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Symptomatological variation of Pestalotiopsis Leaf spot and Blight disease of Cashew under western part of West Bengal

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Cashew is an important tree crop in western part of West Bengal. The purpose of this study was to identify and describe the pathogen that causes cashew leaf spot and blight disease in the western part of West Bengal during the Rabi and Pre-Kharif seasons. Four different types of leaf spot and blight symptoms (Sym 1, Sym 2, Sym 3, and Sym 4) caused by *Pestalotiopsis* have been found during a field survey. During this research, Syms 1 and 4 were shown to be the most common amongst them. The Sym-1 isolate was identified as *Pestalotiopsis* sp. by molecular method through sequencing of ITS (with primers ITS1 and ITS4) region. Among the different isolates, Sym-1 exhibited the longest middle cell, apical cell, basal cell, apical appendages, and basal appendages, along with the greatest conidial length and width across the symptom types. A hierarchical cluster analysis based on conidial morphology of four *Pestalotiopsis* sp. isolates demonstrated that sym-1 was distinctly different from the others, forming a separate cluster (cluster-1), while the remaining isolates were grouped together in cluster-2.The average relative humidity and the maximum and minimum temperatures were shown to be the important predictors associated with the predictions of Pestalotiopsis leaf spot and blight disease risk or no-risk condition in cashew. The conjoint influence of temperature between 21 to 24°C and the average relative humidity more than 80% was found to be highly congenial for incidence and spread of Pestalotiopsis leaf spot and blight disease of cashew in lateritic region of West Bengal. The Binary Logistic Regression model excelled the multiple regression model for successful prediction of disease risk and no risk period.

Keywords : Binary logistic, disease- risk, leaf spot, *Pestalotiopsi*s sp., relative humidity, temperature

INTRODUCTION

The cashew is a significant tropical tree crop that comes in second or third place among the main edible nuts which are traded internationally. The primary producer countries include Brazil, India, Mozambique, and Tanzania (Menge *et al.* 2013). Grown in more than 32 countries worldwide, the cashew tree (*Anacardium occidentale* L.) is the most productive nut crop (Agu, 2006). Africa contributes 40% of the total raw cashew nut production, with West Africa accounting for 80% of this African production (Ah-You *et al.* 2007). Nowadays, cashew is one of the nation's most export-focused horticulture products, and the number of processing facilities is growing constantly.

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Symptomatological variations in cashew leaf spot and blight diseases

The cashew tree has significant environmental value and socioeconomic significance. It is utilized in reforestation projects and as a firebreak around forest boundaries (Arauìjo, 2013). Additionally, it provides protective vegetation cover and supports as intercropping with food crops.

Conventional cashew orchards are made up of tall trees that are widely separated and genetically varied. It was unmanaged by fertilization, pruning or chemical treatments. Outbreaks of disease were rare in these circumstances. Nonetheless, a number of diseases have been identified in kernels, mature plants, and seedlings. Cashew orchards are generally planted as monocropping systems which promotes the spread of diseases due to the lower genetic variability in monocultures, compromising plant defence mechanisms. Additionally, the often close spacing between trees facilitates disease transmission .Fungal diseases can affect every part of cashew trees and cause significant losses, especially when they damage immature tissues. This damage can result in flower loss and nut death, ultimately reducing yield and nut quality (Veloso, 2018). For example, gummosis, caused by *Lasiodiplodia* spp. in Nigeria, reduced production by 40–50%, while recent outbreaks of *Fusarium oxysporum* in Tanzania had severe consequences, quickly destroying cashew trees (Tibuhwaand Shomari, 2016).

Although cashews hold significant economic importance, there is a lack of comprehensive data on the diseases that threaten and reduce their production, and this information has not yet been adequately documented or made available. Nowadays, only a few reports are available on cashew leaf spot and blight disease in India. Our aim was to identify the pathogen and to study the symptomatological variation of Pestalotiopsis leaf spot of cashew. We also studied the relationship between weather variables and Pestalotiopsis leaf spot disease severity.

MATERIALS AND METHODS

Survey and isolation of pathogen

Samples were taken from orchards of Bidhan Chandra Krishi Viswavidyalaya's Regional Research Sub-Station (RRSS) which is situated in Jhargram, West Bengal. The samples were collected from August 2021 to March 2022. From randomly chosen trees along the diagonal transect of every orchard visited, infected leaf samples were collected. In each locality, five to ten orchards were surveyed. Information including the sampling date, locality name, and geographic coordinates were recorded for every sample. After collection, the samples were taken to the lab and kept at 4°C in plastic bags or envelopes.

Isolation and identification of the causal fungus

Naturally infected cashew leaves were randomly collected from multiple orchards at the Regional Research Sub-Station (RRSS) of Bidhan Chandra Krishi Viswavidyalaya in Jhargram, West Bengal. After carefully washing for five minutes under running tap water, the samples were surface sterilised with 1% NaOCl for one minute followed by washing in sterilized water after that the surface sterilized samples were divided into tiny pieces and placed on a medium of potato dextrose agar (PDA) supplemented with 100 mg/L of streptomycin. After being sealed, the plates were incubated for three days at 25±2°C in the dark. The hyphal tips from the growing fungal colonies were transferred onto fresh agar plates to obtain pure isolates. Every three months, the pure cultures were periodically sub-cultured and maintained at 4°C on PDA slants.

Morphological characterization

After being cultured on PDA, the fungal isolates were incubated at 25±2°C. The morphology of the colony was assessed after seven days, and the colour of the colony was categorized using Raynor's (1970) system. By measuring the length and width of specific conidia from conidial suspensions made in sterile distilled water (SDW), the size of the spores was measured. Conidial size, median cell colour, length, and apical appendage's thickness and length were measured and this morphological and cultural traits used to initially identify the isolates. These characteristics were contrasted with descriptions in *Monochaetia* and *Pestalotia* (Guba, 1961), as well as research by Keith *et al.* (2006), *Das et al.* (2010), Watanabe *et al*. (2010), Maharachchikumbura *et al.* (2011), and Strobel *et al.* (1996).

