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Assessment of the current status and characterization of mango anthracnose (C*olletotrichum gloeosporioides)* isolates in Wolaita zone, south Ethiopia

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Abstract

Mango anthracnose (*Colletotrichum gloeosporioides*) poses a significant threat to small-scale mango producers in the Wolaita zone of southern Ethiopia. This study investigated the disease's incidence and severity, along with characterizing the pathogen and its impact on mango production in the area. Disease incidence was determined by examining eight randomly selected mango plants per farm field, while severity was assessed by measuring the percentage of leaf/fruit area infected on eight randomly selected plants. Laboratory analysis focused on characterizing *C. gloeosporioides* isolates collected from infected plant material. Conidial morphology was examined by measuring the length and width of 20 conidia per isolate. Cultural characteristics, including colony growth, color, form, and diameter, were also documented for each isolate grown on potato dextrose agar. Results revealed a high incidence of mango anthracnose in the surveyed orchards, ranging from 60.5% to 84.0% on leaves and 70.8% to 79.0% on fruits. Disease severity also varied significantly between locations, with leaf severity ranging from 39.75% to 76.75% and fruit severity ranging from 40.25% to 57.15%. Morphological analysis of *C. gloeosporioides* isolates revealed an average conidial length of 10.65 µm and width of 4.46 µm. Colony morphology on potato dextrose agar exhibited variability, with an average diameter of 34.47 mm and the lowest mycelial growth of 18.18 mm. The study highlights the significant threat of mango anthracnose to mango production in the Wolaita zone. The observed variability in disease severity and pathogen characteristics underscores the need for location-specific disease management strategies. Future research should

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focus on developing effective and affordable control measures for mango anthracnose in the Wolaita zone, considering the observed variability in disease severity and pathogen characteristics.

Keywords: Anthracnose, cultural characterization, disease intensity, isolate, mango

Introduction

Mango (*Mangifera indica* L.), a member of the *Anacardiaceae* family, stands as a cornerstone of fruit production in tropical and subtropical regions worldwide (Ploetz, 2004). Originally cultivated in the Indian subcontinent and southern Asia, mango has transcended geographical boundaries to achieve global acclaim, with India reigning as the top producer and Mexico dominating the export market (Ploetz, 2004). Revered as the "King of fruits," mango's esteemed status is deeply rooted in its exceptional qualities, encompassing its remarkable diversity, delectable flavor, exceptional nutritional and medicinal value, and profound cultural significance (Ploetz, 2004). For millions, particularly in developing nations, mango is more than just a fruit; it is a dietary staple, deeply intertwined with cultural traditions and livelihoods (Evans et al., 2017).

Cultivated in over 87 countries, mango production represents a vital agricultural sector globally (Kuhn et al., 2017). Although developing countries produce most of the world's mangoes (about 98%), developed countries are responsible for 80% of global mango imports (Normand and Lauri, 2018). In Ethiopia, mango cultivation is predominantly concentrated in the western, southwestern, and rift valley regions, with Arbaminch, Dilla, and Wolaita emerging as major production hubs (Kumar et al., 2021). The mango industry in this area plays a significant role in generating income for smallholder farmers and contributing to the local economy (Zhang and Lee, 2020).

Despite its significance, mango production in Wolaita zone faces a myriad of challenges, including abiotic stresses, pests, and diseases, all of which affect yield and fruit quality (Evans et al., 2017). Among these challenges, anthracnose, caused by the fungal pathogen *Colletotrichum gloeosporioides* Penz., stands out as a formidable threat to mango production worldwide (Freeman et al., 2015; Kumar et al., 2021). This disease is prevalent in mango-growing regions globally and poses a significant threat to mango production, leading to substantial economic losses and jeopardizing the livelihoods of millions of farmers. This devastating disease affects

various parts of the mango tree, including leaves, flowers, fruits, and branches, leading to reduced yields, poor fruit quality, and even tree death in severe cases. In Ethiopia, mango production grapples with additional constraints, such as ageing orchards, inadequate management practices, reliance on seedling trees, and a lack of awareness about effective disease management strategies. These factors contribute to the declining trends in mango quantity and quality observed (Muhammad et al., 2017).

The primary sources of *C. gloeosporioides* inoculum include infected plant parts such as branch terminals, mummified inflorescences, flower bracts, and leaves (Muhammad et al., 2017). Conidia, the asexual spores of the fungus, are dispersed by rain splash and require free moisture for infection. Upon contact with susceptible host tissue, the conidia germinate and produce specialized infection structures called appressoria. Melanization, the process of melanin deposition in the appressorium wall, strengthens the structure and facilitates the penetration of the host cuticle by infection pegs. The presence and prevalence of Melanized appressoria have been used as indicators for predicting infection periods and the need for disease management interventions (Muhammad et al., 2017). Recent research has also highlighted the role of specific fungal enzymes, such as cutinases and pectinases, in the infection process. These enzymes degrade the structural components of the plant cell wall, allowing the pathogen to breach host defenses and colonize plant tissues (Trejo-López et al., 2022).

Anthracnose infection can occur at various stages of mango development, from flowering to fruit maturity. Infection during early fruit development can lead to fruit drop, while infections at later stages can result in fruit lesions, discoloration, and rot, rendering the fruit unmarketable. Accurate identification of *C. gloeosporioides* is crucial for effective disease management. While morphological characteristics such as conidial morphology, appressoria development, colony color, and the presence or absence of setae have been traditionally used for identification, these traits can be influenced by environmental factors and repeated subculturing, leading to variations (Muhammad et al., 2017). In 2010, Alemayehu et al. (2014), characterized isolates of Colletotrichm gloeosporioides, and evaluated the efficacy of essential oils against the pathogen in three areas of southern Ethiopia, i.e., Arbaminich, Dilla, and Wolayta. Amsalu et al. (2016), conducted study during the 2013 and assessed the prevalence, incidence and severity of mango anthracnose in southwest Ethiopia (Jimma). However, limited research has been conducted on the prevalence and impact of this destructive pathogen, despite Wolaita area has a significant

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role in mango production. By characterizing the pathogen and thoroughly assessing its incidence and severity in both field conditions and local market environments among small-scale farmers, this research addresses a critical gap in knowledge. The insights gained will inform the development of effective and sustainable disease management strategies, ultimately aimed at safeguarding mango production and enhancing the livelihoods of farmers in the area. Understanding the specific dynamics of mango anthracnose in this context is essential for formulating tailored interventions that consider local agricultural practices and environmental factors.

