

# First Report of Fungal Pathogens Causing Post-harvest Crown Rot Disease in Bananas from Coastal Districts of Odisha

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## ABSTRACT

From March 2022 to February 2023, a study was carried out in the coastal districts of Odisha state to investigate crown rot disease samples, symptoms, and morphological traits of these pathogens in three banana varieties: Champa (AAB), Deshi patakpara (AAB), and G9 (AAA). Jajpur district had the highest disease incidence from March to June (2022) (Patakpara: 96%, G9:100%, Champa: 82%), whereas Mayurbhanj district had the lowest incidence between November and February (2022–2023) (Patakpara:18%, G9:22%, Champa:12%). Incidence of disease peaked in the summer, followed by the rainy season, and was minimal in the winter. Tissue blackening, softening, and a white mycelium or orange tinge beneath the crown's surface were typical symptoms of crown rot. 4500 banana hands, 1230 crown fragments, and 474 fungal colonies were used in the investigation's year-long data collection. Using cultural and morphological methods, these colonies were isolated, characterized, and identified, with *Fusarium* and *Colletotrichum* being the most prominent genera. *Fusarium* genera comprised 79.7% of the fungi that were identified, with *Fusarium equiseti* (37.3%), *Fusarium verticillioides* (35.4%), and *Fusarium oxysporum* (27.2%) among its species. *Colletotrichum* genera, as represented by 20.2% of the sample, was identified as *Colletotrichum musae*. White mycelia with orange pigmentation, slender macroconidia, and oval to obovoid-shaped microconidia lacking chlamydospores were all characteristics of *Fusarium verticillioides*. *Fusarium equiseti* colonies developed a russet coloration and included oval, hyaline microconidia and curved, hyaline macroconidia, and 2-5 septa with conspicuous foot cells. *Fusarium oxysporum* colonies initially produced white colour which changed to violet colour and produced fusiform macroconidia, 2-4 septa, and ellipsoidal to cylindrical microconidia. Both *Fusarium equiseti* and *Fusarium oxysporum* included chlamydospores. Black, acervulus-like masses, white to orange aerial mycelium, ellipsoidal aseptate conidia, and discernible oil globules were all present in *Colletotrichum musae* cultures.

**Key words:** Banana, Crown rot, *Fusarium verticillioides*, *Fusarium equiseti*, *Fusarium oxysporum*

## Introduction

The banana (*Musa paradisiaca* L.), one of the world's most important food sources, generates income and contributes to global food security. They initially

adapted to a variety of subtropical climatic conditions from the humid tropical habitat. Bananas and plantains (*Musa* spp.) are the world's two most important fruit crops, with yearly production totaling over 148 million tonnes in 135 different countries

(FAOSTAT, 2021). Every year, 30.86 million tonnes of bananas are produced worldwide, with India accounting for approximately 21% of that total (Musapedia). Bananas are grown in Puri, Khurdha, Cuttack, Bhadrak, Anugul, Dhenkanal, and Kalahandi in the Indian state of Odisha. Odisha cultivates the Champa, Patkapura, Batisha, Dakhina Sagar, and Chini types. While ripening, bananas can be affected by several kinds of environmental factors and are susceptible to pathogenic microorganisms, which can result in a number of diseases and reduce fruit quality. Some of the postharvest diseases that affect bananas are crown rot, anthracnose, black end, cigar-end rot, and ripe rot. Banana crown rot is by far the most significant economic disease, claim Kruss *et al.* (2000). The main postharvest disease of bananas is crown rot, a complex fungal disease that significantly reduces the quality of organic fruit (Alvindhia, 2013). *Fusarium verticillioides*, *Lasiodiplodia theobromae*, *Thielaviopsis paradoxa*, and *Colletotrichum musae* are the main fungi responsible for banana crown rot disease (Alvindhia *et al.*, 2000a, 2002b). Crown rot caused by *Fusarium* spp. is regarded as the most significant postharvest disease and is in charge of the enormous loss of banana fruit all over the world (Triest and Hendrickx, 2016; Li *et al.*, 2021). The softening and blackening of the fruit tissue are the initial symptoms of crown rot, which is not apparent on the crown surface until after packaging and shipment from the producing to the consuming countries (Kamel *et al.*, 2016; Triest and Hendrickx, 2016). The entire banana fruit may become infected when crown rot begins to develop (Triest and Hendrickx, 2016).

