EPIDEMIOLOGY AND CONTROL OF *PSEUDOCERCOSPORA* FRUIT AND LEAF SPOT DISEASE OF SWEET ORANGE (*Citrus sinensis* (L) Osbeck)

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DECLARATION

I, John Yangyuoru Kupagme, do hereby declare that the work herein presented is the result of my own investigation and that except other people's work, which have been duly acknowledged, this thesis has never been presented to this university or elsewhere for any degree.

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DEDICATION

TO THE GLORY OF GOD, AND MY LATE FATHER, MARK KUPAGME

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LIST OF ABBREVIATIONS/ACRONYMS

1. FAOSTAT= Food and Agricultural Organization Statistics
2. MoFA=Ministry of Food and Agriculture
3. RCBD=Randomized Control Block Design
4. MT=Metric tonnes
5. L=Litres
6. USDA=United States Development of Agriculture
7. PFLS=*Pseudocercospra* Fruit and Leaf Spot
8. EPPO= European and Mediterranean Plant Protection Organization
9. ml=millilitres
10. g=Grams
11. BC=Before Christ

12. US=United States

13. GEPC= Ghana Export Promotion Council

14. FAO=Food and Agricultural Organization

15. WHO=World Health Organization

16. GAIN= Global Agricultural Information Network

17. TSS=Total soluble solids

18. Kg=Kilogram

19. Ha=Hectares

20. NGO=Non-Governmental organization

21. CC=Citrus canker

22. CABI=Centre of Agriculture and Bioscience International

23. CMI=Commonwealth Mycological Institute

24. CAB= Centre of Agriculture and Bioscience

25. PDA=Potato Dextrose Agar

26. MEA=Malt Extract Agar

27. V8A=V-8 Juice agar

28. cjPDA=Carrot juice-PDA

29. PCA=Potato Carrot Agar

30. RH=Relative Humidity

31. FOHCREC=Forest and Horticultural Crop Research Centre

32. LSD=Least Significant difference

33. H_2O=Water

34. UNCTAD=United Nations Conference on Trade and Development

35. mm=Millimetres

36. cm =Centimetres

37. ms^1 =Metres per second

38. SE=Standard Error

39. IDM=Integrated Disease management

40. AILAP= Agricultural Improvement and Land Access Program

ABSTRACT

Citrus plants are regarded as the world's second fruit crop consumed by volume next to banana, providing food nutrients, foreign currency, raw materials for agro-industries and source of employment. The production and productivity of citrus in tropical Africa including Ghana is threatened by a number of diseases. *Pseudocercospora* leaf and fruit spot PFLS of citrus caused by a fungus *P. angolensis* is the most destructive disease of citrus in most citrus growing areas. The disease was observed for the first time in Kwaebibirem District of the Eastern Region of Ghana in 2013. A study was conducted on the epidemiology of *Pseudocercospora* Fruit and Leaf spot (PFLS) disease of citrus in the humid semi-deciduous tropical rainforest of Ghana where citrus is produced to assess the fungistatic potential of some medicinal plant extracts to be used for control of the disease. A Randomized Control Block Design (RCBD) was established to determine the critical infection period of citrus. Selected Late Valencia fruits from 20 trees under natural infection were subjected to 18 different treatments. Some were exposed throughout the season, others covered throughout the season and the rest exposed fortnightly to the local environmental conditions. The roles of some climatic factors such as rainfall, temperature, humidity and aero-mycoflora in the infection and development of the disease were also studied. Data were analysed by ANOVA and LSD statistically using it to perform mean separation test at ($P \leq 0.5\%$) probability level. Field inspections revealed that fruits and leaves symptoms were surrounded by prominent yellow halos. High premature defoliation and fruit drops were common. Young fruits leaves

were more susceptible compared to the mature ones. Field survey data after harvest indicated that incidence of the disease was very high (93.9%) in July compared with remaining months of the year (57.75%). The trend of disease incidence and severity decreased beyond July. July recorded the highest disease incidence and severity (94.9% and 67.75%) respectively. *In vitro* studies on the effects of ethanolic extracts of five medicinal plants with antifungal properties namely, *Zingiber officinale, Allium sativum* (L.), *Moringa oleifera, Azadirachta indica,* and *Carica papaya* were also carried out with the view to their replacing the use of chemicals (Mancozeb plus Carbendazim). *Allium sativum* recorded the highest percentage inhibition (90.45%) followed by *Zingiber officinale* (52.46%), *Azardirachta indica* (45.99%), *Moringa oleifera* (37.35%) and *Carica papaya* (36.67 %). For humanhealth and eco-friendly reasons, the use of botanicals, such as ethanolic extracts of *Allium sativum*, is recommended for field trials within the periods of June and August in a semi-deciduous area to see if the active compounds in these extracts withstand environmental conditions.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Citrus (*Citrus sinensis* (L) Osbeck) is an important tropical and subtropical evergreen fruit crop widely cultivated around the world with various benefits to man and next to banana in terms of global production volume (FAOSTAT, 2016). The sweet orange groups belonging to the family Rutaceae are the most desirable and most important citrus species for fresh and juice consumption purposes worldwide, with about 112,714.108 Mt produced in 2016, followed by Mandarin (27 Mt), lemon / lime (13 Mt) and grapefruit / pummel (5 Mt) (FAOSTAT, 2016). Sweet orange is Ghana's most consumed perennial fruit crop (MOFA, 2011). Citrus production can be divided into four main groups, namely sweet oranges (*C. sinensis* (L) Osbeck), mandarins (*C. reticulata*), grapefruits (*C. paradisi*) and citrus limes (*C. aurantifolia*), (Liu *et al*., 2012).

Economically, citrus has manifold benefits such as source of food, peels for the production of agro-chemicals, source of income for people in the rural areas where citrus is grown and other actors along the citrus value chain. With strong linkages among the various actors on the citrus value-chain, the citrus industry is one of the avenues for the stimulation of employment and economic growth and development of the Ghanaian economy. Citrus has some essential oils, which are extracted from the peels used in the pharmaceutical, perfumery and other industries (Jazet-Dongmo *et al*., 2002). Citrus fruits are known to have a variety of vitamins, minerals, fiber, carotenoids, flavonoids, and limonoids, which are of health benefits to humans. They have a reduced energy density than other fruits and are free of sodium and cholesterol unlike other fruits (Guthrie *et al*., 1995; Whitney *et al*., 2009; USDA, 2011).

Despite the importance of sweet orange in the economy of Ghana, in 2004 a new devastating fruit and leaf spot disease of citrus was observed in the Eastern region causing 50-100% yield

loss. A decade later, Brentu *et al*., (2013) identified the disease as *Pseudocercospora* fruit and citrus leaf spot (PFLS) disease caused by *Pseudocercospora angolensis*. Currently, PFLS has reached epidemic levels in all growing areas (Eastern, Central and Ashanti regions) in the country and almost collapsing the citrus industry in Ghana. The disease is characterized by necrotic leaf and fruit spots with light brown centers and dark brown margins surrounded by a yellow halo. Affected fruits become hard with deep cracks in the rind. These cracks are predominantly observed on young fruits compared to old ones. All citrus varieties grown in the country are known to be susceptible to *P. angolensis* (Brentu *et al*., 2013). Every fruit infected by the disease is not suitable for consumption or processing and the diseased fruit may eventually drop leading to yield loss, which may reach total loss of 100% (Brentu *et al*., 2013). This destructive disease primarily attacks the leaves, fruits and young seedlings that cause many spots leading to premature fruit and leaf drop (Kuate *et al*., 1997). Consequently, PFLS is flagged by Europe and the Mediterranean region as a highly rated (A1) quarantine disease (EPPO, 2013), leading to banning of export of citrus fresh fruit from Ghana.

Lawson (2014) studied the aetiology, importance and controlled PFLS using a combination of carbendazim (40 g) and mancozeb (30 ml) fungicides per 12L of water in Ghana. For effective control of PFLS one has to repeat the fungicides application every four weeks starting from leaf flushing up to few weeks to harvesting. This has made control of the disease dependent on the use of synthetic fungicides with its consequent adverse effects on human health, the ecosystem, and the retention of chemical residue in the fruits. Even though this disease is not new in Ghana, little is known about its epidemiology. There is the need to study its epidemiology to determine the critical infection period (s) of the disease to aid in reducing drastically the number of fungicide applications per the fruit phenology. This could

reduce the pesticide residue in the fruit making it safer for human consumption as well as rendering the production of citrus environmentally friendly.

The world is now moving from chemical control to biocontrol of pest and diseases because of the pesticide residue problems of food safety. This calls for the need of an alternative control measure to replace the carbendazim and mancozeb, currently in use plant extracts with antifungal properties against vegetative growth of *P. angolensis* in the laboratory was accessed with the view to future prospect of managing PFLS under field conditions in Ghana.

The general objective of this work was therefore to study the epidemiology of PFLS disease of citrus in the humid semi-deciduous tropical rainforest of Ghana where citrus is produced and to assess the potential of some medicinal plant extracts for use as biofungicides for control of the disease.

The specific objectives were to:

1. Determine the critical infection period of the disease.
2. Assess the inhibitory effect of five medicinal plant extracts namely; *Zingiber officinale, Allium sativum* (L.), *Moringa oleifera, Azadirachta indica,* and *Carica papaya* on mycelia radial growth of *P. angolensis*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The citrus plant. Its origin, distribution and challenges

2.1.1 The citrus plant

The citrus genus is known to be the largest in the family of *Rutaceae* and subfamily *Aurantioideae*. It is also known to be a highly patronized horticultural product in the world with high demand. Citrus are evergreen flowering plants with various sizes with reference to the species and cultivar, and tree height ranges from 3-15m (Manner *et al.,* 2006). Citrus grows in the tropics and subtropics without irrigation with varying yields depending on variety and growing conditions. Mature trees of oranges and grapefruits can bear fruits of various shapes, which have high quantity of juice fragrance and flavour in commercial situations. (Manner *et al.,* 2006; MOFA, 2007). Citric acid is contained in the juice together with complex mixed with other acids, oils, and sugars, giving it the characteristic usual flavor (Albrigo and Carter 1977; Ranganna *et al*., 1983). There is always a variation in the total sugar and acidity levels during maturation of the fruits and this results in high sugar and low acidity. This change makes the consumption of this fruit more pleasurable and hence the sweet taste. Colour change and increase in fruit size is also very conspicuous in matured fruits. These changes also account for the sour or acidic taste one will get from consuming an unripe citrus fruit.

The dietary significance of citrus fruits extends far beyond the supply of vitamin C as many people generally think (Nagy and Attaway,1980). Citrus fruits are also known for their abundance of macronutrients, such as easy sugars and nutritional fibres, and are a source of many micronutrients, including folate, thiamine, niacin, vitamin B6, riboflavin, pantothenic

acid, potassium, calcium, phosphorus, magnesium, and copper, essential for maintaining health and normal growth (Rouseff and Nagy, 1994; Economos and Clay, 1999). Citrus has a very low average energy value and is free of sodium and cholesterol, which may be relevant for consumers concerned about weight-related issues (Guthrie *et al.,* 1995).

2.1.2 Origin and spread of citrus to other parts of the world

Citrus is a significant and popular fruit of southeastern Asia (Swingle, 1943; Webber et al., 1967; Gmitter and Hu, 1990). Even though these uncertainties about this location, researchers believe that the origin of citrus dates back to 4000 BC, (Ceiba-Geigy, 1975). It is speculated that ancient Chinese documents show that earlier reference to citrus was during the reign of Ta Yu, popularly known as Yu the great (around 2205 to 2197 BC) when citrus fruits, especially mandarins and pummelos, were considered highly prized tributes and were only available to the imperial court (Webber, 1967; Nagy and Attaway, 1980). A gradual process with many actors has been the distribution of citrus to other parts of the world. Some Arab traders are also thought to have used paths to Africa leading to the central Mediterranean basin while the crusaders took some of the fruits to Italy, Spain and Portugal around 1000AD (Scora, 1975). The Dutch merchants later introduced planting materials to South Africa in 1654 (Oberholzer, 1960). Citrus is presently grown in the world's tropical and subtropical areas in over 137 nations on six continents, producing annual world fruit industry revenues of about US$ 105 billion (Ismail and Zhang, 2004). Adverse weather conditions such as hurricanes, freezing, drought and diseases led to the introduction of citrus into some new places in the world such as Florida to Brazil to serve as an alternate production site in order to meet the high demand of North America and Europe (Plattner and Perez, 2012; Morton, 1987; Gmitter *et al.,* 1992). Currently, citrus is estimated to be the fastest growing tree crop

in Ghana with increasing demand for fresh fruits from both the domestic and regional markets (MoFA, 2011). In Ghana, annual production of citrus is estimated at 550,000 metric tonnes and available data indicates that 20,000 metric tonnes citrus were exported in 2006 (GEPC, 2006). The citrus industry in Ghana is believed to employ about 10,000 smallholder farm families.

Citrus, like any other fruit is attacked by several pathogens that influence the quality and yield of fruits before and after harvest. Pathogenic fungi such as *Penicillium* spp. (blue and green mould) (Droby *et al*., 1989), anthracnose disease caused by *Colletotrichum gloeosporioides* Penz (Whiteside *et al*., 1988; Davies and Albrigo, 1994), and acidic rot caused by *Geotrichum candidum* Link ex Pers (Howard, 1936; Whiteside et al., 1988) are some of the significant post-harvest issues causing market losses and therefore industry constraints.

2.2 Global production, consumption and trade of citrus

Among total global gross citrus production, *C. sinensis* (sweet orange) take a heavy toll (about 2/3) of the harvested fruits (UNCTAD, 2004). Currently, eight of the ten known edible citrus species are commercially grown and five are of major economic importance (Salunkhe and Desai, 1984). More than 104 million tonnes of citrus is produced and about 15 million tonnes are traded annually (UNCTAD, 2004). The total area under citrus production in Africa is estimated at 1.3 million hectares; 44 000 hectares of which are in South Africa and 4 500 hectares in Ethiopia (FAO, 2010) (Table 1). After the introduction of citrus in Ethiopia (Seifu, 2003), citrus cultivation is spread across the nation (Lipsky, 1962).

Total world production of citrus was estimated at 116 million tonnes (Mt) in 2007.

Table 1: Table showing the area under cultivation and volumes of citrus produced in Ethiopia, Africa and South Africa compared with the rest of the world for the period 1985-2004

Country	Total area harvested (ha)	Growth of total area harvest (%	Total production Mt/year	Growth of production (%)	References
World	4,908,106-7,090, 356	30.8	64,053,474-103,685,840	37.6	FAO, 2004
Africa	1,009,277-1,325,135	23.84	6,821,085-11,088,509	38.5	FAO, 2004
South Africa	35,400-69 200	48.8	706,228-1,712,149	58.75	FAO, 2004
Ethiopia	3 115 – 4 800	35.1	23,600 –29,800	20.8	CACCE, 2003; FAO, 2004

Source: FAO (2004)

This production volume increased after 3 years to 122.5 million tonnes in 2010 with about 8.7 million hectares harvested; oranges were 50%–62% of the total area harvested and total production (FAOSTAT, 2007) (Fig. 1). In spite of this increase in production volume of citrus, the industry still faces various challenges throughout the world, especially in the tropical regions of the world. US lemon output (2011–2012) was 10.6 million tonnes, with Florida contributing 65 % and California 32 % (Plattner and Perez, 2012). Citrus production in the United States has declined, apparently due to negative climatic occurrences such as hurricanes, freezes, and drought. Over the past 30 years, there has been a steady rise in annual per capita citrus consumption.

