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RESEARCH ARTICLE

Importance, etiology and management of the tear stain disease symptoms of mango fruits in Ghana

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Abstract

The tear stain symptom refers to slightly raised blisters which reduce the aesthetic value of mango fruits in Ghana. The disease incidence, severity and percentage of exportable mango fruits on infected mango trees were determined in the coastal savannah zone of Ghana in 2019. Samples of disease fruits were collected and the causative agent isolated and identified using phenotypic and genotypic characteristics. Selected copper based fungicides, namely, Cuprous oxide (750 g/kg), Copper oxychloride (500 g/kg) and Mancozeb (800 g/kg) either solely or in combination with Acibenzolar-S-methyl were applied in the field to determine their effect on the disease incidence and severity and on the percentage of exportable fruits. The disease incidence ranged from 9.4% to 11.0% in the major season and 37.3% to 45.0% in the minor season. Severity ranged from 0.08 to 0.1 in the major season and 1.4 to 2.1 in the minor season. The fungus isolated showed the characteristic short conical spores with rounded edges, an indication that it was *C. gloeosporioides*. The combination of gene sequences of the ITS region and the partial actin gene identified the fungus as *C. siamense*. All fungicides evaluated were able to reduce the disease incidence and severity on treated trees and resulted in a higher percentage of exportable fruits compared to non-treatment control trees.

Keywords Mango · Anthracnose · Tear stain · *Colletotrichum siamense*

Introduction

Mango (*Mangifera indica*) is one of the most important non-traditional exports fruit crops from Ghana (Ablormeti et al. 2021). Total production of mango has been estimated to be about 110,000 tonnes. Out of this, about 40,000 tonnes are consumed locally, making the crop an important food security crop for the country. In terms of the Ghana's total GDP, mango exports contribute a modest 0.3%, however, with the ever increasing demand for the crop in international markets, the crop has a potential of contributing more to the country's GDP in the near future. It has been estimated that about 30% of Ghana's total mango production is lost yearly to poor postharvest practices (MOAP 2016). Other factors, such as pests and diseases infestation, contribute to reduced yields in the field. Therefore, when these problems are addressed, volumes of production and exports are likely to rise.

In Ghana, one common disease of mango is tear stain. The symptoms of the disease are characterised by the aggregation of numerous tiny spots that are slightly raised and are rough to touch. The pattern created by the symptom on the fruit surface sometimes resemble the back covering of an alligator, causing the symptoms to be described as an alligator skin effect (Nelson 2008). The nature of the blemishes reduce the aesthetic value of the fruits and hence their marketing, especially in international markets.

The tear stain disease symptom is very common on mango fruits produced in the minor season in the Coastal savannah areas of Ghana, where huge quantities of the crop are produced for both the local and export markets. A casual look and reports by farmers show that several fruits, intended for international markets are rejected due to the blemishes caused by the disease. Work, however, has not

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been carried out to determine the importance of the disease in these major mango producing areas of Ghana.

The etiology of the tear stain disease of mango in Ghana is uncertain. It has been attributed to expansion and contraction of fruits, in response to temperature changes. Others are of the view that it represents healing scars from wounds created by insects feeding activities. Elsewhere, the disease has been ascribed to *Colletotrichum gloeosporioides* (Nelson, 2008), another possibility of the etiology of the disease in the country. Different species of *Colletotrichum* have been identified on mango in Ghana and have all been shown to produce the characteristic, dark brown slightly sunken spots, known as anthracnose on mango fruits in Ghana (Honger et al. 2014). Currently, none of the species have so far been associated with the tear stain symptoms.

Copper based fungicides such as Funguran (copper hydroxide) and copper oxychloride are one of the major group of fungicides, recommended for the control of diseases affecting mango in Ghana (EPA 2015). Copper-based fungicides have been shown to be as effective as other synthetic fungicides (Honger 2013). One advantage in the use of copper is that it is accepted in organic production systems worldwide, and can therefore be used for the control of diseases on fruits that are intended to be sent to international markets. Several of these fungicides are available on the Ghanaian markets, but have not been evaluated extensively, to determine their effect on the disease incidence and severity of the tear stain disease symptoms on mango, in the country.

Given the paucity in information as regards the tear stain disease symptoms in Ghana, this work was carried out to determine the determine the disease incidence and severity and its effect on mango disease marketing, to identify the causal agent of the disease and to evaluate some selected copper-based fungicides for the control of the disease in the field.

Materials and methods

Field survey and collection of diseased mango fruits.

Field survey for the prevalence, disease incidence and severity was carried out in commercial mango orchards the major (May–June) and Minor (December) mango productions seasons of 2019. The surveys were carried out in five different locations in Ghana where mango farms are heavily concentrated. These were Asutuare and Dodowa in the Greater Accra Region, Akuse, Somanya and Kpong in the Eastern Region and Juapong in the Volta Region of Ghana. The districts where these localities were found were selected with the help of National Agricultural Extension agents after which the district extension agents were consulted for the selection of localities where the orchards were located. Mango farms (2–100 acres) in each locality were selected at random and assessed for the disease incidence and severity. Diseased mango fruits were collected at random from farms selected at Akuse, Somanya and Kpong for isolation of causal agent of the disease.

