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Screening of black pepper varieties against anthracnose under nursery conditions

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Abstract

Pathogen causing anthracnose of black pepper was isolated from symptomatic leaf sample and was identified as *Colletotrichum gloeosporioides* on the basis of morphological, cultural and molecular characterization. Later, pot culture experiment was conducted in greenhouse (year 2020-21) to determine resistance/susceptibility of seven different black pepper varieties *viz.*, Arakkulamunda, Doddigya, Karimunda, Malligesara, Panniyur-1, Poonjarmunda and Uddagare, against anthracnose in nursery condition. It was observed that no variety was resistant but Karimunda variety was found to be highly tolerant against the disease. Whereas, Poonjarmunda and Panniyur-1 were classified as susceptible and highly susceptible, respectively.

Keywords: anthracnose, *Colletotrichum gloeosporioides*, *Piper nigrum*, *pollu* disease

Introduction

The diseases that most commonly cause huge losses to black pepper are anthracnose, foot rot and slow decline. Anthracnose, also known as '*pollu* disease' (caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc) is an economically important disease which prevails in field and nurseries. It chiefly affects leaves, spikes and berries thus severely affecting the productivity (Sankar & Kumari 2002). Symptoms on leaf include brownish to greyish, circular to irregular lesion surrounded by yellow halo. Later these spots coalesce and several tiny black dot like structures known as acervuli forms on the papery textured lesion (Jayakumar *et al*. 2009). Dark brown lesions on spikes progresses upwards and if stalk is infected, whole spike shrivel, dries, and shed off at early stage (Biju *et al*. 2013). The disease is seen mainly after monsoon season and spread by rain splashes. Santhakumari & Rajagopalan

(2000) reported 100% crop loss due to spike shedding and damage of black pepper berries due to this disease. Therefore, attempts were made to evaluate seven cultivars/varieties *viz*., Arakkulamunda, Doddigya, Karimunda, Malligesara, Panniyur-1, Poonjarmunda and Uddagare, for resistance/susceptibility against anthracnose.

Materials and methods

Isolation and identification pathogen

The pathogen was isolated from diseased leaves (local variety) exhibiting characteristic anthracnose symptoms on potato dextrose media (PDA) in the year 2020. Samples were collected from the orchard, Assam Agricultural University, Jorhat-13 (Assam) 26.7248° N Latitude, 94.1956°E Longitude and 86.6 M above sea level. After isolation and purification the pathogen was identified based on morphological, cultural and molecular characters. Genomic DNA was isolated using fungal DNA isolation kit and the purity and concentration was analysed using Denovix DS-11 spectrophotometer. The ITS region was amplified using universal primers (ITS1 and ITS4) and Taq DNA Polymerase 2x Master Mix RED on Gene Amp PCR System 9700. The PCR amplicon obtained was subjected to Exo-SAP purification, sequenced and subjected to BLAST analysis. The first ten sequences in the NCBI database that showed highest identity were used for analysis including phylogenetic tree construction.

Evaluation of cultivars/varieties

Pot experiment was conducted (2020-2021) at the green house, Department of Plant Pathology, AAU, Jorhat. Seven cv/var *viz*., Arakkulamunda, Doddigya, Karimunda, Malligesara, Panniyur-1, Poonjarmunda and Uddagare (five replications each) were screened for anthracnose disease resistance. Two month old cuttings raised in earthen pots were artificially inoculated with *C. gloeosporioides* by spraying spore suspension plus pin prick method. At first, wound was made using sterilized hypodermic needle (3-4 wound per leaf) followed by spraying with conidial suspension (5 x 10^5 conidia ml^{-1} water) using hand sprayer. After inoculation plants were bagged by using sterilized transparent polythene bags for 72 hours to create favourable conditions for pathogen. Empirical phenotype group was made according to the reaction of different black pepper varieties against anthracnose (Table 1). Per cent disease index (PDI) was calculated by referring disease rating scale developed by Biju *et al. (*2013). PDI was determined using the formula given by Mayee and Datar (1986), and the PDI was calculated at weekly interval.

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of grades of each leaf}}{\text{Total no. of leaves observed x maximum grade}} \times 100$$

Table 1. Empirical phenotype group in accordance to reaction of different black pepper varieties against anthracnose in the nursery

Disease reaction	Group range description
Resistant	No anthracnose symptoms (0% DI)
Highly tolerant	01-19% DI
Tolerant	20-40% DI
Moderately susceptible	40-55% DI
Susceptible	55-85% DI
Highly susceptible	More than 85% DI

