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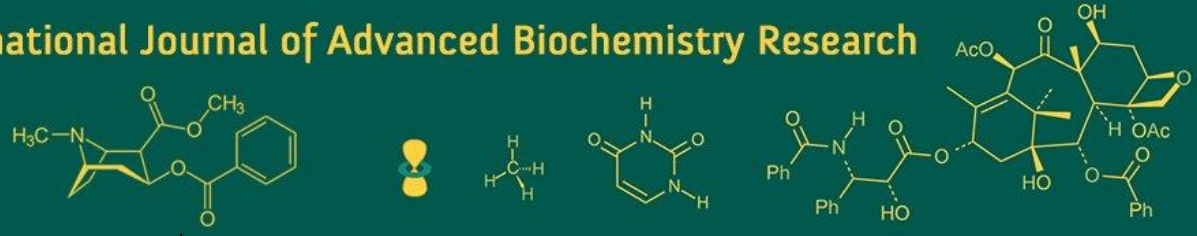

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Cultural and physiological characterization of *Pestalotiopsis* sp. causing grey leaf blight of coconut

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Abstract
Coconut (*Cocos nucifera* L.) is referred to as a 'KalpaVriksha' in ancient Indian literature, which implies a tree that gives all of life's basic necessities. Grey leaf blight, caused by *Pestalotiopsis* sp., is one of the most serious fungal diseases affecting coconut. Grey blight symptoms include minute yellow or brown dots encircled by a greyish ring on the leaflets. The cultural features of the pathogen were examined on five non-synthetic/semi-synthetic (Potato dextrose agar, Oatmeal agar, Bennet's agar, Host leaf extract agar and Sabouraud's dextrose agar) and two synthetic solid and liquid media (Richards' agar and Czapek's (Dox) agar). In which *Pestalotiopsis* sp. exhibits the highest myceial growth and sporulation on Potato dextrose agar (90 mm), Oatmeal agar (90 mm), and Sabouraud's dextrose agar (90 mm) and the least myceial growth and sporulation on host leaf extract agar (84 mm) and Richard's agar (67.50 mm). The pathogen was incubated at various temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C) and pH (3, 4, 5, 6, 7, and 8), in order to determine the optimum pH and temperature for their growth. Physiological studies revealed that the ideal pH and temperature for Pestalotiopsis sp. growth are 5 to 6 and 20 to 25 °C, respectively.

Keywords: *Pestalotiopsis*, agar, pH, mycelia, temperature

Introduction
Coconut palm (*Cocos nucifera* L.) is a member of the family Arecaceae (palm family) and the only species of the genus *Cocos*. The coconut palm is endearingly called 'Kalpa Vriksha, 'meaning the tree of heaven. It provides food, fuel, cosmetics, medicine and building materials, among many other uses. Coconut is a major plantation crop grown in the world in an area of 11,906 thousand hectares with a production of 67128 million nuts. In India, coconut is produced in an area of 2150.89thousand hectares with a production of 21288.24 million nuts. Coconut is grown in 18 Indian states and three union territories. In India, the four southern states of Kerala, Tamil Nadu, Karnataka, and Telangana account for more than 90% of the area and production of coconut (CBD, 2019) [3]. Coconut contributes more than Rs. 34100 crores to the country's GDP, in addition to 3975 crores in export revenues. Coconut provides a living for 80 million people worldwide, including more than 10 million in India (Anon, 2018) [2].
Over a dozen and a half fungus from India have been documented to cause various coconut leaf diseases. Around 40 leaf disease-causing fungus have been reported from the world's major coconut-growing countries. The most prevalent disease recorded from 28 nations was grey leaf blight (Joseph and Radha, 1979; Koshi, 2000; and Doraiswamy *et al*., 2003) [7, 8, 4]. A preliminary field survey was done in the major coconut growing region of Karnataka to gather grey blight infested leaves (*Pestalotiopsis* sp.). In this article related to study culture characteristics of the pathogen which helps in understanding the nature and variability of the pathogen, and physiological studies help to know the ideal pH and temperature of a pathogen to thrive and cause palm infection.

