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Influence of Media and Temperature on the Growh of *Colletotrichum Spp* Isolated from Avocados

Wamala Joseph Ronald[1]

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DECLARATION

I Wamala Joseph Ronald hereby declare that this research report titled "Influence of media and temperature on the growth of *Colletotricum spp* isolated from Avocado" is entirely my original work and it has never been submitted to any institution of higher learning for the award of a Degree. Furthermore, all sources of information used in this research report have been cited and referenced. I take full responsibility for the integrity of the research work presented in this report and assert that it has not been submitted, in part or in whole, for any other academic evaluation or publication.

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APPROVAL

This research report has been submitted to the Faculty of Agriculture and Environmentwith my approval as a Supervisor.

Signature…… Date……

Geofrey Kawube, PhD

Senior Lecturer, Department of

Faculty of Agriculture and Environment,

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DEDICATION

I dedicate this report to my late parents Mr. Musuuza Leonard and Mrs. Nakalinzi Jane for the commendable work of bringing me into this world. The moral and life principles you instilled in me have brought me this far. My brothers and sisters, relatives, friends and all other persons who have supported me throughout my academic journey

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LIST OF ABBREVIATIONS AND ACRONYMS

PDA:	Potato Dextrose Agar
MEA:	Malt Extract Agar
RBA:	Rose Bengal Agar
RT:	Room Temperature
FAO:	Food and Agriculture Organisation
MAAIF:	Ministry of Agriculture, Animal Industry and Fisheries
PDM:	Parish Development Model
NaOH:	Sodium hydroxide
HCl:	Hydrochloric acid
ANOVA:	Analysis of Variance
LSD:	Least Significant Difference
CRD:	Completely Randomized Design

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ABSTRACT

Colletotricum spp are of a significant concern in the Avocado industry in Uganda due to their ability to cause Anthracnose which results substantial pre- and post-harvest crop losses. In this study, the influence of media and temperature on the growth of Colletotricum spp isolated from avocado was investigated. The aim of the study was to determine the best media and optimum temperature for the growth of Colletotricum spp. The study was done using three different solid media; Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Rose Bengal Agar (RBA) and six different temperatures. All treatments were designed in a CRD with three replications and data on mycelial radial growth was collected daily for 7 days. Experimental data was analysed using ANOVA in Genstat Version 13 and mean values were separated by LSD at α = 0.05% level of significance. Results revealed that the growth of Colletotricum spp was significantly (P<0.001) influenced by both media and temperature. The highest radial mycelial growth was observed on PDA (64.27mm) followed by MEA (58.30mm) and RBA (34.74mm) after 7 days. Temperature plays an important role in infection and disease development. Best growth of Colletotrichum spp was recorded at 28°C (65.44mm) after 7 days followed by RT°C (58.41mm), 25°C (53.46mm), 30°C (52.72mm), 20°C (45.19mm) and 15°C(39.39mm). The study provides valuable information about the growth patterns of Colletotrichum spp and can serve as a foundation for further investigation into the biology and etiology of Colletotrichum spp attacking avocados in Uganda.

Keywords*: Colletotrichum spp, Anthracnose, solid media, temperature, Uganda.*

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CHAPTER ONE
INTRODUCTION

Fruits are increasingly becoming important worldwide, with the global aggregate production of major tropical fruits estimated at approximately 100 million tonnes in 2018 (Altendorf, 2019). The major fruit producing countries are in Asia and in Pacific region, followed by Latin America, the Caribbean, and Africa (OECD/FAO,2019). In Africa, the total land area under tropical fruit production is estimated at 40,226 ha, which represents 18% of global organic land for tropical fruit production. The main fruits produced in Africa are; pineapple, mango, banana, tomatoes and avocado among others (FAO,2022).

The government of Uganda has stepped up efforts to modernise the agricultural sector through increased productivity and consequently commercialisation (MAAIF and MFEPD, 2000) of non-traditional crops to increase its export earnings. Avocado (*Persea americana)* is one of the crops being promoted to achieve this goal. It is a native crop of tropical America, particularly Mexico and Central America. Its first cultivation was recorded by the early Spanish explorers from Mexico to Peru. The crop was introduced in Zanzibar in 1892 (Juma *et al*., 2019). In Uganda, farmers exported about 4 tonnes of avocado in 2013, but production has expanded so greatly that exports reached a record high of 469 tonnes in 2020 and in 2021, avocado exports totaled up $7 billion (UN Comtrade). Although the crop is widely grown in Uganda, the date of its introduction in the country is not well documented.

