KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF RESEARCH AND GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

IDENTIFICATION OF THE MAJOR FOLIAR FUNGAL DISEASE OF *COLOCASIA ESCULENTA* (L.) SCHOTT. AND ITS MANAGEMENT IN THE KUMASI METROPOLIS

BY

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AUGUST, 2010

IDENTIFICATION OF THE MAJOR FOLIAR FUNGAL DISEASE OF *COLOCASIA ESCULENTA* (L.) SCHOTT. AND ITS MANAGEMENT IN THE KUMASI METROPOLIS

THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE, MASTER OF SCIENCE IN PLANT PATHOLOGY

BY

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AUGUST 2010

DECLARATION

I declare that, except for references to other people's work which have been duly cited, this work is the result of my own original research and that this work has, neither in whole nor in any part, been presented for a degree elsewhere.

…… ……..

Dr. Charles Kwoseh Joshua Sarpong Asraku

(SUPERVISOR) (STUDENT)

DATE…… DATE……...

……

Prof. Richard Akromah

(HEAD OF DEPARTMENT)

DATE…….

DEDICATION

To God Almighty, my son, Oheneba Tutu Asraku who was born at the final stage of my studies. May his birth bring light into our life.

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ABSTRACT

Colocasia esculenta (L.) Schott. is a major delicacy in southern Ghana with high carbohydrate and protein contents. Recent decline in the production of *C. esculenta* in swampy fields has resulted from leaf blight disease, making the disease a threat to food security. A survey was conducted to assess the disease problem and also assess farmers' knowledge. The disease occurred on every *C. esculenta* field surveyed and disease incidence was between 80-90%. Five potted plants were inoculated with each fungal isolate obtained from farmers' fields and observed for symptom expression. Symptoms appeared as small dark-brown lesions which extended rapidly until death of leaves. The pathogenicity test showed that *Curvularia* sp. was responsible for the leaf blight disease. A study of disease progress on tagged leaves on swampy *C. esculenta* fields indicated that leaf blight appeared on leaves just as they unfurl and spread rapidly, debasing the leaf within 18 to 21 days. In a field trial, Topsin M 70 WP (Thiophanate methyl), Sundomil 72 WP (Metalaxyl 8% and Mancozeb 64%) both at 45 g/15l and water (control) sprayed at two-week intervals were evaluated against leaf blight disease incidence, severity, the disease progress and corm yield. The effect of the fungicides on leaf area, area of leaf infected by the disease, lesions per plant and number of leaves that died as a result of the disease, were also investigated. Fungicides-treated *Colocasia* plants showed significantly reduced disease incidence, disease severity and disease progress that resulted in increased corm yield. There were no differences between fungicides-treated *Colocasia* plants. There was a negative correlation between area of leaves infected, lesions per plant, number of leaves infected and the corm yield. The results from the field trial showed that leaf blight disease could be effectively managed with fungicides in swampy fields.

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CHAPTER ONE

1.0 INTRODUCTION

The economy of Ghana is growing with a corresponding increase in population. This has resulted in high demand for staple crops and vegetables, including *Colocasia esculenta* (L.) Schott. *Colocasia esculenta* is consumed as a staple crop in West Africa, particularly in Ghana, Nigeria and Cameroon. In 2005, Ghana produced 1.8 million metric tonnes as highest *Colocasia* producer next to Nigeria (FAO, 2005). The crop is grown by smallholder farmers where the main system of farming is traditional with the use of rudimentary tools.

Colocasia esculenta is next to yam in importance in oriental economies. It serves as both a vegetable crop and a root tuber. The corm is an important component of the diet with a very high starch content which is nutritious, containing dietary fibre and easily digested (Stephens, 1994). The corm is eaten fried, boiled, baked, or converted into breadstuffs. *Colocasia esculenta* has more carbohydrate and protein than potato, and has a pleasant nutty flavour (Stephens, 1994). The fried corm is a major delicacy in many areas in southern Ghana. *C. esculenta* leaves serve as a vegetable and is rich in vitamins and minerals (Coursey, 1968; Plucknett and de la Pena, 1971). They are also good sources of thiamine, riboflavin, niacin, iron, phosphorus, zinc, potassium, copper, and manganese (Onwueme, 1994).

Urbanization slashed down production of *C. esculenta* but recent decline in growth and development has resulted from pests and diseases (Hao, 2006). Diseases of *Colocasia* significantly reduce the number of functional leaves and have led to yield reduction of about 50% worldwide (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1999). *C. esculenta* is affected by a number of infectious diseases caused by fungi, bacteria, nematodes, and viruses as well as noninfectious or abiotic factors. According to Ooka (1994), among these diseases,

fungal diseases of *C. esculenta* are the most significant. Diseases caused by fungi are the most prominent, aided by climatic conditions which favour the growth of *C. esculenta.*

Leaf blight has been responsible for the serious decline in yields of *C. esculenta* in Hawaii (Santos, 1993; Raynor and Silbanus, 1993). *Colocasia* leaf blight has also contributed to the decline in *C. esculenta* production in Ghana (personal observation) but its causal agent has not been identified. In Solomon Islands, Papua New Guinea, Hawaii, Taiwan and American Samoa, the disease is known to be caused by *Phytophthora colocasiae* Rac (Ooka, 1994; Hao, 2006) and has severely constrained *C. esculenta* production in these countries. The disease is capable of compelling farmers to abandon their crop fields and rely on other staple crops (Jackson, 1996). The pathogen can cause over 95% reduction in the supply of *C. esculenta* to the public market (Gurr, 1996).

Leaf blight diseases pose a serious threat to food security and national economies worldwide. Disease levels in recent years have caused tremendous decline in corm yields of *C. esculenta* and there is a corresponding loss of revenue to *C. esculenta* farmers. In Ghana, the rapid decline in *Colocasia* production is threatening the survival and existence of the crop and extinction is eminent. The nation's poverty reduction plan through sustainable agriculture is therefore undermined. This has called for more effective control methods to manage the disease. There are no packages for control options such as cultural and biological control. In addition, *Colocasia* rarely flowers and sets seeds. Therefore, resistant cultivars are not available. The use of chemicals to combat diseases and pests is the fastest method of control and can be used to control fungal pathogens effectively in wetlands. Therefore, it became necessary to research into more effective chemical control strategies with minimal risk to humans and the environment, for the survival and sustainable production of *C. esculenta*.

In Ghana, there is scanty documentary evidence on diseases of *C. esculenta* and how they are managed (Theberge, 1985).

The objectives of the present study were to:

1. identify the major foliar fungal pathogen(s) of *C. esculenta* leaf blight and
2. develop effective management strategy for the leaf blight disease.

CHAPTER TWO 2.0 LITERATURE REVIEW 2.1 DESCRIPTION OF *COLOCASIA*

Colocasia is a genus of six to eight species of flowering plants in the family Araceae, native to tropical Polynesia and southeastern Asia (Wagner *et al*., 1999). There are more than 200 cultivars which fall under two main groups namely, wetland and upland *Colocasia.* However, *C. esculenta* is a mainly wetland herbaceous perennial plant with heart-shaped leaves. The petioles, emanating from corm, are thick, succulent and often purplish and attaches near the centre of the leaf. The mature corms usually weigh 0.5 - 6 kg. In its raw form, the plant is toxic due to the presence of calcium oxalate. The toxin is destroyed by cooking or can be removed by steeping the roots in cold water overnight.

(Onwueme, 1994; http://en.wikipedia.org/wiki/Colocasia. Last accessed September, 2009).

2.2 PROPAGATION AND GROWTH CONDITIONS

Colocasia esculenta is grown in all parts of the tropics and subtropical regions (Onwueme, 1977). It can also be grown in a well-drained soil if supplied with abundant water. They do best in moist or wet soil, rich in organic material or compost. The plant does best in slightly acidic soil of pH 5.5-6.5 (Onwueme, 1978). It is able to form beneficial associations with vesicular-arbuscular mycorrhizae, which therefore facilitate nutrient absorption (Kay, 1973).

It requires a planting distance of 60 by 90 cm or 90 by 90 cm.

C. *esculenta* does best in partial shade, but tolerates full sun if it gets plenty of water. Growth is best at temperatures between 20°C and 30°C (Onwueme, 1978). One particular useful characteristic of *C. esculenta* is that some cultivars are able to tolerate salinity and in Japan and Egypt, *C. esculenta* has been used satisfactorily as a first crop in the reclamation of saline soils (Kay, 1973).

Flowering and seed set in *C. esculenta* are relatively rare under natural conditions (Wilson, 1979). Most plants complete their field life without flowering at all, and some cultivars have never been known to flower. For many years, this characteristic was a great hindrance to *C. esculenta* improvement through cross pollination. However, the problem was solved when it was discovered that gibberellic acid (GA) could promote flowering in *C. esculenta* (Wilson, 1979). *C. esculenta* has long been propagated vegetatively.

