DoI: https://doi.org/10.58537/jorangrau.2024.52.4.04

J. Res. ANGRAU 52 (4) 28-45, 2024

CULTURAL AND MORPHOLOGICAL CHARACTERIZATION OF *ALTERNARIA BRASSICAE* OF MUSTARD IN ALLAHABAD UTTAR PRADESH

***SHAILENDRA KUMAR, S. M. PRASAD and H. K. KEHRI**

*Department of Botany, Kutir P.G. College, Chakkey, Jaunpur -222146

Date of Receipt : 18-09-2024 Date of Acceptance : 23-11-2024

ABSTRACT

Alternaria brassicae isolates were collected from the black leaf spot of mustard during the winter season (2019-2021) in the various regions of the Allahabad district. *In vitro*, results show significant variability in growth pattern, sporulation, conidial length, width, and septa number in different culture media, temperature, pH, and relative humidity. The conidial length of the isolates II-2C20, III-5C20, V-2A20, V-7A20, and I-6A1y21 was the shortest, measuring 14 ìm. The isolate I-4B21 and II-7C21 had the longest conidial length, ranging from 72 ìm to 72.8 ìm, respectively. The average conidial length ranged from 30.1 ìm to 50.9 ìm, with mustard isolate I-6A1y21 having the lowest value and I-4B21 having the highest. The highest average width was 15.86 ìm. The best mediums for 31 isolates were Potato Dextrose Agar, Meal Agar, Corn Meal Agar, Czapex dox agar, Carrot meal agar, and V-8 juice Agar. The highest radial growth was shown in the OMA medium by I-6A1y21 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 I-6B121 sporulated the most ($41.75x10^5$/ml) whereas the isolate I-4A21 sporulated the least ($0.5x10^5$/ml) in OMA and CA medium. The radial growth and sporulation are optimal at 24°C, moderate at 18°C and 30°C, and lowest at 6°C and 36°C. Isolate III-3B20 and II-5C20 showed the least average radial growth and sporulation at all pH levels, while isolate I-6A1y21 showed maximum growth. The pH 7 is more favourable for radial growth and sporulation. The study found that radial growth and sporulation increased in relative humidity between 80-100%, while decreased in 20-40%, with isolate I-6A1y21 showing the highest growth while II-UN120 and III-5C21 exhibited the least. Among the 31 isolates, 9 isolates II-UN120, IV-5C20 I-4A21, I-6B21, I-6A1y21, II-8B21, III-6C21, IV-4B21, and IV- 6AY21 were found to be high degree of infection as the spot produced by them were more than 10 mm in diameter. Statistical analyses were conducted using Microsoft 365 Excel and SPSS 28.0 version on Windows 10, using ANOVA and Duncan's multiple range test of homogeneity at a P=0.05 level.

Keywords: *Alternaria brassicae*, cultural growth, leaf spot diseases, morphological variations, mustard crop, Allahabad mustard crop.

*Corresponding Author E-mail i.d: chaudharishailendra.1@gmail.com; Part of Ph.D. thesis submitted to Kutir P.G. College, Chakkey, Jaunpur

INTRODUCTION

Mustard is an economically important genus of the family Brassicaceae and this family includes oil seed crops like mustard (*Brassica juncea*) *and* rape (*Brassica campestris*). These crops are grown all over the world under a wide range of agro-climatic conditions including India. These crops are commercially significant in both national and international trade because they provide edible oil, which is the primary cooking medium in Northern India. Global estimates for the area, production, and yield of rape seed-mustard in 2018–2019 and 2019–2020 were 34.86 million hectares (mha), 69.07 million tonnes (mt), and 1980 kg/ha and 35.95 million hectares, 71.49 million tonnes, and 1990 kg/ha, respectively (DRMR, 2020-21). While in respect to India, estimates for the area, production, and yield of rape seed-mustard in 2018–2019 and 2019–2020 were 6.12 mha, 9.26 mt, and 1511 kg/ha and 6.86 mha, 9.12 mt, and 1331 kg/ha, respectively (DRMR, 2020-21). The fungal diseases, including white rust, downy mildew, powdery mildew, stem rot, clubroot, damping-off, and Alternaria blight, are recognized as major causes of the losses in mustard production. Among several diseases, Alternaria blight, also referred to as Alternaria dark spot or Alternaria leaf spot, is the most devastating disease, which is caused by the *Alternaria brassicae*. The symptom of Alternaria blight which appear as brown to black circular spots on upper and lower parts of mustard leaf. *Alternaria brassicae* caused losses of up to 47% and 20.28% in Indian mustard (*Brassica juncea*) (Chattopadhyay, 2008; Singh *et al*., 2021). Alternaria blight is a global headache and could reduce yield up to 70% in rapeseed mustard (Pandey *et al*., 2023). Alternaria leaf blight (black spot) on various crucifers, such as oil rapeseed, cabbage, cauliflower and mustard has been documented in different countries including United States, United Kingdom, several other European countries (Blagojevic *et al*., 2020) and India.

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 (Punia *et al*., 2021). The main taxonomic criteria for defining fungal species are the morphological properties of conidia and conidiophores, as well as occasionally host plant relationships (Fatima *et al*., 2019). 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). In view of the above facts, a present study has been undertaken to investigate the *Alternaria brassicae* and serverity of *Alternaria brassicae* disease in mustard in Allahabad districts.

MATERIAL AND METHODS

31 infected leaf samples were collected from various regions of Allahabad (25.4683° N, 81.8546° E), and the total geographical area is approximately 5,482 km². Infected leaves were collected, sterilized (4% NaOCl), washed, and placed on Potato Dextrose Agar (PDA) medium. After 4-5 days of incubation at +24°C, fungal colony growth was observed in PDA plates, which were isolated and preserved at 4°C. Streptomycin antibiotics were used to prevent bacteria growth in *Alternaria brassicae* culture medium, identified by single spore technique and calibrated using an ocular micrometre (Patel *et al*., 2023).

Thirty-one single-spore cultures of *A. brassicae* were examined for their cultural diversity at Six different culture media, seven distinct pH values (5, 6, 7, 8, 9 and 10), four different RH conditions (20-40%, 40-60%, 60-

80% and 80-100%), and six different temperatures (6^0C, 12^0C, 18^0C, 24^0C, 30^0C, and 36^0C).

A. brassicae colonies were cultured in PDA medium with pH 7.0 for 10 days, examining humidity's impact on mycelial growth and sporulation. Six different culture media (PDA, CMA, CZA, V-8J, OMA, and CA) were developed, and colony growth and sporulation were observed at different conditions.

The haemocytometer, a counting-chamber device, was utilized to measure the conidial concentration of each culture after 10 days of inoculation for sporulation observations.