Pathogenicity tests

Tests for pathogenicity were performed using the methodology outlined by Keith *et al.*(2006). Detached cashew leaves were inoculated directly. The leaves were surface sterilised before being inoculated by soaking them in a 0.5% sodium hypochlorite for two minutes, then washing them with sterile distilled water (SDW) and letting them air dry in a laminar flow hood. After that, the leaves were put inside plastic containers lined with damp, absorbent cotton. Actively growing mycelial discs (3 mm in diameter) from fungal cultures that were

5–7 days old were inoculated to the leaves pre-wounded with a sterile needle. Five days after the inoculation, the length of necrotic lesions was observed, which were incubated at room temperature (25±2°C). Only PDA discs were used to inoculate the control samples. Three separate tests were performed on each isolate. Infected tissues were again re-isolated on PDA medium and checked for the formation of pathogen-specific colonies in order to validate Koch's postulates.

Molecular characterization

Molecular identification was carried out through a series of steps including DNA extraction, amplification and sequencing. The modified cetyltrimethylammonium bromide (CTAB) approach, which was based on the Borges *et al.* (2009) methodology, was used to extract DNA. The ITS 1 primer (5'-CTTGGTCATTTA GAGGAAGTA-A-3') and the ITS-4R primer (5'-TCCTCCGCTTATTGATATGC-3') were used for the amplification. PCR conditions included an initial denaturation at 95°C for 1 minute, followed by 30 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 58°C for 15 seconds, and extension at 72°C for 10 seconds, concluding with a final extension at 72°C for 7 minutes (Herliyana *et al* ,2022). After loading 3 μL of the amplified DNA onto a 2% agarose gel and running it at 100 volts for 30 minutes, the PCR products were examined by electrophoresis. A UV transilluminator was used to view the resultant DNA bands. After then, the amplified products were sent for sequencing.

Epidemiological studies

Observations on incidence and severity of Pestalotiopsis disease were recorded from 2nd week of December 2021 to 16th June of 2022 at fifteen days interval based on "0-5" Pestalotiopsis leaf spot and blight disease rating scale (Keith and Zee, 2008) as mentioned in Table 1.

The impact of various meteorological variables, such as average temperature (both maximum and minimum), relative humidity, and rainfall (mm), the growth and progression of Pestalotiopsis leaf spot and blight disease in field conditions was examined by using a variety of statistical techniques. From the second week of December to the second week of June 2022, Percent Disease Index (PDI), which measures the severity of the disease, was calculated at 15-day intervals using a 0–5 disease grading scale. The Statistical Package for Social Sciences (SPSS-25) was then used to correlate the PDI values with meteorological data from NASA POWER CERES (https://power.larc.nasa.gov).

Correlation analysis

The time-series data on disease severity was examined in relation to weather factors in order to evaluate the impact of weather variables on the development of Pestalotiopsis leaf spot and blight disease.

Multiple regression analysis

A linear multiple regression model was built to predict disease severity and multiple correlation coefficients were computed to assess the influence of different independent factors on disease development (Percent disease Index). The effect of various environmental factors on disease progress of Pestalotiopsis leaf spot and blight disease of cashew was determined by using multiple linear regression analysis with the prediction equations as:

Y= b0 + b1 X1 + b2 X2 + ...+ b6 X6.

where, Y is the percent disease index, b0 is the constant and b1 to b6 are the regression coefficients of X1 to X6, respectively. The coefficient of multiple determinations was used to evaluate the adequacy of the fitted regression models.

Binary logistic regression

Every two weeks, data on disease severity was collected and transformed into binomial values based on cumulative disease incidence (%). A value of 0 denoted no increase (no disease), while a value of 1 signified an increase in disease severity (disease present). A binary logistic model was used to analyse the linear and nonlinear correlations between interaction variables in the nearest neighbour system after the data had been converted into a risk (1) and no-risk (0) matrix.

RESULTS AND DISCUSSION

Symptomatological studies of Pestalotiopsis leaf spot & blight disease

The disease caused by *Pestalotiopsis* sp. appears as the formation of spots and blighted areas on the leaves of both seedlings and mature plants. Both seedlings and mature plants are susceptible to the diseases. Four different categories of distinctive symptoms (sym-1, sym-2, sym-3, and sym-4) associated with Pestalotiopsis leaf spot and blight disease were discovered during the survey (Fig.1). Reddish-brown, sunken patches appeared on the upper leaf surface of the sym-1 type. In sym-1 type, reddish brown depressed spots appeared on the leaf's top surface and spots coalesces at the edges and interveinal regions followed by drying and withering of leaves (Fig.1 a). The symptoms of sym- 2 included the development of minute, angular to irregular lesions on the mature leaves, as well as lesions surrounded by a dark brown to black border and, in extreme cases, defoliation; sym- 3 included irregular reddish- or reddish-brown patches along the veins and interveinal regions; sym-4 included regular to irregular necrotic lesions on the leaf from midrib to the leaf edge; the infected leaves gradually blighted and withered, and pycnidia masses of conidia appeared on the surface of both pale grey to necrotic lesion areas (Fig.1). Sym-1 and Sym-4 were found to be the most prevalent types during the study period. Joshi (2009) also reported similar symptoms in Maharashtra. Furthermore, Freire *et al*. (2002) observed Pestalotia leaf spot in cashew in Brazil. In India, Adhikary *et al*. 2023) also observed Pestalotiopsis leaf spot in Cashewnut plant. *Pestalotiopsis* species are usually regarded as weak or opportunistic diseases that do little harm on ornamental plants. *Pestalotiopsis* species are common phytopathogens that cause a variety of plant diseases, according to recent research. Several post-harvest diseases that cause large financial losses include canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, and fruit rots. Apple, tea, blueberry, coconut, chestnut, ginger, grapevine, guava, hazelnut, mango, orchid, peach, and wax apple are among the crops that are impacted (Chen *et al*. 2012; Chen *et al*. 2018 Evidente *et al*. 2012; Ismail *et al*.2013; Joshi *et al*. 2009; Keith *et al*. 2006; Maharachchikumbura *et al*. 2013; Ren *et al*. 2013).

Table 1: "0-5" disease rating scale for Pestalotiopsis leaf spot (Keith and Zee,2008)
Disease score Description

Disease score	Description leaf area infected and covered by spot	Disease severity (%)
0	No disease symptom	0
1	1-5 %	1-5%
2	6-20 %	6-20%
3	21-35%	21-35%
4	36-60 %	36-60%
5	61-100%	61-100%

Description of conidia of Pestalotiopsis sp.

