KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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DEPARTMENT OF CROP AND SOIL SCIENCES

SCREENING OF PEPPER VARIETIES AND ACCESSIONS FOR RESISTANCE TO ANTHRACNOSE AND MANAGEMENT OF SEED-BORNE PATHOGENS OF PEPPER

BY

ERIC GYASI (B.Sc. (HONS.) AGRICULTURE)

AUGUST, 2012

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DISSERTATION SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN CROP PROTECTION (PLANT PATHOLOGY)

AUGUST 2014

DECLARATION

I hereby declare that this submission is my own work towards the Master of Science in Crop Protection (Plant Pathology) and that, to the best of my knowledge it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this thesis to my uncle, Mr. Joseph K. Asomaning who has been a rock of stability throughout my life, and also to my late brother, Enoch Amponsah and my entire family.

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I am most grateful to the gracious and ever merciful God Almighty for the abundant grace and protection throughout this programme.

I am also indebted to my supervisors, Dr. Charles Kwoseh of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST and Dr. Emmanuel Moses of CSIR- Crop Research Institute (CRI), Fumesua, Kumasi for their advice, time, encouragement and their constructive criticisms. I appreciate every input they made and also count myself blessed to have been associated with them. May the Almighty God richly bless you. My profound gratitude also goes to the entire lecturers of Faculty of Agriculture who have baked me to become who I am today.

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ABSTRACT

Three main studies including a survey was conducted in two pepper growing communities in the Ashanti Region namely Ofoase-Kokoben and Konongo-Obenemasi to assess farmers' perception and knowledge of the anthracnose disease by the use of structured questionnaires. Laboratory experiments took place at the Plant Pathology Laboratories of Faculty of Agriculture, KNUST, Kumasi and Crop Research Institute, Fumesua. Detection of fungi on pepper seed samples from farmers in Ofoase-Kokoben and Konongo-Obenemasi communities was done by direct plating on moist blotters in the laboratory. The efficacies of three botanical extracts namely garlic, ginger and neem, were also tested alongside Mancozeb, a synthetic fungicide for the control of seed-borne pathogens of pepper. Resistance of pepper accessions (CRI OO5, CRI 007 and CRI K82) and varieties (Shito adope, Legon 18, Meko hwam, Ohene nsatea and African bird's eye) were evaluated in an anthracnose disease hotspot both in the minor and major seasons. The African bird's eye was used as the check.

Analysis of the questionnaires showed that, 72.5 % of farmers in the studied communities perceived anthracnose disease as one of the major diseases affecting pepper production. The farmers do not use certified seeds; about 75 % used seeds saved from previous harvest, 20 % used seeds obtained from friends and 5 % obtained their pepper seeds from the local markets. They do not treat their seeds before sowing. Nine genera of fungi including 12 species were identified from the pepper seed samples collected. *Colletotrichum capsici* and *C. gloeosporoides* were found to be associated with the anthracnose disease in the studied community. However, *C. capsici* was found to be the most predominant. Soaking the pepper seeds for 24 h in garlic extract was very effective as mancozeb in controlling *Colletotrichum* species present on the seeds. The results gathered from the screening of the accessions and varieties on the field also showed that, all the accessions and varieties were not resistant to the disease. Breeding for resistance is, therefore, necessary to increase farmers' income and to

ensuring food security. Further research involving the evaluation of a greater number of varieties and accessions from breeding programmes is also necessary if resistant varieties are to be identified for pepper production in Ghana.

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CHAPTER ONE

1.0 INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is a herbaceous perennial dicotyledonous plant of the family Solanaceae. It is also called cayenne, chili, bird eye or red pepper (Tindall, 1983).

Capsicum contains approximately 20 to 27 species, of which five are domesticated (Tong and Bosland, 1999). They are *C. annuum*, *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz and Pav., and are cultivated in different parts of the world. Among the five species of cultivated *Capsicum*, *C. annuum* is the most commonly cultivated worldwide (Tong and Bosland, 1999) followed by *C. frutescens* (Bosland and Votava, 2003). Chili has many culinary advantages and contains chemicals such as steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements (Bosland and Votava, 2003). Many chili constituents are important for nutritional value, flavour, aroma, texture and colour. Chilies are low in sodium and cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E (Bosland and Votava, 2003). Fresh green chili peppers contain more vitamin C than citrus fruits and fresh red chili has more vitamin A than carrots (Marin *et al*., 2004; Osuna-García *et al*., 1998). Capsaicinoids are alkaloids that make hot chili pungent.

FAO (2010) estimated world production of *Capsicum* peppers in 2001 at 21.3 million tons from a harvested area of 1.6 million ha (average yield 13.4 t/ha). Production has increased on average 3.9 % per year during the last 10 years led by a steady increase in global demand. China is the largest producer with 10 million tons, followed by Mexico (1.9 million tons) and Turkey (1.5 million tons) which in total account for more than 70 % of the world chili pepper production (MiDA, 2010). Production of pepper in tropical Africa is estimated at 1 million tons with

Nigeria (715,000 tons from 90,000 ha) and Ghana (270,000 tons from 75,000 ha) as the largest producers (FAO, 2010).

Capsicum pepper is Ghana's most popular second vegetable after tomato (Schippers, 2000). Chili peppers have been widely produced in Ghana for local consumption but have been increasingly exported to the European market in recent years. Ghana is the fifth largest exporter of chili pepper to the European Union, where demand has been increasing annually by 17 % on average since 2000 (FAO, 2010). There is a high demand for chili pepper from Ghana compared with chili peppers from other countries (MiDA, 2010). Farmers can take advantage of this high demand to increase their production.

Despite the nutritional value of the fruit and their economic importance to the Ghanaian economy in providing income to farmers and foreign exchange to the country, pepper production is affected by a number of constraints. Among these constraints, diseases are prominent. Pepper mosaic virus is very widespread in pepper producing areas in Ghana (Ofei Bonsu, personal communication). In addition to losses caused by viruses due to susceptibility of most of the cultivated varieties, fungal diseases, particularly anthracnose, affect pepper production severely (Ofei Bonsu and Moses, personal communication). Losses in yield due to anthracnose could be as high as 84 % (Thind and Jhooty, 1985). Fruit drop, dieback and blackened sunken lesions are very common in pepper fields in most parts of the country. Pepper-producing farmers rely heavily on chemical fungicides to control anthracnose outbreak on their farms often without any success.

In several pepper producing areas in Ghana, farmers over dependence on chemical fungicides to reduce losses is of great concern. Developing appropriate control measure to reduce losses due to anthracnose could go a long way to increase yield of farmers and also eliminate chemical and mechanical expenses of controlling the disease (Agrios, 2005). A more realistic approach to reducing losses due to anthracnose and other fungal diseases is to identify resistant cultivars or genotypes for farmers. This will minimize the dependence on chemical fungicides for the control of fungal diseases. The use of resistant varieties in controlling anthracnose disease has been reported by Yoon and Park (2001); AVRDC (1999); Hong and Hwang (1998); Pae *et al*. (1998); Kim *et al*. (1987); Park *et al*. (1987) and Kim *et al*. (1986).

Pepper anthracnose is a seed-borne disease (Pring *et al*., 1995). Controlling pathogens on seeds through the development and application of appropriate treatment regimes has been reported to bring about reduction in losses (Korpraditskul *et al*., 1999; Jeyalakshmi and Seetharaman, 1998).

The main objective of this experiment was to identify anthracnose resistant pepper cultivar(s) for cultivation or breeding purposes.

The specific objectives were;

i. to document farmers' perception and knowledge of pepper anthracnose disease in Ofoase-Kokoben and Konongo-Obenemasi communities in the Ashanti Region of Ghana with the aim of using this information to develop control measures ii. to inventory seed-borne pathogens of pepper associated with farmers' own saved seeds from the two communities iii. to evaluate the efficacy of aqueous botanical products against seed-borne pathogens of pepper with emphasis on the anthracnose pathogens.

iv. to screen a number of pepper varieties and accessions in an anthracnose hotspot for resistance.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of pepper anthracnose disease

Anthracnose disease is believed to have been first reported in India on chilis from the Coimbatore of Madras Presidency (Sydow, 1913). The disease has since been identified in all chili producing regions of the world and has become a serious constraint in chili production whenever the crop is grown.

