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Monilinia fructicola (G. Winter) Honey as the first report of brown rot of peach, (*Prunus persica* (L) Batsch.) from Meghalaya of North East India

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Abstract
Peach, (*Prunus persica* (L) Batsch.) is one of the most important stone fruit commercially grown in the hills including Meghalaya. During Feb-June, 2020 in a roving survey we have found infection on twigs and leaves of peach at Umiam and Pepbah of Ribhoi and East Khasi Hills district of Meghalaya. The infected part showing symptoms of twig blight, blossom blight, cankers and fruit rot. Samples of young and matured leaf, blossom, twigs etc with infection were collected. We studied the detailed symptomatology, isolated and purified the associated causal organism. Through Koch's postulate we confirmed the pathogenic association of the causal agent. Further through cultural, morphological, and micrometry study the microorganism was identified as *Monilinia fructicola* (G. Winter) Honey and this is the first report from the Meghalaya of North East India.

Keywords: Brown rot, Meghalaya, *Monilinia fructicola*, peach

Introduction
India is ranked as the second largest producer of fruits in the world. Fruits production is estimated to be around 97.97 million tons in 2019-20 compared to 96.45 million tons of 2017-18. The leading fruits produced are apple, banana, citrus, mango, grapes and stone fruits (peach, nectarine, plum, apricot and cherry). Peach (*Prunus persica* (L) Batsch.) is one of the most important stone fruits commercially grown in the hills of UP, Himachal Pradesh, Kashmir. It is also an important fruit crop of Meghalaya and other North Eastern Hill (NEH) region. Because of its refreshing, health promoting, and delicious qualities, peach fruit is popular all over northern India. It is also a rich source of vitamin A, iron, proteins, sugar and minerals. The growth potential in peach cultivation is highly exciting for agro-processing, development of value-added products and employment generation through entrepreneurship development in the rural areas. From Meghalaya, occurrence of powdery mildew (*Podosphaera pannosa*) and shot hole (*Wilsonomyces carpophilus*) diseases of peach has been recorded (). But in a routine field visit we have observed severe infection of twigs, blossom, leaves and fruits of peach in an around of Ribhoi and East Khasi Hills district of Meghalaya during Feb-June, 2020. Growers reported serious losses due the same. So, we started systematic study on the disease to identify the actual causal agent/s and characterization, so that it help us to develop management strategies in due course of time.

Materials and Methods
Ethics statement
Infected leaf, blossom and twigs showing system of the infection were collected with prior approval of the growers of Ribhoi and East Khasi Hills district of Meghalaya in the month of Feb-June, 2020 in a plastic zipper bag and brought to the laboratory and stored in refrigerator at 4°C for further use.

Study on symptomatology
Collected samples were carefully observed for detail study of symptom developed on the different plant parts and compared with relevant literature.

Isolation of the Pathogen:
As symptoms were more prominent on the leaves, so, we used the leaf sample for isolation of the causal agents. From leaf sample, a small fragment from the border of healthy and diseased parenchymal tissue was aseptically excised and surface sterilized with sodium hypochlorite (5.0%) for 1 minute, followed by 70% ethanol, and then washed with double distilled sterile water. The excised sections were dried in sterilized blotting paper. Once the sections got dried, they were inoculated in petri plates (90 mm diameter) having potato dextrose agar (PDA) medium. Inoculated plates were incubated at ambient temperature (22-25 ℃) in alternate 12 hrs light and dark condition. Regular observation on initiation of any growth from the inoculated parts were observed carefully.

Purification of the isolated culture
During our study as we observed the initiation of fungal growth from the infected plant part in PDA plates, so, by hyphal tip culture method the fungus was purified in PDA plates. Purified culture was then transferred to PDA slants and stored in refrigerator (Samsung, RS20NRPS5/2009) at 4 ^{0}C for further studies.