To evaluate pathogen variability based on conidial characteristics, the micrometric measurement approach was used to analyse the conidial morphology of Pestalotiopsis leaf spot and blight-infected leaves with various symptom types. Conidial length (CL), conidial breadth (CB), middle cell length (LMC), apical cell length (LAC), basal cell length (LBC), apical appendage length (LAA), basal appendage length (LBA), and the conidial length-to-breadth ratio (CL/CB) were measured. Five-celled conidia with a single hyaline basal appendage and two hyaline apical appendages

Table 2 : Conidial characteristics of different symptoms of Pestalotiopsis leaf spot and blight disease of Cashew

Pestalotiopsis isolates on various symptom types	Conidial Characteristics									
	Conidial Length (CL)	Conidial Breadth (CB)	CL/CB	Length of Middle cell	Length of Apical Cells	Length of Basal Cell	Lengthof Apical Appendeges (LAA)	Length of Basal Appendeges (LBA)	LAA/ LBA	No. of Appendages
Symptom-1	23.85	6.12	3.90	12.29	4.77	4.44	14.82	5.19	2.90	2
Symptom -2	19.01	4.26	4.46	7.20	4.32	4.44	11.73	4.23	2.81	2
Symptom -3	17.58	4.59	3.83	6.77	4.53	4.53	11.88	3.66	3.34	2
Symptom -4	19.94	4.92	4.05	8.68	4.65	4.71	12.54	3.87	3.33	2
SE(m)	0.69	0.138	0.14	0.356	0.133	0.166	0.473	0.253	0.20	
CD	2.01	0.406	0.40	1.05	NS	NS	1.388	0.743	NS	
CV	9.03	7.358	8.88	10.79	7.723	9.703	9.818	15.792	17.89	

Table. 3: Relationship between PDI and Disease progress rate with weather variables

Date	PDI	Disease progress rate	Tmax	Tmin	Trange	RH	PPT
16.12.21	6	0.4	23.90	14.72	9.18	85.73	80.94
01.01.22	14	0.54	21.97	11.01	10.95	80.73	7.62
16.01.22	24	0.67	22.78	12.01	10.77	81.10	21.77
01.02.222	32	0.54	21.88	10.66	11.22	78.45	9.6
16.02.22	34	0.13	23.99	10.4	13.6	71.18	30.24
01.03.22	40	0.4	27.20	13.47	13.73	70.30	12.51
16.03.22	36	-0.27	30.64	16.39	14.26	64.35	0.5
01.04.22	32	-0.27	36.35	20.88	15.47	64.66	0.93
16.04.22	24	-0.53	39.75	23.88	15.86	60.87	1.55
01.05.22	22	-0.13	42.41	26.20	16.20	49.12	3.05
16.05.22	16	-0.4	38.95	25.56	13.39	60.27	39.86
01.06.22	12	-0.27	40.95	26.94	14.00	54.17	19.45
16.06.22	4	-0.53	39.43	28.04	11.37	57.75	17.41

Table 4: Pearson correlation coefficient of Pestalotiopsis leaf spot disease severity and progress rate with the weather variables

	Pestalotiopsis leaf spot of cashewnut	
Weather variables	Disease severity (PDI%)	Disease progress growth rate
Tmax	-0.271	-0.889**
Tmin	-0.465	-0.857**
Trange	0.453	-0.625*
RHavg	0.062	0.835**
RF	-0.496	0.241

Table 5: Multiple Linear Regression of Pestalotiopsis leaf spot disease severity and progress rate with the weather variables

MODEL	EQUATION	R^2	R^2adj
MLR backyard method for prediction of Pestalotiopsis leaf spot and blight disease severity	Y(PDI)=-11.375-1.519(T min)**+4.757(T range)**------- Eqn.1	0.822	0.786
MLR backyard method for prediction of Pestalotiopsis leaf spot and blight disease progress rate	Y(Disease progress rate)= 1.578*-0.046(Tmin)**-0.054 (T range) -----Eqn, 2	0.792	0.750

Table 6: Binary Logistic Regression Model and Canonical Discriminant Function

Model	Equation
Binary Logistic Regression Model	Y (0/1) = -286.67 + 9.9 (T_{max}) .... (3) Cox and Snell R^2 : 0.749

Table 7: Validation of binary logistic regression model prepared with 2021-22 dataset for the Pestalotiopsis leaf spot and blight disease /no-disease prediction during 2021-22

Binary value (No-disease/Disease)	Actual	Predicted	Percent success
No-disease (0)	7	7	100.00
Disease (1)	6	6	100.00
Overall			100.00

Table 8: IdentificationoftheITSsequencesofthePestalotiopsis isolateusingNCBI-BLAST

Scientific Name	Strain	Max Score	Total Score	Query Cover	E value	Per.Id entity	Acc.L en	Accession
Neopestalotiop sischrysea	FZXM 038	953	953	100%	0	100. 00%	577	KU534 876.1
Pestalotiopsis sp.	SMS13	953	953	100%	0	100. 00%	581	KR085 971.1