Colletotrichum is one of the most important plant pathogens worldwide, causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Bailey and Jeger, 1992). Among these hosts, chili (*Capsicum* spp.), an important economic crop worldwide (Poulos, 1992), is severely infected by anthracnose which may cause yield losses of up to 50 % (Pakdeevaraporn *et al*., 2005). Thind and Jhooty (1985) also reported that the disease can also cause yield losses of up to 84 % in the tropics. Economic losses caused by the disease are mainly attributed to lower fruit quality and marketability. Although infected fruits are not toxic to humans or animals, severely affected fruits showing blemishes are generally considered unfit for human consumption. This is because the anthracnose causes an unpleasant colour and taste in chili products. Anthracnose of chili has been shown to be caused by more than one *Colletotrichum* species including *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc., and *C. coccodes* (Wallr.) S. Hughes (Than *et al*., 2008; Pakdeevaraporn *et al*., 2005; Sharma *et al*., 2005; Voorrips *et al*., 2004; Nirenberg *et al*., 2002; Kim *et al*., 1999 Johnston and Jones, 1997 and Simmonds, 1965). Surveys conducted have revealed *C. capsici* as the most predominant species in the major chili growing areas in India (Ramachandran *et al*., 2008). Park *et al*. (1987) observed that *C. gloeosporioides* was the predominant species on chili in Korea and has the widest host range among solanaceous crops and biotypes have been reported on hosts. *C. acutatum* causes severe fruit and foliar damage to pepper in several tropical regions (Hong and Hwang, 1998). *C. coccodes* is the least aggressive species and is more commonly found in the temperate regions (Hong and Hwang, 1998).

2.2 Conditions for pepper anthracnose disease development and mechanism of transmission

The pathogens that cause the anthracnose disease of pepper are seed-borne and may also persist on alternative hosts such as other solanaceous crops (tomato, potato, eggplant), cucumber, and many other cultivated crops and weeds (Pring *et al*., 1995). These pathogens will also readily persist in crop debris and in weeds, in some cases (e.g. *C. coccodes*) as resistant fungal structures called sclerotia (Pring *et al*., 1995). The pathogens will increase in number under continuous cultivation of pepper, tomato or potato. Secondary cycles of anthracnose development during the growing season arise from spores produced on diseased fruits or leaves. Water-splash or winddriven rain is required for dispersal of fungal spores or microsclerotia on soil particles (Roberts *et al*., 2001). Wounds in fruit are not required for infection, but wetness is needed for spore germination and infection (Isaac, 1992).

The optimum temperature for fruit infection is 20-24 °C with fruit surface wetness, although infection may occur from 10 to 30 °C (Roberts *et al*., 2001). However, the longer the period of fruit surface wetness, the greater the anthracnose severity. Fruits that are at or near the soil surface are the most likely to become infected by rain-splash or direct soil contact. Overhead irrigation will favour development of anthracnose because of increased relative humidity and increased duration of dew period (AVRDC, 2004).

2.3 Symptoms and damage caused by *Colletotrichum* species on pepper

Anthracnose causes extensive pre- and post-harvest damage to chili fruits, causing anthracnose lesions. Even small anthracnose lesions on chili fruits reduce their marketable value (Manandhar *et al*., 1995). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection (Jeffries *et al*., 1990). Appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Bailey and Jeger, 1992). Appressoria that form on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Prusky and Plumbley, 1992). Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits (Isaac, 1992). Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses (Isaac, 1992). Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scattered or in concentric rings on the lesions.

2.4 Causal agents of chilli anthracnose

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose (Cannon *et al*., 2000; Freeman *et al*., 1998; Simmonds, 1965). *Colletotrichum* species causing anthracnose of chili have been reported from different countries and regions. Although these species have been the subject of numerous investigations, there remain many gaps in the knowledge of the disease process and understanding of the complex relationships between the species involved (Simmonds, 1965). Kim *et al*. (2004) reported that different species cause diseases of different organs of the chili plant. For example, *C. acutatum* and *C. gloeosporoides* infect chili fruits at all developmental stages, but usually not the leaves or stems, which are mostly damaged by *C. coccodes* and *C. dematium*. Leaf anthracnose of chili seedlings caused by *C. coccodes* was first reported in chili growing in a field in Chungnam Province of Korea in 1988 (Hong and Hwang, 1998). Different *Colletotrichum* species may also play an important role in different diseases of mature stages of chili fruit. For example, *C. capsici* is widespread in red chili fruits, whereas *C.*

acutatum and *C. gloeosporoides* have been reported to be more prevalent on both young and mature green fruits (Kim *et al*., 1999; Hong and Hwang, 1998). Anthracnose caused by *C. coccodes* does not result in severe epidemics on chili fruits (Hong and Hwang, 1998).

2.5 Disease cycle and epidemiology of pepper anthracnose

Environmental factors play a major role in the development of disease epidemics. The relationships among rainfall intensity, duration and crop geometry and the dispersal of inoculum possibly lead to different levels of disease severity (Dodd *et al*., 1992). Generally, infection occurs during warm, wet weather. Temperatures around 27 °C and high humidity (a mean of 80 %) are optimum for anthracnose disease development (Roberts *et al*., 2001).

Colletotrichum species utilize diverse strategies for invading host tissues, which vary from intracellular hemibiotrophy to subcuticular intramural necrotrophy (Bailey and Jeger, 1992). *Colletotrichum* species produce a series of specialised infection structures such as germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae (Perfect *et al*., 1999). These pathogens infect plants by either colonising subcuticular tissues intramurally or being established intracellularly. The pre-infection stages of both are very similar, in which conidia adhere to and germinate on the plant surface, producing germ tubes that form appressoria which in turn penetrate the cuticle directly (Bailey and Jeger, 1992). Following penetration, the pathogens that colonise the intramural region beneath the cuticle invade in a necrotrophic manner and spread rapidly throughout the tissues (O′Connell *et al*., 1985). There is no detectable biotrophic stage in this form of parasitism. In contrast, most anthracnose pathogens exhibit a biotrophic infection strategy initially by colonising the plasmalemma and cell wall intracellularly. After the biotrophic state, intracellular hyphae colonise one or two cells and subsequently produce secondary necrotrophic hyphae (Bailey and Jeger, 1992). These pathogens are, therefore, regarded as hemibiotrophs or facultative biotrophs (Kim *et al*., 2004). For example, *C. gloeosporioides* on avocado, chili and citrus can produce both types of colonisations: intracellular biotrophy at an early stage and intramural necrotrophy later (O′Connell *et al*., 2000). Although the mechanisms developed by *Colletotrichum* species appear similar in pre-penetration events, there are differences between species in the later mechanisms such as spore adhesion, melanisation and cutinisation in penetration of the plant cuticle by the appressoria. The hostpathogen interaction of *C. acutatum* appears to be more biotrophic than that of some other species such as *C. gloeosporioides* (Wharton and Diéguez-Uribeondo, 2004).

There are only a few detailed studies on penetration and colonisation by *Colletotrichum* species on chili. Kim *et al*. (2004) noticed that there was no biotrophic infection vesicle found during the infection process of *C. gloeosporioides* in susceptible chili (*C. annuum* cv. jejujaerae).

2.6. Management of chili anthracnose disease

Agrios (2005) and Bailey (1987) recommended integrated management techniques, as no single specific management programme could eliminate chili anthracnose. Effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic resistance (Wharton and Diéguez-Uribeondo, 2004).