Materials and methods

Description of the study area

This field study was conducted in four districts in the Wolaita Zone of southern Ethiopia, an area known for having favorable mango cultivation conditions. Districts - Offa, Humbo, Kindo Koisha, and Boloso Bombe- were purposively selected based on their high potential for mango production and the significant land area dedicated to mango orchards. Offa, with a mean temperature of 23°C and annual rainfall of 1150 mm, sits at an altitude of 2000 meters above sea level. Its coordinates are 6° 44' 60" N, 37° 29' 59" E. Humbo is characterized by a mean temperature of 20°C and annual rainfall of 1295 mm and is located at 1800 meters above sea level. Its coordinates are 6°73’125” N, 37°74’742” E. Kindo Koisha experiences a mean temperature of 21°C and annual rainfall of 924 mm and is situated at an altitude of 1170 meters. Its coordinates are 6°49’60” N, 37°29’59” E. Lastly, Boloso Bombe, with a mean temperature of 24.4°C and annual rainfall of 1520 mm, is located at an altitude of 1267 meters. Its coordinates are 7°08′15.1"N, 37°34'54.1"E (WZFEDD, 202; BBDANRDO, 2016).

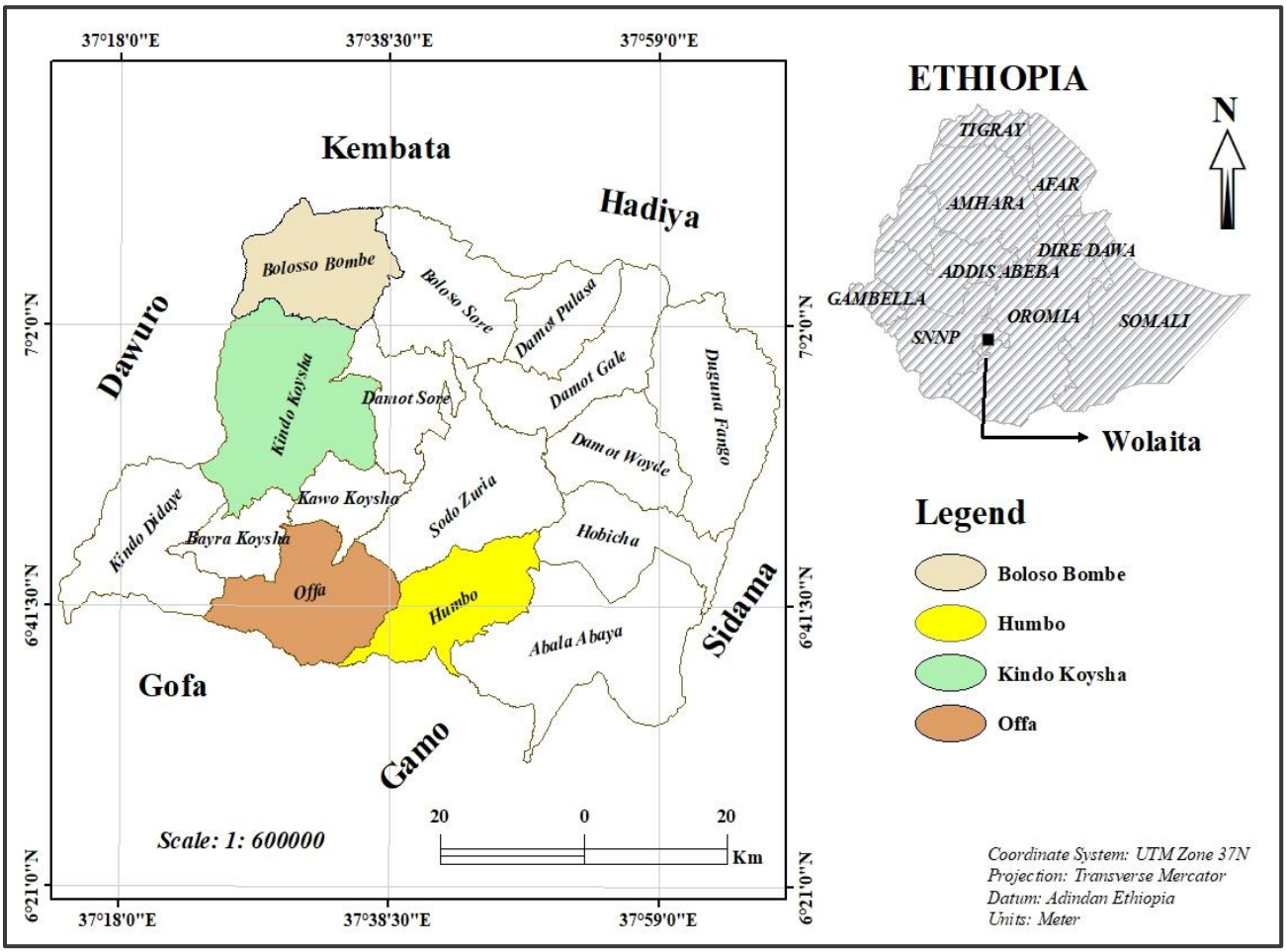

Figure 1. Map of the study areas.

Study area selection and sampling strategy

Four districts, namely Boloso Bombe, Kindo Koisha, Humbo, and Offa, were purposefully selected to represent the variability in mango farming practices, altitude, climatic conditions (temperature and humidity), and mango crop availability within the Wolaita Zone. In each district, six kebeles (smaller administrative units) with notable mango production were chosen. Sampling locations were marked using GPS to record altitude and coordinates. Within each kebele, three points were randomly selected and marked along a diagonal line within a designated area, forming a triangular sampling zone. From the center of this zone, eight mango trees were randomly selected for disease assessment, resulting in a total of 48 trees assessed per district (6 kebeles x 8 trees). This strategy ensured a representative sample across different microclimates and management practices within each district.

