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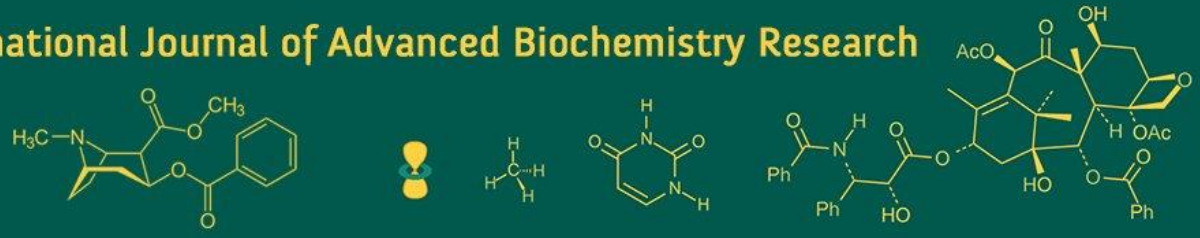

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Cultural and morphological variability among the isolates of *Alternaria porri*

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Abstract
Onion (*Allium cepa* L.) indeed holds significant importance globally, not just as a culinary staple but also for its numerous health benefits. As a member of the Alliaceae family, it has been cultivated for centuries and is widely recognized for its medicinal properties. The fungus associated with naturally infected onion leaves was isolated by adapting standard tissue isolation technique on potato dextrose agar medium. Similarly, different isolates of *Alternaria porri* were obtained from diseased plants from different onion growing area of Konkan region and rest of the Maharashtra. Pathogenic ability of the fungus isolated from diseased plant tissue was carried out on susceptible variety of onion (Cv. Alibag Local) in pots under glass house conditions by following Koch's postulates and the pathogenic fungus was identified as *A. porri* (Ellis) Cif. Identification of the causal organism inciting purple blotch disease in onion was done based on morphological characters of the isolated fungus. The mycelium was composed of smooth, septate, short, simple or branched, sub fasciculate hyphae and light brown to dark brown in colour. The conidia of the pathogenic fungus were generally solitary, straight or curved, obclavate or with the body of conidium ellipsoidal tapering to the beak with 4-10 transverse and zero to few longitudinal septa and with beak flexuous, pale, thick and tapering. The conidia were pale to dark brown and muriform with size ranged from 67.3 – 131.4 x 17.7 - 22.3 µ. All isolates of *A. porri* exhibited a wide range of variability in respect of the cultural characteristics *viz*., colony diameter, colony colour, growth rate, colony elevation and topography, colony margin and zonation. All isolates of *Alternaria porri* exhibited a wide range of variability in respect of the morphological characteristics *viz*., hyphal width, conidial shape, conidial size, beak length and number of transverse and longitudinal septa.

Keywords: Onion (*Allium cepa* L.), *Alternaria porri*, Purple blotch disease, Pathogenic variability, Morphological and cultural characteristics

Introduction
Onion (*Allium cepa* L.) indeed holds significant importance globally, not just as a culinary staple but also for its numerous health benefits. As a member of the Alliaceae family, it has been cultivated for centuries and is widely recognized for its medicinal properties. One of the key components responsible for the characteristic pungency of onion bulbs is Allyl-propyl-di-sulphide which is not only responsible for the onion's flavour but also contributes for its medicinal properties. Allyl-propyl-di-sulphide possesses a different beneficial effect including anti-cancerous antibacterial, antifungal, anti-helminthic, anti-inflammatory, antiseptic and antispasmodic properties (Augusti, 1996) [8]. The onion contains a variety of bioactive compounds, such as organosulfur compounds, flavanols, ascorbic acids and carbohydrate prebiotics. Additionally, its by-products contain a higher concentration of flavonoids compared to the bulb. The major organosulfur compounds found in onions are diallyl monosulfide, diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide, while quercetin, kaempferol, anthocyanin and luteolin are considered as the main flavonoids. Ascorbic acid and fructo-oligosaccharides are also recognized as bioactive compounds. These bioactive compounds in onions have strong antioxidant potential for neutralizing the oxidative stress of cells (Sagar *et al*., 2022) [26].
Maharashtra accounts for a significant share of India's onion farming and output. The state cultivates onions for approximately 43.98% of the total onion production area in India. In onion cultivation, Maharashtra ranks first with an area of 667.44 thousand hectares.

Maharashtra has the lowest onion productivity of 12.89 tonnes/ha with 8602 thousand tonnes of production. This distinction reveals a critical knowledge gap in agricultural extension, as farmers in Maharashtra fall behind scientifically suggested modern agricultural techniques. In Maharashtra, onion cultivation is practiced across multiple seasons including *Kharif*, late *Kharif* and *Rabi*, ensuring a continuous production cycle throughout the year. Several districts in Maharashtra like Nashik (251.45 thousand hectare), Ahilyanagar (175.50 thousand hectare), Pune (76.87 thousand hectare), Solapur (48.50 thousand hectare), Sambhaji Nagar (38.89 thousand hectare), Beed (37.82 thousand hectare), Dharashiv (16.12 thousand hectare), Jalgaon (12.41 thousand hectare), Buldhana (5.61 thousand hectare) and Akola (5.14 thousand hectare) are key contributors to the state's onion cultivation. Additionally, the Konkan region of Maharashtra, encompassing districts like Raigad and Ratnagiri, are also suitable for cultivating onions particularly white onions due to the prevalence of lateritic soil. Raigad contributes about 0.28 thousand hectares white onion cultivation area with 0.33 thousand tonnes production and 1.20 tonnes/ha productivity. Ratnagiri contributes about 0.06 thousand hectares white onion cultivation area with 0.23 thousand tonnes production and 3.65 tonnes/ha productivity. Raigad and Ratnagiri districts soil is well-suited for onion cultivation, contributing further to the agricultural diversity and productivity of Maharashtra (Anonymous, 2024) [6].

Amongst the important diseases, purple blotch disease incited by *Alternaria porri* is the major hurdle in successful and fruitful cultivation of onion. The pathogen *Alternaria porri* is classified under the Deuteromycetes class, Moniliales order, Dematiaceae family and *Alternaria* genus. It specifically targets the leaf tissue, which in turn disrupts the stimulus for bulb initiation and delays the process of bulbing and maturation. In the case of severe infection, flower stalks can become completely girdled with necrotic tissue, leading to their collapse and a total loss of seed production capacity. In India, Ajrekar (1921) [3] initially reported the disease in Bombay State, identifying the causal agent as *Macrosporium* sp. Later, Pandotra (1964) [23] discovered that causal organism as *Alternaria porri*. Since then, the disease has been detected in several other regions where onions are grown. The purple blotch disease affects both aboveground and underground parts of plants under field conditions. This disease results in a significant reduction in leaf production, ranging from 62-92%, as observed. Additionally, it causes about 59% reduction in bulb yield and 97% reduction in seed yield. The yield loss of onions due to purple blotch disease in India under favourable conditions can vary significantly, ranging from 5.0 to 96.5% (Gupta and Pathak, 1988) [17].

