KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

STUDIES ON FUNGAL STORAGE ROT AND SEED-BORNE PATHOGENS OF ONION AND THEIR MANAGEMENT

BY

BARNABAS AYINEDENABA ADONGO

(BSc. HONS. AGRICULTURE)

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DISSERTATION PRESENTED TO THE SCHOOL OF GRADUATE STUDIES

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OF

MSc. CROP PROTECTION (PLANT PATHOLOGY) DEGREE

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DECLARATION

I hereby declare that, except for specific references which have been duly acknowledged, this Project is the result of my own research and has not been submitted either in part or whole for other degree elsewhere.

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Dr. Charles Kwoseh	……	……..
(Head of Department)	Signature	Date

DEDICATION

I dedicate this thesis to my late father, Mr. Adongo Akurigu.

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ABSTRACT

Surveys of fungi associated with postharvest deterioration of onion bulbs in four major markets in Kumasi Metropolis were conducted. Rotten onion bulbs obtained from the four markets: Abinchi, Anloga, Kwadaso and Central markets were infected by five fungal species: *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Rhizopus stolonifer* and *Fusarium oxysporum.* Of these, *Aspergillus niger* and *Penicillium* sp. were the most frequently isolated fungi. *Aspergillus flavus* was the least encountered fungus. *Rhizopus stolonifer* and *Aspergillus niger* were the most pathogenic. Black mould, Blue mould, Soft rot, Neck rot and Basal plate rot were the major postharvest diseases identified in a dry season survey in the markets. Incidences of these postharvest diseases were high in the wet season. Black mould enjoyed the highest incidence in all the markets. The lowest incidence was recorded for Blue mould. Seed-borne mycoflora of 37 samples of farmer-saved onion seeds from Bawku in the Upper East Region of Ghana were studied. Nine different fungal species were identified and isolated from the seeds. The most frequently encountered fungi species were *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Penicillium* sp. and *Fusarium verticilloides* with percentage occurrence of 33.3, 32.5, 25.8, 3.1 and 3.0 %, respectively. Efficacy of aqueous leaf extracts of Pawpaw (*Carica papaya*), Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*), Cassia (*Cassia alata*) and Tobacco (*Nicotiana tabacum*) in managing seed-borne fungi of Bawku Red were studied *in vitro* and *in vivo*. All the aqueous leaf extracts significantly ($P < 0.05$) inhibited the radial mycelial growth of the test fungi (*Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Botrytis* sp. and *Fusarium oxysporum*) *in vitro*. The highest percentage growth inhibition was achieved with aqueous Pawpaw leaf extract. In the *in vivo* test, all aqueous leaf extracts significantly ($P < 0.05$) reduced all the seed-borne fungi. Lastly, three fungicide/insecticide chemical seed dressants: Seed Power, Seedrex and Seed Star were evaluated for effectiveness in the management of seed-borne pathogens of onion. Seedrex was identified as the most effective seed dressant. However, seed germination was not significantly enhanced.

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CHAPTER ONE

1.0 INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown in Ghana and worldwide. In Ghana, commercial production of onion is done in the Northern and Upper East Regions especially, around Bawku and Bolgatanga. Intense onion production is also found in the Kwahu South District, Mankessim and Berekum areas of southern Ghana (Norman, 1992).

Onion is cultivated for its pungent or mild flavours and forms an essential ingredient in the diet of many people. According to Norman (1992), onion is relatively high in food value, intermediate in protein, rich in calcium and riboflavin. The distinctive characteristic of onion is due to the presence of alliaceous odour which accounts for its use as food, salad, spices, condiment and in medicine (Raju and Naik, 2007).

Onions form an essential part of our daily diet. Therefore, there is a relatively constant consumer demand for it all year round. However, onion production in Ghana is seasonal. Storage of onion, therefore, is important to make it available to consumers at reasonable price during the off-season and to afford the producers an opportunity to receive reasonable prices.

Several factors including sprouting, drying and rotting affect the storage life of onion in the markets and homes (Currah and Proctor, 1990). Bulb rotting, particularly, caused by fungal species, causes severe losses during storage. Losses in storage are high when poor storage methods are practised. Losses as high as 60 % during storage due to moulds have been reported (Tanaka, 1991). Losses in the range of 10 – 50 % due to

bulb rot could occur during storage within three months when varieties susceptible to rot organisms are handled (Matthananda, 1992). Observations in Ghanaian markets and homes indicate that, losses due to microbial attacks could be higher than what have been reported by other workers because of poor conditions under which onions are stored.

Ghana does not produce all its total requirements of onions annually but imports significant amounts of onions from Burkina Faso to fill demand gaps of the country. Finding solutions to postharvest losses caused by fungal organisms during storage could help improve availability of onion produced locally in the Ghanaian markets. By effectively controlling losses in the local onion industry, the country's dependence on imported onions could be reduced.

The seeds of onion, like any other seeds, can also serve as a source of inoculum for disease development in the field and later during storage of the bulbs. Onion production is, therefore, limited by fungal infections which cause considerable pre- and post-harvest losses that are largely seed-borne (El-Nagerabi and Ahmed, 2003). It is, therefore, expected that various pathogenic fungi would be associated with onion seeds and can contribute to the rot of bulbs during storage.

Despite the losses from onion bulb rot and seed-borne pathogens, little studies have been done in Ghana to identify the fungal genera or species responsible for the storage rot and the possible contribution of the onion seeds to the storage rots. The objectives of the study, therefore, were to:

i. determine the incidence of postharvest diseases of onion in selected local markets,

ii. identify fungal pathogens associated with storage rot of onion bulbs,

iii. identify seed-borne fungal pathogens of onion,

iv. evaluate the efficacy of some botanicals against fungi associated with both seed and bulb rot of onion *in vitro* and *in vivo* and

v. evaluate some fungicides for seed treatment of onion.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The origin and botany of onion

Onion (*Allium cepa* L.) is one of the oldest vegetables known to man and a major vegetable crop in West Africa. The centre of origin of the crop is situated in the region covering the Near East and Central Asia. The early Europeans introduced the crop to West Africa. Onion was introduced into Ghana from Burkina Faso and Northern Nigeria. The popular cultivar, Bawku, was introduced into Ghana around 1930 and was first grown at Bugri, near Bawku (Obeng-Ofori *et al*., 2007).

Allium cepa belongs to the family *Alliaceae*. The onion is a biennial crop grown as an annual. It is characterised by a pungent alliaceous compound, ally-propyl disulphide. The onion bulbs consist of thickened bases of leaves attached to a small conical stem. The bulbs vary from flat to round in shape. The leaves are long, round and hollow, often blue in colour. The flowers are small in terminal umbels and corolla colour is often greenish white (Obeng-Ofori *et al*., 2007; Norman, 1992).

2.2 Food value and uses of onion

The onion bulb contains 88 % water (Obeng-Ofori *et al*., 2007). A 100 g edible portion of onion contains 31calories energy; protein, 1.5 g; fat, 0.6 g; total sugar, 7.2 g; other carbohydrates 0.3 g; vitamin A, nil, thiamine, 0.04 mg; riboflavin, 0.02 mg, niacin, 0.1mg; vitamin C, 7 mg; Fe, 0.5 mg, Mg, 16.5, K, 150 mg; Na, 7 mg (Obeng-Ofori *et al*., 2007; Norman, 1992).

The mild types of onion are eaten raw whilst the pungent types are cooked. The pungent types are an essential ingredient in stews, gravy and soups. Those grown as

spring, salad or bunching onions are eaten raw with salad. The crop has some medicinal properties (Obeng-Ofori *et al.*, 2007).

2.3 Production of onion

According to the United Nations Food and Agriculture Organization estimates, there are seven million acres of land in the world producing over 37MT of onion each year. Onion is produced in 170 countries in the world. China ranks first in the world with respect to onion production followed by India, USA, Turkey, Pakistan, Iran, Indonesia, Vietnam and Myanmar (Kabir, 2001).

In terms of productivity, Korea Republic is the highest (67.25MT/ha) followed by USA (53.91MT/ha), Spain (52.06MT/ha) and Japan (47.55MT/ha). India being a second major onion producing country in the world has a productivity of 10.16 MT/ha (FAO, 2008) and Niger and Nigeria have 35.58MT/ha and 14.79MT/ha, respectively (FAO, 2008). Most onion production in West Africa is concentrated in Burkina Faso, Northern Nigeria, Niger, Northern Ghana and Senegal. Niger and Burkina Faso export onions to neighbouring West African states, whereas Senegal, exports onions to France (Norman, 1992)

In Ghana, onions are grown commercially in the Northern and Upper regions, especially around Bawku and Bolgatanga. Other production areas in Ghana are Ashaiman, Dawhenya, Akatsi, Nsawam, Prestea, Koforidua, Kwahu, Mankessim and Berekum Districts (Obeng-Ofori *et al*., 2007; Norman, 1992). Small-scale production is carried out in and around many urban centres.

2.4 Onion production constraints

The production of onion in Ghana is associated with low yields and poor storability. These problems are attributed to unavailability of technical knowledge on agronomic practices and postharvest techniques, incidences of pests and diseases, and lack of improved varieties and quality seeds (Shah *et al.*, 2011; Abbey and Oppong-Konadu, 1997). Other constraints to onion production include inadequate credit facilities, research and market information to farmers (Abbey and Oppong-Konadu, 1997).

The low and variable yields of onion as compared to those of other countries in Africa are, to a large extent, attributed to diseases. Postharvest losses, in particular, are mainly caused by both pre- and postharvest diseases such as Neck rot, Black mould and Fusarium basal plate rot. Bulb rot contributes 10-50 % of storage losses of different onion varieties within three months of storage under local conditions (Matthananda, 1992). During storage, some losses occur due to sprouting, drying and rotting (Currah and Proctor, 1990).

Poor quality onion seeds have been implicated as being carriers of some of the pathogens associated with bulb rot during storage. Bulb rotting is caused by a number of microorganisms. Among them are fungi which are the major causal agents responsible for storage losses (Padule *et al.*, 1996; Currah and Proctor, 1990).

2.5 Fungal diseases of stored onion bulbs

2.5.1 Neck rot

Neck rot, as the name implies, causes a soft rot in the neck and upper regions of infected bulbs. A black mass of sclerotia, the resting bodies of the fungus, develops below the dry outer skin of the decaying tissue. The sclerotia which are hard, dry

reproductive structures may be white at first but turn black with age. A grey mould of sporulating bodies may also develop on the surface of the decaying fresh scales (Brewster, 1994). These symptoms usually develop two to three months after the apparently healthy but infected bulbs are placed in store. Two pathogens, *Botrytis allii* Munn and *Botrytis byssoidea* J. C. Walker, are the causal agents, the former being the most common (Brewster, 1994). Occasionally, the leaf pathogen *Botrytis squamosa* J.C. Walker causes Neck rot on white-skinned bulbs.

Botrytis allii produces spores on the leaf tissue and these can invade the senescent tissues that occur at the tip of ageing onion leaves. The fungus then spreads down the leaf into healthy tissues. It is latent in fresh green leaves, causing no disease. By sequentially invading successive leaves when they start to senesce, the pathogen ultimately enters those leaves which swell at the base to form the outer fleshy scales of the bulb. It then remains symptomless in the bulb for many weeks after harvest. Infection may also enter the bulb neck at harvest time if damaged neck tissue is exposed to spores, but such infection is avoided by rapid drying at the necks after harvest (Brewster, 1994).

There is no evidence of spread from bulb to bulb in store and trials have shown a one – to – one relationship between the number of bulbs rotting in store and the number of infected plants present at harvest. The apparently progressive increase in the number of bulbs that rot in store is simply the result of differences in the time to start rotting between individual pre-infected bulbs. The pathogens also invade the inflorescence of onions and can diminish seed yield. Infected seed heads produce seeds which carry the infection (Brewster, 1994).

The disease is seed-borne, so its control is crucial. By applying a coating of the systemic benzimidazole fungicide (Benomyl) to seeds (1g a.i per kg seed), infection of the cotyledon from inoculum on the seed coat is prevented. A given percentage of infected seed results in a higher percentage of infected bulbs in a wet growing season than in a dry season. High humidity during the season of leaf growth favours spread from leaf to leaf and therefore, from plant to plant.