The observations were made regarding the symptomatology, disease incidence, and disease severity. Seventy randomly selected plants were used to record disease incidence data per 100 m^2 area of every survey area. To determine the frequency and severity of the disease the five lowest (oldest) leaves on each plant were selected. The following formula were used to determine the disease severity and incidence using the systematic sampling method.

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plant in sample unit}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Total infected leaf area per plant}}{\text{Total leaf area of infected plant}} \times 100$$

This formula was used to determine the percent disease index (PDI)

$$\text{PDI} = \frac{\text{Sum of all numerical rating}}{\text{Total number of leaves examined x maximum disease rating}} \times 100$$

A pathogenicity test was conducted on mustard plants using conidial suspension and mustard seeds were sown in the botanical garden of the University of Allahabad, from October 2019 to 2021. After seven weeks, plants should emerge from dense plants, creating a 25-30 cm distance between them, and the leaf area should be sprayed with an optimal inoculum concentration (1.0 $x10^5$ conidia /ml).

Microsoft 365 Excel and SPSS version 28.0 on Windows 10 were used for conducting the statistical analyses. The data in the table were analysed by single-way analysis of variance (ANOVA). Means were separated by Duncan's multiple range test of homogeneity at the P=0.05 level.

RESULTS AND DISCUSSION

The morphological and cultural features of various isolates

A survey was carried out from February of 2019 to 2021 to document the incidence and severity of Alternaria leaf blight in mustard and rapeseed, as well as to gather disease samples from various mustard and rapeseed-growing in Allahabad regions (Figure 2). According to data at different locations, the disease severity and incidence on different cultivars varied from 5 to 50% and 15 to 85% respectively**.** Barwa II and Morahu had the highest disease incidence (85%), with a 45% to 50% disease severity on cultivar Coral PAC-432 (Hybrid) and Pusa mustard 26 (NPJ 113), respectively. The isolate II-UN120 from the Phulpur region exhibited the lowest disease incidence (15%) and severity (5%) on the Pusa mustard 26 (NPJ 113) variety of mustard. It was followed by 25% disease incidence on Pusa mustard 27 (EJ 17) collected from Kotawa with 5% disease severity (Figure1) (Table 2 &3).

The color of conidia on PDA remained consistent, with colonies ranging from light brown to dark brown, and mycelia ranging between grey and brown. Significant morphological variability ($P < 0.05$) was

observed in 31 isolates of *A.brassicae* single-spore cultures with respect to conidia length, conidia breadth, beak length, and septum number. The isolates II-2C20, III-5C20, V-2A20, V-7A20, and I-6A1y21 had the lowest conidial length, measuring 14 ìm, while the isolates I-4B21 and II-7C21 had the longest conidial length, between 72 ìm and 72.8 ìm, respectively. The average conidial length varied between 30.1 ìm and 50.9 ìm; the values were lowest in the isolate I-6A1y21 and greatest in the isolate I-4B21 at 50.9 ìm. The average conidial width varied between 8.4 ìm and 15.86 ìm, with the isolate I-4B21 having the highest average width at 15.86 ìm and the isolate IV-6Ay21 having the lowest average width at 8.4 ìm. The number of transverse septa varied from 3.0 to 7.0, with the isolate II-7C21 having the highest number at 7.0 and the isolate V-1B20 having the lowest number at 3.0. The number of vertical septa ranged from 1.0 to 4.0, with the isolate II-7C21 having the highest number at 4.0 and the isolate II-2C20 having the lowest number at 1.0 (Table 1 & 4).

(Saha *et al*., 2016) who requested that substantial variability was observed among the *A. brassicae* isolated from several locations.

Radial growth on different culture media

The radial growth of *A. brassicae* in various cultural media ranged from 11.46 mm to 71.20 mm. The radial growth ranges of various *A. brassicae* isolates on different culture media are as follows: 28.28 mm to 52.35 mm on PDA, 12.56 mm to 42.16 mm on CMA, 37.4 mm to 71.20 mm on OMA, 23.33 mm to 62.66 mm on CZA, 11.46 mm to 32.30 mm on CA, and 27.26 mm to 49.73 mm on V-8J media are, respectively (Table 5). The highest radial growth (71.20 mm) was shown in the OMA medium by I-6A1y21 isolates, while the lowest radial growth (11.46 mm and 11.56 mm) is shown in the CA medium by I-6A1y21 isolates, while the lowest radial growth (11.46 mm and 11.56 mm) is shown in the CA medium by the II-7C21 and II-8B21 isolates.The OMA medium showed maximum radial growth for *A. brassicae* isolates, while CA medium showed the least growth (Figure 3 & 4).

Sporulation on different culture media: The average sporulation range in PDA medium was 16.50 x 10^5 to 29.50 x 10^5/ml, among them the maximum sporulation (29.50 x 105 /ml) was shown by the isolate I-6A1y21, while the isolate II-5C20 and IV-5C20 had the least sporulation (16.25 x 10^5/ml) in PDA medium. The sporulation range in CMA medium was 5.25 x 10^5/ml - 14.50 x 10^5/ml

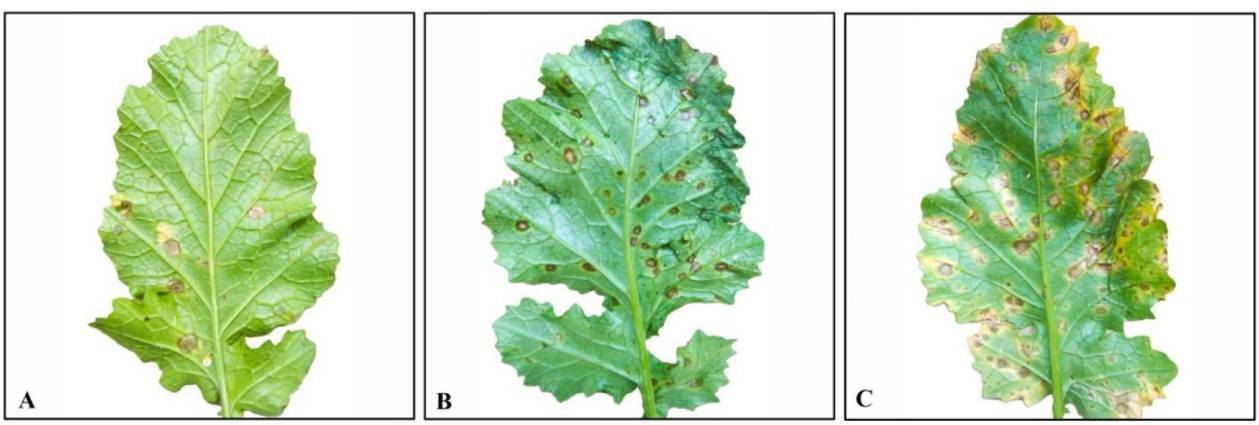

Fig. 1. Disease severity on mustard leaf per plant : **A**) A leaf with minimum grade of infection (10-25%) **B**) A leaf with moderate grade of infection (50-70%)
C) A leaf with maximum grade of infection (70-100%)

