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Research Article

Epidemiological Insights into Mango Anthracnose Disease Invited by *Colletotrichum gloeosporioides*

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ABSTRACT

Mangifera indica L., commonly known as mango, is a vital fruit crop in Pakistan, renowned for its rich nutritional profile and phytochemical composition. However, mangoes are susceptible to various phytopathogenic diseases, with anthracnose being a significant concern. This study investigated the impact of environmental factors, specifically temperature and humidity, on the development of mango anthracnose. Seven mango varieties (Duseri, Anwarlatore, Sindhri, Langra, Sammar, White Chunsa, and Fajr) were selected and surveyed in the Bahawalpur region, including Mubarakpur, Yazman, Ahmadpur East, Lalsuhanra, and Bahawalpur City. Tagged leaves exhibiting anthracnose symptoms were monitored to assess the disease's growth rate in response to environmental factors. Our findings suggest that optimal conditions in the Bahawalpur region facilitate the proliferation of mango anthracnose. Significantly, changes in rainfall patterns, potentially linked to global warming, contribute to the spread of anthracnose disease.

Keywords: Anthracnose, Fungal Disease Management, Agricultural Epidemiology, Phytopathogenic Fungi, Tropical Fruit, Disease rate.

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INTRODUCTION

Mango (*Mangifera indica* L.) is consumed by the majority of people in developed and developing countries and is known as the "King of Fruits" for its delicious taste, nutritional content, and therapeutic benefits (Deressa et al., 2015). It has originated from tropical Asia and belongs to the Anacardiaceae, Mangifera, and Spanidales families and genera. Most mango-producing countries produce 98 % of world mangoes and trade up to 80 % (Bally, 2006). The fruit has a source of vitamins C and A, protein (6.3%), amino acids (43.7–54.5%), carbs (58–80 %), sugars (15 %), and fatty acids (3.6–6.7 %). The seeds of mangoes were used to manufacture flour and lipids which are further processed and used for the preparation of butter (Awa et al., 2012).

Green mangoes are utilized for the preparation of pickled and jam items. The bark and leaves of the bowel disease and large intestine inflammation (Duricova, 2017) mango tree are therapeutic (Archibald et al., 2003). Studies revealed that mango fruit contains anti-inflammatory phytochemicals, especially for infections including inflammatory. Pakistan ranked sixth for the production of mango crop worldwide. The 4-5 % of the total production of the mangoes was exported (Badar et al., 2015), (Hammad Badar et al., 2019).

The fruit was damaged by a lot of pathogens such as bacteria, fungi, nematodes, and abiotic factors such as physiological environmental, and nutritional deficiencies factors (Jiskani et al., 2007). The production of mango crop faced various mango malformation, anthracnose, bacterial black spots, powdery mildew, cankers, and other complications worldwide. The diseases that were caused by phytopathogens in Pakistan include phimosis, Alternaria, anthracnose rot, and stem-end (Khanzada et al., 2018). Anthracnose is the world's most important growing zone disease that affects mango each year (Monteon Ojeda et al., 2012). The *C. gloeosporioides*, is the causal agent of mango anthracnose disease. It produces a big outbreak during postharvest flowering. The anthracnose disease caused a huge loss to the agricultural industry. Due to excessive humidity and heavy rain, anthracnose is the most common and prevailing mango disease. Disease losses in an unmanaged orchard might reach 50 % to 100 % under optimal conditions (Khaskheli, 2020). The symptoms on twigs, leaves, petioles, fruits, and flower clusters initially. The brown angular lesions formed on leaves and fruits turn brown to black spots. Panicle symptoms include little black to dark brown spots that damage flowers and reduce production (Nelson, 2008).

