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### DISTRIBUTION AND IDENTIFICATION OF ANTHRACNOSE OF PAPAYA CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES* IN THE CENTRAL RIFT VALLEY OF ETHIOPIA

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#### ABSTRACT

Papaya (*Carica papaya* L.) is one of the most important fruit crops in Ethiopia. Despite its importance, papaya production is hampered by different biotic and abiotic factors. Among biotic factors, anthracnose is an important disease, causing serious damage to papaya, especially in the postharvest stage. The surveys were conducted in eight *kebeles* (smallest administrative unit) of three districts (Adama, Adamitulu Jidokombolcha, and Dugda) in the East Shewa Zone of the Oromia regional state, Ethiopia from August to September 2016 and August to September 2021, to assess the prevalence, incidence, and severity of papaya anthracnose disease. Disease incidence and severity on leaves and fruits were determined using random sampling techniques. Cultural and morphological characterization of the causal agent of papaya anthracnose was also done. The survey results showed that all the eight *kebeles* assessed during 2016 and 2021 were affected by papaya anthracnose disease. The overall mean incidences of the disease were 63.9% on the leaves and 40.3% on the fruits while average disease severities were 24.8% and 22.8%, respectively. Disease incidences in different *kebeles* ranged from 60 to 66.7% on leaves and from 30 to 48.9% on fruits. Disease severity also varied from 21.1 to 29.8% on leaves and from 20 to 26.7% on fruits. Disease incidences and severities were generally higher in Wolda Kelina *kebele*. In 2021, the assessment result indicated that in Dugda and Adamitulu Jidokombolcha districts anthracnose was a major papaya production constraint. Based on field symptomatic assessment as well as cultural and morphological characterization, *Colletotrichum gloeosporioides* was identified as the causal agent of the disease threatening papaya production in the surveyed areas. These findings provide information on the magnitude of the damage due to papaya anthracnose disease and will be useful to devise disease management strategies. It is necessary to investigate the reactions of the available papaya cultivars against the pathogen and select disease tolerant papaya materials.

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#### INTRODUCTION

Papaya is a popular and economically important fruit tree

of tropical and subtropical countries (Da Silva et al., 2007).

Papaya is believed to be native to southern Mexico and

neighboring Central America. Papaya grows well in areas with elevations ranging from sea level to 1600 m; but preferably grows best below 1500 m. Papaya requires ample sunshine and thrives best in areas with mean temperatures of 21-33 °C. Cool temperatures reduce growth and yield, and adversely affect the fruit quality by impeding the accumulation of sugars which render the fruit relatively insipid. Nutritionally, papaya fruit has tremendous nutritional value and contains 1.5% protein, 0.1% fat, 7.1% carbohydrates, 35% calories per 100 g edible fruit, calcium, iron, sodium, potassium, β-carotene, vitamin B2, niacin and vitamin C (Samson, 1986). It is also recommended by dieticians as supplementary food because of its purgative nature, low calories, negligible fats and rich medicinal ingredient to cure hepatogastric disorders (Sharma, 2015).

Papaya is one of the most important fruit crops in Ethiopia due to its great economic potential. It is important cash crop for gardeners, small-scale producers, commercial farmers, traders and processors. Currently, the production of papaya is entirely for domestic consumption. Ethiopia ranked 23<sup>rd</sup> in global papaya production; it accounts less than 4% of the world's papaya production (FAO STAT, 2019). To date, the production area of papaya is increasing and producers are well aware of the importance of this fruit. Papaya is commercially cultivated in about 3485 hectares of land with annual production of about 54,355 tons (Anonymous, 2018).

However, papaya suffers a great loss due to various diseases. In Ethiopia, diseases such as anthracnose (*Colletotrichum gloeosporioides*), Phytophthora (*Phytophthora palmivora*), powdery mildew (*Oidium caricae*), black spot (*Asperisporium caricae*) and papaya ring spot virus are seriously affecting the production and productivity of the crop. Papaya being perishable fruit is highly susceptible to fungal pathogens that attack the fruits during pre- and post-harvest operations and become significant limiting factor to attain best possible marketable production since it can cause 25-40% fruit losses during final product (Sharma et al., 1998). Anthracnose is an important disease in fruit trees, causing serious damage, especially in the post-harvest stage (Arauz, 2000). Papaya Anthracnose refers to a group of fungal diseases characterized by the development of dark,