Determination of disease incidence and severity

Mango fruits, totalling 400 were randomly selected from at least 20 trees per farm, were inspected for the presence of the disease symptoms (Fig. 1a and b) and a farm was marked as free from the disease (0) or infected (1). A subsample of 50 fruits per farm was taken and the surface area of the fruit covered by the disease symptom was visually rated on a scale of 0–5, 0 = no infection, 1 = up to 5% of fruit surface area covered, 2 = 6–10% of fruit area affected, 3 = between 11 and 20% of fruit area covered, 4 = 21–50% of fruit area affected and 5 more than 50% of the fruit surface area covered (Lakshmi et al. 2011). Data obtained were used to calculate the disease prevalence, incidence and severity as follows:

$$\text{Disease prevalence} = \frac{\text{No of farms with disease symptoms}}{\text{Total numberof farms surveyed}} \times 100$$

$$\text{Disease incident} = \frac{\text{No of infected fruits}}{\text{Number of fruits inspected}} \times 100$$

$$\text{Disease severity} = \frac{\sum fX}{\sum x} \text{where,}$$

f = number of fruits with a particular rating, x = a particular rate based on the percentage of fruit surface area covered by disease lesion. Data collected from farms within the same locality were bulked and means and standard errors calculated for the different localities.

Isolation and morphological characterisation of *Colletotrichum* species

Isolation of the causal agent was carried out in the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana. The causal agent was first isolated on water agar (20 g/l) and sub-cultured on potato dextrose agar (39 g/l). Each medium was autoclaved at 121 °C and after cooling were dispensed into clean sterilized Petri dishes. Diseased fruits from the field were first washed with soap and rinsed under running water. Piece of the fruit tissues (5 × 3 mm) were then excised from the blisters on some of the fruits, surface sterilised with 1% sodium hypochlorite,

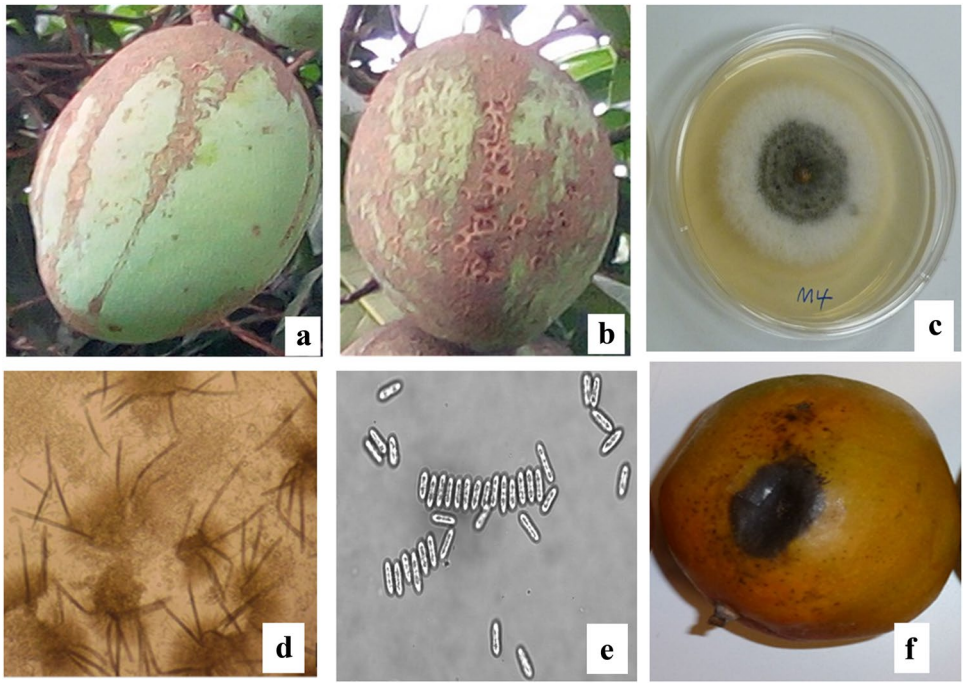

Fig. 1 Symptoms and cultural and morphological characteristics of the causal agent of tear stain disease of the tear stain disease of mango. **a** typical symptoms on a fruit with slight blisters in the field; **b** fruits showing the pronounced blister-like symptoms in the field, **c** symptoms induced on fruits artificially inoculated with the pathogen, **d** mycelial growth on PDA, **e** acervuli showing the presence of setae, **f** short conical spores with rounded edges

