

**PREVALENCE, INCIDENCE AND MOLECULAR PHYLOGENY OF A FUNGUS
(*PHYTOPHTHORA COLOCASIAE*) CAUSING TARO LEAF BLIGHT IN GHANA**

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DECLARATION

This thesis is the product of research undertaken by ABUBKAR YUSIF in the Department of Nuclear and Radiation Processing of the School of Nuclear and Allied Sciences, University Of GHANA, under the supervision of Dr. ANDREW SARKODIE APPIAH, Dr. SAMUEL AMITEYE, and Dr. NUSRAT AFFUL.

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DEDICATION

I dedicate this research work firstly, to the Almighty Allah who has kept me safe through the turbulence of my life and through the period of this work. This work is dedicated to the Yusif Family; first and foremost, to my lovely wife, Nasibatu Abdul Rahman and to my mother, Mariam Abdul Karim, for her spiritual, moral and motherly support throughout the period of study. You have indeed been such an influence and inspiration to me in my academic pursuit. I also dedicate this work to my departed father Yusif Abubakar, May Almighty Allah bless him with heaven. Lastly, to my able son Yusif Abubakar who I named after my father.



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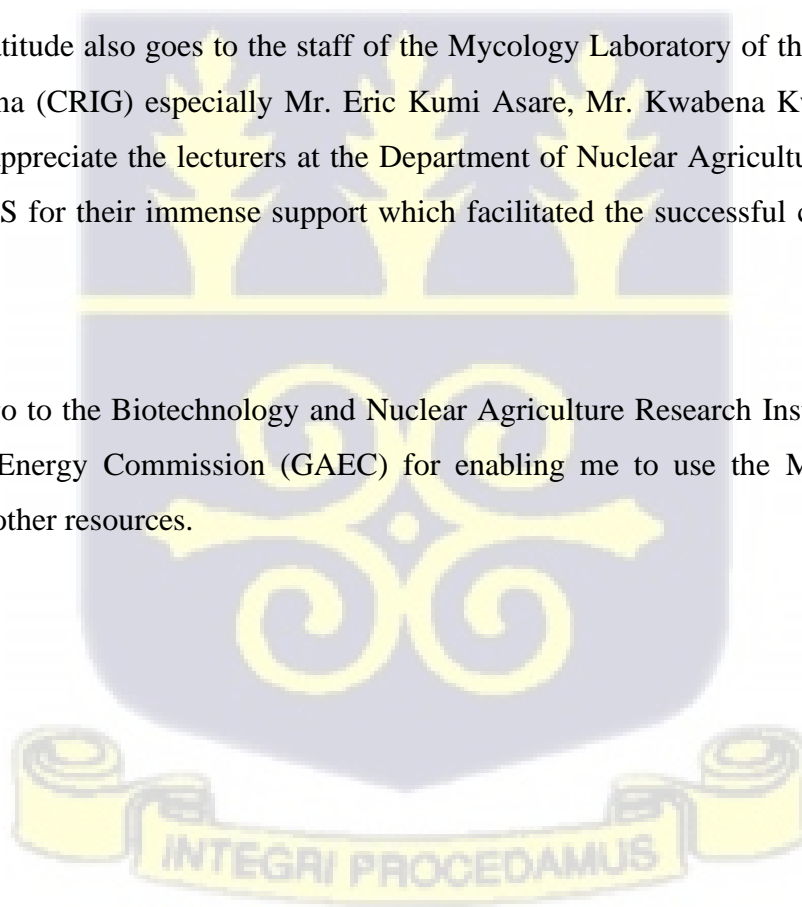


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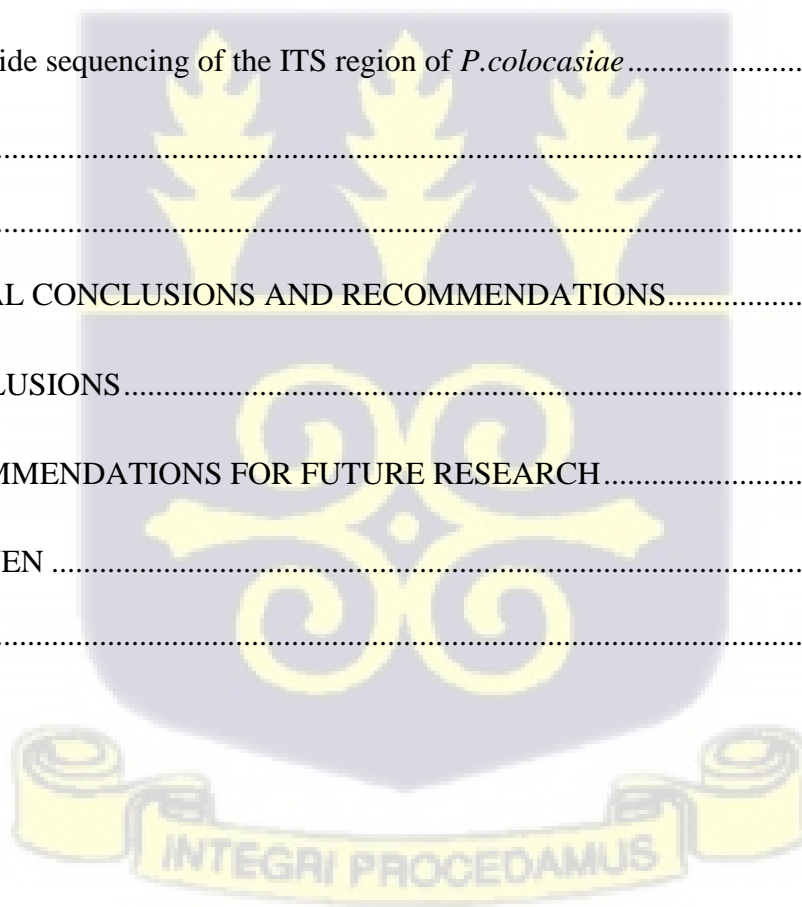
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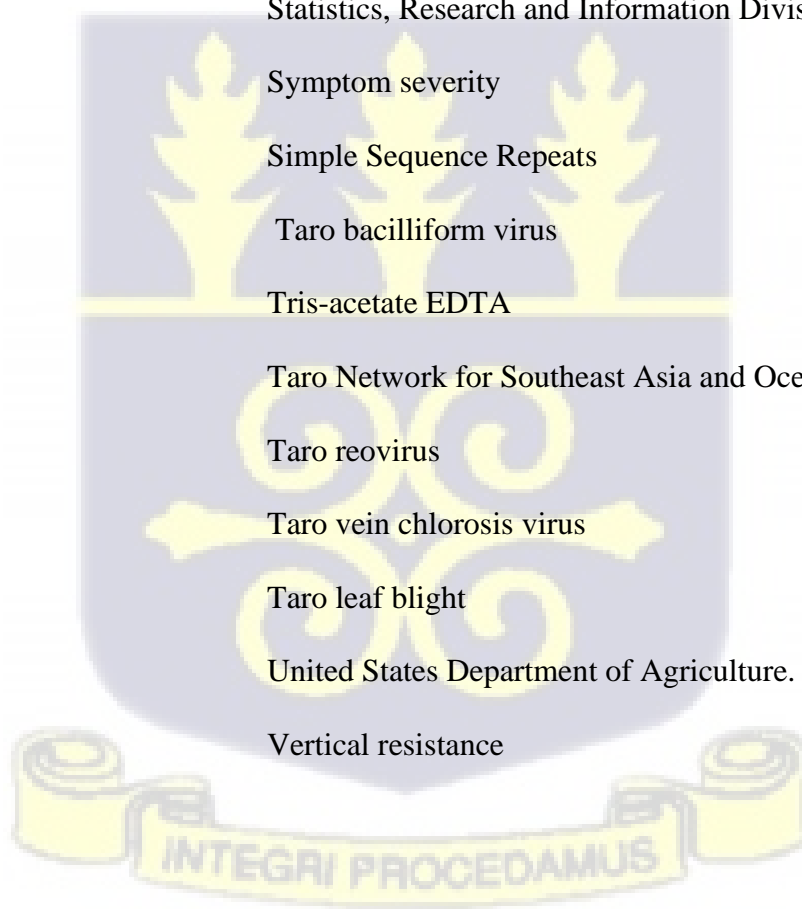


LIST OF ABBREVIATIONS

ABVC	Alomae – Bobone virus complex
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance
APHIS	Animal and Plant Health Inspection Services
BAF	Biosecurity Authority of Fiji
BNARI	Biotechnology Nuclear and Agriculture Research Institute
CABI	Center for agriculture and bioscience international
CBDV	Colocasia bobone disease virus
cDNA	Complementary Deoxyribonucleic acid
CePaCT	Centre for Pacific Crops and Trees
CMI	Commonwealth Mycology Institute
CRIG	Cocoa Research Institute of Ghana
CSIR	Council for Scientific and Industrial Research
CTA	Technical Centre for Agricultural and Rural Cooperation
CTAHR	College of Tropical Agriculture and Human Resources
CTCRI	Central Tuber Crops Research Institute
DESA	Department of Economic and Social Affairs
DI	Disease incidence
DNA	Deoxyribonucleic acid
DsMV	Dasheen mosaic virus
EDTA	Ethylenediaminetetra acetic acid
EtBr	Ethidium bromide

FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
FCFA	franc CFA
GAEC	Ghana Atomic Energy Commission
g-DNA	Genomic DNA
GDP	Gross Domestic Product
GLB	Genomic lysis buffer
GSSP	Ghana strategy support programme
HR	Horizontal resistance
HR	Hypersensitivity response
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
ITS	Internal transcribed space
MINADER	Ministry of Agriculture and Rural Development
MoFA	Ministry of food and Agriculture
MRACLS	Multi-Round Annual Crop and Livestock Surveys
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
NGO	Non-governmental organization
PCR	Polymerase chain reaction
PGRRI	Plant Genetic Resources Research Institute
PNG	Papua New Guinea

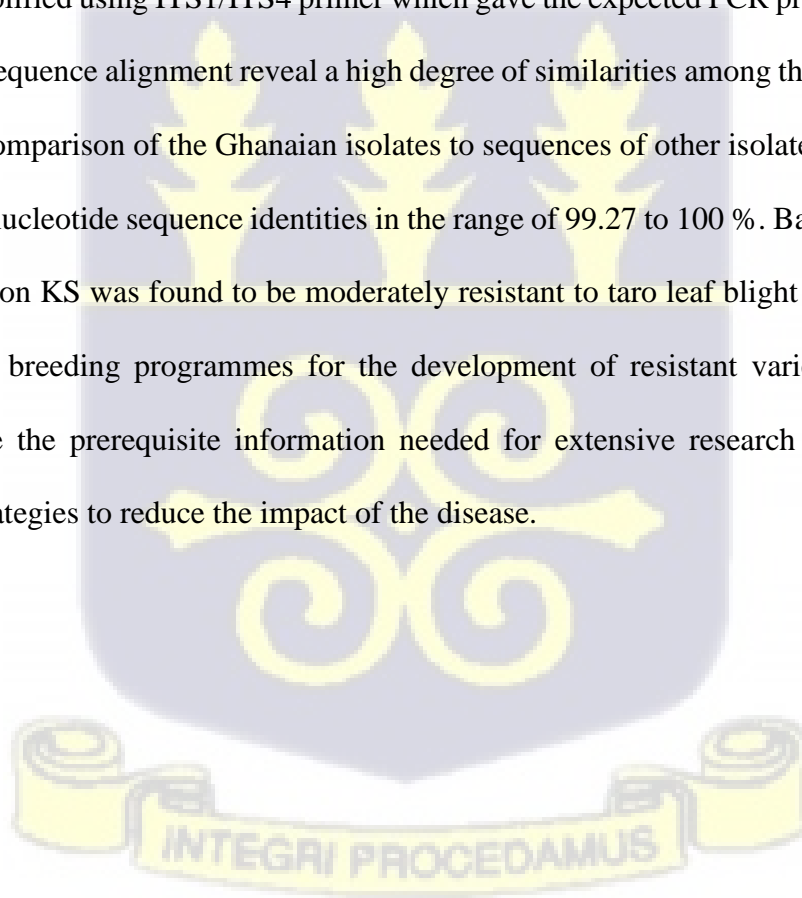
PR	Pathogenesis-related
RAMS	Random amplified microsatellite markers
RAPD	Random amplified polymorphic DNA
RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
RGC	Regional Germplasm Centre
SAR	Systemic acquired resistance
ScoT	Start codon targeted polymorphism
SNAS	School of Nuclear and Allied Sciences
SPC	Secretariat of the Pacific Communities
SRID	Statistics, Research and Information Division
SS	Symptom severity
SSR	Simple Sequence Repeats
TaBV	Taro bacilliform virus
TAE	Tris-acetate EDTA
TANSAO	Taro Network for Southeast Asia and Oceania
TaRV	Taro reovirus
TaVCV	Taro vein chlorosis virus
TLB	Taro leaf blight
USDA	United States Department of Agriculture.
VR	Vertical resistance



ABSTRACT

Taro (*Colocasia esculenta* (L.) Schott) is an e staple crop for millions of citizens in some developing nations. The crop popular dish in southern Ghana because of its high carbohydrate and protein content. Taro leaf blight caused by the fungus *Phytophthora colocasia* is a devastating disease of taro in many parts of the world where the crop is grown. Serious outbreaks of taro leaf blight in Samoa in 1993, as well as in Cameroon, Ghana, and Nigeria in recent years, have demonstrated the disease's catastrophic impact on the livelihood and food security of small farmers and local communities that rely on the crop. In several countries, it is responsible for up to 80 % of taro yield loss. In Ghana, the disease causes a 25–50 % reduction in yield. The objective of this research was to determine the prevalence, incidence, geographical distribution and molecular diversity of the fungus causing the blight disease of taro in Ghana. A survey was conducted to examine the extent of the posed by the pathogen as well as to ascertain the disease awareness level of farmers. The disease was in all taro fields surveyed, with average incidence of 79.26%. The highest disease incidence and severity on the farmer's field was observed in Anyinam whilst the least incidence was observed in Atimpoku in the Eastern Region. Field trials were set up in an RCBD and replicated three times. Ten taro accessions namely; Adenta (AD), Akim tafo (AT), Atimpoku (AP), Fawade (FW), Gyinyasi (GY), Haatso (HT), Kumasi tafo (KT), Koforidua (KF), Kasoa (KS) and Weija (WJ) were used in the study. The taro accessions were obtained from farmers in the Ashanti, Eastern, Greater Accra and Central region. The effect of the infection was assessed by measuring disease incidence and severity. Accession FW had the highest incidence of 96.67 ± 5.77 and the least was KS with 60 ± 52.9 . Disease severity was significantly higher in accession Gyinyasi (GY) with 1.69 ± 0.27 and the least in accession Atimpoku (AP) with 0.70 ± 0.27 . The effect of the disease on yield was also assessed. Gyenyasi (GY) was the accession

with the highest yield of 71.47 kg/ha and the least yield was recorded in accession KT with 41.00 kg/ha. The average yield of all the accessions was 56.64 kg/ha as compared to a control yield of 198.67 kg/ha. Fungal isolation was done from the diseased leaves collected during the survey and morphologically identified under the light microscope. Koch's postulate was conducted to confirm the isolated fungus. Fungal cultures placed on healthy leaves of fifteen potted taro plants per accession resulted in 100 % infection. DNA of the *P. colocasiae* was successfully extracted from the different fungal isolates. Molecular detection of the pathogen using Polymerase Chain Reaction (PCR) and sequencing were also be carried out using Sanger sequencing. Nine DNA template of the *P. colocasiae* from the samples collected from Midie farm 1, 2 and 3 (M1, M2, M3), Kasoa farm 1, 2, and 3 (K1, K2, K3) and Akim Tafo farm 1, 2, and 3 (T1, T2, T3) were successfully amplified using ITS1/ITS4 primer which gave the expected PCR product size of ~870 bp. Nucleotide sequence alignment reveal a high degree of similarities among the isolates from the three regions. Comparison of the Ghanaian isolates to sequences of other isolates available on the NCBI revealed nucleotide sequence identities in the range of 99.27 to 100 %. Based on the current findings, accession KS was found to be moderately resistant to taro leaf blight disease and could be employed in breeding programmes for the development of resistant varieties. The current findings provide the prerequisite information needed for extensive research and development management strategies to reduce the impact of the disease.



CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Taro (*Colocasia esculenta* L. Schott), which is locally called ‘aborbe’ or ‘mancanikokoo’ in Ghana, is often grown in home gardens, rain-fed uplands, forests, paddy fields, and along rivers (Bammite *et al.*, 2018). Taro is a root and tuber crop planted for consumption of its tuber (Aboubakar *et al.*, 2008). The plant is a part of the Araceae family in Arales order, and its members are monocots (Van Wyk, 2005; Henry, 2001). Araceae contain fewer genera and over thousand species scattered around the globe (Vinning, 2003; Merlin, 1982). The genera include *Alocasia*, *Amorphophallus*, *Colocasia*, *Cyrtosperma* and *Xanthosoma* consumed by humans as a source of carbohydrate (Matthews, 1995). The genus *colocasia* is said to be generated from colcus or qolqas, an old Egyptian term for taro (De Candolle, 1885). Taro is a temperature-sensitive crop grown in the areas of Africa, the Mediterranean, Asia and the Pacific (Matthews, 2006). The crop does well in rainy areas and most varieties are drought intolerant. The taro plant is about 1-2 meters tall and has a globular shape of leaves. The plants can grow in hilly environments if there is a considerably spread of rainfall of about 175cm during the growing period. Basically, annual rainfall level of 250cm is suitable for the growth of taro. High rainfall is important in the first 20 weeks of growth for good leaves development to enable the crop withstand the harmattan (dry) season can be withstood till harvesting (Lebot, 2009). *C. antiquorum* (syn. *C. esculenta*, *C. affinis*, *C. fallax*, *C. virosa*, *C. mannii*, and *C. gigantea*) is one of six species of taro found in India according to (Hooker, 1894). The species are allogamous and occurs in different morphs and form a significant portion of human nutrition (Ivancic *et al.*, 2003). *Colocasia* species are polymorphic. The two major cultivars are categorised into *Colocasia esculenta* (L) Schott var. *esculenta* which is

characterised to have a huge pipe-like corms with smaller number of cormels known as dasheen whiles *Colocasiae esculenta* (L) Schott var. *antiquorum* with haracteristic small globe-like central corm usually surrounded by a lot of cormels also termed as eddoe (Lebot and aradhya, 1991; Purseglove, 1972). *C. esculenta* Var. *antiquorum* is the most cultivated and a significant food plant in Ghana (Ackah et al., 2014). Cultivars are distinguished mostly by leaf pigmentation, plant structure, corm shape, number of cormels, cormel shape and cormel pigmentation (Quero-Garcia et al., 2010). The chromosomal number in *C. esculenta* is 14 and there are two cytotypes: triploid with 42 chromosomes and diploid with 28 chromosomes (Coates *et al.*, 1988). Triploids are sterile, but diploid cultivars are fertile. Triploids could have emerged as a result of autopolyploidy, according to cytogenetic, morphological, and biochemical investigations (Isshiki et al., 1999).

Worldwide, taro is positioned fourteenth in the hierarchy amidst staple crops with around 12 million tonnes cultivated globally from about 2 million hectares with mean yield of 6 tonnes/ha (FAOSTAT, 2010). Ghana, Nigeria, China, Cameroon and Papua New Guinea lead the production with more than 10 million tonnes of taro in 2014 (Otekurin *et al.*, 2021). Africa produce about 60% of taro in the world (Mweta *et al.*, 2010). Traditionally, taro is a source of income and food for the livelihood of rural people (Ackah *et al.*, 2014). It is an important crop for food security and revenue generating through exportations (Revill *et al.*, 2005). Normally, taro is underused because of its subsistence cultivation as compared to cassava and potatoes (Ugwu, 2009). Taro is primarily grown for because of its leaves and the corms but the flower, cormels, rhizomes and the stalk can also be consumed (Lakhanpaul *et al.*, 2003; De Candolle, 1885). Locally, the corm of taro is cooked by either boiling, roasting, frying, baking, or it can be crushed and moulded into balls and consumed with soup (Lewu *et al.*, 2010). it is a good constituent for food preparation because it

can be turned into flour, and it has starch and mucilage content (Liu *et al.*, 2006). According to Darkwa and Darkwa, (2013), cakes and chips are prepared from a mixture of taro flour and cereals is adopted in Ghana. The mix processing of cassava and taro improved the nutritional content of gari; food product from cassava which is traditionally eaten by West Africans (Bamidele *et al.*, 2013). The corms are rich in carbohydrate with approximately 70-96 % (Naidoo *et al.*, 2015; Aboubakar *et al.*, 2008). According to Igbabul *et al.*, (2014) the fermented taro flour has decreased the anti-nutritional factors, improved nutrients and can be the processed of taro flour into different food products.

The newly emerged leaves of a growing taro plant are used as vegetable and are significant in folic acid, vitamin A, vitamin C, Vitamin E, riboflavin and minerals including iron and potassium (Amagloh and Nyarko, 2012). Medicinally, taro contains a soluble fibre which aids in human digestive health (Amagloh and Nyarko, 2012). Taro can be consumed because of its ability to fight against tuberculosis, ulcer and reduction of lung congestion and fungal infections (Nath *et al.*, 2016).

Despite the numerous benefits of the crop, its production is constrained by diseases and pests. Globally, it is assumed that diseases lower yield of the crop by over 10 %, which equates one half of a billion tonnes produced annually (Hunter *et al.*, 1993). These epidemics lead to reduction in food availability, rising cost of food cost and a threat to rural lifestyle and national food security. Fungal diseases in particular, pose a huge danger to taro production, especially throughout the wet and humid seasons (Lin *et al.*, 2014). Taro leaf blight (TLB) caused by *Phytophthora colocasiae*, accounts for 25 to 50 % loss of taro output in several countries including Ghana (Mishra, 1997) The disease was first identified by Marian Raciborski in 1900 from Java on taro crops. In Africa,

the disease was initially discovered in Ethiopia and Equatorial Guinea in 2005. Subsequently in Cameroon (Fontem and Mbong, 2011), Nigeria (Bandyopadhyay *et al.*, 2011) and Ghana (Omane *et al.*, 2012), the disease is a strong devastating impact on lives and food security of subsistence farmers' who rely on taro as sources of income and food (Singh *et al.*, 2012). The disease damages the entire crop from the leaves, corms, petioles and cormels, leading to extensive reduction in yield (Maheshwari *et al.*, 2007). *P. colocasiae* is a foliar pathogen which also causes postharvest rotting of corms. A petiole rot is also based on the susceptibility of varieties (Brooks, 2008). Apart from *P. colocasiae*, taro root rots caused by *Ceratocystis fimbriata* also causes rot of corms worldwide (Harrington *et al.*, 2011).

TLB starts as a small, water-soaked flecks which extent to form dark brown lesion which is transmitted to clean and healthy crops. Wind-blown rain and dew distribute inoculum in the form of spores to surrounding plantations. The disease can also be transferred by taro planting material. The fungus has been found to linger on the tops of the plants for up to three weeks after harvest (Jackson, 1999). The surface of leaves are infected within 3-5 days of the first symptoms depending on the atmospheric circumstances. The disease spreads in conditions of cloudy weather with rainfall at irregular intervals with temperature is around 28°C. under such favourable conditions there is high increase in disease occurrence, and the whole crop field display a 'blighted' appearance (Misra *et al.*, 2008). Secondary infections cause rapid leaf destruction, which can happen in as little as 10–20 days in highly susceptible cultivars. The disease produces a massive reduction in the number of photosynthetic leaves and can result in yield reductions of up to 50 % (Jackson, 1999; Trujillo, 1967; Trujillo and Aragaki, 1964).

1.2 Problem statement

The major constraints of taro cultivation in West Africa and for that matter Ghana are erratic rainfall, pests and diseases attacks, restricted resources, preference for cereals and other root crops as source of dietary energy, relatively lower sales of taro and little research attention for crop improvement (Ofori and Kien 2004). Among these production constraints, the highest-ranking worry in taro cultivation is pests and disease attacks. The crop is attacked by at least ten main diseases and pests globally (Kohler *et al.*, 1997).

The prime important disease TLB caused by *Phytophthora colocasiae* could lead to 50 % loss in corms (Jackson, 1999, Trujillo, 1967, Singh *et al.*, 2006, Trujillo and Aragaki, 1964) and leaf destruction by as high as 95 % in susceptible varieties (Nelson and Brooks, 2011). The TLB disease in general poses challenges of low yield and poor quality of the harvested produce. In view of this problem of taro leaf blight disease in Ghana, farmers are increasingly losing interest in taro cultivation and virtually abandoning the crop leading to a looming threat of food insecurity and loss of farmer income.

In spite of the widespread prevalence of the disease and extensive crop damage, there is absolutely little data on the disease occurrence and severity in Ghana's taro-growing regions. Another, problem of taro production and utilization is that the germplasm discovered in farmers' fields and wooded regions are getting gradually eroded because of prolonged droughts, extensive deforestation and woeful lack of research attention for taro improvement (Boampong *et al.*, 2019). bBreeding for taro improvement have not yielded the expected impact because the available germplasm is poorly characterized and evaluated. In addition, the identification and molecular

phylogeny of *P. colocasiae* species have not been established to aid crop improvement efforts in taro in Ghana and many countries across sub-Saharan Africa.

Consequently, although taro is a major root plant grown in Cameroon, Nigeria, Ghana and Burkina Faso (Ndaeyo *et al.*, 2003 and Sanou *et al.*, 2012) where the crop has significant impact on the lives of people, there is not much documented information sharing within the sub region to help increase in the improvement of taro.

1.3 Justification of the study

In Ghana, the TLB disease has been so severe that it has pushed many farmers to abandon their taro fields with some of them converting their farms into vegetable farming (Omane, 2011). In spite of the widespread nature of the disease and the damages it causes to the taro crop, there is limited information about the disease's occurrence and severity in taro growing areas of Ghana. To properly establish long-term disease management techniques, it is necessary to first determine the epidemic's scope. Accession of taro that show high level of blight could be screened from germplasm, which are the farmers' field, home gardens and river banks that could serve as a foundation to facilitate and speed up the development of resistance to *P. colocasiae* for effective management strategy of the disease in Ghana. Research success will encourage scientists establish effective sustainable measure methodology to curb the spread of the fungus. Success in this regard will enable increased taro production, boost food security and substantial enhancement in farmer's income. Curtailing the spread of the disease by developing a strategy of managing the *P. colocasiae* through DNA techniques will revitalize the interest of farmers for commercial scale cultivation of the crop which would ultimately boost the exportation of taro product and increase

the nation's per capita income. For the purpose of crop improvement, the establishment of a basic collection of taro germplasm will help to facilitate research works in Ghana.

According to Nath *et al.*, (2016) screening of taro cultivars for *P. colocasiae* resistance has been investigated in leaf blight disease prone environment. Although this method has since been the standard for assessing taro cultivars for leaf blight tolerance trait, it depends on the prevalence and evenly spread of pathogen inoculum and favourable environmental factors for disease development. It is therefore, very important that research efforts are intensified towards the screening of available Ghanaian germplasm to identify *P. colocasiae* species to speed up the search for resistant cultivars or tolerant traits.

1.4 Objectives of the study

The main objectives of the study were to evaluate the incidence, severity, and phylogenetic structure of *P. colocasiae* in order to gain an excellent knowledge of the pathogen's molecular phylogeny and aid in the quest for long-term solutions to the taro leaf blight disease in Ghana.

The specific objectives were to:

1. assess farmers' knowledge on leaf blight disease.
2. conduct a disease survey to determine the prevalence, incidence and severity in four regions of southern Ghana.
3. screen taro accessions on the field for resistance to the blight disease.
4. undertake molecular detection, sequencing and phylogenetic analysis of Ghanaian isolates of *P. colocasiae*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Centre of origin and distribution of taro

Archeological records show that taro is among the world's oldest domesticated and important commodities (Lebot, 2009). The origin of taro is traced back to Asia, North eastern India and the Mediterranean region (Hanson and Imamuddin, 1983; Ivancic, 1992). The generally accepted view is that, the origination and domestication of taro began in eastern India and spread to Southeast Asia before spreading to the rest of the world (Kuruvilla and Singh, 1981; Yen and Wheeler, 1968). The theory that taro originated in Asia and spread throughout the Pacific is supported by archives of prehistoric human habitation and traditions in the Pacific Islands (Greenwell, 1947; De la Pena 1970; Cable, 1984; Matthews, 1995). From its centre of origin, the crop was distributed to Eastern Asia and the Pacific Islands; then westward to Egypt and the Eastern Mediterranean and eventually dispersed from southward and westward to East and West Africa, from where taro reached the Caribbean and America (Lee, 1999; Onwueme, 1978). The cultivation of taro led to its spread to more than 65 countries across the world (USDA, 2001). Taro has been positioning the fourteenth most consumed food worldwide. Africa produces approximately 60 % and Asia and the Pacific countries produce 40 % of taro volumes (FAO, 2003; Mweta *et al.*, 2010).

Molecular analysis using isozyme established two gene complexes for planted taro in Asia and Pacific (Lebot and Aradhya, 1991). Molecular markers such as SSR (Simple Sequence Repeats) (Srivastava, & Gupta, 2008) and AFLP (Amplified Fragment Length Polymorphism) (Kreike *et al.*, 2004) have proven the existence of these two distinct gene flows. The implication of the two

district gene pools is that taro was domesticated in Asia and the Pacific. As a result, taro is also considered a Pacific native plant. The period taro dispersed in West Africa is unknown. However, it is speculated that taro became established in Senegambia by AD 1500, long ago before the Portuguese arrived in West Africa (Blench, 2009). The first introduction of taro to East Africa is believed to be at least 2,000 years ago and thento Egypt via Arabia (Bown, 2000). Furthermore, tannia (*Xanthosoma sp.*) was imported to Central and West Africa throughout the 16th and 17th centuries by the Portuguese (Bown, 2000).

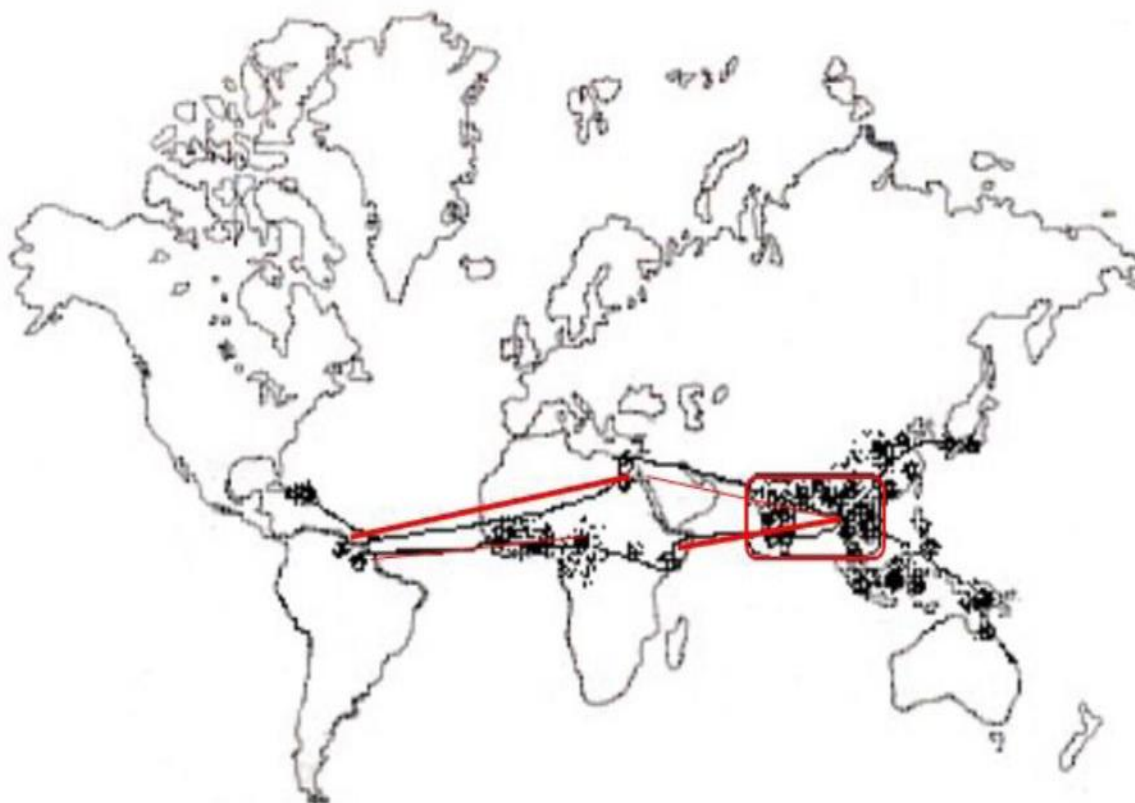


Figure 2.1 Origin and distribution of Taro

Source: (Wickramasinghe *et al.*, 2009)

Key: The dot shows movement of settlers from one location to another

2.2 Taxonomy and botany of the crop

Taro is a monocotyledonous plant that belongs to the genus *Colocasia* and the subfamily *colocasioideae* of the Araceae family (Hay, 1996; Plucknett, 1983). The epithet *colocasia* is allegedly deduced from *colcus* or *qolqas*, former Egyptian name for taro (De Candolle, 1885). Hooker (1894) identified six Indian taro species: *C. antiquorum* (syn. *C. esculenta*), *C. affinis*, *C. fallax*, *C. virosa*, *C. mannii*, and *C. gigantea*. *Colocasiae* species are polymorphic with the two major cultivars categorised into *Colocasiae esculenta* (L) Schott var. *esculenta* which is characterised to have huge pipe-like corms with smaller number of cormels known as dasheen while *Colocasiae esculenta* (L) Schott var. *antiquorum* consist of small globe-like mother corm surrounded by a lot of cormels referred to as eddoe (Lebot and Aradhya, 1991; Purseglove, 1972). Although cultivated taro is classed as *C. esculenta*, the species is polymorphic. (FAO, 1999; Purseglove, 1972).

Taro is a herbaceous and tuberous plant with a strong short caudex, producing flowers and leaves together. Typically, leaves are extended arrows or heart-shaped, with a cluster of them facing earthward (Prajapati *et al.*, 2011). The plant has 1-2-meter-tall erect stems with orange, red-black, or multi-layered pigmentation. The adventitious and narrow root system produces a large amount of good carbohydrate from the corm (Reyad-ul-Ferdous *et al.* 2015). The plant propagates itself through tubers and corms, and it also produces a cluster of 2–5 fragment inflorescences in the leaf axil (Diwedi *et al.*, 2016). Taro plants characteristically exhibits profusion of periderm, big thinwalled parenchymatous cells that store food. They also have badly formed and few vascular tissues with and the inclusion of latex and mucilage cells, and ergastic compounds like druses and raphides.

Raphides are associated with taro acidity or itchiness. Leaves of taro seem to be peltate and grow from the corm's apical bud, while the root grows downward. Cormels, daughter corms, and runners (stolons) grow horizontally. The root system is fibrous and primarily restricted to the top one metre of soil (Miyasaka, 1979).

Colocasia esculenta var. *esculenta* contains a large pipe like central corm which is surrounded few cormels. This type is termed 'dasheen' taro. The dasheens have 30cm long and 15cm in diameter below ground surface edible part of the crop. On the other hand, *C. esculenta* var. *antiquorum* is an oracle cloud corm that is ringed by cormels (stem tubers) and daughter corms. This type of taro is referred to as the eddoe. In both eddoe and dasheen kinds of taro, the core corm is the plant's main stem structure (Fig. 2.2).

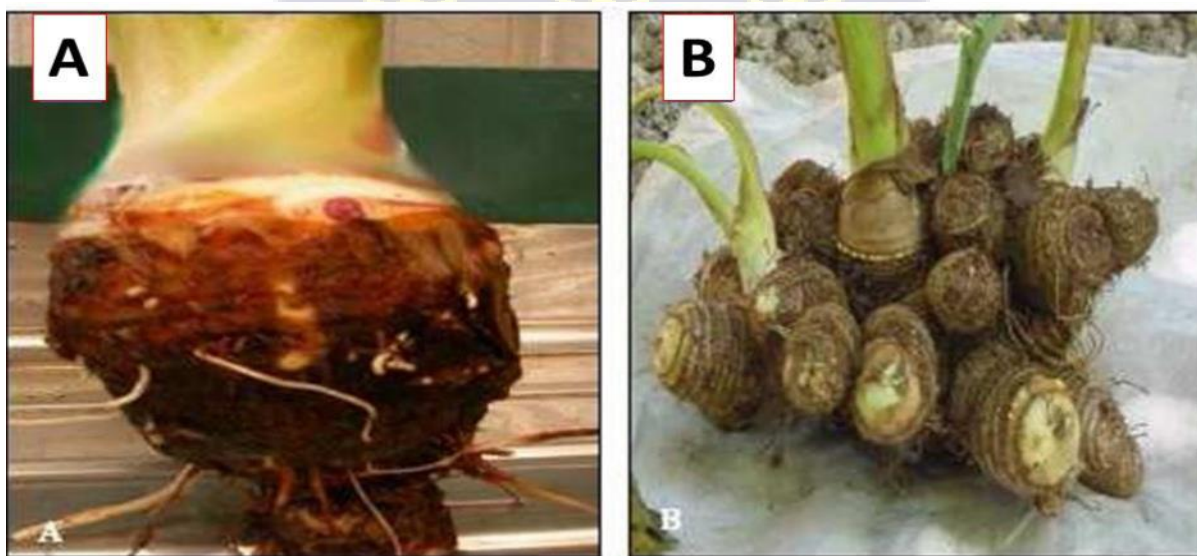


Figure 2.2 The corm structure distinguishes the two major taro cultivars. (A) *Colocasia esculenta* var. *esculenta* (dasheen) has a big central corm, whereas (B) *Colocasia esculenta* var. *antiquorum* (eddo) has a thin central corm with several fairly large.