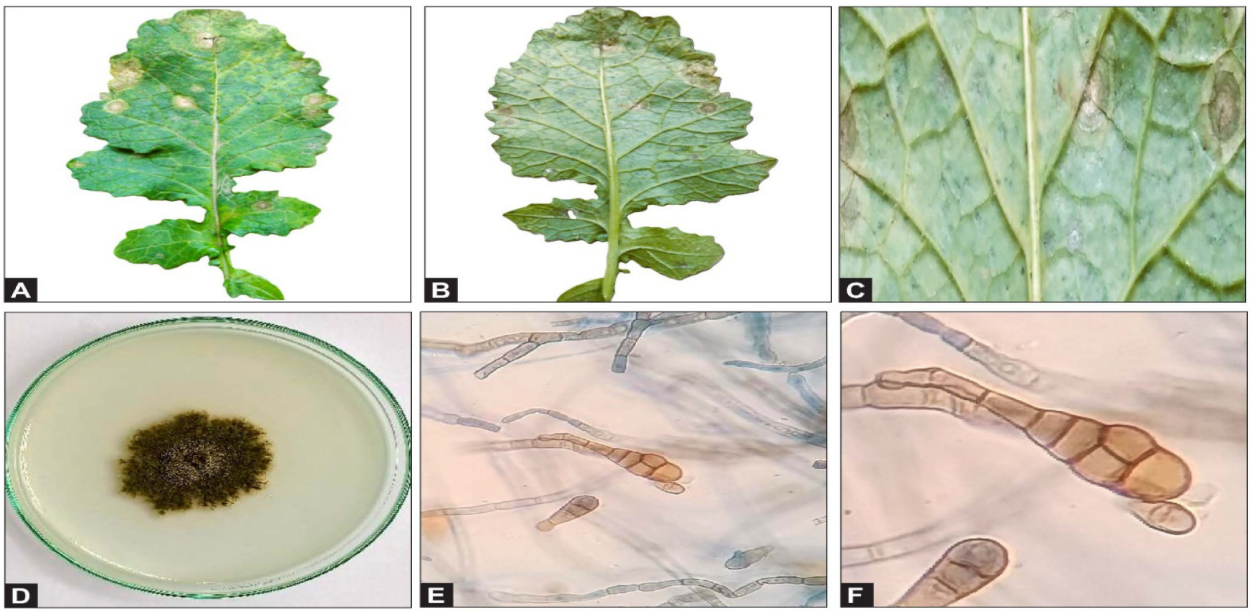

Fig. 2 Survey of field, morphological and cultural characteristic of *A.brassicae* isolate I-4B121.**A** Dorsal view of an infected leaf with pathogen, **B** ventral view of infected leaf, **C** Leaf with concentric ring, **D** Growth on culture medium, **E** Fungal hyphae with spore, **F** A single spore of *Alternaria brassicae* isolate I-4B121.

among them, with the isolate I-6A1y21 was most sporulated (14.50 x 10^5/ml), while the isolates I-7B21 and II-3B21 was sporulated least (5.25 x 10^5/ml) in CMA medium. The magnitude of sporulation in OMA medium is 16.25 x 10^5/ml - 30.25 x 10^5/ml, in which maximum sporulation (30.25 x 10^5/ml) was observed in isolate I-6A1y21 while the least sporulation (16.25 x 10^5/ml) was reported in the II-3B21 isolate of *Alternaria brassicae*. The sporulation range in CZA medium was 10.50 x 10^5/ml–23.50 x 10^5/ml, in which the maximum sporulation (23.50 x 10^5/ml) was found in isolate I-6A1y21 while the least sporulation (10.50 x 10^5/ml) was observed in the isolate IV-5C20. In CA medium, the range of sporulation was 3.95 x 105/ml–8.2 x 10^5/ml. The maximum sporulation (8.25 x 10^5/ml) was shown by the isolate I-6A1y21, while the least sporulation (3.95 x 10^5/ml) was shown by isolate IV-4B21 in CA medium (Table 6).

The previous investigation confirmed the finding of current study which showed that high sporulation and radial growth of *A. brassicae* on PDA medium across a variety of tested conditions (Prasad *et al.*, 2019). It has also been reported that culture medium affect the morphology and development of conidia (Kumar and Sharma, 2017).

Radial growth at different temperatures

The radial growth range of different *A.brassicae* isolates at different temperatures was as follows: 1.75 mm to 9.18 mm at 6°C, 11.40 mm to 23.73 mm at 12°C, 15.20 mm to 33.36 mm at 18°C, 28.28 mm to 52.35 mm at 24°C, 27.28 mm to 38.11 mm at 30°C, and 4.64 mm to 13.65 mm at 36°C temperature, respectively.

Among 31 isolates, four isolates, such as I-6A1y21, III-7B21, I-4B21 and IV-4B21, showed the highest radial growth at 6°C temperatures, (9.18 mm), (8.7 mm), (8.46 mm), and (8.23 mm), respectively. While four isolates, III-1B20, II-6C21, II-5C20 and III-

5C20, showed the least radial growth, (1.75 mm), (2.6 mm), (2.76 mm) and (2.78 mm), respectively, at 6°C temperatures. The maximum (23.73 mm and 33.36 mm) and minimum radial (11.40 mm and 15.20 mm) growth was shown by the isolate I-6A1y21 and the isolate I-7B21 at 12°C and 18°C, respectively. The isolate I-6A1y21 showed the maximum radial growth (52.35 mm, 38.11 mm, and 13.65 mm), while the least radial growth (28.28 mm, 27.28 mm, and 4.64 mm) was shown by the isolates I-11A21, III-3B20, and II-6C21 at 24°C, 30°C, and 36°C temperatures, respectively. Results show that radial growth of 31 *A. brassicae* isolates is influenced by very high and very low temperatures, with optimal growth at 24°C.

Sporulation at different temperatures

The cultures were cultivated at six distinct temperatures to determine the conidial concentration of each isolate. Each isolate of *Alternaria brassicae* showed different sporulation at different temperatures on 10 days after incubation. Sporulation on 10 days of 15 isolates I-6B21, I-6A1y21, II-4II20, UN120, III-3B20, II-6C21, II-7C21, II-8B21, III-6C21, IV-4B21, V-7A20, I-4A21, I-4B21, I-7B21 and III-7B21 was higher at 24°C (range: 20.41 x 10^5/ml–29.50 x 10^5/ml), and among them, the isolate I-6A1y21 showed the highest sporulation (29.50 x 10^5/ml), while the isolate II-4II20 showed the lowest sporulation (20.41 x 10^5/ml). At 24°C, the overall sporulation range of all 31 isolates was 16.50 x 10^5/ml - 29.50 x 10^5/ml. Out of these, isolate I-6A1y21 showed the highest sporulation (29.50 x 10^5/ml), while the isolates IV-5C20 and II-5C20 showed the least sporulation (16.50 x 10^5/ml). The range of sporulation of 31 isolates at 18 °C was 5.5 x 10^5/ml - 12.25 x 10^5/ml; among them, isolate I-6A1y21 showed the highest sporulation (12.25 x 10^5/ml), while the isolates III-1B20 and IV-5C20 showed the least sporulation (5.5 x 10^5/ml). At 30°C the sporulation range of 31 isolates was 11.50 x 10^5/ml - 19.75 x 10^5/ml; among them, the higher sporulation (19.75 x 10^5/ml) was observed in the isolate I-6A1y21 while the least sporulation (11.50 x 10^5/ml) was found in the isolate III-1B20.

