ISOLATION OF FUNGI ASSOCIATED WITH DISEASED LEAF OF GUAVA (*Psidium guajava* L.)

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc.) IN PLANT BIOLOGY AND BIOTECHNOLOGY

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CERTIFICATION

We certify that this research work was carried out by **Roselyn Omotola IKOTUN (Miss)** of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Nigeria.

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Head of Department.

DEDICATION

This work is dedicated to God Almighty for His guidance, help and strength. I also dedicate this work to my family, whose unwavering support and encouragement have been of help in my academic pursuit.

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ABSTRACT

The study of the fungi associated with leaf spot disease of *P. guajava* (guava) was conducted in order to compare the radial mycelial growth of the isolated fungi on both potato dextrose agar (PDA) and corn meal agar (CMA). Fungi associated with the diseased guava leaves were isolated using the direct plating method while the radial mycelial growth of the isolated fungi was done using radial mycelial measurement technique. *Pestalotia psidii* and *L. theobromae* were identified as the fungi associated with the diseased guava leaves. Cultural description shows *P. psidii* to form whitish to grey mycelium with sparse black sporulation on PDA but grows very slowly on CMA with rarely visible mycelial growth on the culture plate. *Lasiodioplodia theobromae* on the other hand developed cottony mycelia that transition from white to black as the colony matures on PDA and produces less whitish to dark, septate mycelia on CMA which barely turns black as the colony matures. The results the radial mycelial growth of *P. psidii* and *L .theobromae* on corn meal agar shows an increase in the average mycelial growth of *P. psidii* from 3.25cm on day 3 to 5.20cm on day 7 while *L. theobromae* was observed to increase from 3.63cm on day 3 to 5.63cm on day 7. On PDA the average mycelial growth of *P. psidii* increases from 5.05cm on day 3 to 8.5cm on day 7 while *L. theobromae* was observed to increase from 3.90cm on day 3 to 7.45cm on day 7. Findings of this study has shown *P. psidii* and *L. theobromae* to be the major fungi associated with leaf spot disease of guava leaves and also shown the growth medium PDA to be more suitable for the *in vitro* radial mycelial growth study of *P. psidii* and *L. theobromae.*

CHAPTER ONE

INTRODUCTION

1.1 Background Study

Guava is a popular fruit enjoyed all over the world. Its exact origin is uncertain, but it's thought to come from tropical areas, especially from places like Mexico and the Caribbean, where it's also called "sand plum." Guavas have been known since the 15th century. Today, they are grown commercially in several countries, including India, Mexico, Hawaii, and Egypt (Amusa *et al.,* 2005)

Guava trees thrive in tropical and subtropical climates, particularly where summers are dry and winters are short. Trees that experience some winter weather usually produce more fruit than those in warm areas. While guava trees can handle both high humidity and dry conditions, young plants are more vulnerable to drought and cold. Additionally, they don't do well in frost. Guava trees can be cultivated at sea level up to about 1,500meters, and some types can even grow well at higher elevations at 2500 m (Mercadante *et al.,* 1999). Guava trees can grow well in many types of soil, from heavy clay to gravel and even limestone. However, the best soil for guavas is well-drained and fertile, with a pH between 4.5 and 9.4. It's also important that the soil has low salt levels and a high water table. To grow guava trees, people usually use seeds, but they can also be propagated through cuttings, grafting, air layering, and budding. In India, air layering and marching were once common methods, but now grafting and budding are preferred because trees planted using the old methods often only last 2 to 3 years. Typically, guava cuttings are planted in July and August

Guava (*Psidium guajava*), a member of the Myrtaceae family, has been cultivated in Nigeria since the early 17th century and is among the most widely consumed fruits in the country (Mishra *et al.,* 2000). Its popularity is largely due to its year-round availability, placing it just after mango, banana, and citrus fruits in importance (Malleswari, 1996). This medium-sized tree can reach heights of about thirty feet and requires less attention compared to banana and citrus plants (Byresh, 2007). Guava is more drought-resistant than many other fruit trees and can endure summer temperatures as high as 46°C (Taiwo, 2005). The plant adapts well to various soil and climatic conditions, thriving throughout Nigeria (Annon, 2005). It can be cultivated in plains, provided that young trees are adequately protected from frost, cold, and wind during their initial two to three years of growth. Additionally, it flourishes in areas with limited rainfall, such as northeastern Nigeria, as long as there are irrigation facilities available.

Guava tree is susceptible to attack by many pathogens mainly fungi aside bacteria, algae and other physiological deficiencies or disorders. Several studies carried out by shown that about 177 pathogen were reported on various parts of the guava tree of which 167 are fungal pathogens, 3 algae, 3 bacteria, 3 nematodes and 1 epiphytes. Thus making guava crops suffer from various fungal diseases such as seed rot, anthracnose, wilt and cercospora spot (Younis *et al.,* 2004; Ismail *et al.,* 2010 and Dwivedi and Neetu, 2012).

Despite all these studies, the optimal growth condition for these fungal pathogens are not well understood. Research has shown that growth medium can significantly impact fungal growth and isolation (Youssef *et al.,* 2015).

1.2 The Guava Tree

The guava tree is a small tree or shrub that grows about 2 to 7meters tall. Its leaves grow opposite each other, are always green, thick, and have short stems. The leaves are irregular in shape, ranging from oval to elliptical, and are about 7 to 15cm long and 3 to 5cm wide. They have visible veins that run from the center out to the edges. The flowers, which are 2 to 5cm wide, grow in clusters near the leaves and have 4 to 5 white petals along with many stamens (Azzolini *et al.*,2005)

Guava is a type of berry that belongs to the myrtle family and the genus *Psidium*. The fruit can be round, oval, or pear-shaped, measuring 5 to 10cm across and weighing between 50 to 200 grams, depending on the variety. The outer layer of the fruit, called the exocarp, is thin and light yellow with a hint of pink. Right beneath it is the mesocarp, which is the thick, fleshy part that can be white, yellowish, or dark pink. This part is juicy, tangy, and flavorful. Inside the fruit is the endocarp, which is also juicy and slightly darker, containing many small yellowish seeds. The pulp inside can be about 6mm across and holds between 112 to 535 seeds (Azzolini *et al.*, 2005).

Table 1.1: Scientific classification of guava

Kingdom	Plantae
Division	Spermophyta
Sub-division	Angiosperm
Class	Dicotyledons
Order	Myrtales
Sub-order	Myrtineae
Family	Myrtaceae
Genus	*Psidium*
Species	*guajava*
Botanical name	*P. guajava*

Source: Beulah, *et al.*, (2024)

1.2.1 Origin and habitat

Guava, one of the most gregarious fruit, is popular throughout the world. The actual origin of the fruit is unknown. However, guavas are believed to be native to area ranging from tropical America (Mexico) to Caribbean region where it is also known as “sand plum”. Guava has a documented history from around fifteenth century. Nowa-days, the commercial cultivation of guava is carried out in many countries such as India, Mexico, Hawaii and Egypt. Guava trees grow best in tropical and sub-tropical climate having dry summers and short winters. Trees which encounter winters are known to give better yield than those which are in tropics. It can survive both in high humidity as well as drought conditions though young crops are susceptible to drought and cold conditions. Moreover, guava tree is less resistant to frost. Cultivation of guava trees is carried from sea level to 1500m. However, some varieties even thrive at 2500m (Mercadante *et al.*, 1999). Less rainfall and low humid conditions are favourable for flowers to blossom and fruits to grow whereas high temperature during fruit development causes low yield by increasing fruit drop. Thus, the ideal temperature for growth and propagation of guava tree is 23–26 °C. Though the tress is drought resistant, it still requires 1000–2000mm of well distributed rainfall especially during its flowering stage (June–September).