blotted dry with sterile paper tissues and plated on water agar. Other cleaned diseased fruits were stored in cardboard for two weeks at 25–27 °C and 65% RH, till the blister-like spots expanded to produce dark-brown spots on the fruits surface. Pieces of the fruit tissues (5 × 3 mm) were then taken from the advancing edge of the disease symptoms and plated also on water agar. At 7 days, when enough growth of a fungus that grew was obtained, it was sub-cultured on PDA and incubated for a further 7 days. Isolates that grew were identified to the genus level based on cultural and morphological features. *Colletotrichum* species which were consistently isolated from the expanded brown lesions and from very few of the blister-like spots were maintained. Single spore cultures of the selected isolates were produced and plugs (8 mm in diameter) were placed on fresh PDA plate and incubated at 28 °C. Diameter of colony growth was measured daily for 7 days and the seven-day average of mean daily growth was calculated to represent the mycelial growth rate (millimetres per day). Spore sizes were measured under the microscope and means for 20 individual conidia per isolate was calculated (Prihastuti et al. 2009). The slide culture technique after Johnston and Jones (1997) was used to stimulate appressorial growth after which the dimensions of the appressoria produced were also measured. *Lasiodiplodia* and A*spegillus* species were isolated from few of the samples of the blister-like symptoms. *Lasiodiplodia* is known to cause soft rot of mangoes in Ghana while *Aspergillus* species were considered saprophytes on mango fruits in Ghana. These two fungal species were therefore not selected for any further studies in this work.

Pathogenicity test

Pathogenicity of isolated *Colletotrichum* species was tested on physiologically matured mango fruits. Spore suspension of the fungi (1×10^7 spores/ml) was used to inoculate, disinfected healthy mango fruits, by placing the 15 µl of the suspension on a filter paper disc attached to the fruit surface. Sterile distilled water served as control. The inoculated fruits were placed in plastic containers on benches in the laboratory at 25–27 °C and 65% RH. When symptoms were observed, the pathogen was re-isolated from the induced symptoms to complete Koch's postulates.

Molecular characterisation

Polymerase chain reaction and sequencing of products

Five of the isolates of the *Colletotrichum* species were selected at random and DNA was extracted using the Sigma's GenFlute Plant Genomic DNA Miniprep Kit (St. Louis, MO, USA), following the manufacturer's instructions. DNA extracted was used as templates in PCR. The PCR were carried out using two primers pairs; ITS1/ITS4, to amplify the entire internal transcribed spacer region (White et al. 1990) and ACT512F/ACT783R to amplify a part of the actin gene (Carbone and Kohn 1999). PCR reaction mixtures and conditions were after Honger et al. (2014). Amplification products were separated by 1.5% w/v agarose gel (Invitrogen, Carlsbad, CA), stained with gel red. Separation was achieved with a 100 V and

was run for 1.5 h. The presence and size of bands were observed under UV light. The amplified products were purified and sequenced directly at ETON Bioscience Laboratory at Raleigh in North Carolina. Nucleotides were analysed and consensus strands generated with the aid of Bioedit software.

Phylogenetic analysis

The concatenated nucleotide sequences of the ITS region and the actin gene of 31 ex-types and isolates of *Colletotrichum*, made up of 26 isolates of confirmed identities (downloaded from EMBL database) and 5 obtained from this study (Table 1), were aligned using Clustal W. Maximum Parsimony analysis was performed on the multiple sequence

Table 1 Isolates of *Colletotrichum* used in the study with their accession numbers

Species	Strain identification	Host	Country	GenBank accession numbers	
				ITS	ACT
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX009443
C. aeschynomenes	ICMP 17673*, ATCC 201874	*Aeschynomene virginica*	USA	JX010176	JX009483
C. alatae	CBS 304.76*, ICMP 17919	*Dioscorea alata*	India	JX010190	JX009471
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX009572
C. aotearoa	ICMP 18537*	*Coprosma sp.*	New Zealand	JX010205	JX009564
C. asianum	ICMP 18580*, CBS 130418	*Coffea arabica*	Thailand	FJ972612	JX009584
C. asianum	ICMP 18696	*Coffea arabica*	Thailand		JX 009,576
C. clidemiae	ICMP 18658*	*Clidemia hirta*	USA	JX010274	JX009537
C. cordylinicola	MFLUCC 090551*	*Cordyline fruticosa*	Thailand	JX010226	HM470235
C. fructicola	ICMP 18581*	*Coffea arabica*	Thailand	JX010165	FJ907426
C. gloeosporioides	IMI 356878* ICMP 17821	*Citrus*	Italy	JX010152	JX009531
C. gloeosporioides	CORCG4	*Orchis sp.*	China	HM034808	HM034800
C. gloeosporioides	CORCG5	*Orchis sp.*	China	HM034809	HM034801
C. horii	NBRC 7478* ICMP 10492	*Diospyros kaki*	Japan	GQ329690	JX009438
C. kahawae	IMI 319418*	*Coffea arabica*	Kenya	JX010231	JX009452
C. musae	CBS 116870* ICMP 19,119	*Musa sp*	USA	JX010146	JX009433
C. nupharicola	CBS 470.96* ICMP 18187	*Nuphar lutea subsp. polysepala*	USA	JX010189	JX009486
C. psidii	CBS 145.29* ICMP 19120	*Psidium sp*	Italy	JX010219	JX009515
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009447
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009562
C. siamense	ICMP 18578* CBS 130417	*Coffea arabica*	Thailand	JX010171	JX907423
C. siamense	BMLI15	*Coffea arabica*	Thailand	FJ972614	FJ907422
C. siamense	**ALSKN-CG1**	***Mangifera indica***	**Ghana**	**MT450687**	**MT452577**
C. siamense	**ALSKN-CG2**	***Mangifera indica***	**Ghana**	**MT450688**	**MT452578**
C. siamense	**ALSKN-CG3**	***Mangifera indica***	**Ghana**	**MT450689**	**MT452579**
C. siamense	**ALSKN-CG4**	***Mangifera indica***	**Ghana**	**MT450690**	**MT452580**
C. siamense	**ALSKN-CG5**	***Mangifera indica***	**Ghana**	**MT450691**	**MT452581**
C. theobromicola	CBS 124945* ICMP 18649	*Theobroma cacao*	Panama	JX010294	JX009444
C. ti	ICMP 4832*	*Cordyline sp.*	New Zealand	JX010269	JX009420
C. trichellum	CBS 217.64	*Hedera helix*		GU227812	GU227970
C. xanthorrhoea	BRIP 45094* ICMP 17903	*Xanthorrhoeae preissii*	Australia	JX010261	JX009478