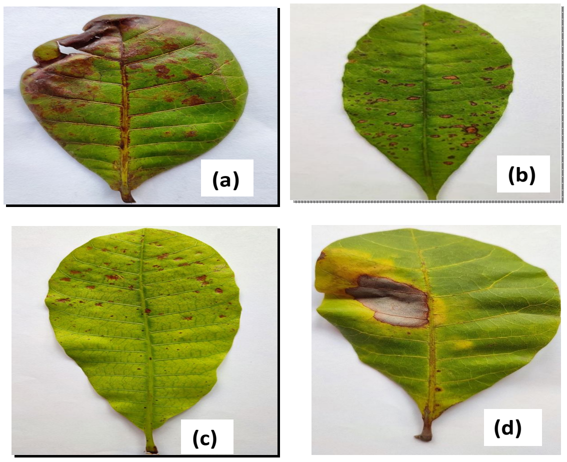

Fig. 1 : (a)- Symptom 1, (b) – Symptom 2, (c)- Symptom -3, (d) – Symptom 4

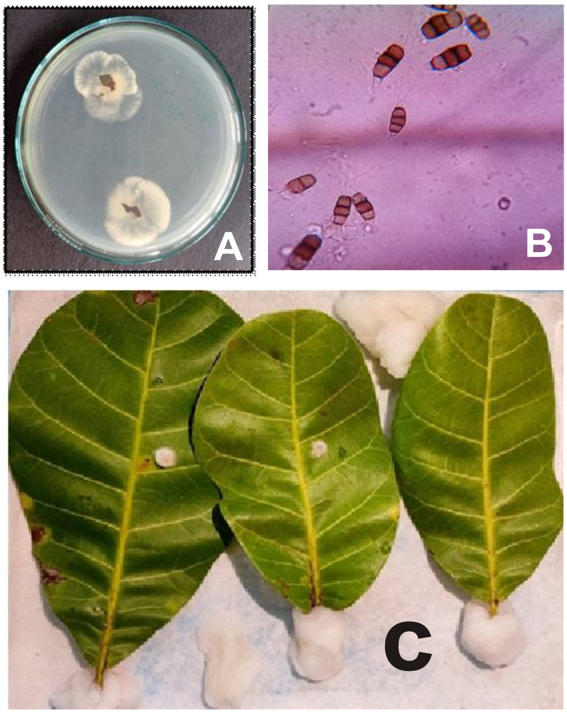

Fig. 2 : (a) Growth of pathogen in Petri plate (b) Microscopic

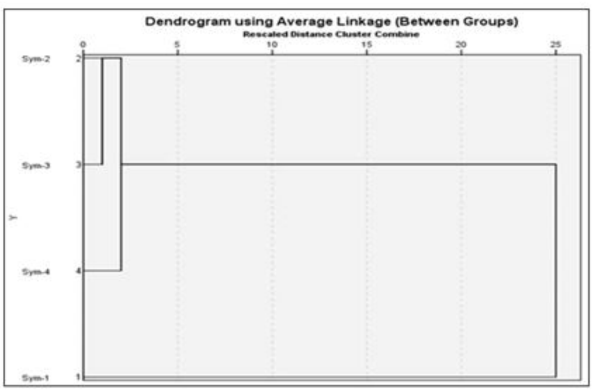

Fig. 3: Hierarchical cluster analysis of *Pestalotiopsis* sp.causing four different types of leaf spots and blight symptoms on cashew based on conidial morphology

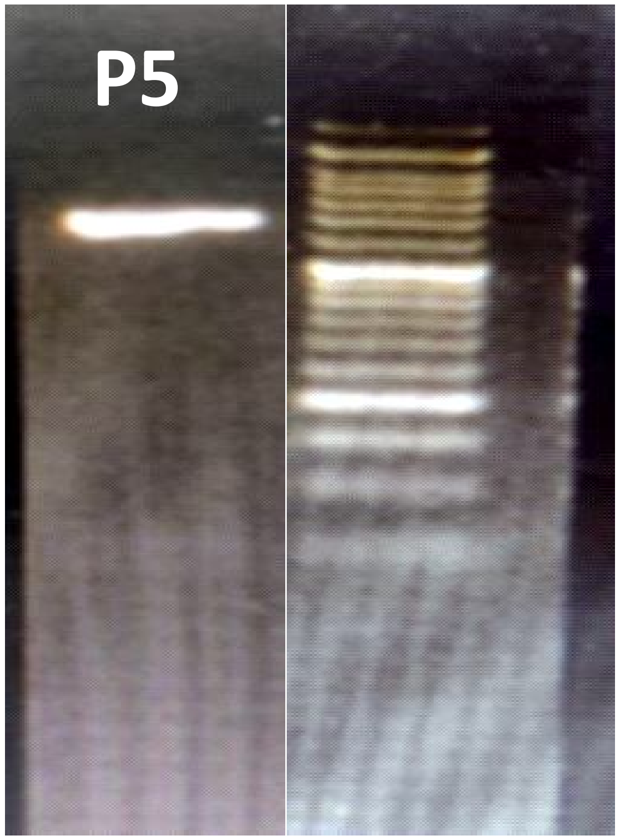

Fig. 4: Agarose gel electrophoresis of the amplified product of ITS regions of the test pathogen (*Pestalotiopsis* sp.)

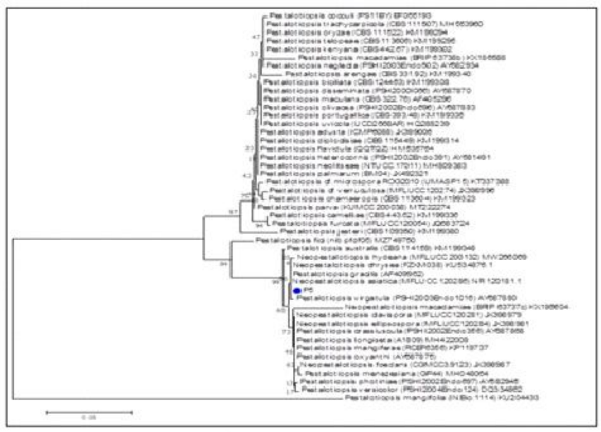

Fig. 5: Evolutionary relationships of the ITS sequence of the *Pestalotiopsis* sp. obtained in the current research work with popular *Pestalotiopsis* sp. and *Neopestalotiopsis* sp.

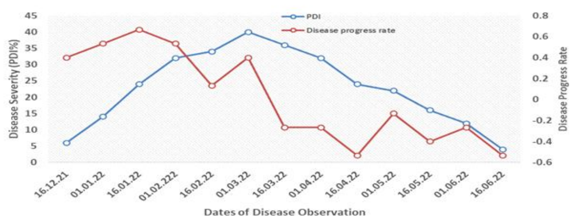

Fig. 6: Cashew *Pestalotiopsis* leaf spot and blight disease severity and disease progress rate during January to June, 2022.