2.3 IMPORTANCE OF *COLOCASIA*

Colocasia esculenta is a major food staple and it remains an important crop to many cultural and agricultural traditions worldwide (Ooka and Brennan, 2000). It serves both as a vegetable crop and as a root tuber. The entire plant can be eaten. The corm is eaten fried, boiled, baked, or converted into breadstuffs. Nutritionally, *C. esculenta* corms contain 63-85% water, 1.33.0% protein, 0.2-0.4% fat and appreciable quantities of Vitamins B and C. The leaf contains 87.2% water, 3.0% protein, 0.8% Fat and 6.0% carbohydrates (Coursey, 1968; Onwueme, 1994). The protein is richer in total sulphur-bearing amino acid than that of other root tubers (Parkinson, 1984). Typical of leaf vegetables, *C. esculenta* leaves are rich in vitamins and minerals. They are good sources of thiamin, riboflavin, iron, phosphorus, and zinc (Coursey, 1968; Onwueme, 1994) as well as vitamin B6, vitamin C, niacin, potassium, copper, and manganese (Plucknett and de la Pena, 1971). The juice extracted from the petioles is rubefacient, stimulant, and styptic, and is elsewhere used in treatment of earache (Stephens, 1994). Juice from the corms is used externally for treatment of baldness and internally as a laxative and an antidote to wasp stings (Stephens, 1994). *Colocasia* species are used as food plants by the larvae of some Lepidoptera species including *Palpifer murinus* (Hampson) and *Palpifer sexnotatus* Moore (Stephens, 1994).

2.4 DISEASES AND PESTS OF *C. ESCULENTA*

C. esculenta is affected by a number of infectious diseases caused by fungi, bacteria, nematodes, and viruses and noninfectious or abiotic problems caused by poor soil nutrients. Although, *C. esculenta* is susceptible to at least 23 pathogens, only a few cause serious reduction in growth and production (Ooka, 1990). Ooka (1994) reported that among these disease classes, fungal diseases are the most important.

Plant rot disease, reported to be caused by a species of the genus *Phytophthora*, now plagues crops and subsequently kill the entire plant in cases of severe fungal infection throughout the United States and in West Africa (Hao, 2006). Leaf spot disease caused by *Cladosporium colocasiae Sawada* has been reported in Ghana (Awuah, 1995). Pathogens that plaque *C. esculenta* include *Pythium* species, *Phytophthora colocasiae* (Racc.), *Cladosporium colocasiae* (Sawada), *Sclerotium rolfsii* Sacc., *Curvularia* species, *Rhizopus stolonifer* (Ehrenb.), *Fusarium solani* (Mart.) Sacc., *Colletotrichum gloeosporioides* (Penz.) and *Corynespora cassiicola* (Berk and Curtis) Wei. which cause leaf spot and leaf blight diseases.
Others, while currently not economically significant, have the potential to become so (Ooka, 1994).

Ooka (1994) reported that major pests of *C. esculenta* are the Mole cricket (*Gryllotalpa africana* Palisot De Beauvois), Crayfish (*Procambraus clarkii* Girard), and the Apple snail (*Pomacea canaliculata* Lamarck). According to Jackson (1980), *Colocasia* beetle belonging to the genus *Papuana* (Coleoptera: Scarabaeidae) tunnel into the soil at the base of the corm, leaving large holes that degrade the eventual market quality and value of the corm. The wounds that they create while feeding promote the attack of rot-causing organisms.

Sato and Hara (1997) reported that *Colocasia* root aphid, *Patchiella reaumuri* (Kaltenbach) is a tiny sucking insect found primarily on roots and corm. When populations are high, it can also be found on the aboveground portions of the plant around the base of leaf sheaths and on young leaves. Plants infested with root aphid appear stunted, the leaves may be yellow and the roots and corm may rot. When *Colocasia* seedlings are planted with infested *Colocasia* root aphid, it will never provide adequate yield (Sato and Hara, 1997).

A slightly less affliction of C. *esculenta* is the alomae/bobone virus disease complex and the dasheen mosaic virus disease occurring world-wide (Rodoni, 1995). The alomae virus disease is caused by a complex of two or more viruses acting together. The two viruses that are definitely involved are the taro large bacilliform virus (TLBV) which is transmitted by the plant hopper, *Tarophagus proserpina* (Kirkaldy), and the taro small bacilliform virus (TSBV) which is transmitted by the mealybug, *Planococus citri* Rossi. (Rodoni, 1995)

2.5 *CURVULARIA* SPECIES AND THEIR SIGNIFICANCE

Most species of *Curvularia* are ubiquitous facultative pathogens of plants and of the soil in tropical and subtropical areas, while the remaining few are found in temperate zones (Smith *et al*., 1989). *Curvularia* produces rapidly growing, woolly colonies on potato dextrose agar at 25°C. From the front, the colour of the colony is white to pinkish grey initially and turns olive brown or black as the colony matures. From the reverse, it is dark brown to black (de Hoog *et al*., 2000; Sutton *et al*., 1998).

The septate hyphae, conidiophores, and conidia of *Curvularia* are visible under light microscope. Conidiophores are simple or branched and are bent at the points where the conidia originate. The conidia (8-14 x 21-35 µm) are straight or pyriform, brown, multiseptate, and

have dark basal protuberant hila. The central cell is typically darker and enlarged, compared to the end cells in the conidium (de Hoog *et al*., 2000; Sutton *et al*., 1998; St-Germain and Summerbell, 1996).

The number of the septa in the conidia, the shape of the conidia (straight or curved), the colour of the conidia (dark or pale brown), existence of dark median septum, and the prominence of geniculate growth pattern are the major microscopic features that help in differentiation of *Curvularia* spp. (Larone,1995).

Smith *et al*. (1989) describes damping-off of seedlings crown, root lesioning, and blighting as potential symptoms of *Curvularia* infections in plants. *Curvularia* species affect many species of grasses worldwide (Smith *et al*., 1989; Weng *et al*., 1997). This fungus has been reported on grasses such as *Oryza, Paspalum, Pennisetum, Sorghum, Triticum* and *Zea* causing severe blight (Srivanesan, 1987).

2.6 BLIGHT DISEASE AND CAUSAL AGENT(S)

Cladosporium colocasiae causes leaf spot and leaf blight (Awuah, 1995). *Phytophthora colocasiae* causes blight disease and the pathogen is responsible for *C. esculenta* blight in Papua New Guinea, American Samoa and some parts of Asia (Trujillo, 1967). Blight is a symptom of *Curvularia* species (Smith *et al*., 1989) and is known to have caused blight disease in White clover and zoysiagrass, a common turfgrass used in home lawns and golf courses.

2.7 IMPACT OF BLIGHT DISEASE ON WORLD'S PRODUCTION OF *COLOCASIA*

Colocasia leaf blight has been recorded in a number of countries in the Pacific region. The disease significantly reduces the number of functional leaves and can lead to yield reductions of the magnitude of 50% (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1999). In Ghana,

the disease has been in existence for about two to three years and has brought significant changes to cropping patterns (personal observations).

Wherever the disease occurs, growers are forced to abandon *Colocasia* and rely on other root crops (Jackson, 1996). In Hawaii, prior to the arrival of *Colocasia* leaf blight, there were approximately 350 different varieties of *Colocasia* in the country. Today, there are less than 40 different varieties of Hawaiian *Colocasia* (Trujillo, 1996).

Colocasia leaf blight has contributed to significant changes in dietary patterns and cropping systems in Micronesia where cassava has become the main staple instead of *Colocasia* (Barrau, 1961; Jackson, 1996). In Pohnpei, *Colocasia* now ranks behind yams, banana, imported rice and breadfruit as a staple crop (Primo, 1993; Raynor and Silbanus, 1993).The majority of the *Colocasia* varieties that existed are no more (Trujillo, 1996) and leaf blight has been responsible for the serious decline in *Colocasia* as a crop plant (Santos, 1993; Raynor and Silbanus, 1993).

It has been reported that the epidemic of *Colocasia* leaf blight in Bougainville, Papua New Guinea resulted in the death of about 3000 people (Putter, 1993). The disease has severely constrained *Colocasia* production in American Samoa (Gurr, 1996). Within a year of disease incidence, it had caused over 95% reduction in the supply of *Colocasia* to the public market. Prior to the blight disease outbreak, *Colocasia* was the major export earner in American Samoa and over 90% of households were growing the crop (Gurr, 1996). Paulson and Rogers (1997) reported that only 1% of the total supplies of *Colocasia* in June, 1993 were available to the local market for sale in June, 1994.

Blight diseases pose a serious threat to food security and national economies worldwide. Major examples are the southern Corn leaf blight caused by *Exserohilum turcicum* formally known

as *Helminthosporium maydis* (Nisikado and Miyake) and *Colocasia* leaf blight, caused by *Phytophthora colocasiae* (Trujillo, 1967; Jackson, 1999).