Source: (Onwueme, 1999)

The combination of cormels and daughter corms accounts for a sizable portion of the edible eddoe taro. Subsidiary shoots are produced by daughter corms, but cormels remain inactive and only develop new shoots after the main plant get rotten. Each of the cormels and daughter corm contains terminal, axillary buds and numerous scale leaves surrounding the body. Mature corms normally weigh 0.5 - 6 kg. When fresh from the soil, the plant is toxic due to calcium oxalate in the corm and the needle-like raphides in the cells of the plant. The toxicity is eliminated by cooking or soaking the corm in ice-water overnight (Onwueme, 1999)

Internally, corms, cormels, and daughter corms are all very similar. The outer surface layer is made up of thick brownish periderm. Starch-filled ground parenchyma is found within the periderm. Vascular bundles and laticifers protrude from the ground parenchyma. Idioblasts cells containing raphides or bundles of calcium oxalate crystals are also found in ground tissue and nearly every other part of the taro plant. According to Bradbury and Nixon (1998), acidity is triggered by a protease bonded to raphides and constitutes a functional complex known as 'Nature's poisoned spear'. The density and woodiness of corm increase as the plant ages. Taro plants occasionally generate runners, which grow by side to side on the soil surface and give rise to new shoots (Onwueme, 1996). Each corm's surface is marked with rings indicating the attachment points of scale or senesced leaves. Axillary buds can be found on the corm at nodal positions. The apex of the corm denotes the plant's growing point and is directly adjacent to the ground level. Actively growing leaves sprout from the corm apex in a whorl. These leaves are the only viewable part of the plant above ground. They determine the height of the plant in the field. Each leaf has erected petiole and a large lamina. The petiole is bulged out at its bottom where it fastens to the corm, allowing it to clasp around the corm's apex. The petiole is thickest at its foundation, thinnest near

its adhesion to the lamina. Internally, the petiole has a spongy texture and excessive air spaces, which supposedly helps in exchange of gases when the plant is grown in marshy or deluged situations. The connection of the petiole to the lamina is peltate in most taro types, which means that the petiole is glued somewhere in the middle of the lamina rather than at the edge. This peltate leaf bonding differentiates taro from tannia, which has a hastate leaf. Lamina is oblong-ovate in shape, with a rounded lobe and sinus. Taro leaves consist of three major veins that radiate from the point where the petiole and leaf meet. The remaining veins surface from the three main veins, resulting in reticulate leaf venation. (Onwueme and Johnston, 1998; Purseglove, 1972). The wide, ovate leaves that grow to a height of 1–2 m is referred to as the "elephant head."

Occasionally, flowering occurs in taro. The inflorescence shoots arise from the leaf axils, or hub of closed leaves. The flower comprises of a short peduncle, a spadix, and spathe. Botanically, the spadix is a spike with a fleshy central axis to which the tiny sessile flowers are bonded. The spadix is 6-14 cm long, with female flowers at the base, male flowers near the tip, and sterile flowers in between, in the region squeezed by the spathe's neck. The extreme tip of the spadix with no flowers, is termed as the sterile appendage. The sterile appendage is a taxonomic feature that clearly delineates dasheen and eddoe taro. The sterile appendage of eddoe kinds is longer than the male part of the spadix, while the appendage of dasheen types is shorter. The spathe is a broad yellowish bract that sheaths the spadix and is around 20 cm long. The lower part of the spathe scarves tightly around the spadix, completely obfuscating the view of the female flowers. The zenith of the spadix is rolled inward, but one side is open to expose the male flowers on the spadix. A short neck region separates the top and bottom segments of the spadix, which corresponds to the zone of the sterile flowers on the spadix (Onwueme, 1999). Pollination of taro is facilitated by insects. Under natural

circumstances, fruit set and seed production occur on a sporadic basis. Fruits are 3-5 mm in diameter and lies at the lower part of the spadix. Self-pollination (Carson and Okada 1982) and cross-pollination (Patel et al. 1984) have been suggested, but floral morphology and male and female part development favor cross-pollination (Jackson and Pelomo 1980). Discovery of gibberellic acid (GA) has helped in the inducement of taro to promote flowering (Wilson, 1979).

2.3 Cytogenetics and genetic diversity of taro

A fundamental pair of 14 chromosomes has been determined for taro (Kokubugata and Konishi, 1999), as well as the bigger colocasoid species (Cusimano *et al.*, 2012). Karyotypes of *C. esculenta* are diploid ($2n = 28$ chromosomes) and triploid ($3n = 42$ chromosomes) (Coates et al., 1988; Yen and Wheeler, 1968). Triploid cultivars are infertile, but diploid varieties are viable. Dasheen types are usually diploids, while eddoe types are triploids (Quero-Garcia et al., 2010; Ivancic and Lebot, 2000). Chromosome numbers of $2n = 22, 26, 28, 38,$ and 42 have been found in many different places. Triploids may have arisen as a result of autopolyploidy, according to cytogenetic, morphological, and biochemical research (Isshiki *et al.*, 1999). Kreike *et al.* (2004) discovered diploids and triploids in Asia, while all accessions in the Pacific were diploids. Triploids appear to have evolved in reaction to the environmental situations on the hills in north eastern India, where diploids and triploids are prevalent (Kuruvilla and Singh, 1981). Tetraploid chromosomes can also appear on rare occasions (Isshiki et al., 1999), however at very low frequency (Matthews 2004). Generally, the genetic variation is narrow because taro farmers preferentially select for non-flowering types.

The number of morphological markers that indicate various phenotypes is limited, difficult to detect, and altered by environmental parameters. Several publications have attempted to use

isozymes to explain taro genetic diversity (Lebot and Aradhya, 1991) and RAPD markers (Irwin et al., 1998). Studies show that the greatest genetic variability of taro is found in Indonesia. Later, Isozymes (Lebot *et al.*, 2004) and AFLP (Kreike *et al.*, 2004) markers were then employed to examine the genetic variation of accessions from Vietnam, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea, and Vanuatu. These studies resulted in the establishment of two secondary domestication centers. The first center was discovered in Southeast Asia, and the second in Melanesia. Other studies have been undertaken inside countries: Vanuatu (Sardos *et al.*, 2012), Papua New Guinea (Singh et al., 2008), India (Lakhanpaul *et al.*, 2003), and Cuba (Manzano et al., 2001). However, worldwide characterization and its dispersal patterns of the genetic diversity of taro incorporating African and American genotypes is yet to be conducted (Chair et al., 2016).

Germplasm collections of the taro can be found in a number of research institutions in everywhere around the world such as the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria; the Philippine Root Crop Research and Training Center in Beybey, Philippines; the Koronivia Research Station in Fiji; the Bubia Agricultural Research Centre in Papua New Guinea; and a variety of other spots across Oceania. In Ghana, the Plant Genetic Resources Research Institute (PGRRI) Bunso of the CSIR is in charge of germplasm conservation and characterisation (Asomani *et al.*, 2017).

2.4 Edaphic, rainfall conditions and propagation of taro

Taro is grown in Africa, Mediterranean, Asia and the Pacific (Matthews, 2006). Taro does well in rainy areas. It is often grown in home gardens, rain-fed uplands, forests, paddy fields, and along rivers (Bammite et al., 2018). The plant is well-suited to tree crop and agroforestry systems, and

some varieties are especially well-suited to poor land and soil conditions, such as inadequate drainage. Taro is grown using two different production strategies. Taro can be cultivated in flooded or wetland areas where water is abundant, cool and continuously flowing for optimal level of dissolved oxygen. Warm, stagnant water with low oxygen levels causes the taro's basal rot. Airflow gaps in the petiole allow buried regions to sustain gaseous exchange with the environment under flood circumstances. The second system is dryland or upland taro production. Taro farming in the upland or dryland is primarily rain-fed, with sprinklers or furrow irrigation used to augment rainfall and keep the soil moist. Cultivation is normally done at the beginning of rainy season. Taro thrives best in areas where 6-9 months of rainfall is received and considerably well spread with about 175 cm during the growing period. Generally, 250 cm rainfall annually is sufficient for the growth of taro. The crop also thrives well in hilly environments where annual rainfall levels are high at least 250 cm. Such rainfall pattern enables the taro crop to receive enough water to mature (Varin and Vernier, 1994; Pardales and Villanueva, 1984; Pena, 1978). Nonetheless, most varieties are drought intolerant. Naturally, taro is mainly grown in soil that holds water or clay soils with a pH range of 5.5-6.5 (Weightman, 1989). Production of taro is usually limited to location around rivers or streams, zones with considerable rainfall and areas with stagnant water sources. The ability of some taro cultivars to resist saline is a particularly useful trait. Indeed, taro has been successfully employed as a first crop in the rehabilitation of saline soils in Japan and Egypt (Kay, 1973).

Taro is mainly vegetatively propagated; however, the entire life cycle includes both sporophyte and gametophyte generations. There is virtually minimal genetic heterogeneity within cultivars due to vegetative/clonal propagation although somatic mutations may occur (Ivancic, 1992; Kuruvilla and Singh, 1981; Strauss *et al.*, 1979; Shaw, 1975) For this reason, sexual hybridization of taro is now very much relied on and methods for pollinating and growing seedlings have been

developed for materials with wider genetic diversity (Tyagi *et al.*, 2004; Singh *et al.*, 2001; Hanson and Imamuddin, 1983). The vegetative part used for planting are categorized into four parts: the side suckers which comes from lateral growth of mother plant or runners, small corms which found attached to the mother plant, apical meristem (1-2) of the corm with 15-20 cm of the petioles joined and corm pieces formed whereby the big corms is divided into smaller pieces or peeled. These parts are excellent because they form the edible parts of the crop and they establish quickly when use as planting material. Minisett technique is also used to generate multiple planting materials in taro (Pena, 1978). Seed and meristem tissue culture can also be used to generate plantlet for propagation (Wilson, 1990; Jackson, 1977).

2.5 Major taro growing regions and production volumes

Africa is the world's greatest taro producer, followed by Asia and Oceania. China, Japan, the Philippines, and Thailand are the top producers in Asia, while Papua New Guinea, Samoa, the Solomon Islands, Tonga, and Fiji are the top producers in Oceania. (FAO, 1999). In Africa, Nigeria, Ivory Coast, Ghana, Congo and Cameroon are major taro producers. Other important taro producing African countries are Gabon, Egypt, Rwanda, Burundi, Zaire, Central African Republic, Comoros Island, Sao Tome and Principe, Madagascar and Mauritius. Taro contributes greatly to food security in both West Africa and the Pacific producing regions. Taro serves as a remarkable food in dry season before crops like yam and cassava are harvested in West Africa. The crop fills in the gaps in seasonal food fluctuations (Rashmi *et al.* 2018). Comparatively, taro contributes higher dietary energy in the Pacific countries than in West Africa (Ofori, 2019; FAO, 2008). Taro cultivation serves a source of revenue for small scale farmers in West Africa and the Pacific regions. Taro production helps to alleviate poverty for a number of vulnerable groups in the

producing regions. Taro exports are a significant source of foreign cash for some Pacific countries. In West Africa, particularly southern and eastern Nigeria, southern Ghana and Benin (Irvine 1974), taro is classified as an important non-traditional export commodity (Ofori, 2019).

According to the FAO (2012), global taro production in 2005 totaled more than 11.7 million metric tons from 1.78 million hectares of land, with an average global yield of 6.5 tons per hectare. Six leading taro growing countries are Nigeria, Ghana, China, Cambodia, Côte d'Ivoire and Papa New Guinea (FAO, 2012). The highest volume of taro is produced in Africa, where Nigeria and Ghana produce 5.1 and 1.8 million metric tons covering an area of 667,000 and 270,000 hectares respectively. Consistently, in the last three decades, Africa has recorded increasing contribution to the total global taro production volumes, which stood around 10 million tonnes per annum (FAO, 2012). The mean global production of the crop in the recent decade (2003–2012) was more than double that of three decades ago (1983–1992), due to increased output in Africa. Nigeria, Ghana and Cameroon accounted for a significant increase in the production of taro. These three countries together contributed 41 % of the average global output within 1983 and 1992, also 62 % of the average output between 1993 and 2002, 68 % of the mean volume between 2003 and 2012. In the years 2008 – 2012, West Africa contributed 50 % of global production of taro. These production levels established West Africa as the highest taro producing region in the world (FAO, 2013). As of 2017 Nigeria was the leading producer of taro with an estimated 3.25 metric tons representing one-third of total global production. China was second who produces 1.95 metric tons, Cameroon was third and Ghana was fourth about 1.8 and 1.5 metric tons respectively (Temesgen & Retta 2015).

2.6 Food, nutritional and health benefits of taro consumption

Taro is one of the few major staples whose leaf and subterranean components, as well as the corms, are consumed (Lee, 1999). The entire plant can be eaten. The petiole and the tender leaves are used as vegetables and consumed with boiled corm and any other foods (Linus, 2003). In parts of Africa, the petioles and the leaves are used as a soup thickener when groundnuts are expensive. Dried petiole and leaves are preserved during periods of dry season because of scarce as it used in making meal. Taro leaves can also be blended with smashed cowpea to prepare a common meal called “Koki” which is popular among Cameroonians. The corm of taro is cooked by boiling, roasting, frying, baking, or crushed and moulded into balls and consumed with soup (Lewu *et al.*, 2010). In parts of West Africa, cooked corm can be crushed and used as weaning diet. The corms are suitable for making mashed appetizer weaning food called ‘mpotompoto’ for babies in Ghana (Onwulata and Konstance, 2002). The corms are used to prepare traditional dishes and thus, make taro a delicacy for most traditional rites (Mbong *et al.*, 2013). Taro is a fine constituent in food preparation because of ease of turning it into flour with appreciable starch and mucilage content (Liu *et al.*, 2006). Cakes and chips formed as a result of from mixing taro powder and cereals is adopted in Ghana (Darkwa and Darkwa, 2013). The mixed processing of cassava and taro enhance the nutritional content of gari, a food product from cassava which is traditionally eaten by West Africans (Kapoor, Singh & Kumar, 2021).). According to Igbabul *et al.*, (2014), fermentation of corm before processing into flour decrease anti-nutritional factors, improve nutrients and increase use of taro flour into different food product. In Asia, it was observed that babies fed on poi, a type food prepared from taro, suffered less from diarrhoea, pneumonia, enteritis and beriberi than babies fed with rice and bread (Miller, 1971). Kelby (2012) revealed nutrient content of poi as hypoallergenic, rich in calcium, potassium, phosphorus, magnesium, B vitamins, vitamin A and

C, and carbohydrate. The protein, fats and ash content of taro are relatively low (Mawoyo, 2017). Taro flour, starch and mucilage are good components and are integrated as functional ingredients in food formulations (Liu *et al.*, 2006).

Taro starch is simple and easy to digest. The starch grains are very thin and hypoallergenic in nature, meaning they have a low chance of causing allergic reactions. They are also gluten-free (Jane *et al.*, 1992; Kochhar, 1998).

Taro corms consist of approximately 70-96 % carbohydrates of which a substantial proportion is starch with 17-28 % amylose, the other fraction being mainly amylopectin (Naidoo *et al.*, 2015; Aboubakar *et al.*, 2008; Moorthy, 2002; Oke, 1990). The mucilage content of taro corm is approximately 7-10 % (Huang *et al.*, 2010; Hong and Nip, 1990). *C. esculenta* corms contain approximately 63-85% water, 1.3-3.0 % protein, 0.2-0.4 % fat and minerals. The leaf content contains 87.2 % water, 3.0 % protein, 0.8 % Fat and 6.0 % carbohydrates (Onwueme, 1994). Taro contains 3.54 - 7.78 % of ash with good mineral contents (Nijoku and Ohia, 2007; Mbofung *et al.*, 2006). Taro contain lessfat and protein, although taro protein is less in isoleucine, tryptophan and Methionine but contains higher amount of sulphur-bearing amino acids rather than the proteins of other root tubers (Parkinson, 1984). The corm also contains elements such as zinc, ascorbic acid, vitamin A, vitamin B1, vitamin B2, and vitamin B3, vitamin B6, vitamin C, niacin, potassium, copper and manganese (Jirarart *et al.*,2006). Taro also serves as a superb birthplace of carotene, calcium, phosphorous, iron, riboflavin, thiamine, and dietary fibre (Opara, 2001; Bradbury and Holloway, 1988). Taro contains significantly greater amount of vitamin B-complex than full milk (Lee, 1999).

Taro foods are suitable for persons who are hypersensitive to cereals and infants who are lactose intolerant (Darkwa and Darkwa, 2013). Corms and leaves of taro are rich trypsin inhibitors (de Oliveira *et al.*, 1977). These bioactive compounds in sufficient amounts enhance good health and diseases prevention (Padula *et al.*, 2013; Rouphael *et al.*, 2012). Phenolic compounds in taro including (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, gallic acid, chlorogenic acid and possible traces of flavanols and proanthocyanins enhance antioxidant activities (Agbor-Egbe and Rickard, 1990). Many studies have confirmed that the consumption of phenolic compounds lower the chance of cardiovascular diseases and development cancers development (Mengane, 2015; Padula *et al.*, 2013; Abad-García *et al.*, 2009). In addition, bioactive compounds have been found to have soothing ability, antibacterial, antifungal, antimicrobial, anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antihypertensive, and antiviral activities (Mengane, 2015; Padula *et al.*, 2013; Abad-García *et al.*, 2009). The juice of the corm is a softening, purgative, and painkiller. Leaves of *C. esculenta* plants have been shown by phytochemical analyses to have anthocyanins, mainly cyanidin-3-rhamnoside, cyanidin 3-O-glucoside, and pelargonidin 3-O-beta-D-glucoside. These anthocyanins have cell strengthening abilities and control hepatoprotective of lipid peroxidative movement (Cambie and Ferguson, 2003; Kowalczyk *et al.*, 2003; Noda *et al.*, 2002).

Medicinally, consumption of taro helps to boost and build a strong immune system, lowers blood pressure, acts as weight and tiredness reducer, prevents cell damage, builds powerful bone, and brace proper thyroid function (Misra *et al.*, 2002; Nip *et al.*, 1989). Taro consumption prevents the expansion of cell lines in breast and prostate diseases, and utterly restricts movement of tumor cells (Kundu *et al.*, 2012). Externally, corm juice is used to treat baldness and serves as a laxative

and remedy for wasp stings. (Stephens, 1994). Nonetheless, taro foods possess acidity effects (Akpan and Umoh, 2004). These acidity factors can be diminished by immersion and acidification during preparation (Sharma *et al.*, 2016). Long duration cooking and peeling of the thick skin layer are superb ways of significantly minimizing acidity (Kaushal *et al.*, 2015; Crabtree and Baldry, 1982). Acridity can be also lessened by baking or ethanol extraction. These techniques help in enhancement of texture, cooking quality, palatability and digestibility of taro product (Kaushal *et al.*, 2015).

Taro root preparations are also used in traditional medicine to treat rheumatism and acne. Leaf extracts are used on cuts to induce blood clotting, neutralizing snake poison and as medicine for purging (Kubde *et al.*, 2010; Thinh, 1997). Taro plant extracts act as stimulant, rubefacient, styptic for treatment of buboes, otalgia, adenitis, and also act as laxative, demulcent, and urodynamic. In terms of health-promoting qualities such as antidiabetic, antidepressant, and anthelmintic effects, taro leaves have been announced to be potent (Dnyaneshwar *et al.*, 2018; Lin and Huang, 1993). Taro is also rich in soluble fibre that helps in the controlling of gastrointestinal motion and improving the quality of dietary mass and feces after ingestion because of its hydrophilic nature (Saladanha, 1995). *C. esculenta* contains a wide range of healing qualities. Anti-lipid peroxidative action, antimetastatic, antifungal, mitigating, and antibacterial, antihepatotoxic, and diabetic-friendly effects have been described. Individuals from some clans commonly utilize taro corm to treat physical ailments. Corm juice are derived for an expectorant and astringent are effective nervine tonic (Soumya *et al.*, 2014). Patients with peptic ulcers, pancreatic disease, chronic liver disease, inflammatory bowel disease, and gall bladder disease benefit from taro starch (Emmanuel-Ikpeme *et al.*, 2007) and its phytochemicals proof and anticancer effects (Huang *et al.*, 2004).

Industrially, the crop is used for production of taro chips, bread, vegetable taro burger and infants' food which is found in large supermarkets due to its high digestibility (Linden, 1996). Furthermore, active chemicals such as flavonoids and triterpenoids are extracted from Colocasia leaves and also Vicenin-2, iso-vitexin, iso-vitexin 3-O-glucoside, vitexin 3-O-glucoside, iso-orientin, orientin-7-O-glucoside, luteolin 7-O-glucoside are flavonoids available in the condensed Colocasia leaves which are used medically (Iwashina et al., 1999).

2.7 Socio-cultural and economic importance of taro

Taro is a crop known originally from the south-east Asian zone and disseminated to many countries across the globe. Taro cultivation and use have mainly developed along with the evolution of the cultures of the people from Asian and Pacific countries. The importance of the crop underlines its function as a popular ingredient in the socio-cultural life of people in the growing regions (Matthews, 1998). Taro crop has been important in attempts aimed at achieving food security in Africa and presents rich economic and socio-cultural significance (Darkwa and Darkwa, 2013; Mwenye, 2009; Onyeka, 2014). Traditionally, taro is known to be a primary plant for royalty, donations, feasting and fulfilment of other social responsibilities. In Samoa and Tonga regions, taro is at times used for barter trading serving as a form of currency. Similar status is accorded to yam in West Africa. Many sections of the plant are utilized in making conventional medicines (Onwueme 1999). The socio-cultural attachment to taro has elevated the crop to become an emblem of cultural recognition in many regions. The people of Pacific Island ancestry continue to eat taro anywhere in the world, not because there are no other food options, but rather to stay connected to their heritage. This ethnic commitment to taro has yielded a profitable taro foreign

market to tribal Pacific Islanders living in Australia, New Zealand and western North America. (Ofori, 2019; Onwueme, 1999).

Colocasiae. esculenta remains one of the most underutilised root tubers in Africa because less research attention has been devoted to taro production and use compared to the significantly larger research resources that drive cassava, potatoes or sweet potatoes improvement (Ugwu, 2009). In West Africa especially Ghana, taro has been defamed as food for the poor, therefore, it is cultivated mainly for subsistence with little surplus for income to small scale farmers. In Tonga, for example, tubers account about half of the nation's calorie consumption, with taro contributing 40 % of the calories. Similarly, taro provides roughly 10 % of people's dietary calories in the Solomon Islands, while other tubers provide 30 %. Furthermore, prior to the disastrous occurrence of taro leaf blight disease (TLB), nearly the population's dietary intake in Samoa came from tubers, with taro being part of the tubers (CTA, 2003). In horticulture, taro is cultivated as a decorative herb for beautification and it is sold as attractive aquatic plant. It was awarded the Royal Horticultural Society's Award of Garden Merit in the United Kingdom. (Dastidar, 2009; <http://en.wikipedia.org/wiki/Colocasia>. Last accessed February, 2021).

Industrially, there is the potential for taro to be used to produce alcohol as a fuel. Starch produced from taro can also be used as a feedstock in cosmetic and plastic manufacturing industries. Other products such as flour have been used largely in infant formulations in the United States and form a significant ingredient in canned baby foods (Lee, 1999). Moreover, cost effective and highly nutritious compatible foods and noodles are produced from taro and cowpea composites (Ikpeme-Emmanuel *et al.*, 2009). Taro is used in laboratory anthocyanin analysis, mainly with reference

abaxial and adaxial anthocyanin fixation (Hughes *et al.*, 2014). Because of increased industrial demand for starches, extensive research on underutilised traditional crops like taro, which have received little attention in this regard can be improved into a potential raw material for the production of tuber starches (Hoover, 2001). In animal husbandry, Griffin (1982) reported the production of taro silage and application livestock feed particularly for pig. In addition, production of chicken fodder using taro leaves is cost effective and contains substantial amount of amino acids and thus, can be used to substitute cereals in chicken feed (Anaeto and Adighibe, 2011).

2.8 Constraints of taro production

In spite of the importance of taro in the livelihoods of populations in of the growing regions and beyond, various constraints such as poor soil fertility and nutrient deficiency, high temperatures, erratic rainfall and prolonged drought, scarcity of better varieties, competition from weeds, and the presence of pests and diseases predominantly the most critical soft rots disease and leaf blights (Akwee *et al.*, 2015; Talwana *et al.*, 2009; Tumuhimbise *et al.*, 2009). These constraints translate into very low average yields obtained from the crop (Carmichael *et al.*, 2008; Ooka, 1990). Other obstacles are scarcity of land for large scale cultivation, lack of clean disease-free planting materials, lack of extension services, inappropriate agronomic practices, lack of high yielding, improved cultivars, ineffective marketing, and limited and uncoordinated research (Onwueme, 1999). The production challenges, generally cause very low yield levels of taro in West Africa compared to yields obtained in the agriculturally more resourced Pacific and Asian regions (FAO 1987). The negative impact of pests and diseases ranks very high among the challenges that hinder optimal performance of the taro plant. Leaf and corm rot diseases can reduce yield by 40-90 %

(Doku 1984). Corm rot in storage has been reported to cause about 75 % corm loss during bad infection (Nwufu, 1988; Nwufu and Fajola 1981).

2.9 Pests of economic importance in taro cultivation

Different pests for example insects, snails and nematodes cause a huge yield reduction in yields of taro (Lebot, 2009). Pests of particular attention are the insects, among which the most important are taro beetles and leaf hoppers which reduce production levels considerably. The taro beetle (*Papuana spp.*) belonging to the genus *Papuana* involve the species *P. woodlarkiana*, *P. biroi*, *P. huebneri*, and *P. trinodosa*. The adult beetle is black and lustrous, with a size of 15-20 mm. Many species have horns on their forehead. The usual life cycle is 22 to 25 weeks long. Adult beetles swarm from their nesting grounds to the taro field, tunnelling into the soil just below the taro corm's base. The beetles then feed on the developing corm, producing big holes that predispose rot-causing microorganisms to attack the crops. The feeding operation might force the damaged plants to wilt and probably die (BAF, 2014; SPC, 2002). Taro beetle multiplication has been discovered in a wide variety of plants (Sar *et al.*, 1997). The need to check the spread and damage caused by these devastating insect pests has led to stern quarantine laws on transfer of cultivars through regions. Markets for taro from certain producing countries have also been constrained by pest issues (Liloqula and Samu 1996).

Planthopper *Tarophagus Proserpina* (Kirk) (*Homoptera, Delphacidae*) is the transmitter of the bobone virus. This insect feeds exclusively on taro and cause heavy infestation that results in wilt later death of the plant. Sap gobbling and oviposition cause sap bleeding, which forms small red encrustations on petioles (Jackson, 1978). The caterpillars of other insect pests, *Spodoptera litura*.

and hornworms (*Hippotion celerio*) can be quite destructive as well. The adult armyworm moth is 15-20 mm and wingspan of 30-38 mm. Larvae of the insect consume the leaves by shredding the interveinal leaf surface and skeletonizing the leaves. The caterpillars of the hornworm (hawk moth), however, eat the greater leaf area and cause the leaf to appear ragged. Other pests of taro are the aphids (*Aphis gossypii*). These pests are small and pear-shaped. Aphids pierce leaves to obtain sap and making the plant to wilt and grow stunted. Sato and Hara (1997) reported that colocasia root aphid, *Patchiella reaumuri* (Kaltenbach) is a small sucking insect predominantly found on corm which causes corm rot.

Furthermore, Spider mites (*Tetranychus spp.*) are also known to suck the sap in leaf tissues usually between the main veins and make the taro plant to turn from white to pale yellow speckling. The spiralling whitefly (*Aleurodicus disperses*) causes damage by piercing the leaf and sucking the sap resulting in blacken leaf, degrading photosynthesis and early death of the plant when infestations are too severe. Another group of pests are the mealy bugs (*Pseudococcidae*) which have a long feeding tube for piercing the plant and juice sucking. Direct feeding causes wilting, yellowing, stunting, and deformed leaves. Mealy bugs are also vectors of taro viruses. Additionally, the giant African snail (*Lissachatina fulica*), is also an important pest. It is a huge, noticeable creature that conceal within the day and feeds at night. Plants destroyed by the snails to show severe harsh symptoms and malformed foliage. The weight of large numbers of snails can cause the branches or stems of host plants to break. The snail's slime path can also be used to identify it (Sundar, 2016; Carmichael *et al.*, 2008).



2.10 Important diseases of taro

Colocasia esculenta is susceptible to fungi, bacteria, nematodes, and viruses, which cause a variety of infectious diseases. Even though taro is sensitive to at least 23 diseases, only a few of them cause severe growth and yield reductions (Ooka, 1990). Bacterial soft rot (*Erwinia chrysanthemi*) and bacterial leaf spot are among the most economically important bacterial diseases of taro. These diseases cause a stinking, creamy-white corm soft rot, and plant wilt (Kidanemariam, 2018; Carmichael *et al.*, 2008; Ooka, 1990). Nematodes (*Hirschmanniella miticausa*) cause diseases such as Mitimiti disease. *Hirschmanniella miticausa* are intestinal parasite of taro worm that was initially discovered in Solomon Islands. The disease attacks the vascular system of taro plants and causes a very severe corm rot making the corm look reddish, giving it an appearance of uncooked fatty meat (Mortimer *et al.*, 1981). The Root knot (*Meloidogyne spp.*) also attack roots and forms galls or knots on the root of the plant while the lesion nematode (*Pratylenchus coffeae*) act as endo- and ecto-parasite that attack the roots, punches and penetrates the epidermis and the cortex of the plant (Smiley, 2010; Gowen *et al.*, 2005). Sign indication of virus infection was first announced by Johnston (1960). Viruses are among the most economically important pathogens of taro and infection can result in significant yield losses. Some examples of virus diseases of taro are Dasheen mosaic virus (DsMV), Taro bacilliform virus (TaBV), Colocasia bobone disease virus (CBDV), Taro vein chlorosis virus (TaVCV) and Taro reovirus (TaRV). These viruses have been found to cause yield loss of up to 20 % and reduce the qualities of the taro plant. After the discovery of taro viruses, there was restriction on the international movement of germplasm, which had serious impact on the accessibility to ergonomically elite lines including selected traditional cultivars.

Dasheen mosaic virus (DsMV) was first reported in 1970 from Florida, USA and subsequently assigned under the family *Potyviridae*, genus *Potyvirus* (Zettler *et al.*, 1970). The virus is flexuous and rod shaped and invades both edible and ornamental aroids. The virus is transfer by aphids, through vegetative portion and also through a mechanically process using plant sap (Babu *et al.*, 2011; Elliott *et al.*, 1997; Zettler *et al.*, 1970). The associated disease is distinguished by chlorotic and fluffy mosaic patterns on the leaf, as well as leaf disruption and crippled plant growth. The disease decreases corm yield by 20 to 60 % (Nelson, 2008; Rana *et al.*, 1983). Taro bacilliform virus (TaBV), a badnavirus causes vein chlorosis and down rolling of the leaf blades. TaBV is a plant pararetrovirus, family *Caulimoviridae* and genus *Badnavirus* (Bömer *et al.*, 2017; Bhat *et al.*, 2016). Another important virus, a cytorhabdovirus is the colocasia bobone disease virus (CBDV) which causes terrible stunting, resulting in deformed, thickened, and brittle leaves that may or may not unfold. Galls can form on the petioles and bigger veins. The virus is much more devastating when co-infection with taro bacilliform virus (TaBV) occurs. Instances of double infection give rise to the development of alomae disease, a composite of two or more viruses acting simultaneously. Alomae disease could also be caused by the taro large bacilliform virus (TLBV) which is transmitted by the plant hopper, *Tarophagus proserpina*, and the taro small bacilliform virus (TSBV) transmitted by the mealybug, *Planococcus citri*. Taro vein chlorosis virus (TaVVCV) is a nucleorhabdovirus and causes distinct veinal chlorosis symptoms whereas Taro reovirus (TaRV) co-infects taro plants together with other viruses but usually found to induce no symptoms in infected plants (Revill *et al.*, 2005a, b).

Another group of taro diseases that cause considerable economic losses is fungal associated diseases. Pathogens of *Colocasia esculenta* incorporate *Pythium* species, *Phytophthora colocasiae* (Rac.), *Cladosporium colocasiae* (Sawada), *Sclerotium rolfsii* (Sacc.), *Curvularia* species,

Rhizopus stolonifer (Ehrenb.), *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum*, *Colletotrichum gloeosporioides* (Penz.) and *Corynespora cassiicola* (Berk and Curtis) Wei, *Phytophthora nicotianae*, *Phytophthora citricola*, (Carmichael et al., 2008; Ooka, 1994) and black rot (*Ceratocystis fimbriata*). These fungal pathogens cause considerable loss in yield taro worldwide (Harrington *et al.*, 2011). Fungal diseases manifest mainly as diseases of the leaves, corms and roots. The most devastating fungal disease of the leaves is created by the fungus *Phytophthora colocasiae* (Liwqula *et al.* 1980). Oomycete water mould, *Phytophthora colocasiae* is a pathogen that is implicated to cause TLB. The pathogen causes round, water soaked, necrotic spots on the leaves and petioles and the entire collapse of the plant (Deo *et al.*, 2009). TLB was long reported in West Africa where it continues to destroy taro cultivation, and adversely influencing means and food security of rural sections. Coleman et al., 2003 indicated that an outbreak of the disease in the Solomon Islands after World War II resulted in a permanent shift to other crops. TLB epidemic across the American Samoa and Western Samoa regions in 1993-1994 caused drastic reduction in export and loss of several millions of dollars (USDA, 2001). *Pythium* species reported as the cause of corm rot in taro (Jackson and Gollifer, (975). Corm soft rot (*Pythium spp.*), *Pythium* root and corm rots appear to be the highest dispersed disease of taro (Ooka, 2014). Plant rot is a disease caused by *Phytophthora* species, affect the crop and later execute the whole plant. Extensive fungal infection have been reported in the United States and West Africa (Hao, 2006).

Taro corm rot can also be caused by the soil-borne fungus *Athelia rolfsii* which infects taro at the soil level and cause corms and roots to rot and leaves to wilt. Furthermore, another disease of considerable importance is the brown leaf spot (*Cladosporium colocasiae*) or ghost spot of older leaves. On either leaf surface, the symptoms appear as reddish-brown, round or irregular, diffuse

patches or blotches, sometimes with dark, diffuse centers. Awuah () reported in Ghana, this leaf spot disease caused corm rot. Also of interest is the Shot hole (*Phoma spp.*) fungus that produces big lesions on the leaves, with the centers breaking out as the spots mature, giving the appearance of a shot hole (Sundar, 2016).

2.10.1 PHYTOPHTHORA SPECIES

Phytophthora is a genus of plant-damaging oomycetes, Phylum oomycota, a fungus-like ancestry in the kingdom chromista (Levesque *et al.*, 2008). Species of *Phytophthora* have the potential to cause massive economic harms in crops as well as plant destruction in natural environments. Even though 100–500 undiscovered *Phytophthora* species are expected to live, only about 170 species are listed (Brasier, 2009). *Phytophthora spp.*, predominantly dicotyledonous plant diseases, are found in soil and water all over the world. The pathogens affect a diverse array of agricultural, recreational, and forest plants. *P. infestans* is the most well-known *Phytophthora* species, as it is the pathogen in charge of potato late blight and the Irish potato crisis of 1845, which saw 25 % of Ireland's 8 million people die through starvation and emigration. *P. infestans'* host range is confined to solanaceous crops since many *Phytophthora* species are host-specific, but some species have a wider host range. *P. cinnamomi* is published to infect over 900 species of woody and herbaceous plants while *P. nicotianae* attacks more than 250 plant species including onions (Nowicki *et al.*, 2011). *P. capsici* infects *Cucurbitaceae* fruits for example cucumbers and squash. *P. citricola* foster root rot and stem cankers in citrus trees. *P. fragariae* is also known to cause red root rot affecting strawberries and *P. kernoviae* is a pathogen associated with diseases in beech, rhododendron, shrubs such as oak and holm oak and other trees.

P. megakarya, among the cocoa black pod disease organisms, is widespread and accountable for the majority of cocoa crop losses in Africa. *P. multivora* was found in the investigation of isolates with *P. cinnamoni* dieback infections in Southwest Australian woodlands. The creature was located in a variety of taxa and was named multivora (Scott et al., 2009). *P. palmivora* induces fruit rot of coconuts and betel nuts. *P. ramorum*, on the other hand, infects over 60 plant genera and over 100 host species, causing rapid oak death in a number of tree species as well as ramorum blight in a number of aesthetic and shrub species (APHIS, 2006). *P. alni* also give rise to root and collar rot in alders whereas Rhododendron root rot is generated by *P. cactorum*, which affects rhododendrons, azaleas, and other Rhododendron species. *P. cactorum* further creates bleeding canker in hardwood trees, as well as commercial crops including apple, pear, and strawberry (<http://wikki.bugwood.org>). *P. nemorosa*, a new pathogen confirmed to cause cankers and leaf blight of forest trees (Hansen, 2003). *P. hydropathica* is an oomycete plant pathogen that lives in water, places such as irrigation and river water. *P. hydropathica* has been found infecting a variety of plants, including Catawba rhododendrons, English Roseum rhododendrons, mountain laurels, and Laurustinus trees (Vitale et al., 2014; Loyd et al., 2014; Hong et al., 2008). *P. sojae* is a soil-borne plant pathogen that induces stem and root rot of soybean and a major cause of crop loss. Even though plant diseases produced by this genus are strenuous to control chemically, the main management technique is to develop resistant cultivars (Schmitthenner, 1985.). *P. colocasia* infects primarily leaves, petioles and corms of *colocasia spp.* and *Alocasia marcorhiza* (Singh et al., 2012).



