

Morpho-cultural characterization of major post harvest fungal pathogens causing rot in cucumber

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Abstract

Cucumber (*Cucumis sativus* L.) is an important vegetable crop, but its post-harvest shelf life is significantly reduced by fungal pathogens causing fruit rot during storage and transit. The present study aimed to cultural and morphological characterization of major post harvest fungal pathogens associated with cucumber rotting using potato dextrose agar (PDA). Five fungal genera, namely *Aspergillus*, *Fusarium*, *Rhizopus*, *Colletotrichum*, and *Alternaria*, were identified based on cultural and morphological characteristics. Eight *Aspergillus* isolates showed black colonies with globose conidia (3.91–4.31 μm) and high sporulation. Ten *Fusarium* isolates produced micro- and macroconidia (7.1–9.2 \times 2.0–2.4 μm and 31.4–44.3 \times 3.2–4.3 μm) along with chlamydospores. Eleven *Rhizopus* isolates exhibited rapid growth with globose sporangiospores (5.0–6.9 μm) and characteristic stolons and rhizoids. Two *Colletotrichum* isolates produced cylindrical conidia (\approx 12.3 \times 4.4 μm) with acervuli and setae. One *Alternaria* isolate showed muriform conidia (37.5 \times 8.9 μm). Considerable variability in colony morphology, pigmentation, and sporulation indicated high pathogen diversity. These findings provide a basis for developing effective strategies to manage post-harvest losses in cucumber.

Keywords: Cucumber, post harvest diseases, morpho-cultural characterization, *Aspergillus*, *Fusarium*, *Rhizopus*, *Colletotrichum*, *Alternaria*

Introduction

Cucumber (*Cucumis sativus* L), a widely cultivated warm-season vegetable, belongs to the Cucurbitaceae family and has a chromosome number of 2n=14. This is one of the most popular and favourite vegetable crops in different parts of the world. The family has two subfamilies which includes 118 genera and 825 species (Judd *et al.*, 2008) [12]. Most species are climbing perennial herbs and are edible fruits (Heywood *et al.*, 2007 and Judd *et al.*, 2008) [10, 12]. The origin of cucumber is India and now this crop is grown globally.

Cucumber fruits grown in the field during transit and storage are affected by post-harvest diseases leading to spoilage of the fruits. Post harvest spoilage of fruits is mainly caused by fungi. Fungi, especially moulds are important pathogens of fruits and vegetables particularly under tropical and sub-tropical conditions (Adebayo and Diyaolu, 2003) [1]. Several pathogenic fungi such as *Alternaria tenuis*, *Alternaria alternata*, *Aspergillus* spp., *Bipolaris* spp., *Botrytis cinerea*, *Cladosporium tenuissimum*, *Cladosporium cladosporioides*, *Corynespora cassiicola*, *Choanephora cucurbitarum*, *Curvularia* sp. *Didymella bryoniae*, *Fusarium equiseti*, *Fusarium solani*, *Geotrichum candidum*, *Penicillium oxalicum*, *Phytophthora capsici* and *Rhizopus nigricans* have been reported (Steekelenburg, 1986; Blancard *et al.*, 1995; Gevens *et al.*, 2006; Naureen *et al.*, 2009) [7, 9, 18, 22] to be associated with rots of both immature and mature cucumber fruits.

It is estimated that about 20-30 per cent of all fruits and vegetables produced is lost each year due to spoilage resulting from post-harvest diseases (Kader, 2002; Agrios, 2005; Barth *et al.*, 2009) [2, 6, 13]. Economic losses due to post harvest diseases may be up to 50 per cent or even higher in developing countries (Park *et al.*, 2008) [19].

Materials and methods

1. Cultural and morphological identification of major post harvest fungal pathogens of cucumber on potato dextrose agar media

1.1 Cultural characteristics

Cultural characteristics of all those fungi associated with cucumber rotting were studied, using PDA as basal culture medium. For the purpose, autoclaved and cooled PDA medium was filled (20 ml/plate) in sterilized glass petri plates (90mm) and allowed to solidify at room temperature. On solidification of PDA, these plates were inoculated aseptically by putting in the centre a 5mm fungal culture disc separately of the test fungi, obtained from their actively growing 7 days pure culture and incubated at 27 \pm 2 $^{\circ}$ C. After a week of incubation. Observations on cultural characteristics viz., colony character such as colony type, colony colour, colony margin, colony elevation, topography and zonation and morphological characteristics study was done, by observing under research microscope.