Guava tree blossoms twice a year, once in March for summer crop later in August for winter crop. The tree bear flowers for 45days. Winter crop due to its high productivity and fruit quality is preferred over summer crop. Guava fruit takes over 125days to ripen after the growth of fruit. Since guava is a climacteric fruit, thus, the fruit is harvested at maturity when the skin is green. Harvesting time lasts for 8–10weeks (Bose *et al.*, 2001).

Guava trees do equally well in all types of soils ranging from heavy clay to gravel or even limestone. Nonetheless, well drained, fertile soil with pH 4.5–9.4 is ideal for cultivation of guava tree. Moreover, the soil should have high salt concentration and high-water table level. Generally, propagation of guava trees is carried out via seed through cuttings, grafting, air layering and air budding. Air layering and marching were the common methods of plantation followed in India which has been replaced by budding and grafting methods as the trees planted in former fashion doesn’t stand beyond 2–3years. Plantation of guava tree cutting is done usually during the month of July and August. Guava cutting is hard and thus is planted under mist. Usually, 6–12.5mm thick half ripened cutting with roots is the propagating material. If plantation is practised through seeds, they germinate within 2–3weeks of sowing. However, some varieties can even germinate in the 8th week of sowing. Guava tree cuttings are planted 5–6m away from each other. The trees are fast growers and bears fruit after 2–4years of its plantation. They are long lived, produce fruits up to 40 years, however, the yield decline after 15th year.

Figure 1.1: Leaves of guava with its fruits.

Source: iStock photo,(2025)

The better understanding morphology of guava to better knowledge of plant parts like roots, stem, tree size, leaves, flowers and fruits.

A. **Tree and stem:-** The guava tree is a small, medium size tree or shrub with multiple branches arises from ths stem, that can reach a height of 2 to 7 meters. Its bark is smooth, thin, and copper-colored, and it sheds off easily. Underneath the outer layer of bark is a greenish skin that becomes visible when the outer bark is removed. The trunk of the tree can grow to a diameter of 25 cm when the tree reaches to full maturity. The twigs of the tree are four-sided (quadrangular) and curve.

B. **Leaves:-** The scientific name for guava leaves is *P. guajava.* Guava leaves are characterized by their opposite phyllotaxy, leathery texture, and elliptical to oval shape. They typically measure 7-15 cm in length and 3-5 cm in width. The leaves have prominent parallel veins that originate from the midrib and extend towards the edges (Azzolini *et al.*, 2005)

The stages of guava leaf based on their characteristics below: -

i. Young leaves: These are newly sprouted leaves that are small, tender, soft, and light green. They are actively growing and developing, in initial stages the guavas leaf colour is red to cupper colour due to presence of more Anthocyanin. After maturity of leaves the Anthocyanin colour are degrade and formation of chlorophyll pigmentation.

ii. Mature leaves: These leaves have reached their full size and are a darker shade of green. They are rigid and have distinct veins, and the leaves textures are goes to hardness, formation of more chlorophyll pigments on the leaves.

iii. Senescent leaves: These are older leaves that display signs of aging such as yellowing, browning, and wrinkling. Eventually, they drop off the plant.

iv. Shedding leaves: As leaves age and complete their life cycle, they naturally detach from the plant and fall to the ground. This process supports the guava tree's renewal and growth. For better understanding these stages is essential for monitoring the health and progress of the guava plant, as they reflect various aspects of leaf development and replacement in the plant's life cycle (Azzolin*i et al.*, 2005)

C. **Flowers**: The guava plant produces flowers either singly or in groups of two or three on the new growth of the current season, at the junctions of the leaves. Typically, the branches that bear flowers are a few centimeters long and have four to five pairs of leaves (Dasrathy, 1951). When a flower forms and sets on the branch, the terminal bud stops growing until the next growing season (Gardener *et.al*, 1952). Flowering bud on the guava plant is a combination of different types, and the flowers appear on the sides of the flowering shoot. Both the main shoot and side can bear flowers, as observed by various researchers. It has been noted that not all parts of the shoot produce buds, and they may appear sporadically along the branch (Shrivastav, 1962a). The flowering period of guava plants can last between 25 to 45 days, depending on factors such as the specific cultivar, the season, and the location where the plant is grown.

D. Fruits:- Guava fruit is typically round, ovoid, or pear-shaped, measuring 5–10 cm in diameter and weighing between 50–200 g, depending on the variety. The outer layer of the fruit, known as the exocarp, is thin, light yellow with a touch of pink. Directly beneath the exocarp is the mesocarp, the fleshy part of the fruit. The mesocarp is granular and can be thick white, yellowish, or dark pink in color, extending up to 3–12 *mm*. It is juicy, acidic, and flavorful. Further inside the fruit is the central pulp, known as the endocarp. The endocarp is juicy, slightly darker in color, and contains stony yellowish seeds. The pulp itself is 6 mm in diameter and can contain anywhere from 112–535 seeds. Within the pulpy endocarp are two types of cell wall tissues: stone cells and parenchyma cells. Stone cells made of lignified woody material give the fruit its characteristic gritty texture and are resistant to enzymatic digestion. Guava fruit has a relatively short shelf life of about 3–5 days at room temperature due to its high respiration rate and intense metabolism (Azzolini *et al.,* 2005).

1.2.2. Distribution and ecology

Psidium guajava is a native of tropical America and is found on a variety of soil. It needs full or partial sunlight for growth. It is a fruit yielding plant and cultivated for its fruit and medicinal properties.

Guava may have originated either from tropical America or from Asia, and is now widespread throughout the tropics and subtropics. It is naturalized in the Old World Tropics and in the West Indies. Guava can grow under a wide range of environmental conditions. It is reported as an invasive weed in many countries (mainly in the Pacific Islands and along the Pacific rim). Guava can be found in open areas, such as savannah/shrub transitional zones, or in frequently disturbed areas (Orwa *et al*., 2009). In some places, it can form dense thickets with more than 100 trees per hectares, and it can cause pasture abandonment and land degradation (Orwa *et al.,* 2009). There are no recent world statistics for guava production. In India, guava production was above 3 million tonnes in 2012-2013. The main producers of mango and guava were India (21 million tonnes), China (4 million tonnes), Kenya (2.7 million tonnes), Thailand (2.6 million tonnes), and Indonesia (2.3 million tonnes) (Tiwari, 2013).

Guava is a very versatile species. It is found from sea level up to an altitude of 1500-2000m in the tropics but produces better below 800-1000*m*. Guava thrives in places where average annual temperatures are about 23-28°C but can grow within 15-45°C. It does well in places where night temperatures drop to 10°C during winter. Quiescent trees survive light frosts. Guava grows in areas where annual rainfall ranges from 1000 to 2000mm. Rainfall should be evenly distributed over the year for optimal fruit production. However, rainfall during fruit ripening causes the fruit to lose flavour and split open. Guava is among the most drought resistant tropical fruit crops. It grows on a wide range of soils provided they are relatively well-drained. Guava withstands acidic soils and is tolerant of shade (Orwa *et al., 2009).*

1.2.3. Ethnobotany

Guava fruit is an ethnomedicine. It has special importance in the traditional system of medicine. In Indian Ayurveda, it is considered as an important herbal medicine for dysentery and diarrhea. In Traditional Chinese Medicine system, it is used to treat many diseases. It has been used since ages to improve the health of humans.