2.10.2 *PHYTOPHTHORA COLOCASIAE*: FIRST REPORTS AND DISTRIBUTION

TLB, originated by *Phytophthora colocasiae*, a foliar oomyceteous diseases agent is reported as greater impediment in taro cultivation around the globe (Brooks, 2008). Unlike Pacific regions, there has been less information of TLB disease in other areas. In most cases in the Pacific, growers to abandon taro wherever the disease had occurred and cultivate different root crops (Jackson, 1993). The infection has resulted in the extinction of more than 60 % of known types in Guam (Hunter *et al.*, 1998). Similarly, there were over 300 different taro types thought to exist before the disease arrived in Hawaii, but only a few survived the disease's impact (Trujillo, 1996). TLB extended to Cameroon in West Africa in 2010, causing severe harvest losses of up to 90 % (Guarino, 2010). The TLB epidemic caused very high market prices for taro and limitation of planting stuff. The disease spread rapidly to other West African countries including Nigeria and Ghana (Bandyopadhyay *et al.*, 2011; Omane *et al.*, 2012). A disastrous cascade effect resulted from the decreased food and household earnings, increasing poverty, and even hunger.

Raciborski (1900) was first to study TLB in Java and the first to name the causative *P. colocasiae* Racib. The genesis of *P. colocasiae* is still uncertain (Zhang *et al.*, 1994). However, given that Asia is the world's center of origin for many wild and farmed taro species, Ko (1979) speculated that Asia could be the origin of *P. colocasiae*. Trujillo (1967) had earlier postulated Southeast Asian to be the origin of the pathogen. The presence of A1/A2 mating type ratio of roughly 1:1 is one of the indicators of a fungus's center of origin, such as *Phytophthora* (Zentmyer, 1988). China has recently provided evidence for *P. colocasiae* Asian origin (Zhang *et al.*, 1994), earlier reports showed that only the A2 copulatory type was observed (Ho *et al.*, 1983). There were 136 A1, 102 A2, and 42 A0 mating types among 280 strains of *P. colocasiae* collected from Hainan Island.

Such studies shows that Hainan Island is part of the centre of origin of *P. colocasiae*. Ann et al. (1986) screened and discovered that all 799 isolates from blight-infected *C. esculenta* farms in Taiwan had comparable colony morphology and behaved as A2 mating types. A suggestion that the fungus is not native to Taiwan. In Papua New Guinea (PNG), Hawaii, Samoa and Guam (Fullerton *et al.*, 2000) only mating type A2 were identified. Only A1 mating types were previously reported from India (Narula and Mehrotra, 1981) even though lately, A2 mating strains have also been found from India (Misra *et al.*, 2011; Tyson and Fullerton, 2007). Aside *P. colocasiae*, few other species of *Phytophthora* viz., *P. araceae* (Coleman) Peth, *P. palmivora* Butler, *P. parasitica* Dast. var. *pipernia* Dast, *P. nicotiana* Bredade Ham. Var. *parasitica* Dastur, have been reported to infect taro (Umabala and Ramarao, 1972; Narasimhan, 1927). However, it is unknown what function these species have in terms of severity, harm or epidemiological factors.

Phytophthora. colocasiae is thought to spread through vegetative propagated material and potentially through soil. The Oomycete is now largely scattered worldwide in Asia where is mostly in Bangladesh, Brunei, China, India, Indonesia, Irian Jaya, Japan, Korea, Malaysia, Peninsular Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Africa it can be found Cameroon, Equatorial Guinea, Ethiopia, Ghana, Nigeria, Seychelles, in North America it can mostly found USA Central America and Caribbean includes Brazil, Cuba, Dominican Republic, Puerto Rico, Trinidad and Tobago, South America it can be commonly found in Argentina, and Oceania includes American Samoa, Guam, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, Solomon Islands (Omane *et al.*, 2012; Bandyopadhyay *et al.*, 2011; CMI, 1997; Weston, 1918).



2.10.3 BIOLOGY OF PHYTOPHTHORA COLOCASIAE

2.10.3.1 LIFE CYCLE OF THE PATHOGEN

In 1890, Raciborski identified the cause of TLB as *P. colocasiae*, which he discovered in Indonesia. The mycelium of the pathogen is hyaline, coenocytic and inter- or intra-cellular. The hyphae are slender, long and unbranched. The fungus grows best at a pH of 6.5 and a temperature of 28°C (Sahu et al., 2000). The sporangiophores are thin, unbranched, and exceedingly narrow at the tip, with a length of up to 50 μ m. The sporangia are long, lemon- or pear-shaped, and range in length from 38-60 \times 18-26 μ m. The sporangia grow directly or indirectly based on the environmental situations. In indirect germination which usually occurs in water at optimum temperature of 20-21°C, as many as twelve reniform, biflagellate zoospores are freed, converted into cysts and germinate 30 minutes after release. Cytoplasm of each sporangium divides into 15–20 zoospores, which flow out via the end pore and use their propellers to swim over water. This is a quick process that takes less than a minute starting with divergence in the cytoplasm to the release of zoospores. Within 10 minutes, the zoospores land on leaf surfaces, lose their flagella, and form a spherical cyst that sprouts to form a germ tube (Trujillo, 1965; Misra, 1996; Fullerton and Tyson, 2001). Direct germination occurs 5-6 hours at optimum condition of 20-28 °C. The fraction of sporangia that sprout immediately is usually substantially lower than that of sporangia that germinate via zoospore formation. (Thankappan, 1985; Misra, 1999; Putter, 1976).

According to Misra (1996) zoosporangial length exceeds 100 μ m and width exceeds 50 μ m. The zoosporangium might produce another zoosporangium depending on the weather circumstances. The zoosporangium can germinate either directly or indirectly by creating one or more germ tubes or zoospores. Zoosporogenesis, or indirect zoosporangial germination, begins with the almost

spontaneous union of cleavage capsules and the release of zoospores. The zoospores are ejected from the discharge vesicle and manage to escape by breaking through a thin plastic barrier. The zoospores encyst with a thick cell wall within 20 minutes after being released. Within 30 minutes after encystment, the cysts proliferate (Fig. 2.3). *P. colocasiae* is a dangerous pathogen because of its abundance of zoosporangia, zoospores, and cysts. Notably, amphigynous antheridium, in particular remains at the base of the spherical and yellowish oogonium for a considerable length of time after the oospores have formed. The oospores are also spherical with between 20 and 28 μm diameter and lie freely in the oogonium (Misra *et al.*, 2008). *P. colocasiae* produces pectolytic enzymes such as polygalacturonase, pectin methyl transesterase, and poly methyl galacturonase, which play a key role in *P. colocasiae* pathogenesis. (Aggarwal and Mehrotra, 1986). The majority of infections occur between the hours of midnight and dawn. Infections occur only during the day when the weather is consistently wet. During infection, germ tubes formed by sporangia or encysted zoospores either perforate the epidermis directly or enter through stomata. The Oomycete spreads intercellularly after penetrating the mesophyll (Fig. 2.4). The initial symptoms normally manifest in 24 hours, and symptomatic progression is fastest at temperatures ranging from 25 to 30 °C in gloomy and/or rainy situations. However, at 35 °C, symptom growth is silenced. (Singh *et al.*, 2012).

Sporangia can travel with the water across a whole field and into adjacent paddies in wetland taro. The pathogen can also survive as mycelium in dead or dying plant tissues and infected corms for a period of time. During dry periods, they can stay alive as encysted zoospores or as chlamydospores in soil (Quitugua and Trujillo, 1998). *P. colocasiae* mycelium has a short life in soil, usually lasting less than five days. In the absence of a living host plant, however, encysted zoospores can live for several months. Most sporangia in vegetative planting material only live a

few days, although a few have been shown to live for up to two weeks (Fullerton and Tyson, 2004). Oospores and chlamydospores can behave as survival structures in diseased plant tissues or soils, but they are rarely found in the field (Nelson *et al.*, 2011).

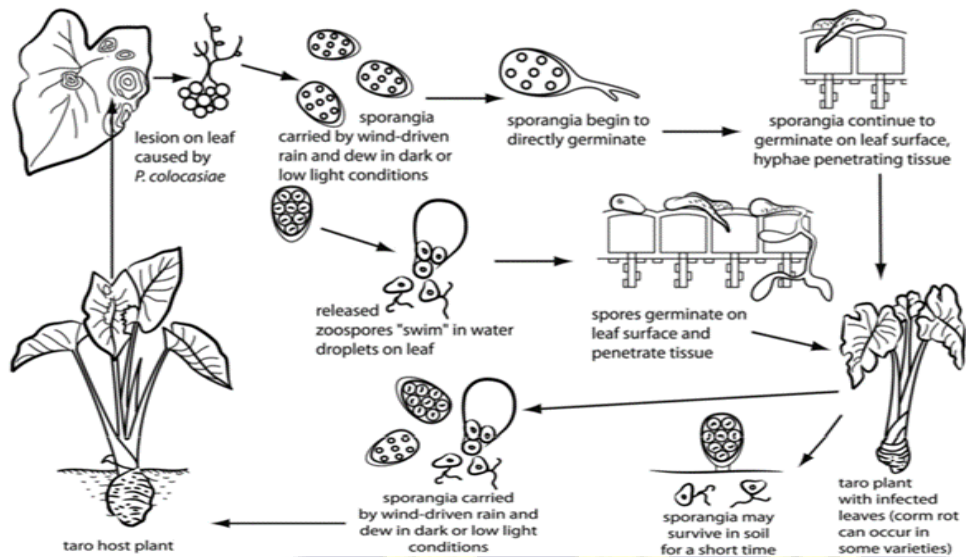


Figure 2.3 *P. colocasiae* sporangia germinate both straightforwardly and via zoospore generation. (Singh *et al.*, 2012).



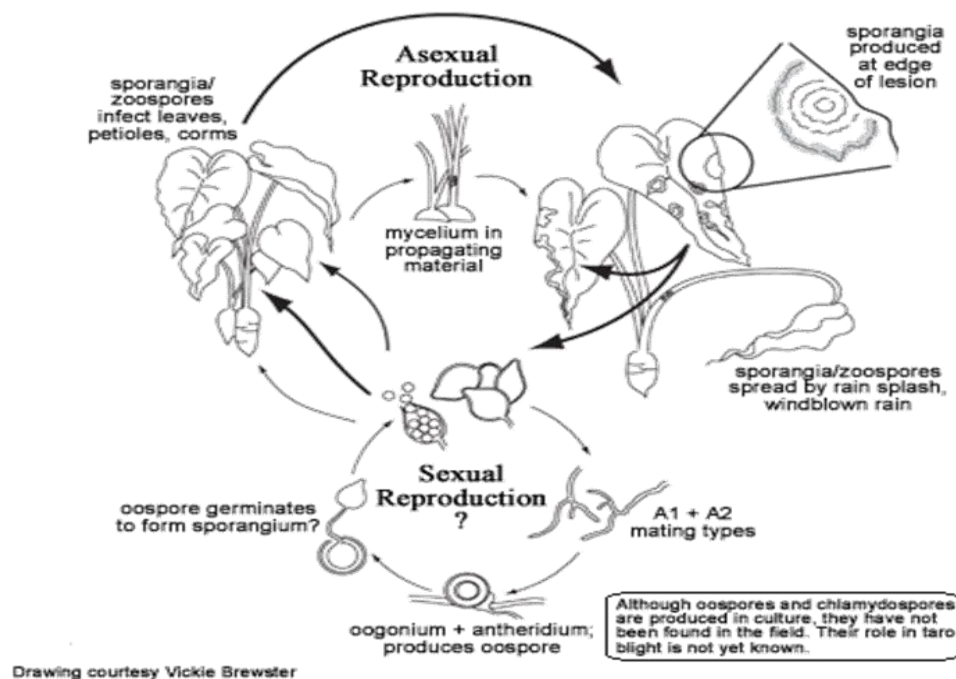


Figure 2.4 The disease cycle and epidemiology of taro leaf blight

2.10.3.2 GENETIC VARIABILITY OF *PHYTOPHTHORA COLOCASIAE*

Phytophthora colocasiae is a diploid heterothallic Oomycete that requires opposing breeding types (A1 and A2) to produce oospores (Tyson and Fullerton, 2007). Heterothallic *Phytophthora* species rapidly produce oospores when two concurrent mating types are paired (intra- or inter-specific). (Ko, 1979). Depending on the constancy of A1 and A2 mating types, multiple strains are likely to conjoin and evolve quickly. While oospore creation can be easily influenced in culture between opposing mating types, there is no proof that this event occurs daily basis in nature. The scope of genetic variability in *P. colocasiae* is undisclosed, but sexual reproduction is related to increased genetic differences in other *Phytophthora* species, including increased variability in virulence and

combativeness (Fullerton and Tyson, 2004). Recently, Lebot *et al.*, (2003) investigated isozyme diversity among *P. colocasiae* isolates from Southeast Asia and the Pacific region, discovering that TLB is caused by a multitude of different and genetically varied isolates across this broad geographic range. Variabilities both within and between countries occurs due to the diploid and heterothallic nature of *P. colocasiae*, different genetically variable isolates are likely to hybridize and unfold counting on the recurrence and presence of A1 and A2 mating kinds. In a study of Pacific cell lines, only one mating type (A2) was identified throughout the zone (Tyson and Fullerton, 2003). Neuter know as A0 mating type variants were also discovered in Indonesia, Thailand, and Papua New Guinea, with no oospores generated with either assay. *P. colocasiae* exhibits a high level of genetic diversity, regardless of the lack of a sexual cycle. Isoenzyme and RAPD studies of strains from Thailand, Vietnam, the Philippines, Indonesia, and Papua New Guinea (TANSAO, 2001) indicated significant genetic heterogeneity within and between countries.

2.10.3.3 EPIDEMIOLOGY AND SYMPTOMS OF TARO LEAF BLIGHT

Phytophthora colocasiae is largely a foliar pathogen that also damages the hosts' petioles and corms and causes corms to decay after harvest (Brooks, 2008). Early leaf infections are common in areas where rain, dew, or guttation droplets collect. Infections begin as water-soaked sores that quickly grow into big brown patches (Nelson *et al.*, 2011). The appearance of these lesions is characterized by a distinct day/night cycle. The lesions grow during the night by forming 3–5 mm wide water-soaked border. During the day, this margin dries out, and the next night, a newer water-soaked zone forms (Fullerton and Tyson, 2001) Infected petioles are rare, but they do happen in sensitive cultivars. Small, brown, elongated patches appear at the commencement of the infection.

In wet weather, the spots can broaden and soften until the weight of the leaves breaks the petioles. (Brooks, 2005). As the spots grow, they congregate and swiftly demolish the leaf. Cores of lesions turn papery and drop out in dry weather or in some improve varieties, giving them a "shot-hole" appearance (Nelson *et al.*, 2011). Leaves of sensitive cultivars collapse in around 20 days, but non-infected plants take 40 days (Jackson *et al.*, 1980; Jackson and Gollifer, 1975a). As a result, photosynthesis in sensitive plants is severely decreased, resulting in smaller leaves and corms.

Corm rots typically develop quickly after harvest, with the whole corms putrefying in 7–10 days. Rots typically begin in areas damaged throughout harvest when the petiole bases and suckers are removed, particularly during or after humid, hot climates. The diseased tissue is light-brown in color, hard, and frequently defined border in early phases. The rotted corm tissue may be attacked by *Lasiodiplodia theobromae* and turn black in advanced stages of corm rot (Jackson and Gollifer, 1975b). *P. colocasiae* causes significant yield losses, and considerable attention is needed to handle leaf blight before beginning taro farming. In locations where leaf blight is severe, the use of tolerant cultivars can significantly lessen the destruction caused by the disease. TLB shows small, water-soaked flecks that expand in perimeter to mould dark brown lesion also transfer to clean and healthy crops. The surface area of the leaf is affected within 3-5 days after the first symptoms based on the atmospheric conditions. Within cloudy weather conditions where rainfall occurs at irregular intervals and temperature around 28 °C, there is highly increase of the disease and the whole field display a blighted appearance (Misra *et al.*, 2008). Secondary infections cause speedy leaf destruction, which can happen in as little as 10–20 days in highly prone types. A healthy leaf has a lifespan of around 40 days. The disease causes a large drop in the number of functioning leaves and can result in yield losses of up to 50 % (Jackson,

1999; Trujillo, 1967; Trujillo and Aragaki, 1964). Wind-driven rain and dew disseminate inoculum in the form of spores to surrounding plants and crops.

2.10.3.4 CULTURAL CONTROL METHOD OF *PHYTOPHTHORA COLOCASIAE*

Cultural control, which entails removing all contaminated leaves, results in full defoliation of the crop, which has a negative impact on productivity (Adams, 1999). Control strategies include the disposal of contaminated leaves, the use of clean corms, and crop rotation (Mundkur, 1949). They discovered that even when disease risks were high, the plant density could be doubled and produce could still be boosted. Mulching with paddy straw increases yield, improves weed management, and reduces disease severity marginally. Other cultural measures suggested include postponing planting on the same site for at least three weeks, avoid replanting near older diseased ones, and stopping the spread of pathogen-carrying corms or suckers from one crop to the next (Jackson, 1999). According to preliminary data, fertilizer treatment may have also aided the plant's resistance to leaf blight (Filial et al., 1996). Intercropping taro with other crops can help reduce disease in some cases. In taro monocrops, disease severity was consistently higher than in a taro/maize intercropping system (Hunter *et al.*, 1998; Amos and Wait, 1997). In Samoa, trials to determine the effect of planting time, intercropping, fertilizer, and leaf removal on the occurrence and severity of the disease were equivocal (Chan, 1997). Planting time can be changed so that the critical stage of plant growth and the best climatic conditions for pathogenicity do not overlap. In a field experiment at the Regional Centre of CTCRI, Bhubaneshwar (India) to examine the effect of planting date on the prevalence of leaf blight and tuber yield. Misra (1988, 1989, 1990) discovered that planting tubers in the month of May gives higher yield and avoided much of the blight damage (Misra and Chowdhury, 1995)

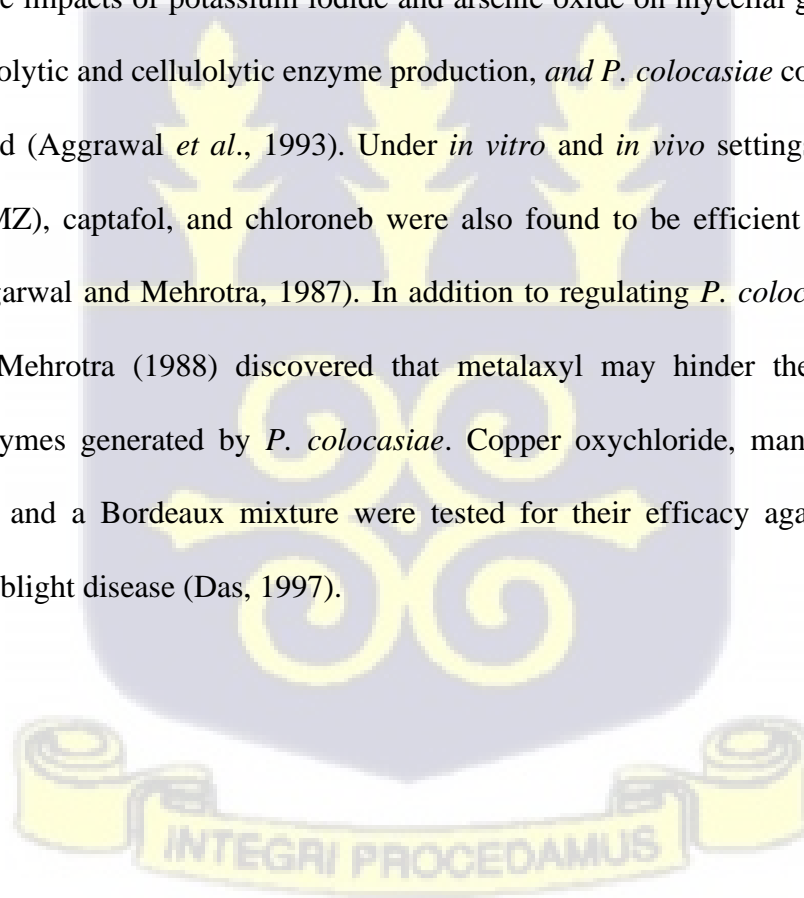
2.10.3.5 BIOLOGICAL CONTROL METHOD OF PHYTOPHTHORA COLOCASIAE

Biological control agents applied foliarly have the capacity to prevent taro crops from infection. Example, plants sprayed with the fungus *Trichoderma* showed significant decrease in number of infected leaves and disease severity (Palomar et al., 2001). Nerula and Mehrotra (1981, 1987) investigated the *phyllo plane microflora* of *C. esculenta* in connection to *P. colocasiae* and discovered that *Myrothecium residuum*, three *Streptomyces* organisms, and two bacterial isolates were hostile to *P. colocasiae* in dual culture plates. *In vivo*, the bacteria lowered disease incidence by up to 43 %, with *Streptomyces albidoflavus* reducing infection by 90-93 % and *S. diastaticus* reducing infection by 76 %. *Botrytis cinerea* had the best control of plant infection, with a 33 % reduction. *Trichoderma viride*, *T. harzianum*, *Gliocladium virens*, and an unknown sterilised fungal culture were observed to have prospective antagonism against *P. colocasiae* (Pan and Ghosh, 1997; Sawant *et al.*, 1995). The mycoparasitic or hyperparasitic actions of these isolates on *P. colocasiae* were caused by morphological changes such as winding of hyphae, creation of haustorian-like structures, disorientation of host cell components, and permeation into host hyphae. Rhizobacteria isolated in colocasia rhizosphere soil have been shown to totally suppress *P. colocasiae* growth in vitro. *T. viride* obscured the population of *P. colocasiae* by up to 88.88 %, whilst *T. harzianum* and *T. pseudockei* effectively lowered the population of *P. colocasiae* by up to 77.77 and 88.88 %, respectively (Misra et al., 2008)

2.10.3.6 CHEMICAL CONTROL METHOD OF PHYTOPHTHORA COLOCASIAE

chemical control of taro leaf blight disease utilizing copper oxychloride has been described in the Solomon Islands (Jackson *et al.*, 1980) Copper oxychloride fungicides are used to combat taro leaf

blight in the Pacific Islands (Semisi et al., 1998; Cox and Kasimani, 1990; Aggarwal and Mehrotra, 1987). In Fiji, copper fungicides have proven to be particularly efficient in eradicating taro leaf blight disease (Parham, 1949), in India (Mundkur, 1949) and Hawaii (Bergquist, 1972, 1974; Trujillo and Aragaki, 1964; Paris, 1941). Mancozeb (e.g., Dithane M45), Polyram, Benlate, Perinox, and Dyrene have also been reported to be beneficial in the management of this disorder (Bergquist, 1972; Maheswari *et al.*, 1999). In Samoa, a research program to evaluate chemical control (Hunter and Iosefa, 1993) proposed that phosphorous acid (Foschek), which had been demonstrated to be effective against TLB, be alternated with Mancozeb to save money and limit the risk of the Oomycete acquiring resistance. There was also no considerable variation in disease control between phosphorus acid formulations (Foschek, Agri-Fos 400, and Foli-R-Fos) (Nelson *et al.*, 2011). The impacts of potassium iodide and arsenic oxide on mycelial growth, sporangial production, pectolytic and cellulolytic enzyme production, and *P. colocasiae* control on taro have been investigated (Aggrawal *et al.*, 1993). Under *in vitro* and *in vivo* settings, metalaxyl (e.g., Ridomil Gold MZ), captafol, and chloroneb were also found to be efficient in eliminating *P. colocasiae* (Aggarwal and Mehrotra, 1987). In addition to regulating *P. colocasiae* in the field, Aggarwal and Mehrotra (1988) discovered that metalaxyl may hinder the cellulolytic and pectinolytic enzymes generated by *P. colocasiae*. Copper oxychloride, mancozeb, metalaxyl, captafol, ziram, and a Bordeaux mixture were tested for their efficacy against the taro var. antiquorum leaf blight disease (Das, 1997).



2.10.3.7 RESISTANT CULTIVARS AND GENETIC RESOURCES METHOD OF CONTROLLING *PHYTOPHTHORA COLOCASIAE*

In most production systems, the utilization of resistant cultivars presents an enormous protracted management strategy towards TLB. Resistance is divided into two categories namely vertical and horizontal. Vertical resistance (VR), also known as monogenic resistance, is a form of resistance regulated by one or a few main genes that allows absolute control over a pathogen's race (Singh *et al.*, 2001). It is frequently distinguished by a hypersensitive reaction in the host. A gene-for-gene association has been observed in several of cases (Robinson, 1996). As a result, new pathogen races emerge that can attack previously resistant plants. As a matter of fact, VR is also known as non-durable resistance (Singh *et al.*, 2002). The genetic management of VR against TLB may not be overly complicated and can be passed down through the generations (Patel *et al.*, 1984).

Horizontal resistance (HR), on the other hand, is regulated by a small number of genes and does not involve a gene-to-gene connection. It is effectual against all kinds of a disease and has a prestige for being long-lasting, hence named durable resistance. Unlike VR, this sort of resistance does not provide perfect control, but it does limit the pathogen's progress within the plant and frequently lowers sporulation. Using a variety of host-pathogen modelling techniques and genetic testing, the resistance mechanism in taro against TLB is categorized under the HR (Singh *et al.*, 2001; Ivancic *et al.*, 1996; Robinson, 1996). Due to the high heterozygosity of taro genotypes, studying inheritance in a standard Mendelian method is difficult (Ivancic, 2000). The physiological and pharmacological processes of resistance and host defense responses in the taro and *P. colocasiae* pathosystems have not been thoroughly investigated (Ho and Ramsden, 1998). Sharma *et al.* (2009) recently used suppressive subtractive hybridization, cDNA libraries, Northern blot

analysis, high throughput DNA sequencing, and bioinformatics to describe defensive scheme genes in taro caused by *P. colocasiae* infection. Two potential resistance genes and a transcription factor were discovered among the elevated sequences using these genomic methods. TLB-resistant genotypes had higher total expression of these genes than those that were vulnerable to the disease. The conservation and exploitation of plant genetic resources are linked by characterisation of germplasm and the evolutionary process in viable ecosystems. The advancement of molecular and biochemical methodologies assists researchers in not only identifying genotypes but also assessing and utilizing diversity (Whitkus et al., 1994). Plant breeding and ex situ conservation of gene banks would benefit from understanding the comparative genetic variation within taro genotypes (Ochiai et al., 2001; Irwin et al., 1998; Wolff and Peter-van Rijn, 1993). The Secretariat of the Pacific Community (SPC) manages a library of taro cultivars with varied levels of TLB resistance at the Centre for Pacific Crops and Trees (CePaCT, previously the Regional Germplasm Centre (RGC)). These variants are the result of breeding programs in Hawaii, Papua New Guinea, and Samoa. There are additional taro varieties from Asia in the CePaCT that have been tested for TLB resistance in their home countries. A new initiative in Hawaii gathered nearly 300 taro genotypes from Nepal, Thailand, Vietnam, Indonesia, Myanmar, China, Japan, and the Philippines, as well as seven sites in Micronesia, Melanesia, and Polynesia. TLB resistance levels were found to vary (CTAHR, 2009). The Plant Genetic Resources Research Institute (PGRRI) of the Council for Scientific and Industrial Research (CSIR) of Ghana has a germplasm of taro cultivars such as KA/019, SAO/020, SAO/006, ELO/002, KA/035 and EX BUNSO which are Ghanaian land races and are tolerant to TLB, some genotypes from Somoa which include BL/SM/16, BL/SM/10, BL/SM/116, BL/SM/80, BL/SM/115, BL/SM/151, BL/SM/132 and BL/SM/158 which are also

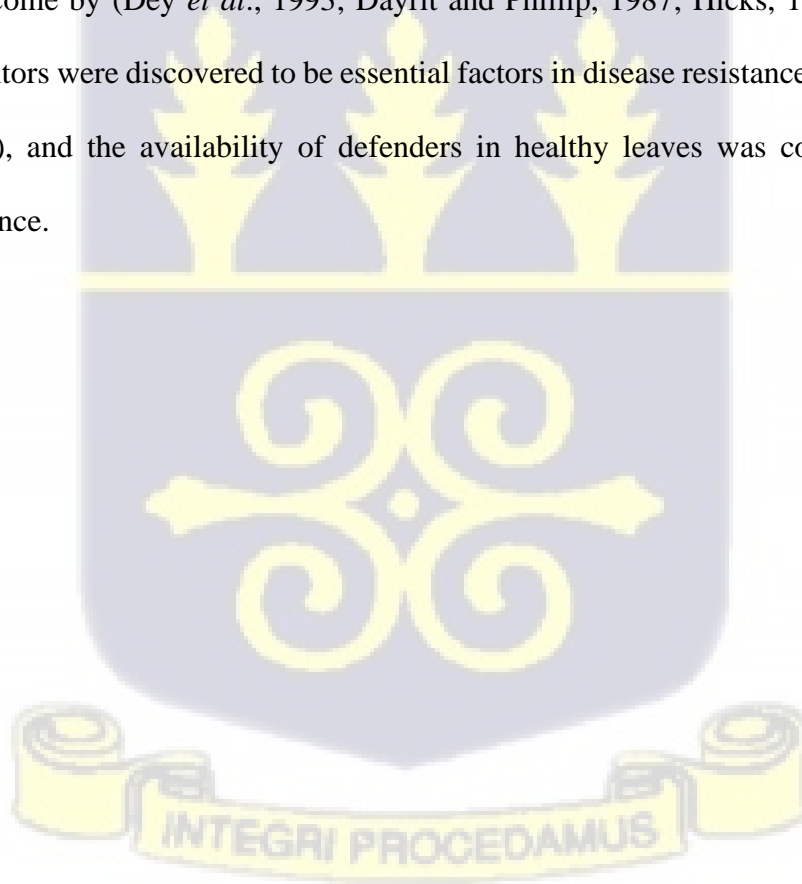
tolerant and CE/MAL/32 and CE/MAL/14 from Malaysia are Resistant to the *P. Colocasiae* (Boampong *et al.*, 2019)

2.10.3.8 BREEDING FOR RESISTANCE CULTIVARS AGAINST *PHYTOPHTHORA COLOCASIAE*

Breeding for resistance for the control of Taro leaf blight has proven extremely cost-effective approach (Singh *et al.*, 2010; Iramu, 2003). The taro breeding effort in Papua New Guinea has mostly relied on a modified recurrent selection method with a high premium on TLB resistance. In 1994, cycle-1 was created by combining a resistant foundation populace with outstanding, high-yielding, and tasty local taro cultivars (Okpul *et al.*, 1997). Some partly superior genotypes with undesired wild traits were obtained from cycle-1. In 1996, these partially improved genotypes were intercrossed to form Cycle-2. In 2001, cycle-2 produced three new types (NT 01, NT 02, and NT 03) (Singh *et al.*, 2010; Okpul *et al.*, 2002), and one variety (NT 04) was generated from cycle-3 by intercrossing chosen cycle-2 genomes (Singh *et al.*, 2006). TLB has been reduced in Papua New Guinea owing to the creation of these high-yielding taro varieties. The varieties overperformed in farmers' fields, yielding more than 50 % as much (approximately 9 t/ha) as the celebrated variety "Numkowec" (roughly 6 t/ha) used as a control (Okpul *et al.*, 2002). In several parts of Papua New Guinea, these breeders' lines have been generally embraced (Guaf and Komolong, 2006). A program was started in Samoa to examine and evaluate exotic taros and PSB-G2, Pwetepwet, Pastora, and Toantal were shown to be more resistant to leaf blight than the other cultivars tested in the field. During the years 1996–1998, these four cultivars were multiplied and tested in trials. The severity of disease recorded for each cultivar was not significantly different, according to a pilot trial. The largest corms were generated by Pastora, followed by PSB-G2, Pwetepwet, and Toantal (Hunter and Pouono, 1998).

Variety 'Ahina' was discovered to be resistant to blight by Deshmukh and Chibber (1960). When compared to sensitive varieties, 'Ahina' generated fewer sporangia. At Simla, Paharia and Mathur (1964) screened 20 varieties. They discovered that variety 'Poonam Pat' is immune to blight, variety 'Sakin' is resistant, and another seven varieties are moderately resistant. Misra (1988, 1989, and 1990) examined 43 colocasia cultivars where 'Jankhri' and 'Muktakeshi' were found which are extremely resistant, five were resistant, 12 were moderately resistant, and the rest were moderately to highly prone to *P. colocasiae* (Goswami et al., 1993). Choudhury and Mathura Rai (1988) used wild colocasia plants to breed resistance and selected resistant lines from them. Five of the twenty genotypes examined in Arunachal Pradesh were tolerant to blight.

In other colocasia-growing regions, cultivars with a respectable level of blight resistance have proved hard to come by (Dey *et al.*, 1993; Dayrit and Phillip, 1987; Hicks, 1967; Paris, 1941). Proteinase inhibitors were discovered to be essential factors in disease resistance in taro by Ho and Ramsden (1998), and the availability of defenders in healthy leaves was connected with the increased resistance.



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CHAPTER THREE

INCIDENCE, SEVERITY, AND FARMERS' KNOWLEDGE OF LEAF BLIGHT IN MAJOR GROWING REGIONS

3.0 INTRODUCTION

Taro is an important food crop in Africa, Asia, and Latin America (Nebiyuq, 2003). Taro is also a communal and food security crop, for multiplicity of humanity in Sub-Saharan Africa (Muluaem, 2012). Ghana is the third-largest source of taro plants in Sub-Saharan Africa, after Nigeria and Cameroon, and fourth in the universe. Taro (*Colocasia esculenta*) is grown virtually every portion of the moist tropics in over 65 nations throughout the world, and it is a crucial staple food and carbohydrate source for residents in a few subtropical and tropical zones (Tsedalu *et al.*, 2014). It's predominantly grown in thriving nations, on smaller farms with limited technology rather than big plantations (Mwenye, 2009). The crop is the world's 14th most prevalent tuber vegetable, with a crop productivity of 12 million tonnes derived from around 2 million hectares of land and a yield of 6.5 tonnes per hectare (Rao *et al.*, 2010, Hollyer, 1997). Taro is an economically important grown mostly in Ghana's transitional and forest zones, namely the Bono, Ashanti, Western, and Eastern Regions (Acheampong, 2014). The plant contributes to food security because it is a versatile crop in which both the corms and the leaves (known colloquially as 'kontomire' in Ghana) are used in various forms as meals. (Sagoe, 2006; <http://agricinghana.com>). Taro plants are more nutritious than other root crops such as cassava, potato, and yam, and are widely grown by poor farmers (Onyeka, 2014). The leaves are full of fibre and contain key nutritional compounds such as vitamins A and C. The leaves are also beneficial to one's health and are prescribed for persons with stomach problems, diabetics, and the elderly (Plucknett, 1970). The plant is high in nutrients

like niacin, thiamine, and riboflavin, as well as a manuscule portion of dietary fiber and vital minerals like calcium, phosphorus, magnesium, and potassium. (Niba, 2014; Onyeka, 2014; Eleazu, 2013). Potassium is plentiful and iron is scarce among the minerals. Sugar content in corms changes depending on maturity and storage conditions; sugars are converted to starch as corms grow, and starch is converted back to sugars when stored corms sprout (Agbor-Egbe and Rickard, 1990; Maga, 1992). In the pharmaceutical sector, starch can be utilized as a replacement for maize as a particular binding substance (Subhadhirasakul et al., 2001). Corms chips, which are manufactured from the corm, are also widely available on Ghanaian streets, where they are purchased and consumed as appetizers and desserts (Owusu-Darko et al., 2014).

Taro tubers can be uteded to make 'ampesi' or 'fufu' during the off-seasons of crops such as yam and plantain. In Ghana, women constitute the laargest proportion intaro plant production and trading, and for most them, it is an alternate source of income. The plant adds to Ghana's GDP in a minor way as a significant world trade commodity (Onyeka, 2014). Taro beetles (*Papuana spp.*), the Alomae Bobone virus complex (ABVC), decreased soil fertility, whiles *Phytophthora colocassia* Racib-caused taro leaf blight (TLB) reduce yield. The effects these hindrances to production have led to the crop's diminishing productivity (Sar *et al.*, 1998). Changes in nutritional patterns and preferences for foreign foods, as well as the advent of alternative crop species having superior competitiveness, such as Chinese taro (*Xanthosoma sagittifolium*) and sweet potato, have reduced taro output (Joughin and Kalit, 1986; Bourke, 1982; Waddell, 1972).

Despite the multiple socioeconomic advantages of taro plants, the crop's output continues to fall year after year due to a variety of restrictions, one of which is the taro blight disease caused by *P. colocasia* Racib, a fungus-like Oomycete. The pathogen is the most significant disease-causing organism that induces taro blight disease worldwide. This disease is the primary cause of massive yield losses of up to 100% of both corms and leaves (Fontem and Mbong, 2011; Trujillo *et al.*,

1997). The taro leaf blight epidemics has the ability to significantly food supply, raise food prices, increase poverty among rural inhabitation and endanger national food security. *P. Colocasia* spreads through diseased vegetative plant parts and potentially infected soil. This vegetal pathogen has now developed throughout Africa, East Asia, the Americas, the Caribbean, and the Pacific, also other taro-growing region on the earth, with varying degrees of severity. (Omane *et al.*, 2012; Bandyopadhyay *et al.*, 2011). Taro production in 2005 was barely 4 million pounds, according to the United States Department of Agriculture (USDA, 2006), the lowest since data became available in 1946. Rainy weather, TLB, and taro pocket rot (another *Phytophthora* species-related disease) were all contributors to the low production (USDA, 2006). The struggle against plant disease is a never-ending battle for farmers. Plant disease epidemics have altered the course of history in some localities where they have had a terrible impact and remain the considerable concern, particularly individuals whose livelihoods are dependent on their crops. Plant diseases have caused hundreds of thousands of deaths due to devastation of staple food crops and subsequent famine. Studies have shown that plant disease has general impacts on the farmers as well as the environment (Anon, 1998).

3.1 Objectives of the study:

The main objectives of this research were to evaluate taro farmers' perspective and management measures for controlling leaf blight pathogens.

Specific objectives of the study were:

1. To determine the level of awareness of farmers on *P. colocasia* diseases that infect taro crops.
2. To assess taro leaf blight incidence and severity in major *Colocasia* growing areas in the regions.

3.2 MATERIALS AND METHODS

3.2.1 Study area and location

The research was carried out in the Ashanti, Greater Accra, Central, and Eastern regions of Ghana.

