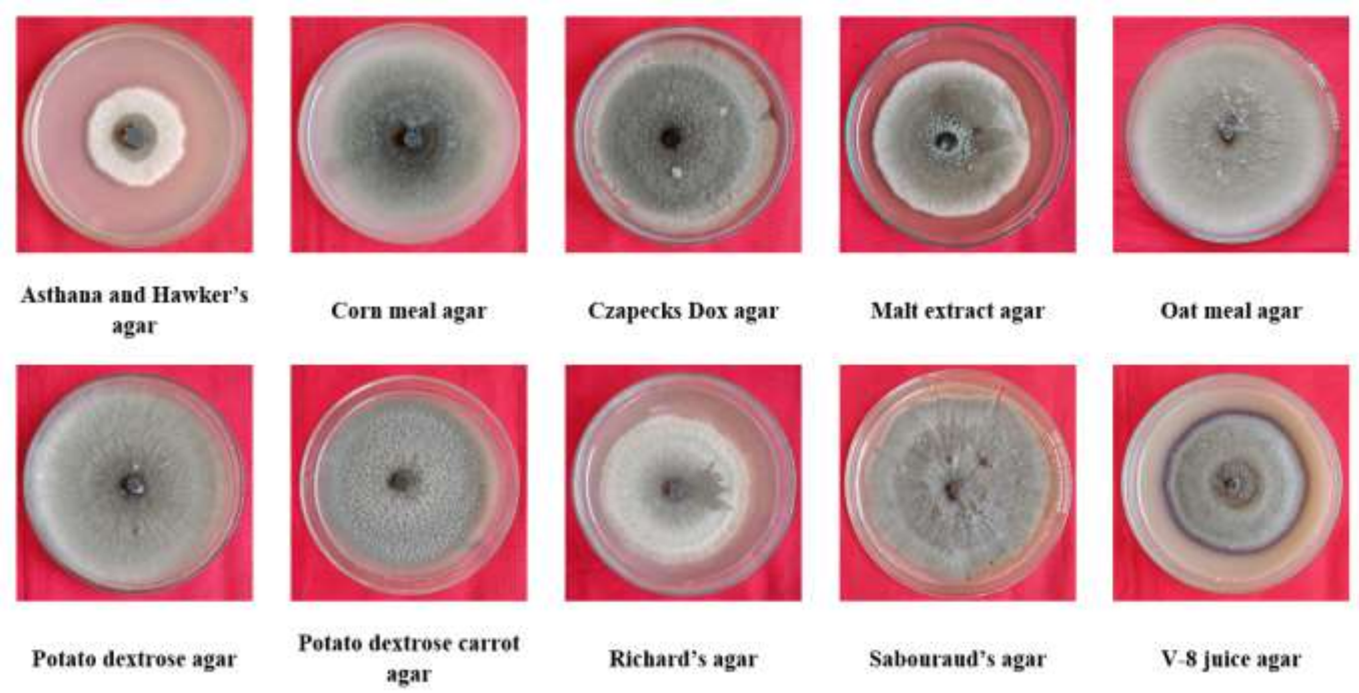

Plate 1: Effect of solid culture media on mycelial growth and sporulation of *A. sesame*