Materials and Methods
Cultural studies
The cultural features of single spore isolation of pathogens were examined on five non-synthetic/semi-synthetic (Potato dextrose agar, Oatmeal agar, Bennet's agar, Host leaf extract

agar and Sabouraud's dextrose agar) and two synthetic solid and liquid media (Richards' agar and Czapek's (Dox) agar). The composition and the production of the aforementioned synthetic and semi-synthetic media were derived from Ainsworth and Bisby's 'Dictionary of the Fungi' by Ainsworth (1971) [1] and Tuite (1969)'s [2] plant pathological methods, fungi, and bacteria.

Each of the above-mentioned mediums was put aseptically into 90 mm diameter Petri plates. After solidification, five mm discs of the *Pestalotiopsis* sp. were selected from an actively growing culture using a cork borer and a single disc placed at the center of the Petri plate. Each experiment was duplicated three times and incubated for 12 days at 27 °C. The colony diameter, colony colour, and sporulation were noted as cultural characteristics. The sporulation was graded as follows.

The sporulation was graded as follows.

Sl. No.	Score	Grade	Conidia/microscopic field (40x)
1	++++	Excellent	>75
2	+++	Good	50-75
3	++	Moderate	25-50
4	+	Poor	1-25
5	-	No sporulation	---

Physiological studies

Growth tests at different temperature

Block of agar containing mycelia of the *Pestalotiopsis* sp.was inoculated onto potato dextrose broth (PDB). One block of agar containing mycelium of the tested *Pestalotiopsis* sp.was inoculated into the media. The isolate was incubated at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C. The mycelium of *Pestalotiopsis* sp.was filtered by using the known weight of dried filter paper. The mycelium was dried for two days at 60 °C. Then, the dried weight of the mycelia was determined.

Growth tests at different pH

Thirty ml of potato dextrose broth (PDB) was placed in a 150 ml conical flask and the pH was adjusted by adding HCl or NaOH. The tested pH values were 3, 4, 5, 6, 7, 8 and 9. The media were autoclaved at 15 psi and 120 °C for 15 minutes. One block of agar containing mycelium of the tested *Pestalotiopsis* sp. was inoculated into the media. The inoculated media were incubated at 25 °C for seven days. The mycelium of *Pestalotiopsis* sp. was filtered by using the known weight of dried filter paper. The mycelium was dried for two days at 60 °C. Then, the dried weight of the mycelia was determined.

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

Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the standard statistical procedure adopted for completely randomized design and interpretation of data were carried out following Walter (1997) [13]. The level of significance used in 'F' and 'T' test was P = 0.05 and P = 0.01. Critical differences were calculated wherever 'F' test was significant.

Results

Cultural studies

The results of the isolate revealed that amongst the all solid media tried, substantially highest mycelial growth was achieved in Potato dextrose agar, Oatmeal agar and Sabouraud's dextrose agar (90.00 mm), the next best in order of merit Czapek'sdox agar (89.00 mm) followed by Bennet agar (87.00 mm) and the minimum radial growth was achieved in Richard's synthetic agar (67.50 mm). Excellent sporulation was reported in all solid media that were tried except for Richard's synthetic agar and host leaf extract (Table 1 and Fig.1).

Physiological studies

For this present study, the influence of physiological conditions on *Pestalotiopsis* sp. For particular pH and temperature, growth was analyzed by obtaining dry mycelial weight from the fungal inoculated potato dextrose broth shown in table 2

The maximum dry mycelial weight obtained at pH 6 (300.00 mg) followed by pH 5 (290.00 mg) and the least dry mycelial weight obtained at pH 9 (106.66 mg) followed by pH8 (126.66 mg) among the various pH studies results were depicted in fig. 2.

The maximum dry mycelial weight obtained at 25 °C (296.77 mg) followed by 20 °C (280.00 mg) and the least dry mycelial weight obtained at 5 °C (103.33 mg) followed by 10 °C (115.00mg) among various temperatures (Fig. 3).