1.2.4. Traditional uses of *P. guajava*

Psidium guajava, commonly known as guava, has been widely used in traditional medicine for centuries. It's various parts, including the leaves, fruits, bark, and roots are employed in treating numerous ailments due to their therapeutic properties. Among these, the leaves are most frequently utilized, followed by the fruits, bark and roots. In some cases, the entire plant is used for its medicinal benefits. This section elaborates on the traditional uses of different parts of the *P. guajava* plant, reflecting it's significance in folk medicine.

Guava Leaves

The leaves of guava hold a prominent place in traditional medicine and are often used in the form of decoctions, infusions, or poultices. In India, the leaves are valued for their febrifuge and antispasmodic properties and are used to alleviate rheumatism. Across regions such as Columbia, Mexico, Maya, Nahuatl, Zapotec, the USA, and Mozambique, guava leaves are traditionally used to treat diarrhea and stomachaches. In the USA, they are also recognized for their antibiotic properties.

Externally, guava leaf poultices or decoctions are applied to wounds, ulcers, and toothaches. In South Africa and the Caribbean, they are widely used to manage chronic conditions such as diabetes and hypertension. In Latin America, Central and West Africa and South East Asia, guava leaf decoctions serve as remedies for sore throats, laryngitis and swelling of the mouth. The decoctions are also applied externally for skin ulcers, vaginal irritation, and discharge. In Trinidad, guava leaves are used for cleansing the blood, treating bacterial infections, and alleviating dysentry and diarrhea.

In the Pacific Islands, such as New Guinea, Samoa, Tonga, Niue, Futuna and Tahiti, boiled preparations of guava leaves are used to soothe itchy rashes caused by scabies. The leaves are also regarded as astringents and remedies for lung problems. In several countries, including Panama, Cuba, Costa Rica, Mexico, Nicaragua, Venezuela, Mozambique, Guatemala, and Argentina, guava leaves are applied to reduce inflammation. In Uruguay, leaf decoctions are used as vaginal and uterine washes, particularly in treating leucorrhoea.

Guava Bark

The bark of *P. guajava* is another vital component in traditional medicine. In the Philippines, the bark is used as an astringent to treat ulcers, wounds and diarrhea. Decoctions and poultices prepared from the bark are employed for similar purposes in Panama, Bolivia, and Venezuela. In Congo and Kinshasa, the bark is used as an antiamoebic agent, often in the form of infusions and decoctions.

In Mexico, a combination of bark and leaves in the form of decoctions or poultice is used to treat fever, respiratory infections, wounds, dehydration, and skin ailments. The bark is also used to expel the placenta after childbirth and address conditions such as vaginal hemorrhage and toothaches.

Guava Roots

The roots of guava are used traditionally in countries like Fiji and Senegal to prepare juices or decoctions for treating diarrhea, coughs, stomachaches, dysentery, toothaches, indigestion, and constipation. In the Philippines, root decoctions and poultice are employed as astringent to heal ulcers, wounds and diarrhea. The roots are valued for their ability to address both internal and external health issues effectively.

Guava Fruits

The fruits of *P. guajava* are extensively used for their benefits. In Brazil and Fiji, the ripe fruits are mashed or prepared as decoctions to treat anorexia, diarrhea, cholera, digestive problems, dysentery, indigestion, and constipation. They are also used to address inflamed mucous membranes, laryngitis, and sore throats. The fruit is applied externally for skin problems and ulcers and is considered beneficial for vaginal discharge.

The Whole Plant

In some traditional practices, the entire guava plant is utilized for its medicinal properties. In Tahiti and Somoa, infusionsz decoctions, or pastes made from the whole plant or young shoots are used as skin tonics. They are believed to help with painful menstruation, miscarriagesz uterine bleeding, and premature labour in women.

The whole plant's versatility and medicinal properties make it an integral part of traditional medicine systems in these regions (Gupta *et al.*,2010)

1.2.5. Medicinal uses Of guava

Guava plant has many medicinal properties. It has antibacterial and anti-fungal properties. Leaves act as astringent, anti-inflammatory and antiseptic. Tea made from the leaves are used to treat dysentery, diarrhea etc. Leaves can be chewed raw to get rid of gum and teeth problems. Leaf paste can be applied to the fresh wound as it is anti-bacterial and antiseptic in nature. It is also used to treat a common cough and cold, gonorrhea, stomach ache and skin problems. Leaves are also hepatoprotective and used to treat liver injury due to medicines.

The decoction made from the bark of this plant is also used against ringworms, ulcers, diarrhea and dysentery. The oil extracted from seeds possesses anti-inflammatory activity.

All parts of this plant have medicinal uses. The plant parts have hepatoprotective, antioxidant, antidiabetic, antimicrobial, anti-inflammatory, antispasmodic, analgesic and anti-cancer properties (Maan and Onkar, 2019)

1.3 Economic Importance of Guava

Guava is a fruit that's packed with Vitamin C, even more than citrus fruits like oranges (Alagumani, 2005). It also has good amounts of Vitamin A and other important nutrients. The fruit contains various acids, including citric, lactic, malic, oxalic, and acetic acids, along with a tiny bit of formic acid (Alagumani, 2005).

Ripe guava is often enjoyed as a dessert. You can also use it to make things like jellies, jams, juices, baby food, syrups, and even wine (Basseto *et al.,* 2005). It can be sliced and eaten with cream and sugar, or added to cakes and pies. It is also used in some dishes, like "sinigang" (Silva *et al.,* 2005). Parts of the guava tree have medicinal uses too. The bark and leaves can help during childbirth to remove the placenta. The leaves can be brewed into tea or made into a mixture to help with stomachaches and intestinal issues. Chewing the leaves can relieve toothaches, and crushed leaves can be applied to sore spots like rheumatism. Additionally, they're used for dyeing and tanning (Millins *et al.,* 2000).

The bark of the guava tree is sometimes used in certain cosmetics for conditions like hystero-epilepsy. The wood is fairly strong and durable, making it useful for handles and in carpentry (Silva *et al.,* 2005).

Guava fruit is best enjoyed when it's perfectly ripe and fresh from the tree. It has a sweet smell and a tasty flavor that's both sweet and a bit tangy. You can eat the whole fruit, including the thin, papery skin that blends well with the flesh *(Shivanand, 2002)*. Guava is considered one of the most delicious fruits. It has more proteins, carbohydrates, and minerals than apples and contains 50-60 times more Vitamin C. Only the Indian gooseberry has more Vitamin C. Unlike most fruits, guava is available year-round, earning it the nickname "Poor man's apple" due to its high calorie content. Since our bodies can't store Vitamin C, we need to consume it daily for good health. An adult needs about 50-70 milligrams of Vitamin C each day, which can easily be met by eating one or two guavas (Stephen *et al.*, 2002).

The ripe guava's skin can be used to make tasty salads and puddings, and it's excellent for making jellies, jams, ice cream, and guava paste. It can also be canned in sugar syrup or turned into fruit butter. These products are delicious and have health benefits, being refreshing and gentle on the stomach. Unripe guavas can help with diarrhea (Alice, 2014). Guava juice is great for making drinks and can be a substitute for orange or tomato juice for babies. Guava products are in high demand both in India and internationally, which presents opportunities for export.

Guava could become even more popular with the rise of canning and processing industries (Silva *et al.,* 2005). It has great potential as a profitable fruit crop in India, where it's grown in places like Bihar and introduced to other areas. In tropical countries, wild guava is often sour and used for processing rather than eating fresh. However, in India, many varieties are juicy and sweet, making them more enjoyable to eat fresh, with only a small amount being preserved.

1.4. Diseases of Guava

I. Wilt: This is caused by fungi like *Fusarium* sp. it is a serious disease guava disease sometimes encountered, especialy in alkaline soils. The symptoms are browning and wilting of the leaves, discolouration of the stem and death of the branches along one side. Sometimes the infection girdles the entire stem and the whole plant may wilt. In severe cases the entire tree may die.