2.6.1 Cultural practices

Pathogen-free chili seed should be planted and weeds eliminated. Crops should be rotated every two to three years with crops that are not alternative hosts of *Colletotrichum*. Transplants should be kept clean by controlling weeds and solanaceous volunteers around the transplants. The field should have good drainage and be free from infected plant debris. If disease was previously present, crops should be rotated away from solanaceous plants for at least two years (Roberts *et al*., 2001). Choosing cultivars that bear fruit with a shorter ripening period may allow the fruit to escape infection by the fungus. Wounds in fruit from insects or other means should be reduced because wounds provide entry points for *Colletotrichum* species and other pathogens such as bacteria that cause soft rot. At the end of the season, infected plant debris from the field must be removed or deep ploughed to completely cover crop diseases (Agrios, 2005).

2.6.2 Use of resistant cultivars

The use of resistant varieties not only eliminated losses from diseases, but also eliminated chemical and mechanical expenses of disease control (Agrios, 2005). Some genetic resources resistant to anthracnose in chili have been independently reported from different countries and regions of the world (Yoon and Park, 2001; AVRDC, 1999; Hong and Hwang, 1998; Pae *et al*., 1998; Kim *et al*., 1987; Park *et al*., 1987; Kim *et al*., 1986). No strong resistance has been found in *Capsicum annuum*, which is the only species grown worldwide (Park, 2007). Management and control of the anthracnose disease are still under extensive research (Yoon *et al*., 2004). Among disease control management, the use of resistant cultivars is the cheapest, easiest, safest and most effective means of controlling the disease. This is not only to eliminate losses from the disease but also decrease the cost of chemical and mechanical control, as well as reduce contamination of the environment from the use of toxic chemicals. However, management of disease through breeding of pathogen-resistant cultivars has only had limited success due to frequent breakdown of resistance under field conditions. Commercial cultivars of *C. annuum* resistant to the pathogens that cause anthracnose have not yet been developed (Park, 2007). Nevertheless, high levels of resistance to the *Colletotrichum* species that infect chili have been found in some species of *Capsicum*, for instance, *C. baccatum*. Current research is focusing on introgression of this resistance into susceptible commercial cultivars of *C. annuum* (Pakdeevaraporn *et al*., 2005;

AVRDC, 2003). Although there are currently extensive researches on disease control management including breeding programmes for resistant cultivars to anthracnose, the current status of the chili anthracnose disease still requires improvement.

2.6.3 Use of chemicals

Chemicals are the most common and practical method to control anthracnose diseases. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991). The fungicide traditionally recommended for anthracnose management in chili is Manganese ethylenebisdithiocarbamate (Maneb) (Smith, 2000), although it does not consistently control the severe form of anthracnose on chili fruit. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have been labelled for the control of anthracnose of chili, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Lewis and Miller, 2003; Alexander and Waldenmaier, 2002). The disease can be controlled under normal weather conditions with a reasonable spray programme. However, there are numerous reports of negative effects of using chemicals on farmers' income and health, and toxic contamination to the environment, particularly in developing countries (Voorrips *et al.*, 2004).

2.6.4 Use of biofungicides

The control of chili anthracnose fruit rot has, for many years, relied on chemicals and resulted in many undesirable problems. There is a need to incorporate alternative control components that are effective in the field. Biological control of fruit rot and dieback of chili with plant products tested in many laboratories and field trials showed that the crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii* (Roxb.) W. Wats) oil, *Ocimum sanctum* Linn. leaf extract, neem

(*Azadirachta indica* A. Juss) oil and leaf extract and garlic (*Allium sativum* L.) leaf extract could restrict growth of the anthracnose fungus (Korpraditskul *et al*., 1999; Jeyalakshmi and Seetharaman, 1998). Several reports have been made on garlic as one of the botanicals effective against the fungus *Colletotrichum* species (Syed *et al*., 2012; Ines *et al*., 2008; Shovan *et al*., 2008). Charigkapakorn (2000) also found that sweetflag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage offered effective control of the fungus. Ginger extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, anti-ulcer, gastric anti-secretory, anti-tumor, anti-fungal, antispasmodic, anti-allergenic, and other activities (Foster and Yue, 1992). A survey was conducted in Jaffna, Sri Lanka, to determine the fungicidal properties of ginger rhizome extract. The growth inhibition on *Fusarium*, *Colletotrichum* and *Curvularia* species by ginger rhizome extract were 70.0, 71.0 and 64.2 %, respectively (Krishnapillai, 2007). Investigation was also carried out to test the potency of some plant extracts for the control of yam tuber rot caused by *Fusarium oxysporum* Schlecht, *Aspergillus niger* Tiegh and *A. flavus* Link. Hot water extract obtained from ginger (*Zingiber officinale* Rosc.) was fungitoxic against the fungi. The extract suppressed the growth of *Fusarium oxysporum*, *Aspergillus niger* and *A. flavus* (Okigbo and Nmeka, 2005). Ines *et al*. (2008) using different botanicals including garlic, neem, malunggay and dithane to control anthracnose disease of mango seedlings reported that garlic showed the least mean number of infected leaves followed by neem and concluded that the use of garlic and neem botanical extracts in controlling anthracnose of mango seedlings had been found promising.

2.6.5 Biological control

Biological control methods for chili anthracnose have not received much attention. The potential for biological control of *Colletotrichum* species had been suggested in 1976 by

Lenné and Parbery (1976). Jeger and Jeffries (1988) also stressed the possibilities of biological control of post-harvest fruit diseases by using *Pseudomonas fluorescens*. Antagonistic bacterial strains (DGg13 and BB133) were found to effectively control *C. capsici*, the major anthracnose pathogen in Thailand (Intanoo and Chamswarng, 2007). It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Maymon *et al*., 2004; Jeffries and Koomen, 1992). *Trichoderma* species have been applied to control *Colletotrichum* species in chili (Boonratkwang *et al*., 2007), strawberries (Freeman *et al*., 2001), and citrus in Belize (Moretto *et al*., 2001) with concomitant disease reduction. Other biological control agents have been tested for efficacy against *C. acutatum* such as *Bacillus subtilis* (Ehrenberg) Cohn and *Candida oleophila* (Wharton and Diéguez-Uribeondo, 2004).

2.7. Botanicals used in the study

Fungi are one of the factors in storage seeds which reduce seed viability. Natural toxicants from plants have been used for pest control more than any other chemicals until recently. Flowers, cloves, leaves, bark, root and seed extracts were used for pest control. Garlic, neem and ginger were the botanical extracts used in this study together with mancozeb which is a synthetic fungicide.

2.7.1 Mancozeb (Chemical fungicide)

Mancozeb belongs to thiocarbamate fungicides containing Manganese and Zinc, basically it is ethylene bisdithiocarbamates. It is very effective against seed-borne diseases especially blight, seed rot, damping off and wilt etc (SinoHarvest, 2005).

2.7.2 Botanical fungicides

2.7.2.1 Garlic (*Allium sativum* L.)

The common kitchen herb, the garlic is also a culinary spice. This perennial herb is known for its white coloured bulb that is composed of small white cloves that have a very peculiar odour and tangy taste. The various forms of sulphur compounds found in garlic are responsible for the unique smell. Garlic has been scientifically proven to be a powerful natural antibiotic, antiviral and antifungal agent (Michelle, 2003). The main active ingredient of garlic is the sulphur compound allicin, which in turn produces other sulphur compounds, including ajoene, allyl sulfides, and vinyldithiins (Koscielny *et al*., 1999).

2.7.2.2 Neem (*Azadirachta indica* A. Juss)

Neem is a miraculous tree with myriad of uses. The bitter taste of neem is due to the presence of an array of complex compounds called limnoids (triterpenoids) (Sindhumol, 2007). Ten limnoids have been isolated and identified in neem seeds viz., salanin, salannol, salannol acetate, diacetyl salanin, 14-Epoxy azaradion, gedunin, nimbine, D-acetyl nimbenin, azadirachin and azadirachtin (Sindhumol, 2007). Of these, azadirachtin is the most active compound (Sindhumol, 2007).

2.7.2.3 Ginger (*Zingiber officinale* Rosc.)

The primary active ingredients in ginger are the pungent principles that give the plant its special aroma and flavour. The active ingredients in ginger are gingerols and shogaols, gingerdiones and zingerone (Joe and Terry, 2005). Studies have shown that gingerols have a similar structure to capsaicin, the active ingredient of *Capsicum* (Danny, 2001).