A. Importance of Avocado

Avocado is consumed as fresh, in sandwich filling or in salads. It can also be used in ice creams and milk shakes. Avocado is popular because of its high nutritive value; the pulp is rich in proteins (up to 4%) and fat (up to 30%), but low in carbohydrates. The fat part of avocado is widely used in the preparation of cosmetics. Furthermore, Avocados have the highest energy value (245 cal/100 g) of any fruit and it is also rich in several vitamins (Vitamis A, Ascorbic acid, Niacin, Riboflavin and Thaimine) and minerals such as Calcium, Chlorine, Copper, Magnesium, Phosphorus, Sulphur and Sordium (Saurindra, 1999). Additionally, consumption of avocados supplies dietary fiber, which is linked to lower incidence of cardiovascular disease and obesity (Ashande,2020; Ngbolua et al., 2019). Furthermore, it has also been reported that three antifungal compounds have been isolated from the peel extracts of immature fruits of the Green cultivar. These can be an alternative approaches in the management of fungal diseases. The most interesting avocado healthy content is its monounsaturated fatty acids that are potential to decreasing the risk of coronary heart disease, cataracts, diabetes, benign prostatic hypertrophy, prostate and other cancers and macular degeneration (Eyres et al., 2001; Kawagishi et al., 2001; Birkbeck, 2002). It is also a source of phytochemicals that function as antioxidants, phytoestrogens and anti-inflammatory agents and through other protective mechanisms (Ajila & Rao, 2013; Slavin & Lloyd, 2012; Pogonici & Butnariu, 2022). Avocado fruit is mostly farmed for the fresh market, although the pharma, cosmetics and vegetable oil industries are demanding more of it (Kimaru, 2018). Avocado seed oil can be used to produce biodiesel through a process of trans-esterification. It is also a better alternative for biodiesel production when compared to waste cooking oil since it requires no further conversions for changing fatty acids to esters. The avocado seed is rich in starch and thus can be used as suitable raw-material for bioethanol, pigment and starch production. Due to its high starch, the fruit is a potential raw material for the production of bioplastics as well as a substrate for manufacture of bacterial culture media as opposed to potatoes, cereals, and cassava that double as staple food. Avocado seeds can be a source of antioxidants relevant which prevent enzymatic browning to increase product shelf life (Baidhe, E., Kiggundu, N. & Banadda, N., 2020). Avocados play a very important role in the economies of a household; it is a source of income to those who grow and sale it, the traders, especially the women, who sale it in the markets (Kitagawa, 1994).

B. Avocado Production Constraints in Uganda

In Uganda, the yields of avocados are still very low when compared to other global tropical fruit producing giants. The low yields are caused by several abiotic and biotic factors (Ddamulira *et al*., 2019). These range from lack of clean planting materials, poor agronomic practices to price fluctuations which lead to low harvest and resulting to food insecurity and low income for farmers. Biotic factors such as pests like the fruit fly (*Bactrocera invadens), Ceratitis cosyra*, *Ceratitis rosa* and *Ceratitis capitata* exist as well as the false codling moth (*Thaumatotibia leucotreta*). Diseases of the genera *Phytophthora*, *Ralstonia*, *Xanthomonas*, *Fusarium* as well as *Colletotricum spp* have posed serious threats(FAO, 2017).

Diseases and pests pose the greatest challenge to Avocado production, with the estimated avocado losses of : 10-20% (pre-harvest); 20-30% (post-harvest); and up to 100% for perishable crops and export crops (OECD/FAO,2016;FAO,2017;Viljoen et al., 2017). Among all the diseases attacking tropical fruits, Anthracnose is the major disease limiting Avocado production worldover. The disease is caused by many fungal strains in the genus *Colletotrichum*, with the most dominant being *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. (Giblin, 2007; FAO. 2017; Zakaria, 2021). The pathogen attacks avocado at all stages of its value chain from production to stores (Sharma *et al*.,2017). The symptoms are seen on the leaves, twigs, flowers and fruits.

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Black or dark brown sunken lesions containing conidial masses form on the surface of diseased fruits. Larger lesions can form when smaller lesions combine. Consumers find these surface-level black or dark brown lesions unappealing, which greatly lowers the market value of such fruits (Zakaria, 2021). The fruits rot so quickly after harvest due to anthracnose rendering marketable fruits unattractive and worthless (Zakaria, 2021). Affected fruits cannot be sold or exported since they don't meet the required quality standards (Belder,2012), resulting into huge losses to the farmers and the government (Kimaru, 2018). This disease has made tropical fruit production non-attractive to both farmers and home gardeners (Kiaya, 2014). Furthermore, this has impaired the export of avocados to European markets because of failure by the exporter to meet the phytosanitary standards set by the importing countries.

Therefore, if the population is to benefit from production of avocado in Uganda. There is an urgent need to control the disease. This will require understanding the cause, spread and survival of the causal entity.

C. Statement of the Problem

Avocado production and marketing in Uganda is being constrained by Anthracnose disease, causing serious economic and nutritional losses (Katongole, 2023). The disease is caused by fungal pathogen in the genus *Colletotricum*, which is so diverse with several strains. This makes understanding its epidemiology complex. Management of any disease requires in-depth study of its biology and etiology, which require culturing and isolation of pure cultures in the laboratory. However, colony characteristics and growth of *Colletotricum spp* varies with media on which it is grown and there is no universal media upon which all fungi can grow. Furthermore, temperature plays a big role in initiation and growth of fungi. Different fungal species and isolates of Colletotrichum genera behave differently in terms of growth and sporulation under different temperatures. Currently, there is lack of information on the cultural requirements for the growth of *Colletotricum spp*. This study aims at determining the most suitable media and temperature for the growth of *Colletotricum spp*. isolated from Avocado. Results from this study will be a basis of further investigation into the biology and etiology of *Colletotricum spp* attacking avocados in Uganda.

D. Research Objectives

- *General Objective*

To assess the influence of media and temperature on the growth of *Colletotricum spp* isolated from avocado

- *Specific Objectives*

- To determine the effect of different media on the growth of *Colletotricum spp*
- To determine the effect of different temperature on the growth of *Colletotricum spp*

- *Research Hypotheses*

- The growth *Colletotricum spp* varies from culture medium to another
- All temperatures have different effects on the growth of *Colletotricum spp*

E. Justification

This investigation into the effect of media and temperature helps to understand the optimum temperature and media for the growth of the *Colletotricum spp*. This information is important for researchers who may wish to investigate further the strains causing Anthracnose disease. It is also important in investigating the distribution of the pathogen in the country. These results of the study also have implications for development of management strategies against Anthracnose disease attacking Avocados in Uganda.