The range of sporulation of 31 isolates at 12 °C temperatures was 2.15 x 10^5/ml - 9.25 x 10^5/ml; among these, the isolate I-6A1y21 showed maximum sporulation (9.25 x 10^5/ml), while the isolate IV-6Ay21 showed the least sporulation (2.15 x 10^5/ml). The sporulation range of all 31 isolates at 6 °C was 1.08 x 10^5/ml - 3.25 x 10^5/ml; among these, isolate I-6A1y21 showed maximum sporulation (3.25 x 105/ml), while the isolate II-4II20 showed the least sporulation (1.08 x 10^5/ml).The range of sporulation of 31 isolates at 36 °C was 1.15 x 10^5/ml–3.95 x 10^5/ml; among these, isolate II-4II20 showed the least sporulation (1.15 x 10^5/ml), while the isolate I-6A1y21 showed maximum sporulation (3.95 x 10^5/ml). The study found that sporulation of all isolates was influenced by very high and very low temperatures, with the highest sporulation at 24°C, moderate at 18°C and 30°C, and least at 6°C, 12°C, and 36°C.

Similar findings were observed by Singh *et al*. (2021), who reported that maximum mycelial growth and sporulation were recorded at 25°C, 30°C, 20°, and 35°C, and a minimum at 40°C temperatures. The present study proved that different temperatures were favorable for the mycelial growth and sporulation of various *A. brassicae* isolates, indicating cultural variation among them.

Radial growth at different pH level

The various isolates of *A. brassicae* showed different rates of radial growth at various pH level**.** Radial growth on 10-days-old cultures of all 31 isolates was higher (range: 37.06 mm - 49.77 mm) at pH 7.0. Among them, isolate I-6A1y21 showed the highest radial

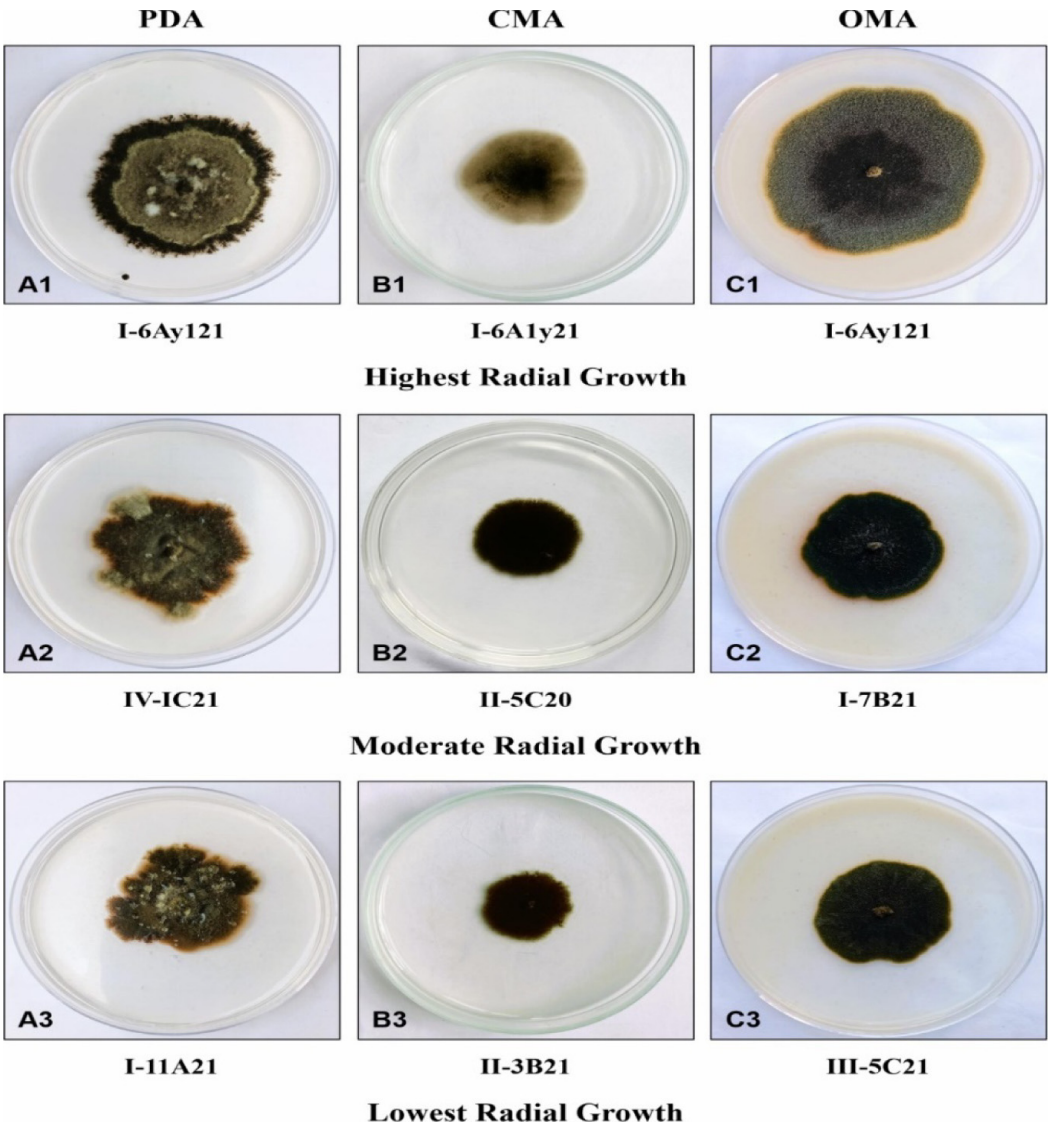

Fig. 3. The effect of culture media on 31 isolates of *Alternaria brassicae*. Of these, A (1) The isolate I-6Ay121 showed the highest radial growth, while C (3) Isolate III-5C21 showed the least radial growth on OMA medium

growth (49.77 mm), whereas the isolate III-3B20 showed the least radial growth (37.06 mm) and at pH 8.0 was from 18.22 mm to 22.76 mm, and among them, isolate I-6A1y21 showed the highest radial growth (22.76 mm), while the isolate I-4B21 showed the least radial growth (18.22 mm). The radial growth range of all isolates at pH 5.0 was from 11.75 mm to 17.26 mm, and among them, the isolate I-6A1y2 showed the highest radial growth (17.26 mm), while the isolate III-6C20 showed the least radial growth (11.75 mm). At pH 6.0 the range of radial growth was from 21.33 mm to 38.34 mm; among 31 isolates, the isolate II-UN120 showed the least radial growth (21.33 mm), while the isolate I-6A1y21 showed the highest radial growth (38.34 mm). All isolates showed radial growth ranging from 16.43 mm to 21.24 mm at pH 9.

