

Original Research Article

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## Morphological and Cultural Studies of *Peziotrichum corticolum* (Masse) Subramanian Causing Black Banded Disease of Mango

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### ABSTRACT

#### Keywords

Black banded disease, Mango, *Peziotrichum corticolum*, Cultural, Morphological.

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Black banded disease of mango, caused by *Peziotrichum corticolum* (Masse) Subramanian, is becoming severe for the last three to four years in all the mango growing regions. Morphological and cultural characteristics of the pathogen were studied. The identity of the fungus was confirmed. The fungus showed maximum radial growth on host extract dextrose agar on 12<sup>th</sup> day after incubation at 27±1°C. Culture of *P. corticolum* exhibited diversity with respect to cultural characters like type of the growth, mycelial colour, pigmentation and colony margin. Maximum dry mycelial weight of the fungus was recorded on 14<sup>th</sup> day after incubation. Dry mycelial weight of the fungus was more in Richards' broth (538.00 mg) than potato dextrose broth (442.33 mg).

### Introduction

In the basket of Indian fruit market, mango (*Mangifera indica* L.) shares a big place. It is described as the “king of fruits” or “super fruit” and known for its strong aroma, delicious taste and high nutritive value. It belongs to the family *Anacardiaceae*. It is considered to be originated in Indo-Burma region. Mango is a most important crop among the tropical and sub tropical fruit crops. India ranks first among world's mango producing countries accounting for 2.51 mha area having an annual production of 18.43 mt with the productivity of 7.3 t/ha. Mango crop contributes 20.7 % production share of major fruit crops in India. In Karnataka, the area under mango cultivation is 1.80 lakh ha with a

production of 1.75 mt and productivity of 9.7 t/ha (National Horticultural Database, 2014).

Although India is the largest producer of mango, the productivity is low. This may be due to the wide range of climatic conditions, environmental situation in which the mango grows and the diversity of the associated disease problems. There are more than 140 diseases, insect pests and physiological disorders, known to cause damage to the crop at all stages of its development, right from the plant in the nursery to the fruit in storages or transit, yet there are few diseases which are of great economic importance (Om Prakash, 1998).

Earlier black banded disease of mango was considered as a minor disease but for the last three to four years it is becoming severe in all the mango growing regions. The causal fungus *Peziotrichum corticolum* (Massee) Subramanian was described on the bark of trees in Poona, India by Massee (Hughes, 1980). Later on, it has been reported from Goa, West Bengal, Karnataka, Maharashtra, Bihar, Orissa, Andhra Pradesh, Kerala, Andaman & Nicobar Islands and Tamil Nadu in mild to severe form (Naqvi, 2004; Ploetz and Om Prakash, 1997 and Om Prakash and Srivastava, 1987).

The pathogen grows superficially on the bark of trees forming large, dark black, irregular, girdle-like infection patches and hence the name. Pandey and Dinesh (2010) found black velvety growth of fungus on the midribs and veins of leaves also (Pandey and Dinesh, 2010).

As the information available on the causal fungus *Peziotrichum corticolum* is very less, morphological and cultural studies were conducted in this present study.

### **Materials and Methods**

The present investigations on black banded disease of mango were conducted during 2014-2015 at the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. It is situated in northern transitional zone (Zone 8) of Karnataka state at 15°15'N latitude, 75°7'N longitude and at an altitude of 774.0 m above mean sea level. The materials used and methodology adopted during the course of investigations are presented here.

### **Collection and isolation of the pathogen**

The twigs of mango (*Mangifera indica* L.) showing the typical symptoms of the disease

were collected from silver jubilee orchard, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The standard tissue isolation procedure was followed to isolate the pathogen. The infected bark bits were surface sterilized with sodium hypochlorite (1.0%) solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any, then aseptically transferred to sterilized Petri plates (1-2 bits of infected bark portion per Petri dish) containing Potato Dextrose Agar (PDA). The Petri plates were incubated at room temperature ( $27 \pm 1^{\circ}\text{C}$ ) and observed periodically for the growth. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at  $27^{\circ} \pm 1^{\circ}\text{C}$  for 12 days. Then such slants with pure culture were used for further studies.

### **Identification of the pathogen**

Identification of *Peziotrichum corticolum* was achieved by studying morphological characters of the same pathogen as described by Mukherjee and Litz (2009) and Patil and Dangat (2012). Koch postulates were proved to identify and to confirm the pathogenicity of isolated pathogen.