## Materials and Methods

### Survey and Sampling

A study on banana crown rot disease was carried out in ten districts of Odisha (latitude 19.38 N to 22.00 N, longitude 85.05 E to 86.91 E) between March to June 2022 (Summer), July to October 2022 (Monsoon), and November 2022 to February 2023 (Winter). Markets, storage spaces, and farmers' storage were used to collect 4500 samples of three different banana varieties. Climate information, such as humidity and temperature, was recorded. At the Institute of Agricultural Sciences, SOADU, Bhubaneswar's Department of Plant Pathology, diseased fruits were examined for the isolation of fungi

that cause crown rot. The laboratory investigation of isolated fungus continued.

### Isolation of the associated fungus

Using agar plates and standard blotting paper techniques, fungal pathogens associated with banana crown rot were identified. The diseased fruit tissue was cut into 6 mm fragments and surface-disinfected with 0.1% mercuric chloride (HgCl<sub>2</sub>) solution after exhibiting the expected symptoms. The sterilized tissues were placed on Petri plates with Potato Dextrose Agar (PDA) medium with Streptomycin sulfate (25 milligrams per liter<sup>-1</sup>) to prevent bacterial growth after being rinsed with sterile distilled water. Following ten days of incubation at 28 ± 2 °C, fungal colonies were examined using a compound microscope. Three sheets were cut, sterilized with 70% ethanol, and dried by air for the blotting paper technique. After being wet with sterile water and dried on, they were put in a Petri dish that had already been sterilized. Fungal growth was seen under a stereomicroscope during the ten-day incubation period at 28 ± 2 °C.

### Purification and preservation of the isolated fungal isolates

Each sample's fungi were quantified and identified using an optical microscope. Using either hyphal tip or single spore methods, pathogens were isolated. Colonies with similar phenotypic traits were selected and subsequently purified on PDA. Single-spore isolation was carried out using a method similar to that of Choi *et al.* (1999) with a few modifications. Spores from sporulating mycelium were placed into a tube with 10 ml of sterile distilled water, serially diluted to 10<sup>-8</sup>, and then spread over the PDA plates which were subsequently incubated at 28 ± 2 °C overnight. In contrast, for non-sporulating mycelia, individual hyphal tips were painstakingly removed under the microscope with a sterile needle and transferred to new PDA plates. At 4 °C, pure cultures were kept on PDA slants.

### Identification of the isolated fungus based on morphological and cultural characters

On the Potato Dextrose Agar (PDA) medium, triplicates of each pathogen isolate were cultured. Colony diameter, culture color, borders, and overall Petri dish look were among the important factors evaluated. For ten days, colony diameter was observed. Following this time, the size, color, and zonation of

the conidial mass were observed. The morphology of mycelium, conidia, conidiophore, and chlamydospore were observed and measured using a digital microscope (5.1Mp IS-500 CMOS) from ESAW Optoscopes in order to isolate fungus.

## Results and Discussion

### Survey and Disease Incidence of Banana Crown Rot Disease

Samples were collected from ten districts in Odisha: Mayurbhanj, Balasore, Bhadrak, Jajpur, Kendrapara, Jagatsinghpur, Cuttack, Puri, Ganjam, and Khordha. A total of 4500 samples were collected, including three different banana types with greenish-yellow skin: Champa (AAB), Deshi patakapur (AAB), and Grand Naine (AAA). These samples revealed modest crown rot symptoms that appeared during the summer, monsoon, and winter seasons of 2022–2023. From March to June 2022, the weather was unpredictable, with temperatures ranging from 32°C to 40 °C and an average relative humidity of 72% to 76%. July to October 2022 experienced temperatures between 27°C and 34 °C with an average relative humidity of 78% to 88%. The temperature during the winter varied between 24 °C to 30 °C, with an average relative humidity of 63% to 71%. Information on the incidence of various postharvest diseases was gathered when disease symptoms showed on the surface of ripened fruits. The formula below was used to determine disease incidence: Percentage of disease incidence= (Number of infected fruits “ Total Number of fruit samples) × 100