Pathogenicity test to prove Koch's postulate
The healthy leaves of peach were selected for pathogenicity test and the test was conducted by by detached leaf technique (Ward, 1959). The healthy leaf lamina were surface sterilized with 70% ethanol to remove the unwanted dirt particles. 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.
The healthy leaves were inoculated with the freshly cultured 7 days old mycelial disc (size of 5.0 mm) of the isolated culture in both abaxial and adaxial surface of the leaves with five replications. Two discs per leaf were used to place on the adaxial and abaxial surface of healthy surface sterilized leaves. Plugs from sterile PDA agar were used as a negative control. 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. Inoculated leaves were incubated for 7 days in a sterilized glass container at room temperature.

Observation recorded
Observations of symptom development on the artificially inoculated leaves were recorded carefully at 24 hrs interval by naked eye and magnifying glasses. Lesion length were measured as regular intervals. Re-isolation of the associated organism was made in fresh PDA medium and incubated at 22-25 °C for 7 days. Re-isolated organism was thoroughly studied for cultural, morphological and microscopical characteristics and identification was made by comparing with the available relevant literature.

Study on cultural and microscopical characteristics
The organism when it was proved as the causal agent of the disease, the cultural characteristics like mycelial growth rate, colour (both front and back side), pigmentation etc. on PDA media were also recorded. Growth rates were expressed as mm of growth per day and the mean of 3 replicate colonies.
The microscopical characteristics like colour, shape, size (50 moniloid cells) etc. of inoculum were studied in details by light microscopy (Leica, ICC 50) study and photographed digitally. The observation recorded were compared with the relevant literature and identification was made as per the synoptic key of Lane (2002) [7].

Result and Discussion
Symptomatology
Symptom on leaves were dark brown to black discoloration on the leaf tip (Plate 1). Symptoms were visible prominently in the upper surface of leaves but without any symptom on the lower surface. The infected portion appeared rotten. In blossom blight, two phases of disease occurs: The first phase of brown rot blossom blight occurs early in the season when stone fruit tress start blooming. Blooms start wilting and turns brown. The wilted blossoms often remain attached to the gummy ooze. Phase two occurs right before harvest time. The ripening fruits develop tan spots which turns into gray-brown masses of fungal spores. Fruits cling to the branch or fall in the ground and become shrivelled and mummified. In case of twig blight, twig develop oozing cankers.

Pathogenicity test
Infection on leaves were observed after 4 days of inoculation. Initial symptoms on inoculated leaves was development of mycelial growth on surface of inoculated portion whereas in the opposite side development of water exudate was observed (Plate 2). Later after 2-3 days, the leaves turned into brown colour and showed discolouration. Symptoms appeared in midrib which progressed towards the upper tip portion of the leaf (Plate 3). 70% of inoculated leaves developed typical brown rot symptoms after 4 days of incubation and rest of the leaves developed symptoms within one week, while all the control un-inoculated leaf remained healthy. Minor grey sporodochia were found sparsely distributed on the leaf surface after 8-9 days of inoculation (Plate 3). Symptom produced in artificial inoculation were similar with the natural infection. Re-isolation of the fungus was done in PDA media and purified.

Study on cultural and microscopical characteristics
Cultural characteristics
In PDA media, mycelia or colony initially appeared as white (Plate 4a) then to grey in later stage with yellow tinge (Plate 4b). Produces abundant spores in concentric ring (Plate 5). Growth rate was 11 mm in first 24 hrs and 80-85 mm after 7 days of plating at temperature of 22-25 ℃. Colony when observed from reverse side of PDA plates, showed margin non lobed. Upper surface of colony "rosetted", i.e., showing mycelium in distinct layers (petals) on top of each other. Lower surface of colony showing yellowish brown of rosetted isolates (Plate 6).

Morphological characteristics
Conidia under microscope were one-celled, hyaline, lemon-shaped (Plate 7). Conidia were found to produce in moniloid chain, short cylindric to rounded and conidiophore were acropetalous branched chains (Plate 7). Conidial size were recorded as (7.22-) 12.8-20.7(-25.8) X (4.77)-9.8 – (13.0) µm. i.e., normally the length varies from 12.8-20.7 µm. It may extend upto 25.8 µm with a minimum of 7.22 µm. And its width varies from 9.8 to 13.0 µm with a minimum of 4.77 µm.

