STUDIES OF THE INFECTION OF CULTIVARS OF SHALLOT (*ALLIUM ASCALONICUM* L.) IN GHANA BY *ASPERGILLUS NIGER* VAN TIEGHEM AND THE EFFECT OF THE HOST ON SOME ASPECTS OF THE BIOLOGY OF *A. NIGER.*

A Thesis presented by

YAHAYA BUKARI B.Sc. (HONS), M. PHIL.

(10088032)

In part fulfilment of the requirements for the Ph. D. Degree of the University of Ghana.

From: DEPARTMENT OF BOTANY, UNIVERSITY OF GHANA, LEGON.

JULY 2014.

STUDIES OF THE INFECTION OF CULTIVARS OF SHALLOT (*ALLIUM ASCALONICUM* L) IN GHANA BY *ASPERGILLUS NIGER* VAN TIEGHEM AND THE EFFECT OF THE HOST ON SOME ASPECTS OF THE BIOLOGY OF *A. NIGER.*

A Thesis presented by

YAHAYA BUKARI B.Sc. (HONS), M. PHIL.

(10088032)

In part fulfilment of the requirements for the Ph. D. Degree of the University of Ghana.

From: DEPARTMENT OF BOTANY, UNIVERSITY OF GHANA, LEGON.

JULY 2014.

DECLARATION

I, the undersigned, Yahaya Bukari, declare that this thesis is the result of my own research carried out in the Department of Botany, University of Ghana, legon, under the supervision of Emeritus Prof. G.C. Clerk.

This work has never been presented either in part or completely, for any degree of this University or elsewhere. References cited have been fully acknowledged.

……………………………….. ……………………………………….

YAHAYA BUKARI EMERITUS PROF. G.C. CLERK

(Student) (Supervisor).

DEDICATION

This thesis is dedicated to my loving mother, Madam Zenabu Dabonne, to the memory of my late father, Mr. Yorda Bukari, and to my sisters and brothers.

ACKNOWLEDGEMENT

My first thanks go to Almighty God who has guarded and guided me throughout this programme.

I wish to express my deepest appreciation to my supervisor Emeritus Prof. G.C. Clerk for his guidance and deepest interest throughout the course of this work. I am forever indebted to him for all his time and patience for me during this course. May Almighty God continue to bless him and his entire family.

I will like to thank the Head of Department, Prof. G.K. Ameka, for his interest in my work and also for making available the department vehicle during my trips to Anloga in the Volta Region. I am also grateful to Prof. G.T. Odamtten, Prof. L. Enu-Kwesi, Prof I.K. Asante, Dr. Ebenezer Owusu and all the lecturers of the Department of Botany for their encouragement. May God bless them all.

My sincere thanks go to Mr. Kofi Baako, a chief technologist of the Department for his personal interest in my work and who used much of his time to help me in my work and I am grateful for all his encouragement during the course of this work. I will like to thank all the technicians of the Department as well, especially, Mr. George Akwettey and the former Chief technologist Madam Alberta Banson. My thanks also go to the technicians of the Ecological Laboratory of the University of Ghana, especially, Mr. Prince Owusu.

My appreciation also goes to the drivers of the Department Messrs. G. O. Agyeman and Raphael Atutra. I am grateful to Messrs Stephen Akpedonu and A. Orizi of Anloga who helped me in the collection of soil samples and procurement of shallot bulbs.

Finally my thanks go to all who helped me in the typing of this thesis: Gilbert Boateng, Diana Ayegbe, Esther H. Quacoo, Monica Esinam Gasika, Taquiyya Najib and Benedict Quagraine.

There are many others who contributed in diverse ways during this programme who cannot individually be mentioned here because of the number. I thank them all.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

Page

Page

Page

Page

Page

Page

Page

Page

Page

LIST OF FIGURES

LIST OF PLATES

Page

LIST OF APPENDICES

Page

ABSTRACT

The relationship between five randomly selected isolates of *Aspergillus niger* from naturally infected bulbs of shallot and two shallot cultivars (*Allium ascalonicum* L.) was investigated. The two cultivars were “Pale-brown” and “Pink”- named for the colours of their scale leaves. *A. niger* causes bulb rot of shallots which have important uses in food and traditional medicine in Ghana The relationship showed by the cultivars were similar in some aspects and different in many other aspects, indicating physiological differences between the cultivars.

In the field, the Pale-brown plants were bigger (mean length of 3.62±0.06 cm and mean width of 2.71±0.05 cm) with greener and more lush leaves. Chemical analysis of the typically larger bulbs of Pale-brown cultivar showed higher mean concentrations of calcium (0.164%), magnesium (0.054%), nitrogen (1.456%), phosphorus (0.406%), potassium (1.142%) and sodium (0.179%). In comparison, the respective percentage values for the Pink cultivar were 0.145, 0.046, 1.192, 0.367, 0.979 and 0.175. The mean length and the mean width of the Pink bulbs were also 3.26±0.08 cm and 2.08±0.08 cm respectively.

Any nutrients secreted by the scale leaves and swollen leaf bases were not able to stimulate germination of conidia of *A. niger* in distilled water droplets placed on them. The conidia did not also germinate in very dilute (1:40) droplets of Potato Dextrose Broth. Higher concentrations of Potato Dextrose Broth, galactose, glucose and sucrose induced appreciable levels of conidium germination. When that happened, the different types of leaves of the bulbs showed differences in their influence on the conidia. Conidium germination was better on the scale leaves than on the swollen leaf bases while percentage germination on the corresponding organs was better and germ tubes were longer on the Pale-brown cultivar than on the Pink cultivar.

The mycelium of *A. niger*, in addition, grew better in extract of bulbs of the Pale-brown cultivar and rotted bulbs of the wound-inoculated Pale-brown cultivar faster than the Pink bulbs. Conidiophores produced in various tests were larger on Pale-brown bulbs than on Pink bulbs.

Conidia formed by *A. niger* growing on ground bulbs of the two cultivars showed some similarities and differences when they were stored in atmospheres of different relative humidities, from zero to 100% R.H., provided and maintained with appropriate concentrations of sulphuric acid. The two sets of conidia lived longest at 60 to 100% R.H. and died quickest at zero to 50% R.H. At the best survival humidity of 100% R.H., 52.1, 51.2 and 35.8% of conidia of *A. niger* Isolates 1, 3 and 5 respectively formed on the Pale-brown cultivar medium were still alive on the 200th day. The corresponding values for conidia formed on Pink cultivar medium were 43.8, 42.3 and 32.3 per cent, respectively. At each percentage viability assessment, conidia formed on the Pale-brown cultivar medium showed higher survival potential. There was positive correlation between spore longevity and the length of the germ-tubes.

The two shallot cultivars clearly stimulated fungi in the neighbourhood of their bulbs and roots over 60 days. Studies using the soil dilution plate method identified *Aspergillus* as the dominant genus in the phyllosphere, rhizosphere and non-rhizosphere soils. The remaining genera were *Fusarium, Penicillium* and *Rhizopus*. The *Aspergilli* were *A. flavus*, *A. niger*, and *A. terreus*. *A. niger* was the most abundant, constituting more than half the fungus population. Application of

Urea and Potassium fertilizers separately to the soil did not alter the pattern of the influence of the shallot cultivars on the fungal populations. The pattern was also the same in different farm soils under continuous cultivation with different crops.

Because of the restraining effects of various sorts of the Pink cultivar on *Aspergillus niger*, it was considered to be the better cultivar for expanded shallot cultivation in Ghana and suitable for breeding improved stocks to reduce the threat of *A. niger* to the shallot industry.

I INTRODUCTION AND LITERATURE REVIEW

Shallots (*Allium ascalonicum*) and onions *(Allium cepa*) are among the most important vegetables in Ghana. Shallots were introduced in Ghana in 1800 from Anecho in neighbouring Republic of Togo and cultivated in the country for more than a century before the introduction of onion in 1930 (Adomako, 1959). Shallots, anyway, are preferred to the onions because of their shorter growth cycle, better tolerance to disease and drought stresses, longer shelf life and because of their distinct flavour (Brewster, 1990; Currah and Proctor, 1990; Grubben, 1994; Pathak, 1994; Sumiati, 1994; Abbey *et al.,* 1998). Furthermore, shallot appears to contain more flavanoids and phenols than other alliaceous plants (Yang *et al*., 1955). Finally shallot plants produce clusters of several bulb splits that number from two to twelve pieces, with an ideal marketable size compared to the single bulb produced by onion plants.

Shallots and onions are used in many households and almost daily. They are grown primarily for the bulbs although the green leaves may also be consumed. They can be used raw in salads and more importantly serve as essential ingredients in various dishes. In certain regions of West Africa, the green leaves are ground, moulded into balls and used for seasoning dishes. The bulbs of shallots and onions have antimicrobial properties which can reduce contamination by bacteria, protozoa or helminthes in salads.

In traditional medicine shallot is used to treat boils, wounds, coughs, bronchitis, asthma, gastro-intestinal disorders and headache. The juice of the bulbs is rubbed on the body of a person with fever. Also, in some parts of Ghana, they are used as antidote for snake bite. It is known that the scent of onions and shallots repels snakes and they are planted near homes to achieve that purpose (Sinnadurai and Abu, 1977).

Both shallot and onion bulbs contain sulphosides, the most important being cystein sulphoside. Upon bruising the bulb, these are degraded by allinase to release pyruvic acid and alkyl-thiosulphinates which turn into sulphides and disulphides. The volatile allinase product is the well known lachrymatory factor in onions and shallots. (Nabos, 1971). The degradation of sulphoxides can be influenced by external conditions such as boiling and frying. The combined taste of sulphides and caramelized sugars give flavours to fried onions.

The shallot bulb, as reported by Soitout (1969) contains 81.8% moisture. A 100g dry weight of the bulb contains 67cal. total energy, 9g total protein; 0.3g fat; 15.4g total carbohydrates; 0.7g fibre; 0.6g ash, 36mg calcium; 45mg phosphorus; 0.8mg iron; 12mg sodium; 334mg sulphur; 5I.U. vitamin A; 0.04mg thiamine; 0.02mg riboflavin; 0.3mg niacin and 2mg ascorbic acid. The corresponding data given by Soitout (1969) for onion bulbs were 88.0% water and 31cal. energy; 1. 5g total protein; 0.6g fat; 7.2 g total sugar; 0.g carbohydrates; 0.4 mg thiamine; 0.02mg riboflavin; 0.1mg niacin; 7mg vitamin C; 30mg calcium; 0.5g iron; 16.5mg magnesium; 35mg phosphorus and 7mg sodium in a 100g of edible portion of the bulb.

The high water content of the bulbs certainly affects the shelf life of onions and shallots as well as other vegetable crops as growth of bacteria and fungi is greatly encouraged. Bulb rot in storage is largely caused by *Erwinia* sp (Norman, 1992) and *Pseudomonas* sp. (Clerk, 2012. Personal communication) and the fungi *Aspergillus niger* and *Fusarium solani* (Clerk, 1974).

Stomata are a significant target for plant pathogenic bacteria. The motile *Pseudomonas* and *Erwinia* cells enter shallot bulbs by the stomata and sub-stomatal chambers are sites of initial proliferation. There is evidence that bacteria generally also cause infection by penetrating wounds of even minute proportions. Indeed, Crosse *et al.* (1972) demonstrated that exposure of the vascular system of shoots of apple (*Malus silvestris* Mill.) apex insured xylem penetration by *Erwinia amylovora* and subsequent systemic infection. Entry of bacteria through leaf traces provide the rationale for reports of dormant bud infections (e.g. Baldwin and Goodman, 1963; Dowler and Peterson, 1967; Dueck and Quame, 1973).

Upon entry into vascular plants, extensive multiplication by bacteria occurs either intercellularly or in the xylem. Fox *et al*. (1971) and Fox (1972) recorded penetration of wounds and subsequent spread through electron micrographs of potato tuber tissues infected by *Erwinia carotova* var *atroseptica*. Spread was primarily between storage parenchyma cells and restricted infection to xylem tissues. Wallis *et al*. (1973) also observed the same phenomenon in their ultra-structural histopathology of cabbage (*Brassica olereceae* var *capitata*) leaf infected with *Xanthomonas campestris*. Generally bacteria do not multiply in phloem tissue, probably according to Goodman (1976), due to high soluble concentration of the phloem which is inhibitory to bacterial replication.

The dry rot of *Erwinia* infection of shallots is visualized as the result of limited degradation of the tissues and desiccation of the unmacerated dead tissues. The genus *Pseudomonas*, on the other hand, contains species with remarkably diverse biochemical capacities that ensures more intense degradation. The ability to carry out some of these activities depends on plasmids (Nester *et al*., 1975).

The symptoms of the respective shallot rots caused by *Fusarium solani* and *Aspergillus niger* in storage are very distinctive. Shallot bulbs infected by *F. solani* are covered by a fluffy dirty white mycelium of the fungus whereas *A. niger* produces flat mycelium bearing masses of black conidia, for which it is given the name, 'black mould'. Black mould is by far the more common infection. In both, colonization of the bulbs proceeds rapidly and infection spreads quickly among bulbs in storage even though the conidia in the two fungi which constitute the major dispersal agents possess different physiological characteristics. Whereas the macro-conidia and microconidia of *F.solani* germinate very well, 98.4 and 90.5 per cent, respectively, in distilled water in six hours at the optimum temperature (28 ^{o}C) (Halm, 1971), *A. niger* conidia do not germinate in distilled water (Kesse, 1995). In spite of this, *A. niger* is cosmopolitan flourishing on all sorts of substrates. It is common in soil (Khan *et al*., 2001; Thom and Raper, 1945), parasitic on plants of 37 genera (Farr *et al*, 1989), contaminant of harvested fruits and vegetables (Sneh *et al.*, 1991) and all sorts of plant products (Pitt and Hopking, 1997; Peronne *et al.,* 2001; Perfect *et al.,* 2009) , probably because conidial germination is stimulated by very low concentrations of sugars and amino acids (Kesse, 1995).

A. niger is equally notable in industry as a very useful microorganism in industry because of its ability to carry out various metabolic processes. It is the source of many enzymes, for example, amylase, amyloglucosidase, cellulases, glucoamylase, invertase, lactase pectinases, proteases etc. (Bennett, 1985; Ward, 1989). Besides the production of useful enzymes certain strains of *A. niger* are used in industry in the preparation of citric acid and gluconic acid used in the food industry. The most important organic acid produced by fungi is citric acid. Both have been assessed as acceptable for daily intake by the World Health Organization (WHO) and Food and Drug Administration (FDA) of the United States of America (USA) (Schuster *et al*, 2002). The annual production of citric acid by fermentation of *A. niger* is presently 350,000 tonnes (Ward, 1989). About 70% of it is used in the food industry while 10% is used to produce cosmetics and pharmaceutical products (Bigellis, 1991). Another value of *A. niger* in the biotechnology industry is the production of magnetic isotope-containing variants of biological macro-molecules for Nuclear Magnetic Resonance (NMR) analysis (Staiano *et al.*, 2005).

The rotting of bulbs by *A. niger* is accompanied by infiltration of the tissues by the mycotoxins, fuminisin B2 and ochratoxins, synthesized by the fungus (Abarca *et al*., 1994; Noonimabc *et al*., 2009; Edwin *et al*., 2010). Such contaminated bulbs are unsuitable for consumption (May and Adams, 1997; Schuster *et al.*, 2002; Noonimabe *et al*., 2009; Al-Abdalall, 2009). Fuminisins and ochratoxins may cause immunotoxicity, carcinogenicity and hepatoxicity (Louthrenoo *et al*., 1990).

Oxalic acids, kojic acids and cyclic pentapeptides are another set of products of *A. niger* of moderate to high acute toxicity. Oxalic acid causes pulmonary oxalosis (Nakagawa *et al*., 1999).

The third category of *A. niger*-related hazard to health of humankind is the result of direct contact with the human body. There are two major cases of great concern to persons who, by vocation, are continually associated with shallots.

Superficial contact allergy to fungal spores depends on prior sensitivation to specific antigen. Later exposure to larger quantities of the same spores may give rise to skin irritation of the type observed in a farmer who had smut, *Ustilago maydis* (Preininger, 1937-1938) allergy. Similarly, one of the characteristics lesions seen in the ‘ maladie des Cannes de Provence” occurs on the shoulders of the workmen who carry bundles of the reeds affected by fungi especially *Papularia sphaerosperma (*Mandoul *et al*., 1954) on their shoulders. Also, *Alternaria tenuis* is thought to cause allergic eczema in dogs and horses (Allen, 1945). Louthrenoo *et al.* (1990) provided evidence of such relationship between *Aspergillus niger* and humankind.

All types of fungal spores and the associated structures, such as conidiophores, which achieve the air-borne state can theoretically be inhaled. *A. niger* is naturally included. The range of air-borne spore concentration is vast. The concentration depends on the fungus species, time of the day and season of the year. Even the concentration of outside and air spora of enclosed area differ considerably. *Aspergillus* spores, for instance, occur in much smaller numbers in the outside air and probably rarely exceed 500 per m^3 but within a farm building following the shaking of the mouldy hay, Lacey and Lacey (1964) found up to 21 million per m^3

No comparative data are yet available for conidia of *A. niger* in the atmosphere outside and inside market stalls in Ghana handling shallots and onions. A significantly high concentration of spores in the air of the buildings will consequently increase intake by those worker there, even if exposure times are shorter than outside. Definitive estimation of the actual numbers in future studies is essential considering the fact that *A. niger* conidia are encountered among the important fungal spores involved in respiratory allergy in humankind (Austwick, 1966; Edwards and Al Zubaidy, 1977).

Whatever the circumstance, persons persistently handling *A. niger*-infected shallots bulbs will be exposed to another medical hazard: otomycosis. It is a subacute or chronic superficial fungal infection of the external auditory canal and auricle. *A. niger* is the most common cause, with occasional cause by *Aspergillus flavus* and *Aspergillus fumigatus* (Berjak and Arya 1970; Than *et al*., 1980; Mugliston *et al*., 1985; Poulose *et al*., 1989; Lucente, 1993; Yehia *et al.*, 1990). The disease is worldwide in distribution. Otomycosis is more prevalent in warm, humid climates, particularly in the rainy season as opposed to dry season. The most common form of the disease is termed ' otitis externa', first described by Chandler (1968). By the estimation of Osguthorpe and Nielson (2006) chronic otitis externa affects 3.0-5.0 per cent of the population.

Cases of otitis externa described in the reports in the preceding paragraph mention a number of symptoms including redness and swelling of the external ear canal, ear pain always accompanied with a sensation of irritation and itching, a feeling of pressure and fullness inside the ear, a thin and watery discharge from the ear, swollen and sore lymph nodes in the throat and above all the presence of growth of projecting *Aspergillus c*oniodiophore from the walls of the ear canal.

Above all, an important consequence of rotting of shallot bulbs in storage is the loss of planting material. For, unlike onions which flower and produce viable seeds for propagation, shallot genotypes rarely flower in tropical regions. Many shallot genotypes are, therefore, only clonally propagated (Jones and Mann, 1963; Currah and Proctor, 1990; Messiaen *et al*., 1994).

There is no information on the magnitude of post-harvest losses of shallot bulbs in Ghana. Hayden (1989) estimated a 30 per cent post-harvest loss of onions (*Allium cepa*) in Sudan, and Fedaku (1989) recorded the same level of loss (25-35 per cent) in Ethiopia, but for all horticultural crops in general. It is probable that the average yearly level shallot production of 34,262.8 tonnes, calculated from the productions data of the Ministry of Agriculture of the Government of Ghana reproduced in Table 1, might suffer approximately the same level of post-harvest loss.

Table 1: Production of shallot (*Allium ascalonicum*) in Ghana in 2005-2009 (Library record: Ministry of Agriculture)

Year	Area of Cultivation (Hectare)	Yield (Metric Tonnes)
2005	4,000	30,000
2006	4,300	30,014
2007	4,800	34,000
2008	4,900	39,000
2009	4,950	40,000

There are two sets of microflora of stored plant products: field microflora and storage microflora. The field species appear on the products during development, maturation and before harvest. The storage species are the post-harvest invaders together with the viable field species transferred from the field into storage.

Field fungi of Red Creole cultivar of onion (*Allium cepa*) included species of *Aspergillus*, *Cladosporium*, *Fusarium* and *Rhizopus* with *Aspergillus niger* constituting 65 per cent of fungal isolates of the dry season crop (January – March, 1989) and 74 per cent of the rainy season crop (June – August, 1989) (Teye, 1994). Most of the species survived many weeks of storage, thereby transformed into storage fungi as well. *Penicillium cyclopium* was the only new colonizer during storage of the bulbs worthy of note. In this instance, the pre-harvest and post-harvest bulbs provided physiologically similar substrates which supported practically the same fungal communities.

In circumstances where the substrates and the ambient environmental conditions differ, clearly distinct fungal communities with different component species would develop. For example, the field fungi of maize (*Zea mays*) grains include species of *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium* which invade the maturing grains when the moisture content is high and they are metabolically active (Christensen, 1957). These fungi require moisture level in equilibrium with a relative humidity of 90% or more. The storage fungi arrive after harvest and mostly xerophilic as the grains, by then, contain very little water. The *Aspergillus* species are the most important storage fungi of maize grains (Mislivec and Tuite, 1970). They are xerophilic and they grow at low water activity (0.70 – 0.75 aw) (Magan and Lacey, 1987).