Spores may also emanate from infected debris in soil or in dumps of onion waste. The disease survives for just two years in soil debris. Therefore, control is possible by crop rotations of three or more years, provided seed-borne infection is eliminated. It is also likely that infected bulbs planted for seed production are a source of inflorescence and ultimately seed infection.

The neck rot pathogen is rapidly destroyed by temperatures of 30 °C or above. Therefore, if onions are topped in the field, they should be quickly taken into store and sealed at the neck by blowing warm air over the bulbs to destroy any inoculum invading the neck. The pathogen cannot infect dry senescent leaf tissue, so that in dry climates, onions field dried with the foliage intact are not at risk. Treatments with fungicidal foliar sprays or fungicidal treatments to harvested bulbs are not effective in disease control (Brewster, 1994).

2.5.2 Black mould

Black mould, caused by *Aspergillus niger* Van Tiegh, is a common onion postharvest disease under hot and humid storage conditions. Infected bulbs show black discolouration on the neck, shallow lesions on the outer scales, and streaks of black

mycelia and spores beneath the outer dry scales. In advanced stages of the disease, the fungus produces enzymes that soften the onion bulb tissue, favouring infections by secondary bacterial organisms. Infections spread from bulb to bulb by direct contact, through bruises or wounds, by mechanical means or by air-borne spores. Spores can germinate within three to six hours under high relative humidity, but germination is inhibited below 75 % relative humidity (Sumner, 1995).

Sporulation can take place in 24 h after infection (Salvestrin and Letham, 1994). The optimum growth temperature of *A. niger* ranges from 28 to 34 °C, and growth is inhibited below 17 and above 47 °C (Sumner, 1995). Thus, Black mould is prevalent when onions are stored under ambient high temperatures (> 30 °C) and humidity (> 80 % relative humidity) (Musa *et al.*, 1973).

Chemical and cold treatments can effectively control onion Black mould. Bulb treatment with a mixture of diethofencarb and carbendazole, thiabendazole or imazalil (Grinstein *et al.*, 1992) or fumigation with sulphur dioxide (Thamizharasi and Narasimham, 1993) can control black mould during storage. However, chemical treatment of bulbs is undesirable due to the potential health hazards. The bulbs should, therefore, be protected from moisture during and after harvest to prevent black mould development. Prompt and thorough curing and good ventilation during storage are also recommended.

2.5.3 Basal plate rot

Fusarium basal plate rot (FBR), caused by *Fusarium oxysporum f. sp. cepae,* is an important soil-borne disease of onions worldwide (Christopher, 2000). This disease begins in the field and continues after harvest as storage rot. Fusarium basal rot is a root and bulb fungal disease of onions in temperate and subtropical areas (Brayford, 1996). Losses from Fusarium basal rot can occur in the field and/or during storage (Christopher, 2000). In addition to onion, Fusarium basal rot affects other *Allium* species such as shallot (*Allium cepa* L. *var. ascalonicum* Backer), Welsh onion (*A. fistulosum L.*) and chives (*A. schoenoprasum* L.) (Havey, 1995; Kodama, 1994). *Fusarium oxysporum f. sp. cepae* invades the plant through roots and the basal stem plate via the soil. The disease progresses from slight discolouration of the basal plate to total necrosis, death of older leaves and the entire plant, and eventual rot of the internal bulb scales.

The visual symptoms of Fusarium basal plate rot can be observed on plant leaves, roots, basal stem plate and bulb scales of small seedlings, mature plants and dormant bulbs. Bulbs infected in the field may develop a symptomatic white to pinkish mould during storage. Symptoms on leaves of small seedlings are difficult to observe. If the environmental conditions are conducive to pathogen growth, *Fusarium oxysporum f. sp. cepae* will kill young seedling before visual symptoms are observed (Tahvonem, 1981). In addition, FBR can cause delayed seedling emergence, seedling damping off and stunted growth of seedlings (Entwistle, 1990). On mature plants, the first above-ground symptoms of FBR would be chlorosis of leaves. This chlorosis leads to tip necrosis and eventually progresses to entire leaf necrosis and plant death (Wall *et al.*, 1993; Havey, 1995; Brayford, 1996). The infection within the basal plate also causes root death and root abscission. A noticeable symptom of FBR is the separation of roots

from bulbs at the stem plate during uprooting. Within the basal plate, *Fusarium oxysporum f. sp. cepae* causes a brown discolouration of the basal plate tissue. Once the entire basal plate is destroyed, the stem plate can be easily removed from the rest of the bulb.

In severe cases, *Fusarium oxysporum f. sp. cepae* infects the basal portion of the bulb scales and white mycelium can be observed on the basal portion of exterior bulb scales. Symptoms are reduced at temperatures between 8 and 15 °C. In addition, FBR provides a mode of entry for secondary pathogens to infect the bulb scales. Losses to FBR can occur in the field and/or storage (Christopher, 2000). In Bangalore, India, the incidence of FBR ranged from 20 to 80 % of bulbs infected using bulb infection method (Somkuwar *et al.*, 1996). For onion cultivars grown in Brazil, the incidence of FBR ranged from 12 to 75 % of bulbs inoculated with *Fusarium oxysporum f. sp. cepae* as seedlings (Stadrick and Dhingra, 1996). FBR reduces the number of marketable bulbs in the field as well as reduce the weight of bulbs at harvest.

2.5.3.1 Control of Fusarium basal plate rot

Fusarium oxysporum f. sp. cepae can be controlled through host plant resistance, crop rotation, solarisation, biological control and fungicide application. Resistant cultivars are available for intermediate and long-day onions but not available for short-day onions (Christopher, 2000).

Losses to Fusarium basal plate rot can be significantly reduced through the use of FBR-resistant cultivars. Under field conditions, spring-planted cultivars such as Dawn, Impala and La Nina showed high levels of Fusarium basal plate rot resistance in Southern New Mexico. A crop rotation of four years with a non-susceptible host is

recommended before planting another onion crop in the field (Havey, 1995; Entwistle, 1990). Crop rotation with maize or spring wheat will reduce soil inoculum levels and onion bulb loss to FBR in the following years.

Field solarisation can decrease the incidence of Fusarium-caused disease (Katan *et al.*, 1980). Biological control using fungal and bacterial antagonists such as *Trichoderma species, Pseudomonas fluorescens and Bacillus subtilis* were effective against *Fusarium oxysporum f. sp. cepae* under *in vitro* conditions (Rasendrau and Ranganathan, 1996). For the chemical control option, seeds, sets and transplants can be treated with fungicides such as Benomyl to reduce losses to FBR and seeds treated (Koycu and Ozer, 1998).

2.5.4 Blue mould of onion

The disease is caused by various *Penicillium* species (Sumner, 1995; Maude, 1990). The later stage of the disease at harvest and during storage is called Blue mould and the earlier seedling stage is commonly called *Penicillium* decay (Sumner, 1995). Therefore, *Penicillium* causes both field and storage disease problems. Primary decomposition of onion bulbs in storage is usually due to infection by *Penicillium* rather than other pathogenic organisms (Bodner *et al.*, 1998, Bottcher and Gunther, 1994)

Onions with signs of disease are usually discarded before planting, so the seedling stage of the disease is usually of minor importance (Sumner, 1995). When infected onions are planted, the subsequent seedlings may appear wilted, chlorotic and/or stunted in growth (Sumner, 1995). The development of new roots is usually reduced in

number and the roots themselves are often stunted in growth. If the plants continue to survive they remain weak in growth and new bulb production is poor (Sumner, 1995).

Early symptoms are visible as pale yellowish blemishes, watery soft spots or purplish red stains on the outside of the bulb (Sumner, 1995). Initial infections are visible at the base of the bulbs as masses of blue-green spores (Bodner *et al.*, 1998, Sumner, 1995). Later infection may be hidden within the tissue and therefore are not noticeable until the dry scale around the infected onion is removed (Sumner, 1995). Onions that are infected with *Penicillium* are light in weight. Tissue decaying as a result of infection by *Penicillium* usually has a musty odour (Sumner, 1995). Affected onion bulbs may show little external evidence of disease until decay is advanced (Sumner, 1995).

2.5.4.1 Management of blue mould of onion

Onion bulbs need to be protected from physical damage in the field, during harvest and in storage regardless of what causes the physical damage (Sumner, 1995). Reducing physical damage to onion will reduce the potential for infection by *Penicillium*. Free moisture on bulbs at harvest and during storage should be removed through prompt drying (Sumner, 1995). Avoid planting infected onion bulbs or seeds. Treating onions with fungicides and disinfecting solutions such as sodium hypochlorite may be recommended (Sumner, 1995). Iprodione, a dicarboximide fungicide is the most common product registered for treatment of onion. The recommended rate is dipping bulbs for 30 minutes in a solution of 2 g of Iprodione per litre of water. No fungicide treatments are recommended if the bulbs are going to be consumed directly, although consumption of infected bulbs is not recommended since some *Penicillium* sp. produce substances toxic to humans (Jay, 2000).

2.6 Major Seed-borne pathogens of onion

2.6.1 *Aspergillus niger*

Aspergillus niger Van Tiegham causes black mould on the onion bulbs. It occurs on both coloured and white onions in the field, during transit or during storage and has been reported in the United States of America, the United Kingdom, Australia, Spain, Chile, Japan, India, Nigeria, Sudan and Turkey (Köycü and Ozer, 1997; Sumner, 1995). The disease was also observed on 10 % of the total dry onion shipments inspected in the New York market during 1972 – 1984 (Ceponis *et al.*, 1986). This pathogen attacks many fruits and vegetables through wounds or during ripening (Sumner, 1995).

The conidia are black, spherical, irregularly shaped and are borne in chains. Conidiophores arise from long, broad thick-walled, mostly brownish, sometimes branched foot cells. The conidiophore axis swells to form a vesicle on which prophiales are formed. Phialides (sterigmates) are borne in clusters from the prophialides. Spore cluster can be seen without magnification.

The symptoms of black mould, caused by *Aspergillus niger* begin to appear at germination stage of seeds, continue until the storage and also in store. The pathogens reduce seed germination, seedling emergence and vigour (El-Nagerabi and Ahmed, 2001; Ozer and Köycü, 1997; Hayden and Maude, 1992; Tanaka, 1991; Gupta *et al.*, 1984). *A. niger* generally causes significant reduction in germination of seeds, in severe cases, root and shoots cannot develop because of pre-emergence damping-off (Gupta and Mehra, 1984). The development of symptoms is closely related to temperature. *Aspergillus niger* has negative effects on seedling development at 30 and 35 °C, but fails to develop on seedlings grown at 13 and 15 °C (Hayden and Maude,

1992). Any visual symptom is not observed on set bulbs developing from contaminated seeds. However, visual symptoms can be seen on mature bulbs in the fields and in store. *Aspergillus niger* firstly appears as a small black spore masses under the outer dry scales of the bulb spread as strip lying from the base to neck parts. When the outer covering (dry scale) is removed, the spore masses are observed. The fungus grows on the inner scales of the bulbs in similar manner. In severe instances, spore masses cover all over the surface of the bulb tissues.

In many developing countries, bulbs are stored on mats, straw huts or stack, which may leak during the rainy season. This condition causes high humidity coinciding with optimal temperature for the growth and pathogenicity of *A. niger* (Maude and Burchile, 1998; Coskuutuna and Ozer, 1997; Hayden *et al.,* 1994). Transmission of this pathogen from naturally contaminated seeds to set bulbs is possible, without showing any symptoms during the seedling development. When these sets are examined, *A. niger* can be isolated easily from the roots and bulb tissue (Köycü and Ozer, 1997). It seems that the onion set is a source for the latent infection of this pathogen.

Hayden and Maude (1994) observed that *A. niger* could also be transmitted from contaminated seeds to stored bulbs. This fungus is also soil- and air-borne. It was determined that it could be transmitted from contaminated soil to seedlings and sets in temperate and hot climatic conditions (Köycü and Ozer, 1997; Hayden *et al.,* 1994).

