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SURVEY, ISOLATION AND CULTURAL CHARACTERIZATION OF *ALTERNARIA BRASSICAE (BERK.)* SACC., FORM THE NATURALLY INFECTED FIELDS OF CAULIFLOWER IN AGRA UTTAR PRADESH INDIA

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Abstract

One of the most destructive fungi, *Alternaria brassicae* (Berk) Sacc., is responsible for the *Alternaria* blight, also known as the leaf blight disease of cauliflower, which causes a serious qualitative and quantitative yield loss in cabbage and cauliflower at the curd formation and seed setting stages. Thus the current study was carried out to determine the disease occurrence and to conduct in-depth studies of the morphological and cultural characteristics of the fungi. Survey was taken up in districts Agra UP India. Shamshabad recorded the highest disease incidence at 35%, while Malpura recorded the lowest at 8%. From the disease samples, 11 isolates were recovered and purified.All the cultures did well using potato dextrose agar.There were variations in the sporulation and mycelial growth. The study of cultural and morphological characteristics of various isolates of Alternaria spp. showed that the mycelia were septate and that conidia were formed in chains from the conidiophores. Both longitudinal and transverse septa make up conidia, which are beaked.

Kewwords **:** Cauliflower, *Alternaria brassicae*, *Leaf blight disease*, *Cultural variability*, *Potato Dextrose Agar*.

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Introduction

Brassica oleracea L (Cauliflower) is mainly vegetable crop cultivated in both temperate and tropical regions (Hossain and Hossain, 2010). It is the fifth most favorite vegetable crop in India after tomato, brinjal, cabbage and onion occupy an important place among the fresh vegetables in world vegetables. In India, *Brassica oleracea* is cultivated on large average and the major growing states of India are Punjab, Sikkim, Uttar Pradesh, Tamil Nadu, Rajasthan, Uttaranchal and West Bengal (Patel *et al*., 2018). There is approximately 90% water contains cauliflower curd thus it has the affinity to lose humidity in the process of evaporation or transpiration.Since the cauliflower curd is not covered by a thick, waxy layer, water loss is high. Low relative humidity (RH) and high outside temperatures increase the rate of water loss from the curd, which leads to a loss of crispness and subsequently browning (Serrano and Rolle, 2018). It is recognized to have health-promoting qualities and contains high quantities of vitamins C and B, iron, calcium, and phosphorus (Serrano and Rolle, 2018). An aborted floral meristem's head (the white curd) is typically the only part consumed, the stalk and several thick, green leavesare either discarded or used in vegetable broth (Patel *et al*., 2018).

The curd in cauliflower is the edible part which is used as delicious vegetable part. There is hardly any house where it is not regularly used single or mixed with potato as vegetable due to high phosphorus and ascorbic acid content in the curd. It is either used single or mixed with potato as fried or in curry from. During last three decades efforts are being made to improve the quality of cauliflower in various parts of the country the cauliflower head or curd is an undeveloped flower bud consisting of various florets. (Serrano and Rolle, 2018). In India, species *Brassica oleracea*, is cultivated both in hills or plains and from 11°N to 35°N. It is also normally grown in Nilgiri hills and in south northern Himalayas. In the plains of northern IndiaIn the hills across the world, it is cultivated from late August or early September to late February or early March, and from March to November. The latitude range between 11 and 60° N, with average temperatures between 5-8°C and 25–28°C, is where cauliflower grows best. During the vegetative growth cycle, cauliflower can survive temperatures between 10°C and 40°C for a few days.

Area of productivity and production of cauliflower in India and in worldwide are 22,840,000 and 8,573,000 Mt, 1,258,000 ha and 433,000.9 ha, and 18.2 and 19.8 Mt·ha^{-1}, respectively (Anonymous, 2014). China, India, Mexico, France, Italy, Poland, the United States, Pakistan, Germany, and Egypt are the top ten producing nations in the world. In India highest production of cauliflower occurs in West Bengal, Maharashtra, Bihar, Madhya Pradesh, Odisha, Gujarat, Haryana, Chhattisgarh, Jharkhand, Assam, and Uttar Pradesh (Anonymous, 2014).

