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Research Article

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Morphological and cultural variations of *Alternaria brassicae* isolates from mustard crop of Allahabad, Uttar Pradesh

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ABSTRACT

Mustard is the most economically important genus of the family Cruciferae. It is an oil seed crop grown under a wide range of agro-climatic conditions in India. Among the various fungal diseases occurring on mustard, *Alternaria* leaf spot caused by *Alternaria brassicae* is one of the devastating diseases reported to cause 10-70% yield losses. Variations in morphological and cultural characteristics among 31 representatives in Allahabad geographical isolates of *A. brassicae*. Different isolates showed high-level variability *in vitro* concerning growth pattern, sporulation, conidial length, width, and number of septa. Conidia of Allahabad isolate (II-1b21) was the smallest in size with the lowest number of horizontal and vertical septa. Conidia of Allahabad isolate (IV-8A21) and (II-7C21) showed the largest in size with the highest number of horizontal and vertical septa. Substantial variation was found in mycelial growth and sporulation among these isolates in different culture media, temperature, pH and relative humidity. However, Potato Dextrose Agar, oatmeal agar, Czapex dox agar, Carrot meal agar and V-8 juice Agar were best for all the cultures. The highest radial growth was shown in the OMA medium by III-6C20 isolates, while the lowest growth was shown in the CA medium by II-7C21 and II-8B21 isolates. Out of all of them, the isolate from Barwa (I-6B121) sporulated the most ($41.75x10^5$/mL) whereas the isolate from Shekhsarwa (I-4A21) sporulated the least ($0.5x10^5$/mL). The various isolates showed varying rates of mycelial development and sporulation at various pH values. Phoolpur isolate (UN120), Sahso (II-4II20), Bhandra Naini isolate (III-5C21), SHUATS Naini isolate (III-6C20), Among the 31 isolates,9 isolates II-UN120 from Phoolpur, PRG, IV-5C20 from Malak har har Soraon, I-4A21 from Shekhsarwa, I-6B21 from Barwa PRG, I-6A1y21 from Barwa PRG, II-8B21 from Devnahri Phoolpur, III-6C21 from Sarangpur, IV-4B21 from Phaphamau, and IV- 6AY21 from Morahu were found to be high degree of infection as the spot produced by them were more than 10 mm in diameter. A dendrogram was created using the Unweighted Pair Group Method with Average Means (UPGMA) to analyze the morphological and cultural characteristics of *A. brassicae* isolates on nutrient media. This dendrogram identified two major clusters with 90% similarity. One cluster (group I) comprised 24 isolates while another cluster (group II) comprised of remaining seven isolates. Isolates of Karchana, Meja, and Koraon were found to be more similar to each other whereas Handia, Phoolpur, and Soraon isolates were distantly related to each other.

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INTRODUCTION

Mustard, a crucial genus in the Cruciferae family, includes oil seed crops like mustard, rape, and vegetables like cauliflower and cabbage, grown globally under diverse agro-climatic conditions. These crops are economically important in local and international trade as they yield edible oil (30-48%), which is used as the main cooking medium in northern India.

Alternaria was first described in 1817 by Nees with A. *tenuis* as a type of species which was later renamed as A. *alternata*. The fungus that causes infections on the mustard is *A. brassicae*. Identification of this fungus (Berkeley, 1836) and named it *Macrosporium brassicae* Berk (Verma & Saharan, 1994) which was later renamed as *A. brassicae* (Berk.) Sacc. by Saccardo (1886). In India, Butler (1918) first reported *Alternaria* blight of rapeseed on mustard (*Brassica campestris* var. Sarson) in 1901 at Tirhoot near Pusa, Bihar.

Dark black spots on leaf of different crucifers have been reported in India (Meena *et al.*, 2010) and other country. This pathogen has the potential to survive in the infected seed, in plant-diseased debris, in soil and on weed hosts for several months at different temperatures and Relative humidity (Kumar & Gupta, 1994). This is the most threatening disease and yield losses ranging from 30-47% have been reported from India mustard (Chattopadhyay *et al.*, 2008).

The main taxonomic criteria for defining fungal species are the morphological properties of conidia and conidiophores, as well as occasionally host plant relationships (David, 1991). Brassica are affected by *Alternaria* blight depending on the season, the location, and even the specific crop within a region. There have been reports of variations in the morphological traits of A. *brassicae* isolates from various parts of India (Goyal *et al.*, 2011).

The purpose of this study was to determine the morphological and cultural growth characterization A. *brassicae* isolates collected from infected leaves of mustard plant.

MATERIALS AND METHODS

Thirty one infected leaf samples are collected from different regions of Allahabad like Karchhana, Koraon, Phulpur, Sahso, Bara, Sankargarh, Meja, Sadar, Soraon, Handia and the total range of Allahabad is approximately ~5,482 km^2. The pathogen *Alternaria brassicae* isolates were obtained from the black leaf spot of mustard during the winter season from Feb 2019 to March 2021 (Figure 1). After collection of infected leaves they were placed in an ice bath that is maintained at 4 °C. The infected leaves were incised into 2 mm pieces and sterilized with 4% Sodium hypo-chloride (NaOCl) solution for 1-2 minutes, washed thoroughly with sterile distilled water (SDW) 3-4 times and placed on Potato Dextrose Agar (PDA) medium containing culture plates and in BOD incubator for 4-5 days. Fungal colony growth was observed in PDA plates containing diseased leaf pieces after 4-5 days of incubation at 24-25 °C. Streptomycin was added in the culture medium during pouring to escape the growth of bacteria. The mycelia from the margins of the leaf spot pieces which appeared to have formed separate colonies on the media were then aseptically placed into separate Petri plates that contained PDA medium. There, they grew for 7 days at +24 °C in the BOD incubator. The pathogen was isolated using a single spore isolation technique based on Simmons (2007) assessments of the conidiophore and conidial morphology. The fungal pathogen cultures that were successfully isolated have been preserved at 4 °C on PDA slants. The calibration of fungal spores was done by using an ocular micrometre (Meena *et al.*, 2005). Morphological variability was examined among the 31 isolates of A. *brassicae* their single-spore cultures of A. *brassicae* were subjected to an *in vitro* study of their cultural variability in different conditions, like temperature, relative humidity (RH), duration of light, pH, and culture media. Thirty-one single-spore cultures of A. *brassicae* were examined for their cultural diversity at seven distinct pH values (5, 6, 7, 8, 9 and 10), four different RH conditions (25, 50, 75, and 100%), and six different temperatures (5, 10, 15, 20, 25, and 30 °C). PDA medium with a pH of 7.0 was inoculated with a 4 mm mycelial disc of developing A. *brassicae* colonies. Once inoculated, Petri plates were cultured for 28 days at seven different temperatures and 100% relative humidity. Every treatment was conducted three times. Various humidity solutions were maintained using Goyal (2011) method to examine the impact of relative humidity on mycelial development and sporulation. Six different culture media Corn meal agar (CMA), Czapex dox agar (CZA), V-8 juice agar (V-8J), Oatmeal agar (OMA), and Carrot agar (CA) were developed, and pH was adjusted to (5, 6, 7, 8, 9 and 10) according to culture, to study the impact of culture media on mycelia growth and sporulation. The colony's radial growth was observed every day for 10 days following inoculation at various temperatures, RH, pH, and culture conditions. Consider the cultures that were grown in the seven different media plates and find out the conidial concentration of each isolate. Ten millilitres (10 mL) of sterile distilled H_2O was poured into culture culture-containing plate. The culture surface was gently scrapped with the help of a sterile glass slide to make a conidial suspension. The haemocytometer was used to determine the conidial concentration of each culture. The haemocytometer consists of a thick glass, microscope slide with rectangular indentation that forms a precision volume chamber. It is a counting-chamber device and is usually used for counting of blood cells after 28 days of the inoculation observations for sporulation were performed. A pathogenicity test was performed on mustard plants for symptom production by spraying the leaves with a conidial suspension of A. *brassicae*. The seed of mustard was sown in fields of the botanical garden, Department of Botany, University of Allahabad, Prayagraj Uttar Pradesh during October 2019-20 and October 2020-21. After seven weeks some plants should emerge from the dense plants and a distance of 25-30 cm should be created between them. The leaf area was sprayed with an optimal inoculum concentration of $1.0x10^6$ conidia/mL. The data were aggregated for the isolates' mycelial growth (on the 10^{th} day) and sporulation (on the 25^{th} day) to cluster 31 A. *brassicae* isolates based on observations for diversity in culture. Thus, based on information about the isolates'

