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## First report of *Diplodia seriata* de Not. causing black rot of apple (*Malus domestica* L. Borkh.) in Meghalaya, North Eastern India

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### Abstract

In a survey conducted during January-May, 2020, apple trees showing characteristic symptoms of frog-eye leaf spots, canker and twig die back that in severe cases causing dead branches and severe defoliation have been observed from apple plantation of Umiam, Meghalaya. Isolation of the associated causal fungal agent was done in selective media and its cultural, morphological as well as microscopical studies were carried out. Furthermore, confirmation of the putative fungus was done by conducting pathogenicity test to prove Koch's postulate. From the available literature, the fungus associated with the disease symptom have been identified as *Diplodia seriata* causing black rot of apple. To the best of the knowledge, this is the first report of isolation and pathogenicity confirmation of black rot of apple caused by *D. seriata* from Meghalaya, India.

**Keywords:** Apple, black rot, characterization, *Diplodia seriata*, Meghalaya

### Introduction

Apple (*Malus domestica* L. Borkh., Fam. Rosaceae) is the fourth most important horticultural crop grown in temperate parts of India having an area of 306.0 thousand ha with production of 2371.0 thousand MT and productivity 7.7 MT/ha (Bhat, 2019) [2]. The crop has been recently introduced in the Meghalaya state of India but until date, there is no record on the organized cultivation area for the crop. Therefore, literature on pests and diseases of apple for the state is rare. In search for literature on diseases of apples showed that the fruit crop is infected with wide range of diseases such as fire blight, apple scab, alternaria blotch, bitter rot, powdery mildew etc. that causes severe yield loss.

In a fixed plot survey conducted during January - May, 2020, in the apple plantation of Umiam, Meghalaya severe infestation on the leaves of growing plants showing extensive (50-100%) defoliation and dead branches (Plate 1) with severity of 30.0-35.0% have been observed. To identify the causal agent of the observed disease, we conducted a thorough *in vitro* study and proved the pathogenicity test. Further study has identified the pathogen responsible for the disease.

### Material and Methods

**Collection of sample:** Leaf samples (both mature and young leaves) showing diseased symptom of brown spots were collected in an ice box and brought to the Plant Pathology Laboratory of School of Crop Protection, CPGSAS, Central Agricultural University, Umiam, Meghalaya for further study. Collected samples were washed under running tap water to remove the dirt and impurities. Excess water from the samples were removed by soaking in sterilized blotting paper. Samples were then kept under moist chamber and continuous moisture were maintained by periodic spraying in the moist chamber with sterile distilled water. Moist chamber with samples were kept at room temperature of 25±2 °C. Observation on growth of associated causal agents were recorded at an interval of 24 hours up to 7 days of incubation.

### Isolation and pure culture of the causative agent

The diagnostic study for putative causal agent was done by growing it on Potato Dextrose Agar (PDA) media (HiMedia Laboratories Pvt. Ltd.). Collected diseased samples, including fusions of healthy and infected portions were cut into small bits (1-2 cm) and surface sterilized

with 5% sodium hypochlorite (NaOCl) solution for 30 seconds followed by rinsing thrice with sterile distilled water (SDW) for one minute in each and blot dried. Sample bits in five (5) numbers each were placed on petri plates containing PDA medium and incubated (Bio-Technics India, BTI 06) under 12:12 diurnal photoperiod at  $25 \pm 2$  °C for 5-7 days. Observation on growth of microorganism were recorded at an interval of 24 hrs.

### Purification and maintenance of culture

Purification of fungus was done by hyphal tip culture method. The pure culture was maintained by periodical sub culturing in PDA slants and storing in refrigerator (Samsung, RS20NRPS5/2009) at 4 °C throughout the period of study. Macro and micro characteristics of the organism was studied. As during the study, fungal organism was found to grow on the media so, the morphology, growth characteristics and conidial characteristics (shape, size, colour, etc.) were studied in details.