Alternaria blight disease severity in cauliflower occurs after initiation of curd formation and continues till seed pods are set. After infection, it is difficult to control the disease by cultural or chemical methods. In cauliflower, symptoms appear after mid-season in the generative phase. The disease primary occurs like wet soft lesions on cauliflower curd; lesions enlarge into a watery rotten mass of tissues and

covered by a dark appearance. Infection of stem, curd head, branches and inflorescence show symptoms of wilting and later dies. The affected curd of cauliflower loses its turgidity and fails to develop flowering shoots. Leaves become yellow and often drop and abscise. Since pathogen causes considerable loss to the production of this crop hence considering the economic importance of cauliflower and reduction in its production due to *Alternaria* blight disease in India. The present experimentation and investigation is undertaken on biotic and abiotic elicitors and induced resistance in cauliflower against *Alternaria brassicae.*

One of the most harmful fungal diseases, *Alternaria* blight, commonly known as the cauliflower leaf blight disease, significantly reduces the yield of cauliflower and cabbage at the time of curd formation and seed setting. It is caused by *Alternaria brassicae* (Berk) Sacc. and *A. brassicicola* (Schw.) Wilt. Although the disease is present everywhere, it is more common in subtropical and temperate regions.Since no proven source of resistance has been noted in any of the hosts to date, managing the disease is very difficult. In all of India's cauliflower and cabbage growing regions, the yield loss caused by this infection ranges from 5 to 30%.In Uttar Pradesh, the incidence and severity of the disease were 10–40% and 26%, respectively. *Alternaria brassicae* (Berk.) Sacc. is an ubiquitous fungus having a wide host range. It causes black leaf spot in cauliflower leaves and curd (Selvamani *et al*., 2014). The severity of *Alternaria* blight of cauliflower is seriously affected by variability of temperature and relative humidity (Awasthi and Kolte, 2003). It is considered as one of the most destructive diseases of vegetables in relatively cool and moist areas of the world and causes considerable damage. Various attempts to manage *Alternaria* leaf spot disease with the combination of effective fungicides, botanicals and bioagents are reported (Chavan *et al.*, 2015 and Devi *et al*., 2019).

In all of India's developing areas for cauliflower, the production loss caused by this pathogen ranges from 5 to 30%. In Uttar Pradesh, the incidence and severity of the condition were 10–40% and 26%, respectively (Saha *et al*., 2016). In most cases, *Alternaria* attacks its host's aerial parts.Naturally occurring small, round, dark spots are the first indications of Alternaria infection in plants. The round patches, which are often grey, gray-tan, or nearly black in appearance as the infection advances, may get as big as a half inch in diameter or more..

Spores in most *Alternaria* species are beaked and multicelled. The cells are divided longitudinally and transversely. Normally *Alternaria* species are saprophytes and abundant in the environment, still some plant are pathogenic, cause diseases on a great variety of economically important crops like oil-seed crops, cereals, vegetables and fruits (Pitt and Hocking, 2009).

Material and Methods

(1) Survey of *Alternaria brassicae* (Berk.) Sacc., from naturally infected plants

Cauliflower curd and leaves showing blight disease (*Alternaria* blight) symptoms were collected sampled in different region of Agra (Achhnera, Runkata, Bichpuri, Iradatnagar, khandauli, Shamshabad, Malpura, Fatehabad, Bah, Fatehpursikari, Kiraoli etc.) Uttar Pradesh, India.