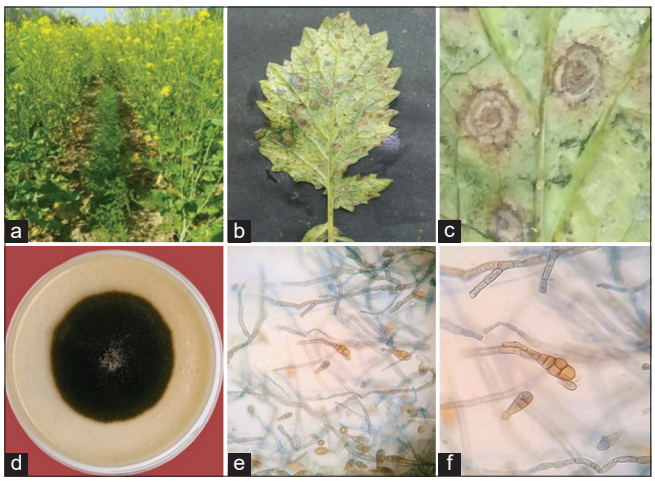

Figure 1: Morphological and cultural characteristics of *Alternaria brassicae* isolate. a) An agriculture fields of mustard crop infected with pathogen, b) An infected leaf, c) Leaf with concentric ring, d) Growth on culture medium, e) Fungal hyphae with spore and f) A single spore of *Alternaria brassicae*

mycelial development and sporulation, distinct dendrograms were developed. SAS Version 9.1 statistical software was used to do the hierarchical approach of clustering (Johnson & Wichern, 1996) to produce the dendrograms.

RESULTS

The Morphological and Cultural Characteristics of Various Isolates of *A. brassicae*

All 31 *A. brassicae* isolates that were collected from different region of Allahabad. These isolates showed variations based on morphological and cultural characteristics (Table 1). Among these Naini mustard isolate (IV-6AY21) having the lowest average width at 8.4 μm. The average number of transverse septa varied from development rates. Using a compound microscope, morphological assessments of *A. brassicae* were conducted on both the host and in medium (PDA). There was minimal change in the colour of the conidia growing on PDA and the colonies. The *A. brassicae* isolates range in colour from light brown to dark brown. The mycelia colour varies between grey and brown. Among the isolates, the conidia shared similar characteristics, such as a smooth, rough surface and a light grey or brown colour. The majority of the conidia had lengthy beaks and were obpyriform in shape. In terms of conidia length, conidia width, beak length, and septum number the 31 *A. brassicae* single-spore cultures displayed significant ($P<0.05$) morphological variability (Table 2). The Jhansi, Naini, Ghoorpur, Hanumanganj, and Barwa isolate (II-2C20, III-5C20, V-2A20, V-7A20 and I-6A1y21) had the lowest conidial length, measuring 14μm, while the Thanpur Sahso and Sekhsarwa isolate (I-4B21, II-7C21) had the longest conidial length, between 72 μm to 72.8 μm respectively. The average conidial length varied between 30.1 and 50.9 μm; the values were lowest in the Barwa mustard isolate (I-6A1y21) and greatest in the Sekhsarwa mustard isolate (I-4B21), at 50.9 μm. The average conidial width varied between 8.4 and 15.86 μm, with the Barwa mustard isolate (I-4B21) having the highest average width at 15.86 μm and the Morahu isolate (IV-6Ay21) having the lowest average width 8.4. Average number of transverse septa varied from 3.0 to 7.0 with the Thanpur Sahso mustard isolate (II-7C21) having the highest number at 7.0 and the Narayanpura Jhusi mustard isolates having the lowest number at 3.0. The Average number of vertical septa varied from 1.0 to 4.0 with the Thanpur Sahso mustard isolate (II-7C21) having the highest number at 4.0 and the Kahimapur Jhusi mustard isolate (II-2C20) having the lowest number at 1.0. At last, it was found that the Jhusi and Barwa isolate (I-6A1y21, IV-6Ay21, V-IB20 and II-2C20 isolates, respectively) had the smallest conidia size and smallest number of septa while the longest conidial size and highest number of septa is found in Sahso mustard isolate (II-7C21) and sekhsarwa isolate (I-4B21) respectively. Conidia were examined under a 40X magnification microscope to show size variability. They could be divided into two groups: small (<43.4) and long (>43.4 μm), but not based on their geographical origin. The small group include from Barwa (I-6A1y21and IV-6Ay21) Jhusi (V-IB20, V-2A20 and II-2C20) Naini (III-IB20 and I-1B20) Hanumanganj (V-7A20) and Bara ghoorpur (III-5C20) while long group include Sahso (II-7C21) and Sekhsarwa (I-4B21).

A dendrogram (Figure 2) was constructed based on data for morphological and cultural characteristics of *A. brassicae* isolates on nutrient media from the similarity coefficient by using the Unweighted Pair Group Method with Average Means (UPGMA). This dendrogram identified two major clusters with 90% similarity. One cluster (group I) comprised 24 isolates from Narayan Daspura Jhusi (V-1B20), Mubarakpur kotwa (I-1B20),

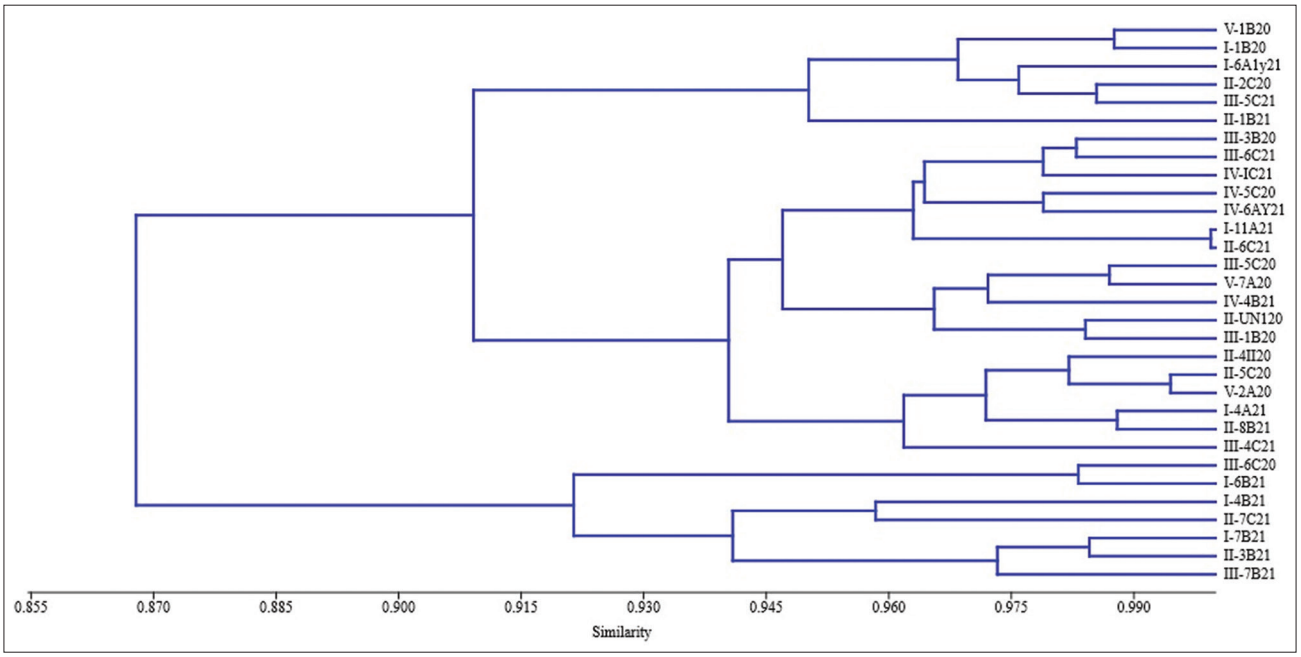

Figure 2: Dendrogram showing Comparison of conidial size and septation of 31 *Alternaria brassiace* isolates collected from the different geographical regions of Allahabad