Results and Discussion

Morphological and cultural characterization

The pathogen was identified as *C. gloeosporioides*, on the basis of morphological-cultural characteristics. The observations on culture were taken after 8 days of isolation. The colony was initially whitish in colour but later on it turned grey. In culture abundant mucilaginous mass of orange-pinkish colour and spores were observed (Fig. 1a). Light microscopy revealed that the mycelium of the pathogen was hyaline and septate. Isolate produced hyaline, cylindrical conidia (13.21-16.62 µm x 3.2-4.53 µm) with oil globule in the centre (Fig 1b). Growth of isolate on PDA started after 3

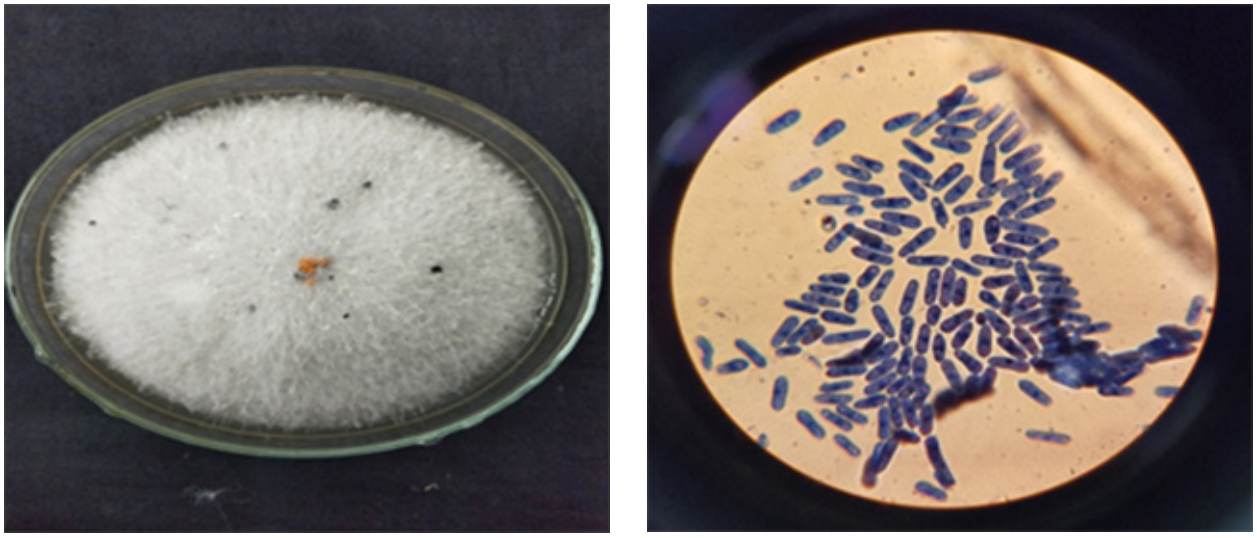

Fig. 1. (a) Culture obtained from diseased part (b) Hyaline-cylindrical spores with oil globule in centre.

days of isolation and full growth was achieved on 8th day. The cultural characteristics, spore morphology, pigmentation and pathogenicity of the isolated pathogen was same as reported by Sankar (2002) and Bandgar *et al*. (2018).

Molecular characterization

The isolate showed significant similarity with *C. gloeosporioides* based on nucleotide homology and phylogenetic analysis (Fig. 2). Mills *et al.* (1992) for the first time used DNA sequence data for distinguishing six different species of *Colletotrichum* and they identified variation in ITS1 region of nrDNA (nuclear ribosomal DNA).

Evaluation of cultivars/varieties

No variety was found to be resistant against anthracnose. Among the seven varieties, only Karimunda (2.70% DI) was classified as highly tolerant and four cultivars *viz*., Arakkulamunda, Doddigya, Malligesara,