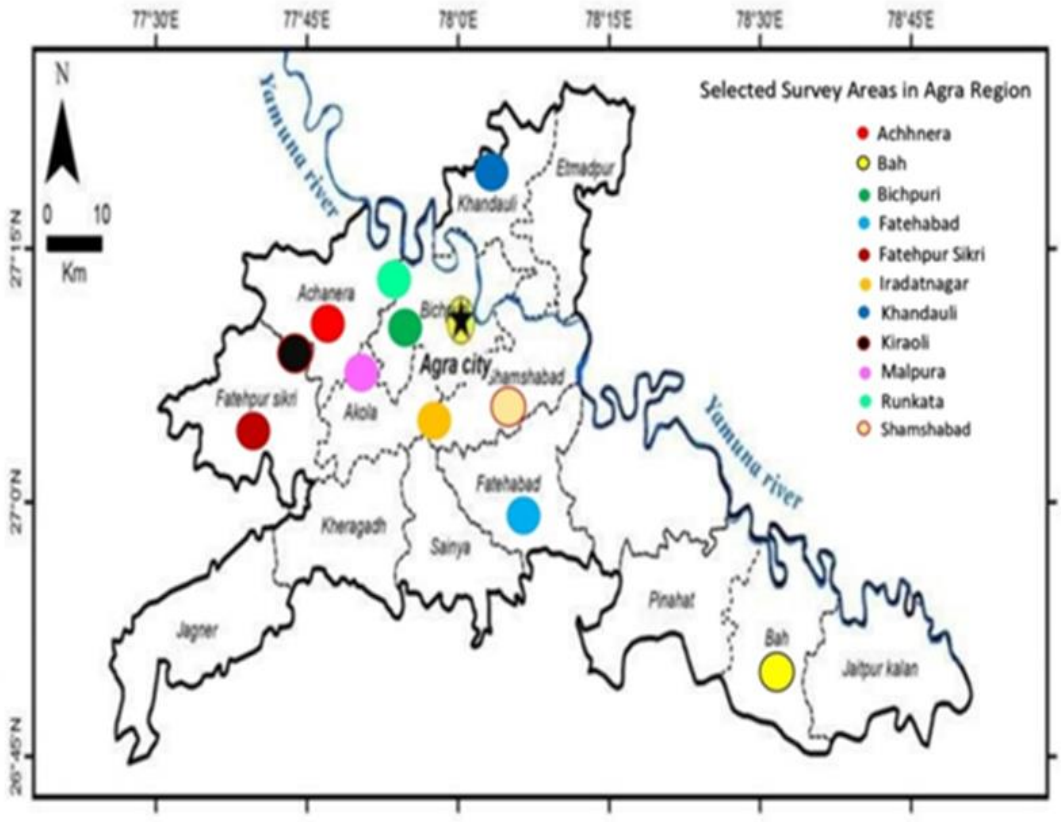


Fig. 1 : Selected Survey Areas in Agra Region (U.P.)

Table 1: *Alternaria brassicae* isolates infecting *Brassica* species at different geographical locations.

***A. brassicae*isolate**	**Host**	**Date of collection**	**Location**	**Latitude and longitude**	**Plant part**
Ach C-1	Cauliflower	15-Feb- 2022	Achhnera	27° 15' N, 77° 54'E	Leaf
Run C-1	Cauliflower	22-Jun-2022	Runkata	27° 14' N, 77° 52'E	Leaf
Bip C-1	Cauliflower	28-Feb-2021	Bichpuri	27° 17' N, 77° 91'E	Inflorescence
Khc C-1	Cauliflower	23-Feb-2022	Khandauli	27° 8' N, 77° 54'E	Inflorescence
Smd C-1	Cauliflower	24-Jan-2021	Shamshabad	27° 010'N,78°16 ' E	Inflorescence
Fad C-1	Cauliflower	8-Mar-2022	Fatehabad	27°09' N,78°17'E	Leaf
Mal C-1	Cauliflower	1-Feb-2021	Malpura	27° 17' N, 77° 98'E	Leaf
Bh C-1	Cauliflower	1-Feb-2022	Bah	26° 89' N, 78° 57'	Inflorescence
Irn C-1	Cauliflower	3-Mar-2022	Iradatnagar	26° 87' N, 80° 92'E	Leaf
Fat C-1	Cauliflower	10-Feb-2022	fatehpursikri	27° 17' N, 77° 44'E	Leaf
Kir C-1	Cauliflower	21-Jan-2021	Kiraoli	27º 13' N, 77º 78'E	Inflorescence

Fig. 2: Leaf blight symptoms on Leaf of cauliflower

Fig. 3: *Alternaria* blight symptoms on Inflorescence

Fig. 4 : Infected Farmer field of cauliflower during the survey

Fig. 5 : Infected Farmer field of cauliflower during the survey

(2) Identification using slide culture technique:

The fungus culture was identified by morphological and microscopic methods. (Abdel-Fattah *et al*., 2007). The microscopic characteristics for morphological characterization in accordance with the common taxonomic key are determined using slide culture (Singh *et al*., 2012, Singh *et al*., 2013).