2. Materials and methods

2.1. Collection of disease specimens for isolation

Leaves of onion crop exhibiting typical symptoms of purple blotch disease were collected from various locations of Maharashtra, in clean and dry paper bags, labelled with relevant details and brought to the laboratory for further investigation.

2.2. Preparation of potato dextrose agar (PDA) medium

The common basal medium i.e. Potato Dextrose Agar (PDA) medium was used for various *in vitro* studies and maintenance of culture of fungal pathogen and bio-agents. For making 1 litre of PDA, 200g of peeled potatoes were chopped and boiled in 500 ml of distilled sterile water till desired extract was obtained. Agar-agar (20 g) was dispensed and boiled to melt in remaining 500 ml of distilled sterile water. The potato extract was collected by filtering through clean muslin cloth and the filtrate was added to the molten agar-agar to which 20 g of dextrose was added and the final volume was made to 1000 ml by adding more distilled sterile water. The pH of the medium was adjusted to 6.5 and then it was sterilized in an autoclave at 1.054 kg/cm^2 (121 ^{o}C) pressure for 20 minutes.

2.3. Isolation and purification of the fungal isolates

Isolation of fungus associated with diseased plant tissue was carried out by standard tissue isolation technique. Naturally infected onion leaves showing typical symptoms of purple blotch disease were collected from different parts of region of Maharashtra and brought to the laboratory. The onion leaves naturally infected with the disease were washed thoroughly with water, blot dried and cut with sharp sterilized blade into small bits (5 mm), keeping half healthy and half diseased portion intact. These cut pieces of leaf sample were surface sterilized in 1% sodium hypochlorite (NaOCl) solution for 1-2 minutes, rinsed thrice in sterilized water and dried with sterilized blotting paper. These surface sterilized leaf bits were then inoculated on the cooled and solidified Potato Dextrose Agar medium in Petri plates under aseptic conditions of laminar-air-flow cabinet. Inoculated plates were then inoculated in BOD incubator for incubation at 27 ± 2 ^{o}C temperature for seven days.

Pure culture of the fungus obtained from diseased tissue was sub cultured on PDA slants and incubated at 27 ± 2 ^{0}C. The slants with the fungal culture were preserved in refrigerator at 4 ^{0}C and maintained in pure form for their further use. Well grown pure culture of the fungus was maintained on PDA slants by periodic sub culturing. Using a similar procedure, a total of ten isolates of associated fungus were isolated, purified, multiplied and labelled as specified in the subsequent table and they were stored in the refrigerator for further investigation.

Table 2.1: List of *Alternaria porri* isolates collected from different locations of Konkan and other parts of Maharashtra

Sr. No.	Isolate	Location/ Tehsils	District
1	ApPu	DOGR, Pune	Pune
2	ApJa	Jamkhed	Ahilyanagar
3	ApAl	Alibag	Raigad
4	ApKa	Kalamb	Dharashiv
5	ApDh	Dharashiv	Dharashiv
6	ApPe	Pen	Raigad
7	ApDa	Dapoli	Ratnagiri
8	ApBa	Barshi	Solapur
9	ApLa	Latur	Latur
10	ApRa	Ranjangaon	Pune

2.4. Pathogenicity of causal organism

Pathogenicity test of the isolated fungus was carried out on onion cultivar ‘Alibag Local’ using standard Pin prick method of inoculation. Forty days old seedlings of onion were transplanted in pots having autoclaved potting mixture of soil : sand : FYM (2:1:1), in greenhouse. The plants were watered regularly and kept for the further growth. Pure sporulating culture of *Alternaria porri* isolated from

diseased onion leaves was used to prove its pathogenic ability. Seven days old pure culture of *Alternaria porri* was used to make spore suspension through homogenization of mycelial mass in sterile distilled water followed by filtration through muslin cloth. Test plants were washed with distilled sterile water for removing dust deposited on leaves and then spray inoculated (automizer) with the spore-cum-mycelial suspension of the test fungus following Pin prick method of inoculation. For maintaining requisite relative humidity, all inoculated plants were covered with polythene bags for few days. Plants sprayed only with distilled sterile water were served as control. Both inoculated and non-inoculated seedlings were maintained in greenhouse by watering regularly with distilled sterile water and observed for the development of the disease symptoms. After development of typical purple blotch symptoms on artificially inoculated plant, it was subjected to reisolation to prove Koch's postulates.

From the leaves of onion seedlings showing typical purple blotch symptoms which were artificially inoculated and diseased, reisolation of associated fungus was done aseptically on PDA medium. After a week of incubation at 27±2 ºC, the cultural and morphological characteristics of the reisolated fungus were observed and it were compared with the characteristics (cultural and morphological) of the *Alternaria porri*.

2.5. Identification of the causal fungus

Based on typical symptoms (naturally and artificially diseased) of purple blotch, morpho-cultural characteristics, microscopic observations and pathogenicity test etc., the test pathogen was identified as *Alternaria porri* and it was confirmed by comparing its authentic descriptions / characters (Ellis, 1971) [15]. Further, the pure culture was sent to chief Mycologist, Agharkar Research Institute, Pune for confirmation of the fungus up to species level.

2.6. Morphological and cultural variability among isolates of *Alternaria porri*

2.6.1. Cultural variability

The cultural traits of the ten isolates of *Alternaria porri* were examined using PDA as the primary culture medium. Sterilized glass Petri plates (90 mm dia.) were filled with autoclaved and cooled PDA medium (20 ml/plate), which was then allowed to solidify at room temperature. Once the PDA gets solidified, each plate was inoculated separately and aseptically with a 5 mm diameter culture disc containing one-week-old pure culture. Each isolate was replicated three times and then placed in an incubator at 27 ± 2 ºC for 7 days. Observations on cultural characteristics such as colony diameter (mm), colony colour (front and rear), growth rate (mm/day), colony elevation and topography, colony margin, zonation etc., were documented.

2.6.2. Morphological variability

For morphological characteristics, the temporary mounts on clean glass slides in a drop of lactophenol cotton blue stain, pure culture (two weeks old) of *Alternaria porri* isolates were prepared separately, covered with cover slip and observed under microscope (at 40x magnification). Observations on mycelium, size and shape of spores, septation etc. were recorded. Measurements of various structures of *Alternaria porri* isolates were attempted by using Digital Image Analyzer, under five random microscopic fields (40x), using Micam 3.0.2 software.