1.2 Morphological characteristics

For studying morphological characteristics, the temporary mounts on sterile glass slide in a drop of Lactophenol cotton blue stain, of the pure culture (7 days old) of the test fungi were separately prepared, covered with cover slip and observed under research microscope. Observations on hyphae, mycelium, spores/ conidia, conidiophores and special structures. in respect of their size, shape, colour, conidial septation, etc. were recorded. Sporulation scoring was done using the grading system given by Vandana *et al.* (2022 [23]) which as follows:

Sl. No.	Score	Grade	Conidia/microscopic field (40x)
1	++++	Excellent	> 75
2	+++	Good	51-75
3	++	Moderate	26-50
4	+	Poor	1-25
5	-	No sporulation	-

Results and discussion

1. Cultural and morphological identification of major post harvest fungal pathogens of associated with cucumber rotting

Cultural and morphological identification of major post-harvest fungal pathogens associated with cucumber rotting was carried out using potato dextrose agar media. All the isolates of five fungal pathogens, namely *Aspergillus*, *Fusarium*, *Rhizopus*, *Colletotrichum* and *Alternaria* were studied for their colony characteristics, pigmentation, growth pattern, and microscopic features.

2. Morpho-cultural characteristics of *Aspergillus* on potato dextrose agar

Eight isolates of *Aspergillus* sp. (As-1 to As-8) exhibited considerable variability in colony colour, pigmentation, type of growth, conidial morphology, conidial size, and sporulation intensity. The colony colour of the isolates ranged from black to black with white margins. Pigmentation varied from white, whitish grey, light brown to whitish brown. All the isolates exhibited powdery growth with regular margins on PDA, showing uniformity in growth pattern but variation in pigmentation intensity

among the isolates. Microscopic examination revealed that the conidia were predominantly globose in shape, while a few isolates (As-2 and As-6) produced globose to sub-globose conidia. The mean conidial size showed slight variation among the isolates, ranging from $3.91 \times 4.06 \mu\text{m}$ (As-3) to $4.31 \times 3.93 \mu\text{m}$ (As-6). Special reproductive structures such as vesicles and phialides were present in all the isolates, confirming their characteristic *Aspergillus* morphology. Sporulation intensity varied among the isolates, with most isolates exhibiting excellent sporulation (++++) on PDA. A few isolates (As-3, As-5 and As-8) showed comparatively lower but still good sporulation (+++). The observed variability in cultural and morphological traits among the *Aspergillus* isolates highlights the diversity present within the pathogen population collected for the present study (Table 1, Fig. 1). The present findings on the cultural and morphological characteristics of *Aspergillus niger* on potato dextrose agar (PDA) are in close agreement with earlier reports. Diba *et al.* (2007) [8] observed distinct variations in colony colour, texture, and growth pattern of *Aspergillus* isolates on PDA, supporting its reliability as a medium for morphological characterization.

Table 1: Morpho-cultural characteristics of *Aspergillus* isolates

Sl. No.	Isolate	Colony colour	Pigmentation	Type of growth	Conidia shape	Mean conidial size (μm)	Special Structures (Vesicles and phialides)	Sporulation
1	As- 1	Black with white margin	White	Powdery with regular margin	Globose	4.11×3.90	Present	++++
2	As- 2	Black with white margin	Whitish grey	Powdery with regular margin	Globose to Sub globose	4.21×3.98	Present	++++
3	As- 3	Black	Light brown	Powdery with regular margin	Globose	3.91×4.06	Present	+++
4	As- 4	Black with white margin	Light brown	Powdery with regular margin	Globose	4.05×4.01	Present	++++
5	As- 5	Black with white margin	Light brown	Powdery with regular margin	Globose	3.96×4.05	Present	+++
6	As- 6	Black	Light brown	Powdery with regular margin	Globose to Subglobose	4.31×3.93	Present	++++
7	As- 7	Black	Whitish brown	Powdery with regular margin	Globose	3.98×4.12	Present	++++
8	As- 8	Black	White	Powdery with regular margin	Globose	4.07×3.94	Present	+++

+ (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)