Results and Discussion

The fungus was recognised using the common taxonomic key, which takes into account the colour of the colony and the shape of the hyphae and conidia. Colonies were fast growing, black toolivaceous-black or greyish colour when incubated on PDA plates at 30 °C for 7 days (Fig. 1a). Septate hyphae, condiophore structure and development of condia was seen under the microscope (Fig. 1b,c). Conidia were foundin chains with a conspicuous beak, smooth, greyish-olive, with 7-8 cross septa and longitudinal ones, slightlyconstricted at the septa (Fig. 1d)

Pathogen was recognized according to the morphology of hyphae and conidia, colony colour and standard taxonomic key. Colony was fast growing, and colour change to black to olivaceous-black or grayish when incubated on PDA plates at 25 °C for seven days (Fig. 6). In the microscope fungus were seen the development of conidia. Septet hyphae, structure of conidiophores (Fig. 7). Conidia were found in chains with a conspicuous beak, smooth, grayish-olive, with 6-8 cross septa and longitudinal ones, slightly constricted at the septa (Fig. 7) (Dhaliwal & Singh, 2019).

Survey and frequency of *A. brassicae* in different Cauliflower Fields

The survey was done in mainly cauliflower growing regions of Agra, Uttar Pradesh during cropping season (Oct to Feb) (Table 1). The disease occurrence various cauliflower field to another cauliflower field in different areas of Agra region. The major disease symptoms were observed on leaves and curd which are dark brown to black circular spots covering the whole leaves and curd. After that these spots become large, covered with black spores in concentric zonation and destroyed the cauliflower inflorescence (Fig. 2 to 5). The average frequency of disease ranged from 8 to 35 % in different cauliflower fields (Table 2). The highest disease frequency was recorded in as Shamshabad as 35 % and minimum in Malpura as 08 % (Table 2)

Table 2 : Survey of Alternaria blight disease in cauliflower during 2021-2022

S.N	Area in Agra region	Isolate code	% Frequency
1	Achhnera	Ach C-1	18-22
2	Runkata	Run C-1	25-30
3	Bichpuri	Bip C-1	15- 25
4	Khandauli	Khc C-1	11-20
5	Shamshabad,	Smd C-1	25-35
6	Fatehabad	Fad C-1	25-30
7	Malpura	Mal C-1	08-15
8	Bah	Bh C-1	17-24
9	Iradatnagar	Irn C-1	12-20
10	Fatehpur sikri	Fat C-1	15-26
11	Kiraoli	Kir C-1	15-22

Cultural Variability of Different Isolates of A. brassicae

Culture-grown petri dishes were directly observed to record cultural variability such as colony colour, mycelial

growth, zonation, pigmentation on medium, and texture, and slides of 9-day-old cultures examined under a microscope to document sporulation. On potato dextrose agar, all of these isolates were analyzed for morphological and cultural differences.) Following the agar's solidification, each isolate's 5 mm culture petri plate was injected onto the aforementioned PDA media. For the development, these inoculated petri plates were maintained in BOD at 25 -1 C. At 3–4 days following inoculation on the aforementioned selective nutrient media, radial development was seen (Saha et al., 2016)