3. Results and discusssions

3.1. Isolation and purification of the fungal isolates

Leaves of onion showing typical symptoms of purple blotch disease were collected from naturally infected fields of Konkan region and other onion growing regions of Maharashtra. Collected diseased samples were subjected to isolation by using standard tissue isolation technique under aseptic conditions on potato dextrose agar (PDA) medium. The fungal growth emerged around the inoculated bits of diseased host tissue was cottony and olivaceous brown in colour. Initially, the fungal growth was light to dark olivaceous with greenish or brownish tinge which gradually turned to whitish gray with zonation in old cultures. The fully developed colony of isolated fungus was round, sporulating on PDA and spreading outward (Plate I).

Similar procedure was followed to obtain isolates of *Alternaria porri* from diseased plants collected from different onion growing area of Konkan region and rest of the Maharashtra. The cultures of isolates of *Alternaria porri* were purified by using hyphal tip isolation technique on potato dextrose agar medium. The pure cultures of ten isolates were allotted designation as Ap (*Alternaria porri*) indicating name of the pathogen. These isolates were further designated as depicted in Table 3.1. The pure cultures of all the ten isolates were maintained by regular sub-culturing at every 30 days interval on Petri plates containing potato dextrose agar medium. Pure cultures slants of all the isolates were preserved in refrigerator for further research work.

Front View

Rear View

Plate I: Pure culture of *Alternaria porri*

Table 3.1: Isolates of *Alternaria porri* causing purple blotch disease of onion

Sr. No.	Isolate	Location/ Tehsils	District
1	ApPu	DOGR, Pune	Pune
2	ApJa	Jamkhed	Ahilyanagar
3	ApAl	Alibag	Raigad
4	ApKa	Kalamb	Dharashiv
5	ApDh	Dharashiv	Dharashiv
6	ApPe	Pen	Raigad
7	ApDa	Dapoli	Ratnagiri
8	ApBa	Barshi	Solapur
9	ApLa	Latur	Latur
10	ApRa	Ranjangaon	Pune

The findings of the current study are closely aligned with the previous reports of Bhandekar *et al*. (2019) [10] who isolated *Alternaria porri* from infected leaves of onion showing typical purple blotches by standard tissue isolation technique on PDA medium. Gore *et al*. (2020) [16] isolated *A. porri* from the diseased leaves collected from a naturally infected onion field showing typical symptoms of purple blotch disease of onion by using standard tissue isolation technique on PDA medium. Singh and Tiwari (2020) [31] conducted a survey on purple blotch disease of onion in major onion growing districts of Bihar and further isolated *A. porri* from infected onion leaves. Anand (2021) [5] also isolated *A. porri* from onion leaves infected with purple blotch disease.

The current investigation's findings are closely aligned with previous reports of Khandagale *et al*. (2022), Chandan *et al*. (2023), Ishak *et al*. (2023), Devi and Lal (2024), Patel and Arsia (2024) and Asghar *et al*. (2025) [20, 11, 19, 13, 24, 7].

3.2. Pathogenicity of causal organism

Pathogenic ability of the fungus isolated from diseased plant tissue of onion was carried out on susceptible variety of white onion (Cv. Alibag Local) in pots under glass house conditions. The study revealed that the fungus produced typical symptoms of purple blotch disease in the form of small (2-3 mm in diameter), water-soaked spots on the leaves, which soon turned into whitish grey centres. After inoculation, the first typical symptom of the purple blotch disease developed within a week. In advanced stages of disease development, these spots then expanded, coalesced, formed a zonate pattern and colour of which changed from brown to purple, spreading upward and downward. It was observed that the symptoms caused on artificially inoculated plants were the same as those seen on naturally infected plants. However, onion plants which were maintained as a control and sprayed simply with distilled sterilized water showed no symptoms of the purple blotch disease (Plate II).

Inoculated Control

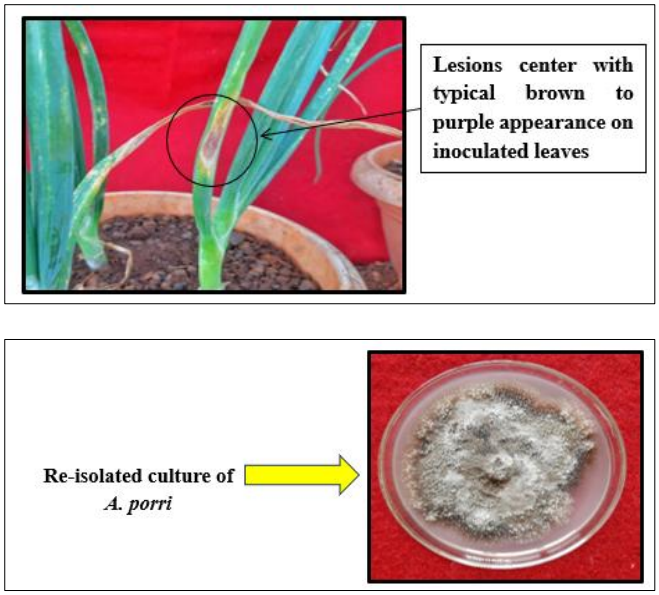


Plate II: Pathogenicity of *Alternaria porri* on onion Cv. Alibag Local

A similar fungus was re-isolated from artificially inoculated onion plants which produced purple blotch disease symptoms. The morphological and cultural characteristics of re-isolated fungus were examined and contrasted with those of the original culture, which was isolated from the tissues of naturally infected onion plants. Koch's postulates were thus proved on a susceptible cultivar of white onion (Cv. Alibag Local) which confirmed the pathogenic potential of the isolated fungus. Based on morphological and cultural characteristics, the pathogen was confirmed as *Alternaria porri*.

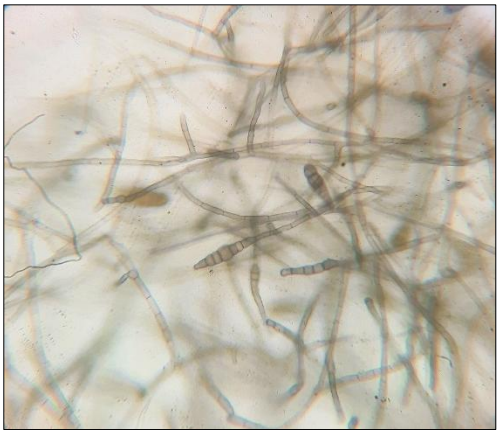
The results of current investigations are consistent with those of previous researchers, Behera and Das (2018) [9] who proved the pathogenicity of *A. porri* inciting purple blotch disease of onion by spraying spore cum mycelial suspension from a ten-day-old culture of *A. porri* on onion seedlings. Bhandekar *et al.* (2019) [10] proved the pathogenicity of *A. porri* by inoculating seventeen-day-old onion seedlings. They created a fungal suspension by diluting a seven-day-old culture to a concentration of 4×10^4 spores/ml of water and used for inoculation. Shree *et al.* (2020) [29] proved the pathogenic potential of *A. porri* inciting purple blotch disease of onion by spraying the spore cum mycelial suspension of 1×10^8 spores/ml on 30 days old onion plants. Khandagale *et al*. (2022) [20] also proved the pathogenicity test of *A. porri* by spraying the spore cum mycelial suspension 1×10^8 CFU/ml on 25 days old onion plants. After 5-6 days of inoculation, the inoculated plant showed symptoms as water-soaked lesions that usually have a white center.