The map below shows the study areas

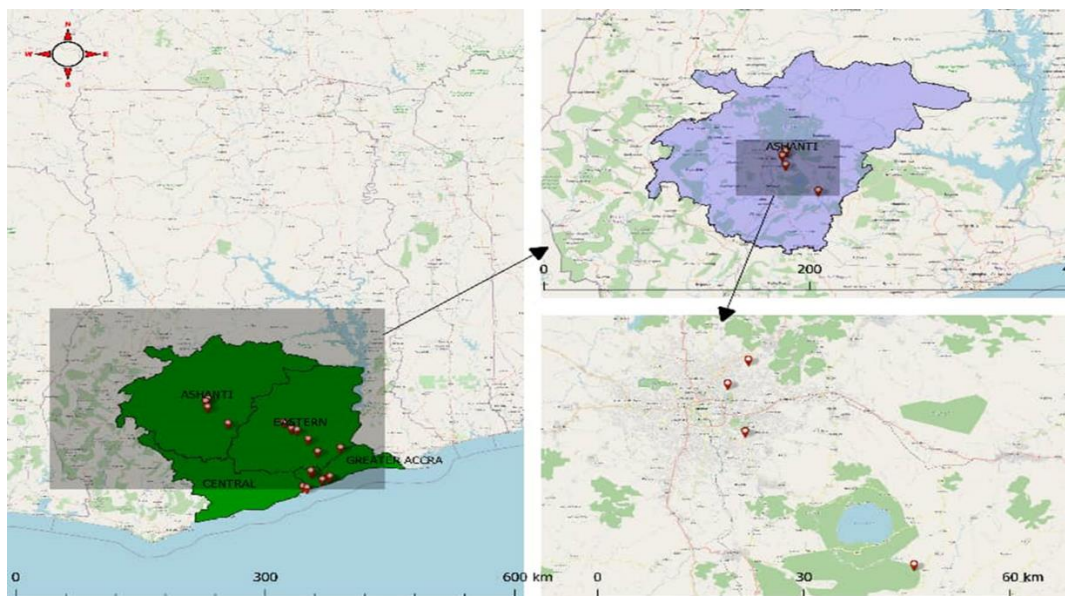


Figure 3.1 The area covered in the study Ashanti, Greater Accra, Central and Eastern region.

3.2.2 Ashanti Region

The Ashanti Region is located in southern Ghana and is the third largest of the now 16 administrative regions (originally 10) with only a total land area of 24,389 km² representing 10.2% of the total land area of Ghana. However, in terms of population, the Ashanti regions is the most densely inhabited region, with population of 4,780,380 representing 19.4 % of the toatal population of the nation according to 2011 census (Ghana Statistical Service, Population and Housing Census 2010). The Ashanti Region lies between longitude 0°15-2°25 West and Latitude 5°50 –7°40 North. The region shares boundry on the north with the Brong Ahafo Region on the west by Western Region, south by Central Region, and on theeast by Eastern and Volta Regions. The region receives twice as much rainfall as the rest of the country, with the highest rainfall level occurring in May/June and October. The average annual rainfall is between 1100 and 1800 millimeters. The average annual temperature in the region varies from 25.5°C in the southern regions to 32°C in the northern districts. Humidity is high, with an average of 85 % in the southern regions and 65 % in

the northern. The Guinea savanna comprises the northern half, while moist semi-deciduous woodland dominates the southern parts. The Guinea Savanna is made mostly of short, fire-resistant deciduous trees. Rivers in the woodlands can also be discovered along the Afram River and in the savanna zone's streams. The region covers around 3,180 sq km, or 22.5 % of Ghana's forest reserves, with 2,340 sq km (65 %) being exploited and 1,240 sq km (32%) being protected. Monoculture, shifting cultivation, and mixed cropping with short fallow periods usually less than 5 years are practiced. Monocultures of plantation of tree crops such as cocoa, oil palm, citrus, and mango are cultivated. During the early stages of development, however, these plantation crops are intercropped with food crops. Approximately 77 % farmers that are less than 1.2 hectares in size (MoFA last retrieved June.2021).

3.2.3 Greater Accra

In terms of landmass, the Greater Accra region is the smallest, with a total surface area of 4,450 km². the region, between 5° 30' North and 6° 03' North latitudes and 0° 30' West and 0° 35' East longitudes. It is flanked on the north with the Eastern Region, on east the Volta Region, and the west with Central Region. The Gulf of Guinea, which stretches 220 km² from Langma near Kasoa in the west to Ada in the east, is to the south of the region. Except where the geography is disturbed by hills, the region is primarily a low-lying undulating coastal plain with heights rarely exceeding 76.2 m above sea level. The Shai Hills, which reach a height of 304.8 m, are located in the region's north-eastern corner. On the western end, the Togo series and the Cape Coast formations have rounded low hills ranging in height from 121.92 m to 152.4 m. The Akwapim ranges, which reach heights of around 213.36 m, intrude into the eastern side of the region (Botsyo et al., 2020; www.ghana.gov.gh/greater Accra Retrieved June, 2021).

The region receives major rains April to July. The main season, and the minor rains from August to October the period of minor rainy season. The average annual rainfall ranges from 800 mm on the coast to 1270 mm in the interior. Thick overcast weather and high-intensity storms are typical of rainfall. The valley bottoms are constantly flooded and the coastal wet plains are usually inundated as a result of the occasional spillage of the Weija dam. This has impact on agricultural productivity in the region. The annual average temperature varies between 25.1 °C in August to 28.4 °C in the warmer months of February and March. The Relative humidity is around 75 % at 6:00 and 15:00, the average duration values are around 94 % and 69 %, respectively (MoFA, retrieved June, 2021). The Greater Accra region is Ghana's second most populated region with 4,010,054 in 2010, behind the Ashanti Region, representing 16.3 % of the country's overall population. (Ghana Statistical Service, Population and Housing Census 2010).

3.2.4 Central region

The central Region is located in the south-western corner of Ghana. The region is bordered by the Ashanti Region on the north, on the east by the Eastern Region, on south by the Greater Accra Region, and on the west by Western Region. The Gulf of Guinea serves as a southern border. The region has a total boasts a 150 km² shoreline, making it Ghana's longest. The region has a total land area of 9,830 km² (3,794 sq. mi), which is 4.1 % of the overall land area of Ghana (Ghana Statistical Service, Population and Housing Census 2010). The entire cultivable land area in the region is estimated to be 7,864 km² (about 80 % of total land area). Only 3,932 km² (or 40 % of the Region's land area) is cultivated. The region lies between the coordinates of latitude 5°30'0.00" N and longitude-1°00'0.00" W, and the land rises between 250 metres and 300 metres above sea level. The temperature is normally warm, ranging from 24 to 34°C Celsius. It also has a relative

humidity of between 50 % and 85 %. Rainfall in the region is bi-modal, ranging from 800 to 1500 mm on the average, with the coast receiving the least amount of rainfall. April to July is the major season, and September to November is the minor season (MoFA/central region retrieved June, 2021; [www.ghana.gov.gh/central region](http://www.ghana.gov.gh/central%20region) Retrieved June, 2021).

3.2.5 Eastern region

Lake Volta borders the Eastern region on the east, Bono East Region and Ashanti Region on the north, Ashanti Region on west, Central Region and Greater Accra Region on south. The Eastern Region is ranked third most populated region with a population 2,633,154 inhabitants with a farming population of 270,103. The Eastern Region covers 19,323 km² (7,461 sq mi), accounting for around 8.1 % of Ghana's total landmass (Ghana Statistical Service, Population and Housing Census 2010; Eastern Region" GhanaDistricts.com, 2013. Retrieved June 2021). Agriculture covers about 90.0 % of land in Eastern Region. Land rotation, sole cropping, inter-cropping, mixed cropping and mixed farming are practiced in the Region. Shifting cultivation is done by one year cropping followed by two to three years fallow period or crop rotation. There is intensive land use with high rate of both inorganic and organic fertilizers. It lies within longitude 0° 30" East and 1° 30" West and latitude 5° 30" North and 7° 22" north. Major rainy season is from March to July while minor season covers September to November. The dry season, which lasts from November through February, is marked by cold, dry weather. Annual rainfall of the region ranges between 1500mm and 2000mm in the forest zone and 900mm from to 1300mm in the savannah zones respectively. The rains last 85 and 130 days. The humidity within the region is fairly moderate between ranging 65-95 % and temperature range of 22-33 °C. Semi-deciduous rain forest covers the southern and central parts of the region, transitional savanna covers the northern parts behind the Kwahu Scarp,

and Coastal savanna covers the eastern boundaries behind the Akwapim Range (SRID MRACLS, 2010; MoFA/eastern region retrieved, June, 2021).

3.2.6 Selection of farmers

In each region, farmers were selected using purposive sampling methods. A total of 50 farmers were questioned about their perspectives on the disease, its occurrence, cropping systems, disease severity, and disease management choices. The farmers consist of males and females. The farmers interviewed were those who had taro farms at the time of the study and those who sell the corms. In the places where *C. esculenta* is produced, personal observations were made on the symptomatic plants in farmers' fields, wetlands along streams, and home drainage channels. Diseased leaf samples were as well taken for pathological investigation.

3.3 Data collection

3.3.1 Field survey

The primary data was collected by means of structured, self-administered interviews and field observation guided by a questionnaire (appendix 10.0). The questionnaire comprised open and closed ended questions which were written in the English language and administered in both English and local languages (Akan Twi). The data was collected during the major cropping season of taro from March to July 2019. The survey questionnaire comprised three categories of questions based on:

1. Socio-economic background of the farmer (sex, age, educational background, years of cultivation).

To measure the socio-economic characteristics of the respondents' relevant questions were asked

on their age, educational levels, land size, farming experience. The socio-economic characteristics of the respondents was operationalized as follows

Age: The respondents were asked to give their actual age in years. The actual age in years was used to determine the average age of the respondent. The actual age was later grouped.

Sex: The respondent was asked to indicate their sex by ticking either 'male' or 'female'.

Educational levels: The educational levels of the respondents were determined by asking the respondents to tick against any of these: 'no formal education', 'primary school', 'junior high school', 'senior 'high school' and 'tertiary education' as it applies to them.

Farming experience: Respondents were asked to provide a year-by-year breakdown of their actual farming experience. The actual farming experience was used to determine the mean number of years of experience.

2. Farming history and agronomic practices (land size, sources of planting materials, fertilizer application, etc.): To determine the farm history and agronomic practices employed by the farmer, important questions were asked on land size, fertilizer application, weeding, source of planting materials etc. The activities were carried out as follows

Farm size: This was ascertained by asking the respondents the size of land they cultivated. The farmers were asked to indicate the size of land they are able to farm during the major cropping seasons.

Sources of planting material: In ascertaining where farmers get their planting materials, the respondents were asked to identify where they got their planting materials as follows; 'market', 'research institution', 'NGO', 'farmers' fields', 'ministry of food and agriculture (MoFA).

Fertilizer application: This was done by asking the respondents if they applied fertilizer on the taro crop by ticking 'yes', or 'no' against their preferred answer

3. Disease knowledge and control: Farmers knowledge on disease incidence, causes, effects on the crop, management and control were ascertained. To evaluate the farmer's knowledge on disease, relevant questions on disease incidence, effect of the disease on the crop, control and management of the disease, symptoms and season in which there is more effect of the disease were posed.

Disease incidence: The prevalence and incidence of taro leaf blight of *C. esculanta* was confirmed by asking the respondents to tick against 'yes' or 'no'. This helps the interviewer to know if farmers' field have been attacked by disease.

Seasonal effect of disease: To ascertain when the disease is severe; either in major, minor, dry season or all the seasons, the farmers were asked to indicate any of the seasons in which the disease mostly affect the taro plants.

Symptoms of the disease: To ascertain that the disease is leaf blight, a list of symptoms known to be the signs of taro leaf blight was suggested to the farmers, to choose the type they have observed on their taro farms.

Effect of leaf blight disease: The appearance of the taro plants after leaf blight infection was determined by asking the respondent to tick against any of these: 'rotting of corms', 'drying of leaves', and 'reduced number of leaves' etc. as it appears within their jurisdiction.

Control of disease: Disease management strategies adopted by the farmers were ascertained by asking the respondents to tick whether 'yes ', or 'no' if they control the taro leaf blight on their farms.

Method of disease control: Methods of disease control was confirmed by asking the respondent the methods they employed to control the spread of the taro leaf blight disease in their farms. The respondents were asked to select the approach used, whether they used pesticides, weedicides, rouging or other control methods.

3.3.2 Assessment of incidence and severity of taro leaf blight on farmers' fields in major taro-growing areas in Ghana's four regions

A survey was carried out in a few selected localities based on availability of farms in as in the four major taro-growing regions listed below; Ashanti region (Fawade, Old tafo, Tafo estate, Gynyasi), Central region (Kasoa), Eastern region (Atimpoku, akim tafo, koforidua, anyinam, osino, Sekyere, Somanya) and Greater Accra region (Adenta, Haatso, Weija, Medie, Ashale botwe, Kwafo krom) between March and July, 2019. The prevalence and severity of the taro leaf blight disease of taro were documented. In each community, the distance between the selected farms was 0.5-1 km. Taro farms were chosen at random and screened in each location. On each farm, 50 taro plants were randomly picked and investigated for TLB occurrence and severity in a diagonal orientation across the farm. The taro farms chosen for scoring ranged in size from 0.10 to 1.0 hectares. Table 3.1 shows the distributions of farms that were screened. personal observations were performed on diseases plants in farmers' fields, marshland along streams, and backyard drainage channels where *C.esculenta* is prevalent. Pathological examination was undertaken on diseased leaf samples gathered from farmer's crops. On the taro plants, disease signs included leaf lesions with yellow and red liquid droplets in the center of the lesion and dried solid, brown granules on the leaf lamina, commonly with a white ring of sporangia around the edges.

A subjective score scale of 0 to 5 was used to assess disease severity., where; 0 means no symptoms; 1 mild symptoms, only leaf margins affected; 2 one quarter of a leaf affected; 3 completely half of a leaf affected; 4 three quarter of a leaf affected; and 5 entire leaf affected.

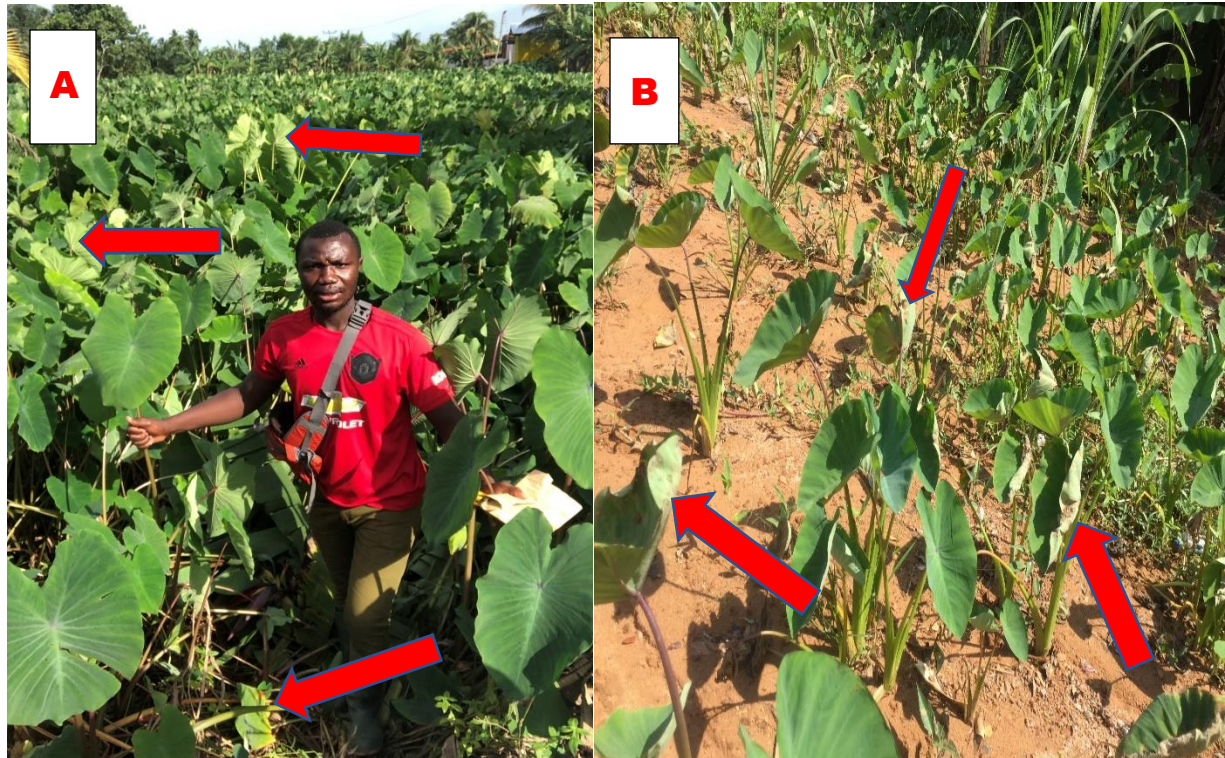


Figure 3.2 Farms A and B infected by taro blight disease at Anyinam and weija in the Eastern and Greater Accra region respectively.

Table 3.1 Farms screened for disease incidence and severity in the four regions

Region	City/Towns	Number of farms surveyed
Ashanti	Fawade	5
	Gyenyasi	5
	Old tafo	2
	Tafo estate	3
Eastern	Anyinam	6
	Akim tafo	3
	Sekyere	4
	Osino	3

	Koforidua	2
	Atimpoku	1
	Somanya	1
Greater Accra	Adenta	1
	Ashale botwe	2
	Haatso	2
	Weija	3
	Medie	3
	Kwafo krom	1
Central	Kasoa	3
<hr/>		
Total		50
<hr/>		

Source: field survey, 2020

The disease incidence and severity were calculated using the formula recommended by the CSIR-Crops Research Institute Kumasi, Ghana, as shown below:

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100 \%$$

The accessions were tested for taro leaf blight incidence ranging from 0% to 100%.



$$\text{Disease severity (S)} = \frac{\text{Area of leaf affected}}{\text{Total area of leaf}} \times \text{the rating value}$$

Total area of leaves

Disease severity is rated using 0-5 scoring scale

3.4 DATA ANALYSIS

Data on taro farmers socio-demographic characteristics, farm history and agronomic practice and knowledge and perceptions on diseases and their control practices were analysed using Microsoft excel software (2010 edition) and all questionnaire results were examined using IBM Statistics SPSS (Statistical Package for Social Scientists) version 25.0. The results were also quantified in percentages and depicted in tables and pie charts. Data on disease incidence and severity was gathered, and calculations were performed on the data for each disease incidence and severity. They were summed up into mean percentages, and the outcomes were published in tables and graphs.

3.5 RESULTS AND DISCUSSION

3.5.1 DISEASE INCIDENCE AND SEVERITY ON THE FARMER'S FIELD

There were varying levels of disease incidence (DI) within the farmer's field across the four regions. Anyinam had the highest DI with 95 % followed by Sekyere which recorded 90% incidence of taro leaf blight disease. Atimpoku recorded the least DI with 50%. All towns within the four regions surveyed had disease incidence above 50 %, suggesting high level TLB disease in the regions (figure 3.4). Eastern region had the highest disease incidence followed by Ashanti region with Greater Accra region recording the least of DI 65 % among the four regions (figure

3.5). Although 50 were assessed in the Greater Accra region, the DI within the taro farms was low. The low disease incidence in the Greater Accra region could be due to the prevailing weather conditions including erratic rainfall patterns experienced in the region. Weather conditions have been reported to influence TLB incidence (Otieno, 2020). Likewise, the high disease incidence observed in the Eastern and Ashanti regions could be attributed to the high rainfall patterns observed in the regions. Disease severity in all the four regions was 2.0 and above (Figure 3.6) indicting the devastative nature of TLB disease. TLB disease was most severe in Anyinam (Eastern region) which recorded the highest disease severity with 4.25 followed by Sekyere (Eastern region) and Koforidua (Eastern region) which recorded 3.92 and 3.87 respectively. Atimpoku had the least severity with 2.0. Eastern region and Ashanti regions recorded the highest disease severity of 3.43 and 3.4 respectively while the least severity among the regions was recorded in the Central region with 2.43 (Figure 3.7). According to Venier et al. (1998), the most important environmental element determining the prevalence and severity of fungal diseases on plants is generally the amount of moisture in the atmosphere. Therefore, the relatively high disease severity in the Eastern and Ashanti regions could be due to high inoculum pressure produced as a result of favourable environmental conditions required for fungal growth. Furthermore, disease incidence in a particular field may be high but severity may be lower and vice versa. This can be attributed to differences in the climatic condition within the regions in which the surveys were conducted. Rainfall, temperatures and humidity has significant influence in the severity and incidence of TLB disease. According to Chiejina and Ugwuja (2013), one of the most important factors influencing the propagation of an epidemic is the environment, with the potential to instigate or cause retardation. TLB disease spreads faster when there is intermittent rainfall, high humidity but the rate of spread decreases when temperatures are high. The main determinants of the taro leaf blight

disease cycle and epidemiology are rainfall, humidity, and temperature. It is therefore, not surprising to see high disease incidence and severity in the Ashanti and Eastern regions compared to the Greater Accra and Central regions of Ghana. According to Ayogu et al. (2015), TLB outbreaks are aided by favourable temperatures and regular phases of leaf wetness, especially in the humid tropics, which favor pathogen dissemination, infection, and disease development. Disease severity also depends on the host plant and effectiveness of the fungus. According to Norman (1992) and Park (1990), the degree of interaction between the host, the pathogen, and the environment determines the severity of the disease.



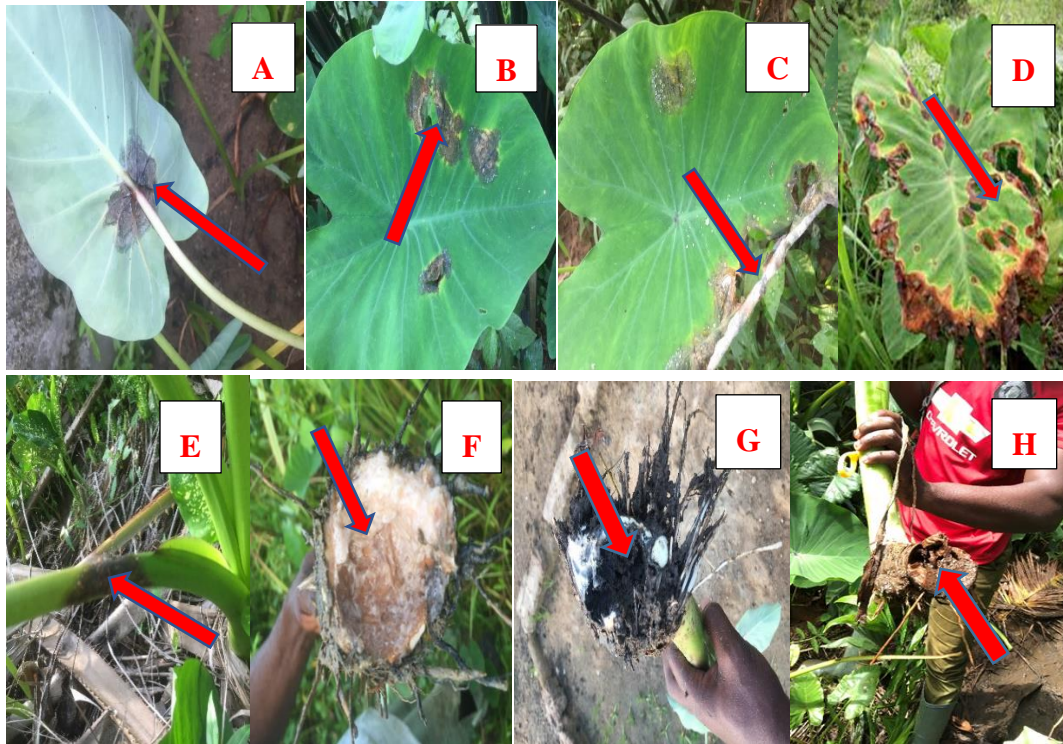
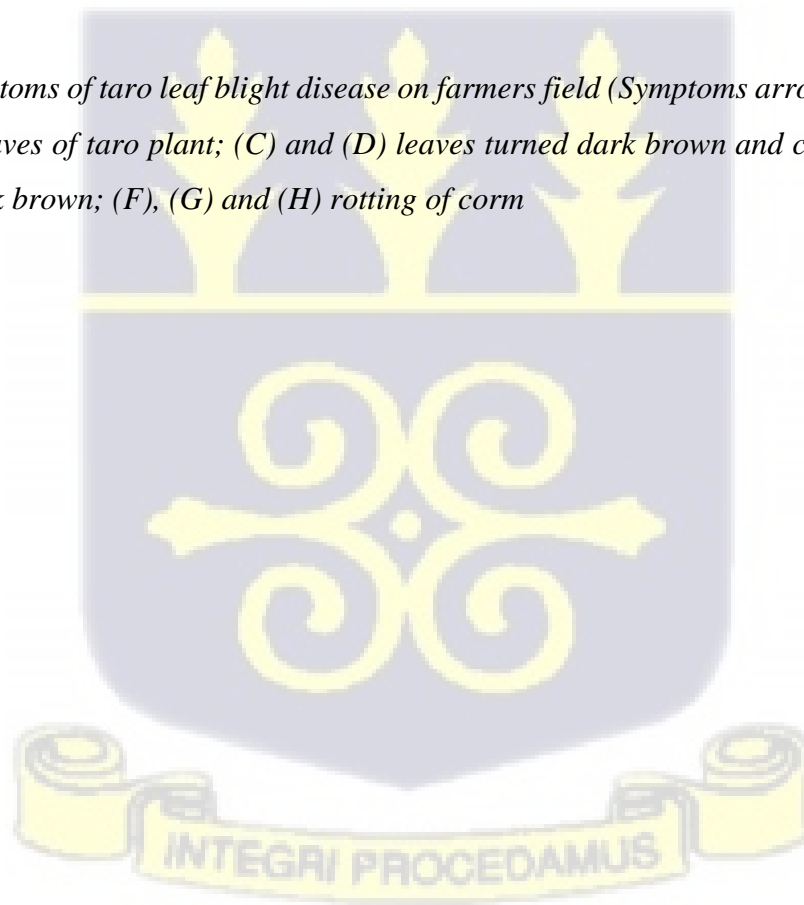


Figure 3.3 Symptoms of taro leaf blight disease on farmers field (Symptoms arrowed). (A) And (B) lesion on the leaves of taro plant; (C) and (D) leaves turned dark brown and creating holes; (E) petiole turn dark brown; (F), (G) and (H) rotting of corm



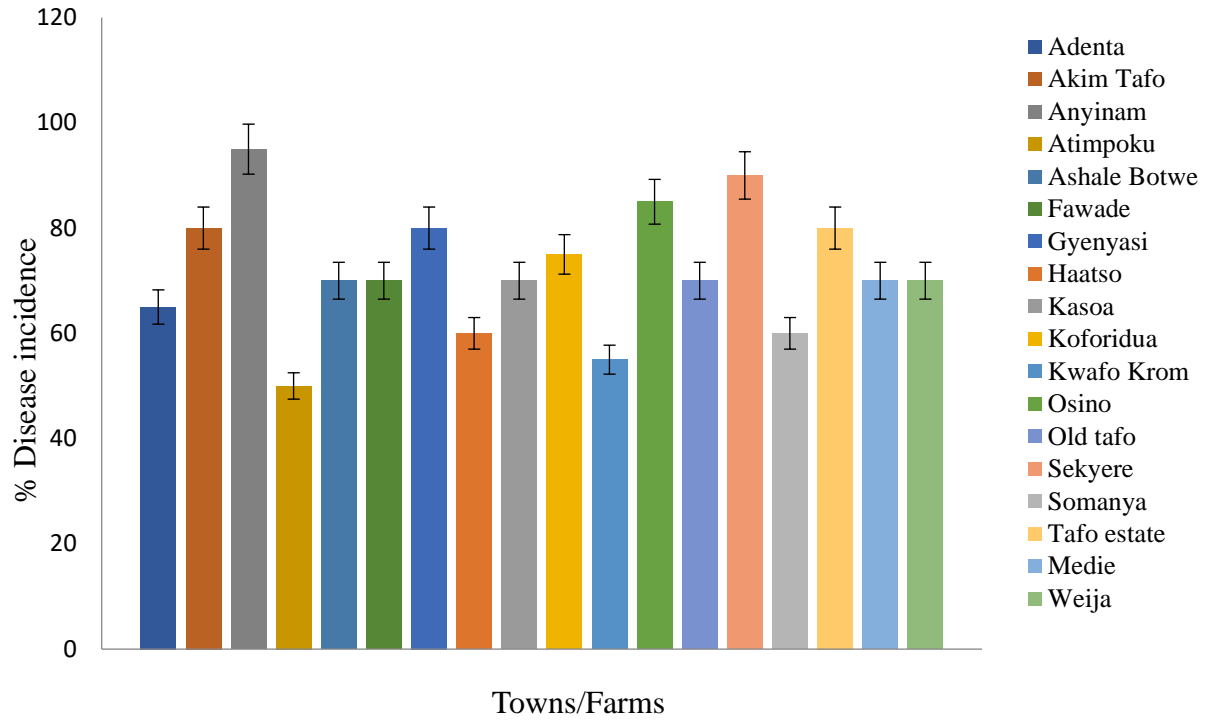


Figure. 3.4: Disease incidence observed among taro farms within the various towns in four regions.

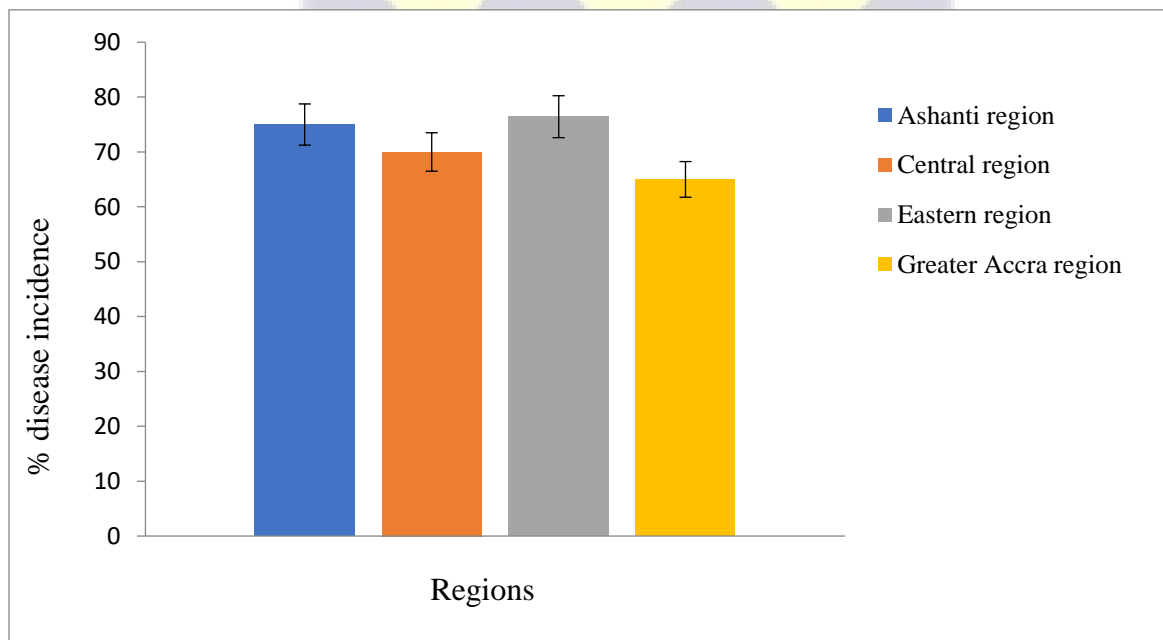


figure 3.5: Taro leaf blight incidence in the four regions studied

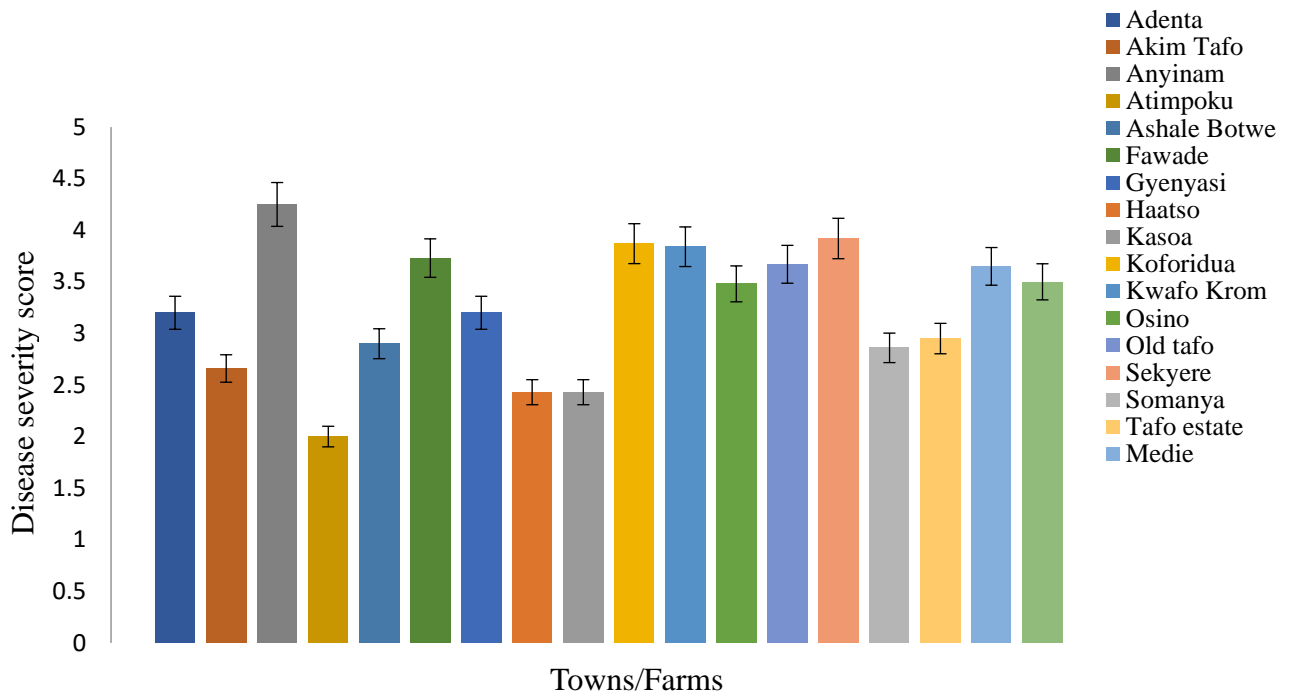


Figure 3.6. Taro leaf blight severity observed in taro farms within the various towns in the four regions.

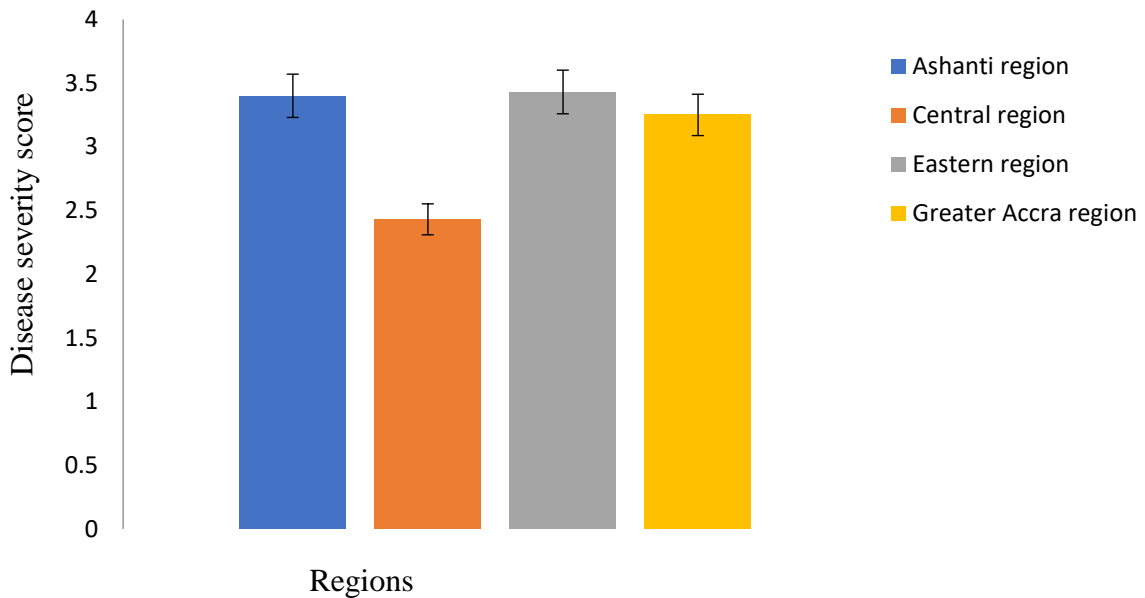


Figure 3.7: Taro leaf blight severity recorded in the four regions

3.5.1 Demographic characteristics of respondents

The majority of interviewees (60.0 %) are males, while 40.0 % are females, according to Table 3.2. This is most likely due to women's participation in agricultural produce buying and selling rather than farming. This was evident in areas such as kejetia, kosoa, anyinam where food crops are normally sold at markets. This could possibly be due to the inconvenient nature of taro farming activities for women. Most of the respondents were able-bodied men who had engaged in agricultural production and are married. According to Egbule (2010), married people dominate cocoyam production in the rural region. Most respondents were above 50 years suggesting that the young people are perhaps not interested in taro farming. According to Ogunleye and Oladeji, (2007), age could influence productivity and farm decision making process, farmers should have noticed the recent effects of TLB disease and may be willing to utilize information to help them adapt to TLB effect on their farm, as well as take rational decisions in disease management.

Table 3.2 Percentage distribution of taro farmers based on gender, age, level of educational level and farming experience within Ashanti, Greater Accra, Central and Eastern regions.