In the tropical Sudan, its incidence in the air increased progressively in onion crops during the growing season (Hayden *et al.,* 1994). Mechanical wounds during harvesting, packing and storage are the other transmission means of the pathogen. Air contamination is important for the infection of seed stalks and flowers. *Aspergillus niger* can utilise the vulnerability of the flowers to penetrate the onion seed. When *A.*

niger spores reach the mature capsule prior to flower opening, the possibility of seed infections is the highest. The pathogen has also the capability of saprophytic parasitation on the senescing onion flowers and systemic invasion of other parts on onion plant and seed, to maintain its survival and reproduction (Sirois and Lorbeer, 1998). The optimum temperature for fungal growth is 28-34 °C; and growth is inhibited at 47 °C. Thus, the disease is more common in hot climates (30-35 °C) or under warm storage conditions (24-30 °C). Spores germinate well at relative humidity of 80-86 %. Free moisture must be present on the onion for 6-12 h for infection to occur (Sumner, 1995; Hayden and Maude, 1992).

2.6.1.1 Control of *Aspergillus niger*

Cultural practices include the thinning-out of seedlings produced from seeds in the seed bed and in the field, thereby reducing spread of the pathogen within crop canopy; avoidance of continuous cropping of onions on the same site, removal and incineration of onion leaves from the field after harvest; minimum disturbance of the foliage is checked during the growth of the crop to prevent the release of *A. niger* conidia; regular ventilation of stores is done to maintain humidity level at less than 80 % (Hayden *et al.*, 1994). The cultivar Akgun 12 revealed tolerance to infections of pre- and post-emergence damping-off and set rot after seed infestation with *A. niger* in controlled pot experiment (Ozer, 1998). The seeds of the cultivar Rossa Savones also exhibited resistance to the pathogen during germination (Ozer *et al.*, 1999).

Onion seeds should be treated with fungicides to help prevent seed rot and damping-off (Sumner, 1995). Results of *in vitro* studies previously suggested that Carbendazim, Benomyl, Thiram, Benomyl + Thiram, Prochloraz and Tebuconazole (Köycü and Ozer,

1998) were the best chemical products in controlling the pathogen. Among these, Benomyl and Thiram were used as treatment for reducing seed-borne *A. niger* of onion (Hayden *et al.*, 1994). It was reported that treatment of *A. niger*-infested onion seeds with Benomyl dust (1g ai/kg seed) or foliar spray of Thiram (0.4 g ai/ha) to plants grown from infected seeds under temperate (UK) conditions reduced the incidence of *A. niger* in the harvested crops. However, when seeds were naturally infected with this pathogen, the treatment of Benomyl + Thiram to seeds (2.5+2.5 g ai/kg seed) or soaking the seeds in hot water (15 min at 60 °C) reduced the incidence of black mould on bulbs grown in the field soil of Sudan that had not previously been used for onion production. In addition, these treatments were less effective in crops produced in fields regularly used for onion production (Hayden *et al.*, 1994). It was suggested that Prochloraz (0.90 cc ai/kg seed) and Thiram (1.35 g ai/kg seed) were the most effective chemicals for controlling *A. niger* infections from seeds and soil respectively (Köycü and Ozer, 1998).

Alternative compounds or treatments to pesticides have been evaluated. El-Nagarabi and Ahmed (2003) reported that dip and spray treatments of seedlings with a commercial product of *Trichoderma* (Promat) prevented *A. niger* infection of the bulbs. El-Nagarabi and Ahmed (2001) found that surface disinfection of onion seeds with 10 % garlic water extracts and sterile distilled water at 60 °C reduced seed infection, pre- and post-emergence damping-off and also enhanced the seedling growth in the field. Ozer *et al.* (2002) found that incorporation of the stalks of sunflower, alfalfa and Hungarian vetch, especially, sunflower stalks to soil after the harvest, suppressed seed rot by *A. niger* in naturally infested soil.

2.6.2 *Botrytis aclada* Fresen (Syn.: *B. allii* Munn)

Botrytis aclada is the causal agent of neck rot disease in onion. The pathogen has been considered as dominant species causing disease in the United Kingdom (Maude and Presly, 1977), Germany (Rudolph and Brautigam, 1990), New Zealand (Stewart and Franicevic, 1994), Korea Republic and Poland (Tylkowska and Dorna, 2001).

Mycelium is septate, branched and hyaline when young. Sclerotia are frequently formed on natural substrata, but they are less in culture. The mature sclerotium has a narrow rind and round cells and not thick-walled empty cells and a large medulla of filamentous hyphae loosely arranged in gelatinous matrix. When germinated, it produces abundant conidiophores with conidia. Conidiophores emerge from the ruptured rind originated in the medulla (Sadeh *et al.*, 1985). Sclerotia often form on the shoulders of affected bulbs and may be up to 10 mm in length. Sometimes they occur as solid crust around the neck area (Lacy and Lorbeer, 1995). Conidia and conidiophores take on a smoky gray appearance in mass. Conidia are narrowly ellipsoidal and hyaline. They are borne on brown rather short (about 1 mm) conidiophores with side branches at the tips, each of which has many appullae that swell gradually at the tips to form conidia on fine denticles (Lacy and Lorbeer, 1995).

The disease generally appears after the bulbs are stored. The fungus grows down through the inner scales and partially causes decay of the bulbs before external injury appears. Infected tissues usually appear soft and watery at first, but later turn brown and become spongy and light in weight. The disease reduces seed yield, and seed quality (germination, conductivity and field emergence). Quality of seeds produced by infected plants is significantly reduced (Tylkowska and Dorna, 2001; Rudolph, 1990).

A major source of the pathogen is the samples of infected seeds (Tylkowska and Dorna 2001; Maude and Presly, 1977). The pathogen *B. aclada* remains viable up to three and a half years in storage at 10 °C and 50 % RH (Maude and Presly, 1977). Seeds from 5 % non-healthy onion bulbs may be infected with the pathogen under favourable meteorological conditions (Tylkowska and Dorna, 2001).

The fungus can be transmitted from infected seeds to seedlings and bulbs. The fungus which remains attached to the cotyledons can attack the living tissues of the leaves of seedlings emerging from the soil. No symptom of the disease is observed on these leaves. Fungus produces conidiophores and conidia only after the leaves senesce and become necrotic. It invades the leaves at the tip parts, then grows downwards in the tissues and invades the neck of the onion bulbs at harvest; it penetrates deep in the neck tissue of maturing bulbs. The disease is mainly spread by conidia formed abundantly on conidiophores on plants in the field under high humidity. However, all seed infection may not result in seedling or bulb infections (Stewart and Franicevic, 1994).

The disease does not spread from infected bulbs to healthy ones during storage but the infected ones rot. The amount of neck rot in store is directly related to the percentage infection of onion seeds (Stewart and Franicevic, 1994). Maximum seed infection occurs in the stage of full bloom. Cool and rainy weather conditions are important for the infection of flowering shoots (Rudolph, 1990). *Botrytis aclada* is pathogenic on onion umbels and causes flower blight. In humid conditions, *B. aclada* forms lesions on onion seed stalks and expands to girdle the stalks and abort the umbels. The pathogen needs free moisture in order to colonise uninfected tissues and long period of continuous free moisture at 12 °C induce a substantial amount of blighting of florets

and immature seed capsule. Dry infected debris may remain in the soil surface after the crop has been cleared and in some cases rotted bulb in the field may release sclerotia into the soil. However, fungus does not survive in the soil on debris or as sclerotia for more the two years (Maude *et al.,* 1982).

The disease is prevalent in the areas with cool, moist weather conditions before and during harvest. It develops most rapidly between 15-20 °C. Fungal growth slows greatly at temperatures below 3 °C, but neck rot can continue to develop even at 0 °C over several months of storage.

2.6.*3 Fusarium oxysporum f. sp. cepae*

Fusarium oxysporum f. sp. cepae causes the Basal plate rot of onion. The pathogen exists in nearly every onion-growing area of the world (Köycü and Ozer, 1997; Havey, 1995). Incidence of Fusarium basal rot generally occurs during the development of seedlings and bulbs, and in storage and ranges from 2.9 to 80 %, depending upon the time of year, environmental conditions, cultivars and level of inoculum (Köycü and Ozer, 1997)

This fungus can be transmitted from the seeds to onion sets. Other pathogens such as *Aspergillus niger* in seeds, which grows very rapidly, can inhibit the development of *F. oxysporum* in artificial media. The pathogen also is a natural component of the soil microflora (Ford *et al.,* 1970). *Fusarium oxysporum* is transferred from seed to soil and it permeates the soil for a longer or shorter period and then to host as local or systemic infection. However, it is known that *Fusarium* sp. have a poor ability to compete with other microflora in natural soil (Ford *et al.,* 1970). The pathogen can be spread by infected debris, infected soil, irrigation water, farm equipment and onion transplant. Mechanical wounding resulting from cultivation from hand weeding and from clipping

plant roots before transplanting also spread the pathogen. However, the pathogen can cause the disease on unwounded bulbs also. In Colorado, Fusarium basal rot is often associated with maggot infestation, especially seed-corn maggot (*Delia platura* Mergen). However, they generally appear to be secondary invaders of diseased bulbs in onion fields (Everts *et al.*, 1985). The optimum soil temperature for the development is between 28 and 32 °C; but the disease can occur at soil temperature range of 15-32 °C (Kodama, 1994). The optimum P^H for growth is 6.6, but growth can occur at a P^H range of 2.2 to 8.4.

2.6.3.1 Control of *Fusarium oxysporum f. sp. cepae*

The disease can be controlled through crop rotation, host plant resistance, biological control and fungicide application. Crop rotation with a crop such as maize or spring wheat reduces soil inoculum levels and onion bulb rot. A crop rotation of four years with a non-susceptible host has been recommended (Havey, 1995).

Disease management strategies should be based on the relationship between onion maggots, seed-corn maggots and Fusarium basal rot and the importance of minimising stress and injury to the bulbs should be emphasized (Everts *et al.*, 1985). Various fungal antagonist such as *Beaveria bassiana*, *Trichoderma* and bacterial antagonists (*Pseudomonas fluorescens* and *Bacillus subtilis*) under *in vitro* conditions inhibited mycelial growth of the pathogen (Rajendran and Ranganathan, 1996).

A combination of *Trichoderma viride* and *Pseudomonas fluorescens* were most effective for reducing Fusarium basal rot incidence under pot and field conditions (Rajendran and Ranganathan, 1996). Different fungicides such as Benomyl,

Carbendazim, Iprodione, Metoxymethyl, Mercury chloride, Thiram and Prochloraz have been tested for eradication of *F.oxysporum f. sp. cepae* on onion seeds with varying degrees of success (Barnoczkine-Stooilova, 1998; Köycü and Ozer, 1998).

In greenhouse trials, Roberts *et al.* (1989) found that Maneb resulted in development of the highest percentage of healthy plants, alone and in mixture with Thiophonate-methyl gave the highest percentage germination in artificially infected soil with *Fusarium oxysporum f. sp. cepae.* It was also reported that Carbendazim and Benomyl protected seedlings from infection by this pathogen (Abd-El Razik *et al.,* 1990). In the following years, Benomyl + Thiram (1.50 +4.05 g ai/kg seed) and Prochloraz (1.35 cc ai/kg seed) were found to control infection by *F. oxysporum* on seeds and in soil. Organic amendments of naturally infected soil by this pathogen with stalks of sunflowers, alfalfa and Hungarian vetch reduced the incidence of the disease on the sets developed from seeds (Abd-El Razik *et al.,* 1990).

2.7 Other seed-borne pathogens of onions

Other fungi isolated from onion seeds include *Alternaria porri* (Ellis), *Aspergillus species, Beaveria bassiana, Botrytis byssoidea, B. cinerea* Pers., *B. squamosa, Cladosporium allium-cepae, Colletotrichum circinans* (Berkeley), *C. gloeosporioides* (Penz.), *Rhizoctonia solani* J.G. Kuhn, *Rhizopus stolonifer, Stemphylium botryosum* (Wall.) Simons, *Trichoderma* sp. *and Alternaria porri. Botrytis byssoidea, B. cinerea, B. squamosa* and *Stemphylium vesicarium* sometimes cause several lesions on leaves, and also reduce seed yield (Tylkovoska and Dorna 2001; Köycü and Ozer, 1997; Boff *et al.,* 1995; Aveling *et al.,* 1993).

Lesions may develop on the leaves by *A. porri* and *S. vesicarium* and may develop on floral parts of seed onion. Seeds may not develop or are shrivelled (Aveling et *al.*, 1993). It is not yet known as to whether *A. porri* is transmitted from infected seeds to seedlings, sets and bulbs (Aveling, 1993). Wet weather during the periods from anthesis to seed harvest appears to favour disease development (Crowe *et al.*, 1995).