Table 1: Morphological and cultural features of Alternaria brassicae isolates (31) collected from different regions of Allahabad Uttar Pradesh

Isolate code	Plant parts	Survey site	Geographical data	Appearance of culture on PDA plates	Hyphae	Conidia			
						Colour	Surface	Shape	Beak
I.1B20	Leaf	Mubarakpur kotwa,	25.4356° N, 81.7555° E	Brownish black	Septate and brownish	Dark brown	Rough	Long pear shape	Long
II.2C20	leaf	Kahimapur, Jhusi, PRG	25.4453° N, 81.9562° E	Grey compact and light white at the upper surface	Septate and light grey	Light grey	Rough	Long obpyriform	Long
II.UN$_1$20	leaf	Phoolpur, PRG	25.5510° N, 82.0884° E	Dark brown and leathery growth	Brown with septations	Brown	Rough	Elongated pyriform	Long and septate
II-4II20	leaf	Sahso, PRG	25.4848° N, 81.9805° E	A light brown, concentric ring at centre with a mild whitish upper surface	septation with brownish	Dark Brown	Mild rough	Long obpyriform	Long and septate
II-5C20	leaf	Babuganj, PRG	25.5214° N, 82.0428° E	Brown with fuzzy-type growth	Septate and brown	Brown	Rough	Elongated with pyriform	Long and septate
III-1B20	leaf	Govindpuram, Naini, PRG	25.3830° N, 81.8446° E	Dark brown compact with leathery at the centre	Septate and dark brown	Brown	Rough	Obpyriform	Long and septate
III-3B20	leaf	Sarangapur, PRG	25.3494° N, 81.8429° E	Brown and sooty growth	Septate and brown	Brown	Rough	Elongated pear-shaped	Highly elongated and septate
III-5C20	leaf	Shimra, Ghoorpur, PRG	25.3096° N, 81.8156° E	Brown, sooty growth and concentric ring-like structure in culture	Brown and septate	Brown	Rough	Obpyriform	Long
III-6C20	leaf	SHUATS, Naini, PRG	25.4120° N, 81.8476° E	Brown woolly colony	Brown and septate	Brown	Rough	Obclavate and septate	Long
IV-5C20	leaf	Malakharhar, Soraon, PRG	25.5380° N, 81.8261° E	Dark brown colony	Brown and septate	Brown	Rough	Obpyriform	Long
V-1B20	leaf	Narayan Daspura, Jhusi, PRG	25.4627o N, 81.9475o E	Light brown at the periphery and deep brown in centre	Brown and septate	Brown	Rough	spatulate	Long
V-2A20	leaf	Chamnpur, Jhusi, PRG	25.4279677oN, 81.8980579oE	Brown and fuzzy growth	Brown and septate	Brown	Rough	Club – shaped	Long
V-7A20	leaf	Sundripur kla Hanumanganj, PRG	25.4142° N, 82.0261° E	Grey whitish compact colony growth	Grey and septate	Grey	Rough	Obovate	Long
I-4A21	leaf	Shekhsarwa, PRG	25.25'559o N 81.42'295o E	Brown and Woolly colony	Brown and septate	Brown	Rough	Obovoid	Long
I-4B121	leaf	Sekhsarwa, PRG	25°25'55.9"N 81°42'29.5"E	Dark brown with scattered and compact growth	Brown and septate	Brown	Rough	Obpyriform	Long
I-6B121	leaf	Barwa, PRG	25°25'55.9"N 81°42'29.5"E	Dark green with a concentric ring	Brown and septate	Green	Rough	Pyriform	Long
I-6A1Y21	leaf	Barwa, PRG	25°25'55.9"N 81°42'29.5"E	Dark brown with scattered and compact growth	Brown and septate	Brown	Rough	Ovate	long
I-7B21	leaf	Bhagwatpur, PRG	25.4322° N, 81.7083° E	Brown with woolly growth and pearl-like structure at centre	Brown and septate	Brown	Rough	Obpyriform	Long
I-11A21	leaf	Peepalgaon, PRG	25.4224° N, 81.7709° E	Light brown with scrubby dusted colony	Brown and septate	Brown	Rough	Obpyriform	Long
II-1B21	leaf	Andhawa, jhusi, PRG	25.4238° N, 81.9169° E	Light brown with whitish at the whole periphery	Brown septate	Brown	Rough	Pyriform	Long
II-3B21	leaf	Sherdeeh, jhusi, PRG	25.4549149° N, 81.9180762° E	Whitish cottony and compact colony	Light grey septate	Light grey	Rough	Pyriform with broad breadth	Long
II-6C21	leaf	Kaserua, Jhusi, PRG	25.4786699° N 81.9353613° E	Dark brown at the centre with whitish at corner	Brown and septate	Brown	Rough	Obpyriform	Long
II-7C21	leaf	Thanpur, sahso, PRG	25.495969° N 82.0101305° E	Greyish compact colony	Grey and septate	Brown	Rough	Club-shaped	Long
II-8B21	leaf	Devnahri, Phoolpur, PRG	25.5068047° N 82.0113325° E	Greyish compact whitish at the centre	Grey and septate	Grey	Rough	Elongated	Long and globose at tip
III-4C21	leaf	Mamabhanja Talab, Naini PRG	25.3706773° N 81.8386209° E	Brown compact and wheel-shaped growth from centre to periphery	Brown and septate	Brown	Rough	Club-shaped constricted at the bottom	Long

(*Contd...*)

Table 1: (Continued)

Isolate code	Plant parts	Survey site	Geographical data	Appearance of culture on PDA plates	Hyphae	Conidia			
						Colour	Surface	Shape	Beak
III-5C21	leaf	Bhandra, Naini, PRG	25.356599° N 81.8461002° E	Light grey whitish at the periphery	Grey and septate	Grey	Rough	Club shaped	Long
III-6C21	leaf	Sarangapur, PRG	25.356599° N 81.8461002° E	Brownish and circled growth	Brown and septate	Brown	Rough	Obpyriform	Long
III-7B21	leaf	Bigahiya, Ghoorpur, PRG	25.3275513° N 81.8208703° E	Greyish, concentric in centre	Grey and septate	Grey	Rough	Obpyriform	Long
IV-IC21	leaf	Teliyarganj, Barud khana, PRG	25.4990823° N 81.8548549° E	Dark brown and compact colony	Brown and septate	Brown	Rough	Club- shaped	Long
IV-4B21	leaf	Phaphamau, Belakachhar, PRG	25.5201477° N 81.8280953° E	Whitish with scrubby dusted colony	Light brown and septate	Light brown	Rough	Obpyriform	Long
IV-6AY21	leaf	Morahu, patelbasti, PRG	26.7734286 N 82.6452016 E	Brown compact and smoothy	Brown and septate	Brown	Rough	Club -shaped	Long

Table 2: Comparison of conidial size and septation of Alternaria brassiace isolates collected from the different geographical regions of Allahabad