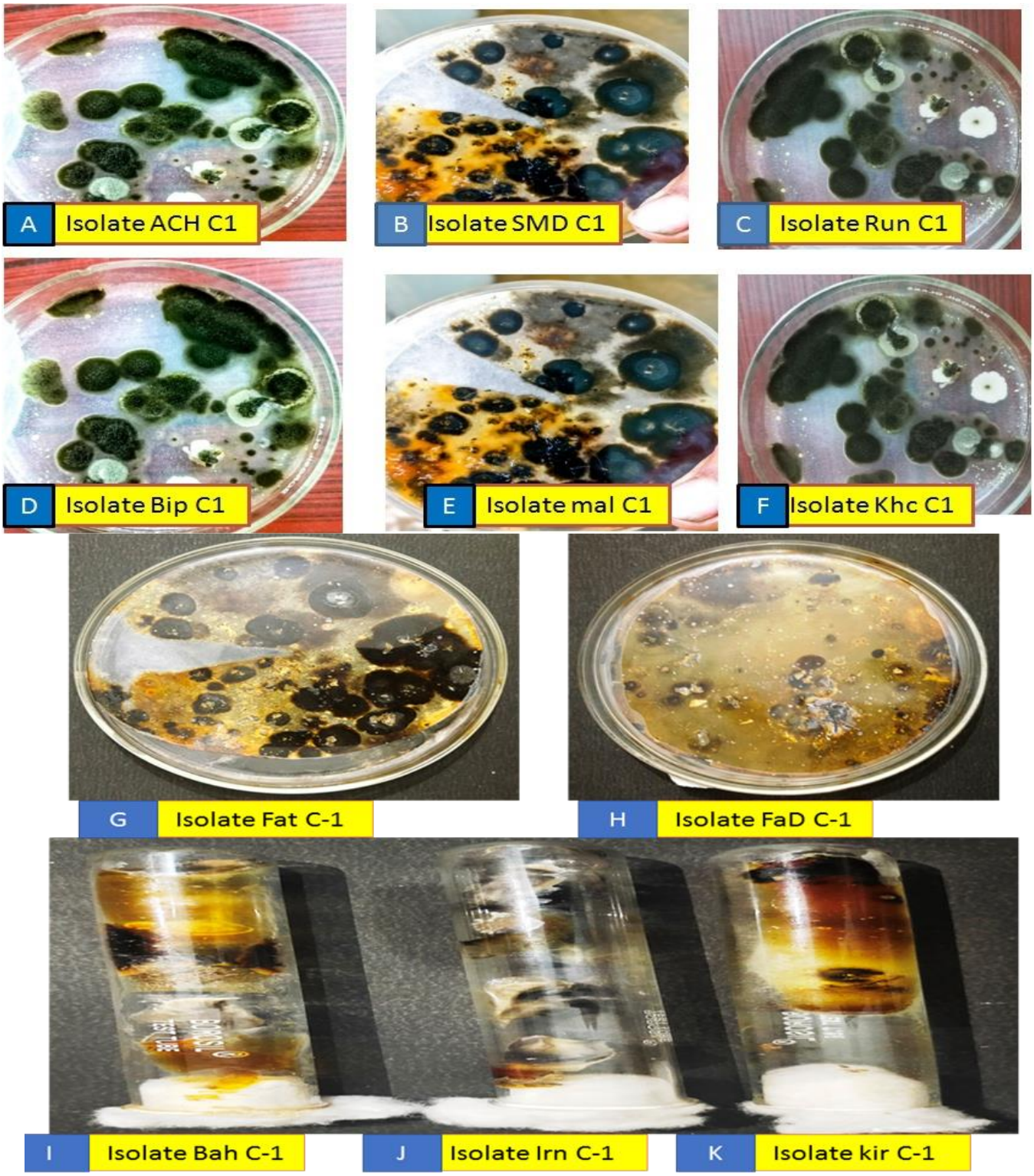
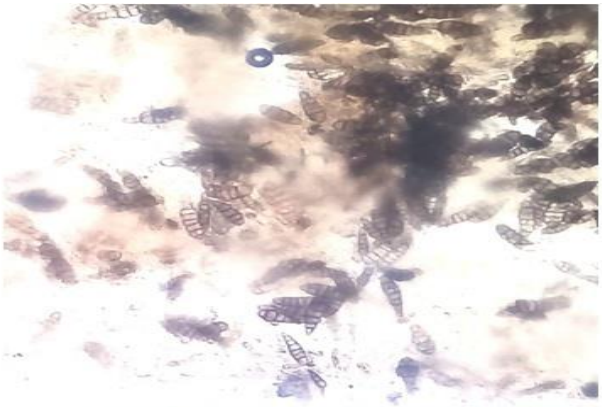