Variable	% respondents
A. Gender	
Male	60.0
Female	40.0
B. Age	
10-19	0.0
20-29	4.0
30-39	10.0
40-49	26.0
50-above	60.0
C. Education	

Non formal	22.0
Primary school	30.0
Junior high school	40.0
Senior high school	8.0
Tertiary	0.0

D. Farming experience (years)

Less than 1	22.0
1-5	22.0
5 and above	56.0

Source: Field Survey, 2020

Majority (40.0%) of the respondents have had junior high school education, about 30.0% had completed primary school while 22.0 % had no formal education. About 8.0% completed senior high school education while none of the farmers had tertiary education. This implies that some of the respondents possessed some form of literacy thus the utilization or adoption of information on TLB disease will be welcomed. According to Ringler, et al., (2012), formal education increases disease awareness and the likelihood of adaptation. Furthermore, Williams et al. (1984) emphasized the value of education in farmer education and understanding and utilization of extension recommendations.

The results show that majority (56.0%) of the farmers had more than 5 years farming experience. About 22.0% of the farmers had been growing taro for less than 1 year while the remaining 22 % had between 1- and 5-years' experience in taro cultivation (Figure 3.8). This implies that a greater number of the respondents have been practicing agriculture long enough and could therefore have noticed significant effects of TLB disease as it affects their livelihood, and thus will be interested in information on TLB disease. Adebisi et al. 2019) stated that long farming experience could

influence farmer's willingness to learn and adopt new agricultural practices more quickly. Furthermore, taro farmers are likely to have accumulated experience in taro production, marketing, and related information because of their comparatively long years of agricultural experience. Several information is believed to have been made available to them, either intentionally or unintentionally, particularly through friends, neighbours, and extension workers.

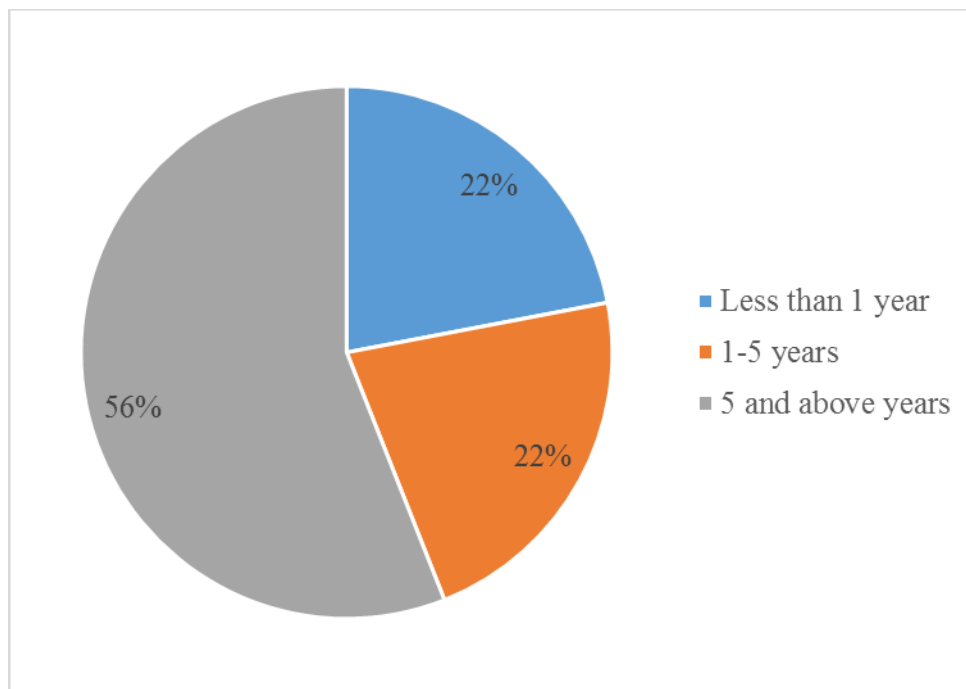


Figure 3.8 Experience of respondents in taro production in the studied region

3.5.2 FARM CHARACTERISTICS AND AGRONOMIC PRACTICES OF RESPONDENTS

As shown in Table 3.3, about 64.0 % of the taro farmers cultivate less than 1 acre of taro crop while 20.0 % cultivate between 1-3 acres of land with the remaining 16.0 % cultivation more than 3 acres. This demonstrates only small size of land is use in cultivation of taro. This little farmland could be an indication of neglect and underutilization of the taro crop. The unfavourable conditions

such as disease infection, erratic rainfall patterns, crop going extinct, use of farm land for other purposes and inadequate resources like farming machine, labour may be responsible for the decrease in taro production.

Majority (62 %) of the farmers collect their planting materials for planting from their colleague farmers as shown in Table 3.3. About 18 % and 16 % take their sources from research institution and MoFA respectively. The least percentage of farmers (2.0 %) takes their sources from an NGO. However, the respondents indicate that it is costly getting planting materials from the research institutions and organizations. They, therefore, choose to obtain their planting materials from other farmers at no cost. The parts of taro used as planting materials are the suckers, small corms, apical corm with petiole attached and corms cut into pieces. Onwueme, (1999) revealed that apical corm attached to petiole used as a planting material establishes faster than the other parts as they need to be nursed before planting. As a result of this most of farmers end up picking diseased plantlet to their fields rather taking screened ones from research institution. Of the 50 farmers interviewed, none of them confirmed that they do apply fertilizer on their taro crops, especially those who rely upon taro for a living. Some experts even claim that fertilizers impair the quality and shelf life of taro. However, Fertilizers, manures, and composts have been proven to work well with taro. The types and amounts of fertilizer that are advised vary greatly from place to another. John et al. (2004), indicated that poultry manure contains vital nutrient elements that promote high photosynthetic activities which stimulates root and vegetative growth. In addition, poultry manure is a source of nitrogen which promotes plant growth, increases the number and length of internodes, resulting in an increase in plant height with time. Saigusa et al. (1999), Gasim (2001) and Dauda et al. (2008) made similar observations

Table 3.3 Percentage distribution of taro farmers based on size of farm, age, source of planting material and fertilizer application in Ashanti, Greater Accra, Central and Eastern region

Variable	% taro farmer's response
A. Size (acre)	
Less than 1	64.0
1-2	20.0
Above 3	16.0
B. Source of planting materials	
Market	2.0
Farmers	62.0
Research institutions	18.0
MoFA	16.0
NGO	2.0
C. Fertilizer application	
Yes	0.0
No	100.0

Source: Field survey, 2020

3.5.3 Disease awareness and management

Table 3.4 shows that all 50 farmers interviewed admitted the presence of diseases which confirmed 100 % disease presence on their taro farms. Farmers interviewed believed that dying of their crop is as the result of chemical compounds (weedicides and other pesticides) from nearby vegetable growers flow into the environment. The responders are of the view that taro has soft leaves and thus allergic to chemicals. Farmers, on the other hand, were able to recognize TLB disease

symptoms, but they have no idea of what causes the disease. TLB has caused a lot of reduction and physiological changes of the plant which affects the yield of the taro crops on the fields surveyed. The results of this study is in line with IFPRI-PBS, (2006) and GSSP Report, (2010) that revealed the effect of fungal disease on crop growth in Ghana. Guarino, (2010) reported that TLB has spread to Cameroon across West Africa, where it has culminated in harvest losses of up to 90 %.

Majority (42.0 %) of the farmers indicated the disease is severe in the major rainy seasons. However, 34.0 % of the farmers responded that disease spreads faster during all the seasons (major rainy, minor rainy and dry season). Twenty-two (22.0 %) and 2.0 % of the farmers indicated that TLB spread in both minor raining and dry season respectively (Table 3.4). The disease struck both young and old plants, according to the farmers. The relatively high incidence displayed in the rainy season agree with the findings of Aggarwal and Mehrotra (1987) who reported that high relative humidity and frequent rainfall are important factors that favour the development of *Phytophthora* leaf blight. Thankappan (1985) also observed that TLB outbreaks are aided by optimum temperatures and regular bouts of leaf wetness, specifically in the humid tropics, which favour pathogen dissemination, infection, and disease development. Despite rapid spread and increased severity of TLB disease in the wet season, Tarla et al. (2014) reported increased yield in dry season than the wet season because disease reduce yield drastically during the raining season. A list of symptoms of the taro leaf blight disease was presented to each farmer to ascertain whether they had observed any on their farms, as shown in Table 3.4. Most of the respondents (64.0 %) gave the common symptom observed being water-soaked lower leaf surface or dry and greyish appearance. Twenty percent (20.0 %) of the farmers observed rapid spots enlargement on leaves making the crop become purplish brown to brown in colour, 12.0 % responded that their taro crop

demonstrates early spots on the leaves and 4.0 % said the crop latterly become bright orange or reddish brown. This implies that the farmers have good knowledge of the disease symptoms. The symptoms were similar to that that reported by Nelson et al. (2011). None of the farmers upon first enquiry attributed symptoms they observed on their farms to a fungus. On corm rot, the respondents disputed the fact that the disease is caused by a fungus. Instead, they justified that corms rot as a result of overgrowth when corms are not harvested and left in the water for long period. The farmers interviewed also confirmed that the disease physically affects and changes the looks of taro plant on their field as listed in the questionnaire. Although the farmers gave multiple responses but most of the farmers testify that the disease causes huge corm rot as yield is their major concern. Fourteen percent indicated that TLB disease makes the leaves of the plant dies off. Whilst 8% admitted the disease physically challenges the crop by reducing the number leaves on the plants (Table 3.4). The defoliation reduces the activities of photosynthesis and eventually leads to decrease in yield. In addition to the reduced yield, the fungus also causes postharvest decay of taro corms. The results of this study in tandem with the results of Maheshwari et al. (2007), who indicated that the disease affects the entire crop, including the leaves, corms, petioles, and cormels, causing considerable foliage destruction and reduced yield.

Majority (84.0 %) of the farmers said they do not control the disease whiles 16.0 % control the disease (Table 3.4). The main reason for not controlling the disease most by the majority of farmers is the fear that the use of weedicides, pesticides and other chemical to control disease would burn down their crops. This means that the vast majority agreed never had any extension education on how to manage the TLB disease. According Nhemachena (2007), exposure to extension services influence the capacity of farmers to adapt to disease effects.

The method of controlling the disease and adaptive strategies employed towards adapting to TLB disease by the respondents is found in Table 3.4. The results revealed that 60.0 % of the farmers use rouging method, about 30.0 % employ other methods such weed control about 6.0% used weedicides while 4.0 % used pesticide to control the disease. Therefore, most farmers remove disease crops to widen space between two taro crops. This was in line with the earlier report by Hawkins et al., (1998) in which wide plant spacing was used to decrease the transmission of disease. Other methods such as selection of sites flanked by forest as obstacle to disease spread and the use of fertilizer to encourage early maturity of the crop before the outbreak of TLB have also been reported.

Table 3.4 Percentage distribution of taro farmers based on TLB disease presence, season of TLB disease, effect of TLB disease, control and method of control in Ashanti, Greater Accra, Central and Eastern region.

Variable	% of farmer's responses
A. Presence	
Yes	100.0%
No	0.0%
B. Season	
Major raining	42.0%
Minor raining	22.0%
Dry season	2.0%
All the above	34.0%
C. Effect	
Drying of leaves	14.0%
Rotting of corms	78.0%

Reduced number of leaves	8.0%
D. Control	
Yes	16.0%
No	84.0%
E. Method of control	
Weedicides application	6.0%
Removal of infected plant	60.0%
Pesticide application	4.0%
Other	30.0%
F. Symptoms	
Early spots often occur and accumulates on leaves	12.0%
The spots expand rapidly becoming reddish brown to brown in colour	20.0%
The lower leaf surface is water-soaked or dry and greyish	64.0%
The plant become bright orange or reddish brown	4.0%

Source: Field survey, 2020

*multiple responses

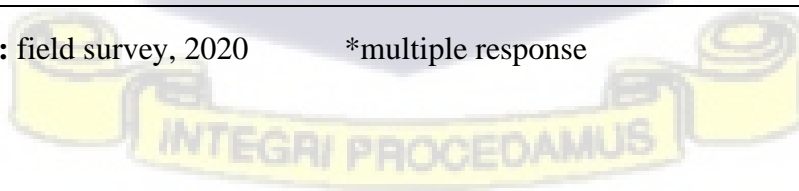
According to 72.0 % of the famers, all the parameters listed such as lack of water, lack of nutrient and lack of weed control has a drastic effect on the taro crop and its yields (Table 3.5). About 18.0 % of farmers claimed that the crop is most affected when there is lack of water, while others indicated that lack of nutrient and weed control also have effect on the crop. Bussell and Bonin (1998) revealed in their studies that water increases the structural features of a taro plant. In addition, Faamatuainu (2018) reported that nitrogen fertilizer provides the required nutrient for the growth of taro crop. Most of the Farmers (52.0 %) attributed the abnormalities in taro crop to the soil factors. Twenty four percent of the respondents are of the view that the challenges are not caused by either the soil or the climate. Twenty percent of the farmers linked the abnormalities to the climatic conditions of the area of study (Table 3.5). Very few (2.0 %) of the respondents suggested other factors such as lack of rainfall, nutrient and late harvest. The results revealed that

58.0 % of the farmers indicated that about 50 % of losses in yield occurs after harvest, 40.0 % claimed that less than 50 % of yield are lost after harvest and 2.0 % admitted that the whole yield can be lost after harvest. These findings attest to the devastating effect of taro leaf blight on farmer’s field. Most taro sellers (54.0 %) revealed that there is shortage of corm whiles 46.0 % indicates availability of corm (Figure 3.6).

Table 3.5 Percentage distribution of taro farmers based on limitations, causes of abnormalities and yield losses in Ashanti, Greater Accra, Central and Eastern region

Variable	% of farmer’s response
A. Limitations	
Lack of nutrient	2.0%
Lack of water	18.0%
Lack of weeds control	8.0%
All the above	72.0%
B. Cause of abnormalities	
Soil	52.0%
Weather	20.0%
Not sure	24.0%
Other	4.0%
C. Harvest losses	
Less than half the crop	40.0%
About half the crop	58.0%
The whole crop	2.0%

Source: field survey, 2020 *multiple response



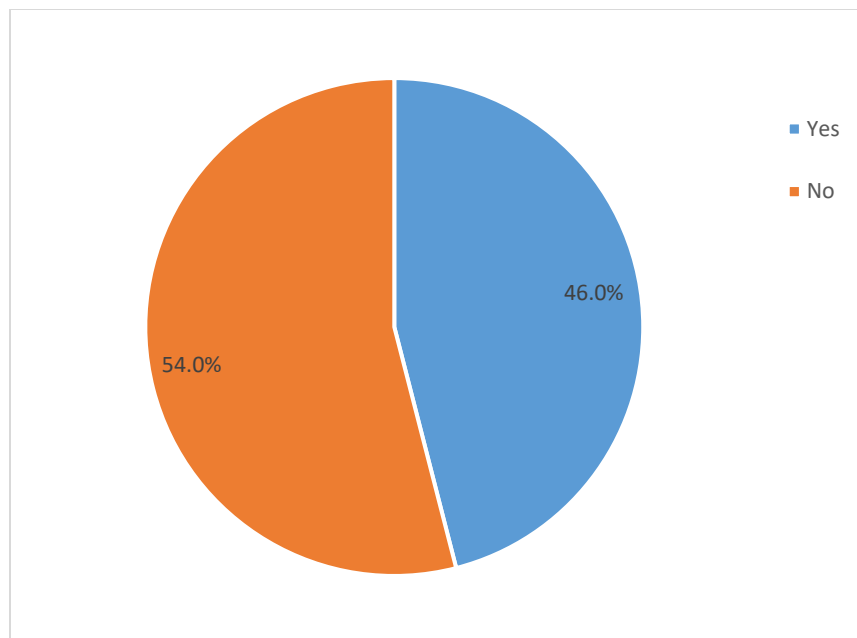


Figure 3.9 Percentage distribution of farmer's response based on availability of taro corm in the four regions

Approximately, farmers sell about half of an acre yield of taro corm after harvest for 1200 Ghana cedis. Currently, a tuber of taro which weighs 2.5 kg cost 15 Ghana cedis averagely while 5 pieces of smaller corms cost 20 Ghana cedis. This implies that taro farming and its business is profitable due to the high demand for the corms which are in short supply. The shortage of the crop is greatly as a result of the TLB disease. Urbanization has also had negative effect on taro cultivation so the crop faces possible extinction.

Additionally, it was observed during the survey that most of the farmers used monoculture system of farming in cultivation of *C. esculenta*, and some also performed mixed cropping, producing *C. esculenta* as the principal crop while intercropping sugar cane or maize during the dry season. Monoculture suffers from the disease more than mixed cropping. According to the interviewees, practicing of mixed cropping as adaptive measure decrease spread of the TLB which in line with

the findings of Ayogu *et al.* (2015) and Amosa and Wati, (1997). In their study, severity of disease was consistently higher in a taro monoculture system than in a taro/maize cropping system. Asraku (2010) also reported that TLB disease symptoms were highly severe in mono cropping than mixed farming system. Some farmers have adapted the practice of adjusting the time of planting of their taro so as to meet the first rain. These adaptation strategies are mostly used by the farmers to minimise the negative effects of TLB disease. Adjustment of planting dates in order to adapt to disease effects on crops has been reported (Ugwoke *et al.*, 2010).



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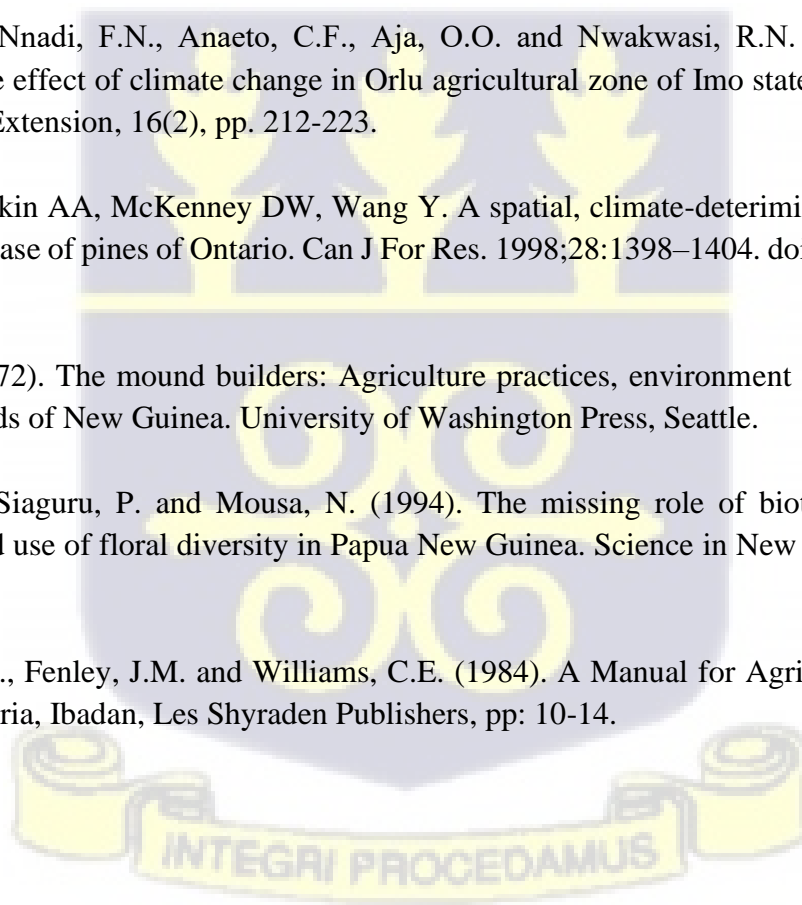
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CHAPTER FOUR

INCIDENCE OF TARO LEAF BLIGHT DISEASE AND EFFECT ON YIELD IN FIELD GROWN TARO (*COLOCASIA ESCULENTA* (L.) SCHOTT)

4.0 INTRODUCTION

The natural habitat of taro is the edge of water courses and in marshy areas where few crops would triumph (Wanyama and Mardell, 2006). Taro can be planted from three kinds of planting materials. These are the tops which are the petioles with root corm attached serve as the most common planting material followed by suckers which grow from sides of the taro roots or small cormels and usually have pointed base and the runners which emerge from corms and rung over the surface of the ground is also serve as an excellent planting material (FAO, 1999). Taro is widely grown in Nigeria, China, Cameroon, and Ghana, where annual rainfall levels are at least 2000 mm. The crop thrives in hot, humid climates with temperatures exceeding 21°C. It is a lowland crop since it is sensitive to frost (Scot et al., 2011). In West Africa, particularly in Ghana, Nigeria, and Cameroon, taro is grown as a crucial macroeconomic food crop and vegetable (Demie, 2018). It is mostly cultivated for its corm, a starchy subterranean stem. Taro is a moist-loving plant that may be grown in both non-flooded and flooded circumstances (Plucknett et al., 1970). Deep planting is essential for optimum corm set, because shallow soils hamper corm and root development. Due to the extreme variability and unpredictability of rainfall, as well as the high expense of irrigation, the ability of the soil to store water becomes an important thought in upland taro cultivation. Taro may be grown in a variety of soil conditions, good results are achieved on deep-well-drained, friable loamy soils with pH 5.5 to 6.5. It is best to avoid stony or rocky soils because these type soil produce mutilated corms and make harvesting more difficult. According to Hill et al., (1998), the

crop has a salinity tolerance threshold (95 % of maximum growth) of 4 mM NaCl (Miyasaka et al., 2003). Reynolds (1977), established that there were five developmental growth stages of *Colocasia esculanta* which are the first month of establishment comprising root formation and leaf production high - speed root and shoot development, with corm development commencing one to four months later During four to six months, the root and shoot growth reaches its pinnacle, with a rapid increase in corm production. During the six to nine-month senescence period, root and shoot growth will slow while corm size grows and during eight to ten months, there is a phase of decreased corm weight, possibly due to rot, and the commencement of continued vegetative growth with enhanced root and branch growth. Flooded farming has some benefits over dry-land cultivation in that they have high produce and it controls weeds (FAO, 1999). The crop matures within six to twelve months and twelve to fifteen months after planting for dryland farming and wetland cultivation respectively (Tumuhimise, 2009). The crop is harvested whenever the height of the plant has diminished and the leaves have turned yellow. In flooded taro cultivation, the signals are frequently less apparent than in dryland cultivation. Even in mechanized production systems, harvesting is typically done with hand instruments. After loosening the soil around the corm, the corm is pulled up by grasping the base of the petioles (FAO, 1999).

In Ghana, existing production levels of taro is slightly low Ghana was the second-largest producer of colocasia after Nigeria in 2005, with 1.8 million metric tons. (FAO, 2005). On a global basis, taro yields 6000 kg/ha as compared to 14746 kg/ha of potato (*Solanum tuberosum* L.) and 13,628 kg/ha of sweet potato (*Ipomoea batatas* L.) FAO (1991). According to Singh et al., (2012) cocoyam farmers in most African countries use minimal inputs. Regardless of its financial abilities as food and cash crop and its nutritional worth, the crop is under exploited and poorly understood. Onyeka (2014) stated that there is non-existence of history and solid understanding on taro

production even though the crop is furnishing extensively to food security, earnings to many households. Simongo et al. (2016) stated that taro is a very significant crop in the life and culture of the highlanders not only as one of their staple foods but also indispensable part in the performance of holy activities and rituals. In Cameroon and other West African countries, taro is a cultural food which is deemed sacred and is greatly honored (Carnot et al., 2016). The corms are used in a wide variety of industries to make high fructose syrup and alcohols (Vishnu et al., 2012). In biodegradable plastics, toilet formulations or aerosol production, it is used as filler (Nip, 1997). Proposed to mimic oil droplet in food emulsions such as mayonnaise, taro starch contributes to reducing the risks of cardiovascular diseases by reducing the consumption of oil (Nip, 1997).

TLB poses a serious warning to food security in national economies where it is grown. It has resulted in considerable nutritional and farming system changes (Singh et al., 2012). Prior to the outbreak of leaf blight, taro was a key export earner in countries like American Samoa, with over 90 % of families farming the crop. After the outbreak, only 1 % of the total supply of *Colocasia esculenta* were available to the local market (Asraku, 2010). The majority of varieties of taro that existed have been lost primarily through infection by the pathogen. There were roughly 350 different types of taro in Hawaii previous before the emergence of taro leaf blight, which overtime as a result of TLB disease became less than 40 different varieties (Asraku, 2010). The use of diseased corm planting material enhances the disease incidence in successive taro crops. Other factors like, density of plants, temperature and humidity are among factors influencing infection and spread of TLB disease (Power et al., 2011).

The agronomic abilities and value of taro stays unidentified considering the fact that it remained underutilized and abandoned crop in country as a result of little awareness on the crop, which has resulted in unsafe reduction in economic livelihoods and a loss of genetic diversity (Akwee, 2015). In the last three decades, taro production in Africa has continuously attained a growing share of the world's cocoyam production, which is now at 10 million tonnes each year (FAO, 2012). This increase largely depends on cultivating extra land rather than boosting crop yields. This contradicts predictions of FAO that the 70 % upsurge in the world's agricultural output required to grow yet another 2.3 billion people by the year 2050 must be achieved by improving yields and cropping intensity on existing farmlands, as a substitute rather than enlarging the area under cultivation (FAO, 2009).

4.1 Objectives of the study:

The objective of this study was to assess how taro accessions react to the taro leaf blight disease in the field and to determine how the accessions respond to the disease.

Specific objectives of this study were to:

1. Examine the prevalence and severity of taro leaf blight infection on a taro accession in the field.
2. Assess the impact of taro leaf blight on yield.



4.2 MATERIALS AND METHODS

4.2.1 Study area and location

The research was carried out at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) farms between July 2019 and March 2020. Field studies were carried out on the institute's research farm located at Kwabenya, Accra on latitude 5° 4'N, longitude 0° 13'W with Ochrosol (Ferric Acrisol) soil type, derived from quartzite schist. Within the coastal savannah agro-ecological zone, the land is well irrigated and has an altitude of 76 meters above sea level. GAEC has an equatorial climate with two wet seasons and two dry seasons. From November to March, there is a dry season with monthly rainfall of roughly 32 mm. This is followed by a monsoon season from April to June, with an average monthly rainfall of nearly 125 mm. From July through August, there is a brief dry period before the rainy season begins. The mean annual rainfall is 830 mm. March and April have the maximum mean monthly temperature of over 30°C, while August has the minimum mean monthly temperature of around 26°C. The greatest monthly mean relative humidity does not surpass 75 %, while the lowest is around 60 % (Dickson and Benneh 2004). The Onyasia River's large valley lies on the eastern side, while swampy conditions prevail on the site's north-east side. Small localized swamps form during the wet season and can last well into the dry season. Because the topsoil is all sandy, surface run-off is relatively low in this area. However, water migration over the clay horizon beneath the sandy topsoil may occur during severe rains (Akaho *et al.*, 2003).



4.2.2 Planting materials

Three hundred taro suckers taken within various locations in the four agro ecological regions of Ghana; Ashanti region, Greater Accra region, Central region and Eastern region and grouped into accessions based on the town it was collected. The taro accessions were Fawade (FW), Gyenyasi (GY), Kumasi Tafo (KT), Adenta (AD), Haatso (HT), Kasoa (KS), Koforidua (KF), Akim Tafo (AT), Weija (WJ), and Atimpoku (AP). Following the assessment of the disease incidence and symptom severity, the pathogen was detected from the samples collected from the four regions.

4.2.3 Field layout and experimental design

Experimental area measuring 3,600m² (60m by 60m), was cleared with a machete, hand ploughed, and harrowed twice using hoes. Three hundred taro suckers were planted in 60-centimeter-deep holes, each sucker firmly inserted by hand utilizing Brooks' procedures. (2011). Three replicates of a Randomized Completely Block Design (RCBD) were used to arrange the plants, each replicate measuring 20 × 20m² with 2m between blocks. Plants were spaced 1.0 m apart. Watering with about one litre per plant was done in the morning and evening using a sprinkler, about one liter per plant.

4.2.4 Agronomic practices undertaken

General agronomic procedures applicable in the taro fields were carried out, to maintain plant in the field. Experimental field was slashed, ploughed and harrowed after which the lay-out was done. The planting materials which were obtained from demonstration fields within the four regions were used for planting. Each block size was 20 m x 20 m and suckers were planted at 1.0 m x 1.0

m apart suckers were independently placed gently housed in each hole and soil was pushed around and firmed. Weeding was done by hand using the hoe when needed. At least weeds were controlled every three to four weeks. Irrigation was done mainly in the dry season, i.e. from mid- March 2020 to mid of August 2020. During this period the field was irrigated every three days.



Figure 4.1 Experimental field at BNARI showing taro plants.



4.2.5 Data collection

Initial spots that give rise to secondary infections (Jackson, 1999) were carefully observed to confirm the disease. Disease incidence and severity were recorded every two weeks from the onset of the first symptom to the harvesting of the crop. Yield was also recorded after the corms were harvested. The data on the above parameters was taken from March-September, 2020.

4.2.6 Estimation of disease incidence

Total number of suckers infected were investigated, number of leaves infected and the disease incidence were recorded at two weeks intervals from the start of the first symptom till the crop was harvested. The disease incidence was recorded one month after planting. The subsequent readings were taken every two weeks representing a single week. The data was recorded for eight weeks. Old leaves that were touching the ground and new half furled leaves were not graded. TLB disease symptoms which includes, yellow and crimson liquid drips with a dry solid in the midst of the lesion, brown particles on the leaf lamina, with a white sporangia ring around the edge of the lesions which subsequently become papery and may fall out, giving the appearance of a 'shot hole' were carefully studied to confirm the condition.

Disease incidence was determined according to the formula of Opara et al (2012) as follows:

$$\text{Disease incidence (DI)} = \frac{\text{Number of leaves affected per accession}}{\text{Total number of leaves sampled per accession}} \times 100$$

The accessions were tested for taro leaf blight incidence ranging from 0 % to 100 %.

4.2.7 ESTIMATION OF DISEASE SEVERITY

The severity of TLB on the taro accessions was measured by scaling the portions of the leaves affected by the disease. There severity was determined by how much damage the disease had done to the plant in terms of reduction in the surface area of the leaves which leads to the significant loss in photosynthesis.

Disease severity was scored per accession and this was done using a subjective score scale of 0 to 5, modified after CSIR-Crops Research Institute (Kumasi, Ghana) Anonymous (2008) as follows:

0→ No disease symptom, all leaves are healthy

1 → moderate symptom expression on taro plant leaf (s) covering 1/8 of total leaf (s) area

2→ TLB symptom expression on taro plant leaves covers 1/4 of the total leaf (s) area. 3→ TLB symptom expression on leaves and tissues spans half of the taro plant's total leaf (s) area.

4→ TLB symptom expression on leaves and tissues covers 3/4 of the taro plant's total leaf (s) area.

5→ TLB Symptoms on the leaf and in the tissues covers completely the entire leaf (s) area of taro plant leading complete death of taro plant to the point of no recovery and corm rot.

Using the scores, the disease severity for the taro accessions were calculated following the formula proposed by CSIR-Crops Research Institute (Kumasi, Ghana)

Disease severity = $\frac{\sum (\text{Number of plants scored for each rating} \times \text{the rating value})}{\text{The total number plants scored}}$

The total number plants scored

Rating value is 0 to 5-point scale.

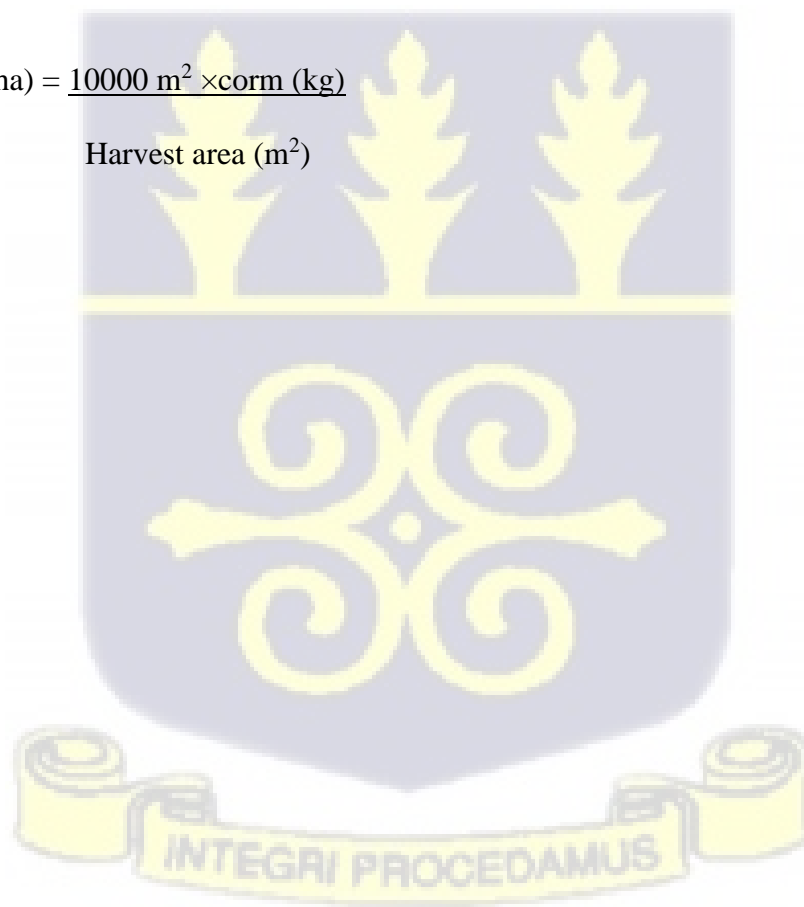


4.2.8 ESTIMATION OF CORM OF YIELD

Corm yield of the taro crops on the field were harvested immediately the crop reached maturity. Corms from the accession were harvested in November 2020. The rotten corms as well as healthy corms were weighed using a scale. In this case only the corms were considered as the final yield of the taro crop. Corms from the taro plants were selected and their weights taken with the aid of ADAM AFP – 3100L scale. Total yield of both corms and cormels were bulked together and their means from each plant were determined. Both the disease-free and diseased corms of individual accessions were weighed and recorded as the corm yield.

The corms were initial weighed in kilogram (Kg) converted to kilogram per hectare (Kg/ha) using the following formula:

$$\text{Corm yield (kg/ha)} = \frac{10000 \text{ m}^2 \times \text{corm (kg)}}{\text{Harvest area (m}^2\text{)}}$$



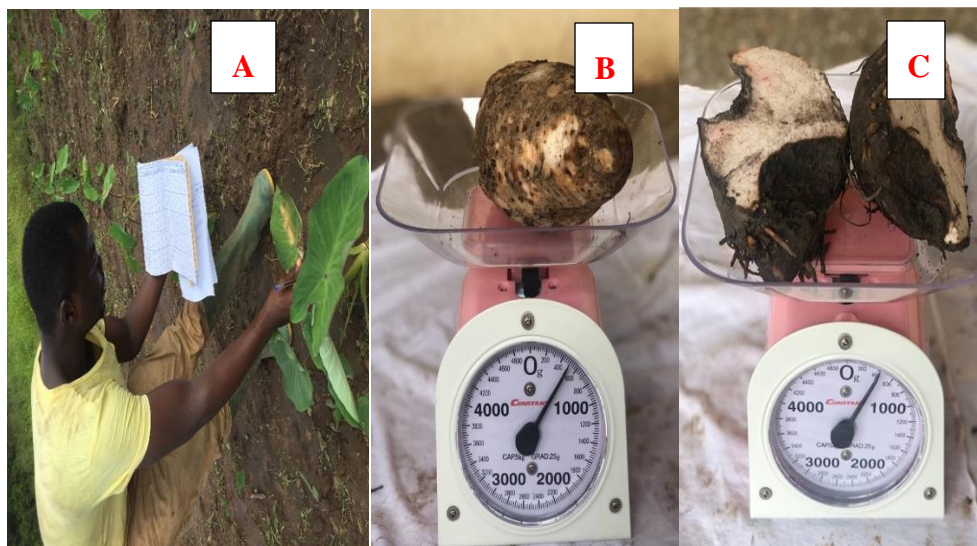


Figure 4.2 (A) Shows the examination of TLB disease and scoring for disease's prevalence and severity; (B) Weighing of disease-free corm yield. (C) Weighing of disease affected corm yield harvested from the field on a scale.

4.3 Data analysis

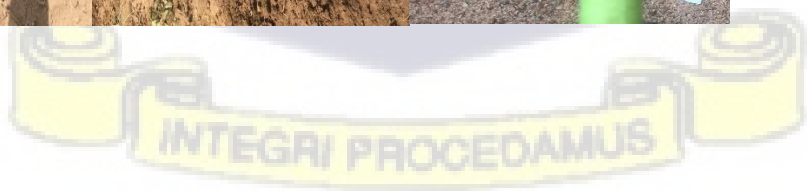
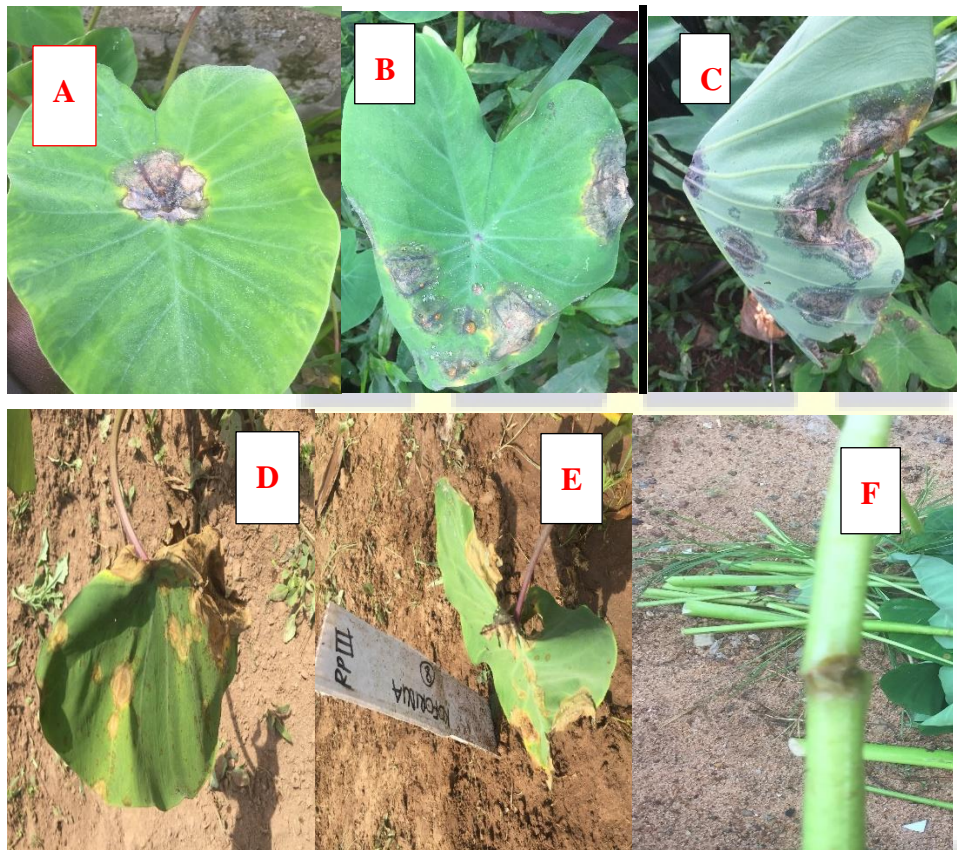
Disease incidence, severity and yield was analyzed using Minitab Statistical Software (19th Edition) and Microsoft Excel Software (2013 edition). Quantitative data was subjected to Analysis of variance (ANOVA) and mean was subjected to the Fisher pairwise comparison for mean separations.