It has long been known that numerous diffusible compounds of low molecular weight leak from actively growing plants and their release is not metabolically mediated. Leakage is the loss of compounds along electro-chemical potential gradients by simple diffusion. On the other hand, by an active metabolic process, poorly diffusible, high molecular weight compounds are also transported across membranes. These compounds are energy-carrying metabolites which act as an ecologically favourable matrix for the evolution of complex biological communities.

In soil, roots and other subterranean structures become covered by a slime or mucilage. Root mucilages can be classified into two types. The first is secreted by outer root cap cells as droplets containing sloughed cells which cover the root tips of axenically grown roots (Miki *et al*., 1980; Rougier, 1981). The second is a firm, mucilagenous layer overlying the epidermal cells and coating the root hairs (Oaddes, 1978).

Miki *et al*. (1980) recommended the use of the terms root cap mucilage' and 'epidermal mucilage' to designate the two types of mucilage on axenically grown roots. The term 'mucigel', first introduced by Jenny and Grossenabcher (1963) to describe the mucilagenous material at the surface of roots grown on non-sterile soils, which could be applied to both types of mucilage detected on axenic roots, and thereby to the mucilagenous material of soil-grown developing shallot bulbs. The mucilages contain in addition, slime of bacterial cells on the plant surfaces as well as products of decomposing root hairs and exfoliated epidermal cells.

The mucigel occupies a key position at the plant structure-soil interface and may well be of significance to plants, soils and the soil microflora. The following are the most important contributions to root growth proposed: (a) root hairs and their associated mucigel may help anchoring of the young seedling in the soil (b) the adsorption of fine clay particles to mucigel constituents in the first in the formation of stable micro aggregates around plants roots (Turchenek and Oades, 1976): (c) reduction of friction between the growing roots tip and the soil (Oades, 1978: Rougier, 1981): (d) protection of root from desiccation (Greenland, 1979), toxic ions (Horst *et al*., 1982) or metal injury (Barlow, 1975; Drew, 1979); (e) improvement of the root-soil contact facilitates water and nutrient movement of the dry soil or water-stressed plants, there is an increased production of mucigel by the roots which results in a cylinder of soil tightly adhering to the roots maintaining a hydraulic conductivity between root and soil (Mambiar, 1976; Martin, 1977; Drew, 1979; Foster, 1982); (f) mucilage adsorps ions and exerts some selectivity on the uptate of ions (Rovira *et al.*, 1983): and (g) acid phosphatase and esterase in mucigel (Felipe *et al*., 1979) hydrolyze organic phosphate esters in the soil into inorganic phosphate which could then be assimilated by plants.

The acid phosphatase and esterases and the vast range of organic compounds including amino acids, auxins, carbohydrates, enzymes, flavones, growth factors, nucleic acid derivatives, organic acids, phenol derivatives and vitamins and the various microorganisms they influence have been extensively reviewed (e.g. Oades, 1978; Foster, 1982; Rougier and Chaboud 1985). The composition of mucigel is generally specific for a plant species and even cultivars of a species. Consequently, host species or cultivars have recognizable characteristic microflora.

Whether or not it is utilized as a food source by microorganisms, mucigel is a favourable ecological microhabitat which promotes their rapid growth and special activities. For example, mucigel compounds play a role in the recognition of maize roots by *Phytophthora cinnamon*, zoospores (Hinch and Clarke, 1980; Irving and Grant, 1984). Umali-garcia *et al.* (1980) considered that mucilage may be helpful for the nitrogen- fixing bacterium *Azospirillum* in regulating transport of oxygen originating from the soil; the process of formation of nodules by legume roots is triggered off by vitamin B, in the roots exudate which stimulates the growth of the *Rhizobium* population close to the root; the interaction can only proceed when the *Rhizobium* cells covert tryptophan in the root exudate to Indole Acetic Acid (IAA), and the bacterial slime polysaccharide stimulates the production of the enzymes polygalacturonase (PG) by the legume root cells which then finally catalyze the breaking of the linkages in the cellulose microfobrils at the apex of the root hair leading to the development of the infection thread. Mucigel may represent a favourable site for the establishment and development of mycorrhizae (Reid and Bowen,1979), and the mucigel may suppress or eliminate activities of soil pathogens, first by being inhibitory to the pathogens or secondly by either parasitism, competition or antibiosis by beneficial mucigel species.

An exhaustive search of the pertinent literature failed to reveal any information on pathogenesis of *A. niger* on shallot bulbs in storage. Teye (1994), however, identified *A. niger* in her studies as a formidable storage fungus of bulbs of the Red Creole and Texas Grano cultivars of onion (*Allium cepa*) in Ghana. She reported that bulbs of the onions still retained viable *A. niger* colonies after they had been cured for 30 days, then irradiated with 0.5 Gy gamma rays and then stored at room temperature for 90 days.

Woldestadik (2003) noted marked differences in the extent of rotting of three cultivars of shallot, DZ- Sht-78, DZ-Sht-91 and Fedis in Ethiopia. The rotting affected 44 percent of Fedis bulbs and about 26 percent of DZ-Sht-91 bulbs. That of DZ-Sht-78 lay about median between these two extremes. The report did not, however, specify the cause of rotting.

Resistance or susceptibility of onion and shallot bulbs to post-harvest fungal contaminants may be determined by chemical antimicrobial defense mechanisms. For example, Smalley and Hansen (1962) observed that growth of *Penicillium corynbiferum*, which attacks garlic (*Allium sativum L.)* was less inhibited by garlic extracts than was the growth of 17 species of *Penicillium* from other hosts.

As has been mentioned earlier at the beginning of this chapter, the compound alliin (S-allyl-L-cysteine-sulfoxide) is converted by the enzyme allin-lyase to allicin (Diallyl-disulfide-oxide). Eventually, allicin is converted to diallyl- disulphide in plants of the genus *Allium*. The role of allicin and related compounds in antimicrobial defence mechanism in shallot remains to be determined.

The classical case of pre-formed inhibitory compounds emanating from the leaves of high plants and preventing the germination of potential parasites is that of the onion metabolites protocatechuic acid and catechol which confer resistance to smudge caused by *Colletotrichum circinans* in yellow-scaled onions by virtue of the sporostatic activity of these compounds (Walker and Stahmann, 1965). In this case not only germination is inhibited but the substance caused lysis of the spores, particularly if any of them do germinate. Shallots have not been studied for these compounds.

Aspergillus niger has been considered, on the one hand, as a strict saprophyte (Commonwealth Institute, 1966; Farr *et al.*, 1989) and, on the other, a serious facultative parasite . Indeed, Lorbeer *et al*. (2000) gave a detailed account of their findings on the pathogenesis of *A. niger* attacking onion plants (*Allium cepa*). They inoculated the seeds of onion with *A. niger* conidia, sowed them in sterile soil and followed the course of infection of the various organs of the plants. The hyphae invaded the roots, the basal disc and the leaves. In a subsequent experiment, flowers of different ages were inoculated with *A. niger* conidia. The hyphae were eventually detected in all parts of the flowers including the ovules and developing seeds.

Saprophytes and facultative parasites employ the same enzyme systems to dismantle the various plant cell constituents. Obligate parasites (Van Sumere *et al*., 1957) and mycorrhizas (Williamson and Hadley, 1970) also have the ability to produce cell wall degrading enzymes. The production of their enzymes, however, must be under adequate control lest the invaded tissues become macerated and the host cells killed.

Based on the concept of primary cell wall structure, pectic components of unaltered cell walls will be attacked first rendering other wall polymers susceptible to enzymatic hydrolysis. The pectic enzymes have been grouped according to the following criteria: (a) the mechanism by which the α-1, 4 glycosidic bond is split, (that is, hydrolytic or lytic) (b) enzyme specificity for a substrate, that is, pectin or pectic acid, and (c) position in the pectic chain at which cleavage occurs, that is, random or terminal point of attack (Bateman and Millar, 1966; Rombouts and Pilnik, 1972). The enzymes specific for pectin are referred to as pectinmethylgalacturonases. In contrast, the polygalacturonases are specific to pectic acid. If the enzyme attacks the terminals of the pectic chains and releases only monomeric products, it is termed an exopolygalacturonase. If the attack is random and oligomers are released, the enzyme would be called an endopolygalacturonase. Different activities of the enzymes release different reaction products.

The multi- enzyme hypothesis of enzymatic decomposition of cellulose recognizes a C_1, C_x and β-glucosidase (Reese, 1963). Cellulose is an insoluble crystalline substance in its native form. Microorganisms capable of utilizing native cellulose are believed to produce an enzyme designated C_1 which acts on native cellulose by destroying its crystalline structure and exposing the glucose chains to β-1, 4 endoglucanases, termed C_x enzymes. The C_x enzymes degrade the glucan chains to cellobiose. Conversion of cellulose to glucose also requires a cellobiase or β-glucosidase (King and Vessal, 1969).

Similarly, there are specific enzymes which break down other cell components in herbaceous plants like shallots. Glucanase, oxylanase, mannonase, mannosidase, glucosidase which hydrolyze the hemicellulose fraction of the plant cell wall. Phospholipases hydrolyze the acylester and phosphate ester bonds of phospholipids of cell membranes (Gatt and Barenholz, 1973). While proteolytic enzymes hydrolyze peptides in proteins. Primary classification of proteolytic enzymes is based on the catalytic mechanism of the active site (Smith, 1960; Matsubara and Feder, 1970).

Invading pathogenic fungi of aerial parts of plants first come in contact with the cuticle covering of the epidermal cells and penetrate by enzymatic dissolution. Cuticle also covers the cells in the substomatal cavity. In addition, cuticular layer is present in the palisade and spongy mesophyll cells (Sitholey, 1971).

Cuticle has a framework of cutin, a polymer of crosswise esterified mono-, di-, and, trihydroxy fatty acids linked by peroxide bridges (carboxycutin). Other components are waxes, cellulose and pectin (Van den Ende and Linskens, 1974).

Cutin is attacked by a number of cutinolytic enzymes. The principal enzyme is cutin esterase which by hydrolytic cleavage of ester links delivers substrates for oxidizing enzymes. For instance, the product oleic acid is acted on by oleic oxidase, which in turn, produces substrates for linoleic oxidase. Accompanying the oxidizing enzymes is the peroxidase, carboxycutin peroxide, which catalyzes the release of oxygen from carboxycuttin by loosening the peroxide bridges (Van den Ende and Linskens, 1974).

These enzymes make it possible for microorganisms like *Spilocaea oleagina* which live sub or intracuticularly to use the cuticle as a food source (Ruinen, 1966) and decay of cutin and cuticular components by microorganisms in their environment (de Vries *et al*., 1967).

Aspergillus niger may damage the host plant by other means. It produces the phytotoxic compounds called malformins. The toxin cause curvatures and grotesque plant malformations (Curtis, 1958; Postlethwait and Curtis, 1959). Malformin is a polypeptide. There are two types of malformin- ‘old’ and ‘new’ malformins. ‘Old' malformin is a neutral cyclic pentapeptide (

D-leucyl-L-iso-leucyl-D-cysteinyl-L-Valyl-d-cysteine anhydride) and the ‘new’ malformin contains an allo-isoleucine (Narumo and Curtis, 1961; Takahashid and Curtis, 1961). Many plant species are not susceptible to malformin (Curtis, 1961).

Shallot plants take averagely ten weeks from the time of sprouting to reach maturity. By that time the green leaves have turned pale green or brown and lost much of their turgidity. The plants are pulled and laid out on the ground for some days to dry the leaves thoroughly. Because of the small size of the bulbs, they are separated into many lots of manageable sizes and the leaves of each lot plaited together into a “pigtail” by which the bunch is handled. Spread of *A. niger* among the bulbs in the bunch and, indeed, the store houses happens in two ways. One is by mycelial growth whereby the hyphae from an infected bulb reaches healthy contact bulbs. The second and faster process involves air transport of the essential dispersive conidia.

The conidia are passively liberated by blow-off, conidia blown off their conidiophores by wind, and by shake-off, when the conidiophores are agitated. An important attribute of a spore from the point of dispersal is its retention of the power to germinate. The fact that the spore in the air can remain in suspension for a long time is of no significance if at the end of its journey it is incapable of germination.

Very great differences have been found in the survival potential of different kinds of fungal spores. The viability of all spores decrease with time and the rate of loss of vigour is dependent on the inherent characteristics (genome) of the spore, and upon environmental conditions, especially, with light, temperature and humidity (Gottlieb, 1950; Hawker, 1950; Cochrane, 1958).

Generally, longevity increases with decreasing light intensity, and blue light appears to be the most active portion of the visible spectrum. Rotem and Aust (1991) found that exposure to sunlight affected the survival of conidia of *Alternaria macrospora* and *Botrytis cinerea* in the same way as ultraviolet light. One protection mechanism against injurious rays is offered by the pigments of some spores, and many correlations between colour of spores and resistance to light have been made. For example, Dillon-West and Halman (1932) showed among early mycological physiological studies that white and orange spores of *Puccinia graminis tritici* are more easily killed by ultraviolet light than are grey and red spores.

Kesse (1995) studied the longevity of conidia of four *Aspergillus* species isolated from mouldy maize grains in atmospheres of zero to 100% R.H. Interestingly, she recorded four different % R.H viability relationships. Incidentally, the conidia of all the four species did not germinate at 100% R.H. and the experimental conidia were stored at zero, 20, 40, 60, 80 and 100% R.H in light for 56 days.

A. clavatus conidia survived longest at 0.0 and 100% R.H. and lost viability quickest at 20 – 80% R.H. *A. flavus* conidia survived longest at 0.0 – 40% and 100% R.H. and lost viability quickest at 80% R.H. *A. niger* conidia survived longest at 100% R.H and viability decreased with decreasing percentage relative humidity, and, *A. tamarii* conidia survived longest at 0.0% R.H. and viability decreased with increasing percentage relative humidity.

The work of Kesse (1995) did not compare longevity in light and dark. The period of study of 56 days was also rather short. For, many investigations have shown that the physiology of some fungal species dramatically changes beyond a certain age. Hahne (1925), for instance, indicated that spores of *Tilletia levis* and *Tilletia tritici* less than two years old germinated in dark or light whereas spores more than two years old could not germinate in dark. Later, Gassner and Niemann (1955) also reported the importance of age in the response of smut spores to light. Teyegaga and Clerk (1972), on the other hand, showed that in dark, *Cercospora canescence* conidia lost viability sooner at high relative humidities than at low, whereas conidia exposed to light survived longer at medium humidities close to 40 % R.H. than at high and low humidities. Survival of *A.niger* conidia should be re-examined.

There are commonly cultivars of many crop plant species. In a particular species, the cultivars differ from each other in many ways, including form, function, and response to the environment and reaction to diseases. A good understanding of *A. niger* decay of shallot is needed in order to develop management strategies that would increase returns to producers. In an environment where *A. niger* is ubiquitous, any shallot cultivar that would exhibit an appreciable level of resistance to the fungus could be preferred to ensure enhanced productivity. It is also assumed that a resistant cultivar would not support the usual prolific sporulation associated with *A. niger* and would be a less capable source of the bulb infection units – the conidia.

The thesis contains results of investigations carried out mainly on the:

(a) growth, vegetative morphology and chemical composition of the bulbs of the two shallot cultivars, Pale brown and Pink, cultivated in Ghana;

(b) pathogenesis of five *A. niger* isolates, isolated from infected shallot bulbs;

(c) relationship between the soil fungi and the shallot bulbs during development;

(d) effects of Potassium and Urea fertilizers on the phyllosphere and rhizosphere mycoflora of the shallot plants and the response of bulbs of 60 day old plants formed in the treated soils to inoculation with *A. niger*.

(e) Influence of shallot cultivars on longevity of *A. niger* conidia.

II. MATERIALS AND GENERAL METHODS

(i) MATERIALS

a. Shallot bulbs.

Pale-brown and Pink cultivars of shallot (*Allium ascalonicum*) cultivated in Ghana were used in this investigation. In Anloga district where the bulbs were periodically obtained, there are three shallot growing seasons in a year: a major season from April to August and two minor seasons, January to March and September to December. New harvested bulbs were purchased in bulk at the end of March, August and December in order to standardize the ages of the experimental bulbs. The bulbs were stored in a refrigerator at 4^{o}C and used within 30 days of purchase.

b. Isolates of *Aspergillus niger*

Five isolates of *Aspergillus niger* obtained from naturally infected shallot bulbs were used in this investigation. Bulbs of each cultivar showing signs of *A. niger* infection purchased from the Agbogbloshie were placed in open Petri Dishes standing in desiccators with internal atmosphere of 100% R.H. provided by distilled water. The bulbs were examined after 5 days incubation at 32^{o}C, and sub-cultures were made of as many *A. niger* colonies on the bulbs as possible on Potato Dextrose Agar. The isolates were sub-cultured on two further occasions to obtain pure cultures.

By numerous cultural tests, five isolates with clearly distinguishable characteristics as shown in EXPERIMENT E of the Results were selected for the various pathogenicity and conidial longevity tests. Stock cultures of the five isolates were maintained on PDA in McCartney tubes at $4^{o}C$ in the refrigerator. The stock cultures were sub-cultured fortnightly.

c. Soil Sample.

Sandy loam soil samples with similar history were obtained from the shallot farms at Anloga were used as planting material where necessary. Shallots are grown on raised beds (Plate 1). Because of the annual flooding by the sea, the farmers neutralize the salty silt left by the floods by adding river sand and organic manure consisting mainly of poultry manure and cow dung (Plate 2). At an advance stage of growth, the shallot plants are inter-cropped with egg plants, pepper and tomato (Plates 3 and 4). Because of the dense cropping, the soil samples sometimes needed in large quantities, could be conveniently collected from freshly prepared plots before planting.

PLATE 1. Photograph showing first stage of preparation of raised shallot plots at Anloga

Plate 2. Photograph of a prepared plot at Anloga showing manure heaps to be incorporated into the sandy soil.

x 1/10

PLATE 3. Photograph showing pure stands of 30-day old (TOP) and 60-day old (BOTTOM) plants of the Pink cultivar of shallot (*Allium ascalonicum*).

X 2/5

Plate 4. Photograph showing 60-day Pink cultivar of shallot plants (*Allium ascalonicum*) inter-planted with pepper plants (*Capsicum annum*) at Anloga.

d. Grains of Maize, Seeds of Cowpea and Tubers of Cassava, Irish Potato and Sweet Potato.

Sound grains of maize (*Zea mays* L.), seeds of cowpea (*Vigna unguiculata* L. (Walp.) and tubers of cassava (*Manihot esculenta* L.), Irish Potato (*Solanum tuberosum*) and Sweet Potato (*Ipomea batatas* L.) used in the preparation of culture media were purchased from the Madina Market and stored at 4^{o}C in the refrigerator until needed.

e. Chemicals

Chemicals were purchased from Oxoid Ltd. London and Accra Chemists Ltd, Accra.

(ii) **GENERAL METHODS**

a. **Characteristics of growing plants**.

Bulbs of both cultivars of shallot, Pale-brown and Pink, were kept in a refrigerator of a temperature of 4^{o}C for 60 days before planting. This was done to break the dormancy of the bulbs. Bulbs of each cultivar approximately the same size, weighing between 6.55g and 7.14g were selected for planting. Each bulb was planted in a 20cm by 12 cm black polythene bag filled with sandy-loam soil of the same weight from Anloga. The bulbs were each watered with 300 ml of water every day, and the plants they produced after sprouting were kept in the open for 60 days after which the lengths of leaves were measured with a meter rule while the width at the broadest point were measured by means of a Vernier caliper.

b. Elemental composition of bulbs

To determine the biochemical composition of the bulbs, they were cut into pieces and air dried for two days. After that the samples were milled and used for the analysis.

(i) Estimation of total Nitrogen

The Micro-Kjeldahl method of AOAC (1984) was used for the nitrogen determination. The procedure involved digestion, distillation and titration.

Digestion

An amount of 0.5g of the sample was weighed into each of three 250ml digestion tubes. A small scoop of selenium reaction mixture and 8ml of concentrated sulphuric acid were added to each and whirled. The tubes were then placed on a pre-heated digestion block. The samples were heated continuously until they were clear. They were then removed from the block digester and allowed to cool.

