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## Morphological and cultural characterization of *Phyllosticta zingiberi* (Ramkr.) causing leaf spot disease of ginger

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**Abstract:** Isolation of ginger (*Zingiber officinale*) leaf spot pathogen from the UBKV farm field was done in the laboratory. The isolated pathogen was identified as *Phyllosticta zingiberi* on the basis of morphological characters as documented in taxonomic keys. The microscopic observation revealed that the pycnidia were globose to sub-globose with dark brown colour measuring  $124.16 \mu\text{m} \times 2.35 \mu\text{m}$  in average. The pycnidio spores were hyaline, oval to bullet shaped, monoguttulate measuring  $4.02 \mu\text{m} \times 2.35 \mu\text{m}$  in average. Among the different media tested for growth highest growth was recorded in Oat meal agar ( $26.44 \text{ cm}^2$ ) followed by malt extract agar ( $24.04 \text{ cm}^2$ ) which was statistically *at par*. The temperature of  $25^\circ\text{C}$  favoured maximum growth ( $24.20 \text{ cm}^2$ ). However, higher sporulation was observed in  $30^\circ\text{C}$ . Among the different carbon source tested, mannitol supported the highest growth of the pathogen ( $27.67 \text{ cm}^2$ ).

**Keywords:** Ginger, Leaf spot, Mannitol, *Phyllosticta zingiberi*, Pycnidia

### INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an important tropical spice belonging to the family Zingiberaceae. The plant possesses a combination of many attributes and properties there of making its utility diverse. Ginger is prone to many serious diseases, which are caused by fungal, bacterial and nematode infections. Among them the major diseases causing hindrance in ginger production includes soft rot (*Pythium spp.*), yellows (*Fusarium spp.*), Rhizome rot complex (fungi, nematode/ insect interactions), leaf spot (*Phyllosticta sp.*) and storage rots (*Pythium spp.*, *Fusarium spp.* etc.) (Rajan *et al.*, 2002; Senapati and Ghose, 2005). Leaf spot diseases appears and remains prevalent during the growth period of the crop and may have little or severe effect depending on the environmental conditions. The major leaf spot diseases are *Phyllosticta*, *Helminthosporium*, *Cercospora*, *Pyricularia*, *Rhizoctonia* *Septoria* leaf spots, etc. Among these *Phyllosticta* leaf spot caused by *Phyllosticta zingiberi* Ramakr. is considered to be the most destructive appearing in mild or severe form in all the ginger growing tracts of the country (Sood and Dohroo, 2005; Singh, 2015). During the recent years the disease has become significantly important due to severe leaf spotting which destroys the chlorophyllous tissues causing significant reduction in yield (Iyer, 1988; Singh *et al.*, 2000). Sarma *et al.* (1994) has recorded 13-66 percent yield loss depending upon its severity. Sood and Dohroo (2005) recorded 48.3 percent loss in mother

rhizome and 65.9 percent in yield of fresh rhizome when the severity of *P. zingiberi* was 58.3 percent. Singh (2015) recorded nearly 80% disease incidence in untreated control plot infected with leaf spot disease of ginger and lowest disease of 21.3% in 0.1% carbendazim treated plots. Again Bandyopadhyay *et al.* (2015) recorded PDI of 34.24 of leaf spot disease of ginger in untreated control plots whereas 3 sprayings with 0.1% hexaconazole produced the lowest PDI of 12.12 So keeping in view of the losses caused by leaf spot pathogen and sparse information available on different aspects of the disease an attempt was made to study the morphological and cultural characters of *P. zingiberi* causing leaf spot disease of ginger.

### MATERIALS AND METHODS

**Isolation, identification and pathogenicity:** Isolation of the pathogen was done on potato dextrose agar (PDA) medium from the diseased leaf samples collected from the UBKV farm field. The inoculum of the isolated fungus was sprayed on healthy leaves of ginger plant raised in green house condition for proving pathogenicity (Ramakrishnan, 1942).

**Morphological characters:** The morphological characters of *P. zingiberi* were studied with respect to colour, shape and size of pycnidia and pycnidiospores. For this purpose, fourteen days old culture of isolate grown on PDA was studied. For pycnidial and conidial dimensions fifty pycnidia and pycnidiospores were selected for study at random from slides. The study was done with the help of Olympus CX41 model

microscope fitted with Moticom 3.0 MP. The captured images were measured microscopically with the help of Moticom software and average dimensions were worked out.

