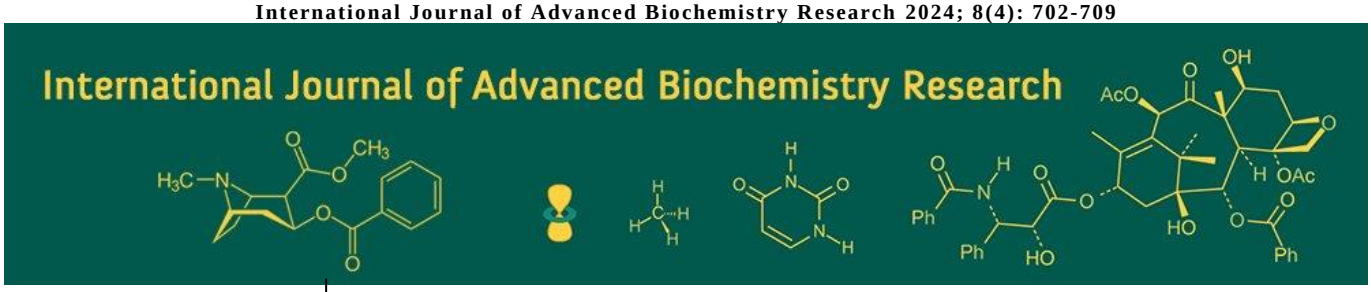

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Insights into cultural and physiological characterization of *Phyllosticta zingiberi* Ramakr. isolates causing leaf spot disease on ginger (*Zingiber officinale* Rosc.)

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Abstract
Ginger (*Zingiber officinale* Rosc.) is a perennial monocotyledonous herb belonging to the the family Zingiberaceae. The leaf spot disease in ginger caused by *Phyllosticta zingiberi* Ramakr. Occur in mild to severe form in all ginger growing areas of Karnataka. Investigation was carried out to study the cultural and physiological characteristics of the pathogen. Among different solid media tested, maximum mycelial growth was recorded on Potato dextrose agar and Richard's agar followed by Sabouraud's dextrose agar. Among the different temperature regime tested, maximum mean mycelial growth of 90.00 mm was observed at 30 °C followed by 25 °C (84.70 mm). When exposed to different light regimes, the highest mean mycelial growth of 88.10 mm was recorded when the isolates were exposed to alternate cycles of 12 hours light and 12 hours dark followed by complete dark (85.50 mm). Highest mean mycelial growth of the pathogen was recorded at pH 4.00 (86.30 mm) followed by pH 5.00 (86.00 mm). Further, maximum mean mycelial growth was recorded in media containing dextrose (83.76 mm) followed by mannitol (82.66 mm) and sucrose (76.06 mm). Highest mean mycelial growth of 90.00 mm was recorded in potassium nitrate followed by sodium nitrate (89.34 mm) and ammonium molybdate (78.16 mm) was recorded from among the different nitrogen source.

Keywords: Ginger, leaf spot, media, temperature, light regimes, pH, carbon and nitrogen sources

Introduction
Ginger (*Zingiber officinale* Rosc.) is a perennial monocotyledonous herb belonging to the the family Zingiberaceae. Ginger is an important commercial crop throughout the subtropics and tropics and cultivated for its aromatic rhizomes which are used both as spice and medicine cultivated worldwide (Sharma, 10) [10]. India is considered as "The Land of Spices" from ancient times. The country ranked first in terms of production and consumption of ginger and accounts for about 21 per cent of the global share of ginger production, followed by China (20.5%), Indonesia (12.7%), Nepal (11.5%) and Thailand (10%) (Indian Horticulture Database, 4) [4].