During digestion, organic and inorganic nitrogen were converted to ammonium salt by wet oxidation in the presence of concentrated sulphuric acid with a suitable catalyst that promoted the oxidation of organic matter.

$$\text{Sample} + \text{Conc. } H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2$$

Distillation and Titration

This step involved the separation of ammonium salt from the concentrated H_2SO_4. In strong alkaline solution, gaseous ammonium is distilled and trapped in standard acid solution. Distilled water was first used in the distillation apparatus to clean it for a few minutes. The Kjeldahl extract was diluted with about 30ml of distilled water. Exactly 10ml of 0.1 N HCl was put in a conical flask containing two drops of Tashiro indicator and placed under a condenser. The tip of the condenser was dipped into the receiving acid in order to avoid any loss of ammonia. The digestion tube was fixed to the distilling end and about 15ml of 40% NaOH added automatically. This was distilled for about four minutes within which about 40ml of the distillate was collected in a conical flask. The flask was lowered and the tip of the condenser washed with distilled water. The flask containing the distillate was removed and titrated with 0.1N NaOH to obtain a green end point. Percentage nitrogen was calculated using the formula:

$$\% \text{ N in soil sample} = \frac{(\text{a}-\text{b}) \text{ x } 0.01 \text{ X } 14 \text{ X v X } 100}{1000\ X\ w\ X\ al}$$

where v = final volume of digestion= 100ml

w = weight of sample taken in grams

al = aliquot of solution taken for analysis

0.01 = Molarity of acid

14 = Molar weight of Nitrogen

(ii) Estimation of Calcium, Iron, Magnesium and Potassium levels

The wet oxidation method (IITA, 1981) was used in the determination of calcium, iron, magnesium and potassium levels. Half a gram of sample was weighed into each of three Erlenmeyer flasks. Five millilitres of concentrated sulphuric acid were added to each flask and the flask placed on pre- heated sand bath in a fume chamber. Thirty percent Hydrogen peroxide (H_2O_2) was added drop by drop until the solution turned colourless. The flasks were then removed from the sand bath and the contents allowed to cool. Each was diluted with distilled water and filtered into 100ml volumetric flask and made up to the volume mark with distilled water. Potassium levels were determined using the flame photometer while calcium, iron and magnesium levels were determined from readings on the Atomic Adsorption Spectrometer (Perkin Elmer Atomic Absorption Spectrometer, Model PinAAcle 900T)

(iii) Total Phosphorus determination(Bray 1 Method)

The Bray method (Bray and Kutz, 1945) was used for phosphorus determination. This process involved in the digestion of the sample, titration with ammonium in the presence of P- nitrophenol to give a yellow colour and spectrophotometer used to read the optical density by which the amount of phosphorus was calculated.

Digestion

Half gram of the sample was weighed into a 125ml Erlenmeyer flask, 5ml of the digestion mixture (1.5 parts of $HClO_3$) added and placed on a pre-heated sand bath. The sample was heated until it fumed and H_2O_2 was added drop by drop until the solution turned colourless. The sample was taken off to cool and diluted with distilled water. It was filtered into 100 ml volumetric flask and made up to the mark with distilled water.

Titration and reading on the spectrophotometer.

Aliquots of 2ml of the digested samples were dispensed into 50ml volumetric flasks. A volume of 30ml of distilled water was added to each flask and a drop of P- nitrophenol added. A few drops of ammonium solution were added until the colour turned yellow. Eight millilitres of the ascorbic acid mixture were added and the volume made to the mark with distilled water. The optical densities of the samples were read using the spectrophotometer. The reading was used in the calculation below to obtain the phosphorus concentration in the sample.

$$P\ (ppm) = \frac{\text{Spectrophotometer reading x Volume of sample}}{\text{sample weight x volume of aliquot}}$$

c. Soil analysis

The levels of sodium, potassium, calcium, magnesium were determined using the wet oxidation method, phosphorus using the Bray 1 method and that of nitrogen using the Micro-Kjeldahl method. These methods are described in Experiment **b.**

d. Preparation of and composition of nutrient media.

Potato Dextrose Agar (PDA) was prepared with 200g of tuber of Irish potato, 10g dextrose, 12g Agar and 1000ml distilled water. The Peeled tubers were washed and cut into smaller pieces. An amount of 200g was weighed and boiled in 500ml distilled water in an aluminium pan until they started to break up. The fluid was collected in a 500ml beaker by using a muslin sieve, left to stand and cool and then poured into a 1L measuring cylinder and topped up to the 1000ml mark. An amount of 10g of Dextrose and 12g of Agar were added and the mixture was transferred into a 2L Erlenmeyer flask and heated in a water-bath to melt the agar. The medium was subsequently dispensed into medicinal flats and autoclaved at 121^{o}C for 30 minutes.

Potato Dextrose Broth (PDB) was also prepared with 200g of tuber of Irish potato, dextrose, 10g; Agar, and 1000ml distilled water. This provided a liquid medium (broth) as was done in the case of PDA without the Agar.

Sabouraud Dextrose Agar was prepared with 10g peptone, 20g Dextrose and 1.5g Agar. They were dissolved in 1L of distilled water in a 2L Erlenmeyer flask and heated in a water-bath to dissolve the agar completely. The medium was then dispensed into medicinal flats and autoclaved.

Czapek Dox Broth medium was prepared by dissolving Sucrose, 30.0g; Sodium nitrate, 3.0g; Dipotassium phosphate, 1.0g; Potassium chloride, 0.5g; Magnesium sulphate, 0.5g; and Ferrous sulphate, 0.01g in 1L distilled water in a 2L Erlenmeyer flask. Aliquots of 250ml each were dispensed into four medicinal flats and autoclaved.

Ground shallot bulb medium was prepared by grinding five peeled shallot bulbs in a mortar into a paste. The paste was spread evenly in a Petri Dish and autoclaved.

Shallot bulb Extract. Healthy shallot bulbs without the covering of scale leaves, were ground in a mortar and the fluid strained with a muslin cloth. The extract represented undiluted standard concentration which was diluted to different strengths as desired for some conidium germination tests.

e. Methods of sterilization.

i. Erlenmeyer flasks, pipettes and Petri dishes were immersed in potassium dichromate solution ($K_2Cr_2O_7$), 100g; conc. H_2SO_4;distilled water, 1000ml); for 48 hours. They were next washed under running tap and rinsed twice with distilled water. They were air-dried and then sterilized in an electric oven for six hours at 160˚C. Pipettes were wrapped in brown paper.

ii. Glass slides and solid watch glasses were washed with detergent, rinsed under running tap and stored in 70% ethanol. The slides were flamed-sterilized just before use.

iii. Media and distilled water were autoclaved for 30 minutes at a temperature of 121˚C at a pressure of 1.1kg per square centimeter (6.9x103 Pa). Cotton wool plugs were temporary covered with aluminum foil caps to prevent the penetration of any condensed water during autoclaving.

iv. The inoculation room and lamina flow chamber were sprayed with 10% aqueous Dettol solution, 20 minutes prior to plating of the agar media and inoculation. Inoculation of all other media was carried out also in the pre-treated inoculation room.

v. Forceps, inoculation needles and loops, and scalpels were flamed to red-heat and air-cooled before use.

f Soil mycofloral studies.

Soil from freshly collected farm plots at Anloga were used in this experiment. A small quantity of soil weighing 1.0g was put in 10 ml of sterile distilled water in a McCartney tube. The tube was shaken vigorously to ensure a thorough dispersion and the mixture used to prepare dilution series down to 1:1000 using sterile distilled water. One millilitre of this final dilution was mixed with 20 ml of either sterile cooled molten PDA or Sabouraud Agar containing a few drops of 1.0% streptomycin solution in each of the three replicates sterile Petri dishes. The mixture was mixed by a gentle circular motion and allowed to set. The plates were incubated at 30^{o}C. Colonies of fungi which developed were counted after 5 days and the fungal isolates identified.

g Colonization of surface of bulbs and roots of shallot growing in soil by fungi.

Shallots plants of the two cultivars were raised in Anloga farm soil in potting plastic bags in the greenhouse of the Botany Department for this investigation. At sampling time, non-rhizosphere soil was collected by removing 2 cm-long core soil with a sterile No. 6 cork borer (1cm in diameter) from the root-free region of the potting bag. Plugs of soil samples from the bags of each treatment were pooled, pulverised and thoroughly mixed. Phyllosphere and Rhizosphere soils were collected by loosening up the soil and gently pulling off the potting bag. The plants were carefully lifted to preserve as much of the root system as possible. Excess soil was then removed from the roots by gentle shaking of the plants. The closely adhering soil remaining on the bulbs and roots constituted the phyllosphere and rhizosphere soils, respectively. The bulbs were separated from the root systems using a sterile scalpel and the two placed in separate transparent polythene bags. The bags were then vigorously shaken to dislodge the respective adhering soils.

Soil dilution plate method was used to isolate the fungi in the soils. Serial dilutions were prepared for each of the soils by adding one gram of soil to 100ml sterile distilled water contained in 250ml Erlenmeyer flask. The soil suspension was vigorously shaken by a mechanical shaker(Whirli Mixer Tm, Wm/250/SCP/2) for 30 minutes. Immediately following dispersion, a suspension of 1:1,000 was made. One millilitre of this dilution was transferred into a sterile 9cm-diameter Petri dish and 20 ml of cooled (40^{o}C) molten

Sabouraud Agar, supplemented with Rose bengal (0.05 g/l) to slow down growth of fast growing species, and a few drops of streptomycin (0.002g/l) to prevent bacterial growth on the plates. The inoculum was evenly dispersed in the agar medium by oscillating the dish gently by a circular movement after addition of the cool agar medium before it set. The Petri dishes were incubated in an inverted position at 32±2ºC for 5 days. The fungal species which developed were identified and the number of colonies of each species recorded. Counts of fungal population were used to calculate the quantity of fungi in unit soil.

h. Conidium germination tests

Slide method

Conidia were carefully gathered with sterile inoculating loop and transferred into the germination liquid medium in McCartney tubes. The spore suspensions were shaken after adding a drop of Tween 80 by hand for 10 minutes to give uniform dispersion. The number of spores in suspension for every germination test was strictly standardised to 400,000-500,000 per millilitre of medium with the aid of a haemocytometer. Sterile Petri dishes, each containing a sterile glass slide lying on moist filter paper formed the incubation chamber. Using a sterile dropping pipette, three individual drops of the spore suspension (about 0.01ml in volume) were placed on each slide and the Petri dish closed and incubated at 32±2ºC for 24 hours. At the end of the incubation period, the spores were stained with cotton blue (0.01%) in lactophenol. If observation could not be made immediately, drops of N/40 formaldehyde were added, in addition to the stain to make sure the development of the spore was arrested. Percentage germination for any treatment was based on 400-500 observed conidia was recorded. A conidium with discerniable germ-tube was considered to have germinated. The lengths of 20 randomly selected germ-tubes were also measured with an eye-piece graticule. Using the same method, conidial suspension drops were placed on small pieces (1.0 x 1.0cm) of either scale leaves or swollen leaf bases lying on the slides.

i. ***Aspergillus niger*** **growth on shallot bulbs on sale at the vegetable stores**

Commercial shallot bulbs sold on the markets showed varying degrees of *A. niger* contamination. Bulbs of both Pink and Pale-brown cultivars were kept in desiccators for three days. The bottom of the desiccators was filled with water to provide 100% R.H. The percentage of bulbs showing external *A. niger* growth in each case was recorded. The coloured papery scale leaves were carefully detached, examining for further growth on them and beyond.

j. ***A. niger*** **culture growth tests.**

Some experiments involved growth of *A. niger* on solid agar media in Petri dishes and in liquid media in 250 ml Erlenmeyer flasks. A Petri dish contained 20 ml of the medium and a 250ml Erlenmeyer flask contained 30 ml of the medium. The diameter of the growing colony on the agar medium was measured periodically along two diameters at right angles to each other drawn at the bottom of the Petri dish. Mycelium produced in the liquid medium in the Erlenmeyer flask was harvested after the desired period of incubation on a pre-weighed filter paper, dried at 80^{o}C for 12 hours and weighed. The dry weight of the mycelium was then determined.

k. Growth of *A. niger* isolate in aqueous extracts of the bulbs.

An amount of 40g of the fleshy leaf base of the bulb was blended in 20ml of distilled water and the mixture was strained with muslin cloth to obtain clean extract free from residues. Thirty millilitres of the extract of each was put into a 250ml Erlenmeyer flask and the initial pH of was recorded. The medium was autoclaved and inoculated with a 3 mm diameter *A. niger* culture disk taken from the growing edge of the culture. The flask was incubated at 32±2^{o}C for 5 days. The pH of the medium was measured again. The mycelium was harvested on a pre-dried filter paper, dried at 80^{o}C for 24 hours and weighed. The dry weight of the mycelium was calculated.

l. Humidity chambers

Wide-mouthed glass jars with screw-cap lids.

Solid watch glasses, each measuring 3.7x 3.7 cm with a well 1.6 cm in diameter and 1.0 cm deep, served as Van Tieghem cells for conidium longevity test. Dry conidia of each of the different *A. niger* isolates were placed separately in wells of the watch glasses which were carefully placed in wide-mouthed glass bottles containing different concentrations of Sulphuric acid.

Petri dishes

Petri dishes were used for some of the experiments which required 100 % R.H. A shallow pool of distilled water was put in a clean Petri dish to provide an atmosphere of 100% R.H.

Desiccators

Desiccators were also used as humidity chambers to incubate shallot bulbs .The edge of the lid of the desiccator was smeared with petroleum jelly to make it air-tight when placed in position.

m. Maintenance of constant humidities

Constant relative humidities ranging from 10 to 90% were maintained with solutions of sulphuric acid (H_2SO_4) of different concentrations according to the data of Solomon (1952) and as shown in Table 2. Nominal zero per cent R.H. was maintained with anhydrous calcium chloride. Water provided 100% R.H.

Table 2: Sulphuric acid (H_2SO_4) solutions for maintaining Constant Humidity

(Data of Solomon, 1952)

% R.H.	Weight of Sulphuric acid (g)	Volume of distilled water (ml)
100	0.00	100.00
90	17.91	82.09
80	26.79	73.21
70	33.09	66.91
60	38.35	61.65
50	43.10	56.90
40	47.71	52.29
30	52.45	47.55
20	57.76	42.24
10	64.45	35.55

n. Study of rot development of wound-inoculated bulbs

The outer brown scales of the bulb were removed and the ‘naked’ bulbs, surface-sterilized by immersion in 5% sodium hypochlorite solution for 10 minutes. The bulbs were rinsed twice in two changes of sterile distilled water.

A shallow wound was made at the equator of the bulb with a sterile No 1 cork borer into which a 3 mm *A. niger* culture disc was placed face down. The wound was covered with the “lid” and sealed with petroleum jelly. The bulbs were placed in watch glasses and incubated at a relative humidity of 100% at 32±2°C. The diameter of rot that developed was measured daily along two planes at right angles to each other and the mean calculated.

o. *In Vitro* production of Pectic enzymes.

The procedure adopted by Tortoe (1997) was followed. Czapek-Dox medium containing 1% Pectin was prepared. For each exercise, fourteen 250ml Erlenmeyer flasks were each filled with 35ml of the Czapek-Dox medium and autoclaved. Two of the flasks were taken after autoclaving, their contents pooled together and the initial pH determined. Each of the remaining 12 flasks were inoculated with a 3mm culture disc of the isolate of *A. niger*. The flasks were incubated at 32±2°C. Three flasks were withdrawn after 3, 6, 9 and 12 days, respectively, and the mycelium harvested, using filter paper. The harvested mycelia were dried at 80°C for 18 hours and weighed to obtain the dry weight.

p. Maceration test

The pooled culture filtrates of Section ‘**o**’ above were divided into two lots. With the No. 8 cork borer, plugs of Irish potato tuber were removed and placed in distilled water in a clean Petri dish. The plugs were carefully cut into 0.5 mm thin discs with a surgical scalpel.The discs were washed in sterile distilled water and drained on filter paper. Two millilitres of culture filtrate were put in a mini- Petri dish and the pH adjusted to pH 5.0 by adding 0.5ml of 0.1M Citrate Buffer of pH 5.0. Six of the discs were placed in the preparation. At 5-minute intervals, a disc was picked and gently pulled from two opposite ends with a pair of blunt forceps. The disc was placed back in the medium if it was still firm and did not pull apart. It came to a time that each of the six disc were pulled apart. Each of the discs were pulled apart at a particular time. The time was noted and the disc discarded.

The enzyme activity was calculated by the formula:

(1/ Mean time of maceration of 6 discs) x 100.

q Determination of productivity of *A. niger* on ground shallot bulb

Ground shallot paste described in Section ‘d’ was inoculated with a 3mm culture disc of *A. niger* and incubated at 32±2^{o}C. The degree of sporulation was determined after 10 days. Four discs were removed from the plate with a No. 7 cork borer from four symmetrical positions equidistant from the centre of the culture. Each disc was placed in a McCartney tube holding 10ml of distilled water, containing Tween 20, and shaken vigorously to disperse the spores. The number of spores per ml. of suspension was determined with a haemocytometer.

r Conidial Longetivity test.

Conidia of *A. niger* were removed in the dry state into clean watch glasses and spread into thin layer in the watch glasses. The watch glasses with their load of conidia were placed in a series of shallow pools of sulphuric acid of different concentrations in wide screw glass jars (8.5cm high and 10.0 cm in diameter). The sulphuric acid solution provided respective relative humidities of 10, 20, 30, 40, 50, 60, 70, 80, and 90% R.H as indicated in Table 2, while other jars containing anhydrous calcium chloride provided a nominal 0% R.H. and distilled water maintained 100% R.H. The jars were incubated on the laboratory bench under normal day-night light regime. The problem of irregular electric light delivery did not allow the setting of tests under continuous light and continuous dark over a protracted period.

Viability of test conidia was determined after desired intervals, by germinating samples of conidia transferred from the jars into PDB at 32±2^{o}C for a period of 24 hours, after which the percentage germination was determined and germ-tubes lengths measured.

s. Application of Potassium and Urea fertilizer

Various amounts of 50, 100, 150 and 200g of Potassium fertilizer (Multi-K) were used to prepare different concentrations of the fertilizer. Each was thoroughly dissolved in 15 L of water and stored in plastic gallons. An aliquot of 300ml of each preparation was applied weekly to the respective plants. In the case of Urea fertilizer, two levels of concentrations were used. Each of the potting bags in one set was treated with 1.98g and in the other set 3.88g of Urea per bag.

Samples of shallot bulbs of the different treatments were uprooted at intervals of 20, 40 and 60 days for various assessments.

t. Photography

The photographs in this thesis were taken with a Sony Cyber-Shot camera, model No. DSC-W330.

u. Statistical Analysis

Experimental results where necessary, were analysed statistically with SCHEFFE'S CALCULATED CONFIDENCE LIMITS AT 95% PROBABILITY. (Kershaw, 1973).

v. Experimental precautions.

i. Glassware were kept scrupulously clean. Particularly, glassware already in circulation in the laboratory were first scrubbed with detergent to remove all traces of chemicals and grease.

ii. In the assessment of conidia percentage germination, conidia were counted in all suspension drops of each treatment.

iii. When measuring the length of germ-tubes, those at the edge of the suspension drops were avoided as these were under more aerated condition than the submerged germ-tubes.

iv. Similarly, conidia in clumps which might have been influenced by chemical substances of adjacent conidia, were not included in the assessment of percentage germination and measurement of lengths of germ-tubes.

v. In any of the *A. niger* growth experiments, in the event of the incidence of bacterium or fungus contamination on any of the replicate cultures, the entire set of replicates was discarded and the exercise repeated.

vi. In order to avoid cross contamination, experiment with only one *A. niger* Isolate was set up on any particular day.

vii. Metal screw-caps of glass containers (McCartney tubes, medicinal flats) were kept loose during autoclaving and then tightened after autoclaving.

III. EXPERIMENTAL DETAILS

SHALLOT CULTIVARS IN GHANA

EXPERIMENT A. Morphology of the two shallot cultivars

There are two distinctive cultivars of shallots (*Allium ascalonicum*) cultivated in Ghana. They have been designated “Pale-brown” and “Pink” cultivars in this thesis. Their distinct characteristics were studied in Experiment A and the observations made are supported by histograms (Figs 1 and 2) and photographs (Plates 5, 6 and 7).

EXPERIMENT B. Elemental composition of the bulbs of the two shallot cultivars

The morphological differences between the two cultivars were identified in EXPERIMENT A. Differences in chemical composition of the two cultivars may also exist. The levels of the components may influence the growth of *Aspergillus niger* in the bulbs and affect pathogenesis. Five batches of bulbs of Pale-brown cultivar and Five batches of Pink cultivar were purchased in the same month, October 2012, and analyzed. They were purchased from the markets of Anloga in the Volta region and of Agbogbloshie, Kaneshie, Makola and Mallam Atta in Accra. Bulbs of approximately the same size were selected for the analysis. For any test five bulbs were taken, sliced and pooled. The chemical components identified and their concentrations are shown in Tables 3 to 8.

ASPERGILLUS NIGER INFECTION OF THE SHALLOT BULBS.

EXPERIMENT C. *Aspergillus niger* growth on bulbs of fresh stocks of the two shallot cultivars in market stalls.

It was noticed that bulbs of both cultivars purchased on any occasion included samples bearing the spreading dark colonies of *A. niger*. In order to quantify these natural infections, shallot bulbs were purchased from Agbogbloshie market in Accra and Anloga market in the Volta region in August 2011 and January 2014, respectively. One hundred bulbs each of the two cultivars, Pale-brown and Pink, were selected from the produce obtained from Agbogboshie market. The same was done for the produce purchased from Anloga market. Each was meticulously scrutinized and *A. niger* growth detected at various locations of each bulb was recorded. The percentages of these sides bearing *A. niger* growth for each cultivar are shown in Table 9 and example of colonization of the scale leaves is depicted in Plate 8.