Most of the time the infection only damages the outer surface of the fruit but sometimes it can also spread to the pulp of the fruits. The fungus develops acervuli and covers lesions with orange to salmon-pink conidia in the early stages (Arauz, 2000). The management of Anthracnose is reduced by using Bacillus strains (Fatima et al., 2023), (Maalik et al., 2023), (Malik et al., 2024) by the production of lipopeptides (Mahmood et al., 2022), (Mahmood et al., 2023b) *Trichoderma harzianum* also as a biological control (Mahmood et al., 2024) by using essential oils (Mahmood et al., 2023a), (Ahmad et al., 2023) because chemical pesticides can be harmful. There were a lot of plant species that previously tested against *C. gloeosporioides* including *A. indica*, *M. indica*, *Z. officinale*, *A. sativum*, *C. longa*, and *C. sativa* (Ajay Kumar, 2014).

To analyze environmental variables that affects the infection and disease development to control mango anthracnose disease and reduce infection promptly. The goal of the study is to investigate the environmental factors that affect anthracnose disease in different varieties of mango

MATERIALS AND METHODS

Selection of varieties of experiment design

After careful consideration of the environmental factors and cultivars that were planted in different areas of southern Punjab, such as Bahawalpur, Ahmadpur East, Yazman, Lalsuhanra, and Noorpur Nuranga, five orchards were selected for this study, the seven varieties were selected for the study was Duseri, Langra, Anwar Ratole, White Chunsa, Sammar, Sindhri, and Fijri. The period selected for research was from September 2021 to the end of August 2022. It was appropriate to conduct the research on the selected orchards and the data was recorded as well as the severity of the disease severity on the different varieties of mango.

Tagging of Leaves

The infected leaves from selected orchards of mango plants of different varieties were marked and tagged. A total of 49 plant leaves were marked with tags (seven plants from each variety) to collect information regarding anthracnose disease of mango and to check the influence of environmental effects damage the mango plant. To maintain data from each cultivar tagging of plants and leaves was done with great attention and care. Each leaf of a variety was marked with its different codes, such as SinL1, SinL2, SinL3, SinL4, and SinL5. The plant leaves were observed before and after blossoming and throughout the investigation.

Observation of Anthracnose disease in mango

The plant leaves that pose the symptoms of anthracnose disease were selected and observed throughout the duration. During the survey procedure, stringent measures are taken to ascertain that the plants under examination have not been subjected to chemical or bio-pesticide applications. Furthermore, meticulous observation and documentation of anthracnose symptoms are conducted for comprehensive record-keeping.

Disease incidence

The incidence of disease is also known as an estimation of a specific disease that occurs within a specific period. The disease incidence of anthracnose disease in mango was determined by using the equation for the whole percentage as described by (Akhtar and Alam, 2002). The results and discussion section describe mango anthracnose's restricted disease incidence.

$$Disease\ Incidencce = \frac{No. of\ diseased\ leaves}{Total\ no. of\ leaves} \times 100$$

Isolation of fungal pathogen

The pathogen was isolated from the diseased leaves of plants. The collected infected leaves were washed with 1% NaOCl solution and three washing with distilled water for 1 minute respectively. Following washing, leaves were placed on the potato dextrose agar (PDA) media for isolation, and placed the plate in an incubator at 26 ℃ for 7 days. After 7 days the colony growth and morphology were observed and microscopic features were also observed.

Microscopic identification of the pathogen

The microscopic analysis revealed the presence of mycelium belonging to a particular fungus species, observed at varying magnifications, specifically 10X, 40X, and 100X. The species identification was established through careful assessment of colony morphology, growth patterns, and spore characteristics. These findings were subsequently summarized in a chart, delineating the respective traits and properties of the identified species

Determination of Infection Frequency

The following formula was used for the determination of the colony count of the pathogen was used to calculate the frequency of pathogen infection:

$$Infection\ frequency\ (IF) = \frac{No. of\ bits\ colonized}{Total\ no. of\ cultured} \times 100$$

Recording of Meteorological Data

By accumulating meteorological data consisting of low and high temperatures, relative humidity, rainfall, and effects of environmental factors on the disease anthracnose on mango was observed. The data was collected by using the data recorders that were placed at various sites. The data on weather were collected from September 2021 to the end of August 2022. Throughout the entire study, the average data for each month was recorded to assess the effect of environmental factors on the incidence of pathogen infection.

