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Morphological Characterization of Fungal Leaf Spot Diseases of Mango (*Mangifera Indica L.*) and *in Vitro* Evaluation of the Effect of Antagonists on Its Mycelial Growth

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Abstract: The incidence of fungal leaf spot diseases on mango (*Mangifera indica*) in Oda Bultum University Mango farm and application of fungicide and a biological control measure was investigated. In addition microbial biocontrol and fungicide control against *C. gloeosporioides* developed for mangoes has been less reported in Ethiopia. Common mango disease control mechanisms in Ethiopia are utilization of pesticides. However, pesticides are overused and misused. As a result there is an urgent need to reduce the use of synthetic chemicals. Biological control offers an alternative to the use of pesticides. *Invitro* evaluation and testing of *T. harzianum* showed maximum mycelial growth inhibition (74.4% and 73.5%) on *C. gloeosporioides* isolates 1 and 3 respectively. The minimum percent of mycelial growth inhibition (71.5%) of *T. harzianum* was observed on isolate 1. *Pseudomonas fluorescens* showed maximum mycelial growth inhibition on isolate 2 (74.1%), followed by isolate 1 (70.4%). Least percent of mycelial growth inhibition by *P. fluorescens* was observed on isolate 3 (69%). *B. subtilis* showed maximum mycelial growth inhibition on isolate 3 (56.1%), followed by isolate 1 (51.5%). Least percent of mycelial growth inhibition by *B. subtilis* was observed on isolate 2 (48.6%). *In vitro* evaluation of fungicides against the three isolated test pathogen revealed that at 1000 PPM, all fungicides showed highest percentage inhibition (72.8% to 90.14%). The highest percentage inhibition was observed at the concentration of1000 PPM on isolates 2 (90.14%) and 3 (88.13%) and the lowest percentage inhibition was observed at the concentration of 200 PPM on isolates 1 (71.46%) and 3 (77.18%). The highest percentage mycelial growth inhibition by Sancozeb 80% WP on the isolates was 90.14% at a concentration of 1000PPM, whereas the lowest percentage mycelia growth inhibition of 83.06% was recorded at 200PPM. The highest percentage mycelial growth inhibition by Ridomil on the isolates was (83.16%) at 1000 PPM and the lowest percentage mycelial growth inhibition was recorded on isolate 1 (72.83%) at 200 PPM. Among the two fungicides Sancozeb was the most effective fungicide to inhibit the growth of the isolates with 87.98-90.14% inhibition.

Keywords: Biocontrol, *Colletotrichum gloeosporioides*, Epiphytes, Mango, Leaf Spot, *in Vitro*, Mycelial Growth, Microbial Antagonists

1. Introduction

Ethiopia is one of the world's major food exporters and fruits and vegetables are mainly shipped to various countries such as the United States, the European Union, China and Japan. Therefore the agricultural sector plays an essential role in Ethiopian economy. Agricultural production used 80% of the workforce and 20% of the land area [1] making it the main source of income for rural people who rely heavily on this sector. To increase the product yield, pesticides are overused and misused in spite of health concerns, environmental contamination and export bans [2]. There is an urgent need to find alternative ways to replace or reduce the use of synthetic chemicals. Biological control, using microbes to prevent and/or suppress plant diseases, offers an

alternative to the use of pesticides [3].

The agricultural based economy is a core business in Ethiopia and food export is one of the main sources of income for the Ethiopian population. However, pesticides are overused and misused. As a result there is an urgent need to reduce the use of synthetic chemicals. Biological control offers an alternative to the use of pesticides. Mango (*Mangifera indica* L.) is widely planted in Oda Bultum University, Ethiopia and is one of the major cash crops for local export. However, mango suffers from various diseases especially anthracnose, a fungal disease caused by *Colletotrichum gloeosporioides.*

Mango (*Mangifera indica* L.) is a tropical tree crop belonging to the family of *Anarcadiaceae*. Mango occupies relatively the same position in the tropics as enjoyed by the apple in temperate America and Europe [4]. The cultivars of mango commonly found in Ethiopia are West and East Hararge Regions, West Welega regions.

Mango (*Mangifera indica* L.) is widely planted in many parts of Ethiopia and it is one of the major fruits consumed domestically as well as exported internationally. The mango variety Kent, Keitt, Tommy Atkins, and Apple Mango are the most popular variety for export due to its sugary flavor and soft texture. The export value of mango was 22.8 million USD per year which makes it one of the major cash crops in Ethiopia [5]. However mango suffers from various diseases caused by many phytopathogens. Anthracnose, caused by a fungal pathogen – *Colletotrichum gloeosporioides*, is a severe disease which can cause serious losses at various stages of mango production ranging from the blossom period to post-harvest. Although chemical treatment using various fungicides presented high efficiency against *C. gloeosporioides,* the emergence of fungicide resistant isolates of this pathogen has been reported [6]. Hence there is an urgent need to tackle this problem.