EXERCISE D. Influence of micro-habitats of shallot bulbs on the development of *Aspergillus niger* conidiophores.

As a sequence, the morphology of *A. niger* at the various sites was studied. The various sites constituted different micro-habitats which might affect *A. niger* differently. That supposition was investigated in this Experiment. Samples of the fungus taken from the different sites were mounted on glass slides in Lactophenol cotton blue and studied under high power of the microscope. Quantitative data obtained are tabulated in Table 10

EXPERIMENT E. Culture characteristics of the five test isolates of *Aspergillus niger*.

Variation is a common phenomenon among microbial species. This produces variance with distinctive characteristics. Any investigation that incorporates variants yields a better information on the subject matter. This research therefore, selected five of the numerous *A. niger* isolates obtained from naturally infected bulbs of the two shallot cultivars and used them as the test fungi for this experiment. The growth rate, colour of the culture and habit of growth of the mycelium were used to distinguish these five Isolates, designated 1,2,3,4 and 5.

Table 11 contains the details of the characteristics of the five Isolates and Plate 9 shows the cultures, growing on Potato Dextrose Agar in Petri dishes.

EXPERIMENT F. Germination of conidia of the five test *Aspergillus niger* Isolates and pattern of growth on the swollen leaf bases and the scale leaves and fate of the germ tubes.

The conidia of the five Isolates of *A. niger* were taken from five day old respective cultures and suspended in Potato Dextrose Broth (PDB) of different concentrations to find their germination capacity in the different media and to study the growth habit of the germ-tubes. The germination media used for each isolate were Potato Dextrose Broth of four dilutions viz,1:10, 1:20, 1:30 and 1:40 v/v (PDB: sterile distilled water). Distilled water constituted control medium. Conidia in these suspensions were incubated for 12 hours in light intensity of 76 lux. The suspension drops were incubated on surfaces of detached parts as follows: (a) outer surface of the external leaf scale (b) inner surface of the external leaf scale (c) outer surface of the internal leaf scale (d) inner surface of the internal leaf scale (e). outer surface of the outermost swollen leaf base.

The bulb parts were separately placed in 5% sodium hypochlorite solution in sterile Mc-Cartney tubes for five minutes to surface sterilize them and then rinsed in sterile distilled water and air dried on sterile filter paper. Six square pieces, each measuring 5x 5 mm, were cut from each specimen and inoculated. For each, the desired test surface was inoculated with a drop of spore suspension. Three pieces for each treatment were withdrawn after 12 hours, stained with lactophenol cotton blue and used in the germination assessment. The results obtained are presented in Tables 12 to 16. The remaining three pieces were withdrawn after 24 hours, stained and the pattern of growth of the germ-tubes studied. Since there was good germination especially at the higher concentrations of PDB, a subsequent germination test was carried out to find out the level of germination which could be supported by individual selected sugars, galactose, glucose and sucrose solutions of concentrations of 0.125, 0.25, 0.5 and 1.0 percent. In this experiment the outer surfaces of the external and internal leaf scales and the outermost swollen leaf base were used. The spore suspensions were incubated for 12 hours and the results are tabulated in Tables 17 to 31. In both experiments the lengths of germ tubes of 20 germinated conidia in each case were measured and the mean calculated.

EXPERIMENT G. Germination of conidia of the five test *Aspergillus niger* isolates in aqueous extracts of the two shallot cultivars.

In the event of bruising of the bulb, the conidia of *A. niger* will come in contact with exudates from these wounds. The next germination test was, therefore, carried out to find whether these exudates are rich in nutrients that may support good germination of the conidia and growth of the germ-tubes as in the case of Potato Dextrose Broth. The conidia were germinated in the fluids expressed from ground bulb, which constituted standard concentrations and dilutions of ½, 1/4, 1/8 and 1/16 of the fluid.

Distilled water was the control medium. Spore suspension drops on sterile slides were incubated in humid Petri dishes at 32±2^{o}C under light illumination of 76 lux. There were three replicate slides for each treatment. The results, both percentage germination and mean length of germ-tubes, are shown in Tables 32 to 36 and Fig 3.

EXPERIMENT H. Shallot bulb inoculation tests using the two shallot cultivars and the five test *Aspergillus niger* Isolates.

Good germination of the conidia and healthy growth of the germ-tubes occurred at the higher concentrations of the expressed fluid of the bulbs in the preceding experiment. This suggested the probable absence of toxic compounds in the fluid. The germ-tubes will also probably grow well in the tissues of the bulbs of both cultivars. This suggestion was examined in this experiment. Bulbs of approximately the same size were surface sterilized by immersing in 10% sodium hypochlorite solution for five minutes and rinsed in sterile distilled water after the covering pigmented scale leaves had been removed. Bulbs of each cultivar were divided into two sets. One set was surface inoculated by placing 3 mm diameter culture discs, face down on the bulbs. The second set was wound-inoculated as described in " Materials and General Methods" with 3mm diameter culture discs of the *A. niger* isolates. Both sets were stored in humidity chambers with internal atmosphere of 100% R.H. The diameters and depths of rots which developed after 3 days were measured and recorded. There were four replicates in each treatment. The mean values of the measurements appear in Table 37.

EXPERIMENT I. Rotting of wound-inoculated bulbs at different relative humidities using the two shallot cultivars and the five test *Aspergillus niger* isolates.

Humidities prevailing in shallot stalls rarely attain 100% R.H. The rate of rotting occurring under those conditions may, therefore, be slower than those recorded in Experiment H. The relation between the percentage relative humidity of the environment and the rate of rotting was investigated in this experiment. The same procedure was followed but, on this occasion incubating different batches of the inoculated bulbs at 50, 60, 70, 80, 90 and 100% R.H for 10 days and the effect of the different relative humidities on the extent of rotting of the bulbs, on the development of the conidia apparatus and the degree of sporulation of the *A. niger* isolates are reported in Tables 38-47. Plate 10 is a photograph of one set of the inoculated bulbs at the end of the incubation period.

EXPERIMENT J. *In-vitro* production of pectic enzymes by the five test *Aspergillus niger* Isolates

Rotting by *A. niger* is the symptom of the degradation activities of the pectic enzymes it produces. The differences in the rate of rotting by the different Isolates of *A. niger* observed in the previous experiment possibly reflects differences in the ability to produce these enzymes. This was verified by examining the positive correlation between growth rates and corresponding enzyme activity of the filtrates of *A. niger* isolates . In this experiment, tests were carried out to:

(a) establish growth rate of the *A. niger* strains in Czapeck Dox medium

(b) establish enzyme activities of the Czapeck Dox medium filtrate of the *A. niger* isolates.

(c) find out whether the order of growth performance in the Czapeck Dox medium of the *A. niger* isolates will be altered by change in substrate, by growing the Isolates in Bean Extract, Cassava Dextrose Broth, Oat Extract, Potato Dextrose Broth and the Sweet Potato Broth.

There were four replicate 250ml Erlenmeyer flasks in every test, each containing 30 ml of the medium. The inoculated flasks were incubated for 8 days under 12- hour day-night cycle, at 32±2^{o}C.

The results of the numerous tests are tabulated in Tables 48a-e, 49a-e, 50, 51, 52.53, 54a-e and 55. Because the volume of the culture filtrate diminished very drastically during growth of the cultures, the replicate filtrates were pooled on the 12th day to provide adequate volume of fluid for the determination of a single final pH for any particular treatment.

THE SOIL *ASPERGILLUS NIGER*-SHALLOT BULB COMPLEX.

EXPERIMENT K. Mycoflora of the bulbs and the rhizosphere of shallot plants raised in soil of Anloga farms amended with Urea fertilizer.

Knowledge of the response of *A. niger* in soil to the presence of the shallot plants is imperative for complete understanding of *A. niger*-shallot plant association. If organs of shallot plants secrete stimulatory compounds, growth of *A. niger* in the vicinity will be much stimulated. It is not uncommon for certain fungal species in soil to be associated with specific living roots.

Pertinent experiments were designed to study the sort of relation between *A. niger* and the two shallot cultivars in soil.

Anloga shallot farmers routinely add manure to the freshly prepared shallot plots prior to planting. Manure adds nitrogen compounds to soils. The effects of known quantities of nitrogen was studied by adding to the soil different concentrations of Urea. The bulbs of each of the two shallot cultivars were planted in several black polythene bags (20 x 12 cm). Each bag contained 1200g of Anloga farm soil. For each shallot cultivar, the bags were divided into three batches of 15 bags each and the bags were planted with a single bulb each. Urea powder weighing 1.94g and 3.88g respectively were added to the bags of two batches. Bags of the third batch received no urea. The potted bulbs were placed in the green house under a 12-hour day/night cycle.

The densities of fungi on the surface of the developing bulblets, in the rhizosphere and of those in the non rhizosphere soil were determined at 20, 40 and 60 days after planting. By the 20th day, the parent bulb had disintegrated and the bulblets had become independent and self-sustaining. At sampling time, three bags of each treatment were withdrawn for the estimation of fungal populations. Non rhizosphere soil was collected at a distance of 2.0 cm from the developing bulbs to a depth of 2.0 cm with a sterile cork-borer. Samples of the replicate bags were pooled, pulverized and thoroughly mixed. One gram was then taken and used to estimate the fungal population by the soil dilution plate method. Rhizosphere soil estimation of the fungal population, also by the soil dilution plate method, was collected from gently uprooted plants as described under General Methods. The same method was used to determine the density of fungal population of soil particles adhering to the surfaces of the bulbs which in essence was phyllosphere population. There were three replicate Petri plates for each determination. The results, presented as number of Colony Forming Unit (CFU's) x 10^3 per g of soil are recorded in Tables 60 to 62. The results also include data on the stage of development of the bulbs at the estimation time. Plates 11, 12 and 13 are examples of photographs of colonies of fungal species of Anloga soil and of rhizosphere soils of 40 day old growing bulbs.

EXPERIMENT L. Response of bulbs of the two shallot cultivars formed in Anloga farm soils amended with Urea fertilizer to *Aspergillus niger* inoculation using *A. niger* Isolates 1 and 2.

It was expected that an additional supply of nitrogen to the shallot plants would result in an enhanced metabolism and growth. A more robust plant that would be less susceptible would be one of the important consequences. Experiment L studied the relative susceptibility of bulbs formed in soils amended with the different concentrations of Urea used in the preceding Exercise.

Following the procedure used in Experiment H, bulbs harvested on the 60th day after planting were wound inoculated with mycelium of *A. niger* Isolates 1 and 2. Samples were stored at 50, 60, 70, 80, 90 and 100% relative humidity for three days after which the extent of infection was assessed. There were three replicate bulbs for each urea concentration.

Tables 63a-c contain measurements of diameters of the rotted areas recorded.

EXPERIMENT M: Mycoflora of the bulbs and the rhizosphere of shallot plants raised in soil of Anloga farms amended with Potassium fertilizer.

Experiment K was repeated in this investigation but with different objective. Potassium as a fertilizer is well documented. It also has a unique characteristic of increasing the permeability of plant cell membranes. Higher concentrations will in the first place cause an increased leakage from the bulbs and roots into the soil encouraging greater fungal colonization of the surfaces of the plants. Secondly, the increase in cell membrane permeability on the application of potassium may cause faster penetration of cell degrading enzymes of invading fungal parasites into host tissues.

The effect of Potassium fertilizer applied at concentrations of 0.00, 3.33, 6.67, 10.00 and 13.33 g/l on the density of fungi colonizing the shallot plant surfaces was the subject of Experiment M.

The effect of the Potassium fertilizer at these concentrations on the susceptibility of 60 day old bulbs to *A. niger* infection constituted EXPERIMENT **N: 'Response of bulbs of the two shallot cultivars formed in Anloga soils amended with Potassium fertilizer to *Aspergillus niger* inoculation using *A. niger* Isolates1 and 2'** following the procedure of Experiment L. The results are tabulated in Tables 64 to 70.

EXPERIMENT O. Mycoflora of the bulbs and rhizosphere of plants of the two shallot cultivars grown in soils of farms of non-alliaceous crops.

The results of Experiment K and M revealed overwhelming preponderance of *A. niger* growth around the shallot plants. It was considered necessary to verify whether this pattern of colonization would be replicated in farm soils which had supported non-alliaceous crops, namely, cassava (*Manihot esculenta*), groundnut (*Arachis hypogea*) and maize (*Zea mays*). Bulbs of the two shallot cultivars were used according to the procedure described in Experiments K and M but without fertilizers. The results are recorded in Tables 72 to 74.

ROLE OF THE DIFFERENT SHALLOT CULTIVARS IN THE PERSISTENCE OF *A. NIGER* IN THE ECOSYSTEM

EXPERIMENT P. Conidiation of *Aspergillus niger* Isolates 1, 3 and 5 on ground bulb tissue of the two shallot cultivars and the germination capacity of the conidia.

Growth of *A. niger* on the subterranean organs of the shallot plants was overwhelming. The bulbs and the roots will contribute largely to persistence of *A. niger* in the soil. Innumerable quantities of *A niger* conidia, are produced by hyphae growing on the post-harvest bulbs as shown in Plate 8. These, as dispersal units, are likely to contribute, to a far greater measure, to the persistence of the fungus in the ecosystem, provided, they live long. For any shallot cultivar to play this role well, the bulb should be a suitable medium substrate for growth and congenial for sporulation. The comparative suitability of the bulbs of the two cultivars was investigated in the following experiments using *A. niger* Isolates 1, 3 and 5.

(a) Vegetative growth on ground bulb tissues of the two shallot cultivars.

(b) Degree of sporulation on ground bulb tissue.

(c) Germination capacity of the conidia in nutrient solutions.

Rate of vegetative growth was provided by daily measurements of diameters of the cultures. There were four replicates Petri dishes for each test. The measurements are recorded in Tables 75 to 77.

Discs of identical diameters of the cultures were shaken in similar identical quantities of distilled water at the end of incubation period of 7 days. The density in the suspensions were determined using a Haemocytometer. The calculated densities are shown in Table 78.

Vigour of the conidia at harvest, 7 days after inoculation, was tested by suspending them in Bean Meal Extract, Potato Dextrose Broth and Sweet Potato Extract and incubating them on glass slides at 32±2^{o}C. The percentage germination and mean length of germ-tubes were recorded after 12 hours. Tables 79 to 81 contain the results of this test.

EXPERIMENT Q. Longetivity of conidia formed by *Aspergillus niger* Isolates 1, 3 and 5 at different relative humidities.

Finally, the longetivity of the conidia of *A. niger* Isolates 1, 3 and 5 was investigated. Dry conidia were removed from seven day old cultures and the samples kept in solid watch glasses standing in bottles with screw caps. The bottles contained 0.5 cm deep solutions of Sulphuric acid of different concentrations which provided and maintained 10, 20, 30, 40, 50, 60, 70, 80, and 90% Relative humidity. Zero and 100% Relative humidities were provided by and maintained by dry anhydrous Calcium chloride and distilled water, respectively. The preparations were kept at 32±2^{o}C under 12- hour day night cycle. Some of the conidia of each treatment were suspended in Potato Dextrose Broth at 32±2^{o}C after specific intervals. The percentage of the conidia which germinated after 12 hours represented the percentage viability of the sample. Germ-tube of the germinated conidia were also measured as an indicator of the vigour of the germinated conidia. When a conidia sample registered zero percentage germination, samples for that particular watch glass were used in the next two consecutive germination tests to confirm the zero results before tests were discontinued. The experiment was run over a total period of 200 days from January 2012 to July 2012. The results are presented in Appendices B to V and Fig 4.

IV. RESULTS

EXPERIMENT A. Morphology of the shallot cultivars

The two shallot cultivars, Pale-brown and Pink, have many similarities. There are also many distinct differences by which they can be identified.

Features common to the bulbs of the two cultivars observed were:

1. Conical shape of the bulbs as shown in Plate 5,
2. Two inner and outer, coloured scale leaves;
3. White-coloured swollen leaf bases;
4. Swollen leaf bases of same thickness;
5. Small cylindrical discs.

The differences between the bulbs of the two cultivars were as follows:

1. Pale-brown and Pink pigmentation of the scale leaves of the Pale-brown and Pink cultivars respectively
2. Larger bulbs of the Pale-brown cultivar.
    i. The respective mean lengths of the bulbs of the Pale-brown and Pink cultivars were 3.62±0.06 and 3.26±0.08cm which by the calculated Scheffe's Confidence limit were significantly different at the 5% level of probability, and the corresponding ranges were 3.1 to 4.18 and 2.66 to 3.83cm as shown by the histograms in Fig. 1.
    ii. The respective mean widths of the bulbs were 2.71±0.05 and 2.08±0.08cm, which by the calculated Scheffe's confidence limit, were significantly different

at 5% level of probability, and the corresponding ranges were 2.50-3.08 and 1.65-2.51cm as observed in the histograms in Fig. 1

3. As a consequence of the difference in sizes, the respective mean weights of the bulbs of the Pale-brown and Pink cultivars were 8.79±0.27 and 5.98±0.29g, which by the calculated Scheffe's Confidence Limit were significantly different at the 5% level of probability, and the corresponding ranges were 6.48 to 10.95 and 4.43 to 7.82g according to the data in Appendix A.
4. The growing plants produced by these bulbs could be easily differentiated.
    i. Pale-brown cultivar plants were larger than Pink cultivar plants as observable in Plate 6 of 30 day old potted plants. The histograms in Fig.2 contain details of the lengths and widths of mature leaves of 60-day old plants
    ii. The lengths of the mature leaves of the Pale-brown and Pink cultivars ranged from 34.3 to 49.9 and 33.9 to 46.2cm, respectively, with corresponding means of 40.96±4.5 cm and 38.67± 3.1cm respectively.
    iii. The width of the mature leaves of the Pale-brown and Pink cultivars ranged from 4.33 to 6.10 and 3.54 to 5.98mm, respectively, with corresponding means of 5.38±0.9mm and 4.84±0.7mm.
    iv. In the field the Pale-brown cultivar crop looked greener and more lush than the Pink cultivar of the same age crop as shown in Plate 7.

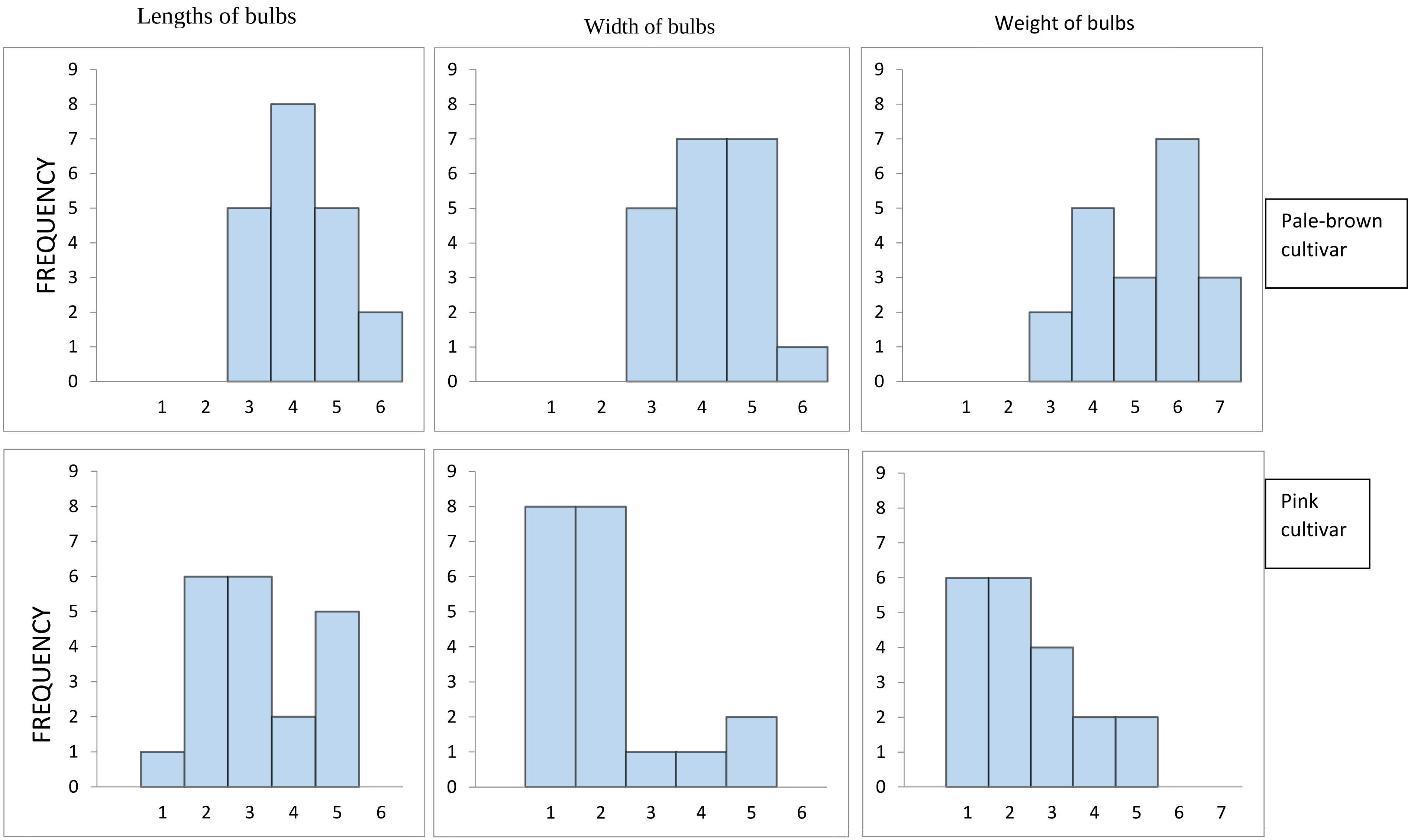
X 1 $^{1}/_{4}$

Plate 5. Photographs of bulbs of Pale- brown (TOP) and Pink (BOTTOM) cultivars of shallot (*Allium ascalonicum*)

X 1/8

PLATE 6. Photograph of 30-day old plants of the Pale-brown (TOP) and Pink (BOTTOM) cultivars of shallot (*Allium ascalonicum*).