The findings of present study were also in close conformity with previous reports of Kim *et al*. (2022), Akter *et al*. (2022), Ishak *et al.* (2023), Abo-Zaid *et al.* (2024) and Asghar *et al*. (2025) [21, 4, 19, 2, 7].

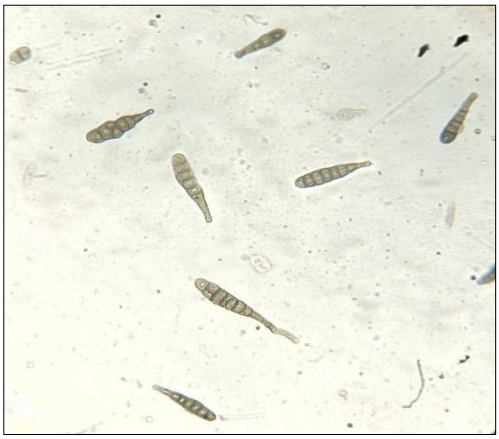
3.3. Identification of the pathogen

Identification of the causal agent inciting purple blotch disease of onion was done based on morphological characters of the isolated fungus (Plate III, IV & V). The mycelium was composed of smooth, septate, short, simple or branched, sub fasciculate hyphae and light brown to dark brown in colour. Conidiophores were emerged singly or in

groups, straight or flexible, occasionally geniculate, septate and varied in colours from light to mid-brown. The conidia of the fungus were solitary, straight or curved, obclavate or with the body of conidium ellipsoidal tapering to the beak with 4-10 transverse and zero to few longitudinal septa and with beak flexuous, pale, thick and tapering. The conidia were pale to dark brown and muriform with size ranged from 67.3 – 131.4 x 17.7 - 22.3 µ. These morphological characteristics of the purple blotch disease causing fungus were matched to the fungus description on the official website, www.mycobank.org and it was verified and identified as *Alternaria porri.* The Chief Mycologist of Agharkar Research Institute, Pune further verified the fungus and identified it as *Alternaria porri*.

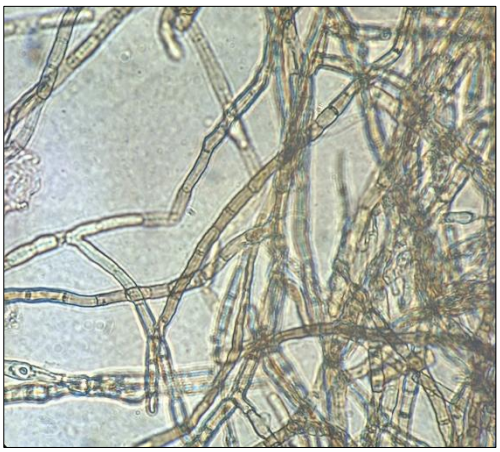


Mycelium

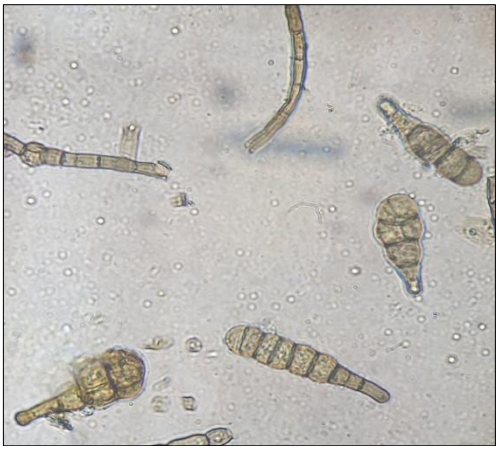


Conidia

PLATE III: Microphotographs of *Alternaria porri under* 40x

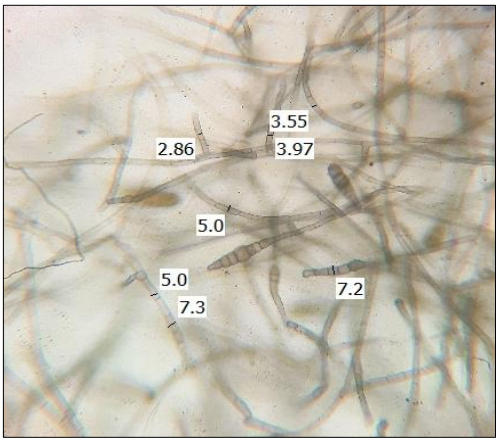


Mycelium



Conidia

Plate IV: Microphotographs of *Alternaria porri under* 100x



Mycelium

Conidia

Plate V: Micrometry of *Alternaria porri* under 40x

3.4. Morphological and cultural variability among the isolates of *Alternaria porri*

3.4.1. Cultural variability among the isolates of *Alternaria porri*

The results illustrated in Table 3.2 and Plate VI revealed that all the ten isolates of *A. porri* collected from different locations exhibited great cultural variability on PDA medium in respect of colony diameter, colony colour, growth rate, colony elevation and topography, colony margin and zonation.

3.4.1.1. Colony diameter

The results revealed that after eight days of incubation, the radial mycelial growth of the test isolates ranged from 57.50 mm (ApPe) to 90.00 mm (ApAl and ApRa). Mycelial growth was significantly highest in the isolate ApAl and ApRa (90.00 mm), followed by the isolates ApPu, ApKa, ApJa, ApDa, ApLa, ApDh and ApBa with mean colony diameter of 83.50, 82.50, 80.50, 77.00, 76.50, 75.00 and 59.50 mm, respectively. Least colony diameter of 57.50 mm was recorded in isolate ApPe.

3.4.1.2. Colony colour (Front and Rear)

The test isolates of *A. porri* grown on PDA medium exhibited a wide range of front and rear colony colours. The test isolates showed front colony colour variation as whitish gray (ApPu, ApJa and ApKa), greyish black (ApAl), greenish grey with white margin (ApDh), greyish white (ApPe, ApBa and ApLa), greyish white at center, surrounded by blackish grey (ApDa) and greyish white with white margin (ApRa).

The isolates showed rear colony colour variation as greyish black (ApPu, ApPe and ApBa), greyish brown (ApJa, ApAl, ApKa and ApDa), blackish grey with white margin (ApDh), blackish grey with white at center (ApLa) and greyish white (ApRa).

3.4.1.3. Colony growth rate

The results of the present study indicated that, the growth rate of ten isolates of *A. porri* on PDA medium varied from 7.15 to 11.25 mm/day. The highest growth rate of 11.25 mm/day each was recorded in isolates ApAl and ApRa. It was followed by ApPu, ApJa, ApDa, ApLa, ApKa, ApDh and ApBa with mean colony growth rate of 10.45, 10.06, 9.74, 9.56, 9.43,9.41 and 7.51 mm/day, respectively. Least colony growth rate of 7.15 mm/day was recorded in isolate ApPe.