2.8 SOURCES OF INOCULUM OF *CURVULARIA* BLIGHT AND ENVIRONMENTAL FACTORS INFLUENCING BLIGHT DISEASE

Inoculum in the form of spores is spread by wind-driven rain and dew to adjacent plants and nearby *Colocasia* plantations (Jackson, 1999). The disease can also be spread by planting materials and the fungus has been reported as remaining active on planting material for about three weeks after harvest (Jackson, 1999). Mostly, *Colocasia* planting material for the next crop comes from the crop being harvested (Ooka and Brennan, 2000). The use of planting material from infected corms increases leaf blight disease incidence in subsequent *Colocasia* crops (Ooka, 1994).

Density of plants, temperature and humidity appear to be the most important factors influencing infection and spread of blight disease (Ivancic *et al.*, 1996). The number of plants grown in a given space affects *Colocasia* disease prevalence and yield (Ooka and Brennan, 2000). High plant density may make it easier for insect pests to move among plants and if sunlight and air circulation are too restricted, blight disease can occur more readily (Ooka and Brennan, 2000). Plants growing in extremely hot and humid environments show higher susceptibility to blight disease than those growing under normal conditions (Ivancic *et al*., 1996). Absence of certain important soil nutrients such as calcium and phosphorus can also exacerbate the disease (Tilialo *et al.*, 1996).

2.9 SIGNS AND SYMPTOMS OF *CURVULARIA* LEAF BLIGHT

The disease is mainly a foliar disease. Initial symptoms of the disease are small brown watersoaked flecks on the leaf that enlarge to form dark brown lesions, often with a yellow margin. Secondary infections lead to rapid destruction of the leaf, which may occur in 10–20 days or less in very susceptible varieties (Hunter *et al*., 2001). The normal longevity of a healthy leaf is about 40 days (Ooka and Brennan, 2000). The disease significantly reduces the number of functional leaves and can lead to yield losses (Trujillo and Aragaki, 1964; Trujillo, 1967; Jackson, 1999).

2.10 MANAGEMENT OF LEAF BLIGHT DISEASE OF *COLOCASIA ESCULENTA*

Blight disease of *C. esculenta* not managed early led to yield reduction of more than 50% (Jackson, 1999). Unmanaged blight disease also caused changes in cropping patterns of *C. esculenta* (Barrau, 1961; Jackson, 1996) and consequently, the existence of *C. esculenta* was jeopardized. The survival of the crop and genetic data base became threatened and extinction was therefore eminent and inevitable. Various management strategies have been used to control *Colocasia* leaf blight.

2.10.1 CULTURAL CONTROL

Various cultural methods have been recommended for the control of *Colocasia* leaf blight. Removal of infected leaves has been effective during the early stages of disease development in a number of countries (Hunter *et al*., 2001). According to Jackson *et al*. (1980), regular roguing of diseased leaves in plots affected by a severe blight did not eradicate the pathogen. Disease increased rapidly after roguing ceased and corm yields were greatly decreased. Roguing of infected leaves does not eradicate the pathogen but may delay the start of epiphytotics (Ashok and Mehrotra, 1987). Wide spacing of plants has been reported to reduce

disease severity but this appears to have a negligible effect when conditions favour disease development (Hunter *et al*., 2001). Attempts to decrease the effect of *Phytophthora colocasiae* by wider spacing than the traditional spacing (76 X 76 cm) were unsuccessful (Jackson *et al*., 1980).

Other cultural methods that have been recommended include delay planting on the same land for a minimum of three weeks, avoiding plantings close to older infected ones and preventing the carry-over of corms or suckers which can harbour the pathogen from one crop to the other (Jackson, 1999).

Amosa and Wati (1997) reported that disease incidence and severity of *colocasia* leaf blight was lower in *Colocasia*/Maize intercropping system than those grown in monoculture. The effect of planting density and relative time of planting on *Colocasia*/Rice intercropping system yielded similar results (Agyekum, 2004). A trial to investigate the effect of planting time, intercropping, the role of fertilisation on the incidence and severity of the disease and the effect of leaf removal was inconclusive (Chan, 1997). Fertilizer treatment may help the plant cope with leaf blight (Tilialo *et al.,* 1996).

2.10.2 CHEMICAL CONTROL

The use of fungicides such as Copper and Copper metalaxyl-based compounds is the most reliable and popular with farmers because of the quick and effective action (Adejumo, 1997). Jackson (1996) reported that blight disease can be controlled by spraying with Copper fungicides. Ashok and Mehrotra (1987) observed in field trials that excellent control of *Colocasia* leaf blight was obtained when plants were treated with Chloroneb and Captafol,

good control with Metalaxyl, fair control with Copper oxychloride and poor control with Thiophanate-methyl and Zineb.

Field experiments conducted to study the effect of fungicides in controlling leaf blight caused by *P. colocasiae* in *C. esculenta* revealed that 0.2% Metalaxyl and Mancozeb as Ridomil MZ-72 was the most effective treatment, followed by 0.2% Captafol, Bordeaux mixture (1% Copper sulphate and lime) and 0.25% Mancozeb (Ashok and Saikia, 1996). A significant increase in yield was recorded for all treatments over the untreated control.

The frequency and time of spray application have been reported to affect the effectiveness of fungicides (Adegbola, 1993). Bergquist (1974) confirmed the effect of fungicide rate, spray interval, timing of spray application and precipitation in relation to control of leaf blight disease of *C. esculenta*. In an experiment conducted by Bergquist (1974), *C. esculenta* was sprayed with Mancozeb at rates of 4.48, 2.24 or 1.12 kg/ha at intervals of 5, 7, 10 or 14 days at drier and wetter sites. Rate of fungicide had no effect in the drier sites, while at wetter sites, the highest rate of 4.48 kg/ha was the most effective. Spraying every 5 days was significantly more effective than spraying every 14 days. Applications of fungicide at 7-day intervals gave substantial disease control.

2.10.3 USE OF RESISTANT VARIETIES

Relatively, there are very few varieties of *C. esculenta* and this is believed to be as a result of diseases (Wall and Wiecko, 1998). Most farmers who traditionally grow *C. esculenta* cannot afford the extra costs required for fungicides and labour involved in leaf removal and spraying (Hunter *et al*., 2001). Host resistance is probably the most valuable control in agriculture (Erwin

and Ribiero, 1996). Resistant varieties are not only environmentally friendly but also require little additional disease control inputs from farmers.

In Samoa, four *C. esculenta* cultivars screened and evaluated for their resistance to *Colocasia* leaf blight, for their yield and eating quality performed well and gave positive results (Iosefa and Rogers, 1999; Hunter and Pouono, 1998).

2.10.4 BIOLOGICAL CONTROL

Several potential biocontrol agents have been reported on various plants. These include *Aspergillus niger* (Van Tieghan), *Penicillium* spp. and *Trichoderma viride* (Peri) (Odamtten, 1977; Figuerdo and Medeiros, 1977; Frais and Garcia, 1981). *Bacillus* spp. (Odigie and Ikotun, 1982) and *Anoplolepis longipes* (Jerdon) (McGregor and Moxon, 1985).

Effect of soil application, seed treatment, and foliar spray of rhizobacterial cultures that were isolated from *C. esculenta* on *Phytophthora* blight reduced the disease incidence and severity and increased the yield, compared to untreated pathogen-inoculated control plants (Sriram *et al.*, 2003).

Biological control agents may be used judiciously as a complement to chemical application and cultural practices. In such a situation, compatibility with the synthetic fungicide would be desirable, as it is often possible to schedule both in control programmes (Coffey, 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODS

A survey and two experiments, namely pot and field experiments, were conducted.

3.1 FIELD SURVEY: Assessment of *Colocasia* leaf blight incidence, severity and farmers' knowledge of the disease in major *Colocasia* growing areas in Kumasi

Questionnaires (Appendix 3) were administered through interviews to 50 *C. esculenta* farmers at random in the major growing areas in Kumasi Metropolis to assess the perception of the farmers on the disease, disease occurrence, cropping system, disease severity and disease management options. Both male and female farmers were interviewed.

Personal observations were made on the symptom types on plants in farmers' fields, swamps along streams and backyard drainage channels where *C. esculenta* are grown in the Kumasi Metropolis. Diseased leaf samples were collected from farmers' fields for pathological analysis.

3.2 Isolation and identification of fungi associated with *Colocasia* leaf blight

All laboratory work was conducted at the Plant Pathology Laboratory, Department of Crop and Soil Sciences, KNUST, Kumasi.

3.2.1 Preparation of culture media.

Three different culture media, namely; Potato Dextrose Agar (PDA), Oat Meal Agar (OMA) and Water Agar (WA) were used to isolate fungi.

Potato Dextrose Agar was prepared by weighing 200 g of clean peeled potatoes using an electronic balance. The peeled and chopped potatoes were boiled with 500 ml of water in a

pyrex beaker for 30 minutes. With the aid of a funnel and cheese cloth, the potato suspension was sieved into beaker and 20 g each of agar and glucose were added. The mixture was then amended with 400 mg of Chloramphenicol and topped with water to 1litre. The resultant suspension was stirred thoroughly, transferred into a conical flask, stoppered with non-absorbent cotton wool and autoclaved at 15 psi, 121^0C for 20 minutes.