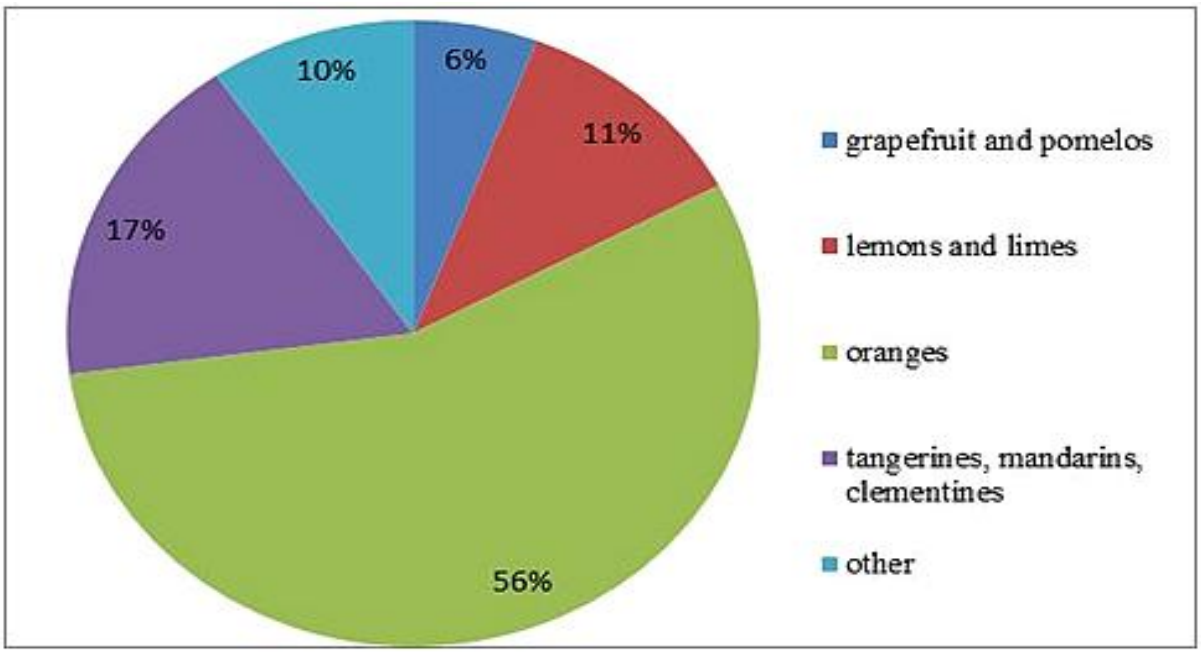

Fig.1: Estimated supply and consumption of citrus by region, per person per day, and the percentage of citrus consumed that is oranges and mandarins. (FAOSTAT, 2007).

However, in the least developed sub-Saharan Africa countries and Southeast Asia, (which usually have the largest percentage of malnourished people with nutrient

deficiencies (WHO, 2009), citrus consumption is also low. Due to high population growth, individuals in low-income African and Asian nations have struggled to withstand the increasing development of the citrus sector in China and are consuming about one-fourth as much citrus as advanced nations (FAOSTAT, 2007). From a statistical point of view, populations consume only 8 g / person / day in least advanced nations, which is six times lower than the world average production. Brazil is one of the world's largest orange growing nations, and output is found mainly in the port sector, which accounts for about eightieth of Brazil's production and about five hundredths of total global production (GAIN, 2010).

2.3 Sweet orange

Sweet oranges (*Citrus sinensis* L. (Osbeck) from the Family Rutaceae are the most preferred among all the other nine edible species of citrus due to its taste, nutrients, cosmetic, pharmaceutical and market value. Sweet orange is produced in many countries around the world especially in warm and tropical weathers. Among all the other edible species of citrus, sweet orange account for about 56% of the world production (FAOSTAT, 2007). Sweet orange contributed 49.6 million metric tonnnes to world production of oranges in 2016-2017 (FAOSTAT, 2007). Different cultivars of sweet orange have different periods or seasons of maturation. This results in the seasonal maturity names; early, mid and late seasons. The seed numbers per fruit is also one of the indices for classifying the different cultivars of this fruit. The seed number per fruit of sweet orange ranges from 0-30 and this varies in the different varieties. Ortanique has between 1-10 seeds, Parson brown (10-20), Pineapple (15-25), Red navel (0-6), Temple (15-20), Navel (0-6), Hamlin (0-6), Blood (4-10), Ambersweet (10-20) and Valencia (0-10).

2.3.1 Varieties of Sweet orange

2.3.2 Valencia (Late)

Valencia orange, a variety of sweet orange, which was first, hybridized by William Wofskill, an American agronomist and land developer in the mid-19th century on his farm in Santa Ana, South California, United States. Valencia is the world's leading sweet orange variety with a wide range of climate adaptability. The production of fruit is substantially less than the production of early varieties. It accounts for about 50% of the crop in Florida. Sweet orange is noted for its excellence in fruit quality, primarily due to the development of deep orange peel, juice colour and a high level of total soluble solid (TSS) (Gmitter *et al*., 1992; Davies and Albrigo, 1994). When compared to other varieties, Valencia's outstanding internal quality makes it attractive for both production and fresh markets.

2.3.3 Sanguinelli (blood orange)

This variety is not commercially cultivated in Florida because of the red flecks of pigment (anthocyanins) found in the flesh of the fruit late in the season. As the development of the pigment depends on the extended periods of cool weather, the fruit of this variety is more likely to attain the greatest amount of red pigment in the northern areas of Florida. According to (Ziegler and Wolfe, 1975, Gmitter *et al.,* 1992; Davies and Albrigo, 1994), red pigmentation differs with weather and can be severe when growing blood oranges in temperature-sensitive areas. In spite of the drawback, the variety is equally economically important as others due to their commercial value.

2.3.4 Hamlin

Hamlin was known to be a seedling by chance in an orchard or grove planted in the 1879 near Deland by Judge Issac Stone, which was later, purchased by A.C. Hamlin, hence the name (Morton, 1987; Gmitter *et al*., 1992). Hamlin became very popular during the 1894-95 freeze and gradually replaced 'Parson Brown' as Florida's major early season cultivar. It became Florida's most commonly cultivated sweet orange early-season variety. Hamlin can generally be harvested before freezing occurrences take place in Florida. Based on pounds of solids per acre, it is the most productive orange due to its elevated yield, although solids per box are smaller than for midseason varieties and Valencia. Fruits of this variety are medium-small, round and contain few or no seeds in most cases. The tiny fruit nature of this variety, especially during heavy crop years, can be an issue for the new fruit market. Although this variety's fruit quality is lower than many other cultivars, its elevated productivity, fewer or no seeds and early fruiting makes this cultivar popular in Florida (Ziegler and Wolfe, 1975; Gmitter *et al.,* 1992).

2.3.5 Navel Oranges

Navel oranges are mostly grown in the Mediterranean region, before they were introduction to Brazil by the Portuguese. It was introduced in 1870 to the United States. They are suitable for Mediterranean and subtropical environments where excellent external and internal color is obtained. According to Ziegler and Wolfe, 1975, ' Washington navel, ' during the nineteenth century before its introduction to the United States in 1870 was the most economically significant navel cultivar, which originated in Brazil as a mutation. Navel oranges are well noted for their fresh consumption since bitterness often developed when used for juice, making it unpalatable if not consumed within a short time (Ziegler and Wolfe, 1975; Gmitter *et al.,* 1992; Davies and Albrigo, 1994). Extreme tropical climates are not well suited for navel production, limiting its global adaptability. A tiny, secondary fruit inserted in the stylar end of the main fruit also characterizes them. Fruits are typically large, commercially seedless and early ripening. Navels have a distinct flavor, easy to peel and lower in acid content as compared to other varieties of sweet orange. The most popular among the navel oranges is the Washington navel, but there are a number of other selections such as Cara cara (red navel) available.

2.3.6 Pineapple sweet orange

Pineapple sweet orange came from a seedling planted by Rev. J.B. Owens in 1860 near Citra, Florida. With good internal quality and juice colour, this variety was the leading midseason variety. Unlike Hamlin, seedy, well-sized pineapple sweet orange fruit grows with a reddish-orange color.

2.4 Orange juice production and market

In a general context, juice is described as the extractable cell or tissue liquid content (Merriam-Webster, 1981). Where cells or tissues in this regard is limited to only plants and orange for that matter. The juice sector is the largest end user / consumer of fruit juices and concentrates "semi-finished form." According to statistics, world citrus consumption per capita is estimated to be 12.2 kg per year for oranges, 1.8 kg lemons and limes and 0.7 kg grapefruit (Ismail and Zhang, 2004). Convenience is one of the driving forces behind the fast growth of this industry. One can easily hold, drink a can of bottled fruit drink anytime anyway but this is not the case for fruit consumption. Orange juice is one of Brazil's primary production chains, representing almost 17.8 million tonnes of annual manufacturing. Records show that 1.0 million tonnes of orange juices were produced in 2009, which is equal to 57% and 80% of world exports (Deser, 2009). Florida is the biggest producing state of orange juice in the United States, and likewise São Paulo is Brazil's largest producer. The worldwide market for commercial citrus juice consumption is growing exponentially fast and this could be due to convenience. In 1995, about ten billion gallons were consumed which resulted in a market of more than US$ 35 billion in sales. In the year 2000, a growth of about 25% was seen in this number. USA alone consumes 1/3 of total markets of fruit juice worldwide (Tillotson, 1997). Orange juice processors in Florida and São Paulo control an average of 89% of the U.S. market, while processors in São Paulo provide an average of 84% of the European market (Foreign Agricultural Service, 2012). The biggest importers of orange juice in the world are the United States, Canada, Japan, Russia and China (USDA, 2009). This is quite surprising because Florida is the major producer of citrus which contributes to the US world production in volume. This could imply that the demand for the

juice is higher than the supply. About 30% of Ghana's annual food harvest is wasted due to the lack of storage facilities and underinvestment in the nation's food processing industry (Boapeah, 1993). The high production of perishable fruits in Ghana and failure on the part of the Government in establishing processing firms have made individuals use their own processing skills and techniques to process perishable fruits.

2.4.1 Citrus production in Ghana

In Ghana, citrus production is located in the Central, Eastern, and Ashanti regions and is progressively expanding towards the Western and Ahafo regions. The area of land under cultivation in both Ashanti and Eastern regions alone is estimated to be about 26,000 to 30, 000 ha (MOFA, 2007). Dewdney and Timmer (2009) found that Ghana has about 60,000 ha of commercial citrus mainly for domestic consumption and some for organic juice export to Europe. Citrus is a major cash crop and one of Ghana's most famous fruit plants (MOFA, 2007; Dewdney and Timmer, 2009). Fresh citrus fruits are eaten locally and large quantities are exported to neighboring countries such as La Cote d'Ivoire, Burkina Faso, Nigeria and Togo during the main and minor harvesting seasons (MOFA, 2007). About 90% of annual production in Ghana is consumed fresh whilst only 10% is processed into juice and concentrate for the local market and sometimes exports to Europe in the form of organic juice (GEPC, 2006). The average yield in million tonnes per hectare of orange trees under rain fed conditions in 2010 cropping season was 35.0 Mt/Ha (MOFA, 2011). Citrus cultivation is a significant source of income for many rural individuals as well as fruit retailers in Metropolitan regions and towns (*Asare-Bediako* et al., 2013). This is one sure way by which jobs can be created to reduce the alarming rate of unemployment in the country. Some citrus varieties grown in Ghana include Sweet orange (*Citrus sinensis*) and

Tangerine (*Citrus reticulate*) (Blanco). Other available varieties are Grape fruits (*Citrus paradisi*), Lemons (*Citrus limon*), Lime (*Citrus aurantifolia*), Tangors, Tangelos and Ortanique (AILAP, 2006). The following orange varieties are cultivated in Ghana periods of maturation: Early maturing (August – October) Ovaletto, Skkan; Mid-season (October – January) Obuasi, Mediterranean sweet and Red Blood; Late maturing (March – April) Late Valencia, Olinda and Frost Valencia (AILAP, 2006). Red Blood oranges and Valencia oranges are the predominant varieties found in Boamadumasi in the Ejisu - Juaben Municipality. The Blood and Valencia oranges are popularly known as Bar red and Water neck respectively by the farmers in this locality.

The University of Ghana Forest and Horticultural Crops Research Centre (FOHCREC)-Kade was established in 1957 to provide resistant rootstocks for study into quality citrus fruit cultivation and disease management, particularly viral diseases such as Citrus Tristeza Virus (CTV). These rootstocks for the experimental trials at the Centre were obtained from local varieties gathered from other agricultural stations and farms in the country. FOHCREC over the years has been very instrumental in the rapid development of the citrus industry in Ghana by researching into citrus fruit quality, diseases and by supplying quality planting materials especially the non local varieties to over 90% of farmers in Ghana in collaboration with NGOs and others (MOFA, 2007).

Nonetheless, the citrus industry in Ghana is relatively underdeveloped despite its agro-economic and nutritional value. The production and development of the crop in the country is confronted with many problems especially, with pests and diseases. In sub-Saharan Africa, citrus production is severely hampered by the pathogen *Pseudocercospora angolensis* (Seif *et al.,* 1984b). Yield losses due to this pathogen can reach 50%-100% (total loss of crops) if climatic circumstances are favourable for the growth of the causative fungus and timely efficient control measures are not taken (Seif, 1994).

2.4.2 Importance of citrus to Ghana and the economy

Citrus in Ghana serves both as a foreign exchange earner (for the country) by exporting fresh fruit to neighboring countries such as La Côte d'Ivoire, Burkina Faso and Togo, as well as an alternative source of income for local individuals through jobs and commerce. Ten thousand, seven hundred and twenty-nine (10,729) metric tons of fresh oranges were exported from the country in 2010, generating US$ 654,000 in foreign exchange (MoFA, 2011). The citrus industry in Ghana is also believed to employ about 10,000 smallholder farm families excluding those in the retail of processed citrus products. Ghana produces approximately 250,000 tonnes of citrus per year, representing 40% of fruit production and 36% of citrus exports. Ghana's citrus industry is the country's largest exporter of fresh fruit, worth more than 200 million GHS per year (Sakyi, 2010). Citrus contains several omnipresently found carotenoids (tetraterpenoids) in crops; more than 600 carotenoids were recognized and about 50 are present in human diets (Britton, 1995 ; Khachick *et al.,* 1997). Carotenoids have many uses and health benefits, some of which have been described as serving as antioxidants and these have beneficial effects on the immune system (Britton, 1995; Katsuura *et al.,* 2009; Matsumoto *et al.,* 2007), encourage bone development and health (Uchiyama and Yamaguchi2004), boost cell-to-cell interaction (Stahl *et al*., 1998), improve eye health (Stahl *et al*., 1998). Worldwide vitamins are estimated to have deficiency affects on about 209 million women and children and are the leading cause of preventable blindness (WHO, 2009). The healthier the people the healthier the nation and that culminates to a strong economy. Oranges have a lot of health benefits that helps improve the health condition of the people of Ghana and the economy as well. Oranges are known to contains vitamins such as riboflavin, thiamine, niacin, folate, vitamin A, vitamin B6, and vitamin B5 in addition to the vitamin C. The mineral content of orange peels consists of 10 mg of calcium and tiny amounts of iron, magnesium, potassium, phosphorus,

zinc, copper and selenium (Gargulinski, 2011). The quantity of sweet orange and the values exported from Ghana in 2000 and 2009 are undeniable facts that the citrus industry has a tremendous economic influence (Table 2).