Microorganisms associated with post harvest spoilage of fruits have engaged the attention of many mycologists for years [7]. It has been reported that *Macrophoma mangiferae* Higorami and Sharma caused foliage blight of young seedlings and young grafted plants, while *Botryodiploidia theobromae* was the agent of die-back and bark canker, and the gray blight of leaves is caused by *Pestalotiopsis mangiferae* [8]. Epiphytic microbes that live on plant surfaces without causing any symptoms to the plants are well known for their efficacy to inhibit plant pathogens [9, 10]. In this study bacteria and fungus are focused on because they are able to produce diverse groups of bioactive substances and they can persist under a wide range of environmental conditions which is the key for future industrial application. Among bacterial species, *Bacillus* species are particularly favored because they are best known for producing secondary metabolites [11].

The attention of mycologists is presently focused on control measures of mango diseases [7]. The use of chemicals to control plant pathogens, especially foliage pathogens, has had only limited success in the past in Africa due to lack of suitable methods of application, or lack of an effective chemical and prohibitive cost. There is also the added concern about chemical residue in the environment and the development of resistance by the pathogen [12].

Biological control of plant pathogens could reduce or eliminate some of those concerns. It is also potentially more durable and much cheaper [12]. More recently, an increasing number of reports have focused on the potential of *Bacillus subtilis* as a bio-control agent [13]. The presence of endospores in *Bacillus* sp. would allow it to persist on the leaf surface of mango, especially in the hot tropics of Africa. In this study, the fungi responsible for leaf spot diseases of mango were isolated, identified and pathogenicity tests carried out. The potential to use *Bacillus subtilis* as a biocontrol agent was also investigated.

Colletotrichum gloeosporioides has been extensively studied because it can infect a wide range of crops and cause serious economic losses [14]. Although fungicides have been reportedly used to control *C. gloeosporioides*, the pathogen has become resistant to fungicides [15]. Clearly the use of fungicides is not sustainable. Rather than the heavy use of fungicides alone, biocontrol could be used to reduce, or, together with chemicals, to avoid fungicide-resistance which may occur in plant pathogens. Biocontrol of *Colletotrichum* has been widely reported on various tropical fruit crops for example banana [16], grape [17] and papaya [18]. However, mango has received much attention in biological control research because of its worldwide economic value [19]. The *Bacillus* species has been in the spotlight for biocontrol research for decades due to its various modes of actions [20]. Four modes of action in biocontrol include (1) competition for nutrients and space, (2) production of antibiotics, (3) direct parasitism and (4) inducing plant resistance [21]. Various bioactive substances produced by *Bacillus* spp. were purified and reported for their inhibitory effect against *C. gloeosporioides*. [22] isolated and identified potential metabolites produced by *B. subtilis* which strongly inhibit spore germination of *C. gloeosporioides*. [23] showed that *Bacillus* were able to inhibit pathogens with more than one mode of action such as secreting protease enzymes and producing antibiotics.

However; research on biocontrol of mangoes has never been done in Ethiopia particularly in Oda Bultum University Mango farm West hararge region. The overall aim of this work was to expand the knowledge of using biocontrol and to find new microbial biological control agents (BCAs) and fungicides for fungal leaf spot disease on mangoes in Oda Bultum University.

2. Materials and Methods

2.1. Experimental Site

The study was conducted in Oda Bultum University Biology and Animal Science Department Laboratory rooms situated in Eastern part of Ethiopia. These sites are among the sites where vast areas of natural *mango plants* are found and these sites are known for the large quantity of mango production. Oda Bultum University, one of the study sites is situated in Western

Hararge, Oromia Regional State, Ethiopia.

2.2. Survey Area and Collection of Leaf Spot Diseased Specimens

Mango plants showing typical symptoms of leaf spot diseased were collected in plastic bags and envelopes from three different sampling sites of mango growing areas from Oda Bultum University mango farm, from Chiro Town and farmers farm of Chiro Zuria woreda, West Hararge Ethiopia. The sampling areas lie between $34^0 18' 43''$ to $43^0 0' 4''$ E Latitude and $10^0 09' 24''$ to $30^0 18' 43''$ N longitude with altitudes ranging from 1033 to 2322 masl. The diseased samples were kept in refrigerator at, 4°C for further studies.