sunken spots or lesions, often with a raised rim, on affected foliage, stems and fruit. The fungus produces, hyaline one celled, ovoid to oblong, sometimes curved dumbbell shape conidia, 10-15 μm in length and 5-7 μm in width. The fungus is favored by high temperature and humid or moist weather. The pathogen affects non-wounded, immature green fruit in the field. Thus, papaya anthracnose has a latent stage in its development that is similar to many anthracnose diseases of tropical fruits (Adikaram, 1991). Therefore, this paper presents the prevalence, incidence and severity of papaya anthracnose; identification of the present status of post-harvest diseases of papaya and characteristic feature of the pathogen in the Central Rift valley of Ethiopia. The results of this study could be used for the development of integrated anthracnose management.

## MATERIALS AND METHODS

### Description of study areas

The study was conducted in three districts (Adama, Dugda and Adamitulu Jidokombolcha) in the East Shewa zone in the Oromia regional state of Ethiopia during August and September 2016 and August and September 2021. The geographic coordinates of the locations were taken using Global Positioning System (GPS) receiver. The surveyed locations had altitudes ranging from 1399 to 1671 meters above sea level.

### Sampling, data collection and analysis

A multi-stage sampling technique was used to select sample units (districts, *kebeles*, farms, plants, and leaves and fruits). Three main papaya producing districts were selected from the Central Rift Valley areas. The *kebele* selection from each district was based on papaya area coverage and annual production. A total of twelve papaya producing farms (three farms per *kebele*) were selected and the prevalence, incidence and severity of papaya anthracnose disease during 2016 were recorded. Samples were collected using random sampling method on intact leaves and fruits. Similarly, quick assessment was conducted during 2021, to identify the status of papaya diseases with special focus on anthracnose. Disease prevalence was assessed as the percentage of *kebeles* in which papaya anthracnose disease symptoms were observed to the total number of *kebeles* surveyed in each district using the following equation (Amata et al., 2009).

$$\text{Prevalence \%} = \frac{\text{Number of } kebeles \text{ with the disease records}}{\text{Total number of } kebeles \text{ surveyed in each district}} \times 100$$

Five representative papaya plants were selected randomly diagonally across the field in each farm to determine disease incidence (DI) and severity of leaves and fruits. The incidence and severity data were the mean values of leaf or fruit infection. Incidence on foliage or fruit was assessed using six randomly

selected intact leaves or fruits on upper, middle and lower parts of the canopy of each plant. The incidence was calculated by counting visibly infected leaves or fruits expressed as a percentage of the total number of leaves or fruits sampled using the following equation (Cooke et al., 2006).

$$DI (\%) = \frac{\text{Number of infected leaves or fruits}}{\text{Total number of leaves or fruits assessed}} \times 100$$

Assessment of severity on the foliage and fruits was done on the same samples used for disease incidence. Severity on leaves and fruits was estimated based on a 0-5 rating scale, where 0 = no symptom, 1 = 0-20%, 2 = 20-40%, 3 = 40-60%, 4 = 60-80% and 5 = 80-100% of leaf or fruit area infected (Dickman and Alvarez, 1983).

#### Isolation of fungal pathogen

Fungal strains were isolated from symptomatic (anthracnose infected) papaya leaf and fruit samples which were collected from surveyed farms and brought to the pathology laboratory at Melkassa Agricultural Research Centre. Infected leaf and fruit samples were carefully cut in to small pieces of tissue using sterile scissors and scalpel. The tissues were then sterilized by 10% sodium hypochlorite for one minute, rinsed three times with sterile distilled water and dried on sterilized filter paper for 10 minutes. Sterilized leaf or fruit peel pieces were aseptically placed on potato dextrose agar (PDA) plates and incubated at 25±1°C for two days. The growth was further purified on another Petri plate with PDA for morphological characterization.

#### Cultural and morphological characteristics

Fungal growth on PDA was determined after five days of incubation. Colony development was recorded as presence or absence of fungal growth from each isolated leaf or fruit tissue in each plate. Colony color and density were assessed by visual observation after seven days of growth. Isolates were examined for their mycelia and conidial characters by mounting on slides with the aid of

a stereomicroscope.