### **Maintenance of the cultures**

The fungus was sub-cultured on Potato dextrose agar (PDA) slants and allowed to grow at  $27 \pm 1^{\circ}\text{C}$  for 16 days, such slants were preserved in refrigerator at  $5^{\circ}\text{C}$  and maintained.

Sub-culturing was done once in a month, such cultures were used throughout the study; virulence of the fungus was maintained by passing through the host after every three months.

### **Proving the pathogenicity**

Mango seedlings of aged three to four years were grown in the earthen pots filled with sterilized soil. Pathogenic culture of *P. corticolum* was grown on PDA for 15 days. Injury on seedlings was made by removing upper layers of the bark with the help of a sharp, sterilized knife. With sterilized spatula, mycelial disc was inoculated on the injury. Then the inoculated area was added with small quantity of sterile distilled water and covered with the help of transparent adhesive tape. Two controls were maintained as explained here under.

Control I: Injury + media + sterile distilled water

Control II: Sterile distilled water

All plants were covered with polythene bags for 48 hr to maintain humidity. After 48 hr of incubation, polythene bags were removed and the plants were kept in greenhouse. Observations were made for symptom development periodically. Re-isolation was made from the spots which were showing typical symptoms on inoculated plants. The isolated culture was compared with the original culture to confirm the pathogen.

### **Cultural characteristics of *P. corticolum* on different solid media**

Dharwad isolate of *P. corticolum* was grown on different media viz., Potato dextrose agar, Host extract dextrose agar, Czapek's malt agar, Richards' agar, Oat meal agar, V-8 juice agar and Potato carrot agar and observations were recorded for colony characters viz., radial growth in (mm), colour, type of growth, pigmentation, colony margin and sporulation of the pathogen, when the maximum growth was attained on any one of the tested media. The composition and preparation of the above

mentioned synthetic and semisynthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Ainsworth (1971) and plant pathological methods, fungi and bacteria by Tuite (1969).

For Host extract dextrose agar (HEDA), bark from the healthy green twigs was peeled (200.0 g) and boiled in 500 ml water for 30 min. Extract was collected by filtering through muslin cloth. Dextrose (20 g) was added in this extract and mixed well. The agar agar was melted in 500 ml water. Both the solutions were mixed and the volume was made up to 1000 ml and then autoclaved at 1.1 kg/cm<sup>2</sup> pressure for 15 minutes at 121°C.

### **Cultural characteristics of *P. corticolum* on liquid media**

Two liquid media namely, potato dextrose broth and Richards' broth were used to study growth phase of *P. corticolum*. The composition and preparation of both liquid media used were the same as that of solid media except that the agar was not added. Thirty ml of broth was added into each of 100 ml conical flask and sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 minutes at 121°C. After sterilization, these flasks were allowed to cool and then inoculated with 5 mm disc from 15 days old culture and incubated at room temperature. Each treatment was replicated three times. The culture was filtered through Whatman No. 42 filter paper of 9.0 cm diameter, which was dried to a constant temperature at 60°C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to remove traces of salts likely to be associated with it. Three flasks were harvested at 48 hrs after incubation and subsequent harvesting was done at an interval of two days up to 20 days. The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed

immediately on a digital balance. The difference between final and initial weight of filter disc was taken as the weight of the mycelial mat. The data were analyzed statistically.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

### **Spore Morphology**

The spores produced in the black velvety growth of fungus on the host bark were observed. Length and breadth of spores were measured using motic images in computer (400 X). The average size of the spore was calculated.

## **Results and Discussion**

### **Symptomatology**

The typical black banded symptoms were observed on twigs, branches, leaf petiole, midrib and veins of leaves. Symptoms were rarely produced on main trunk of the tree. Inflorescences were found to be free from symptoms. On branches and twigs, symptoms are characterized by typical black, irregular, superficial velvety fungal growth. The size of fungal growth increased with the advancement of the disease which resulted in large, black coloured, girdle like velvety bands. Velvety appearance of bands is due to fungal mycelium which aggregate together and erect, perpendicular to bark.

The young spreading mycelium near the periphery of the infection bands is white or nearly hyaline. In infected trees, symptoms are more conspicuous on young branches and twigs than on old branches. The velvety mycelial growth drops off in the summer months leaving light black coloured, bands in the affected portions. On leaves, black velvety

fungal growth can be seen on midrib and veins. Girdling of petioles by black velvety fungal growth is also common (Fig. 1).