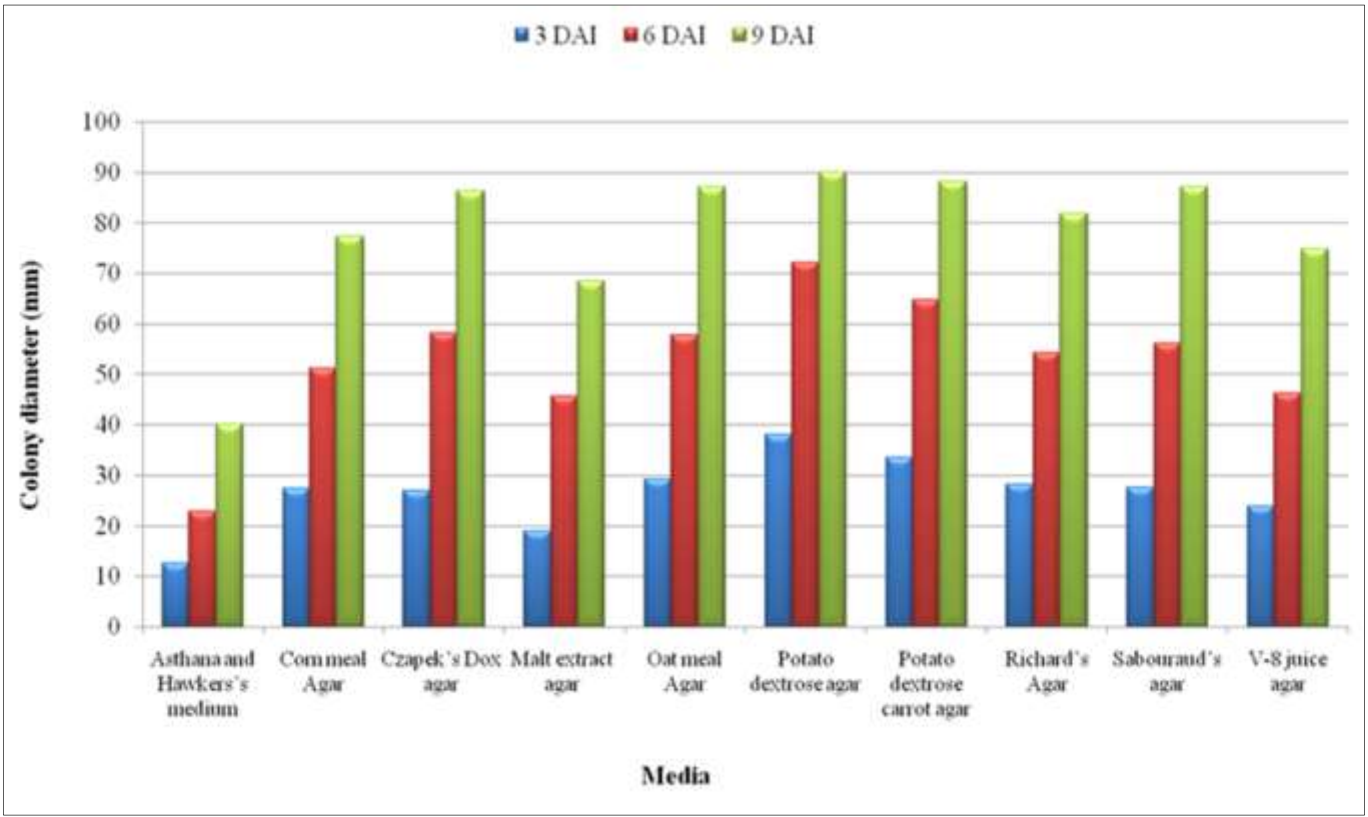

Fig 1: Effect of solid culture media on mycelial growth and sporulation of *A. sesami*