Control: The infection can be minimized by soil drenching with Brasicol and spraying of Bavistin (0.1%) around the roots and leaves at an interval of 15 days.

II. Anthracnose: This is caused by fungi like *Gloesporium psidii*, and *Glomerella psidii*. The affected plants showed signs of die back from the tip of the branch.

Control: Spraying the trees with Copper Oxychloride, Cuprous Oxide or Difolatan controls the disease.

III. Cercospora Leaf Spot: This is caused by *C. sawadae*, water-soaked patches under the leaf are the characteristic symptoms of the disease.

Control: Spraying copper oxychloride at 0.3 per cent can reduce the infection.

IV. Scab: This is caused by *Pestalotia psidii*.The fungus mainly attacks the unripe fruits to produce dark scabby lesions, 2-4mm in diameter. The scab disfigures the fruits and their market value is considerably reduced.

Control: Application of Zineb 20g or Chlorthalonil-20g/10 litres of water at the time of flowering and then subsequent sprays at 15 days interval helps to control the disease effectively (Misra, 2016).

V. Canker: The disease mostly affects green fruits, with leaves very occasionally being affected. The initial sign of infection on fruit is the emergence of tiny, brown or rust-colored, circular, necrotic patches that are not broken. At an advanced stage of infection, these areas tear up the epidermis in a cyclic fashion. A depressed area can be seen inside the lesion, and its margin is high. On fruits rather than leaves, the crater-like look is more obvious.

VI. Algal leaf and fruit spot: The alga stains the fruit and leaves, which lowers the plant's ability to photosynthesize. Small, shallow brown lesions first form on leaves; as the disease advances, the lesions grow to a diameter of 2–3 mm. On leaves, there may be tiny spots or large regions of colour. They could be strewn around or crowded. The most frequently affected sections of a leaf are its tips, margins, or regions close to the mid vein. The growth of fruits causes cracks to appear regularly on older blemishes. Only a few layers of cells beneath the epidermis are susceptible to fruit penetration (Misra, 2016).

1.5. Literature Review

1.5.1. Fungi associated *P. guajava* leaves and the influence of culture media on their growth

In a study conducted by Valentino *et. al* (2015), aimed at investigating the phytopathogencity of fungi associated with crown rot of guava, eight species of fungi, including *A. fumigatus, A. niger, A. tamarii, A. japonicus, A. flavus, Fusarium sambucinum, F. verticilloides*, and *Lasiodiplodia theobromae* were isolated. Out of which five species (*A. fumigatus, A. niger, A. tamarii, A. japonicus*, and *A. flavus*) were phytopathogenic, causing crown rot on the wounded surface of guava fruits. According to the authors, the lesions caused by these fungi consisted of dark brown to black discoloration on the infected area of the guava fruits. Kotan *et al.*, (2009) characterized *Aspergillus* species as wound-invading pathogen that causes decay on stored citrus fruits damaged by insects, animals, early splits, and mechanical harvesting. This also confirmed the reports of Chuku *et al.*, (2008), Mathew *et al*., (2010), Akinmusire (2011) and Amadi *et al.*, (2014), that *A. flavus, A. flumigatus and A. niger* are causal agents of postharvest spoilage in fruits including guava and tomatoes. Furthermore, members of genus *Aspergillu*s are one of the major fungi species producing aflatoxin which were classified as toxin and carcinogenic compounds causing serious health implications, thus making fruits unfit for human and animal (Shenasi *et al*., 2002). No signs of crown rot in guava were observed in fruits treated with *F. sambucinum, F. verticilloides* and *Lasiodiplodia theobromae* indicating their non- pathogenicity.

According to Pitt and Hocking (1999), *F. verticillioides* is an endophytic fungi are capable of living in host plants without causing any symptoms (Petrini *et al*., 1992). As stated by Munkvold and Desjardins (1997), this symptomless infection can exist throughout the plant in leaves, stems, roots, grains, and the presence of the fungus is in many cases ignored because it does not cause visible damage to the plant. In a previous study by Rubini (2005), species of *Fusarium* and *Lasiodiplodia* along with other species of fungi were isolated as endophytes of cacao and were identified as potential antagonist of *Crinipellis perniciosa*, a causal agent of Witches' Broom Disease.

Contrary to present results of phytopathogenicity test, Cardoso (2002) and Punithalingam (1980) cited *Lasiodiplodia* as one of the causal agent of stem end rot, dieback, root rot, fruit rot, blights, gummosis, stem necrosis, leaf spot, and witches'broom disease of tropical crops. Misra & Pandey (1999) also reported the phytopathogenicity of several *Fusarium* species causing wilt to guava plants. In addition, *Fusarium* are fumonisin producers, which are phytotoxic, damaging a wide variety of crops and have emerged as a highly visible animal and human health safety concern since they have been associated with many animal diseases such as leukoencephalomalacia and has been evaluated as possibly carcinogenic to humans (Fandohan *et al*., 2003).

Further study was on the pathogencity of guava anthracnose, showed *Pestalotia psidii*, *Colletotrichum gloeosporioides*, and *Botryodioplodia theobromae* as causal organism of guava anthracnose. The growth of *B. theobromae* was the fast growing fungus followed by *P. psidii* and *C. gloeosporioides*. All the pathogens grew well at 28-30°C with the exception of P. psidii at 30°C.

Endophytic fungi such as *Rhizopus arrhizus, Candida albicans, Epidermophytum floccosum, Aspergillus niger, Mucor circinelloides* and *Lichtheimia corymbifera* associated isolated from *P. guajava* fruits according to Sandhu *et al. (*2014) showed with little modification in terms of cultural and microscopic characteristics such as shape, size, texture, colour, pattern and arrangement of mycelium, conidial arrangement and types of spore with reference to fungal atlas (Watanabe, 2002)and similar report (Kidd *et al.*, 2016).

Symptoms observed were gray/light brown lesions surrounded by dark brown borders on leaves and brown,raised, corky, necrotic lesions on the exocarp of fruit which progressed as the fruits matured. Seventeen isolates from infected fruit, six isolates from lesions on leaves, and nine isolates from additional crops surrounding the guava trees were collected. The main fungi consistently isolated from symptomatic leaves and fruit were *Pestalotiopsis spp*. Twenty-three isolates of *Pestalotiopsis* were obtained from the diseased samples, and an additional nine were collected from other hosts, including wild guava, blueberry (*Vaccinium corymbosum*), waiawi (*Psidium cattleianum*), and tea (*Camellia sinensis*).

1.5.2. Effect of cultural medium on fungal morphology

Fungi such as *A. candidus, A. niger, A. sulphureus, A. versicolor, P. corylophilum, P. expansum, Penicillium sp., Acremonium kiliense, Chaetomium funicola, and F. oxysporum* was isolated from a decaying vegetable waste of *P. guajava* leaves. The pure cultures of these fungi were cultured on Czapek Dox agar, Lignocellulose agar and Potato Dextrose agar. Findings showed that all three media supported fungal growth, but to different extents. Six fungal species showed the best growth on Lignocellulose agar (LCA) after 7days. *A.candidus, A.niger, A.sulphureus, A.versicolor, P.corylophilum* and *P.expansum* were the six fungal species that shows the best growth on LCA. On the other hand, *Penicillium sp*. and another unidentified fungus grew better on Potato Dextrose agar (PDA), while *C. funicola* and *F. oxysporum* grew most on Czapek's Dox+ Yeast extract agar (CYA). The color of the fungal colonies varied depending on the media. For instance, *F. oxysporum* had magenta pink colonies on PDA, white on CYA, and clear (hyaline) on LCA. *A. versicolor* showed different colors on each medium ranging fromwhite to orange with green spores in the center on PDA, white with reddish exudates on CYA, and colorless on both sides on LCA. These color changes show that the type of medium affects the appearance of fungi (Sharma and Pandey, 2010).