Discussion

Fungi require nutrients and food for their growth and reproduction. So, for artificial culturing of fungi on different media containing different compositions are used to know their growth with respect to particular media. Various media were used to understand which media supports the fungus best growth during *in vitro* culturing of the fungus. The radial growth of the fungus was measured on different solid media used under the study. So other media, including synthetic and semi-synthetic media, were used against different isolates of *Pestalotiopsis* sp. Among seven different media used in which *Pestalotiopsis* sp. significantly shows the highest mycelial growth and sporulation was observed in Potato dextrose agar (PDA), Oatmeal agar and Sabouraud's dextrose agar (SDA), the next best in order of merit was Czapek's dox agar and minimum radial growth was observed in Richard's synthetic agar and host leaf extract. Potato dextrose agar, Oatmeal agar and Sabouraud's dextrose agar were best suited to almost all isolates which may be because they contain a high ratio of carbohydrates and nitrogen sources which supports to the growth of the fungus. Similar results were also recorded by Pruthviraj (2018) [9], Fovo *et al.* (2017) [5] and Rokade (2009) [10].

From the results of the effect of different pH and temperature on the fungus' growth and maximum dry mycelial growth was observed at pH 6 and 25 °C temperature and the least dry mycelial growth were obtained at pH 9 and 5 °C temperature. Therefore, optimum pH and temperature for the *Pestalotiopsis* sp. werebrought under this study was 5 to 6 and 20 to 25°C, respectively. Similar results were also obtained in *P. palmarum*, *P fici* and *P. guepinii* (Zahra Ibrahim El-Gali, 2017) [14], followed by Sawant and Raut (1995) [11] in *P. mangiferae* and Hopkins and Mc-Quilken (2000) [6] in *P. sydowiana*.

Table 1: Cultural characteristics of *Pestalotiopsis* sp. of on different solid media

Sl. No.	Different Media	Mycelium				Sporulation
		Radial growth (mm)#	Colour	Type of growth	Pigmentation	
1	Host leaf extract agar	84.50	Light white	Flat circular	-	+
2	Oatmeal agar	90.00	White	Flat circular	Black	++++
3	Potato dextrose agar	90.00	White	Flat circular	Black	++++
4	Sabouraud's dextrose agar	90.00	White	Flat circular	Black	++++
5	Bennet's agar	87.00	White	Flat circular with wavy margin at the periphery	Black	++++
6	Richard's agar	67.50	Dull white	Flat circular	Black	+++
7	Czapek's (Dox) agar	89.00	White	Flat circular with wavy margin at the periphery	Black	++++
S.Em. ±		**0.99**				
CD at 1%		**3.03**				

#Mean of three replication

Sporulation	conidia/microscope field (40X)
++++	>75
+++	50-75
++	25-50
+	1-25
-	No conidia

Table 2: Effect of different pH and temperature on growth of *Pestalotiopsis* sp. causing grey leaf blight of coconut

Sl. No	pH	Dry mycelial weight(mg)#	Temperature (°C)	Dry mycelial weight(mg)
1	3	133.33	5	103.33
2	4	230.00	10	115.00
3	5	290.00	15	178.33
4	6	300.00	20	280.00
5	7	253.33	25	296.77
6	8	126.66	30	253.33
7	9	106.66	35	212.90
S.Em.±		2.53		2.51
CD @ 1%		7.75		7.70

(#Mean of three replication)

