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Original Research Article

Distribution of Resident Mycoflora of *Piper longum* and their Influence on Plant Health and Causing Leaf Spot Disease by *Botrayodiplodia theobromae*

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A B S T R A C T

India is known for different medicinal plants with their different medicinal value and its various uses that had been developed and practiced from the ancient time . Among the cultivable medicinal plants in India Pippali (*Piper longum*) is one of the most important crop which suffers from leaf spot disease (fig.-1) caused by *Botrayodiplodia theobromae*. During the study different steps was carried out likesymptoms, isolation, and identification of various pathogensresponsible for causing foliar diseases in *P. longum*under Pusa, Bihar. Diseased leaves of *P. longum* showing symptoms of leaf spot were frequently collected from herbalgarden of RAU, Pusa, Bihar. Microscopic examination of the tissue from phylloplane(infected and healthy leaves) and rhizospherefrequently revealed the association of fungi like *B.theobromae*, *Fusarium pallidoroseum*, *Trichoderma harzianum*, *Aspergillus fumigatus.*, *Penicillium* sp., *Pythium* sp., *A.niger.*, *Cladosporium* sp.and *A.flavus* .All the fungal species were frequentlyisolated, purified and multiplied on PDA. *Botrayodiplodia theobromae*were found to produce typical symptoms ofdisease in pathogenicity tests using the detached leaftechnique and under pot condition and again reisolatedfrom the infected host, thereby pathogenicity of the fungus was established.The identity of the fungus was further confirmed from the IndianType Culture Collection (I.D. No. 8867.12), Division of Plant Pathology, IARI, New Delhi.

Introduction

The *Piper longum* (long pepper) is an important medicinal herb used as ayurvedic medicine. *P. longum* is described in the ayurvedic and unani systems of medicine as a valuable drug used for treatment of various kinds of ailments Viswanathan, 1995). The fruits of the plant are very well- known medicine for diseases of the respiratory tract, viz. cough, bronchitis, asthma, etc; as counter irritant, analgesic when applied locally for muscular pains and inflammation and general tonic. Fruits, spikes are cylindrical, oblong, berries red or black when ripe, globose with aromatic odour and pungent taste (Sumy *et al.,* 2000; Banerjee *et al*, 1999). Mainly spikes of the plant, dry roots and stem just above the ground are used as medicine for the cure of various diseases. It is also used as an antidote to snake-bite and scorpion-sting (Sumy *et al*,

2000). Like other crops long pepper has also been reported to be affected by number of fungal diseases showing symptoms of rotting of leaves and vines caused by *Colletotrichum gloeosporioides* and necrotic spots and blights on leaves caused by *Collectotrichum* and *Cercospora* sp. (Kurion *et al.*, 2007). Various mycoflora present in the rhizosphere and phylloplane largely determine the health status of a plant. Hence, in the present study, distribution of resident mycoflora and their influence on *Piper longum* plants were evaluated.

Materials and Methods

Isolation and identification of rhizosphere and phylloplane mycoflora

Rhizosphere mycoflora: The rhizosphere soils were collected from *Piper longum* plants. 10^{-5} dilution were prepared from 10 gram of each rhizosphere soil and 1 ml of each dilution was plated on Maritn's Rose Bengal Agar medium and incubated at 28 $\pm 2^0$C for 5-6 days. Each fungal colony which appeared on Rose Bengal Agar plates was transferred to PDA slants and purified by hyphal tip method. The fungal isolates were identified based on morphological and cultural characters.

Phylloplane mycoflora: For isolation of leaf surface mycoflora vigorous washing of leaves was done in sterilized water to prepare stock suspension of phylloplane mycoflora. After that, serial dilution were prepared from the stock suspension. Thereafter each dilution was plated on PDA plates and incubated for 6-7 days at 28 ± 1^0C. Then, each colony was transferred on PDA slants separately, purified and multiplied. The fungal isolates were identified on the basis of morphological and cultural characters

Effect of various rhizospheric fungal isolates on crop health: Each fungal isolates obtained from rhizosphere of *Piper longum* were multiplied separately on sand-maize meal (9:1) medium for 25 days. The earthen pots were prepared by using 1kg soil in each pot and the inocula @ 50g/kg soil of different fungi were added in different pots maintaining three replication for each fungus. After 24hrs planting was done in each pot (one seedling in each pot) and plants were regularly monitored for health condition and data on crop health (disease) was taken up to 45 days after planting and fresh weight of leaves + stem was taken after 45 days. On the basis of the result, all the rhizosphere isolates were found to be non-pathogenic.