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Post-harvest disease assessment

To evaluate the post-harvest implications of anthracnose, mango fruits were sampled from local markets in each of the four districts. Specifically, markets in Bombe, Bele, Tebela, and Gesuba were selected. Ten mango sellers were randomly chosen at each market, and sixty fruits were randomly sampled (6 fruits per vendor) to assess post-harvest disease incidence and severity. This market survey was replicated three times to account for potential variations in fruit sources and handling practices.

Disease assessment

Field assessment**:** Eight mango trees were randomly selected within each designated field plot. To capture the vertical distribution of the disease intensity (disease incidence and severity), assessments were conducted at three different height levels: upper, middle, and lower canopy strata. This approach provided a comprehensive understanding of disease pressure within the tree canopy.

Market assessment**:** Sixty (60) mango fruits, randomly selected from ten (10) different vendors at each market, were examined for anthracnose symptoms. This sampling strategy aimed to represent the overall disease incidence in marketed mangoes from each district.

The disease incidence, both in the field and at markets, was calculated using the formula proposed by Suharban et al. (1985). This standardized approach allowed for a direct comparison of disease pressure across different locations and stages of the mango supply chain. Disease incidence, representing the percentage of infected mango plants, was meticulously assessed in both field and market settings.

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased fruits (leaves)}}{\text{Total number of assessed fruits (leaves)}} \times 100 \quad \text{eq. (1)}$$

Leaf severity assessment

Disease severity on mango leaves was assessed using a modified 0-5 scale adapted from Suharban et al. (1985). This scale correlated the percentage of leaf area affected by anthracnose lesions with a corresponding numerical rating (Table 1).

Table 1. Leaf severity assessment

Rating	Description
0	No visible spots on the leaf surface.
1	1-20% of the leaf area is covered in lesions (equivalent to 1-5 spots).
2	21-40% of the leaf area affected (6-10 spots).
3	41-60% of the leaf area exhibiting lesions (11-15 spots).
4	61-80% of the leaf surface is covered in lesions (16-25 spots).
5	More than 80% of the leaf area is damaged by anthracnose (>25 spots).

To ensure a representative assessment of each tree, leaves were examined at three vertical strata (upper, middle, and lower canopy). The average severity score across these three strata provided a comprehensive picture of disease severity for each mango tree.

Fruit severity assessment

An adapted 0-4 scale, based on the work of Corkidi et al. (2006), was utilized to assess the severity of anthracnose on mango fruits (Table 2).

Table 2. Fruit severity assessment

Fruit severity scale	Percentage of fruit area affected	Description
0	0-1%	No visible disease symptoms
1	1-5%	Slight disease presence
2	6-25%	Moderate disease severity
3	26-50%	Severe disease
4	51-100%	Very severe disease

For each field plot, eight mango trees exhibiting anthracnose symptoms were randomly selected. The average fruit area covered by lesions was meticulously assessed at the physiological maturity stage of the fruit. This stage was chosen, as it represents the point of peak susceptibility to anthracnose infection.

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To facilitate statistical analysis, the numerical severity ratings for both leaves and fruits were converted into a percentage severity index using the formula developed by Mayee and Datar (1986). This transformation provided a standardized measure of disease severity, allowing for direct comparisons between different sampling locations. Means of the severity from each plot were used in data analysis.

$$PSI = \frac{S_{nr}}{N_{pr} \times M_{sc}} X\,100 \quad \text{..... eq. (2)}$$

Where, S_{nr} - is the sum of numerical ratings, N_{lr} - is the number of (leaves/fruits) rated, and M_{sc} – is the maximum score of the scale.

Sample collection for laboratory analysis

Following the protocols outlined by Kumar et al. (2021), symptomatic mango fruits were collected from both household gardens and commercial orchards situated within the districts of Boloso Bombe, Humbo, Kindo Koisha, and Offa. These samples were then transported to the Plant Protection Laboratory at Wolaita Sodo University's College of Agriculture for further analysis. Upon arrival at the laboratory, the samples underwent a rigorous cleaning procedure.

Isolation and identification of the pathogen

The collected samples were thoroughly washed with tap water to remove any dirt or debris. The surface was then sterilized with 70% ethanol for three minutes, followed by a three-minute soak in 1% sodium hypochlorite solution. Then, the samples were rinsed three times with sterile distilled water to remove any residual disinfectant. Sections of infected mango fruit tissue were carefully cut into small pieces. These pieces were then placed on sterile paper towels to dry. Once dried, the pieces were transferred onto potato dextrose agar plates for incubation. The PDA plates containing the tissue sections were incubated at room temperature (25^0C) for five days. Incubation under these conditions allowed for the selective growth of fungi, resulting in visible colonies. To obtain pure cultures, isolated colonies were carefully transferred or sub-cultured onto fresh PDA plates. Pure cultures were identified based on their visual appearance and microscopic examination, as described by Agostini and Timmer (1992).

Morphological characterization of *Colletotrichum gloeosporioides*

Macroscopic characterization

After five to seven days of incubation, each *C. gloeosporioides* isolate grown on PDA media was examined for colony morphology. This included observing the colony's color, shape, size, and margin characteristics. The colony diameter was measured daily for seven days to calculate the average daily growth rate (mm/day) (Nagamani et al., 2006). The color of each *C. gloeosporioides* isolate's colony was assessed from the front surface, and the pigment types from the back of the petri plate were identified using an RGB color chart (Anonymous, 2005). To examine the conidia, a small amount of each *C. gloeosporioides* isolate was placed on a microscope slide and mixed with a drop of lactophenol blue stain. The slide was then covered with a coverslip and observed under a compound microscope (Nagamani et al., 2006). The length and width of 20 conidia per isolate were measured using an ocular micrometer (μm) at 40x magnification of a compound microscope (Dugan, 2006).