2.4.3 Challenges in citrus production in Ghana

Citrus production in Ghana is confronted with numerous challenges, which in turn impede the development of the industry and the country's economy. Among the various challenges are: lack of certified bud wood and rootstocks, damage caused by insect pests (largely fruit flies), land tenure systems, limited extension services, lack of ready market and fungal, viral and bacterial disease (citrus spot) (MOFA, 2007).

Gummosis, Tristeza and Fruit spots are among the country's known citrus diseases, a major threat to Ghana's citrus industry (MOFA, 2007). The emergence of a new spot disease is a serious threat to the industry. The devastating effect of the disease on citrus trees in Ghana has led to individual farmers quickly replacing their citrus trees with other cash crops predominantly oil palm, Para- rubber and cocoa in the major citrus growing areas such as Kwaebibirem Municipality.

Table 2. Sweet orange export volumes and value for Ghana from 2000 – 2009

Year	Volume exported (MT)	Value (USD$)	Year	Volume exported (MT)	Value (USD$)
2000	1,243	248.8	2005	5,846	3,865
2001	1,336	126.4	2006	6,283	462
2002	15,474	671.9	2007	3,674	333
2003	4,304	329.1	2008	10,991	1,647
2004	741	3.9	2009	11,028	875

Source: GEPC (2009).

2.5 Citrus fruit diseases

Most of the post-harvest losses and decline of citrus fruits can be attributed to diseases occurring either between flowering and fruit maturity during post harvest handling or cultural methods and storage practices. Nearly all infections during preharvest are primarily induced by fungal pathogens such as *Phytophthora* spp. *Botrytis cinerea* Pers ex Fr, *Diplodia natalensis* Pole-Evans, *Phomopsis citri* Faw, and *Alternaria citri* Ellis and Pierce (Browning *et al*., 1995; El-Ghaouth *et al*., 2002). Citrus farmers in various parts of the citrus producing regions are faced with many production constraints and challenges. These challenges do not only include diseases and pests but also other factors such as lack of adequate knowledge or information regarding the orchard management, high cost of transportation due to poor road infrastructura or network, lack of inputs, low prices and poor

marketing strategies. Even though all the above-mentioned constraints can be managed at a considerable rate, pests and diseases will take more input to manage, which often results in greater yield loss and increase in the cost of production. Worldwide, post-harvest fruit and vegetable losses were estimated at 25% annually (Wisniewski and Wilson, 1992). This proportion is likely to increase in developing nations to 50% where there is insufficient crop protection and inappropriate fruit handling (Coursey and Booth, 1972). In Ethiopia such an estimation is considered to be cautious. A larger proportion of this could be expected due to poor handling, the lack of cool storage facilities and insufficient post-harvest treatment (Eyob, 1997).

In Ghana, most the citrus diseases farmers encounter are; *Alternaria* rot, Anthracnose, *Armillaria* root rot, Citrus Canker, Citrus Blast, Black spot, Blue Mould, *Botrytis*, Exocortis, Greasy spot, Greening, Gummosis, Melanose, Post bloom fruit drop, Psorosis, Scab, *Septoria* Spot and Tristeza.

2.5.1 Major citrus postharvest diseases, epidemiology and control

2.5.2 Citrus Canker disease

Citrus canker (CC), caused by the pathogenic bacterium *Xanthomonas axonopodis*, is a serious disease in the family of most commercial citrus varieties and some other species. Citrus canker's Asian, or A-strain, is believed to have originated in Southeast Asia or India (Civerolo, 1984) and spread throughout the rest of the world to Japan, Southern and Central Africa, the Middle East, Australia, New Zealand, Pacific Islands, South America and the Southeast United States (Gottwald *et al*., 2002b). Citrus canker has been eradicated from countries such as South Africa, Australia, the Fiji Islands, Mozambique, New Zealand and

the USA (Koizumi, 1985). In Uruguay and Brazil, active eradication / containment programs are being implemented. Citrus canker is the most destructive of all agricultural pests and diseases that threaten citrus (Das, 2003) (Fig. 2).

In 1984-1986, about 20 million seedlings were destroyed in Florida at a cost of over $ 25 million as a result of the bacterial disease (Schoulties *et al*., 1987). The main source of inoculum citrus canker is cankerous leaves, twigs and branches. The pathogen can survive for about 6 months in infected leaves (Rao and Hingorani, 1963).

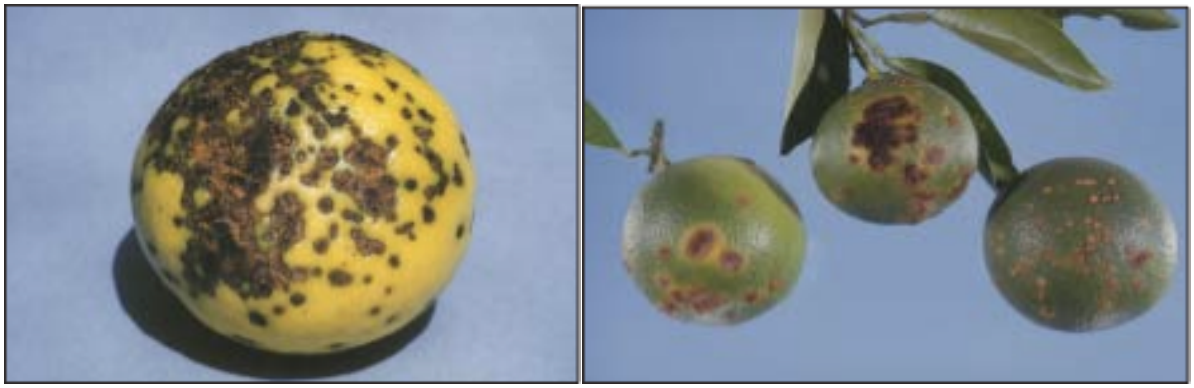

Fig.2: Fruit symptoms of Citrus canker on sweet orange (left) and grapefruit (right). Photo credit (left) x 0.5: Jeffrey Lotz

2.5.3 Symptoms and infection process

All young and old above-ground tissues of citrus are susceptible to *Xanthomonas axonopodis*. The bacterial pathogen enters plant tissues through natural openings (stomata) and wounds, as bacteria cannot penetrate intact skin like in the case of fungi. The earliest symptoms on leaves appear as slightly raised tiny blister-like lesions within a maximum of one week (7 days) after inoculation under optimum temperatures between 20-30°C in the presence of water (Koizumi, 1985). Under less optimum conditions, symptoms may take more than 60 days to appear (Loucks, 1934; Goto, 1992). As the lesions age, they turn tan to brown, and water-soaked margins appears surrounded by a chlorotic halo. The center of the

lesion becomes raised and corky. Lesions are usually visible on both sides of a leaf. Eventually, the centers of leaf lesions become crater-like and may fall out, creating a shot-hole effect. Defoliation and twig dieback become a problem as the disease intensifies on a plant. Early fruit drops and blemish are the major economic impacts of the citrus canker disease. Wind-driven rain is the primary dispersal mechanism for Citrus canker, similar to other bacterial diseases. It is believed that a drop of rainwater can carry a maximum load of bacteria of about 10^7 to 10^8 cells. Inoculum-contaminated equipment and hands can transmit *Xanthomonas axonopodis*. Long-distance spread normally occurs by movement of infected or exposed citrus plant materials, but circumstantial evidence points to occasional long-distance transport by unusual storm events such as tornadoes and tropical storms.

The pathogen is not systemic in the host plant. Citrus canker bacterial can remain viable as long as the host cells in the vicinity of the lesion remain viable, though the bacterial titer eventually drops considerably. However, reports on inoculum longevity outside host tissue are inconsistent. *Xanthomonas axonopodis* may persist for several weeks on non-host plant material, with some exceptional reports of longer persistence (about 8 months) in the root zone of certain grasses under infected trees in Japan (Goto *et al.,* 1975). This prolonged persistence of this pathogen can be related to the intact polysaccharide slime coating on the cells per 5ml. Once the citrus plant is infected, exposed leaves or fruit drop to the ground, the bacterial population declines to a non-detectable level in 1-2 months because of antagonism and competition with saprophytic microorganisms (Goto, 1992). In most areas of the world where Citrus Canker is endemic, disease management and control methods involve the use of resistant varieties, windbreaks to hinder inoculum dispersal and timely applications of copper-containing bactericides.

2.5.4 Citrus Melanose

Citrus Melanose is one of the most commonly observed diseases of citrus fruits worldwide. It occurs in many citrus growing regions of the world and infects many citrus species. It affects young leaves and fruits of certain citrus species or varieties when the tissues grow and expand during extended periods of rainy or humid weather conditions (Scot, 2008). Citrus Melanose is caused by the plant-pathogenic fungus *Diaporthe citri* (anamorph = *Phomopsis citri).* Citrus Melanose can create severe fruit rind blemishes, but does not normally affect the pulp. On leaves, the small, black, raised lesions are often surrounded by yellow halos, which can cause leaf distortion in most cases. On fruits, the disease produces a superficial blemish, which is unlikely to affect the overall yield of processing fruit, but causes external blemishes, which reduce the fresh aesthetic value of fruit intended for the market. This disease is generally of minor economic importance on foliage but is significant on the fruits due to the reduction in aesthetic, (Whiteside, 2000; Scot, 2008). The disease typically attacks sweet orange (*Citrus sinensis),* grapefruit (*C. paradisi)* and pummelo (*C. grandis).*

Diaporthe citri is a fungus that causes two distinct diseases on Citrus species. In the perfect (teleomorphic) stage, this fungus causes melanose, disease characterized by superficial fruit and foliar lesions, and in the imperfect (anamorphic) stage, it causes *Phomopsis* stem-end rot, a post- harvest disease of this economically important crop (Whiteside, 1988). Melanose on leaves results in ruptured cuticle and an exuded gummy substance, which turns brown and hardens. Severely infected leaves become pale green to yellow, can be distorted and fall from the tree with time. The yellowish margin disappears leaving the hardened gummed areas with a sandpaper-like texture. Infected areas on the leaf may be scattered, aggregated, or in streaks, depending on the location of water transported by the inoculums prior to

infection. Melanose on fruits produces scattered specks while young fruits remain small but abscise prematurely with time. This disease affects young leaves and fruits of other citrus species when the tissues grow and expand during extended periods of rainy or humid weather conditions (Scot, 2008). Melanose infects the entire genus Citrus but grapefruit and lemons tend to be more susceptible. Melanose infected fruits have a normal taste, even with the lesions on the rind, but their poor appearance reduces their market value (Whiteside, 2000; Scot, 2008). Ascospores are dispersed by wind over longer distances. The more dead wood that exists in an orchard, the more ascospores will be produced to serve as source of inoculum. Fruits become susceptible to infection from about 3–5 months after petal fall, depending on the area. Approximately 8–24 hours of continuous exposure to moisture on leaf or fruit surfaces is required for infection to occur, depending on air temperature (Scot, 2008).

Cultural treatments of pruning and burning dead wood reduces inoculum, is one way of reducing Melanose (Timmer and Kucharek, 2012). Even though pruning and burning may be carried out consistently, fungicide spraying will still be required to produce blemish-free fruit for good market value purposes. Effective control of Citrus Melanose can be achieved by using copper products or strobilurins (Timmer, 2003, 2012).

2.5.5 Greasy Spot

Greasy spot is one of the common diseases of citrus of economic importance, which reduces the aesthetic market value. The disease is caused by the fungus *Mycosphaerella citri* Whiteside. (anamorph, *Stenella citri-grisea* Fisher). It is known to be one of the most important foliar fungal diseases of citrus in the Caribbean Basin, including Florida, the

Caribbean Islands, Eastern Mexico, and Central America (Hidalgo *et al*., 1977; Timmer and Gottwald, 2000; Mondal and Timmer ,2006). *Mycosphaerella citri* infect both leaves but also fruits and produces greasy spot rind blotch that affects the external quality and marketability of the fruit (Timmer and Gottwald, 2000). Greasy spot affects all species of citrus but is most severe on lemon and grapefruit. *M. citri* produces ascospores in pseudothecia in the decomposing leaf litter (Whiteside, 1970; Timmer *et al*., 2001). Airborne ascospores are spread by wind and sometimes rain to the surface leaves of the citrus plant. They then germinate and mycelium grows epiphytically for weeks prior to penetration through the stomata (Whiteside, 1970; Timmer and Gottwald, 2000).

Initial symptoms appear on leaf as yellow mottles on the lower surface of the leaves. Growth of hyphae inside the leaf tissue causes cellular swelling, which results in blister formation on the lower leaf surface. These infected leaves often drop before the development of a dark greasy appearance on the lesions. Susceptible varieties (lemons and grapefruit) show early symptoms than on Valencia oranges and tangerines (Timmer *et al*., 2001). Infected fruit has tiny points black specks, which occur between the oil glands. On susceptible host such as grapefruit, larger and coalescent specks are sometimes produced giving rise to greasy spot rind blotch.

Another serious characteristic of greasy spot rind infection is that living cells adjacent to the specks often retain a green colour for much longer than normal (Timmer *et al*., 2001). Relative humidity from 90% and above is essential for ascospores germination and growth of germ tube. Longer periods of leaf wetting (6 hours at most) or high humidity with temperatures high enough to permit leaf penetration which occur almost nightly from June to early October, but are less frequent at other times of the year are mechanisms that trigger infection of the pathogen. Foliar susceptibility to the infection is usually throughout their

lifetime (Timmer *et al*., 2001). Due to the deep penetration and superficial growth of the fungus, single fungicides application not only protects the leaf from future infections but also kills the superficial fungus growth already present (Timmer *et al*., 2001).

2.5.6 Citrus Black Spot

This disease is caused by the plant pathogenic fungus *Guignardia citricarpa* Kiely; anamorph - *Phyllosticta citricarpa* (McAlpine) van der Aa (syn. *Phoma citricarpa* McAlpine); Teleomorph-*Guignardia citricarpa* Kiely (Kiely, 1948a, 1949; Kotzé, 1981, 2000). *Guignardia citricarpa* Kiely originated collectively with its host, *Citrus* L., from South East Asia (Smith *et al.,* 1997). First report of this disease was in Africa in the year 1929 in South Africa (Doidge, 1929). Today, the disease is widespread across the world and occurs in Argentina, Australia, Bhutan, Brazil, China, Ghana, India, Indonesia, Kenya, Mozambique, Nigeria, Philippines, South Africa, Swaziland, Taiwan, United States (Florida), Uruguay, West Indies, Zambia and Zimbabwe (European Union, 1998; Baayen *et al.,* 2002; Paul *et al.,* 2005; Lemon and McNally, 2010; Schubert *et al.,* 2010). Despite the widespread of this disease, no reports have been made from Mediterranean countries (Schutte, 1995; Kotzé, 2000). Yield losses and cost for chemical control can be significant (Kotzé, 1981; Smith *et al*., 1997). Citrus black spot has a wide host range and for that reason affects all species of cultivated citrus and their hybrids, with the exception of sour orange and its hybrids (Kotzé, 1981).