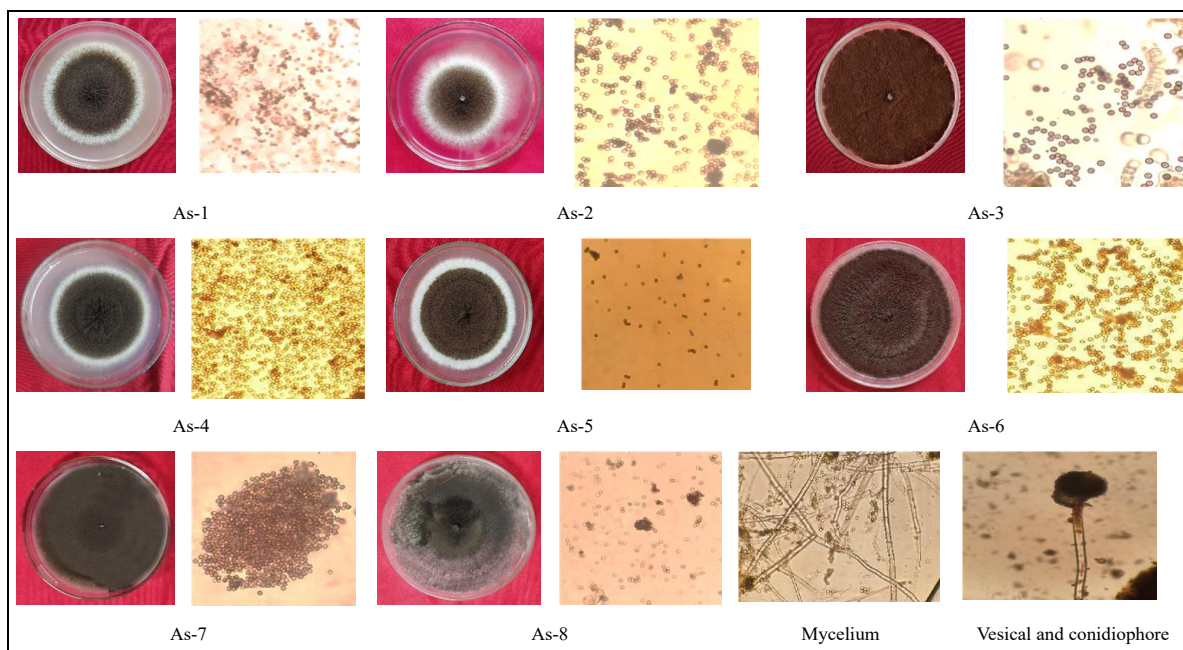


Fig 1: Cultural and Morphological characteristics of *Aspergillus* isolates

3. Morpho-cultural characteristics of *Fusarium* on potato dextrose agar

Ten isolates of *Fusarium* sp. (Fu-1 to Fu-11) exhibited considerable variability in colony colour, pigmentation, type of growth, conidial morphology, mean conidial size, and sporulation intensity. The colony colour of the isolates ranged from white to brownish white, while pigmentation varied from white to whitish grey. Most isolates exhibited fluffy growth with regular margins, whereas a few isolates (Fu-5, Fu-6 and Fu-8) showed flat growth with regular margins on PDA. Such variations in growth pattern and pigmentation clearly reflected cultural diversity among the isolates. Microscopic examination revealed that all isolates produced both microconidia and macroconidia. The microconidia were pyriform in shape, while the macroconidia were spindle to sickle-shaped. The mean microconidial size varied among the isolates, ranging from $7.1 \times 2.0 \mu\text{m}$ (Fu-6) to $9.2 \times 2.4 \mu\text{m}$ (Fu-10). Similarly, the mean macroconidial size showed considerable variation, ranging from $31.4 \times 3.2 \mu\text{m}$ (Fu-1) to $44.3 \times 4.3 \mu\text{m}$ (Fu-6), with intermediate values recorded for the remaining isolates.

The mycelium was septate and hyaline. The Chlamydospores were thick-walled, spherical to oval resting spores formed terminally or intercalarily on hyphae. All isolates exhibited good to excellent sporulation on PDA. The majority of the isolates showed excellent sporulation (++++) while a few isolates (Fu-6, Fu-8 and Fu-9) exhibited slightly reduced but still good sporulation (+++). The observed variability in cultural and morphological characteristics among the ten *Fusarium* isolates highlights the inherent diversity present within the pathogen population collected for the present investigation (Table 2, Fig. 2 and Fig. 3). The cultural characteristics of *Fusarium* spp. on potato dextrose agar (PDA) in this study were consistent with earlier reports, showing white colonies with good sporulation as noted by Jhamaria (1972), Raghuwanshi (1995), and Mandhare (1997). Identification was based on septate mycelium and the presence of both macroconidia and microconidia. The macroconidia were fusiform, sickle-shaped, and multi-septate, while microconidia were single-celled, pyriform to reniform, aligning with descriptions by Khuna (2022) and Aldakil *et al.* (2019) [4, 14].

Table 2: Morpho-cultural characteristics of *Fusarium* isolates

Sl. No.	Isolate	Colony colour	Pigmentation	Type of growth	Conidia shape		Mean conidial size (μm)		Sporulation
					Micro conidia	Macro conidia	Microconidia	Macroconidia	
1	Fu-1	White	Whitish grey	Fluffy with regular margin	Pyriform	Spindle to Sickle	7.4×2.1	31.4×3.2	++++
2	Fu-2	Brownish White	Whitish grey	Fluffy with regular margin	Pyriform	Spindle to Sickle	8.1×2.3	33.8×3.5	++++
3	Fu-3	White	White	Fluffy with regular margin	Pyriform	Spindle to Sickle	8.9×2.4	36.2×3.7	++++
4	Fu-4	White	White	Fluffy with regular margin	Pyriform	Spindle to Sickle	8.6×2.6	38.9×3.9	++++
5	Fu-5	White	White	Flat with regular margin	Pyriform	Spindle to Sickle	8.3×2.7	41.6×4.1	++++
6	Fu-6	White	Whitish grey	Flat with regular margin	Pyriform	Spindle to Sickle	7.1×2.9	44.3×4.3	+++
7	Fu-7	White	White	Fluffy with regular margin	Pyriform	Spindle to Sickle	8.8×2.0	36.8×4.1	++++
8	Fu-8	White	Whitish grey	Flat with regular margin	Pyriform	Spindle to Sickle	7.6×2.1	39.5×4.2	+++
9	Fu-9	White	White	Fluffy with regular margin	Pyriform	Spindle to Sickle	8.4×2.3	42.1×4.1	++++
10	Fu-10	White	White	Fluffy with regular margin	Pyriform	Spindle to Sickle	9.2×2.4	34.6×5.0	++++