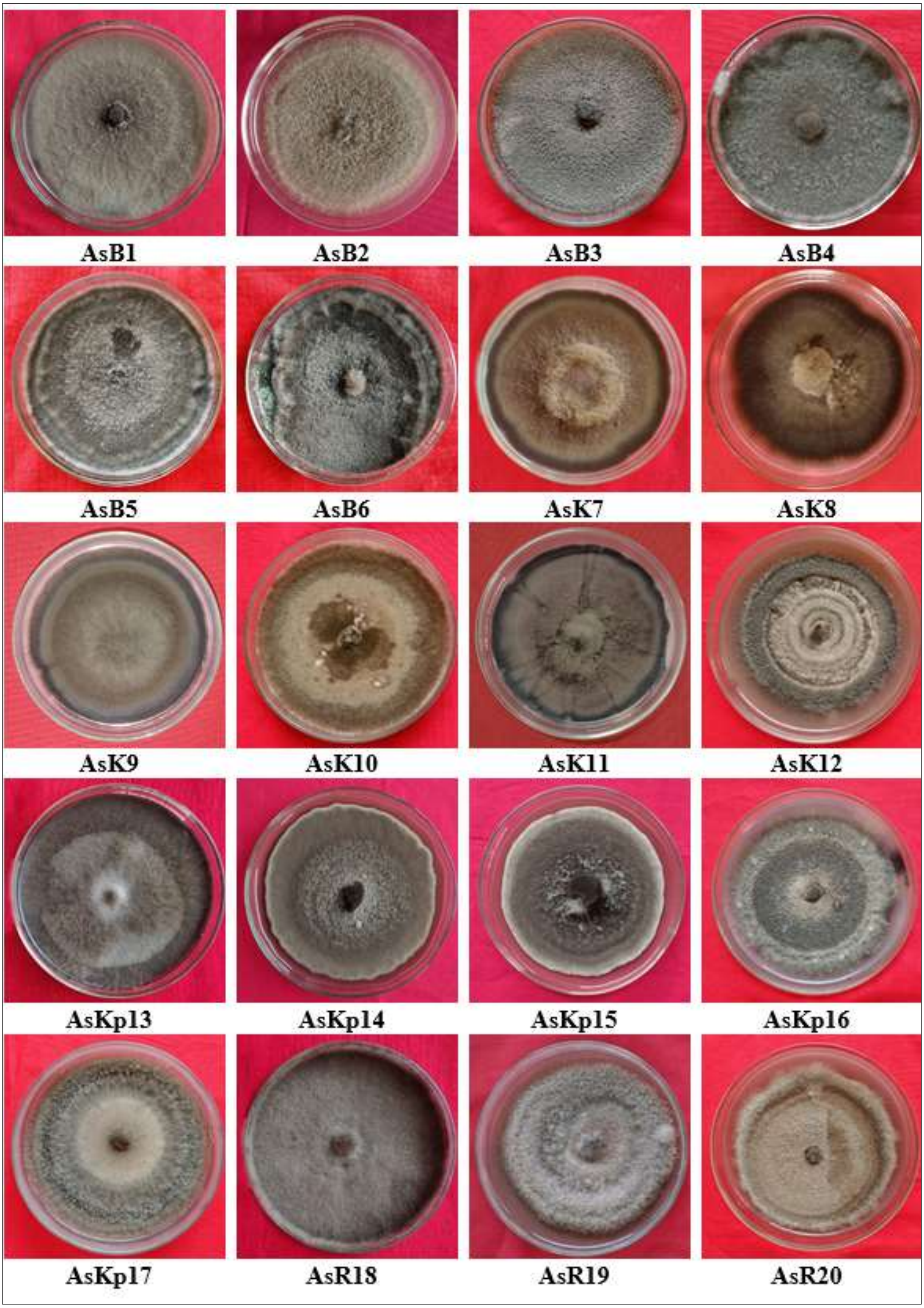

Plate 2: Cultural variability in different isolates of *Alternaria* spp.

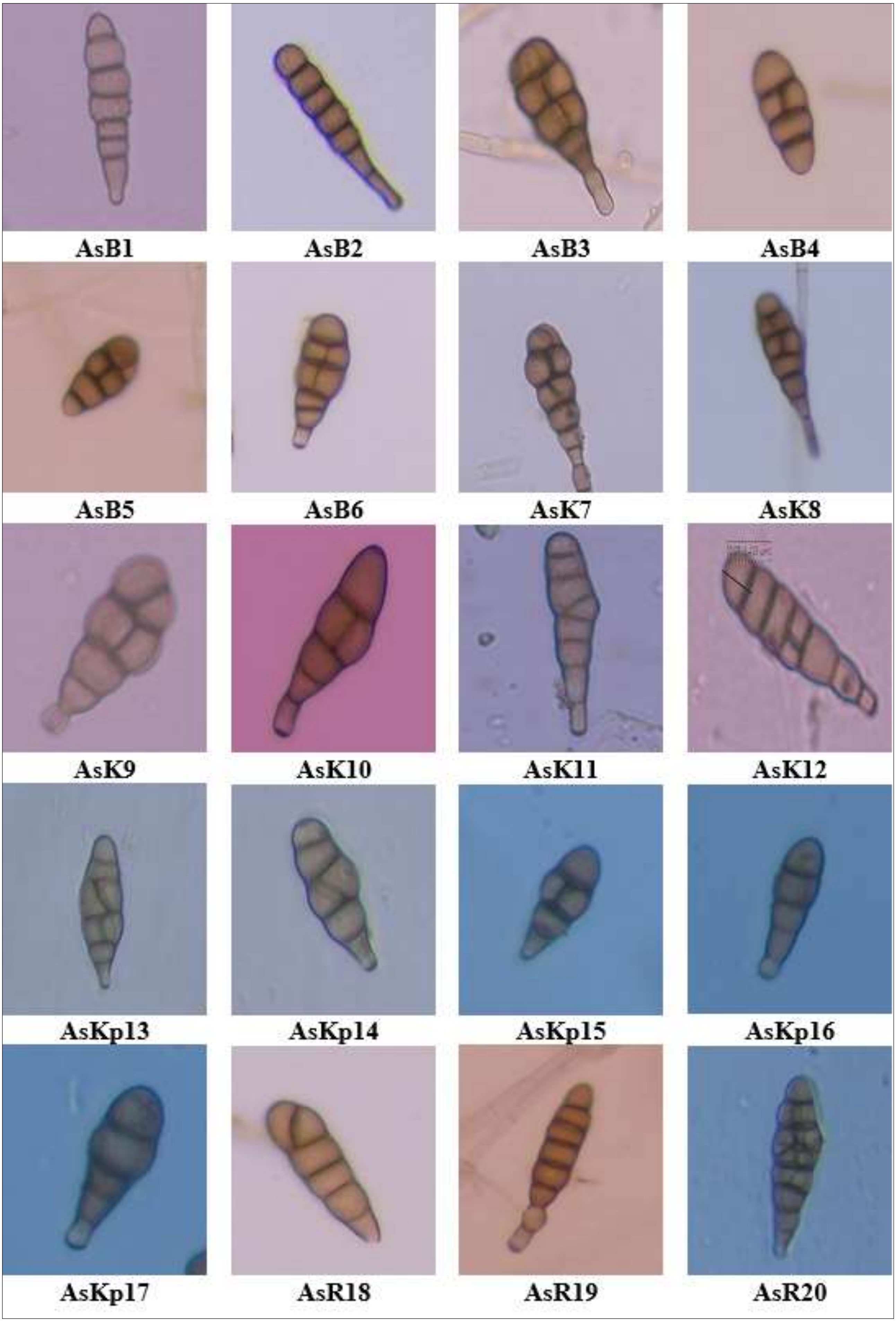

Plate 3: Conidial variability of *A. sesami* isolates (40X)

Conclusion
The diversity of cultural and morphological characterization observed in the present investigation emphasizes the variability in *A. sesami* isolates. Variability among the isolates may be attributed to long-term influence of weather conditions of particular location and ability of pathogen to adopt the varieties developed in specific situation.

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