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CHAPTER TWO
LITERATURE REVIEW

A. Colletotrichum spp as plant Pathogens and agents of Postharvest Disease

Colletotrichum spp also known by its sexual stage name *Glomerella*, is one of the most commercially significant genera of fungi (Dickman, 2000). It causes anthracnose disease, which affects a variety of hosts, particularly tropical and subtropical crops as well as fruit trees, causing a lot of yield losses. At any stage, the stems, leaves, flowers, and fruits of plants might be infected by Colletotrichum spp infections. The blackening of tropical fruits, notably bananas and mangoes in fruit bowls, is a symptom of Colletotrichum spp anthracnose that is well-known to many people(Tang *et al*., 2005)

Various *Colletotrichum spp* or biotypes have the potential to combine to generate an infection complex in a single host (Hassan *et al*., 2018). The two species most frequently linked to fruit anthracnose, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, are widely dispersed and infect a variety of subtropical and tropical crops, including bananas (*Musa spp*), papaya (*Carica papaya*), mangoes (*Mangifera indica*), avocados (*Persea americana*) and passion fruit (*Passiflora edulis*)(Zakaria, 2021). Other hosts of *Colletotrichum spp* include woody and herbaceous crops, ornamentals, fruits and conifers. Among the crops where *Colletotrichum acutatum* infections are commercially significant include almond, citrus, lupin, olive, and strawberry. The primary characteristics that set *Colletotrichum acutatum* apart from other Colletotrichum spp were its propensity for producing ellipsoidal, fusiform conidia that were either directly carried in the mycelium or formed in brilliant orange masses. For some strains, a pink to red chromogenic colony shape is frequently seen. The grey type isolates exhibit more varied spore morphology than those of the pink to red type, according to a recent re-examination of the holotype and paratype materials (Freeman *et al*., 1998)

The most common species producing anthracnose disease in nurseries, especially in tropical and subtropical areas, is *Colletotrichum gloeosporioides*. It has a fairly broad variety of hosts, yet occasionally host-specific strains might appear that only infect a certain type of plant host (or only cause very minor symptoms in other "non" hosts). But frequently, a *Colletotrichum gloeosporioides* isolate from one host plant species will also be able to cause anthracnose in another host plant species(Sanders & Korsten, 2003).

Anthracnose appears primarily as water-soaked spots that become sunken, turn brown to black, and enlarge to 5 centimeters or more in diameter. Pinkish-orange areas are formed by the conidial masses that cover the lesion center and these lesions are referred to as "chocolate spots". As the fruit ripens, these spots rapidly enlarge up to 20 mm in diameter, to form the characteristic circular sunken lesions. Infected fruit is of much reduced quality and much of it becomes worthless (Sultan *et al*., 2017).

B. Cultural Characteristics of Colletotrichum spp on growth Media

In terms of morpho-cultural characteristics, there is generally substantial variability among the fungal species, particularly between those from the genus *Colletotrichum spp*. This behavior is typically brought on by environmental factors, especially those pertaining to the various media types, ambient temperature and brightness (Maia & Moore, 2011). Even when employing sub-colonies that originated from the same colony, certain fungal species exhibit cultures with features that are significantly distinct from the original.

For example, Kimaru *et al*.,(2018) reported that after being inoculated, *Colletotrichum gloeosporioides* isolates extracted from Avocado expanded quickly on the PDA medium, filling the whole area of the Petri dish in 10–12 days. The isolates' mycelial colors on the upper side of the culture ranged from white grey to whitish cream to greyish pink. The bottom cultures also had creamish grey, greyish orange, and grey. Radial diameter of all the isolates ranged from 0.3 to 0.93 cm and 2.37 to 4.5 cm. Mycelia with a cottony texture were found in 24 isolates, while those with a velvety structure were found in 22 isolates.

C. Impact of Anthracnose Disease on the Different crops' Production

- *Anthracnose disease on cereals*

Sorghum anthracnose caused by *Colletotrichum sublineolum* is considered to be one of the most devastating diseases affecting cultivated sorghum (Sserumaga *et al*., 2013). Severe losses in many parts of the world have been attributed to high levels of incidence and severity of this disease due to conducive environments for proliferation of the pathogen. Estimates of yield losses from this disease in Uganda do not exist, but losses in excess of 50% have been reported on susceptible sorghum cultivars for anthracnose (Thomas *et al*., 1996). The high severity and incidence of anthracnose suggest that sorghum anthracnose is a major threat to production in virtually all agro-ecologies of Uganda (Sserumaga *et al*., 2013). *Colletotrichum graminicola* is the causal agent of maize (*Zea mays*) anthracnose. The most common symptoms are leaf blight, top dieback and stalk rot although it can infect all parts of the plant.(Baroncelli & Thon, 2019).

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- *Anthracnose disease on root tubers*

One of the most challenging and destructive diseases, causing heavy losses in yam is Anthracnose. It is seen as an important pathogen component of the anthracnose disease complex (Okon *et al*., 2022). Anthracnose has been implicated in yam tuber yield losses ranging from 50 to 90% under favorable conditions for pathogen infection, establishment, and disease development (Okon *et al*., 2022; Abang *et al*., 2003). Infection and disease symptoms are concentrated on leaves, though yam petioles, stems, and tubers are also known to be infected (Okon *et al*., 2022). Cassava production is strongly limited by many diseases where cassava anthracnose disease (CAD), caused by *Colletotrichum spp*., is considered one of the most destructive cassava leaf diseases especially in main cassava producing countries worldwide. CAD is characterized by cankers on stems, branches, fruits, leaf spots, and diebacks on aerial parts of diseased plants (Alves *et al*., 2020)