Oatmeal agar was prepared by weighing 72.5 g of Oatmeal powder in 1litre of distilled water and heated to boiling until the medium was completely dissolved. Fifteen grams (15g) of agar was added and the resultant suspension autoclaved at 15 psi, 121^0C and maintained for 20 minutes (http://www.sigmaaldrich.com/life science/tissue culture protocols. Last accessed December, 2009).

Water agar was prepared by putting 15g of agar in 1 litre of distilled water. The resultant suspension was stirred thoroughly, transferred into a conical flask, which was then stoppered with non- absorbent cotton wool and autoclaved 15 psi, 121^0C for 20 minutes.

3.2.2 Isolation of fungi from diseased leaf samples

Fungi isolates were obtained from different *C. esculenta* fields in major growing areas in the Kumasi Metropolis. Pieces of diseased leaves and petioles were cut with a scalpel blade and cultured on different culture media in 9.0 cm Petri dishes. The cultures were maintained at room temperature and subcultured till pure cultures were obtained.

3.2.3 Identification and scoring of fungi for frequency of occurrence

Identification of fungal isolates were done with the aid of a light Microscope and Standard identification manuals (Barnett and Hunter, 1972; Watanabe, 2000). Fungal isolates were

scored for frequency of occurrence in percentage (%). The suspected etiologic agent of *C. esculenta* leaf blight was described.

3.2.4 Sterilization of soil

Black soil was sifted to remove stones, plastic materials and plant debris. The soil was steam sterilized in a barrel at 100^0C for two hours. The sterilized soil was left in the barrel overnight to cool before used.

3.3 EXPERIMENT 1: Proof of pathogenicity of fungi isolates associated with *Colocasia* leaf blight on potted plants in the plant house

The experiment was conducted at the Department of Crop and Soil Sciences, KNUST, Kumasi.

3.3.1 Raising and potting of *Colocasia* seedlings

Minisett technique was used to raise uniform *Colocasia* seedlings. *Colocasia* corms were cut to 20 g pieces and each planted in steam sterilized moist saw dust. Sterilized black soil was dispensed into 1.5 litre size plastic pots. Two-week-old seedlings were potted in the sterilized black soil with one seedling per pot. Four-week-old established potted seedlings were then inoculated with pure cultures of the fungi isolates separately.

3.3.2 Preparation of inoculum of fungi isolates for pathogenicity test

For each fungal isolate, 10-day-old culture on PDA was added to 50 ml distilled water and agitated using Waring blender at low speed for two minutes to release spores. The suspension was filtered using cheese cloth to remove PDA. Spores were counted using Haemocytometer and 472 spores/ml of suspension was obtained. Each potted plant was inoculated by atomising 10 ml of the suspension until run off with each fungal isolate on the potted *Colocasia* leaves

using a 100 ml gun sprayer. Inoculated plants were enclosed in polyethene bags and maintained in the plant house. The polyethene bags were then removed and the plants rated for disease after five days. Five uninoculated plants served as the control.

3.4 EXPERIMENT 2: Confirmation of pathogenicity of *Curvularia* species on potted *Colocasia* plantlets in a moist chamber

Four-week-old established potted *Colocasia* seedlings were inoculated with spore suspension of pure cultures of *Curvularia* species. The inoculated plants were not covered with polyethene bags but a beaker of water was placed in the chamber to create humified environment. Inoculum and number of spores were obtained and applied as above.

The proof of pathogenicity test based on Koch's postulates was used to confirm the causal organism of the disease. Koch's postulates are:

1. Constant association between diseased plants and the suspected agent
2. The microorganism must be isolated from a diseased host and grown in pure culture.
3. The cultured microorganism should cause similar disease when introduced into a healthy host.
4. The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

3.5 EXPERIMENT 3: Field assessment of *Colocasia* leaf blight and its management

3.5.1 EXPERIMENTAL SITE

The experiment was carried out on a farmer's field at Gyinyase, a suburb of Kumasi in the Ashanti Region of Ghana from May to December, 2009. The area was marshy with sandy loam soil. The field used had been successively cropped more than thrice to *C. esculenta* and has been a hotspot for *C. esculenta* leaf blight.

3.5.2 LAND PREPARATION AND PLANTING OF *COLOCASIA* SEEDLINGS

An experimental area (hotspot for leaf blight disease) measuring 608 m^2 was cleared using machete, allowed to dry and then burnt. Herbicide (Sunphosate) was applied three weeks after burning. The area was demarcated into three blocks each with five plots. The area of each plot was 6 x 4.5 m^2. Blocks and plots were separated from each other by a distance of 1 m.

Disease-free young seedlings of *C. esculenta* weighing about 20-30 g obtained from a farmer's field were used for planting. The plants were cut to a height of 20 cm for uniformity and were planted in rows at a spacing of 60 cm x 90 cm. Weeds were controlled as and when necessary by hoeing. Plants were planted on the flat.

3.5.3 EXPERIMENTAL DESIGN AND TREATMENTS

The experimental design used was randomised complete block design (RCBD) with five replications. Three treatments, namely; spraying of Topsin M 70 WP (Thiophanate methyl) and Sundomil 72 WP (Metalaxyl 8% and Mancozeb 64%) at the manufacturers' recommended rates. Water was used as the control. Natural infestation was the main source of inoculum.

3.5.4 DATA COLLECTED

Ten established plants (3-5 leaf stage) per plot were selected across the diagonals of the plot and were monitored every seven days for seven weeks for disease incidence (I) and disease severity (S). For the treated plants, fungicides were applied at a rate of 3 g/litre and plants were sprayed till run off. Spraying was done once every two weeks. The control plants were also sprayed with water till run off. Fungicide-treated and water-treated leaves were scored for disease based on the area of leaves infected by the disease and number of leaves per plant infected by the disease. Lesions per plant, leaf area and number of leaves dead due to disease were also ascertained. Disease incidence and severity were computed as follows:

Disease incidence (I)

$$I = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100\ \%$$

Disease severity (S)

$$S = \frac{\text{Area of leaves affected}}{\text{Total area of leaves}} \times 100\ \%$$

Colocasia leaf blight progress was also monitored on individual selected *Colocasia* leaves. Five newly unfurled leaves from selected untreated plants (control) were tagged and monitored every three days for four weeks for symptom initiation and subsequent progression of symptoms, using the syndrome scale (Horsfall and Cowling, 1978) below;

0 No disease

1 Presence of lesions less than 10 cm^2 of leaf area

2 Presence of lesions 11 - 30cm^2 of leaf area

3 Presence of lesions 31 - 60cm^2 of leaf area

4 Presence of lesions 61 - 90cm^2 of leaf area

5 Presence of lesions more than 90 cm^2 up to 25% of leaf area.

6 Coalesce of spot more than 25% of the leaf covered

7 Coalesce of spot more than 50% of the leaf covered

8 Coalesce of spot more than 75% of the leaf covered

9 Collapse of petiole accompanied by complete leaf blight

Areas of leaves were measured by using non-destructive methods, using the formula W_P x L_{PA} (Chan *et al*., 1993; Lu *et al*., 2002) where;

W_P = leaf width passing the petiole-attaching point

L_{PA} = length from the petiole-attaching point to the apex of leaf (Appendix 4).

This method allows successive measurement of the leaf area in all developmental stages and the plant canopy is not damaged. Measurements are done directly by passing through one well defined point. Area of leaves (Ala) infected by the disease were assessed using the maximum length and breath of the affected leaf area.

3.5.5 ASSESSMENT OF *COLOCASIA* YIELD AFTER FIELD TREATMENTS

C. esculenta yield was assessed eight months after planting to compare mean yields of fungicide-treated and water-treated plants. Pearson correlation matrix was run between yields, number of leaves affected, number of leaves dead due to disease, lesions per plant, leaf area and area of leaves affected.

3.6 DATA ANALYSIS

Data collected were analysed using Analysis of variance (ANOVA). All count data were square root transformed ($\sqrt{x} + 0.5$) where, x is the mean values and LSD at 5% was used to compare

mean differences. All statistics were performed by using Genstat statistical package (3rd edition, 2008).

CHAPTER FOUR

4.0 RESULTS

4.1 FIELD SURVEY: Assessment of *Colocasia* leaf blight incidence, severity and farmers' knowledge of the disease in major *Colocasia* growing areas in Kumasi

Of the 50 farmers interviewed in the Kumasi Metropolis, 64% of them were males and 36% were female with ages between 40 and 80 years.

About 80% of the farmers practiced monoculture with *C. esculenta* and 20% practiced mixed cropping, cultivating *C. esculenta* as the main crop intercropped with sugar cane or maize in the dry season (Table 1). The disease was more pronounced in monoculture than in mixed cropping, according to the respondents. All farmers cultivate *C. esculenta* annually with 60% of them on commercial bases and 40% for subsistence (Table 2). All farmers' fields surveyed were swampy or marshy.

