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Research Paper

Influence of weather parameters on Anthracnose in black pepper (*Piper nigrum* L.) in the upper Brahmaputra valley zone of Assam

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ABSTRACT

Black pepper (*Piper nigrum* L.) production faces several challenges due to various diseases, with anthracnose being the most significant. It is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., a fungal plant pathogen that leads to severe infections in black pepper plants, both in nurseries and in the field. The occurrence of anthracnose disease is highly influenced by weather conditions. Epidemiological studies were conducted at Assam Agricultural University, Jorhat, Assam from 2019 to 2021 to determine the impact of weather factors such as temperature, rainfall, rainy days, and relative humidity on anthracnose incidence in seven different black pepper varieties. Upon analyzing the recorded data, it was found that rainfall, minimum temperature, rainy days, and morning relative humidity are the most significant contributors to disease occurrence. However, the role of maximum temperature, evening relative humidity, and bright sunshine hours was statistically non-significant. Data from 2019 and 2020 were further analyzed using stepwise multiple regression to estimate anthracnose incidence in individual black pepper varieties. These regression models were subsequently validated using data of 2021. The root mean square error values varied between 0.0001 and 0.0011, indicating that the models are acceptable.Top of Form

***Keywords*:** Anthracnose, black pepper, disease incidence, rainfall, rainy days, relative humidity

Black pepper (*Piper nigrum* L.), also known as "Black gold" in India, holds a prominent position in the spice industry. It is cultivated to produce black, white, and green pepper. For many years, India enjoyed the top position in black pepper production, but it is now facing competition from other countries. In India, the cultivation of black pepper is primarily practiced in southern states, but some states in North-East India, particularly Assam, are also involved in its production. Black pepper cultivation in Assam covers an area of 3,300 hectares, producing 2713 tons of peppercorn (Department of Agriculture & Farmers Welfare, 2023). Black pepper is susceptible to several diseases, including anthracnose, foot rot, charcoal rot, and slow decline. Among these diseases, anthracnose, also known as 'pollu disease,' is economically significant, affecting plants in both the field and the nurseries. It is caused by the fungal pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. This disease primarily affects aerial parts such as berries, leaves, and spikes (Sankar and Kumari, 2002). Symptoms on leaves include gray to brown-colored lesions surrounded by a yellow halo. As the disease progresses, these lesions coalesce, and several acervuli (asexual fruiting bodies) form on the papery-textured lesion (Jayakumar *et al*., 2009). If the infection reaches the spike and stalk, the entire spike shrivels, dries, and sheds prematurely. Cracks on the pericarp of berries have also been reported (Biju *et al*., 2013). Anthracnose disease incidence in Assam has been reported to range from 50% to 60% (Verma *et al*., 2022).

The effective disease management often relies on measures such as using resistant varieties and chemicals. However, these chemicals are not only expensive but also pose hazards to humans, biodiversity, and the environment. Their indiscriminate use exerts selection pressure on pathogens, leading to the development of resistance (Abdelaal *et al*., 2014). If the disease status could be known in advance, it would be possible to curtail the indiscriminate use of chemicals. In this context, the use of weather-based