Effect of phylloplane fungi on leaves using detached leaf technique: Various fungi obtained from phylloplane of *Piper longum* were studied for their pathogenic effect by detached leaf technique as described by Benedikz *et al*. (1981) with minor modification. All the phylloplane fungal isolates were brushed on the underside of the excised leaves and leaflets. The inoculated leaves were placed, inoculated side up, on several layers of wet blotting paper in a Petri plates to maintain high humidity and kept in incubator for 10 days.

Result and Discussion

The pathogen was isolated from infected leaves and stem of *Piper longum.* The infected plants parts leaves and other parts were placed on sterilized potato dextrose agar medium by leaf tissue method (Rangaswami, 1972).

Rhizosphere and phylloplane fungi

Various rhizosphere fungal isolates obtained from *Piper longum* were identified on the

basis of morphological and cultural characters as *Cladosporium* sp., *Trichoderma harzianum* (Plate -1), *Penicillium* sp. (Plate -2), *Aspergillus fumigates* whereas the phylloplane isolates obtained from *Piper longum* were identified on the basis of morphological and cultural characters were- *Aspergillus fumigatus, A. niger, A. flavus, Trichoderma harzianum. Pythium* sp., *Penicillium* sp., *Fusarium pallidoroseum,* and *Botryodiplodia theobromae.*

Effect of various rhizospheric mycoflora on crop health of *Piper longum* under pot condition

The data showing the effect of various rhizosphere mycoflora isolates on *Piper logum* plants (table.1.) showed that *Trichoderma harzianum* had promising growth promoting effect on plants with fresh weight 5.31gm. This was followed by *Penicillium* sp. and *Aspergillus fumigatus* which also exhibited vigorous plant growth with fresh weight 5.232gm, and 4.419gm respectively. After 45 days of planting, where as *Cladosporium* sp. resulted in poor growth 2.22 g fresh weight of the plants.

Effect of various phylloplane fungi on leaf

Various fungi obtained from phylloplane of *Piper longum* were studied for their pathogenic effect using detached leaf technique and result presented in Table 2.

It is clear from the Table 2 that, among various phylloplane isolates, only *Botryodiplodia theobromae* was pathogenic. Other isolates showed non pathogenic effect during research. It was also observed that inoculation with *Botryodiplodia theobromae* showed brown to black circular spot on leaves after 15 days interval and after 20 days the lesion gradually merged and entire leaves showed burnt appearance. The pathogenic identity was further confirmed from the Indian Type Culture Collection (I.D. No.8867.12) for the fungus *B. theobromae*. Division of Plant Pathology, IARI, New Delhi. Review of literature revealed that *P. longum* is a new host of *B. theobromae*.

The other isolates like *Aspergillus fumigatus, A.flavus, A. niger, Trichoderma harzianum, Pythium* sp., and *Penicillium* sp., showed no effect on leaves after inoculation by respective isolates. On the basis of these studies various isolates were divided into pathogenic and non pathogenic category.

Effect of various phylloplane isolates on *Botrayodiplodia theobromae*

Effect of various isolates with *Botrayodiplodia theobromae* was studied as per method mentioned above and data presented in Table 3.

The Table 3. clearly indicates that all the phylloplane isolate were quite effective in suppressing the growth of pathogenic *Botrayodiplodia theobromae* in dual culture.

The most effective isolates showing strongest antagonistic activity was *Aspergillus fumigatus* where the pathogen was confined to growth of 27 mm and thus inhibited by 70%.

Likewise other isolates such as *Trichoderma* sp. and *Penicillium* sp. also exhibited marked antagonistic effect, restricted the growth of pathogen to 40 mm and 51.5 mm, and thereby inhibited by 55.56% and 42.78% respectively. The other isolates *Aspergillus niger* and *Pythium* sp. were, although inhibitory to the pathogen but with milder antagonistic effect.

Effect of various phylloplane fungal isolates in relation to effect of *Diplodia theobromae* on crop health under pot conditions

Various phylloplane fungal isolates were studied in relation to the effect of *Botryodiplodia theobromae* on crop health under pot conditions and data are presented in Table 4.