Isolate code	Conidial Length in μm				Conidial breadth in μm				Number of Transverse septa	Number of vertical septa
	Least length	Median length	Maximum length	Average length	Least breadth	Median breadth	Maximum breadth	Average		
I-1B20	16.8	32.33	39.2	30.6	5.6	8.4	11.2	8.4	3.0	2.0
II-2C20	14.0	34.6	39.2	31.44	5.6	11.2	14	10.5	3.0	1.0
II-UN120	15.5	39.13	44.8	35.2	5.8	10.64	11.2	9.21	3.0	3.0
II-4II20	16.5	42	50.4	38.58	5.8	9.8	11.2	8.9	4.0	2.0
II-5C20	16.8	43.4	50.4	39.48	8.4	10.2	11.2	9.93	4.0	2.0
III-1B20	14.0	39.2	47.6	35.7	5.6	11.2	11.2	9.31	3.0	2.0
III-3B20	22.4	33.6	44.8	34.3	5.6	11.2	14	11.05	6.0	3.0
III-5C20	14.0	33.6	54.6	36.75	5.6	8.4	14	9.33	4.0	2.0
III-6C20	22.4	46.2	53.2	43.05	11.2	14	19.6	14	6.0	2.0
IV-5C20	16.8	39.2	47.6	36.3	8.4	11.2	14	11.2	5.0	1.0
V-1B20	16.8	30.8	39.2	30.4	8.4	9.8	9.8	9.3	3.0	2.0
V-2A20	14.0	44.8	50.4	39.2	2.8	14	14	10.26	4.0	2.0
V-7A20	14.0	37.8	56.6	36.9	6.25	11.2	11.2	9.55	4.0	3.0
I-4A21	20.44	39.2	56	39.41	8.4	11.2	18.2	12.6	4.0	2.0
I-4B21	28	42	72	50.9	8.4	11.2	14	12.6	6.0	2.0
I-6B21	22.4	39.2	67.2	42.7	8.4	16.8	22.4	15.86	6.0	2.0
I-6A1y21	14	30.8	42	30.1	8.4	11.2	13.72	11.06	3.0	2.0
I-7B21	23.8	44.8	67.2	46.55	8.4	11.2	14	11.2	4.0	3.0
I-11A21	23.8	33.6	44.8	34.65	8.4	14	15.4	12.66	4.0	2.0
II-1B21	26.6	32.2	36.4	32.2	8.4	14	16.8	13.06	3.0	2.0
II-3B21	26.6	42	63	46.9	9.8	10.64	11.2	10.54	5.0	3.0
II-6C21	26.6	28	40	34.65	11.2	12.6	14	12.6	4.0	2.0
II-7C21	21	44.8	72.8	49.35	8.4	11.2	14	11.2	7.0	4.0
II-8B21	25.2	39.2	47.6	38.5	8.4	11.2	16.8	12.13	4.0	2.0
III-4C21	22.4	43.4	53.2	41.05	9.8	11.2	14	11.2	5.0	2.0
III-5C21	16.8	32.2	39.2	31.15	8.4	11.2	12.08	10.56	3.0	2.0
III-6C21	21	25.2	58.8	35	8.4	11.2	14	11.2	5.0	3.0
III-7B21	26.6	44.8	58.8	45.15	8.4	11.2	14	11.2	4.0	2.0
IV-IC21	14	33.6	53.2	34.4	8.4	11.2	12.6	10.73	5.0	2.0
IV-4B21	25.2	33.6	50.4	36.5	5.6	8.4	11.2	8.4	4.0	1.0
IV-6AY21	14	42	44.8	36.05	8.4	11.2	14.0	11.2	4.0	2.0

Barwa (I-6A1y21), Kahimapur Jhusi (II-2C20), Shimra Ghoorpur (III-5C21), Andhawa jhusi (II-1B21), Sarangapur (III-3B20), Sarangapur (III-6C21), Teliyarganj Barud khana (IV-IC21), Malakharhar soraon (IV-5C20), Morahu patelbasti, (IV-6AY21), Peepalgaon (I-11A21), Kaserua Jhusi (II-6C21), Malakharhar Soraon (III-5C20), Sundripur kla Hanumanganj (V-7A20), Phaphamau Belakachhar (IV-4B21), Phoolpur (II-UN120), Govindpuram Naini (III-1B20), Sahso (II-4II20), Babuganj (II-5C20), Chamnpur Jhusi (V- 2A20), Shekhsarwa (I-4A21), Devnahri Phoolpur (II-8B21), Mamabhanja Talab Naini (III 4C21) while another cluster (group II) comprised of remaining seven isolates from SHUATS Naini (III-6C20),

Barwa (I-6B21), Sekhsarwa (I-4B21), Thanpur sahso (II-7C21), Bhagwatpur (I-7B21), Sherdeeh jhusi (II-3B21), Bigahiya Ghoorpur (III-7B21). Group I was further sub-clustered into two, of which the first sub-cluster (group IA) had six isolates Narayan Daspura Jhusi (V-1B20), Mubarakpur kotwa (I-1B20), Barwa (I-6A1y21), Kahimapur Jhusi (II-2C20), Shimra Ghoorpur (III-5C21), Andhawa jhusi (II-1B21), and the second cluster (group IB) included eighteen other isolates from Sarangapur (III-3B20), Sarangapur (III-6C21), Teliyarganj Barud khana (IV-IC21), Malakharhar soraon (IV-5C20), Morahu patelbasti, (IV-6AY21), Peepalgaon (I-11A21), Kaserua Jhusi (II-6C21), Malakharhar Soraon (III-5C20), Sundripur kla Hanumanganj (V-7A20), Phaphamau Belakachhar (IV-4B21), Phoolpur (II UN120), Govindpuram Naini (III-1B20), Sahso (II-4II20), Babuganj (II-5C20), Chamnpur Jhusi (V-2A20), Shekhsarwa (I-4A21), Devnahri Phoolpur (II-8B21), Mamabhanja Talab Naini (III-4C21). Another time, group IA was split up into two clusters: group IAa and group IAb. Group IAa had five isolates from Narayan Daspura Jhusi (V-1B20), Mubarakpur Kotwa (I-1B20), Barwa (I-6A1y21), Kahimapur Jhusi (II-2C20), Shimra Ghoorpur (III-5C21), while Group IAb include only one isolate Andhawa jhusi (II-1B21). Two isolates of group I Shimra Ghoorpur (III-5C21), and Andhawa jhusi (II-1B21) did not share any cluster. Once again group IB is subdivided into two clusters: IBa and group IBb. Group IBa had twelve isolates Sarangapur (III-3B20), Sarangapur (III-6C21), Teliyarganj Barud khana (IV-IC21), Malakharhar soraon (IV-5C20), Morahu patelbasti, (IV-6AY21), Peepalgaon (I-11A21), Kaserua Jhusi (II-6C21), Malakharhar Soraon (III-5C20), Sundripur kla Hanumanganj (V-7A20), Phaphamau Belakachhar (IV-4B21), Phoolpur (II-UN120), Govindpuram Naini (III-1B20) while Group IBb include six isolate Sahso (II-4II20), Babuganj (II-5C20), Chamnpur Jhusi (V-2A20), Shekhsarwa (I-4A21), Devnahri Phoolpur (II-8B21), Mamabhanja Talab Naini (III-4C21). Group IBa was further divided into two sub-clusters Group IBa1 and Group IBa2. Group IBa1 had seven Sarangapur (III-3B20), Sarangapur (III 6C21), Teliyarganj Barud khana (IV-IC21), Malakharhar soraon (IV-5C20), Morahu patelbasti, (IV-6AY21), Peepalgaon (I-11A21), Kaserua Jhusi (II-6C21) while Group IBa2 include Malakharhar Soraon (III-5C20), Sundripur kla Hanumanganj (V-7A20), Phaphamau Belakachhar (IV-4B21), Phoolpur (II-UN120), Govindpuram Naini (III-1B20). Again group IBb was split up into two clusters: group IBb1 and Group IBb2. Group IBb1had five isolates Sahso (II-4II20), Babuganj (II-5C20), Chamnpur Jhusi (V-2A20), Shekhsarwa (I-4A21), Devnahri Phoolpur (II-8B21) while Group IBb2 include only one isolate Mamabhanja Talab Naini (III-4C21). Group II was further sub-clustered into two, of which the first sub-cluster (group IIA) had only two isolates SHUATS Naini (III 6C20), Barwa (I-6B21), and the second cluster (group IIB) included five other isolates from Sekhsarwa (I-4B21), Thanpur sahso (II 7C21), Bhagwatpur (I-7B21), Sherdeeh jhusi (II-3B21), Bigahiya Ghoorpur (III-7B21). Another time, group IIB was split up into two clusters: group IIBa and group IIBb. Group IIBa had two isolates Sekhsarwa (I-4B21), and Thanpur sahso (II-7C21), while group IIBb included three isolate Bhagwatpur (I-7B21), Sherdeeh jhusi (II-3B21), Bigahiya Ghoorpur (III-7B21).