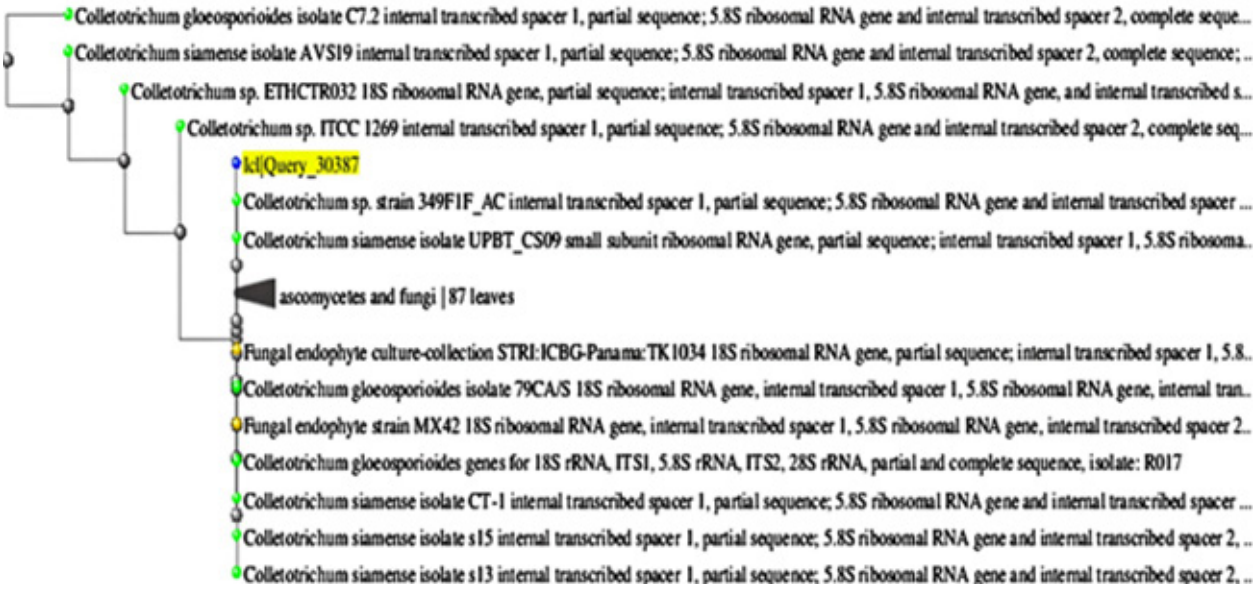

Fig. 2. Phylogenetic tree of isolated fungus with other known isolates of *Colletotrichum gloeosporioides* based on the ITS sequence.

uddagare were classified under tolerant group. High percent disease index (88.23% & 69.06%) was recorded in Panniyur-1 (highly susceptible) and Poonjarmunda (susceptible) respectively (Table 2).

Pathogen isolated from the symptomatic leaves was identified as *C. gloeosporioides* on the basis of morphological, cultural and molecular characters. When the purified pathogen was inoculated in different varieties, small minute lesion with yellow halo was formed in all varieties after 3 days of inoculation (DAI) (Fig. 3). Later, lesion started increasing in size in Arakkulamunda, Doddigya, Malligesara, Panniyur-1, Poonjarmunda and Uddagare but in Karimunda no increase in lesion was observed (Fig. 4). In Panniyur-1 the lesion covered whole leaf in 50 DAI. Lesion was papery in texture

Table 2. Black pepper varieties grouped according to the reaction against anthracnose

Variety	PDI (%)			Reaction
	2020*	2021*	Mean	
Arakkulamunda	37.1±0.61	33.9±0.03	35.5	Tolerant
Doddigya	32.7±2.02	24.62±0.57	28.66	Tolerant
Karimunda	3.4±1.67	2.01±0.10	2.70	Highly tolerant
Malligesara	40.03±1.88	34.03±0.32	36.55	Tolerant
Panniyur-1	89.64±0.26	86.82±0.21	88.23	Highly susceptible
Poonjarmunda	71.01±0.37	67.11±1.02	69.06	Susceptible
Uddagare	27.21±0.84	25.21±0.08	26.27	Tolerant