Data analysis

The collected data on anthracnose incidence and severity were analyzed using descriptive statistics to calculate the mean values for each district, enabling a quantitative comparison of disease prevalence across different geographical locations. Laboratory data, including mycelial growth diameter per week and daily growth rate, were statistically analyzed using one-way ANOVA in SAS software (version 9.2, 2008). To further investigate significant findings from the ANOVA, the Least Significant Difference test was employed at a 5% significance level.

Results and discussion

Disease intensity of mango anthracnose at the farmers' fields

Mango plants were susceptible to a variety of diseases and insect pests. Mango anthracnose was present throughout the entire investigated area, although to varying degrees. The mean incidence of mango anthracnose differed significantly between the districts (Figure 2). Boloso Bombe and Humbo exhibited the highest disease incidences, with 84.0% and 80.75% on leaves and 78.0% and 79.0% on fruits, respectively. Kindo Koisha and Offa had lower incidences, with 65.0% and 60.5% on leaves and 70.8% and 75.5% on fruits, respectively. This variation in disease incidence highlights the influence of local factors such as climate, orchard practices (pruning, sanitation,

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and fungicide applications), mango varieties, and soil conditions on disease incidence. These findings are consistent with previous studies on mango anthracnose. For instance, Sanders et al. (2000) reported a 32% incidence in South Africa, while Arauz et al. (1994) recorded an incidence rate of 65% of mango anthracnose in Costa Rica. Arauz et al. (1994) further noted that mango anthracnose disease incidence could reach almost 100% in fruits cultivated in humid environments. Similarly in Ethiopia, Chala et al. (2014) documented a 37% incidence of mango anthracnose in the Wolaita Zone and a 77% incidence in Southern Ethiopia. Likewise, Ayantu et al. (2014) reported a range of 41% to 72% severity on leaves and 36% to 74% severity on fruits in Southwestern Ethiopia. The widespread presence of mango anthracnose in the Wolaita Zone, particularly in Boloso Bombe and Humbo, underscores the need for effective disease management strategies in these regions.

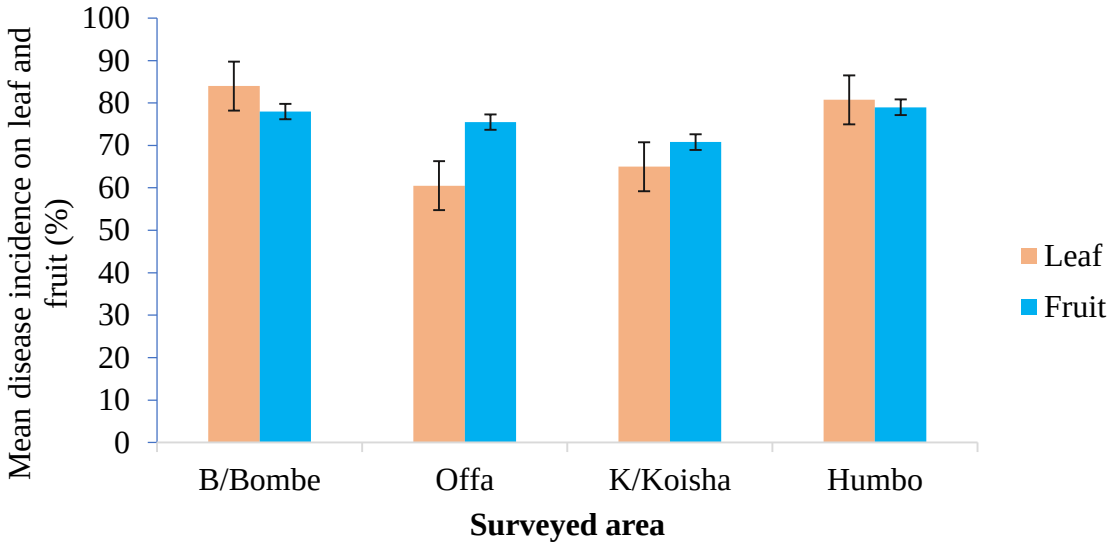

Figure 2. Mean disease incidence of mango anthracnose on the leaves and fruits in assessed districts

The survey conducted in the Wolaita zone of South Ethiopia revealed a significant incidence of mango anthracnose, a fungal disease that poses a severe threat to mango production. The analysis displayed variation in disease severity across different districts, which can be attributed to their distinct agroecological conditions. Notably, Boloso Bombe district was identified as a critical area for mango anthracnose, recording the highest average severity level of 76.75 % on leaves. This frightening figure highlights the extent of the problem in this area and underscores the

urgent need for targeted management strategies. Following Boloso Bombe, the districts of Humbo and Kindo Koisha reported anthracnose severities of 70.0 % and 56.75 %, respectively, reflecting a worrying trend that could affect local mango yields. Conversely, the Offa district showed a considerably lower severity of 39.75 %, suggesting that either environmental factors or existing control measures may be more effective in this area.

When assessing the impact on mango fruits, the mean anthracnose severity among the surveyed farmer fields ranged from 40.25 to 57.15 %. Boloso Bombe again led with a severity level of 57.15 %, while Humbo and Kindo Koisha recorded lower values of 52.00 % and 45.25 %, respectively. Offa district emerged with the least severity at 40.25 %, indicating a potential for better overall fruit health and possibly highlighting differences in cultivation practices or the resilience of local mango varieties.

The overall average severity of mango anthracnose reached a concerning 60.81 % on leaves and 49.66 % on fruits across all surveyed districts. These findings underscore the critical challenges faced by mango producers in the region. The consistently high levels of anthracnose severity are indicative of a pressing need for research into effective disease management practices and the development of resistant mango varieties. Encouragingly, the lower severity observed in some districts such as Offa opens avenues for further investigation into their management practices, which may serve as a model for improving disease resilience in more severely affected areas. To sum up, heightened awareness and proactive strategies are essential to combat the detrimental effects of mango anthracnose, ensuring the sustainability and productivity of mango farming in the Wolaita zone of South Ethiopia. The research finding aligns with existing literature that underscores the damaging impacts of anthracnose disease on mango production. Chala et al. (2014) emphasized the disease's potential to severely impair mango production. The detrimental impact of anthracnose on fruit quality and quantity, particularly in humid tropical regions, has been well documented (Chala et al., 2014; Arauz, 2000). Premature fruit drop, a direct consequence of anthracnose infection, further exacerbates the problem (Chala et al., 2014; Dodd et al., 1992).