Table 1: Cropping system of *C. esculenta* farmers in fields visited during the survey

Cropping system	Percentage (%) number of farmers /cropping system
Monoculture	80
Mixed cropping	20
Total	100

Table 2: Farming system of *C. esculenta* farmers in 50 fields

Farming system	Percentage (%) number of farmers/ farming system

Subsistence farming	40
Commercial farming	60
Total	100

The major problem encountered by *C. esculenta* growers included urbanization, inadequate planting materials, weeds, excessive rain making farm lands inaccessible and quite recently diseases. About 90% of the farmers ranked disease as the major constraint halting the cultivation of *C. esculenta* (Table 3).

Table 3: Major problems encountered by *C. esculenta* farmers in the 50 fields surveyed

Problem encountered	Percentage (%) number of farmers responded/problem
Diseases	90
Urbanization	2
Planting materials	4
weeds	2
Excessive rainfall	2
Total	100

Plate1. Advancing *Colocasia* leaf blight symptom

The farmers observed that, the disease attacked both young and old plants. The disease was also observed in both wet and dry seasons. However, it was reported that the disease spread at a faster rate in the rainy season than in the dry season. About 70% of *C. esculenta* disease affected mainly the shoot that show symptoms as leaf burns, small brown lesions which extend until death of leaves (Plate 1) and 30 % affected the corms.

The blight disease was present in all swampy fields and infected *C. esculenta* at all developmental stages of the plant. According to the farmers, the diseased plants had averagely smaller photosynthetic leaf area, resulting in reduced corm yield. In addition, the diseased leaves were also rendered useless as a vegetable.

Of the farmers interviewed, 20% believed that the disease was caused by chemical (weedicides and other pesticides) drift from near-by vegetable growers. Farmers were, however, able to identify the blight disease but 80% did not have any idea about the cause of the disease. All the *C. esculenta* farmers spoken to did not manage the disease in any way. It was observed that the

disease occurred in every field surveyed and 90% of the plants were infected with the leaf blight disease. Disease symptoms were conspicuous on both upper and lower epidermis of the leaves.

Clasdiosporium colocasiae leaf spot (Plate 2) disease was moderate on the semi-upland planting and moderate to severe on all upland plantings. The disease was, however, absent on swampy fields.

Plate 2. Advancing *Colocasia* leaf spot symptom

4.2 Isolation and identification of fungi associated with *Colocasia* leaf blight

Pure cultures of fungal isolates identified using standard reference manuals (Barnett and Hunter, 1973; Watanabe, 2000) from the different *C. esculenta* fields are presented in Table 4.

Table 4. Mean frequency of occurrence (%) of fungi isolates associated with *Colocasia* blight disease in farmer fields in the Kumasi Metropolis

Fungi isolates	Mean frequency of occurrence (%)
Penicillium sp.	23
Cladosporium colocasiae	7
Fusarium sp.	3
Aspergillus niger	30
Pythium sp.	2
Curvularia sp.	20
Rhizopus stolonifer	15

Of the fungal isolates identified, *Aspergillus niger* occurred most with an average of 30% and *Pythium* species. least occurred with an average of 2% (Table 4). Occurrence of *Penicillium* and *Curvularia* species were 23% and 20%, respectively.

4.3.1 Experiment 1: Proof of pathogenicity of fungal isolates associated with *Colocasia* leaf blight on potted plants in the plant house

Two out of five potted plants inoculated with *Aspergillus niger* produced leaf yellowing symptoms (Plate 3). All five potted plants inoculated with *Curvularia* sp. produced conspicuous brown lesions on both lower and upper epidermis which later spread to the entire

surface of the leaf (Plate 5 and 6). The infected leaves died 14 – 21 days after initial infection (Plate 7). Uninoculated control plants showed no symptoms (Plate 4).

Plate 3: Potted *Colocasia* plant showing leaf yellowing symptoms

Plate 4: Control

Plate 5: Potted *Colocasia* plant showing blight symptoms on lower epidermis of the leaf

Plate 6: Potted *Colocasia* plant showing blight symptoms on upper epidermis of the leaf

Plate 7: Potted *Colocasia* plant showing leaf blight symptoms

4.4.2 Experiment 2: Confirmation of pathogenicity of *Curvularia* species on potted *Colocasia* plantlets in a moist chamber

Colocasia plantlets produced symptoms seven days after innoculation with *Curvularia* sp. (Plate 8). The blight disease progressed and covered the total leaf area and subsequently death of leaf occurred. New unfurl leaves were also infected (Plate 9).

Pathogenicity test carried out indicate that symptoms produced by *Curvularia* sp. were similar to those observed on the farmer's field (swampy fields). Spores of *Curvularia* sp. were constantly associated with severe blighting of *C. esculenta. Penicilium* sp., *Fusarium* spp., *Pythium* sp., and *Rhizopus stolonifer* did not produce symptoms of leaf blight.

Plate 8: *Colocasia* plantlets inoculated with *Curvularia* sp. in a moist chamber showing leaf blight symptoms after seven days

Plate 9: Advancing leaf blight symptoms on *Colocasia* plantlets in a moist chamber after 14 days

Plate 10: Advancing leaf blight symptoms on *Colocasia* plantlets in a moist chamber 21 days after inoculation with *Curvularia* sp.

Plate 11: Death of *Colocasia* leaf in a moist chamber after 28 days after inoculation with *Curvularia* sp.

4.5 EXPERIMENT 3: Field assessment of *Colocasia* leaf blight and its management

4.5.1 *Colocasia* leaf blight incidence under field condition

Water treated *C. esculenta* plants (control) recorded disease incidence of 50% as against 48% and 40% in *C. esculenta* plants treated with Topsin M and Sundomil, respectively, in the first week. Disease incidence increased gradually to 86% with water-treated *C. esculenta* plants as against 64% in Topsin M-treated plants in the seventh week. However, Sundomil-treated plants recorded disease incidence of 36% in the seventh week (Fig. 1).

Fig. 1: *Colocasia* leaf blight incidence over a seven-week period under natural infection in the field following treatments

4.5.2 *Colocasia* leaf blight disease severity under field condition

Disease severity in water-treated plants (control) was 3.0% in the first week and increased to 14.3% in the seventh week (Fig. 2). For Topsin M-treated plants, disease severity was 2.7 % in the first week and decreased in the second and third weeks. It then increased in the fourth week from 2.1 % to 3.8 % in the seventh week (Fig. 2). Sundomil-treated plants had the least severity of 1.6% in the first week, increased slightly in the second week and decreased in the third week. Severity decreased from 1.3 % in the fourth week to 1.1 % in the seventh week (Fig. 2).