All commercially grown citrus species and cultivars have been observed to be susceptible to the disease. Lemon on the other hand is particularly susceptible, and has been observed that when citrus black spot appears in an unaffected area, its symptoms are first observed on lemons (Kotzé, 2000), with the exception of the epidemic in Florida. Generally, infections

remain in the latent phase until the fruit is mature (Kiely, 1949; Kotzé, 1981). At this stage, the fungus may grow further into the rind producing several black spot symptoms months after infection, throughout maturity and harvest. *Guignardia citricarpa* appears as sunken lesions with varying size, shape and colour, depending on stage of fruit or leaf development. It is the fruit that suffers the greatest damage from black spot. A yellow halo can be associated with the lesions (Wager, 1952). Pre-mature fruit drop may occur as a result of severely necrotic infections (Kotzé, 2000). Fruit symptoms are broadly classified into three categories; hard spots, freckle spots and virulent spots (Kiely,1949). Pycnidia appear as small black dots, which are visible with a hand lens but can be confused with acervuli of *Colletotrichum* spp (Kotzé, 1981).

2.5.6.1 *Pseudocercospora* fruit and leaf spot

This disease is a major phytosanitary disease of citrus caused by the pathogenic fungus *Phaeoramularia angolensis*, (Kirk, 1986). This disease causes premature abscission and defoliation of affected trees. On the other hand, young leaves and fruit appear to be more susceptible than older mature leaves (Sief and Hillocks, 1999). Affected fruits ripen prematurely and drop or dry up and remain on the tree (Kuate, 1998). Seif (1995), highlighted the economic importance of the *Pseudocercospora* fruit and leaf spot and its devastating effect on citrus. The most devastating effects of the disease on citrus are premature abscission of young fruits and leaves, and the development of fruit lesions, which render the fruit unmarketable (Seif and Hillock, 1993). *Pseudocercospora* fruit and leaf spot is known to be a serious production constraint causing about 20-100% yield loss to farmers especially in highlands areas (of above 200 m elevation), where the disease incidence and severity are very high (Seif and Hillocks, 1993; Diarri-Diallo, 1995; Kuate, 1997; Kuate *et*

al., 2002). The severe disease outbreak during the late 1980's led to farmers in Trans-Nzoia, Kenya, to replace their citrus with cereals and vegetables (Seif, 1995). This disease is also known to affect the yield and quality of essential oils (Kuate *et al*., 2003). Citrus leaves, fruit peels and flower petals, organs contain valuable essential oils that can be extracted using various techniques (Huet, 1991).

2.5.6.2 Origin, history and distribution of *Pseudocercospora* fruit and leaf spot disease of citrus

This disease was first reported in Angola and Mozambique in 1952 (De Carvalho and Mendes, 1953). It spread to other parts of Africa since its first report Table 3). The disease is somehow restricted primarily to the humid tropics in Africa between altitudes of 80 and 1500 m (Brun, 1972; Seif and Kungu, 1989). The disease is reported in Yemen as the only country outside the Africa continent where the disease is found (Kirk, 1986; Seif, 2000; Pretorius, 2005). The species was first described as *Cercospora angolensis*, causing a leaf spot on *Citrus sinensis* in Angola (De Carvalho and Mendes,1953). It was later reported by Emechebe (1981), as *Phaeoisariopsis* sp. on citrus in Nigeria and from other citrus-growing areas in Africa (Seif and Hillocks, 1993). *Cercospora angolensis* was later transferred to the genus *Phaeoramularia* by *(*Kirk, 1986) due to the presence of pale-brown conidia produced in chains and the scars at the conidiogenous loci being conspicuous and slightly pigmented (Pretorius *et al*., 2003). (Braun, 1999) assigned it to a new genus, *Pseudophaeoramularia*, because the scars on the conidiogenous cells were unthickened, i.e. their conidiogenous loci do not fit with those of the former genus *Phaeoramularia* (now *Passalora* emend (Crous and Braun, 2003). Crous and Braun (in Pretorius *et al*., 2003) used the technique of molecular analyses and reassess conidiogenesis and the structure of the conidiogenous loci.

It was also established that the conidiophore morphology is not distinct from that of the genus *Pseudocercospora*.

The disease as reported by EPPO (2009), is an A1 quarantine pest for Europe and the Mediterranean region, and also of concern for other warm humid regions where citrus is grown, such as Florida, USA. *Pseudocercospora* fruit and leaf spot disease of citrus has now spread to about 21 countries in sub-Saharan Africa, including Yemen in the Middle East (Yesuf, 2001). The disease is currently present in different countries in Africa and as shown in (Table 3).

Table 3. Chronology of occurrences of *Pseudocercospora* fruit and leaf spot of citrus in different African countries.

Country	Year	Reference
Angola	1952	De Calalho and Mendes (1952)
Mozambique	1952	De Calalho and Mendes (1952)
Zaire	1966	Brun (1972)
Central African Republic	1968	Brun (1972)
Cameroon	1969	Menyonga (1971)
Gabon	1971	Brun (1972)
Congo	1971	Brun (1972)
Cote d'voire	1972	Brun (1972)
Togo	1972	Brun (1972)
Zambia	1973	Kirk (1986)
Nigeria	1978	Emechebe (1981)
Burundi	1980	IAPSO (1985)

Zimbabwe	1982	Maramba (1982)
Uganda	1983	Kirk (1986)
Kenya	1984	Seif and Whittle (1984a)
Comoros	1985	Kirk (1986)
Yemen	1986	Kirk (1986)
Tanzania	1990	National Agricultural Research Laboratories, Kenya
Ethiopia	1990	Eshetu (1995)
Republic of Guinea		Kuate (1998)
Ghana	2013	Brentu *et al.,* (2013)
Rwanda		Kuate (1998)
Sierra leone	2010	Harling (2010)

Source: Modified after Seif, A.A. and Hillocks, R.J. (1998) and Kuate, J. (1998)

2.5.6.3 Symptoms of the Disease

The disease has been observed to attack all known citrus species, which includes, grapefruit (*Citrus grandis* [L.] Osb), lemon (*Citrus lemon* [L.] Burn. F.), lime (*Citrus aurantifolia* [Christm] Sw), orange (*Citrus sinensis* [L.] Osb), pomelo (*Citrus paradisi* Macf.), and mandarin (*Citrus reticulata* Blanco) (Kuate, 1998; Pretorius, 2005). The susceptibility of

citrus species to the disease varies from one species and cultivars to another. Grapefruit, oranges, pomelo and mandarin are known to be highly susceptible, while lemon and lime are the least susceptible respectively (Seif, 1994; Kuate, 1998). Susceptibility may have the tendency to differ within the same cultivar between leaves and fruit, or with different periods of the year (seasonal change), or even with locations (Kuate, 1998). There has not been the report of any alternate host (Chung and Timmer, 2007).

2.5.6.4 Symptoms on Leaves

The pathogenic fungus produces circular, mostly solitary spots that are about 10 mm in diameter with light brown or greyish spots on leaves of citrus. These spots are usually surrounded by a prominent yellow halo as reported by (Seif and Hillocks, 1993; Kuate, 1998). According to (Emechebe, 1981) a single leaf can have about 40 lesions when citrus is heavily infected. Lesions on lower leaf surface sporulate more quickly than those on upper leaf surface and this could be due to the prevailing conditions (Emechebe, 1981). It is known that, leaf lesions produce more conidia than those on fruit and therefore constitute the main source of inoculum for primary and secondary infections in endemic areas (Seif and Hillocks, 1993). During the periods of rains giving rise to high relative humidity, the spots of young and new leaves may coalesce and resulting in general chlorosis. When the petioles of leaves are infected, it results in premature defoliation of the citrus plant. Brun (1972), cited by Kuate *et al.,* (2003), states that the disease symptoms show four developmental stages: The first stage has the appearance of a lighter point on infected leaves. In stage two, lesion becomes brown with appearance of a yellow halo within 4-6 days after infection is established. Stage three results in the central lesion increase in size to about 3 to 4mm within 25 to 30 days.

In the fourth and final stage, central lesion dries, leaving a circular hole on the leaf surface.

Kuate *et al*., (undated), however, reported that symptoms on young leaves develop in five successive stages; stage one: A depressed circular area appears on the leaf. This can be seen on both surfaces of the leaf. Stage two: The lesion becomes brown (tanning). Stage three: Appearance of a downy structure, black-grey in colour. These are conidiophores bearing numerous conidia. After scraping, they can be seen under a microscope. The beginning of a yellow halo can also be observed. Stage four: The central lesion has increased in size; tissues die in that area and yellow halo is already formed. Stage five: The centre of the lesion dries (grey in colour) and becomes surrounded by a brown crown. Sometimes, the centre of the lesion becomes perforated, leaving a hole in the leaf. Some lesions especially on grapefruit reach the last stage (5) within ten days.

Young lesions, at a glance look similar to those of canker (caused by *Xanthomonas campestris* pv. citri (Hasse) Dye), but differ in structure (Sief and Hillocks, 1993; Kuate, 1998). Even though Canker lesions on leaves also produce a yellow halo, they are distinguished by a water-soaked margin around the spot (Brlansky, 1988), as the flat lesions caused by other bacterial pathogens of Citrus (Duan *et al*., 2009). Individual leaf lesions of *Pseudocercospora* fruit and leaf spot would have some similarities with those induced by *Alternaria* species, but they tend to be more in quantity and join together. *Pseudocercospora* fruit and leaf spot affect almost all citrus species, but *Alternaria* sp. affects only some tangerines and hybrids (Chung and Timmer, 2007).

2.5.6.5 Symptoms on Fruits

Symptoms of affected fruits show circular areas are to irregular, discrete or coalescent, usually surrounded by yellow halos (Seif and Hillocks, 1993; Kuate, 1998; Chung and Timmer, 2007; CABI and EPPO, 2009; Kuate, Undated). Kuate, in 1998 reported that spots are mostly up to 10 mm in diameter (Kuate, 1998). However, Pretorius (2005) reported that

most spots measure up to 8 mm in diameter. Young fruits about the size of a golf ball are usually very susceptible to infection (Pretorius, 2005). Unlike leaves, which have reduced susceptibly with age, fruits can be attacked at all growing stages, (Yesuf, 2013). Infection often results in hyperplasia on young fruits, producing raised tumour-like growths surrounded by a yellow halo; which later develop central necrosis and collapse (Kuate, 1998). According to Pretorius, (2005), young fruits, symptoms often commence with nipple-like (tumour-like) swellings without a yellow halo. Affected fruits often ripen prematurely and drop or dry up and remain on the tree with time (Kirk, 1986; Kuate, 1998). In some instances, the lesions crack and exude fruit juice, which sometimes become sticky. Young leaves and fruits appear to be more susceptible than older mature leaves (Sief and Hillocks, 1998;1999), but the host species and variety varies the susceptibility (Bella-Manga *et al*., 1999) and location (Derso, 1999) as cited in CAB International, (2011). Usually, infected fruits are predisposed to secondary infections by *Colletotrichum gloeosporioides* Penz. and *Phoma* spp (De Carvalho and Mendes, 1952; Seif and Kungu, 1990), thus it is common to find a dark brown to black sunken margin of anthracnose around the fruit spots.

2.5.6.6 Factors influencing symptoms expression and disease severity on fruits and leaves of citrus

It is believed that conidia produced on previous year's lesions infect young leaves, hereby starting a new disease cycle (Emechebe, 1981). This could be because, during wet or humid weather, new disease-free flushes of leaves are formed, while older leaves may contain a varying number of non-sporulating lesions. These lesions sporulate 3-5 weeks after the start of the rainy season and symptoms on young leaves appear 2-3 weeks later. Prolonged wet weather conditions followed by dry spells, coupled with moderately cool temperatures of 22-26°C favour the spread of the disease (Emechebe 1981; Kungu *et al*., 1989; Seif and

Hillocks, 1993). In the absence of conidia, substantial moisture is required for their production and for new infections (Emechebe, 1981). The disease causes severe damage in humid regions at elevations above 200 m (Kaute, 1998). According to Diallo (2001), the disease was serious on trees of the highlands of Guinea, whereas the lowland areas appeared to be disease-free. The disease is serious at altitudes above 600 m in Kenya and severity is mostly high in Western Kenya (Seif and Hillocks, 1993). The fungus *Pseudocercospora* fruit and leaf spot grows well at elevations from sea level to 2000m. This means the lower the elevation, the higher the risk of infection by *Pseudocercospora* fruit and leaf spot. Hence, citrus plantations at elevations below 700-800 m are mostly at risk. Even though *Pseudocercospora* fruit and leaf spot causes damage everywhere, the disease incidence and severity vary (Ragazzi, 1997). Disease incidence varies with the amount of rainfall (Kuate *et al*., 1994).

2.5.6.7 Nature and Biotypes or Species of the Causal Pathogen

Leaves and fruits infection are most likely caused by conidia although the mechanism involved is unknown. The pathotype specialization of this fungus is also unknown (Ragazzi, 1997; Kuate, 1998). The pathogenic fungus is only known from its asexual stage (anamorph). If teleomorph exists, it is likely to belong to the Dothideales, possibly a species of *Mycosphaerella* (CABI and EPPO, Undated; Kuate, 1998). According to Ragazzi's (1997) report, variations in response to the disease, both among citrus species and within citrus cultivars suggested that certain degree of genetic variation in the fungus is possible. Survival mechanisms of the pathogenic fungus in natural conditions are unknown (Kuate, 1998). It is reported that the fungus probably survives in dormant lesions on infected material until the onset of conditions conducive to sporulation (CAB International, 2011).

2.5.6.8 Morphology of *Phaeoramularia angolensis*

The morphology of *Phaeoramularia angolensis* consists of the following; Conidiophores macronematous, mononematous and fasciculate or forming loose synnemata 12-45 µm wide, arising from a usually large stroma (30-60 µm in diameter), simple, septate, smooth, pale-brown to brown, (60-240) µm high, 4.5-7 µm wide. Conidia acrogenous, becoming acropleurogenous, solitary or catenate, borne in simple or branched chains of 2-4 conidia, cylindrical to narrowly obclavate, rounded at the apex, truncate at the base, straight or slightly flexuous to more or less curved, smooth, hyaline to very pale-brown, septate, 24-79 µm long, 4-5 (-6.5) µm wide at the base, the basal and, when present, the apical scar slightly thickened and pigmented (Kirk, 1986; CMI, 1986; CABI and EPPO, 2009) (Fig.3). The pathogenic fungus can be recognized on its host by dense tufts (synnemata) of light chestnut-coloured, multiseptate conidiophores, 27-240 by 3-7 µm, which arises from a stroma and emerge through stomata on the lower leaf surface bearing conidia singly or in chains of two or four. The conidia are hyaline, cylindrical to slightly flexuous, 1-6 (usually 3or 4) septate and 23-87 by 3-7 µm (Emechebe, 1981; Seif and Hillocks, 1993). In culture medium, the fungus grows well and sporulates on malt agar (DeCavalho and Mendes, 1952), orange fruit peel extract agar, orange leaf peel extract agar (Emechebe, 1981), V-8 juice agar (V8A), mycophyl agar (Ndzoumba, 1985) and on potato- dextrose agar (PDA) (Ndzoumba, 1985; Kaute and Fouré, 1988). However, it has been recently reported that the fungus is a slow grower on potato carrot agar (PCA), malt agar (MA) and carrot juice-PDA (cjPDA) incubated at 25^{o}C under continuous light (Seif and Hillocks, 1993). Colony surface on plates shows greyish colouration in appearance, often velvety with an elevation at the central point, forming a gnarled mat-like substance and a dark-green colouration beneath the colony when plate is turned upside down. The colony diameters of a 14-day old culture on PCA, cjPDA and MA were 5, 4, and 3.5 mm corresponding to daily growth rates of 0.61,

0.50 and 0.43 mm/day respectively with no sporulation observed. Ndzoumba (1985) on the other hand, obtained abundant sporulation of the fungus on PDA, V8A and mycophyl agar at 25°C regardless of the amount of light present (continuous light or alternating light and darkness). Conidia produced on artificial media were comparable to those formed on diseased tissues (Emechebe, 1981). On the contrary, the synnemata formed in artificial culture were more erratic than those produced on lesion surfaces. Conidiophores from young cultures were more loosely aggregated, while the synnemata produced in older cultures reassembled more closely than those produced under natural conditions.