The observed variations in disease severity across different locations likely stem from differences in environmental conditions, particularly temperature and rainfall. These factors are known to influence the development of plant diseases (Harikrishnan and Delo Rio, 2008; Tarekegn et al., 2006; Estrada et al., 2000). Rain, in particular, plays a crucial role in the

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germination, release, and dispersal of fungal spores, making humid regions more susceptible to severe anthracnose outbreaks. The widespread prevalence and severity of mango anthracnose in the Wolaita zone necessitate immediate attention. Implementing effective disease management strategies is crucial to mitigate the economic losses faced by farmers and ensure the sustainability of mango production in this region.

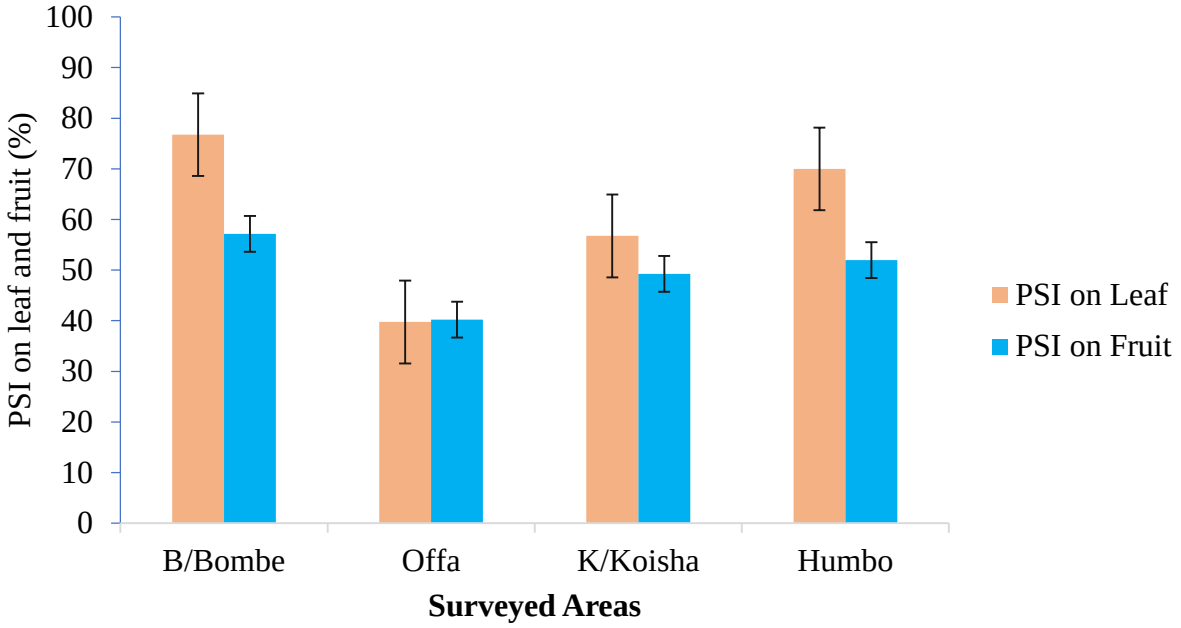

Figure. 3. Mean disease severity of mango anthracnose on leaves and fruits in assessed districts

Disease intensity of mango anthracnose at the local market

The investigation into mango anthracnose extended beyond the fields into the bustling marketplaces of the Wolaita zone. The findings revealed a disconcerting truth: this fungal disease continues to plague mangoes even after they have been harvested, impacting their quality and marketability. Researchers surveyed four major markets: Bombe, Bele, Tebela, and Gesuba, representing different districts within the zone. Alarmingly, mango anthracnose was present in all four markets, underscoring its pervasive nature.

The incidence of mango anthracnose, indicating the percentage of infected mangoes, ranged from 75.50% to 92.00% across the local markets (Figures 4 and 5). In the Bombe and Tebela local markets, the highest incidence rates were recorded, marking them as a potential hotspot for disease concentration. Beyond its mere presence, the severity of anthracnose infection, measured

by the extent of damage to the mangoes, raised significant concerns. The severity ranged from 68.50 to 79.00 % (Figure 4), with Bombe, Tebela, and Bele markets exhibiting the most severe infections.

Interestingly, the study revealed a higher incidence of anthracnose on mango fruits in marketplaces compared to farmer fields. This suggests that the journey from farm to market exacerbates the disease. Several factors contribute to this phenomenon. Firstly, the ripening process itself weakens the mango's natural defenses, making it more susceptible to latent anthracnose infections. As mangoes soften and sweeten, they become easy targets for the opportunistic fungus. Secondly, inadequate handling and transportation methods during the post-harvest phase play a critical role in amplifying the disease. Farmers often rely on rudimentary transportation methods like donkey carts and baskets, which, while economical, lack the necessary protection against physical damage and disease spread. Overfilling these containers further exacerbates the problem, creating a conducive environment for fungal growth.

The findings resonate with previous study conducted across Ethiopia's mango-growing regions. These studies consistently identify post-harvest diseases, particularly anthracnose, as a major bottleneck to the mango trade, both domestically and internationally (Chala et al., 2014). The dual nature of anthracnose, affecting mangoes both before and after harvest, makes it a formidable adversary (Prusky et al., 2009). Its ability to rapidly develop during storage and ripening further compounds the problem, leading to significant post-harvest losses (Prusky et al., 2009). The widespread presence and severity of mango anthracnose in Wolaita's marketplaces demand immediate attention. Addressing this challenge requires a multi-pronged approach, focusing on improving pre-harvest disease management, implementing strict post-harvest handling protocols, and adopting safer and more hygienic transportation methods. By tackling this issue head-on, we can safeguard the quality of mangoes, enhance their market value, and ensure the livelihoods of those who depend on this vital fruit.