CHAPTER THREE 3.0

MATERIALS AND METHODS

Three main experiments were conducted in this study:

(i) A survey conducted in Ofoase-Kokoben and Konongo-Obenemasi, pepper growing communities in the Ashanti Region of Ghana to document farmers' perception and knowledge on the anthracnose disease.

(ii) Laboratory experiments: Seed health tests and treatment of the pepper seeds with appropriate aqueous botanical extracts and Mancozeb.

(iii) Field experiment: Screening of pepper varieties and accessions for resistance to anthracnose in a hotspot in the minor and major seasons at Konongo in the Asante Akim Central Municipality in the Ashanti Region of Ghana.

3.1.0 SURVEYS: Surveys conducted in Ofoase-Kokoben and Konongo-Obenemasi, pepper growing communities in the Ashanti Region of Ghana to document farmers' perception and knowledge of pepper anthracnose disease

3.1.1 Surveys to document farmers' knowledge and perception on pepper anthracnose disease

Surveys were undertaken in two major pepper growing communities in the Ashanti Region of Ghana. The two communities were Konongo-Obenemasi in the Asante Akim Central Municipality and Ofoase-Kokoben in the Amansie East District in the transition and forest agroecological zones, respectively. Farmers were interviewed by administering questionnaires

(Appendix 1) to find out their knowledge on anthracnose and other diseases and pests of pepper. Twenty farmers were interviewed in each of the two communities. The farmers were selected at random in each community and the interview was done with the aid of well-structured questionnaires. Pepper seed samples were also collected from the farmers interviewed in each community.

3.2.0 Seed health test and treatment of the pepper seeds with aqueous botanical extracts and Mancozeb

The laboratory work was conducted at the Plant Pathology Laboratories of the Faculty of Agriculture, KNUST, Kumasi and Centre for Scientific and Industrial Research-Crops Research Institute (CRI), Fumesua. The survey was conducted in June, 2011.

3.2.1 Seed health tests

Sets of three blotters moistened with sterile distilled water were placed in Petri dishes. Twenty-five seeds were placed on the moist blotters in each Petri dish following techniques described by International Seed Testing Association (ISTA) (2005). *Capsicum annuum* var. Meko hwam seed samples were used for this test. A total of 200 seeds were examined from each sample. The plated seeds were then incubated at 24 ±1 °C under alternating cycles of 12/12 h of near ultra-violet light and darkness for seven days. On the eighth day of incubation, the seeds were examined for the presence of fungal growth with the aid of a stereo microscope. Fungi were identified with identification manuals (Mathur and Kongsdal, 2003; Kulshrestha *et al*., 1976; Barnett and Hunter, 1972).

Fungi species that could not easily be identified on blotter were plated further on potato dextrose agar (PDA). The potato dextrose agar was prepared from scratch. The potato was scrubbed clean and was cut with a kitchen knife into pieces without peeling them. Two hundred grams of the pieces were taken and were rinsed rapidly with water and were boiled until soft.

It was then mashed and the pulp was squeezed with fine sieve. 20 g of dextrose and 20 g of agar were then added and then boiled till dissolved. It was then made up to 1 L before autoclaving for 20 min to kill any organisms in it. Fungal species growing on the PDA were identified based on their spore characteristics using a compound microscope following description of Mathur and Kongsdal (2003).

3.2.2 Studies on efficacy of botanical extracts in controlling seed-borne pathogens of pepper

3.2.2.1 Preparation of aqueous ginger rhizome extract

Rhizomes of ginger bought from the commercial area, KNUST, were peeled and washed thoroughly with water. They were blended into a fine paste with an electric blender (Binatone, BLG-401, Hong Kong) at 4000 rpm for 10 min. Sixty grams of the blended ginger was put into a beaker and 40 ml of sterile distilled water added and stirred thoroughly with a glass rod to obtain an aqueous extract concentration of 60 % (w/v) (Plate 1(b)).

3.2.2.2 Preparation of aqueous garlic extract

Bulbs of garlic also bought from the commercial area, KNUST, were peeled and washed thoroughly with tap water. They were blended into a fine paste with an electric blender (Binatone, BLG-401, Hong Kong) as described above. Sixty grams of the garlic paste was put into a beaker and 40 ml of sterile distilled water added and stirred thoroughly with a glass rod to obtain an aqueous extract concentration of 60 % (w/v) (Plate 1(a)).

3.2.2.3 Preparation of aqueous neem extract

Fresh leaves of neem obtained from Ayeduase behind Shalom Hostel, near KNUST, were washed thoroughly with tap water. The fresh leaves were blended into a fine paste with an electric blender (Binatone, BLG-401, Hong Kong) at 4000 rpm for 10 minutes. Sixty grams of the neem paste was put into a beaker and 40 ml of sterile distilled water was added and stirred thoroughly with a glass rod to obtain an extract concentration of 60 % (w/v) (Plate 1(c)).

3.2.2.4 Preparation of Mancozeb solution

Mancozeb was obtained from an agrochemical shop in Kejetia, Kumasi. Mancozeb solution was prepared by following the manufacturer's recommendation. About 7.5 g of the mancozeb fungicide powder was taken and dissolved in 1 L distilled water. After that 40 ml was taken for the experiment (Plate 1(d)). The Mancozeb was used as a check.

3.2.2.5 Treatment of the pepper seeds with the prepared aqueous botanical extracts

Capsicum annuum var. Meko hwam seed samples with the highest level of seed-borne pathogens were used in the study. The seeds were soaked in each of the prepared aqueous extracts separately for 24 h. Some of the seeds were also soaked in distilled water for 24 h which also served as a control. The soaked seeds were then air dried for 24 h in the laboratory. The air-dried seeds were platted on blotters following the ISTA (2005) procedure.

3.2.2.6 Experimental Design and Data Analysis

The data collected were the frequency of pathogens examined on the pepper seeds soaked in the distilled water, Mancozeb solution and the botanical extracts. The experimental design used was completely randomized design (CRD) with three replications and GENSTAT statistical package edition 9 (2007) was used to analyse the data. Differences in treatment means were evaluated for significance, using the least significant difference. Data on the control of *Colletotrichum* species and *Aspergillus* species by the botanical extracts, were transformed, using square root transformation ($\sqrt{x}$ + 0.5) before analysis was done.

Plate 1(a). Garlic and its aqueous extract

Plate 1(b). Ginger and its aqueous extract

Plate 1(c). Neem leaf aqueous extract

Plate 1(d). Mancozeb (synthetic fungicide)

Plate 1: Botanicals and Mancozeb used for the treatment of the pepper seeds

3.3.0 Screening of pepper varieties and accessions for resistance to anthracnose disease in disease hotspot area in the minor and major seasons

3.3.1 Experimental site and description

The experiment was conducted at Lowcost, a suburb of Konongo in the Asante Akim Central Municipality in the Ashanti Region of Ghana. The site has bimodal rainfall pattern. The area had been under cultivation of varieties of vegetables including pepper, tomato, okra and garden eggs for over ten years. The site was near the Oweri River, making watering easy in the minor season. The experiment was carried out between July and December, 2011 in the minor season.

3.3.2 Pepper varieties and accessions used and sources

The planting materials used were the accessions CRI 005, CRI 007 and CRI K82 and the varieties Shito adope, Meko hwam, Ohene nsatea, Legon 18 and African bird's eye. African bird's eye was used as a check. Seeds of all the varieties and accessions except Meko hwam and Ohene nsatea were provided by the Horticulture Division of CSIR-Crop Research Institute, Kwadaso, Kumasi. Seeds of Meko hwam and Ohene nsatea were obtained from farmers in Ofoase-Kokoben.

3.3.3 Nursing of pepper seeds

The pepper seeds were sown in a plastic tray containing sterilized soil. Thinning was done to remove some of the seedlings to avoid competition for nutrients.