Table 1-3 gives a thorough analysis of diseased samples collected from various district sites between 2022 and 2023. The Jajpur district had the highest percentage of disease incidence (Patakpara: 96%, G9: 100%, Champa: 82%) during the summer (March to June 2022), followed by the Bhadrak. Mayurbhanj districts had the lowest percentage of disease incidence (Patakpara: 50%, G9: 56%, Champa: 36%) followed by the Jagatsinghpur district. The monsoon season of July to October 2022 saw the highest disease prevalence in the Ganjam district (Patakpara: 78%, G9: 86%, Champa: 66%) followed suit were the Jajpur districts, whereas Mayurbhanj districts once more showed the lowest disease incidence (Patakpara: 38%, G9: 46%, Champa: 28%), closely followed by Jagatsinghpur district. Ganjam district continued to lead in the winter season (November to February 2022-23) (Patakpara: 50%, G9: 54%, Champa: 38%), Cuttack district followed closely, and the Mayurbhanj district had the lowest disease incidence (Patakpara: 18%, G9: 22%, Champa: 12%), followed by Balasore district. According to a survey conducted in Odisha over the course of three seasons, crown rot disease is mostly more prevalent in the summer, followed by the monsoon season, and least prevalent in the winter. This is in accordance with research by Lukezic *et al.* (1967) and Shillingford (1978), who observed that the incidence of crown rot disease in bananas is frequently higher during the summer (March to September) and decreases during the winter (October to February) in Honduras and Jamaica, respectively.

### Symptomatology and Fungal Isolation

Initially exhibiting fungal mycelial growth on the

**Table 1.** Percentage of disease incidence during the summer season (March to June 2022) of different banana varieties.

| Banana varieties | Patakpara (AAB)               |                           |                        | G9(AAA)                       |                           |                        | Champa (AAB)                  |                           |                        |
|------------------|-------------------------------|---------------------------|------------------------|-------------------------------|---------------------------|------------------------|-------------------------------|---------------------------|------------------------|
|                  | Total Bananas sample observed | Number of Banana infected | % of Disease incidence | Total Bananas sample observed | Number of Banana infected | % of Disease incidence | Total Bananas observed sample | Number of Banana infected | % of Disease incidence |
| Mayurbhanj       | 50                            | 25                        | 50                     | 50                            | 28                        | 56                     | 50                            | 18                        | 36                     |
| Balasore         | 50                            | 39                        | 78                     | 50                            | 44                        | 88                     | 50                            | 29                        | 58                     |
| Bhadrak          | 50                            | 46                        | 92                     | 50                            | 48                        | 96                     | 50                            | 38                        | 76                     |
| Jajpur           | 50                            | 48                        | 96                     | 50                            | 50                        | 100                    | 50                            | 41                        | 82                     |
| Kendrapara       | 50                            | 38                        | 76                     | 50                            | 44                        | 88                     | 50                            | 31                        | 62                     |
| Jagatsinghpur    | 50                            | 31                        | 62                     | 50                            | 37                        | 74                     | 50                            | 22                        | 44                     |
| Cuttack          | 50                            | 40                        | 80                     | 50                            | 47                        | 94                     | 50                            | 33                        | 66                     |
| Khordha          | 50                            | 33                        | 66                     | 50                            | 42                        | 84                     | 50                            | 29                        | 58                     |
| Puri             | 50                            | 37                        | 74                     | 50                            | 45                        | 90                     | 50                            | 35                        | 70                     |
| Ganjam           | 50                            | 42                        | 84                     | 50                            | 46                        | 92                     | 50                            | 37                        | 74                     |