In India, major ginger growing states are Kerala, Sikkim, Meghalaya, West Bengal, Orissa, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Arunachal Pradesh, Uttarakhand and Himachal Pradesh. During cultivation, the crop is severely affected by various diseases of fungal, bacterial and viral origin and reduces its potential yields drastically (Dohroo, 3) [3]. Some of the important diseases include rhizome rot (*Pythium* spp., *Fusarium* spp.), leaf spot (*Phyllosticta zingiberi*), nematodes such as root knot nematode (*Meloidogyne* spp.), burrowing nematode (*Radopholus similis*), reniform nematode (*Rotylenchus reniformis*), stunt nematode (*Tylenchorhynchus* spp.) and lesion nematode (*Pratylenchus* spp.) (Sagar, 9) [9].

Among the diseases of ginger, occurrence of leaf spot disease caused by *Phyllosticta zingiberi* Ramakr. is increasing severely in all ginger growing areas of Karnataka. The disease symptoms are observed on leaves as oval or elongated spots varying in size ranging 9-10×3-4 mm. Some are small and roundish being a millimeter in length and half in breadth. The spots are almost white in the centre and have a dark brown margin, surrounded by a yellow halo.

The central portion is thin and papery and more often torn up (Ramakrishnan, 6) [6]. The disease causes significant reduction in yield due to its severe leaf spotting which destroys the chlorophyllous tissues which leads to 13 to 66 per cent yield losses (Singh, 11) [11].

There is no evidence of scientific work regarding cultural and physiological studies of ginger leaf spot. Therefore, the present study was undertaken to know the growth of the pathogen causing ginger leaf spot disease using different cultural and physiological parameters for the knowledge of nutritional pattern and factors influencing the growth of the pathogen.

Materials and Methods

Isolation

The disease specimens collected from the field during the survey was used for isolation of the pathogen. Five isolates *viz*., MND, HSN, RNP, HNP and PYP were isolated from Mandya, Hassan, Arakalgud, Holenarasipura and Periyapatna respectively and these isolates were used for further study.

Studies on the effect of different solid media on the growth of the pathogen

The effect of solid media on the growth of *P. zingiberi* was studied on nine different solid media *viz*., Potato dextrose agar (PDA), Richard's agar, Oat meal agar, Corn meal agar, Malt extract agar, Czapeks Dox agar, Sabouraud's dextrose agar (SDA), V - 8 juice agar and Ginger leaf extract agar. The isolates were incubated at 28 °C for 7 days. The radial growth (mm) of the fungal mycelia was recorded 8 days after inoculation.

Studies on the effect of temperature on the growth of the pathogen

The effect of temperature on growth of *P. zingiberi* was studied on potato dextrose agar. Five isolates were exposed to 4 different temperature levels *viz*., 20, 25, 30 and 35 °C and were replicated thrice. The radial growth of the fungus was recorded on 8th day after incubation.

Studies on the effect of pH on the growth of the pathogen

The effect of pH on growth of *P. zingiberi*. was studied on potato dextrose agar at six levels of pH *viz*., 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The adjustment of pH was done adding 0.1N Sodium hydroxide and 0.1N Hydrochloric acid.

Studies on the effect of light on the growth of the pathogen

The effect of light on growth of *P. zingiberi* was studied by exposing the pathogen to different light regimes. The plates were incubated at 28 °C for 7 days under continuous light (fluorescent light of 40 watts), alternate cycle of 12 hour light and 12 hour dark and continuous darkness. The radial growth of the fungus was recorded 8 days after incubation.

Studies on the effect of different carbon and nitrogen sources on the growth of the pathogen

Growth of *P. zingiberi* was studied on Richard's agar by replacing the carbon and nitrogen compound present in that media. The following carbohydrates were used as carbon sources *viz*., dextrose, fructose, maltose, sucrose, mannitol, lactose, starch and cellulose and nitrogen compounds such as ammonium nitrate, ammonium molybdate, ammonium oxalate, ammonium sulphate, potassium nitrate and sodium nitrate were used as nitrogen sources.