3.3.4 Land preparation and field layout

The land was first cleared with a cutlass and burnt when dried. The field measured 35 m x 18 m and was divided into three blocks each measuring 35 m x 5 m. In between each plot was a 1.5 m space. Ridges were made across the slope. The ridges measured 0.5 m x 5 m and the interval between the ridges was also 1 m. Each accession or variety was replicated four times.

3.3.5 Transplanting and maintenance of pepper seedlings

Sunphosate was sprayed on the emerging grasses before transplanting was done. Healthy five-week-old seedlings raised from the seeds were transplanted onto the 0.5 x 5 m ridges.

Weed control was effectively done at two weeks intervals using a hoe. Fertilizer, (NPK, 1515-15) was applied two and four weeks after transplanting. Each plant received 5g of the NPK. Water from the Oweri River was used to irrigate the field in the minor season.

3.3.6 Disease assessment

The plants were monitored regularly to record the first symptoms and signs of anthracnose. Disease assessment was conducted 12, 14 and 16 weeks after seedlings were transplanted. Incidence of anthracnose in plots was recorded. Anthracnose disease severity was also recorded using disease assessment scale.

Disease severity was scored using a 0 – 5 scale described by Paul *et al*. (2008). Details of the disease severity scale used are presented in Appendix 2.

The experiment was repeated at the same location, using the same genotypes in the major season between February and July, 2012.

CHAPTER FOUR

4.0 RESULTS

4.1 SURVEY: Farmers perception and knowledge of anthracnose disease in OfoaseKokoben and Konongo-Obenemasi in the Ashanti Region of Ghana

Eighty percent of the farmers interviewed ranked pests and diseases as their second major constraint after credit acquisition (Table 4.1). Marketing and transportation were not constraints to farmers interviewed in the studied communities. Planting materials were not a major constraint to the farmers interviewed and only 5 % of the farmers interviewed were identified to experience that constraint.

Table 4.1: Identified constraints of pepper farmers in Ofoase-Kokoben and Konongo-Obenemasi in the Ashanti Region of Ghana

Constraints	No. of farmers who experienced constraint/ community		Total respondents (%)
	Ofoase-Kokoben	Konongo-Obenemasi	
Capital	19.0	16.0	87.5
Pests and diseases	15.0	17.0	80.0
Land acquisition	2.0	3.0	12.5
Marketing	0.0	0.0	0.0
Planting materials	1.0	1.0	5.0
Transportation	0.0	0.0	0.0

Farmers interviewed were all major pepper growers but also cultivate other crops. The other crops cultivated included tomato, okra, garden eggs, water melon, maize and plantain on a small scale (Fig. 4.1). Eighty percent of the farmers interviewed cultivated the *Capsicum annuum* var Meko hwam (Fig. 4.2).

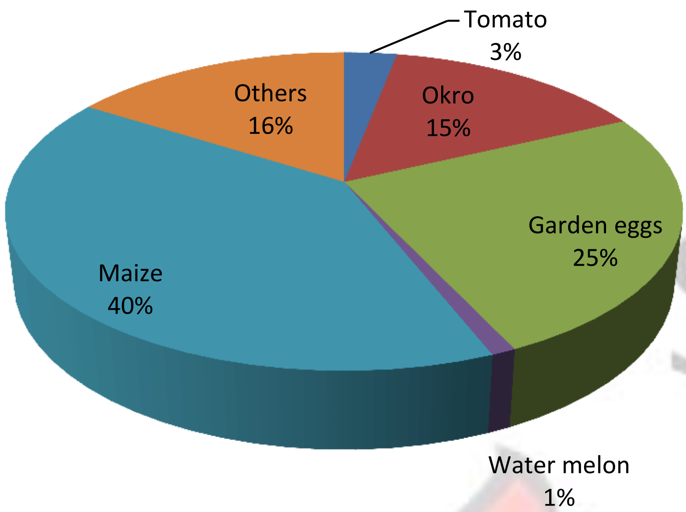

Fig 4.1. Percent distribution of other crops produced by the pepper farmers in Ofoase-Kokoben and Konongo-Obenemasi

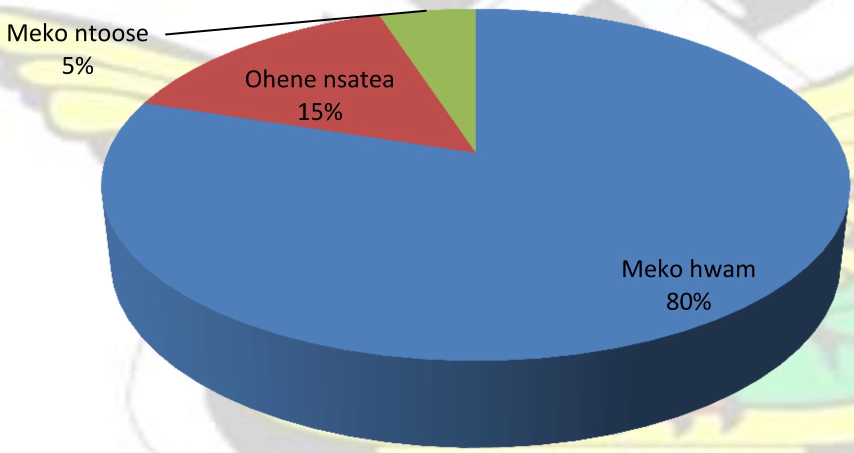

Fig 4.2. Percent distribution of varieties of pepper cultivated by pepper farmers in Ofoase-Kokoben and Konongo-Obenemasi

The livelihood of the farmers interviewed in the studied communities depends on the income accrued from the sale of the pepper produced. One of the setbacks was the menace of pests and diseases. Some of the insect pests that commonly attacked the pepper crop in the studied communities were aphids, caterpillars and grasshoppers (Fig 4.3).

In terms of diseases, pepper mosaic virus (PMV), pepper anthracnose and leaf spot were common in the communities. Twenty-nine of the interviewed farmers representing 72.5 % perceived the pepper anthracnose disease as a major problem (Fig 4.3). According to the farmers, they lose half of their produce and even more when the disease is severe in some seasons.

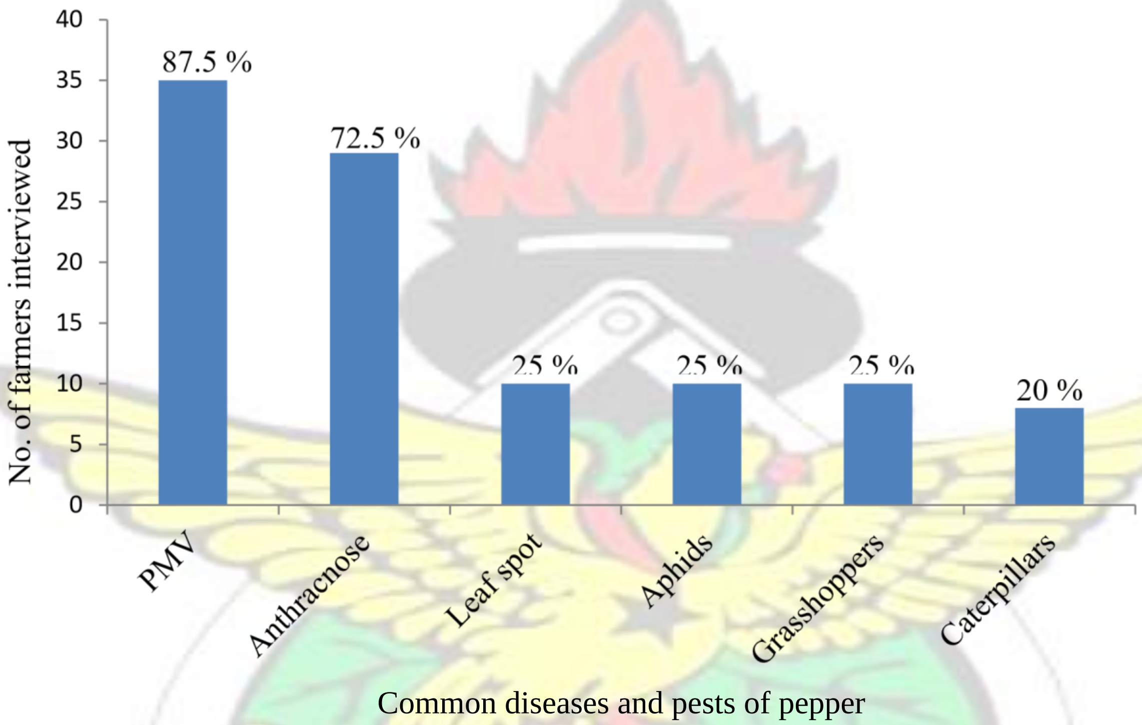

Fig. 4.3. Distribution of diseases and pests of pepper identified in Ofoase-Kokoben and Konongo-Obenemasi by farmers