In a study conducted to isolate *Pisolithus c*ultivated in a liquid Melin-Norkrans Modified medium (MNM) at PH 6.5 and incubated at 25°C. The fungus growth pattern was affected by agitation speed (Costa *et al*., 2020). The study observed that optimal growth of *Pisolithus* occur at 200rpm for 14 days producing the highest mycelium dry mass, while excessive agitation (250rpm) led to fungal cell damage which result in low biomass production.

The influence of agitation speed on the morphology of filamentous fungi in submerged liquid culture has been widely studied, with significant findings reported for species such as *Blakeslea trispora* (Mantzouridou *et al*., 2002), *Cordyceps militaris* (Park *et al*., 2002b), *Paecilomyces sinclairii* (Kim *et al*., 2003), and *Aspergillus niger* (Kelly *et al*., 2004). These studies highlight that filamentous fungi are morphologically complex organisms whose structural characteristics vary depending on several factors, including the stage of their life cycle, the type of growth environment (surface vs. submerged culture), the composition of the culture medium, and the physical conditions in which they grow (Papagianni, 2004).

One of the most notable effects of agitation speed in submerged culture is its impact on fungal pellet formation and mycelium dry mass production. When filamentous fungi grow under conditions of low agitation, they tend to form larger pellets, but this often results in reduced overall mycelium dry mass. This reduction is likely due to limited nutrient diffusion into the inner regions of the pellets, leading to lower metabolic activity in those areas (El-Enshasy *et al.*, 2006). On the other hand, higher agitation speeds promote greater fragmentation of the mycelium, leading to smaller pellet formation and potentially enhancing nutrient uptake and biomass production.

Regardless of the agitation speed applied, the dry mass accumulation of the D216 isolate of Pisolithus sp. was observed to continue until approximately 14 days of growth. Beyond this period, mycelial growth became negligible, which could be attributed to factors such as changes in pH within the culture medium or the depletion of essential nutrients. Interestingly, this growth pattern represents an improvement compared to the typical growth duration reported in the literature for *Pisolithus sp*., which generally requires between 20 and 30 days for inoculant production (Fernandes *et al.*, 2014; Costa *et al.*, 2015).

In contrast, some other fungi with faster growth rates, such as *A. niger* and *Ceriporiopsis subvermispora*, are known to achieve peak mycelium dry mass production within a significantly shorter period, typically between four to five days (Gupta *et al.*, 2003; Kelly *et al.*, 2004). This rapid growth suggests that different fungal species exhibit distinct metabolic and morphological adaptations to submerged culture conditions, with variations influenced by species-specific physiological traits, nutrient availability, and the physical parameters of the culture system.

1.6. Aim and Objectives 0f Project Research

Aim

The aim of the study is to isolate fungi associated with diseases leaves of *P.guajava* and able to compare the growth of the isolated fungi on two media

Objectives

The objective of this research is to:

1.To identify the fungi associated with diseased leaves of *P. guajava* using their morphological features

2.To determine the *in vitro* mycelial growth of the isolated fungi on both potato dextrose agar and corn meal agar.

CHAPTER TWO

MATERIALS AND METHODS

2.1.1. Source of material

The diseased leaves used in this study was collected from an orchard within the University of Benin (UNIBEN) campus. The samples were identified by Prof. Henry A. Akinnibosun, the voucher specimen was deposited in the University of Benin Herbarium with the voucher number UBH-P378.

2.1.2. Steriliization

All Petri dishes and glasswares was washed with detergent, rinsed with tap water and finally with distilled water and allowed to dry. They were sterilised in a pressure and allowed to steam for 30 minutes.

2.2. Experimental Methodology

2.2.1. Preparation of potato dextrose agar

30g of potato dextrose agar (PDA) was measured into a conical flask and 500ml of water was added into the conical flask. After heating, additional 500ml of water was added to make it up to 1litre. The medium was the sterilised by autoclaving at 15psi (121°C) for 15minutes. Chloramphenicol of 10mg per 200ml was introduced at pouring to inhibit the growth of bacteria. Inoculation and transfer of culture were carried out on sterile inoculating bench after wiping with methylated spirit.

2.2.2. Preparation of corn meal agar

9g of corn meal agar (CA) was measured into a conical flask and 500ml of water was added into the conical flask.The medium was sterilised by autoclaving at 15psi (121°C) for 15 minutes. Inoculation and transfer of culture were carried out on sterile inoculating bench after wiping with methylated spirit.

2.2.3 Isolation of fungi

Isolation of fungi associated with leaf spot disease was done by using the direct plating technique. This was done by cutting small portions of diseased leaves showing symptoms of the leaf spot disease of the guava plant with a new scalpel blade. These portions of leaves were surfaced sterilised using 50% sodium hypochlorite solution for two minutes and rinsed with distilled water three times.The leaves were blotted dry with sterile filter paper, then the leaves was plated in Petri dishes containing potato dextrose agar (PDA) medium and corn meal agar(CA) medium.

These were incubated at the temperature of 28°C ± 2°C for 7 days. After incubation, different colonies of fungi associated with the leaf spot were each sub-cultured using a flamed inoculating loop onto sterile plates containing PDA and CMA.

2.2.4. Identification of Isolates

Fungal cultures were examined macroscopically and microscopically using a compound light microscope. It was examined and identified based on their cultural and microscopic characteristics according to the method described by Barnett and Hunter (1998) and Nyongesa *et al* (2015).

2.2.5 Sub-culturing Of microorganism

Freshly prepared PDA and CMA was poured into an already sterilized petri dishes, then it was allowed to solidify. From the old plates containing the microorganism, the fungal cultures was sub-cultured by carefully taking a fresh growing portion with a sterilized needle into a fresh PDA plates.

2.2.6 Comparative study of the radial mycelia growth of isolated fungi on potato dextrose agar and corn Meal Agar

The centre of each three replicates PDA and CMA plates was inoculated with a 0.5cm diameter mycelial disc cut from the 7days culture of *P.psidii* and *L.theobromae*. The microorganism identified was incubated at room temperature for 7days, the radial mycelial growth was measured daily after 3days of incubation at the end of the incubation period. The radial growth of mycelium was measured along the intersecting lines and the mean of the two measurements was recorded for each three replicates of the respective culture media.

Plate 2.1: Diseased leaf of *P.guajava*

CHAPTER THREE

RESULTS

3.1. Description of the diseased leaf of *P.guajava*

Leaf spot disease of *P.guajava* was characterized by development of small necrotic patches on the leaf surface. These spots can begin as pale yellow, light brown or reddish discoloration that expand into irregular shapes with defined margins.

3.1.1. Description of *P. psidii* on PDA

Pestalotia psidii appears as small dark brown spots surrounded by a yellow halo but when cultured on a media, it forms whitish to grey mycelium with sparse black sporulation after few days of incubation.