The taxonomic classification of the fungus is

After comparing with the relevant literature for the cultural, morphological and microscopical features, the fungus was confirmed as *Monilinia fructicola* (Sonoda, 1982; Corazza *et al*., 1999; Washington and Pascoe, 2000; Lane, 2002; OEPP/EPPO, 2002; Cote 2004) [12, 3, 15, 7, 4]. The taxonomic classification of fungus falls under Kingdom: Fungi, Division: Ascomycota; Class: Leotiomycetes, Order: Helotiales; Family: Sclerotiniaceae; Genus: *Monilinia*: Species: *M. fructicola*. Earlier the species was reported from North, Central and South America, Australia and New Zealand (CABI / EPPO, 1999). It was also reported that, *M. fructicola* occurs most frequently on peach and nectarine, *M. fructigena* is usually found on apple and pear, and *M. laxa* is most commonly found on apricot and almond (EPPO/CABI, 1997). In a study by Poniatowska (2013) [10] reported that there are 30 species within the genus of *Monilinia* with having great economic importance in stone fruit production. *M. fructicola*, *M. laxa* and *M. fructigena* are common pathogens in Europe, while *M. fructicola* is known mainly in some countries of Asia, North, Central and South America, as well as in Australia and New Zealand. From the earlier study, it was understood that the pathogen is an importance hindrance factor for fruit production including peach throughout the world. The present study is the first report of its occurrence in the state Meghalaya of North Eastern Region of India. This will help the researcher to formulate a management strategies of the disease.

Plate 1: Dark brown to black discoloration on the leaf tip

Plate 2: Water exudates and black discoloration of infected leaves

Plate 3: Infection on leaves showing dark brown to black discolouration during pathogenicity test (Left: adaxial surface and b. abaxial surface)

Plate 4a: Growth of *M. fructicola* in initial days on PDA medium

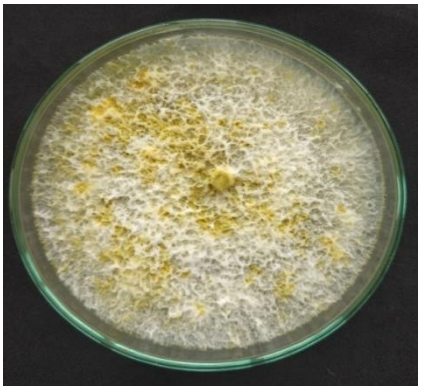

Plate 4b: Yellowish tinge on growth of *M. fructicola* on PDA

Plate 5: Front view of *M. fructicola* on PDA medium after seven days with colour change

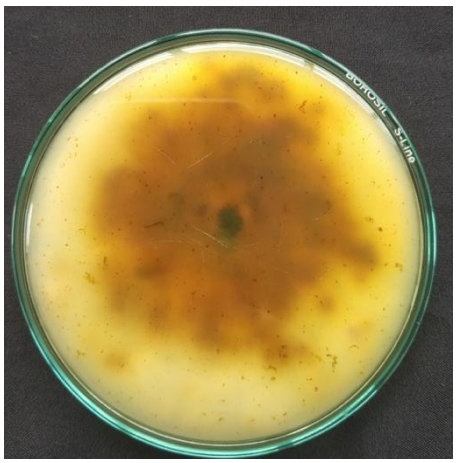

Plate 6: Reverse side of *M. fructicola* on PDA medium with change of colour and sporulation

Plate 7: Conidia of *M. fructicola* (X40) (Left: Conidia in chain, Centre: Conidiophore with conidia, Right: Lemon shaped conidia)

Conclusion

In this present study, Brown rot disease caused by *M. fructicola* was observed in peach leaf which can cause symptoms like blossom blight, twig blight, cankers, leaf shot-hole and brown rot of fruits. As this disease affects the fruit, it can cause serious economic losses in the time of harvest giving lesser yield. Therefore, as the disease is reported for the first time in Meghalaya, this report can be helpful for studying proper management practices to increase the productivity in future.

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