## RESULTS AND DISCUSSION

### Symptoms of anthracnose on papaya and conditions favoring the pathogen

The symptoms of anthracnose disease on papaya referred to a group of fungal diseases characterized by the development of sunken spots or lesions, often with a water sock, on affected foliage, stems and fruit (Figure1). Disease symptoms were not clear at the time of harvest, but appeared when the fruits were ripening or became ripe (Figures 1 and 2). From the pictures taken during field assessment, the pathogen showed irregular symptoms on leaf and immature fruit part. Similar findings were reported by different authors (Dickman and Alvarez, 1983; Rohani, 1994).

Symptoms of anthracnose occur on branches, leaves, flowers and fruits, which may cause coalescence of lesions (Dickman and Alvarez, 1983). Infection involves the fruit tissue, which become softer with the diseased portion eventually falls off or readily separated from the uninfected parts of the fruit. Small, irregular-shaped water-soaked spots on the leaves may also be seen (Rohani, 1994). Anthracnose reached highest disease incidence and severity in areas where relative humidity and rainfall were highest and the air temperature was warm and conducive for fungal development. In tropical and subtropical areas, the growing season is continuous and the disease cycle is perpetually repetitive.



a



b

Figure 1. Papaya anthracnose symptoms on fruits; (a) picture taken from Dugda district; and (b) picture captured from Adamitulu Jidokombolcha district

With physiological maturation, papaya is believed to have antifungal compounds, reducing sugar increase, and other compounds, which are favorable to reactivation of infection (Prusky, 1996). Similarly, the survey result showed that the symptoms of anthracnose increased as the maturity of the fruit increased.

Anthraco­nose spores infect the plant tissues in the early then go dormant until around harvest time. The nature of the pathogen is actively invading the papaya fruit when the fruit ripe and the infection are quiescent.

Under high humidity and temperatures (above 22 °C) the production of orange mucilage, with hyaline conidia was observed in the center of the lesions (Bailey and Jeger, 1992). When the fruit is stored for long period in cold storage, disease symptoms may appear on green unripe fruit (Rohani, 1994). In assessed locality, during survey period the season was summer and in general the months starting from June to December are known for high rainfall, high relative humidity and medium temperature. So, in Ethiopia those months are most conducive to major papaya diseases.

### Management of papaya anthracnose disease

Weed control and appropriate use of pesticides may reduce the epidemics of this disease in the farm (Oniha and Egwari, 2015). The weed population and diversity in papaya orchards were observed during the 2016 and 2021 survey periods. The over saturation of weeds have direct effect on papaya by utilizing the nutrient, and indirectly reduce air circulation. This can be a cause for the increment of relative humidity and the temperature which are conducive for the disease epidemics.

From this survey, it was noticed that farmers located in the Central Rift Valley areas especially in Adamitulu Jidokombolcha and Dugda districts did not practice to manage papaya anthracnose disease. As it is seen in Figure 3, the papaya field was seriously affected by weeds which resulted in the suffocation of the field and anthracnose disease was very devastating. This is the knowledge gap the farmers have regarding papaya disease identification and possible management options.



a



b

Figure 2. Papaya anthracnose symptoms on fruits (a) taken from Wonji, Adama district (b) Shubi Gamo, Dugda district.



a



b

Figure 3a, b. Severely infected field by anthracnose and black spot in Dugda district, 2021.

Melkassa Agricultural Research Centre (MARC) conducted a study on time of removing infected plants parts for disease control at Tibila farm. The result showed that the removal of infected plants parts at every two weeks significantly reduced the build-up of papaya anthracnose. Thus, it was identified that sanitation measures are important components of integrated disease management strategy against papaya anthracnose (Yesuf et al., 2001).

Synthetic fungicides are currently used as the primary means for the control of plant diseases. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens and high development cost of new chemicals (Lee et al., 2007). Available literature suggests that benomyl or thiobendazole is among the most important fungicides used to reduce anthracnose diseases of papaya. Also, hot water treatment and control of atmosphere are considered as supplements to proper temperature and relative humidity (Broadrick et al., 2012).

Moreover, 36 papaya accessions were obtained from the National Fruit Improvement Program at MARC and evaluated for resistance/tolerance to anthracnose in 2001/2002. The result showed that four papaya

accessions were found resistant against papaya anthracnose (*C. gloeosporioides*). These materials along with the available information were provided to the Breeding Program for further studies (Yesuf et al., 2001).