2.5.6.9 Epidemiology

Information on epidemiology of the disease cycle of this pathogenic fungus is lacking (Emechebe, 1981; Kuate and Fouré, 1988; Seif and Hillocks, 1993; Kuate, 1998). Transmission of this disease is most likely to be via air-borne conidia or infected plant materials. Long-distance dispersal of the fungal spores is by windborne conidia (De Carvalho and Mendes, 1952). Rain splash or drops are known to be the primary source of local dispersal (Seif *et al*., 1989). Transportation of infected fruits and propagating materials has played some role in the dissemination of the fungus in Africa (CAB International, 2011). Even though insect transmission may occur; no report of such is recorded CAB International, 2011; CABI and EPPO, undated). It has also been reported that the disease is mainly restricted to the humid tropics in Africa (Brun, 1972), between altitudes 80 and 1500 m (Brun, 1972; Seif *et al*., 1989). Prolonged wet or humid conditions accompanied by dry spells favour development of the disease (Emechebe, 1981; Kungu, *et al*., 1989) coupled with moderately cool temperatures of 22- 26 ^{o}C (Kungu *et al*., 1989). During the start of the rains, new disease-free flushes of leaves are formed, while older leaves harbour a varying

number of non-sporulating lesions. Non-sporulating lesions begin to sporulate between 3 to 5 weeks after the start of the rains and new symptoms on young leaves become visible within 2 to 3 weeks later (Emechebe, 1981). This implies that conidia produced in the previous season infect the new flushes, which then begins a new disease cycle (Seif and Hillocks, 1993). Leaves become no longer receptive to the fungus after 5 to 8 weeks, but fruits remain susceptible throughout their development (Kuate and Fouré, 1988; Kuate *et al.,* 1994).

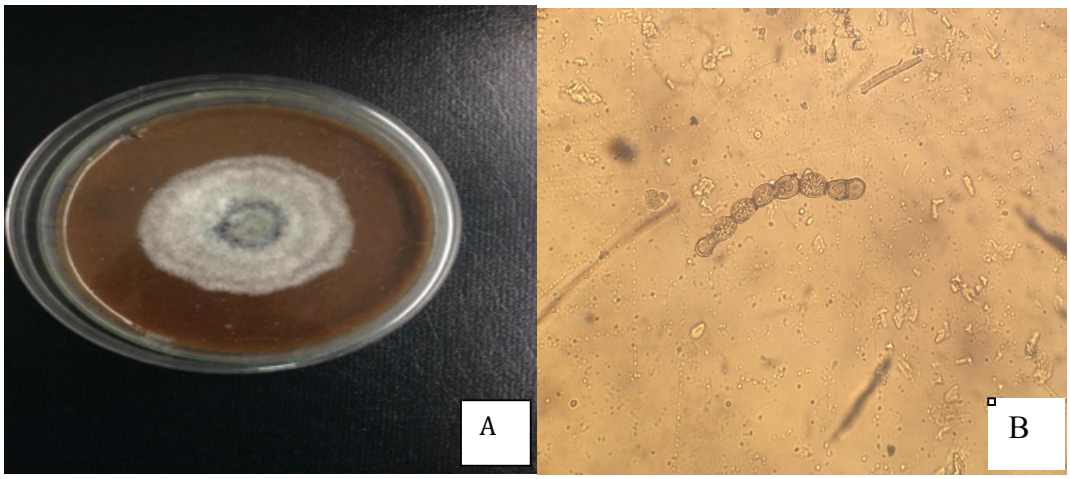

Fig.3: Cultural and morphological characteristics of the isolated pathogen obtained on Malt Extract Agar (MEA): greyish-whitish colony surface growth (A) × 0.5 and long chained conidia (B) × 400

2.5.7 Disease Management

It is expedient to prevent the introduction of a disease than to wait for it to be established before taking mitigation measures. *Pseudocercospora* fruit and leaf spot is considered an A1 level restricted plant pathogen in Europe (EPPO, 2009). Highly restrictive measures already have taken by most citrus-growing countries in the EPPO region for the import of citrus

from outside the region would certainly be screened by Quarantine Regulation to protect against the introduction of the disease (CABI and EPPO, Undated). Avoiding the transportation of infected seedling, fruits and other planting materials from contaminated areas is a key measure for mitigation the spread of the pathogen in and from Africa (Kuate, 1998). According to Vicent and García- Jiménez (2008), the relative aridity of the Mediterranean climate makes it unsuitable for wind-disseminated pathogens like *Pseudocercospora angolensis*.

2.5.8 Host Resistance

The use and development of resistant varieties would benefit growers with small orchards or a few trees, who cannot afford fungicide treatments Yesuf (2007). Citrus species and varieties of various kinds differ in susceptibility to the pathogen and the devastating effects of the disease (Kuate, 1998; Bella-Manga et al., 1999; Sief and Hillocks, 1999). Progress in this effort is hindered by an absence or unavailability of suitable resistant varieties, loss of apparent resistance in different ecological zones or in different seasons, and the need to evaluate the susceptibilities of leaves and fruits (Kuate, 1998). Genetic engineering could improve appropriate resistance by offering motivating solutions, using differences of susceptibility between species and cultivars. On the contrary, this procedure may be more costly and time-consuming than conventional breeding methods, but new hybridization and tissue culture techniques such as using somatic hybrids are promising (Kuate, 1998).

2.5.9 Cultural Control, farm hygiene and sanitation

The elimination of neglected orchards or infected tress to reduce inoculums is one effective way of managing the pathogen (Pretorius and Holtz, 2008). Below are some effective disease management practices recommended by Seif and Kungu (1989).

a. Discouraging inter-planting in affected orchards composed of mature producing

trees, fostering a microclimate of relatively cool temperatures and high relative humidity (RH), and thus preventing disease development.

b. All fallen fruits and leaves in affected orchards should be collected and destroyed by burying or burning. This may significantly decrease the inoculum population in the field.

c. Careful pruning of shoots to permit light penetration into and free aeration within the tree canopy, thus making the environment in the phyllosphere less favorable for disease development, i.e. shorter leaf-wetness period, lower RH, moderate temperatures.

d. Planting of windbreaks around the citrus orchards to decrease the impact of wind, which is the primary dispersal agent for spores.

2.5.9.1 Chemical control

The use of Perenox (cuprous oxide) and Benlate (benomyl) have proven to be the most effective against *P. angolensis* (Menyonga, 1971; Rey *et al*., 1988). Mancozeb, tridemorph, triadimenol and propiconazole were also found to be effective against the pathogen (Rey *et al*., 1988). The applications of Mancozeb in the wet season were not effective in Zimbabwe (Pretorius and Holtz, 2008). Resistant strains development may occur with the use of benomyl only (Kuate, 1998). The alternative application of Benomyl and copper-based fungicides may be applied at two-week intervals beginning a week after the onset of rains (Seif and Hillocks, 1993). In addition, three fortnightly treatments with copper-based fungicides, followed by one of benomyl, can be made when the fruits are of golf ball sizes (42.67mm). Wet and humid weather stimulates spore production and favours infection (Kuate *et al*., 1994) thus; spraying after rainfall, rather than on a fixed calendar is

recommended (Seif and Hillocks, 1997). The newer Triazole fungicides, Fluzilazole provided the best control of the disease in the field, but tebuconazole was not as effective as a copper hydroxide formulation (Seif and Hillocks,1997). Later report by Pretorius and Holtz (2008) showed that a Trifloxystrobin + Mancozeb + mineral spray oil combination, applied in November, January and March, provided the best control of the disease on foliage in Zimbabwe.

Chemical treatments using benzimidazoles are efficient, but too expensive and a high risks of resistance and negative effects on the environment Kuate (1998). Chemical application on citrus trees is a challenge for most small-scale farmers due to lack of application equipment like motorized sprayer and chemicals (Yesuf, 2001). Recent research has shifted their focus to the likelihood of using natural oils, which should be comparatively accessible, inexpensive, and environmentally friendly in place of synthetic chemicals. Extract of oils from the skin of fruits of more resistant species of Citrus: *Citrus latifolia* and *Citrus limon*, reduced the growth of an isolate of the pathogen more than the extracts of susceptible species (Jazet *et al*., 2002). Oil extracts from the leaves of two *Eucalyptus* species had minimum inhibitory concentrations of 6000 and 6500 ppm respectively (Jazet *et al*. 2008). Among the plants extracts reported so far, *Cymbopogon citratus* was most effective at inhibiting fungal growth in the laboratory, with activity at 600 ppm comparable to that of any known fungicide Tchinda *et al*., (2009).

The evidence of effective disease management with inorganic chemicals cannot be denied over the past decades. However, this effectiveness also comes with several deleterious effects, which causes more harm to humans, animals and the ecosystem (Segura and Abakerli, 1999). Non-judicious use and high frequency of application of inorganic chemicals has resulted in several high risks and health hazards, which results in ecological

imbalance, destruction of naturally biologically important microorganisms and increased level of pesticide residues in food cycle (Mahapatra and Tiwari, 1994).

2.4.9.2 Botanical control (the use of medicinal plant extracts)

Plant extracts of medicinal value have gained special recognition and significance in recent times as a strategy for combating diseases and a remedy for developing ecologically safe medicines for humans, plants and animals (Ranjana *et al*., 1999). Phytotoxicology is not a major issue of great concern in the application of plant extracts on food crops against pathogens because the plants and constituents are less phytotoxic, more systemic, bio degradable and induces the metabolism of host to resist pathogen infection through the production secondary metabolites (Malik, 1987; Gupta, 1997).

A number of higher plants have been identified to have active ingredients efficacious against fungi, bacteria and viruses of different families (Gohil and Vala, 1996; Ushiki *et al*., 1996; Hussain *et al*., 2000; Parveen and Kumar, 2000). The extracts of *Azadirachta indica* A. Juss, *Polyalthia longifolia* Benth and Hook, F., *Ocimum tenuiflorum* Benth and Hook, *Catharanthus roseus* L., and rhizome of *Zingiber officinale* L. inhibited growth of *Alternaria triticina* (Parveen and Kumar, 2000). It is also reported that extracts of *Allium sativum* L. bulb, *Aegle marmelos* L. leaf and *C. roseus* flower have inhibitory properties against the germination and mycelia growth of *Alternaria solani*, a pathogenic fungi causing tomato and potato blight (Vijayan, 1989). Extract from garlic and neem oil also reduced disease incidence of leaf blight in onion caused by *A. alternata* during a field evaluation (Kannan and Subbaraja, 1999). As small as 10% extract of *Lawsonia inermis* L., *Eucalyptus globulus* Labill., *A. indica* and bulb extract of *A. sativum* reduced damping off in chilli (Kurucheve and Padmavathi ,1998). The leaves, seeds, stem and roots of *Carica papaya* are known to be highly potent as antifungal, antibacterial as well as other ethnobotanical values

(Ekaiko, *et al*., 2015). The phytochemical properties of these plant parts are capable of suppressing the germination of spores and mycelia growth of fungi and other pathogenic organisms of economic importance. *Moringa oleifera*, a small evergreen tree crop has also been reported to have some bioactive compounds in the leaves responsible for the inhibition of *Saccharomyes cerevisae, Candida albicans* and *Candida tropicalis* (Patel *et al.*, 2014) (Fig.4). The use of plant extracts to control pathogenic fungi is more environmentally friendly than the use of chemicals, which does more harm than good.

Fig.4: Cultures showing inhibitory effects of Allium *sativum* (A), *Carica papaya* (B), *Azachdiracta indica* (C), distileed water with plate turned upside down to indicate the level of invasion of *P.angolensis* (D). × 0.5

2.5.9.3 Economic importance of *Pseudocercospora* fruit and leaf spot

Pseudocercospora angolensis is a citrrus disease of major economic importance, with the most devastating effect being the premature abscission of young fruits and leaves. Development of few lesions decreases the aesthetic nature of the fruits and therefore rendering it unmarketable. A high yield loss estimated between 50-100% is a common phenomenon in most disease-affected areas (Menyonga, 1971; Brun, 1972; Seif and Kungu, 1989; Seif and Hillocks 1993; Kuate, 1998; Chung and Timmer, 2007). Desiccation of shoots and loss of leaves can have a considerable devastating effect on the tree, which subsequently affects fruit yields (Kuate, 1998). Losses as a result of *Pseudocercospora* fruit and leaf spot infection in Kenya have been estimated at US$ 4 million per year (Seif, 1994). According to Yesuf (2001), complete crop failure has also been reported in severely attacked citrus orchards in Ethiopia mainly on grapefruit and sweet orange varieties. *Pseudocercospora* fruit and leaf spot is also known to be a serious production constraint causing 20-100% yield loss to growers especially in highlands (of above 200 m elevation) where the disease incidence and severity are very high (Kuate, 1998; Kuate *et al*., 2006). According to Huet, (1991), valuable essential oils can be extracted using a variety of techniques from citrus leaves, fruit peels and flower petals, organs attacked by this disease. Farmers in some areas of Ethiopia have abandoned citrus orchards or replaced them with other crops (Seif and Hillocks, 1993; Kuate *et al*., 1997; Kuate, 1998; Kassahun *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

This experiment was carried out in the Kwaebibirem Municipality where a designated field was set up and regular visits and survey were carried out. Major laboratory work on this research was done at the Forest and Horticultural Crops Research Centre (FOHCREC), Kade. The Research Centre is located at 120 km northwest of Accra (60 09 N, 00 55 W) (Gray, 1987) and 135.9 m above sea level. The Centre covers a land area of 1090 hectares and has moist semi-deciduous tropical rainforest (Taylor, 1967) with two wet and two dry seasons.The area has a mean annual rainfall of about 1650 mm (Obeng, 1959). The Centre is known to have about 80 hectares of citrus plantation with 35 different species.