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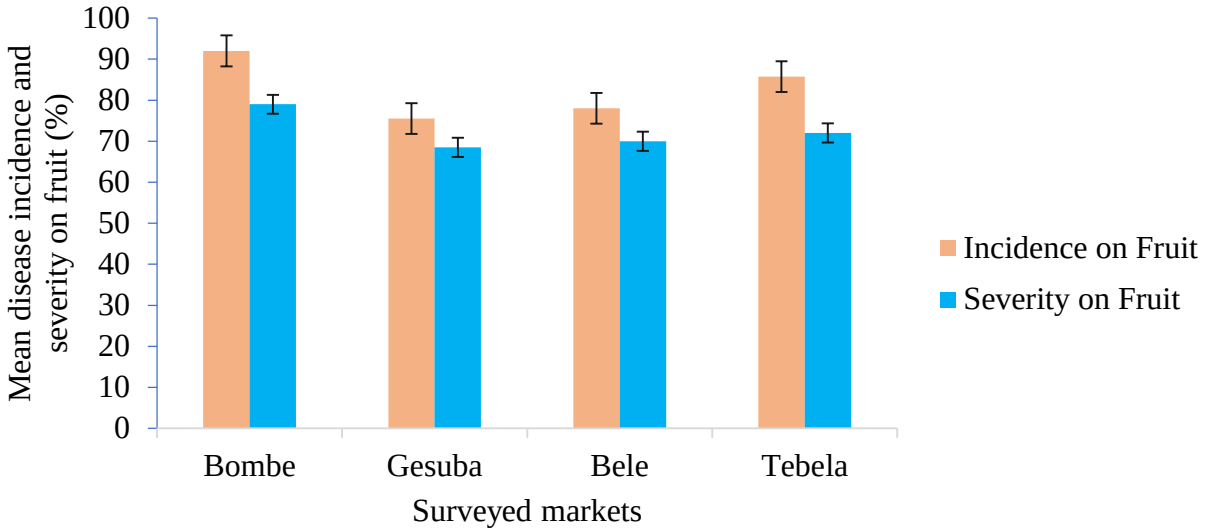

Figure. 4. Mean disease incidence and severity of mango anthracnose on mango fruits in different markets in Wolaita zone

The investigation revealed a concerning trend of sensitive mango anthracnose severity in marketplace settings. Across all surveyed markets, the average severity of this fungal disease was significantly higher in fruits compared to those observed in the field. This shocking observation underscores the vulnerability of mangoes to anthracnose during the post-harvest phase. The average severity of mango anthracnose in marketplaces ranged from 68.5 to 79 %, highlighting the substantial damage inflicted by this disease on fruit quality (Figures 4 and 5). The severity of this disease is likely to increase at the consumer level, which is a significant concern. If ripe mangoes are stored for extended periods after purchase, even for a few days, it creates a conducive environment for pathogen growth, accelerating disease development and leading to significant losses. This finding aligns with previous research by Akem (2006), who revealed that post-harvest losses in mango fruits could reach a staggering 97%. This study emphasized the interplay of various factors influencing mango losses, including mango variety, geographical location, cultivation practices, and prevailing environmental conditions. In addition to these factors, mango diseases play a critical role in impacting yield and fruit quality.

The results consistently demonstrated a higher incidence and severity of anthracnose in marketplace fruits compared to those examined directly in farmers' fields (Figure 5). This pattern was particularly evident in the Boloso Bombe district and its corresponding market, where both disease incidence and severity were most pronounced. This clear link between farm and market

suggests a direct relationship between anthracnose infection in the field and its subsequent manifestation in the marketplace. The majority of mangoes sold in these markets originate from local farms, carrying latent infections that remain dormant until triggered by the ripening process.

These latent infections, acquired before harvest but lying dormant until specific conditions arise during ripening, are a major contributor to the high disease prevalence and severity observed in marketplaces. Coupled with inadequate post-harvest handling practices, these latent infections pose a significant threat to mango quality and marketability. Anthracnose exemplifies this phenomenon of quiescent infection, capable of infecting and decimating yields of various tropical and subtropical fruits (Tronsmo et al., 2020; Prusky et al., 2009). The fungus can lie dormant within the fruit, only to emerge with a vengeance during ripening, catching consumers off guard and causing significant economic losses. Therefore, mitigating post-harvest anthracnose losses requires a comprehensive approach that addresses both pre-harvest and post-harvest factors. An implementing effective disease management strategy in the field, coupled with improved handling, storage, and transportation practices, is crucial to safeguarding mango quality and ensuring the livelihoods of those who depend on this valuable fruit.