4.4 RESULTS AND DISCUSSION

4.4.1 Symptoms of TLB disease observed on the experimental field

All the accessions produced leaves uniformly within the fourth week after planting. However, all the ten accession of taro plantlets exhibited some degree of taro leaf blight disease symptoms two weeks after the emergence of leaves indicating fungal infections (Figure 4.3). The accessions displayed varying degrees of *P. colocasiae* disease incidence which ranged from highly resistant

to highly susceptible. There was a significant difference ($P < 0.05$) in disease incidence between the taro crops in week 1 and week 2. The disease symptoms which were observed on the leaves were mostly observed on mature leaves. Initial infections formed water-soaked lesions which developed to form large brown spots on the leaves with reduction of surface area of leaves. There was formation of shot-holes with brown spots forming on the petioles. Corm rot and yellow patches were also observed on some of the leaves. Similar symptoms in taro have been reported by Nelson *et al.*, 2011 Helen and Jackson, 2005 Misra *et al.*, 2008 Jackson and Gollifer (1975).



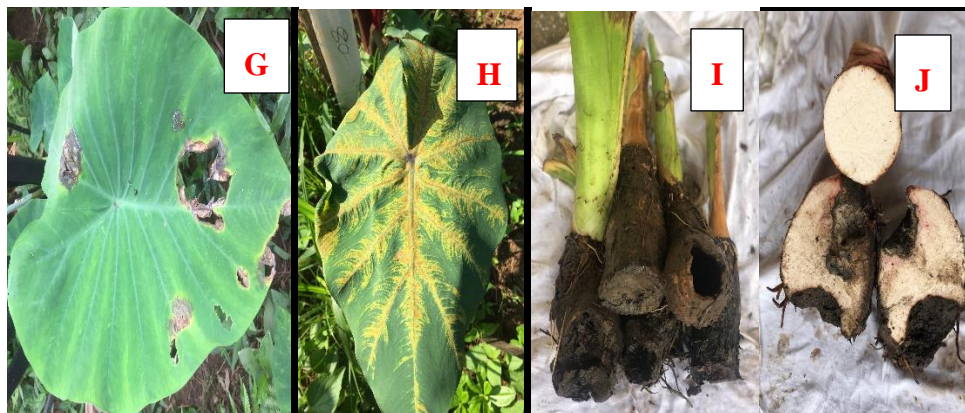


Figure 4.3 Taro crop displaying symptoms of TLB infections (A) Water-soaked lesion and slight yellowing of leaves (B) Enlargement of lesion, reddish brown occur at the margin of the leaves; (C) Underneath spot occur after lesion (D) Reduction in leaf area and the leaves turn reddish brown; (E) Tearing apart, starting from lesion to brown parts of the leaf (F) Rotten of leaf stalk (petiole); (G) Lesions become papery and break apart in the middle, resulting in a noticeable "shot-hole" look (H) Yellowing of leaf ribs (I) Rotten corms becomes dark (J) Dissection of taro corm showing the degree of damage.

4.4.2 Disease incidence and severity of field-grown taro crops

4.4.2.1 Disease incidence

The taro accessions displayed different degrees of disease incidence (DI) from the first week to eighth week (Figure 4.3). In the first week, taro accessions AD and AP had low levels of disease incidence; 46.67 % each. Accessions AT, HT, FW and GY also had 50 % each of disease incidence. However, from the first to the eighth week, DI increased steadily, except KS which had a decreased diseased incidence from 93.33 % at sixth week to 60 % at the seventh and eighth week.

There was a steady increase in disease incidence from 46.67 % to 96.67 % between the first week and eighth week after planting (Figure 4.4). This could be as a result of the corresponding increase

in fungal load and extreme susceptibility of the accessions. In spite of the fact that the DI generally shoot from week one to eighth, KS decrease in the sixth, seventh and eighth week. This can be attributed to the plant's defense mechanism where plant cells (genetic makeup) defend themselves to avert the spread of infections by pathogen following a previous infection with the disease. The genetic composition for symptoms recovery are Vertical Response (VR) known to be hypersensitive reaction which controls one or few genes major genes and Horizontal Resistance (HR) which is control by minor genes (Singh et al., 2001; Robinson, 1996; Ivancic et al., 1994). TLB disease resistance can also be attributed to enzymes produced by the taro crop. Ho and Ramsden (1998) reported that peroxidase enzymes play no part in taro's defense mechanisms, however proteinase inhibitors are essential for TLB resistance. Moreover, symptoms recovery in TLB disease can be assigned to changes in climatic conditions which is a major player in the disease resistance. According to Misra et al. (2008), despite heavy humidity and rain, low temperatures below 20°C and high temperatures over 28°C inhibited fungal sporulation and lessened severity. Agrios (2005) also reported that the number of spores generated in a unit plant area and the number of spores emitted in a certain time period are affected by temperature.

At week 3, 4 and 5, accession KS had the highest percentage disease incidence of 100 % whereas the same KS recorded the lowest percentage of 60 % at week 7 and 8. Week 1 and 2 recorded two different levels of disease incidence, for example in week 1, AD, AT, AP, FW, GY, HT, KS and KT was moderately susceptible with a disease incidence of 46.67, 50, 46.67, 50, 50, 50, 56.67 and 56.67 % respectively whereas KF and WJ were highly susceptible with an incidence of 70 and 63.335 respectively. Some of these accessions had changes in disease incidence at week 2, for example accessions AD, AT, AP, FW and KS were still at the level of moderate susceptibility with disease incidence of 43.33, 56.67, 50, 51.67 and 56.67 % respectively whereas GY, HT, KF, KT

and WJ were highly susceptible with incidences of 73, 60, 65, 63.33 and 70 % respectively. The differences in percentage disease incidence exhibited by the accessions can be linked to each distinct location of origin influenced the disease in a unique manner probably due to other factors like climate peculiar to each environment of origin. The present results have shown the effect of region of origin of taro on disease incidence. This fact was supported by findings of Chiedina and Ugwuja (2013).

Accessions AD and AP were observed to be moderately resistant with AD having 46.67 % and 43.33 % and AP with 46.67 % and 50 % at week 1 and 2 respectively, while the same accessions, FW and AP increased to highly susceptible percentages of 96.67 % and 94.44 % discretely at week 8. Accessions KF and WJ remained highly susceptible with increased disease incidence from 70 to 93.33 % and 63.33 to 85.51 % respectively from week 1 to week 8. Accession KS was moderately susceptible with 56.57 % at week 1 and 2 and increased slightly to 60 % each in week 7 and 8. However all the taro accession used were susceptible to the TLB disease at the end of week 8. The varied level in DI disease incidence through week 1 to 8 could be as a result of genetic differences among the taro accessions and it concurred with the findings of Miyasaka et al., (2012) that taro cultivars differ in their incidence to corm rot. It could also be as a result of weather influence such as increased or decreased amount of rainfall. Campbell and Benson, (2012) reported similar results that factors involved in plant fungal epidemic included favorable environment, susceptibility of host and virulent pathogen. Sarkar et al., (2017) in a study on field management of TLB using promising germplasm also observed that taro leaf blight disease incidence correlated with meteorological parameters.

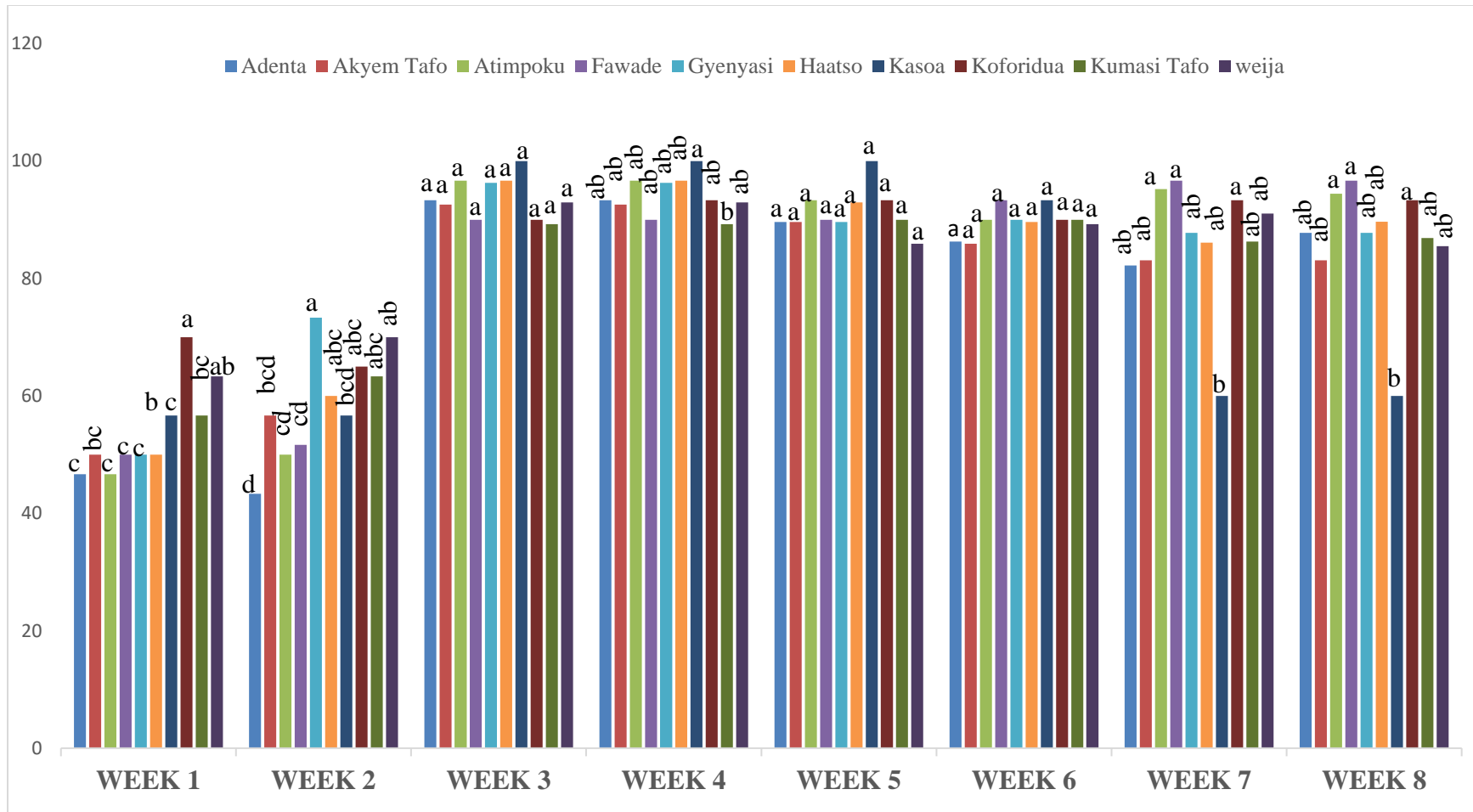
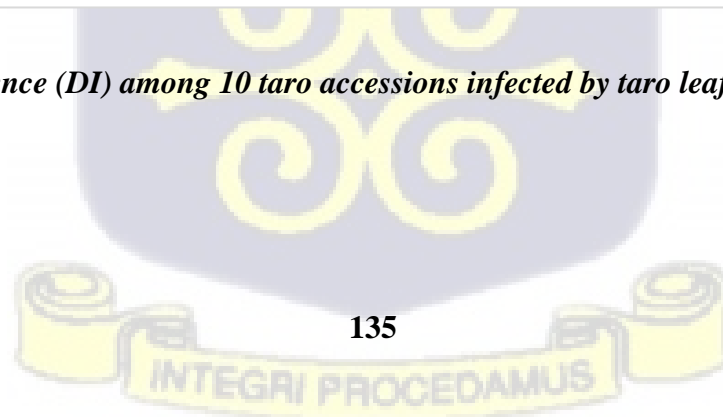


Figure 4.4 Fungal disease incidence (DI) among 10 taro accessions infected by taro leaf blight disease over 8 week period.



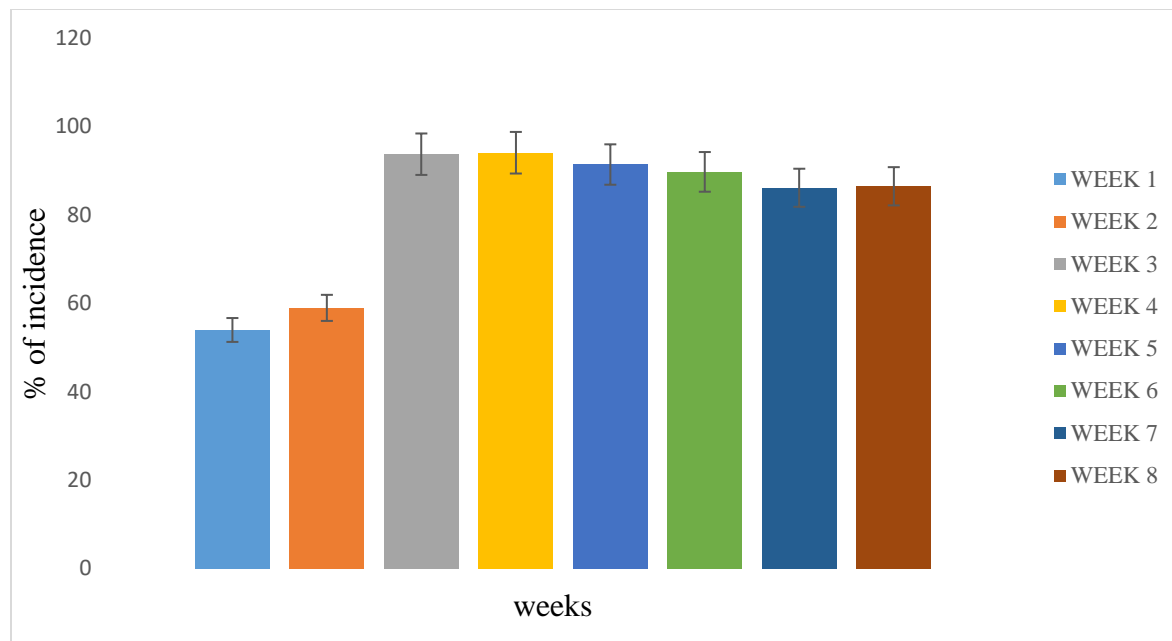


Figure 4.6 Incidence of taro leaf blight disease throughout an 8-week period.

The taro accessions included in this study exhibited various degrees of symptoms which mimicked TLB disease infection by the end of the fourth week after planting. The symptoms were identical to those noticed by Nelson *et al.* (2011), Brooks (2005), Fullerton and Tyson (2001), Jackson *et al.* (1980) and Jackson and Gollifer (1975) on TLB disease-infected taro plants

The taro accessions used in the study exhibited various degrees of TLB disease incidence (DI) which increased steadily from week 1 and week 8. The highest DI was found in KS (100 %) at week 3, 4 and 5. There was progressive increase of DI with the plant's age in most of the accessions studied which could be attributed to reduced immunity normally increasing with age. These facts supported the findings of Nwanosike *et al.* (2015) that most fungal diseases depended on stage of plant growth and they tended to increase with age. The increase in disease incidence with increasing age was also supported by the earlier work of Shakywar *et al.* (2013) in which increasing disease levels usually occurred in the late growing season as a result of increasing age and the

susceptibility of plant tissues. Several authors have also reported increased fungal disease incidence and with plant age (Tyson and Fullerton, 2015; Charles *et al.* 2016). They attributed this to more cell death than cell division that increased the susceptibility of the plant to diseases as well as increased accumulation of wastes as plant ages. The rapid increase in DI from week 1 to week 8 could be due to an increase in build-up inoculum coupled with the susceptibility nature of accessions (Jackson, 1999; Putter, 1976). The consistent increase in disease incidence could also be as a result of the prevailing favorable weather conditions of high rainfall, relative humidity and temperature during the respective weeks.

Similar results were reported by Chikkaswamy and Rabin (2014) that powdery mildew disease caused by fungus *Phyllactinia corylea* commonly occurred during September to March in tropical regions. Although DI generally ballooned from week 3 to week 8, at weeks 7 and 8, KS demonstrated a significant reduction in DI with 60 % and this could be due to symptom improvement. This could be related to the plant's tendency to shed diseased leaves as a result of systemic acquired resistance (SAR) which is characterized by a local hypersensitivity response (HR) that results in programmed cell death in the region of infected cells (Kombrink and Schmelzer, 2001), Changes in cell wall structure and composition that prevent infections from penetrating further, as well as local and systemic production of pathogenesis-related (PR) proteins (van Loon, 1997). The findings of this investigation corroborated the findings of the previous study of Haelapur (2005) in which prevalence of TLB disease grew over time before stabilizing. It was also in tandem with the research of Chowdhury and Hossain (2011) which stated that a decrease in incidence could be caused by new leaf growth and flashes that were not impacted by the pathogen as a result of the plant obtaining resistance as a result of enhanced immunity.

4.4.2.2 Disease severity

Generally, the symptoms severity (SS) for all plants increased from first to third week and decreased from fourth to eighth week (Table 4.5). The decrease in symptom severity of all the accessions from fourth week to the eighth week indicated that there was a mild fungal infection at the developmental stages of the taro crops. The generally low severity exhibited among the taro accessions is possible that this is due to accession's reduced vulnerability to TLB disease. Hiraida, (2016) in his study, linked low disease severity of a cultivar to the cultivar being less prone to diseases. The findings of Adamako et al. (2016) supported the current findings in which low TLB disease severity was linked to clean planting materials and good agricultural practices. It could also be as a result of external factors like temperature, rainfall and relative humidity being unfavorable to the pathogen. The influence of infection by weather conditions such as high rainfall, temperature and humidity has been reported (Hiraida, 2016).

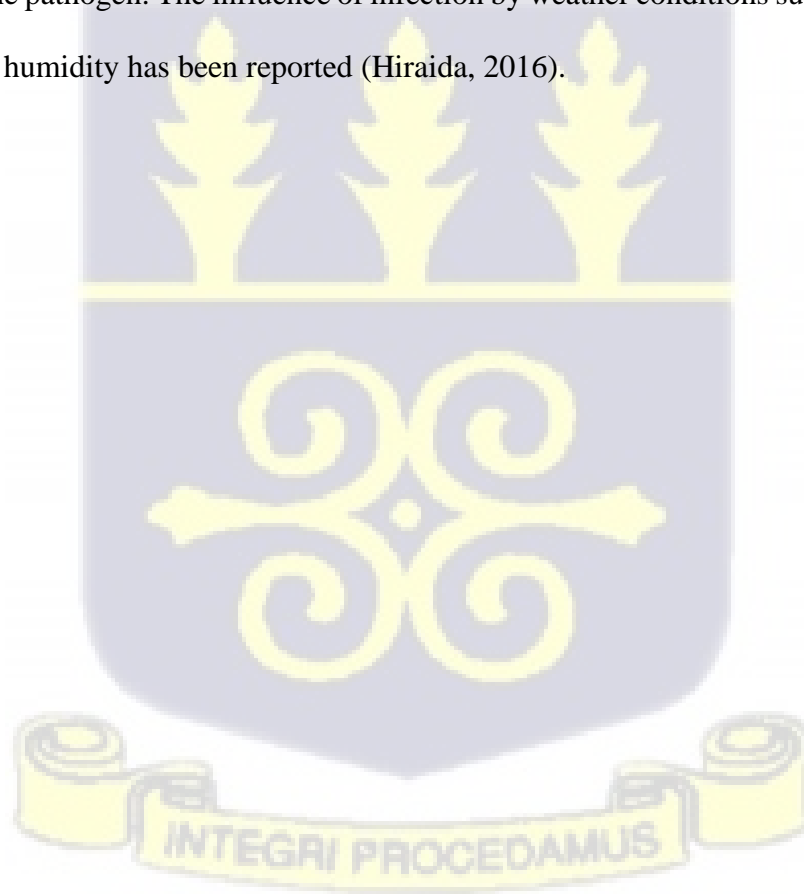


Table 4.1 severity of disease symptoms for all plants in all 10 accessions

Accessions	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
ADENTA	2.33±0.71 ^{bc}	4.21±0.23 ^{ab}	4.58±0.44 ^a	3.38±0.23 ^{abc}	2.39±0.35 ^a	1.57±0.17 ^a	1.73±0.45 ^a	1.48±0.57 ^{ab}
AKYEM TAFO	2.07±0.49 ^{bcd}	3.23±0.78 ^{bc}	4.49±0.45 ^a	3.29±0.35 ^{abc}	2.29±0.25 ^a	1.42±0.16 ^a	1.40±0.15 ^a	1.32±0.24 ^{ab}
ATIMPOKU	1.53±0.70 ^d	2.42±1.20 ^c	4.69±0.45 ^a	2.78±0.50 ^c	2.21±0.44 ^a	1.53±0.40 ^a	1.11±0.54 ^a	0.70±0.26 ^c
FAWADE	1.98±0.64 ^{cd}	4.41±1.02 ^a	4.58±0.40 ^a	3.19±0.71 ^{abc}	2.58±0.48 ^a	1.53±0.06 ^a	1.49±0.17 ^a	1.48±0.09 ^{ab}
GYENYASI	2.43±0.10 ^{bc}	4.12±0.33 ^{ab}	4.51±0.79 ^a	3.23±0.46 ^{abc}	2.32±0.13 ^a	1.56±0.35 ^a	1.48±0.18 ^a	1.69±0.27 ^a
HAATSO	3.39±0.16 ^a	4.00±0.06 ^{ab}	4.77±0.35 ^a	3.45±0.18 ^{ab}	2.54±0.32 ^a	1.70±0.27 ^a	1.41±0.43 ^a	1.48±0.09 ^{ab}
KASOA	2.70±0.20 ^{bc}	3.72±0.35 ^{ab}	4.63±0.47 ^a	3.18±0.47 ^{abc}	2.69±0.10 ^a	1.76±0.04 ^a	1.13±0.98 ^a	1.01±0.92 ^{bc}
KOFORIDUA	2.16±0.39 ^{bc}	3.94±0.41 ^{ab}	4.52±0.17 ^a	3.14±0.09 ^{ab}	2.50±0.28 ^a	1.73±0.021 ^a	1.57±0.17 ^a	1.31±0.17 ^{ab}
KUMASI TAFO	2.75±0.52 ^{bc}	4.09±0.60 ^{ab}	4.36±0.18 ^a	3.61±0.09 ^a	2.37±0.04 ^a	1.62±0.20 ^a	1.61±0.32 ^a	1.42±0.20 ^{ab}
WEIJA	2.81±0.22 ^{ab}	4.44±0.64 ^a	4.12±0.33 ^a	2.97±0.15 ^{bc}	2.56±0.24 ^a	1.59±0.18 ^a	1.37±0.32 ^a	1.31±0.12 ^{ab}
Mean severity	2.42±0.52	3.86±0.62	4.52±0.18	3.22±0.24	2.45±0.15	1.60±0.10	1.43±0.20	1.32±0.28

Each value shows mean of three replicates, and values following the same superscript(s) are not significantly different at ($p>0.05$) according to fishers pairwise comparison.

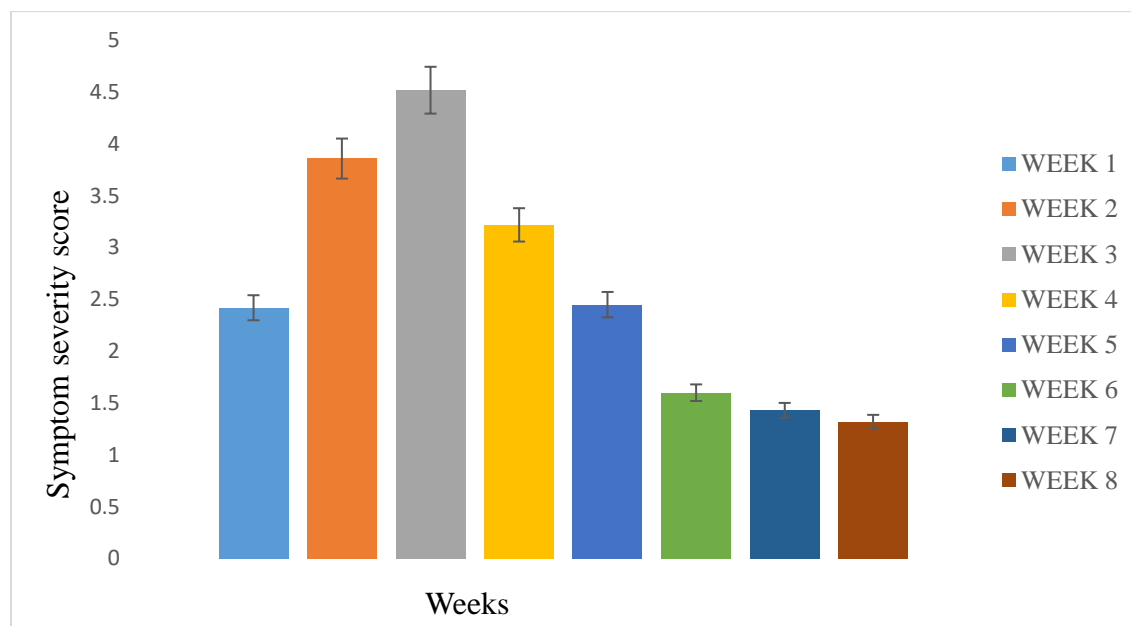


Figure 4.7 Disease severity showing the level increase from week 1 to the fourth week and decrease from week 3 to week 8.

At week 3, the accessions recorded their highest mean symptoms severity. HT was the accession with the highest severity with 4.77 while WJ recorded the lowest mean of SS with 4.12. The mean severity of all accessions at week 1 was 2.42 and increased to 4.52 at the fourth week. However, at week 1 HT recorded the highest severity (3.39) while AP had the lowest (1.53). The eighth week recorded the least mean SS (1.32) for all accessions. HT and AD scored 1.47 as the accession with the highest mark. Nonetheless, AP recorded 0.7 SS which is the lowest mark in the eighth week (Table 4.1). This present finding of lower disease severity at initial stages of growth and increased severity with age could be related to increased inoculum and plant age. The result corroborated the report by Hiraida (2016). According to the author, Taro uses non-specific ways to eradicate pathogens during the early stages of TLB infection by elevating antifungal levels, which protects

cells from oxidation and facilitates recovery during inflammation. Adhiambo et al. (2019). also reported a parallel effect that plants with a high vulnerability to fungal infections are particularly susceptible after flowering. Therefore, decreased severity at the eighth week suggested a decreased susceptibility to taro leaf blight by the accessions. Similar findings by Brook (2008) indicated that as plants get older, the diameter of TLB lesions declines. The varied levels of disease severity among the taro accessions showed that there were different levels of inherent properties to reduce the impact of the disease. Thus, various taro accessions showed varying degrees of disease resistance.

In general, accession KS recovered moderately and resisted the TLB disease. KS had the lowest disease incidence in the seventh and eighth week. The disease incidence for accession KS dropped from 100 % at week five to 60 % at week eight. Furthermore, the disease severity of the same accession decreased from 4.63 at week three to 1.01 at week eight. This could be attributed to genetic make-up of the accession KS that makes it tolerant to TLB described by Nath et al. (2013) and Miyasaka et al. (2012). Furthermore, Omege et al. (2016) ascribed the differences in disease severity among taro plants to genetic factors.

4.4.3 Corm yield

Most of corms after harvesting exhibited some dark appearance representing corm rot. Individual corms from the field displayed varying degree of corm rot; the corms of individual taro showed 25, 50 and 100 % rots (Figure 4.6). Nelson et al., (2011) came to a same conclusion that for incredibly fragile taro cultivars, taro leaf blight reduced taro corm output by 50% or more. The corm after harvesting was seen to decay very fast and turned dark within one week. The finding

was supported by Jackson and Gollifer, (1975) that Corm rots occur quickly after harvest, and entire corms can deteriorate in as little as 7–10 days. The decayed corm tissue can be invaded by *Lasiodiplodia theobromae* and turn black in advanced stages of corm rot.

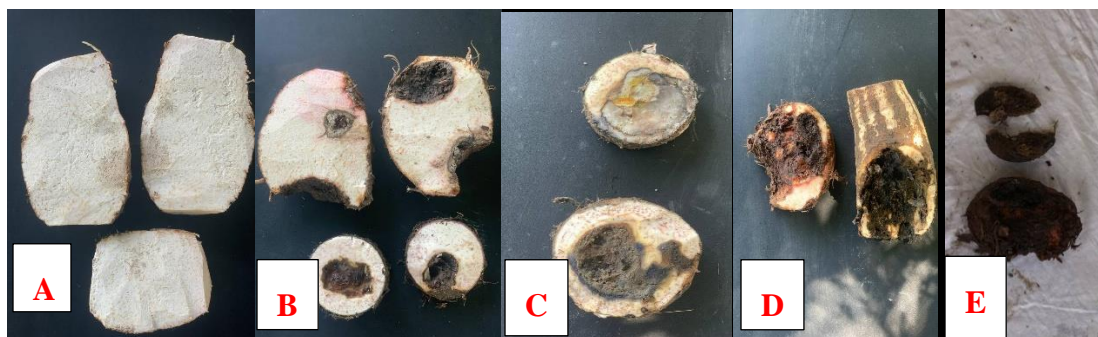


Figure 4.8 Showing TLB development in corm;(A) Clean taro corm free from disease; (B) approximately 25 % portion of corm rot; (C) 50 % corm rot; (D) 100 % corm rot and (D) corm turning black after some days of harvest.

The data on the yield shows that, GY produced the highest yield with 71.47 kg/ha, AP recorded the second highest yield with 61.50 kg/ha while KT recorded the least yield with 41kg/ha. The rest of the accession AD, AT, FW, HT, KS, KF and WJ recorded 55.20, 58.20, 56.6, 51.73, 56.10, 57.70 and 56.90 kg/ha respectively (Figure 4.8). The experiment was affected by long period of drought and could be a great contributory element for smaller yield. In addition, many corms got rotten, presumably due to increased soil temperature as a result of the drought. Mishra and Singual, (1992) observed that extremely higher temperature restricts most of the biological activities as well as increasing the potentials of roots damage (root rot). All the accessions had a reduction in yield indicating that they were seriously infected by the TLB disease (Figure 4.8) which reduced the number and area of functional leaves where photosynthesis will take place. Jackson (1999) reported that taro leaf blight significantly reduced the amount of functioning leaves, resulting in a

global yield reduction of around 50 %. Similar result was reported by Nelson et al., (2011) that TLB can reduce taro corm yield by 50 % and up to 95 % leaf yield or more for highly susceptible taro cultivars in Hawaii. Genotypic differences among the accessions could possibly be one of the contributing elements for the differences of corm yield due to variations in the utilization of resources. Thus, one accession could better utilize resources available to it than the other. Goenaga and Chardon, (1995) established that in many components of taro plant, there are varietal differences in nutrient uptake and dry matter accumulation.

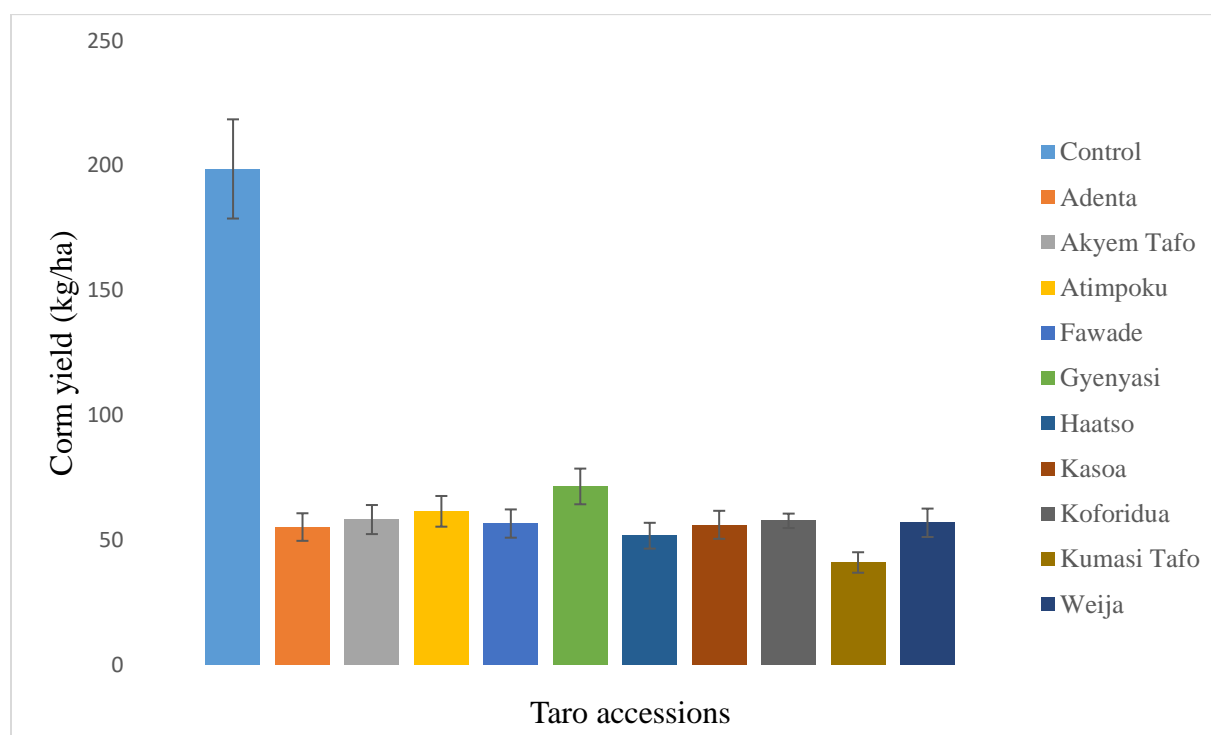


Figure 4.9 Corm yield of ten taro accessions after harvesting.

Generally, the yield of all the accessions is lower as compared to the yield of the control taro crop which was not affected by TLB disease. The mean yield of the affected accessions was 56.64kg/ha while the mean yield of the control taro crops was 198.67kg/ha. The yield of the accession is almost equal to one quarter to the yield of the control showing a significant reduction in corm yield due to infection by TLB disease (Figure 4.8).

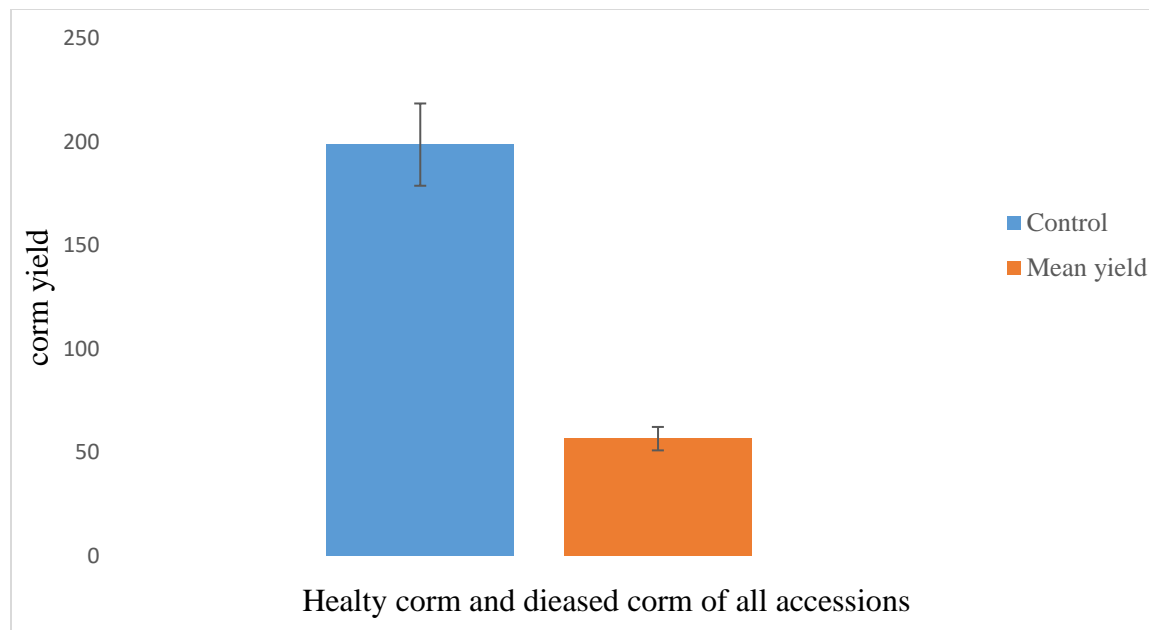


Figure 4.9.1 Mean corm yield of healthy taro compared to the average yield of all TLB-affected taro plants.