Results and Discussion

Effect of different solid media on the growth of the pathogen

Nine different solid media were used to study the cultural characters of *P. zingiberi*. Mean radial growth of all the isolates on different solid media are presented in Table 1, Plate 1. Among the 9 different solid media tested, highest mean mycelial growth of 90.00 mm was observed on Richard's agar and potato dextrose agar followed by Malt extract agar (89.00 mm) and Sabouraud's dextrose agar (89.00 mm), V-8 juice agar (86.50 mm) and Oat meal agar (86.00 mm). Lowest mean mycelial growth of 82.00 mm was observed on Ginger leaf extract agar (82.00 mm) followed by Corn meal agar (84.50 mm) and Czapeck's Dox agar (85.00 mm) When compared among all the nine different solid media, maximum mycelial growth of all five isolates was recorded on Potato dextrose agar and Richard's agar as Richard's agar media contains all three major compound for fungus growth i.e. carbon, nitrogen, phosphate. Among five isolates, four isolates showed maximum radial growth on malt extract agar. Least mycelial growth was observed on Czapeck's Dox agar followed by ginger leaf extract agar.

The result was in accordance with (Rai 5) [5] in which the highest growth of *Phyllosticta zingiberi* was recorded in malt extract agar whereas lowest growth was observed in corn meal agar, followed by Czapeck's dox agar.

Table 1: Mean radial growth of *P. zingiberi* isolates on different solid media

Solid media		Mean mycelial growth (mm)					
		MND	HSN	RNP	HNP	PYP	Mean value (mm)
V-8 juice agar		87.50	85.00	80.00	90.00	90.00	86.50
Corn meal agar		85.00	85.00	85.00	82.50	85.00	84.50
Czapeck's Dox agar		80.00	90.00	90.00	75.00	90.00	85.00
Sabouraud's dextrose agar		90.00	90.00	90.00	90.00	85.00	89.00
Ginger leaf extract agar		80.00	85.00	82.50	77.50	85.00	82.00
Malt extract agar		90.00	85.00	90.00	90.00	90.00	89.00
Oat meal agar		90.00	90.00	90.00	80.00	80.00	86.00
Potato dextrose agar		90.00	90.00	90.00	90.00	90.00	90.00
Richard's agar		90.00	90.00	90.00	90.00	90.00	90.00
F		**					
S.Em ±	I	0.03					
	M	0.05					
	I*M	0.10					
C.D. @ 1%	I	0.13					
	M	0.17					
	I*M	0.38					

*I-Isolates, M-Different media, I*M- Interaction

Effect of different temperature on colony diameter of the pathogen

The growth of *P. zingiberi* at four different temperature levels *viz*., 20, 25, 30 and 35 °C was studied on potato dextrose agar. Observations were recorded after 7days of incubation and mean mycelial growth of *P. zingiberi* isolates are presented in Table 2, Plate 2.

Maximum mean mycelial growth of 90.00 mm was observed at 30 °C followed by 25 °C (84.70 mm) and the lowest mean radial growth of 56.12 mm was observed when exposed to 20 °C followed by 35 °C (77.46 mm).

Table 2: Effect of different temperature on radial growth of *P. zingiberi*

Temperature		Mean mycelial growth of the isolates (mm)					
		MND	HSN	RNP	HNP	PYP	Mean value (mm)
20^0		49.30	52.70	59.30	59.30	60.00	56.12
25^0		90.00	78.50	85.00	90.00	80.00	84.70
30^0		90.00	90.00	90.00	90.00	90.00	90.00
35^0		70.00	78.30	77.30	81.70	80.00	77.46
F		**					
S.Em±	T	0.02					
	I	0.03					
	T*I	0.11					
C.D.@1%	T	0.08					
	I	0.10					
	T*I	0.40					

I-Isolates, T- Temperature regimes, T*I- Interactions

These results are in line with (Zimowska, 12) [12], wherein he reported that the temperature range of 16 °C to 28 °C was considered optimal for the growth of the fungus *P. plantaginis* which caused leaf spot in *Plantago lanceolata.* (Rai, 5) [5] also reported that the temperature of 25 °C favoured maximum growth (24.20 cm^2) whereas temperature of 35 °C recorded lowest growth of *P. zingiberi* on PDA. Similar type of result was found by (Ramakrishnan, 6) [6] who have reported 25 °C to be the best temperature regimen for the mycelial growth of the pathogen *P. zingiberi*. Highest growth of *P. musarum* causing freckle disease in banana was recorded in 30 °C which is closely followed by 25 °C (Ravikumara, 8) [8].