### **Proving pathogenicity**

Fungus was isolated from infected bark of mango tree and pure culture was obtained by subsequent sub-culturing and such culture was used for pathogenicity test as described in “Material and Methods” (Fig. 2).

On tenth day of inoculation, typical black velvety fungal growth was seen on twigs. On 13<sup>th</sup> day, area between velvety fungal growths became black. The boundary of black fungal growth was light white in colour. The fungus was reisolated and pathogenic culture thus obtained was compared with the original culture of *P. corticolum*.

### **Identification of the pathogen**

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The fungus in the present study produced septate mycelium. Isolated fungal culture was white. After twenty days, white colour changed to brown and subsequently black colour. Fungal hypha was white in early days later it changed to brown colour (Fig. 3).

### **Morphological and cultural studies of the pathogen**

#### **Spore morphology**

In the present study, conidia of *P. corticolum* obtained from infected bark were measured and compared with respect to their spore morphology. The conidia were single-celled, pale brown, globose, smooth-walled and measured 11.25 - 17.36  $\mu$  in diameter. Further conidia were transferred to water agar and germination of conidia was observed (Fig. 3).

### **Mycelium morphology**

Mycelium of *P. corticolum* obtained from infected bark and culture was septate, brown in colour and measured 3.2 - 6.82  $\mu$  in width (Fig. 3).

### **Cultural characteristics of *P. corticolum* on different solid media**

Diversity in cultural and morphological characters of *P. corticolum* were studied in five non synthetic / semi synthetic and two synthetic media at room temperature  $27 \pm 1^\circ\text{C}$  as described in materials and methods. Colony characters and sporulation of the fungi were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungi differed significantly.

Maximum radial growth of *P. corticolum* was recorded on host extract dextrose agar (90.00 mm), which was found to be significantly superior to all other media followed by oat meal agar (83.3 mm), V-8 agar (75.3 mm), potato carrot agar (74.70 mm), Czapek's malt agar (72.00 mm) and potato dextrose agar (66.7 mm).

The least radial growth was recorded in Richards' agar (64.7 mm). Potato dextrose agar (66.7 mm) and Richards' agar (64.7 mm) were on par with each other. Further same on par results were found for V-8 agar (75.3 mm) and potato carrot agar (74.70 mm). The non synthetic / semi synthetic media recorded maximum growth compared to synthetic media.

Mycelium colour varied from white to light gray. The growth varied from flat to sparse. Pigmentation in the media also varied from brownish yellow to gray (Table 1 and Fig. 4). Spores were not produced in any media used for study.

### **Cultural characteristics of *P. corticolum* on different liquid media**

The experiment was conducted to ascertain the period for the maximum growth of the fungus by dry mycelial weight method, using two liquid media viz., potato dextrose broth and Richards' broth, starting from the 2<sup>nd</sup> day to 20<sup>th</sup> day. The results obtained are presented in the table 2 and figure 5.

It is evident from the data that there were significant differences in the different incubation periods. Dry mycelial weight of *P. corticolum* recorded gradual increase in potato dextrose broth and Richards' broth, starting from second day (33.67 mg and 79.33 mg respectively) and reached peak growth on 14<sup>th</sup> day (442.33 mg and 538.00 mg respectively) and remained significantly superior to remaining treatments. Later the dry mycelial weight declined to reach 292.67 mg and 401.00 mg respectively on 20<sup>th</sup> day of incubation. Mean dry mycelial weight of *P. corticolum* recorded maximum (490.17 mg) on 14<sup>th</sup> day of incubation. The dry mycelial weight on 12<sup>th</sup> and 16<sup>th</sup> day in potato dextrose broth remained on par with dry mycelial weight on 20<sup>th</sup> day in Richards' broth. Similarly on par results were recorded on 14<sup>th</sup> day in potato dextrose broth and 10<sup>th</sup> and 18<sup>th</sup> day of incubation in Richards' broth.

The description of typical black banded disease symptoms observed in this study, were in accordance to previous workers Reddy *et al.* (1961); Om Prakash and Srivastava (1987); Mukherjee and Litz (2009) and Patil and Dangat (2012). *Peziotrichum corticolum* (Masse) Subramanian was isolated and pure cultured. Identification of the same fungus was confirmed on the basis of morphology and pathogenicity in accordance with the description given by Subramanian (1956); Mukherjee and Litz (2009) and Patil and Dangat (2012).