#### **Distribution and economic importance of anthracnose disease of papaya**

The assessment conducted during field surveys showed that anthracnose was observed in all the surveyed locations during 2016 and 2021. Nevertheless, the prevalence, incidence and severity varied across the locations. Some studies in the country indicate that different diseases have been recorded on papaya (Yesuf et al., 2001), of them; anthracnose, root rot (*Phytophthora sp.*), black spot and dieback have become important. During the 2016 survey, the major diseases that were threatening papaya production were anthracnose (*C. gloeosporioides*), black spot (*A. caricae*), powdery mildew (*O. caricae*) and *Phytophthora (P. palmivora)*.

The survey results indicated that prevalence of papaya anthracnose was relatively higher at Wonji and Wolda Kelina *Kebeles* than at Melka Woba and Shubi Gamo *Kebeles* (Table 1). Accordingly, 100% anthracnose prevalence was recorded in Wonji and Wolda Kelina *Kebeles* while 93.3% prevalence was recorded in Melka Woba and Shubi Gamo *Kebeles*, respectively.

Table 1. Papaya anthracnose disease in major papaya growing districts of Ethiopia, 2016

District	Kebele	Farm	Prevalence (%)	Incidence (%)		Severity (%)		
				Fruit	Leaf	Fruit	Leaf	
Dugda	Shubi Gamo	F-1	80	40.0	53.3	23.3	28.0	
		F-2	100	33.3	66.7	23.3	26.7	
		F-3	100	40.0	66.7	20.0	20.0	
		Mean	93.3	37.8	62.2	22.2	24.9	
	Wolda Kelina	F-1	100	53.3	66.7	36.0	28.0	
		F-2	100	53.3	66.7	21.3	34.7	
		F-3	100	40.0	66.7	22.7	26.7	
		Mean	100	48.9	66.7	26.7	29.8	
	District Average			96.7	43.4	64.5	24.5	27.4
	Adama	Melka Woba	F-1	100	30.0	66.7	20.0	20.0
F-2			100	40.0	66.7	20.0	23.3	
F-3			80	20.0	46.7	20.0	20.0	
Mean			93.3	30.0	60.0	20.0	21.1	
Wonji		F-1	100	26.7	66.7	24.7	24.7	
		F-2	100	66.7	66.7	20.0	20.0	
		F-3	100	40.0	66.7	22.7	25.3	
		Mean	100	44.4	66.7	22.4	23.3	
District Average			96.7	37.2	63.4	21.2	22.2	
Overall Mean			96.7	40.3	63.9	22.8	24.8	

The disease incidence of *C. gloeosporioides* was recorded from fruits and leaves in Shubi Gamo (37.8% and 62.2%), Wolda Kelina (48.9% and 66.7%), Melka Woba (30% and 60%) and Wonji (44.4% and 66.7%). The severity of anthracnose from papaya fruits and leaves was 22.2% and 24.9% in Shubi Gamo, 26.7% and 29.8% in Wolda Kelina, 20.0% and 21.1% in Melka Woba and 22.4% and 23.3% in Wonji, respectively.

The survey result showed that anthracnose disease and its causal pathogen is re-emerging in the country. Even though the production of papaya is high, the quality of fruits matters most. In all surveyed areas for papaya anthracnose, the incidence and severity infection percentage on the leaves were higher than that of the fruits (Table 1). High incidence on the fruit was recorded at Wolda Kelina *Kebele* (48.9%) and lowest at Melka Woba *Kebele* (30%) and on the leaves the highest record was for Wolda Kelina and Wonji (66.7%) and the lowest at Melka Woba (60%) respectively. The severity for fruit was recorded highest at Wolda Kelina (26.7%) and lowest at Melka Woba (20%). On the leaves, severity was recorded highest at Wolda Kelina (29.8%) and lowest at Melka Woba (21.1%). Over all, Wolda Kelina showed highest anthracnose incidence record for fruit and leaves (48.9%) and (66.7%) and highest severity record for fruit and leaves (26.7%) and (29.8%) respectively. The surveyed location was conducive

to the papaya plant as well for the pathogen. On the other hand, Melka Woba showed less incidence on the fruit and leaves (30%) and (60%) and less severity on the fruit and leaves (20%) and (21.1%), respectively. So, these stated that climatic factors contribute to the deterioration of commercial papaya fruits.

