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Effect of different media and temperature on mycelial growth of *Pestalotiopsis* sp., incitant of leaf spot and blight disease of cashew in western part of West Bengal

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ABSTRACT: Cashew leaf spot and blight disease caused by *Pestalotiopsis* sp. in the western region of West Bengal, where cashews are grown, is one of the most prevalent and emerging foliar disease and primarily affects older leaves. It severely affects the yield of the crop. The knowledge of the influence of temperature and nutritional factors on mycelial growth, sporulation and pathogenic fitness is of utmost need for understanding the physiology of this dreaded pathogen. Cultural and physiological studies of *Pestalotiopsis* sp. were conducted at Plant Bacteriology Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya during 2021-2022. The cultural studies were conducted to know the effect of different media on growth and sporulation of *Pestalotiopsis* sp. Among the different media, the maximum mycelial growth of *Pestalotiopsis* sp. was observed on Oat meal agar (OMA) medium (8.9 cm) followed by Potato dextrose agar (PDA) at 6 days after inoculation. Irrespective of different media, the highest mycelial growth rate (13.84 mm / day) and AUMPC (174.72) of *Pestalotiopsis* sp. was recorded at 22ºC followed by 27˚C. However, highest sporulation was observed on potato dextrose agar (3.14×10^{-5}/ mm^2) followed by oat meal agar (2.35×10^{5}/ mm^2) whereas it was least on Czapeck's dox agar medium (7.85×10^{-4}/ mm^2). The present findings thus, indicated that 22-25ºC was found to be the favorable temperature for mycelia growth of *Pestalotiopsis* sp. causing leaf spot and blight disease of cashewnut. Pathogenicity testing of *Pestalotiopsis* sp. was done on three different hosts (Mango, cashew and jackfruit) through detached leaf assay. Among these three hosts, jackfruit is found to be the most susceptible host for *Pestalotiopsis* sp sym-1 (cashew) isolate.

Keywords: Leaf spot, Blight, Growth rate, *Pestalotiopsis* sp., Sporulation.

INTRODUCTION

Cashew, (*Anacardium occidentale* L) belonging to the Anacardiaceae family, originally native of Brazil but was brought to India around five centuries ago (Khatoon *et al.,* 2017). It has now become a significant cash crop, with India being the world's second-largest producer, closely following Brazil (Shanthi and Vittal, 2012). With an annual cashew nut production of approximately 35.000 tonnes, provides income for more than 45.000 households (Ah-You *et al*., 2007). In contemporary times, cashew has emerged as one of the foremost horticultural crops in the nation with a strong focus on exports (Wonni *et al.,* 2017). Furthermore, the country has witnessed a steady rise in the establishment of processing facilities each year. Beyond its socio-economic significance, the cashew tree also plays a role in ecology and environmental conservation. It serves as a fire barrier along forest boundaries or in afforestation plans (Araújo, 2013). Additionally, it provides a protective vegetable cover and permits intercalated food crops. Cashew is unfortunately threatened by a wide range of biotic and abiotic stresses, which results in significant output losses. The most harmful biotic constraints are diseases and pests, which lower the supply of cashew nuts in both quality and quantity (Audouin, 2014). There are more than twelve diseases that affect cashew plants globally. The most significant diseases among cashew-producing nations that cause serious damages include anthracnose foliar blight, fruit rot, and gummosis of twigs and trunk (NARI, 2009). Cashew leaf spot and blight incited by *Pestalotiopsis* sp. in the western region of West Bengal, where cashews are grown, is one of the most prevalent foliar diseases and primarily affects older leaves (Patsa *et al.,* 2023). The changeable weather condition interacting with high density planting in cashew coupled with orchard management system of the area trigger the pathogenic fitness of *Pestalotiopsis* sp. and poses a serious threat of crop production in this region. Although *Pestalotiopsis* sp. fungus is cosmopolitan in nature and it requires several specific compounds for their growth (Maharachchikumbura, *et al.,* 2011). Considering the limited research conducted on fungal diseases affecting cashew plants specially cashew leaf spot and blight caused by *Pestalotiopsis* sp. under

western part of West Bengal and influence of temperature and nutritional condition on mycelial growth, sporulation and pathogenic fitness of this dreaded pathogen. The aim of this study was to know effect of different media and temperature on mycelial growth and sporulation of *Pestalotiopsis* sp.