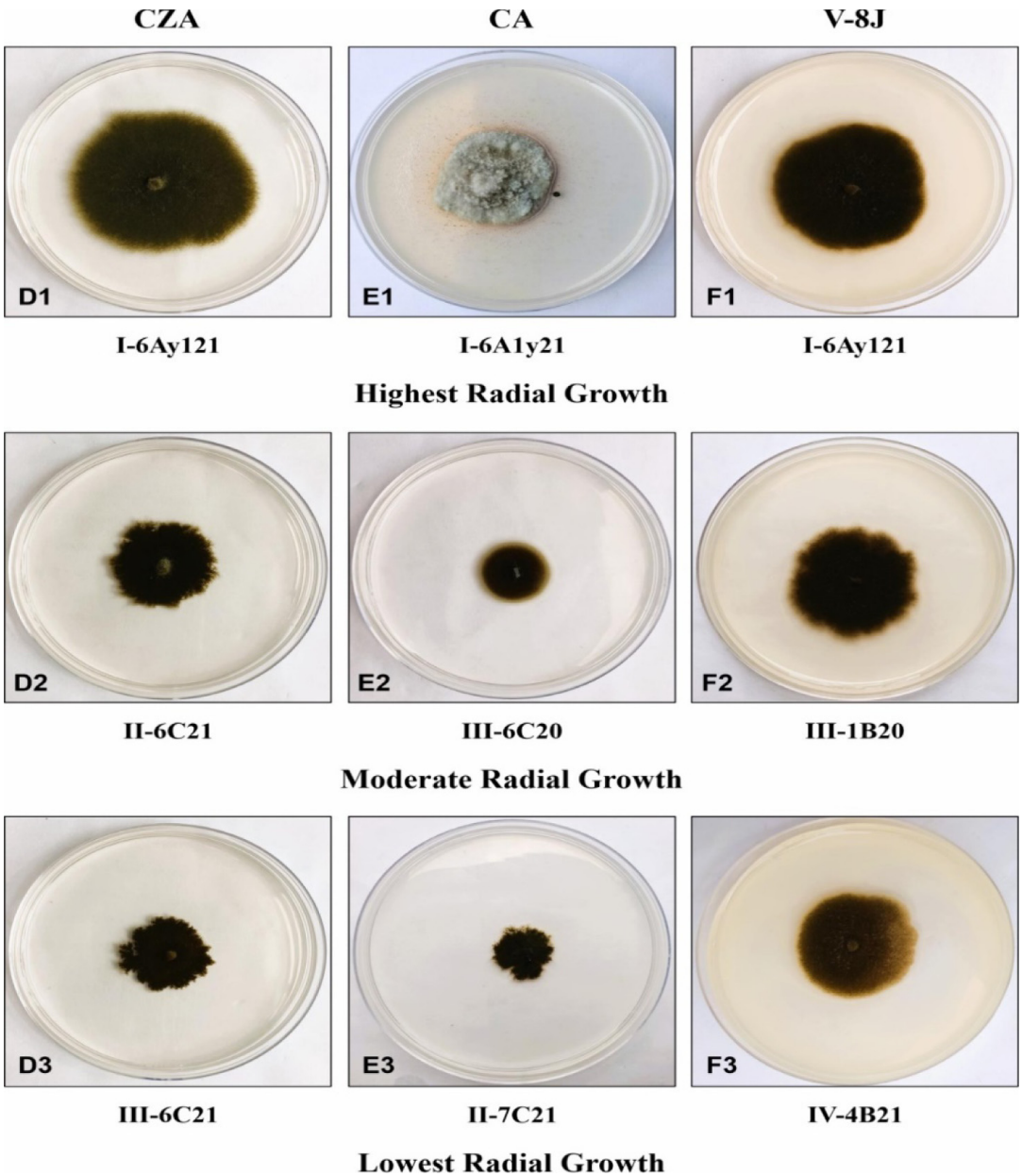

Fig. 4. The effect of culture media was observed on 31 isolates of *Alternaria brassicae*. Of these, — D (1) The isolate I-6Ay121 showed the highest radial growth, while F (3) Isolate IV-4B21 showed the least radial growth on V-8J medium.

Among all of these, only the isolate I-6A1y21 shows more radial growth (22.26 mm), while the isolate IV-IC21 shows the least radial growth (16.43 mm) at pH 9. The radial growth range of all isolates at pH 10 was 8.53 mm to 10.06 mm; the isolate I-6A1y21 exhibits greater radial growth (10.06 mm) among 31 isolates, whereas the isolates I-6B21 and II-2C20 exhibit the least radial growth (8.53 mm). The isolate III-3B20 showed the least average radial growth in all six different pH levels, while the isolate I-6A1y21 showed the maximum average radial growth in all six different pH levels. The pH 7 is more favorable for radial growth of all

isolates, while pH 6, 8, and 9 are moderately suitable for radial growth. Above and below a pH of 7, radial growth appears to decrease.

Sporulation at different pH level

The various isolates of *A.brassicae* showed different rates of sporulation at various pH level. Sporulation of all 31 isolates on 10-days-old culture was higher (range:16.66 $x10^5$/ml -35.50 $x10^5$/ml) at pH 7.0. Among them, the isolate I-6A1y21 showed the highest sporulation (35.50 $x10^5$/ml) whereas the isolate III-5C21 showed the least sporulation (16.66 $x10^5$/ml).The sporulation range of all 31 isolates at pH 8.0 was 10.25 $x10^5$/ml to 18.15 $x10^5$/ml and among them, the isolate I-6A1y21 showed the highest sporulation (18.15 $x10^5$/ml), while the isolate I-1B20 showed the least sporulation (10.25 $x10^5$/ml). The sporulation range of all 31 isolates at pH 5.0 from 9.58 $x10^5$/ml – 17 $x10^5$/ml and among them, the isolate I-6A1y21 showed the highest sporulation (17 $x10^5$/ml) while the isolate I-1B20 showed the least sporulation (9.58 $x10^5$/ml). Sporulation of 31 isolates at pH 6.0, the range was from 13.66 $x10^5$/ml to 27.50 $x10^5$/ml out of these, isolate I-11A21 showed the least sporulation (13.66 $x10^5$/ml) while the isolate I-6A1y21 showed the highest sporulation (27.50 $x10^5$/ml). Among all of these, only isolate I-6A1y21 shows more sporulation (10.08 $x10^5$/ml and 7.25 $x10^5$/ml) while the isolates II-5C20 and II-2C20 shows the least sporulation (5.58 $x10^5$/ml and 1.66 $x10^5$/ml) at pH 9 and 10, respectively. The isolate II-5C20 showed the least average sporulation in all six different pH levels, while the isolate I-6A1y21 showed the maximum average sporulation in all six different pH levels. The study found that pH 7 is the most suitable for sporulation of all 31 *Alternaria brassicae*, followed by pH 6, with moderate sporulation at pH 5 and 8.