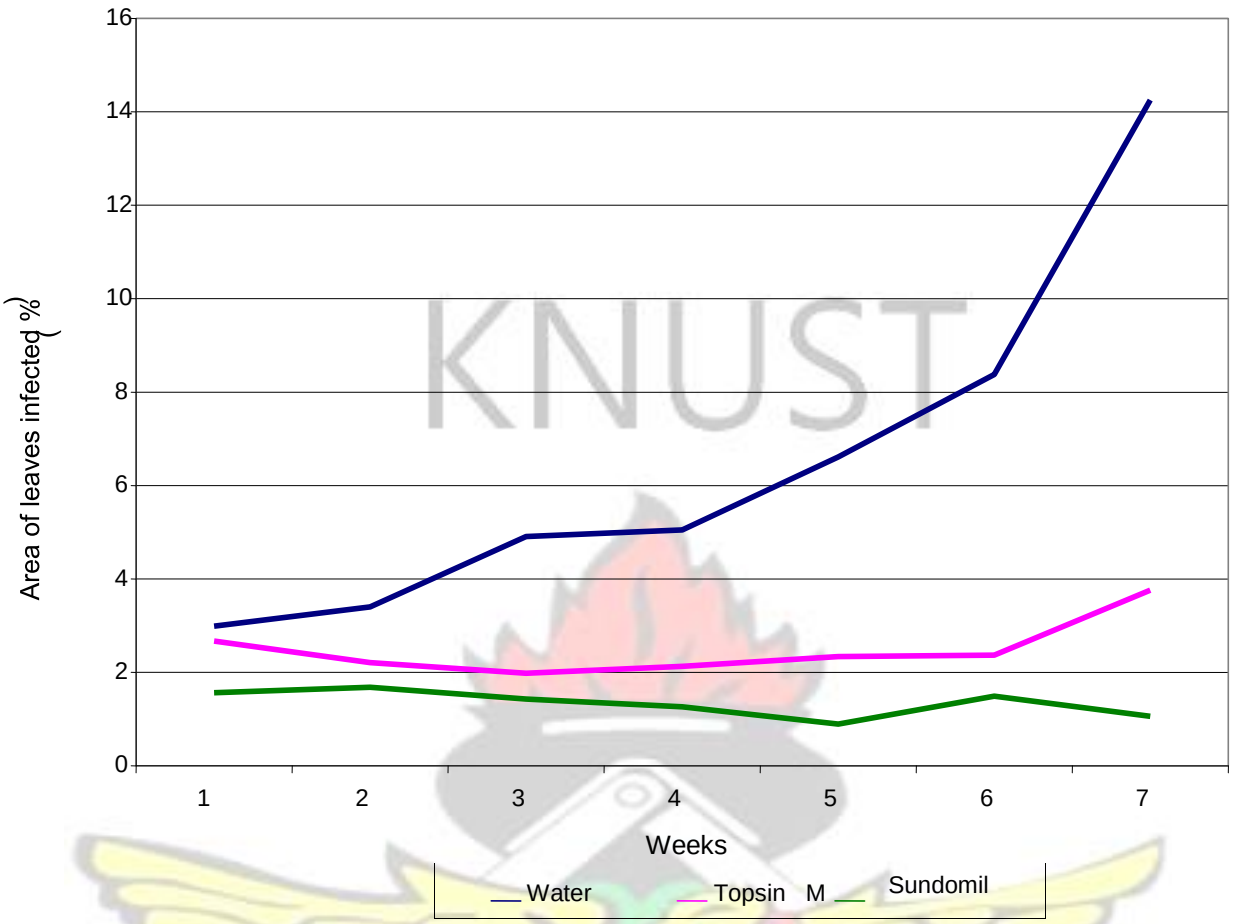

Fig. 2: *Colocasia* leaf blight severity over a seven-week period under natural infection in the field following treatments

4.5.3 *Colocasia* leaf blight disease progress under field condition on water treated plants

Generally, on the field-grown naturally-infected *Colocasia* plants, the disease appeared as tiny lesions. Conspicuous symptoms were observed three days after unfurling. Severity increased on an average of 34.3% in all five tagged plants as most of the lesions coalesced after nine days. Death of leaves occurred within 15 to 18 days (Table 5). Lesions closer to the petiole caused petiole dislodge and leaves died earlier (Table 6).

Table 5: Progress of field-infected *Colocasia* leaf blight disease on five tagged leaves over 21 days on water treated plants

	Leaf blight disease severity (%) on five tagged leaves					
Number of days of leaf blight disease appearance	1	2	3	4	5	Mean
3	0.2	0.0	0.5	0.7	1.0	0.5
6	1.7	12.0	21.5	24.0	24.0	16.6
9	27.0	26.0	31.5	47.0	40.0	34.3
12	61.0	46.0	51.0	60.0	80.0	59.6
15	100.0	85.0	90.0	80.0	90.0	89.0
18	100.0	100.0	100.0	100.0	97.0	99.4
21	100.0	100.0	100.0	100.0	100.0	100.0

Table 6: Progress of field-infected *Colocasia* leaf blight disease score on five tagged leaves over 21 days on control plants

Number of days of leaf blight disease appearance	*Colocasia* leaf blight score (scale 0-9) on five tagged leaves					
	1	2	3	4	5	Mean
3	1	0	0	1	1	1
6	3	2	2	3	3	3
9	5	5	5	5	5	5
12	7	6	7	7	7	7
15	8	8	8	8	8	8
18	9	9	9	9	9	9
21	9	9	9	9	9	9

*0 = No disease; 9 = Collapse of petiole accompanied by complete leaf blight (Horsfall and Cowling, 1978; Cole, 1981)

Disease scored 1 (presence of disease less than 10 cm^2 of the leaf area) on the third day after the leaf completely unfurled. Disease scored an average of 7 (coalesce of spots more than 50% of the leaf covered) on the 12^{th} day after the leaf unfurled. Disease score increased to 9 on day 18 (Table 6).

4.5.4 Area of leaves infected with *Curvularia* leaf blight

In the first and second weeks, there were no significant differences (P<0.05) between treatment means. There were, however, significant differences (P<0.05) between treatments in the third to the seventh weeks (Table 7). All fungicide-treated leaves differed significantly (P<0.05) from water treated leaves (control). For the Topsin M and Sundomil-treated leaves, there were no significant differences between them.

Table 7: Area of *C. esculenta* leaves infected (Ala) with leaf blight over seven weeks

	Mean area of leaves infected (Ala) in cm over seven weeks						
Treatments	1	2	3	4	5	6	7
Topsin M	3.64	3.56	4.05	4.02	4.45	4.45	5.70
Sundomil	3.02	3.04	3.49	3.38	3.31	3.39	3.50
Water	3.72	4.77	6.13	6.90	6.86	7.94	9.74
Lsd(5%)	NS	NS	1.52	1.14	2.77	1.78	2.47
CV(%)	41.30	16.00	18.60	8.30	21.50	6.90	14.10

2

4.5.5 Lesions on *C. esculenta* leaves per plant (Lpp) following treatments

There were no significant differences (P<0.05) between treatment means in the first four weeks. Sundomil-treated plants differed significantly (P<0.05) from the control plants from the fifth to the seventh week (Table 8). There were significant differences (P<0.05) between fungicide-treated plants.

Table 8: Lesions on *C. esculenta* leaves per plant taken over seven weeks

	Mean lesions per plant (Lpp) over seven weeks						
Treatments	1	2	3	4	5	6	7
Topsin M	1.18	1.33	1.77	1.55	1.45	1.59	1.40
Sundomil	1.22	1.26	1.25	1.45	1.02	1.05	1.07
Water	1.32	1.22	1.55	1.67	1.63	1.79	1.64
Lsd(5%)	NS	NS	NS	NS	0.28	0.18	0.26
CV(%)	7.90	10.40	9.90	11.00	10.90	6.60	4.00

4.5.6 Number of *C. esculenta* leaves infected with leaf blight disease per plant (Nla) after treament

There were no significant differences (P<0.05) between the fungicides-treated plants and control plants in the first four weeks. There was, however, significant difference (P<0.05) between Sundomil-treated plants and control plants from the fourth week to the seventh week **(**Table 9). For the Topsin M and Sundomil-treated leaves, there were no significant differences (P<0.05) between them in the sixth and seventh week.

Table 9: Number of leaves infected per plant over seven weeks

	Mean number leaves affected per plant over seven weeks						
Treatments	1	2	3	4	5	6	7
Topsin M	0.18	1.06	1.21	1.20	1.17	1.17	1.19
Sundomil	0.99	1.05	1.01	1.08	0.96	1.06	1.01
Water	1.07	1.07	1.19	1.21	1.21	1.28	1.29
Lsd(5%)	NS	NS	NS	NS	0.11	0.15	0.28
CV(%)	5.70	4.00	5.00	3.80	5.60	5.60	8.20

4.5.7 Number of leaves dead due to leaf blight disease (Ndd) after treatment

There were no significant differences (P<0.05) between treatment means in the first two weeks. From the third to the seventh week, Topsin M-treated plants differed significantly (P<0.05) from water-treated plants. There were no differences between fungicide treated plants (Table 10)

Table 10: Number of leaves dead due to disease over seven weeks

	Mean number leaves dead due to disease (Ndd) over seven weeks						
Treatments	1	2	3	4	5	6	7
Topsin M	0.79	0.91	0.92	0.90	0.86	0.86	0.77
Sundomil	0.72	0.81	0.86	1.07	0.77	0.78	0.72
Water	0.78	0.92	1.11	1.29	1.12	1.06	1.12
Lsd(5%)	NS	NS	0.15	0.18	0.26	0.20	0.14
CV(%)	5.70	7.30	6.00	5.50	10.00	7.40	5.50

4.5.8 Leaf Area of *C. esculenta* plants

There were no significant differences (P<0.05) between fungicide-treated plants and water treated plants from the first to the sixth week. There were significant differences (P<0.05) between Sundomil-treated plants and control in the seventh week. However, there was no significant difference (P<0.05) between Topsin M-treated-plants and the control. Mean leaf area values were higher in treated plants than the untreated plants from week one to week seven (Table 11).

Table 11: Summary of mean leaf area (La) from week one to week seven 3

	Mean leaf area (La) (cm)						
Treatments	La1	La2	La3	La4	La5	La6	La7
Topsin M	22.80	26.00	27.70	27.90	28.70	29.10	29.20
Sundomil	24.00	25.30	29.20	31.30	29.80	31.80	34.40
Water	24.60	21.70	28.40	30.60	28.50	27.40	26.00
Lsd(5%)	NS	NS	NS	NS	NS	NS	8.20
CV(%)	5.80	4.40	7.60	4.80	4.90	6.90	6.30

4.6 Corm yield

The yield of *Colocasia* ranged from 19.28 to 25.95 Tonnes/hectare with the Sundomil-treated plants recording the highest (Table 12). There were significant differences ($P<0.05$) between fungicide-treated plants and water-treated plants. Fungicide-treated plants also differed significantly from each other (Table 12).

