BIBLIOGRAPHY

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Adviser: Andres A. Basalong, MSc.

ABSTRACT

The study was conducted primarily to describe the symptoms of *Colletotrichum* species associated to stems/twig, leaves and berries of Arabica coffee and determine the cultural and morphological characteristics of the fungi.

Result of the study showed that stems/twig infected by *Colletotrichum gloesporioides* designated as isolate A showed brown lesion along the length of the stems/twig and can also produce cankers on petioles that causes severe defoliation. Leaves infected by *Colletotrichum coffeanum* designated as isolate B showed symptoms of small and irregular, brown, dark-brown or black spots. The infected part darkens as it ages and characterized generally as large brown leaf blight while berries infected by *Colletotrichum kahawae* designated as isolate C showed symptoms of circular watery, dark sunken lesion that appear on the surface of the pulp.

The colony diameter of *Colletotrichum gloesporioides* in PDA after 7 days was 35.7mm while *Colletotrichum coffeanum* and *Colletotrichum kahawae* grew up to 40.5mm and 32.3mm. *Colletotrichum gloesporioides* exhibited a color of white mycelia in PDA, while *Colletotrichum coffeanum* and *Colletotrichum kahawae* had white to black mycelia. The size of conidia of *Colletotrichum gloesporioides* is 11.3µm x2.19 µm for *Colletotrichum coffeanum* is 11.6µm x2.16µm and *Colletotrichum kahawae* is 11.3µm x1.6µm.

Three species of *Colletotrichum* are associated to Arabica coffee should be validated through pathogenicity test.

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INTRODUCTION

Coffee has the great demand in the world market and it is good for business. Coffee is the most important raw material traded throughout the world behind crude oil, and the most important export article for the nations that grow it. Coffee is the most precious beverage by Filipinos (Daguio, 2000).

Coffee is one of the major products which is favored by people all over the world. It has become the major source of foreign exchange after oil for many developing countries, and it has been sustained in their economies. It supports lives of people who cultivate it.

The universal demand for coffee is due to the invigorating effect and to some extent the medicinal value derived from it. Coffee assists in assimilation and digestion, it reduces the amount of blood circulation, and whopping cough (Cresencia, 1979). The impact of coffee to human health revolves largely around caffeine. Caffeine is the most active stimulant of xanthine alkaloids. It also acts as diuretic, vasodilator, laxative and appetite suppressant. Caffeine is sold commercially in medications, diet pills, as the counter stimulants, and medicines administered to hyperactive children, where ironically, it has a calming effect.

Coffee contains the alkaloid caffeine (1, 3, 7 trimethylxanthine), which is the most active stimulant among the xanthine alkaloids. Caffeine is sold commercially in pain medications, stimulants and it has a calming effect to hyperactive children. Moreover, moderate consumption of coffee has been reported to reduce risk of colon cancer, cirrhosis, gallstones, Parkinson's disease and asthma (Vinson, 2001).

Farmers started growing coffee many years ago but up to the present there is still very low supply due to attack of insect pests and diseases. Anthracnose is one of the major diseases infecting coffee. It is caused by several species or strains of *Colletotrichum*. This pathogen is capable of infecting leaves, berries, stem bark and twigs of the coffee plant. *Colletotrichum coffeanum* invades the main body of the plant but does so without any signs of disease. However, when the plant set fruit, the fungus becomes aggressive and the diseases become apparent. *Coffee coffeanum* affects green or immature coffee berries and the coffee flower at any stage in its development. Infected berries often show dark sunken pits that spread and coalesce to cover the whole berry. As the fungus sporulates, a pale pink crust of conidia appears on the lesion. Depending upon the timing of the infection, the bean can also become infected. Some cases, a milder scab like disease occurs. Losses of up to 75 % of a crop have been reported.

Previous reports of the *Colletotrichum* spp. infecting coffee in some growing areas indicated that species associated to the leaves do not infect coffee berries. Likewise species found infecting berries cannot infect the leaves.