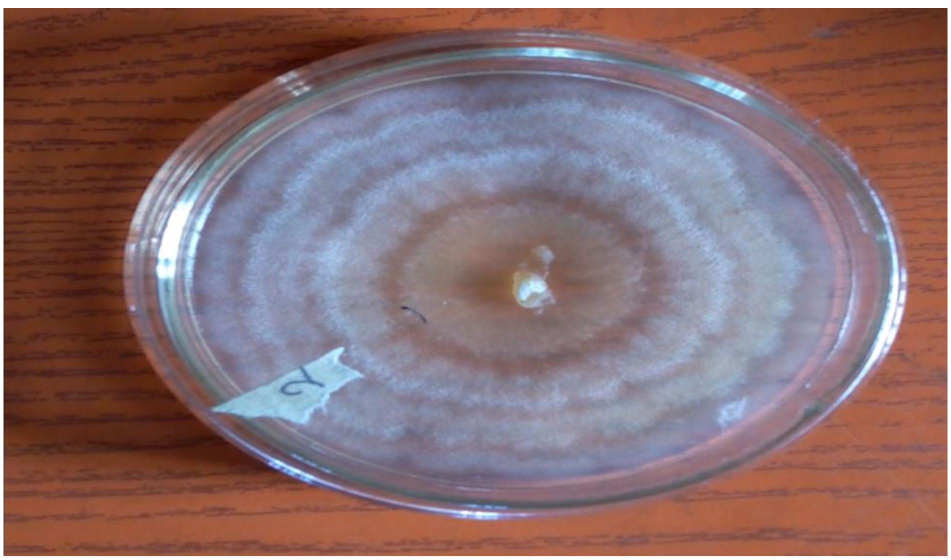

Plate 3.1: Culture plate of *P. psidii* on PDA after 7 days of incubation under room

3.1.2 Description of *P. psidii* on CMA

Pestalotia psidii grows very slowly on CMA with rarely visible mycelial growth on the culture plate. No sign of black sporulation was noticed on the culture plate after 7 days of growth.

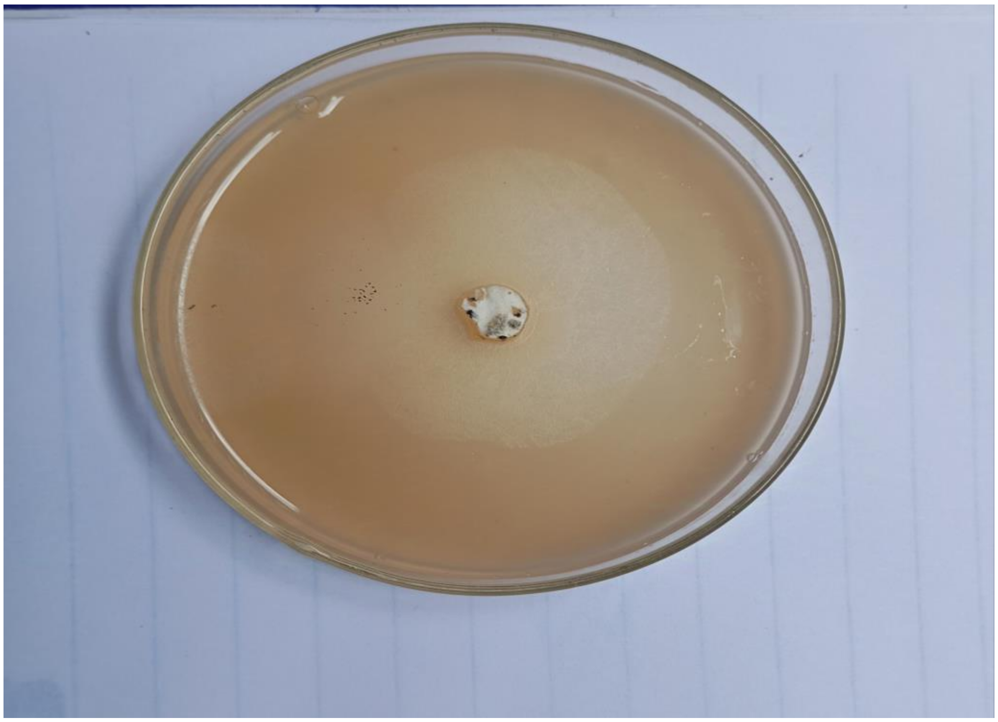

Plate 3.2: Culture plate of *P. psidii* on CMA after 7 days of incubation under room temperature

3.1.3 Description of *L.theobromae* on PDA

Lasiodiplodia theobromae produces dark, septate mycelia. The fungus develop cottony mycelia that transition from white to black as the colony matures.

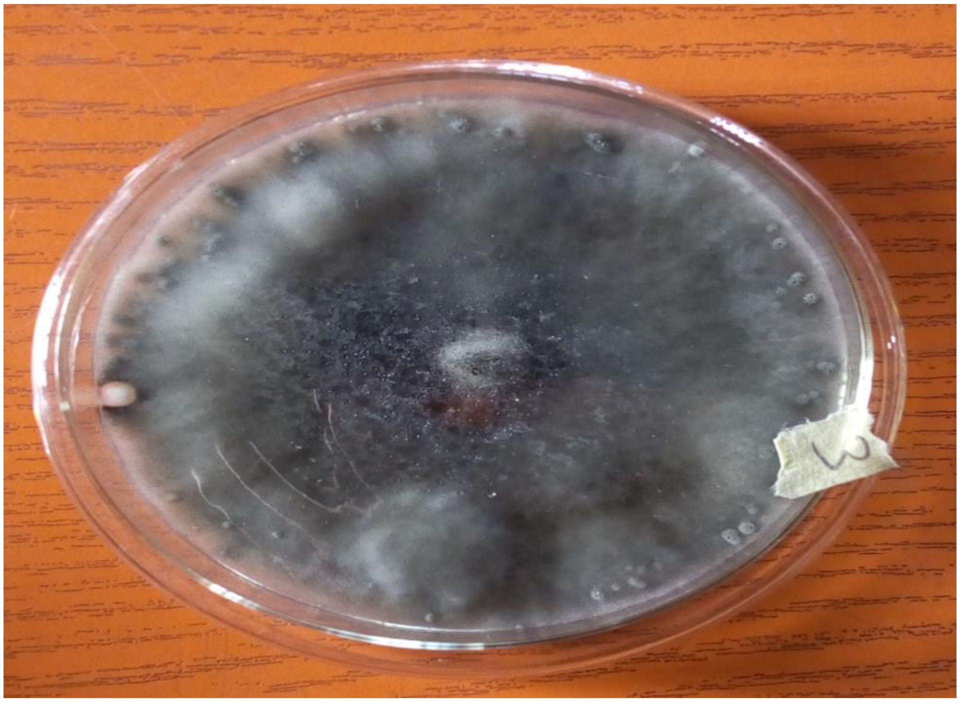

Plate 3.3: Culture plate of *L. theobromae* on PDA after 7 days of incubation under room temperature

3.1.4 Description of *L. theobromae* on CMA

Lasiodiplodia theobromae produces less whitish to dark, septate mycelia on CMA which barely turns black as the colony matures.