The drastic yield loss of the taro accessions from the field showed the effect of the TLB disease on the field. The significant loss in the corm yield confirmed that historically, TLB disease is the most important and damaging taro disease on the globe, and it is responsible for significant taro crop losses around the world. With reference MINADER/DESA (2010), who reported that TLB caused 100 % yield losses in many production fields and an average of 80 % national production was lost to the disease epidemic in 2010. At least, 413,051 tonnes of taro tubers were lost, estimated at 70 billion FCFA in Cameroon. The study also confirmed that Trujillo et al., (1997), who described the devastating magnitude of the taro leaf blight epidemic in American Samoa in 1993-1994, was correct. Elsewhere, Taro production plummeted from 357,000 kg (786,000 lb) per year prior to the pandemic to fewer than 5,000 kg (11,000 lb) by the end of 1995. (Brooks, 2008). Similarly, Western Samoa (now Samoa) exported taro worth US\$3.5 million in 1993, accounting for 58 % of the country's agricultural exports. Taro exports were worth less than \$60,000 in 1994.

(Nelson et al., 2011). The TLB disease has overwhelming impact in human society and food safety and according to Fisher et al. (2012), preventing the spread of fungal infections in the world's five most significant crops could feed more than 600 million people per year.



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CHAPTER FIVE

MOLECULAR DETECTION, SEQUENCING AND PHYLOGENETIC ANALYSIS OF GHANAIAN ISOLATES OF THE *PHYTHOPHTHORA COLOCASIAE*.

5.0 INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is a highly diversify crop from the Araceae family, Taro is farmed within a wide range of settings throughout the tropics and subtropics. In terms of consumption, the plant is the second most staple root crop after sweet potato (Singh *et al.*, 2006) and it is the fourth root crop base on weight production after sweet potato, yam, and cassava (Bourke and Vlassak 2004). Taro is gradually being replaced by more productive root crops such as cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* (L.) Lam.). As a result, most of the genetic diversity of taro is being eroded (Caillon *et al.*, 2006). Taro is grown as a staple crop up to an altitude of 2,200 m (Bourke *et al.*, 1998). Taro may have evolved in the Indo-Malayan region, either between Eastern India and Bangladesh (Purseglove, 1988; Plucknett, 1983) or even in Southern China (Cable, 1984). The origins of wild taros and their domesticated counterparts, as well as their dissemination routes, are still debated (Yen, 1995; Matthews and Terauchi, 1994). Taro displays a wide array of agro-morphological diversification. Clonally stable traits are utilized as markers for varietal recognition and genetic heterogeneity evaluation. IBPGR (1980) established the first taro descriptor list, which was used in earlier attempts to characterize the germplasm (Akus *et al.* 1989; Levett *et al.* 1985) but currently an edited version (IPGRI, 1999) created by IPGRI in conjunction with TaroGen is being used. In addition, the TANSO network

generated a descriptor list based on significant agro-morphological characteristics that can be used to choose national core samples for a regional core collection in all of the partner nations (TANSAO, 1998).

Taro production in the world is projected to be 11.8 million tonnes per year (Vishnu *et al.*, 2012). It is grown on roughly 2 million hectares worldwide, with an average yield of 6 tonnes per hectare (Singh *et al.*, 2012). The majority of global output comes from emerging countries with small-scale production systems and few foreign resource inputs (Singh *et al.*, 2012). Taro is beneficial to resource-poor farmers and consumers in terms of food security, nutrition, culture, and revenue generation even though it is understudied (Sharma *et al.*, 2008). Taro production, on the other hand, has been dropping in recent years, according to Wagih *et al.* (1994). TLB (*Phytophthora colocassia* Racib.), taro beetles (*Papuana spp.*), the Alomae – Bobone virus complex (ABVC), and decreased soil fertility all have a negative impact on yield. Their effects have had a role in the crop's decline in productivity (Sar *et al.* 1998). The introduction of other crop species with better comparative advantages, such as Chinese taro (*Xanthosoma sagittifolium*) and sweet potato, as well as changing dietary habits and consumer preferences for unusual cuisines, have all had a negative influence on taro production (Joughin and Kalit, 1986; Bourke, 1982; Waddell, 1972). According to Omane *et al.* (2012), Bandyopadhyaya *et al.*, (2011) and Ooka, (1994), several infectious diseases produced by fungus, bacteria, nematodes, and viruses, as well as non-pathogenic or abiotic causes, have afflicted taro. *Phytophthora colocasiae*, a biotic factor, has caused a significant drop in taro output in the Solomon Islands, Papua New Guinea, Hawaii, Taiwan, American Samoa, Nigeria, Ghana, and Cameroon. According to Ooka (1994), fungal diseases of taro are the most significant because, they benefit from favourable climatic conditions

for taro cultivation. CABI (2014), also indicated the presence taro leaf blight in Cameroon, Equatorial Guinea, Ethiopia, Ghana and Seychelles. The most devastating disease of taro is *P. colocasiae* Raciborski leaf blight, which is a serious constraint to taro production around the world (Sahoo *et al.*, 2007; Misra 1999; Jackson *et al.* 1980). *P. colocasiae* can infect the taro plant at any stage, causing considerable harm to the foliage. Initial symptoms appear as small, water-soaked circular patches emerges on the edges of the leaves. As the disease progresses, these spots expand, coalesce, and turn dark brown with yellow edges, eventually destroying the entire leaf. Temperatures of 20°C to 25°C, with a relative humidity of 90 % to 100 %, are conducive to epidemics (Trujillo, 1965; Thankappan, 1985; Mishra *et al.*, 2010.)

Pathogen diversity can affect the development and application of management strategies such as resistant cultivars. As a result, knowing how the causative pathogen varies might help you explore different disease control strategies. *Phytophthora* species have traditionally been identified based on host differences, biochemical tests, morphological, and molecular traits (Appiah *et al.* 2003; Appiah 2001; Erwin and Ribeiro 1996). Individual genotypes can be tracked using molecular markers, and population diversity can be studied using molecular markers. The genetic diversity of *P. colocasiae* has been determined using a multitude of genetic markers (Nath *et al.*, 2016). Molecular markers have recently become a tool for not only identifying and authenticating species, but also for differentiating between them. *Phytophthora* species have been effectively evaluated using DNA-based approaches such as restriction fragment length polymorphism (RFLP), amplified fragmented length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and gene sequencing (Yang *et al.* 2008; Ochwo *et al.* 2002; Förster *et al.* 2000). Lebot *et al.* (2003) discovered diversity in *P. colocasiae* isolates from Indonesia, Papua New Guinea, the Philippines, Thailand, and Vietnam using isozyme and RAPD analyses. Similarly, using RAPD,

AFLP, and Start codon targeted polymorphism (ScoT) analysis, multiple studies (Nath *et al.* 2012, 2013 and 2015; Mishra *et al.*, 2010)) discovered variations between *P. colocasiae* isolates from India. Zietkiewicz *et al.* (1994) first described the random amplified microsatellite markers (RAMS) technique, in which the DNA between the distal ends of two closely placed microsatellites is amplified and the PCR products are separated electrophoretically. According to Acosta (2007), Because of the variety in the number of repeat units, SSR markers have a range of polymorphism. SSR markers are also highly reproducible, locus-specific, and sensitive to discriminating between related individuals as compared to other markers (Pervaiz *et al.* 2009; Bindler *et al.*, 2007). The establishment of successful disease control techniques requires accurate pathogen identification and characterisation. The use of PCR-specific markers for *P. colocasiae* genomic DNA could allow for early diagnosis of taro leaf blight before symptoms manifest. Plant pathogen detection and characterization have benefited greatly from the use of PCR-based molecular biology techniques (Milgroom and Fry, 1997). A speedy and efficient genomic DNA isolation approach for *P. colocasiae* with reasonably high quantity and quality is required to take advantage of these methods (Aamir *et al.*, 2015; Hussain *et al.*, 2014; Mishra *et al.*, 2008)

5.1 Objectives of study:

The main objectives of the study was to asses the genetic variation of some Ghanaaian isolates of *P. colocasiae*.

The specific objectives of the study were to

- (1) Isolate the fungus from the diseased tissues of taro and grow it on a culture media.
- (2) Identify the fungus using the light microscope and fungi descriptions manuals

- (3) Identify *P. colocasiae* in samples collected from symptomatic taro plants using polymerase chain reaction (PCR).
- (4) Sequence and phylogenetic analysis the resulting PCR amplicons of the Ghanaian isolates of the *P. colocasiae*.

5.2 MATERIALS AND METHODS

5.2.1 Study area

Phytophthora colocasiae isolates were cultured at the Mycology Laboratory of the Cocoa Research Institute of Ghana (CRIG), DNA extraction, gel electrophoresis, polymerase chain reaction (PCR), and greenhouse experiments were carried out at the Ghana Atomic Energy Commission's Biotechnology Nuclear and Agriculture Research Institute in Kwabenya, Greater Accra Region. Sequencing was done at Inqaba Biotechnology Laboratory in South Africa.

5.2.2 Sampling and isolation of *P. colocasiae* isolates

Taro leaves with indications of taro leaf blight disease were harvested from 10 farms in the Ashanti, Greater Accra, Central, and Eastern regions. Fifteen (15) diseased leaves were collected from the four (4) regions. The samples were well labelled and kept in brown paper bags to minimize tissue damage during sample transfer from the farm to the Mycology Laboratory of the Cocoa Research Institute of Ghana, new Akim Tafo. Surveys were carried out from March to July, during the main cropping season, when the weather conditions were ideal for disease growth. The leaf samples were first rinsed under running tap water, to eliminate all debris. Small pieces of leaf tissue (about 1 cm) were cut with scissors and scalpels from the expanding borders of infected area lesions, surface sterilised in 70 % ethanol for 30 seconds, and rinsed three times with sterile

distilled water. The leaves were blotted dry on Whatman filter paper in laminar flow chamber. The dried leaf tissues were first plated on a prepared water agar medium (500ml of distilled water and 10g of agar powder) with inoculation pin and incubated at 28°C for 14 days.

The morphology of *P. colocasiae* colonies was investigated on V8 juice agar medium (200ml of V8 + 800ml of sterile distilled water + 20g of Agar +2.5g of calcium carbonate). After that, the mixture was autoclaved for 15 minutes at 121°C. Petri dishes containing the medium at a rate 15 ml per plate were autoclaved was allowed to cool and harden under sterile conditions. A 3mm disc was taken from the margin of an actively growing colony of *P. colocasiae* on water agar media and deposited in the center of the V8 juice medium, with chock borer and inoculation pin. The diameter of each isolate was measured after 7 days following incubation. The identification of the fungi was done using a compound microscope (Motic china) and fungal description manuals. (Mathur and Kongsdale, 2003; Barnett and Hunter, 1972).

5.2.3 PROOF OF PATHOGENICITY OF *P. COLOCASIAE* ON POTTED PLANTS IN THE SCREEN HOUSE

The proof of pathogenicity test based on Koch's postulates was used to confirm the disease's causing organism. The Minisett method was employed to raise homogenous *Colocasia* seedlings in a greenhouse at BNARI. The corms were split into 20 g pieces and planted in damp sawdust that had been steam sterilized. Sterilised black soil was dispensed into 1.5 litre size plastic containers. Seedlings were potted in black bags when they were two weeks old, one seedling per pot. Pure cultures of the *P. colocasiae* isolates were then inoculated into four-week-old established potted seedlings using a cock borer and inoculation pin. The inoculated plants were placed under tree to create humidified environment and also to prevent direct sunlight. The inoculated plants

were monitored for symptom development. Lesion was observed after 7 days. All of the resulting lesions were re-isolated according to Koch's postulates.



Figure 5.1 Potted taro leaf inoculated with the Akim Tafo isolate of *P.colocasiae*

5.2.4 Preparation of *P. colocasiae* suspension for DNA extraction

All pure fungus cultures hyphae and spores were scraped from the surface of culture media with heated sterilized surgical blades. 200 μ l of double distilled water was poured on the petri dish containing the fungus and a surgical blade was used to scrap the isolated fungus into 1.5 ml Eppendorf tubes. The operation was performed in sterilized condition under laminar flow hood. Two hundred microliters (200 μ l) of the samples were discharged into a new Eppendorf tube and 800 μ l genomic lysis buffer (GLB) was added. The mixture was vortexed for 30 seconds before being incubated in a water-bath for 30 minutes.

5.2.5 Genomic DNA extraction

A genomic DNA extraction kit was used to extract total DNA from the *P. colocasiae* according to the manufacturer guidelines (Zymo kits). The protocol is designed for up to 200 μ l of biological liquid sample and other cell suspensions containing less than 5.0×10^6 cells as well as lysates derived from proteinase K digested samples.

Four volumes of genomic lysis buffer were added to each volume of liquid samples (4:1). The samples were briefly mixed by vortexing, allowed to stand at room temperature for 5-10 minutes and 1ml of the supernatant was transferred to new tubes. Zymo-Spin™ 11C column was placed in new collection tubes. Two hundred microlitres (200 μ l) of DNA pre-wash buffer was added to the spin column and it was centrifuged at 10000 \times g for one minute. Five hundred microliters (500 μ l) of g-DNA wash buffer were added to the spin column and it was centrifuged at 10000 \times g for one minute. The spin column was transferred to a clean micro centrifuge tube. DNA elution buffer ($\geq 50\mu$ l) was added to the spin column. The set up was incubated for 2-5 minutes at room temperature and then centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was stored for molecular based application at $\leq -20^\circ\text{C}$ for future use.

5.2.6 Analysis of the quality and quantity extracted DNA

The purity of the extracted genomic DNA was determined using agarose gel electrophoresis. Gel electrophoresis was conducted with 0.8 % of agarose gel. Agarose powder (0.24g) was weighed and dissolved in 30ml of 1XTAE (Tris-acetate EDTA) buffer boiled in a microwave until the agarose was completely dissolved. The mixture was allowed to cool for a while and 2 μ l of ethidium bromide (EtBr) was added. The mixture was poured into a gel tank with a comb and was allowed to solidify. Two microliters (2 μ l) of loading dye and 7 μ l of the DNA was mixed and

electrophoresed at 90V until the bromophenol had migrated about two-thirds of the way down the gel. The DNA was visualized under Gene Flash Bio-imaging System (Syngene, Cambridge, UK) and subsequently stored at -20°C until use.

5.2.7 Polymerase chain reaction (PCR) amplification

ITS region of *P. colocasiae* isolates was amplified by using the universal primers set ITS1 (5' TCCGTTGGTGAACCAGCGG 3') and ITS4 (5' TCCTCCGC TTATGATATGC 3') (White et al., 1990) and PCSP- RF (5' CAGATGAAGAGGTCCTGTGAGG 3') and PCSP- RR (5' AGGGAGTTGGCACAACCATT 3') (Nath et al., 2014). 2X Master mix was prepared by taken 250µl of the buffer 20µ of primer, 2µ of the DNA template was used and Nucleases-free water was used to adjust the volume. PCR amplification was run in the thermal cycler by providing a specific program of initial denaturation at 94°C for 5 minutes followed 35 cycles of denaturation at 94°C for 1 minute, annealing at 60 °C for 1 minute and extension at 72 °C for 1 minute and final extension at 72 °C for 10 minutes and held at 4°C until it was removed. The PCR amplicons were run on 1 % (w/v) agarose gel as described previously. The amplification was done by using the Nexus Mastercycler (Eppendorf AG, Germany). Ten microliters (10 µl) of the PCR products were mixed with 2X loading dye (Bromophenol blue) and electrophoresed at 90V 1 kbp DNA ladder marker (Bio Labs) was used to verify the amplicon product size. The bands were visualized under UV trans illuminator (Cambridge, UK).

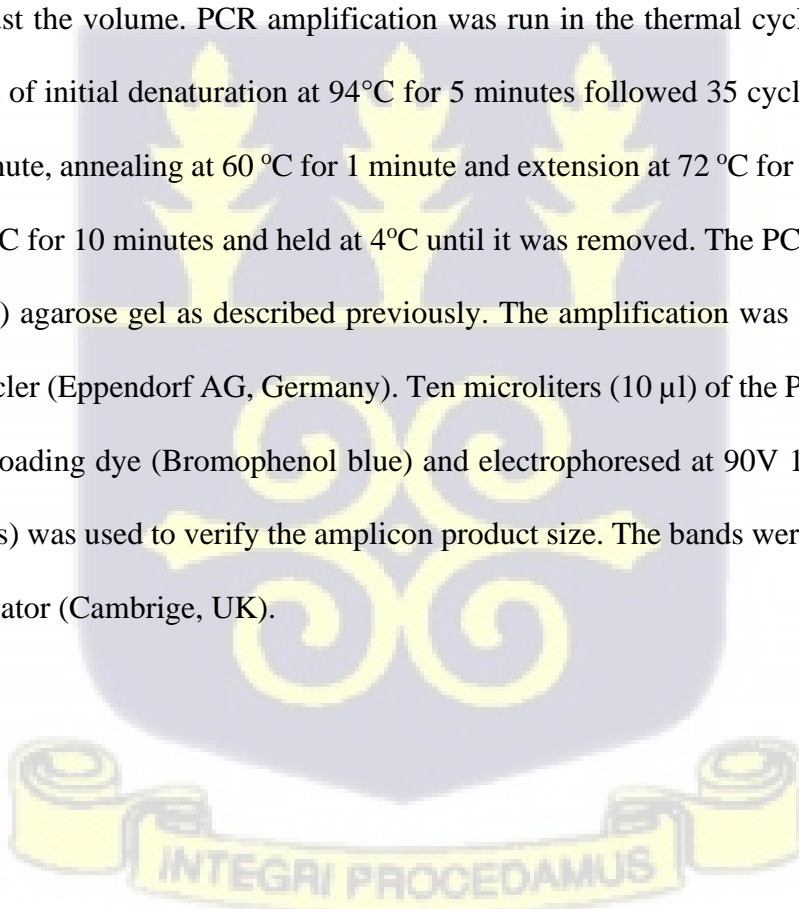


Table 5.1 Oligonucleotide primers used for the amplification of *P. colocasiae*

Agent	Primer name	Direction	Primer sequence	Amplicon size	Reference
<i>P. Colocasiae</i> White <i>et</i> <i>al.</i> , 1990	ITS1	Forward	5'-TCCGTAGGTGAACCTGCGG-3'	830bp-870bp	
	ITS4	Reverse	5'-TCCTCCGCTTATTGATATGC-3'		
<i>P. Colocasiae</i> <i>et al.</i> ,	PCSP-RL F	Forward	5'-GGTGTGGACTTTGTGAGTTTCAG-3'	206bp	Nath 2014b
	PCSP-RL R	Reverse	5'-AAGGGAGTTGGCACAACCATT		

5.2.8 Amplicon sequencing

The forward and reverse primers (ITS1 and ITS4) were used as sequencing primers to sequence PCR products that produced unambiguous single bands in both directions at Inqaba Biotechnology Laboratory, Pretoria, South Africa.

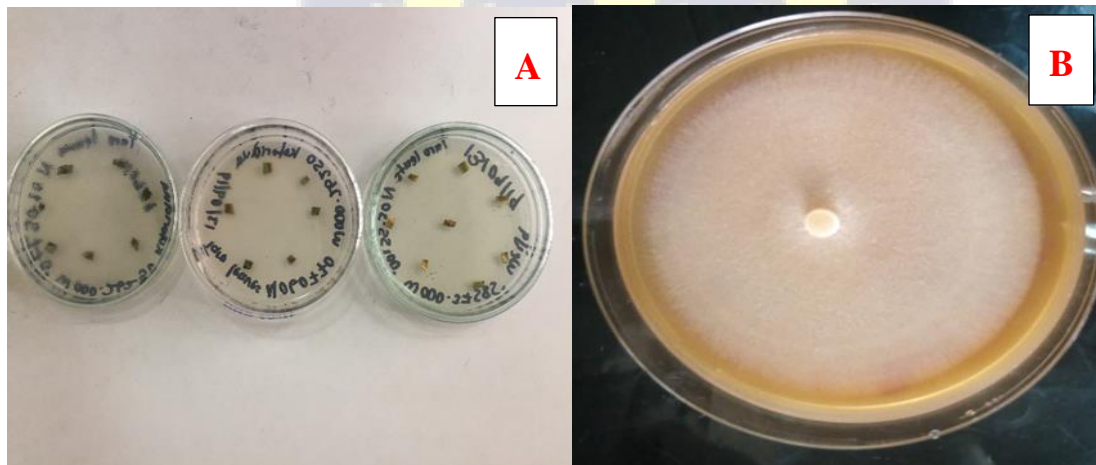
5.2.9 Sequence analysis

The sequenced chromatograms were manually verified for base miscalls and then trimmed at the 5' and 3' ends to remove low-quality sequences. Complete homology was ensured by comparing forward and reverse sequences. After that, the sequences were edited, aligned, and compared to other published isolates using Geneious 9.0 (Biomatters), ClustalW (Thompson *et al.*, 1994) and Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) Geneious Tree Builder was used to create cluster dendrograms using the Neighbor-joining method (1000 bootstrap replicates), and TreeView was used to examine and print the visual phylograms (Page, 1996).

5.3 Result and discussions

5.3.1 Pathogen identification

A total of nine isolates of *P. colocasiae* were successfully isolated from the 15 samples obtained from various farms across the four regions. Based on mycelial and sporangial characteristics, the isolates were identified as *P. colocasiae*. *P. colocasiae* isolates were identified by the formation of semi-papillate sporangia that are caducous and had a pedicel length of 4–10 μ m. Aseptate and hyaline mycelium was discovered.



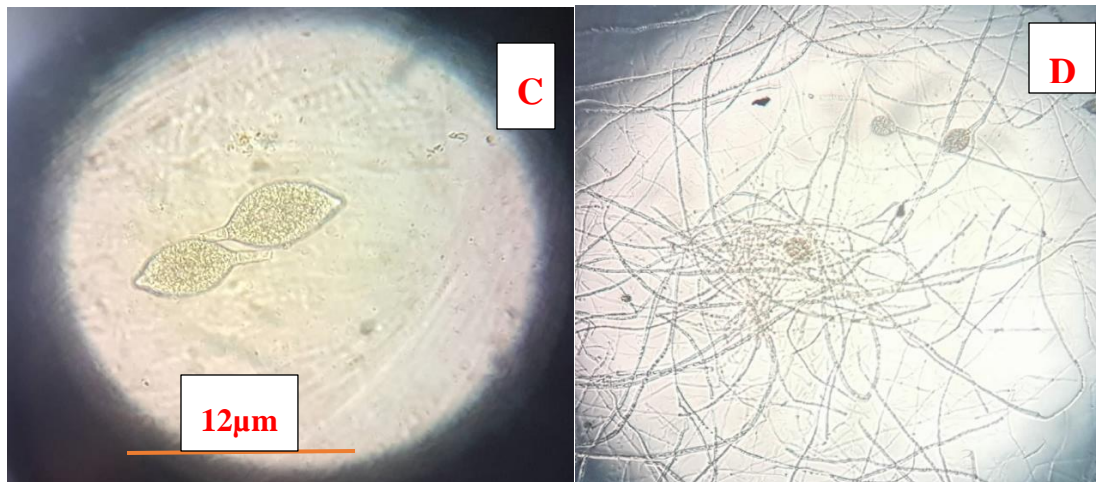
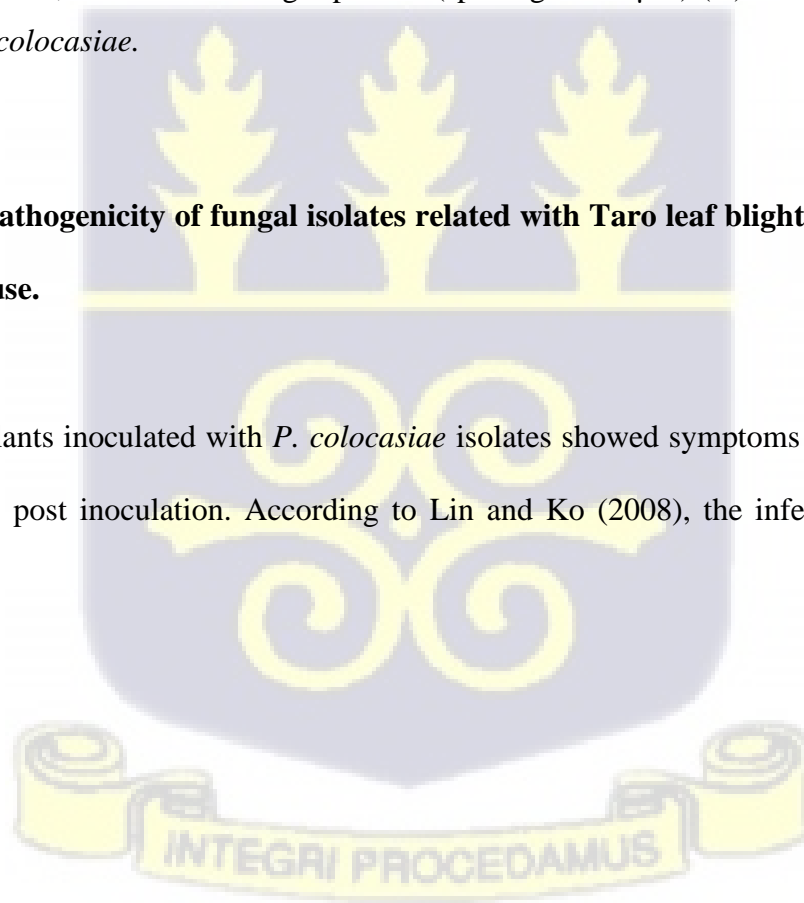


Figure 5.2 (A) Leaf discs from symptomatic of taro leaves on a medium (B) Colony of *P. Colocasiae* isolates grown on a medium (C) Sporangium of *Phytophthora colocasiae* with an apical papillum and a basal, intermediate-length pedicel (sporangium 12µm) (D) Microscopic image of mycelium of *P. colocasiae*.

5.3.2 Proof of pathogenicity of fungal isolates related with Taro leaf blight on potted plants in the plant house.

All the potted plants inoculated with *P. colocasiae* isolates showed symptoms of taro leaf blight after two weeks post inoculation. According to Lin and Ko (2008), the infected area initially



showed water-soaked lesions that eventually turned brown.



Figure 5.3 A and B showing symptoms of taro leaf blight disease after two weeks post inoculation with *P. colocasiae* isolate from the laboratory

5.3.3 Genomic DNA isolation and Gel electrophoresis

Genomic DNA of *P. Colocasiae* was successfully isolated from the nine cultures Kasoa 1 (K1), Kasoa 2 (K2), Kasoa 3 (K3), Medie 1 (M1), Medie 2 (M2), Medie 3 (M3), Akim Tafo 1 (T1), Tafo 2 (T2), and Tafo 3 (T3) and the bands were successfully checked by the using gel electrophoresis.



Figure 5.4 Isolate DNA of *P. colocasiae* observed in 0.8 % gel, bands 2-9 are the DNA of the *P.colocasiae* isolates. The lane 1 is the control, lane 2 3 4 is kasoia farm 1 2 3 4 lane 5 6 7 8 is medie farm 1 2 3 and lane 10 11 12 13 is Akim tafo farm 1 2 3

5.3.4 PCR amplification

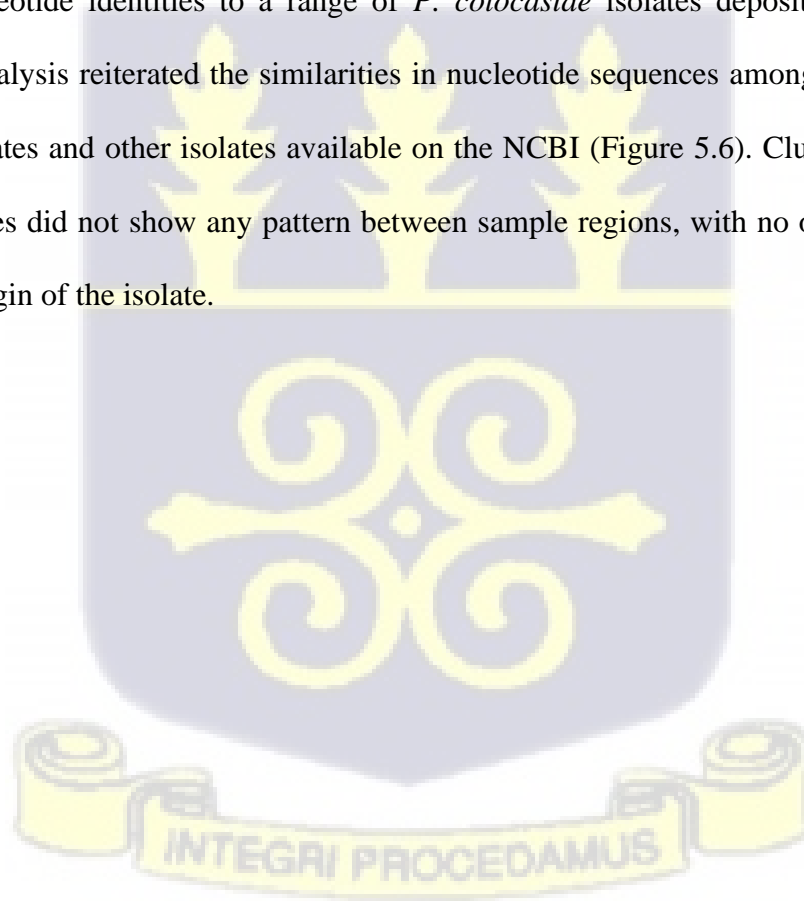
DNA from all *P. colocasiae* cultures was successfully amplified using ITS1/ITS4 primer pair. PCR products of 870bp was amplified from each sample using the universal primer pair ITS1 and ITS4 as shown in Figure 6 below. This results is in line with work done by Al-abedy et (2020) who amplified fungal products by using ITS1 and ITS4. Quality and quantity of PCR amplicons were successfully 1 % agarose gel.



Figure 5.5 Sharp and clear bands of 870bp amplified PCR products of internal transcribed spacer (ITS) region of *P. colocasiae* isolates (1)medie farm 1, (2) Medie farm 2, (3) Medie farm 3, (4) kasoia farm 1, (5) kasoia farm 2, (6) kasoia farm 3, (7) Akim tafo farm 1, (8) Akim tafo farm 2, (9) Akim tafo farm 3.M= 1Kbp DNA ladder marker. NC: Negative control (no DNA template added). 11= positive control (a known *P. colocasiae* positive sample).

5.3.5 Nucleotide sequencing of the ITS region of *P. colocasiae*

Nucleotide sequences obtained from the nine amplicons of the *Phytophthora* isolates were trimmed. After trimming, sequences of 736bp were compared. The alignment of sequences revealed a high degree of similarity among the Ghanaian isolates from the three regions (Greater Accra, Eastern and Central). The isolates also showed close similarity to nucleotide sequences of isolates from other countries with identities ranging from 99.27 to 100 %. BLAST analysis revealed high degree of similarity between the Ghanaian isolates and those available on the National Center for Biotechnology Information (NCBI) with nucleotide sequence identities in the range of 99.27 to 100%. Kasoa isolates 1 and 3 and Akim Tafo isolate 3 showed 99.56 to 99.85 %, Medie isolates 1, 2, 3 and Tafo isolate 2 showed 99.71 to 100 % whilst Tafo isolate 1 showed 99.27 to 99.56 % nucleotide identities to a range of *P. colocasiae* isolates deposited on the NCBI. Phylogenetic analysis reiterated the similarities in nucleotide sequences among the Ghanaian *P. Colocasiae* isolates and other isolates available on the NCBI (Figure 5.6). Clustering within the Ghanaian isolates did not show any pattern between sample regions, with no obvious clustering based on the origin of the isolate.



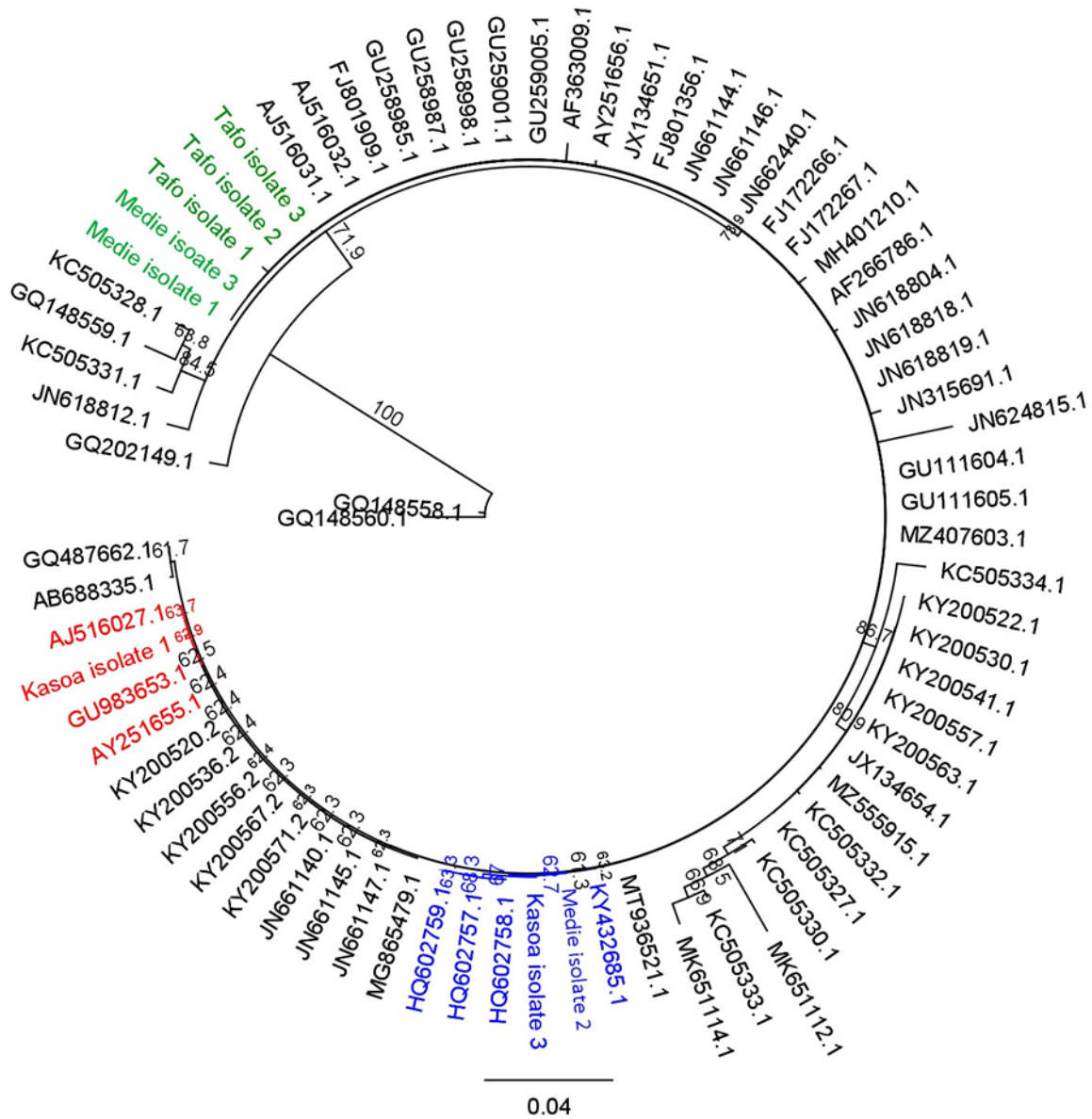


Figure 5.6 Phylogenetic tree (neighbor-joining) of an ~736 nt fragment of ITS gene of *P. colocasiae*. Numbers at the nodes denotes the percentage of 1000 bootstraps iterations supporting the branches. Nodes with <60% bootstrap support were collapsed.

Nucleotide alignment of a segment of the 736 bp nucleotide of the nine Ghanaian isolates of *P. colocasiae* revealed only a few single nucleotide changes in Kasoa isolate 2, Kasoa isolate 3 and Akim Tafo isolate 1 (Figure 5.6).