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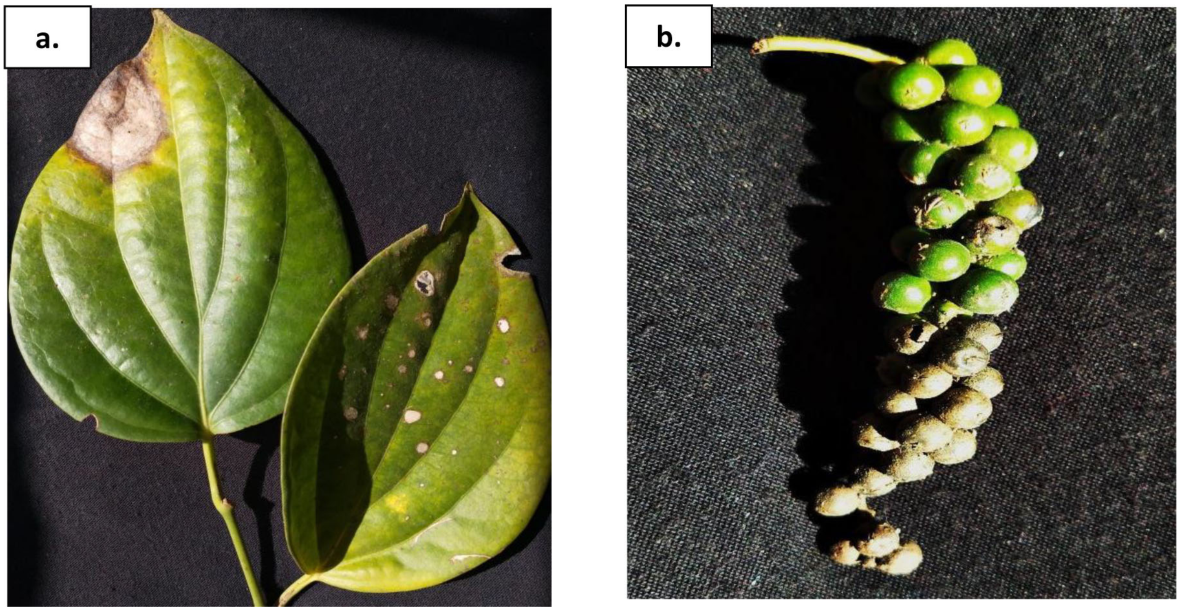

Fig 1: **(a.)** Symptoms of anthracnose on black pepper leaves **(b.)** Symptoms on berries

regression models can assist in advising farmers to implement timely anthracnose management plans, thereby reducing both the economic and environmental costs. Several disease prediction models have been developed to explore the influence of weather factors on host-pathogen interactions. These models encompass downy mildew in pearl millet (Kumar *et al*., 2010), anthracnose in betel vine (Sahoo *et al*., 2012), early blight in potato (Saha and Das, 2013), alternaria leaf spot in apple (Huang *et al*., 2022), anthracnose in soybean (Bhatt *et al*., 2022), fusarium wilt in chickpea (Khanna *et al*., 2022), nursery wilt in black pepper (Verma and Chakrawarti, 2022), and alternaria blight in Rapeseed-mustard (Yadav *et al*., 2022).

In the present study, attempts were made to investigate the role of different weather parameters on the occurrence of anthracnose in commonly grown black pepper varieties in the Upper Brahmaputra Valley Zone of Assam. Our goal was to develop a regression model to estimate disease incidence based on weather variables.

MATERIALS AND METHODS

An experiment was carried out during 2019-21 at the orchard of Assam Agricultural University (AAU), located in Jorhat (26.7248° N, 94.1956° E, 116 m), which is in the Upper Brahmaputra Valley Zone of Assam. The soil of the experimental field is red laterite with a pH of 5.5. In the experimental field, seven different black pepper varieties, namely Arakkulamunda, Doddigya, Karimunda, Malligesara, Panniyur-1, Poonjarmunda, and Uddagare, were planted in July 2019. The planting was organized in accordance with Randomized complete block design with four replications. Recommended horticultural practices were followed without applying fungicides, allowing natural epiphytotics to occur.

Diseased samples were collected from the black pepper orchard at AAU, Jorhat. The pathogen was isolated from diseased leaves exhibiting characteristic anthracnose symptoms on potato dextrose media (PDA) (Fig 1). After isolation and purification, the pathogen was identified based on morphological, cultural, and molecular characters. Genomic DNA was isolated using a fungal DNA isolation kit. The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction, sequenced, and subjected to BLAST analysis.

Daily data on various weather factors, including maximum (T_{max}) and minimum (T_{min}) temperature, bright sunshine hours (BSH), rainfall (RF), morning relative humidity (RH I), evening relative humidity (RH II), and rainy days (RD), were obtained from the Department of Agrometeorology at AAU, Jorhat-13, Assam.

Disease scoring (%)

To assess the disease reaction under natural conditions, a rating scale developed by Verma *et al*. (2022) was utilized. This scale categorizes disease severity into the following ranges: Resistant (no anthracnose symptoms), highly tolerant (01-19), tolerant (20-40), moderately susceptible (40-55), susceptible (55-85), and highly susceptible (more than 85). The number of vines displaying anthracnose symptoms was counted, and the percent disease incidence (PDI%) was calculated using the following formula:

$$Disease\ incidence\ (\%) = \frac{No.of\ infected\ plants}{No.of\ plants\ observed} \times 100$$

The PDI for each variety was recorded at weekly interval, during 2019-2021.