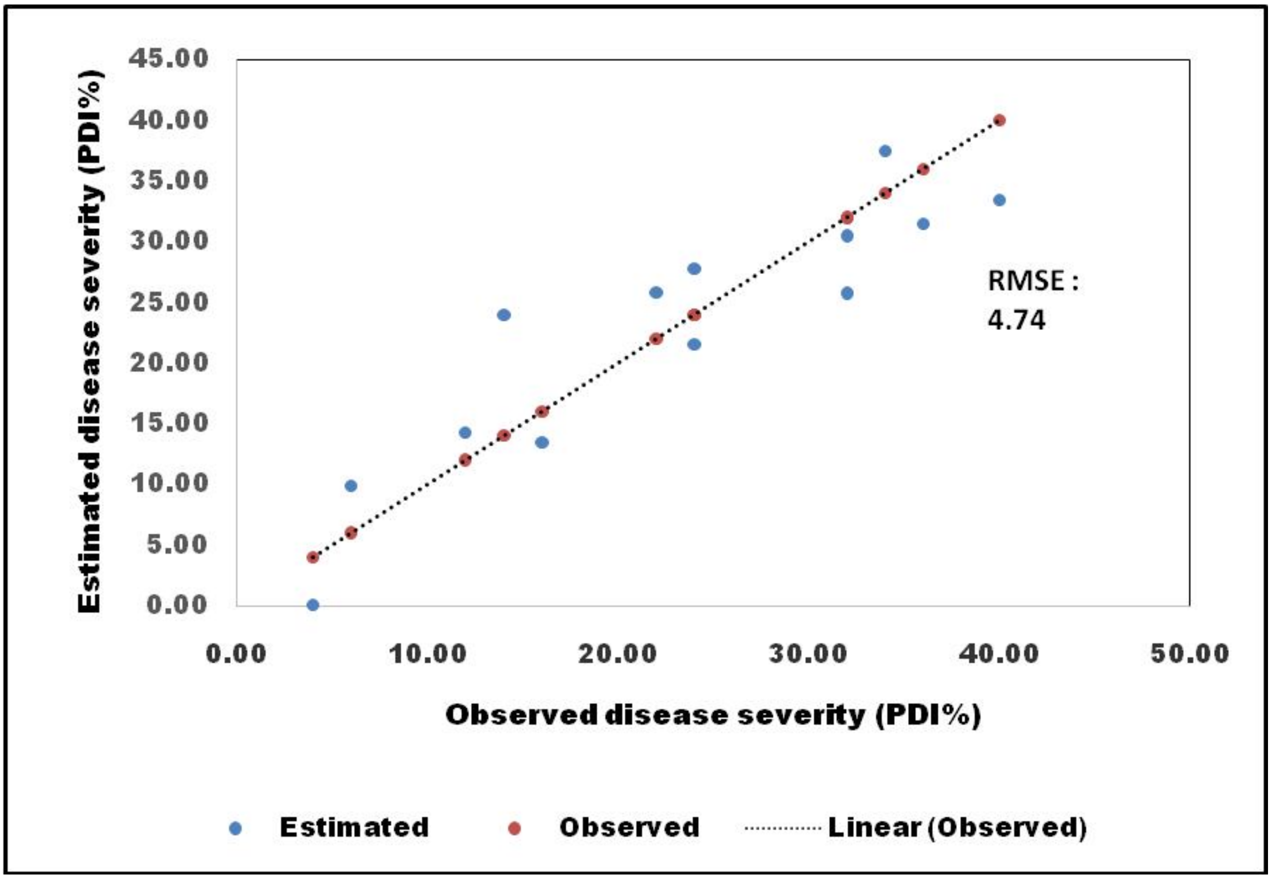

Fig. 7 : Relationship between predicted and observed disease severity of Pestalotiopsis leaf spot and blight in Cashew

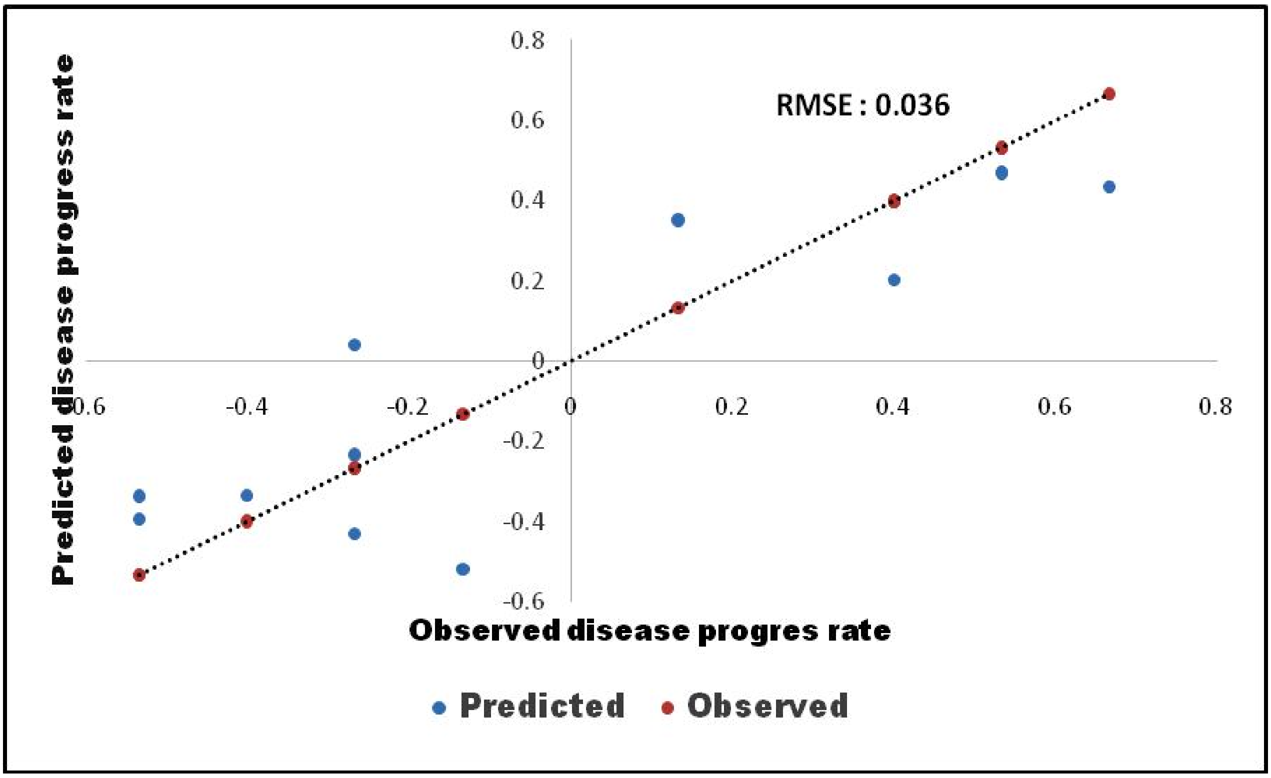

Fig. 8: Relationship between predicted and observed *Pestalotiopsis* leaf spot and blight disease progress rate in Cashew