* Mean of five replications, ± Standard error

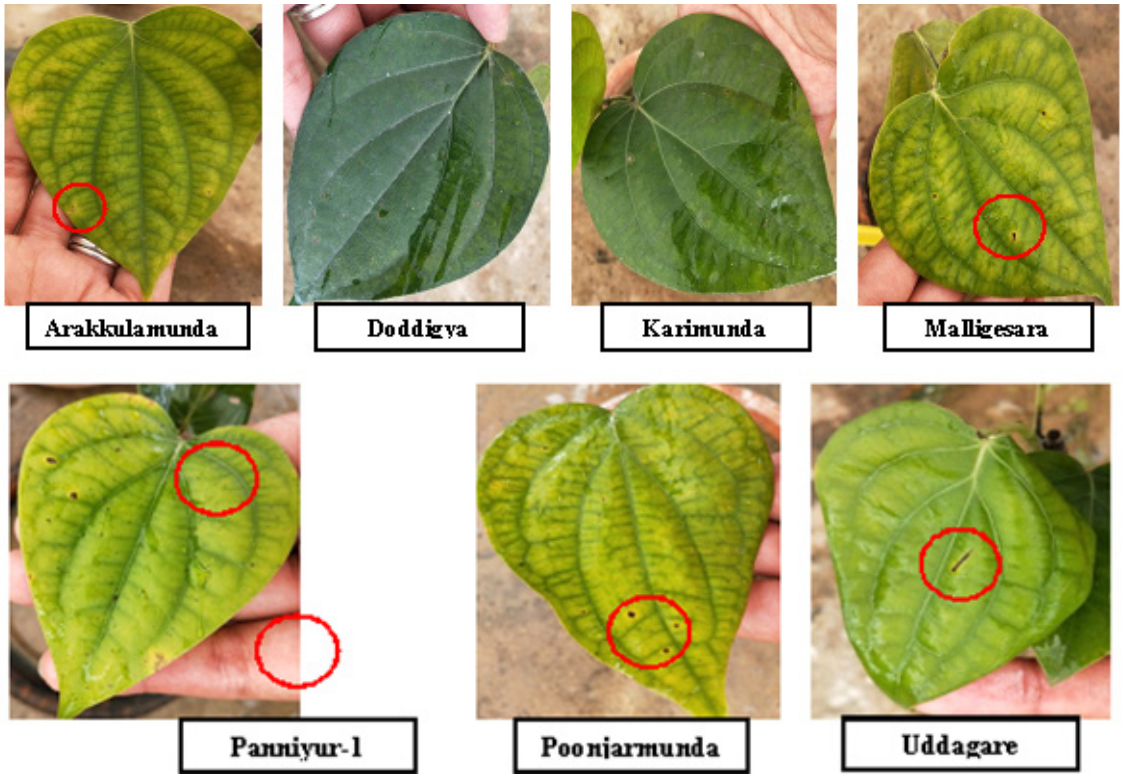

Fig. 3. Leaves of different cultivars/varieties of black pepper showing symptoms three days after inoculation with *Colletotrichum gloeosporioides*

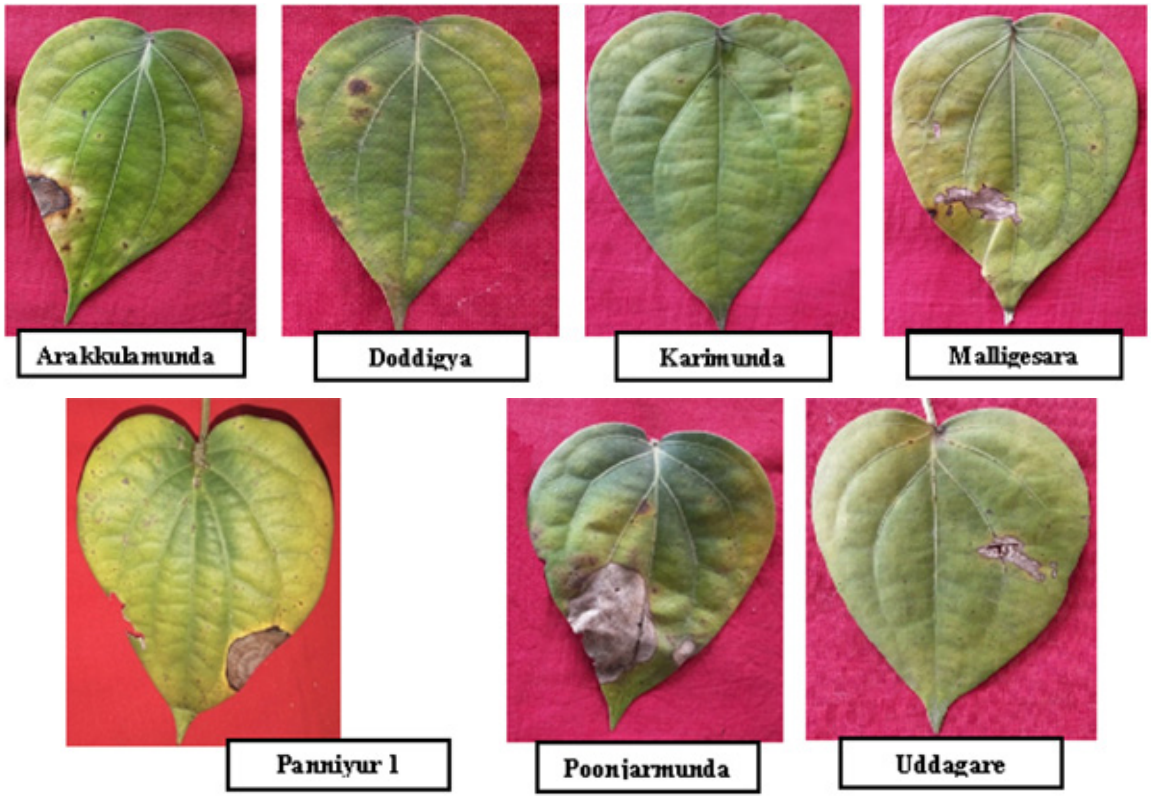

Fig. 4. Leaves of different cultivars/varieties of black pepper showing symptoms twenty days after inoculation with *Colletotrichum gloeosporioides.*

with several minute dot like acervuli in the center, surrounded with thin yellow halo. Per cent disease index was found to be highest in Panniyur-1 and lowest in Karimunda. Similar results were obtained by Divya and Sharda (2014) in the case of Panniyur-1 and Karimunda for resistance/susceptibility against *Phytophthora capsici* Leon.

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