Analysis of data

All the parameters were transformed into weekly mean, except the rainy days and rainfall, which were used as totals for individual week. The weekly PDI data were expressed as cumulative PDI (%). The cumulation start from the same date, for all weeks. Pearson correlation analysis was carried out to investigate how

Table 1: Disease reaction and per cent disease incidence (PDI) for 2019 and 2020

Variety	Reaction to anthracnose	PDI (%)	
		2019	2020
Arakkulamunda	T	35.1±0.31	34.2±0.23
Doddigya	T	30.7±1.16	26.7±0.17
Karimunda	HT	13.4±0.97	10.1±0.16
Malligesara	T	42.7±1.21	33.4±0.62
Panniyur-1	S	81.1±0.25	86.8±0.11
Poonjarmunda	S	74.0±0.27	68.3±1.62
Uddagare	T	29.2±0.19	24.8±0.18

*T= Tolerant, HT= Highly Tolerant, S= Susceptible
Here, ± Standard error

different weather conditions affected the interaction between black pepper plants and the anthracnose pathogen. Significant weather parameters were considered in the development of our regression model. Stepwise multiple regression analysis was employed to create models for estimating anthracnose incidence in various black pepper varieties. The weekly data, collected in 2019 and 2020, included anthracnose incidence as the dependent variable and various weather factors as independent variables. All the statistical analyses were conducted using SPSS (Statistical Package for Social Sciences), a software program developed by Stanford University in California.

Model validation

For validation of the models, the anthracnose incidence for different varieties was estimated by fitting the predictor variables of the 2021 dataset into respective models and comparing the estimated disease incidence with the actual disease incidence during the year. To determine the performance of models Root mean square error (RMSE) was determined by following formula:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (predicted - actual)^2}{N}}$$

Where, N is the number of samples.

RESULTS AND DISCUSSION

Isolation and pathogen identification

Fungal colonies on PDA after 14 days of incubation were cottony, dense, white to grey, and with salmon pink conidial mass in centre. Conidia (*n* = 50) were 13.6 to 19.8 µm × 4.2 to 6.4 µm, cylindrical, hyaline, single-celled, smooth-walled, and with rounded ends. Conidiophores were aseptate, hyaline, and branched. Based on morphological features, the isolate was identified in the *Colletotrichum gloeosporioides* species complex (Weir *et al*. 2012). The ITS (OK505611) sequence was deposited in the NCBI GenBank database. A BLAST analysis of ITS sequence revealed high level (100%) similarity to *C. gloeosporioides*.

Influence of weather factors on black pepper anthracnose incidence

The anthracnose disease was prevalent at high levels during the monsoon period, i.e., June to August (2019-20). During the pre-monsoon period (March to May), a low disease incidence was observed. The highest peak in mean disease occurrence was in July (2019-20), during which the mean monthly RF, RD, and RH I ranged quite high (83.5-89.6 mm, 15-20 days, and 88.8-92.1% humidity, respectively), and it was at its lowest in March. The disease reaction and PDI are presented in Table 1. The anthracnose disease was significantly and positively correlated with T_{min}, RF, RD, and RH I ($p < 0.05$) in all the varieties (Table 2). This indicates that the pathogen, *C. gloeosporioides*, prefers high minimum temperatures and atmospheric moisture for its growth and sporulation.

Multiple regression model

Weather factors which were found to be statistically significant were considered for model preparation using stepwise multiple regression analysis. The analysis was carried out based on the two years data (2019 and 2020) for the estimation of anthracnose. For black pepper anthracnose, RF, T_{min}, RD and RH I showed 68.2, 72.0, 82.3, 78.4, 68.0, 84.2, 82.1 percent variability in Arakkulamunda, Doddigya, Karimunda, Malligesara, Panniyur-1, Poonjarmunda and Uddagare, respectively. Models for each variety are presented in Table 3. To find out the Y value (Disease incidence) one has to put values of RF, T_{min}, RD and RH I only. High R^2_{adj} value shows the high efficiency of developed model to estimate the anthracnose incidence.