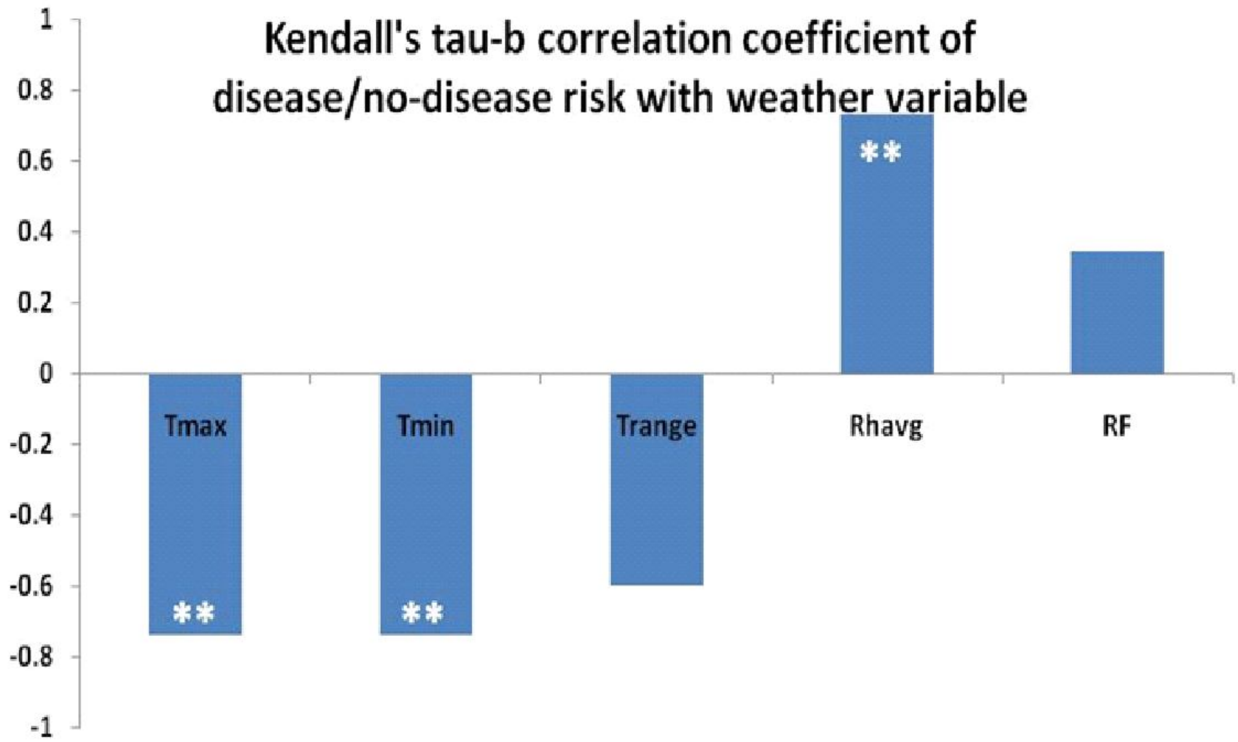

Fig . 9: Kendall's tau-b correlation coefficient of disease /no disease risk with weather variables

were used to identify field isolates of *Pestalotiopsis* (Fig. 2 a,b,c). *Pestalotiopsis* sp. had fusiform, straight conidia with one hyaline cell at the top and one at the bottom, and three pigmented middle cells. Sym-1 type conidia were consistently smooth, with length (L) and width (W) of 22.11 ± 2.82 µm and 6.12 ± 0.43 µm, respectively, and a L:W ratio between 3.83 and 4.46 (Table2). There were usually one basal appendage and two apical appendages. Using Guba's monograph, the isolated fungi were provisionally identified as *Pestalotiopsis* sp. based on morphological and cultural traits. *Pestalotiopsis* funereal generated five-celled spores with three to four apical appendages and one basal appendage, according to (Sousa *et al.* 2004; Watanabe *et al*. 2010) have also studied the morphology of *Pestalotiopsis* sp. Similar conidial traits in *Pestalotiopsis* spp., which produce coconut grey blight, were also reported by **(**Mishra and Akkin, 2024**)**. In comparison to other isolates, sym-1 had the longest middle cell, apical cell, basal cell, apical appendages, and basal appendages, as well as the largest conidial length and breadth among the symptom kinds. Based on conidial morphology, a hierarchical cluster analysis of four *Pestalotiopsis* sp. isolates revealed that the sym-1 isolate was significantly different from the others and formed a distinct cluster (cluster-1), while the other isolates were grouped together in cluster-2 (Fig.3).

Molecular identification through amplification of complete internal transcribed spacer (ITS) of Pestalotiopsis sp. Isolates

Modern taxonomy extensively utilizes ITS region sequencing for identification and variability studies because of its accuracy, ease of use, and ability to generate sufficient variability to distinguish different microorganisms.ITS sequencing, for instance, has been used to examine the genetic diversity and demographic history within and between the genera *Pseudopestalotiopsis*, *Neopestalotiopsis*, and *Pestalotiopsis* linked to *Camellia sinensis* that are grown in China, as well as a few isolates from Japan, France, Turkey, and Thailand (Chen *et al*. 2018). The internal transcribed spacer (ITS) sections (White *et al.* 1990) are specifically targeted by the rDNA primers ITS1/ITS4, which were used in this investigation to extract and amplify the total DNA from the fungal isolate (Fig.4). Sanger sequencing technology was used to sequence the amplified

DNA forward, and then it was trimmed. NCBI's Basic Local Alignment Search Tool (BLAST) tool (http://www.ncbi.nlm.nih.gov/) were used for identification. The resulting sequence was 100% identical to *Pestalotiopsis* sp. (KR085971.1), an endophytic fungus from *Pinus kesiya* var. l*angbianensis*, and *Neopestalotiopsis chrysea* (KU534876.1), which was previously isolated from a blueberry dieback twig.

Molecular variability of Pestalotiopsis sp. Isolate

ITS sequences of several *Pestalotiopsis* sp. and *Neopestalotiopsis* sp. were obtained from the NCBI nucleotide database and aligned with the ITS sequence of the P5 isolate in order to analyse the molecular variability of the isolate in relation to frequently occurring related species and the Pestalotiopsis genus.The alignment was performed using MEGA software version 11. Trimming of the aligned sequences was performed to remove the overhang regions at the beginning and the end of the alignment to obtain uniform length of alignment of the various isolates. A dengogram was generated using MEGA11 software package using the Neighbor-Joining method (Saitou and Nei,1987) by eliminating all positions containing gaps and missing data. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein ,1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*. 2011) and are in the units of the number of base substitutions per site.

All the 47 sequences generated two major clusters of which cluster B contained *P. mangifolia* sequence which is totally different from other sequences and mostly worked as outgroup. Under cluster A, two clear subclusters were formed of which subcluster A1 contained only *Pestalotiopsis* sp. sequences whereas A2 contained both *Pestalotiopsis* sp. and *Neopestalotiopsis* sp. Sequences (Fig.5)**.** The ITS of the isolate P5 is having highest similarity with *Pestalotiopsis grandis*, *Neopestalotiopsis asiatica* and *N. chrysea*.