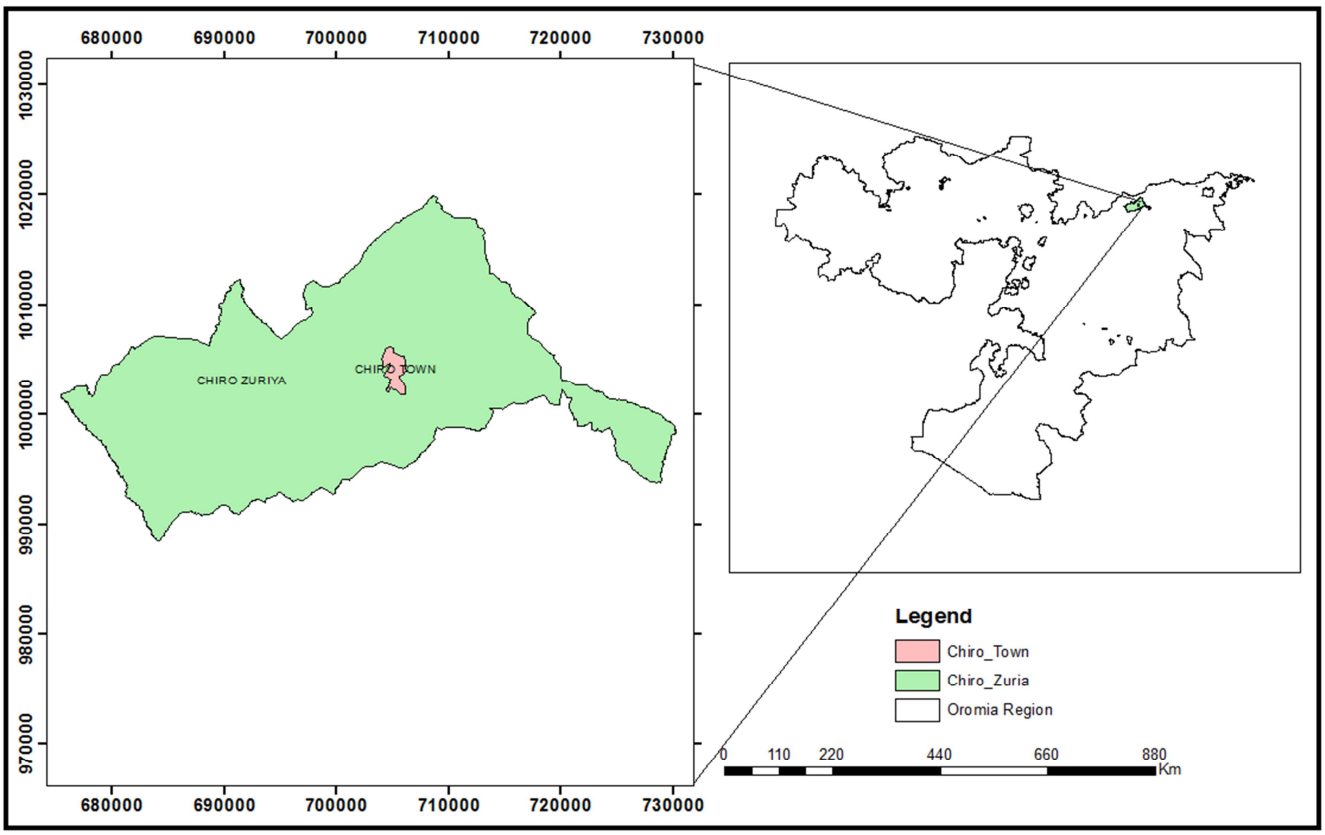


Figure 1. *Map of Study Site.*

2.3. Sample Collection and Isolation

Prior to sample collection, attempts were made to identify clear external symptoms and sign of infestation on part of the tree. Samples were then collected from the symptomatic part of *mango* trees. The samples then transferred to laboratory and store at, 4°C.