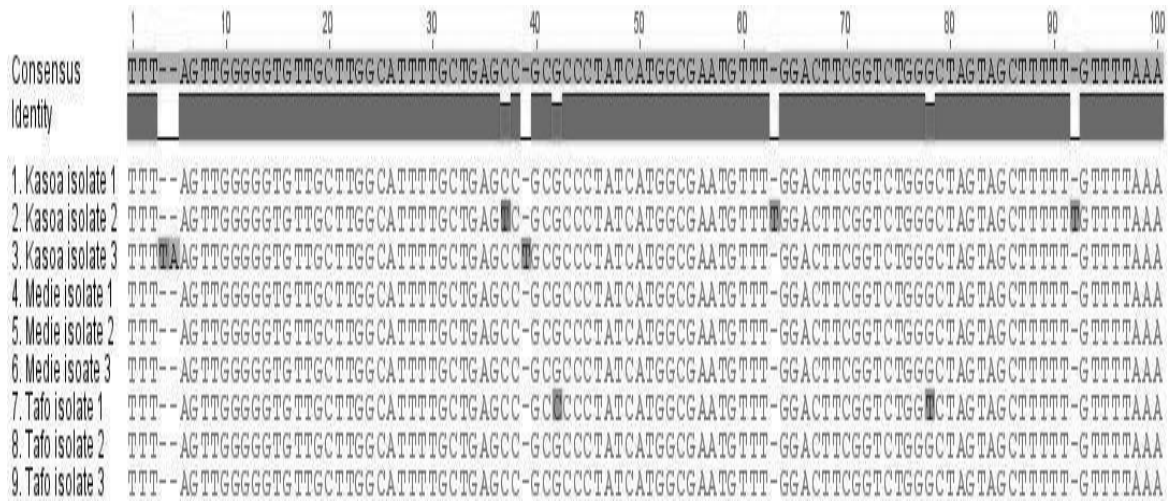
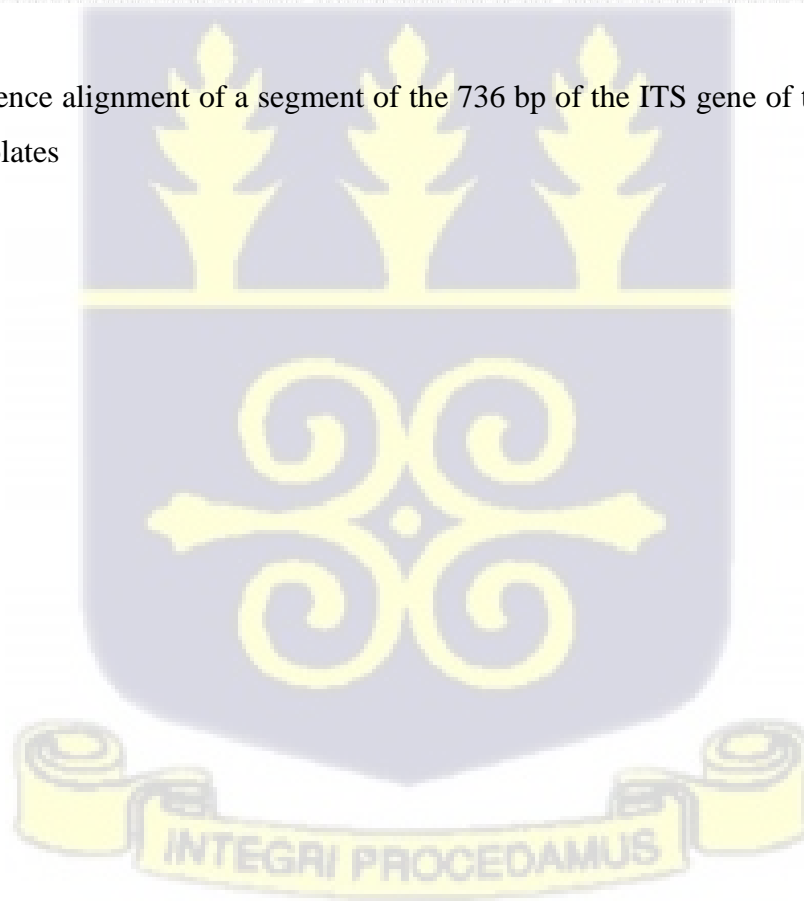


Figure 5.7 Sequence alignment of a segment of the 736 bp of the ITS gene of the nine Ghanaian *P. colocasiae* isolates



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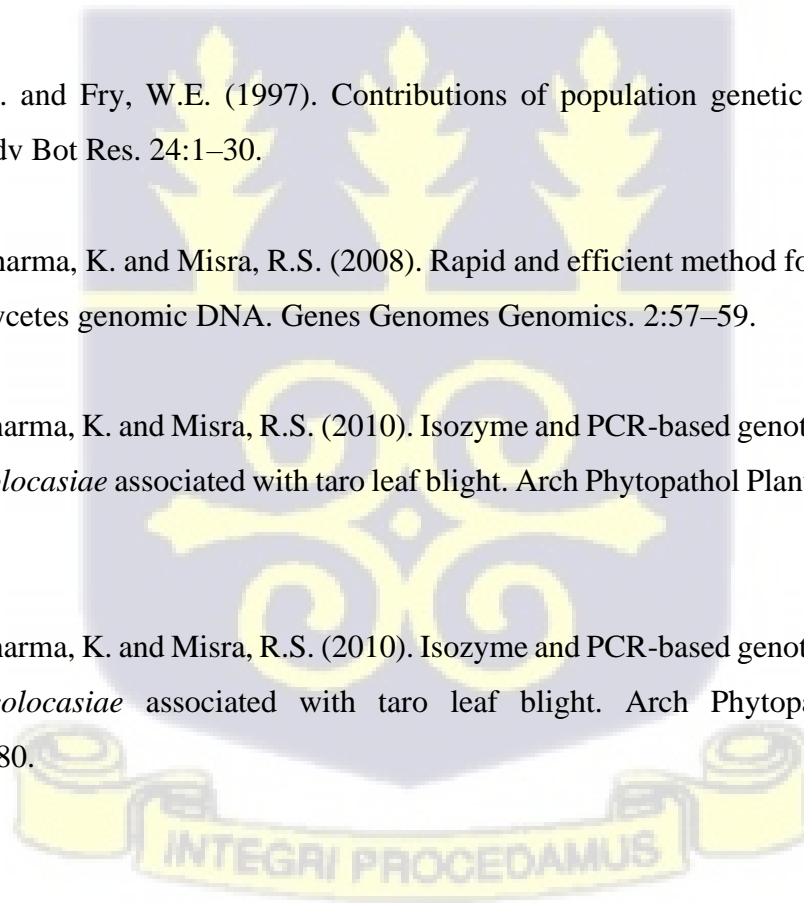
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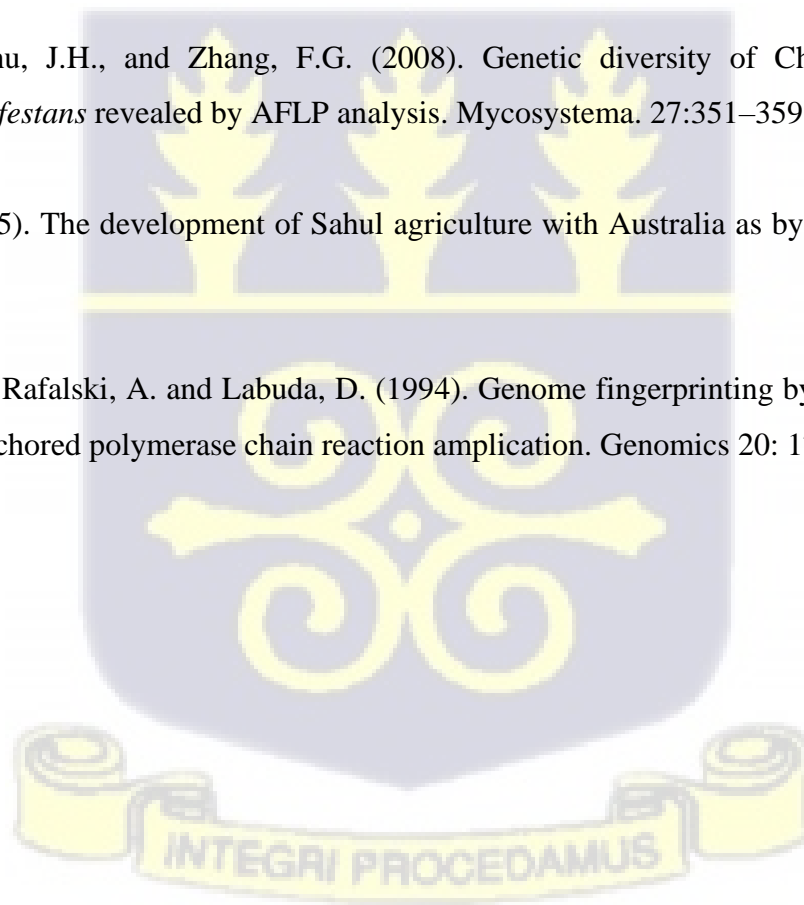
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CHAPTER SIX

6.1 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1.1 CONCLUSIONS

On the basis of the results obtained from this research, the following conclusions are presented:

1. Greater proportion of the farmers are male, had low level of education had no knowledge about taro leaf blight and its adaptation strategies.
2. The farmers confirmed the presence of the disease but they did not really know what chemical to use in its control.
3. Disease incidence and severity on the farmer's field revealed that different taro farms in Anyinam in the Eastern region had high incidence of the TLB disease and the lowest incidence was Atimpoku in Eastern region.
4. Incidence of the TLB generally increased in all accessions through the first to eighth week except KS which displayed decreased incidence at the seventh and eight weeks.
5. Accession AP recorded the highest TLB disease incidence and KS recorded the lowest incidence at eighth week. Accessions GY recorded highest disease severity while the accession AP recorded the lowest severity.
6. TLB disease caused corms of most of the accessions to rot and the rotting increased at around 5 days after harvest. The corms became black and the yield from all the accessions reduced drastically as compared to the control.
7. Accession KS exhibited gradual recovery and resistance to the TLB infection. It recorded low disease incidence and severity at the eighth week.

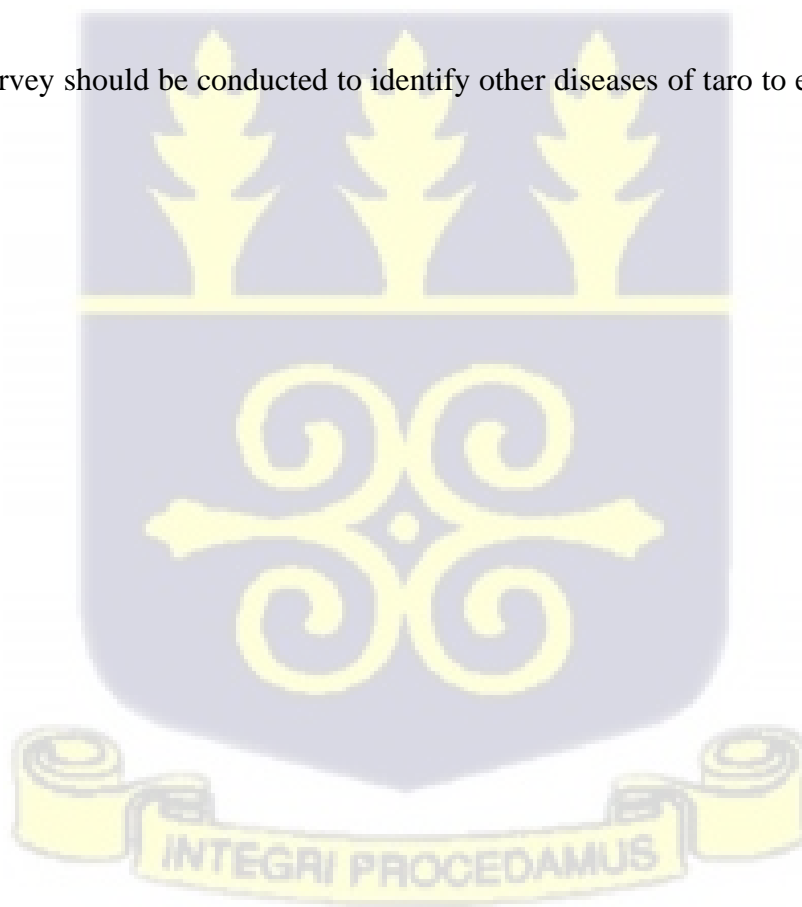
8. Accession GY recorded the highest yield whilst accession KT recorded the lowest yield. The mean yield of all the accessions in the three replicate is lower than the mean yield of the control.
9. Out of the fifteen samples of diseased leaves used for culturing *P. colocasiae*, culturing was successful with samples from Medie farm 1,2 and 3(M1, M2, M3), Akim tafo farm 1,2 and 3(T1, T2, T3) and Kasoa farm 1,2and 3 (K1, K2, K3).
10. Koch's postulate confirmed the presence of the TLB disease on a potted plants which gave 100% disease incidence and severity.
11. 11. The fungus was found in all samples analyzed by polymerase chain reaction (PCR) with the expected PCR product amplicon size of ~ 500bp.
12. 12. Sequence alignment reveals that Ghanaian isolates from the three locations have a significant degree of similarity. (Greater Accra, Eastern, Central) and other isolates in other countries with identities ranging from 99.27 to 100%.
13. Accessions K1, K3 and T3 showed 99.56-99.85%, M1, M2, M3 and T2 showed 99.71-100% and accession T1 showed 99.27-99.56% to other *P. colocasiae* sequence ITS sequences available on the NCBI.

6.1.2 RECOMMENDATIONS FOR FUTURE RESEARCH

On the basis of the findings of this study and prior TLB taro studies, the following are recommended for consideration in any future work on taro.

- (i) Based on the major findings, the study therefore recommends that awareness should be created by extension agents using appropriate means of dissemination of information on TLB disease among farmers. Moreover, private and public agencies should invest in taro research to assist in solving farmer's problems.

- (ii) Accession KS showed tolerance/resistance to the taro TLB disease; therefore, they could be utilized in future breeding programmes for creation of *P. colocasiae*-resistant genotypes.
- (iii) Molecular characterization of a greater number of TLB pathogen isolates should be conducted and their genetic diversity together with their pathogenicity be studied in detail for effective realization of sustainable prevention of the blight disease and for combating the taro leaf blight menace.
- (iv) Taro crop research must expand since taro suffers from a lack of attention from Governments, International Agricultural Research Centers, and other root and tuber groups in order for it to continue to play the role it has in ensuring food security and farmer economic empowerment.
- (v) It is obvious that *P. colocassia* causes significant yield losses; consequently, appropriate attention is essential to manage leaf blight before using tolerant accessions in locations with high blight incidence.
- (vi) A nationwide survey should be conducted to identify other diseases of taro to enhance resistance breeding.



CHAPTER SEVEN

APPENDICES

Table 7.1 Disease incidence and severity for fifteen farms at Ashanti region (Fawade, Gyenyasi, Old tafo, Tafo estate)

Town	Farms	Disease incidence (DI %)	Disease severity (DS)
Fawade	Farm 1	10.3	0.47
	Farm 2	10.0	0.47
	Farm 3	13.6	0.75
	Farm 4	19.5	1.50
	Farm 5	16.6	0.54
Total	five farms	70.0	3.73
Mean		14.0	0.746
Gyenyasi	Farm 1	12.35	0.67
	Farm 2	9.8	1.00
	Farm 3	12.7	0.91
	Farm 4	12.45	0.33
	Farm 5	32.7	0.32
Total	5 farms	80.0	3.23
Mean		16.0	0.646
Old tafo	Farm 1	40.0	1.34
	Farm 2	30.0	2.33
Total	Two farms	70.0	3.67
Mean		35	1.835
Tafo estate	Farm 1	29.33	1.13
	Farm 2	30.7	0.85
	Farm 3	19.97	0.97
Total	Three farms	80.0	2.95
Mean		26.67	0.983

Disease incidence = [Number of infected plants / total plant scored x100]

$$\text{Disease severity} = \frac{\sum (\text{Number of plants scored for each rating} \times \text{the rating value})}{\text{Total plants scored}}$$

(CSIR- Crops Research Institute Kumasi, Ghana)

Table 7.2 Disease incidence and severity for twenty farms at Eastern region (Anyinam, Akim tafo, Sekyere, Osino, Koforidua, Atimpoku, Somanya)

Town	Farms	Disease incidence (DI %)	Disease severity (DS)
Anyinam	Farm 1	12.7	0.60
	Farm 2	20.0	0.75
	Farm 3	16.0	1.25
	Farm 4	11.0	0.61
	Farm 5	20.0	0.55
	Farm 6	15.30	0.45
Total	Six farms	95.0	4.25
Mean		15.83	0.708
Akim tafo	Farm 1	26.33	1.00
	Farm 2	30.0	0.78
	Farm 3	23.67	1.88
Total		80.0	3.66
Mean		26.67	1.22
Sekyere	Farm 1	17.25	0.94
	Farm 2	19.25	1.45
	Farm 3	27.0	0.75
	Farm 4	26.5	0.78
Total	Four farms	90.0	3.92
Mean		22.5	0.98
Osino	Farm 1	33.0	1.48
	Farm 2	28.0	0.98

	Farm 3	24.0	1.02
Total		85.0	3.48
Mean		28.33	1.16
Koforidua	Farm 1	35.0	1.65
	Farm 2	40.0	2.22
Total		75.0	3.87
Mean		37.5	1.935
Atimpoku	Farm 1	50.0	2
Somanya	Farm 1	60.0	2.86

Disease incidence = [Number of infected plants / total plant scored x100]

Disease severity = $\frac{\sum (\text{Number of plants scored for each rating} \times \text{the rating value})}{\text{Total plants scored}}$

(CSIR- Crops Research Institute Kumasi, Ghana)

Table 7.3 Disease incidence and severity for twelve farms at Greater Accra region (Adenta, Ashale botwe, Haatso, Weija, Medie, kwafo krom)

Town	Farm	Disease incidence (DI %)	disease severity (DS)
Ashale botwe	Farm 1	23.0	1.00
	Farm 2	47.0	1.90
Total	Two farms	70.0	2.90
Mean		35.0	1.45
Haatso	Farm 1	40.3	0.77
	Farm 2	17.7	1.66
Total	Two farms	60.0	2.43
Mean		30	1.215

Weija	Farm 1	16.7	1.26
	Farm 2	30.0	0.66
	Farm 3	23.3	1.58
Total	Three farms	70.0	3.5
Mean		23.33	1.167
Medie	Farm 1	26.9	0.87
	Farm 2	19.77	1.21
	Farm 3	23.33	1.57
Total	Three farms	70.0	3.65
Mean		23.33	1.217
Adenta	One farm	65.0	3.2
Kwafo krom	One farm	55.0	3.5
Adenta	One farm	65.0	3.2

Disease incidence = $[\text{Number of infected plants} / \text{total plant scored} \times 100]$

Disease severity = $\frac{\sum (\text{Number of plants scored for each rating} \times \text{the rating value})}{\text{Total plants scored}}$

(CSIR- Crops Research Institute Kumasi, Ghana)

Table 7.4 Disease incidence and severity for three farms at Central region (Kasoa)

Town	Farm	Disease incidence (DI %)	Disease severity (DS)
Kasoa	Farm 1	32.3	1.17
	Farm 2	12.85	0.88
	Farm 3	24.85	0.38
Total		70.0	2.43
Mean		23.33	0.81

Disease incidence = [Number of infected plants / total plant scored x100]

Disease severity = $\frac{\sum (\text{Number of plants scored for each rating} \times \text{the rating value})}{\text{Total plants scored}}$

(CSIR- Crops Research Institute Kumasi, Ghana)

**Table 7.5 Disease incidence and severity for fifty farms within the four region of Ghana
(Ashanti region, greater Accra region, central region, eastern region)**

Regions	Towns	Farms	Disease incidence (DI %)	Disease severity (DS)
Ashanti	Fawade	5	70.0	3.73
	Gyenyasi	5	80.0	3.23
	Old tafo	2	70.0	3.67
	Tafo estate	3	80.0	2.95
Total		15	300.0	13.58
Average			75.0	3.40
Eastern	Anyinam	6	95.0	4.25
	Akim tafo	3	80.0	3.66
	Atimpoku	1	50.0	2.00
	Koforidua	2	75.0	3.87
	Osino	3	85.0	3.48
	Sekyere	4	90.0	3.92
	Somanya	1	60.0	2.86
Total		20	535.0	24.04
Average			76.43	3.43
Greater Accra	Adenta	1	65	3.2
	Ashale botwe	2	70	2.9

	Haatso	2	60	2.43
	Medie	3	70	3.65
	Weija	3	70	3.5
	Kwafo krom	1	55	3.84
Total		12	390.0	19.52
Average			65.0	3.25
Central	Kasoa	3	70.0	2.43

Table 7.6 Percentage distribution of respondents according to their social-economic characteristics n=50

Variable	Frequency	Percentage%
Age (years)		
10-19	0	0.0
20-29	2	4.0
30-39	5	10.0
40-49	13	26.0
50-above	30	60.0
Gender		
Male	30	60.0
Female	20	40.0
Educational level		
Non formal	11	22.0
Primary school	15	15.0
Junior high school	20	40.0
Senior high school	4	8.0
Tertiary	0	0.0

Farming experience		
Less than 1	11	22.0
1-5	11	22.0
5 and above	28	56.0

Source: Field Survey, 2020

Table 7.7 Percentage distribution of respondents according to their Farm characteristics and agronomic practices n=50

Variable	Frequency	Percentage%
Farm Size(acre)		
Less than 1	32	64.0
1-2	10	20.0
Greater than 3	8	16.0
Planting materials		
Market	1	2.0
Farmers	31	62.0
Research institutions	9	18.0
MoFA	8	16.0
NGO	1	2.0
Fertilizer application		
Yes	0	0.0
No	50	100.0

Source: Field survey, 2020



Table 7.8 Percentage distribution of respondents according to their Disease awareness and management n=50

Variable	Frequency	Percentage%
Disease presence		
Yes	50	100.0
No	0	0.0
Disease season		
Major raining	21	42.0
Minor raining	11	22.0
Dry season	1	2.0
All the above	17	34.0
Symptoms		
Early spots often occur on the leaf and accumulates.	6	12.0
The spots enlarge rapidly becoming purplish brown to brown in color	10	20.0
The lower leaf surface is water-soaked or dry gray appearance present	32	64.0
The plant become bright orange or reddish brown	2	4.0
Symptoms	Frequency	Percentages
Early spots often occur on the leaf and accumulates.	6	12.0
The spots enlarge rapidly becoming purplish brown to brown in color	10	20.0
The lower leaf surface is water-soaked or dry gray appearance present	32	64.0
The plant become bright orange or reddish brown	2	4.0
Disease effect		
Drying of leaves	7	14.0

Rotting of corms	39	78.0
Reduced number of leaves	4	8.0
Disease control		
Yes	8	16.0
No	42	84.0
Method of disease control		
Weedicides application	3	6.0
Removal of infected plant	30	60.0
Pesticide application	2	4.0
Other	15	30.0
Limitation		
Lack of nutrient	1	2.0
Lack of water	9	18.0
Lack of weeds control	4	8.0
All the above	36	72.0
Cause of abnormalities		
Soil	26	52.0
Weather	10	20.0
Not sure	12	24.0
Other	2	4.0
Harvest losses		
Less than half the crop	20	40.0
About half the crop	29	58.0
The whole crop	1	2.0
Availability		
Yes	27	46.0
No	23	54.0

Source: field survey, 2020

*multiple response

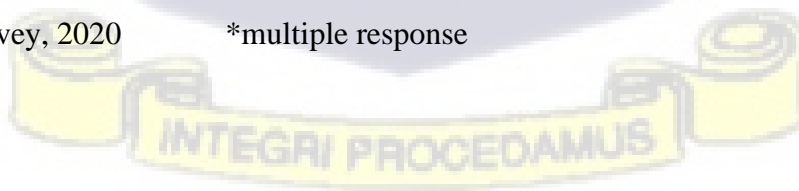


Table 7.9 ANOVA for TLB disease incidence for all accession at week 1

Source	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	1586.7	176.30	3.78	0.006
Error	20	933.3	46.67		
Total	29	2520.0			

Table 8.0 ANOVA for TLB disease incidence for all accession at week 2

Source	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	2320	257.78	3.12	0.016
Error	20	1650	82.50		
Total	29	3970			

Table 8.1 ANOVA for TLB disease incidence for all accession at week 3

Source	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	338.9	37.66	0.72	0.682
Error	20	1040.3	52.01		
Total	29	1379.2			

Table 8.2 ANOVA for TLB disease incidence for all accession at week 4

<i>Source</i>	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	293.3	32.59	0.92	0.527
Error	20	707.0	35.35		
Total	29	1000.3			

Table 8.3 ANOVA for TLB disease incidence for all accession at week 5

<i>Source</i>	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	381.4	42.38	0.56	0.812
Error	20	1510.2	75.51		
Total	29	1891.6			

Table 8.4 ANOVA for TLB disease incidence for all accession at week 6

<i>Source</i>	DF	<i>Sum of square</i> SS	<i>Mean square</i> MS	F-Value	P-Value
Accession	9	158.2	17.57	0.20	0.991
Error	20	1741.5	87.07		
Total	29	1899.6			



Table 8.5 ANOVA for TLB disease incidence for all accession at week 7

<i>Source</i>	DF	<i>Sum square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	2941	326.8	0.89	0.551
Error	20	7351	367.6		
Total	29	10293			

Table 8.6 ANOVA for TLB disease incidence for all accession at week 8

<i>Source</i>	DF	<i>Sum square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	2825	313.9	0.88	0.556
Error	20	7106	355.3		
Total	29	9931			

Table 8.7 ANOVA for TLB disease severity for all accession at week 1

<i>Source</i>	DF	<i>Sum square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	7.375	0.8194	3.76	0.007
Error	20	4.362	0.2181		
Total	29	11.737			

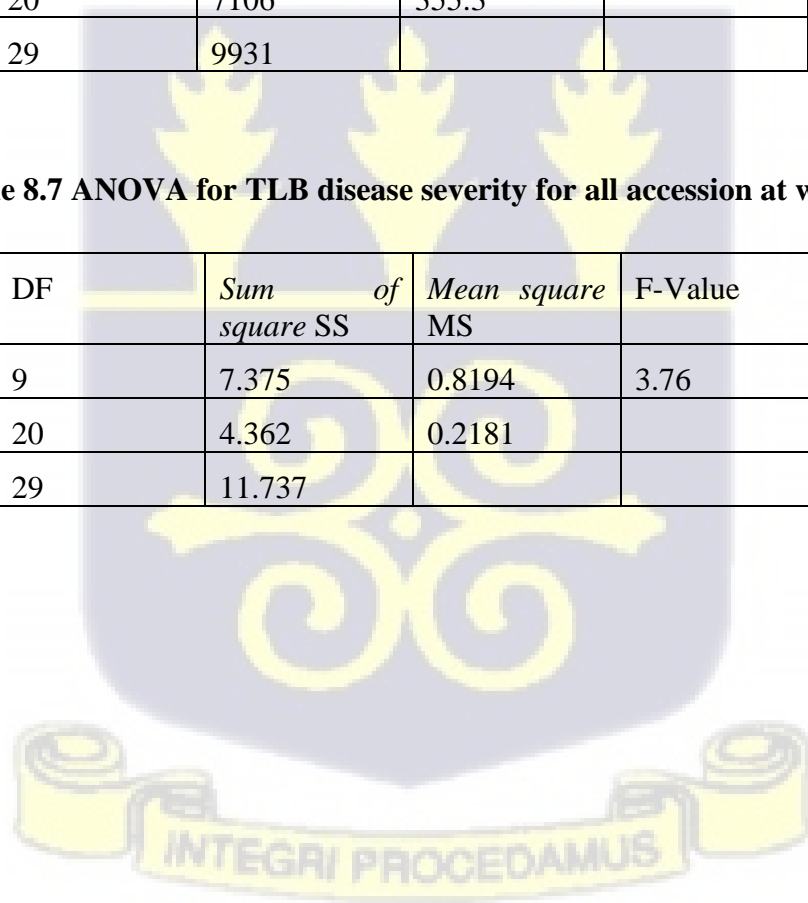


Table 8.8 ANOVA for TLB disease severity for all accession at week 2

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	10.211	1.1345	2.62	0.035
Error	20	8.661	0.4330		
Total	29	18.871			

Table 8.9 ANOVA for TLB disease severity for all accession at week 3

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	0.8989	0.09988	0.53	0.838
Error	20	3.7871	0.18935		
Total	29	4.6859			

Table 9.0 ANOVA for TLB disease severity for all accession at week 4

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	1.525	0.1695	1.19	0.354
Error	20	2.852	0.1426		
Total	29	4.377			

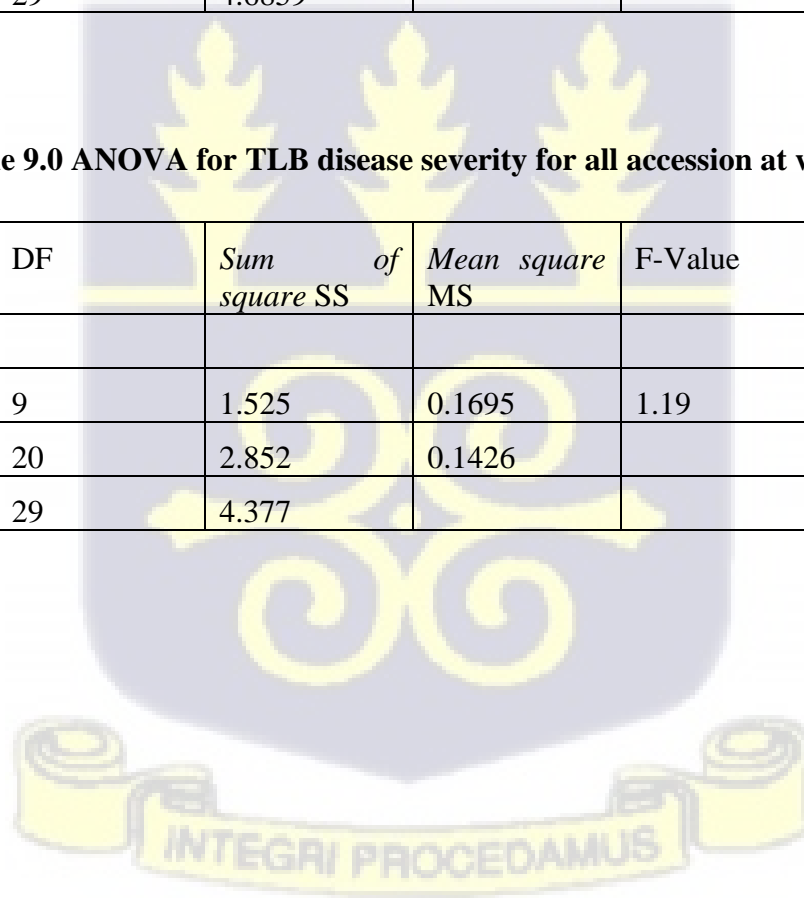


Table 9.0 ANOVA for TLB disease severity for all accession at week 4

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	0.6277	0.06974	0.80	0.622
Error	20	1.7457	0.08728		
Total	29	2.3733			

Table 9.2 ANOVA for TLB disease severity for all accession at week 6

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	0.2977	0.03307	0.62	0.766
Error	20	1.0663	0.05332		
Total	29	1.3640			

Table 9.3 ANOVA for TLB disease severity for all accession at week 7

<i>Source</i>	DF	<i>Sum of square SS</i>	<i>Mean square MS</i>	F-Value	P-Value
Accession	9	1.028	0.1143	0.59	0.791
Error	20	3.882	0.1941		
Total	29	4.910			



Table 9.4 ANOVA for TLB disease severity for all accession at week 8

Source	DF	Sum of square SS	Mean square MS	F-Value	P-Value
Accession	9	2.115	0.2350	2.03	0.090
Error	20	2.314	0.1157		
Total	29	4.429			

9.5 Preparation of water ager

500ml of sterile distilled H₂O

10g of agar powder (oxid)

Autoclave 121°C for 15minutes

9.6 Preparation of V₈ Agar (1L)

800ml of sterile distilled H₂O

200ml of V₈

20g of agar

2.5g of CaCO₃

Autoclave for 121°C for 15minutes

9.7 DNA extraction

1. 200µl of *phythophthora colocasiae* suspension
2. Addition of Genomic lysis buffer and vortex
3. Addition of DNA pre-wash buffer and centrifuge two times
4. Addition of g-DNA wash buffer and centrifuge
5. Addition of DNA elusion buffer and centrifuge
6. Store eluted DNA or use immediately

9.8 Gel electrophoresis

Agarose

1/100x100=0.5g (0.5g agarose dissolve in 1Xtae buffer and pour into 50ml tank)

1xTAE buffer

Ethidium bromide (Etbr)

Loading dye

DNA samples

Gel comb

9.9 TAE buffer

Tris base

Acetic acid

EDTA (Ethylenediaminetetraacetic acid)

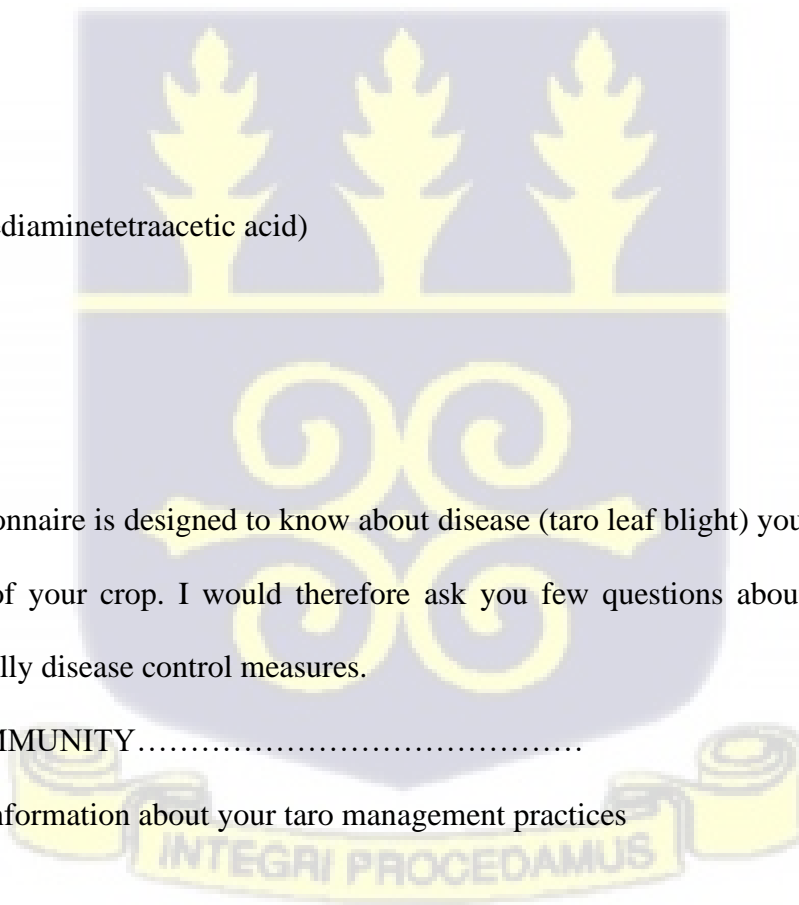
Deionize H₂O

PH 8.3

10.0 This questionnaire is designed to know about disease (taro leaf blight) you encounter during the production of your crop. I would therefore ask you few questions about your production practices especially disease control measures.

NAME OF COMMUNITY.....

Please provide information about your taro management practices



1. Gender

A) Male B) Female

2. How old are you?

A) 20-29 years B) 30-39 years C) 40-49 D) 50-above

3. Highest Education level

A) Non formal B) Primary C) JHS D) SHS E) Tertiary

4. How many years have you grown your taro crop?

A) < 1 year B) 1 – 5 years C) Above 5 years

5. What is the size of your land?

A) < 1 acre B) 1 – 2 acres C) Above 3 acres

6. Where did you obtain your corms?

A) Market B) Farmers C) Research institution D) MoFA E) N.G.O F) Other

7. Did you encounter any disease?

A) Yes B) No

8. How does it look like?

A) Drying of leaves B) Rotting of corms C) Reduced number of leaves.

9. Have you observed any of the following symptoms?

A) Early spots often occur on the leaf and accumulates. Yes/no

B) The spots enlarge rapidly becoming purplish brown to brown in color. Yes/no

C) The lower leaf surface is water-soaked or dry gray appearance present. Yes/no

D) The plant become bright orange or reddish brown. Yes/no

10. What do you think could be the cause of these abnormalities?

A) Weather B) Soil C) Not sure D) Others

11. What other limitation may cause the yield lost?

A) Lack of nutrients B) Lack of water. C) Lack of weeds control D) All the above.

12. Did you apply any fertilizer?

A) Yes B) No

13. If yes, at what rate did you apply it?

.....

14. Did you control the disease?

A) Yes B) No

15. How did you control the disease in your field?

A) Weedicides application B) Removal of infected plant C) Pesticide application D) Others

16. If no, why?

A) High cost of pesticide B) No effect after use C) Not sure

17. In which season is the disease severe?

A) Dry season B) Major raining season C) Minor raining season D) All the above.

18. How many acres do you harvest?

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19. What is the maximum weight of the corm you harvested?

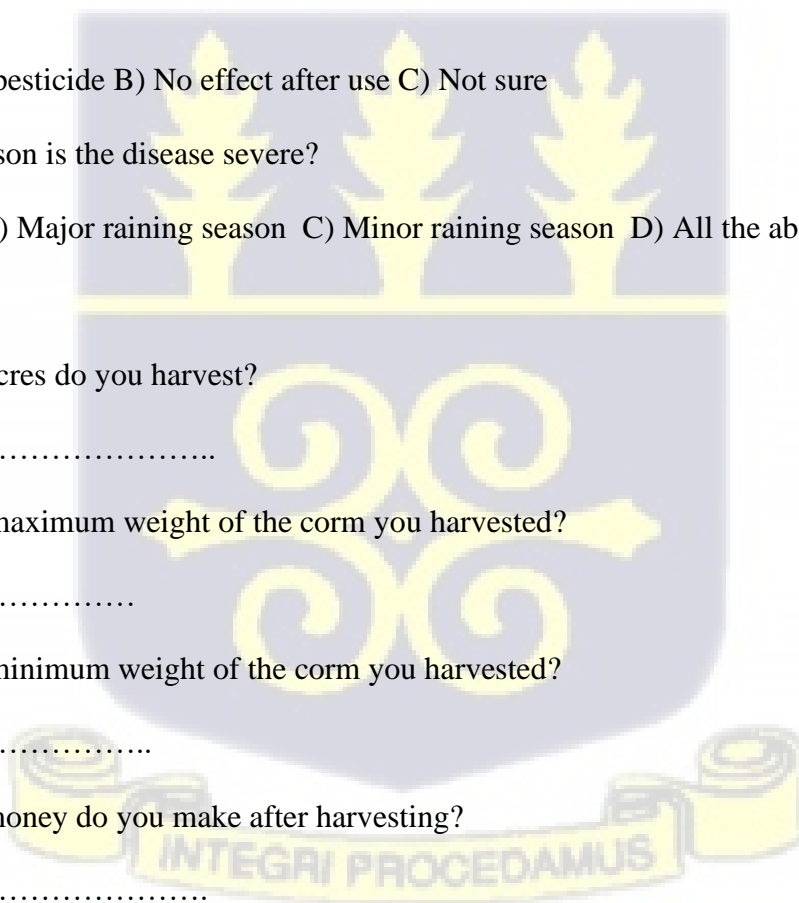
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20. What is the minimum weight of the corm you harvested?

.....

21. How much money do you make after harvesting?

.....



22. What losses did the disease cause?

A) Less than half the crop B) About half the crop C) The whole crop

23. Do you or have you ever sold taro before?

A) Yes B) No

How much is a tuber of taro

.....

24. How has the disease affected your livelihood?

.....

25. If yes, do you have a constant supply?

A) Yes B) No

26. If no, what could be the cause of the shortage?

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