crown’s outer surface, banana crown rot symptoms can spread internally and lead to fruit deterioration. Other symptoms include tissue darkening and softening along with the penetration of white mycelium or orange colour molds beneath the crown’s surface (Fig. 1). The identified fungus genera were *Fusarium* (MRR1) and *Colletotrichum* (MRR2). *Fusarium verticillioides* (MRRS1), *Fusarium equiseti* (MRRS2), and *Fusarium oxysporum* (MRRS3) all represented the *Fusarium* (MRR1) genera. *Colletotrichum* (MRR2) included one species, *Colletotrichum musae* (MRRS4), identified from banana cultivars (G9, Champa, and Patakpara) displaying crown rot symptoms in commercial markets. Based on their morphological and cultural traits, all genera were distinguished. Similar crown rot symptoms were described in a study by Lassois *et al.* (2010). *Fusarium* was determined to be

the leading genus by Kamel *et al.* (2016), accounting for nearly 55% of all detected fungi and having an 80% prevalence in the samples that were looked at. Kamel observed 285 *Fusarium* strains, encompassing nine different species, with the *F. incarnatum-equiseti* complex being the most predominant at 53%. Other noteworthy species included *F. verticillioides* (12%), *F. sacchari* (12%), *F. proliferatum* (7%), and *F. solani* (6%). In Windward Island banana farms, *F. oxysporum*, *F. verticillioides*, and *F. graminearum* are the three major pathogens in crown rot disease that Knight (1982) identified.

**Morphological and Cultural Identification of Isolated Fungus**

For each isolate, fungal colonies were seen on PDA media at 28 °C. 4,500 banana samples totaling 1,230

**Table 2.** Percentage of disease incidence during the monsoonseason (July to October 2022) of different banana varieties.

| Banana varieties<br>Districts | Patakpara (AAB)                        |                                 |                              | G9(AAA)                                |                              |                              | Champa (AAB)                           |                                 |                              |
|-------------------------------|--|---------------------------------|------------------------------|--|------------------------------|------------------------------|--|---------------------------------|------------------------------|
|                               | Total<br>Bananas<br>sample<br>observed | Number<br>of Banana<br>infected | % of<br>Disease<br>incidence | Total<br>Bananas<br>sample<br>observed | No. of<br>Banana<br>infected | % of<br>Disease<br>incidence | Total<br>Bananas<br>sample<br>observed | Number of<br>Banana<br>infected | % of<br>Disease<br>incidence |
| Mayurbhanj                    | 50                                     | 19                              | 38                           | 50                                     | 23                           | 46                           | 50                                     | 14                              | 28                           |
| Balasore                      | 50                                     | 24                              | 48                           | 50                                     | 28                           | 56                           | 50                                     | 17                              | 34                           |
| Bhadrak                       | 50                                     | 30                              | 60                           | 50                                     | 31                           | 62                           | 50                                     | 17                              | 34                           |
| Jajpur                        | 50                                     | 33                              | 66                           | 50                                     | 36                           | 72                           | 50                                     | 26                              | 52                           |
| Kendrapara                    | 50                                     | 29                              | 58                           | 50                                     | 31                           | 62                           | 50                                     | 22                              | 44                           |
| Jagatsinghpur                 | 50                                     | 20                              | 40                           | 50                                     | 25                           | 50                           | 50                                     | 16                              | 32                           |
| Cuttack                       | 50                                     | 31                              | 62                           | 50                                     | 33                           | 66                           | 50                                     | 24                              | 48                           |
| Khordha                       | 50                                     | 28                              | 56                           | 50                                     | 26                           | 52                           | 50                                     | 20                              | 40                           |
| Puri                          | 50                                     | 30                              | 60                           | 50                                     | 35                           | 70                           | 50                                     | 21                              | 42                           |
| Ganjam                        | 50                                     | 39                              | 78                           | 50                                     | 43                           | 86                           | 50                                     | 33                              | 66                           |