Model validation

Models were validated with the separate data set recorded during 2021. The regression models were validated satisfactorily with low RMSE which shows that the developed models have

Table 2: Correlation coefficient between black pepper anthracnose incidence and weather variables (2019 and 2020)

Weather parameters	Arakkulamunda	Doddigya	Karimunda	Malligesara	Panniyur-1	Poonjarmunda	Uddagare
T_{max}	-0.01	-0.02	-0.01	-0.01	-0.02	-0.02	-0.01
T_{min}	0.66*	0.68*	0.60*	0.77*	0.82*	0.80*	0.68*
RF	0.88*	0.86*	0.82*	0.88*	0.94*	0.92*	0.86*
RH I	0.82*	0.84*	0.84*	0.84*	0.85*	0.85*	0.82*
RH II	0.11	0.12	0.12	0.11	0.12	0.21	0.20
RD	0.88*	0.88*	0.79*	0.85*	0.88*	0.86*	0.87*
BSH	-0.42	-0.41	-0.41	-0.43	-0.41	-0.44	-0.42

*Significant at P = 0.05

Table 3: Models for estimation of anthracnose in different black pepper varieties

Variety	Equation	R^2	R^2_{adj}
Arakkulamunda	$Y = -36.7 + 0.12\ (RF) + 0.35\ (T_{min}) + 0.87\ (RH\ I) – 0.60\ (RD)$	0.68	0.67
Doddigya	$Y = -48.7 + 0.17\ (RF) + 0.73\ (T_{min}) + 0.78\ (RH\ I) – 0.90\ (RD)$	0.72	0.72
Karimunda	$Y = -12.8 - 0.03\ (RF) + 0.26\ (T_{min}) + 0.25\ (RH\ I) + 0.12\ (RD)$	0.82	0.82
Malligesara	$Y = -18.7 + 0.20\ (RF) - 0.27\ (T_{min}) + 0.23\ (RH\ I) – 0.42\ (RD)$	0.78	0.77
Panniyur-1	$Y = -10.38 + 0.20\ (RF) + 0.16\ (T_{min}) + 0.69\ (RH\ I) – 0.73\ (RD)$	0.68	0.67
Poonjarmunda	$Y = -13.25 + 0.108\ (RF) + 0.43\ (T_{min}) + 0.54\ (RH\ I) – 0.24\ (RD)$	0.84	0.84
Uddagare	$Y = -13.2 + 0.19\ (RF) + 0.41(T_{min}) + 0.36\ (RH\ I) – 0.22\ (RD)$	0.82	0.82

* Y (dependent variable) = Disease incidence; RF = Weekly average of rainfall (mm); T_{min} = weekly average of minimum temperature (°C); RH I = Weekly average of morning relative humidity; RD = Number of rainy days