+ (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)

4. Morpho-cultural characteristics of *Rhizopus* on potato dextrose agar

Eleven isolates of *Rhizopus* sp. (Rh-1 to Rh-11) exhibited considerable variability in Distinct differences were observed with respect to colony colour, pigmentation, type of growth, sporangiospore shape, sporangiospore size, and sporulation intensity. The colony colour among the isolates ranged from whitish grey, greenish white to white. Pigmentation varied from white to no distinct colour, with most isolates producing white pigmentation, while a few isolates (Rh-5, Rh-8 and Rh-10) showed absence of distinct pigmentation. The majority of the isolates exhibited raised and fluffy growth with irregular margins, whereas a few isolates (Rh-8 and Rh-10) showed flat growth with irregular margins on PDA. Morphologically, all isolates produced globose to sub-globose sporangiospores. The mean sporangiospore size showed variation among the isolates,

ranging from $5.0 \times 4.1 \mu\text{m}$ (Rh-3 and Rh-8) to $6.9 \times 6.2 \mu\text{m}$ (Rh-11). Intermediate sporangiospore sizes were observed in other isolates, indicating variability in spore dimensions. Sporulation intensity also varied among the isolates. Most of the isolates exhibited excellent sporulation (++++) on PDA, while moderate sporulation (+++) was observed in isolates Rh-8 and Rh-10. A distinguishing feature of this species was the presence of stolons. The columella was cylindrical and hyaline, while the rhizoids were root-shaped, ranging from hyaline to dark brown and arose abundantly from which the sporangiophores developed (Table 3, Fig. 4 and Fig. 5). Similar features have been reported by Jimeta *et al.* (2022) and Mphahlele (2011) [11, 17], who identified *R. stolonifer* as a major post-harvest soft rot pathogen of cucumber and other fruits. Sandya and Karigar (2025) [21] also described *R. stolonifer* on PDA as producing fluffy, cottony colonies with hyaline, aseptate mycelium, closely supporting the present observations.