Fig. 6 (A to K) : Colony morphology of Isolate on PDA Medium

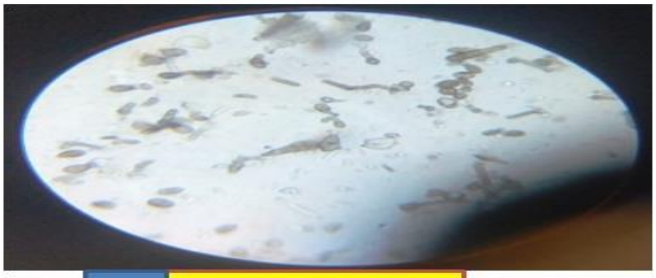
The colony of *Alternaria brassicae* grow on potato dextrose medium. These culture are collected in different areas in Agra Uttar Pradesh. The islolate 1Smd C-1Ach C-1, Khc C-, Mal C-1 Bip C- 1 growth in maximum growth in potato dextrose Agar medium and isolate Run C-1 Fat C-1, Fad C-1, Bah C-1, Irn C-1, Kir C-1 minimum growth in potato dextrose Agar medium.

A Isolate Smd C-1



B Isolate Ach C-1

C Isolate Run C-1

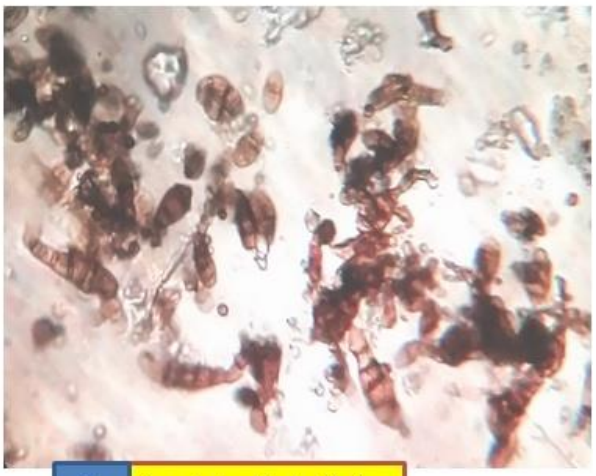


D Isolate Kir C-1

E Isolate Fad C-1

F Isolate Bip C-1

G Isolate Mal C-1



H Isolate Bah C-1

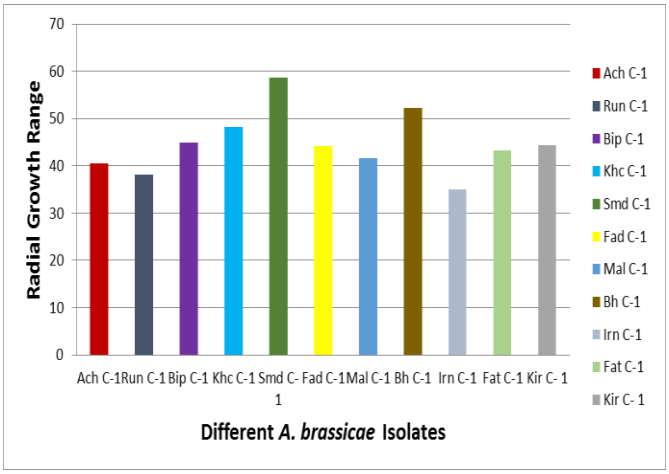
Fig. 7 (A to H) : Conidia of *Alternaria brassicae* under microscope

Radial Growth of *Alternaria brassicae* Isolates

The radial development of eleven different isolates of *A. brassicae* was seriously different on PDA media. Radial growth of *A. brassicae* isolates in PDA media various from 37.15 to 58.75 mm. The Smd C–1 isolate noticed highest radial growth on PDA media. The lowest radial growth was exhibited by Run-C-1 isolate.

Table 3 : Effect of different media on radial growth of *A. brassicae* isolates.