Radial growth on different Relative humidity

The various isolates of *Alternaria brassicae* at different relative humidity showed different rates of radial growth**.** The maximum radial growth occurred at 80-100% relative humidity in all 10-days-old cultures (range: 33.15 mm-46.12 mm), and among them, isolate I-6A1y21 showed the highest radial growth (46.12 mm), while the isolate V-7A20 showed the least radial growth (33.15 mm). At RH 60-80%, the range of radial growth was from 18.30 mm to 24.12 mm. Isolate I-4A21 showed the least radial growth (18.30 mm), while the isolate I-6A1y21 showed the highest radial growth (37.24 mm). All isolates showed radial growth ranging from 11.78 mm to 16.24 mm at RH 40-60%. Among all of these, isolate I-6A1y21 isolate shows more radial growth (16.24 mm), while the isolate I-11A21 shows the least radial growth (11.78 mm). The radial growth range of all 31 isolates at 20-40% was 4.17 mm to 10.25 mm; among all of these, the isolate I-6A1y21 exhibited greater radial growth (10.25 mm) whereas the isolate II-UN120 and the isolate II-4II20 exhibited the least radial growth (4.17 mm). The observation found that the radial growth was more in relative humidity between 80-100%, while the least radial growth was observed in relative humidity between 20-40%.

Sporulation on Relative humidity

The various isolates at different relative humidity showed different rates of sporulation, and the maximum sporulation occurred at 80-100% relative humidity in all 10-days-old cultures (range: 11.58 x 10^5/ml - 34.50 x 10^5/ml). Among these, isolate I-6A1y21 showed the highest sporulation (34.50 x 10^5/ml), while the isolate II-6C21 showed the least sporulation (11.58 x 10^5/ml). At RH 60-80%, the range of sporulation was from 10.50 x 10^5/ml to 23.5 x 10^5/ml; out of these, isolates IV-5C20 and II-

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	Mubarakpurkotwa, PRG	25.4356° N 81.7555° E	Brownish black	Septate and brownish	Dark brown	Rough	Long pear shaped	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 withseptations	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	Govindpur 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	Long 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	25.4627^o N 81.9475^o E	Light brown at the periphery	Brown and septate	Brown	Rough	spatulate	Long
V-2A20	leaf	Chamnpur,Jhusi, PRG	25.4279677^{o}N 81.8980579^{o}E	Brown and fuzzy growth	Brown and septate	Brown	Rough	Club shaped	Long
V-7A20	leaf	Sundripurkla, PRG	25.4142° N 82.0261° E	Grey whitish compact colony growth	Grey and septate	Grey	Rough	Obovate	Long
I-4A21	leaf	Shekhsarwa, PRG	25.25'559^{o}N 81.42'295^o E	Brown and Woolly colony	Brown and septate	Brown	Rough	Obovoid	Long
I-4B121	leaf	Sekhsarwa, PRG	25.25'55.9 ^{o}N 81.42'29.5 ^{o}E	Dark brown with scattered and compact growth	Brown and septate	Brown	Rough	obpyriform	Long

Isolate code	Plant parts	Survey site	Geographical data	Appearance of culture on PDA plates	Hyphae	Conidia			
						Colour	Surface	Shape	Beak
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-6A$_1$Y21	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	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 septate
III-4C21	leaf	MamabhanjaTalab, 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	Long
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

The severity of the disease was measured on 0-5 scale as follows:

Table 2. The grade and degree (severity) of infection

S.No.	Grade	The degree of infection
1.	0	Healthy leaves
2.	1	Between 1 to 10% of the leaf surface is infected
3.	2	Between 11 to 25% of the leaf surface is infected
4.	3	Between 26 to 50% of the leaf surface is infected
5.	4	Between 51 to 75% of the leaf surface is infected
6.	5	Mare than 75% of the leaf surface is infected

8B21 showed the least sporulation (10.50 x 10^5/ml), while the isolate I-6A1y21 showed the highest sporulation (23.50 x 10^5/ml). All 31 isolates showed sporulation ranges from 7.33 x 10^5/ml to 16.25 x 10^5/ml at RH 40-60%. Among all of these, only isolate I-6A1y21 showed the highest sporulation (16.25 x 10^5/ml), while the isolate III-5C21 showed the least sporulation (7.33 x 10^5/ml). The sporulation range of all isolates at 20-40% was 1.96 x 10^5/ml to 4.15 x 10^5/ml; among all of them, isolate I-6A1y21 exhibits the highest sporulation (4.15 x 10^5/ml) among all of these, whereas the isolates III-5C21 exhibit the least sporulation (1.96 x 10^5/ml). The RH (80-100%) is the standard scale for sporulation; below this scale, the sporulation gradually decreases. The pathogen produces fewer conidia in lower humidity conditions. The sporulation significantly decreased when RH dropped below 70%, and no sporulation was observed at RH levels below 50 (Thakur, 2024). The previous study supports the present study that *Alternaria brassicae* showed high radial growth and sporulation in relative humidity more than 50% and significantly showed reduction below 50% relative humidity.

Pathogenicity test

It was found that all 31 of the *A.brassicae* isolates showed pathogenic behaviour. Among the 31 isolates, 9 isolates II-UN120, IV-5C20, I-4A21, I-6B21, I-6A1y21, II-8B21, III-6C21, IV-4B21 and IV-6AY21 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-1B2, I-4B21, II-3B21, II-7C21 and III-7B21 showed least degree of infection as the spot produced by them were 1mm -5 mm in diameter. Seventeen isolates II-2C2, II-4II20, II-5C20, III-1B20, III-3B20, III-5C20, III-6C20, V-1B20, V-2A20, V-7A20, I-7B21, I-11A21, II-1B21, II-6C21, III-4C21, III-5C21 and IV-IC21 were found to be moderately degree of infection as the spot produced by them from 6 mm to 10 mm in diameter.

Table 7 showing pathogenicity test on mustard 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 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 (+++).