Plate 7. Photographs of plots of 60-day old plants of shallot (*Allium ascalonicum*) distinguishing between the plants of the Pale-brown cultivar (TOP) and the Pink cultivar (BOTTOM).

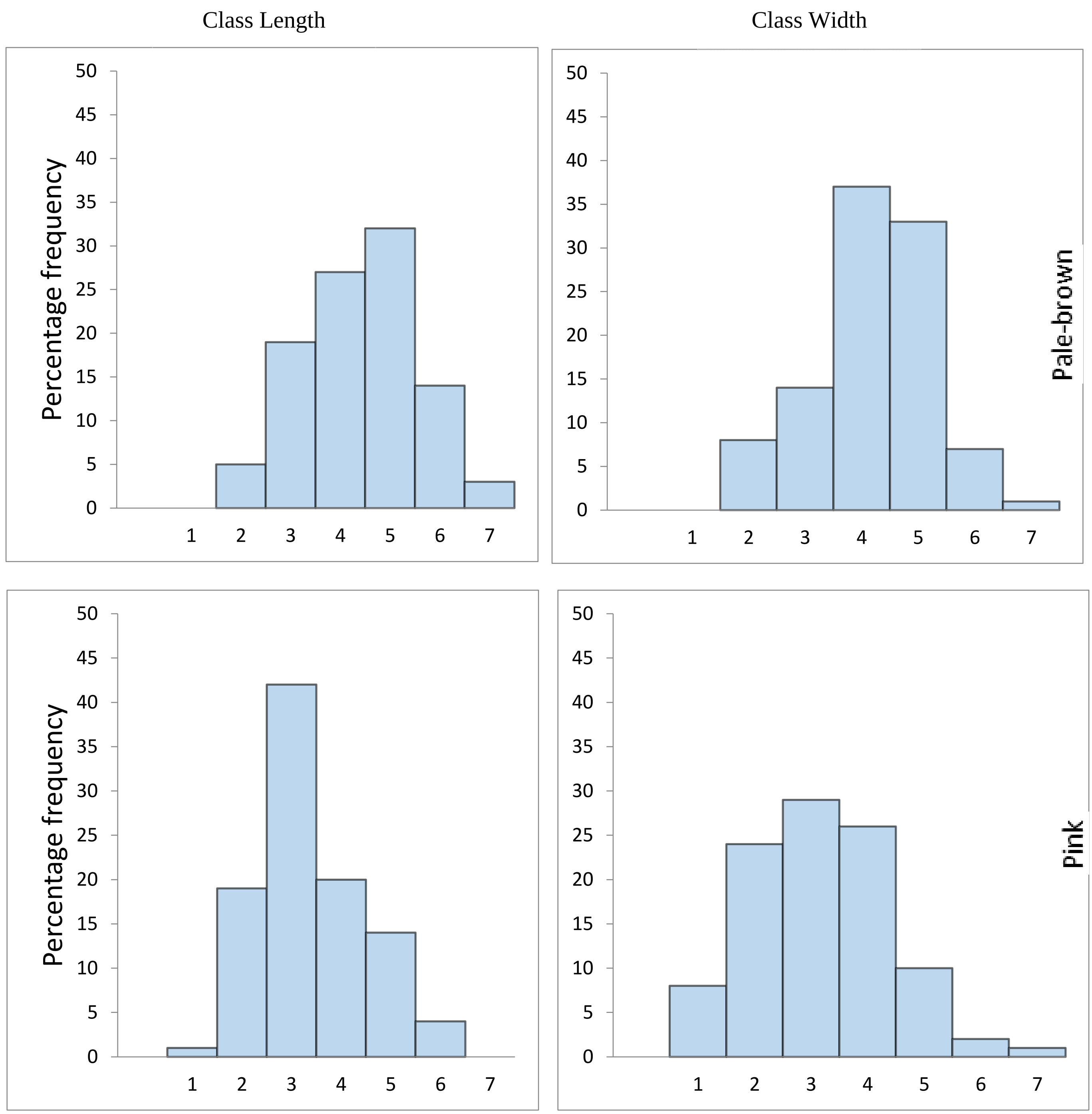


Key: 1: 2.51-2.80 cm 4: 3.41-3.70 cm
2: 2.81-3.10 cm 5: 3.71-4.00 cm
3: 3.11-3.40 cm 6: 4.01-4.30 cm

Key: 1: 1.61-1.90 cm 4: 2.51-2.80 cm
2: 1.91-2.20 cm 5: 2.81-3.10 cm
3: 2.21-2.50 cm 6: 3.10-3.40 cm

Key: 1: 4.01-5.00 g 4: 7.01-8.00 g 7: 10.01-11.00g
2: 5.01-6.00 g 5: 8.01-9.00 g
3: 6.01-7.00 g 6: 9.01-10.00 g

Figure 1: Length, width and weight of both Pale-brown and Pink cultivars of Shallot (*Allium ascalonicum*) purchased from the Anloga market.



Key: 1: 30.1-33.0 cm 4: 39.1-42.0 cm 7: 48.1-5.0 cm
2: 33.1-36.0 cm 5: 42.1-45.00 cm
3: 36.1-39.40 cm 6: 45.1-48.0 cm

Key: 1: 35.1-40.0 cm 4: 55.1-60.0 cm
2: 40.1-45.0 cm 5: 60.1-65.0 cm
3: 45.1-50.0 cm 6: 65.1-70.1 cm ($x10^{-3}$)

Figure 2: Class-lengths and class-widths of the mature leaves of the Pale-brown and Pink shallot (*Allium ascalonicum*) cultivars

EXPERIMENT B. Elemental composition of the bulbs of the two shallot cultivar

Six major chemicals, as indicated in Tables 3 to 8, were detected in bulbs of both the Pale-brown and pink shallot cultivars. The chemicals were of different quantities in the following descending order: Nitrogen>Potassium>Phosphorus>Sodium>Calcium>Magnesium. As shown in Table 8, the percentages of Nitrogen in the Pale-brown and Pink cultivars were 1.456±0.007 and 1.192±0.05 respectively. The mean percentage of magnesium, in comparison, for the pale-brown and pink cultivars were 0.054±0.00 and 0.046, respectively.

This higher level of Nitrogen and Magnesium in the Pale-brown cultivar than in the Pink cultivar was applicable to all the chemicals except for the rare occurrence of higher concentrations of Sodium in Tests 2 and 3 involving bulbs purchased from the Anloga and Mallam Atta markets as reported in Tables 4 and 7. Even with these, the values for the two cultivars were extremely close. According to the data of the Anloga lot recorded in Table 4, values for the pale-brown cultivar for Tests 1, 2 and 3 were 0.183, 0.181 and 0.186 per cent, respectively and for the pink cultivar were 0.181, 0.185 and 0.186 per cent, respectively.

Similarly, information in Table 7 on the Mallam Atta products indicated 0.183, 0.185 and 0.190 per cent Sodium, respectively for Tests 1, 2 and 3 of the pale-brown cultivar, while the pink cultivar had 0.183, 0.189 and 0.192 per cent, respectively.

It is noteworthy that the values for any chemical were different for the different tests, howbeit within reasonable ranges.

Table 3: Major chemical elements (%) of bulbs of two cultivars of shallot (*Allium ascalonicum*) purchased from Agbogbloshie Market, Accra in October 2012.

	Test					
	1		2		3	
Chemical Elements (%)	Pale brown cv.	Pink cv.	Pale brown cv.	Pink cv.	Pale brown cv.	Pink cv.
Calcium	0.192	0.162	0.188	0.188	0.178	0.174
Magnesium	0.051	0.041	0.041	0.039	0.051	0.050
Nitrogen	1.643	1.245	1.595	1.432	1.621	1.319
Phosphorus	0.373	0.412	0.394	0.363	0.423	0.369
Potassium	1.183	1.134	1.178	1.123	1.180	1.134
Sodium	0.181	0.163	0.164	0.143	0.171	0.169

Table 4: Major chemical elements (%) of bulbs of two cultivars of shallot (*Allium ascalonicum*) purchased from Anloga Market, in October 2012.

	Test					
	1		2		3	
Chemical Elements (%)	Pale brown cv.	Pink cv.	Pale brown cv.	Pink	Pale brown cv.	Pink cv.
Calcium	0.150	0.124	0.148	0.121	0.158	0.124
Magnesium	0.049	0.037	0.049	0.040	0.043	0.037
Nitrogen	1.599	1.233	1.589	1.229	1.588	1.228
Phosphorus	0.399	0.343	0.401	0.341	0.400	0.336
Potassium	1.089	1.045	1.094	1.048	1.093	1.054
Sodium	0.183	0.181	0.181	0.185	0.185	0.186

Table 5: Major chemical elements (%) of bulbs of the two cultivars of shallot (*Allium ascalonicum)* purchased from Kaneshie Market, Accra in October 2012.

	Test					
	1		2		3	
Chemical Elements (%)	Pale brown cv.	Pink cv.	Pale brown cv.	Pink	Pale brown cv.	Pink cv.
Calcium	0.149	0.096	0.153	0.133	0.152	0.123
Magnesium	0.057	0.051	0.062	0.061	0.058	0.053
Nitrogen	1.245	0.882	1.452	1.250	1.352	1.084
Phosphorus	0.375	0.296	0.429	0.377	0.498	0.354
Potassium	1.096	0.738	1.017	0.873	1.025	0.934
Sodium	0.178	0.167	0.169	0.167	0.168	0.167

Table 6: Major chemical elements (%) of bulbs of the two cultivars of shallot (*Allium ascalonicum*) purchased from Makola Market, Accra in October 2012.

Chemical Elements (%)	Test 1 Pale brown c.v.	Test 1 Pink c.v.	Test 2 Pale brown c.v.	Test 2 Pink	Test 3 Pale brown c.v.	Test 3 Pink c.v.
Calcium	0.193	0.193	0.191	0.187	0.187	0.185
Magnesium	0.062	0.048	0.062	0.059	0.059	0.044
Nitrogen	1.632	1.013	1.453	1.233	1.214	1.112
Phosphorus	0.401	0.394	0.367	0.345	0.389	0.383
Potassium	1.201	1.111	1.211	1.081	1.093	0.073
Sodium	0.182	0.173	0.193	0.188	0.185	0.171

Table 7: Major chemical elements (%) of bulbs of the two cultivars of shallot (*Allium ascalonicum*) purchased from Mallam Atta Market, Accra in October 2012.

	Test					
	1		2		3	
Chemical Elements (%)	Pale brown cv.	Pink cv.	Pale brown cv.	Pink	Pale brown cv.	Pink cv.
Calcium	0.140	0.110	0.147	0.121	0.142	0.110
Magnesium	0.055	0.050	0.060	0.046	0.059	0.046
Nitrogen	1.224	1.211	1.263	1.199	1.276	1.214
Phosphorus	0.389	0.395	0.379	0.400	0.411	0.393
Potassium	1.212	1.111	1.236	1.019	1.226	1.112
Sodium	0.183	0.183	0.185	0.189	0.190	0.192

Table 8: Means of the values of the major chemical elements (%) of the bulbs of the two cultivars of shallot (*Allium ascalonicum*) recorded in Tables 3 to 7

.

Chemical	Pale-brown cv.		Pink cv.	
	Range	Mean	Range	Mean
Calcium	0.149-0.193	0.164±0.01	0.096-0.215	0.145±0.02
Magnesium	0.041-0.062	0.054±0.00	0.037-0.061	0.046±0.00
Nitrogen	1.124-1.643	1.456±0.07	0.882-1.432	1.192±0.05
Phosphorus	0.367-0.498	0.406±0.01	0.296-0.412	0.367±0.01
Potassium	1.025-1.236	1.142 ±0.03	0.738-1.134	0.979±0.08
Sodium	0.168-0.193	0.179±0.00	0.143-0.192	0.175±0.01

EXPERIMENT C. *Aspergillus niger* growth on bulbs of fresh stocks of the two shallot cultivars in market stalls.

The bulbs of the two cultivars of shallot were seemingly readily prone to *A. niger* infection as shown by the data in Tables 9. The fungus did not only invade the exposed surfaces of the bulbs but also, despite the cover of the scale leaves, invaded the surfaces of the swollen leaf bases and even the contact surfaces of the bulblets. The extent of colonization, however, varied with the different sites. Other factors which influenced infection were the source of bulbs and the type of cultivar.

Remarkably, for all the sites of colonization, bulbs of the pale-brown cultivar showed higher number of infected bulbs than bulbs of the Pink variety. The difference in number of bulbs involved may be marginal as in the category of " Contact surfaces of mini-bulbs" where the respective numbers of infected bulbs for the pale-brown and pink cultivars for Agbogbloshie market were 64 and 64,and the Anloga market 68 and 65 out of the 100 observed bulbs in each case. The greatest difference was observed on the adaxial surface of the outer scale leaf with the corresponding values out of 100 bulbs of 80 and 68 for the Agbogbloshie bulbs, and 86 and 73 for the Anloga bulbs.

Colonization of the different parts of the bulbs showed all sorts of combinations and permutations. The photograph in Plate 8 shows an example of the colonization of the scale leaves of the bulb.

Table 9: *A. niger* growth on the bulbs of the two shallot cultivars at the time of purchase from Agbogbloshie Market, Accra (August 2011) and Anloga market, Volta region (January 2014).

Site of bulb with A. *niger* growth	Percentage, out of 100 bulbs, with A. *niger* growth purchased from			
	Agbogbloshie Market		Anloga Market	
	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
Abaxial surface of outer brown papery leaf scale	68	58	76	69
Adaxial surface of outer brown papery leaf scale	80	68	86	73
Abaxial surface of inner brown papery leaf scale	84	82	86	80
Adaxial surface of inner brown papery leaf base	70	68	80	76
Abaxial surface of Outermost fleshy leaf base	72	69	78	65
Contact surfaces of mini-bulbs	64	64	68	65

X 2

Plate 8. Photograph showing colonies of *Aspergillus niger* on the inner and outer scale leaves of naturally infected shallot bulb (*Allium ascalonicum*) of Pale-brown cultivar on sale at the Agbogbloshie market

EXPERIMENT D. Influence of micro-habitats of shallot bulbs on the development of *Aspergillus niger* conidiophores

An examination of the colonies of *A. niger* growing at the different locations with the high power of the microscope revealed an influence of the microhabitat on the colonies. The unusually rapid transportation of the protoplast of the mature hyphae to the growing apices rendered the empty hyphae unstainable and, therefore, difficult to observe morphological details. Reliable quantitative data were provided by the thick-walled conidiophore. The length of the conidiophores at the different locations were measured and their means calculated.

The values of the mean conidiophore lengths are tabulated in Table 10. All the four sets of conidiophores showed significantly different mean conidiophore lengths, ranging from 1125.8±9.2 to 2124.8±12.5 µm on bulbs of the pale-brown cultivar and 799.8±10.2 to 1720.5± 11.5µm on bulbs of the pink cultivar. These values clearly point to the development of larger conidiophores in bulbs of the pale-brown cultivar. The possible reasons for this observation will be discussed later in detail under "General Discussion".

Table 10: Influence of the micro-environment of bulbs of the two shallot cultivars purchased from Anloga, Volta region on the development of the conidiophores of *A. niger*.

Site of bulb with *A. niger* growth	Mean length of 50 Conidiophore±Standard Error (µm)on	
	Pale-brown cv.	Pink cv.
Abaxial surface of outer brown scale leaf	2124.8±12.5 [a]	1720.5±11.5 [b]
Abaxial surface of inner brown scale leaf	1855.3±10.8 [a]	1480.3±12.3 [b]
Abaxial surface of outermost fleshy leaf base.	1125.8±9.2 [a]	799.8±10.2 [b]
Contact surface of mini bulbs	1024.6±8.8 [a]	1000.8±11.8 [b]

By the calculated Scheffe's Confidence Limit, pairs of values in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Experiment E. Culture characteristics of the five test isolates of *Aspergillus niger*

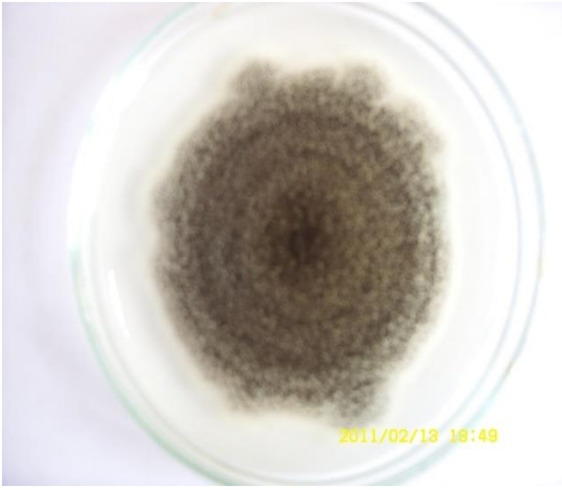
The isolates were obtained from bulbs of both shallot cultivars and selected on the basis of the three characteristics stated in Table 11. They differed by the rate of growth on Potato Dextrose Agar Petri plates. Isolate 1 grew fastest attaining a mean culture diameter of 6.98±0.08 cm in 7 days. It was followed by Isolates 3, 4, 5 and 2 in descending order. The slower growing isolate attained a mean culture diameter of 4.35±0.04 cm only. Values for the five Isolates significantly different from each other are indicated in Table 11.

Of the two remaining characteristics each isolate had its peculiar combination. Other characteristics of the Isolates will undoubtedly emerge in the course of this investigation

Table 11: Characteristics of *A. niger* Isolates 1, 2, 3, 4 and 5 as test fungi in Experiments F-J, L, N, P and Q.

Isolate	Mean Culture Diameter (cm) 7 days at 32±2^{o}C	Growth habit after of mycelium on Petri plate	Colour of mycelium
1	6.98±0.08 [a]	Flat mycelium	Brown black
2	4.35±0.04 [b]	Presence of Zonation	Brown
3	6.40±0.08 [c]	Frequent sectoring	Brown black
4	6.28±0.07 [c]	Flat mycelium	Black
5	5.98±0.06 [d]	Fluffy growth	Black

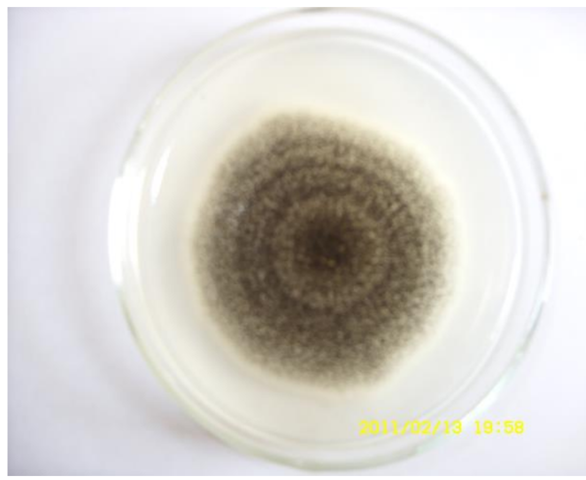
By the calculated Scheffe's Confidence Limit, pairs of values in horizontal rows bearing the same letters are not significantly different at 5% level of probability.



1

2

3



4

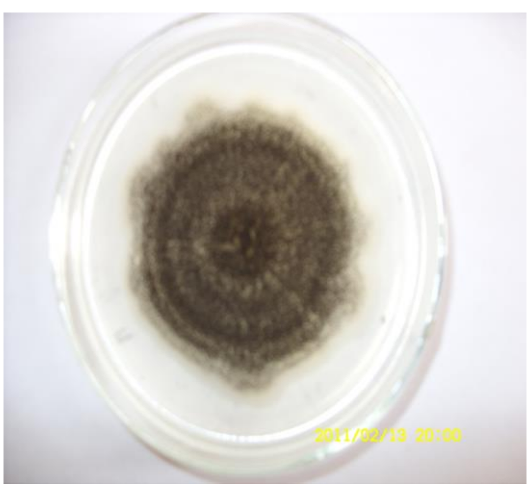


PLATE 9: Photographs of cultures of *Aspergillus niger* Isolates 1, 2, 3, 4 and 5

X $^{2}/_{3}$

EXPERIMENT F. Germination of conidia of the five test *Aspergillus niger* Isolates and pattern of growth on the swollen leaf bases and the scale leaves and fate of the germ tubes.