It appears from the table that the *Trichoderma harzianum* was highly effective in promoting the growth of plant, completely suppressed the pathogenic effect of *Botryodiplodia theobromae* and registered maximum fresh weight (5.346 g). Likewise isolates of *Aspergillus fumigatus* and *Penicillium* sp. were also found to inhibit the pathogenic effect of *Botryodiplodia theobromae* with concomitant increase in fresh weight i.e. 4.806 and 4.485 g as compared to control (1.822 g fresh weight). The isolate of *A. niger*, although suppressed the pathogenic effect of *Botryodiplodia theobromae* but showed slow crop growth. Isolate of *Pythium* sp. favoured the development of disease caused by *Botryodiplodia theobromae.*

Fig.1 Symptoms of leaf spot

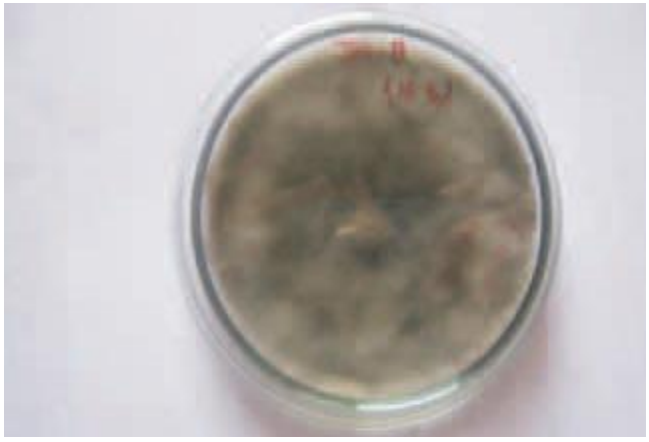


Fig.2 Pure culture of *Botryodiplodia theobromae*

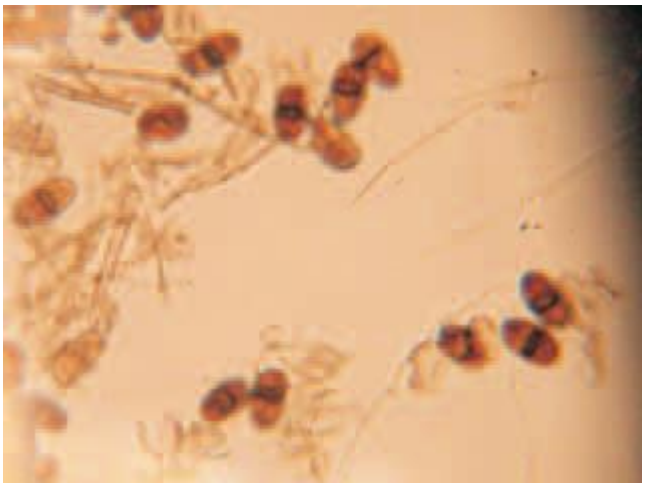


Fig.3 Microscopic view of spores of *Botryodiplodia theobromae*

Table.1 Effect of various Mycoflora on crop health under pot condition

Sl.No.	Fungi isolate	Effect on crop (upto 45 day)	Fresh wt (gm) (stem+leaves) after 45 days of planting
1.	*Cladosponium* sp.	Poor crop growth	2.22
2.	*Trichoderma harzianum*	Healthy and vigorous growth	5.31
3.	*A. fumugous*	Healthy and vigorous growth	4.41
4.	*Penicillium* sp.	Healthy and vigorous growth	5.23
5.	Control	Normal growth	3.25
	CD@ 5%		1.31
	CV %		0.34

Table.2 Effect of various phylloplane fungi on leaf using Detached leaf technique

Fungal isolates	Effect on leaves after 15 days of inoculation
Botryodiplodia theobromae	Brown to black circular spot developed on leaves and after 20 days the lesion gradually merged and entire leaves showed burnt appearance
Aspergillus fumigates	No effect
A. niger	No effect
A. flavus	No effect
Trichoderma harzianum	No effect
Pythium sp.	No effect
Penicillium sp.	No effect