Fusarium sp. and *Rhizoctonia solani*, known seed-borne fungi cause damping-off where onions are grown as a continuous crop in seed beds, field and or garden (Sumner, 1995). *Fusarium moniliforme* and *R. solani* are pathogenic and cause pre- and post- emergence damping-off of seedlings (Abd-El Razik *et al.*, 1990).

Penicillium cyclopium may appear on the wounded or unwounded bulbs in the field and storage (Sumner, 1995). It has been suggested that *A. niger* and *A. fumigatus* compete for the same niche in the onion leaf mycoflora, but *A. niger* is the more competitive (Hayden *et al.*, 1994). *Colletotrichum circinans* causes the smudge on the bulbs of white onion cultivars. *C. gloeosporioides* is known as the causal agent of twister or anthracnose disease (Boff, 1996; Hill, 1995). *Rhizopus nigricans* and *R. stolonifer* cause mushy rot on onion bulbs, particularly in the neck regions and require wounds to invade the onion bulbs (Abdel-Sater and Eraky, 2001; Sumner, 1995). Although all these fungi were isolated from the surface of onion seeds, it is not known whether they are important in the disease cycle and transmission. Seed-borne infection is relevant only if infected seeds germinate and transmit the pathogen to plant which then acts as primary disease source in crops (Maude, 1980; Neergaard, 1979).

2.8 Description and active ingredients of the various botanicals

2.8.1 Neem (*Azadirachta indica* A. Juss)

Neem (*Azadirachta indica*) belongs to the family *Meliaceae* (mahogany family). Neem contains many compounds which have been divided into two major classes; Isoprenoids and others (Devakumar and SukhDev, 1996). The Isoprenoids include diterprenoids and triterprenoids and their derivatives such as nimbin, salanin and azadirachtin. The non-isoprenoids include proteins (amino acids), carbohydrates, sulphur compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds (Devakumar and SukhDev, 1996; Kraus, 1995). According to Kausik *et al.* (2002), sulphur containing compounds such as cyclic trisulphide and tetrasulphide isolated from Neem leaves have antifungal activity against *Trichophyton mentagrophytes.*

2.8.2 Tobacco (*Nicotiana tabacum* L.)

Tobacco belongs to the family *Solanaceae* and a very important economic crop. Tobacco has been used as a pain reliever, laxative, for the treatment of worm infestation, dental caries, arthritis, skin diseases and ulcers. The use of Tobacco as a medicine has been descended because of the addictive tendency and harmful effects of nicotine present in it. Nicotine is a liquid volatile, colourless alkaloid found in the plants of *Solanaceae*. It constitutes approximately 0.6 to 3.0 % of the dry weight of tobacco (Chitra and Sivaranjani, 2012).

Tobacco leaf is a good bio-resource due to abundant phenolics and other bioactive substances (Chitra and Sivaranjani, 2012). According to Bazinet *et al.*, (2005) and Riuz *et al.* (1998), tobacco leaf is rich in polyphenols which possess various bio-activities and affects the colour and quality of Tobacco leaf. Suleimana (2011) reported that

Tobacco extracts had fungitoxic effects that controlled the mycelial growth of *Aspergillus viride* and *Penicillium digitatum* (Pers.Ex St.Am) Sacc.

2.8.3 Cassia (*Cassia alata* Linn.)

Cassia alata is one of the most important species of the genus *Cassia* which is rich in anthraquinones and polyphenols. The leaves of *C. alata* have been qualitatively analysed for the presence of primarily five pharmacologically active anthraquinones; rhein, aleo-emodin, chrysophanol, emodin and physcion as well as the flavonoid, kaempferol (El-Mahmood and Doughan, 2008; Moriyama *et al.*, 2001).

Idu *et al.* (2007) observed that preliminary phytochemical analysis of *Cassia alata* showed the presence of phenols, tannins, anthraquinones, saponins and flavonoids. Odunbaku and Lusanya, (2011) also corroborated this study; they further stated that the plant also had alkaloids and cardenolides. Sharma *et al.* (2010) also reported in their study that preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone glycosides, phenolic compounds; saponin glycoside and while aqueous extract showed presence of glycosides and phenolic compounds, saponin glycoside. These anthraquinone derivatives are well known to exhibit a variety of biological activities such as antimicrobial, antitumour, antioxidant, antifungal, cytotoxic and hypoglycaemic activities.

Abubacker *et al.* (2008) stated that aqueous extract of *Cassia alata* can bee used as a potential antifungal agent. They observed that the aqueous extract of *Cassia alata* had effect on *Aspergillus flavus, A. parasiticus, Fusarium oxysporum* and *Candida albicans*

(Robin) Berkhout. The leaves contain a fungicide, chrysophanic acid which is a common ingredient in soaps, shampoos and lotions (Ajose, 2007).

2.8.4 Pawpaw (*Carica papaya* L.)

Pawpaw belongs to the family *Caricaceae* and several species of have been used as a remedy against a variety of diseases (Mello *et al.,* 2008). Pawpaw is a perennial plant and it is distributed over the whole of the tropical areas of the world. The leaves contain active components such as papain, chymopapain, cystatin, tocophenol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates (Noriko *et al.,* 2010). Aqueous extracts of leaves and seeds are known to have antifungal activity against *Colletotrichum gloeosporioides* (Bautista-Banos *et al.,* 2002).

The high level of natural self-defence compounds in the tree bark makes it highly resistant to insect and disease infection (Joel, 2007). The seeds of Pawpaw according to Okoye (2011) have phenolic compounds which have antimicrobial potentials. Phenols, according to Oakenful (1981), have been extensively used in disinfections and remain the standard with which other bactericides are compared. The antifungal activity of Pawpaw leaves may be due to the action of the proteolytic enzyme, papain, which is the major component of Pawpaw latex. This enzyme, according to Olahan and Amadi (2006), has an adverse effect on the protein component of fungal cells and as a result hinders the growth and other activities of the cells.

2.8.5 Moringa (*Moringa oleifera* Lam.)

The leaves of Moringa are eaten in African countries such as Ghana, Ethiopia, Nigeria, East Africa and Malawi (Oluduro, 2012). The Moringa tree is cultivated for foods and

medicinal purposes (Olson, 2002). According to Thilza (2010), the Moringa leaf is a natural antihelmintic, antibiotic, detoxifer, outstanding immune builder used in the treatment of malaria and malnutrition. According to Oluduro (2012), the leaves of Moringa contain alkaloids, tannins, flavonoids and phenols. It is reported by Nwangburuka *et al.* (2012) that pre-treatment of seeds before storage with Moringa leaf extract reduces fungal infection and maintains the vigour of okro seeds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Surveys on incidence of onion postharvest diseases in four selected markets in the Kumasi Metropolis

Two surveys were carried out in four selected markets in the Kumasi Metropolis namely; Central, Anloga, Kwadaso and Abinchi markets to document incidence of onion postharvest diseases. The surveys were done separately, one in the dry season (January-February) 2012 and the other in the wet season (June-July) 2012. At each market, 50 onion bulbs were selected randomly from 10 onion traders and the type of diseases or rot (blemish) on each bulb recorded after careful visual observation of symptoms on the selected bulbs. The percentage disease incidence (PDI) for each disease was determined by using the formula (Raju and Naik, 2007) below.

$$PDI = \frac{\text{Number of infected bulbs}}{\text{Total number of bulbs}} \times 100$$

3.2 Assessment of fungal pathogens associated with storage rot of onions

3.2.1 Collection of onion samples

Onion bulbs showing symptoms of rot and discolouration were randomly collected from various markets in the Kumasi Metropolis. The onion samples were collected from various traders in the four selected markets by sorting and selecting the onion bulbs with symptoms of rot and discolouration. These bulbs were brought to the Plant Pathology Laboratory of Crop Research Institute, Kumasi, for microbial or pathological analysis.

3.2.2 Preparation of Potato Dextrose Agar (PDA) for isolation of fungi

Two hundred grammes (200g) of peeled potato, 20 g of glucose, 20 g of agar and 1L of distilled water were used to prepare the PDA. The peeled potato tubers were cut into pieces with a sterile knife, weighed, washed and boiled in 500 ml of the distilled water. The potato was then mashed in the boiling water and sieved three times through cheesecloth to obtain the potato extract. The 20 g of glucose was then added to the potato extract and stirred thoroughly, using a magnetic stirrer. The agar was then melted in the potato-glucose mixture in a beaker and was then topped with distilled water to 1 litre. The PDA was sterilised in an autoclave at 121 ^{o}C at 0.98kg/cm^{3} for 20 minutes. The PDA was left to cool to about 45 ^{o}C and it was dispensed into sterilised Petri dishes.

3.2.3 Isolation and identification of rot-inducing fungi from infected onion bulbs

The infected bulbs were stripped of their outer dry scales and small pieces of the infected onion bulbs were removed with a sterile knife and surface sterilised in 1 % bleach for one minute. The pieces of the scale tissues were then rinsed three times in sterile distilled water and allowed to dry in a sterile laminar flow cabinet. The scales were plated on PDA in 90 mm-diameter sterilised Petri dishes and incubated at 28 °C for seven days in an incubation room.

The developing fungal colonies were sub-cultured on fresh PDA plates to obtain pure cultures. The fungal isolates were identified, based on their cultural and morphological characteristics including shapes of spores or conidia (Barnett and Hunter, 1998). The frequency of isolation of each fungal species was recorded. The percentage occurrence was calculated using the following formula:

$$\% \text{ Occurrence} = \frac{\text{Number of times a fungus was encountered}}{\text{Total fungal isolations}} \times 100$$

3.2.4 Pathogenicity test using fungal isolates

Fresh healthy onion bulbs were removed of their outer dry scales. The inner fresh scales were swabbed with cotton wool soaked in 70 % ethanol aseptically. Holes were made in the bulbs, using 3 mm-diameter sterile cork borer and the tissue plugs were pulled out and exchanged with 3 mm diameter mycelial disc of each of the cultured isolated fungi. The fungal disc was inserted into the hole created and onion plug placed back. The wounded area was sealed with Vaseline to prevent extraneous infections. The bulbs were incubated for four weeks at 28 °C in the incubation chamber. Three replications containing two bulbs were prepared for each treatment (fungus). The control consisted of sterilised 3 mm PDA disc placed in holes made in healthy bulbs. Observation for rot (symptoms) development was made and the degree of pathogenicity of each fungus determined by measuring the extent of the rot (mm) on the inoculated bulbs with a ruler.

3.3 Seed health test of onion

Bawku Red seeds were collected from 37 farmers at Bawku in the Upper East Region of Ghana. The farmer-saved onion seeds were used for the seed health test.

The seed health test was conducted, using the Standard Blotter Method (Mathur and Kongsdal, 2003). The seed-borne fungal infections were determined by plating 400 seeds on moistened three-layer filter paper to provide enough moisture in the Petri dishes. Each Petri dish contained 25 seeds. The plates were incubated for seven days at 25±2 ^{o}C in alternating cycles of 12 h near-ultraviolet light (NUV) and darkness, and then examined for fungal infection (Mathur and Kongsdal, 2003). Identification of

fungi based on their habit characters and morphological characteristics was done, using stereo and compound microscopes. Sub-culturing for pure cultures of some of the fungal isolates was done as and when necessary, for proper and accurate identification. The mean percentage of isolations for each fungus was based on 400 seeds.

3.4 *In vitro* antifungal activity of botanicals on seed-borne fungi of onion

3.4.1 Source of fungi

The seed-borne fungi: *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Botrytis* sp., *Fusarium verticilloides* and *F. oxysporum* were isolated from naturally infected onion seeds, plated on PDA and continuously sub-cultured to obtain pure cultures as described in section 3.3 above.

3.4.2 Preparation and dispensing of aqueous leaf extracts

Fresh leaves of Pawpaw (*Carica papaya*), Neem (*Azadirachta indica*), Tobacco (*Nicotiana tabacum*) and Moringa (*Moringa oleifera*) were collected from various fields around KNUST campus. Seed Star (20 % metalaxyl, 20 % imidacloprid and 4 % anthraquinone; Nova Agro Limited) was used as the positive control. The fresh leaves of Pawpaw, Neem, Tobacco and Moringa were separately and carefully washed under running tap water. They were cut into pieces with a sterilised sharp knife. The aqueous extract of each leaf sample was prepared by blending 100 g of leaves of each plant separately in 70 ml of distilled water in an electric blender. The leaf extracts were sieved, using a cheese cloth to remove debris. The aqueous leaf extract were dispensed into sterilised Petri dishes containing PDA. To every 15 ml of the PDA, 5 ml of the aqueous leaf extract of each plant as well as a solution of Seed Star were added and thoroughly mixed by swirling gently the content of the Petri dishes.