Radial Growth in Different Cultural Media

Six different types of culture media were used to determine the radial growth and sporulation of 10-day-old culture of all 31 different isolates (Table 3). Mycelial growth pattern and sporulation of thirty-one isolates of A. *brassicae* were studied in five different culture media like Corn meal agar (CMA), Czapex dox agar (CZA), V-8 juice agar (V-8J), Oatmeal agar (OMA), and Carrot agar (CA). The radial growth pattern of A. *brassicae* in different cultural media varied from 1.10 mm to 6.30 mm and 3.45 mm to 19.78 mm respectively. The circumference and diameter (Growth pattern) from 2.10 mm to 5.05 mm and 6.59 mm to 15.85 mm on PDA, 1.27 mm to 4.05 mm and 3.99 mm to 12.71 mm on CMA, 3.75 mm to 6.30 mm and 11.77 mm to 19.78 mm on OMA, 2.30 mm to 5.75 mm and 7.22 mm to 18.05 mm on CZA, 1.10 mm to 3.10 mm and 3.45 mm to 9.73 mm on CA, 2.72 mm to 4.80 mm and 8.55 mm to 15.07 mm are respectively. The highest diameter and circumference were shown in the OMA medium by III-6C20 isolates, while the lowest growth is shown in the CA medium by II-7C21 and II-8B21 isolates. In all the isolates the highest growth was observed in Oat Meal Agar (OMA) medium while the lowest growth was observed in Carrot Agar (CA) medium.

A dendrogram (Figure 3) was constructed based on data on the radial growth of 10 days culture of 31 A. *brassicae* isolates on six different nutrient media from the similarity coefficient by using Unweighted Pair Group Method with

Table 3: Measurement of radial growth of Alternaria brassiace in different culture media

Isolates	PDA	CMA	OMA	CZA	CA	V-8J
I-1B20	4.85	2.40	4.77	4.08	1.80	3.90
II-2C20	3.40	3.40	4.12	3.30	2.60	3.65
II-UN120	3.30	3.05	6.90	3.75	2.35	4.80
II-4II20	3.75	3.15	5.60	3.10	2.50	3.70
II-5C20	4.50	2.75	3.80	2.70	1.52	3.95
III-1B20	3.90	2.85	5.30	2.95	1.95	3.80
III-3B20	3.20	2.90	3.95	3.25	1.95	4.10
III-5C20	3.15	4.05	5.35	5.75	1.80	4.15
III-6C20	3.75	2.65	6.30	3.40	2.05	4.70
IV-5C20	3.60	2.65	4.80	2.75	3.10	3.05
V-1B20	3.75	2.50	3.95	3.10	1.50	3.77
V-2A20	3.70	2.45	4.20	4.40	2.20	3.85
V-7A20	4.55	1.75	4.25	3.50	1.42	3.75
I-4A21	3.75	2.35	4.05	3.60	2.00	2.85
I-4B21	3.36	1.75	3.95	2.75	1.60	3.05
I-6B21	2.10	2.25	4.65	3.35	1.47	3.60
I-6A1y21	2.85	2.21	4.80	2.85	2.02	3.67
I-7B21	2.80	1.45	4.05	4.20	1.62	3.75
I-11A21	2.85	2.50	4.60	3.55	1.97	3.65
II-1B21	5.05	1.87	4.60	4.65	1.72	3.85
II-3B21	3.80	1.27	4.45	3.10	1.27	2.85
II-6C21	3.50	2.30	5.45	4.10	1.60	3.75
II-7C21	2.95	1.30	4.60	2.45	1.10	2.90
II-8B21	3.05	1.45	4.10	2.90	1.10	3.65
III-4C21	4.10	2.22	4.30	3.70	1.80	3.90
III-5C21	3.78	2.10	3.75	3.55	1.95	4.15
III-6C21	3.17	2.80	4.50	2.30	2.22	4.50
III-7B21	3.80	2.28	4.20	3.95	1.53	4.05
IV-IC21	4.12	2.35	4.10	3.65	1.97	3.65
IV-4B21	3.70	1.32	3.75	2.40	1.20	2.72
IV-6AY21	2.98	2.54	4.05	2.85	1.57	4.10

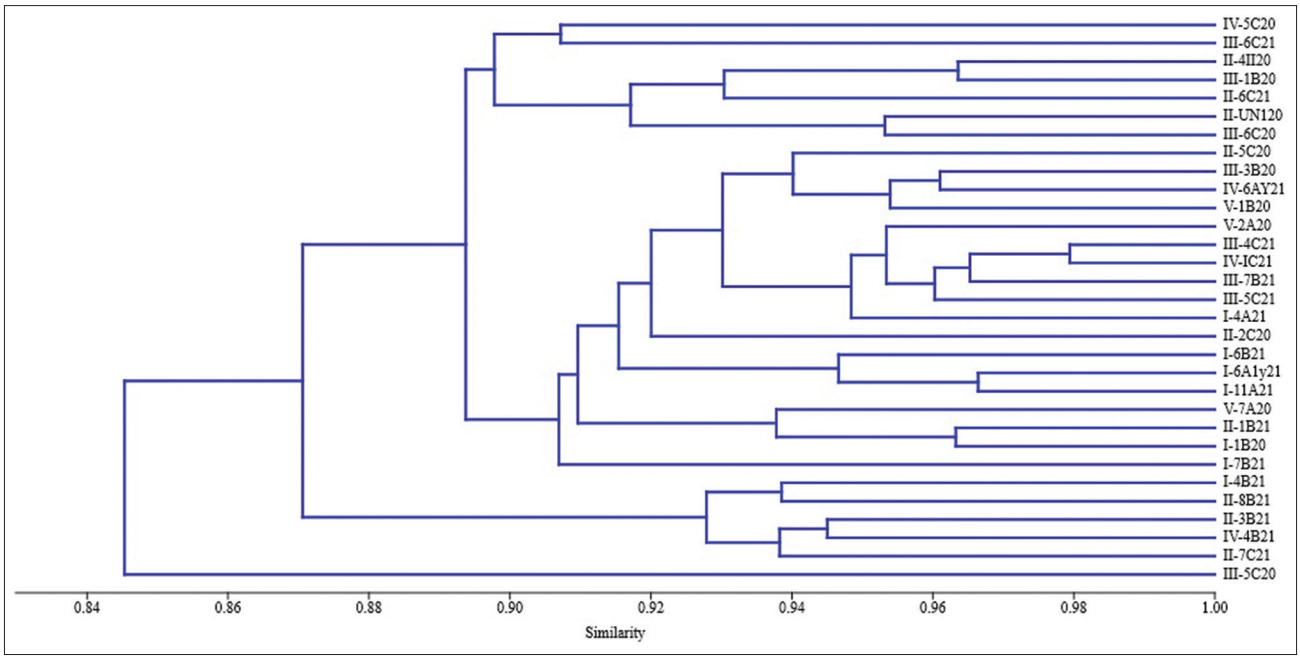

Figure 3: Dendrogram showing radial growth of 10 days old culture of 31 *Alternaria brassiace* isolate (on six different nutrient media) collected from the different geographical regions of Allahabad