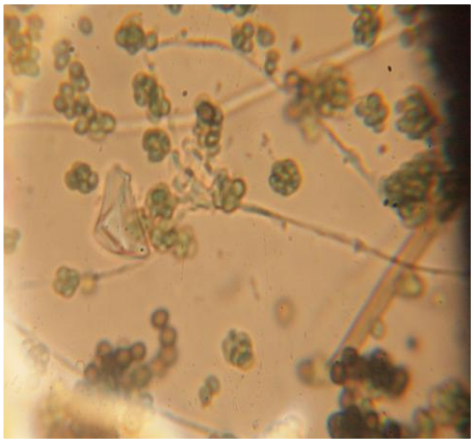
Table.3 Effect of various phylloplane mycoflora on the growth of *Botrayodiplodia theobromae* in dual culture

Fungal isolates	Radial growth of pathogen in Dual plate culture (in mm)	`Growth %	Inhibition %
Aspergillus fumigatus	27	30	70
Trichoderma sp.	40	44.44	55.56
Pythium sp.	74	82.22	17.78
Penicillium sp.	51.5	57.22	42.78
A. niger	68	75.55	24.45
Control	90	100	-
SEm (±)	3.33		
CD (at 5%)	10.27		
CV (%)	9.87		

Table.4 Effect of various phylloplane mycoflora on the pathogenic effect of *Botryodiplodia theobromae* under pot condition

Sl.No.	Fungal isolate	Effect on plants upto 45 days of planting	Fresh wt (gm) of (Stem + leaves) upto 45 days of planting
1.	*Fusarium* pallidoroseum	Drying of leaves started after 20 days of planting, and stopped after 30 day	3.032
2.	*Aspergillus fumigatus*	Healthy and vigorous growth of plant	4.806
3.	*A. flavus*	Drying of leaves started after 20 days of planting, but developed slowly, checked after 30 days	3.522
4.	*Penicilium* sp.	Healthy growth of plant	4.485
5.	*Trichoderma harzianum*	Healthy and vigorous growth of plant	5.346
6.	*A. niger*	Plant looked healthy but slow growth	2.355
7.	*Pythium* sp.	Drying started from margin gradually affected the entire leaf gave blighted appearance	1.809
8.	Control (Only *Botryodiplodia theobromae)*	Drying started from margin gradually affected the entire leaf, gave burnt appearance	1.822
9.	CD		0.979

Plate – 1. Microscopic view of *Trichoderma*



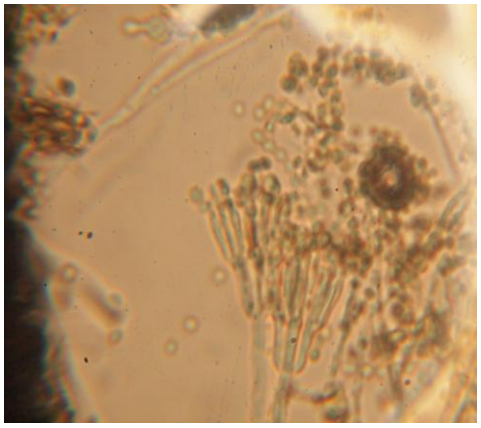
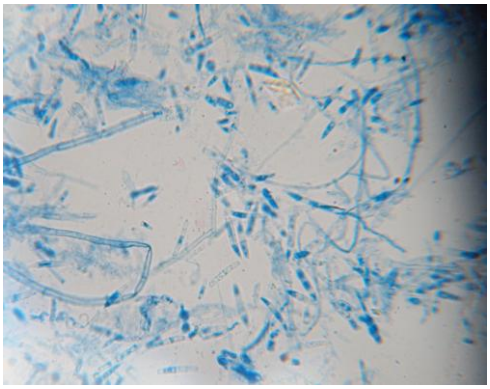


Plate –9. Microscopic view of *Fusarium* sp**.**

Plate – 10. Microscopic view of *Fusarium* sp.



