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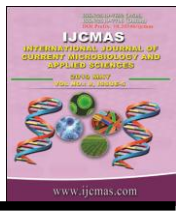

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First Record of *Colletotrichum boninense* causing Anthracnose Disease in *Piper longum* Linn. in Karnataka and *in vitro* Biological Control

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A B S T R A C T

Piper longum Linn. (Piperaceae) is a valuable medicinal herb and due to its high therapeutic composition, there is a need to protect the plant from severe diseases as they may hinder the medicinal properties. In this paper we report the Anthracnose disease of *P. longum* caused by *Colletotrichum boninense* for the first time. The morphological characteristics of the pathogen have been described and its identity was confirmed by molecular characterization. Also *Trichoderma virens* was subjected to antagonistic test against *C. boninense* in vitro and the results showed 64.28% inhibition by *T. virens* and hence *T. virens* can be recommended as a potential biocontrol agent against Anthracnose disease of *P. longum.*

Introduction

Piper longum, commonly called as Indian long pepperor Thippali is a medicinal herb, which trails either on ground or climb on trees. It is an indigenously growing plant in India and is also cultivated in the tropical and subtropical regions of Asia and Pacific islands (Tripathi *et al.*, 1999). Being a shade loving crop, can be cultivated as intercrop in coconut and areca nut gardens and even in rubber plantations (Maheswari, 2015). The dried form of spikes of this plant makes pippali, while its root is known as pippalimulam (Sivarajan and Balachandran, 1994). *P. longum* is a panacea for various ailments such as, asthma, acute and chronic bronchitis, abdominal complaints, fevers, leucoderma, urinary discharges, tumors, piles, diseases of the spleen, inflammations, leprosy, insomnia, jaundice, hiccough and tuberculous glands (Kurian and Shankar, 2007). Heavy loss in yield and quality of *P. longum* plants occur due to different diseases like leaf spot by *Botryodiplodia theobromae* [*Lasiodiplodia theobromae*] and rot caused by *Fusarium pallidoroseum* (Anupam and Jha, 2014), *Cercospora* leaf spot by *Cercospora piperata*

(Asthana and Mahmud, 1947; Rao, 1962). In recent years there is an increasing demand for controlling plant diseases in a more eco-friendly way by using biocontrol agents rather than the traditional way of using chemical pesticides. Chemical pesticides are also found to be toxic to normal micro flora of the rhizosphere (Molli et.al, 2016). Hence, the use of biocontrol agents is found to be promising in protecting the rhizosphere or spermosphere by inhibiting the pathogen (Marx, 1972) and by competing with the pathogen for limiting the growth nutrients (Chet, 1979; Couteaudier and Alabouvette, 1990). Studies were undertaken on the diseases of *P. longum*in Karnataka which lead to discover the infection of *Colletotrichum boninense* causing Anthracnose disease for the first time in it. Further *Trichoderma virens* was subjected to antagonistic test against *C. boninense in vitro* and the findings are discussed in this communication.

Materials and Methods

Field study and survey

Field survey was carried out in and around Bangalore. During periodic survey symptoms of Anthracnose disease were observed on *P. longum* plants. The severity of the disease was recorded by following 0-9 scale given by Mayee and Datar (1986). Further, the scores based on the scales were converted into Per cent Disease Index (PDI) by using formula given by Wheeler (1969).

Pathogenicity assay

The pathogenicity of purified Isolate-1 was tested and proved by Koch's Postulates (Stammler, 2013). Severe symptoms of Anthracnose disease were observed 12 to 15 days post inoculation and the disease intensity was recorded. The symptoms were observed and compared with the original symptoms. The fungi was reisolated from artificially inoculated leaves and compared with original culture isolates.

Isolation and identification of the pathogen

A large number of Anthracnose infected *P. longum* leaf samples were collected and standard tissue isolation procedure was followed to isolate associated causal pathogens (Aneja, 2003). Further, the pure cultures of the fungi were obtained by hyphal tip method. Identification until genus level was performed using identification manual (Barnet and Barry, 2003). Molecular characterization was carried out to confirm the identity of the isolate until species level. The obtained gene sequence was subjected to BLAST to find percentage identity of related species.

In vitro screening of *Trichoderma virens* against *Colletotrichum boninense* by dual culture method

T. virens cultures with GenBank accession number: MK275662.1 (Soma and Sundararaj, 2018) which were previously isolated and identified was utilized for *in vitro* experiments. Dual culture method (Skidmore and Dickinson, 1976) was followed to analyze the degree of antagonism exhibited by *T. virens* against the pathogen *C. boninense*. Triplicates of test plate and a control plate of the pathogen were maintained. Test plate with *T. virens* against *C. boninense* and control plate with the pathogen alone was noted for 5 days and tabulated. The diameter of the colony of the test organism i.e., *C. boninense* was measured and compared with that of the control plate to calculate percent inhibition. The percent inhibition of radial mycelial growth of the pathogen was calculated using the formula as given by Singh *et al.*, (2002). The degree of antagonism of *T. virens* against *C. boninense* was determined according to the classification given by Bell *et al.*, (1982).