Average Means (UPGMA). This dendrogram identified two major clusters with 84% similarity. One cluster (group I) comprised 30 isolates from Malakharhar soraon (IV-5C20), Sarangapur (III-6C21), Sahso (II-4II20), Govindpuram Naini (III-1B20), Kaserua Jhusi (II-6C21), Phoolpur (II-UN120), SHUATS Naini (III-6C20), Babuganj (II-5C20), Sarangapur (III-3B20), Morahu patelbasti (IV-6AY21), Narayan Daspura Jhusi (V 1B20), Chamnpur Jhusi (V-2A20), Mamabhanja Talab Naini (III-4C21), Teliyarganj Barud khana (IV-IC21), Bigahiya Ghoorpur (III 7B21), Shimra Ghoorpur (III-5C21), Shekhsarwa (I-4A21), Kahimapur Jhusi (II-2C20), Barwa (I-6B21), Barwa (I-6A1y21), Peepalgaon (I-11A21), Sundripur kla Hanumanganj (V-7A20), Andhawa jhusi (II-1B21), Mubarakpur kotwa (I-1B20), Bhagwatpur (I-7B21), Sekhsarwa (I-4B21), Devnahri Phoolpur (II-8B21), Sherdeeh jhusi (II-3B21), Phaphamau Belakachhar (IV-4B21), Thanpur sahso (II-7C21) while another cluster (group II) had only one isolates from Malakharhar Soraon (III-5C20). Group I was further sub clustered into two, of which first sub-cluster (group IA) had twenty five isolates Malakharhar soraon (IV-5C20), Sarangapur (III 6C21), Sahso (II-4II20), Govindpuram Naini (III-1B20), Kaserua Jhusi (II-6C21), Phoolpur (II-UN120), SHUATS Naini (III-6C20), Babuganj (II-5C20), Sarangapur (III-3B20), Morahu patelbasti (IV-6AY21), Narayan Daspura Jhusi(V-1B20), Chamnpur Jhusi (V 2A20), Mamabhanja Talab Naini (III-4C21), Teliyarganj Barud khana (IV-IC21), Bigahiya Ghoorpur (III-7B21), Shimra Ghoorpur (III-5C21), Shekhsarwa (I-4A21), Kahimapur Jhusi (II-2C20), Barwa (I-6B21), Barwa (I-6A1y21), Peepalgaon (I-11A21), Sundripur kla Hanumanganj (V-7A20), Andhawa jhusi (II-1B21), Mubarakpur kotwa (I-1B20), Bhagwatpur (I-7B21) and the second cluster (group IB) included five other isolates from Sekhsarwa (I-4B21), Devnahri Phoolpur (II-8B21), Sherdeeh jhusi (II-3B21), Phaphamau Belakachhar (IV-4B21), Thanpur sahso (II-7C21). Another time, group IA was split up into two clusters: group IAa and group IAb. Group IAa had seven isolates from Malakharhar soraon (IV-5C20), Sarangapur (III-6C21), Sahso (II-4II20), Govindpuram Naini (III 1B20), Kaserua Jhusi (II-6C21), Phoolpur (II-UN120), SHUATS Naini (III-6C20) while group IAb include eighteen isolates Babuganj (II-5C20), Sarangapur (III-3B20), Morahu patelbasti (IV-6AY21), Narayan Daspura Jhusi (V-1B20), Chamnpur Jhusi (V-2A20), Mamabhanja Talab Naini (III-4C21), Teliyarganj Barud khana (IV-IC21), Bigahiya Ghoorpur (III-7B21), Shimra Ghoorpur (III 5C21), Shekhsarwa (I-4A21), Kahimapur Jhusi (II-2C20), Barwa (I-6B21), Barwa (I-6A1y21), Peepalgaon (I-11A21), Sundripur kla Hanumanganj (V-7A20), Andhawa jhusi (II-1B21), Mubarakpur kotwa (I-1B20), Bhagwatpur (I-7B21). In group IAb three isolates Babuganj (II-5C20), Kahimapur Jhusi (II-2C20), Bhagwatpur (I-7B21) further formed separate individual clusters while isolates from Sarangapur (III-3B20), Morahu patelbasti (IV-6AY21), Narayan Daspura Jhusi (V-1B20), Chamnpur Jhusi (V-2A20), Mamabhanja Talab Naini (III-4C21), Teliyarganj Barud khana (IV-IC21), Bigahiya Ghoorpur (III-7B21), Shimra Ghoorpur (III-5C21), Shekhsarwa (I-4A21),Barwa (I-6B21), Barwa (I-6A1y21), Peepalgaon (I-11A21), Sundripur kla Hanumanganj (V-7A20), Andhawa jhusi (II 1B21), Mubarakpur kotwa (I-1B20), formed a cluster with 60% similarity. Group IAa comprised seven isolates Malakharhar soraon (IV-5C20), Sarangapur (III-6C21), Sahso (II-4II20), Govindpuram Naini (III-1B20), Kaserua Jhusi (II-6C21), Phoolpur (II-UN120), SHUATS Naini (III-6C20) with 90% similarity coefficient. In group IB only one isolate from Thanpur sahso (II-7C21) further formed separate individual clusters while isolates from Sekhsarwa (I-4B21), Devnahri Phoolpur (II-8B21), Sherdeeh jhusi (II-3B21), Phaphamau Belakachhar (IV-4B21) with 95 similarity coefficients.

Fungal Sporulation in Different Culture Medium

On the other hand, the cultures were grown in six different media to find out the conidial concentration of each isolate. Each isolate of A. *brassicae* on the ten days showed nearly identical sporulation on various mediums (Table 4). Sporulation on 10 days of three isolates [SHUATS Naini (III-6C20), Jhusi (V-IB20), Barwa (I-6B21) range (14.75x10^5/mL - 41.75x10^5/mL)] and four isolates [Andhawa Jhusi (II-IB21), Phaphamau (IV-4B21), Jhusi (V-1B20), Hanumanganj (V-7A20) range (14.0x10^5/mL - 22.75x10^5/mL)] was higher on Oat meal Agar and Czepax Dox Agar medium respectively. Out of all of them, the isolate from Barwa (I-6B121) sporulated the most (41.75x10^5/mL) whereas the isolate from Shekhsarwa (I-4A21) sporulated the least (0.5x10^5/mL). The two isolates Barwa (I-6B121) and Hanumanganj (V-7A20) sporulated higher on OMA (41.75x10^5/mL) and CZA (22.75x10^5/mL) medium respectively. The maximum average sporulation of isolate Barwa (I-6B121) on a different culture (14.60 x10^5/mL) while the minimum average sporulation (2.75x10^5/mL).

Effect of Different Temperatures on Mycelial growth of *A. brassicae* Isolates

Mycelial growth pattern and sporulation of thirty-one isolates of A. *brassicae* were studied in six different media like 6, 12, 18, 24, 30 and 36 °C. Mycelial growth on 10 days of 25 isolate Phaphamau (IV-4B21), Phoolpur (UN120), Andhawa Jhusi (II-1B21), Devnahri Phoolpur (II-8B21), Simra Ghoorpur (III-5C20), Mubarapur kotwa (I-1B20), Sahso (II-4II20), Govindpuram, Naini (III-1B20), Simra ghoorpur (III-5C20), SHUATS Naini (III-6C20), Jhusi (V-2A20), Hanumanganj (V-7A20), Sekhsarwa (I-4A21), Barwa (I-6B121), Barwa (I-6A1y21), Bhagwatpur (I-7B121), Peepalgaon (I-11A21), Andhawa jhusi (II-1B21), sherdeeh jhusi(II-3B21), Kaserua Jhusi (II-6C21), Thanpur sahso (II-7C21), Mamabhanja Naini (III-4C21), Sarangpur (III-6C21), Bigahiya ghoorpur (III-7b21), Teliyarganj barud khana (IV-IC21), Morahu (IV-6AY21) was higher at 24 °C (range:14 mm - 20.5 mm) and among them, SHUATS Naini (III-6C20) isolate showed highest growth (20.5 mm) while the Sekhsarwa (I-4A21) isolate showed least growth (14 mm). The mycelial growth of the remaining Six isolates Babuganj (II-5C20), Sarangpur (III-3B20), Narayan-daspur Jhusi (V-IB20), Sekhsarwa (I-4B21), Bhandra Naini (III-5C21) and Phaphamau Belakachhar (IV-4B21) was higher at 30 °C (range:15 mm - 19.5 mm) and out of these, Sekhsarwa (I-4B21) isolate showed highest growth (19.5 mm) while the Bhandra Naini (III-5C21) isolate showed least mycelial growth (15.07 mm).

Table 4: Effect of different culture media in fungal sporulation.