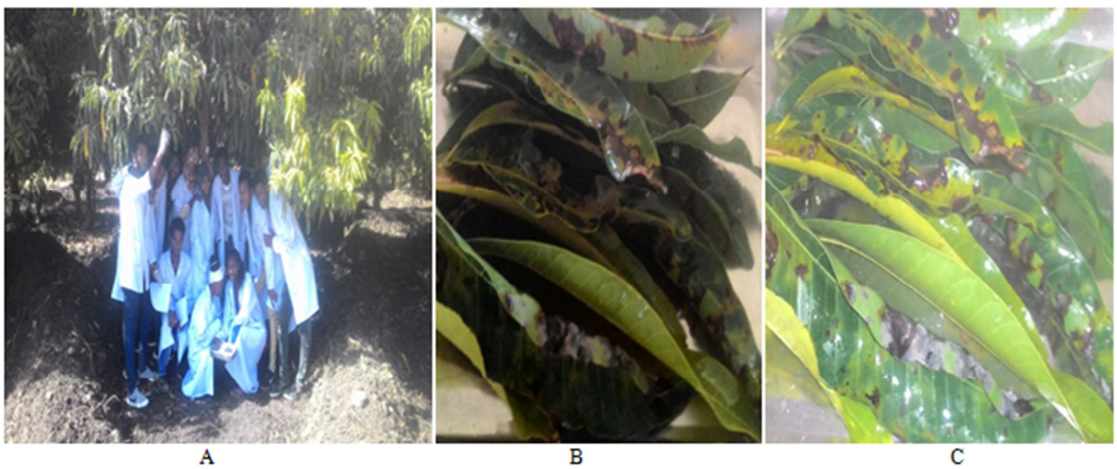


Figure 2. *Collection leaf spot samples from Mango plant (A. OBU; B. Chiro Town; C. Chiro Zuria Woreda).*

Isolation was made from sample collected from different parts of the tree with disease symptom. Two different isolation techniques were used. This involves, direct transfer of sterilized symptomatic mango leaf (2-3 mm) on PDA (Potato dextrose Agar). Incubating the pieces of leaf (5 mm) with symptoms of infection on moist chamber is the other isolation technique used. Potato Dextrose Agar (PDA, Oxid Ltd. Basingstoke, Hants England) containing 1.8 g Agar and 2 g glucose, is the growth media used for fungal isolation. The PDA used for the isolation was treated with streptomycin to minimize bacterial contamination. Small plug of resultant mycelial growing on symptomatic wood on PDA were then transferred onto PDA growth medium. Similarly, the fruiting structures grown on the piece of leafs were picked up with needle and transferred onto PDA. These isolates were incubated at room temperature. Following this, segments of the resultant mycelial growth were again transferred onto new media (PDA) to get pure culture to be used for further study. All fungal isolates were kept in fridge where the temperature is adjusted to about 5-4°C. A Piece of fungal mycelial from edge of clean colony was transferred on to Water Agare (WA) containing three to four sterilized pine needle to initiate spore production [24].

2.4. In Vitro Evaluation of Antagonistic Activity of Trichoderma, Pseudomonas and Bacillus Species Against Fungal Leaf Spot (C. Gloeosporioides) Isolates

One species of *Trichoderma harzianum*; and two species of bacteria namely *Pseudomonas. fluorescens* and *Bacillus Subtilis* from culture collection of mango farm soil were used to evaluate the antagonistic activities against the test isolates. Dual culture method [25] was employed to evaluate the antagonistic activities of *T. harzianum*, *S. fluorescens* and *B. Subtilis*. Four mm diameter mycelial disc from the periphery of 10 day old culture of the antagonists was placed on the plate at four locations, approximately 3 cm from the center. Similarly *Pseudomonas fluorescens* isolate was assessed for potential antagonistic activity against the isolates on PDA using dual culture technique [26]. Four mm diameter mycelial disc was cut from an actively growing *C. gloeosporioides* culture and placed on the surface of fresh PDA medium at the center of the Petri plates. A loopful of actively growing *Pseudomonas fluorescens* and *Bacillus Subtilis* isolate were streaked on the plate at four spots, approximately 3 cm from the center. Plates inoculated with isolates and without antagonists was acts as control All *in vitro* tests of antagonism were performed three times. All plates were incubated at, 27±1°C for 7 days. Growth inhibition was observed every two days and the final measurements were recorded at the 7 th days for incubation. Degree of antagonism was determined by measuring the radial growth of the isolate by the *Trichoderma s*pecies, *Pseudomonas fluorescens* and *Bacillus Subtilis* in dual culture in relation to growth of the control and percentage of inhibition was calculated by using the following equation [27].