3.4.3 Inoculation of amended PDA

The different amended PDA plates were inoculated at the centre with 5 mm disc of each test fungus (seed-borne fungi). The test fungi were obtained by plating infected onion seeds on PDA and sub-culturing them to obtain five-day old pure cultures. The 5 mm disc of the test fungi was obtained using a sterilised cork borer. The inoculated plates were incubated at 25±2 ^{o}C for a number of days until the growth in the negative control plates covered the entire Petri dishes. The medium with inoculum disc but without any extract served as a negative control.

Experimental design and Data analysis

Percentage inhibition of mycelial growth by the leaf extracts was calculated using the formula below (Ogbebor *et al.,* 2005).

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

Where;

C – Mycelial diameter of the control

T – Mycelial diameter of the treatment

3.5 *In vivo* antifungal activity of botanicals on seed-borne fungi of onion

The efficacy of the aqueous leaf extracts of Neem, Moringa, Pawpaw, Tobacco and Cassia were tested on onion seeds with high natural infections of *A. niger, A. flavus, F. verticilloides, F. oxysporum* and *Penicillium in vivo*. The aqueous leaf extracts were prepared as described in section 3.4.2 above. The onion seeds were soaked in the aqueous leaf extracts separately for different durations viz: 1, 2, 6 and 24 h. Untreated naturally-infected seeds soaked in water served as control. The efficacy of the extracts was tested using the blotter method described in section 3.3 above. The seeds were then examined under stereo and compound microscopes for fungal growth and with the aid

of laboratory reference, the various fungi were identified and their frequency of occurrence was recorded.

3.6 Evaluation of seed dressants for onion seed treatment

Three fungicide-insecticide seed dressants namely, Seed Star (20 % metalaxyl, 20 % imidacloprid and 4 % anthraquinone), Seedrex (15 % carbendazim, 12 % chlorothalonil and 33 % permethrin) and Seed Power (20 % metalaxyl, 20 % imidacloprid and 4 % anthraquinone) were evaluated for their effects on onion seed-borne fungi *in-vivo* by the slurry method of seed treatment. Slurry of the seed dressants were obtained by dissolving 2 g of each chemical in 6 ml of sterile distilled water. Twenty grammes of naturally-infected onion seeds were uniformly mixed with the slurry of each of the three seed dressants for one hour. The seeds were then air-dried under ambient conditions. For each seed treatment, 400 seeds were randomly selected and then plated in Petri dishes using the moist blotter method (ISTA 2003). Twenty-five seeds per plate were placed in the plastic Petri dish of 90 mm diameter containing three well moistened blotters. The plates were then incubated at 25±2 °C under alternating cycle of 12 h of darkness and 12 h of light near ultra violet (NUV) for seven days. The seeds were then examined under stereo microscope for the presence of associated seed-borne fungi and germination. With the aid of pertinent literature and compound microscope, the examinations were made. The total number of seeds infected by specific seed-borne fungus was scored to determine percentage of seed infection (Mathur and Kongsdal, 2003).

CHAPTER FOUR

4.0 RESULTS

4.1 Surveys on incidence of onion postharvest diseases in four selected markets in the Kumasi Metropolis.

Table 1: Incidence of postharvest diseases of onion in four selected markets in Kumasi Metropolis during the dry season

	Postharvest disease incidence (%)				
Markets	**Black mould**	**Blue mould**	**Soft rot**	**Neck rot**	**Basal plate rot**
Abinchi	16.2	0.2	1.8	3.0	8.2
Central	6.0	0.2	2.8	2.8	1.0
Anloga	1.6	0.0	0.6	2.0	0.2
Kwadaso	0.6	0.2	2.4	0.2	0.2
Mean	**6.1**	**0.2**	**1.9**	**2.0**	**2.4**

In the dry season survey of the markets to determine the incidence of postharvest diseases of onion, five diseases were encountered and they are Black mould, Blue mould, Soft rot, Neck rot and Basal plate rot (Table 1). All the markets recorded very low incidences of the postharvest diseases (Table 1) with the exception of Abinchi market that had 16.2 % for Black mould. The most predominant disease during the dry season survey was Black mould, followed by Basal plate rot (2.4 %), then Neck rot, Soft rot and the least by Blue mould (Table.1).

Table 2: Incidence of postharvest diseases of onion in four selected markets in Kumasi Metropolis during the wet season

Markets	Postharvest disease incidence (%)				
	Black mould	**Blue mould**	**Soft rot**	**Neck rot**	**Basal rot**
Abinchi	22.5	2.0	32.3	10.4	6.0
Central	25.2	3.0	28.2	11.4	7.8
Anloga	31.3	2.1	17.0	12.6	5.3
Kwadaso	27.7	1.2	18.0	12.0	6.8
Mean	**26.7**	**2.1**	**23.9**	**11.6**	**6.5**

The survey, in the wet season, recorded increases in the postharvest diseases at the various markets (Table 2), compared with the dry season evaluation, Anloga market recorded the highest incidence of Black mould and Soft rot diseases (31.3 and 12.6 %), respectively (Table 2). Similarly, Abinchi market recorded the highest incidence (32.3 %) of Soft rot and the Central market recorded the highest incidence of Basal plate rot (7.8 %). In all the four markets surveyed, Black mould was the most predominant (26.7 %), followed by Soft rot (23.9 %), Neck rot (11.0 %), Basal plate rot (6.5 %) and the lowest incidence of 2.1 % by Blue mould (Table 2)

4.2 Assessment of fungal pathogens associated with storage rots of onion

Table 3: Percentage occurrence of isolated fungi associated with storage rots of onion bulbs for the selected markets

Fungus	Frequency of isolation from rotten bulbs (%)
Aspergillus niger	35.0
Aspergillus flavus	9.0
Penicillium sp.	27.0
Rhizopus stolonifer	14.5
Fusarium oxysporum	14.5

A total of four fungal genera and five species namely, *Aspergillus niger, A. flavus, Penicillium* sp., *Rhizopus stolonifer and Fusarium oxysporum* were isolated from the infected onion bulbs collected from the selected markets (Abinchi, Central, Anloga and Kwadaso) in the Kumasi Metropolis (Table 3).

Aspergillus niger recorded the highest percentage frequency of isolation (35.0 %), followed by *Penicillium* (27.0 %), then *Rhizopus stolonifer* (14.5 %) and *Fusarium oxysporum* (14.5 %), with *A. flavus* recording the least of 9.0 % (Table 3).

Table 4: Pathogenicity of isolated fungi on onion bulbs determined by the diameter of rot after 21 days of incubation

Treatment	Diameter of rot on onion bulb (mm)
Aspergillus niger	40.0
Aspergillus flavus	21.0
Penicillium sp.	16.0
Rhizopus stolonifer	43.0
Fusarium oxysporum	37.0
Control	12.0

In the experiment to establish pathogenicity of isolated fungi, the highest growth (rot) diameter was recorded with *Rhizopus stolonifer* (43.0 mm) in 21 days when bulbs of Bawku Red were incubated. The lowest growth (rot) diameter of 16.0 mm was recorded with *Penicillium* sp. (Table 4).

4.3 Onion seed health test

Table 5: Percentage occurrence of fungi on onion seeds from Bawku

Fungus	Percentage occurrence
Aspergillus niger	33.3
Aspergillus flavus	25.8
Penicillium sp.	3.1
Rhizopus stolonifer	32.5
Fusarium oxysporum	0.7
Fusarium verticilloides	3.0
Fusarium poae	1.2
Alternaria porri	0.2
Botrytis sp.	0.2

Using the moist blotter method as recommended by the International Seed Testing Association (ISTA), seed-borne mycoflora of 37 samples of farmer-saved onion seeds from Bawku in the Upper East Region were examined. Nine different fungal species were isolated from farmer-saved seeds collected from Bawku (Upper East Region). These fungal species were *A. niger, A. flavus, Penicillium* sp., *Rhizopus stolonifer, F. oxysporum, F. verticilloides, F. poae, Alternaria porri* and *Botrytis* sp. (Table 5) and (Plate 1). The frequently isolated fungi were *Aspergillus niger, Rhizopus stolonifer, A. flavus, Penicillium* and *Fusarium verticilloides* with frequency of isolation of 33.3, 32.5, 25.8, 3.1 and 3.0 %, respectively. The least encountered were *Alternaria porri* and *Botrytis* sp. (0.2 %) each (Table 5).

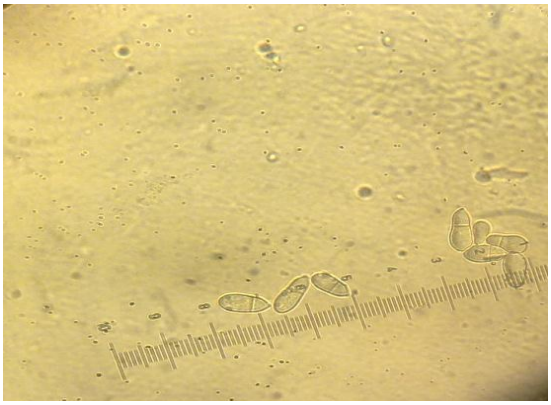

Plate 1a: Spores of *Fusarium poae*

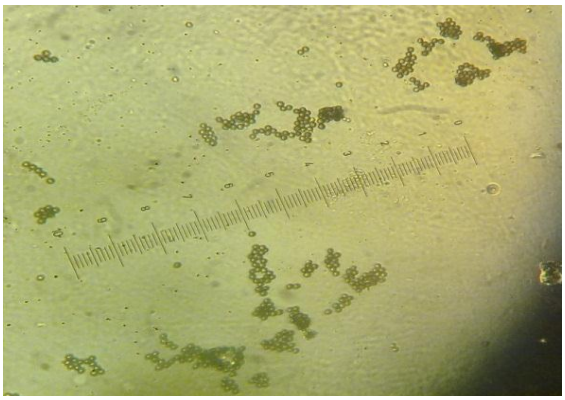

Plate 1b: Spores of *Aspergillus niger*

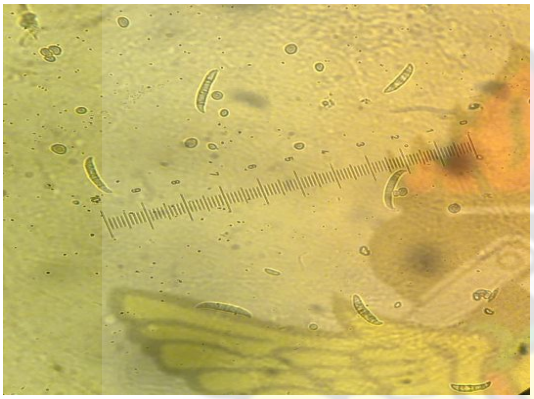

Plate 1c: Spores of *Fusarium oxysporum*

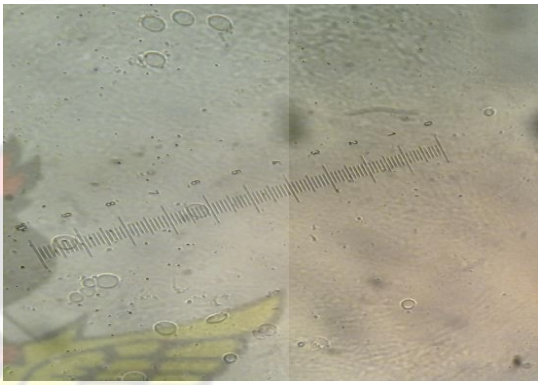

Plate 1d: Spores of *Botrytis* sp.