- *Colletotrichum spp in tropical fruits*

The fungus genus *Colletotrichum spp* are the primary culprits of anthracnose in tropical fruit crops. While the infection is observed from field to storage, it is the postharvest or ripening stages that result in the greatest economic losses. Anthracnose lowers the quality and marketability of fruit by causing black to dark brown deep lesions to grow on the fruit surface. Mango, papaya, banana, avocado, guava, and dragon fruit are among the tropical fruit crops that are most frequently susceptible to anthracnose; these are important exports to many developing nations (Zakaria, 2021). S. K. Kimaru et al. (2020) reported that over 60% of avocado produced in Kenya cannot be marketed because of damage and low quality of fruits associated with anthracnose disease. Anthracnose is also the most important postharvest disease limiting shelf life and export of fresh mangoes fruits where it produces rounds with regular or irregular contours black spots(Kouakou, 2020). This disease is most important and prevalent in all mango growing regions (Lakshmi et al., 2011). Anthracnose disease mainly attacks inflorescences and fruit bodies (both during ripening and post-harvest) and occasionally affects young leaves. Under wet or very humid conditions, the incidence of the disease on mango can reach almost 100 % (Arauz, 2020).

D. Effect of Different Cultural media on the Growth of Colletotrichum spp

According to Leharwan *et al*.(2018), the fungus produces good aerial mycelium on different synthetic and semi-synthetic media as reported where they obtained maximum growth and sporulation of *Colletotrichum gloeosporioides* in Richard's synthetic medium followed by Potato dextrose and Czapek's dox agar media and poor growth without sporulation was recorded in Oatmeal. Lokare & Fatima (2021) in their study reported that among all the solid media tested, maximum mycelial growth was observed in Potato Dextrose Agar Medium (89mm) and in Czapek's Dox Agar Medium (89 mm), which was remarkably greater than the other culture media. It was followed by Sabouraud's Agar Medium (83mm), Mango Leaf Extract Agar Medium (83mm) and Asthana & Hawker's Media (85mm). All growth was recorded at room temperature (24ºC). Additionally, Abera (2016) revealed that the colony characters and growth of *Colletotrichum gloeosporioides* varied on different media which was due to the variation in the nutritional requirement of the fungus. Fungi secure food and energy from the substrate upon which they live in the culture where essential elements and compounds in the medium which are required for their growth and other life process (Lemessa *et al*., 2015). Leharwan *et al*. (2018) reported that among all used media fungal pathogen from mango plant (*Colletotrichum gloeosporioides*) showed better growth on Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Sabouraud's Agar Medium (SBA), Mango Leaf Extract Agar (MLE), Asthana and Hawcker's Medium (AHM), V8 juice and Malt Extract Agar (MEA), respectively. Not all the media good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can growth. Sultan *et al*.(2017) also reported that the growth of *Colletotrichum capsici* was fast in Potato Dextrose Agar medium (PDA) resulting full plate growth (90mm) within 9 days followed by Yeast Extract Potato Dextrose Agar medium (YEPDA), Chilli Extract Agar medium (CEA), Wheat grain Extract Agar medium (WEA) and Malt Extract Agar medium (MEA) which results full plate growth were recorded in 10, 11, 12, and 13 days respectively.

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E. Effect of Different Cultural Temperature on the Growth of Colletotrichum spp

Different weather parameters play an important role in initiation and progression of the disease. Several scholars have reported differing temperatures supporting *Colletotrichum spp* mycelial growth, which makes it difficult to settle for any when culturing the pathogen. For example; Pandey *et al*.(2012) observed that *Colletotrichum spp* pathogen's growth is optimum at 25°C and ceases beyond 35°C. It was also reported that temperature range between 20- 30°C was optimum for the growth and sporulation of *Colletotrichum gloeosporioides* isolated from mango (Leharwan *et al*., 2018). When exposed to various temperature settings, distinct species and isolates within the genus *Colletotrichum spp* behave differently in terms of their growth and sporulation. At 28 ^{0}C and 30 ^{0}C, the mean colony diameter of isolates on solid medium was at its highest, followed by 25 ^{0}C. At 15^{0}C, all of the isolates' growth was halted (Sawant & Borkar, 2014). Sangeetha & Rawal (2010) reported that the maximum mean colony diameter for all the isolates was at 28°C (26.55 mm), followed by 25°C (21.55 mm), which was noticeably greater than other temperatures. The least amount of growth was demonstrated by all isolates at 15°C (5.64 mm). All of the isolates experienced their fastest development between 25 and 28 °C. Isolates of *Colletotrichum gloeosporioides* and *Colletotrichum truncatum* grew best at a temperature between 25 and 27°C. Any isolates incubated above 31°C showed no growth. *Colletotrichum gloeosporioides* isolates grew noticeably more quickly than *Colletotrichum truncatum* isolates. In their study, Thangamani *et al.* (2011) the results revealed that a highest and lowest mean radial growth of 89.41 mm and 37.58 mm for all isolates was found when mycelial growth occurred at 30°C and 15ºC respectively. Sultan et al. (2017) reported that *Colletotrichum spp* varied in its ability to grow under different environmental conditions. However, isolate preferred temperature range of 20°C to 30°C for the growth on PDA media. Therefore, there is need to determine the right temperature and media which supports *Colletotrichum spp* growth on a case by case basis.