[I% =(C-T)/C] x100 Where,

I= percentage inhibition of pathogen by antagonists

C= radial growth measurement of the pathogen in the control plates and

T= radial growth of the pathogen in the experimental plates

2.5. In Vitro Evaluation and Testing of Fungicides Against the Isolates

The Poisoned Food Technique

To test the effect of fungicides against the isolates, the method followed by [28] was employed. In this technique, the growth medium was poisoned with fungal toxicants, Sancozeb and Ridomil. All fungicides were obtained from Chem Tax PLC. (Addis Ababa). The fungicide concentration were prepared as follows, if the formulated product (fungicide) has, say 50% active ingredient, for 1 ppm solution 2 mg of the formulated product was dissolved in a liter solvent [28]. Stock concentrations of the chemicals used were: Ridomil Gold MZ (metenoxam 4%, and Sancozeb 80% WP (mancozeb 80% a.i and inert 20%). The chemicals were added to the autoclaved PDA medium cooled to 45°C. so that the required concentrations were obtained. Duplicate culture plates, without fungicide were used as control.

The isolates were grown on PDA for 10 days at, 27±1°C. Mycelia plugs, 4 mm in diameter, were cut from actively growing margins of the culture by sterile cork borer and transferred aseptically in to the center of the Petri dishes containing the PDA medium with different concentrations ranges from 200-1000 PPM. Inoculated plates were incubated at, 27±1°C for 7 days. Growth of isolates at each concentration was determined by measuring colony diameters in two perpendicular directions on each culture plate in darkness at, 27±1°C. The relative growth reduction for each rate of fungicide was calculated by the equation below [27].

I= [(C – T)/C] x 100, Where

I= is inhibition of radial mycelial growth;

C= is radial growth measurement of the pathogen in control;

T= is radial growth of the pathogen in the presence of the fungicides.

2.6. Characterizations of the Test Pathogen Isolates

2.6.1. Morphological Characterization of the Fungi

Representative sample isolates obtained from the different study sites were selected and used for spore characterization. The spores used for this purpose were obtained from spores produced on the colony growing on PDA and the spore produced on Water Agar (WA) that contained sterilized pine needle. It was incubated for 10 days at room temperature. The spores obtained from different sources were placed on slide and the shape and size of conidia were evaluated under microscope. The lens magnification range was set on 40 x magnification and the eyepiece was set at 10 x magnification

capacity. Fungal classification key was used to determine the identity of the fungi isolated from mango trees and the colony and spore characteristics of these isolates were compared with the characteristics of its close relative [24].

2.6.2. Microscopic Characterization of Conidia

Morphological characterization was based on conidial features. Each isolate was grown on PDA and incubated at, 27±1°C for 10 days. Blocks of cultures were transferred into sterilized water agar containing 20 g/l for sporulation. The morphological characters such as; shape, colour and size (length and width) of the conidia were measured by taking 50 spores for each isolate and number of septations per conidia was observed using microscope. Based on these features the six isolates were identified using key developed by [29]. The spores were observed on slides after staining with lacto phenol cotton blue under light microscope. The sizes of conidia were measured by using ocular and stage micrometers as described in [30]. The measurements of the spores were made by using digital solutions for imaging and microscopy, soft image system. Microphotographs were taken to show the typical spore morphology of the pathogen.

2.7. Source of Prospective Antagonist

Samples of surface soil collected from under a mango tree were plated out for isolation of species of Bacillus. In each case 2 g of the soil was shaken in 10 ml of water and maintained at 90°C for 15 min in a water bath to select for endospore formers. Samples (0.1 ml) of a 10–2 dilution of the soil suspension was spread on PDA and incubated at room temperature (28–30°C) for 48 h before colonies with the characteristic features of Bacillus were isolated and stored in slant cultures at 4°C. Isolates were subjected to microbial analysis and preliminary identification was done by standard procedures [31]. One of the isolates was later confirmed as Bacillus subtilis. Six replicate plates were prepared for each soil sample and the experiment was repeated thrice. Three loops of two – day old culture on PDA were mixed with 5 ml of Potato dextrose broth and 0.1 ml of the suspension was used as inocula for the Bacillus isolates. The concentration of the bacterial suspension used was 106 cfu/ml.

Data Analysis: Analysis of variance (ANOVA) was used to compute and arrived at statistical decision and $p<0.05$ was considered significant. The data are presented as means±SE. Differences between treatments were determined by using least squared means comparisons with ($P<0.05$). Mean comparisons of each treatments was performed by using Duncan's multiple range test ($\alpha=0.05$).

3. Result

Characterization of isolates from diseased mango plants was undertaken from Oda Bultum University mango farm, Chiro Town and farmers'field of Chiro Zuria Woreda, West Hararge region.