S.N.	Isoltes	Radial growth (mm) 10 days after inoculation (PDA)
1	**Ach C-1**	40.55
2	**Run C-1**	37.15
3	**Bip C-1**	45.00
4	**Khc C-1**	48.25
5	**Smd C-1**	58.75
6	**Fad C-1**	44.23
7	**Mal C-1**	41.55
8	**Bh C-1**	52.23
9	**Irn C-1**	35.00
10	**Fat C-1**	43.25
11	**Kir C- 1**	44.35
	Mean	44.66
	SD	6.276692
	SE	1.8924



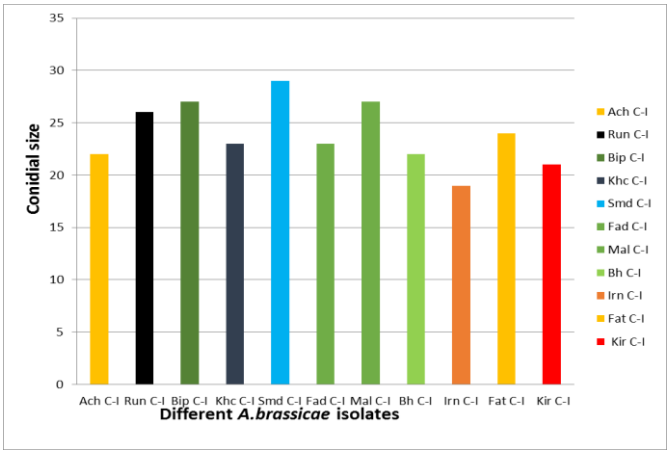
Variability in radial growth of *A. brassicae* isolates on selected nutrient media (Table-3)

Conidial Morphology of *A.brassicae*

In 4 different microscopic fields, the length of 8 conidia from each A. brassicae isolate was measured. The maximum length of conidia ranged from 148 lm (Smd-C I) to 125 lm (Run–C I). Furthermore, the number of transverse and vertical septations in each isolate's conidia was measured. In the isolates Smd-CI and Mal-CI, the highest number of transverse septations was observed at nine, followed by eight in the isolates Khc-CI and Fad-CI. All isolates had between 0 and 2 vertical septations. All of the *A.* brassicae isolates in the current study showed high levels of variability in conidia length, width, and septa, evinceing the existence of variety among the 11 isolates (Table 4). Similar findings were made regarding the conidial length, width, and number of septa in A. brassicae that infected mustard and cauliflower in India. The conidial variations in *A. brassicae* and *A. brassicicola* are one of the most important criteria to differentiate these two species, but not for distinguishing *A. alternata* from *A. brassicae* (Sharma *et al*., 2013).

Table 4 : Conidial morphology of *Alternaria brassicae* isolates of cauliflower

S.N.	Isolates	Conidial Size			
		Length (lm)	Width (lm)	No. of septa	
				Transverse	Vertical
1	**Ach C-I**	125	22	7	1
2	**Run C-I**	140	26	6	1
3	**Bip C-I**	135	27	7	0
4	**Khc C-I**	142	23	8	0
5	**Smd C-I**	148	29	9	2
6	**Fad C-I**	138	23	8	2
7	**Mal C-I**	128	27	9	1
8	**Bh C-I**	144	22	7	0
9	**Irn C-I**	145	19	4	0
10	**Fat C-I**	139	24	5	1
11	**Kir C- I**	126	21	4	1
SD		7.52077	2.8747	1.71044	0.7158
SE		0.670003	0.431679	0.25384	0.715819



Conidial morphology of *Alternaria brassicae* isolates of cauliflower

Conclusion

The research focuses the *A. brassicae*, which causes blight disease in cauliflower, has a wide range of diversity. According to a latest study (Goyal *et al.*, 2011), temperature and place of origin affected the conidial morphology, mycelial development, and sporulation of thirteen isolates of A. brassicae that were collected from different geographical zones. Similar to this, reports of variation in the morphological traits of A. brassicaeisolates from various parts of India (Meena et al., 2005; Kaur et al., 2007; Singh et al., 2007). In terms of mycelial development and sporulation, some researchers have studied the cultural heterogeneity in Alternaria species (Ansari et al., 1989; Patni et al., 2005; Kaur et al., 2007). Winter crops such as cauliflower and mustard are impacted by A. brassicae. The goal of the current study was to better understand the differences in morphology, culture, pathogenicity, and molecular characteristics among the isolates infecting both crops growing in various Indian states. Regardless of the crop or the location, there was a significant difference in the growth, sporulation, and conidial morphology of A. brassicae isolates on various nutritional media. All of the isolates were discovered to be harmful to each host. Despite their

differences in morphology and culture, the 11 isolates of Alternaria brassicae were discovered to be harmful in nature.

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