Results and Discussion

During the survey, the leaves of *P. longum*, showed Anthracnose disease symptoms. The onset of the disease was in June with the highest per cent disease incidence of about 17.5% in September, it gradually decreased during the subsequent months and was found to be nil during April and May. Humid climate is found to be conducive for the infection of this disease. Initially, the lush green colour of healthy leaves gradually changed to pale yellow. These symptomatic leaves primarily showed brown concentric ring shaped spots, which later developed yellow halo around it. Subsequently in the later stages, the leaves abscised and dropped (Figure 1). The transverse sections of such infected leaf, under microscope showed the presence of fungal spores of the pathogen. The pathogen coded as Isolate-1was isolated from these infected leaf samples. Pathogenicity of Isolate-1was confirmed by carrying out the pathogenecity assay. The pathogen exhibited the typical morphology described for *Colletotrichum* sp. taxa, as white margins and circular, greyish and dull orange centres. The size of spores ranged from 9.0 to 12.0 × 3.0 to 5.0 µm, and the conidia were cylindrical, obtuse at both ends (Figure 2) which is similar to the report previously described by Diao *et al.*,(2013).The colonies grew rapidly at 24°C, and the average colony diameter was 51 to 52 mm after 5 days on PDA. Based upon these observations and by using the fungi identification key manual by Barnet and Barry (2003), the causal agent was identified until genus level as *Colletotrichum* sp.

Fig.1 Disease symptoms of Anthracnose on leaves of *Piper longum*

Fig.2 Microscopic view of *Colletotrichum boninense* spores

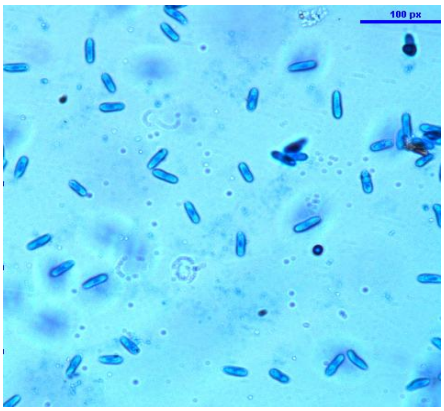


Fig.3 Dendrogram representing the similarity of the pathogen *Colletotrichum boninense* with closely related species

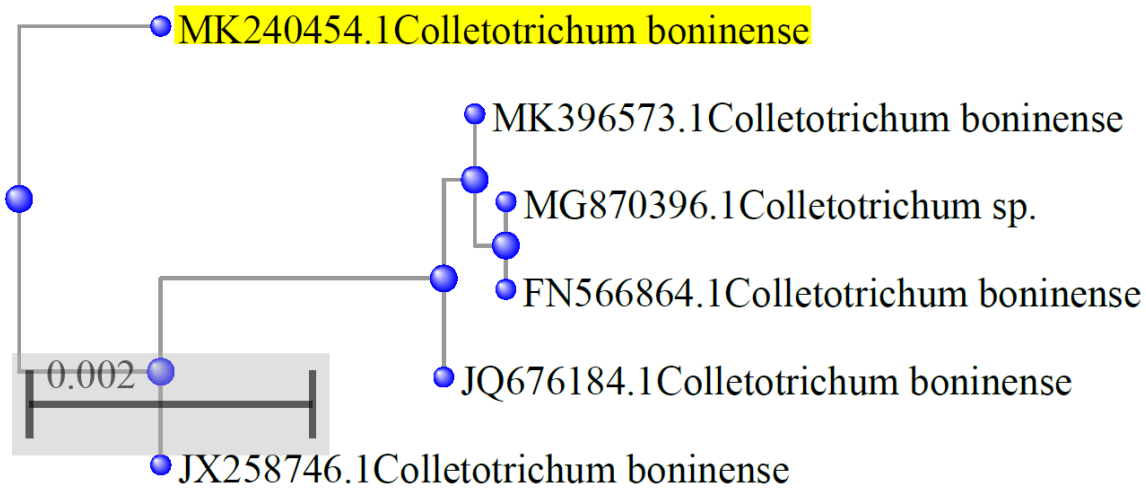
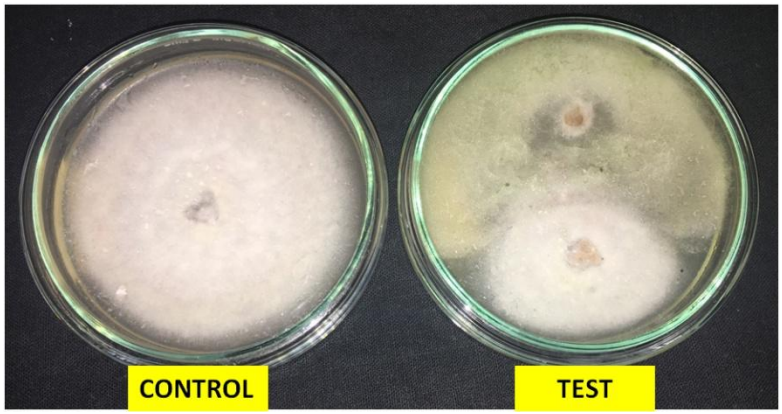


Fig.4 Dual culture of *Trichoderma virens* against *Colletotrichum boninense* in vitro conditions



Further, species level identification by sequencing ITS region and BLAST comparison of the 573-bp product showed 99.29% similarity to that of *Colletotrichum boninense* accession no. MK396573.1 (Parkand Eom, 2019), JQ676184.1 (Su *et al.*, 2012) and JX258746.1 (Cnossen *et al.*, 2012). Dendrogram was deduced for the same and is presented as in Figure 3.

Isolate-1 confirmed as *C. boninense* was deposited to GenBank with the accession number MK240454.1. The percentage of growth inhibition of the pathogen *C. boninense* by *T. virens* showed positive antagonism and percent inhibition was found to be 64.28 %. By this it was observed that *T. virens* exhibited class 2 degree of antagonism where it overgrew at least two third of the medium surface with respect to the pathogen *C. boninense* (Figure 4). Similarly, Ashoka (2005) reported 54.50% of inhibition of *C. gleosporoides* by *T. virens.* Hence, it is concluded that *T. virens* could be used as a potential biological control against Anthracnose disease of *P. longum* caused by *C. boninense.*

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