MATERIALS AND METHODS

Disease sample collection and isolation of pathogen: Cashew leaves showing the typical symptoms of Pestalotiopsis leaf spot and blight disease were collected from the Cashew orchards of Regional Research Sub-Station (RRSS) of Bidhan Chandra Krishi Viswavidyalaya in Jhargram, West Bengal. With sterile gloves on, the leaves were gently broken at the petiole region by hand, bulked in batches of 10 petioles, and placed in sterile bags. These leaves were delivered to the lab in ice and kept there at 4 ºC until processing.

Isolation and purification of pathogen: Along with some healthy portions, the diseased cashew leaf portions were cut into smaller pieces and surface sterilised for 60 seconds with a solution of 1 percent sodium hypochlorite. To completely remove all traces of sodium hypochlorite, these tissue fragments were extensively cleansed in sterile distilled water before being aseptically transferred to sterile potato dextrose agar (PDA) plates. After that, the plates were incubated at 27 °C in a BOD incubator while sporulation and fungus development were periodically monitored. Colonies that formed from the fragments were identified by studying the features of the mycelium and spores under a microscope. They were located and then transferred to fresh PDA slants for later usage.

Multiplication of pathogen: To sustain a pristine culture of the pathogenic microorganism, a 5mm circular section of mycelium from a previous culture was transferred onto a fresh medium consisting of potato dextrose agar. This culture was subsequently placed in an incubator at a temperature range of 25±2°C for a duration spanning between 10 to 15 days, allowing for the fungus to grow under controlled laboratory conditions.

Identification of the Pathogen: Isolates of *Pestalotiopsis* sp. obtained from single spores were grown on PDA (potato dextrose agar) medium. These cultures were then placed in a controlled environment at 24±1 ºC under continuous light conditions, and their cultural characteristics were observed after a 7-day period. To initially identify the pathogen, we compared its morphological features, such as the size of conidia, the colour and length of median cells, the length of apical appendages, using the criteria outlined by Keith *et al.* (2006) for the identification and characterization of *Pestalotiopsis* sp. responsible for causing scab disease in guava.

Pathogenicity test. Fresh and disease-free young cashew leaves were gathered, cleaned extensively with tap water, treated with a 1% solution of sodium hypochlorite, and then rinsed with sterile distilled water. A small disc obtained from the actively developing mycelium in the Petri dish was positioned onto these healthy cashew leaves, which had previously been subjected to injury before inoculation. To serve as a control, another set of leaves was sprayed solely with sterile water. Additionally, a moist cotton swab was positioned at the petiole's base. The leaves that were subjected to inoculation were placed inside a humid chamber and kept at a constant temperature of 24±1 ºC. Observations were made every two days to monitor the development of symptoms. In order to satisfy Koch's postulates, the organism was subsequently retrieved from these artificially inoculated leaves using the same culture medium, and the characteristics of the colony were compared to those of the original culture of the tested pathogen. This pathogenicity test was studied on different host (Cashew, Mango and Jackfruit).

Effect of different media on growth and sporulation of *Pestalotiopsis* sp. The study aimed to assess how different culture media influenced the cultural traits of *Pestalotiopsis* sp. To achieve this, 20 ml of each molten medium was poured into individual 90 mm Petri plates. A 5 mm disc of the fungal culture was obtained using a sterilized cork borer, taken from the edge of a 7-day-old, uncontaminated *Pestalotiopsis* sp. culture that had been cultivated on potato dextrose agar. A single culture disc was positioned upside down at the centre of each Petri plate. These plates were then placed in an incubator at a controlled temperature of 25±2°C, and the growth of the culture was assessed individually for each medium. For each medium, three replicates were maintained.

Study on spore concentration in different media using Haemocytometer. The fungal mycelial disc was impregnated in a sterilized test tube containing 5 ml of sterilized water and vortexed for 1-2 minutes for preparation of spore suspension. The enumeration of spores in spore suspension was measured using Haemocytometer under the microscope.

Statistical analysis. The current laboratory experiments were conducted using a Completely Randomized Design (CRD). The data collected from all the experiments were subjected to statistical analysis using established procedures outlined by Gomez and Gomez (1984), Panse and Sukhathme (1985).

RESULTS AND DISCUSSION

Effect of media and temperature on mycelial growth rate of *Pestalotiopsis* sp.