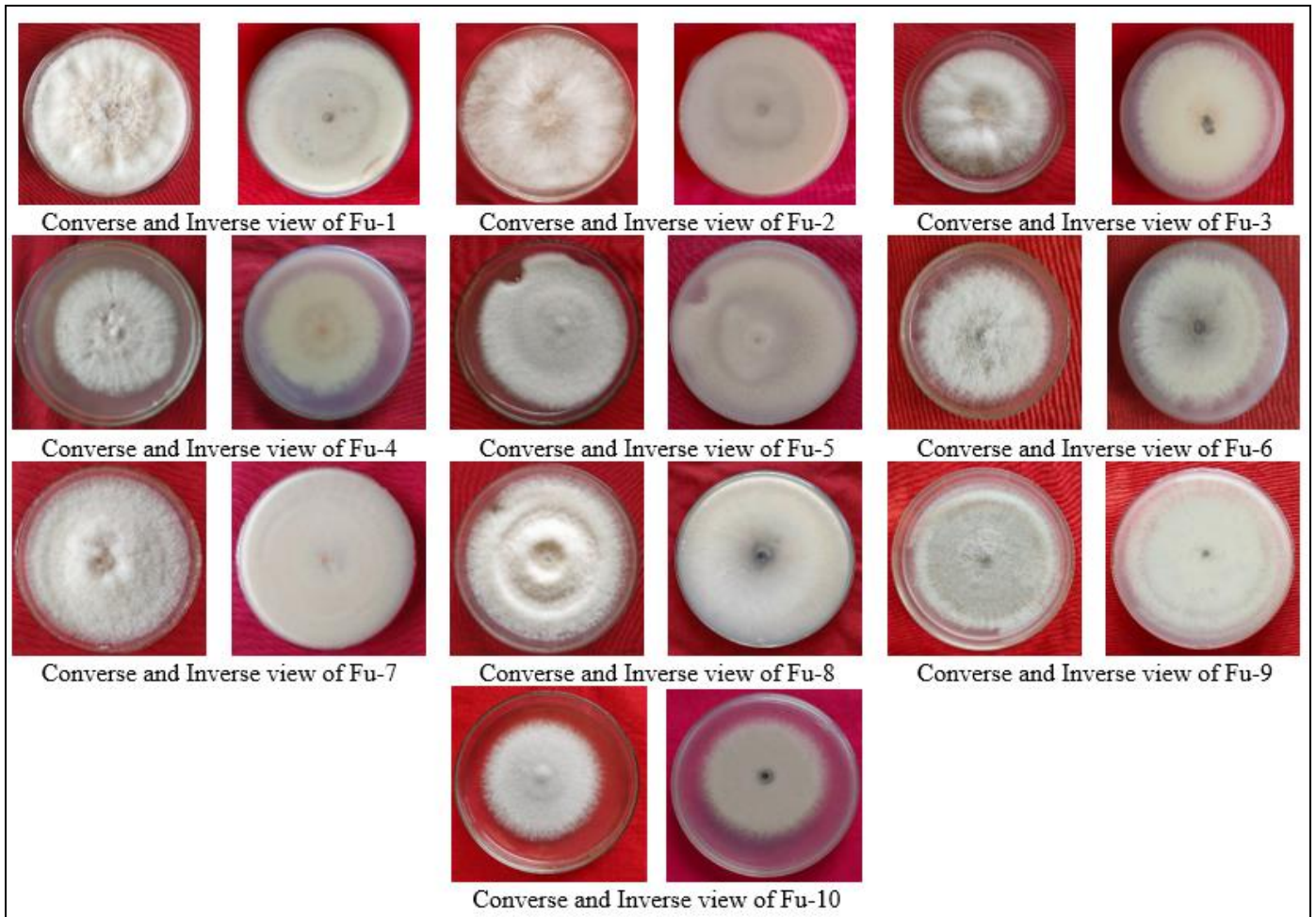


Fig 2: Cultural characteristics of *Fusarium* isolates

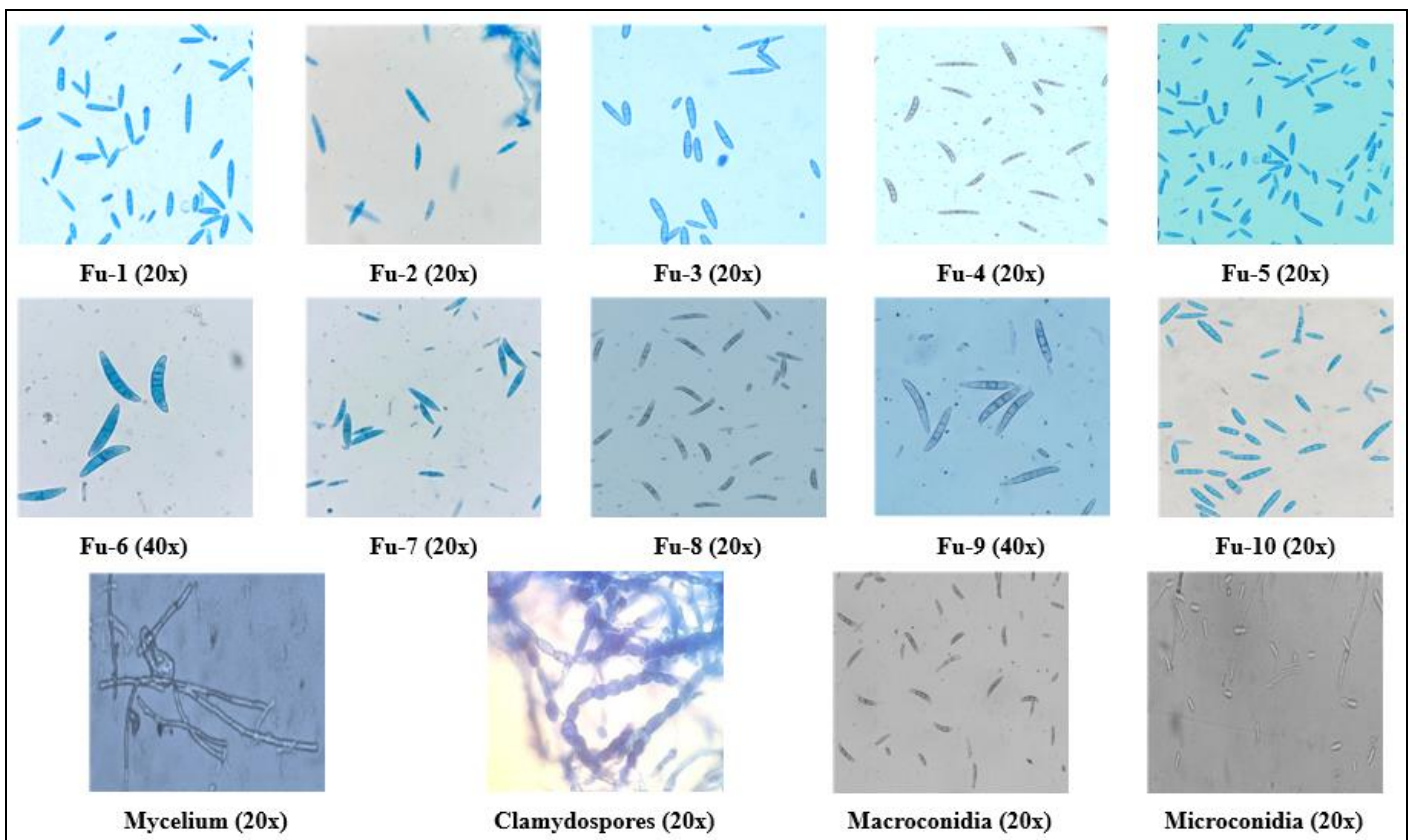


Fig 3: Morphological characteristics of *Fusarium* isolates

Table 3: Morpho-cultural characteristics of *Rhizopus* isolates

Sl. No.	Isolate	Colony colour	Pigmentation	Type of growth	Sporangiospores Shape	Mean Sporangiospore size (μm)	Sporulation
1	Rh-1	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	5.2×4.5	++++
2	Rh -2	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	5.6×4.8	++++
3	Rh -3	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	5.0×4.1	++++
4	Rh -4	Greenish white	White	Raised and Fluffy with irregular margin	Globose to sub globose	5.3×4.4	++++
5	Rh -5	Whitish grey	No distinct colour	Raised and Fluffy with irregular margin	Globose to sub globose	5.7×4.6	++++
6	Rh -6	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	6.1×4.9	++++
7	Rh -7	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	6.4×5.1	++++