### Pathogenicity test

Pathogenicity test was also conducted for all the isolated and purified organism by detached leaf technique (Ward, 1959)<sup>[12]</sup>. Freshly collected leaves were washed under running tap water and excess water was removed by blotting in sterile blotting papers. Petioles of leaves were given a slanting cut with sterile razor blade and the cut end was wrapped with absorbent cotton enriched with 1% sugar solution. Leaf lamina was surface sterilized with 70% ethyl alcohol. Mycelial disc of 3 mm diameter were taken from the periphery of the freshly growing culture of the isolated fungus on PDA medium. Two discs per leaf were used to place on the adaxial and abaxial surface of healthy leaves. Inoculated leaves were kept under moist chamber prepared by placing two layers of sterilized blotting paper on both the part (bottom and lid) of petri dishes. The blotting papers were moistened by sterile distilled water (SDW). Humidity of 90-95% was maintained by spraying SDW during the experimentation period.

### Observation recorded

Observations of symptom development on the inoculated leaves were recorded carefully at 24 hrs interval by naked eye and magnifying glasses. Re-isolation of the associated organism was made in fresh PDA medium. Re-isolated organism was thoroughly studied for cultural, morphological and microscopical characteristics and identification was made by comparing with the available relevant literature.

## Results and Discussion

### Study on symptomatology

During the survey, we have observed similar characteristic symptom like that on the leaves. Symptom on leaves appeared as small, purplish specks that enlarged to form brown spots restricted by veins on both adaxial (Plate 3) and abaxial (Plate 4) surface of infected leaves but with more prominent symptom on the adaxial surfaces. Initially, the spots were round but later became patchy as they grow and coalesce together leading to complete browning of the leaves (Plate 4). All the spots were surrounded by irregular dark purplish to brown margin (Plate 2). Usually, spots were found to form on matured leaves having dark brown centre with concentric rings (Plate 2) that are more prominent on the adaxial surfaces. At later stage, these spots become dry and papery

giving it a “frog-eye leaf spot appearance”. In addition, we have also found cankers and dieback symptoms on shoots (Plate 4) as well as black rot on fruits.

### Cultural characteristics of putative organism

On PDA media, the growth of the fungus appear as fluffy white coloured mycelia that grows denser at centre than peripherals having smooth and regular margin (Plate 5). The fungus covers the whole petridishes (90 mm) within 120 hrs of incubation. The culture from the top showed olive green to grey buff at the centre, while the outer mycelia were fluffy white in colour (Plate 5). Brown to black coloured zonation were observed emerging from centre to the periphery at the rear side of the culture. After two weeks, entire mycelia turn greyish black in colour. During the study, it was found that the fungus on PDA plates started forming light brown (initially) zonation at 3 days of incubation that turns into greyish to dark coloured zonation after 5 days of incubation. The initial zonation forms at 1.2 cm radial distance from the centre, while the consecutive zone formation was observed at 2.20 cm radial distance (Plate 6).

### Microscopical characteristics

Study on microscopical characteristics of the fungal inoculum was done by using compound binocular microscope (Leica ICC50) and photographed digitally. Microscopical characteristics showed hyaline, septate mycelium with width of 8.50 µm and conidia were aseptate, single celled, thick walled, smooth outer surface, oblong conidia that either are round at both the ends or truncate at base (Plate 7). Conidia consisted of smooth outer surface and verrucous inner surface were filled with oil globules with size (13.4 -) 14.5 – 16.6 (- 18.0) X (5.32-) 6.28 – 7.13 9 (- 8.11) µm (n=50) i.e., normally, the length of conidia varies from 14.5-16.6 µm, it may extended up to 18.0 µm with a minimum of 13.4 µm, while, the breadth varies from 6.28-7.13 µm with maximum and minimum size of 8.11 µm and 5.32 µm respectively (Plate 8 and 9).

### Pathogenicity test

Pathogenicity tests conducted during the study by detached leaf technique (Ward, 1959)<sup>[12]</sup> in moist chamber assay showed the development of brown lesions surrounding inoculated area of the leaves three days post inoculation (dpi) (Plate 10). After 5-7 days of post inoculation symptom were observed similar to the symptom observed in field conditions. On re-isolation from the artificially inoculated leaves resulted in yielding the similar fungus as inoculated to prove Koch's postulate.

After comparing with the relevant literature for cultural, morphological and microscopical characteristics, the fungus was confirmed as *Diplodia seriata* (Alves *et al.*, 2006; Phillips *et al.*, 2007; Phillips *et al.*, 2012)<sup>[1, 5, 6]</sup>. The taxonomic classification of the fungus falls under domain: Eukarya, kingdom: Fungi, phylum: Ascomycota, Sub division: Pezizomycotina, Class: Dothidiomycetes, Order: Botryosphaerales, Family: Botryosphaeriaceae, Genus: *Diplodia* and Species: *D. seriata* de Not., while *Botryosphaeria obtusa* (Schwein) Shoemaker is the teleomorphic stage of the fungus. To the best of our knowledge, this is the first report of the isolation and pathogenicity confirmation of *Diplodia seriata* causing canker and die back of apple trees in Meghalaya, India.