The previous investigation confirmed the finding of current study which showed that the fungal pathogen pathogenicity, hosts, and modes of dissemination is essential for maintaining global biosecurity (Fontaine *et al*., 2021).

Table 3. Occurance of Alternaria black spot disease on mustard in Allahabad district (from 2019 to 2021)

S.No.	Isolate	Location	variety	Incidence (%)	Severity (%)
1	I-1B20	Mubarapur - Kotwa	Pusa mustard 27 (EJ 17)	25	5
2	II-2C20	Kahimapur - Jhusi	K-88	45	15
3	II-$UN_1$20	Phoolpur	Pusa mustard 26 (NPJ 113)	15	5
4	II-4II20	Sahso	Pusa Double zero -33(PDZ-33)	35	10
5	II-5C20	Babuganj	Narendra Swarna -08	55	20
6	III-1B20	Govindpuram - Naini	Vardhan (RK1481)	70	25
7	III-3B20	Sarangapur	Coral PAC- 432 (Hybrid)	55	35
8	III-5C20	Shimra - Ghoorpur	Coral PAC- 432 (Hybrid)	55	20
9	III-6C20	SHUATS - Naini	Coral PAC- 432 (Hybrid)	45	15
10	IV-5C20	Malak harhar - Soraon	Coral PAC- 432 (Hybrid)	35	10
11	V-1B20	Narayan Daspura-Jhusi	Narendra Swarna -08	45	25
12	V-2A20	Chamnpur - Jhusi	Narendra Swarna -08	60	25
13	V-7A20	Sundripur kla - Hanumanganj	(NRCHB-506)	50	20
14	I-4$A_2$21	Shekhsarwa I	Pusa Double zero -33 (PDZ-33)	45	15
15	I-4B121	Sekhsarwa II	Pusa Double zero -33 (PDZ-33)	70	35
16	I-6B121	Barwa I	Narendra Swarna -08	55	30
17	I-6A1y21	Barwa II	Coral PAC- 432 (Hybrid)	85	50
18	I-7B121	Bhagwatpur	Pusa mustard 25 (NPJ 112)	50	35
19	I-11A21	Peepalgaon	Vardhan (RK1481)	40	25
20	II-1b21	Andhawa - Jhusi	Pusa mustard 25 (NPJ 112)	35	15
21	II-3B21	Sherdeeh - Jhusi	K-88	60	35
22	II-6C21	Kaserua - Jhusi	Pusa mustard 25 (NPJ 112)	55	20
23	II-7C21	Thanpur - Sahso	(NRCHB-506)	65	25
24	II-8b21	Devnahri - Phoolpur	Coral PAC- 432 (Hybrid)	45	30
25	III-4C21	Mamabhanja Talab - Naini	Laha-101 (Type 101)	45	20
26	III-5C21	Bhandra - Naini	Laha-101 (Type 101)	35	25
27	III-6C21	Sarangapur	Laha-101 (Type 101)	55	30
28	III-7b21	Bigahiya - Ghoorpur	K-88	65	25
29	IV-IC21	Teliyarganj - Barud khana	Pusa mustard 26 (NPJ 113)	75	30
30	IV-4B21	Phaphamau - Belakachhar	Pusa Double zero -33(PDZ-33)	70	35
31	IV-6AY21	Morahu	Pusa mustard 26 (NPJ 113)	80	45

Table 4. Comparison of conidial size and septation of *Alternaria brassicae* isolates collected from the different geographical regions of Allahabad district

Isolate code	Conidial Length in µm				Conidial breadth in µm				No. of Transverse septa	No. 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-6A1 y21	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

Table 5. Measurement of radial growth (mm) of *Alternaria brassicae* in different culture media

Isolates	PDA	CMA	OMA	CZA	CA	V-8J
I-1B20	48.35	24.35	47.7	40.8	18.25	39.4
II-2C20	34.51	34.38	41.36	33.25	26.25	36.6
II-UN120	33.31	30.43	69.26	37.63	23.63	48.26
II-4II20	37.35	31.35	56.23	31.25	25.41	37.26
II-5C20	45.35	27.45	38.38	27.23	15.23	39.56
III-1B20	39.31	28.25	53.25	29.43	19.56	38.23
III-3B20	32.28	29.31	39.4	32.56	19.7	41.13
III-5C20	31.35	40.25	53.6	57.56	18.2	41.7
III-6C20	37.31	26.25	63.25	34.25	20.56	47.26
IV-5C20	36.35	26.45	48.45	27.53	31.13	30.63
V-1B20	37.25	25.36	39.25	31.25	15.1	37.76
V-2A20	44.13	24.45	42.13	44.41	22.2	38.56
V-7A20	45.25	17.38	42.46	35.25	14.26	37.56
I-4A21	37.25	23.25	40.53	36.26	20.23	28.43
I-4B21	34.36	17.26	39.63	27.56	16.2	30.56
I-6B21	41.33	22.45	46.46	33.63	14.76	36.26
I-6A1y21	52.35	42.16	71.20	62.66	32.30	49.73
I-7B21	33.23	14.25	40.25	60.33	16.33	37.65
I-11A21	28.28	25.25	46.23	35.56	19.76	36.66
II-1B21	50.25	18.73	46.25	46.6	17.33	38.56
II-3B21	38.25	12.56	44.28	31.2	12.76	28.56
II-6C21	35.31	23.25	54.4	41.2	16.36	37.56
II-7C21	29.35	13.5	46.36	24.56	11.46	29.38
II-8B21	30.25	14.3	41.23	29.26	11.56	36.56
III-4C21	41.35	22.31	43.25	37.43	18.53	39.25
III-5C21	37.61	21.25	37.4	35.63	19.66	41.58
III-6C21	31.58	28.25	45.38	23.33	22.4	45.08
III-7B21	38.35	22.8	42.2	39.56	15.36	40.63
IV-IC21	41.31	23.4	41.2	36.3	19.75	36.36
IV-4B21	37.25	13.26	37.66	24.2	12.26	27.26
IV-6AY21	29.43	25.45	40.7	28.63	15.76	41.4

Table 6. Effect of different culture media in fungal sporulation.