#### Cultural characters (Growth parameters)

**Suitable media for growth:** In order to find out a suitable media for the growth of the pathogen, six different media i.e. Potato dextrose agar, Czapek-Dox agar, Oat meal agar, Corn meal agar, Potato carrot agar and Malt extract agar were selected. Sood (2002) studied the growth of *P. zingiberi* in different agar media in Himachal Pradesh. Different agar media were also used by Zimowska (2013) for the growth of *Phyllosticta plantaginis*. Growth of *Phyllosticta musarum* causing leaf spot disease of banana was measured in different agar media by Ravikumara in 2011. The pathogen was inoculated on each medium and incubated with three replications at  $25 \pm 1^\circ\text{C}$ . The data pertaining to radial growth of the pathogen i.e colony diameter (cm) were recorded after 8 days. Then the growth was converted to area ( $\text{cm}^2$ ) by the help of the formula  $A = \pi r^2$ .

**Optimum temperature for growth:** The effect of temperature on vegetative growth was investigated by inoculating 9 mm discs of the fungal culture on petriplates containing PDA media. This type of study of mycelial growth of *P. zingiberi* in different temperatures was done by Cerezine *et al.* (1995). Growth of *P. plantaginis* in different temperatures was also studied by Zimowska (2013). Growth of *P. musarum* was studied in 6 different temperatures like 10, 15, 20, 25, 30, 35 C and room temperature by Ravikumara in 2011. The cultures were incubated in different temperature i.e. 20, 25, 30 and  $35^\circ\text{C}$  with three replications. The radial growth of the pathogen i.e colony diameter (cm) was recorded after 8 days. Then the growth was converted to area ( $\text{cm}^2$ ) by the help of the formula  $A = \pi r^2$ .

**Effect of different carbon source on growth:** The fungus was grown in 90 mm petriplates containing 20 ml media amended with different carbon sources such as cellulose, dextrose, fructose, maltose, starch and sucrose. Growth of *Phyllosticta* on different carbon sources was done by Tandon and Bilgrami (1957) and also by Sood (2002). Seven different carbon sources were employed to identify the best carbon that could support good growth and development of *P. musarum* (Ravikumara, 2011). The remaining procedure of plating, inoculation, incubation and recording radial growth of the fungus was same as described earlier.

## RESULTS AND DISCUSSION

The pure culture obtained from the diseased leaves of ginger showing characteristic symptoms of leaf spot was identified as *P. zingiberi* Ramakr. on the basis of morphological characters as documented in taxonomic keys (Ramakrishnan, 1942; Sood, 2002). Further pathogenicity testing proved the pathogenic behaviour of the fungus. The wounded leaves sprayed with fungal suspension (Ramakrishnan, 1942) started producing symptoms from fourth day onwards; initially the spots were small, yellow, round to oval. At later stage the spots became enlarged with irregular spots having white papery centre and yellow halo (Sood, 2002). The colony of the fungus when grown on PDA showed slow growth with smooth surface having regular margin. The colony colour exhibited a pattern of light grey at the margins with olivaceous green zonation. Mycelium was dense, suppressed and light grey in colour. Hyphae of the fungus were hyaline, aseptate and having irregular swellings. Pycnidia produced by the isolate were dark brown, ostiolate, globose to sub-globose measuring  $124.16 \mu\text{m} \times 122.96 \mu\text{m}$  in average. Pycnidiospores were also abundantly released in cirrus which were hyaline oval to bullet shaped, aseptate, uniguttulate and measured  $4.02 \mu\text{m} \times 2.35 \mu\text{m}$  in average (Table 1) which is in accordance with the findings of Ramakrishnan, 1942; Sohi *et al.*, 1964; Shukla and Haware, 1972; Sood, 2002. Ostiolate pycnidia of 63-82  $\mu\text{m}$  in size and aseptate or single celled, hyaline, oval to ellipsoidal conidia with a size of  $5.2-5.5 \mu\text{m} \times 1.8-2.2 \mu\text{m}$  was recorded by Zimowska (2013) in *P. plantaginis*. In *P. musarum* causing leaf spot of banana, the pycnidia were more or less spherical, 46-96.3  $\mu\text{m}$  in diameter on average and the pycnidiospores were hyaline, one celled, sub globose to ellipsoidal and measure  $7.5-15 \mu\text{m} \times 2.5-5 \mu\text{m}$  (Ravikumara, 2011).