Table 1. The meteorological data from the regional agriculture research institute (RARI), Bahawalpur.

Month	Max Temperature. (℃)	Min Temperature. (℃)	RH Percentage Morning
Sep-2021	37.1023	26.4333	78.6333
Oct-2021	34.7097	20.3871	75.129
Nov-2021	29.5333	12.8333	80.6333
Dec-2021	22.5484	7.12903	90.6129
Jan-2022	19.3871	6.77419	90.4516
Feb-2022	22.4516	8.96774	76.8387
Mar-2022	34.0645	17.4516	73.871
Apr-2022	40.6774	22.1935	48.3226
May-2022	43.1613	26.8065	47.5484
Jun-2022	39.8387	25.871	58.6452
Jul-2022	37.0968	26.9032	82.0323
Aug-2022	36.0323	26.6774	82.0968

RESULTS

Identification of Mango Anthracnose based on microscopic observations

The isolation of the pathogen was used to determine the symptoms manifested by the fungal pathogen that is responsible for the disease. The pathogen that causes disease in mangoes possesses the morphological examination through which the identification of causal agent is possible easily. The mango anthracnose was caused by *C. gloeosporioides*.

Mango anthracnose

The acervuli have been attached to the host plant and the conidia was discharged from the structure acervuli. On the PDA medium *C. gloeosporioides* produce greyish-white to pale-brown colonies. The surface of the colonies was round, downy, and fuzzy. The conidia that are typically possessed by fungi are hyaline, one-celled, cylindrical, straight, and obtuse at the apex. Conidia are in size ranges varies 15-20 µm to 5-7 µm and temperatures between 25-30 °C are ideal for the growth of conidia.

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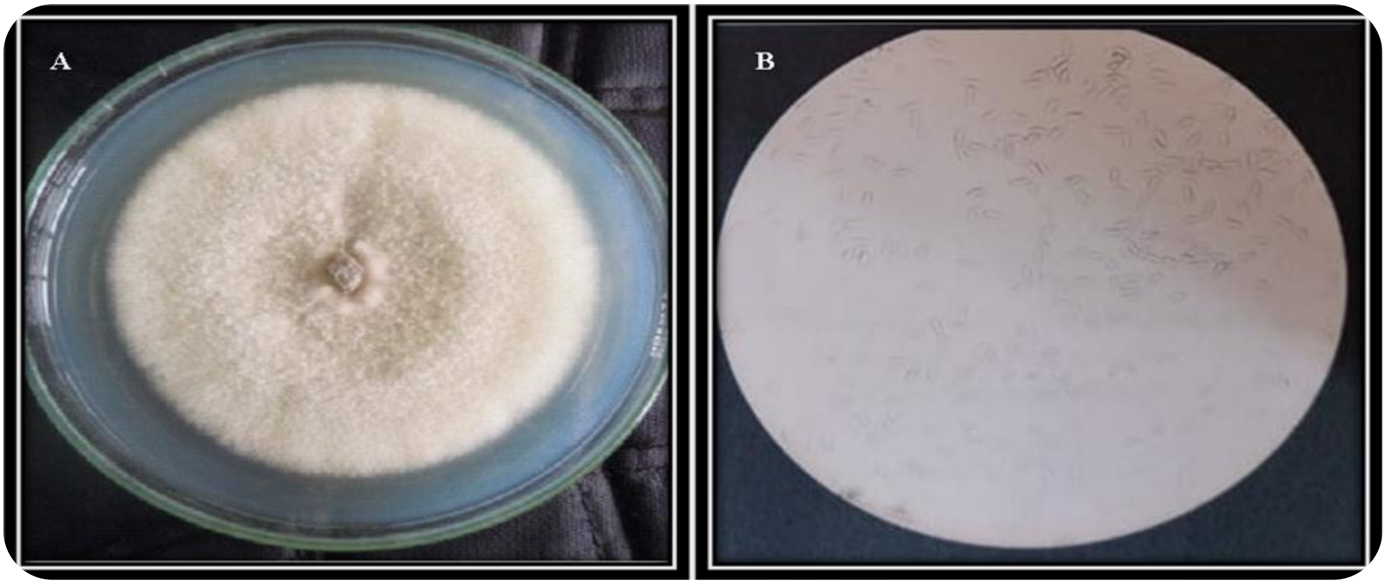

Figure 1: Macro and microscopic features of C. gloeosporioides. A: Colony morphology after 7 days B: Conidia of C. gloeosporioides at 100X magnification.