3.4.1.4. Colony topography and elevation

From the study, it was observed that there was variation in colony growth. Five isolates namely ApPu, ApJa, ApKa, ApPe and ApBa showed rough topography while remaining i.e. ApAl, ApDh, ApDa, ApLa and ApRa showed smooth topography.

Table 3.2: Cultural variability among the isolates of *Alternaria porri*

Isolate code	Colony Diameter (mm)* on 8th day	Colony Colour		Growth Rate (mm/day)*	Topography	Elevation	Margin	Zonation
		Front	Rear					
ApPu	83.50	Whitish grey	Greyish black	10.45	Rough	Raised	Regular	Absent
ApJa	80.50	Whitish grey	Greyish brown	10.06	Rough	Raised	Irregular	Absent
ApAl	90.00	Greyish black	Greyish brown	11.25	Smooth	Flat	Regular	Present
ApKa	82.50	Whitish grey	Greyish brown	9.43	Rough	Slightly raised	Regular	Absent
ApDh	75.00	Greenish grey with white margin	Blackish grey with white margin	9.41	Smooth	Flat	Regular	Present
ApPe	57.50	Greyish white	Greyish black	7.15	Rough	Slightly raised	Irregular	Absent
ApDa	77.00	Greyish white at center, surrounded by blackish grey	Greyish brown	9.74	Smooth	Sparse with fluffy at center	Regular	Present
ApBa	59.50	Greyish white	Greyish black	7.51	Rough	Slightly raised	Irregular	Absent
ApLa	76.50	Greyish white	Blackish grey with white at center	9.56	Smooth	Raised	Regular	Present
ApRa	90.00	Greyish white with white margin	Greyish white	11.25	Smooth	Flat	Regular	Present

*Mean of three replications

The test isolates of *A. porri* grown on PDA medium exhibited a wide range variation in colony elevation. The test isolates showed variation in colony elevation as raised (ApPu, ApJa and ApLa), flat (ApAl, ApDh and ApRa), slightly raised (ApKa, ApPe and ApBa) and sparse with fluffy at center (ApDa).

3.4.1.5. Colony margin and zonation

Seven isolates of *A. porri* showed colony margin as regular that were ApPu, ApAl, ApKa, ApDh, ApDa, ApLa and ApLa and three isolates namely ApJa, ApPe and ApBa showed irregular colony margin.

Concentric zonation's were present in five isolates *viz*., ApAl, ApDh, ApDa, ApLa and ApRa) and in five isolates namely ApPu, ApJa, ApKa, ApPe and ApBa concentric zonation was absent.

3.4.2. Morphological variability among the isolates of *Alternaria porri*

The result presented in Table 3.3 and Plate VIIa to VIId indicated that all ten test isolates of *Alternaria porri* exhibited a significant degree of variation concerning hyphal width, conidial shape, conidial size, beak length and number of transverse and longitudinal septa.

3.4.2.1. Conidia shape

The results showed that the conidia shapes of the test isolates were obclavate to obpyriform (ApPu, ApJa, ApAl, ApPe, ApDa, ApBa, ApDh, ApLa and ApRa) or obclavate to ellipsoidal (ApKa).

3.4.2.2. Hyphal width

Data revealed that the average hyphal width among the isolates ranged from 3.51 to 6.30 µ. The highest hyphal widths were observed in the isolates ApPe (6.30 µ), ApLa (5.49 µ), ApRa (5.10 µ) and ApJa (5.08 µ); those that were medium-sized included ApBa (4.47 µ), ApDa (4.20 µ), ApPu (4.07 µ) and ApAl (4.05 µ), while isolates ApKa and ApDh with smallest hyphal width of 3.68 and 3.51 µ, respectively.

3.4.2.3. Conidia Size

From above results it was observed that the largest conidia based on size was observed in the isolate ApDh (131.40 x 22.30 µ) followed by ApBa (120.50 x 20.34 µ), ApRa (118.50 x 21.10 µ), ApLa (111.66 x 19.06 µ), ApJa (103.61 x 18.50 µ), ApPu (98.33 x 20.18 µ), ApAl (87.75 x 18.80 µ) and ApPe (83.16 x 17.98 µ). In comparison, the conidia size was notably smaller in the isolates namely ApDa (76.60 x 19.50 µ) and ApKa (67.30 x 17.70 µ).

3.4.2.4. Beak length

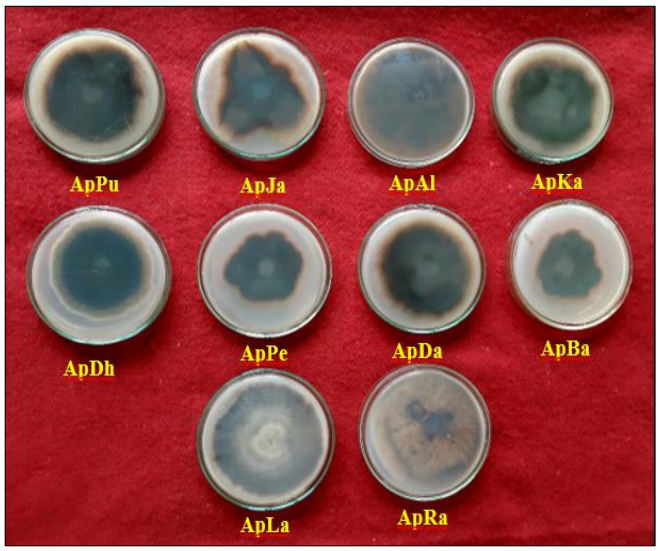
The conidia of test isolates had an average beak length ranged from 17.12 to 40.40 µ. The isolate ApBa had the highest beak length (40.40 µ) and was followed by ApDh (36.72 µ), ApLa (36.44 µ), ApRa (34.04 µ) and ApPu (26.70 µ). The isolates ApJa (25.98 µ), ApAl (21.85 µ) and ApDa (19.32 µ) had medium beak length. Isolates with shorter average beak lengths were ApPe (17.81 µ) and ApKa (17.12 µ).