All the farmers interviewed did not know the causal agent of the pepper anthracnose disease; nonetheless some controlled the disease with some fungicides. Farmers said the pepper anthracnose disease occurred mostly when rainfall was high. All the farmers interviewed also do not treat their seeds before sowing and about 75 % used their own seeds saved from previous harvest as planting material. Twenty percent of the farmers obtained their seeds from friends who saved some of the seeds from previous harvest and 5 % of the farmers also obtained their seeds from the local markets (Table 4.2).

Table 4.2: Sources of planting materials of the pepper farmers in Ofoase-Kokoben and Konongo-Obenemasi

Source of seeds for planting by farmers	% Acquisition by farmers
Farmers' own seeds	75
From friends	20
Local markets	5

4.2.0 Seed health tests and treatment of the pepper seeds with appropriate aqueous botanical extracts and Mancozeb

4.2.1 Identified seed-borne pathogens of pepper seeds in the two studied communities

Colletotrichum capsici was the most frequent fungus identified on the seed samples collected from Ofoase-Kokoben (Table 4.3). *Fusarium solani* and *F. verticilloides* also important pathogens of pepper were identified on 35.3 and 29.4 %, respectively, from seed samples from the Ofoase-Kokoben community. *Aspergillus flavus* were identified on 17.6 % of seed samples collected from the Ofoase-Kokoben community (Table 4.3). Only one sample out of the total number of samples tested was infected by *Colletotrichum gloeosporioides*, *Curvularia pallescens*, *Myrothecium* species, *Macrophomina phaseolina*, *Cladosporium sphaerospermum* and *Penicillium* species and recorded a fungal incidence of 5.9 % each (Table 4.3).

Table 4.3. Incidence of infection of pepper seeds by fungal species from samples from Ofoase-Kokoben

Fungal species	Total number of samples tested	Number of samples infected	Incidence of fungi (%)

Fusarium verticilloides	17.0	5.0	29.4
Fusarium solani	17.0	6.0	35.3
Colletotrichum capsici	17.0	7.0	41.2
Colletotrichum gloeosporioides	17.0	1.0	5.9
Curvularia pallescens	17.0	1.0	5.9
Myrothecium sp.	17.0	1.0	5.9
Macrophomina phaseolina	17.0	1.0	5.9
Rhizopus sp.	17.0	2.0	11.8
Aspergillus niger	17.0	2.0	11.8
Aspergillus flavus	17.0	3.0	17.6
Cladosporium sphaerospermum	17.0	1.0	5.9
Penicillium sp.	17.0	1.0	5.9

Colletotrichum capsici was also the most frequent fungus identified on collected seed samples from Konongo-Obenemasi (Table 4.4). *Fusarium solani* and *F. verticilloides* also important pathogens of pepper were identified on 27.8 and 22.2 %, respectively from seed samples from the Konongo-Obenemasi community. *Aspergillus flavus* were identified on

11.1 % of seed samples collected from the Konongo-Obenemasi community (Table 4.4). Only one sample out of the total number of samples tested was infected by *Curvularia pallescens*, *Myrothecium* species, *Macrophomina phaseolina*, *Aspergillus niger*, *Cladosporium sphaerospermum* and *Penicillium* species and recorded a fungal incidence of

5.6 % each.

Table 4.4. Incidence of infection of pepper seeds by fungal species from samples from Konongo-Obenemasi

Fungal species	Total number of samples tested	Number of samples infected	Incidence of fungi (%)
Fusarium verticilloides	18.0	4.0	22.2
Fusarium solani	18.0	5.0	27.8
Colletotrichum capsici	18.0	6.0	33.3
Colletotrichum gloeosporioides	18.0	2.0	11.1
Curvularia pallescens	18.0	1.0	5.6
Myrothecium sp.	18.0	1.0	5.6
Macrophomina phaseolina	18.0	1.0	5.6
Rhizopus sp.	18.0	2.0	11.1
Aspergillus niger	18.0	1.0	5.6
Aspergillus flavus	18.0	2.0	11.1
Cladosporium sphaerospermum	18.0	1.0	5.6
Penicillium sp.	18.0	1.0	5.6

4.2.2 Control of seed-borne pathogens of pepper with botanical extracts

In the studies to establish the effectiveness of botanical extracts in controlling seed-borne pathogens of pepper, no single botanical product was completely effective against all the major seed-borne pathogens identified. Significant differences were observed between the botanical extracts and water in the control of *Colletotrichum* species and *Fusarium* species (Table 4.5). With the exception of garlic extract, which showed no significance in the control of *Aspergillus* species, the other botanical extracts showed significant difference ($P < 0.05$) in the control of the fungi compared with water. Aqueous garlic extract reduced the frequency of infection of *C. capsici* from an initial level of 7.3 to 0.7 % after 24 h treatment (Table 4.5). The same level of reduction on the infection level of *Colletotrichum* species was achieved with Mancozeb. Garlic extract reduced the infection level of *Fusarium* species from 39.0 to 25.3 % in treatment time of 24 h (Table 4.5).

Ginger and neem extracts were able to reduce infection level of *Aspergillus* species from 2.5 to 0.7 % (Table 4.5). Ginger extract was able to reduce infection levels of *Fusarium* sp. from 39.0 to 7.7 %. This compares effectively with infection reduction achieved with Mancozeb.

Table 4.5. Effect of treatments on the incidence of major seed-borne pathogens of pepper

Treatments	Frequency of fungal infection after treatments (%)		
	Colletotrichum sp.	*Fusarium* sp.	*Aspergillus* sp.
Water (control)	7.3	39.0	2.5
Neem leaf extract	4.1	19.0	0.7
Ginger extract	5.6	7.7	0.7
Garlic extract	0.7	25.3	2.3
Mancozeb	0.7	7.9	0.7
Lsd (5 %)	0.3	5.7	0.5
CV (%)	5.0	22.3	20.5

4.3 Screening of pepper varieties and accessions for resistance to anthracnose disease in the minor and major seasons in the field

The reaction of the pepper genotypes tested for resistance to anthracnose in the minor season is presented in Table 4.6. With the exception of the African bird 's eye (control), symptoms of anthracnose were observed in all of the other genotypes screened (Plate 2). Shito adope was

severely infected than the other susceptible varieties, recording a severity score of 4.0, 12 weeks after transplanting in the minor season (Table 4.6). Sixteen weeks after transplanting in the minor season, CRI K82 and Shito adope were severely affected by anthracnose, recording a severity score of 5.0 (the highest on the scale). CRI 005, CRI 007, Legon 18, Ohene nsatea and Meko hwam were found to be susceptible, 16 weeks after transplanting in the minor season (Table 4.6). The African bird's eye (control) did not develop any of the symptoms of anthracnose 16 weeks after transplanting in the minor season and, therefore, was highly resistant (Table 4.6). Generally, all the genotypes screened were highly susceptible or susceptible to anthracnose except the African bird's eye. Disease severity increased from the twelfth to the sixteenth week.