*Type strain. Isolates with names in bold print were obtained in this study

alignment generated, using MEGA5 (Tamura et al. 2011). Clade stability of the tree resulting from maximum parsimony analysis was assessed by bootstrap analysis with 1000 replicates (Felsenstein 1985).

Effect of selected copper based fungicides on the incidence and severity of tear stain disease

Two copper based fungicides, one synthetic inorganic fungicide and Bion (Acibenzolar-s-methyl), a biostimulant were procured from commercial pesticide shops and evaluated on a 9 year old mango orchard, present at the Soil and Irrigation Research Centre of the University of Ghana, located at Kpong, in the Eastern Region of Ghana. The experiment was carried out in the major and minor seasons of 2020, on the Keitt variety of mango. Six treatments were evaluated in the study these were; cuprous oxide (750 g/kg) at 2 gl^{-1}; copper oxychloride (500 g/kg) at 3 gl^{-1}; Mancozeb (800 g/kg) at 4 gl^{-1}; cuprous oxide (750 g/kg) + acibenzolar-S-methyl at 2 gl^{-1} + 0.17 gl^{-1}, copper oxychloride (500 g/kg) + acibenzolar-S-methyl at 3 gl^{-1} + 0.17 gl^{-1} and non-treatment control. Prior to each experiment, excess leaves, twigs and fruits from the previous seasons' production were pruned off and removed from the orchard. Trees were induced to flower using potassium nitrate. Trees with uniform flowering were selected and the treatments were applied using a mistblower. A randomised complete design with four replicates was used in the trial. Each replicate was made up of three trees. Treatments were applied at bi-weekly intervals, beginning from when more than 50% fruit set was observed, and ended at 7 days before physiological fruit maturity. In all, there were 5 applications of fungicides. Seven days after the last treatment application, all the fruits on each tree were harvested and 200 were selected at random and used to determine disease incidence after which a sub-sample of 50 were picked at random for disease severity assessment. Disease incidence and severity were calculated as described earlier in this work. After that, the fruits from the three trees of each replicate were bulked and separated into exportable, marketable and non-marketable fruits based on the percentage of the surface area covered by the disease symptoms. Percentage exportable fruits was calculated using the formula:

$$\text{Percentage exportable fruits} = \frac{\text{number of fruits that can be exported}}{\text{Total number of fruits}} \times 100$$

Data on disease incidence and percentage exportable fruits were arcsine transformed and subjected to analysis of variance (ANOVA). On the other hand, ANOVA was performed directly on severity indices. Means were separated using LSD at 5%.

Results

Disease prevalence, incidence and severity in different mango growing districts of Ghana

The tear stain disease symptoms were prevalent in the study area. In the major season, the prevalence ranged from 50% in the Asutuare and Juapong areas to 70% in Akuse, in the major season. The prevalence was however, higher in the major season, with the disease being found in all farms inspected in the minor season (Table 2). The disease incidence also ranged from 9.4% in Somanya to the highest of 14.4% in Akuse in the major season. During the minor season, the disease incidence ranged from 37.3% to 45.7% in the study area, with the highest being observed in Dodowa (Table 2). Disease severity also ranged from 0.08 (Somanya)

Table 2 Prevalence of mango tear stain disease and the disease incidence and severity in the indicated areas in the major and minor season of 2019

Area	Prevalence (%) $\pi \pm$ se		Incidence (%) $\pi \pm$ se		Severity $\pi \pm$ se	
	Major season	Minor season	Major season	Minor season	Major season	Minor season
Akuse	70.0	100.0	14.4 ± 0.5	40.9 ± 0.9	0.16 ± 0.01	1.98 ± 0.06
Asutuare	50.0	100.0	9.7 ± 0.2	44.4 ± 1.3	0.13 ± 0.03	1.4 ± 0.06
Dodowa	60.0	100.0	9.9 ± 0.3	45.7 ± 0.1	0.1 ± 0.01	1.5 ± 0.7
Kpong	40.0	100.0	11.9 ± 0.4	42.9 ± 0.9	0.1 ± 0.01	2.08 ± 0.06
Juapong	50.0	100.0	11.5 ± 0.5	42.9 ± 0.6	0.1 ± 20.001	1.6 ± 0.07
Somanya	60.0	100.0	9.4 ± 0.3	37.3 ± 10.5	0.08 ± 0.01	2.1 ± 0.06

$\pi \pm$ se = means + standard error

to 0.16 (Akuse) in the major season while it ranged from 1.4 in Asutuare to 2.1 in Somaya in the minor season (Table 2).