The survey results showed that the disease incidence and severity aggravated on the fruit at maturity and ripening stage, intermediate at the middle fruiting stage and mostly no disease symptom observed at early fruit setting stage and flowering stage. Similarly, on the older leaves high disease incidence and severity was observed but intermediate at the middle part of the leaves and low or none at younger and on newly flushing papaya leaves. Based on the assessment made on papaya anthracnose in the field and identification at MARC pathology lab, it was confirmed that papaya anthracnose (*C. gloeosporioides*) was identified as threats for papaya producers in the surveyed areas of the Central Rift valley of Ethiopia. The quick assessment conducted during the 2021 indicated that in almost all fields, anthracnose was observed as severe.

The result indicated that 64% anthracnose severity was recorded in Malima Bari *kebele* while 12% anthracnose severity was recorded in Bekele GIRRISA, Shubi Gemo and Walda Mekdela *kebeles* of Dugda district (Table 2).

Table 2. Anthracnose disease incidence and severity in ATJK and Dugda districts, 2021.

District	<i>Kebele</i>	Altitude	Anthracnose Disease	
			Severity (%)	Incidence (%)
ATJK	Bochesa	1631	28	100
ATJK	Bochesa	1640	32	100
ATJK	Bochesa	1655	52	100
Dugda	Bekele Girisa	1660	48	100
Dugda	Bekele Girisa	1665	36	100
Dugda	Bekele Girisa	1663	48	100
Dugda	Bekele Girisa	1663	12	60
Dugda	Shubi Gemo	1652	44	100
Dugda	Shubi Gemo	1648	56	100
Dugda	Shubi Gemo	1657	48	100
Dugda	Shubi Gemo	1666	12	60
Dugda	Shubi Gemo	1662	28	100
Dugda	Malima Bari	1628	64	100
Dugda	Malima Bari	1602	48	100
Dugda	Wolda Mekidela	1650	12	60
Dugda	Wolda Mekidela	1648	32	100
Dugda	Wolda Mekidela	1650	36	100
Dugda	Wolda Mekidela	1652	24	100
Dugda	Wolda Mekidela	1671	32	80

Note: ATJK (Adamitulu Jidokombolcha)

### Morphology, culture and spread of papaya anthracnose

During favourable environmental conditions, the pathogen causes total loss and the disease refers to a group of fungal diseases characterized by the development of dark, sunken spots or lesions, often with a raised rim, on affected foliage, stems and fruit (Figure 3). The fungus forms white-brown conidial mass (Figure 4c) that covers the lesion center. Conidia (Figure 5c) are frequently produced in the concentric ring pattern. The infected fruit and senescent leaves

represent important sources of inoculums. The ascospore production in petioles of dried leaves has often been observed, but the importance of the sexual stage of the pathogen is not yet fully understood (Fitzell and Peak, 1984). In this study, the pathogen frequently observed at different crop growth stages as can be seen in figure 4a. If the infected plant is not safely removed from the production orchards, the pathogen easily spread through insect and equipment. The spores of this disease are spread in rainy, humid periods, by rain, splash back, and plant to plant contact.