The Study aimed to describe the symptoms of *Colletotrichum* spp. infection on leaves, stems, and berries of Arabica coffee and determine the species of *Colletotrichum* infecting different parts of Arabica coffee through morphological and cultural characterization.

Diseased samples were collected at the Benguet State University Agroforestry Laboratory from October to February 2011 while the activities were conducted at the Department of Plant Pathology laboratory.

REVIEW OF LITERATURE

Coffee plants belong to the Rubiaceae family. The commercially grown species are *Coffee Arabica, Coffee canefora (Robusta), Coffee liberica, and Coffea exelsa* (Maistri and Rena, 2001). However, several species were mentioned by other authors. Economically, the most important species are *Coffea Arabica* and *Coffee canephora*, with Arabica consisting 70% of the coffee production worldwide (Gilman, 1999).

An understanding of the coffee plant, it make- up and how it grows is essential to understanding how to manage the coffee tree. Management, like the environment and the variety planted, has a very big influence on coffee quality and yield. It is necessary to stress the importance of growing techniques (pricking-out in nurseries, mulching, and irrigation and planting layouts) on the distribution and functions of roots (Winston, 2005). Ringor and Versola (1981) revealed that weeds compete for nutrients and water with newly planted coffee plants. They reduce growth to as much as 50 percent. If left uncontrolled in the plantation area, weeds can delay production for as much as one year. Moreover, they are host of fungal diseases and act as pest shelters, host to insects that transmit diseases, restrict the movement of the water through the plantation area, and hinder efficient harvesting in general. Pest and diseases greatly reduce the coffee yields and its quality. Thus measures should be put in place to prevent and or control pest and diseases. Since coffee is perennial crop, their communities were attack by insects. The control of coffee pests and diseases, therefore, require careful selection of the predator (O' Farrill, 2000) and recommends four means of controlling pest and diseases such as cultural that is by means of weed control, pruning, mulching etc, mechanical method which is done by picking and destroying mechanically infested diseased part of the plant

(Anonymous, 2006) and biological method which involves using predators or parasites to destroy the coffee pests and diseases Since cultural method is the cheapest, safest and easily affordable it should be the first control measure to be used. Other measures should be used only when the cultural measure fails.

Importance of Coffee

Coffee is one of the great social drinks of human culture. Coffee assumption has always been associated with work and activities requiring thought and attention (Macanes, 1990).

Svilaas (2004) also reported that coffee is the number one source of antioxidants. Which have been linked to a number of potential health benefits, including protection against heart disease and cancer.

The day may come when coffee will be gold. This must be the reason for renewed interest to promote coffee in the region. Potentials in the coffee industry may transform the Cordillera as the Arabica coffee capital of the country. Market for coffee in the region is not a problem for current local production could not even supply the growing demands of multiplying shops in Baguio City and other areas in the region (Mondonedo, 2008).

Arabica coffee can also be used as a component of watershed conservation and rehabilitation of denuded watershed as a result of over- logging and swidden farming and can be promoted through the adoption of coffee cultivation as Agro-forestry crop (Alladiw, 2008).

General Characterization of Colletotrichum

The fungus *Colletotrichum* produces colorless or hyaline, one-celled, ovoid, cylindrical, oblong or falcate and sometimes curved or dumbbell shaped conidia in acervuli, *Colletotrichum* acervuli are disc-shaped or cushion shape and waxy having dark, long, sterile hair like (Agrios, 1997 and Quimio, 1999).

Colletotrichum coffeanum is an ascomycete, a member of the sac fungi. One of the features of these fungi is that they generate spores, called conidia that can be easily dispersed by the wind and splashing rain. Spread of *C. Coffeanum* is dependent on water but it can also be spread by animals, by coffee pickers being a particular problem. *Colletotrichum* invades plants but does so without any sign of disease. However when the plants set fruit, the fungus becomes aggressive and the disease becomes apparent (Silva, 2006).