The variables which were involved in the conidia germination in this experiment were; the Isolate of the fungus, the cultivar of the shallot, the component part of the bulb and the media. Some showed a uniform pattern of the influence while other factors showed irregular effect. The following statements summarize the findings reported in Tables 12 to 16.

a. *Aspergillus niger* conidia did not germinate in distilled water
b. PDB dilutions of 1:40 was a very poor germination medium. For example, only 7.4 (Table 12), 4.8 (Table 13) and 10.8 (Table 16) per cent of conidia of *Aspergillus niger* Isolates 1, 2 and 5 respectively germinated on glass slides. Other instances of low germination in the 1: 40 PDB dilution medium showed percentage germination below 6.0 per cent.
c. There was good conidial germination in the higher concentrations of the PDB solutions. Naturally, the best germination was attained in the 1: 10 PDB dilution medium. Percentage germination ranged from 32.0 (Table 15) to 48.5 (Table 16) on glass slides. The majority of the tests gave germination in this medium on shallot bulb tissues between 70 (Table 12) and 93 (Table 15) per cent.
d. Percentage germination then decreased with increasing dilution of the medium.
e. Also, at any PDB dilution, germination was better on suspension drops on pale-brown cultivar bulb tissues than on pink cultivar tissues.
f. Interestingly better percentage was recorded in suspension drops on the scale leaves than on the swollen leaf bases.

g. Mean germ-tube lengths bore the same relationship to medium concentration as percentage germination. The longest germ-tubes were found in the 1:10 PDB dilution medium and germ-tube length decreased with increasing dilution of the medium
h. Slides incubated for 24 hours were thoroughly examined under the microscope after staining with Lactophenol cotton blue. The germ-tubes grew over the epidermal peels without showing any discerniable relationship with the surface architecture of the epidermis.
i. Comparing the performance of the five *Aspergillus niger* isolates, Isolate 3 as shown in Table 14 was stimulated best by the media and Isolate 2 as shown in Table 13 did not grow as well as the other Isolates, giving an indication of the range of response of *A. niger* generally.

The repeat experiments which used Galactose, Glucose and Sucrose at concentrations of 1.25, 2.5, 5.0 and 10.0 g/l gave results with unique features as recorded in Tables 17 to 31.

(a) First, there was again no germination of the conidia of all five isolates of *Aspergillus niger* in distilled water.
(b) The conidia did not also germinate in the media of all the three sugars of concentration of 1.25 g/l.
(c) The three sugars supported conidial germination to different degrees in media of concentrations of 2.5, 5.0 and 10.0 g/l.
(d) For all the three sugars, the concentrations of 10.0 g/l, supported the highest percentage germination and greatest growth of the germ-tubes, albeit to different degrees. The respective values then decreased with increased dilution of the media.

(e) Notably, in this test as with the Potato Dextrose Broth test, the conidia germinated better on the scale leaves than on the swollen leaf base. For instance, germination on the outer scale leaf, inner scale leaf and swollen leaf base in the 10 g/l galactose medium in Tables 17 to 21 on both pale-brown and pink cultivar was 34.3-40.3, 29.2-37.2 and 18.4-23.2 per cent, respectively.

(f) Mean germ-tube lengths bore similar relationship to medium concentration and conidial percentage germination. Mean germ-tube length on the outer scale leaf, inner scale leaf and swollen leaf base in the 10 g/l galactose medium in Tables 17 to 21 of both the pale-brown and pink cultivar was 35.3±3.4 – 57.8±4.0, 31.2±3.3 – 53.7±3.2 and 29.3±3.9 – 50.4±4.9 µm respectively.

(g) The conidia of Isolates 1, 2 and 4 germinated best in the glucose media while those of Isolates 3 and 5 germinated best in the sucrose media. The galactose media were the most unfavourable for the all the *A. niger* isolates.

(h) Whereas conidia of *A. niger* Isolate 1 germinated better than the remaining isolates, performance of Isolate 2 was inferior to the rest.

(i) The germ-tubes of all the isolates in all the media grew in all directions on the swollen leaf base and scale leaves.

Table 12: Germination of conidia of *A. niger* Isolate 1 in light of 76 lux in 12 hours in Potato Dextrose Broth (PDB) suspension drops on different component parts of bulbs of shallot (*Allium ascalonicum*)

		Percentage Germination		Mean Germ-tube Length (µm)	
Component part of bulb	PDB: distilled water mixture (v/v)	Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv.
Outer surface of outer scale leaf	1:10	76.4	78.5	89.6±5.6 [a]	96.3±7.0 [a]
	1:20	73.7	62.3	69.1±3.3 [a]	72.2±3.1 [a]
	1:30	64.8	23.4	62.1±4.8 [a]	55.2±1.4 [a]
	1:40	4.1	0.0	35.9±3.2	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of outer scale leaf	1:10	88.0	88.1	94.3±6.2 [a]	99.3±3.2 [a]
	1:20	71.2	71.3	62.2±5.3 [a]	76.5±3.1 [a]
	1:30	51.9	42.1	60.8±5.8 [a]	59.7±2.9 [a]
	1:40	1.0	0.0	26.5±4.1	-
	Distilled H_2O	0.0	0.0	-	-
Outer surface of inner scale leaf	1:10	89.1	74.9	102.2±4.9 [a]	97.8±2.1 [a]
	1:20	64.0	58.9	65.2±5.3 [a]	60.3±2.7 [a]
	1:30	68.8	33.2	60.8±5.8 [a]	52.1±5.3 [a]
	1:40	3.9	0.0	26.5±4.1	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of inner scale leaf	1:10	87.2	71.9	98.7±3.6 [a]	90.3±2.2 [a]
	1:20	69.9	63.8	55.9±3.8 [a]	63.2±2.3 [a]
	1:30	52.7	47.4	42.1±2.9 [a]	45.4±5.3 [a]
	1:40	2.8	1.3	25.4±2.0 [a]	22.2±1.3 [a]
	Distilled H_2O	0.0	0.0	-	-

By the calculated Scheffe’s Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 12 continued.

Component part of bulb	PDB: distilled water mixture (v/v)	Percentage Germination		Mean Germ-tube Length (μm)	
		Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outermost swollen leaf base	1:10	44.1	43.9	83.9±3.6 [a]	80.2±1.9 [a]
	1:20	21.6	18.9	55.3±4.0 [a]	63.9±2.7 [a]
	1:30	22.3	12.0	27.4±3.8 [a]	35.2±3.8 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Glass slide	1:10	42.9		55.9±2.1	
	1:20	23.7		39.7±2.7	
	1:30	13.7		22.6±4.0	
	1:40	7.4		18.3±1.2	
	Distilled H_2O	0.0		-	

By the calculated Scheffe’s Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 13: Germination of conidia of *A. niger* Isolate 2 in light of 76 lux in 12 hours in Potato Dextrose Broth (PDB) suspension drops on different component parts of bulbs of shallot (*Allium ascalonicum*).

		Percentage Germination		Mean Germ-tube Length (µm)	
Component part of bulb	PDB: distilled water mixture (v/v)	Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv.
Outer surface of outer scale leaf	1:10	69.0	69.5	89.4±4.4 ^{a}	83.8±6.1 ^{a}
	1:20	65.3	47.3	81.9±2.4 ^{a}	72.6±4.2 ^{a}
	1:30	42.3	22.8	71.3±3.8 ^{a}	60.0±2.0 ^{a}
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of outer scale leaf	1:10	77.5	72.7	86.4±2.7 ^{a}	78.9±7.1 ^{a}
	1:20	62.0	58.3	85.6±3.5 ^{a}	70.0±2.7 ^{b}
	1:30	56.8	37.4	68.6±4.2 ^{a}	62.1±2.5 ^{a}
	1:40	5.7	0.0	34.4±2.5 ^{a}	30.2±3.8 ^{a}
	Distilled H_2O	0.0	0.0	-	-
Outer surface of inner scale leaf	1:10	82.4	77.7	80.3±3.2 ^{a}	81.2±2.9 ^{a}
	1:20	60.4	43.9	69.4±4.3 ^{a}	70.3±3.2 ^{a}
	1:30	34.5	22.9	55.4±4.2 ^{a}	54.3±6.0 ^{a}
	1:40	4.5	0.0	34.4±1.8	
	Distilled H_2O	0.0	0.0	-	-
Inner surface of inner scale leaf	1:10	65.4	76.7	77.5±5.5 ^{a}	80.2±2.5 ^{a}
	1:20	62.0	40.5	64.3±3.8 ^{a}	70.2±2.1 ^{a}
	1:30	30.4	23.8	33.4±2.4 ^{a}	43.2±2.8 ^{a}
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 13 continued

Component part of bulb	PDB: distilled water mixture (v/v)	Percentage Germination		Mean Germ-tube Length (μm)	
		Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv.
Outer surface of outermost swollen leaf base	1:10	33.0	30.4	54.4±3.7 [a]	44.2±3.2 [a]
	1:20	32.5	23.3	40.3±4.2 [a]	34.5±3.1 [a]
	1:30	8.6	0.0	21.1±1.9 [a]	20.0±2.0 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Glass slide	1:10	37.1		32.3±3.1	
	1:20	18.5		34.6±2.3	
	1:30	11.0		26.4±3.7	
	1:40	4.8		21.4±2.8	
	Distilled H_2O	0.0		-	

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 14: Germination of conidia of *A. niger* Isolate 3 in light of 76 lux in 12 hours in Potato Dextrose Broth (PDB) suspension drops on different component parts of bulbs of shallot (*Allium ascalonicum*).

Component part of bulb	PDB distilled water mixture (v/v)	Percentage Germination		Mean Germ-tube Length (μm)	
		Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outer scale leaf	1:10	85.6	76.9	102.4±5.7 [a]	93.2±2.5 [a]
	1:20	61.6	60.6	82.7±7.2 [a]	64.7±4.2 [a]
	1:30	53.5	34.5	70.4±5.2 [a]	60.4±3.7 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of outer scale leaf	1:10	77.0	61.3	112.3±7.8 [a]	90.3 ±4.5 [a]
	1:20	45.1	43.0	82.7±2.6 [a]	78.4±5.2 [a]
	1:30	35.6	22.7	54.5±6.3 [a]	60.4±3.6 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Outer surface of inner scale leaf	1:10	90.4	76.8	109.9±7.4 [a]	95.4±4.7 [a]
	1:20	73.7	47.0	87.7±4.7 [a]	85.3±3.9 [a]
	1:30	45.9	32.1	65.8±4.5 [a]	70.4±3.8 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of inner scale leaf	1:10	87.9	79.0	84.9±4.3 [a]	80.4±5.2 [a]
	1:20	56.5	45.8	80.9±4.3 [a]	69.9±4.7 [b]
	1:30	40.8	25.8	67.3±2.4 [a]	60.3±3.5 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 14 continued

		Percentage Germination		Mean Germ-tube Length (µm)	
Component part of bulb	PDB: distilled water mixture (v/v)	Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outermost swollen leaf base	1:10	41.9	29.6	47.2±3.6 [a]	50.0±3.2 [a]
	1:20	20.5	16.6	39.5±5.6 [a]	41.2±4.2 [a]
	1:30	10.7	9.0	20.1±1.5 [a]	31.2±5.1 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Glass slide	1:10	42.2		40.6±4.3	
	1:20	27.7		33.5±2.4	
	1:30	11.9		30.1±1.8	
	1:40	0.0		-	
	Distilled H_2O	0.0		-	

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 15: Germination of conidia of *A. niger* Isolate 4 in light of 76 lux in 12 hours in Potato Dextrose Broth (PDB) suspension drops on different component parts of bulbs of shallot (*Allium ascalonicum*).

		Percentage Germination		Mean Germ-tube Length (µm)	
Component part of bulb	PDB: distilled water mixture (v/v)	Pale brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outer scale leaf	1:10	88.3	88.4	116.0±4.9 [a]	110.0±7.2 [a]
	1:20	62.3	54.4	76.7±3.5 [a]	83.3±2.8 [a]
	1:30	42.0	23.3	45.6±5.2 [a]	67.2±2.1 [b]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of outer scale leaf	1:10	91.8	83.6	120.5±7.9 [a]	99.9±4.6 [a]
	1:20	64.4	45.9	90.5±3.9 [a]	87.4±4.5 [a]
	1:30	41.8	20.0	55.5±3.7 [a]	50.3±3.5 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Outer surface of inner scale leaf	1:10	89.8	80.6	110.4±6.7 [a]	118.4±7.9
	1:20	64.9	52.4	83.2±3.4 [a]	98.4±5.2
	1:30	44.4	32.1	67.3±4.6 [a]	76.5±5.3
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of inner scale leaf	1:10	92.9	86.4	98.4±6.4 [a]	100.1±3.6 [a]
	1:20	56.0	40.7	55.4±4.3 [a]	67.5±4.1 [a]
	1:30	42.1	29.9	45.9±2.8 [a]	50.1±3.7 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 15 continued

Component part of bulb	PDB: distilled water mixture (v/v)	Percentage Germination		Mean Germ-tube Length (µm)	
		Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outermost swollen leaf base	1:10	38.1	26.9	58.7±3.9 [a]	62.1±1.8 [a]
	1:20	26.8	18.9	60.7±3.1 [a]	52.1±4.7 [a]
	1:30	16.4	16.0	45.3±4.2 [a]	40.7±3.6 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Glass slide	1:10	32.0		51.2±3.7	
	1:20	21.2		38.9±4.2	
	1:30	16.1		30.3±3.3	
	1:40	0.0		-	
	Distilled H_2O	0.0		-	

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 16: Germination of conidia of *A. niger* Isolate 5 in light of 76 lux in 12 hours in Potato Dextrose Broth (PDB) suspension drops on different component parts of bulbs of shallot (*Allium ascalonicum*).

		Percentage Germination		Mean Germ-tube Length (µm)	
Component part of bulb	PDB: distilled water mixture (v/v)	Pale-brown cv.	Pink cv.	Pale brown cv.	Pink cv
Outer surface of outer scale leaf	1:10	72.0	77.5	84.0±2.0 [a]	77.2±5.4 [a]
	1:20	55.3	57.3	77.6±6.7 [a]	65.9±7.2 [a]
	1:30	24.9	22.6	45.6±3.5 [a]	55.3±3.3 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of outer scale leaf	1:10	77.5	70.7	92.2±5.8 [a]	83.3±4.9 [a]
	1:20	54.4	60.4	81.2±2.7 [a]	76.4±4.1 [a]
	1:30	28.8	27.3	60.4±4.6 [a]	56.7±3.5 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Outer surface of inner scale leaf	1:10	79.8	83.7	87.3±3.7 [a]	84.3±3.9 [a]
	1:20	60.3	63.1	88.9±4.9 [a]	76.3±2.9 [a]
	1:30	30.7	32.9	70.5±4.7 [a]	65.3±2.8 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Inner surface of inner scale leaf	1:10	64.6	72.7	80.4±2.6 [a]	76.8±8.4 [a]
	1:20	52.7	43.2	69.3±3.7 [a]	70.4±4.6 [a]
	1:30	33.4	33.8	58.9±2.0 [a]	57.7±4.9 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 16 continued.

Component part of bulb	PDB: distilled water mixture (v/v)	Percentage Germination: Pale brown cv.	Percentage Germination: Pink cv.	Mean Germ-tube Length (µm): Pale brown cv.	Mean Germ-tube Length (µm): Pink cv
Outer surface of outermost swollen leaf base	1:10	43.0	38.4	56.8±4.5 [a]	60.4±3.8 [a]
	1:20	32.3	33.1	50.4±4.3 [a]	50.9±4.2 [a]
	1:30	10.6	9.9	35.9±3.1 [a]	42.1±5.2 [a]
	1:40	0.0	0.0	-	-
	Distilled H_2O	0.0	0.0	-	-
Glass slide	1:10	48.5		44.5±5.7	
	1:20	31.6		41.7±4.8	
	1:30	21.0		34.2±1.9	
	1:40	10.8		23.1±3.1	
	Distilled H_2O	0.0		-	

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 17: Germination of conidia of *A. niger* Isolate 1 at 32±2ºC in 12 in light of 76 lux hours in suspension drops of Glucose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	47.3	51.2	68.5±5.3 [a]	61.3±4.5 [a]
	Inner S.L.	45.3	49.3	60.3±5.0 [a]	53.0±4.7 [a]
	S.L.B	40.2	38.5	62.5±4.5 [a]	64.3±6.6 [a]
5.0	Outer S.L.	43.5	41.2	60.4±3.3 [a]	56.5±5.5 [a]
	Inner S.L.	40.2	37.3	53.2±4.2 [a]	50.3±3.1 [a]
	S.L.B	35.2	33.4	50.8±5.0 [a]	51.2±6.7 [a]
2.5	Outer S.L.	18.3	12.5	33.4±4.3 [a]	35.8±4.0 [a]
	Inner S.L.	12.5	15.2	30.2±4.0 [a]	29.3±3.5 [a]
	S.L.B	9.8	10.3	26.5±3.6 [a]	27.3±2.8 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf Inner S.L. = Inner Scale leaf S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 18: Germination of conidia of *A. niger* Isolate 2 at 32±2°C in light of 76 lux in 12 hours in suspension drops of Glucose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(μm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	44.2	42.3	60.8±6.5 [a]	53.4±4.2 [a]
	Inner S.L.	40.2	37.5	56.8±4.8 [a]	50.1±4.0 [a]
	S.L.B	32.3	30.3	50.8±3.9 [a]	46.5±4.5 [a]
5.0	Outer S.L.	40.5	33.2	49.5±5.6 [a]	51.3±5.2 [a]
	Inner S.L.	33.2	30.5	46.3±4.3 [a]	45.2±4.0 [a]
	S.L.B	30.3	22.8	40.0±5.2 [a]	39.8±5.1 [a]
2.5	Outer S.L.	21.3	15.5	30.3±3.0 [a]	23.5±4.0 [a]
	Inner S.L.	23.4	17.2	26.2±2.9 [a]	21.2±2.8 [a]
	S.L. B	10.2	11.1	20.8±3.1 [a]	20.3±2.5 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 19: Germination of conidia of *A. niger* Isolate 3 at 32±2°C in light of 76 lux 12 hours in suspension drops of Glucose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	44.2	46.5	53.4±3.9 [a]	54.3±5.0 [a]
	Inner S.L	43.2	48.3	50.9±4.3 [a]	50.0±4.3 [a]
	S.L.B	32.3	33.2	43.5±5.1 [a]	45.3±5.5 [a]
5.0	Outer S.L.	35.3	37.5	40.3±3.3 [a]	43.5±5.0 [a]
	Inner S.L	34.5	40.2	37.8±4.3 [a]	40.8±4.2 [a]
	S.L.B	26.7	25.2	33.4±4.0 [a]	32.3±3.3 [a]
2.5	Outer S.L.	28.2	15.8	29.3±3.0 [a]	25.8±3.1 [a]
	Inner S.L	26.3	25.2	27.2±2.8 [a]	24.3±2.6 [a]
	S.L.B	10.2	8.5	26.5±2.8 [a]	24.2±2.3 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 20: Germination of conidia of *A. niger* Isolate 4 at 32±2°C in light of 76 lux in 12 hours in suspension drops of Glucose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*)

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(μm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	51.3	45.2	63.4±5.3 [a]	60.8±3.2 [a]
	Inner S.L.	47.3	44.3	60.2±6.2 [a]	58.3±4.3 [a]
	S.L.B	40.2	35.2	53.5±4.3 [a]	55.4±3.2 [a]
5.0	Outer S.L.	42.0	39.3	58.3±4.1 [a]	55.3±3.8 [a]
	Inner S.L.	37.3	36.3	59.2±5.0 [a]	52.0±4.9 [a]
	S .L.B	29.8	28.4	53.1±5.9 [a]	54.8±3.4 [a]
2.5	Outer S.L.	25.2	23.2	50.3±5.1 [a]	48.9±4.1 [a]
	Inner S.L.	20.1	24.2	46.3±5.0 [a]	50.2±3.6 [a]
	S.L.B	16.3	12.5	48.5±4.3 [a]	46.8±3.8 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 21: Germination of conidia of *A. niger* Isolate 5 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of Glucose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(μm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L	46.7	44.4	66.3±4.8 [a]	63.4±4.9 [a]
	Inner S.L.	45.2	40.2	63.8±5.3 [a]	58.0±6.3 [a]
	S.L.B	30.1	33.5	64.5±6.0 [a]	58.3±3.6 [a]
5.0	Outer S.L	32.3	31.5	65.8±3.8 [a]	67.8±3.9 [a]
	Inner S.L.	27.5	28.9	61.2±4.1 [a]	62.0±5.3 [a]
	S.L.B	18.3	20.2	64.5±6.0 [a]	59.3±4.0 [a]
2.5	Outer S.L	21.1	18.3	43.2±2.8 [a]	39.2±4.1 [a]
	Inner S.L.	18.5	18.1	40.3±3.2 [a]	37.8±3.3 [a]
	S.L.B	11.2	10.3	38.3±2.7 [a]	36.3±4.6 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 22: Germination of conidia of *A. niger* Isolate 1 at 32±2°C in light of 76 lux in 12 hours in suspension drops of Galactose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of galactose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L	40.3	38.5	56.9±5.1 [a]	57.8±4.0 [a]
	Inner S.L.	38.3	33.4	52.3±4.3 [a]	53.2±3.2 [a]
	S.L.B	23.2	22.1	50.4±4.0 [a]	49.3±4.4 [a]
5.0	Outer S.L	33.2	30.8	48.8±3.1 [a]	43.1±2.6 [a]
	Inner S.L.	32.1	29.2	43.2±4.3 [a]	38.3±2.9 [a]
	S.L.B	21.2	18.3	40.2±2.9 [a]	33.4±3.0 [a]
2.5	Outer S.L	23.1	19.5	27.8±2.3 [a]	28.3±2.3 [a]
	Inner S.L.	21.3	15.8	30.2±3.1 [a]	24.2±2.5 [a]
	S.L.B	12.3	10.2	25.2±2.9 [a]	20.2±3.0 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 23: Germination of conidia of *A. niger* Isolate 2 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of Galactose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of galactose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	36.1	35.5	44.5±5.0 [a]	35.3±3.4 [a]
	Inner S.L	30.1	34.8	40.2±5.6 [a]	31.2±3.3 [a]
	S.L.B	18.4	20.3	32.3±4.3 [a]	29.3±3.9 [a]
5.0	Outer S.L.	31.2	29.4	40.3±4.2 [a]	41.3±3.0 [a]
	Inner S.L.	32.2	27.5	36.2±4.3 [a]	33.2±3.1 [a]
	S.L.B	18.0	17.3	30.2±3.9 [a]	31.4±2.9 [a]
2.5	Outer S.L.	20.8	14.2	24.8±3.3 [a]	21.2±2.3 [a]
	Inner S.L	15.5	13.8	21.6±3.6 [a]	22.3±2.0 [a]
	S.L.B	9.5	8.3	20.2±2.0 [a]	18.3±1.9 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 24: Germination of conidia of *A. niger* Isolate 3 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of Galactose of different concentrations on parts of bulbs of two cultivars of shallot (*Allium ascalonicum*).