Survey of Pestalotiopsis leaf spot and blight disease

From November 2021 to June 2022, a fixed plot disease survey was carried out at the cashew orchard of RRS Jhargram, BCKV. Observations were made every 15 days to monitor changes and gather data systematically. Beginning in the third week of December 2021 (16.12.2021), Pestalotiopsis leaf spot disease peaked on March 1, 2022 (Table3, Fig. 6). The seasonality and severity of Pestalotiopsis leaf spot in cashew, however, varied significantly.In addition to developing a weather-based disease prediction system for identifying periods of disease risk and no-risk, the aim of this study was to discover important weather parameters that affect the severity of Pestalotiopsis leaf spot and blight disease.

Correlation between weather variables and Pestalotiopsis leaf spot and blight disease severity and progress rate

NASA Power (https://power.larc.nasa.gov) provided daily weather data for RRS Jhargram (Latitude: 22.4563, Longitude: 87.0115) from 16.12.2021 to 16.06.2022, including Tmax, Tmin, Trange, RHavg, and Rainfall. There was no significant correlation between the severity of Pestalotiopsis leaf spot and blight disease and any of these five climatic variables(Table4). However, there was a significantly negative correlation between the disease progress rate and the temperature range ($p<0.05$) as well as the maximum and minimum temperatures ($p<0.01$). On the other hand, the rate at which Pestalotiopsis leaf spot and blight disease progress rate was significantly positively correlated with average relative humidity. According to this study, cashew disease severity was positively correlated with a maximum temperature of 21–27°C and an average relative humidity of greater than 70%. However, when the maximum temperature was between 21 and 24°C and the average relative humidity was more than 80%, the highest rate of disease progression was recorded. The present finding, thus, indicated that the conjoint influence of temperature between 21 to 24°C and the average relative humidity more than 80% was found to be highly congenial for incidence and spread of Pestalotiopsis leaf spot and blight disease of cashew in lateritic region of West Bengal. Furthermore, a few meteorological parameters showed a high degree of association with one another, suggesting that the independent variables under consideration were interdependent. Multiple regression analysis was carried out using the stepwise method in order to reduce data redundancy.

Multiple Regression Analysis (MRA)

To eliminate multicollinearity and identify the major factors impacting the severity and progress rate of Pestalotiopsis leaf spot and blight disease, data from the 2021–2022 season were used for multiple regression analysis (MRA) using the backward method. Five distinct meteorological characteristics were taken into consideration as independent variables, while the severity of the disease and its rate of advancement were addressed as dependent variables.

Table 5 displays the MRA models for the severity and progression rate of the Pestalotiopsis leaf spot and blight diseases in tabular style.

According to equations "1 and 2," minimum temperature (Tmin) and temperature range (Trange) are the primary indicators of the severity and progress rate of Pestalotiopsis leaf spot and blight disease in cashew. Among them, the minimum temperature (Tmin) has the greatest impact on the severity and disease progression(Table5).

The value of final adjusted R2 for eqn.1 is 0.786, which means that the two predictors (minimum temperature and temperature range) conjointly accounted for 78.6% of the variance in the dependent variable, i.e., disease severity and the final value of adjusted R2 for eqn.2 is 0.750 i.e., the two predictors (minimum temperature and temperature range) conjointly accounted for 75% of the variance in the Pestalotiopsis leaf spot and blight disease progress rate of cashew. Hence, these two models could able to successfully explain the risk of Pestalotiopsis leaf spot and blight disease severity and progress rate in cashew up to 78.6% and 75% level of successful prediction, respectively.

Relationship between predicted and observed Pestalotiopsis leaf spot and blight disease progress rate in Cashew
Validation of the MRA model

The MRA models were validated with the same data set on Pestalotiopsis leaf spot and blight disease severity and progress rate with weather variables collected during the year Dec 2021 to June 2022. (Fig. 7) shows the relationship between observed and predicted disease severity of Pestalotiopsis leaf spot and blight (2021-22) based on the MRA model with RMSE value of 4.74. Validation of the MRA models prepared from the 2021-22 datawas done by predicting the disease severity and progress rate of Pestalotiopsis leaf spot and blight in 2021-22 and comparing with the observed disease severity and progress rate of that season (Figs. 7&8) with RMSE value of 4.74 and 0.036.

The high RMSE value (4.74) observed during the validation of the MRA model suggested the need for multivariate and non-parametric analysis to develop a more accurate predictive model for Pestalotiopsis leaf spot and blight disease severity. Consequently, binary logistic regression analysis and canonical discriminant analysis were conducted to establish a weather-based prediction system for assessing disease risk and no-risk conditions in cashew.

Kendall's tau-b correlation coefficient of disease /no disease risk with weather variables

Non-parametric correlation of disease risk/ no-risk period with weather variables

The risk/no-risk period for Pestalotiopsis leaf spot and blight disease was significantly negatively correlated with both maximum and minimum temperatures ($p<0.01$) and temperature range ($p<0.05$) among the five meteorological variables that were examined (Tmax, Tmin, Trange, RHavg, and Rainfall). On the other hand, there was a significant positive correlation ($p<0.01$) between the disease risk/no-risk period and the average relative humidity (Fig.9). Thus, this study emphasizes that the main meteorological factors affecting the forecast of Pestalotiopsis leaf spot and blight disease risk/no-risk periods in cashew are maximum temperature, minimum temperature, and average relative humidity.

Binary Logistic Regression Analysis

Binary logistic regression was used to create a dichotomous model that could forecast whether Pestalotiopsis leaf spot and blight disease would be present (1) or absent (0) in cashew. A given set of covariate values was used to calculate the probability of disease occurrence using the binary logistic model equation Equation '3' in Table6. When the probability exceeded an initial limit of 0.5, the development of the disease was predicted in this model. Based on real values, the binary logistic regression model properly predicted the occurrence of the disease and classified 100% of cases. It was statistically significant. The binary logistic regression model performed better than the multiple regression model in predicting disease risk and no-risk circumstances, according to a comparison of the two models (Table7).

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

REFERENCES

Adhikary P., Sarkar U., Jash S., Ray S.K., Dutta S.2023. 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 WestBengal.*Biol. Forum – Int.J.* **15**: 359-364.

Agu, C.M. 2006. Survey and Effects of Plant Parasitic Root Nematodes of Cashew (*Anacardium occidentale)* in South-Eastern-Nigeria.*J Am Sc*. **3**: 50-54.