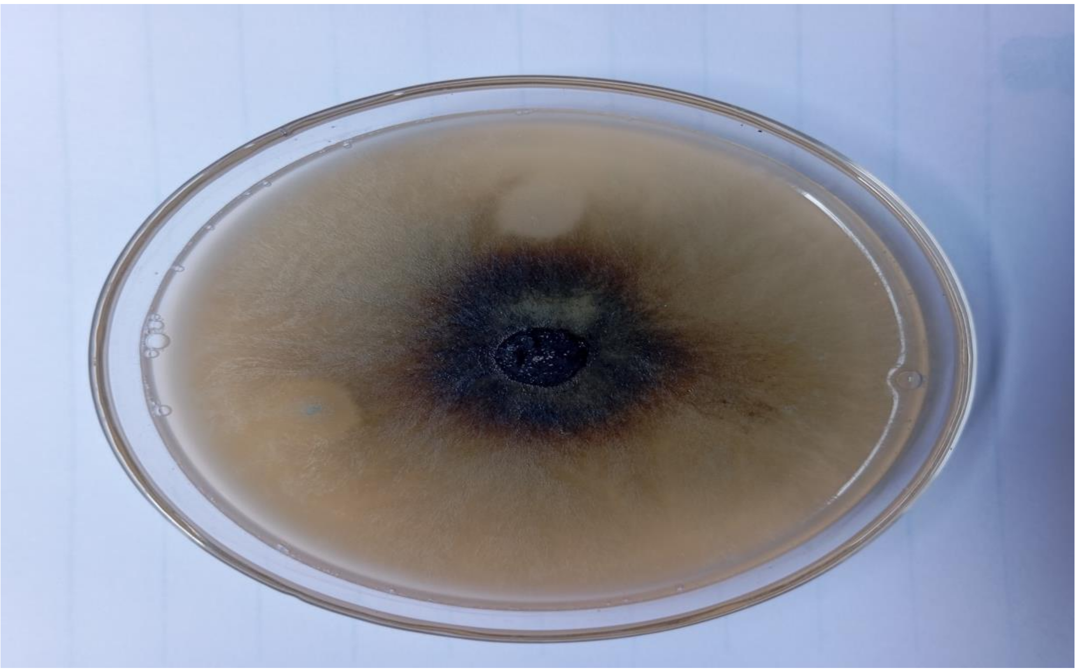

Plate 3.4: Culture plate of *L. theobromae* on CMA after 7 days of incubation under room temperature

3.2 The result of the mycelial growth of *P. psidii* and *L. theobromae* on CMA

The results the radial mycelial growth of *P. psidii* and *L .theobromae* on corn meal agar shows an increase in the average mycelial growth of *P. psidii* from 3.25cm on day 3 to 5.20cm on day 7 while *L. theobromae* was observed to increase from 3.63cm on day 3 to 5.63cm on day 7.

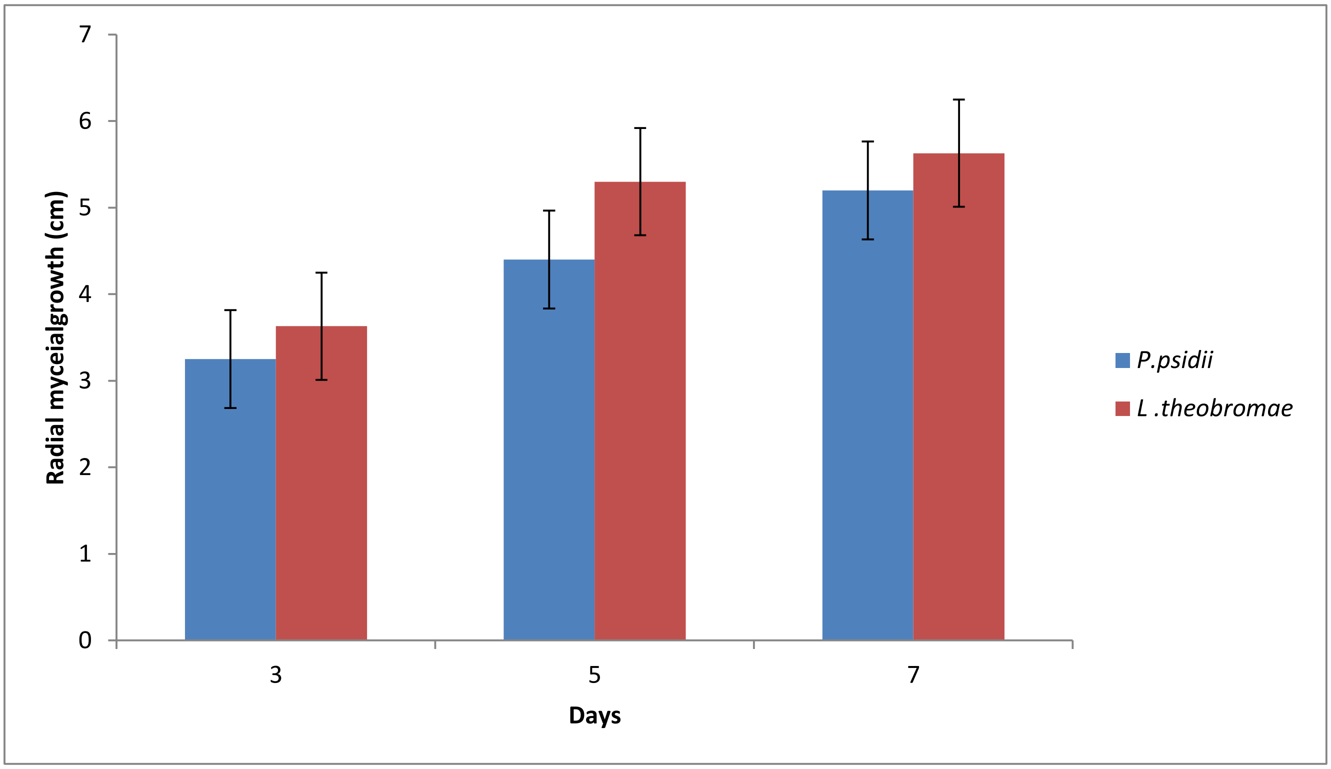

Figure 3.1: Radial mycelial growth of *P.psidii* and *L.theobromae* on CMA

3.3 The result of the mycelial growth of *P. psidii* and *L. theobromae* on PDA

The results the radial mycelial growth of *P. psidii* and *L .theobromae* on potato dextrose shows an increase in the average mycelial growth of *P. psidii* from 5.05cm on day 3 to 8.5cm on day 7 while *L. theobromae* was observed to increase from 3.90cm on day 3 to 7.45cm on day 7.