Table 4.6. Reaction of pepper varieties and accessions to anthracnose disease at 12, 14 and 16 weeks after transplanting to the field in the minor season

	Anthracnose disease severity (Scale 0-5)/weeks	

Pepper genotypes	12	14	16	*Reaction category
CRI 005	3	4	4	Susceptible
CRI 007	3	4	4	Susceptible
CRI K82	3	4	5	Highly susceptible
Legon 18	3	3	4	Susceptible
Shito adope	4	4	5	Highly susceptible
Ohene nsatea	2	3	4	Susceptible
Meko hwam	2	3	4	Susceptible
African bird's eye (Control)	0	0	0	Highly resistant

* Reaction category: 0 = Highly resistant; 1 = Resistant; 2 = Moderately resistant; 3 = Intermediately resistant; 4= Susceptible and 5 = Highly susceptible

Incidence of the disease in the minor season, in the susceptible genotypes was 100 % (Table 4.7). Incidence of the disease in the African bird's eye (Control) was 0 %, 16 weeks after transplanting in the minor season. The disease incidence increased from the twelfth to the sixteenth week after transplanting in the minor season (Table 4.7).

Table 4.7. Anthracnose disease incidence on the pepper genotypes at 12, 14 and 16 weeks after transplanting to the field in the minor season

Pepper genotypes	% Incidence of anthracnose disease/weeks		
	12	14	16
CRI 005	50	100	100
CRI 007	48	80	100

CRI K82	64	100	100
Legon 18	47	70	100
Shito adope	50	100	100
Ohene nsatea	37	69	100
Meko hwam	50	80	100
African bird’s eye (Control)	0	0	0

Plate 2(a). African bird’s eye without symptoms

Plate 2(b). CRI OO5 fruits with symptoms

Plate 2: Degree of susceptibility of the pepper genotypes to anthracnose

Plate 2(c). Legon 18 fruits with symptoms

Plate 2(d). CRI K82 fruits with symptoms

Plate 2(e). Shito adope fruits with symptoms

Plate 2(f). Ohene nsatea fruits with symptoms

Plate 2: Degree of susceptibility of the pepper genotypes to anthracnose

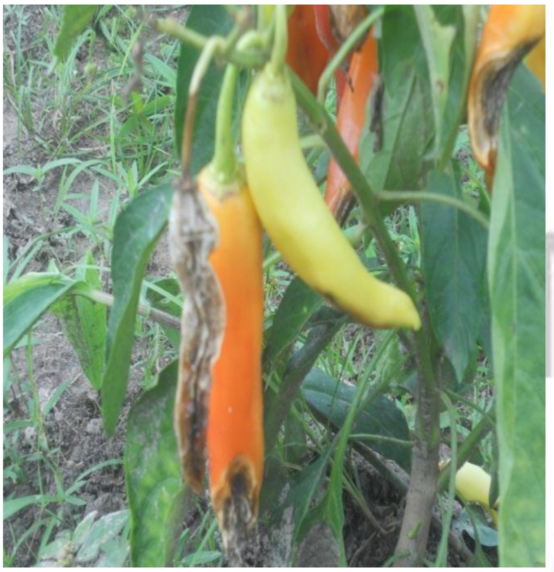

Plate 2(g). CRI 007 fruits with symptoms

Plate 2(h). Meko hwam fruits with symptoms

Plate 2: Degree of susceptibility of the pepper genotypes to anthracnose

In the major season experiment, CRI 005, CRI 007, CRI K82 and Shito adope recorded a disease severity score of 5, 16 weeks after transplanting in the major season indicating that it is highly susceptible (Table 4.8). Legon 18, Ohene nsatea and Meko hwam were found to be susceptible to anthracnose, 16 weeks after transplanting in the major season. The African bird's eye (control) recorded a disease severity score of 1, 16 weeks after transplanting in the major season indicating that it is resistant to anthracnose. Generally, all the genotypes screened were highly susceptible or susceptible to anthracnose except the African bird's eye which was found to be resistant. Disease severity increased from the twelfth to the sixteenth week.

Table 4.8. Reaction of pepper varieties and accessions to anthracnose disease at 12, 14 and 16 weeks after transplanting to the field in the major season

Pepper genotypes	Anthracnose disease severity (Scale 0-5)/weeks			*Reaction category
	12	14	16	
CRI 005	3	4	5	Highly susceptible
CRI 007	3	4	5	Highly susceptible
CRI K82	4	4	5	Highly susceptible
Legon 18	3	3	4	Susceptible
Shito adope	4	4	5	Highly susceptible
Ohene nsatea	3	3	4	Susceptible
Meko hwam	3	4	4	Susceptible
African bird's eye (Control)	0	1	1	Resistant

*Reaction category: 0 = Highly resistant; 1 = Resistant; 2 = Moderately resistant; 3 = Intermediately resistant; 4 = Susceptible and 5 = Highly susceptible

In the major season, incidence of the disease in the susceptible genotypes was 100 % (Table 4.9). Incidence of the disease in the African bird's eye (Control) was 8 %, 16 weeks after transplanting in the major season. The disease incidence increased from the twelfth to the sixteenth week after transplanting in the major season (Table 4.9).

Table 4.9. Anthracnose disease incidence of the pepper genotypes at 12, 14 and 16 weeks after transplanting to the field in the major season

Pepper genotypes	% Incidence of anthracnose disease/weeks		
	12	14	16
CRI 005	80	100	100
CRI 007	70	100	100
CRI K82	80	100	100
Legon 18	55	80	100
Shito adope	80	100	100
Ohene nsatea	54	80	100
Meko hwam	60	100	100
African bird's eye (Control)	0	4	8

CHAPTER FIVE

5.0 DISCUSSION

5.1 SURVEY: Farmers' perception and knowledge of anthracnose disease in OfoaseKokoben and Konongo-Obenemasi in the Ashanti Region of Ghana

In the surveys conducted, majority of farmers (about 80 %), acknowledged the importance of pests and diseases affecting their pepper yields. Pests and diseases were second to credit acquisition when priorities of constraints in pepper production were considered. Farmers in the two communities (about 72.5 %) acknowledged anthracnose as a major disease, and majority of them depended or were over depended on chemical fungicides to control the disease when severe outbreaks occurred, often, with very little success. This information from the farmers implies that other sources of effective management of anthracnose must be found. Marketing and transportation of the pepper were not constraints to the farmers because they have a ready market for their produce and the buyers come to them to buy their produce, hence reducing transportation cost.

Majority of the farmers interviewed (about 80 %) cultivated the *Capsicum annuum* var. Meko hwam. The reason was that unlike Ohene nsatea, Meko hwam can be harvested whilst green and most buyers prefer that variety. However, farmers said ripened fruits attract good price, compared to the unripen fruits. The price of a bag of ripened pepper fruits was twice the price of a bag of unripen pepper fruits.

About 75 % of the farmers use their own saved seeds of pepper for their next season's cultivation to save them from spending their scanty resources in buying seeds for planting. All the famers encountered did not treat their seeds before planting. Continuous use of farmer-saved seeds in the absence of seed treatment could contribute to high incidence of seed-borne pathogens including anthracnose on farmer's fields. According to Isaac (1992), over 70 % of farmers in developing countries use their own saved seeds continuously for planting.

5.2.0 Seed health test and treatment of the pepper seeds with aqueous botanical extracts and Mancozeb

5.2.1 Seed health tests

A number of the pathogens identified on collected seed samples from farmers in this study are pathogens that cause field diseases. *C. capsici* and *C. gloeosporioides* are involved in anthracnose disease. The incidence of infection on seeds by the anthracnose pathogens indicates the possibility of a reasonable transmission of anthracnose disease through infected seeds. *Colletotrichum capsici* was isolated most frequently from the seed samples collected from the studied communities, suggesting that the pathogen is seed-borne. Similar results were reported by Grover and Bansod (1970) and Rout and Rath (1972) on chilli seeds. Four different species of *Colletotrichum* are known to cause anthracnose disease of pepper including *capsici, gloeosporioides, acutatum and coccodes* (Kim *et al*., 2004). Two species, namely *capsici* and *gloeosporioides*, out of the four were identified in causing the disease in the two communities. Ramachandran *et al*. (2008) reported that surveys conducted in India revealed *C. capsici as* the most predominant species in the major chili growing states of Karnataka and Andhra Pradesh. Park *et al*. (1987) also reported that *C. gloeosporioides* was found to be the predominant species on chili in Korea. This clearly suggests that, depending on the geographical location, the predominant species of the fungi may vary.