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CHAPTER THREE
MATERIALS AND METHODS

A. Study location

The study was conducted in G2 Microbiology Laboratory in the Faculty of Agriculture and Environment at Gulu University Main Campus. The campus is located in Laroo division, Gulu city at latitude 2º 46´45.55´´North and longitude 32º 17´5.38´´East in Northern Uganda. The height above sea level is 1100m. Gulu City is one of the eight districts constituting Acholi sub-region and is the historical homeland of the Acholi ethnic group. Gulu is bordered by Lamwo District to the North, Pader District to the east, Oyam district to the South, Nwoya District to the Southwest and Amuru District to the West.

B. Collection of diseased avocado fruits

Fruit samples (avocado) with symptoms of anthracnose disease were collected from Cuk Dire market, Gulu City. They were separately put in clean polythene bags and carried to the laboratory for fungal isolation and further investigations.

C. Preparation of Culture Medium, isolation of infected tissue and culturing

➢ *Culture media preparation*

In this experiment, 3 different types of culture media were used *viz*. Rose Bengal Agar (RBA), Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA). Twenty eight point five grams of RBA, 50g of MEA and 39g of PDA were separately weighed into glass bottles, to which 1 litre of distilled water was added. These were briefly heated for 15 minutes at 150^0C and stirred well with a magnetic stirrer to allow all the solids to dissolve into the water. The pH of MEA and RBA was above 7 and as such a few drops of 0.1M HCl were added to adjust the pH to 7. The pH of PDA was acidic. Therefore, few drops of 0.1M NaOH were added to adjust the pH to 6. The media were then sterilized in the autoclave at 121^0C for 15 minutes and allowed to cool to 47.5ºC. The different media were aseptically poured into 90 mm petri dishes in a Laminar flow hood and allowed to solidify.

➢ *Isolation of the pathogens*

The Avocado samples were washed under running tap water, followed by rinsing with distilled water for 2 minutes. They were further washed with 70% Ethanol for 30 seconds followed by rinsing in distilled water. Small sections (about 0.5 cm^2) of the avocado were cut aseptically at the edge of the diseased part using a sterile surgical blade.

➢ *Culturing*

The diseased piece of the avocado was introduced on PDA media and incubated at a temperature of 25ºC. The mycelial growth was observed for 5 days. Contaminants were observed. Sub-culturing was done to obtain pure culture for subsequent investigations.

➢ *Experimental set-up*

An experiment was setup in the laboratory with two treatments. These were: temperature at 6 levels; room temperature (RT), 15 ºC, 20ºC, 25ºC, 28ºC and 30ºC and, Media at 3 levels: MEA, PDA and RBA. The experiment was set-up in a complete randomized design and replicated three times. The fungal mycelia of *Colletotrichum spp* in the subculture was picked using an inoculation needle and introduced onto the middle of media in the petri-dishes containing the different media. The petri-dishes were, thereafter, incubated at the various temperatures above. The mycelial growth on each plate was monitored for 6 days at 24 hour interval, starting 2 days post inoculation. On each occasion, the diameter of the mycelial growth on each petri-dish was measured using a ruler.

➢ *Data analysis*

Experimental data was analysed using Analysis of Variance (ANOVA) in Genstat Software Version 13. Mean values among treatments were separated by the Least Significance Difference (LSD) at $\alpha = 0.05\%$ level.

➢ *Ethical consideration*

Data was collected at the requited interval and entered and analysed as it was recorded during the experiment. Where information was obtained from literature, the source was cited appropriately.

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Fig 1 Anthracnose Symptoms on Avocado fruits

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CHAPTER FOUR
RESULTS

A. Effect of Culture Media on the Growth of Colletotrichum spp isolated from Avocado

Culture media significantly (P<0.05) influenced the growth of Colletotrichum spp across the 7 days of observation (Table 1). The highest mean radial colony growth was observed on PDA, followed by MEA and least in RBA. Although, PDA influenced growth more than MEA, the growth influence was not significant for all days except for RBA (**Table 2**, **Figure 2**). A similar trend was consistently observed across temperature regimes (**Figure 3**). The interaction of media and temperature had a significant effect (P=0.018) on the growth of Colletotrichum spp (**Table 1**).

B. Effect of Different Temperature on the Growth of Colletotrichum spp Isolated from Avocado

There was a significant difference (P<0.001) in mycelial growth recorded at different temperature regimes (**Table 1**). The highest mean radial growth of mycelia was observed at 28ºC and RT, followed by 25 ºC and 30 ºC, and least under temperature 20 ºC and 15 ºC. This trend was observed up to the 6th day after which there was no significant difference observed in growth during 7 days of incubation (**Table 3**). The difference in growth was observed for 6 days of incubation after which it ceased. A similar trend was observed across all media (**Table 4**, **Figure 3**).

Table 1 F Statistics for analysis of the effects of media and temperature on the growth of *Colletotrichum spp*

Source of variation	DF	SS	MS	VR	F Pr
Media	2	52647.2	26323.6	96.31	<.001
Temperature	5	23156.0	4631.2	16.94	<.001
Media x Temperature	10	5978.9	597.9	2.19	0.018
Residual	304	83090.1	273.3	304	83090.1
Total	321	164965.6			

Table 2 The Effect of Media on Growth of *Colletotrichum spp* Isolated from Avocado

Media	Colony diameter(mm) during days of incubation post media inoculation (from 2 to 7 days)						
	2	3	4	5	6	7	Means
MEA	32.67	48.41	56.90	64.17	71.00	77.11	58.30
PDA	36.89	52.67	64.94	72.56	77.44	81.11	64.27
RBA	17.17	25.67	31.61	39.50	45.17	49.33	34.74
LSD($_{\alpha=0.05}$)	7.38	8.92	9.93	9.69	8.66	7.22	5.02
C.V(%)	3.80	2.70	1.90	1.00	0.40	1.30	1.30