Correlation of disease incidence with environmental variables on different varieties of mango

The epidemiological parameters that were estimated greatly influenced the development of the disease. A statistical study of every epidemiological component influencing the onset of anthracnose disease was conducted, and it was discovered that each of these factors strongly influenced the onset of the disease, providing a positive correlation coefficient for disease onset. For the Sammar, Anwarlatore, Duseri, Langra, Fijri, White Chunsa, and Sindhri varieties, the temperature component correlated positively with co-efficient of 0.5457, 0.4935, 0.5020, 0.4712, 0.5425, 0.4617, and 0.5457, respectively. For the Sammar, Anwarlatore, Duseri, Langra, Fijri, White Chunsa, and Sindhri varieties, the impact of relative humidity was also estimated and shows a negative relationship between two factors (disease incidence and relative humidity) indicated by negative correlation coefficients of -0.2964, -0.3037, -0.3402, -0.3485, -0.3404, and -0.3750, respectively.

Table 2. Correlation of environmental factors with different varieties of mangos in Bahawalpur

VARIETIES OF MANGOES	TEMPERATURE (^{O}C)	RELATIVE HUMIDITY (RH%)
SAMMAR	0.377134711	-0.29645416
	0.457043583	
ANWARLATORE	0.432119392	-0.30370619
	0.493556446	
DUSERI	0.427367756	-0.34029145
	0.50205483	
LANGRA	0.408886909	-0.34850323
	0.471286747	
FIJRI	0.481938796	-0.36842805
	0.542514918	
WHITE CHUNSA	0.410403552	-0.3404346
	0.461787718	
SINDHRI	0.474810174	-0.37509229
	0.545701273	

Effect of Environmental Factors on the Anthracnose Disease

Temperature variable

It is widely known that mango anthracnose turns rachis, florets, leaves, and fruits black. Environmental variables play a significant role in both the outbreak and the spread of this disease from one place to another. The effect of temperature on mango anthracnose development was estimated, and a positive relationship between temperature and disease incidence was observed. For each unit of temperature, the incidence of the diseases Sammar, Anwarlatore, Duseri, Langra, Fajri, White Chunsa, and Sindhri rose gradually by 1.242 %, 1.139 %, 1.257 %, 1.198 %, 1.067 %, 1.061 %, and 1.272 %, respectively. The following regression graph of disease incidence versus temperature for each mango variety is plotted below:

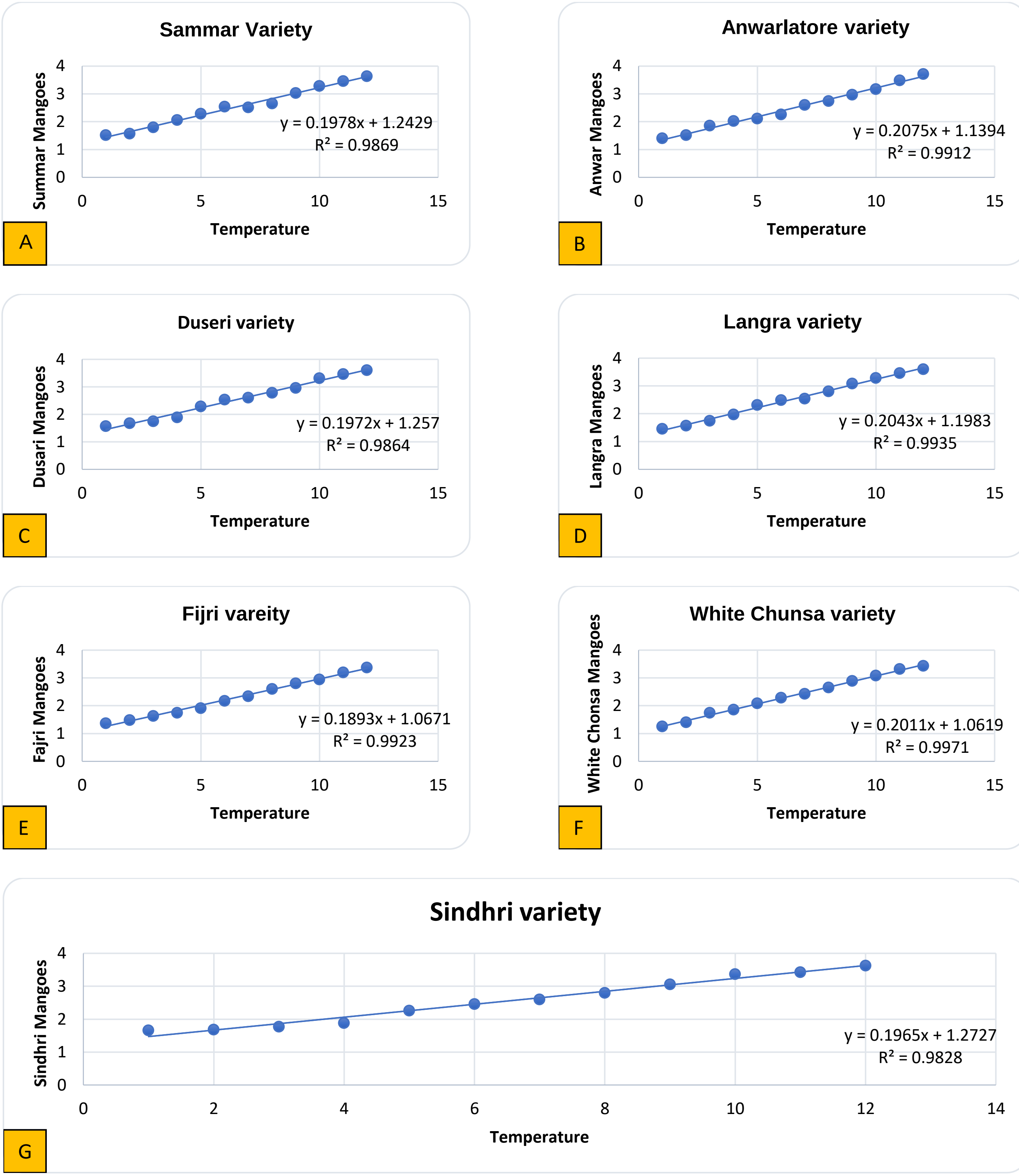

Figure 2: The regression graphs of disease incidence in different varieties of mango crop against temperature (A=Sammar variety; B=Anwarlatore variety; C= Duseri variety; D: Langra variety; E= Fijri variety; F=White chunsa variety; G=Sindhri variety)

Relative Humidity Variable

The effect of relative humidity on disease development was observed when the data were statistically analyzed, the results revealed a negative correlation between the two variables, with increases in disease incidence of about 3.603 %, 3.640 %, 3.768 %, 3.826%, 3.572 %, 3.617 %, and 3.903 % for the Sammar, Anwarlatore, Duseri, Langra, Fajri, White Chunsa, and Sindhri varieties, respectively, for every decreasing unit. Following is a plot of the regression graph of disease incidence against relative humidity for each mango variety:

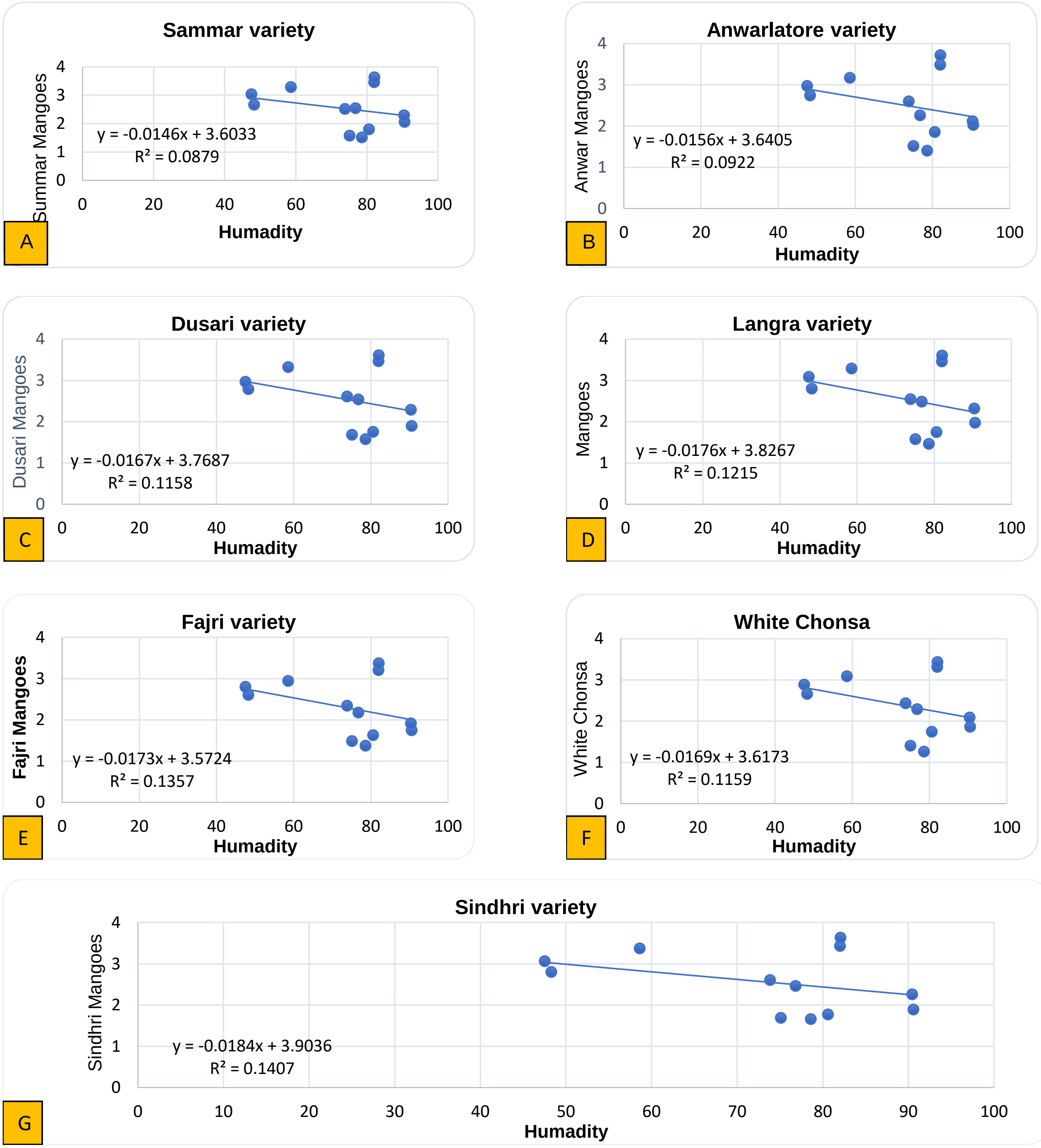

Figure 3: The regression graph of disease incidence in different varieties of mango crop against relative humidity (A=Sammar variety; B=Anwarlatore variety; C= Duseri variety; D: Langra variety; E= Fijri variety; F=White chunsa variety; G=Sindhri variety).

DISCUSSION

The anthracnose was prompted by the fungus *C. gloeosporioides*. It is a facultative parasite that has an incomplete asexual or anamorphic form and can replicate asexually. The growth of conidia is favored by a damp and warm environment.