Effect of light on radial growth of the pathogen

All the isolates of *P. zingiberi* was exposed to different light regimes such as complete dark (24 h), complete light (24 h) and alternate cycles of light and dark (12 h light and 12 h dark) for a period of eight days. Radial mycelial growths of all isolates at different light regimes are presented in Table 3, Plate 3.

The highest mean mycelial growth of 88.10 mm was recorded when the isolated were exposed to alternate cycles of 12 hours light and 12 hours dark followed by complete dark (85.50 mm) and the lowest mean mycelial growth of 81.50 mm was recorded when exposed to complete light for 24 hours.

Table 3: Effect of different light regimes on the growth of the pathogen

Light regimes		Mean mycelial growth of the isolates (mm)					Mean value (mm)
		MND	HSN	RNP	HNP	PYP	
Complete dark (24 h)		75.00	90.00	90.00	90.00	82.50	85.50
Alternate (12 h light and 12 h dark)		90.00	90.00	85.00	90.00	85.50	88.10
Complete light (24 h)		70.00	85.00	77.50	90.00	85.00	81.50
F		**					
S.Em±	I	0.14					
	L	0.12					
	I*L	0.23					
C.D.@1%	I	0.57					
	L	0.49					
	I*L	0.94					

I-Isolates, L- Light regimes, I*L-Interations

From the above investigation, it was reported that the growth of all the isolates varied with different light regimes. The highest mean mycelial growth was recorded when the isolates were exposed to alternate cycles of light and dark, whereas the lowest growth was recorded when exposed to complete light for 24 hours. These results are in line with (Chaithra, 2) [2], wherein they reported that the growth of *Fusarium oxysporum* f. sp. *zingiberi* causing wet rot of ginger produced significantly higher colony growth under 48 h (84.33 mm).

Effect of pH on mycelial growth of the pathogen

Growth of the pathogen *P. zingiberi* was studied at different pH levels (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) on potato dextrose agar. The results are presented in Table 4, Plate 4.

Among the different levels of hydrogen ion concentration tested, the highest mean mycelial growth of 86.30 mm was recorded at pH 4.00 followed by pH 5.00 (86.00 mm) and pH 6.00 (86.00 mm). The lowest mean mycelial growth of 63.50 mm was recorded at pH 9.00 followed by pH 8.00 (81.80 mm).

Table 4: Effect of pH on mycelial growth of the pathogen

pH		Mean mycelial growth (mm)					Mean value (mm)
		MND	HSN	RNP	HNP	PYP	
4.00		87.50	82.50	81.50	90.00	90.00	86.30
5.00		90.00	75.00	85.00	90.00	90.00	86.00
6.00		90.00	82.50	87.50	80.00	90.00	86.00
7.00		87.75	75.00	90.00	90.00	87.50	86.05
8.00		85.50	75.50	85.50	90.00	72.50	81.80
9.00		65.00	62.50	57.50	65.00	67.50	63.50
F		**					
S.Em ±	I	0.12					
	P	0.13					
	I*P	0.29					
C.D.@1%	I	0.47					
	P	0.51					
	I*P	1.13					

I-Isolates, P-pH, I*P-Interaction

The results recorded in the present investigation are similar to the results obtained by (Chaithra, 2) [2], wherein they reported that the growth of *Fusarium oxysporum* f. sp. *zingiberi* causing wet rot of ginger produced highest colony growth of 86.17 mm at 7.00 pH followed by pH 6.50 (84.83 mm).