8	Rh -8	White	No distinct colour	Flat with irregular margin	Globose to sub globose	5.0×4.1	+++
9	Rh -9	Greenish white	White	Raised and Fluffy with irregular margin	Globose to sub globose	6.1×5.6	++++
10	Rh -10	White	No distinct colour	Flat with irregular margin	Globose to sub globose	5.7×4.6	+++
11	Rh -11	Whitish grey	White	Raised and Fluffy with irregular margin	Globose to sub globose	6.9×6.2	++++

+ (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)

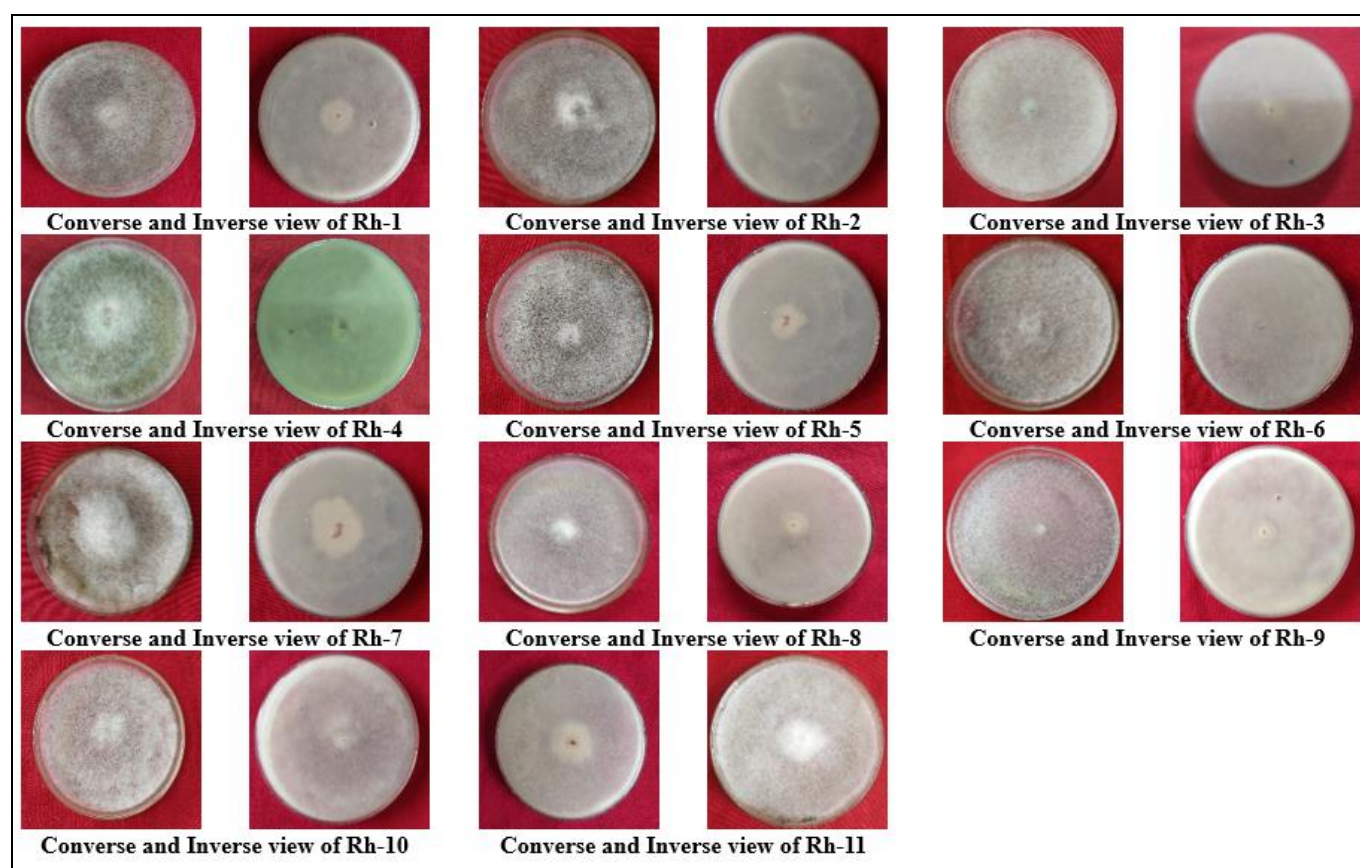


Fig 4: Cultural characteristics of *Rhizopus* isolates

5. Morpho-cultural characteristics of *Colletotrichum* on potato dextrose agar

Two isolates of *Colletotrichum* sp. (Cg-1 and Cg-2) exhibited considerable variability in colony colour, pigmentation, type of growth, conidial shape, mean conidial size, presence of special structures, and sporulation intensity. Two isolates of *Colletotrichum* sp. produced white colonies with whitish grey pigmentation on PDA. The type