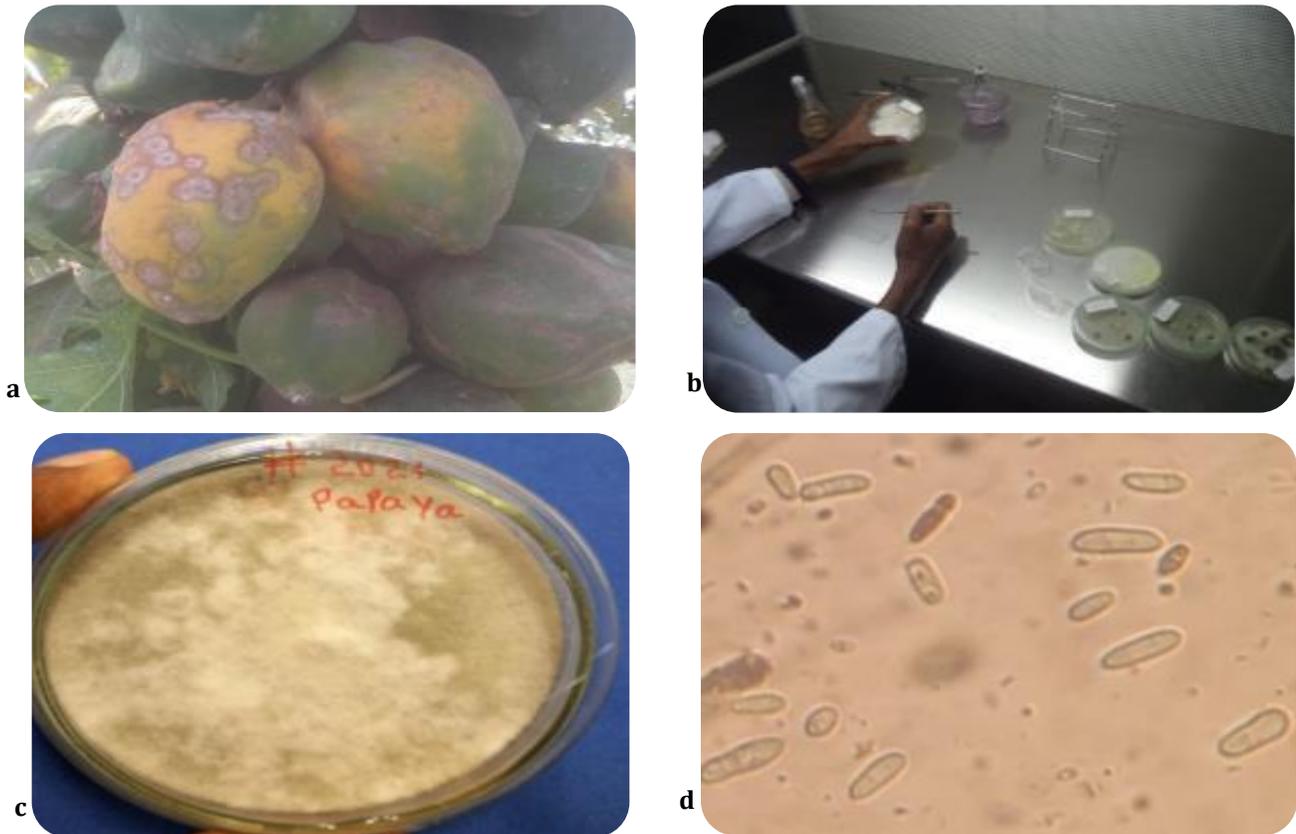


Figure 4. Papaya anthracnose identification based on (a) fruit symptoms: (b) cultural identification in the laboratory: (c) Colony colour on PDA: (d) Spore structure under 40X Microscope.

Spread of the pathogen among fruits and other plant parts and infection of papaya depends heavily on moisture from rainfall. The infective spores are produced on acervuli and are dispersed in splashing water, rain droplets, and wind-blown rain (Singh et al., 2012). In the absence of its host plant, *C. gloeosporioides* can survive as a saprophyte in dead infected papaya tissue or other organic matter (Meah and Khan, 1987). The fungus resumes growth during ripening and causes

the characteristic symptoms. The first symptoms of papaya anthracnose are small round, water soaked and sunken spot on the body of the ripening fruit. *C. gloeosporioides* and *C. capsici* are both pathogens of many economically important hosts. *C. gloeosporioides* is a common pathogen on a variety of tropical crops such as mango, avocado and papaya (Silva-Rojas and Ávila-Quezada, 2011). Two types of conidia were observed: cylindrical and falcate (Figure 5 a). The average length

and width of the conidia were 10.8–15.8  $\mu\text{m}$  and 3–3.02  $\mu\text{m}$ , respectively. Development of dark, sunken spots or

lesions, often with a raised rim, on affected foliage, stems and fruit.