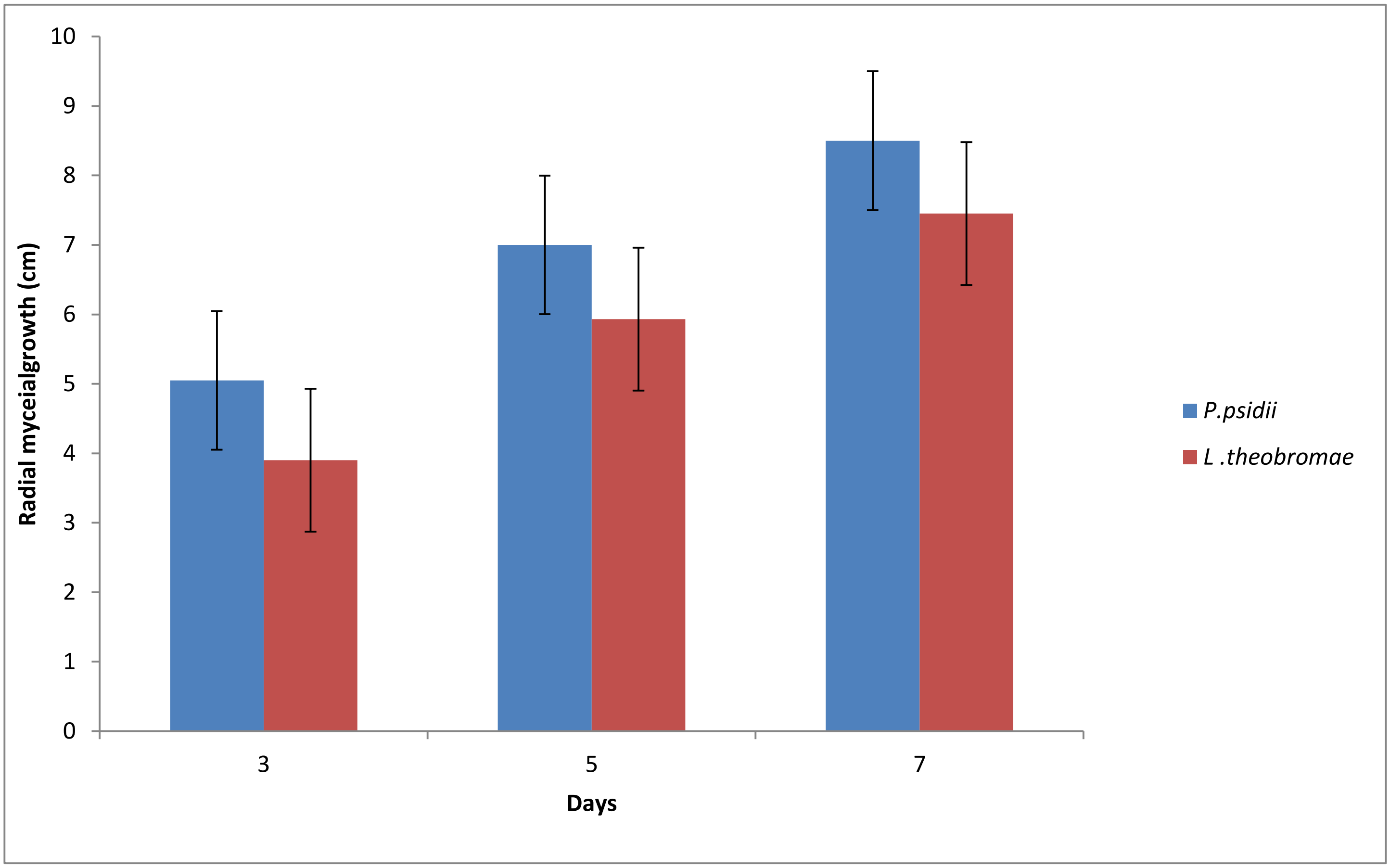

Figure 3.2: The radial mycelial growth of *P. psidii* and *L. theobromae* on PDA

CHAPTER FOUR

DISCUSSION

Psidium guajava is a tropical plant that produces fruit widely valued for its many uses and benefits. It belongs to the family Myrtaceae and grows well in different climates, making it a common crop in many tropical and subtropical regions. Guava is rich in Vitamin C, dietary fiber, and antioxidants, which makes it an important fruit for nutrition and health. It is used in the food industry to make products like juices, jams, and purees, which are enjoyed around the world. Beyond its nutritional value, guava is also important in traditional medicine. The leaves and bark are commonly used to treat conditions like diarrhea, coughs, and infections because of their antibacterial and anti-inflammatory properties. The fruit itself is used in the cosmetic and pharmaceutical industries because it contains special compounds that help improve skin health and prevent diseases (Amusa *et al.*, 2005)

Leaves are vital organ in the plants, serving as the primary site for photosynthesis. The damage caused by fungal infections compromises their ability to perform this essential function, affecting the synthesis and allocation of photosynthetic products critical for plant growth, development and fruit production (Gupta *et al.*, 2010)

In this study, isolation of fungi was done by surface sterilization in order to isolate the pathogen associated with the disease symptoms affecting the diseased leaf. Findings from this study showed *P. psidii* and *L. theobromae* to be associated with the leaf spot disease of *P. guajava.* Cardoso (2002) stated that *Lasiodiplodia* and *Pestalotia* as one of the causal agent of leaf spot, stem necrosis, blight of tropical crops while Valentino *et al. (*2015) reported that *Lasiodiplodia* is non-pathogenic to fruit disease of guava but can be found as one of the causal agent of leaf spot. Perumal *et al*. (2021) also stated that *P. psidii* cause a disease called "Fruit canker" and the disease occur generally on guava fruits but rarely on leaves. In contrast to that, it was recorded by Valentino *et al*. (2015) that *P. psidii* is a causal agent of grey leaf spot and fruit canker of guava.

Findings from this study also shown that CMA is not a suitable growth medium for the laboratory cultivation of *P*. *psidii* and *L. theobromae* compared to PDA.

PDA is rich in nutrients, containing potato extract and dextrose, which provide essential carbohydrates, amino acids, and vitamins that promote optimal growth of fungi. The dextrose in PDA serves as an easily available carbohydrate source, enabling rapid mycelial growth and conidia production. On the other hand, CMA is based on cornmeal, which does not offer the same abundance of nutrients as PDA. Although cornmeal provides some carbohydrates, it is less nutrient-dense, which can limit fungal growth. As a result, *P. psidii* and *L. theobromae* tend to grow slower and less vigorously on CMA.

The pH and osmotic conditions of each medium also influence fungal growth. PDA typically has a pH level that is favorable for many pathogenic fungi, including P. psidii and L. theobromae, which helps support their metabolic functions. In contrast, CMA may have a different pH or osmotic balance that does not support optimal fungal development, leading to slower growth.

Regarding growth rate, both *P. psidii* and *L. theobromae* exhibit faster growth on PDA than on CMA. This is likely because PDA provides more nutrients, which enhances the fungi's mycelial growth and conidia formation. In contrast, the limited nutrients in CMA result in slower growth of the fungi.

In conclusion, PDA is a more suitable medium for cultivating *P. psidii* and *L. theobromae*, as it encourages faster and more robust growth. CMA, however, does not provide the same level of support, making it less effective for studying these fungi in the laboratory.

While previous studies focused primarily on the isolation and identification of fungal species affecting guava, this research contribute to isolation and identification of fungal disease of leaves. It also explains how different culture media influence the fungal growth. The findings from this results provide valuable insight into the major pathogens that causes leaf spot disease of guava.

CONCLUSION

The findings of this study successfully isolated and identified *P.psidii* and *L.theobromae* as the major fungal pathogens of leaf spot diesease of guava. The growth analysis showed that both pathogens grew best on PDA, making it a more suitable medium for their cultivation. Further research can provide insights into the study of guava diseases and provide a basis for developing effective management strategies to mitigate their impact.

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APPENDICES

DAYS	Plate 1 for *P.psidii* on CMA	Plate 2 for *P.psidii* on CMA	MEAN (Plate 1 + plate 2 divided by 4)
DAY 3	3.0 + 3.0	3.5 + 3.5	3.25
DAY 5	4.3 + 4.4	4.4 + 4.5	4.4
DAY 7	5.2 + 5.3	5.3 +5.0	5.2

DAYS	Plate 2 for *L.theobromae* on CMA	Plate 2 for *L.theobromae* on CMA	Mean (Plate 1 + Plate 2 divided by 4)
DAY 3	4.0 + 4.0	3.0 + 3.5	3.63
DAY 5	5.9 + 6.0	4.0 + 5.3	5.3
DAY 7	6.2 + 6.5	4.8 + 5.0	5.63

DAYS	Plate 1 for *P.psidii* on PDA	Plate 2 for *P.psidii* on PDA	MEAN (Plate 1 + plate 2 divided by 4)
Day 3	4.5+5.7	4.5+5.5	5.05
Day 5	7.0+7.5	6.5+7.0	7.0
Day 7	8.5+9.0	8.0+8.5	8.5

DAYS	Plate 1 for *L.theobromae* on PDA	Plate 2 for *L.theobromae* on PDA	MEAN (Plate 1+ Plate 2 divided by 4)
Day 3	4.5+4.4	3.2+3.5	3.90
Day 5	6.5+6.8	4. 5+5.9	5.93
Day 7	8.0+8.3	6.0+7.5	7.45