Mycelial growth, growth rate and area under mycelia progress curve of one isolate of *Pestalotiopsis* sp. were studied on four different media (OMA, PDA, V8 & CDA) at three different temperatures i.e. 22, 27, and 32˚C (Table 1). Among the three different temperature levels, the isolate of *Pestalotiopsis* sp. showed highest growth rate and area under mycelia progress curve (AUMPC) at 22˚C followed by 27˚C while significantly lowest growth rate and AUMPC was observed at 32˚C. After 6 days of inoculation highest mycelial growth was recorded in the isolate of *Pestalotiopsis* sp. in OMA media (8.9 cm) followed by PDA media (8.63cm) and V8 media (6.65 cm) at 22˚C. Irrespective of different media, the highest mycelial growth rate (13.84 mm / day) and AUMPC (174.72) of the isolate

of *Pestalotiopsis* sp. was recorded at 22ºC followed by 27˚C. Whereas, irrespective of different temperatures, the highest mycelial growth rate (15.01 mm / day) and AUMPC (186.36) of the isolate of *Pestalotiopsis* sp. was recorded at OMA medium followed by PDA medium (Table 1). Das *et al*. (2010) reported optimum temperature for the sporulation of the pathogen was 25±2 ºC. Espinoza *et al*. (2008) also recorded similar observation and reported that optimum temperature for mycelial growth of *Pestalotiopsis clavispora* on blueberry was 18 and 25 ºC was 20ºC for *Pestalotiopsis neglecta*. These outcomes were similar to those wheat varieties as reported by Chungu *et al.* (2001), who also noticed a similar link between temperature and duration on the spore germination, on leaf moisture.

Effect of different media and temperature on mycelia growth rate and area under mycelia progress curve of *Pestalotiopsis* sp.

Mycelial growth, growth rate and area under mycelia progress curve of one isolate of *Pestalotiopsis* sp. were studied on four different media (OMA, PDA, V8 & CDA) at three different temperatures i.e., 22, 27, and 32˚C (Table 2). Among the three different temperature levels, the isolate of *Pestalotiopsis* sp. showed highest growth rate and area under mycelia progress curve (AUMPC) at 22˚C followed by 27˚C while significantly lowest growth rate and AUMPC was observed at 32˚C. After 6 days of inoculation highest mycelial growth was recorded in the isolate of *Pestalotiopsis* sp. in OMA media (8.9 cm) followed by PDA media (8.63cm) and V8 media (6.65 cm) at 22˚C. Irrespective of different media, the highest mycelial growth rate (13.84 mm / day) and AUMPC (174.72) of the isolate of *Pestalotiopsis* sp. was recorded at 22ºC followed by 27˚C. Whereas, irrespective of different temperatures, the highest mycelial growth rate (15.01 mm / day) and AUMPC (186.36) of the isolate of *Pestalotiopsis* sp. was recorded at OMA medium followed by PDA medium (Table 2). According to the findings of Keith *et al*. (2006), every strain of *P. microspora* extracted from *P. guajava* demonstrated the ability to thrive within a temperature spectrum spanning 10 to 35°C. Furthermore, the most conducive temperature for growth differed across various strains, fluctuating between 22 and 28°C.Vegh and LeBerre (1992) have found similar observation across several other *Pestalotiopsis* species.

Cultural characteristics of *Pestalotiopsis* sp. on different media

To induce the spore production, isolates of *Pestalotiopsis* sp. was grown on different media like Potato dextrose agar (PDA), Oat meal agar (OMA), Czapeck's dox agar (CDA) and V8 for 30 days. Mycelial growth on different media was recorded. Colony morphology of *Pestalotiopsis* sp. on different media were presented in Plate 3 and Table 3. Colonies on PDA was totally different as compared to colony morphology on other media. White colored wavy mycelial growth with irregular margin was observed in PDA media and dirty white colored profuse mycelium with irregular margin was observed on OMA. On CDA medium, white colored suppressed mycelium was observed with regular margin. Mycelium growth on V8 was moderate and margin are irregular. Microscopic observation was done up to 30 days in all cases to see the spore on the media. Highest sporulation observed on PDA, followed by OMA.

Table 1: Effect of different media and temperature on mycelia growth rate of *Pestalotiopsis* sp. Sym-1 isolate.