Cultural and Morphological characterisation of the isolated fungi

Isolates of the fungus showed similar cultural characteristics. They produced white mycelium which grew to fill an entire 8 mm plate in 7 days (Fig. 1c). After 7 days, numerous bright orange coloured acervuli, which darkened with time, were found in concentric rings in the middle of the culture. The disc-shaped acervuli had numerous black setae on their surfaces (Fig. 1d) and burst to release numerous short conical and hyaline spores (Fig. 1e). The spore sizes were 15.3 µm to 16.1 µm long while the width was from 4.9 µm to 5.3 µm. The appressorial length was 8.9 µm to 9.0 µm with width of 4.8 to 7.0 µm. The growth rate of 1.10 mm/day, was same for all isolates (Table 3).

Pathogenicity test of isolates

All isolates were able to induce anthracnose disease symptoms on the inoculated fruits surfaces. The symptoms on the fruits began as small dark brown spots which were slightly sunken. The spots expanded and covered large areas of the fruits surface by the 7th day after incubation (Fig. 1f). The same fungi were re-isolated from the induced symptoms.

Table 3 Conidia and appressoria size and pathogenicity of *Colletotrichum* isolates from tear stain symptoms on mango fruits

Isolate Designation	[a]Mean conidium size (µm)	[a]Mean appressorium size (µm)	Growth rate (mm/day)
ALSKN-CG1	15.9 ± 0.3 × 4.9 ± 0.1	9.2 ± 0.1 × 5.5 ± 0.1	1.10
ALSKN-CG2	16.0 ± 0.3 × 4.9 ± 0.3	8.9 ± 0.2 × 4.8 ± 0.1	1.10
ALSKN-CG3	15.8 ± 0.1 × 4.7 ± 0.3	8.6 ± 0.2 × 5.7 ± 0.2	1.10
ALSKN-CG4	16.0 ± 0.2 × 4.3 ± 0.2	8.9 ± 0.1 × 5.3 ± 0.3	1.10
ALSKN-CG5	16.0 ± 0.3 × 4.7 ± 0.3	8.9 ± 0.1 × 5.0 ± 0.1	1.10

[a]Mean ± s.e

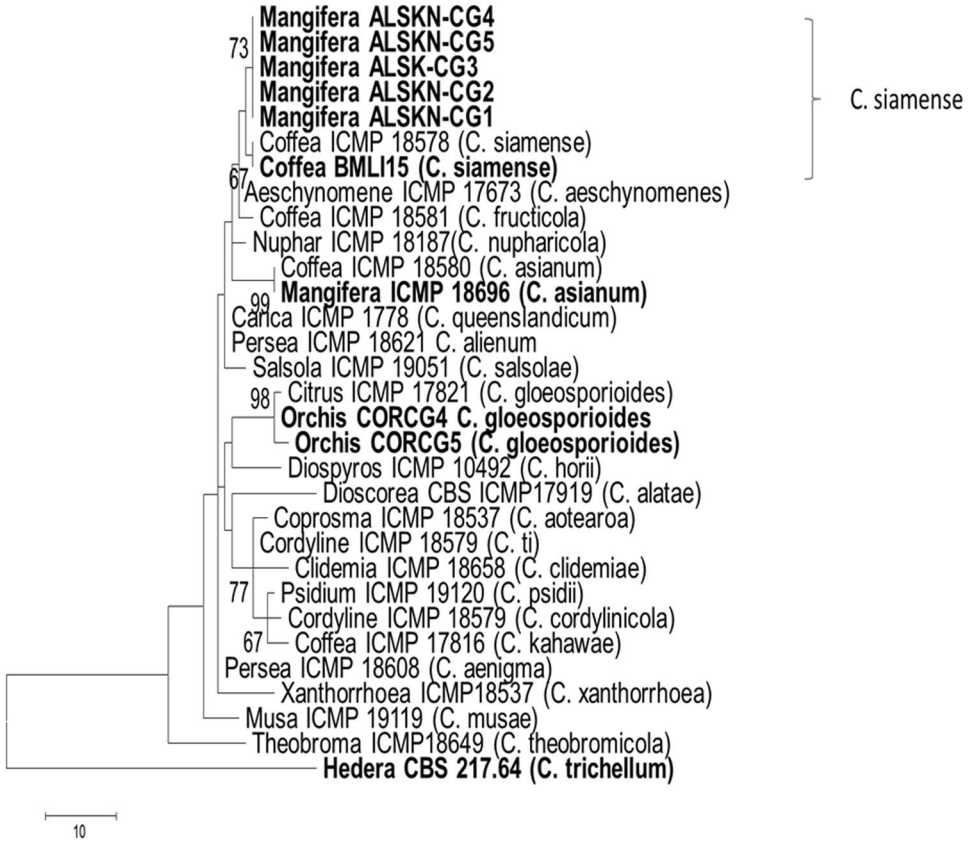

Fig. 2 Maximum parsimony phylogram constructed from the multiple sequence alignment of the combined nucleotide sequences of the ITS region and the ACT gene. *Colletotrichum trichellum* was used as outgroup. With the exception of strains whose names were preceded by ALSKN, all strains are either isolates of confirmed identities (names in Bold) or are ex-type strains. Sequences of ex-type strains or strains with confirmed identities were downloaded from the EMBL database