Earlier *Diplodia seriata* De Not. was reported as the most

widespread disease causing pathogen of apple that can affect leaves, fruits and shoots by causing frog-eye symptom, black rot, cankers and dieback symptoms respectively (Punithalingam and Walker, 1973) [7]. The fungus was first described as *Sphaeria obtusa* by Schweinitz (1832) [8] and renamed as *Physalospora obtusa* by Cooke (1892) [3]. Later, Shoemaker (1964) [10] further renamed it as *Botryosphaeria obtusa*. The connection between teleomorph viz. *Botryosphaeria obtusa* and anamorph *Diplodia seriata* of the disease was established by Hesler (1916) that was confirmed by Shear et al. (1925) [9] and Stevens (1936) [11]. The disease can result in huge losses in apple production by causing defoliation, blighting, and twig dieback, fruit rotting before harvest and in storage as well by production of small, premature, poor quality fruits.



**Plate 1:** Severe defoliation and dead branches of apple trees



**Plate 2:** Irregular brown coloured spots with concentric rings and dark brown margin formed on apple leaves



**Plate 3:** Black rot symptom development on adaxial surface of apple leaves



**Plate 4:** Black rot symptom development on abaxial surface of apple leaves



**Plate 5:** Individual brown spots enlarge and coalesce together resulting into complete browning and shoot dieback of apple



**Plate 6:** Fluffy white coloured mycelia produced by *D. seriata* with olive green to grey buff at the centre



**Plate 7:** Brown to black coloured zonation appear at rear side of the culture





**Plate 8:** Single celled, oblong conidia with rounded base produced by *D. seriata*



**Plate 9:** Attachment of conidia with conidiogenous cells



**Plate 10:** Oblong conidia filled with oil globules



**Plate 11:** Development of brown lesions at three days post inoculation (dpi) under moist chamber assay

### Conclusion

In this present study, it was confirmed that *Diplodia seriata* is the causal agents of black rot and die back of apple in the agroclimatic condition of Meghalaya. As previous study in different places showed that the disease could cause severe defoliation, blight and die back, further studies on effective integrated management practices should be done with the involvement of least cost and less damage to the environment.

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### References

- Alves A, Correia A, Phillips AJL. Multi-gene genealogies and morphological data support *Diplodia cupressus* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Diversity*. 2006; 23:1-15
- Bhat HA. Trends and Growth in Area, Production and Productivity of Apples in India from 2001-02 to 2017-18. *Research Ambition*. 2019; 4(1):13-23.
- Cooke MC. Neglected diagnoses. *Grevillea*. 1892; 20:81-87
- Hesler LR. Black rot, leaf spot, and canker of pomaceous fruits. *Agricultural Bulletin*, Cornell University, New York, 1916.
- Phillips AJL, Crous PW, Alves A. *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusata*. *Fungal Diversity*, 2007; 25:141-155
- Phillips AJL, Lopes J, Abdollahzadeh J, Boibev S, Alves A. Resolving the *Diplodia* complex on apple and other Rosaceae hosts. *Persoonia*. 2012; 29:29-38.
- Punithalingam E, Walker JM. *Botryosphaeria obtusa*. In Sivanesan A, Holliday P (Ed). *Descriptions of pathogenic fungi and bacteria*, Commonwealth Mycological institute, Kew, Surrey, England, 1973.
- Schweinitz LD. Synopsis Fungorum in America boreali media degentium. *Transactions of the American Philosophical Society*, 1832; 4:141-316
- Shear CL, Stevens NE, Wilcox MS. *Botryosphaeria* and *Physalospora* in the Eastern United States. *Mycologia*. 1925; 17:98-107
- Shoemaker RA. Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. *Canadian Journal of Botany*, 1964; 42:1297-1301.
- Stevens NE. Two species of *Physalospora* in England. *Mycologia*. 1936; 28:330-336.
- Ward CH. The detached leaf technique for testing alfalfa clone for resistant to black stem. *Phytopathology*. 1959; 49:690-696.