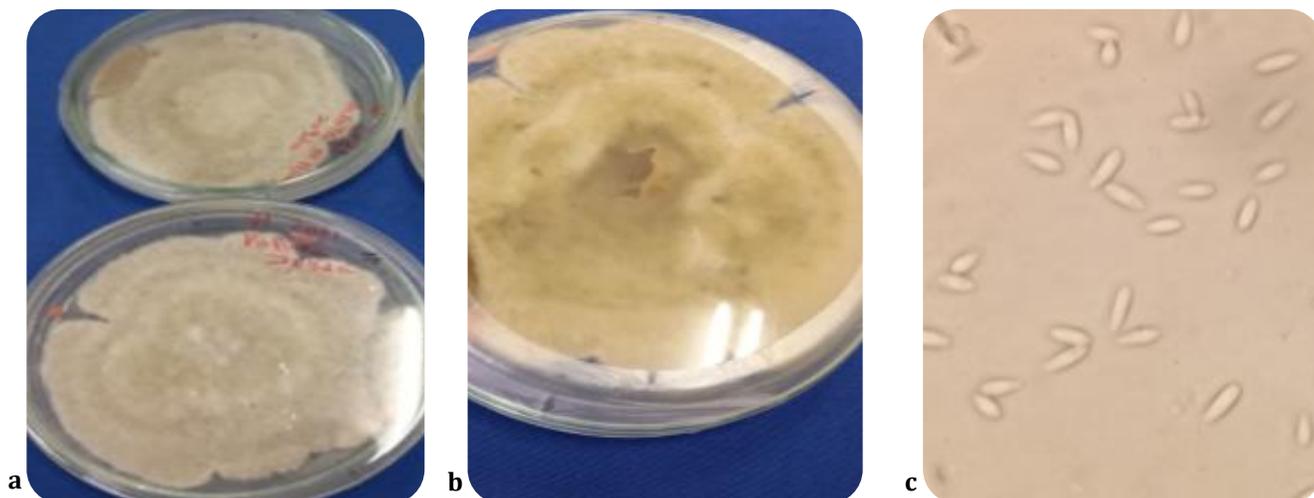


Figure 5. Culture and morphological characters of anthracnose on PDA often with a raised rim and Colony Color (a and b); spore structure of anthracnose (c).

#### CONCLUSION AND RECOMMENDATION

Papaya anthracnose disease was observed in all the surveyed orchards. The disease showed irregular symptoms on papaya leaves and fruits. The symptoms were not clearly visible on unripe fruits at harvest; however, became visible when fruits were ripe. Papaya anthracnose disease prevalence, incidence and severity were also varied across surveyed locations. Disease prevalence ranged from 60 to 100%. Disease incidence on fruits and leaves varied from 20 to 66.7% and 46.7 to 66.7%, respectively. Disease severity ranged from 20 to 36% on fruits and from 20 to 34.7% on papaya leaves. In all the surveyed areas, disease incidence and severity on papaya leaves were higher than that of fruits. Disease incidence and severity on fruits increased with ripening. Papaya anthracnose disease was more severe in areas where there were high humidity, rainfall and temperature. In Ethiopia, months from June to December are therefore conducive for major papaya diseases. Unfortunately, farmers in the surveyed areas were not practicing disease control measures due to knowledge gap on disease identification and possible papaya anthracnose management options. Field sanitation (removal of infected plant parts) at every two weeks interval, hot water treatment of fruits, and synthetic fungicides has been recommended by the research system. Also, four papaya genotypes showed resistance against papaya anthracnose which need to be verified

further.

It is important to give due consideration for major papaya pre- and post-harvest fungal diseases like anthracnose, black spot, powdery mildew and Phytophthora which are seriously affecting papaya plants in Ethiopia. The research community should thoroughly concentrate on resistant breeding to attain and recommend tolerant/resistance papaya variety(ies) against papaya anthracnose which has/have high yield and quality fruits. In future, papaya anthracnose disease control mechanism has to align with IPM system. Training has to be given to papaya producers on major papaya diseases to create awareness on IDM. Disease free and improved planting materials should be provided for the farmers to boost the production and productivity of papaya. Some fungicides should be verified on controlling pre-harvest occurrence of anthracnose as a component of IDM. For reduction of inoculum magnitude sanitation of diseased leaves and fruits, as well as removing dead leaves, fallen fruits, weeds and debris in and around the orchard is one of the components of IPM. Further research should be conducted on postharvest management of anthracnose on papaya fruits. Basic studies on morphological identification should be conducted to characterize isolates. Screening of papaya germplasm should also be intensively conducted to identify tolerant/resistant papaya genotypes. Quantitative and qualitative surveys

are also important to devise management options.

#### **AUTHORS' CONTRIBUTION**

GK and EG designed the study, conducted surveys, performed the experiments, collected and analyzed the data, AD provided technical assistance, EG wrote the manuscript and GK and AD proofread the paper.

#### **CONFLICT OF INTEREST**

The authors declare no conflict of interest

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