Coffee berry disease *Colletotrichum coffeanum* attacks the green tissues at the beginning stage of berry development and often penetrating into interior of the berry destroying the bean. It appears at the berries then move up to the stalk. The berry will have brown patches that seem to have small punctures. Lesions form darker areas of the berry and in time the berry turn ash gray. The disease results in the production of poor berries and difficulty in pulping.

According to Phuong (2010), filamentous fungi of the genus *Colletotrichum* and its teleomorph Glomerella are among the most important plant pathogens world-wide. *Colletotrichum gloesporioides* is so far the most predominant *Colletotrichum* pathogen and can attack about 470 different host genera, Sutton (1980) and Dodd (1992). *Colletotrichum* attacks fruits during development in the field as well as the post harvest.

Infections at the fruiting stage on these crops caused the highest yield losses (Waller, 1992).

Coffee berry disease (CBD) is the anthracnose of green and ripe coffee berries caused by *Colletotrichum kahawae* (Waller and Bridge, 2001). The fungus attacks all parts of the plant including flowers, berries and occasionally, also the branches and leaves. The infected berries mostly shed at an early stage of infection or remain mummified on the stems (Waller, 1992). *Colletotrichum kahawae* also causes "brown blight" symptoms in association with C. *gloesporioides* on ripening berries (Hakiza, 1993). The highest losses are due to premature berry shedding. This disease is the major threat to the production of Arabica coffee in Africa.

Colletotrichum coffeanum is regarded as a saprophyte or weak pathogen of coffee and has been described as being synonyms with *C. gloesporioides* Waller (2007) and Hyde (2009a). This species is very closely related to *C. gloesporioides*, as indicated by their genetic similarities, Screenivasaprasad (1993) and Cannon (2000) . However *C. kahawae* can be distinguished from *C. gloesporioides* by its inability to utilize citric acid and ammonium tartrate as sole carbon sources, by its high pathogenicity on coffee and by molecular tools.

Coffee berry disease has caused severe infection in Kenya and Congo. Varieties of Arabica coffee such as Jamaican Blue Mountain showed infection to these diseases. Control of this disease is choosing the right environment. This disease is a cold temperature disease and may be avoided if temperature never drops to 18.33 ^{o}C. Chemical sprays like Perenox and other copper fungicide in four applications during the year tends to reduce the disease.

MATERIALS AND METHODS

Collection of the Diseased Specimens

Collection of infected coffee plant parts with symptoms exhibiting *Colletotrichum* spp. infection was done on the coffee plantation established at the Benguet State University Agroforestry Project at Bektey, Longlong, La Trinidad Benguet. Infected leaves, stems and berries were randomly collected and were contained separately in plastic bags and labeled for laboratory diagnosis.

Preparation of Culture Medium and Isolation

The Water agar (WA) as culture medium was prepared following the standard procedure. Infected stems, leaves, and berries were cut into 2-3 mm^2 sections which included diseased and healthy tissues, disinfected with 10 % Chlorox for one minute, rinsed with sterile distilled water for 3 times, blot dried in sterile tissue paper, planted in WA and incubated at room temperature. Once fungal growth was observed which usually happens after three days, agar block was cut and transferred into another PDA plate for cultural and morphological characterization of the colonies. Samples of fungal structures were mounted in microscope for characterization of vegetative structures and conidia.