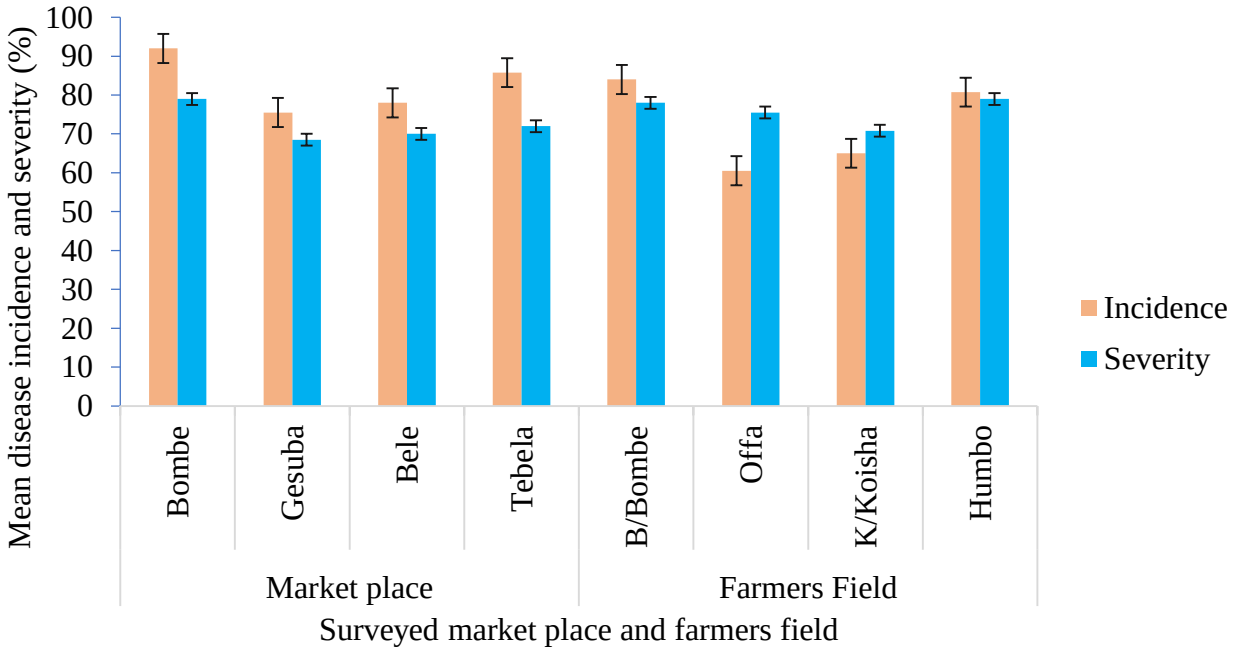

Figure. 5. Mean disease incidence and severity of mango fruit anthracnose in the marketplace and farmers' fields

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Characterization of *Colletotrichum gloeosporioides* Isolates

Morphological characterization

This study investigated the morphological characteristics of eight *Colletotrichum gloeosporioides* isolates obtained from diverse locations within the Wolaita zone of southern Ethiopia (Table 3). Conidial morphology, a key taxonomic feature for this fungal species, was examined. Microscopic examination revealed that all isolates exhibited the characteristic cylindrical conidia with rounded ends, appearing hyaline or colorless (Figure 6). However, despite the consistent cylindrical shape, significant variations were observed in conidial dimensions. Conidial length, for instance, ranged from 7.92µm in the OFA-1 isolate to a substantially larger 13.50µm in the HUM-2 isolate. Similarly, conidial width varied, with the HUM-2 isolates exhibiting the widest conidia at 5.01µm, while the narrowest width of conidia at 3.98µm was observed in the OFA-1 isolate.

This range of conidial sizes within a single species is not unexpected. *Colletotrichum gloeosporioides* is recognized as a species complex, encompassing a wide array of morphologically diverse sub-species and strains (Weir et al., 2012). Microscopic examination confirmed that all isolates exhibited the typical characteristics of *C. gloeosporioides*: hyaline (colorless), cylindrical conidia with rounded ends (Weir et al., 2012). The observed variations in conidial dimensions could be attributed to several factors, including the genetic diversity and geographical origin of each isolate. As highlighted in previous research (Muhammad et al., 2017), has demonstrated that environmental factors such as location and host plant can significantly influence the morphological traits of *C. gloeosporioides*.

This study examined eight *Colletotrichum gloeosporioides* isolates from the Wolaita zone in Ethiopia, focusing on conidial morphology. Microscopic analysis confirmed all isolates displayed typical *C. gloeosporioides* characteristics: hyaline, cylindrical conidia with rounded ends. However, significant variation in conidial dimensions was observed, with lengths ranging from 7.92µm to 13.50µm and widths from 3.98µm to 5.01µm. This size variation is consistent with the understanding of *C. gloeosporioides* as a species complex encompassing diverse strains. The observed differences may be attributed to genetic diversity and environmental influences such as location and host plant.

Table 3. Mean conidial length, width, and conidial shape of eight isolates of *C. gloeosporioides* collected from different districts of Wolaita zone, Southern Ethiopia

Isolates	Length (µm)	Width (µm)	Conidial shape
HUM-2	13.50	5.01	cylindrical
KK-2	12.40	4.81	cylindrical
BB-1	11.75	4.63	cylindrical
BB-2	11.20	4.50	cylindrical
KK-1	10.80	4.32	cylindrical
OFA-2	9.25	4.30	cylindrical
HUM-1	8.43	4.11	cylindrical
OFA-1	7.92	3.98	cylindrical
SE of mean	0.342	0.123	

Where, HUM-1/HUM-2 = Isolate from Humbo district, KK-1/KK-2 = Isolate from Kindo Koisha district, BB-1/BB-2 = Isolate from Boloso Bombe district, OFA-1/OFA-2 = Isolate from Offa district.

Cultural characterization

Eight *Colletotrichum gloeosporioides* isolates were cultivated or cultured on PDA and assessed for variations in colony characteristics (Table 4). All isolates exhibited robust colonial growth, characterized by an elevation in colonial gross. Investigation of colony morphology revealed distinct patterns among the isolates. The predominant colony form observed was circular, followed by spherical shapes with undulating margins. The isolates displayed a striking range of colony colors. The obverse (top) side of the colonies varied from white to dark grey, with Seashell-4 being the most prevalent color. In contrast, the reverse (bottom) side predominantly exhibited Peach-4 and Khaki hues.

The genus *Colletotrichum*, encompassing a large group of fungal plant pathogens, exhibits significant variability in morphology and cultural characteristics, often making species identification challenging (Talhinhas et al., 2021; Bailey and Jeger, 1992). This study investigated the cultural diversity of eight *C. gloeosporioides* isolates, focusing on variations in colony morphology and pigmentation. The observed diversity in colony morphology aligns with previous findings highlighting the significant intra-species variability within *C. gloeosporioides*

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(Gunnell and Gubler, 1992). Furthermore, the variation in colony pigmentation, particularly on the reverse side, supports previous suggestions that this characteristic could be a potential taxonomic marker for differentiating *Colletotrichum* species (Weir et al., 2012). Overall, this study underscores the significant cultural diversity present within *C. gloeosporioides*. The observed variations in colony morphology, color, and pigmentation highlight the importance of integrating morphological and cultural characteristics, alongside molecular data, for accurate identification and characterization of *Colletotrichum* species.