Molecular characterisation

Analysis of the sequences and phylogenetic studies of the ITS region and the partial actin gene

The size of the amplified PCR product of the ITS region and of the partial actin gene obtained from the isolates were approximately 600 bp and 250 bp respectively. After assembling, sequences obtained were 535 bp and 241 bp long for the ITS region and ACT gene, respectively. The most parsimonious tree obtained using the concatenated sequences of the two genes is shown as Fig. 2. The tree length was 166, the consistency, retention and composite indices were respectively, 0.602740, 0.764228 and 0.630718 (0.460630) for all sites and parsimony-informative sites (in parentheses). Shown next to the branches are the bootstrap values in percentages. There were 631 sites in the final data set. The different isolates clustered in different clades, with all isolates obtained from the tear stain symptoms on mango, clustering in a clade containing the *C. siamense* type strain and the other *C. siamense* isolates whose identities are well known. The clade was supported with a high bootstrap value of more than 70% (Fig. 2).

Effect of fungicides on disease incidence and severity and percentage of exportable fruits of mango.

There was significant difference ($p > 0.05$) in the incidence of the tear stain disease on mango fruits in the major season of 2019. The highest incidence was observed on trees that did not receive ant fungicidal treatment while the lowest was observed on trees treated with a combination of cuprous oxide and acibenzolar-S-methyl (Table 4). The difference in disease incidence on all trees that received fungicidal treatments, was however, not significant. Similarly, the disease severity was highest on trees that were not treated with any fungicide while the lowest was on trees treated with cuprous oxide and acibenzolar-S-methyl. However, there was no significant difference in disease severity among fungicidal treatments evaluated in the study (Table 4). There was significant difference ($p > 0.05$) in percentage of exportable fruits on trees that received the different treatments. The highest was obtained on trees treated with Mancozeb while the lowest was obtained on control trees. The percentage of clean fruits on trees treated with Mancozeb was the same as what was obtained on trees treated with all other fungicides except Nordox (Table 4).

Results obtained in the minor season were similar to what was observed in the minor season. The significantly highest disease incidence and severity were obtained on trees that were not treated with any fungicide while the lowest was obtained on trees that received one of the fungicidal treatments. On the other hand, the lowest percentage of exportable fruits was obtained on trees that did not receive any treatment while all fungicidal treatments resulted in the same percentage of exportable fruits (Table 4).

Discussion

The tear stains symptoms on mango fruits in Ghana has been a subject of many discussions. While some people are of the opinion that the symptoms were healing scars from wound created by unknown causes, very few were of the view that they represent another form of the anthracnose symptom. Findings from this research work have shown that the symptoms were caused by known causal agents of anthracnose and hence the symptoms were another form of the anthracnose disease. Typically, anthracnose refers to a dark brown spot which is slightly sunken (Agrios 2005) and has been associated with members of the genus *Colletotrichum* (Ploetz 1998; Arauz 2000). This description is very different from what was described for the tear stain. In fact in this study, the tear stain symptoms were blister-like spots on the infected fruit surface and are rough to touch. However, when infected fruits were incubated for some time, it was observed that the typical dark brown symptoms of anthracnose began to radiate out from the dried tear stain spots. This gives an indication that the tear stain spots were anthracnose symptoms that stopped expanding and eventually developed

Table 4 Effect of fungicides on the disease incidence and severity and percentage of exportable mango fruits in the major and minor season of 2020

Treatments	Major season			Minor season		
	Incidence	Severity	Exportable fruits (%)	Incidence	Severity	Exportable fruits (%)
Curenox	4.2a	0.01a	95.3b	5.8a	0.12a	85.9b
Nordox	4.1a	0.01a	87.6bc	4.1a	0.10a	90.9b
Curenox + Bion	5.6a	0.02a	93.0bc	6.1a	0.12a	83.5b
Nordox + Bion	2.9a	0.01a	94.4bc	2.9a	0.13a	87.7b
Mancozeb	5.0a	0.01a	95.5bc	5.0a	0.13a	84.3b
Control	31.8c	1.20b	38.4a	30.3b	1.90b	28.9a

Means followed by same alphabets in a column are not significantly different

dried blisters in the field and which then resumes expansion after harvest when right conditions for the expansion occur in storage. These observations confirm the reports by Nelson (2008) and Kumar et al (2016) that the tear stain symptoms were another type of anthracnose.