The results of various media evaluated for the growth of the pathogen is presented in Table 2. It was observed that highest growth was recorded in Oat meal agar ( $26.44 \text{ cm}^2$ ) which was statistically at par with malt extract agar ( $24.04 \text{ cm}^2$ ) and potato dextrose ( $22.04 \text{ cm}^2$ ) whereas lowest growth was observed in corn meal agar ( $9.08 \text{ cm}^2$ ). This result matches with the former research done by Sood, 2002 who has reported maximum growth in PDA which was statistically *at par* with malt extract agar. Zimowska (2013) also supports our findings and reported that

**Table 1.** Morphological measurement details of *P. zingiberi*.

Average	Pycnidia				Pycnidiospores				
	Highest length	Lowest length	Highest width	Lowest width	Average	Highest length	Lowest length	Highest width	Lowest width
$124.16 \mu\text{m} \times 122.96 \mu\text{m}$	200.63 $\mu\text{m}$	66.95 $\mu\text{m}$	198.55 $\mu\text{m}$	66.95 $\mu\text{m}$	$4.02 \mu\text{m} \times 2.35 \mu\text{m}$	5.55 $\mu\text{m}$	3.13 $\mu\text{m}$	3.16 $\mu\text{m}$	1.16 $\mu\text{m}$

malt agar medium and the media made with an extract from ribwort leaves are recommended for use in characteristic observations of *P. plantaginis* as very fast sporulation occurs here. Good colony growth with a diameter of 74.67 mm and 68.17 mm was achieved in Potato Dextrose Agar and Oat Meal agar media respectively by *P. musarum* (Ravikumara, 2011).

The mycelial growth of the pathogen grown in different temperatures after 8 days of incubation is presented in Table 3. It was observed that the temperature of 25°C favoured maximum growth (24.20 cm<sup>2</sup>) whereas temperature of 35°C recorded lowest growth of *P. zingiberi* on PDA however, higher sporulation was observed in 30°C. Higher sporulation in higher temperature may be due to the fact that the fungus is exposed to stress condition in higher temperature and it is a common fact that in stress situations fungus produces more number of spores to overcome the stress. The results are in conformity with the works of Ramakrishnan, 1942; Cerezine *et. al*, 1995 and Sood, 2002 who have reported 25°C to be the best temperature regimen for the mycelial growth of the pathogen. Similar type of result was found by Zimowska (2013) who reported that 20°C to 28°C was the optimal temperature for the formation of pycnidia and conidia of *P. plantaginis*. Highest growth of *P. musarum* was recorded in 300 C which is closely followed by 250 C (Ravikumara, 2011).

**Table 2.** Effect of different agar media on the growth of *P. zingiberi*.

Agar medium	Growth in area (cm <sup>2</sup> ) at 192 hours
Cazpek–Dox	15.87
Corn meal	9.08
Malt extract	24.04*
Oat meal	26.44*
Potato carrot	12.94
Potato dextrose	22.04*
SEm ±	1.45
C.V (%)	13.67
CD (P=0.05)	4.58

Figures represented by \* do not differ significantly

**Table 3.** Effect of different temperature regimes on growth of *P. zingiberi* using Potato Dextrose Agar medium

Temperature (°C)	Growth in area (cm <sup>2</sup> ) at 192 hours
20	14.20*
25	24.2
30	10.67*
35	2.45
SEm ±	1.26
C.V (%)	17
CD (P=0.05)	4.37

Figures represented by \* do not differ significantly

**Table 4.** Effect of different carbon source on growth of *P. zingiberi* using Potato Dextrose Agar medium

Carbon Sources	Growth in area (cm <sup>2</sup> ) at 192 hours
Cellulose	14.44
Fructose	23.10*
Maltose	23.78*
Mannitol	27.67*
Starch	18.8
Sucrose	27.29*
Dextrose	24.20*
SEm ±	2.2
C.V (%)	9.13
CD (P=0.05)	3.69

Figures represented by \* do not differ significantly

The carbon source required for the fungal mycelium to attain maximum growth was found to be mannitol (27.67 cm<sup>2</sup>) followed by sucrose (27.29 cm<sup>2</sup>) and dextrose (24.20 cm<sup>2</sup>) which were statistically *at par* with each other and the lowest growth of 14.44 cm<sup>2</sup> was recorded in cellulose (Table 4). The findings corroborates with the study of Sood, 2002 who recorded maximum dry weight of mycelium in mannitol. Among different carbon sources tested, highest growth of *P. musarum* was recorded in glucose which is followed by glycerol, fructose and sucrose (Ravikumara, 2011).

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