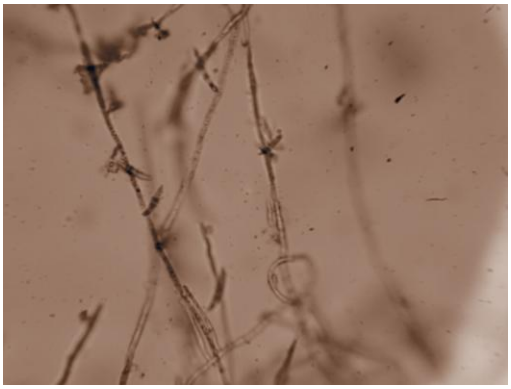
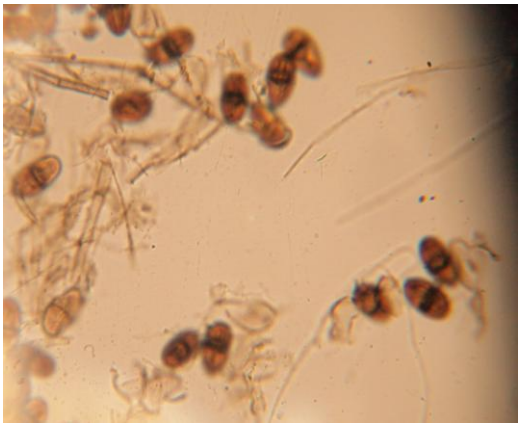
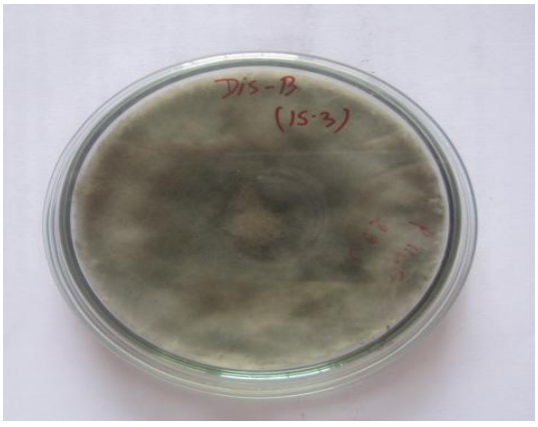


Plate – 11. Microscopic view of *Diplodia* sp**. Plate – 12.** Pure culture of *Diplodia* sp.





Present findings conforms to the findings of several earlier workers who observed the association of various beneficial and pathogenic fungi in the rhizosphere and

phylloplane of different medicinal plants. Various fungal species viz *Colletotricum, Glomerella, Guagnardia* and non-sporulating species etc. were isolated from *Centella asiatica* (Rakotonirian *et al*., 2008). Solanki *et al.,* (2016) studies on leaf blight disease of *Piper longum* and their management by using of safer chemicals and biocontrol agents. Thakur *et al.,* (2012) identified leaf spot disease caused by *Botrytis cinerea* on *Piper longum*. Based on morphological characteristics and pathogenicity tests, the organism was identified as *Botrytis cinerea.* The pathogen was reisolated from the infected leaves, identified and maintained on potato dextrose agar thus, confirming Koch's postulates. *Sagar* and Kumari (2008) reported the presence of sixteen and seventeen species of fungi respectively from rhizosphere of *Centella asiatica* and *Ocimum sanctum* and nine and seven species of fungi from roots and leaves of *Centella asiatica* and *Ocimum sanctum* respectively. Likewise Mohali and Encinas (2001) isolated *Diplodia mutila* from blue-stained Caribbean pine timber (*Pinus caribaea* var. *hondurensis*) in eastern Venezuela and established its pathogenicity *in vitro*. Yandry *et al*., (2006) also isolated various fungi from various medicinal plants like *Baccharis latifolia, Baccharis abtusifolia, Piper barbatum, Borreria laenis, Chuquirage fussieni and Bidens andicola* etc and reported the frequent presence of various fungal species such as *Alternaria, Aspergillus, Epicoccum, Fusarium, Nigrospera* and *Phoma*, which are known to exert beneficial as well as pathogenic effect on plants. Aziz *et al.* (1998) isolated ten fungal genera of different taxonomic groups, of which *Aspergillus flavus, A. parasiticus, A. niger, Fusarium oxysporum* and *Penicillium viridicatum* appeared most often in 89 medicinal plant samples. Noveriza and Quimio (2004) isolated 149 colonies of soil mycoflora belonging to 14 genera from rhizosphere of black pepper (*Piper nigrum*) grown in Batangas and Laguna and found that most dominating genera were *Penicillium, Paecilomyce* and *Aspergillus*.

The conclusion of the present study the rhizosphere of *Piper logum* showed the presence of diverse range fungi, but none was found to exert pathogenic effect on plants. The rhizosphere fungi- *Trichoderma* sp, *Asperguillus fumigatus* and *Penicilliun sp*. were found to exert growth promoting effect on plants. The phylloplane fungi comprised of both pathogenic and nonpathogenic population. The growth promoting attribute of the resident fungi may be exploited for sustainable management of the crop health of *Piper longum*.

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