The disease was found to be very prevalent in the study area, both in the major and minor seasons. Since blemished fruits are considered unfit for the international markets (Arauz 2000) the disease remains a major threat to the profitability of mango farmers in the study area. The disease incidence and severity also appeared to be higher in minor season than in the major season, confirming the reports by farmers. Naturally, fungal disease prevalence correlated positively with environmental factors (Arauz 2000; Agrios 2005), therefore, the high humidity and rainfall that pertains in the major season compared to the minor season in the study area, would have exacerbated the disease in the former than the latter season (Arauz 2000). On the other hand, the short period between the major and the minor season in these areas, makes it impossible for farmers to carry out proper cultural practices such as pruning of trees and cleaning of farms, prior to the minor season. Since the disease is caused by a fungus, poor tree pruning and farm sanitation activities will enhance its proliferation. This could account for the higher disease incidence in the minor season than in the major season. Tear stain disease therefore, remains an important disease of concern as it has the potential of reducing the quantities of mangoes exported to the international markets from the study area, which remains an important source of exportable mangoes from Ghana.

While the nature of the tear stain symptom indicates that it was another type of anthracnose symptom, identifying the causal agent was found to be important in clearing all doubts about its identity. Elsewhere, the tear stain disease symptoms on mango fruits have been attributed to *C. gloeosporioides* (Kumar et al. 2016; Nelson 2008). In this study, the fungus consistently isolated from the disease symptoms produced short conical spores with rounded edges in disc shaped acervuli with setae. Isolates of the fungus caused the typical anthracnose symptoms on the inoculated mango fruits. These morphological characteristics of the fungus and its pathogenicity on the crop confirmed the causal agent as *C. gloeosporioides* (Honger et al. 2014; Arauz 2000; Damm et al. 2010).

Colletotrichum gloeosporioides is a group species made up of several distinct species sharing common cultural and morphological features (Damm et al. 2010). Even, the variability of the sequences of the ITS region, which has been used widely as a bar code for species delineation in fungi, has been found to be inadequate in separating among species in the *C. gloeosporioides* complex (Weir et al. 2012). To be able to achieve this, a polyphasic approach which relies on the use of more than one region has been proposed (Phoulivong et al 2010; Abang 2003). In Ghana, the ITS region has been combined with other gene such as the beta tubulin and glyceraldehyde-3-dehydrogenase gene to differentiate among species within the *C. gloeosporioides* complex (Honger et al. 2014; 2016). Phylogenetic studies involving the nucleotide sequences of the ITS region and partial actin gene showed that the causal agent of the tear stain disease in Ghana was *C. siamense*. The gene tree drawn with the combined sequences of the two gene regions resulted in the clustering of the isolates with the type strain of *C. siamense* in a clade supported by a high bootstrap support. In the same phylogram, the clade made up of type strain of *C. gloeosporioides* and other *C. gloeosporioides* strains of confirmed identities did not include any of the isolates from the tear stain symptoms in the study. This confirms that the isolates were *C. siamense* and not *C. gloeosporioides*. Similar results have been reported in Ghana (Honger et al. 2014).

C. siamense has been reported causing the typical sunken spots characteristic of anthracnose on mango in Ghana (Honger et al. 2014). However, though the fungus was isolated from fruits showing the tear stain symptoms in this study, it could not induce the same symptom on the inoculated fruits. This could be due to the absence of certain factors which may be restricted to the field. In fact, in Ghana, there is higher incidence of tears stain infection in the minor mango season that the major season, which show varied weather conditions (Honger: unpublished data), giving credence to the suggestion that field factors including weather conditions could be involved in the development of the symptoms in the field. However, by inducing the slightly sunken dark spots in this study, it confirms the fungus as pathogenic to mango.

Application of synthetic chemicals, both from organic and inorganic sources has been employed for the control of mango anthracnose disease in several parts of the world (McMillan 1984; Dirou and Stovold 2005; Honger 2013; Nasir et al. 2017). In this study, two copper based fungicides and mancozeb were found to be very effective in reducing the incidence of the disease. Mancozeb is permitted to be used on mango fruits that are exported to European countries where some of the mangoes from Ghana are sent (Arauz, 2000). On the other hand, copper based fungicides are acceptable in all markets, including the organic markets. The effectiveness of these fungicides in this study, therefore, gives options for mango farmers to reduce the effect of the disease on their fruits for export. Recently, the possibility of boosting the natural resistance of susceptible fruits against anthracnose disease, has been investigated by the application of salicylic acid and acibenzolar-S-methyl (Zainuri et al. 2003). In this study, Bion (acibenzolar-s-methyl), was combined with the fungicides to boost their performance. Though, the effect of the biostimulant was not felt, (as the fungicides used without the biostimulant gave same results

as when it was used), it's possible that the results may be felt, with longer use.