It enters into host plant through damaged tissues and develops specialized structures including acervuli, setae, and appressoria through which it interacts with the plant host. The rain droplets act as vectors for its dissemination and throughout the process of infection, the fungus develops asexual entities called acervuli.

Agriculture accounts for 30 % of GDP of Pakistan. The mango fruit is the most eaten fruit that is consumed by the people of the country due to its flavor and it plays a vital role in the food industry (Siraj, 2010). The cultivation of mangoes occurs in every province of Pakistan but in Punjab, the production rate is high as compared to others (Hammad Badar et al., 2019). Multan, Muzaffargarh, Rahim Yar Khan, and Bahawalpur produce the most outstanding mangoes in Punjab. Mangoes have flowery scars. Sometimes, these scars bulge. The skin of mango skin is smooth, waxy, and leathery. During the ripening stage, its color turns light green to yellow, and higher-quality mangoes have less fiber and turpentine flavor.

According to reports of (Karar et al., 2010), 179,000 hectares, the mango is the fourth-largest fruit crop in Pakistan production rate of (1.9 million tonnes) on an area of 179,000 hectares.

Mangoes from Pakistan are exported to approximately 50 countries and are more popular worldwide. Pakistan wants to produce more high-quality mangoes to meet global demand, but climate change has made this challenging. Climate change threatens food and water production worldwide. Global warming affects weather, making climate change studies harder. Climate change affects the growth, development, and prevalence of the disease mango, which requires adjustments in disease management in different places (Ploetz, 2003).

The current study examined how temperature, relative humidity, rainfall, and wind speed affected mango disease. The current study included seven commercial varieties: Sammar, Duseri, Sindhri, Langra, Anwarlatore, Whitechunsa, and Fijri. This study examined how environmental conditions affected several types in the Bahawalpur region. Climate change in Pakistan raises environmental and social concerns. The environment affects fruit and crops (Lioubimtseva and Henebry, 2009).

Mango is susceptible to frost, droughts, floods, windstorms, and seasonal temperature changes. Mangoes grow in frost-free zones. It thrives in cool, dry climates with high temperatures during mango blossom and fruiting. Mango thrives in temperatures between 27 to 37 ℃. Temperature changes affect mango floral disease pathogen proliferation and development, making it hard to predict when they will attack and difficult to manage (Varakumar et al., 2011).

C. gloeosporioides causes mango anthracnose. During mango blossoming, it affects the fruit. It also kills mango panicles, reducing mango fruit production. Fruit productivity dropped to 80 % due to anthracnose (Nazir et al., 2024).

In this investigation, affected components were cultured in vitro on growth conditions to identify the pathogen. Pathogen assault symptoms were then mapped to identify them from other diseases. Anthracnose symptoms during meteorological data collection include strengthening of the infected part, blackening of the peduncle followed by dying back, de-coloration, development of a large black lesion on the lower side, and girding-induced apex death. According to current research, Bahawalpur had a worse mango anthracnose outbreak. Whitechunsa, Sammar, and Anwarlatore were most susceptible to anthracnose, while Langra, Sindhri, and Fijri were least. The ideal anthracnose temperature was 25–30 °C and 80–85 % humidity. Although heavy rain reduces disease prevalence, it increases humidity, which is good for disease. Regular airflow helps, while high-speed wind hurts. In damp settings, pathogens thrive. Wet, cold weather favors anthracnose infections. Data was collected to distinguish the pathogen from other diseases and link it to isolated pathogen symptoms

CONCLUSION

The epidemiological studies on mango Anthracnose disease caused by *C. gloeosporioides* have shed light on the nuanced dynamics of this destructive plant-pathogen relationship. Important factors that influence the spread and prevalence of disease have been identified in current investigations. The findings demonstrate that the mango anthracnose disease is affected by various environmental factors, the host plant's resistance, and the pathogen's genetic diversity. The findings of these investigations can also be used to develop targeted breeding programs, fungicide treatments, and cultural practices for disease management. The output of mango crop can be indefinitely thanks to epidemiological research which helps against new plant diseases and the shifting agricultural landscape.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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