3.1. Disease Survey, Collection and Isolation of Different Isolates

A survey for the diseased specimens of mango plant with typical spot symptoms were collected from different sites in west hararge, Ethiopia during the main cropping seasons of 2018. Incidence of leaf spot disease in the different sites varied from 50.7 to 64.0%, with the greatest incidence in the Oda Bultum University and Chiro Zuria woreda (64.0% and 56.9% respectively). Over all, Mango leaf spot disease was most severe in the Oda Bultum University (32.6%) and least severe in Chiro Town (16.7%) (Table 1). A total of 10 samples from diseased mango plant leaf were collected from different growing areas of west Hararge zone and three samples were selected for further characterization one from each sampling site.

Table 1. *Average disease incidences and severities and their ranges in different sampling sites of The study area.*

Ecological Zone	DI (range) (%)±SD	Average DI (%)±SD	DS (range) (%)±SD	Average DS (%)±SD
Oda Bultum University	50-74.7	64.0±11.1[a]	22.5-50	32.6±8.4[a]
Chiro Town	32-65	50.7±15.0[a]	5-27	16.7±8.1[b]
Chiro Zuria Woreda	37-95	56.9±16.5[a]	5-69	23.7±16.3[ab]
Mean total	39.7-78.9	55.9±14.2	10.8±48.7	24.7±10.93

DI= Disease Incidence; DS= Disease Severity. Values in the same letters are not significantly different at ($p<0.05$).

3.2. In Vitro Evaluation of Antagonistic Activity of Trichoderma Species P. Fluorescence and B. Subtilis Against the Test Pathogen

In dual culture technique seven days after incubation the hyphal growth of *C. gloeosporioides* was found to be inhibited by the hyphae of *Trichoderma* spps. The advancing hyphae of *Trichoderma* species covered the entire medium in the Petri plates, suppressing the growth of *C. gloeosporioides* isolates. *Trichoderma* species tested exhibited inhibition of mycelial growth of *C. gloeosporioides* isolates. The results of this study showed that differences in inhibition of the mycelial growth rate of the test pathogen. *T. harzianum* has rapid growth as compared to all the test isolates of *C. gloeosporioides* and it did not show any clear inhibition zone. *T. harzianum* has rapid growth, in the form of powdery widespread throughout the Petri plates. The interaction was due to the competition for spaces and nutrients rather than forming inhibition zone. *T. harzianum* showed maximum mycelial growth inhibition (*74.4*% and 73.5%) on *C. gloeosporioides* isolates (*1* and *3*), respectively. The minimum percent of mycelial growth inhibition (71.5%) of *T. harzianum* was observed on *isolate 1*.

Similarly, the result of *in vitro* test of antagonistic

activities of *P. fluorescens* against the isolates of *C. gloeosporioides* is indicated in Table 2. Inhibition was clearly shown by limited growth of fungal mycelium in the inhibition zone surrounded by *P. fluorescens*. The antagonistic effects of *P. fluorescens* against *C. gloeosporioides* isolates was in the range of 69.0- 70.4%. *Pseudomonas fluorescens* showed maximum mycelial growth inhibition on isolate *2* (74.1%), followed by isolate *1* (70.4%). Least percent of mycelial growth inhibition by *P. fluorescens* was observed on isolate *3* (69%).

The antagonistic effects of *B. subtilis* against *C. gloeosporioides* isolates was in the range of 48.6- 56.1%. B. subtilis *showed* maximum mycelial growth inhibition on isolate *3* (56.1%), followed by isolate *1* (51.5%). Least percent of mycelial growth inhibition by *B. subtilis* was observed on isolate *2* (48.6%).

Significance difference was observed in the percentage mycelial growth inhibition between the isolates. The control plates without *antagonists* were grown and the Petri plates were covered by the *C. gloeosporioides* isolates. The mean inhibitory effects of the biological control agents showed significant difference among themselves as well as against on the different isolates of the test pathogen. The fungal *T. harzanium* and bacterial *P. fluorescens* biocontrol agents were found to be more effective than *B. subtilis* bacterial antagonist where they showed higher percent of inhibition 74.4% by *T. harzianum* and 74.1% by *P. fluorescens* compared with *B. subtilis* (56.1%).