Table12. Mean Corms yield of fungicide-treated plants and water-treated plants after eight months of planting

Treatments	Mean corms yield (Tonnes/hectare)
Topsin M	22.75
Sundomil	25.95
Water	19.28
Lsd (5%)	0.34
CV(%)	5.20

4.7 CORRELATION MATRICES

Pearson correlation matrix was run for the three treatments to show the relationship between yields, number of leaves infected, number of leaves dead due to disease, lesions per plant, leaf area and area of leaves infected.

4.7.1 Correlation matrix for water-treated plants.

There was a positive correlation (r = 0.2) between leaf area (La) and yield. There were also positive correlations (r = 0.1, r = 0.6 respectively) between area of leaves infected (Ala) and number of leaves dead due to disease (Ndd), and leaf area (La) and number of leaves infected (Nla). There were negative correlations between number of leaves infected (Nla), number of leaves dead due to disease (Ndd), lesions per plant (Lpp), area of leaves affected and yield (Table 13).

Table 13. Correlation matrix for water-treated plants under field conditions

	Yield	Nla	Ndd	Lpp	La	Ala
Yield	1.00					
Nla	-0.04	1.00				
Ndd	-0.65	0.28	1.00			
Lpp	-0.38	-0.29	-0.21	1.00		
La	0.23	-0.09	-0.60	-0.26	1.00	
Ala	-0.11	0.53	0.13	-0.64	0.64	1.00

Nla - number of leaves infected Ndd - number of leaves dead due to disease

Lpp - lesions per plant La - leaf area

Ala- area of leaves infected

4.7.2. Correlation matrix for Topsin M-treated plants

There was a strong positive correlation (r = 0.8) between leaf area (La) and the yield. There were also positive correlations (r = 0.4, r = 0.1) between number of leaves infected (Nla), lesions per plant (Lpp), and area of leaves affected (Ala), respectively. There was negative correlation between area of leaves infected (Ala), lesion per plant (Lpp), number of leaves infected (Nla) and the yield (Table 14.)

Table 14. Correlation matrix for Topsin M treated-plants under field conditions

	Yield	Nla	Ndd	Lpp	La	Ala
Yield	1.00					
Nla	-0.29	1.00				
Ndd	0.45	-0.10	1.00			
Lpp	-0.14	0.43	-0.85	1.00		
La	0.84	-0.58	-0.68	-0.59	1.00	
Ala	-0.03	0.14	0.03	0.12	-0.26	1.00

Nla - number of leaves affected Ndd - number of leaves dead due to disease

Lpp - lesions per plant La - leaf area

Ala- area of leaves affected

4.6.3. Correlation matrix for Sundomil treated plants.

There was a positive correlation (r=0.5) between leaf area (La) and the yield. There was also a positive correlation (r=0.8, 0.3 and 0.7) between number of leaves infected (Nla), lesions per plant (Lpp), number of leaves dead due to disease (Ndd), respectively, and area of leaves infected (Ala). There was negative correlation between area of leaves infected (Ala), lesion per plant (Lpp), number of leaves dead due to disease (Ndd) and the yield. (Table 15).

Table 15. Correlation matrix for Sundomil treated plants under field conditions

	Yield	Nla	Ndd	Lpp	La	Ala
Yield	1.00					
Nla	0.17	1.00				
Ndd	-0.09	0.12	1.00			
Lpp	-0.77	0.09	0.11	1.00		
La	0.47	0.69	0.76	-0.09	1.00	
Ala	-0.04	0.81	0.66	0.27	0.92	1.00

Nla - number of leaves infected Ndd - number of leaves dead due to disease

Lpp - lesions per plant La - leaf area

Ala- area of leaves infected

CHAPTER FIVE

5.0 DISCUSSION

5.1 Field Survey: Assessment of *Colocasia* leaf blight incidence, severity and farmers' knowledge of the disease in major *Colocasia* growing areas in Kumasi

C. esculenta farmers ranked diseases as the major constraint halting the cultivation of *C. esculenta.* Diseases observed on the farmers' fields affected mainly the shoot. This agrees with observation by Ooka (1994). Majority of *C. esculenta* farmers practiced monoculture continually on the same piece of land and this exacerbated the incidence and severity of the disease on farmers' fields. Disease incidence and severity were reduced in mixed cropping fields where *C. esculenta* was intercropped with sugar cane and/or maize. In mixed cropping, the disease developed more slowly probably due to interception of the pathogen and this agrees with the observations by Akanda and Mundt (1996). Following the above, the farmers perceived the disease problem.

5.2 Experiment 1: Proof of pathogenicity of fungal isolates associated with *Colocasia* leaf blight on potted plants in the plant house

Pure cultures of *Penicillium* sp., *Fusarium* sp., *Pythium* sp. and *Rhizopus stolonifer* did not produce *Colocasia* leaf blight symptoms on the leaves and suggest that they are secondary pathogens and are not responsible for the blight disease. Pure cultures of *Aspergillus niger* did not produce leaf blight symptoms similar to those on the field but produced leaf yellowing symptoms, suggesting that it is not responsible for blight disease on the *C. esculenta* fields. Pure cultures of *Curvularia* sp. produced dark brown lesions similar to those on the field, suggesting that it is responsible for blight disease on the *C. esculenta* fields.

5.3 Experiment 2: Confirmation of pathogenicity of *Curvularia* species on potted

***Colocasia* plantlets in a moist chamber**

Inoculation with pure cultures of *Curvularia* sp. obtained from the potted *C. esculenta* plants at the plant house produced leaf blight symptoms. Re-isolation of *Curvularia* sp. from the moist chamber infected plants produced pure cultures of *Curvularia* sp. Pathogenicity test based on Koch's postulates, therefore, produced positive results.

5.4 Experiment 3: Field assessment of *Colocasia* leaf blight and its management

C. esculenta leaf blight disease has been thought to be caused by *Phytophthora colocasiae* (Jackson *et al*., 1980; Bergquist, 1972; Ashok and Mehrotra, 1987). Awuah (1995) published the only documentary evidence of *C. esculenta* disease in Ghana caused by *Cladosporium colocasiae* and remarked on critical suppression of symptom development with Thiophanate methyl. The present study did not review *Phytophthora colocasiae* in *C. esculenta* fields. Contrarily, *Cladosporium colocasiae* produced leaf spot symptoms on upland *C. esculenta* fields as documented by Awuah (1995) and *Curvularia* sp. on swampy fields. Theberge (1985) recognised various diseases of *Colocasia* on farmer fields but noted lack of reports on them in Africa.

5.4.1 Disease incidence and severity of *Colocasia* leaf blight

Presence of the disease on young leaves, just after they unfurl, and the rapid development and spread on leaves when attacked by *Curvularia* sp., suggest that the fungus is a strong pathogen which attacks *C. esculenta* leaves at all developmental stages. According to Awuah (1995), absence of disease on young leaves and the slow development of the pathogen on leaves when infected on upland fields suggest *Cladosporium colocasiae* is a weak pathogen which attack only maturing leaves. It was observed that, disease incidence was higher in the wet season than in the dry season and this agrees with the observations by Plucknett *et al*. (1970) that high humidity and high soil water content (swampy soils) increase susceptibility of plants to the

disease. Disease incidence was higher in the control plants than in all fungicide-treated plants. All fungicides applied subdued the fungus to some extent. The results suggested that the fungicides might have inhibited mycelial growth of the fungus as documented by Ashok and Mehrotra (1988) and hence retarded the growth and spread of the fungus to new tissues.

This study has revealed that fungicide must be applied just after the leaves unfurl to protect the plant from being infected. Das (1997) reported that fungicide application should be done just on the onset of disease which is in line with findings of this study. This observation is important since reduced leaf area in diseased plants reduce yield (Hunter *et al.,* 2001; Cox and Kasimani, 1990). The result was contrary to what was reported by Ashok and Mehrotra (1987) that fungicide application should not start earlier than 90 days after planting since loss of leaves during this period does not affect yield.

5.4.2 Leaf area of *Colocasia esculenta*

Leaf area is a valuable index in evaluating *C. esculenta g*rowth and development (Lu *et al* ., 2002). It is also related to light interception, transpiration, and photosynthesis and thus considered the single most important determinant of dry matter accumulation and yield in *C. esculenta* (Satou *et al*., 1988; Jacobs and Chand, 1992; Chan *et al*., 1993, 1998). The results of this study indicated that the mean leaf area values of treated plants were greater than the untreated plants. Reduced disease severity of treated plants presented an effective leaf area for photosynthesis and hence, increased leaf area and yield. The positive correlation (r = 0.2, 0.8 and 0.4) results between leaf area and yield in water-treated, Topsin M-treated and Sundomil-treated plants, respectively, confirm the studies by Bergquist (1974) and Das (1997).