Fusarium solani and *F. verticilloides* found on about 35.3 and 29.4 %, respectively, of seed samples collected from Ofoase-Kokoben is worth noticing because both pathogens can cause wilt diseases of pepper. Training farmers to develop skills in controlling seed-borne pathogens through the use of appropriate botanicals would possibly go a long way to reduce over dependence on chemical fungicides. Controlling seed-borne pathogens before planting can reduce severity of diseases on the field.

To establish the efficacy of botanical extracts in controlling seed-borne pathogens, aqueous garlic extract and aqueous ginger rhizome extract showed activity against *Colletotrichum* sp. and *Aspergillus* sp., respectively. Whilst garlic extract was very effective in controlling *Colletotrichum* species, it was not quite effective against *Fusarium* sp. Aqueous garlic extract and aqueous ginger rhizome extract have been shown by several workers to possess fungicidal properties. Syed (2012) reported that garlic was equally effective as Mancozeb in controlling *Colletotrichum* species. Ines *et al*. (2008), using different botanicals including garlic, neem, malunggay (*Moringa oleifera*) and Dithene M-45 to control anthracnose disease of mango seedlings reported that garlic and the fungicide were better in controlling the fungi. Shovan *et al*. (2008) also used different botanical extracts to control *Colletotrichum dematium* causing anthracnose disease in soybean and reported that garlic extract appeared to be the best in inhibiting the growth of the fungus. Ginger extract was active in controlling *Fusarium* and *Aspergillus* species. This result is in line with the findings of Fawzi *et al*. (2009) who found ginger to be effective in inhibiting the growth of *F. oxysporum*. Aidoo (2011) also reported that ginger extract was active in inhibiting the growth of *F. oxysporum.* Ginger rhizome extracts have been shown to possess a broad range of biological activity against fungi (Foster and Yue, 1992).

Neem leaf extract was found in this study to be very effective in controlling *Aspergillus* species on seeds of pepper but not *Colletotrichum* species. This was contrary to the findings by Ines *et al*. (2008) using different botanicals including garlic, neem, malunggay and Dithene-M45 to control anthracnose disease of mango seedlings reported that garlic showed the least mean number of infected leaves followed by neem. They concluded that the use of garlic and neem botanical extracts in controlling anthracnose of mango seedlings had been found promising.

In this study, none of the botanical products was found to be capable of controlling all the seed-borne pathogens effectively.

5.3 Screening of pepper varieties and accessions for resistance to anthracnose disease in disease hotspot area in the minor and major seasons

In the studies to identity resistant varieties or accessions to anthracnose disease, none of the evaluated varieties showed any resistant qualities. Variety, Shito adope was found to be highly susceptible to anthracnose disease. Legon 18, an improved variety and the local varieties Ohene nsatea and Meko hwam evaluated in this study were found to be susceptible to anthracnose disease. The accessions CRI 005, CRI 007 and CRI K82 were also found to be highly susceptible to anthracnose disease. Since CRI 005, CRI 007 and CRI K82 are still being improved, it is necessary that these genotypes are further improved to introduce anthracnose resistance genes into them. It is possible that the resistant genes of African bird's eye can be introduced into genotypes being developed to improve their resistance to the anthracnose disease. The severity of the disease was found to increase as the weeks advanced and more fruit tissues were affected. This is because once one fruit is infected, the spread is from the diseased fruits to healthy ones. AVRDC (2004) screened cultivars of pepper for resistance to anthracnose and observed a similar trend where more fruit tissues were infected as the days advanced and concluded that fruits should be harvested promptly since anthracnose spreads as the fruits age. More fruits were infected with anthracnose in the major season than the minor season, probably because heavy rains favour the growth of the fungi.

According to Robert *et al.* (2009), severe losses of the pepper fruits occurred during the raining season because spores are washed or splashed to other fruits, resulting in more infections.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

The major conclusion from this study is that, pepper farmers acknowledged anthracnose disease as a major constraint to pepper production. Anthracnose disease was recognised to be responsible for losses in yields incurred by farmers. Though farmers attempted to reduce loses, the survey revealed that they often were not successful with control using chemical fungicides.

Several of the seed samples tested for the presence of seed-borne pathogens carried high infections of *C. capsici*. This high incidence gives an indication that *C. capsici*; the causal organism of anthracnose of pepper, could be transmitted or could spread largely in the two studied communities through infected seeds. Nine genera of fungi including 12 species were identified from the pepper seed samples collected from the two communities. Two *Colletotrichum* species, namely *capsici* and *gloeosporioides*, were found to be associated with the pepper anthracnose disease in the studied communities. *C. capsici* was found to be the most predominant species of *Colletotrichum* in the studied communities.

Aqueous garlic and ginger rhizome extracts were found to be effective in controlling seedborne *Colletotrichum* sp. and *Aspergillus* sp., respectively. Aqueous neem leaf extract was found to be effective in controlling *Aspergillus* sp.

In the field studies, none of the evaluated varieties and accessions was found to be resistant to the anthracnose disease.

RECOMMENDATIONS

- It is recommended that aqueous garlic and ginger rhizome extracts could be used to manage seed-borne pathogens such as *Colletotrichum* and *Aspergillus* species, respectively.
- None of the varieties and accessions was found to be resistant to the disease, apart from African bird's eye. Breeding for resistance is, therefore, necessary to increase farmers' income and to ensure food security.

- It is also recommended that further research involving the evaluation of a greater number of varieties and accessions from breeding programmes be carried out to quicken the identification and development of anthracnose-resistant pepper genotypes in Ghana.

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APPENDICES

Appendix 1:

SURVEY ON PEPPER PRODUCTION AND THEIR CONSTRAINTS IN PEPPER PRODUCING AREAS IN THE ASHANTI REGION OF GHANA

Name……………………………………………………………

Community…………………………………………………..

District…………………………………………………

Date………………………………………………………..

1. What crops do you cultivate?..

2. Among these crops, which ones are your major crops?..

3. What are the major constraints facing you in farming?

....

....

....

4. What varieties of pepper do you cultivate?..

5. Which pepper diseases do you know affect your plants?.. 6. Do these diseases have an effect on the yield?...

7. If yes, what effect?..

8. What quantity of the yield is affected by the diseases?...

9. Do you use chemicals in treating the diseases you encounter?.......................................

10. If yes, what chemicals do you use? …………………………………………………

11. Where do you obtain the seeds for sowing?...

12. Do you treat the seeds before sowing? ……………………………………………………

13. If yes, with which chemical? …………………………………………………………

Appendix 2: Disease severity scale

Severity scale	Fruit surface affected (%)	Reaction category
0	0	Highly resistant
1	1-20	Resistant
2	21-40	Moderately resistant
3	41-50	Intermediately resistant
4	51-70	Susceptible
5	71-100	Highly susceptible

Appendix 3: Analysis of variance of percent control of *Aspergillus* species after 24 hours Analysis of variance

Variate: Percent control of *Aspergillus* species after 24 h

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Botanicals	4	10.19730	2.54932	31.91	<.001
Residual	10	0.79891	0.07989		
Total	14	10.99620			

Appendix 4: Analysis of variance of percent control of *Fusarium* species after 24 hours Analysis of variance

Variate: Percent control of *Fusarium* species after 24 h

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Botanicals	4	2237.33	559.33	30.07	<.001
Residual	10	186.00	18.60		
Total	14	2423.33			

Appendix 5: Analysis of variance of percent control of *Colletotrichum* species after 24 hours Analysis of variance

Variate: Percent control of *Colletotrichum* species after 24 h

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Botanicals	4	104.98320	26.24580	767.18	<.001
Residual	10	0.34211	0.03421		
Total	14	105.32531			