P. fluorescens

Control

Figure 3. *Mycelial growth inhibition of P. fluorescens.*

Table 2. *In vitro evaluation and testing of T. harzianum P. fluorescens and B. subtilis against mycelial growth of C. gloeosporioides isolates after 7 days on incubation at 27±1°C.*

Isolates	Control Growth (mm)	*T.harzanium* 7DAI		*P.fluorescens* 7DAI		*B.subtilis* 7DAI	
		MG (mm) Mean ± SD	% Inh. Mean±SD	MG (mm) Mean ± SD	% Inh. Mean±SD	MG (mm) Mean ±SD	% Inh. Mean±SD
1	57.50	14.0±0.4^{a}	74.4±0.8^{a}	16.0±0.8^{b}	70.4±1.4^{b}	26.7 ±0.4^{ab}	51.5±0.9^{b}
2	51.00	14.9±1.4^{a}	71.5±2.6^{b}	13.5±0.6^{c}	74.1±1.2^{a}	26.5 ±0.6^{ab}	48.6±0.7^{c}
3	52.45	15.2±0.3^{b}	73.5±0.6^{ab}	17.8±0.7^{ab}	69.0±1.2^{b}	25.3 ±1.0^{b}	56.1±1.0^{a}
Mean	53.63	14.7±0.7	73.13±1.33	16.1±0.7	71.2±1.3	26.2±0.4	52.1±0.9

Values in the same letters are not significantly different at ($p<0.05$). DAI= Days After Incubation

3.3. In Vitro Evaluation of Fungicides Against the Test Pathogen

All fungicides showed varied levels of antifungal activity (Table 3). At 1000 PPM, all fungicides showed highest percentage inhibition (72.8% to 90.14%) against the three isolates. They showed differences in their mycelial growth inhibition at different concentration. The highest percentage inhibition was observed at the concentration of1000PPM on isolates 2 (90.14%) and 3 (88.13%) and the lowest percentage inhibition was observed at the concentration of 200 PPM on isolates 1 (71.46%) and 3 (77.18%) (Table 3).

The highest percentage mycelial growth inhibition by Sancozeb 80% WP on the isolates was 90.14% at a concentration of 1000 PPM, whereas the lowest percentage mycelia growth inhibition of 83.06% was recorded at 200 PPM. The highest percentage mycelial growth inhibition by Ridomil on the isolates was (83.16%) at 1000 PPM and the lowest percentage mycelial growth inhibition was recorded on isolate 1 (72.83%) at 200 PPM.

Table 3. *The evaluations of different concentration of fungicides on mycelial growth of C. gloeosporioides isolates after 7 days of incubation at 27±1°C.*

Isolates	Fungicide	Control	Concentration (PPM) Mycelial growth inhibition (%)			
			200	500	800	1000
1	Sancozeb 80% WP	57.75	83.06±0.7^{a}	85.09±0.4^{a}	85.98±0.9^{a}	87.98±1.9^{b}
	Ridomil	57.75	71.46±0.3^{bc}	71.69±0.7^{e}	72.68±1.8^{e}	72.83±0.3^{e}
2	Sancozeb 80% WP	49.50	85.23±0.5^{a}	86.61±3.4^{a}	87.13±4.6^{a}	90.14±0.6^{ab}
	Ridomil	49.50	76.29±2.9^{a}	80.17±1.1^{b}	82.20±0.3^{ab}	83.16±1.1^{b}
3	Sancozeb 80% WP	57.50	84.64±1.2^{a}	87.04±0.3^{a}	87.62±0.5^{a}	88.13±0.4^{ab}
	Ridomil	57.50	77.18±0.3^{a}	77.39±1.2^{c}	78.70±0.6^{c}	79.85±0.4^{d}

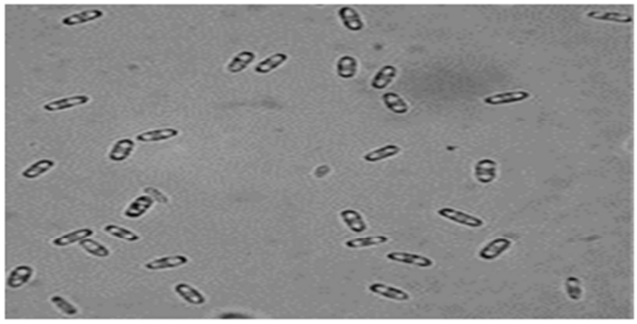
Values with the same letter are not significantly different

3.4. Morphological and Growth Characteristics of the Isolates

The isolation made from each symptomatic sample tree using different isolation methods has resulted into a colony that has identical growth characteristics. At initial stage, the colony has whitish and sparsely distributed mycelial growth. However, as it grew older the color of the mycelia changes into black and it densely covered the whole surface of the Petri dish. The color of the colony can be described as grey to black color at the top and blackish at the reverse side of the Petri dish (Figure 4A).