Table 4. Colonial characteristics of eight *C. gloeosporioides* isolates collected from different origins of Wolaita Zone, Southern Ethiopia

	Colonial color			Colonial gross	
Isolates	Front side	Reverse side	Edge	Elevation	Colony shape
HUM-2	Dark grey	Peach-4	Undulated	Raised	Irregular
KK-2	White	Khaki	Undulated	Raised	All round
BB-1	Seashell-4	Peach-4	Undulated	Raised	Irregular
BB-2	Seashell-4	Peach-4	Undulated	Raised	Irregular
KK-1	White	Khaki	Undulated	Raised	All round
OFA-2	Seashell-4	Peach-4	Undulated	Raised	Irregular
HUM-1	Seashell-4	Peach-4	Undulated	Raised	Irregular
OFA-1	White	Khaki	Undulated	Raised	Irregular

Where, HUM-1/HUM-2 = Isolate from Humbo district, KK-1/KK-2 = Isolate from Kindo Koisha district, BB-1/BB-2 = Isolate from Boloso Bombe district, OFA-1/OFA-2 = Isolate from Offa district.

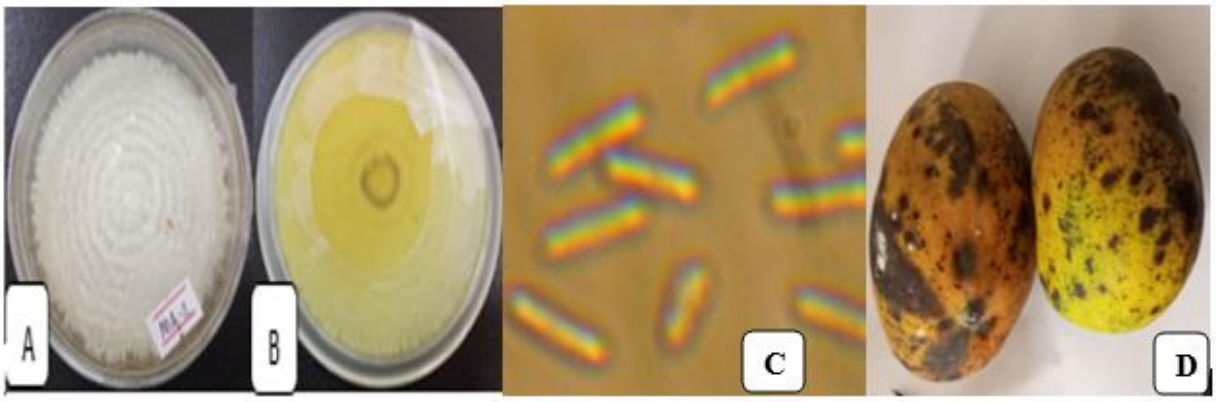

Figure 6. (A & B) Front and reverse side of the colony, (C) Conidia of *Colletotrichum gloeosporioides*, and (D) Infected mango fruits

The growth characteristics of *Colletotrichum gloeosporioides* isolates, specifically examining their mycelial growth patterns on potato dextrose agar, were investigated (Table 5). The researchers meticulously tracked the average colony growth diameter of each isolate over time, revealing notable variations in their growth rates.

Isolate HUM-2 exhibited the most rapid colonization, achieving an average colony growth diameter of 34.47 mm in one week. The isolates exhibited varying growth rates. Isolates KK-2, BB-1, and BB-2 demonstrated robust growth, ranging from 28.18 to 29.96 mm per week. In contrast, isolate OFA-1 grew significantly slower, at 18.18 mm per week. These variations in mycelial growth were found to be statistically significant ($p < 0.01$), underscoring the inherent diversity within the *C. gloeosporioides* species complex. Such differences in growth rate could be attributed to a multitude of factors, including genetic variations among isolates, their adaptation to specific ecological niches, and potential variations in nutrient utilization.

Isolate HUM-2 grew the fastest, at about 4.9 mm each day. Isolates KK-2, BB-1, and BB-2 also grew quickly, at similar rates between 4.03 and 4.28 mm per day. Again, isolate OFA-1 lagged significantly, managing a mere 2.59 mm per day. These findings align with the understanding that *C. gloeosporioides* encompasses a wide array of strains and sub-species, each potentially exhibiting unique physiological and morphological characteristics (Weir et al., 2012). Further research incorporating molecular techniques and exploring the influence of environmental factors on growth patterns would provide a more comprehensive understanding of the factors driving these variations.

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Table 5. Mycelial growth diameter and culture growth rate of *C. gloeosporioides* isolate on Potato dextrose agar (PDA) in Wolaita Zone, Southern Ethiopia.

Isolates	Mycelial Growth (mm/week) after incubation	Growth Rate (mm/day)
HUM-2	34.47[a]	4.94[a]
KK-2	29.96[b]	4.28[b]
BB-1	29.92[b]	4.27[b]
BB-2	28.18[bc]	4.03[bc]
KK-1	27.93[bc]	3.99[bc]
OFA-2	26.06[c]	3.72[c]
HUM-1	23.13[d]	3.30[d]
OFA-1	18.18[e]	2.59[e]
CV (%)	5.10	5.10
LSD (0.05)	2.418	0.345

This means that a column followed by the same letters is not significantly different according to the LSD test at a 5% probability level. HUM-1/HUM-2 = Isolate from Humbo district, KK-1/KK-2 = Isolate from Kindo Koisha district, BB-1/BB-2 = Isolate from Boloso Bombe district, OFA-1/OFA-2 = Isolate from Offa district. CV = Coefficient of Variation, LSD = Least Significant Difference.

Conclusion

This study confirms the widespread presence of mango anthracnose, caused by *Colletotrichum gloeosporioides*, across all surveyed districts in the Wolaita zone of southern Ethiopia. However, the incidence and severity of the disease varied significantly between different districts and markets. Furthermore, the study revealed considerable variability in the morphology and cultural characteristics of *C. gloeosporioides* isolates. This variability was observed in conidial size and shape, as well as colony growth patterns, color, and diameter. These findings highlight the diverse nature of *C. gloeosporioides* populations infecting mango plants in the Wolaita zone. This diversity is likely influenced by environmental factors such as temperature and precipitation, which are known to impact disease development. Further research should be

conducted to investigate the variability of *C. gloeosporioides* isolates across all mango-growing regions of Ethiopia. This will provide a comprehensive understanding of the pathogen's diversity and facilitate the development of effective and targeted disease management strategies.

Conflict of interest

The authors declare that there is no conflict of interest.

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