Table 3.3: Morphological variability among the isolates of *Alternaria porri*

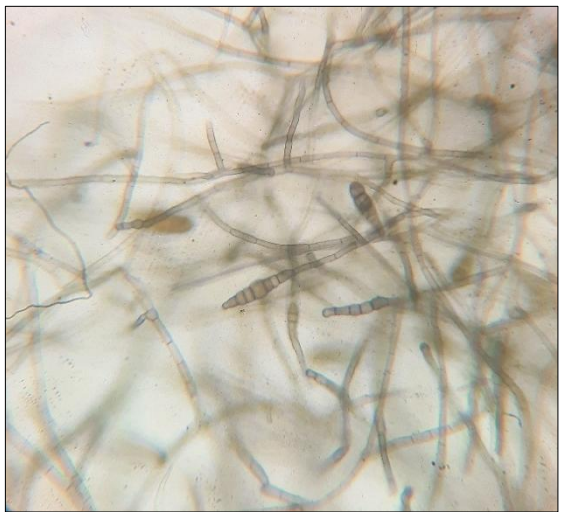
Sr. No.	Isolate code	Average Hyphal Width (µ)	Shape of Conidia	Size of Conidia (µ) L × B	Average Beak Length (µ)	Average No. of Septa	
						T	L
1	ApPu	4.07	Obclavate to obpyriform	98.33 x 20.18	26.70	4-9	0-3
2	ApJa	5.08	Obclavate to obpyriform	103.61 x 18.50	25.98	3-9	0-2
3	ApAl	4.05	Obclavate to obpyriform	87.75 x 18.80	21.85	3-8	0-1
4	ApKa	3.68	Obclavate to ellipsoidal	67.30 x 17.70	17.12	3-6	0-1
5	ApDh	3.51	Obclavate to obpyriform	131.4 x 22.30	36.72	4-8	0-3
6	ApPe	6.30	Obclavate to obpyriform	83.16 x 17.98	17.81	3-7	0-2
7	ApDa	4.20	Obclavate to obpyriform	76.60 x 19.50	19.32	4-7	0-1
8	ApBa	4.47	Obclavate to obpyriform	120.50 x 20.34	40.40	3-8	1-3
9	ApLa	5.49	Obclavate to obpyriform	111.66 x 19.02	36.44	3-6	0-2
10	ApRa	5.10	Obclavate to obpyriform	118.5 x 21.10	34.04	3-7	0-2