of growth was flat with regular margins in both Co-1 and Co-2, indicating similarity in cultural behaviour. Microscopic examination revealed that the conidia were cylindrical in shape. The mean conidial size showed slight variation between the isolates, measuring $12.49 \times 4.50 \mu\text{m}$ in Co-1 and $12.29 \times 4.37 \mu\text{m}$ in Co-2. Special structures such as acervuli and setae were observed in both isolates, which are characteristic features of *Colletotrichum* species.

Both isolates exhibited good sporulation (+++). The overall similarity in cultural and morphological characteristics of the two isolates suggests limited variability within the *Colletotrichum* isolates examined in the present study (Table 4, Fig. 6). The present observations on *Colletotrichum* sp. cultured on potato dextrose agar (PDA) are in close agreement with earlier reports. Kumar *et al.*

(2017) [15] reported that PDA supported significantly maximum growth of *Colletotrichum* sp. isolates compared to other media, while Rite (2017) [20] recorded the highest growth rate (12.15 mm/day) of the pathogen on PDA. Morphologically, the present observations of hyaline, unicellular, cylindrical conidia correspond well with the findings of Bandgar *et al.* (2018) [5].

Table 4: Morpho-cultural characteristics of *Colletotrichum* isolates

Isolate	Colony colour	Pigmentation	Type of growth	Conidia shape	Mean conidial size (µm)	Presence of special structure	Sporulation
Co-1	White	Whitish grey	Flat with regular margin	Cylindrical	12.49×4.50	Acervuli and setae	++++
Co-2	White	Whitish grey	Flat with regular margin	Cylindrical	12.29×4.37	Acervuli and setae	++++

+ (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)