Conidia Measurement

To be able to get the exact measurement of the conidia, a calibrated microscope was used. The ocular micrometer was inserted on the eyepiece of the microscope. While the stage micrometer was placed on the stage of the microscope. Focusing was done on the stage micrometer scale using the low power objective (LPO) and then on the high power objective (HPO) of the microscope. Zero point of the stage micrometer was set to coincide with that of the ocular micrometer. The ocular divisions that cover the space

between the zero and coincident lines were counted. The ocular divisions that cover the space between the zero and coincident lines were counted. Calibration factor (CF) or calibration constant (CC) was calculated using this formula, 10 conidia were been measured by their length and width.

$$CF = \frac{\text{n divisions of stage micrometer}}{\text{n divisions of ocular micrometer}} \times 10 \text{ units / divisions}$$

Data Gathered

1. Diseases symptoms. The appearance of symptoms on infected tissues was characterized.

2. Cultural characteristics of the pathogens. The growths of the pathogen was observed based on the color, and diameter of the colonies observed and the number of days to colony diameter.

3. Morphological characterization of the pathogens. The fungal structures was determined through microscopy based on the appearance and color of the hyphae, mycelium and presence of septation, color of spores in terms of shapes, color and sizes.

RESULTS AND DISCUSSION

Anthracnose Symptoms on Leaves

Anthracnose first appears as small and irregular yellow, brown, dark-brown, or black spots. The spots can expand and merge to cover the whole affected area. The color of the infected part darkens as it ages and characterized generally as large brown leaf blight (Figure 1).

Anthracnose Symptoms on Stem

Symptom on stem or twig appeared as brown lesion along the length of the stem or twig. The disease can also produce cankers on petioles and on stems that causes severe defoliation (Figure 2).

Anthracnose on Coffee Berries

Infected berries exhibited circular watery, dark sunken lesion that appear on the surface of the pulp (Figure 3). The observed symptoms appear to be similar to the scab lesion as describe by Ferreire and Boley (1991).

Figure 1. Coffee twig with Anthracnose *(Colletotrichum gloesporioides)*

Figure 2. Coffee leaf with Anthracnose *(Colletotrichum coffeanum)*

Figure 3. Coffee berries with Anthracnose *(Colletotricum kahawae)*

Cultural Characteristics of *Colletotrichum* Isolates

Colony size. Isolates differed in the diameter of their colony growth in culture media within 1 week (Table 1). *Colletotrichum coffeanum* attained the largest colony diameter of 40.5 mm, followed by *Colletotrichum gloesporioides* at 35.7 mm and the least was isolate *Colletotrichum kahawae* at 32.3mm. Based on the size of colony, *Colletotrichum coffeanum* obtained from infected leaves was fast growing compared to the other isolates.

Colony color. The colony color of *Colletotrichum coffeanum* and *Colletotrichum kahawae* were white to black (Figure 5 and 6) while *Colletotrichum gloesporioides* was white (Figure 4).

Rate of growth. *Colletotrichum coffeanum* was observed to be fast growth as compared to *Colletotrichum gloesporioides* and *Colletotrichum kahawae* which were observed to be moderately fast (Table 1).These observed growth characteristics conforms to their difference in colony size in one week culture.

Morphological Characteristics of *Colletotrichum* Isolates

Table 2 summarizes the morphological characteristics of the *Colletotrichum* spp. isolates.

Mycelia. The mycelia of *Colletotrichum gloesporioides* were observed to be hyaline, septated, and large- walled (Figure 7). Mycelia of *Colletotrichum coffeanum* were hyaline branched, septated, and elongated (Figure 8) while mycelia of *Colletotrichum kahawae* (Figure 9) were slender, septated and hyaline.

Table 1. Cultural characterization of one to four week old isolates of *Colletotrichum* spp. at temperature range of 24- 26 ^{o}C on PDA

CRITERIA	*COLLETOTRICHUM* SPECIES		
	C. gloesporioides	*C. coffeanum*	*C. kahawae*
Colony diameter (mm)	35.7 mm	40.5 mm	32.3 mm
Colony color	white	white to black	white to black
Rate Growth in one week	moderately fast	fast growing	moderately fast