**Table.1** Cultural characteristics of *Peziotrichum corticolum* on different solid media

Sl. No.	Different Media	Colony characters				
		Radial growth (mm) <sup>#</sup> (12 DAI)	Colour	Type of growth	Pigmentation	Margin
1.	Host extract dextrose agar	90.0	White	Flat growth circular	White	Sparse Smooth
2.	Oat meal agar medium	83.3	Grayish off white	Flat growth irregular	Gray	Smooth
3.	Potato carrot agar	74.7	White	Flat growth circular	Gray	Medium Smooth
4.	Potato dextrose agar	66.7	White	Flat growth circular	White	Smooth
5.	V-8 agar	75.3	Yellowish White	Flat growth circular	Yellow	Sparse Smooth
6.	Czapek's malt agar	72.0	White	Flat growth irregular	White	Irregular
7.	Richards' agar	64.7	White	Sparse irregular	Yellow	Sparse Irregular
<b>S.Em.±</b>		<b>0.56</b>				
<b>CD at 1%</b>		<b>2.37</b>				

# Mean of three replications  
DAI= Days after incubation

**Table.2** Growth phase of *Peziotrichum corticolum* in liquid media

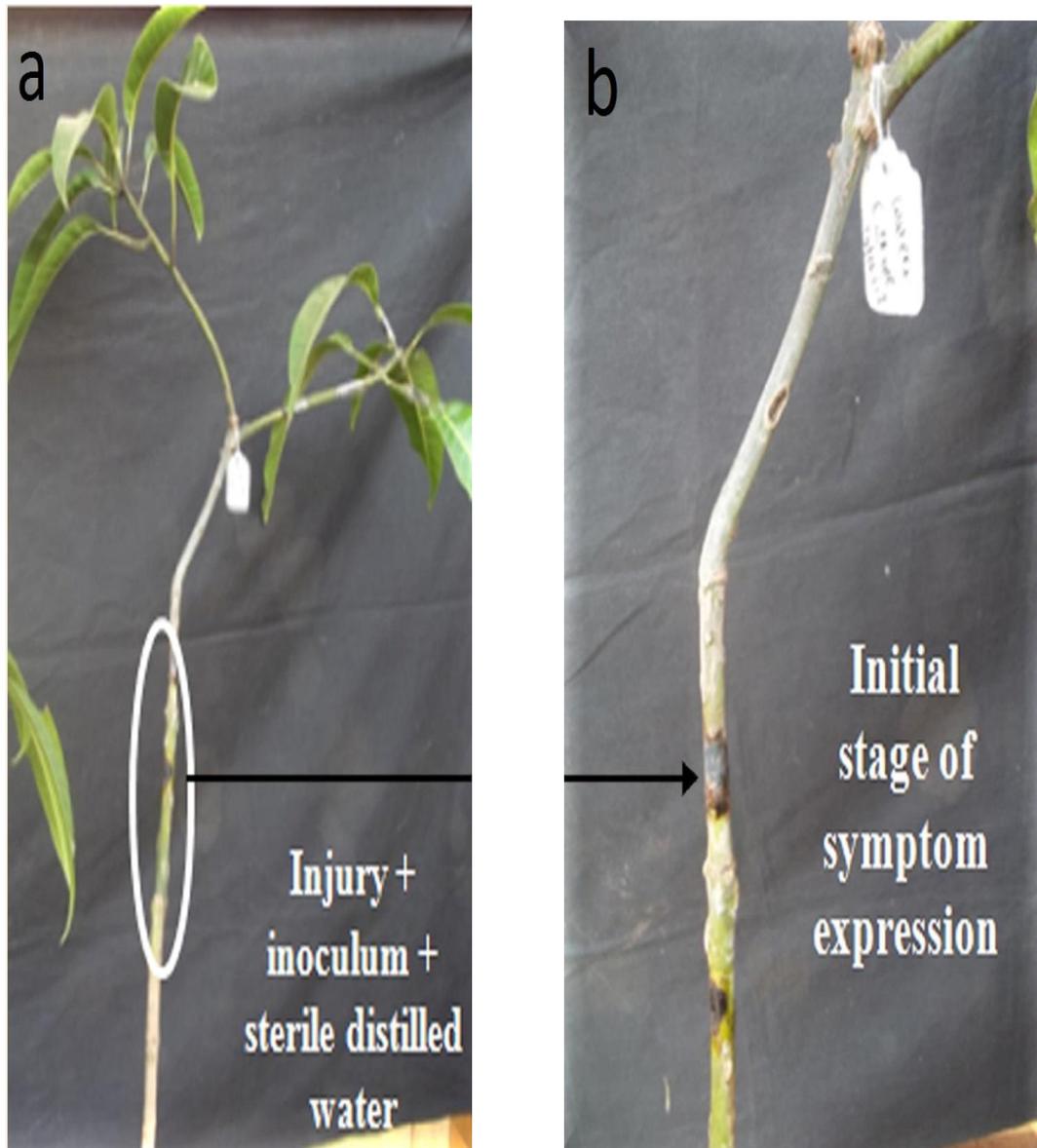
Days after incubation (DAI)	Dry mycelial weight (mg) <sup>#</sup>		Mean
	Potato dextrose broth	Richards' broth	
2	33.67	79.33	56.50
4	65.67	189.00	127.33
6	168.33	282.00	225.17
8	256.67	361.33	309.00
10	332.67	438.67	385.67
12	394.33	512.33	453.33
14	442.33	538.00	490.17
16	400.67	483.00	441.83
18	328.67	444.67	386.67
20	292.67	401.00	346.83
<b>Mean</b>	271.57	372.93	
<b>Source</b>	<b>DAI (A)</b>	<b>Broth (B)</b>	<b>A × B</b>
<b>S.Em ±</b>	1.63	0.73	2.30
<b>CD at 1%</b>	6.23	2.79	8.82