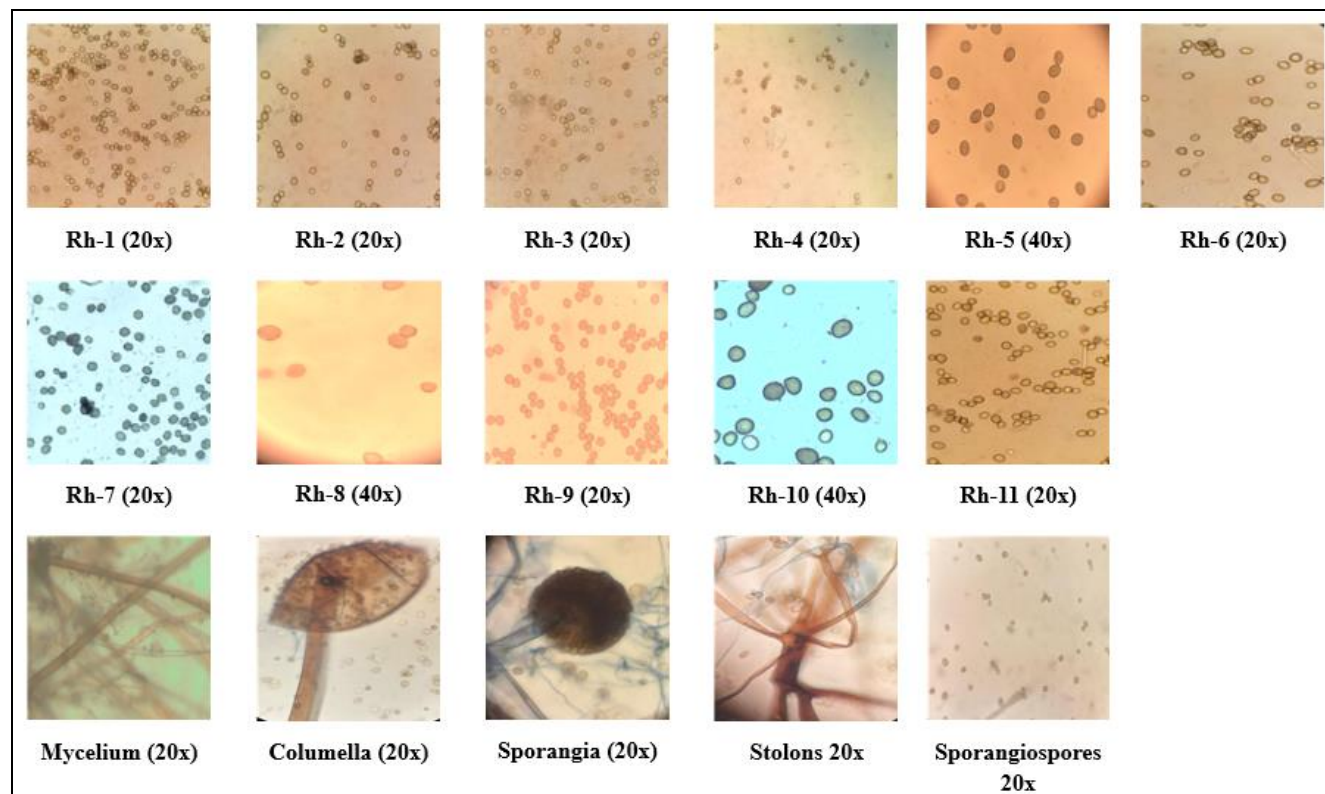


Fig 5: Morphological characteristics of *Rhizopus* isolates



Fig 6: Cultural and morphological characteristics of *Collectotrichum* isolates on potato dextrose agar

6. Morpho-cultural characteristics of *Alternaria* on potato dextrose agar

Cultural and morphological characterization of one isolate of *Alternaria* sp. (A1-1) was studied on potato dextrose agar (PDA). The isolate exhibited distinct cultural and

morphological characteristics, which were assessed based on colony colour, pigmentation, type of growth, conidial morphology, mean conidial size, presence of special structures, and sporulation intensity. The isolate produced olive grey colonies with dark black pigmentation on PDA.

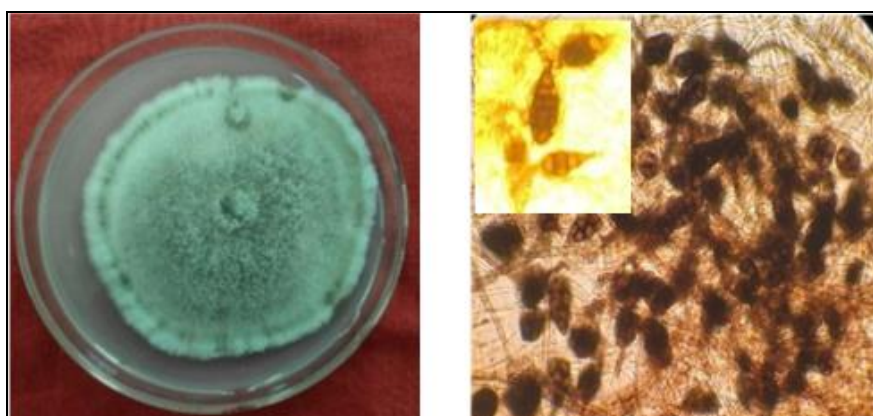
The type of growth was slightly raised and fluffy with regular margins. Microscopic examination revealed that the conidia were muriform in nature. The mean conidial size of the isolate was $37.5 \times 8.9 \mu\text{m}$. Muriform conidia, which are characteristic of *Alternaria* species, were clearly observed. The isolate exhibited good sporulation (+++). The cultural and morphological features observed in the isolate are consistent with the typical characteristics of *Alternaria* species and confirm its identity. The results indicate the presence of *Alternaria* sp. among the fungal pathogens

isolated in the present study (Table 5, Fig. 7). The *Alternaria* sp. isolate exhibited brownish colonies with septate, pigmented mycelium on potato dextrose agar (PDA). Microscopically, it produced brown, muriform conidia with both transverse and longitudinal septa, which are characteristic features of the genus. These cultural and morphological traits closely agree with the descriptions of *Alternaria alternata* reported by Ahmad (2024) and Latinovic *et al.* (2014)^[3, 16].

Table 5: Morpho-cultural characteristics of *Alternaria* isolate

Isolate	Colony colour	Pigmentation	Type of growth	Conidia shape	Mean conidial size (μm)	Presence of special structure	Sporulation
Al-1	Olive grey	Dark black	Slightly raised and fluffy with regular margin	Muriform	37.5×8.9	Muriform conidia	+++

+ (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)



Al-1

Fig 7: Cultural and morphological characteristics of *Alternaria* isolate

Conclusion

The present study identified five major fungal genera, namely *Aspergillus*, *Fusarium*, *Rhizopus*, *Colletotrichum*, and *Alternaria*, associated with post-harvest rotting of cucumber (*Cucumis sativus* L.) using PDA. A total of 32 isolates exhibited considerable variability in colony morphology, pigmentation, growth pattern, conidial characteristics, and sporulation intensity. *Aspergillus* isolates produced globose conidia ($3.91\text{--}4.31 \mu\text{m}$) with high sporulation, while *Fusarium* formed pyriform microconidia and spindle-shaped macroconidia along with chlamydospores. *Rhizopus* showed rapid growth with globose sporangiospores ($5.0\text{--}6.9 \mu\text{m}$) and characteristic rhizoids. *Colletotrichum* produced cylindrical conidia ($\sim 12.3 \times 4.4 \mu\text{m}$), whereas *Alternaria* exhibited muriform conidia ($37.5 \times 8.9 \mu\text{m}$). The observed diversity indicates the involvement of multiple pathogens in cucumber fruit rot, emphasizing the need for accurate identification and effective post-harvest management strategies.

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