Table 3 The Effect of Different Temperature Regimes on Mycelial Growth of *Colletotrichum spp*

Temperature (ºC)	Colony diameter(mm) during 2 - 7 days of post media inoculation						
	2	3	4	5	6	7	Mean
15	17.78	26.67	33.89	42.78	52.11	63.11	39.39
20	26.33	33.89	41.78	48.67	56.11	64.33	45.19
25	21.89	41.78	56.56	64.89	67.00	68.67	53.46
28	36.22	59.22	69.33	74.56	76.00	77.33	65.44
30	33.67	43.89	53.67	58.11	62.22	64.78	52.72
RT	37.56	48.08	51.03	63.44	73.78	76.89	58.41
LSD(α=0.05)	11.58	14.35	17.11	17.06	16.91	16.92	8.02
C.V%	3.80	2.70	1.90	1.00	0.40	1.30	1.30

Table 4 Mean Growth (mm) of *Colletotrichum spp* as Influenced by Temperature and Media

Temperature (ºC)	Media			
	MEA	PDA	RBA	Mean
15	43.06	45.17	29.94	39.39
20	50.50	53.28	31.78	45.19
25	60.67	68.44	31.28	53.46
28	74.72	75.22	46.39	65.44
RT	66.06	69.50	39.67	58.41
30	54.78	74.00	29.39	52.72
Mean	58.29	64.26	34.74	52.43
LSD(α = 0.05)	11.55	11.01	10.79	8.02
C.V%	8.20	0.40	0.40	1.30

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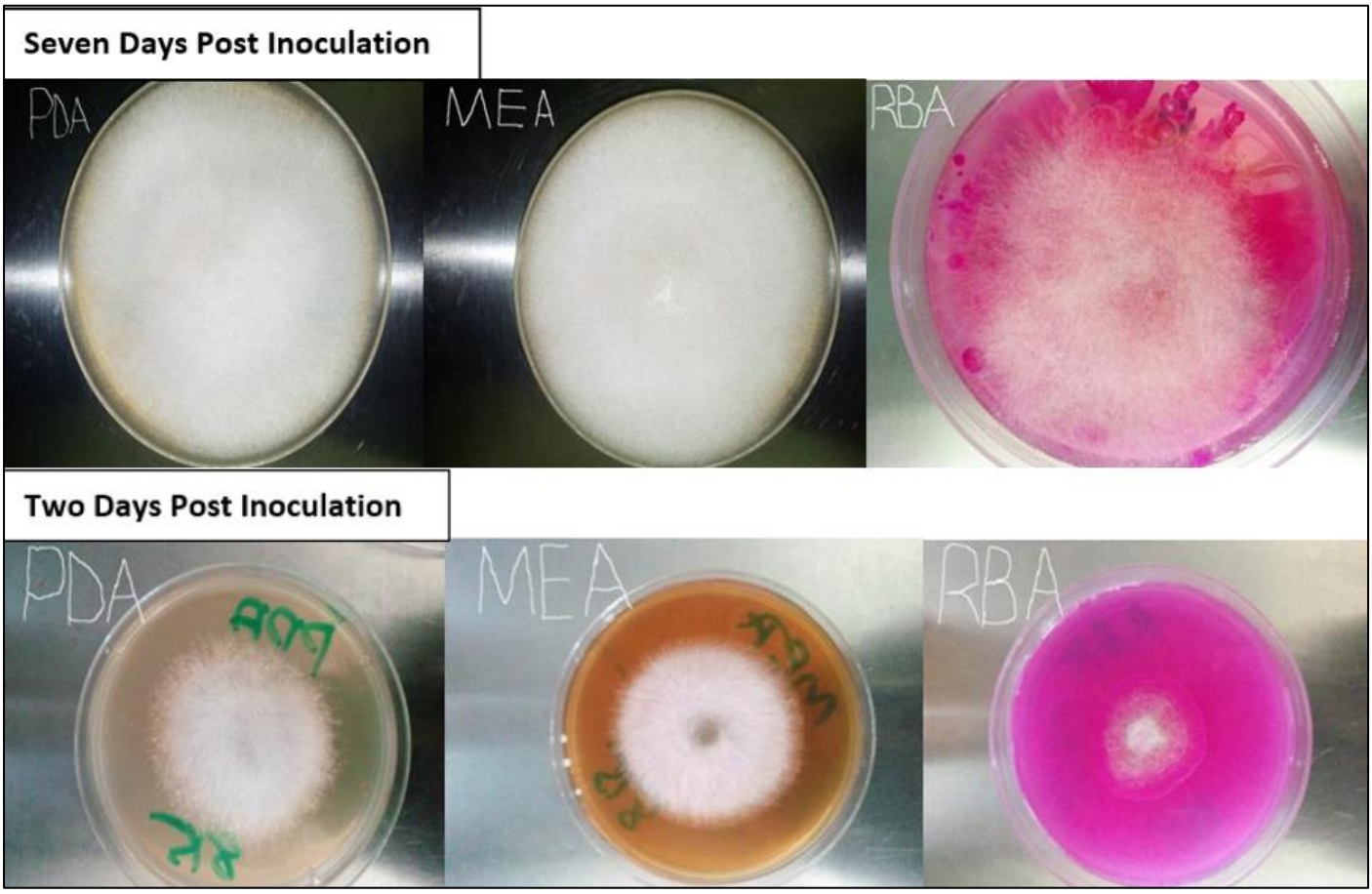