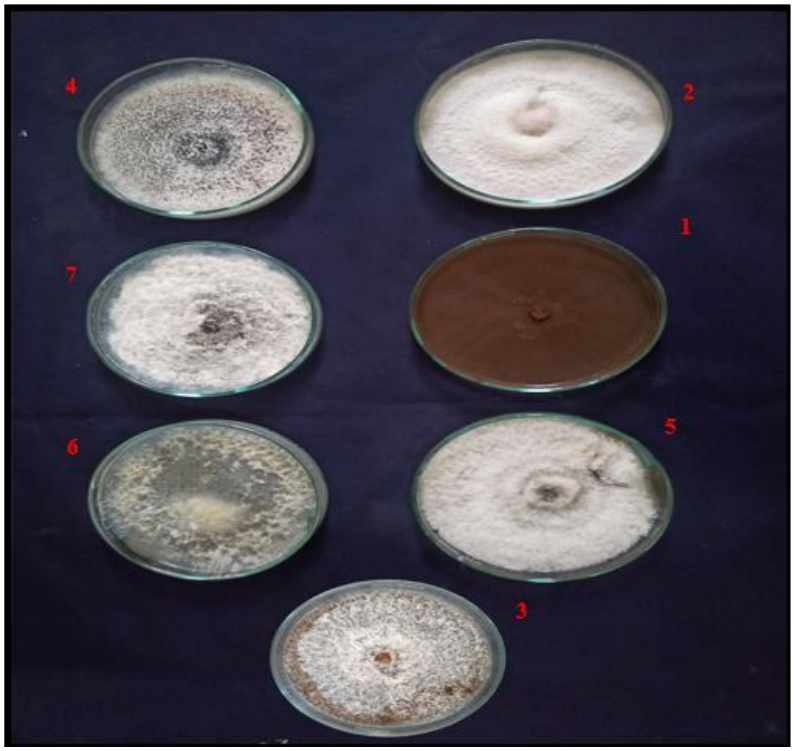

Fig 1: Cultural characteristics of *Pestalotiopsis* sp. on different solid media (1.Host leaf extract, 2. Oat meal agar, 3.Potato dextrose agar, 4. Sabouraud's dextrose agar, 5. Bennet's agar, 6. Richard's agar, 7. Czapek's (Dox) agar).

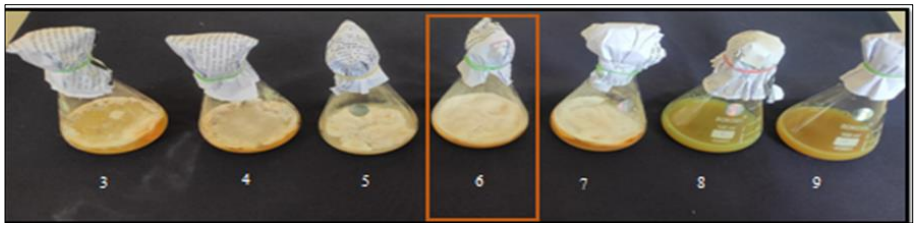

Fig 2: Effect of different p^H on growth of *Pestalotiopsis* sp.

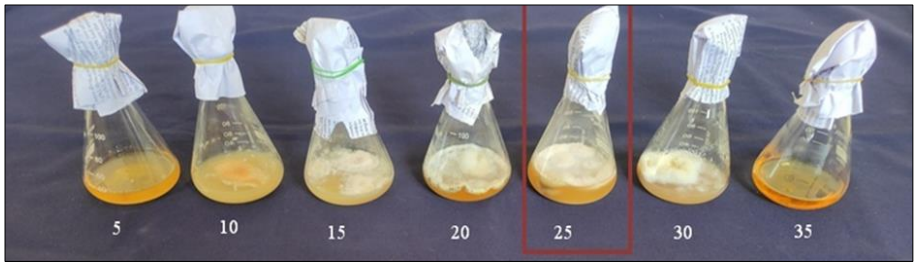

Fig 3: Effect of different temperature (°C) on growth of *Pestalotiopsis* sp.

Conclusion

In conclusion, the cultural and physiological studies conducted on *Pestalotiopsis* sp. shed light on crucial factors influencing its growth and development. Amongst the solid media tested, Potato dextrose agar, Oatmeal agar, and Sabouraud's dextrose agar exhibited the highest mycelial growth, while Richard's synthetic agar showed the least. These findings underscore the significance of nutrient composition in supporting fungal growth. Furthermore, the physiological conditions, particularly pH and temperature, significantly influenced the dry mycelial weight, with optimal growth observed at pH 6 and 25 °C. These results provide valuable insights into the optimal conditions for cultivating *Pestalotiopsis* sp., crucial for various applications in research and industry. Further studies aligning with these findings could contribute to a deeper understanding of fungal biology and enhance cultivation techniques.

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