Concentration of galactose (g/L)	Part of bulb	% Germination		Mean germ-tube length(μm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10	Outer S.L.	34.3	35.8	51.9±6.1 [a]	53.8±6.3 [a]
	Inner S.L.	30.2	32.4	53.2±5.8 [a]	50.2±5.3 [a]
	S.L.B	21.2	20.2	50.4±4.9 [a]	46.3±5.8 [a]
5.0	Outer S.L.	25.3	30.3	42.3±3.9 [a]	40.3±3.8 [a]
	Inner S.L.	24.2	23.4	40.2±4.6 [a]	36.8±4.1 [a]
	S.L.B	19.4	17.2	37.3±5.0 [a]	34.2±3.3 [a]
2.5	Outer S.L.	13.5	12.3	30.5±5.5 [a]	29.3±3.1 [a]
	Inner S.L.	10.9	12.1	28.3±4.3 [a]	26.2±3.1 [a]
	S.L.B	9.3	7.8	20.5±3.1 [a]	22.4±2.5 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 25: Germination of conidia of *A. niger* Isolate 4 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of Galactose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of galactose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L	37.8	36.9	47.2±3.9 [a]	48.8±5.6 [a]
	Inner S.L.	35.2	34.3	46.3±4.1 [a]	45.3±4.0 [a]
	S.L.B	22.2	19.5	44.7±3.5 [a]	43.8±4.0 [a]
5.0	Outer S.L	29.3	26.3	40.3±3.3 [a]	41.2±4.1 [a]
	Inner S.L.	25.2	21.3	36.2±2.8 [a]	35.3±3.1 [a]
	S.L.B	17.9	13.8	30.2±3.1 [a]	28.3±2.5 [a]
2.5	Outer S.L	14.8	10.2	26.3±2.3 [a]	30.2±3.1 [a]
	Inner S.L.	13.2	10.5	24.2±1.9 [a]	22.4±2.3 [a]
	S.L.B	9.3	8.8	21.3±2.1 [a]	19.4±2.0 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 26: Germination of conidia of *A. niger* Isolate 5 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of Galactose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	39.3	37.8	50.1±4.0 [a]	52.3±5.0 [a]
	Inner S.L.	37.2	35.3	47.2±4.4 [a]	48.3±6.3 [a]
	S.L.B	19.8	20.1	44.4±4.8 [a]	45.0±5.5 [a]
5.0	Outer S.L.	32.4	31.2	41.0±3.6 [a]	40.3±3.3 [a]
	Inner S.L.	31.3	26.3	36.3±3.3 [a]	35.3±3.8 [a]
	S.L.B	12.8	17.4	38.2±4.0 [a]	30.3±2.5 [a]
2.5	Outer S.L.	19.3	18.3	33.3±2.5 [a]	29.3±2.6 [a]
	Inner S.L.	18.3	17.3	29.2±3.3 [a]	32.5±2.4 [a]
	S.L.B	10.1	9.9	25.3±2.8 [a]	26.8±3.0 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 27: Germination of conidia of *A. niger* Isolate 1 at 32±2°C in light of 76 lux in 12 hours in suspension drops of sucrose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of sucrose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	49.9	50.0	64.8±7.1 [a]	60.0±5.5 [a]
	Inner S.L.	45.3	47.3	60.3±6.3 [a]	61.3±5.7 [a]
	S.L.B	37.3	40.2	56.8±6.5 [a]	52.4±4.3 [a]
5.0	Outer S.L.	39.3	43.1	50.2±5.3 [a]	48.5±4.1 [a]
	Inner S.L.	40.3	40.8	46.3±5.5 [a]	42.3±3.3 [a]
	S.L.B	30.2	31.1	48.2±4.0 [a]	40.3±4.4 [a]
2.5	Outer S.L.	20.2	19.4	41.3±4.8 [a]	35.6±3.3 [a]
	Inner S.L.	19.3	18.3	36.2±3.9 [a]	37.2±2.8 [a]
	S.L.B	11.1	10.9	30.3±4.4 [a]	27.3±3.7 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf Inner S.L. = Inner Scale leaf S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 28: Germination of conidia of *A. niger* Isolate 2 at 32±2°C in light of 76 lux in 12 hours in suspension drops of sucrose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of glucose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	44.8	43.5	43.3±4.1 [a]	40.3±3.3 [a]
	Inner S.L.	40.3	44.5	42.8±3.3 [a]	42.3±4.2 [a]
	S.L.B	34.5	33.3	40.5±3.1 [a]	41.3±5.0 [a]
5.0	Outer S.L.	36.5	35.5	33.4±4.0 [a]	35.3±3.4 [a]
	Inner S.L.	32.3	30.1	32.8±4.1 [a]	33.2±4.3 [a]
	S.L.B	24.2	26.4	31.4±2.9 [a]	25.4±4.4 [a]
2.5	Outer S.L.	15.3	12.5	26.3±3.3 [a]	27.3±3.2 [a]
	Inner S.L.	14.7	12.5	24.2±3.4 [a]	20.2±2.8 [a]
	S.L.B	10.8	9.2	20.8±2.1 [a]	18.3±2.5 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 29: Germination of conidia of *A. niger* Isolate 3 at 32±2°C in light of 76 lux in 12 hours in suspension drops of sucrose of different concentrations on parts of bulbs of the two cultivars of shallot *(Allium ascalonicum*).

Concentration of sucrose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	47.5	46.5	58.2±3.9 [a]	55.2±3.3 [a]
	Inner S.L.	46.3	44.4	55.3±4.3 [a]	49.3±4.2 [a]
	S.L.B	39.3	40.4	51.2±5.0 [a]	44.3±5.0 [a]
5.0	Outer S.L.	41.2	39.2	45.4±4.5 [a]	40.8±4.1 [a]
	Inner S.L.	40.5	35.3	40.2±2.9 [a]	37.9±5.1 [a]
	S.L.B	31.3	30.8	42.3±3.4 [a]	35.4±4.0 [a]
2.5	Outer S.L.	18.4	17.5	33.4±4.2 [a]	36.3±4.4 [a]
	Inner S.L.	17.4	16.2	31.2±3.9 [a]	30.4±3.3 [a]
	S.L.B	11.8	10.5	30.8±2.8 [a]	27.8±2.5 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 30: Germination of conidia of *A. niger* Isolate 4 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of sucrose of different concentrations on parts of bulbs of the two cultivars of shallot *(Allium ascalonicum*).

Concentration of sucrose (g/L)	Part of bulb	% Germination		Mean germ-tube length(µm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	46.1	44.8	63.8±7.1 [a]	60.2±5.6 [a]
	Inner S.L.	38.9	40.3	52.4±6.2 [a]	55.3±6.7 [a]
	S.L.B	37.7	37.3	50.8±5.8 [a]	51.2±4.9 [a]
5.0	Outer S.L.	33.8	34.5	55.2±5.5 [a]	43.4±4.0 [a]
	Inner S.L.	34.8	33.6	54.3±5.7 [a]	50.5±4.3 [a]
	S .L.B	30.2	29.2	50.3±4.3 [a]	51.0±5.4 [a]
2.5	Outer S.L.	17.9	18.3	51.2± 4.2 [a]	40.4±3.3 [a]
	Inner S.L.	14.5	16.5	43.3±4.7 [a]	36.3±2.8 [a]
	S.L.B	10.8	9.3	37.4±3.0 [a]	34.2±2.5 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

Table 31: Germination of conidia of *A. niger* Isolate 5 at 32±2^{o}C in light of 76 lux in 12 hours in suspension drops of sucrose of different concentrations on parts of bulbs of the two cultivars of shallot (*Allium ascalonicum*).

Concentration of sucrose (g/L)	Part of bulb	% Germination		Mean germ-tube length(μm)	
		Pale-brown cv.	Pink cv.	Pale-brown	Pink cv.
10.0	Outer S.L.	50.1	48.1	49.3±5.0 [a]	50.5±6.1 [a]
	Inner S.L.	48.3	47.8	48.2±6.1 [a]	44.3±5.1 [a]
	S.L.B	40.8	39.9	47.3±5.5 [a]	43.3±4.3 [a]
5.0	Outer S.L.	38.3	41.3	50.2±4.4 [a]	45.2±3.8 [a]
	Inner S.L.	38.0	37.8	46.3±5.0 [a]	40.3±4.1 [a]
	S.L.B	31.3	26.3	44.3±4.1 [a]	42.3±3.3 [a]
2.5	Outer S.L.	20.2	19.5	40.2±3.9 [a]	37.3±4.1 [a]
	Inner S.L.	18.5	15.4	38.3±4.2 [a]	35.3±3.2 [a]
	S.L.B	11.8	11.2	35.2±4.0 [a]	34.1±5.0 [a]
1.25	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-
0.0	Outer S.L.	0.0	0.0	-	-
	Inner S.L.	0.0	0.0	-	-
	S.L.B	0.0	0.0	-	-

* Outer S.L. = Outer Scale Leaf   Inner S.L. = Inner Scale leaf   S.L.B = Swollen leaf base

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability

EXPERIMENT G. Germination of conidia of the five test *Aspergillus niger* isolates in aqueous extracts of the two shallot cultivars.

Conidia of *Aspergillus niger* coming in contact with fluids exuding from wounds on shallot bulbs will germinate very well and the germ-tubes will be exposed to a very congenial medium for growth as indicated by the data in Tables 32 to 36 and Fig 3. With the exception of conidia of Isolate 3 shown in table 34, conidia of the remaining four Isolates registered 100 percent germination in some of the media. Conidia of Isolate 2, the best example (Table 33), attained 100 per cent germination in the pale-brown cultivar extracts of 1/8 dilution to the standard undiluted medium, and in the ¼ extract dilution to the standard undiluted medium of the pink cultivar. It was evident that germination of the *A. niger* conidia in the undiluted extract of the shallot bulbs would greatly aid in initiating invasion in bruised bulbs.

For both cultivars, the extract of the Pale-brown cultivar was superior to that of the Pink cultivar.

Table 32: Germination of conidia of *A. niger* Isolate 1 in fluid of bulbs of the two cultivars of shallot (*Allium ascalonicum*) for 12 hours at 32±2°C.

	Percentage germination in extract of		Mean length of germ tubes of germinated conidia ± S.E in extract of	
Concentration of extract	Pale brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
S	100	93.5	ND	237.8±1.8
$\frac{1}{2}$	95.5	61.9	ND	214.0±2.7
$\frac{1}{4}$	94.1	64.5	ND	200.4±1.3
$\frac{1}{8}$	73.3	64.2	270.5±6.8 [a]	257.5±3.3 [a]
$\frac{1}{16}$	78.4	58.7	197.8±4.3 [a]	198.1±5.2 [a]

ND: Not determined because of extensive growth

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 33: Germination of conidia of *A. niger* Isolate 2 in fluid of bulbs of the two cultivars of shallot (*Allium ascalonicum*) for 12 hours at 32±2ºC.

Concentration of extract	Percentage germination in extract of		Mean length of germ tubes of germinated conidia ± S.E in extract of	
	Pale brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
S	100	100	ND	ND
$\frac{1}{2}$	100	100	ND	ND
$\frac{1}{4}$	100	100	ND	ND
$\frac{1}{8}$	100	93.3	ND	263.5±1.9
$\frac{1}{16}$	96.6	90.9	289.7±5.3 [a]	245.1±6.2 [b]

ND: Not determined because of extensive growth

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 34: Germination of conidia of *A. niger* Isolate 3 in fluid of bulbs of the two cultivars of shallot (*Allium ascalonicum*) for 12 hours at 32±2°C

Concentration of extract	Percentage germination in extract of		Mean length of germ tubes of germinated conidia ± S.E in extract of	
	Pale brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
S	97.2	87.4	244.3±2.9 [a]	158.9±4.6 [b]
$\frac{1}{2}$	94.1	86.0	281.5±4.2 [a]	156.5±8.7 [b]
$\frac{1}{4}$	88.5	84.9	205.7±4.6 [a]	167.7±3.3 [b]
$\frac{1}{8}$	91.2	89.5	188.6±6.3 [a]	152.1±5.6 [b]
$\frac{1}{16}$	90.3	74.1	77.4±2.6 [a]	118.8±8.2 [b]

ND: Not determined because of extensive growth

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 35: Germination of conidia of *A. niger* Isolate 4 in fluid of bulbs of the two cultivars of shallot (*Allium ascalonicum*) for 12 hours at 32±2^{o}C.

Concentration of extract	Percentage germination in extract of		Mean length of germ tubes of germinated conidia ± S.E in extract of	
	Pale brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
S	100	100	ND	ND
$\frac{1}{2}$	100	84.7	ND	148.1±5.4
$\frac{1}{4}$	100	92.9	ND	118.1±1.4
$\frac{1}{8}$	100	92.3	268.5±5.5 [a]	122.9±7.3 [b]
$\frac{1}{16}$	87.7	64.4	239.5±6.8 [a]	106.5±4.3 [b]

ND: Not determined because of extensive growth.

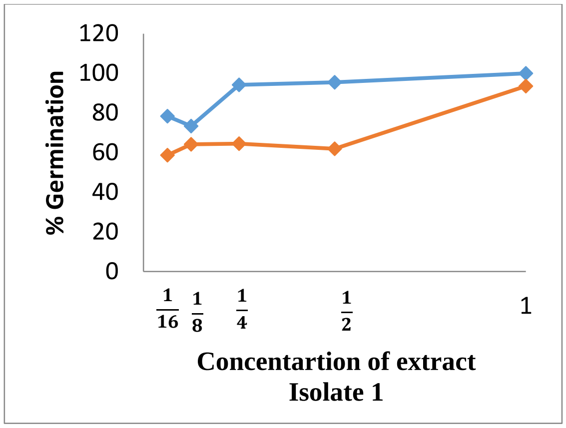
By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

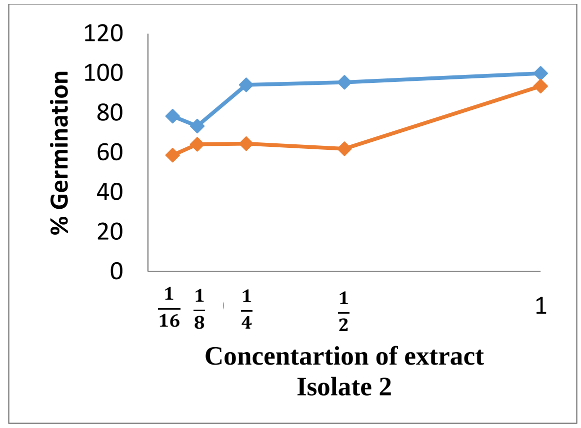
Table 36: Germination of conidia of *A. niger* Isolate 5 in fluid of bulbs of the two cultivars of shallot (*Allium ascalonicum*) for 12 hours at 32±2°C.

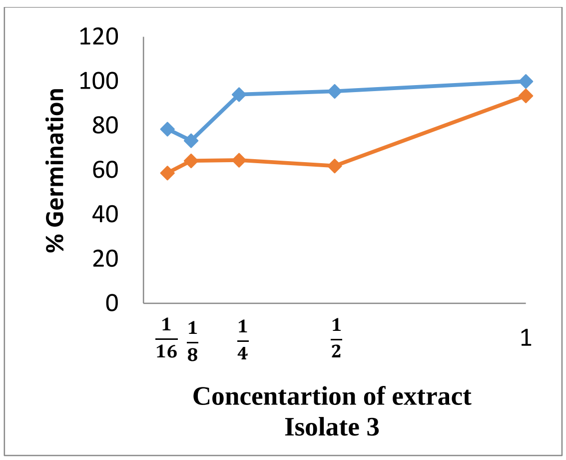
Concentration of extract	Percentage germination in extract of		Mean length of germ tubes of germinated conidia ± S.E in extract of	
	Pale brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
S	100	100	ND	ND
$\frac{1}{2}$	100	96.9	251.9±4.2 [a]	244.7±2.5 [a]
$\frac{1}{4}$	96.2	95.4	243.8±3.9 [a]	218.5±1.9 [b]
$\frac{1}{8}$	100	96.0	248.5±3.1 [a]	200.9±6.4 [b]
$\frac{1}{16}$	77.5	47.7	213.2± 2.3 [a]	204.9±7.2 [a]

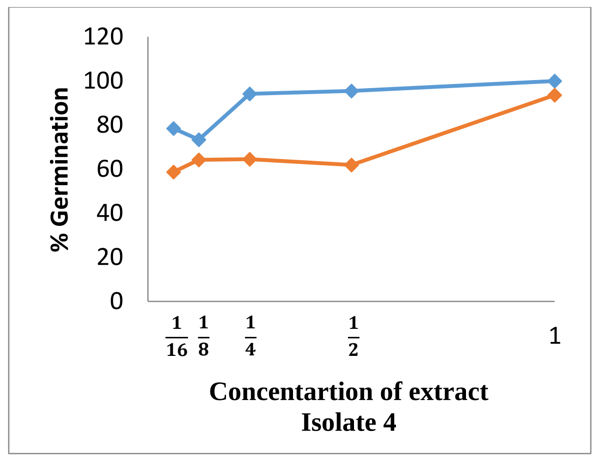
ND: Not determined because of extensive growth

By the calculated Scheffe's Confidence Limit, values of Mean-Germ-tube Length in each horizontal row bearing the same letters are not significantly different at 5% level of probability.









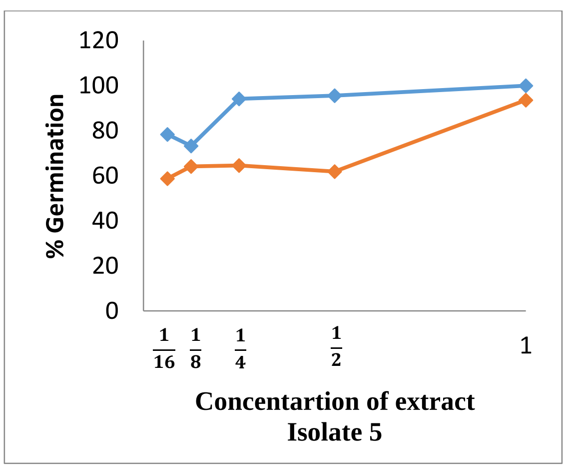


Figure 3: Germination of conidia of the five test *Aspergillus niger* isolates in fluids expressed from ground bulb of the Pale-brown (—◆—) and Pink (—◆—) shallot cultivars.

EXPERIMENT H. Shallot bulb inoculation tests using the two shallot cultivars and the five test *Aspergillus niger* isolates.

It was not possible to incite infection by surface-inoculation. The mycelia of the inocula did not penetrate the intact epidermis of the swollen leaf base of the bulbs of both shallot cultivars and no rotting ensued as reported in Table 37.