Isolate's	Spore concentration (10^5/ ml)					
	PDA	CMA	OMA	CZA	CA	V-8J
I-1B20	5.25	4.07	13.00	8.75	3.25	3.25
II-2C20	3.25	1.50	10.25	12.75	3.50	5.00
II-UN120	4.25	6.25	11.25	7.00	3.25	3.50
II-4II20	6.47	6.75	9.75	11.25	3.75	3.75
II-5C20	5.67	7.50	12.25	12.00	4.75	4.00
III-1B20	7.56	6.50	11.75	13.25	3.00	2.75
III-3B20	3.45	8.00	10.75	11.75	2.50	9.50
III-5C20	7.01	7.25	10.00	11.50	2.75	5.50
III-6C20	2.11	9.00	14.75	8.25	2.25	3.50
IV-5C20	4.33	10.50	11.00	12.50	2.00	2.75
V-1B20	4.83	10.75	17.25	15.75	4.25	6.75
V-2A20	3.45	8.50	11.25	6.75	5.00	5.75
V-7A20	6.45	9.25	9.00	22.75	1.50	6.75
I-4A21	3.75	3.75	10.50	5.00	0.50	3.25
I-4B21	2.75	3.50	2.75	1.25	1.25	2.25
I-6B21	3.00	7.75	41.75	14.25	3.00	3.25
I-6A1y21	9.00	6.25	4.00	10.25	4.00	4.50
I-7B21	3.50	9.75	11.00	9.00	1.75	3.50
I-11A21	4.50	12.00	9.25	6.00	4.75	4.25
II-1B21	6.75	5.75	5.75	14.00	1.25	0.50
II-3B21	4.00	13.00	6.25	12.00	4.50	1.00
II-6C21	5.75	12.50	6.75	5.25	3.25	2.25
II-7C21	9.50	11.75	3.50	10.50	5.25	1.25
II-8B21	7.75	6.25	5.25	4.75	5.00	0.75
III-4C21	8.00	9.00	8.00	8.00	4.25	7.25
III-5C21	4.50	9.50	5.75	10.75	3.50	5.75
III-6C21	6.25	3.00	7.75	2.00	2.75	3.75
III-7B21	8.25	8.75	6.25	7.50	1.25	2.25
IV-IC21	8.50	8.00	8.50	6.25	3.25	16.25
IV-4B21	2.25	7.25	6.25	14.75	4.50	3.00
IV-6AY21	1.50	8.25	7.00	8.50	1.00	1.125

Effect of Different Temperatures on Sporulation of *A. brassiace* Isolates

On the other hand, the cultures were grown at six different temperatures to find out the conidial concentration of each isolate. Each isolate of A. *brassicae* on the 10 days showed sporulation at various temperatures. Sporulation on 10 days of 12 isolate Barwa (I6B121), Hanumanganj (V-7A20), Narayanpur Jhusi (V-IB20), Barwa (I-6A1y21), Phoolpur (UN120), Sekhsarwa (I-4A21), SHUATS Naini (III-6C20), Mubarapur kotwa (I-1B20), Phaphamau (IV-4B21), Sarangapur (III-3B20), Bigahiya ghoorpur (III 7b21), Babuganj (II-5C20),was higher at 24 °C (range: 16.25x10^5/mL - 43.75x10^5/mL) and among them, Barwa isolate (I-6B121) showed highest sporulation (43.75x10^5/mL) while the Narayanpur Jhusi isolate (V-IB20) showed least sporulation (16.25x10^5/mL). The sporulation of 5 isolates: Devnahari phoolpur (II-8b21), Teliyarganj barud khana (IV-IC21), Phaphamau Belakachhar (IV-4B21), Sekhsarwa (I-4B21), and Bhandra Naini (III-5C21) was higher at 30 °C (range: 0.75x10^5/mL - 22.25x10^5/mL) and among them, Phaphamau Belakachhar (IV-4B21) showed the highest sporulation (22.25x10^5/mL), whereas the Sekhsarwa isolate (I-4B21) showed the least sporulation (0.75x10^5/mL). Eight isolates: Bhagwatpur (I-7B121), Peepalgaon (I-11A21), Govindpuram, Naini (III 1B20),Simra ghoorpur (III-5C20), Thanpur sahso (II-7C21), Devnahari phoolpur (II-8b21), Mamabhanja Naini (III-4C21), Sarangpur (III-6C21) had sporulated at 18 °C (range:12.75x10^5/mL - 28.25x10^5/mL) and among them, Mamabhanja Naini isolate (III-4C21) showed the highest sporulation (28.25x10^5/mL) whereas Peepalgaon (I-11A21) showed the least sporulation (12.75x10^5/mL).

Effect of pH on Mycelial Growth and Sporulation of *A. brassicae* Isolates

The various isolates showed varying rates of mycelial development and sporulation at various pH values. Mycelial

growth on 10 days of 14 isolate Barwa (I-6B121), Phaphamau (IV-4B21), Sarangapur (III-3B20), Phoolpur (UN120), Andhawa Jhusi (II-1B21), Devnahri Phoolpur (II-8B21), Simra Ghoorpur (III-5C20), Mubarapur kotwa (I-1B20), Bhagwatpur (I-7B121), Babuganj (II-5C20), Govindpuram, Naini (III-1B20), Jhusi (V-2A20), Morahu (IV-6AY21), Hanumanganj (V-7A20), was higher at pH 7.0 (range:18.05 mm -19.78 mm) and among them, Phoolpur isolate (UN120) showed the highest mycelial growth (19.78 mm) whereas the Sarangapur isolate (III-3B20) showed the least mycelial growth (18.05 mm). The mycelial growth of 12 isolates: Sekhsarwa (I-4A21), Peepalgaon (I-11A21), Sherdeeh Jhusi (II-3B21), Kaserua Jhusi (II-6C21), Sahso (II-4II20), Barwa (I-6A1y21), Thanpur sahso (II-7C21), Mamabhanja Naini (III-4C21), Sarangpur (III-6C21), Bigahiya ghoorpur (III-7b21), Teliyarganj barud khana (IV-IC21), Phaphamau Belakachhar (IV-4B21) was higher at pH 8.0 (range:15.07 mm - 17.58 mm) and among them, Sahso (II-4II20) showed the highest mycelial growth(17.58 mm) while Barwa isolate (I-6A1y21) showed the least mycelial growth (15.07 mm). Five isolates: Narayan-daspur Jhusi (V-IB20), Sekhsarwa (I-4B21), Bhandra Naini (III-5C21), SHUATS Naini (III-6C20) had the highest mycelial growth at pH 5.0 (11.77 mm - 16.69 mm) and among them, Bhandra Naini isolate (III-5C21) showed the highest mycelial growth (16.69 mm) while the SHUATS Naini (III-6C20) showed the least mycelial growth (11.77 mm). Sporulation on 10 days of 16 isolate Govindpuram, Naini (III-1B20), Jhusi (V-2A20), Morahu (IV-6AY21), Hanumanganj (V-7A20), Andhawa Jhusi (II-1B21), Devnahri Phoolpur (II-8B21), Thanpur sahso (II-7C21), Simra Ghoorpur (III-5C20), Mubarakpur kotwa (I-1B20), Bhagwatpur (I-7B121), Babuganj (II-5C20), Barwa (I-6B121), Phaphamau (IV-4B21), Sarangapur (III-3B20) Bhandra Naini (III-5C21) was higher at pH 6.0 (range: 45.95x10^5/mL - 46.17x10^5/mL) and out of these, SHUATS Naini isolate (III-6C20) showed the highest sporulation (46.17x10^5/mL) while the Bhandra Naini isolate (III-5C21) showed the least sporulation (45.95x10^5/mL). The sporulation of 9 isolate: Narayan-daspur Jhusi (V-IB20), Sekhsarwa (I-4B21), Sekhsarwa (I-4A21), Peepalgaon (I-11A21), sherdeeh jhusi (II-3B21), Kaserua Jhusi (II-6C21), Sahso (II-4II20), had highest sporulation at pH 9.0 (range: 35.50x10^5/mL - 44.01x10^5/mL) among them Kaserua Jhusi (II-6C21) showed the highest sporulation (44.01x10^5/mL) whereas the Phoolpur isolate (UN120) showed least sporulation (35.50x10^5/mL). Six isolate: Barwa (I-6A1y21), Mamabhanja Naini (III-4C21), Sarangpur (III-6C21), Bigahiya ghoorpur (III-7b21), Teliyarganj barud khana (IV-IC21), Phaphamau (IV-4B21), Sarangapur isolate (III-3B20) had highest sporulation at pH 10.0 (range:0.75x10^5/mL - 25.75x10^5/mL) among them Barwa isolate (I-6A1y21) showed the highest sporulation (25.75x10^5/mL) whereas the Phaphamau isolate (IV-4B21) showed the least sporulation (0.75x10^5/mL).