Isolates	Spore concentration (10^5/ml)					
	PDA	CMA	OMA	CZA	CA	V-8J
I-1B20	17.24	8.25	18.25	15.5	5.5	12.5
II-2C20	17.5	7.41	20.5	12.5	6.45	13.25
II-UN120	20.5	8.5	21.25	17.25	5.76	12.4
II-4II20	20.41	6.33	19.5	14.5	4.35	10.58
II-5C20	16.5	7.5	22.5	13.25	4.75	9.25
III-1B20	18.5	7.25	24.5	15.65	6.25	11.85
III-3B20	20.5	8.25	23.5	17.5	4.58	9.95
III-5C20	19.5	7.5	21.41	16.41	6.5	10.65
III-6C20	17.25	9.25	18.5	12.5	7.85	12.63
IV-5C20	16.5	10.5	18.25	10.5	7.15	8.95
V-1B20	16.66	10.33	19.5	13.5	5.25	10.85
V-2A20	19.25	8.5	18.5	13.75	4.15	12.85
V-7A20	23.5	9.25	20.25	18.5	5.65	11.65
I-4A21	22.5	6.5	26.5	16.25	7.35	8.45
I-4B21	28.5	5.5	27.5	20.5	4	11.25
I-6B21	21.5	8.5	26.15	15.5	5.15	14.45
I-6A1y21	29.5	14.5	30.25	23.5	8.25	16.66
I-7B21	24.5	5.25	23.25	14.33	6.55	13.56
I-11A21	18.5	7.25	16.5	13.25	4.5	9.55
II-1B21	18.5	6.55	19.5	12.25	4.45	10.56
II-3B21	17.5	5.25	16.25	14.41	4.5	9.35
II-6C21	21.5	9.75	22.5	16.33	5.55	11.95
II-7C21	22.5	8.25	25.5	17.58	7	7.95
II-8B21	22.5	6.25	25.5	18.5	6	9.95
III-4C21	18.33	9.25	28.25	14.41	5.25	7.33
III-5C21	19.5	9.75	23.5	12.5	6.45	10.61
III-6C21	20.58	8.25	26.5	16.41	5.65	7.75
III-7B21	21.5	8.75	25.5	13.33	6.25	14.55
IV-IC21	19.33	7.45	21.5	14.25	4.35	15.45
IV-4B21	22.5	7.25	23.5	14.33	3.95	8.35
IV-6AY21	18.5	8.5	22.25	11.41	5.65	12.35

Table 7 Pathogenicity test 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	+++
I-6B21	+++		

CONCLUSIONS

The survey in Allahabad revealed Alternaria leaf blight incidence and severity varying with cultivars, geographical locations, and micro-environmental conditions, ranging from 5 to 50% and 15 to 85%, respectively. The cultural characteristics of 31 *A. brassicae* isolates reveal high variability in conidial length, width, beak length, and septa number *in vitro*. Among the selected culture media, oatmeal agar was found to be the most favourable medium for *A. brassicae* isolates, exhibiting maximum radial growth and spore production. The optimal temperature for *A. brassicae* isolates' growth and spore production is +24°C, with temperature variation significantly affecting growth and sporulation, with significant reductions observed at lower and higher temperatures. The study found that pH variations significantly impacted radial growth and sporulation, with growth increasing at pH 6 and 7 and decreasing at pH 5, 8, 9, and 10. Significant reduction in growth and sporulation was recorded at 20-40% relative humidity. While 40-60%, 60-80% and 80-100%, relative humidity caused increase in growth and sporulation, however the magnitude of sporulation increase varies isolate to isolate.

REFERENCES

Blagojevic, J. D., Vukojevic, J. B., and Ivanovic, •. S. (2020). Occurrence and characterization of Alternaria species associated with leaf spot disease in rapeseed in Serbia. Plant pathology, 69(5), 883-900.

Chattopadhyay, C. (2008). Management of diseases of rapeseed mustard with special reference to Indian conditions. (in) Sustainable Production of Oilseeds: Rapeseed-Mustard Technology. Agrotech Publishing Academy, Udaipur. pp. 364–388.

DRMR, (2020-2021). 28th Annual AIRCP (R&M) Group Meeting, ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, Pp 3-4.

Fatima, U., Bhorali, P., Borah, S., and Senthil-Kumar, M. (2019). Perspectives on the utilization of resistance mechanisms from host and nonhost plants for durable protection of Brassica crops against Alternaria blight. PeerJ, 7, e7486.

Fontaine, K., Fourrier-Jeandel, C., Armitage, A. D., Boutigny, A. L., Crépet, M., Caffier, V., Gnide, D. C., Shiller, J., Le Cam, B., Giraud, M., Ioos, R., and Aguayo, J. (2021). Identification and pathogenicity of Alternaria species associated with leaf blotch disease and premature defoliation in French apple orchards. PeerJ, 9, e12496.

Goyal, P., Chahar, M., Mathur, A.P., Kuma, r A. and Chattopadhyay, C. (2011). Morphological and cultural variation in different oilseed *Brassica* isolates of *Alternaria brassicae* from different geographical regions of India. Indian J. Agric. Sci. 81 (11): 1052–1058.

Kumar, P., and Sharma, R.C. (2017). "Comparative study of culture media for the growth and sporulation of Alternaria brassicae." Indian Phytopathology, 70(3), 421-427.

Pandey, M. K., Ahmad, M. M., Siddiqui, S., Kumar, A., and Singh, S. K (2023) a. Alternaria Blight–A Serious Affliction of Rapeseed Mustard: A Review.

Patel, S. N., Kumar, P., Raghuvanshi, V. V., Patel, S. K., Siddharth, P., Singh, A., and Milan, A. (2023). Morphological Characterization and Pathogenicity Test of Alternaria Leaf Spot of Chinese Cabbage (Brassica chinensis L.). International Journal of Plant & Soil Science, 35(19), 1616-1625.

Prasad, S., Kumar, R., and Singh, D.P. (2019). "Influence of culture media on the growth and sporulation of Alternaria brassicae." Journal of Plant Pathology, 101(2), 297-302.

Punia, R., Kumari, P., Kumar, A., Rathi, A. S., and Avtar, R. (2021). Impact of weather parameters on alternaria blight of Indian mustard [(Brassica juncea (L.) Czern. & Coss.)]. Bangladesh Journal of Botany, 50(1), 15-19.

Saha, S., Garg, R., Venkataravanappa, V., Mishra, P. K., Rai, A. B., and Singh, R. P. (2016). Molecular and Cultural Characterization of Alternaria brassicae Infecting Cauliflower in Uttar Pradesh, India. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 86, 485-495.

Singh, H. K., Kumar, P., and Singh, S. K. (2021). Yield loss assessment due to Alternaria blight disease in rapeseed and mustard: Yield loss due to alternaria Blight in rapeseed and mustard. Journal of AgriSearch, 8(3), 241-248.

Singh, J., Kumhar, D. R., Kumari, S., and Yadav, A. L. (2021). Effect of physical parameters on the sporulation and mycelial growth of Alternaria brassicae causing Alternaria leaf spot of mustard. Plant Disease Research, 36(2), 184-187.

Thakur, O. (2024). Variability and management of alternaria species causing leaf blight of cauliflower *(brassica oleracea l. var. botrytis)* (doctoral dissertation, dr. yashwant singh parmar university of horticulture and forestry).

Shailendra Kumar, Prasad S. M. and Kehri H. K. 2024. Cultural and morphological characterization of Alternaria brassicae of mustard in Allahabad, Uttar Pradesh. The Journal of Research, ANGRAU. Vol. 52 (4):28-45