**Table 3.** Percentage of disease incidence during the winter season (November to February 2022-23) of different banana varieties.

| Banana varieties<br>Districts | Patakpara (AAB)                        |                                 |                              | G9(AAA)                                |                              |                              | Champa (AAB)                           |                                 |                              |
|-------------------------------|--|---------------------------------|------------------------------|--|------------------------------|------------------------------|--|---------------------------------|------------------------------|
|                               | Total<br>Bananas<br>sample<br>observed | Number<br>of Banana<br>infected | % of<br>Disease<br>incidence | Total<br>Bananas<br>sample<br>observed | No. of<br>Banana<br>infected | % of<br>Disease<br>incidence | Total<br>Bananas<br>sample<br>observed | Number of<br>Banana<br>infected | % of<br>Disease<br>incidence |
| Mayurbhanj                    | 50                                     | 9                               | 18                           | 50                                     | 11                           | 22                           | 50                                     | 6                               | 12                           |
| Balasore                      | 50                                     | 13                              | 22                           | 50                                     | 15                           | 26                           | 50                                     | 9                               | 16                           |
| Bhadrak                       | 50                                     | 18                              | 36                           | 50                                     | 20                           | 40                           | 50                                     | 14                              | 28                           |
| Jajpur                        | 50                                     | 20                              | 40                           | 50                                     | 24                           | 48                           | 50                                     | 16                              | 32                           |
| Kendrapara                    | 50                                     | 12                              | 24                           | 50                                     | 14                           | 28                           | 50                                     | 8                               | 22                           |
| Jagatsinghpur                 | 50                                     | 14                              | 28                           | 50                                     | 16                           | 32                           | 50                                     | 10                              | 20                           |
| Cuttack                       | 50                                     | 23                              | 46                           | 50                                     | 26                           | 52                           | 50                                     | 17                              | 34                           |
| Khordha                       | 50                                     | 16                              | 32                           | 50                                     | 19                           | 38                           | 50                                     | 13                              | 26                           |
| Puri                          | 50                                     | 19                              | 38                           | 50                                     | 19                           | 38                           | 50                                     | 15                              | 30                           |
| Ganjam                        | 50                                     | 25                              | 50                           | 50                                     | 27                           | 54                           | 50                                     | 19                              | 38                           |



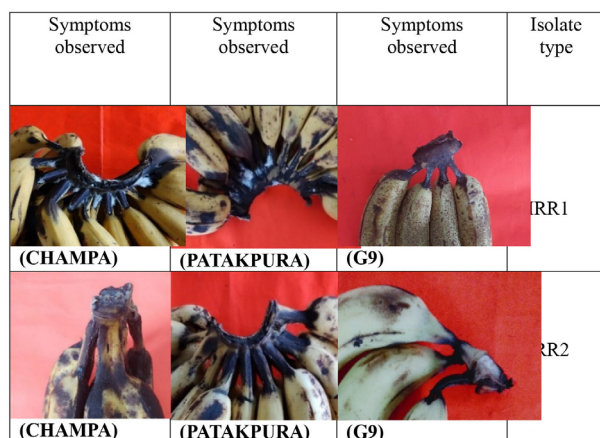


Fig. 1. Crown Rot Symptoms of Banana Varieties

crown fragments were investigated, and 474 representative fungal colonies were isolated. The 474 isolates of the fungal genus were divided into two separate morphological categories. Out of 474 pure cultures, 378 had identical morphology on PDA and produced mycelium colonies that were initially white before turning color. Conidiophores, macroconidia, microconidia, and chlamydo spores were also produced by these cultures. Based on their physical characteristics, all fungal isolates (MRR1) were first grouped within the genus *Fusarium*. Based on distinctive morphological and cultural characteristics, three types of isolates within the *Fusarium* (MRR1) genus were identified and given the names MRRS1, MRRS2, and MRRS3. In the case of MRRS1, 134 of the 378 *Fusarium* genus pure cultures exhibited the same phenotypic characteristics. These cul-