Fig 2 Effect of Different Solid Media on the Growth of *Colletotrichum spp*

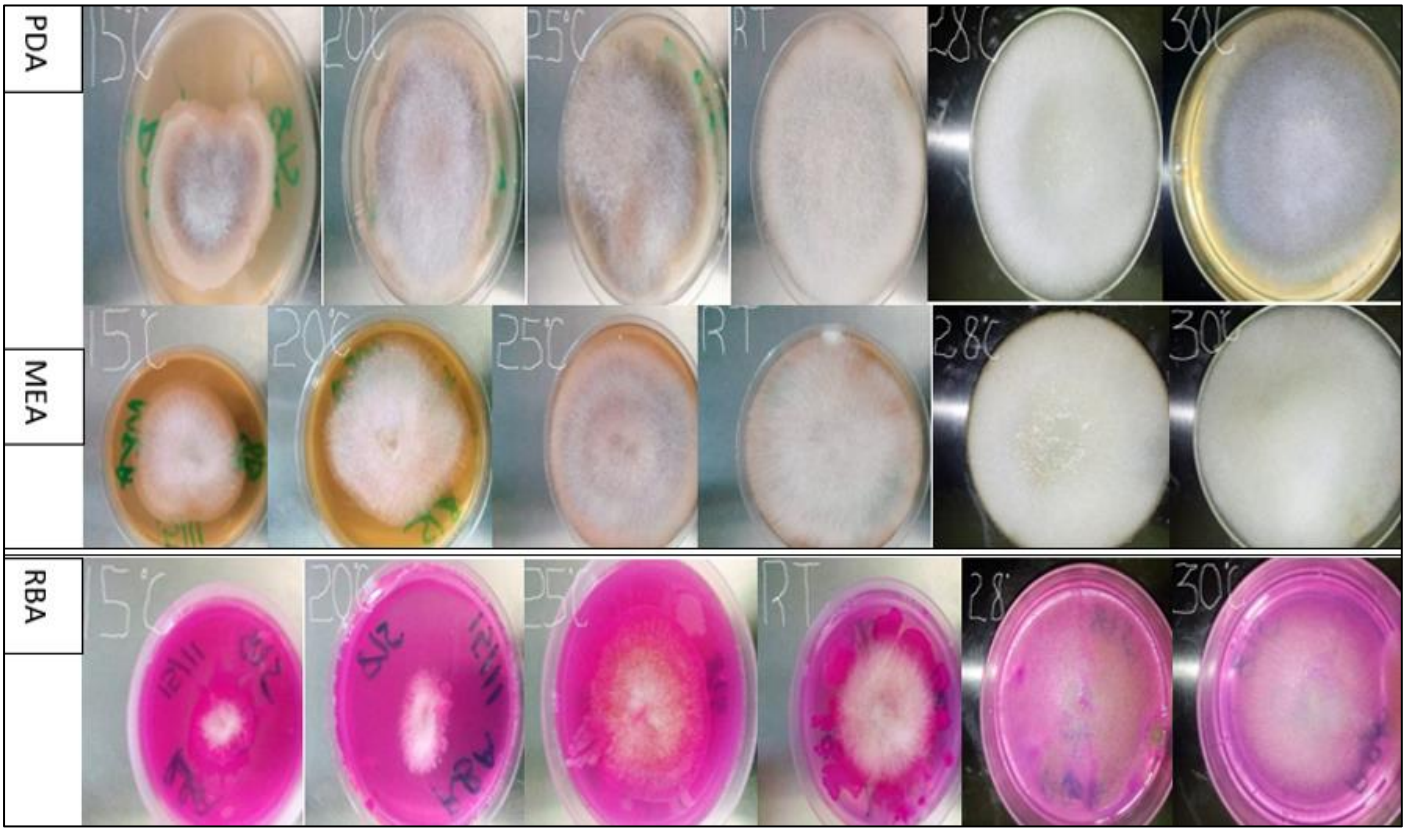

Fig 3 Effect of Different Temperature Regimes on the Growth of *Colletotrichum spp* in Different Solid Media after 7 days of Incubation

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CHAPTER FIVE
DISCUSSION

Although all media supported growth of *Colletotrichum* spp, their difference in growth support gives hope that if best media to support mycelia growth is selected at the right temperature, then culturing *Colletotrichum spp* in the laboratory becomes easy. On that basis, medium RBA, which supported mycelial growth the least, is not suitable for culturing *Colletotrichum spp*. Meanwhile medium PDA which supported the growth of *Colletotrichum spp* most is the best choice for culturing in our G2 Laboratory. However, in its absence MEA can be a good substitute for PDA. Kumar *et al*. (2010) indicated that there is variation in nutrient requirements for the different *Colletotrichum spp* for growth and sporulation. The observed differences in growth of *Colletotrichum spp* on the different media is attributed to the difference in the media nutrient composition - carbohydrates, lipids, proteins and other mineral elements which are needed by the fungi for growth through provision of energy for biosynthesis and cell metabolism (Udhayakumar & Rani, 2010). These results indicate that not all media support *Colletotrichum spp* growth the same way nor is there a universal substrate or artificial medium upon which all fungi can grow because of their difference in nutrient composition (Lokare *et al*., 2021). Therefore, the first hypothesis of this study that the growth *Colletotricum spp* varies from culture medium to another is accepted.