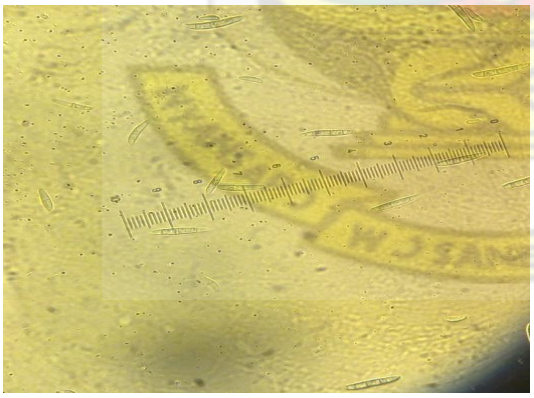

Plate 1e: Spores of *Fusarium verticilloides*

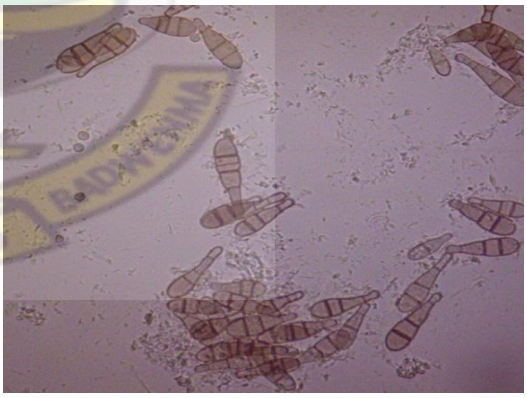

Plate 1f: Spores of *Alternaria porri*

Plate 1: Spores of some fungi associated with the farmer-saved Bawku Red seeds.

4.4 *In vitro* antifungal activity of botanicals on seed-borne fungi of onion

Table 6: Effect of aqueous leaf extracts on radial mycelial growth of onion seed-borne fungi using the food poison technique *in vitro*

Treatment	Percentage (%) inhibition of radial growth of fungi				
	Aspergillus niger	***Aspergillus flavus***	***Rhizopus stolonifer***	***Botrytis* sp.**	***Fusarium oxysporum***
Pawpaw	78.5 (8.9a)	77.5 (8.4a)	70.0 (8.4a)	67.0 (8.2a)	37.5 (6.2b)
Tobacco	65.2 (8.1d)	73.0 (8.6a)	22.5 (4.8c)	62.8 (7.9b)	71.8 (8.5a)
Moringa	52.5 (7.3e)	5.25 (7.5b)	5.00 (2.3d)	23.00 (4.8d)	37.25 (6.14b)
Neem	72.3 (8.5b)	73.5 (8.6a)	44.8 (6.7b)	64.3 (8.5ab)	72.8 (8.6a)
Seed star	68.3 (8.3c)	73.5 (8.6a)	42.3 (6.5b)	26.3 (5.2c)	26.8 (5.2c)
Control	0.0 (0.7f)	0.0 (0.7c)	0.0 (0.7e)	0.0 (0.7e)	0.0 (0.7d)
lsd (5%)	0.1	0.2	0.3	0.2	0.2
CV (%)	1.3	2.2	4.7	2.8	1.9

****The values in the brackets are square root transformed.***

The effects of the various plant aqueous leaf extracts and Seed Star (positive control) on mycelial growth of some seed-borne fungi of onion in vitro are presented in Table 6. All the leaf extracts significantly ($P < 0.05$) inhibited the radial mycelial growth of the test fungi (Table 6). All the aqueous leaf extracts gave highest percentage inhibition of *Aspergillus flavus* when compared with the results of other fungi (Table 6).

Inhibition of all the test fungi, except *F. oxysporum*, was highest with aqueous Pawpaw leaf extract. Crude aqueous extract of Pawpaw leaves inhibited growth of *A. niger* by

78.5 % (Table 6). This was the most effective. The least inhibition (52.5 %) was recorded for aqueous leaf extract of Moringa. Inhibition achieved with the chemical fungicide (Seed Star) was 68.3 %.

Crude aqueous extract of Pawpaw leaves was able to inhibit growth of *A. flavus* by 77.5 %. Percentage inhibition of growth achieved with crude aqueous extracts of Neem, Tobacco, Moringa and the chemical Seed Star were 73.5, 73.0, 55.3 and 73.5 %, respectively (Table 6). There was, however, no significant difference ($P > 0.05$) between the Seed Star, aqueous Pawpaw, Neem, and Tobacco leaf extracts.

Crude aqueous extract of Pawpaw leaves was able to inhibit growth of *Rhizopus stolonifer* sp. by 70.0 %. Percentage inhibition of growth achieved with crude aqueous extracts of Neem, Tobacco, Moringa and Seed Star were 44.8, 5.0, 22.5 and 42.3 %, respectively (Table 6). There was no significant difference ($P > 0.05$) between aqueous Neem leaf extract and Seed Star in their inhibition against *R. stolonifer*. Again, aqueous Pawpaw leaf extract recorded the highest inhibition (67.0 %) against *Botrytis* followed by Neem (64.3 %), Tobacco (62.8 %), Seed Star (26.3 %) and the least inhibition came from Moringa (23.0 %). However, the aqueous Pawpaw and Neem leaf extracts, showed no significant difference ($P >0.05$) between them. Aqueous Neem leaf extract inhibited the growth of *F. oxysporum* by 72.7 %, followed by Tobacco (71.8 %), Pawpaw (37.5 %), Moringa (37.3 %) and the least inhibition was recorded by Seed Star (26.8 %). However, there was no significant difference ($P >0.05$) between the aqueous Neem and Tobacco leaf extracts.

4.5 *In vivo* antifungal activity of botanicals on seed-borne fungi of onion

Table 7: Effect of aqueous leaf extracts on the incidence of individual seed-borne fungi of onion after different duration (1, 2, 6 and 24h).

Trnt	% incidence of seed-borne fungi of onion over different soaking periods/durations in hours																							
	A. niger				*A. flavus*				*R. stolonifer*				*Penicillium* sp.				*F. oxysporum*				*F. verticilloides*			
	1	**2**	**6**	**24**	**1**	**2**	**6**	**24**	**1**	**2**	**6**	**24**	**1**	**2**	**6**	**24**	**1**	**2**	**6**	**24**	**1**	**2**	**6**	**24**
Cassia	4.2c	3.7b	4.9c	5.9e	4.3b	4.5b	3.2d	4.2d	3.7c	3.6c	4.2d	5.2d	0.9b	1.4b	0.7a	1.6b	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a
Moringa	2.9b	2.8a	1.5b	0.7a	3.0a	3.2ab	1.8b	1.7b	3.2b	1.5a	0.7a	1.2b	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a
Neem	3.5b	2.8a	1.9b	1.4b	5.4c	3.2ab	1.0a	2.2b	2.5a	4.8d	1.7b	0.7a	1.2c	1.0a	0.7a	0.7a	1.0b	0.7a	0.7a	0.7a	1.0a	0.7a	0.7a	0.7a
Pawpaw	2.4a	2.5a	0.7a	1.2b	3.7ab	3.1a	1.2a	1.2a	3.5bc	2.8b	1.7b	0.7a	1.0b	1.0a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	0.7a	1.0ab	0.7a	1.0a
Tobacco	3.5b	2.5a	1.9b	2.1c	4.3b	3.1a	2.6c	3.3c	5.3d	5.0d	2.8c	3.5c	1.0b	1.2b	0.7a	0.7a	1.2c	3.5c	1.7b	1.0b	0.7a	1.2b	1.7b	0.7a
Control	5.5d	5.5c	5.5d	5.5d	3.9ab	3.9ab	3.9e	3.9d	6.8e	6.8e	6.9e	6.9e	1.9d	1.9c	0.9b	1.9c	1.2bc	1.2b	1.4b	1.2b	1.4b	1.4b	1.4b	1.4b
lsd (5%)	0.6	0.5	0.5	0.4	0.9	0.9	0.5	0.5	0.4	0.8	0.4	0.3	0.2	0.3	0.1	0.2	0.2	0.3	0.4	0.2	0.3	0.3	0.4	0.3
CV (%)	11.0	9.2	11.7	9.2	15.4	18.6	13.2	12.4	7.6	12.5	10.3	5.7	11.2	18.8	10.0	13.5	15.9	13.3	26.4	17.6	23.7	21.6	26.4	23.7

The values in the table are square root transformed.

In the *in vitro* experiment to determine the effectiveness of botanical extracts against the seed-borne pathogens in the treatment time of 1 hour, Pawpaw leaf extract reduced incidence of *A. niger* from 5.5 to 2.4 % (Table 7). Aqueous leaf extract of Neem was effective against *R. stolonifer* as it reduced its initial incidence of 6.8 to 2.5 %. None of the aqueous leaf extracts significantly ($P < 0.05$) reduced the incidence of *Aspergillus flavus*. *Aspergillus niger, Rhizopus stolonifer, Penicillium* sp. and *Fusarium oxysporum* were significantly ($P < 0.05$) reduced by all the aqueous leaf extracts. Aqueous Neem and Tobacco leaf extracts however were ineffective against *Fusarium oxysporum* incidence.

For the treatment time of two hours, *Aspergillus niger, Rhizopus stolonifer* and *Penicillium* sp. incidences were significantly ($P < 0.05$) reduced by all the aqueous leaf extracts. *Rhizopus stolonifer* in particular was effectively reduced from an initial incidence of 6.8 to 1.5 % by Moringa aqueous leaf extract. Again, there was no significant ($P > 0.05$) reduction in the incidence of *A. flavus* by the extracts. All the aqueous leaf extracts, except Tobacco, were significantly ($P < 0.05$) effective in the reduction of *Fusarium oxysporum* incidence. Pawpaw and Tobacco extracts showed no significant ($P > 0.05$) reduction of the incidence of *Fusarium verticilloides* in the two-hour seed treatment. However, generally, aqueous Moringa leaf extracts was the most effective botanical in the two-hour treatment time.

In the case of the six-hour seed treatment, all the individual seed-borne fungi were significantly ($P < 0.05$) reduced by all the aqueous leaf extracts, except Tobacco which was ineffective in significantly reducing the incidence of *Fusarium oxysporum* and *Fusarium verticilloides*. Cassia, Moringa, Neem and Pawpaw did not exhibit any

significant (P > 0.05) difference in their effectiveness against *Penicillium* sp., *Fusarium oxysporum* and *Fusarium verticilloides* (Table 7).

All the aqueous leaf extracts were significantly (P < 0.05) effective in the reduction of all the individual seed-borne fungi in the twenty-four hour seed treatment (Table 7). However, Tobacco did not significantly reduce *Fusarium oxysporum* incidence (Table 7) and Cassia was also ineffective against *Aspergillus niger*.

Table 8: Effect of different durations of treatment of aqueous leaf extracts on the total incidence of seed-borne fungi of onion

Treatment	Mean (%) seed infection/hour			
	1 h	2 h	6 h	24 h
Cassia	7.3c	6.7c	7.2b	9.0d
Moringa	5.3a	4.6a	2.3ab	2.5b
Neem	7.1c	6.5c	2.5b	2.5b
Pawpaw	5.9b	5.4b	2.1a	1.4a
Tobacco	7.8cd	6.7c	5.6c	5.1c
Control	9.9e	9.9d	9.9e	9.9e
lsd (5%)	0.6	0.6	0.4	0.8
CV (%)	5.4	5.6	5.3	9.9

The values in the table are square root transformed

The one hour treatment of the seeds with various leaf extracts reduced the total incidence of seed-borne fungi within the range of 5.3 to 7.8 % whiles the control recorded an incidence of 9.9 % (Table 11). Moringa aqueous leaf extract recorded the least incidence (5.3 %) followed by Pawpaw (5.9 %). All the aqueous leaf extracts significantly (P < 0.05) reduced the total incidence of the fungi.

The two-hour-seed treatment with the leaf extracts resulted in all the extracts significantly ($P < 0.05$) reducing the incidence of the seed-borne fungi. Pawpaw and Moringa extracts gave the highest reduction of the fungal pathogens. However, Cassia and Tobacco were not significantly different ($P < 0.05$) in reducing the fungal incidence.

Six-hour treatment durations further brought down the total incidence of the seed-borne fungi within the range of 2.1 to 7.2 %. Again, Pawpaw and Moringa were the best treatments, recording the incidence of 2.1 and 2.3 %, respectively. Cassia performed badly and recorded the highest incidence of 7.2 %. The twenty-four hour treatment also resulted in all the leaf extracts significantly ($P < 0.05$) reducing the total incidence of the seed-borne fungi. Pawpaw was the best treatment and reduced the incidence of the fungi to 1.4 %.

In general, the total percentage incidence of the seed-borne fungi decreased with increasing treatment duration for all the various aqueous leaf extracts, with the exception of Cassia which more or less took an opposite trend.