Ah-You N, Gagnevin,L., Joue, E., Rodrigue, N.J., Pruvost, O. 2007. Pathological variation within *Xanthomonas campestris* pv. *mangiferaeindicae* supports its separation into three distinct pathovars that can be distinguished by amplified fragment length polymorphism.*Phytopathol*. **97**: 1568-1577.

Araújo ,J.P.P. 2013. Agronegócio Caju: Práticas e inovações. Brasília: Embrapa, pp. 532.

Borges, A., Rosa, M., Recchia, G., Silva, J.R.Q., Bressan, E.A., Beasey, E.A. 2009. CTAB methods for DNA extraction of sweetpotato for microsatellite analysis. *Sci Agric*. **66** : 529-534. DOI: 10.1590/S0103- 90162009000400015

Chen, F. Y., Lu, L. M., Ni, H. Z., Wang, Y., Wang, Y. G., Li, G. Q. 2012. First report of *Pestalotiopsis mangiferae* and *P. vismiae* causing twig dieback of Myrica rubra in China.*Plant Dis*.**96**: 588-588.

Chen, Y., Zeng, L., Shu, N., Jiang, M., Wang, H., Huang, Y., Tong, H. 2018. *Pestalotiopsis*-like species causing gray blight disease on Camellia sinensis in China. *Plant Dis*. **102**: 98-106.

Das, R., Chutia, M., Das, K., Jha, D. K. 2010. Factors affecting sporulation of *Pestalotiopsis disseminata* causing grey blight disease of *Persea bombycina* Kost., the primary food plant of muga silkworm.*Crop Prot*. **29**: 963-968.

Evidente, A., Zonno, M. C., Andolfi, A., Troise, C., Cimmino, A., Vurro, M. 2012. Phytotoxic á-pyrones produced by *Pestalotiopsis guepinii*, the causal agent of hazelnut twig blight.*J. Antibiot*. **65**: 203-206.

Felsenstein, J.1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*.**39**: 783-791.

Freire, F. C. O., Cardoso, J. E., Dos Santos, A. A., Viana, F. M. P. 2002. Diseases of cashew nut plants (*Anacardium occidentale* L.) in Brazil.*Crop Prot*. **21**: 489-494.

Guba, E. F. 1961. Monograph of Monochaetia and Pestalotia. Harvard Univ. Press, Cambridge, Mass, USA. In: Singh, R.S., Ed. 1987.

Herliyana, E. N., Oktavianto, P., and Siregar, U. J. 2022. Identification and characterization of *Pestalotiopsis* spp. causing leaf spot and leaf blight on jabon (*Neolamarckia* spp.) in Indonesia. *Biodiversitas*. **23** :(12).

Ismail, A. M., Cirvilleri, G., Polizzi, G. 2013. Characterisation and pathogenicity of *Pestalotiopsis uvicola* and *Pestalotiopsis clavispora* causing grey leaf spot of mango (*Mangifera indica* L.) in Italy.*Eur. J. Plant Pathol*.**135** :619-625.

Joshi, S. D., Sanjay, R., Baby, U. I., Mandal, A. K. A. 2009. Molecular characterization of *Pestalotiopsis* spp. associated with tea (*Camellia sinensis*) in southern India using RAPD and ISSR markers.

Keith, L. M., Velasquez, M. E., Zee, F. T. 2006. Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava*, in Hawaii. *Plant Disease*. **90**: 16-23.

Keith, L. M., Zee, F. T. 2008. Guava diseases in Hawaii and the characterization of *Pestalotiopsis* spp. affecting guava. In II International Symposium on Guava and other Myrtaceae. 849:pp. 269-276.

Maharachchikumbura, S. S., Chukeatirote, E., Guo, L. D., Crous, P. W., Mckenzie, E. H., Hyde, K. D.2013. Pestalotiopsis species associated with Camellia sinensis (tea). *Mycotaxon*.**123**:47-61.

Maharachchikumbura, S. S., Guo, L. D., Chukeatirote, E., Bahkali, A. H., Hyde, K. D. 2011**.** Pestalotiopsis—morphology, phylogeny, biochemistry and diversity.*Fungal Divers*.**50**: 167-187.

Maharachchikumbura, S. S., Guo, L. D., Chukeatirote, E., Hyde, K. D. 2014. Improving the backbone tree for the genus Pestalotiopsis; addition of *P. steyaertii* and *P. magna* sp. nov.*Mycol. Prog*.**13**: 617-624.

Menge, D., Makobe, M., Shomari, S., Tiedemann, A. V. 2013. Development and validation of a diagrammatic scale for estimation of cashew blight for epidemiological studies.

Mishra, M., Akkin, N.2024. Morpho-Molecular Characterization and In vitro Management of the *Pestalotiopsis palmarum*

(Cooke) Steyaert Causing the Grey Blight of Coconut. *Int. J. Environ. Clim. Change.***14**: 688-703.

Raynor, R. W. 1970. A mycological Colour Chart. Commonwealth. Mycological Institute, Kew, Surrey, England.

Ren, H. Y., Li, G., Qi, X. J., Fang, L., Wang, H. R., Wei, J. G., Zhong, S. 2013. Identification and characterization of *Pestalotiopsis* spp. causing twig blight disease of bayberry (Myrica rubra Sieb. & Zucc) in China. *Eur. J. Plant Pathol.***137**: 451-461.

Saitou, N., Nei, M.1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees.*Mol Biol Evol.* **4**: 406-425.

Sousa, M. F., Tavares, R. M., Gerós, H., Neto, T. L.2004. First report of Hakea sericea leaf infection caused by *Pestalotiopsis funerea* in Portugal.

Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R. S., Hess, W. M. 1996. Taxol from *Pestalotiopsis microspora*, an endophytic fungus of Taxus wallachiana.*Microbiol.***142** :435-440.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S.2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods.*Mol Biol Evol.***28**: 2731-2739.

Tibuhwa, D.D., Shomari, S. 2016**.** Fusarium wilt disease: An emerging threat to cashew nut crop production in Tanzania. *Asian J. Plant Pathol.* **10** :36–48.

Veloso, J.S., Câmara, M.P.S., Lima, W.G., Michereff, S.J., Doyle, V.P.2018. Why species delimitation matters for fungal ecology: Colletotrichum diversity on wild and cultivated cashew in Brazil. *Fungal Biol.***122**: 677–691.

Watanabe, K., Motohashi, K., Ono, Y. 2010. Description of *Pestalotiopsis pallidotheae*: a new species from Japan. *Mycoscience*.**51** :182-188.

White, T. J., Bruns, T., Lee, S. J. W. T., Taylor, J.1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. **18**:315-322.