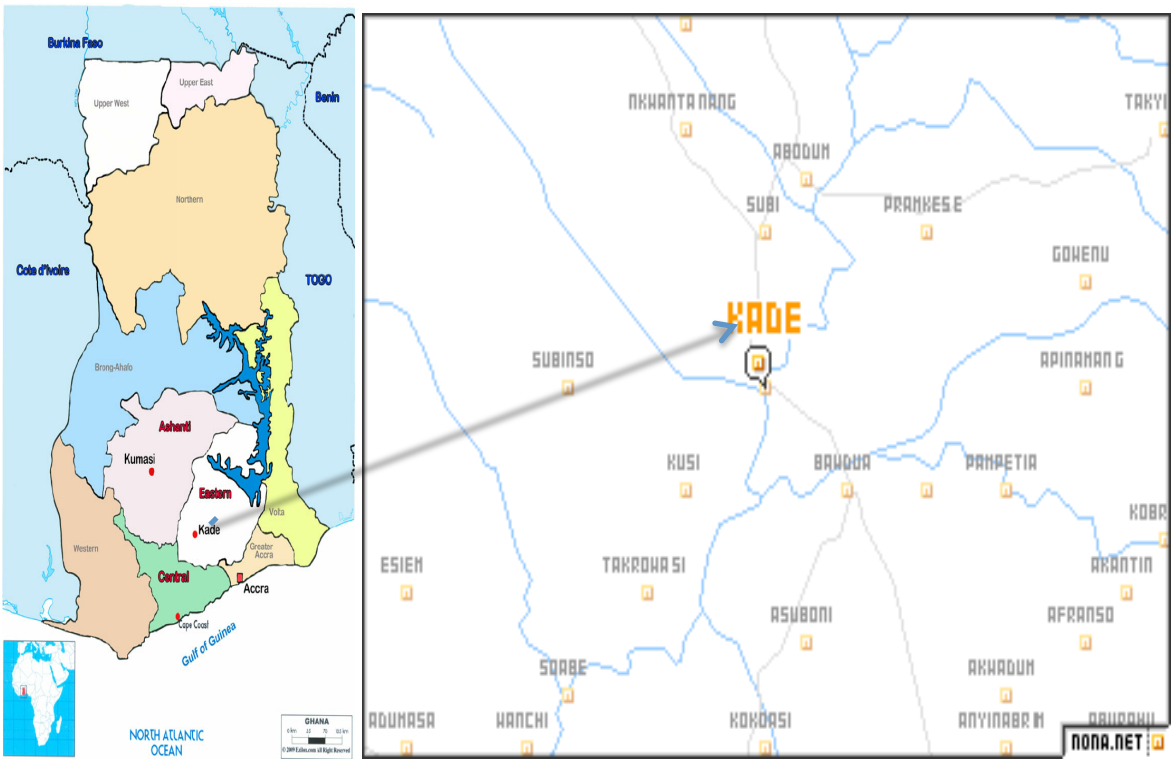

Fig.5: Map of Ghana showing Kade (Experimental site)

3.2 Exposure and non-exposure of sweet orange fruits under natural infection

This experiment was carried out to determine the critical infection period within the fruiting season of citrus under natural infection and the influence of climatic factors (rainfall, temperature, humidity) on the assessment of inoculum on the field, which may influence the infection. Twenty randomly selected sweet orange trees were used. The experimental design employed in this experiment was the Randomized Control Block Design. The selected trees were sprayed with chemical fungicides (Mancozeb and Carbendazin) to as a profilactic treatment or to curtail any infections that might have occurred prior to commencement of the experiment after petal fall. Afterwards, a random selection of five fruits from each of the 20 trees were covered with 80 x130 x240mm waxed paper bags when the fruits were about 2 cm in diameter (Fig.5). Subsequently, a fortnightly removal and exposure to natural infection of the five selected fruits on each 20 trees was followed. After this period of exposure natural infection, the fruits were re-bagged, tagged and assessed for disease incidence and disease severity at maturity. There were 18 treatments and replicated 20 times. The treatments consisted of the following on the table below:

Table 4: A schematic diagram showing various treatments in the experiment

	Exposed	Unexposed	Period
1			First 2 weeks of May 2018.
2			Last 2 weeks of May, 2018
3			First 2 weeks of June, 2018
4			Last 2 weeks of June, 2018
5			First 2 weeks of July 2018.
6			Last 2 weeks of July, 2018
7			First 2 weeks of August, 2018
8			Last 2 weeks of August, 2018
9			First 2 weeks of September, 2018
10			Last 2 weeks of September 2018.
11			First 2 weeks of October 2018.
12			Last 2 weeks of October 2018
13			First 2 weeks of November 2018
14			Last 2 weeks of November 2018
15			First 2 weeks of December 2018
16			Last 2 weeks of December 2018
17			(May-December, 2018)
18			(May-December, 2018)

Legend:

---Exposed

---Unexposed

Fig.6: Late Valencia trees with selected fruits covered with waxed paper envelope according to the various treatments above. × 0.5

3.3.Determination of disease incidence

This was determined by counting both infected and healthy trees on the field. Regular observation was made on all tagged fruits throughout the year. This was scored using a score scale of 0-1 where 0 = no spots on fruit (absence of disease) and 1= spots on fruit (presence of disease). Disease incidence value was computed by the formulae:

$$\text{Disease incidence} = \frac{\text{Number of diseased trees}}{\text{Total number of trees examined}} \times 100\%$$

The data obtained from this were subsequently subjected to analysis of variance using GenStat Statistical Software version 12 and Least Significant Difference (LSD) to separate the means

3.4 Determination of disease severity

Each selected tree on the experimental field served as a plot. During harvest, all *Pseudocercospora angolensis* infected fruits had already fallen before the end of the experiment. Due to this, all tagged fruits that dropped during the experimental set up were recorded and scored for disease severity. Where 0-no lesion; 1-10% of fruit area affected; 2-10-25% of fruit area affected; 3-25-50% of fruit area affected; 4->50% of the fruit area affected.

The score scales were then converted to disease severity index (Kim *et al*., 2000) using the formula:

$$\text{Disease severity index} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of observations x maximum disease score}} \times 100\%$$

Data were subjected to analysis of variance using GenStat Statistical Software version 12 to accomplish Least Significant Difference (LSD) of separated means.

3.5 Determination of environmental factors influencing PFLS

Monitoring of weather factors that affect disease development was done by daily recordings of temperature using thermometer, relative humidity using hygrometer, rainfall using rain gauge and wind-speed using anemometer to establish their effects or relationship on the aforementioned treatments throughout the experimental period (Fig.7). Also to establish the relationship between conidia numbers and the critical infection period for each month and the entire season, sample spore trap from Burkard Agronomics, UK was placed at vantage position in the citrus farm to check and trap conidia and other aero-mycoflora of the site. On a weekly basis, the trapped spores on the tape were removed, counted and their numbers

recorded using a light microscope and haemocytometer at the laboratory at the University Research Centre (FOHCREC) at Kade.

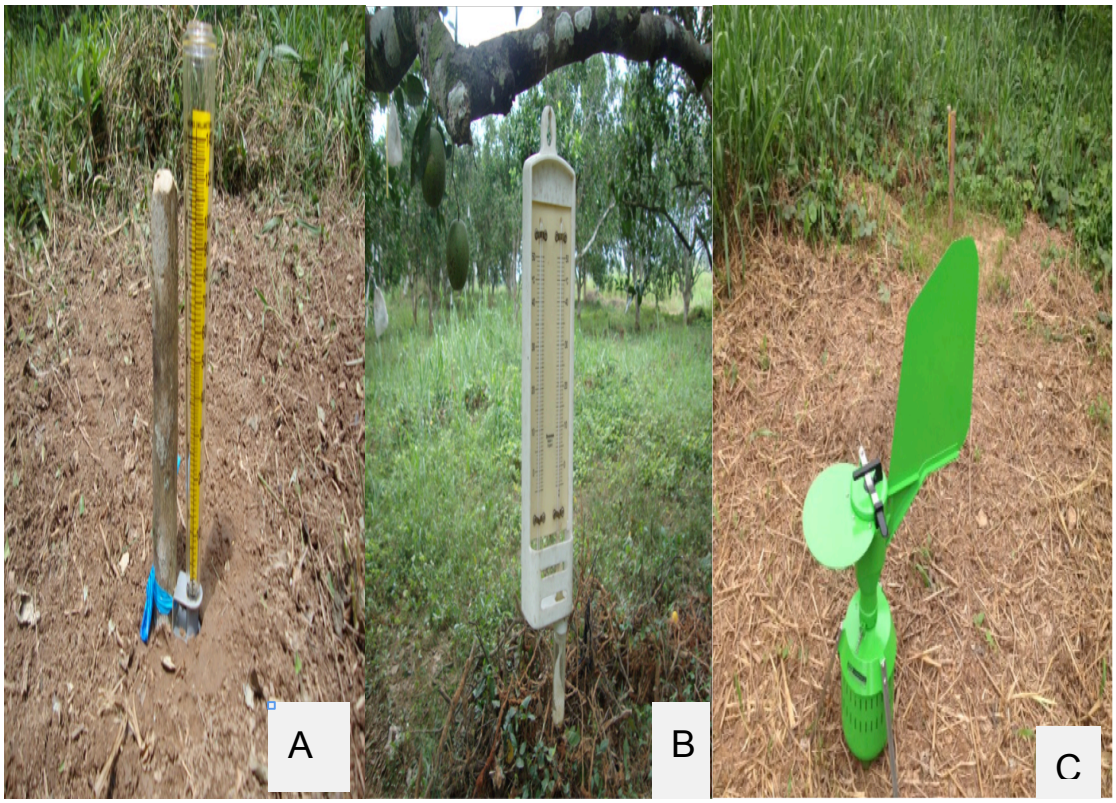

Fig.7: Instruments for recording weather condition and aero-mycoflora **(**× 0.5)

A. Rain gauge for measuring the amount of rainfall.

B. Hygrometer to determine the Temperature and Humidity.

C. Sample spore trap trapping the conidia of the fungus.

3.6 Potential antifungal properties of five medicinal plant extracts

3.6.1 Collection of plant materials

Plant materials selected for this research were collected within the Research Centre and those not available in the field were bought at the Kade market. Plant parts used for this experiment were; *Carica papaya* leaves, *Moringa oleifera* leaves, *Azacdiracta indica* leaves, *Zingiber officinale* and *Allium sativum* (rhizome & bulbs). Plants parts taken were healthy and intact. The samples were washed under running tap water to remove soil and other foreign materials. These plants were selected because they have some broad-spectrum antifungal properties. *C. papaya* leaves, *M. oleifera* leaves and *A. indica* leaves were to air-dried at temperature (32 – 35 °C).

3.6.2 Preparation of plant extracts

The air-dried plant parts were then pulverized into fine powder at the University of Ghana, Department of Plant and Environmental Biology. Pulverized plant parts were then taken to the Department Chemistry for Soxhlet extraction with ethanol. Twenty-five grams (25g) of each of the powdered plant parts were separately extracted in 250ml conical flasks with 90% ethanol (Ethanolic extraction) and concentrated at 40 ^{o}C under reduced pressure using rotary evaporator. Eighty mililitres (80ml) of distilled water was used to dissolve each of the five-powdered extract after rotary evaporation. The conical flasks were plugged with non-absorbent cotton wool and shaken at 120 rpm on a Vortex shaker for 30 minutes.

3.6.3 *In vitro* screening of the five medicinal plant extracts for antifungal properties

The poisoned food technique (Grover and Moore, 1962) was used to assess the inhibitory activity of the five (5) ethanolic plant extract; Carbendazin (0.13g) plus Mancozeb (0.17ml) were used as (a reference check or standard and distilled water as a control) on mycelia radial growth of the fungus in the laboratory. Five different concentrations each of plant extracts were used (1.3%, 2.5%, 3.8% and 5%). Mycelia plugs (10mm discs) of 7-day old actively growing culture of the test fungus were aseptically transferred to the centre of a 9cm Petri plates containing PDA. The test fungus on a poisoned paper on PDA constituted a treatment and each treatment was replicated three times. The test fungus on the paper disc dipped in distilled water on PDA constituted a control. The treatments with their concentrations were:

- *Carica papaya* leaves (1ml of extract: 0.5 H_2O (1.3%), 2ml of extract:0.5 H_2O (2.5%), 3ml of extract:0.5 H_2O (3.8%) and 4ml of extract:0.5 H_2O (5%) were prepared from the medicinal plant extracts of the stock solution volume to distilled water).

- *Moringa oleifera* leaves (1ml of extract: 0.5 H_2O (1.3%), 2ml of extract: 0.5 H_2O (2.5%), 3ml of extract: 0.5 H_2O (3.8%) and 4ml of extract: 0.5 H_2O (5%) were prepared from the medicinal plant extracts of the stock solution volume to distilled water).

- *Azacdiracta indica* leaves (1ml of extract: 0.5 H_2O (1.3%), 2ml of extract: 0.5 H_2O (2.5%), 3ml of extract: 0.5 H_2O (3.8%) and 4ml of extract: 0.5 H_2O (5%) were prepared from the medicinal plant extracts of the stock solution volume to distilled water).

- *Zingiber officinale* (1ml of extract: 0.5 H_2O (1.3%), 2ml of extract:0.5 H_2O (2.5%), 3ml of extract: 0.5 H_2O (3.8%) and 4ml of extract: 0.5 H_2O (5%) were prepared from the medicinal plant extracts of the stock solution volume to distilled water).
- *Allium sativum* (1ml of extract: 0.5 H_2O (1.3%), 2ml of extract: 0.5 H_2O (2.5%), 3ml of extract: 0.5 H_2O (3.8%) and 4ml of extract: 0.5 H_2O (5%) were prepared from the medicinal plant extracts of the stock solution volume to distilled water)
- Carbendazin (0.13g) plus Mancozeb (0.17ml- Reference treatment
- Control (distilled water)

Eleven millimeter (11ml) sterilized filter paper discs were dipped into the plant extracts concentrations for at most 5 minutes and plated onto sterilized PDA. Three of such dipped paper discs were placed almost equidistant from each other on every plate, which served as a plot. The experiment was replicated three times. Actively growing fungal plugs (10mm in diameter) of 7-day-old were aseptically cut out and placed onto the Centre of each filter poisoned paper disc on PDA. Plates were sealed with Para film and incubated at 24°C-26°C and 62-75% RH under continuous fluorescent white light for 14 days. Sterilized filter paper discs dipped into sterile distilled water and placed on PDA served as controls.

The experimental design for the treatment was the randomized complete block design in the Pathology laboratory at FOHCREC with incubation period 7 days under continuous fluorescent white light. Mycelia radial growth of the test fungus was determined by measuring the diameter of the colonies of the fungus from the reverse side of the Petri dishes on the seventh day of incubation using a 30cm plastic ruler. The average diameter of the fungal colonies were measured from the 7^{th} day of incubation to the 14^{th} day and percentage of mycelia growth inhibition was calculated using the formula modified after

(Baka, 2014):

$$\text{Mycelia growth inhibition (\%)} = \frac{\{C-T\}}{C} \times 100\%$$

Where, C = colony diameter (mm) of the control

T =colony diameter (mm) of the test plate.

Percentage inhibition was calculated and an analysis of variance for the different treatments was calculated using Genstat Statistical Software version 12 and Least Significant Difference (LSD) to separate the means.