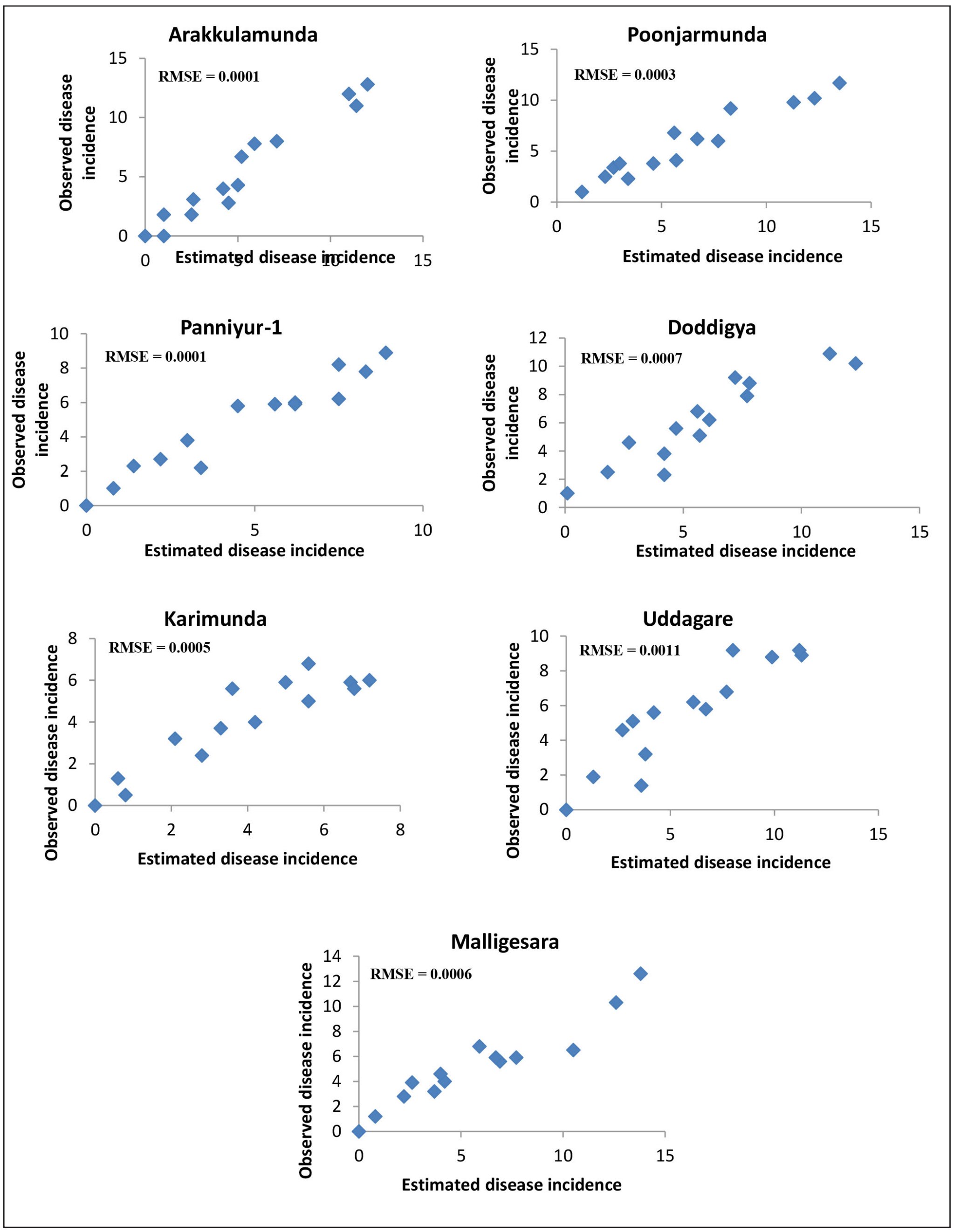

Fig 2: Estimated and observed anthracnose incidence graph based on 2021 data

performed outstandingly in estimation and can be used further to detect the favorable conditions of anthracnose occurrence in black pepper vines. Estimated and observed anthracnose incidences based on 2021 data are graphically represented in Fig 2.

The recent epidemiological studies have illuminated the considerable impact of weather factors on anthracnose incidence. These findings align with the observations made by other researchers, such as Biju *et al.* (2013). Notably, the study identified four influential factors contributing to anthracnose occurrence in the Upper Brahmaputra Valley Zone of Assam, *viz*., with RF (Rainfall), Tmin (Minimum Temperature), RD (Rain Days), and RH I (Morning Relative Humidity). The role of rain splash in dislodging conidia, particularly those embedded in the gelatinous matrix within the acervulus (the asexual fruiting body), has been recognized in previous studies by Estrada *et al.* (1993) and Cartney (1997). Furthermore, it was observed that the highest disease incidence (97.00%), mycelial growth, and conidial germination (72.00%) occurred at 100 percent relative humidity, while the lowest rates were recorded at a relative humidity level of 36.8% (Udhayakumar, 2018). Apart from satisfactory validation, the model also revealed the significance of these four weather parameters in influencing host-pathogen interaction. These well-developed models excel in accurately estimating anthracnose, thereby mitigating the risks faced by black pepper growers in the Upper Brahmaputra Valley Zone of Assam.

CONCLUSION

The anthracnose of black pepper induced by *C. gloeosporioides* was found to be significantly and positively correlated with the minimum temperature, rainfall, rainy days, and morning relative humidity. Developed models (2019 and 2020) performed well during validation (2021). These models will help in guiding the black pepper growers of Upper Brahmaputra Valley in Assam to take the judicious decision for crop protection including prophylactic spray of fungicides against anthracnose.

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Authors contribution: **R. Verma:** Data collection, Data analysis, Conceptualization, Methodology, Visualization, Writing-original draft, Writing-review, **A. Das:** Resources, Conceptualization, Methodology, Supervision, Writing-review and editing, **P. R. Narzary:** Data collection, Writing-review, **S. Sharma:** Resources, Supervision, Writing-review

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