tures covered the PDA plates with cottony flats and white mycelia after 10 days of incubation. subsequently, orange pigmentation emerged, and microscopic examination revealed the presence of macroconidia, which have three septa, being thin-walled, slender, and slightly straight. The apical cells were flattened with minor notching and the basal cells measured 22.14- 45.20  $\mu\text{m} \times 4.13\text{--}6.08 \mu\text{m}$ . chlamydo spores were absent, and microconidia ranged from oval to obovoid and measured 5.45- 25.33  $\mu\text{m} \times 2.74\text{--}4.59 \mu\text{m}$ , thereby confirming their identity as *Fusarium verticillioides* (Fig. 2-3). For MRRS2, the pure cultures initially displayed white colonies that gradually developed a rusty hue near the agar base. Microscopic examination revealed smooth, branching, cylindrical, and septate mycelium. The macroconidia were curved, hyaline, 2-5 septate, and ranged in size from 20.29-38.00  $\times 3.98\text{--}6.17 \mu\text{m}$ , while the microconidia were oval, hyaline, 0-1 septate, and measured 11.11-14.82  $\times 3.28\text{--}5.53 \mu\text{m}$ . chains of spherical, hyaline chlamydo spores measuring 6.59 - 10.28  $\mu\text{m}$  in diameter were observed. *Fusarium equiseti* was found in 141 of the 378 *Fusarium* genus cultures that demonstrated these traits (Fig. 2-3). The colony that MRRS3 initially generated was white, but as time went on, the agar base began to become violet. 103 of the 378 *Fusarium* genus pure cultures shared characteristics, such as smooth, cylindrical, and septate mycelium. Microconidia were hyaline, ellipsoidal to cylindrical, straight or curved, 0-1 septate, and measuring 5.97- 14.49  $\times 3.25\text{--}4.67 \mu\text{m}$ . Macroconidia measured 30.51-39.16  $\times 3.74\text{--}5.21 \mu\text{m}$  were fusiform with

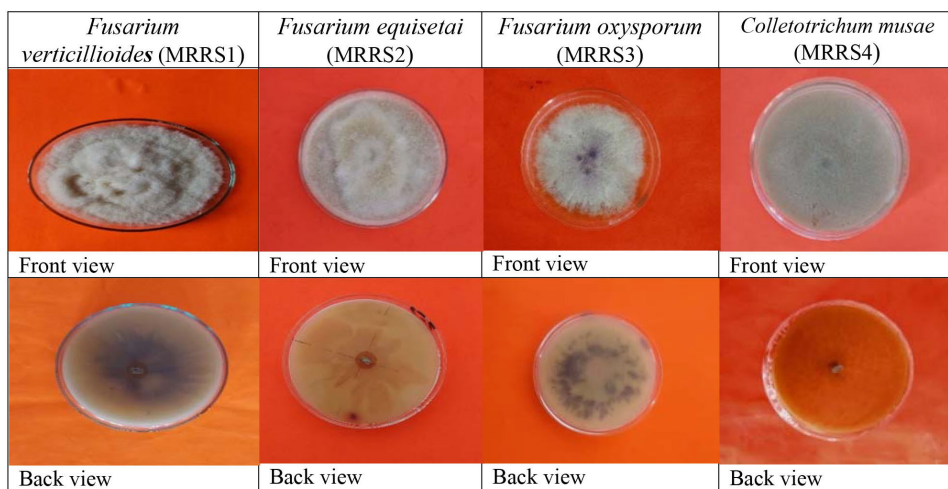





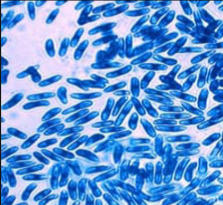
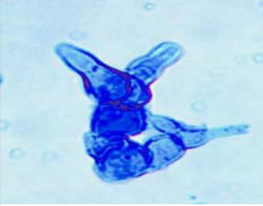
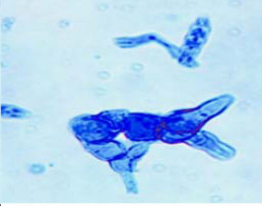
Fig. 2. Morphological characters of *Fusarium verticillioides*, *Fusarium equiseti*, *Fusarium oxysporum*, and *Colletotrichum musae* on PDA medium.