Temperature	Growth rate (mm/ day) of *Pestalotiopsis* sp. at different Media				
	OMA	PDA	V8	CDA	Mean
22 ˚C	18.44	17.53	13.82	10.25	15.01a
27 ˚C	14.46	12.44	10.87	7.93	11.43b
32 ˚C	8.61a	7.63b	6.69c	5.41d	7.09c
Mean	13.84	12.54	10.47	7.87	
Factors	CD	SE(d)	SE(m)		
Temperature (T)	0.44	0.21	0.15		
Media (M)	0.51	0.24	0.17		
T x M	0.89	0.4	0.30		

Table 2: Effect of different media and temperature on area under mycelia progress curve (AUMPC) of *Pestalotiopsis* sp. Sym-1 isolate.

Temperature	Area under mycelia progress curve (AUMPC) of *Pestalotiopsis* sp at different Media				
	OMA	PDA	V8	CDA	Mean
22 ˚C	231.83	206.50	168.93	138.17	186.36a
27 ˚C	186.50	157.47	137.80	103.67	146.36b
32 ˚C	105.83	94.07	80.23	59.83	84.99c
Mean	174.72a	152.68b	128.99c	100.56d	
Factors	CD	SE(d)	SE(m)		
Temperature (T)	2.76	1.33	0.94		
Media (M)	3.192	1.53	1.08		
T x M	5.52	2.66	1.88		

Table 3: Cultural characteristics and sporulation of *Pestalotiopsis sp*. Sym-1 isolate on different media.

Sr. No.	Media	Growth characters	Sporulation(/mm^2)
1.	Potato dextrose agar	Profused, white, wavy mycelia with irregular margin	3.14×10^5
2.	Oat meal agar	Dirty white profused mycelia with irregular margin	2.35×10^5
3.	Czapeck's dox agar	Good growth, suppressed white mycelia with regular margin	7.85×10^4
4.	V8	Moderate growth, margin is irregular, elevated white mycelium	1.57×10^5

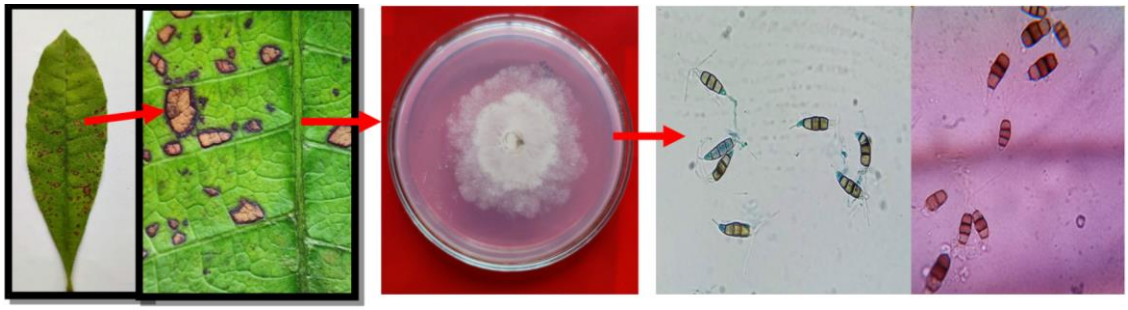

Plate 1: Disease symptom of Pestalotiopsis leaf spot and blight disease of cashew, pure culture and sporulation of the pathogen.

Plate 2: Study of host range of isolates of *Pestalotiopsis* sp. through detached leaf assay.

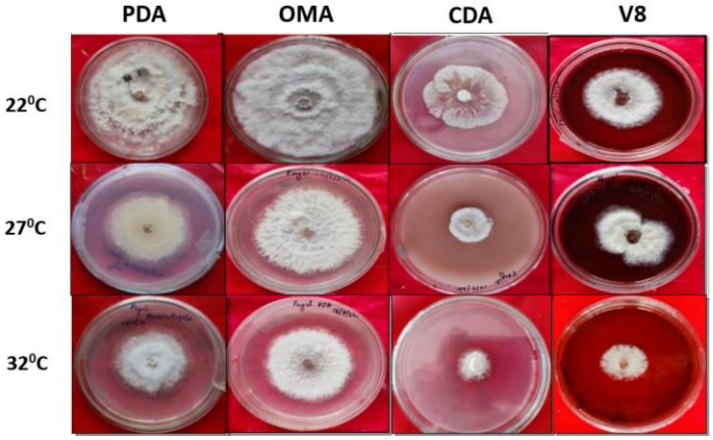

Plate 3: Effect of different media and temperature on mycelial growth of *Pestalotiopsis* sp.