CHAPTER FOUR

4.0 RESULTS

4.1 Epidemiological studies

4.1.1 Exposure and non-exposure of fruits to natural infection

Fruits exposed from June to July showed higher incidence of the disease than any other month throughout the season. It was evident that the critical infection period of this economically important disease is in July and then declined gradually to the end of the season (Table 4). Field observation also shows that the disease only affected fruits below five months of age under natural infection. The mean incidence of the disease was significantly different ($p < 0.001$) among the various periods of exposure as well as the reference treatment. (Carbendazin plus Mancozeb) but the mean incidence was not significantly different ($p>0.001$) among July 1& 2 (93.3%, 93.3%) and the control (94.99%). The mean disease severity on the other hand was observed to be significantly different ($p < 0.001$) among the various treatments except for that of the fruits exposed to natural infection only in July1&2 (56.75%, 58.75%) and the control (67.75%), which were not significantly different ($p>0.001$). The mean disease incidence for the fruits covered throughout the season (Carbendazin plus Mancozeb) was not significantly different ($p>0.001$) from fruits exposed in May 1&2.

4.2 Incidence and severity of PFLS and environmental conditions during the experimental period in a Late Valencia sweet orange farm at FOHCREC, 2018

The disease incidence of *Pseudocercospora* fruit and leaf spot of citrus were recorded in a selected Late Valencia field on citrus plantation number 26. Higher levels of significant

difference ($p<0.001$) were seen in the mean incidence of Late Valencia fruits exposed in August[1], September[1], September[2], October[2], November[1], November[2], December[1], December[2] and the Carbendazim 0.13g + Mancozeb 0.17m. On the other hand, mean disease incidences were not significantly different ($p>0.001$) among fruits exposed in the months of May, June, July and August as well as the control (Table 4). A similar trend was observed in the disease severity of the fruit and leaf spot disease of Late Valencia fruits exposed within the year 2018 citrus season. Mean disease severity for Late Valencia exposed in May[1], July[1], July[2], control and the (Carbendazin plus Mancozeb) was significantly different ($p<0.001$)(Table 4). There was no significant difference ($p>0.001$) among the other treatments exposed to natural infection on the trial field. During the field trial, the following weather elements were recorded per treatment; rainfall, relative humidity, wind speed and temperature. Some months of the year had high rainfall than others. There was a significant difference ($p<0.001$) among the mean disease incidences as it relates to the influence of weather parameters.

Table 5: Incidence and severity of PFLS and environmental conditions during the experimental period in a Late Valencia sweet orange farm at FOHCREC, 2018

Duration of exposure	Disease incidence (%)	Disease severity (%)	Rainfall (mm)	RH (%)	Wind speed (ms^1)	Temp. (°C)
May [1]	2.19a	3.74a	7.47	81.83	4.71	27.02
May[2]	5.94a	15.91bc	8.51	79.83	4.60	27.58
June[1]	49.00fg	21.50cd	7.80	80.10	4.40	27.46
June[2]	64.00hi	19.85bcd	9.58	81.79	3.92	26.34
July[1]	93.90j	56.75f	3.74	82.96	4.00	26.55
July[2]	93.90j	58.75fg	3.15	83.34	3.70	26.60
August[1]	69.00i	35.25e	2.25	79.73	3.60	25.74
August[2]	64.00hi	25.50d	3.24	82.87	3.81	26.37
September[1]	57.50gh	23.00cd	14.33	82.50	4.06	26.67
September[2]	54.00g	19.50bcd	4.86	81.10	3.88	27.13
October[1]	49.00fg	21.75cd	9.10	80.29	4.01	27.33
October[2]	45.00ef	17.75bcd	6.95	78.64	4.27	27.89

November[1]	40.06de	23.57cd	4.05	78.77	4.59	27.68
November[2]	34.19cd	15.70bc	2.22	80.21	3.73	27.65
December[1]	28.00c	36.75e	0.00	75.92	3.20	27.79
December[2]	16.44b	25.46d	0.40	72.71	3.22	27.67
Exposed throughout	94.99j	67.75g	5.38	80.17	3.98	27.01
Covered throughout	8.81ab	11.48ab	5.38	80.17	3.98	27.01

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

1-First two (2) weeks of the month

2- Last two (2) weeks of the month

4.3 Assessment of Aero-mycoflora

4.3.1 Number of conidia responsible for the fruit and leaf spot disease within the experimental field

The numbers of conidia recorded from the beginning of the field trial varied throughout the year. Conidia numbers increased from June and attained a peak in the last two weeks of July and therefore declined gradually towards the end of the year (Fig.7).

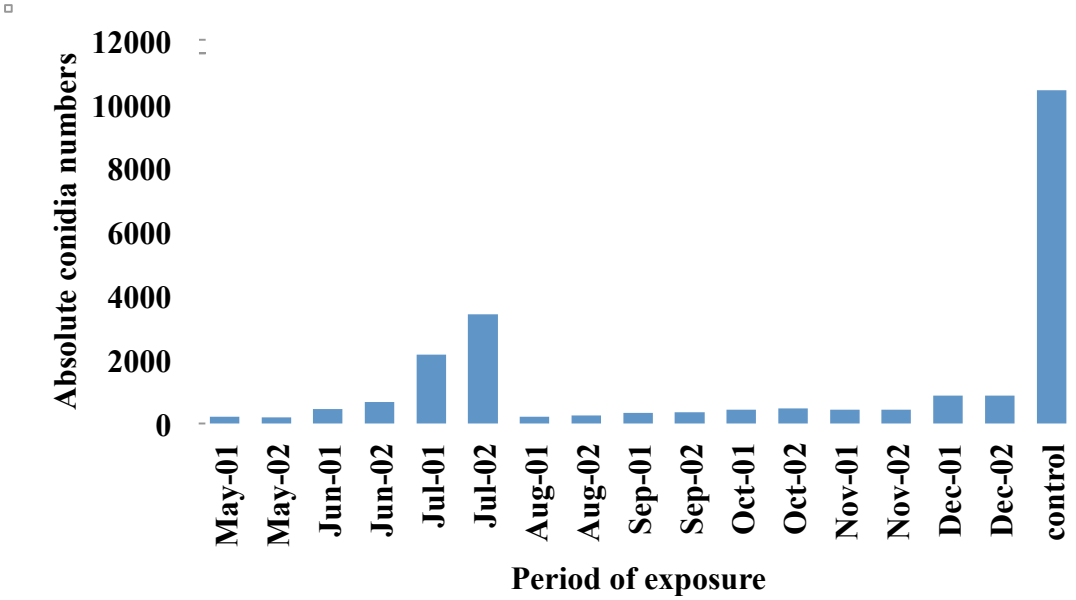

Fig. 8: Relationship between period of exposure of fruits to natural infection and the aeromycoflora of conidia of *P.angolensis* in 2018. (-0.002cm (5%) LSD)

4.4 Sensitivity test of *P. angolensis* rate/concentration of five medicinal plant extracts using food poison technique

4.4.1 Inhibitory effects of *Zingiber officinale* extract on the radial mycelia growth of *P. angolensis*

The reference treatment, which constituted Carbendazin 0.13g + Mancozeb 0.17ml had mean inhibition of mycelia growth of 99.68% after 14 days of in vitro laboratory trials (Table 4). There was a significant ($p<0.001$) difference among the mean mycelia growth of the treatments, 3.8%, 5%, the control and the reference treatment with mean percentages as follows respectively (43.47%, 52.46%, 0.01%, 99.68%). There was no significant ($p>0.001$) difference between mean of mycelia growth inhibition of the 1.3 and 2.5% of the extract.

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%). 1-First two weeks of the given month, 2- last two weeks of the given month.

Table 6: Inhibitory effects of different concentrations of *Zingiber officinale* on the mycelia growth of *P. angolensis* cultured on PDA for 14 days and incubated at 24°C-26°C

Rate/ concentration of *Zingiber officinale* *(*antifungal agent) (%/v)	**Mean radial mycelia growth inhibition (%) ±SE**
1.3	38.38 ±2.588b
2.5	43.47±3.806c
3.8	43.47±6.341c
5.0	52.46±0d
Carbendazin 0.13g + Mancozeb 0.17ml	99.68±0.0129e
Control	0.01±0a

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

4.4.2 Inhibitory effects of *Allium sativum* extracts on the radial mycelia growth of *P. angolensis*

The reference treatment against the fungus recorded the highest percentage value and showed a very high significant ($p<0.001$) difference among the other treatments (99.68%). The control treatment was also significantly ($p<0.001$) lower than all the other treatments (0.01%) (Table 6). There was no significant difference ($p>0.001$) among the 2.5%, 3.8% and 5% treatments. On the other hand, treatment 1.3% was significantly ($p<0.001$) different from all the other treatments (Table 5).

Table 7: Inhibitory effects of different concentrations of *Allium sativum* on the mycelia growth of *P. angolensis* cultured on PDA for 14 days and incubated at 24°C- 26°C

Rate/ concentration of *Allium sativum* *(*antifungal agent) (%/v)	**Mean radial mycelia growth inhibition (%) ±SE**
1.3	69.12 ± 4.120b
2.5	87.89±0.726c
3.8	89.88±1.462c
5	90.45± 1.437c
Carbendazin 0.13g + Mancozeb 0.17ml	99.68±0.0129d
Control	0.01±0a 5.227

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

4.4.3 Inhibitory effects of *Moringa oleifera* extracts on the radial mycelia growth of *P. angolensis*

There was significant difference ($p<0.001$) among the following treatments, 1.3%, 2.5%, control and the reference treatment with mean percentage inhibition of 21.08%, 26.76%, 0.01% and 99.68% respectively. Treatment that constituted 3.8% and 5% of the medicinal plant extract had no significant difference ($p>0.001$) between their mean mycelia growth inhibition (37.35)% and 36.04% respectively (Table 7).

Table 8: Inhibitory effects of four (4) different concentrations of *Moringa oleifera* on the mycelia growth of *P. angolensis* cultured on PDA for 14 days incubated at 24°C-26°C

Rate/ concentration of *Moringa oleifera* (antifungal agent) (%/v)	**Mean radial mycelia growth inhibition (%) ±SE**
1.3	21.08±1.696b
2.5	26.76±2.307c
3.8	37.35±2.391d
5.0	36.04±1.090d
Carbendazin 0.13g + Mancozeb 0.17ml	99.68±0.0129e
Control	0.01± 5.227a

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

4.4.4 Inhibitory effects of *Azadirachta indica* extracts on the radial mycelia growth of *P. angolensis*

The mean percentage inhibition of radial mycelia growth inhibition of *Azadirachta indica* showed no significant difference ($p>0.001$) between the concentrations 1.3% and 2.5% (34.11% and 34.91% respectively. Mean mycelia growth inhibition for concentrations 3.8%, 5%, control and Carbendazin 0.13g + Mancozeb 0.17ml had a significant difference ($p<0.001$) (45.99%, 24.49%, 47.43% and 99.68%) respectively (Fig. 8). Carbendazin 0.13g

+ Mancozeb 0.17ml (reference treatment) recorded the highest inhibition of mycelia growth (99.68%) (Table 8).

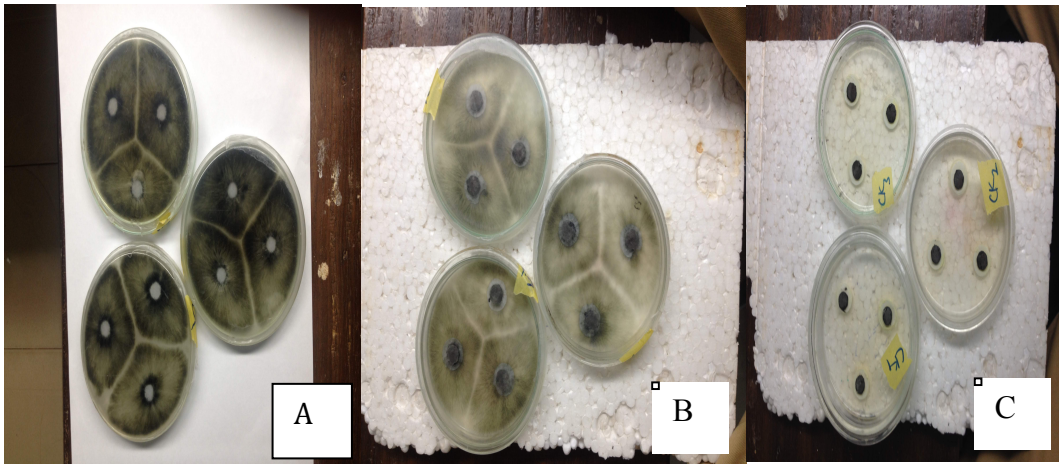

Fig.9: Percentage inhibition growth of *P. angolensis* on (A): Control, (B): *Azacdiracta indica* extract and (C) Carbendazim +Mancozeb (reference treatment), cultured on PDA and incubated at 24°C- 26°C. × 0.5

Table 9: Inhibitory effects of different concentrations of *Azacdiracta indica* on radial mycelia growth of *P. angolensis* cultured on PDA for 14 days and incubated at 24°C-26°C

Rate/ concentration of *Azacdiracta indica* *(*antifungal agent) (%/v)	**Mean radial mycelia growth inhibition (%) +SE**
1.3	34.11 ±2.993c
2.5	34.91±0.663c
3.8	45.99± 5.975d
5.0	24.49±0.223b
Carbendazin 0.13g + Mancozeb 0.17ml	99.68±0.0129d
Control	0.01±0a 5.227

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

4.4.5 Inhibitory effects of *Carica papaya* extracts on the radial mycelia growth of *P. angolensis*

Extract of *Carica papaya* showed no significant ($p>0.001$) difference among the mean mycelia growth inhibition of three of the concentrations used in the experiment (2.5%, 3.8% and 5%) respectively. On the other hand there was significant difference ($p<0.001$) among the other treatments (1.3%, control and Carbendazin 0.13g + Mancozeb 0.17ml), recording mean mycelia radial growth inhibition as follows (20.73%, 0.01%, 99.68%) respectively (Table 9).

Table 10: Inhibitory effects of four (4) different concentrations of *Carica papaya* on mycelia growth of *P. angolensis* cultured on PDA for 14 days and incubated at 24°C-26°C

Rate/ concentration of *Carica papaya* *(*antifungal agent) (%/v)	**Mean radial mycelia growth inhibition (%) +SE**
1.3	20.73±3.080b
2.5	30.37±2.886c
3.8	30.08± 2.309c
5.0	36.67±3.687c
Carbendazin 0.13g + Mancozeb 0.17ml	99.68±0.0129d
Control	0.01± 5.227a

Means followed by the same letters in a column are not significantly different at Fisher's Protected LSD (5%).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Epidemiology of the causal organism of PFLS of Late Valencia

P. angolensis was isolated and identified using the method described by (Lawson *et al.,* 2016). The results obtained from the field trial indicated that the critical period of infection of *P. angolensis* was in July 2018. This was reflected in the incidence and severity of the disease recording the highest among all the months as compared to the control. There was a significantly higher mean disease incidence and severity among all the months except for the first and last two weeks of July (93.9% and 93.9%) respectively. Severely infected fruits do not only become unmarketable (like in the case of Black spot infected fruits) but they also mummified with cracked and hardened large lesions making it unfit for human consumption (Pretorius, 2005). The disease causes serious devastation to the citrus crops and the industry. Consequently, most rural farmers in Ghana have replaced their citrus plants with cocoa (*Theobroma cocao*), oil palm and Para-Rubber plants (Lawson, 2014).