Table 5: Growth of *P. zingiberi* isolates on different carbon sources

Different carbon source		Mean mycelial growth of the isolates (mm)					Mean value (mm)
		MND	HSN	RNP	HNP	PYP	
Dextrose		83.30	83.00	85.00	81.70	85.80	83.76
Fructose		60.70	69.00	75.00	83.30	85.00	74.60
Maltose		53.70	67.70	74.20	80.00	81.70	71.46
Sucrose		62.70	75.00	81.70	76.70	84.20	76.06
Mannitol		78.30	80.00	85.00	83.30	86.70	82.66
Lactose		68.30	61.70	85.80	49.30	71.70	67.36
Starch		63.00	60.70	74.30	58.30	77.70	66.80
Cellulose		49.30	41.70	47.70	31.70	55.30	45.14
S.Em ±	C	0.07					
	I	0.06					
	C*I	0.16					
C.D.@1%	C	0.27					
	I	0.21					
	C*I	0.60					

C-Carbon source, I-Isolates, C*I-Interaction

Effect of different carbon sources on mycelial growth of the pathogen

Growth of five isolates was studied on eight different carbon sources on Richard's agar and the results are presented in Table 5

The maximum mean mycelial growth of 83.76 mm was recorded in media containing dextrose followed by mannitol (82.66 mm), sucrose (76.06 mm), fructose (74.60 mm), maltose (71.46 mm). Least mean mycelial growth of 45.14 mm was recorded in media containing cellulose followed by starch (66.80 mm) and lactose (67.36 mm).

From the present study it was concluded that the highest growth of the pathogen was recorded in media containing mannitol, followed by dextrose. The least mean mycelial growth was recorded in cellulose, followed by starch. The results obtained are in line with (Rai, 5) [5]. They recorded that carbon source required for the fungal mycelium to attain maximum growth was mannitol (27.67 cm^2) followed by sucrose (27.29 cm^2) and dextrose (24.20 cm^2) which were statistically at par with each other and the lowest growth of 14.44 cm^2 was recorded in cellulose. (Ravikumara, 8) [8] also reported that among different carbon sources tested, highest growth of *P. musarum* was recorded in glucose which was followed by glycerol, fructose and sucrose.

Effect of different nitrogen sources on mycelial growth of the pathogen

The effect of six different nitrogen sources on the growth of *P. zingiberi* isolates was studied in Richard's agar. Observations were recorded after 7 days of incubation and results are presented in Table 6, Plate 5.

The highest mean mycelial growth of 90.00 mm was recorded in potassium nitrate followed by sodium nitrate (89.34 mm), ammonium molybdate (78.16 mm) and ammonium nitrate (70.84 mm). The least mean mycelial growth of 17.14 mm was recorded in ammonium sulphate followed by ammonium oxalate (55.22 mm).

Above investigation reported that the maximum mycelial growth was observed when the media contained potassium nitrate as a nitrogen source followed by sodium nitrate. The least mean mycelial growth was observed in ammonium sulphate followed by ammonium oxalate. These results are in line with (Ramjegathesh and Ebenezar, 7) [7] where they reported that potassium nitrate produced maximum mean mycelial growth (9.00 cm) followed by sodium nitrate (7.32 cm) and ammonium molybdate (6.18 cm). (Admassie, 1) [1]

also reported that the mean value which was obtained by sodium nitrate phosphate (18±00 mm) supported the maximum growth followed by potassium nitrate (17±00 mm).