pointed extremities, pedicellate basal cells, hyaline, and 2-4 septate. Chlamydo spores were generated in the chains, spherical, hyaline, and 4.52-9.64 µm in diameter, confirming their identity as *Fusarium oxysporum* (Fig. 2-3). Out of the original 474 pure cultures, the remaining 96 pure cultures exhibited a PDA-like morphology, including white to orange aerial mycelium, black, acervulus-like masses, and dark-orange conidia drops. The hyaline, mostly ellipsoid, aseptate conidia were between 11-17×6-9 µm and contained oil globules. These morphological traits allowed these fungal isolates (MRR2) to be identified as members of the genus *Colletotrichum*, and MRRS4 isolates from *Colletotrichum* genera to be identified as *Colletotrichum musae* (Fig. 2-3). *F. verticillioides*, *Fusarium equiseti*, *Fusarium oxysporum*, and *Colletotrichum musae* all grew on PDA at room temperature (28 ± 2 °C), with average growth rates of 92-94 mm, 96-99 mm, 88-90 mm, and 85-88 mm, respectively following ten days of incubation. Subsequent studies concentrated on MRR1 (Isolate MRRS1, MRRS2, MRRS3) because of the higher prevalence of *Fusarium* genera (MRR1) compared to *Colletotrichum* genera (MRR2). According to Crous *et al.* (2021) and Wang *et al.* (2022), the mycelial colony formation of *Fusarium* species on culture plates follows a specific pattern. They begin as white colonies but eventually change color. These fungi produce various structures, including chlamydo spores, microconidia, macroconidia, and conidiophores.

*Fusarium verticillioides*, which exhibited comparable morphological and culture traits but didn't form chlamydo spores, were found in maize ear rot by Zainudin *et al.* (2017). *Fusarium equiseti* and *Fusarium oxysporum* from Kashmir were shown to have equivalent morpho-cultural characteristics in chili wilt, as reported by Hami *et al.* (2021). In our research, *Fusarium oxysporum* showed originally white colonies that turned violet at the agar base which is supported by Dubey *et al.* (2010), while Hami *et al.* observed initially white colonies that turned peach-brown at the agar base. The current findings about the cultural and physical traits of *Colletotrichum musae* are consistent with Lim *et al.* (2002) investigation.

**Conclusion**

The study, which was carried out in ten districts of Odisha, focused on the post-harvest deterioration of bananas and found a link between such issues, the prevalence of fungi, and seasonal variation. The summer and rainy seasons were when crown rot disease was most prevalent, while winter saw much less of it. *Fusarium* genus accounted for 79.7% and *Colletotrichum* genera for 20.2% of the identified fungal pathogens responsible for crown rot in coastal districts of Odisha state among three banana cultivars, respectively, in the samples examined.

| <i>Fusarium verticillioides</i> (MRRS1)   | <i>Fusarium equiseti</i> (MRRS2)  | <i>Fusarium oxysporum</i> (MRRS3)  | <i>Colletotrichum musae</i> (MRRS4)   |
|---|---|--|---|
|  |  |  |  |
| Macroconidia and Microconidia (100X)  | Macroconidia and Microconidia(100X)   | Macroconidiaand Microconidia(100X)   | Oil globule containing conidia(100X)  |
| Chlamydo spore not found  |  |  | Chlamydo spore not Produced   |
|   | Chlamydo spore (100X)   | Chlamydo spore (100X)  |   |

**Fig. 3.** Microscopic characteristics of *Fusarium verticillioides*, *Fusarium equiseti*, *Fusarium oxysporum*, and *Colletotrichum musae* observed under the microscope.

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**Conflict of Interest:** Authors do not have any conflict of interest to declare.

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