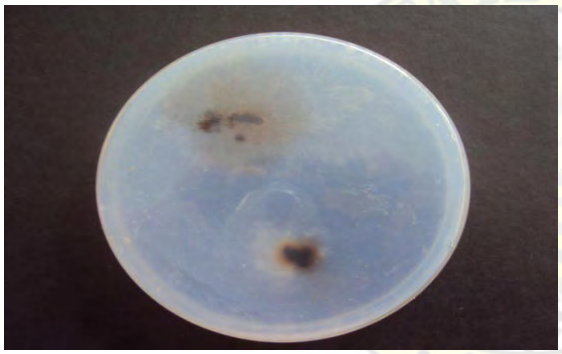


Figure 4. One week old culture of *Colletotrichum gloesporioides* in PDA

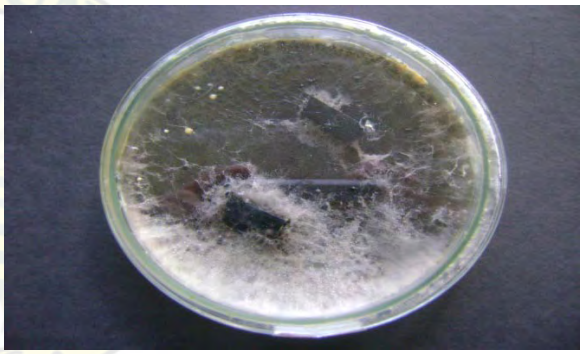
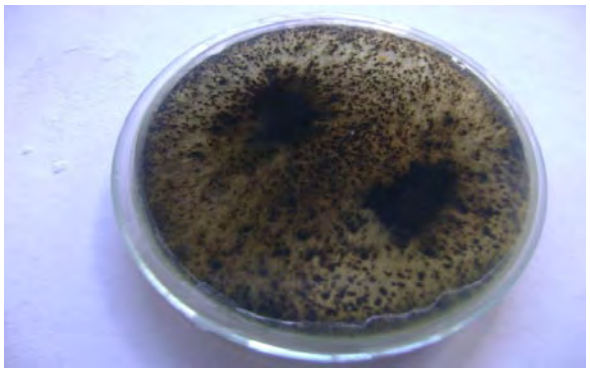


Figure 5. One month old culture of *Colletotrichum coffeanum* in PDA



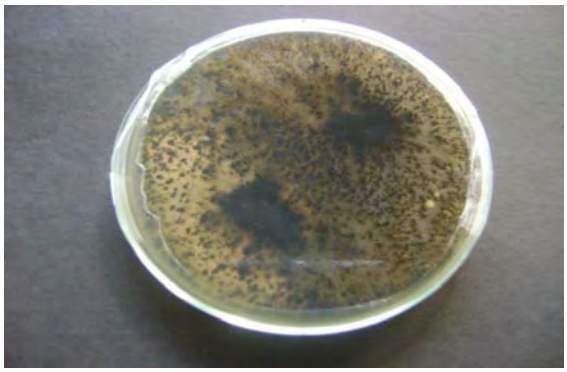


Figure 6. One month old culture of *Colletotrichum kahawae* in PDA

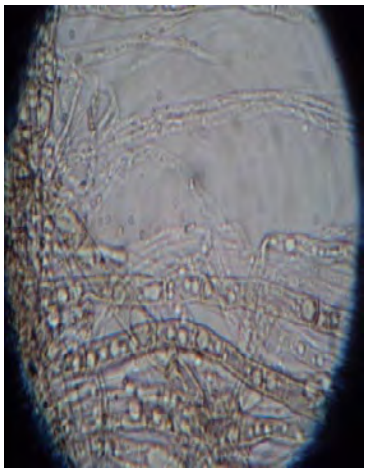


Figure 7. Mycelia of one week old culture of *C. gloesporioides* (400X)

Figure 8. Mycelia of one week old culture of *C. coffeanum (*400 X)