P.angolensis fruit and leaf spot disease has reached an epidemic level in most of the major citrus growing areas of Ghana especially in the Eastern region where citrus is commercially grown. Symptoms begin to appear few weeks after petal fall when the fruits reach a diameter of about the size of a gulf ball. Fruits exposed only at the beginning of the field trial from May showed slight incidence of the disease and progressed to a peak in July. The incidence of the disease then declined towards the end of the year. The same trend was seen in the severity of this devastating disease. Incidence observed on fruits exposed at the beginning of the experiment were not as severely damaged as those exposed thereafter. It was also observed that the susceptibility of both fruits and leaves to the disease also decreased with age of the plant. As the leaves and fruits mature the infection of *P.angolensis*

reduced and increased on the young ones. This could be due to the presence of secondary metabolites in mature plants, which might be toxic to the fungus, or the robust nature of the tissues in the older fruits and leaves. Field study outcome showed that the fruit and leaf spot disease caused by *P. angoleensis* was also first observed in the sweet orange variety and Late Valencia during field trials. This observation from the field agrees with the first observation of this disease in Angola and Mozambique in 1952 (De Carvalho and Mendes, 1953). The disease was also observed on all species of citrus including orange, mandarin, lemon, grapefruit, lime, pomelo (Kuate, 1998; Pretorius, 2005). The critical period of infection by *P. angolensis* causing fruit and leaf spot disease is July even there were pres-sympyomic signs during the first two months before the peak month (July).

5.2 The role of biotic and abiotic factors in the development and spread of *P. angolensis* fruit and leaf spot disease

During field trials (to investigate the epidemiology of the disease) it was observed that the incidence and severity of the disease were dependent on climatic factors, which is subjected to change depending on the weather pattern within a cropping season of the year. Observations made on greasy spots on grape fruit caused by *Mycosphaerella citri* also showed a similar trend in disease cycle (Mondal and Timmer, 2006).). It was observed that during rainy periods, relative humidity was higher; the symptomatic spots on young and new leaves may coalesce and result in general chlorosis. Rain and high relative humidity play a major role in spore germination. Field trials carried out indicated that conidia were generally present throughout the year but their numbers increased from May and peaked in July. The numbers reduced from July till the end of the year. The variation in number of conidia throughout the year could be attributed to the prevailing environmental conditions that either favoured or impeded the production of spores. Wind speed, another parameter

that was taken into consideration in this field trial also played a major role in conidia dispersal and at particular period of the year. Rainfall and high relative humidity enhance spore germination and dispersal, while ideal temperatures, conducive for germination predisposes the host plant to infection. Results obtained by (Baldassari *et al.,* 2006) in Brazil where ascospores numbers of Black spot were also monitored and quantified within a particular period of time and matched with the disease development showed a similar trend of variation of spore numbers observed in this trial. The number of conidia recorded in a month during the year 2018 is not expected to be the same the following year due to local climatic variations. Meteorological data obtained from this experiment showed that highest rainfall for the year 2018 was recorded in the first week of September, followed by second week of June. The first two weeks of the month of May had the highest wind speed of 4.71ms^{-1}, followed by that of the first two weeks of November of 4.59 ms^{-1}. This indicated that even and rapid distribution of conidia is a highly possible situation during these periods of the year. The second week of September recorded the highest rainfall in the year 2018 but the incidence and severity of the disease from September to December was not as high as that of June to August of the same year. This variation is attributed to the variation in wind speed. The average relative humidity for the entire year was 80%. An average temperature, approximately 26°C, was recorded throughout the entire experimental period with slight difference in the various treatments. This present study of the fruit and leaf spot disease caused by *P. angolensis* has shown that the amount of rainfall and its distribution were very important environmental parameters that affect the progress of the disease. Spore trap and weather data indicated that *P. angolensis* needs high moisture and temperatures in above 25°C for disease development (Pretoius, 2005).

On other hand, heavy continuous rain may not be conducive for disease development as seen in the minor season in September, when recorded highest rainfall of 14.33 mm correlated with low disease incidence. The sizes of spores are also determined by the seasonal differences as observed throughout the year as well as climate change. A similar work by Kauserud *et al*. (2011) revealed that, fungal spores produced at the beginning of autumn resulted in increased in size due to greater water accumulation, while spores produced towards the end of autumn decreased in size. In the case of smaller spores due to inadequate water, the spores are easily carried for distribution as compared to greater water accumulated ones. This could be the reason for the spore number distribution throughout the year, which was obtained in this research. All the biotic and abiotic factors played a significant role in the development and spread of *P. angolensis* fruit and leaf spot disease of sweet orange.

5.3 *In vitro* study on the antifungal effect of five plant extracts on growth of *P. angolensis*

The goal of this experiment was to evaluate the anti-fungal activity of medicinal plants in the control of *Pseudocercospora* leaf and fruit disease of citrus. This was made up of in vitro screening of various plants to determine their inhibitory effect on the fungus. The plant extract with the highest inhibitory effect estimated by the measurement of the mycelia radial growth was selected and recommended for the control of *P. angolensis.* These plants were selected because they have some antifungal properties.

Many plants contain active ingredients, which affects the growth and reproduction of microorganisms. This is obviously due to the presence of secondary metabolites in plants that play a role in plant defense mechanisms. Plant extracts have therefore been used to

control the growth of microorganisms. In this present study ethanolic extracts of *A. indica* did not show a significant mycelia growth inhibition as compared to the reference treatment. A number of studies have established the antifungal properties of *A. indica* extracts. For example, Govindachari *et al.* (2000), used neem seed oil to depress by 76 % in the rust pustule count of *P. arachidis* at a minimal concentration. According to Vea and Palmer (2016), neem concentrate and SunSpray Ultra Fine Oil were less effective against *Cercospora* spp. These findings compare favourably with data from this experiment and this could be as a result of physiological difference between the genera of the organism or other factors. In these experiments extracts rather promoted mycelia growth of *P. angolensis* even though it has been reported to contain some antifungal properties. The treatment with the highest concentration recorded the lowest mycelia growth inhibition after the control treatment. This observation in the results indicated that, the antifungal properties of plant extracts are not broad-spectrum agents but species specific. The fact that most plants have inhibitory properties does not imply that all microorganisms are susceptible to their inhibitory effect. The Carbendazim 0.13g + Mancozeb 0.17ml treatment had a highly significant mycelia radial growth inhibition percentage than all the treatments hence its effectiveness against the disease. According to Parveen and Kumar (2000), extracts of *A. indica* and other plants were ineffective against the fungus *Alternaria triticina*.

Results obtained from the *in vitro* laboratory study on the mycelia radial growth inhibition of *Allium sativum* on *P. angolensis* fruit and leaf spot disease showed a significant inhibitory effect at all the concentrations applied as compared to the control (0.01). Silva *et al. (*2014), showed that species of plants namely *Vernonia polysphaera, Syzygium aromaticum,* and *Allium sativum* depressed or inhibited the mycelia growth and conidial germination of *Cercospora coffeicola*, *Colletotrichum gloeosporioides, Fusarium*

oxysforum, Phoma tarda, Rhizoctonia solani, and *Hemileia vastatrix*.

The least concentration of *A.sativum* inhibited growth of *P. angolensis* by 69.12%, which was of higher inhibition rate than the highest concentration of all other plant extracts used in this *in vitro* study. The highest percentage inhibition of *A.sativum* on *P.angolensis*, was 90.45%. This value was not significantly different from that of the reference treatment. Work done by Kurucheve and Padmavathi, (1998), also showed that as low as 10% extracts of *A.sativum* reduced damping off in chilli caused by *Pythium aphanidermatum*. *A.sativum* may therefore broad-spectrum inhibitory effect on both fungi and bacteria species as compared to other plant extracts like *A. indica*. It has also been reported that extracts of *A. sativum* L. bulb, *Aegle marmelos* (L.) leaf and *Cartheranthus. roseus* flower have inhibitory properties against the germination and mycelia growth of *Alternaria solani*, a pathogenic fungus causing tomato and potato blight disease (Vijayan, 1989). In this study, the highest rate/concentration of all the plant extracts used was 5%, in the case of garlic, if the concentration is increased, there could be a higher probability of 100% inhibition of mycelia growth of the pathogenic fungus. There was no significant difference among the other two treatments of the garlic extracts, which constituted of 2.5, 3.8 and 5% with mean mycelia inhibition of 87.89%, 89.88% and 90.45% respectively. The difference between control treatment and all the main treatments was highly significant as compared to other extracts used in this experiment. The Carbendazim 0.13g + Mancozeb 0.17ml treatment was very effective against the fungus. On the other hand, inorganic chemicals have inherent problems that they bring along in spite of their known efficient efficacy in the eradication of pathogens. Ethanolic extracts garlic can be used to control *P. angolensis* fruit and leaf spot if the concentration is increased to an optimum level. Garlic is used as spice in food and for that matter it has no minimum toxicity to human health and the ecosystem, unlike

Carbendazim (0.13) + Mancozeb (0.17) which could be hazardous to both humans and the ecosystem.

Laboratory results from *in vitro* studies on the antifungal effect of *Zingiber officinale* on *P. angolensis* showed a significant difference among the mean radial mycelia growth except for the concentrations of 2.5 and 3.8%, which were not significantly different. *Z. officinale* exhibited some percentage of mycelia inhibition in all the treatments especially in the highest concentration. Parveen and Kumar (2000) reported that *Z. officinale* extracts also showed some mycelia growth inhibition against *A. triticina*. Plants are known to contain phenolic and other secondary metabolic compounds used for defense against potential pathogens. The rhizome of *Z. officinale* and leaves of *C. papaya* showed some inhibitory properties. A study by Singh *et al*. (2011), revealed phenolic compounds in their antifungal properties against six plant pathogenic fungi exhibiting increased fungal activity with increase in the level of phenolic content. This could be the reason for the variation in the inhibitory level of *Z. officinale* as well as the other ethanolic extracts observed in this study. The ethanolic extract of the medicinal plant inhibited the fungus at high concentrations. The ethanolic extract of *A. indica*, on the converse improved growth with increasing concentrations.

In vitro laboratory studies revealed that *Moringa oleifera* has some inhibitory properties against the fungus responsible for the fruit and leaf spot disease of citrus. A significant difference was recognized among all the various concentrations of the extracts except for the concentrations 3.8% and 5%. The radial mycelia growth inhibition showed a similar trend of increasing inhibition with increased concentration. All concentrations of the ethanolic extracts showed a significantly different mycelia growth inhibition growth rate except for the 3.8 and 5%. This small evergreen tree crop has also been reported to have

some bioactive compounds in the leaves responsible for the inhibition of *Saccharomyes cerevisae, Candida albicans* and *Candida tropicalis*. This finding underscores the antifungal property of *M. oleifera* (Patel *et al.*, 2014).

The results from bioassay carried out on the effect of *Carica papaya* leaves on growth of *P. angolensis* yielded a positive result. Even though this extract showed significant differences among the treatments, extracts concentrations of 3.8 and 5% did not show significant difference in their mean mycelia growth inhibition. According to Suprapta *et al.* (2001), out of 15 plant species extracts tested to control *Ceratocystis* fruit rot on Snake fruit (*Salacca zalacca*), the root extract of *Alpinia galanga* and leaf extract of *Carica papaya* significantly inhibited the growth of *Ceratocystis* sp on PDA medium and on Snake fruit.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

- The results of the present epidemiological study of *P. angolensis*, a disease of grave concern to the citrus industry and farmers, have shown that the critical period of infection lies between June and August with July being the peak period of infection. In the experimental trials carried out in 2018, the incidence and severity of *P. angolensis* fruit and leaf spot of Late Valencia was recorded as 93.3% and 57.75% respectively. These values were just lower than that of the control for both incidence and severity, which were recorded as 94.9% and 67.75% respectively. The influence of biotic and abiotic factors on the disease development resulting in the high incidence and severity of this disease cannot be disregarded in this study. Aero-mycofloral distribution, wind speed, temperature, rainfall, relative humidity and insects as well as humans are all possible contributory factors to the spread and development of the disease. Periods of the year with favourable climatic factors that are suitable for the disease recorded high incidence and severity of the disease.

  The core reason behind Integrated Disease Management (IDM) is to keep disease intensity below an economic injury threshold in order to prevent loses. Disease management is all about using effective strategies to deprive the pathogen of at least one parameter on the disease triangle. The results of the of the *in vitro* studies showed that out of the five medicinal plants used against the fungus, four of them showed a significant percentage of inhibition. *Allium sativum* recorded the highest percentage mycelia inhibition (90.45%) at 5% rate/concentration (4ml of garlic extracts: 0.5ml of distilled water), followed by *Z. officinale* (52.46%), *A. indica*

(45.99%), *M. oleifera* (37.35%) and *C. papaya* (36.67%). The control recorded complete colonization by the fungus before the 14th day. Carbendazim 0.13g + Mancozeb 0.17ml recorded no growth at all after 14 days of incubation.

6.2 Recommendation

- Since the critical infection period was known from this study, application of the rightly formulated garlic extracts at the right interval will inhibit to an appreciable level and boost the yield of citrus. Field trials of the plants extracts are strongly recommended on the field as well, since laboratory conditions are not the same as what pertains to the field.

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APPENDICES

APPENDIX 1:Analysis of variance for disease incidence

Variate: INCIDENCE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	19	0.79433	0.04181	2.13	
Plot.*Units* Stratum	17	29.16052	1.71532	87.47	<.001
Month					
Residual	323	6.33424	0.01961		
Total	359	36.28908			

APPENDIX 2:Analysis of variance for disease incidence

Variate: SEVERITY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	19	0.56200	0.02958	1.14	
Plot.*Units* Stratum	17	12.49470	0.73498	28.30	<.001
Month					
Residual	323	8.38834	0.02597		
Total	359	21.44504			

APPENDIX 3: Analysis of variance table for inhibitory effect of *Alium sativum* on mycelia growth (in diameter) of *P. angolensis*

Variate: cause _of_ drop

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	19	3343.8	176.0	0.64	
Plot.*Units* Stratum	5	569893.3	113978.7	415.62	<.001
Month					
Residual	95	26052.4	274.2		
Total	119	599289.5			

APPENDIX 4: Analysis of variance table for inhibitory effect of *Zingiber officinale* on mycelia growth (in diameter) of *P. angolensis*

Variate: Garlic_MGH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	2	47.656	23.828	2.89	
Plot.*Units* Stratum	5	1898.078	379.616	45.98	<.001
Month					
Residual	10	82.558	8.256		
Total	17	2028.291			

APPENDIX 5: Analysis of variance table for inhibitory effect of *Moringa oleifera* on mycelia growth (in diameter) of *P. angolensis*

Variate: moringa_MGH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	2	25.973	12.986	2.01	
Plot.*Units* Stratum	5	19877.731	3975.546	614.82	<.001
Month					
Residual	10	64.662	6.466		
Total	17	19968.366			

APPENDIX 6: Analysis of variance table for inhibitory effect of *Azachdiracta indica* on mycelia growth (in diameter) of *P. angolensis*

Variate: neem_MGH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	2	11.77	5.89	0.23	
Plot.*Units* Stratum	5	17575.45	3515.09	135.66	<.001
Month					
Residual	10	259.12	25.91		
Total	17	17846.35			

APPENDIX 7: Analysis of variance table for inhibitory effect of *Carica papaya* on mycelia growth (in diameter) of *P. angolensis*

Variate: pawpaw_MGH_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plot stratum	2	23.42	11.71	0.59	
Plot.*Units* Stratum	5	20198.87	4039.77	205.00	<.001
Month					
Residual	10	197.07	19.71		
Total	17	20419.35			