L × B = Length × Breadth T= Transverse, L= Longitudinal

Front View

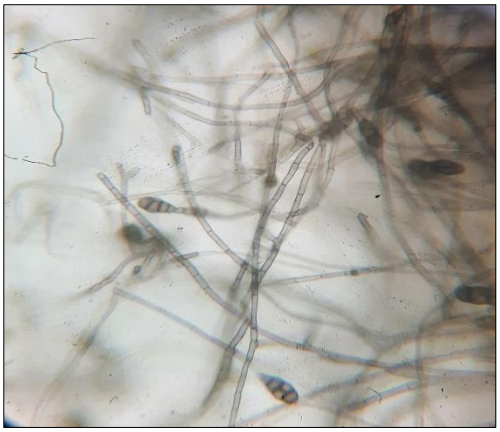


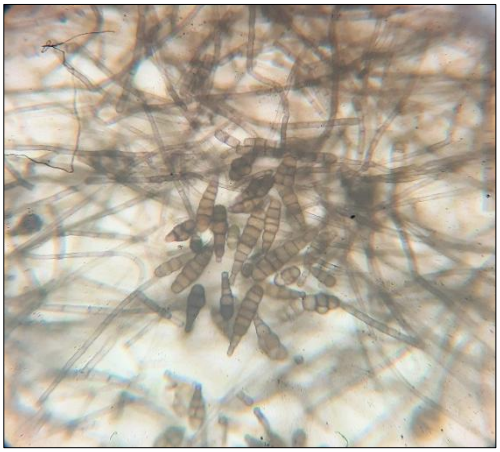
Rear View



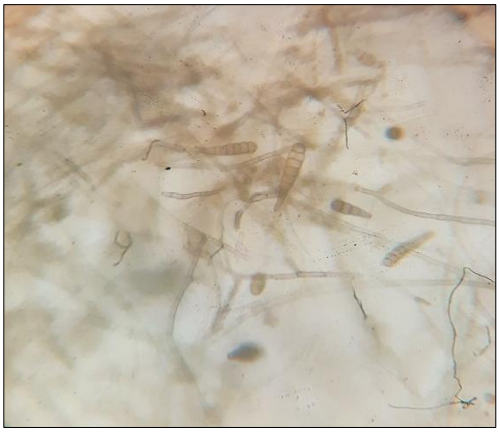
ApPu

Plate VI: Cultural variability among the isolates of *Alternaria porri*

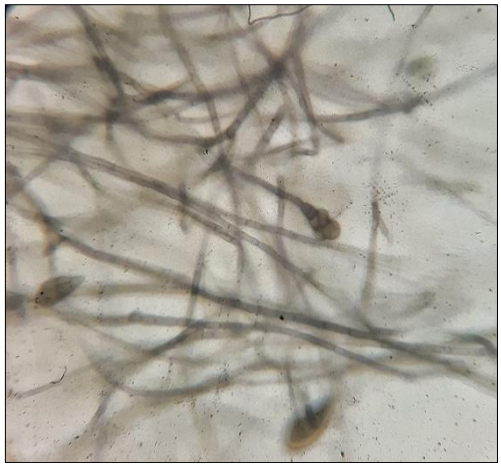


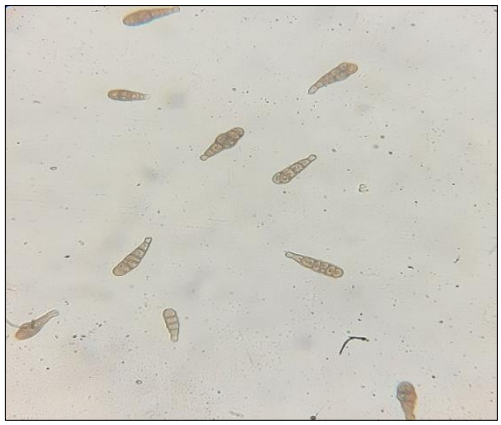


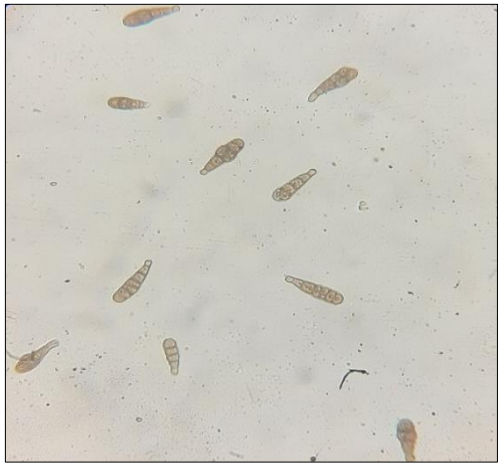
ApJa



ApAl







ApKa

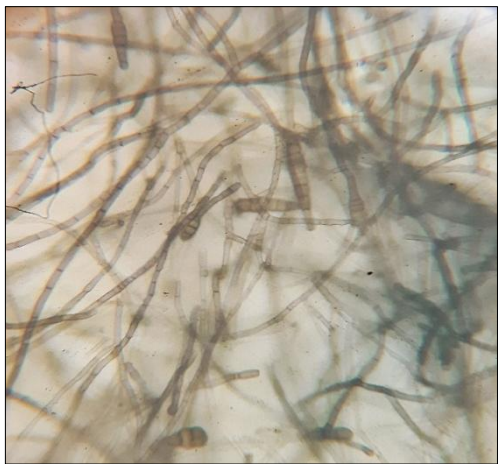
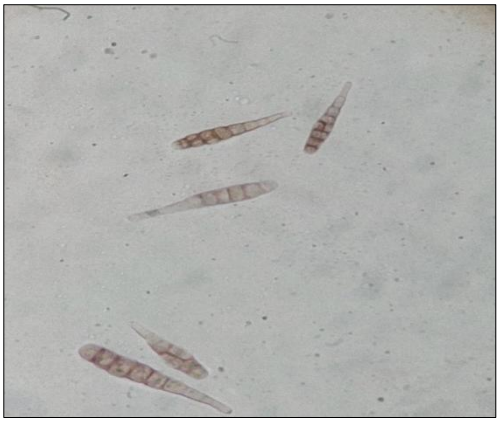
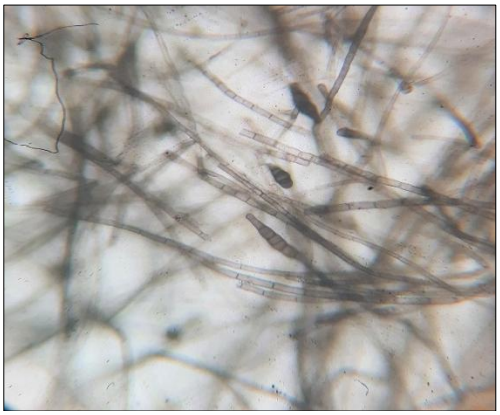
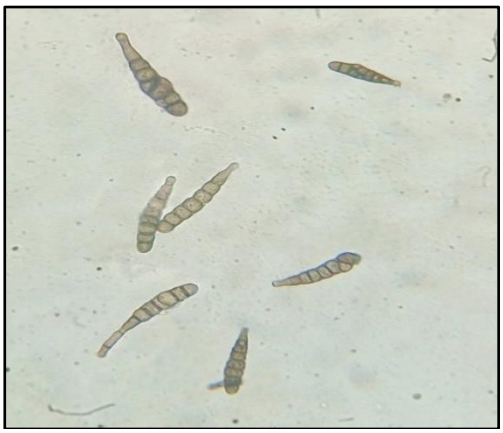


Plate VII (a): Morphological variability among the isolates of *Alternaria porri*



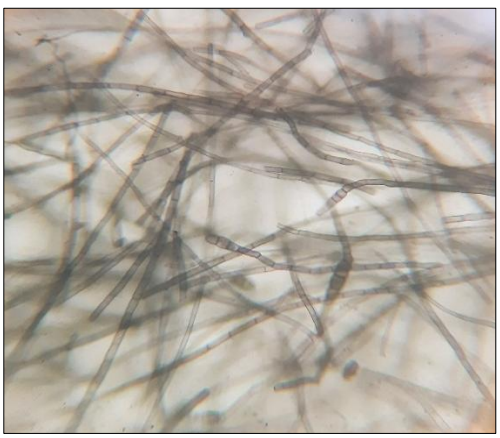
ApDh

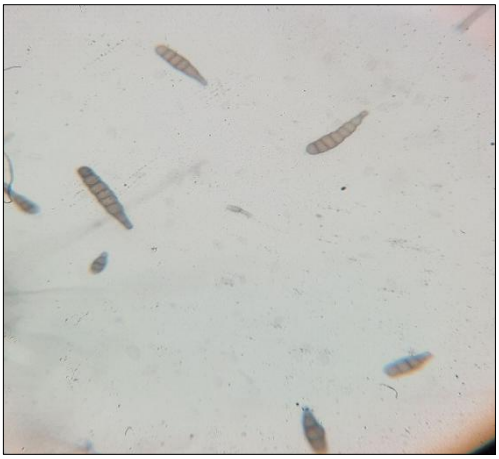




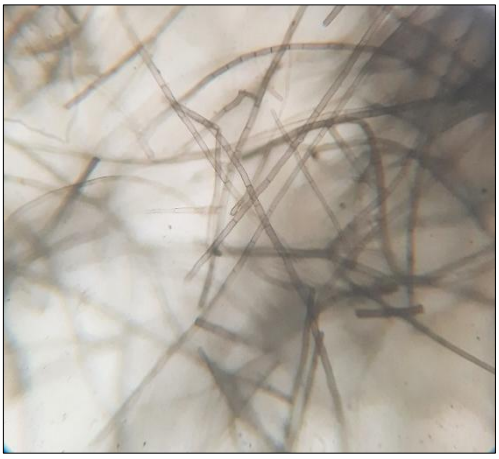
ApPe

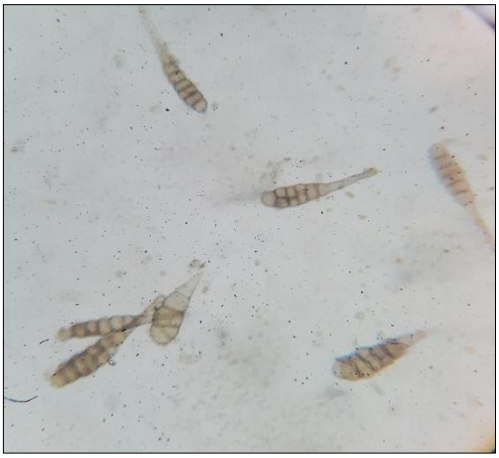
Plate VII (b): Morphological variability among the isolates of *Alternaria porri*