# Mean of three replications

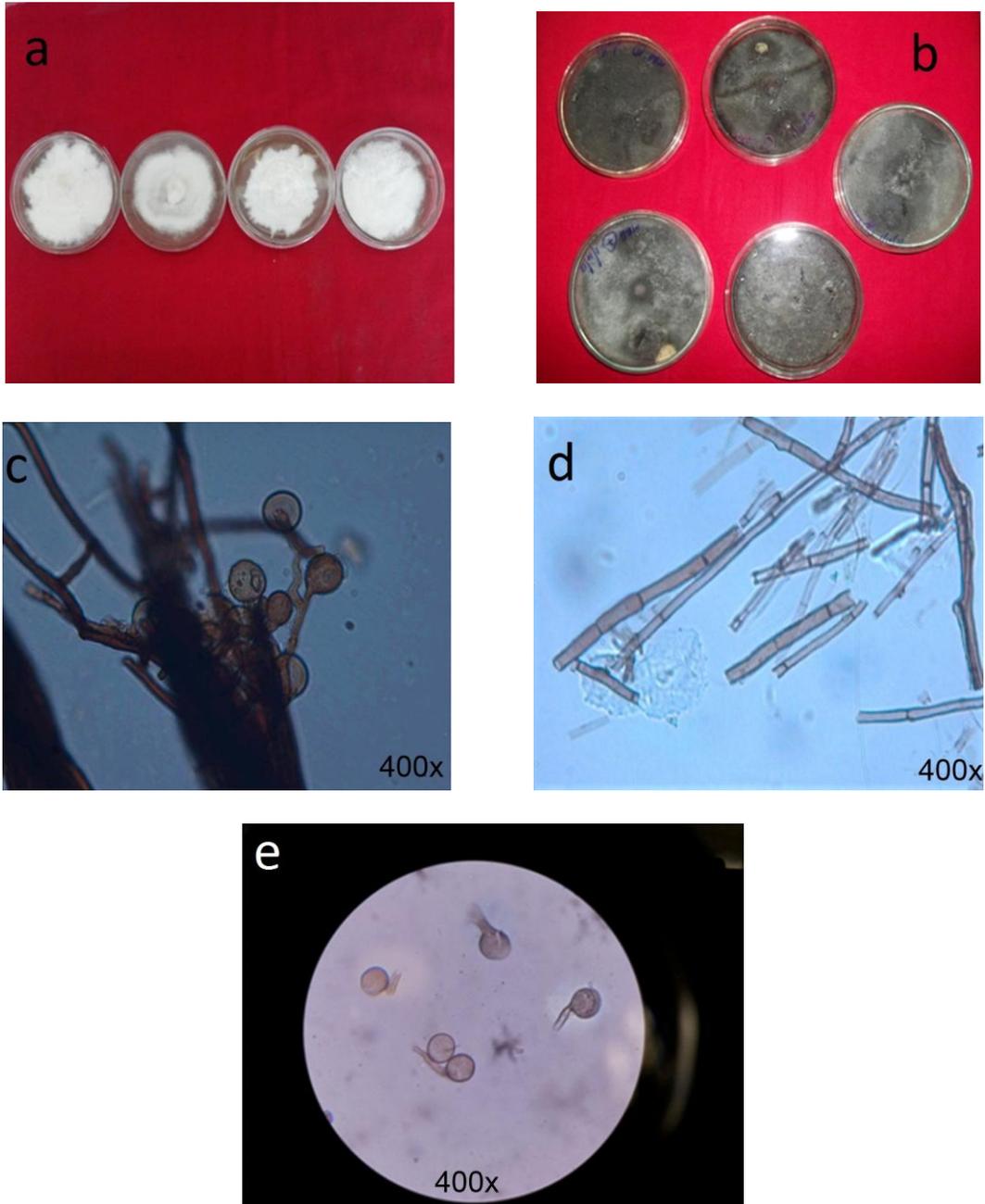
**Fig.1** Symptoms of black banded disease of mango. a) on new branches; b) on old branches; c) on midrib and veins of leaves; d) close view of black velvety symptoms; e) on leaf petiole and f) microscopic view of velvety growth