Table 9: Effect of different soaking durations of Bawku Red in aqueous leaf extracts on the percentage germination of seeds

Treatment	Percentage (%) germination/hour			
	1 h	2 h	6 h	24 h
Cassia	30.0a	36.0a	36.0a	37.0a
Moringa	45.0b	45.0b	60.0cd	61.0c
Neem	61.0d	61.5d	58.5c	65.5d
Pawpaw	60.0d	63.0d	63.0d	65.5d
Tobacco	55.0c	52.0c	60.5cd	62.0c
Control	54.0c	54.0c	54.0b	54.0b
lsd (5%)	3.3	3.4	3.3	3.1
CV (%)	4.4	4.4	4.0	3.7

For the one and two-hour treatments, only aqueous Neem and Pawpaw leaf extracts significantly ($P < 0.05$) increased the percentage germination of the seeds, compared with the control (Table 9). When the seeds were treated for six hours, all the aqueous leaf extracts except Cassia significantly ($P < 0.05$) enhanced the germination of the seeds. Pawpaw recorded the highest percentage germination (63.0 %) followed by Tobacco (60.5 %), Moringa (60.0 %) and Neem (58.5 %). In the 24 hours treatment, all the aqueous leaf extracts except Cassia, significantly ($P < 0.05$) increased the percentage onion seed germination as compared with the control. Aqueous Pawpaw and Neem leaf extracts recorded the highest percentage germination of 65.5 % but there was no significant difference ($P > 0.05$) between them.

4.6 Evaluation of seed dressants for onion seed treatment

Table 10: Effect of seed dressing fungicides on seed-borne fungi and seed germination of onion

Treatment	% Fungal incidence					Total incidence	% Germination
	An	Af	Rhi	P	Fo		
Seed Power	4.9b	6.6d	1.4a	1.9b	1.5b	73.0b	1.6b
Seed Star	5.7c	6.3c	2.7b	2.3b	1.8b	86.0c	0.7a
Seedrex	0.7a	0.7a	1.4a	0.7a	0.7a	2.0a	6.9c
Control	4.9b	1.4b	5.7c	2.9c	2.8c	99.5d	6.9c
lsd (5%)	0.2	0.2	0.4	0.5	0.7	3.2	0.2
CV (%)	3.9	2.4	9.1	15.3	24.9	3.2	3.9

An - (*Aspergillus niger*), Af – (*Aspergillus flavus*), Rhi – (*Rhizopus stolonifer*), P - (*Penicillium* sp.), Fo – (*Fusarium oxysporum*): *The values in the table are square root transformed.

In the experiment to identify an effective fungicide for seed dressing, Seedrex (15 % carbendazime, 12 % chlorothalonil and 33 % permethrin) was found to be more effective than Seed Power (20 % metalaxyl, 20 % imidacloprid and 4 % anthraquinone) and Seed Star (20 % metalaxyl, 20 % imidacloprid and 4 % anthraquinone). It

significantly ($P < 0.05$) reduced the incidence of all the seed-borne fungi viz: *A. niger, A. flavus, Rhizopus stolonifer, Penicillium* sp. and *F. oxysporum.* The incidences of all the seed-borne fungi were reduced to 0.7 % except *Rhizopus* whose incidence was reduced to 1.4 % (Table 10).

Seed Star and Seed Power were ineffective against *Aspergillus niger* and *A. flavus* (Table 10) and recorded higher incidences than even the control. However, the Seed Star and Seed Power were significantly ($P < 0.05$) effective against *Rhizopus stolonifer, Penicillium* sp. and *Fusarium oxysporum,* as compared to the control.

In general, in terms of total incidence, all the three fungicides resulted in significant ($P < 0.05$) reduction but Seedrex reduced the total incidence drastically from to 99.5 to 2.0 % as compared to 73.0 and 86.5 % by Seed Power and Seed Star, respectively.

Germination of seeds, however, was not enhanced by any of the three fungicides (Table 10). Seed Star and Seed Power were observed to have some inhibitory effect on germination since they had lower germination percent than the control.

Plate 2a: Bawku Red seeds treated with Seedrex

Plate 2b: Bawku Red seeds treated with Seed Power

Plate 2c: Bawku Red seeds treated with water (control)

Plate 2d: Bawku Red seeds treated with Seed Star

Plate 2: Plated Bawku Red-treated seeds on blotter paper in Petri dishes.

CHAPTER FIVE

DISCUSSION

5.1 Surveys on incidence of onion postharvest diseases in four selected markets in the Kumasi Metropolis.

From the surveys conducted in this study, Black mould, Neck rot, Blue mould, Soft rot and Basal plate rot were the most important postharvest diseases of onion identified. Among these postharvest diseases, Black mould was the most predominant disease in both the dry and wet season surveys. Raju and Naik (2007), in a similar survey, identified the same diseases as the important post-harvest diseases of onion in Karnataka-India. Ko *et al.,* (2002) and Musah *et al.* (1973) reported that Black mould is a major rot disease when onions are stored under ambient temperatures and therefore, corroborates these findings.

All the postharvest diseases encountered were caused by fungi when isolations were done, except the Soft rot which is caused by bacteria. This finding agrees with the observation made by Padule *et al.* (1996) that fungi are the major causal agents responsible for storage losses of onion bulbs.

According to Sumner (1995), the high incidence of Black mould is attributed to the ability of infections to spread from bulb to bulb by direct contact through bruises or wounds and by air-borne spores. Also, the spores can germinate within three to six hours under high humidity (Sumner, 1995) and sporulation in *Aspergillus niger,* the causal agent, can take place in 24 h after infection (Salvestrin and Letham, 1994).

In general, there was an increase in all the postharvest diseases during the wet season probably because of high relative humidity. This observation, according to Raju and Naik (2002), is due to favourable temperatures and high relative humidity during the wet season. Again, in the wet season, the onion bulbs had been stored for about six months by the traders and, therefore, were more vulnerable to storage rots than those in the dry season which were quite fresh from the farm. Moreover, onion bulbs, being a perishable commodity, retain about 86.8 % of moisture even when in long storage and this forms an ideal medium for proliferation of many storage fungi (Srinivasan *et al.,* 2002).

5.2 Assessment of fungal pathogens associated with storage rots of onion

Onion bulbs being a perishable commodity contain about 86.8 % of moisture and form an ideal medium for proliferation of many storage fungi (Srinivasan *et al.,* 2002). The finding in this study revealed that *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Aspergillus flavus* and *Penicillium* were associated with onion bulb rot in the major markets in Kumasi Metropolis. This finding is in conformity with that of Shehu and Muhammad (2011) who also isolated the above five fungi species from rotten onion bulbs in addition to *A. fumigatus* and *Alternaria porri.*

Ara *et al.* (2008) also isolated *A. niger, F. oxysporum, A. flavus* and *Penicillium* sp. from rotten bulbs of five varieties of onion in Bangladesh. Tyson and Fullerton (2004) also reported *Aspergillus* sp. that causes black mould as major isolate from rotten onions. These fungi in most reports cause severe losses. Losses as high as 35 % have been reported to be caused by *Fusarium oxysporum* (Christopher, 2000).

Aspergillus niger had the highest frequency of isolation (35 %). In fact, this observation is in line with findings of Ko *et al.* (2002) who reported that Black mould caused by *A. niger* is the major disease during storage under ambient conditions in the tropics. *Rhizopus stolonifer* had a faster rate of deterioration of the bulbs and this observation agrees with Shehu and Muhammad (2011) who reported that *Rhizopus stolonifer* was one of the most pathogenic leading to rapid disintegration of the infected onion bulbs. *Aspergillus niger* also had a faster deterioration rate. This observation, however, is in contrast to the findings of Shehu and Muhammad (2011).

5.3 Onion seed health test

In the seed health studies, the fungal isolates; *A. niger, A. flavus, Rhizopus stolonifer, Penicillium* sp., *F. oxysporum, F. verticilloides, F. poae, Alternaria porri* and *Botrytis* sp. were the major fungal species isolated and identified on the onion seeds of Bawku Red variety. Aveling *et al.* (1993) reported isolating the same fungi from infected seeds of onions. It was also observed that *A. niger* was the most encountered fungi among the tested seed samples. This observation corroborates the findings of El-Nagerabi and Abdalla (2004) that *Aspergillus* was the frequently encountered genus in the onion seeds examined in Sudan. The high prevalence of *A. niger* on the seeds could be attributed to its ability to utilize the vulnerability of the flowers to penetrate the onion seeds (Sirois and Lorbeer, 1998).

Although all these fungi were isolated from onion seeds, not all of them are known to be important pathogens in the transmission of onion diseases. Seed-borne infections are relevant only if, the infected seeds germinate and transmit the pathogens to plant which act as primary disease source in crops (Maude, 1980; Neergaard, 1979). Nevertheless,

some of these fungi are pathogens of known onion diseases. For instance, *Aspergillus niger,* in severe cases, prevents the development of root and shoot due to pre-emergence damping-off (Gupta and Mehra, 1984) and also it reduces seed germination, seedling emergence and vigour (El-Nagerabi and Ahmed, 2001; Koycu and Ozer, 1997). Hayden *et al.* (1994) observed that *A. niger* could also be transmitted from contaminated seeds to stored onion bulbs to cause Black mould diseases. Sumner (1995) reported that, *R. stolonifer* on onion seeds cause mushy rot on bulbs, particularly, in the neck region. *Botrytis* sp. causes neck rot disease of onion and *Fusarium oxysporum* causes basal plate rot and could be transmitted from seeds to onion sets (Koycu and Ozer, 1997). *Alternaria porri* and *Botrytis* sp. cause severe lesions or blight on onion leaves and also reduce seed yield. According to Abd-El Razik *et al.* (1990), *Fusarium verticilloides* is pathogenic and causes pre- and post-emergence damping-off of seedlings. The role of the seeds as one of the major sources of fungal infections and disease development in onion production in Ghana, therefore, can not be over-emphasized.

5.4 *In vitro* antifungal activity of botanicals on seed-borne fungi of onion

In the management of seed-borne pathogens of onion, the botanical products from Pawpaw, Neem, Tobacco and Moringa were found to be effective in reducing incidence of the various fungal pathogens on seeds of onion. The aqueous leaf extracts above and the Seed Star (positive control) significantly ($P > 0.05$) inhibited the radial mycelial growth of all the test fungi, with inhibition varying from one extract to another (Table 6). In similar studies, Nwachukwu and Umechuruba (2001) reported that aqueous leaf extracts of Pawpaw, Neem, Bitter leaf and Lemon grass inhibited the radial growth of all the major seed-borne fungi including *A. niger, A. flavus,* and *F.*

moniliforme of African yam bean (*Sphenostylis stenocarpa.* Hochst ex. Rich) seeds *in vitro.*

The highest percent inhibition of radial growth of all the test fungi was in aqueous Pawpaw leaf extract and lowest in Moringa extract (Table 6). This also agrees with the results of Adejumo *et al.* (2000), Owalade and Osikaniu (1999) and Amadioha (1998) who reported the efficacy of extracts from *Carica papaya* (Pawpaw) among other extracts in reducing the mycelial growth of *Erysiphe cichoracearum, Colletotrichum capsici* and *Protomycopsis phaseoli* which compared favourably with the chemical pesticides, Benlate and Ridomil. Ebele (2011) also reported that leaf extracts of *C. papaya* effectively inhibited mycelial growth of *Aspergillus niger, Botryodiplodia theobromae, Fusarium solani* and *Penicillium* sp. Therefore, the sterling performance of Pawpaw in inhibition of radial mycelial growth of the test fungi is consistent with other research findings.

Another observation was that, aqueous Neem leaf extract was second to Pawpaw in inhibiting the radial mycelial growth of the test fungi. This agrees with Babu *et al.* (2008) who reported that aqueous Neem leaf extract among others was highly effective in inhibiting the growth of *Fusarium solani f. sp melongenae,* the incitant of Brinjal (egg plant) wilt.

According to Suleimana (2011), Neem and Tobacco leaf extracts inhibited the vegetative growth of *Aspergillus viride, Rhizopus* sp. and *Penicillium digitatum in vitro*. Tobacco (*Nicotiana tabacum*), according to Akinbode and Ikotun (2008), significantly controlled the growth of *Colletotrichum destructivum in vitro*. The anti-fungal activity of tobacco is due chiefly to the presence of 1-5 % of alkaloid nicotine which was formerly used as an insecticide (Onwueme and Sinai, 1999). These

botanical products tested in this study, particularly, Pawpaw leaf extracts, could, therefore, be depended on in reducing seed-borne infections of onion.