Effect of Relative Humidity on Mycelial Growth and Sporulation of *A. brassicae* Isolates

The various isolates at various RH showed different rates of mycelial growth and sporulation. The maximum mycelial growth occurred at 100% relative humidity in all 10-day-old cultures (range: 1.10 mm - 1.97 mm). The isolates from Devnahri Phoolpur (II 8B21) and SHUATS -Naini (III-6C20) had the lowest growth (1.10 mm) and the most growth (1.97 mm) among them. Therefore under those conditions, there was no variation among them. But compared to isolates from phaphamau (IV-4B21) (40-60%), those from Sarangapur, Sekhsarwa, Barwa and Babuganj required comparatively higher RH (80-100%) for sporulation, whereas some isolates - from Teliyarganj barud khana and Bhandra Naini-sporulate but very few at any RH level. Sporulation on 10 days of 8 isolates Jhusi (V-2A20), Morahu (IV-6AY21), Hanumanganj (V-7A20), Andhawa Jhusi (II-1B21), Devnahri Phoolpur (II-8B21), Babuganj (II-5C20), Barwa (I-6B121), and SHUATS Naini (III-6C20) was highest at 100% RH (range: 0.75x10^5/mL - 22.75x10^5/mL) among them Babuganj (II-5C20) showed highest sporulation (22.75x10^5/mL) while the SHUATS Naini isolate (III-6C20) showed the least sporulation (0.75x10^5/mL). The isolates that sporulated the most at 60% RH were Sahso (II-4II20) and Sarangpur (III-6C21), with 1.6×10^5/mL and 0.85×105/mL, respectively. Isolate Mamabhanja Naini (III-4C21), and Sarangpur (III-6C21) sporulated most at 40% RH 1.25x10^5/mL and 80% 1.40x105/mL respectively. The isolate: Naini (III-1B20), Jhusi (V-2A20), Morahu (IV-6AY21), and Hanumanganj (V-7A20) sporulated at both 40% and 60% RH, 0.625x10^5/mL and 4.25x10^5/mL respectively.

Pathogenicity Test

It was found that all 31 of the A.*brassicae* isolates showed pathogenic behaviour (Table 5). Among the 31 isolates, 9 isolates II-UN120 from Phoolpur, PRG, IV-5C20 from Malak harhar Soraon, I-4A21 from Shekhsarwa, I-6B21 from Barwa PRG, I-6A1y21 from Barwa PRG, II-8B21 from Devnahri Phoolpur, III-6C21 from Sarangpur, IV-4B21 from Phaphamau, and IV- 6AY21 from Morahu were found to be high degree of infection as the spot produced by them were more than 10 mm (>10 mm) in diameter. Five isolates I-1B20 from Mubarakpur kotwa, I-4B21 from Sekhsarwa, PRG, II-3B21 from Sherdeeh, Jhus, II-7C21 from Thanpur Sahso, and III-7B21 from Bigahiyan Ghoorpur showed least degree of infection as the spot produced by them were 1 mm - 5 mm in diameter. Seventeen isolates II-2C20 from Kahimapur Jhusi, II-4II20 from Sahso PRG, II-5C20 from Shimra Ghoorpur, III-1B20 from Govindpuram Naini, III -3B20 from Sarangapur, III-5C20 from Shimra Ghoorpur, III-6C20 from SHUATS Naini, V-1B20 from Narayan Daspura Jhusi, V 2A20 from Chamnpur Jhusi, V-7A20 from Sundripur kla Hanumangan, I-7B21from Bhagwatpur, I-11A21 from Peepalgaon, II-1B21 from Andhawa Jhusi, II-6C21 from Kaserua Jhusi, III-4C21 from Mamabhanja talab Naini, III-5C21 from Bhandra Naini, and IV IC21 from Teliyarganj Barud khana were found to be moderately degree of infection as the spot produced by them from 6 mm to 10 mm in diameter.

Table 5 showing pathogenicity test on diseased leaves, a black leaf spot with a yellow halo was evaluated as plus (+), and no symptoms were recorded as minus (-). The three categories for the symptom's appearance were: black spots measuring between 0.2 and 0.5 cm were ranked as single

Table 5: Testing the pathogenicity of isolates of *Alternaria brassicae* on mustard

Isolates	Degree of infection	Isolates	Degree of infection
I-1B20	+	I-6A1y21	+++
II-2C20	++	I-7B21	++
II-UN120	+++	I-11A21	++
II-4II20	++	II-1B21	++
II-5C20	++	II-3B21	+
III-1B20	++	II-6C21	++
III-3B20	++	II-7C21	+
III-5C20	++	II-8B21	+++
III-6C20	++	III-4C21	++
IV-5C20	+++	III-5C21	++
V-1B20	++	III-6C21	+++
V-2A20	++	III-7B21	+
V-7A20	++	IV-IC21	++
I-4A21	+++	IV-4B21	+++
I-4B21	+	IV- 6AY21	+++

plus (+), 0.6 to 1.0 cm were scored as double plus (++), and spots measuring more than 1 cm were ranked as triple plus signs (+++).

DISCUSSION

Temperature and geographical origin had an impact on the variation in conidial morphology, mycelial growth and sporulation of thirty isolates of A. *brassicae* that were collected from different geographical zones (Goyal *et al.*, 2011). Similar variations have been observed in the morphological traits of A. *brassicae* isolates from various Indian locations (Meena *et al.*, 2005; Kaur *et al.*, 2007; Singh *et al.*, 2007). Regarding mycelial growth and sporulation, some researchers have studied the cultural variability in *Alternaria* species (Ansari *et al.*, 1989; Patni *et al.*, 2005; Kaur *et al.*, 2007). Variability concerning mycelial growth and sporulation of several *Alternaria* species at different temperatures has been reported earlier by many workers (Meena *et al.*, 2005; Singh *et al.*, 2007). Various temperatures were shown to be optimal for the mycelial growth and sporulation of various A. *brassicae* isolates in the present study, indicating cultural diversity among them. The temperature ranges for mycelia growth and sporulation were 25 °C to 30 °C and 15 °C to 35 °C, respectively. Meena *et al.* (2005) and Singh *et al.* (2007), who also discovered variations in the requirements for temperature across various A. *brassicae* isolates of diverse geographical origins, supported these findings. Furthermore, the block's climate, which normally experiences greater temperatures during the rapeseed-mustard crop season than other locations like Babuganj, Phoolpur, and Allahabad may be responsible for a higher temperature that is favourable for the sekhwarwa isolate. Variability was shown among them in these results. It was identical to Meena *et al.* (2005) findings. The number of isolates from Phaphamau, and Sekhsarwa sporulated at 35 °C, and several of these isolates showed increased fecundity at higher relative humidity. These findings suggest that in keeping with current projections for future climate change (Waugh *et al.*, 2003), the existence of these isolates may increase the risk of Alternaria blight on oilseed Brassicas in the future. The enormous variety found among the thirty-one typical isolates of A. *brassicae* further suggests this species' capacity for climate adaptation. However, the fact that each isolate had a distinct optimum pH level indicated variation among them isolates from Phoolpur and Jhusi, Babuganj and Phaphamau regions grew well in neutral situations (pH 7), isolates from Sahso, Teliyarganj and Sekhsarwa in alkaline conditions and isolates from Narayan-daspur Jhusi, Bhandra Naini and SHUATS Naini in acidic environment. The conditions for sporulation, however, were very different. While isolates from Jhusi, Sahso and Peepalgaon sporulated well in an alkaline environment and isolates from Bhagwatpur, Hanumanganj and Phaphamau did so in an acidic pH, isolates from Phoolpur and Jhusi regions sporulated well in a neutral pH. Therefore, it is unable to generalize within a certain range based on the previous situation, which suggests that the pH requirements for various isolates were rather specialized for mycelial development and sporulation. However, the pH requirements for the various isolates of A. *brassicae* examined in this work show that some of them, which originate from the main Brassica growing areas for oilseeds, prefer neutral or acidic pH for mycelial growth after landing on the plant's surface, an A. *brassicae* spore germinates, grows larger and spreads its mycelia, penetrates the epidermis to infect the plant, and necrotizes the tissue (Goyal *et al.*, 2011). Therefore, to develop an oilseed Brassica that is resistant to A. *brassicae*, the plant must have an alkaline pH at the surface to inhibit the formation of the invading pathogen's mycelia. On the same nutritional media, A. *brassicae* did not develop and sporulate abundantly (Lapis & Ricaforte, 1974). According to Ansari *et al.* (1988), Thirty-one isolates of A. *brassicae* in the present research similarly did not exhibit optimal mycelial growth and sporulation on the same medium. Numerous researchers have examined the effects of light and darkness on A. *brassicae* mycelial development and sporulation (Ansari *et al.*, 1989).

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