**Fig.2** Proving pathogenicity. a) Inoculated plant (injury + inoculum + sterile distilled water) and b) Initial stage of symptom expression



**Fig.3** Culture and morphology of *Peziotrichum corticolum*. a) culture up to 20 days; b) culture after 20 days c) spores; d) mycelium and e) germinated spores in agar plate



**Fig.4** Growth of *Peziotrichum corticolum* on different solid media after 12 days. 1) Host extract dextrose agar, 2) Potato dextrose agar, 3) Czapeck's malt agar, 4) Richards' agar, 5) Oat meal agar, 6) V-8 agar and 7) Potato carrot agar



### Pathogenicity

Pathogenicity test was proved following Koch's postulates. As this is the first attempt towards proving pathogenicity against *P. corticolum*, no supporting reference is available for conformity or for comparison.

### Identification

The study on morphological characteristics of *Peziotrichum corticolum* indicated, septate hyphae measuring 3.2 - 6.82 $\mu$  in width, brown in colour. Conidia were single celled, pale brown, globose, smooth-walled; 11.25 - 17.36  $\mu$  in diameter. The findings are in agreement with the findings of earlier workers (Subramanian, 1956; Mukherjee and Litz, 2009; Patil and Dangat, 2012). Based on the mycelial and spore morphology, the fungus under study was identified as *Peziotrichum corticolum* (Massee) Subramanian.

### Cultural studies

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory it is necessary to furnish those essential elements

and compounds in the medium for their growth and other life processes. Neither all media are equally good for all fungi, nor there any universal substrate or artificial medium upon which all fungi can grow. So, different media including, non synthetic, semi-synthetic and synthetic were tried for *P. corticolum*.

### Cultural characteristics of *P. corticolum* on different solid media

Among the non or semi-synthetic and synthetic media used for growth and sporulation of *P. corticolum*, maximum radial growth was recorded on host extract dextrose agar (90.00 mm) and least radial growth was recorded in Richards's agar (64.7 mm). Sporulation was not observed in any media, used for study. Maximum radial growth of the fungus on host extract dextrose agar may be due to host's cell constituents in the media, supporting good fungal growth. Further Naqvi (2004) reported maximum radial growth of *Pestalotiopsis mangiferae* causing grey blight of mango on host extract agar.

### Cultural characteristics of *P. corticolum* on different liquid media

Mean dry mycelial weight of *P. corticolum* was recorded maximum (490.17 mg) on 14<sup>th</sup> day of incubation. Maximum dry mycelial weight of *P. corticolum* was recorded on 14<sup>th</sup> day in both liquid media namely potato dextrose broth and Richard's broth (442.33 mg and 538.00 mg respectively). Later the dry mycelial weight declined to reach 292.67 mg and 401.00 mg respectively on 20<sup>th</sup> day of incubation which may have been due to autolysis of the mycelium, accumulation of toxins and exhaustion of nutrients in the medium after incubation for optimum number of days.

Vinod and Benagi (2009) also reported autolysis of the fungus *Colletotrichum gloeosporioides* after optimum days of incubation. Further Ashoka (2005) reported that dry mycelial weight of *Colletotrichum gloeosporioides* was obtained more in Richards' broth (394.00 mg) than potato dextrose broth (378.00 mg).

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