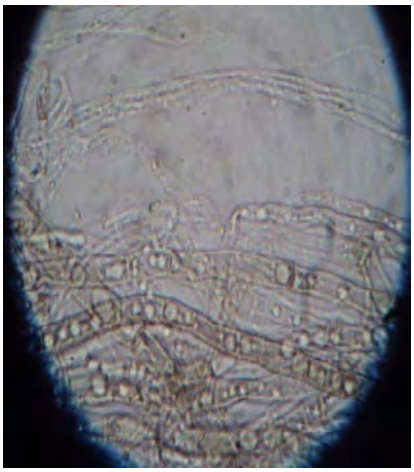


Figure 9. Mycelia of one week old culture of *C. kahawae* (400X*)*

Table 2. Morphological characterization of one to four week old isolates of *Colletotrichum* spp. at temperature range of 24- 26 ^{o}C on PDA

CRITERIA	*COLLETOTRICHUM* SPECIES		
	C. gloesporioides	*C. coffeanum*	*C. kahawae*
Presence of septa on Mycelia	Present	Present	Present
Shape of conidia	Elliptic	Oblong	Cylindrical
Color of conidia	Hyaline	Hyaline	Hyaline
Size of conidia	11.3 μm x 2.19 μm	11.6 μm x 2.16 μm	11.3μm x 1.6 μm

Conidia. Shape of conidia varied between isolates (Table 2). Conidia of *Colletotrichum gloesporioides* was elliptic and oblong, while *Colletotrichum coffeanum*, cylindrical and for *Colletotrichum kahawae*, conidia color were observed similar to all the isolates as hyaline. The elliptic spore and shape observed conforms with the findings of Farreira and Boley (1991) that the spores of coffee berry disease vary from oval to elliptic to some what irregularly clavate. In terms of the conidia size, isolate A has a size of 11.3 µm and a width of 2.19 µm, isolate B is 11.6 µm x 2.16 µm (Figure 10 and 11) and Isolate C 11. 3 µm in length and width of 1.6 µm (Figure 12).

Figure 10. Conidia of isolate *C. gloesporioides* from one week old culture (100X)

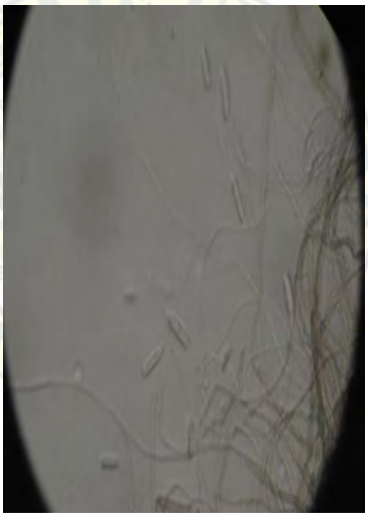


Figure 11. Conidia of isolate *C. Coffeanum* from from one week old old culture (100X)

Figure 12. Conidia of isolate *C. kahawae* from one week old culture (100X)

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted from October to February 2011 at Benguet State University Agroforestry Project. The study found out that there were three different *Colletotrichum* spp. infecting Arabica coffee. Based on their cultural and morphological characteristics the *Colletotrichum* spp. Possess a characteristics similar to that of *Colletotrichum gloesporioides, Colletotrichum coffeanum,* and *Colletotrichum kahawae*. There were cases where two or three types of conidia appeared from a single lesions and in the isolation process.

Conclusion

Based on the finding of the study, it could be concluded that there are three different *Colletotrichum* spp. associated to Arabica coffee. Isolate A which is close to *Colletotrichum gloesporioides* is mounted from the (leaf), isolate B *Colletotrichum coffeanum* from the (twig) and isolate C *Colletotrichum kahawae* from (berries). All the isolates differed in their cultural and morphological characteristics. However, specificity of these isolates in infecting leaves, twigs/stems, and berries were not ascertained.

Recommendation

It is recommended that Pathogenicity test will be done to update earlier identification of the isolates. Pathogenicity shall be done to determine their specificity to the leaves, twigs/stems, and berries of coffee as observed in their result and claimed by previous studies.

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