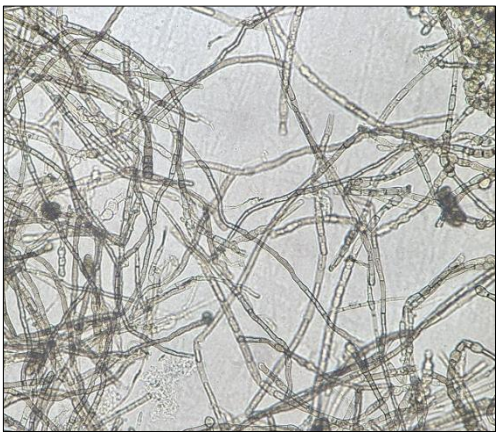


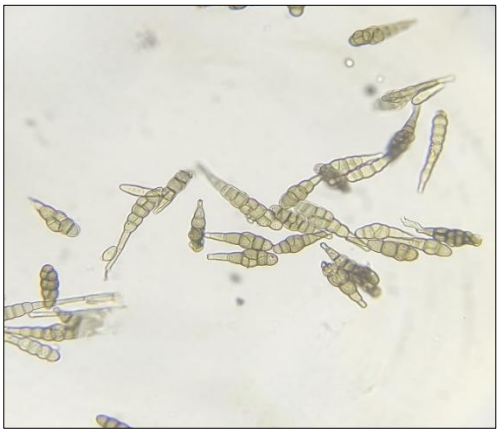
ApDa





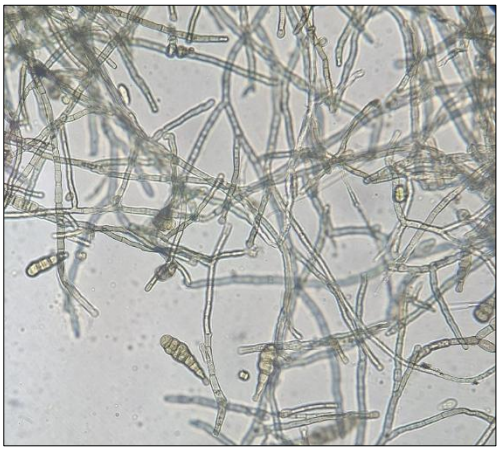
ApBa



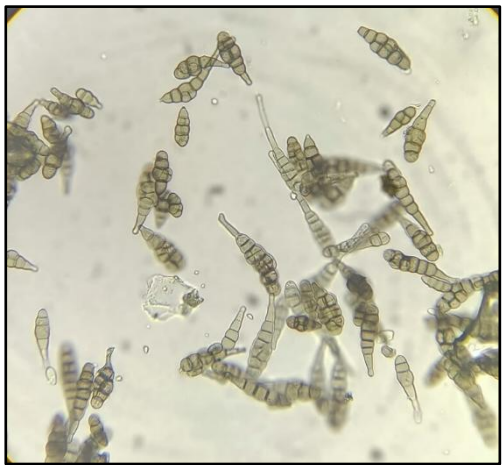


ApLa

Plate VII (c): Morphological variability among the isolates of *Alternaria porri*



Mycelium



Conidia ApRa

Plate VII (d): Morphological variability among the isolates of *Alternaria porri*

3.4.2.5. Septation

The present study revealed that there was variation amongst the isolates with respect to number of transverse and longitudinal septation. The number of transverse septa was ranged between 3-9 and that of longitudinal septa ranged between 0-3.

Variations in cultural characteristics observed in this investigation are in closely consonance with those reported by earlier researchers, Dongre and Borse (2015) [14] observed that *A. porri* colony on PDA medium were initially greyish which later converted to dark brown and black. On the backside of culture plate black colour was prominent. The young hyphae were hyaline slender and septate. Radiating hyphae were initially white in colony later it converted to brown in colour. Mohsin *et al.* (2016) [22] studied the cultural characteristics of *A. porri* and revealed that colony colour of pathogen ranged between light to dark olivaceous and greyish white with irregular and wavy margin of colonies having effuse, fluffy and velvety texture. Hasanuzzaman *et al.* (2016) [18] isolated twenty-seven isolates of *A. porri* from diseased leaf samples from different onion growing regions and observed colony colour light to dark olivaceous and greyish white with irregular or regular with concentric rings and regular without concentric rings. Margin of colonies were entire, irregular and wavy with effuse, fluffy and velvety texture. Isolates impregnated media with colour ranged between grey to brown on the reverse of the plates. Yadav *et al*. (2017) [32] also observed that the colony growth of *A. porri* appeared as creamy white after 3 days of incubation, which eventually changed to greenish grey and finally turned light olivaceous with distinct light green zonation's. The colony was found slightly fluffy with light green raised margins in uniform concentric fashion with radial diameter of 87.43 mm on 10th day of incubation period. Devi and Lal (2024) [13] studied cultural characteristic features of the *A. porri* and observed that colonies look velvety or cottony in appearance with regular to irregular margins and the colony colour from light to dark olivaceous with a greenish or brownish tinge. Asghar *et al*. (2025) [7] studied cultural characteristics of *A. porri* inciting purple blotch disease of onion and observed that the growth of the fungus appeared as circular to irregular, flat or slightly raised colonies with a white to pale gray colour. As the culture matured, it typically turned dark gray or olive-green, often exhibiting a velvety texture.

Variations in morphological characteristics observed in this investigation are in close consonance with those found by earlier researchers, Shahnaz *et al*. (2013) [28] who collected twenty-six mono-conidial isolates of *A. porri* from diseased onion leaves and recorded a beak length of 43.6-10.91 µ, 3 to 12 transverse septa and 0 to 5 longitudinal septa, the average conidial dimensions were 141.2- 81.31 × 22.9-20.14 µ. Priya *et al.* (2018) [25] also observed the size of conidia of six isolates of *A. porri* ranged from 27.13 x 5.6 µ to 101.6 x 17.2 µ with a beak length ranged from 26.60 µ to 90.20 µ. Horizontal septum was 6 to 12 and vertical septa were 1 to 3. Dar *et al*. (2020) [12] also reported that conidia of *A. porri* were normally obclavate, ellipsoid, subcylindrical with multiple transverse and longitudinal septa. The body of the conidium was oblong with its formal end protruded out and the terminal part tapered into a beak and was produced from a bud formed by the conidiophores. Singh and Tiwari (2020) [31] also studied the morphological characters of *A. porri* and found that conidiophores were straight or flexuous, sometime geniculate, septets, pale or mid brown in colour and measured up to 120 µ long and 6-10 µ thick, with one or several conidial scars. Overall length of conidia ranged from 100- 300 µ, 15 – 20 µ thick in the broadest part with 7-12 transverse and zero to several longitudinal septa, beak flexuous, pale, 2-4 µ thick and tapering. Yar *et al*. (2024) [33] studied morphological characteristics of the *A. porri* and observed that the conidia size of *A. porri* was between 100 µ and 300 µ, the diameter was 15 µ to 20 µ, the colour was brown and the conidia were club-shaped, with one tip of the

conidia enlarged and the second tip tapered. The conidia were straight or curved, with 1 to 3 longitudinal and 7 to 9 transverse septa.

The findings of present study are also in close conformity with earlier research reports of Sarnobat *et al.* (2020), Younas *et al.* (2021), Khandagale *et al.* (2022), Sindu *et al.* (2022) and Abo-Elyousr *et al*. (2024) [27, 34, 20, 30, 1].

4. Conclusion

The purple blotch disease of onion incited by *Alternaria porri*, poses a significant challenge to onion cultivation in Konkan region and rest of Maharashtra. Pathogenic ability of isolated fungus was proved on susceptible white onion cultivar Alibag Local and the pathogen was identified as *Alternaria porri*. The wide range of cultural and morphological variability exhibited by all ten isolates of *A. porri* indicated that there might be differences among the *A. porri* population.

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