Table 6: Growth of *P. zingiberi* isolates on different nitrogen sources

Different nitrogen source		Mean mycelial growth (mm) of the isolates					Mean value (mm)
		MND	HSN	RNP	HNP	PYP	
Ammonium nitrate		67.50	77.50	70.00	74.20	65.00	70.84
Ammonium molybdate		90.00	73.30	90.00	87.50	50.00	78.16
Ammonium oxalate		28.30	65.00	72.70	60.80	49.30	55.22
Ammonium sulphate		20.70	11.50	13.30	23.50	16.70	17.14
Sodium nitrate		90.00	86.70	90.00	90.00	90.00	89.34
Potassium nitrate		90.00	90.00	90.00	90.00	90.00	90.00
S.Em ±	N	0.06					
	I	0.06					
	N*I	0.14					
C.D.@ 1%	N	0.23					
	I	0.21					
	N*I	0.52					

N-Nitrogen source, I-Isolates, N*I-Interaction

T_1- V-8 juice agar
T_2- Corn meal agar
T_3- Czapeck's dox agar
T_4- Sabouraud's dextrose agar
T_5- Ginger leaf extract agar
T_6- Malt extract agar
T_7- Oat meal agar
T_8- Potato dextrose agar
T_9- Richard's agar

Plate 1: Mycelial growth of *P. zingiberi* isolates on different solid media

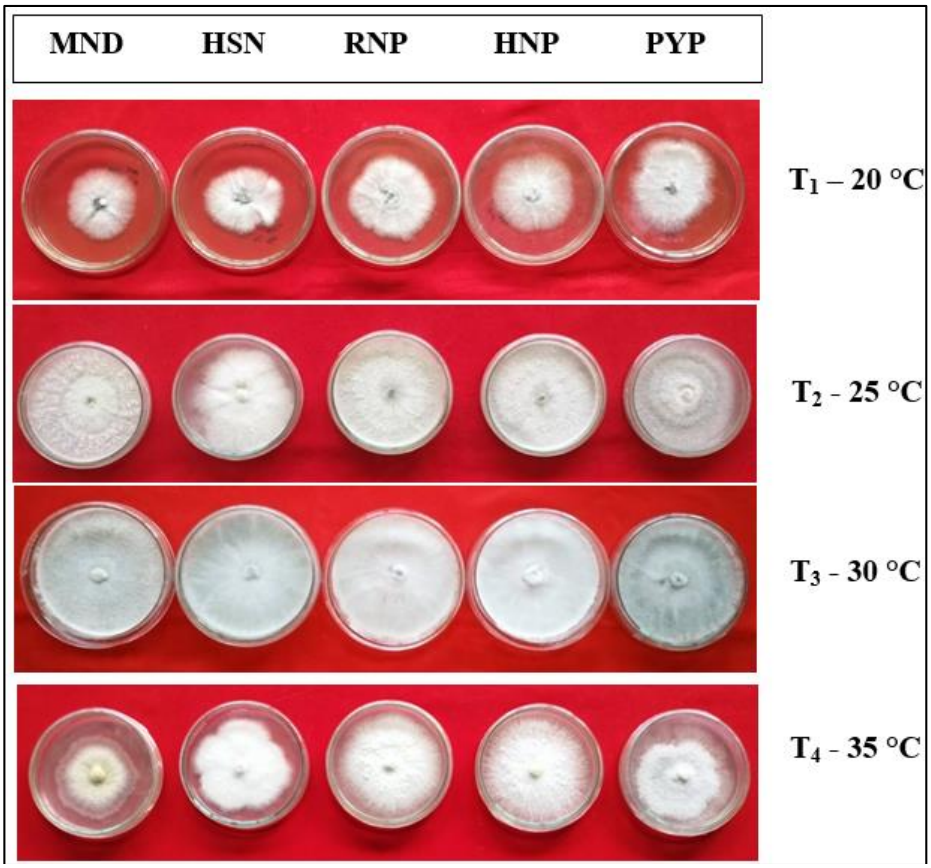
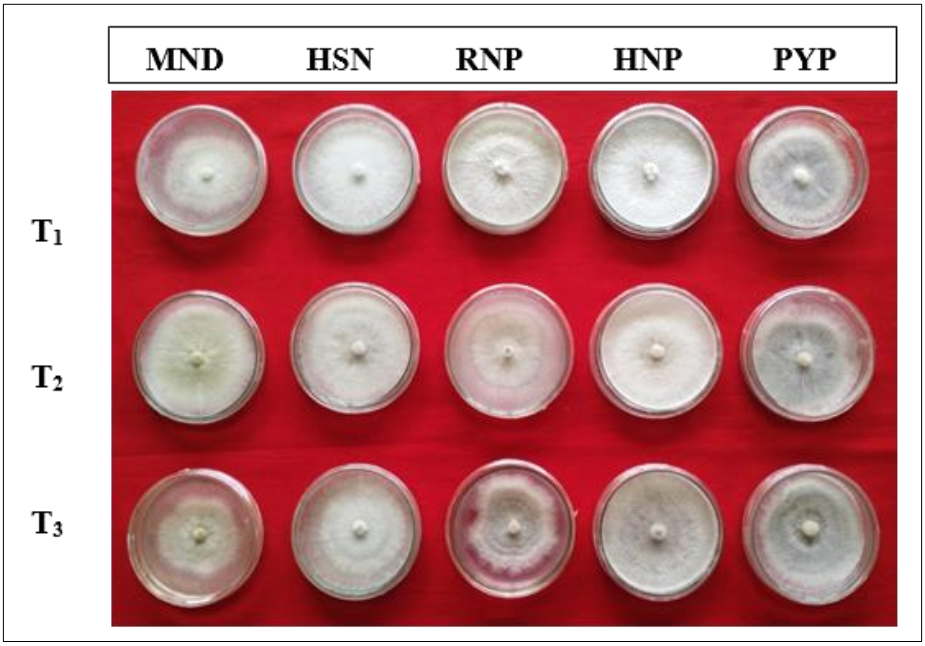