5.5 *In vivo* antifungal activity of botanicals on seed-borne fungi of onion

In this study, it was observed that all the aqueous extracts significantly ($P < 0.05$) reduced the seed-borne fungi tested. However, aqueous Pawpaw, Neem and Moringa leaf extracts, generally, were more effective against the onion seed-borne fungi than the others. This observation agrees with the reports that many plant products contain fungitoxic constituents that have the potential to control plant diseases (Enikuomehin, 2005).

Again, this observation is consistent with the findings of De *et al.* (1999) that some important seed-borne pathogens such as *Fusarium oxysporum, Aspergillus niger, Penicillium* sp., *Phomopsis vexans* and *Aspergillus flavus* are managed by using some botanical plant extracts. The fact that Pawpaw and Neem were highly effective against the onion seed-borne fungi agrees with the report of Nwachukwu and Umechuruba (2001) that Neem and Pawpaw aqueous leaf among other leaf extracts significantly reduced the incidence of seed-borne fungi of African yam bean (*Sphenostylis stenocarpa*) seeds.

Neem, among other botanical leaf extracts, gave the best potential against all tested pathogens; *Aspergillus niger, A. flavus, Penicillium* sp., *Curvularia lunata, Fusarium oxysporum* and *Phomopsis vexans* with 4 % seed infection (Kuri *et al.*, 2011), therefore, the observation in this study is in order. The sterling performance exhibited by aqueous Pawpaw leaf extract against the seed-borne fungi is not strange since, according to Joel (2007), the Pawpaw has high levels of natural self-defence compounds that make it highly resistant to insects and disease infestation. Again,

according Petro *et al.* (2011), Pawpaw leaves are potential source of secondary metabolites (e.g. alkaloids) with antifungal properties.

Also, it was observed in this study that, generally, the total percentage incidence of the onion seed-borne fungi decreased with increasing treatment duration. This trend is attributed to the fact that there was increasingly more absorption of the extracts by the seeds when the treatment duration increased and as a result they had higher extract concentrations that inhibited the fungal growth.

Another observation made was that, all the aqueous leaf extracts, except Cassia, significantly ($P < 0.05$) increased the percentage germination of onion seeds. This is consistent with the findings of Nwachukwu and Umechuruba (2001) who reported of an increase in seed germination of African yam bean seeds when treated with Neem, Pawpaw, Bitter leaf and Basil leaf extracts. The ability of the extracts to increase seed germination could be attributed to the suppression of the incidence of the seed-borne fungi that could have killed the embryo of the seeds.

Finally, in terms of the efficacy of the various aqueous leaf extracts on the individual fungi *in vivo*, it was generally observed that, it varied with the type of leaf extract and the type of fungus. Overall, aqueous Pawpaw, Neem and Moringa leaf extracts were more effective than the rest in the control of all the seed-borne fungi.

5.6 Evaluation of seed dressants for onion seed treatment

To preserve the plant health during the first sensitive stage of their development (germination), seed treatment is usually recommended. In view of this, three

fungicide/insecticide seed dressants were evaluated against onion seed-borne fungi. Among the three seed dressants, Seedrex (15 % carbendazim, 12 % chlorothalonil and 33 % permethrin) was the only effective chemical against all the tested seedborne fungi of onion viz *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Penicillium* sp. and *Fusarium oxysporum*. This observation is consistent with the findings of Ibiam *et al.* (2006) that Bavistin (Carbendazim) among other fungicides was effective against seed-borne fungi of rice. Similarly, Vitavax, Thiram and Mancozeb, according to Huynh and Ashok (2005), eradicated seed-borne fungi of rice *in vivo*. Again, the sterling performance of Seedrex agrees with Aveling *et al.* (1993) that Carbendazim/flusilazole mixture significantly reduced the percentage infection of onion seeds by *Alternaria porri*. Carbendazim which is a systemic fungicide (Hewitt, 1999) and a component of Seedrex probably is responsible for this sterling performance against the onion seed-borne fungi.

According to Agrios (2005), fungicidal seed treatment is an inexpensive method for disease control that can protect the seedlings against a variety of fungal pathogens and improve emergence. Seedrex which is Carbendazim-based in spite of its effectiveness against the seed-borne fungi was unable to enhance seed germination significantly ($P < 0.05$), when compared to the control. This observation, however, contradicts the findings of Aveling *et al.* (1993) that Carbendazim increased seed germination of onion seeds. The inability of Seedrex to enhance the germination of the seeds can be attributed to the fact that the onion seeds had a very low germination percentage due to poor storage by the farmers.

On the other hand, the inability of Seed Star (20 % metalaxyl, 4 % anthraquinone and 20 % imidacloprid) and the Seed power (20 % metalaxyl, 4 % anthraquinone and 20 % imidacloprid) to effectively control the seed-borne fungi of onion could be due to the chemicals not being able to penetrate the seed tissues to kill internal mycelia of the fungi.

Again, the two seed dressants (Seed Star and Seed Power) were observed to have some inhibitory effect on germination of onion seeds by recording no germination at all. According to Neergaard (1977), an effective seed treatment must eliminate pathogens but being non-toxic to seeds. Therefore, Seed Star and Seed Power can be regarded in this study as ineffective seed dressants for onion.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The surveys on onion postharvest diseases revealed that Black mould, Blue mould, Soft rot, Neck rot and Basal plate rot incidences in the four major markets during the dry season were low, compared to the incidences of these diseases during the wet season survey. The postharvest disease with the highest incidence in all the four markets surveyed was Black mould.

Four genera with five fungal species namely; *A. niger, A. flavus, Penicillium* sp., *Rhizopus stolonifer* and *F. oxysporum,* were associated with onion bulb rot in the four markets surveyed in the Kumasi Metropolis. Among these rot-inducing fungi, *A. niger* was the most frequently encountered pathogen. All the isolated rot inducing fungi were found to be pathogenic to onion bulbs, with *Rhizopus stolonifer* being the most pathogenic.

The seeds of Bawku Red were found to be infected with nine fungal species in six genera viz; *Aspergillus niger, A. flavus, Penicillium* sp., *R. stolonifer, F. oxysporum, F. verticilloides, F. poae, Alternaria porri* and *Botrytis* sp. Among these seed-borne fungi of Bawku Red, *Aspergillus niger* was the most frequently encountered fungus and the least encountered were *Alternaria porri* and *Botrytis* sp. Some of these seed-borne fungi of onion were also found to be associated with the onion bulb rots. Therefore, infected onion seeds could serve as inoculum source of the postharvest diseases of onion and contributed to the rot of bulbs during storage.

It was also observed that, the percentage inhibition of radial mycelial growth of all the test fungi *in vitro* was highest in aqueous Pawpaw leaf extract, followed by Neem extract and the lowest was Moringa extract.

Aqueous Pawpaw, Neem and Moringa leaf extracts were effective in reducing the incidence of onion seed-borne fungi *in vivo* with Pawpaw being the most highly effective. Generally, it was also observed that, the total fungal incidence decreased with increasing treatment durations. Also, all the aqueous leaf extracts, except Cassia extract, enhanced the germination of onion seeds.

Among the three seed dressants evaluated, Seedrex was the only fungicide that was significantly effective against all the seed-borne fungi *in-vivo*. Although it recorded the least incidence, it did not enhance seed germination of onion.

6.2 Recommendations

- Aqueous leaf extracts of Pawpaw, Neem and Moringa are easily available, cheaper than chemical fungicides, and environmentally friendly and, therefore, could be used to protect onion seeds against major seed-borne fungi.
- Seedrex was effective in reducing seed-borne fungi of onion seeds; it can be used for onion seed treatment to reduce the incidence of the seed-borne fungi.
- Further studies should be carried out on the anti-fungal properties of the various parts of the Pawpaw plant especially seeds, and also using other methods of extraction.
- Varietal screening of onion genotypes against storage rot should be carried out to determine the variety with good storability.

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APPENDICES

Appendix I: ANOVA table for effect of aqueous leaf extracts on radial mycelial growth of onion seed-borne fungi using the food poison technique *in vitro*

Aspergillus niger

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	193.885411	38.777082	4743.66	<.001
Residual	18	0.147141	0.008175		
Total	23	194.032552			

L.s.d. 0.1

CV% 1.3

Aspergillus flavus

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	202.68066	40.53613	1660.87	<.001
Residual	18	0.43932	0.02441		
Total	23	203.11998			

L.s.d. 0.2

CV% 2.2

Rhizopus stolonifer

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	170.04020	34.00804	646.62	<.001
Residual	18	0.94669	0.05259		
Total	23	170.98689			

L.s.d. 0.3

CV% 4.7

***Botrytis* sp.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	171.02201	34.20440	1281.03	<.001
Residual	18	0.48061	0.02670		
Total	23	171.50262			

L.s.d. 0.2

CV% 2.8

Fusarium oxysporum

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	165.52276	33.10455	2660.57	<.001
Residual	18	0.22397	0.01244		
Total	23	165.74673			

L.s.d. 0.2

CV% 1.9

Appendix II: ANOVA table for effect of different durations of treatment of aqueous leaf extracts on the total incidence of seed-borne fungi of onion

1 Hour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	51.2761	10.2552	67.54	<.001
Residual	18	2.7330	0.1518		
Total	23	54.0091			

L.s.d. 0.6

CV% 5.4

2 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	64.9313	12.9863	95.12	<.001
Residual	18	2.4574	0.1365		
Total	23	67.3887			

L.s.d. 0.6

CV% 5.6

6 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	201.39050	40.27810	579.43	<.001
Residual	18	1.25124	0.06951		
Total	23	202.64173			

L.s.d. 0.4

CV% 5.3

24 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	265.2165	53.0433	211.20	<.001
Residual	18	4.5208	0.2512		
Total	23	269.7373			

L.s.d. 0.8

CV% 9.9

Appendix III: ANOVA table for effect of different soaking durations of Bawku Red in aqueous leaf extracts on the percentage germination of seeds

1 Hour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	2748.833	549.767	108.75	<.001
Residual	18	91.000	5.056		
Total	23	2839.833			

L.s.d. 3.3

CV% 4.4

2 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	2080.833	416.167	78.85	<.001
Residual	18	95.000	5.278		
Total	23	2175.833			

L.s.d. 3.4

CV% 4.4

6 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1971.333	394.267	80.65	<.001
Residual	18	88.000	4.889		
Total	23	2059.333			

L.s.d. 3.3

CV% 4.0

24 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	2372.000	474.400	106.74	<.001
Residual	18	80.000	4.444		
Total	23	2452.000			

L.s.d. 3.1

CV% 3.7

Appendix IV: ANOVA table for effect of seed dressing fungicides on seed-borne fungi of onion

Aspergillus niger

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	60.62358	20.20786	835.88	<.001
Residual	12	0.29011	0.02418		
Total	15	60.91368			

L.s.d. 0.2

CV% 3.9

Aspergillus flavus

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	89.68922	29.89641	2300.72	<.001
Residual	12	0.15593	0.01299		
Total	15	89.84516			

L.s.d. 0.2

CV% 2.4

Rhizopus stolonifer

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	48.53495	16.17832	251.65	<.001
Residual	12	0.77148	0.06429		
Total	15	49.30643			

L.s.d. 0.4

CV% 9.1

***Penicillium* sp.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	10.34882	3.44961	37.93	<.001
Residual	12	1.09141	0.09095		
Total	15	11.44023			

L.s.d. 0.5

CV% 15.3

Fusarium oxysporum

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	9.2208	3.0736	17.06	<.001
Residual	12	2.1622	0.1802		
Total	15	11.3830			

L.s.d. 0.7

CV% 24.9

Appendix V: ANOVA table of effect of seed-dressing fungicides on Total incidence of seed-borne fungi of onion

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	22741.000	7580.333	1749.31	<.001
Residual	12	52.000	4.333		
Total	15	22793.000			

L.s.d.3.2

CV% 3.2

Appendix VI: ANOVA table for effect of seed-dressing fungicides on % seed germination of onion

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	133.14073	44.38024	1842.27	<.001
Residual	12	0.28908	0.02409		
Total	15	133.42981			

L.s.d. 0.2

CV% 3.9