Temperature affects almost every function of the fungi including growth (Godswill *et al*., 2015). The increase in mycelial growth from 25°C-28°C and RT and the decrease thereafter at 30°C could be attributed to increased enzymatic activities which were later lowered due to enzyme denaturation at 30^{o}C. Least mycelial growth recorded at 15°C and 20°C is a result of inactivation and hence reduced enzyme activities at both temperatures. Therefore, the best temperature at which *Colletotrichum spp* can be cultured in G2 laboratory is 28^{o}C. But in the worst case scenario, room temperature is good since the daily average temperature of the laboratory at our institution is slightly above 25^{o}C. From the foregoing, the second hypothesis that all temperatures have different effects on the growth of *Colletotricum spp* is accepted. Reports from several scholars who worked with *Colletotrichum spp*. indicate different optimum temperatures for growth of *Colletotrichum spp*. For instance, Sultan et al. (2017) reported that *Colletotrichum spp* isolates preferred temperature range of 20°C to 30°C for the growth on PDA media. Similarly, Sangeetha & Rawal (2010) reported maximum growth of *Colletotrichum spp* isolates at 28°C while 30°C supported growth of Hassan and Raichur isolates. In another study *Colletotrichum spp isolates* produced maximum radial mycelial growth at 25°C after 6 days (Udhayakumar & Rani, 2010). This highlights the importance of determining the best temperature for growth of the *Colletotrichum spp* on a case by case basis. The positive and significant interaction between media and temperature implies that the best interaction remains that of 28^{0}C and PDA media.

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CHAPTER SIX
CONCLUSION AND RECOMMENDATION

A. Conclusion

In order to culture the fungi in the laboratory, it is necessary to furnish the essential elements and compounds in the medium which are required for their growth and other life process at the right temperature. This study investigated the effect of different media and temperature regimes on the growth of *Colletotrichum spp* isolated from avocado in the G2 laboratory in the Faculty of Agriculture and Environment, Gulu University. The results revealed that *Colletotrichum spp* isolated from avocado can be cultured in the G2 laboratory in the Faculty of Agriculture and Environment, Gulu University. Although several media can support growth of *Colletotrichum spp*, PDA is the best and RBA is the least. This implies that the nutrient composition and form of PDA suits *Colletotrichum spp* growth. In the absence of PDA, MEA can be a good substitute since it fairly supported *Colletotrichum spp* mycelial growth. Optimum temperature is key in supporting growth of an organism. In this study, the highest radial growth of *Colletotrichum spp isolates* were 28°C and RT, followed by 25°C and then 30°C, and least growth was supported by 15ºC and 20ºC. This is true regardless of the media on which the *Colletotrichum spp* is growing.

B. Recommendations

Colletotrichum spp isolated from avocado in the G2 laboratory in the Faculty of Agriculture and environment at Gulu University grows best on PDA at a temperature of 28ºC and at RT. Therefore, for any future work on this pathogen that involves culturing the pathogen, they should use these conditions.

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APPENDIX ONE

➢ *Experimental Data Showing Radial Growth Measured for 6*

Table 5 Experimental Data Showing Radial Growth Measured for 6

TEMPERATURE(°C)	MEDIA	REPLICATES	Day 2	day 3	day 4	day 5	day 6	day 7
RT	MEA	1	48	56	63	74	78	81
RT	MEA	2	46	57	61	73	76	83
RT	MEA	3	45	53	60	71	79	85
RT	PDA	1	48	58	61	73	85	85
RT	PDA	2	50	61	68	74	85	85
RT	PDA	3	51	59	67	73	83	85
RT	RBA	1	18	27	32	47	61	65
RT	RBA	2	12	18	28	42	58	62
RT	RBA	3	20	28	32	44	59	61
15	MEA	1	21	31	38	42	55	68
15	MEA	2	18	30	37	40	52	71
15	MEA	3	24	34	34	46	61	73
15	PDA	1	20	31	43	58	67	75
15	PDA	2	18	21	33	51	64	76
15	PDA	3	15	28	37	48	58	70
15	RBA	1	14	18	26	34	38	45
15	RBA	2	12	21	26	32	36	40
15	RBA	3	18	26	31	34	38	50
25	MEA	1	14	33	52	73	76	79
25	MEA	2	32	51	75	77	79	81
25	MEA	3	31	45	62	74	78	80
25	PDA	1	38	54	77	83	85	85
25	PDA	2	36	60	75	82	83	85
25	PDA	3	4	55	75	85	85	85
25	RBA	1	15	26	31	42	45	47
25	RBA	2	14	28	32	33	34	34
25	RBA	3	13	24	30	35	38	42
30	MEA	1	22	30	32	32	41	50
30	MEA	2	40	50	62	70	78	84
30	MEA	3	38	55	68	70	81	83
30	PDA	1	46	65	80	85	85	85
30	PDA	2	45	56	78	81	85	85
30	PDA	3	55	67	79	85	85	85
30	RBA	1	15	26	31	42	45	47
30	RBA	2	14	28	32	33	34	34
30	RBA	3	13	24	30	35	38	42
28	MEA	1	42	79	85	85	85	85
28	MEA	2	40	68	76	82	85	85
28	MEA	3	41	70	82	85	85	85
28	PDA	1	43	66	81	85	85	85
28	PDA	2	49	70	85	85	85	85
28	PDA	3	44	72	80	84	85	85
28	RBA	1	23	38	48	60	63	65
28	RBA	2	24	40	47	50	54	60
28	RBA	3	20	30	40	55	57	61
20	RBA	1	14	18	21	33	46	50
20	RBA	2	15	17	23	28	37	48
20	RBA	3	20	31	38	42	44	47
20	MEA	1	20	31	45	50	51	56
20	MEA	2	25	36	47	53	71	80
20	MEA	3	41	47	52	58	67	79
20	PDA	1	30	42	47	48	53	62
20	PDA	2	37	43	51	58	64	76
20	PDA	3	35	40	52	68	72	81

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APPENDIX TWO

- *Photos Taken During the Process of Carrying out the Experiment*

Fig 1 Photos Taken During the Process of Carrying out the Experiment