Plate 2: Mycelial growth of *P. zingiberi* isolates on potato dextrose agar at different temperature regimes



T_1- Complete dark (24 hours)
T_2- Alternate cycles of 12 hours light and 12 hours dark
T_3- Complete light (24 hours)

Plate 3: Mycelial growth of *P. zingiberi* isolates at different light regimes

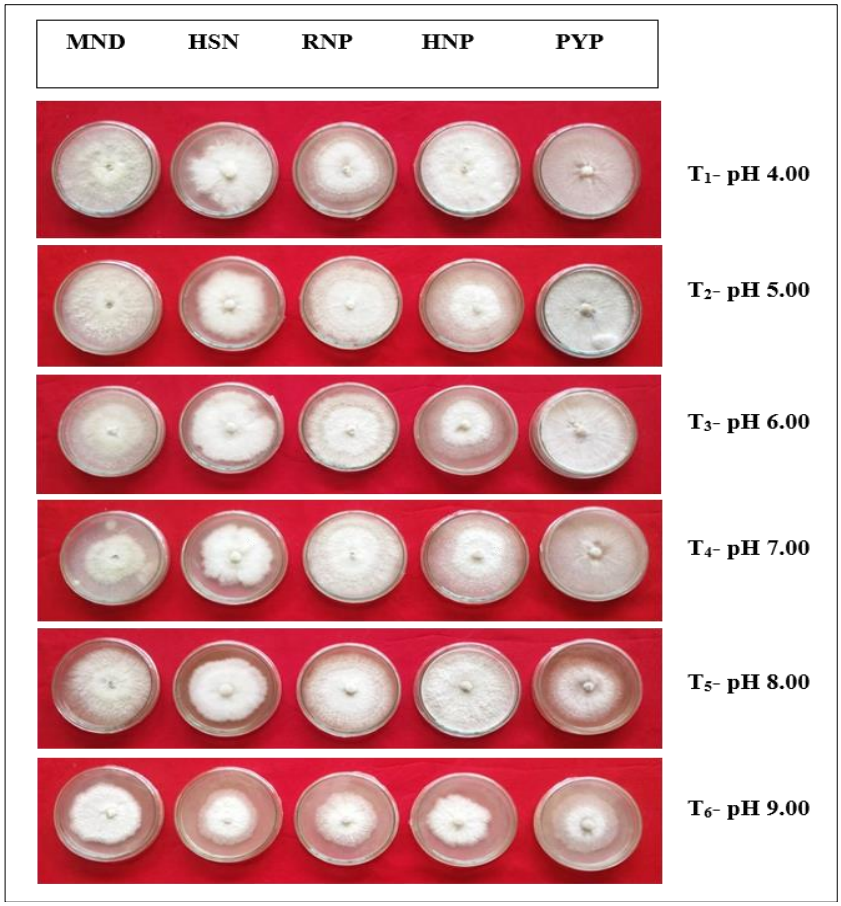
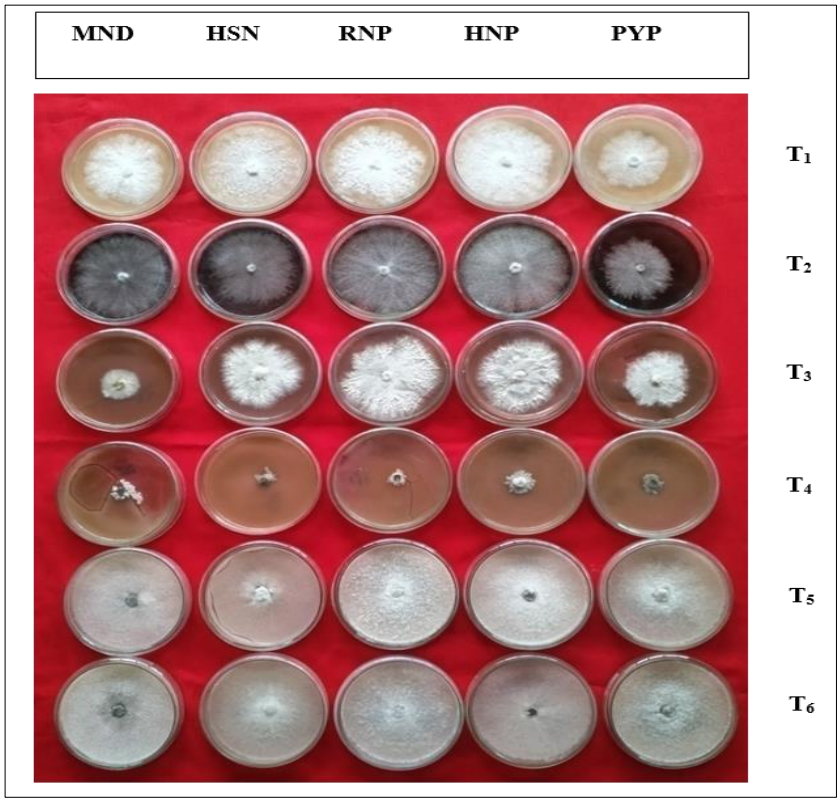


Plate 4: Mycelial growth of *P. zingiberi* isolates at different pH on potato dextrose agar



T_1 - Ammonium nitrate
T_2 - Ammonium molybdate
T_3 - Ammonium oxalate
T_4 - Ammonium sulphate
T_5 - Sodium nitrate
T_6 - Potassium nitrate

Plate 5: Mycelial growth of *P. zingiberi* isolates on different nitrogen sources

Conclusion

The study has strengthened our knowledge to know the cultural and physiological effects on the growth of *Phyllosticta zingiberi* Ramakr. causing ginger leaf spot. The study revealed the favourable cultural and physiological factors of *Phyllosticta zingiberi in vitro* which provide insights that may be useful in developing more effective control measures. Further field studies have to be done to alter the pathogen behaviour in natural field condition and develop strategies to mitigate its impact on ginger production.

Declaration

The authors declare no conflict of interest.

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