Conclusion

Tear stain disease of mango fruits was found to be prevalent in the coastal savannah area of Ghana, where the highest concentration of mango farms are found in the country. More of the disease was found in the minor season than in the major season and this has been attributed to poor cultural practices in mango orchards in the minor season. The causal agent of the tear stain symptoms on mango fruits was identified as *C. siamense* based on the traditional methods and sequence analysis of the ITS region and the actin gene. The pathogen was able to induce the typical dark brown sunken spots associated with anthracnose disease on mango fruit, but not the tears stain symptoms on artificially inoculated fruits. Nonetheless, the identity of the pathogen and it's pathogenicity on mango fruits confirmed that the tear stain disease was another symptom of anthracnose and hence could be controlled using the same strategy employed to control the well-known anthracnose disease symptom. Consequently, three fungicides, namely, Mancozeb, copper oxide and copper oxychloride were found to be very effective against the disease. A combination of the copper based fungicides with Bion (Acibenzolar-S-methyl), a biostimulant, also controlled the disease very well. Findings from this research have provided enough information for the control of the disease in the study area and these must be implemented.

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Declarations

Conflict of interest The author has no conflict of interest to declare.

References

Abang MM (2003) Genetic diversity of *Colletotrichum gloeosporioides* Penz causing anthracnose disease of yam (*Discorea spp*) in Nigeria. Bibl Mycol 197:139

Ablormeti FK, Coleman SR, Honger JO, Owusu E, Bedu I, Aidoo OF, Cornelius EW, Odamtten GT (2021) Management of *Lasiodiplodia theobromae*, the causal agent of mango tree decline disease in Ghana. African Crop Sci J 29:193–207

Agrios GM (2005) Plant pathology, 5th edn. Academic Press, New York, p 952

Arauz LF (2000) Mango anthracnose: economic impact and current options for integrated management. Plant Dise 84(6):600–611

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Damm U, Baroncelli R, Lei C, Kubo Y, O'Donnell R, Weir B, Yoshino K, Cannon PF (2010) *Colletotrichum*: species, ecology and interactions. Int Mycol Assoc 1(2):161–165

Dirou J, Stovold G (2005) Fungicide management program to control mango anthracnose. Primefact 19. Department of Primary Industries, New South Wales (**ISSN 0725-7759**)

E. P. A (2015) List of registered products in Ghana. Environmental Protection Agency, Accra

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Honger JO (2013) Characterisation of the causal agent of mango anthracnose disease in Ghana. Doctoral Thesis, Department of Crop Science, University of Ghana

Honger JO, Offei SK, Oduro KA, Odamtten GT, Tatu SN (2014) Identification and species status of the mango-biotype of *Colletotrichum gloeosporioides* in Ghana. Eur J Plant Pathol 140:455–467

Honger JO, Offei SK, Oduro KA, Odamtten GT, Tatu SN (2016) Identification and molecular characterisation of *Colletotrichum* species from avocado, citrus and pawpaw in Ghana. South African J Plant Soil 33(3):177–185. https://doi.org/10.1080/02571862.2015.1125958

Johnston PR, Jones D (1997) Relationship among *Colletotrichum* isolates from fruit rots assessed using rDNA sequences. Mycologia 89(3):420–430

Kumar P, Kumar GV, Kumar TA (eds) (2016) Current trends in plant disease diagnostics and management practices. Springer international publishing, Switzerland

Lakshmi BKM, Reddy PN, Prasad RD (2011) Cross infection potential of Colletotrichum gloeosporioides Penz, isolates causing anthracnose in sub-tropical crops. Trop Agricult Res 2:183–193

McMillan RT (1984) Control of mango anthracnose with foliar sprays. Proc Florida State Horticult Soc 97:344–345

MOAP (2016) Post-harvest losses in the mango, pineapple and citrus value chains in Ghana. In: Data provided by GIZ

Nasir M, Iqbal B, Idrees M, Sajjad M, Niaz MZ, Anwar H, Shehzad MA, Tariq AH (2017) Efficacy of some organic fungicides against anthracnose and powdery mildew of mango. Pakistan J Agricult Sci 54(3):493–496

Nelson SC (2008) Mango anthracnose (*Colletotrichum gloeosporioides*). College of Tropical Agriculture and Human Resource. Publication PD-48, Manoa

Phoulivong S, Cai L, Chen H, Mckenzie EHC, Abdulsalam K, Chukeatirute EKD (2010) *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Div 44:33–43

Ploetz RC (1998) Anthracnose. In: Ploetz RC, Zetmeyer GA, Nishijima WT, Rohrbach KG, Ohr HD (eds) Compendium of tropical fruit diseases. The American Phytopathological Society, Minnesota, pp 35–36

Prihastuti H, Cai L, Chen H, Hyde KD (2009) Characterisation of *Colletotrichum species* associated with coffee berries in Chiang Mai, Thailand. Fungal Div 39:89–109

Tamura K, Peterson D, Peterson N, Steicher G, Nei M, Kumar S (2011) Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mole Biol Evol 24:1596–1599

Weir BS, Johnston PR, Damm U (2012) The Colletotrichum gloeosporioides species complex. Stud Mycol 73:115–180

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innins MA, Gelfand DH, Snisky JJ, White YJ (eds) PCR protocols: a guide to methods and application. Academic Press, San Diego, California, pp 312–322

Zainuri Z, Irving DE, Dann EK, Coates LM, Wearing AH (2003) Activating mango fruit defense to anthracnose disease. In: Proceedings of Australian postharvest horticulture conference, Brisbane, Queensland, pp 149–150