Lowest no of spores per unit area was observed on CDA. Present studies are in accordance with the findings of Majumdar and Chandra (2019) who reported that synthetic media like Sabourand's Agar (SA), Richard's Agar (RA) and Czapek's Dox Agar (CDA) did not support sporulation of *Pestalotiopsis mangiferae* and highest sporulation observed in PDA. Ebenezer *et al.* (2002) and Bajo *et al.* (2008) also reported same findings from their investigation. Results are further supported by Tandan (1950) who reported that sporulation in *Pestalotiopsis psidii* is abundant on PDA. In the present investigation colony colour and morphological variation of *Pestalotiopsis* sp. was noticed as observed by Keith *et al*. (2006).

Study of host range of isolates of *Pestalotiopsis* sp.

To study the host range of the pathogen associated with Pestalotiopsis leaf spot and blight disease of cashewnut. Isolate of *Pestalotiopsis* sp. was selected based on its

pathogenicity on cashew under *in-vitro* condition. The isolate was artificially inoculated on three fruit crops like cashew, mango and jackfruit through detached leaf assay with mycelial plugs containing condia of isolate of *Pestalotiopsis* sp. in the laboratory and the inoculated leaves were maintained in moist chamber for 12 days at 24±1 ºC. A light brown vascular necrotic lesion was observed at 6 days after inoculation, and white mycelium with small black acervuli was produced on diseased tissues as the age of the lesion increased. Lesion length was measured at 6 days, 9 days and 12 days after pathogen sym-1 (cashew) isolate inoculation. Perusal of the data presented in the table showed that highest lesion length was observed in Jackfruit (5.1 cm) followed by cashew (3.0 cm) after 12 days of inoculation (Table 4). Irrespective of the host range, the progression of the lesion length was observed with the progression of the incubation time, however, comparatively higher lesion length was noticed in jackfruit leaves inoculated with *Pestalotiopsis* sp. sym-1 (cashew) isolate. The present investigation indicated that the jackfruit is the most susceptible host for sym-1 isolate of *Pestalotiopsis* sp., followed by Cashew and Mango. The pathogenicity of *Pestalotiopsis psidii* was proved earlier by Ray, 2005 who confirmed the pathogenicity of *Pestalotiopsis psidii* on guava fruits.

Table 4: Pathogenicity testing of *Pestalotiopsis* sp. Sym-1 isolate on different hosts.

Crops Inoculated	Lesion length (cm) Days after Inoculation			Mean
	6 days	9 Days	12 days	
Mango	0.50	1.50	2.7	1.56
Jackfruit	2.50	4.00	5.1	3.86
Cashew	0.70	1.50	3.00	1.73
Mean	1.23	2.33	3.60	
Sources				
Factors		**SE(m)**	**SE(d)**	**C.D.**
Factor (Crops)		0.11	0.15	0.32
Factor (Days)		0.11	0.15	0.32
Factor (Crops X Days)		0.19	0.27	NS

CONCLUSION

In the current investigation, a total of four different culture media were tested to assess their influence on mycelial growth and sporulation of *Pestalotiopsis* sp. Colony diameter (8.9 cm) was significantly highest on Oat meal agar at 6 days after inoculation. Similarly, the highest mycelial growth rate (13.84 mm / day) and area under mycelial progress curve (AUMPC) (174.72) of *Pestalotiopsis* sp. was recorded at 22ºC followed by 27˚C. Pathogenicity studies were observed on different hosts and jackfruit is found to be the most susceptible host of *Pestalotiopsis* sp. cashew isolate.

FUTURE SCOPE

In future, attempts may be taken for screening of different culture media for secondary metabolite, antioxidant and antimycotic agents production ability of *Pestalotiopsis* sp. Expression of different pathogenicity genes of *Pestalotiopsis* sp. at different media under varying levels of temperature may be evaluated for better understanding the influence of temperature and nutrition on pathogenic fitness of *Pestalotiopsis* sp. – host system.

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Conflict of Interest. The findings presented in this paper originated from my independent research, and there were no conflicting results or contributions from other researchers or scientists.

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