A

B



C

Figure 4. *(A) Mycelial growth on Petri dish and (B & C) Spore morphology of the fungi on Potato Dextrose Agar.*

3.5. Conidial Characteristics of the Test Pathogen Isolates

Description of morphological characteristics of microconidia and macroconidia indicated that the three isolates were grouped into *Colletotrichum* spp, which coincided with their cultural characterization. In all isolates, the shape of the conidia was the same with typically Cylindrical conidia, base rounded. Though closely resembling one another in shape, distinct differences in spore size could be seen among the isolates. Some of them were very long and narrow, while some were fairly broad. The isolates *1* and *3* showed the maximum size of conidial length about 18µm (average 14.5 µm) and 17 µm (average 14 µm), respectively. Minimum size of conidial length was noticed by the isolates *2;* 14 µm (average 12.5 µm) (Table 4).

Table 4. *Conidial size, shape and growth rate at room temperature on PDA for the morphological groups of Colletotrichum spp.*

Isolate	Conidial Shape	Length (µm)			Width (µm)			Growth rate on PDA (mm)		
		Min	Max	Mean*	Min	Max	Mean*	20°C	25°C	30°C
1	Cylindrical	11	18	14.5[a]	3	5	4[a]	7.3	11.5	12.1
2	Cylindrical	11	14	12.5[a]	5	7	6[b]	6.8	10.3	10.5
3	Cylindrical	11	17	14[a]	5	9	7[b]	10.2	12.6	12.5

*The mean difference is significant at the 0.05 level.

4. Discussion

Leaf spot diseases of fungal origin on mango were serious in some areas and some time led to a poor fruit yield as seen in the survey. Among the surveyed areas, Oda Bultum University and Chiro Zuria woreda at the same altitude. The incidence of the disease at Chiro Town was quite low. Similar observations were made by [32] with respect to infection by *Colletotrichum gloeosporioides* the causal agent of mango leaf spot. Many leaf spot diseases manifest more at the onset of the rainy season since the high relative humidity helps in factor development [7]. This confirms the report of Okigbo (2001) that high humidity furthers the disperse of fungal spores of observations were made by [32] with respect to infection by *Colletotrichum gloeosporioides.* The conidial characterstics of the three isolates indicated that they were grouped into *Colletotrichum* spp. Similar studies have been conducted by [33].

The use of specific microbial antagonists has brought remarkable success in the control of plant pathogens [7]. Many *bacillus* species including B. subtilis exhibit antifungal activity against many plant pathogenic fungi [13]. In the present study *B. subtilis* proved to be strong antagonists against *Pestalotiopsis mangiferae, Macrophomam manigferae and Colletotrichum gloeosporioides* on agar plates [34]. The fungal species *T. harzanium* and bacterial *P. fluorescens* biocontrol agents were found to be more effective than *B. subtilis* bacterial antagonist where they showed higher percent of inhibition by *T. harzianum* and by *P. fluorescens* compared with *B. subtilis*. The present study was

similar with the work of [35, 32].

The *in vitro* results indicate that producing antibiotics could play an essential role for *Bacillus* species because wide, clear zones were present without any contact between the test epiphytes and pathogen. Bioactive compounds might have been produced, released and diffused to the test agar medium to inhibit the pathogen. [36] that *Bacillus cereus* and *Pseudomonas fluorescens* were chosen as the most potential candidates based on a series of screening of BCAs from *in vitro* to a field trial conducted in the Philippines. This shows that it may be possible to use *Bacillus* as biocontrol treatment to reduce the development of fungal leaf spot lesions. Also it has potential for post-harvest treatment when synthetic fungicides are strictly prohibited.

Several kinds of microbes were able to effectively inhibit *Colletotrichum* species on mangoes including a filamentous fungus, *Trichoderma harzianum* [37], a yeast, *Cryptococcus* [38] and a filamentous bacterium, *Streptomyces* [39]. However, bacterial biocontrol has been proven to control *C. gloeosporioides* under various conditions such as *in vitro, in vivo*, greenhouse and in the field [17]. [23] showed that *Bacillus* were able to inhibit pathogens with more than one mode of action such as secreting protease enzymes and producing antibiotics.

5. Conclusions

This study has shown that the isolated pathogenic fungi species associated with leaf spot disease of mango are of economical and public health significance. Some strains of *T. harzianum, Pseudomonas fluorescens and B. subtilis* have been reported to have good source of biological control agent for leaf spot disease of mango plant. Rather than relying on importing foreign biocontrol products, searching for indigenous biocontrol should be encouraged in order to reduce costs of crop production, and to avoid the environmental impact of the introduced species as well as the complicated registration of foreign products. The research has shown that three indigenous BCAs isolated from mangoes have the potential to be developed for further study.

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