5.4.3 Area of leaves infected with *Colocasia* leaf blight

Area of leaves affected by the pathogen in the fungicide-treated plants significantly differed ($P<0.05$) from the control plants and this was manifested in the relatively low disease severity in the treated plants and high disease severity in the control plants. This means that the presence of the disease on leaves presented a reduction of leaf area available for effective photosynthesis, thereby resulting in a relatively slower growth rate and reducing yield. These agree with the observations by Lu *et al*. (2002) who reported that reduction of leaf area reduces yield. Leaf blight reduced the cumulative leaf number of *C. esculenta* (Cox and Kasimani, 1987) and also the cumulative leaf area available for effective photosynthesis.

5.4.4 Corm yield of *Colocasia esculenta*

Yield was higher in all plants treated with fungicides at a concentration of 45 g /15 litres at two weeks interval than the control plants. Bergquist (1974) and Das (1997) reported similar results where highest rate of fungicide was more effective than the lowest rate. Sundomiltreated plants performed better (25.95 tonnes/hectare) than Topsin M-treated plants (22.72 tonnes/hectare). According to Das (1997), Metalaxyl and Mancozeb give significantly more effective disease control than the other fungicides.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

C. esculenta farmers exhibited knowledge of *Colocasia* production and perceived the disease problem. The studies have clearly shown that *Curvularia* sp. is the main fungal pathogen that plagues *C. esculenta* in swampy fields. The study reviewed that inoculum in a form of spores of *Curvularia* sp. are responsible for the blight disease. *Cladosporium colocasiae* produced leaf spot symptoms and is associated with upland *C. esculenta* field while *Curvularia* sp. produced leaf blight symptoms, and is associated with swampy *C. esculenta.* The incidence and severity of the leaf blight increased after each down pour of rain, suggesting that high humidity and water availability influenced the disease. There was also a positive correlation between leaf area and the corm yield and a negative correlation between area of leaves affected by the disease, lesions per plant and the corm yield.

In the field trial, *C. esculenta* treated with Topsin M 70 WP (Thiophanate methyl) and Sundomil 72 WP (Metalaxyl 8% and Mancozeb 64%) at a rate of 4 g/15l fortnightly, reduced disease symptoms and produced higher corm yields than the control treatment.

Preventive disease control strategies should be adopted, since blight disease, when present, spreads at a faster rate and fungicide application cannot eradicate the disease but stop the pathogen from spreading to new tissues.

6.2 RECOMMENDATIONS

- Application of fungicides must be done as soon as initial symptoms appear or just at the onset of disease or when leaves unfurl.
- Field trials should be done on *C. esculenta* mixed cropping system with non- susceptible crops such as maize and sugar cane.
- Farmers should be advised to use clean and disease-free planting materials and practice farm hygiene.
- *C. esculenta* should not be cultivated continuously on the same piece of land but rotated with non-susceptible plants.

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APPENDICES

Appendix 1: Summary of disease incidence from week one to week seven

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Treatment 1	50.0	52.0	56.0	68.0	78.0	80.0	86.0
Treatment 2	48.0	51.0	52.0	54.0	58.0	60.0	64.0
Treatment 3	40.0	44.0	46.0	48.0	49.0	52.0	56.0

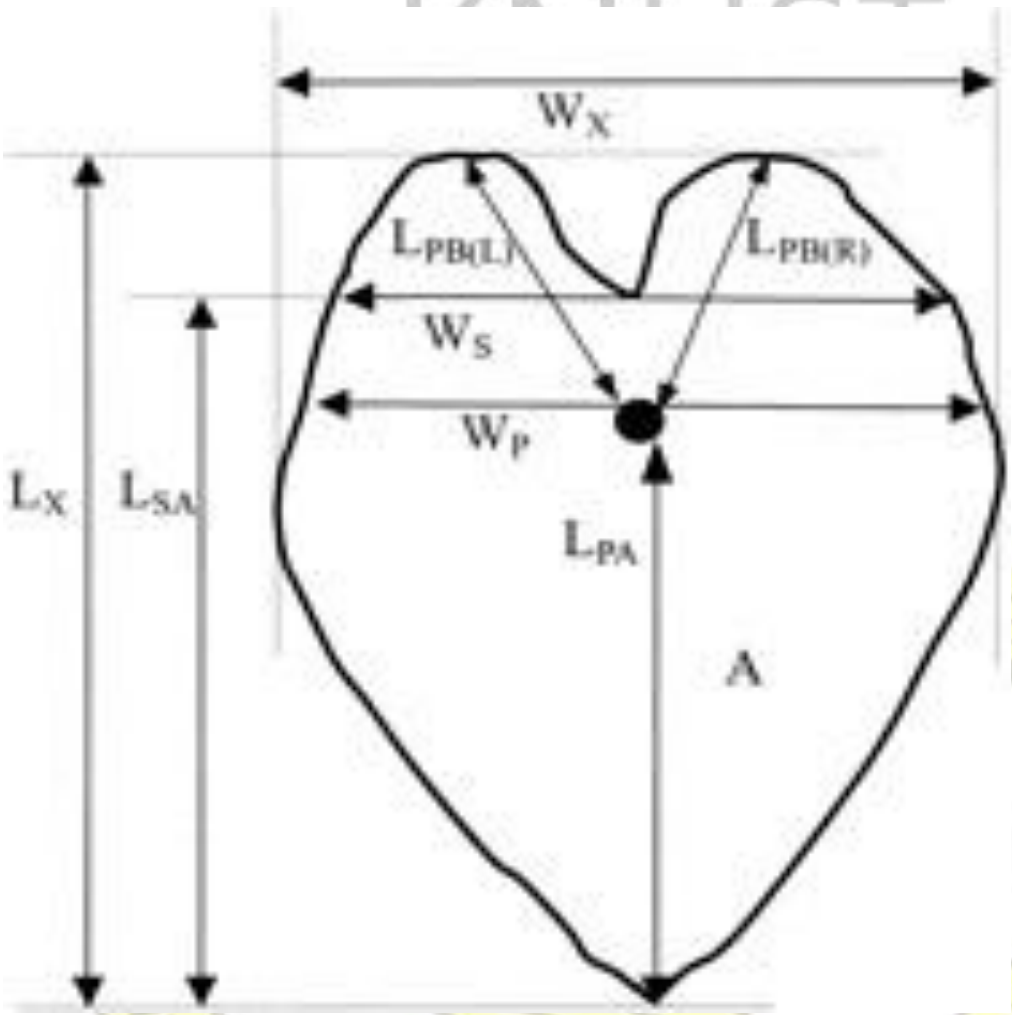
Appendix 2: Summary of disease severity from week one to week seven

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Treatment 1	3.0	3.4	4.9	5.1	6.6	8.3	14.2
Treatment 2	2.7	2.2	2.0	2.1	2.3	2.4	3.8
Treatment 3	1.6	1.7	1.4	1.3	0.9	1.5	1.1

Appendix 3: **Diagram of C. *esculenta* leaf showing positions of quantity descriptors**.

A, leaf area; L_X, maximum leaf length; L_{SA}, length from the sinus base to the apex of leaf along midrib; L_{PA}, length from the petiole-attaching point to the apex of leaf; $L_{PB(R)}$, length from the

petiole-attaching point of leaf to the tip of right lobe; $L_{PB(L)}$, length from the petioleattaching point of leaf to the tip of left lobe; W_X, maximum leaf width; W_P, leaf width passing the petiole-attaching point and perpendicular to L_{PA}; W_S, leaf width passing the sinus base and perpendicular to L_{SA}. (Chan *et al*., 1993; Lu *et al*., 2002)



APPENDIX 4: Questionnaire to assess C. *esculenta* farmers on the perception of the disease

PART A: FARMERS PERSONAL DATA

1. Name.....

2. Sex.....3. Age.....

4. Location of farm.....

PART B: FARM RECORDS (*Tick where appropriate*)

5. What is the history of the land or farm?

.....

6. How long have you cultivated *Colocacia esculenta?*

.....

7. What crop(s) is/are planted with *Colocacia esculenta?*

.....

8. Why do you grow *Colocacia esculenta?*

.....

9. What are the problems encountered in producing *Colocacia esculenta?*

.....

10. Rank problem
.....
.....

11. Which part of the plant is infected?

.....

12. At what stage of the plant is the disease seen or observed?

.....

13. What is the percentage of each disease?

.....

14. Is there any sample of diseased crop? If yes show sample

15. What is the effect of the disease on the plant?

.....

16. What is the cause of the disease?

.....

17. How do you manage the disease?

.....

Appendix 5: Summary of number of leaves per plant from week one to week seven.

	Mean number of leaves per plant (Nlp)						
Treatments	1	2	3	4	5	6	7
Topsin M	1.92	1.97	2.20	1.85	1.71	1.65	1.69
Sundomil	1.96	2.11	2.16	2.09	1.94	2.05	2.14

Water	1.94	1.98	1.99	1.94	1.84	1.73	1.70
Lsd(5%)	0.18	0.11	0.14	0.26	0.32	0.27	0.27
CV(%)	2.40	3.10	5.40	6.20	4.90	5.60	4.60