Wounds were required for infection of the bulbs. Thus wound-inoculated bulbs were rotted by all *A. niger* isolates invading in three days bulb tissues of mean diameters of 10.4±0.4 to 12.5±0.5mm of the pale-brown cultivar and 7.9±0.5 to 11.0±0.4mm of the pink cultivar. Rate of infection of the pale-brown cultivar was consistently faster than that of the pink cultivar. The same trend was found when the depths of the rotted tissues were studied. The invasion went deeper in the tissues of the pale- brown cultivar.

Table 37: Extent of rot of bulbs of shallot (*Allium ascalonicum*) either surface-inoculated or wound-inoculated with mycelium discs of different *A. niger* Isolates and stored at 32±2°C in humid atmosphere (100% R.H.) for 3 days.

	Mean diameter of rot (mm) ± S.E of				Mean depth of rot (mm) ± S.E of			
A. niger	Surface inoculated bulbs of		Wound-inoculated bulbs of		Surface-inoculated bulbs of		Wound-inoculated bulbs	
Isolate	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
1	0.0	0.0	12.1±0.7 [a]	10.4±0.6 [a]	0.0	0.0	2.0±0.0 [a]	1.9±0.1 [a]
2	0.0	0.0	12.3±0.5 [a]	10.8±0.5 [a]	0.0	0.0	2.0±0.0 [a]	1.9±0.1 [a]
3	0.0	0.0	12.8±0.5 [a]	11.0±0.4 [a]	0.0	0.0	2.0±0.0 [a]	2.0±0.1 [a]
4	0.0	0.0	10.4±0.4 [a]	7.9±0.5 [a]	0.0	0.0	1.9.0±0.1[a]	1.6±0.1[a]
5	0.0	0.0	12.5±0.5 [a]	11.3±0.4 [a]	0.0	0.0	2.0±0.0 [a]	1.6±0.1 [a]

By the calculated Scheffe's Confidence Limit, pairs of values for Mean Diameter of rot and mean Depth of rot in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

EXPERIMENT I. Infection of wound-inoculated bulbs at different relative humidities using the two shallot cultivars and the five test *Aspergillus niger* isolates.

The data in Tables 38 to 47 show the various ways in which the course of infection could be influenced by relative humidity. Both the Pale-brown and Pink shallot cultivars could be invaded by *A. niger* isolates at humidities from 50 to 100% R.H. The rates of invasion in 3 days at the two extreme relative humidities were consistently significantly different. The greatest differences, recorded in Table 40, showed a mean diameter of rotted tissue of 9.4±0.3 and 15.2±0.4 mm at 50% and 100% R.H., respectively, caused by *A. niger* Isolate 3 in the Pale-brown cultivar bulbs. The same isolate rotted a region of mean diameter of 13.3±1.0 mm in 3 days in bulbs of the pink cultivar at 100% R.H. almost doubling the diameter of 7.5±0.2 mm at 50% RH. At any storage humidity, greater rotting occurred in bulbs of the pale-brown cultivar than in those of the pink cultivar.

The invading hyphae sporulated, if the storage humidity was favourable. Conidiophores were formed over the rotted areas at 80 to 100% R.H only in 3-4 days after inoculation as indicated in Tables 38 to 42. No conidiophores were formed at 50 to 70% R.H. by the rotted tissue which by the 10^{th} day of incubation were found to have dried up.

Humidity also influenced the degree of sporulation and the morphology of the conidiophores as shown by the information in Tables 43 to 47. The lower the humidity the sparser the crop of conidiophores and the shorter the conidiophores. These observations applied to both Pale-brown and Pink cultivars. However, conidiophores on the Pale-brown cultivar bulbs were slightly taller than those on the bulbs of the Pink cultivar at any particular relative humidity.

Table 38: Infection of bulbs of shallot (*Allium ascalonicum*) wound-inoculated with mycelium of *A. niger* Isolate 1 and incubated at 50-100% relative humidity at 32±2^{o}C.

	Rot development in			
	Pale brown cultivar		Pink cultivar	
Incubation at % R.H.	Mean rot diameter (mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)	Mean rot diameter(mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)
100	14.9±0.9 ^{a}	3	12.8±0.5 ^{a}	3
90	13.2±0.6 $^{a\,b}$	4	11.2±0.3 ^{b}	4
80	11.9±0.4 $^{b\,c}$	4	10.6±0.5 ^{b}	4
70	11.3±0.6 $^{b\,c}$	No sporulation	8.3±0.4 ^{c}	No sporulation
60	10.5±0.5 $^{c\,d}$	No sporulation	8.0±0.2 ^{c}	No sporulation
50	9.4±0.3 ^{d}	No sporulation	7.5±0.2 ^{c}	No sporulation

By the calculated Scheffe's Confidence Limit, values of mean Rot Diameter in each vertical row bearing the same letters are not significantly different at 5% level of probability.

Table 39: Infection of bulbs of shallot (*Allium ascalonicum*) wound-inoculated with mycelium of *A. niger* Isolate 2 and incubated at 50-100% relative humidity at 32±2°C

	Rot development in			
	Pale brown cultivar		Pink cultivar	
Incubation at % R.H.	Mean rot diameter (mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)	Mean rot diameter (mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)
100	13.8±1.4 [a]	3	12.1±1.6 [a]	3
90	12.3±1.7 [a b]	4	11.8±1.3 [a]	4
80	10.5±2.1 [a b]	4	10.2±1.3 [a b]	4
70	9.6±0.8 [b c]	No sporulation	9.1±1.0 [a b]	No sporulation
60	9.1±0.7 [b c]	No sporulation	8.7±0.5 [a b]	No sporulation
50	8.2±0.8 [c]	No sporulation	8.0±0.2 [b]	No sporulation

By the calculated Scheffe's Confidence Limit, values of mean Rot Diameter in each vertical row bearing the same letters are not significantly different at 5% level of probability.

Table 40: Infection of bulbs of shallot (*Allium ascalonicum*) wound-inoculated with mycelium of *A. niger* Isolate 3 and incubated at 50-100% relative humidity at 32±2°C

	Rot development in			
	Pale brown cultivar		Pink cultivar	
Incubation at % R.H.	Mean rot diameter (mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)	Mean rot diameter (mm) ± S.E in 3 days	Time for *A. niger* sporulation (days)
100	15.2±0.4 [a]	3	13.3±1.0 [a]	3
90	14.3±0.3 [a]	4	12.6±0.6 [a]	4
80	12.2±0.5 [b]	4	11.8±0.4 [a]	4
70	10.1±0.2 [c]	No sporulation	9.3±0.6 [b]	No sporulation
60	9.8±0.2 [d]	No sporulation	9.0±0.3 [b]	No sporulation
50	9.4±0.3 [e]	No sporulation	7.5±0.2 [c]	No sporulation

By the calculated Scheffe's Confidence Limit, values of mean Rot Diameter in each vertical row bearing the same letters are not significantly different at 5% level of probability.

Table 41: Infection of bulbs of shallot (*Allium ascalonicum*) wound-inoculated with mycelium of *A. niger* Isolate 4 and incubated at 50-100% relative humidity at 32±2°C.

	Rot development in			
	Pale brown cultivar		Pink cultivar	
Incubation at % R.H.	Mean rot diameter (mm) ± S.E in 3 days	Time for sporulation (days)	Mean rot diameter(mm) ± S.E in 3 days	Time for sporulation (days)
100	14.6±1.3 [a]	3	14.0±1.0 [a]	3
90	14.1±1.0 [a b]	4	13.8±0.9 [a]	4
80	13.6±0.8 [a b]	4	12.5±0.7 [a b]	4
70	12.1±0.6 [b]	No sporulation	11.3±0.5 [a b]	No sporulation
60	11.3±0.3 [c]	No sporulation	10.2±0.5 [b c]	No sporulation
50	9.4±0.3 [d]	No sporulation	9.0±0.4 [c]	No sporulation

By the calculated Scheffe's Confidence Limit, values of mean Rot Diameter in each vertical row bearing the same letters are not significantly different at 5% level of probability.

Table 42: Infection of bulbs of shallot (*Allium ascalonicum*) wound-inoculated with mycelium of *A. niger* Isolate 3 and incubated at 50-100% relative humidity at 32±2°C

	Rot development in			
	Pale brown cultivar		Pink cultivar	
Incubation at % R.H.	Mean rot Diameter (mm) ± S.E in 3 days	Time for sporulation (days)	Mean rot diameter(mm) ± S.E in 3 days	Time for sporulation (days)
100	13.9±0.5 [a]	3	12.4±0.9 [a]	3
90	13.3±0.5 [a]	4	11.6±0.9 [a]	4
80	11.6±0.4 [a b]	4	10.1±0.6 [a]	4
70	10.5±0.4 [b c]	No sporulation	9.9±0.4 [a]	No sporulation
60	9.5±0.5 [c d]	No sporulation	7.9±0.4 [b]	No sporulation
50	8.4±0.2 [d]	No sporulation	7.0±0.1 [b]	No sporulation

By the calculated Scheffe's Confidence Limit, values of mean Rot Diameter in each vertical row bearing the same letters are not significantly different at 5% level of probability.

Table 43: Effect of Relative Humidity on sporulation of *A. niger* Isolate 1 on bulbs of shallot (*Allium ascalonicum*) at 32±2°C under 12 hour day-night cycle in 10 days

	Density of Sporulation on		Mean length of conidiophore (µm) on	
Incubation at % R.H.	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	+++ *	+++	702.1±6.5 [a]	664.3±5.5 [b]
90	++	++	653.4±9.3 [a]	627.8±8.5 [b]
80	+	+	610.5±8.7 [a]	601.0±7.8 [a]

* +++ : Dense

++ : Moderate

\+ : Scanty

By the calculated Scheffe's Confidence Limit, pairs of values of Mean Conidiophore Lengths in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 44: Effect of Relative Humidity on sporulation of *A. niger* Isolate 2 on bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under 12 hour day-night cycle in 10 days

Incubation at % R.H.	Density of Sporulation on		Mean length of conidiophore (µm) on	
	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	+++ *	+++	653.8±10.8 [a]	642.3±6.3 [a]
90	++	++	601.1±4.3 [a]	589.1±7.3 [a]
80	+	+	495.8±8.8 [a]	479.1±6.9 [a]

* +++ : Dense

++ : Moderate

+ : Scanty

By the calculated Scheffe's Confidence Limit, pairs of values of Mean Conidiophore Lengths in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 45: Effect of Relative Humidity on sporulation of *A. niger* Isolate 3 on bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under 12 hour day-night cycle in ten days.

Incubation at % R.H.	Density of Sporulation on		Mean length of conidiophore (µm) on	
	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	+++ *	+++	724.0±6.8 [a]	700.1±6.1 [a]
90	++	++	672.8±7.7 [a]	654.8±9.2 [a]
80	+	+	611.1±6.4 [a]	587.2±4.9 [b]

* +++ : Dense

++ : Moderate

+ : Scanty

By the calculated Scheffe's Confidence Limit, pairs of values of Mean Conidiophore Lengths in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 46: Effect of Relative Humidity on sporulation of *A. niger* Isolate 4 on bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under 12 hour day-night cycle in 10 days

Incubation at % R.H.	Density of Sporulation on		Mean length of conidiophore (µm) on	
	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	+++ *	+++	692.8±7.3 [a]	665.8±7.2 [a]
90	++	++	653.1±10.2 [a]	620.1±6.4 [a]
80	+	+	562.3±6.3 [a]	529.3±6.3 [b]

* +++ : Dense

++ : Moderate

+ : Scanty

By the calculated Scheffe's Confidence Limit, pairs of values of Mean Conidiophore Lengths in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 47: Effect of Relative Humidity on sporulation of *A. niger* Isolate 5 on bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under 12 hour day-night cycle in 10 days

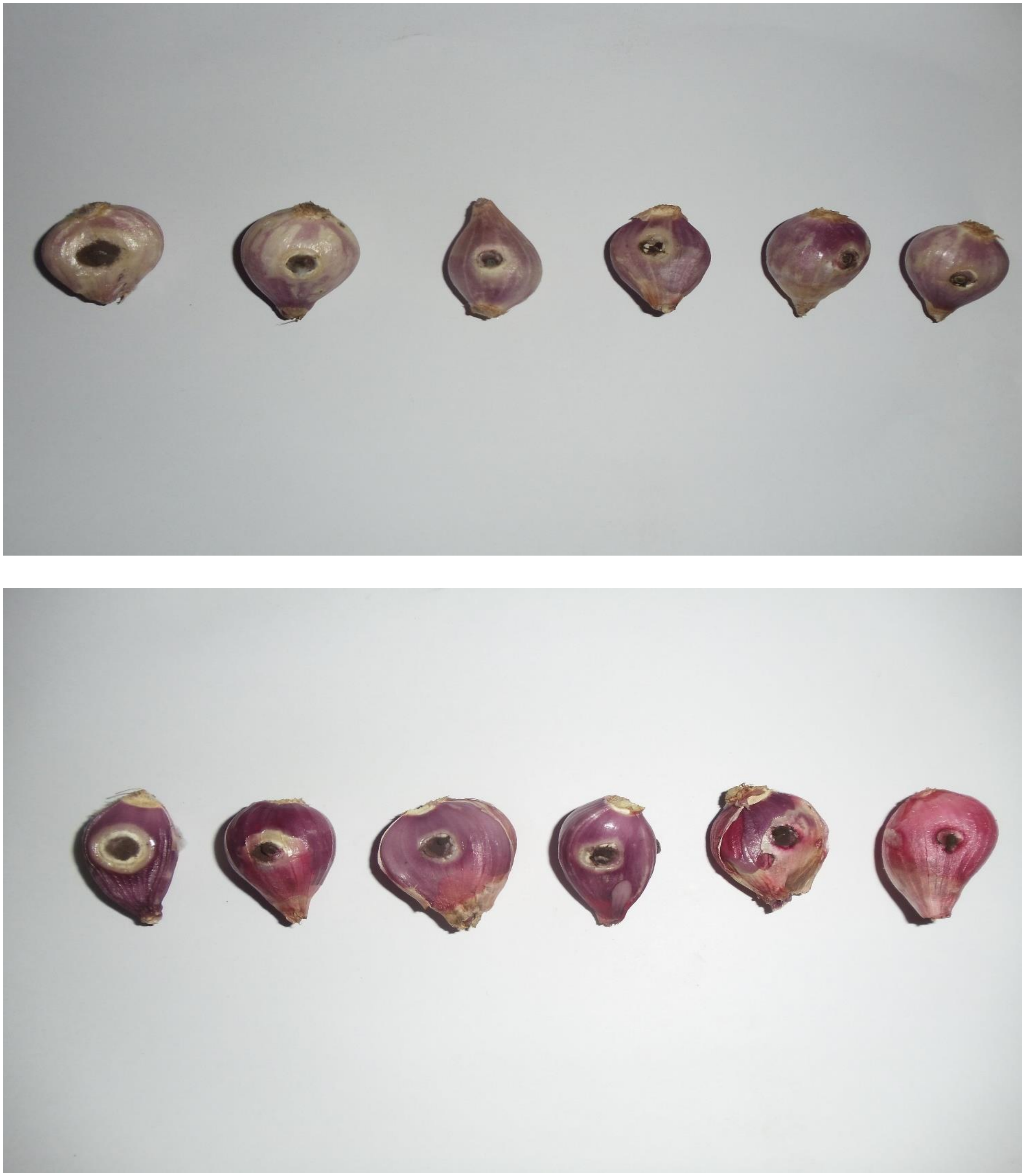
Incubation at % R.H.	Density of Sporulation on		Mean length of conidiophore (µm) on	
	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	+++ *	+++	687.1±9.2 [a]	662.1±4.9 [a]
90	++	++	624.0±8.3 [a]	591.7±10.2 [a]
80	+	+	484.1±6.1 [a]	479.0±8.3 [a]

* +++ : Dense

++ : Moderate

\+ : Scanty

By the calculated Scheffe's Confidence Limit, pairs of values of Mean Conidiophore Lengths in horizontal rows bearing the same letters are not significantly different at 5% level of probability.



X 3/4

Plate 10. Photographs showing wound-inoculated bulbs of the Pale-brown (TOP) and Pink (BOTTOM) cultivators of shallot (*Allium ascalonicum*) and incubated at different relative humidities. (From left: 100, 90, 80, 70, 60 and 50% R.H.)

Experiment J. *In-vitro* production of pectic enzymes by the five test *Aspergillus niger* isolates

The details in Tables 49a-e show the enzyme activity of Czapeck Dox culture filtrate of the *A. niger* Isolates as determined by the disk maceration time method. Enzyme activities attained a peak on the 9th day of growth of all the five *A. niger* isolates. The activities ranges from 7.75 by the enzymes of Isolate 3 and 5 culture filtrates (Tables 49 c and e) to 8.89 by the culture filtrates of *A. niger* Isolates 1, 2 and 4 (Tables 49a, b and d); respectively. Coincidentally, mycelial growth by *A. niger* Isolates 3, 4 and 5 (Tables 48c, 48d and 48e) also reached the peak by the 9th day after inoculation. Isolate 1 (Table 48a) and 2 (Table 48b) however, grew faster and attained peak mycelial dry weight by the 6th day. Enzyme synthesis, therefore, reached a peak when mycelial autolysis had set in.

The rate of production of the macerating enzyme by Isolate 5 (Table 49e) proceeded very fast synthesizing enzymes with enzyme activity of 6.15 in the first three days to the maximum of 7.75 recorded on the ninth day. In contrast, next was Isolate 2 (Table 49b) producing filtrate with enzyme activity of 6.49 and 8.89 respectively within the corresponding 3 and 9 days respectively. Lesser activities were associated with the remaining three *A. niger* Isolates with Isolate 3 being the least (Table 49c) with corresponding rates of 4.52 and 7.75, respectively. In all cases enzyme activity declined slowly when autolysis set in.

Table 48a: Growth of *A. niger* Isolate 1 grown in Czapek-Dox broth medium (pH 7.0) at 32±2^{o}C under a 12-hour day-night cycle in 12 days.

Days after Inoculation	Mycelium Dry weight (g)			
	Replicates			Mean ± S.E
3	0.4511	0.4312	0.4112	0.4312±0.012
6	0.4739	0.4725	0.4767	0.4744±0.001
9	0.4642	0.4567	0.4332	0.4514±0.009
12	0.4346	0.4152	0.4254	0.4251±0.006

Table 48b: Growth of *A. niger* Isolate 2 grown in Czapek-Dox broth medium (pH 7.0) at $32\pm2^{o}C$ under a 12-hour day-night cycle in 12 days.

Days after Inoculation	Mycelium Dry weight (g)			
	Replicates			Mean ± S.E
3	0.3834	0.3683	0.3734	0.3750±0.004
6	0.3765	0.3750	0.3957	0.3824±0.067
9	0.3202	0.2942	0.3512	0.3221±0.017
12	0.3112	0.2912	0.2940	0.2988±0.006

Table 48c: Growth of *A. niger* Isolate 3 grown in Czapek-Dox broth medium (pH 7.0) at 32±2^{o}C under a 12-hour day-night cycle in 12 days.

Days after Inoculation	Mycelium Dry weight (g)			
	Replicates			Mean ± S.E
3	0.3949	0.4012	0.3952	0.3971±0.002
6	0.3994	0.4112	0.4200	0.4102±0.006
9	0.4111	0.4202	0.4359	0.4224±0.007
12	0.4018	0.3993	0.4112	0.4041±0.004

:Table 48d: Growth of *A. niger* Isolate 4 grown in Czapek-Dox broth medium (pH 7.0) at 32±2^{o}C under a 12-hour day-night cycle in 12 days.

Days after Inoculation	Mycelium Dry weight (g)			
	Replicates			Mean ± S.E
3	0.4112	0.3923	0.4008	0.4014±0.005
6	0.4490	0.4502	0.4450	0.4481±0.016
9	0.4653	0.4328	0.4928	0.4636±0.017
12	0.4121	0.4567	0.4346	0.4345±0.013

Table 48e: Growth of *A. niger* Isolate 5 grown in Czapek-Dox broth medium (pH 7.0) at 32±2^{o}C under a 12-hour day-night cycle in 12 days.

Days after Inoculation	Mycelium Dry weight (g)			
	Replicates			Mean ± S.E
3	0.3892	0.4100	0.3995	0.3996±0.006
6	0.4210	0.4120	0.4019	0.4116±0.006
9	0.4520	0.4431	0.4999	0.4650±0.018
12	0.4421	0.4989	0.4210	0.4540±0.023

Table 49a: Mean maceration time of Potato tuber discs by *Aspergillus niger* Isolate 1 filtrate and calculated enzyme activity of the culture filtrate.

Days of growth of culture	Maceration time of discs (seconds)			Enzyme activity
	Sample 1	Sample 2	Mean	
3	22.8	24.2	23.50	4.26
6	11.7	12.5	12.10	8.26
9	11.7	10.8	11.25	8.89
12	11.7	11.7	11.70	8.55

Table 49b: Mean maceration time of Potato tuber discs by *Aspergillus niger* Isolate 2 filtrate and calculated enzyme activity of the culture filtrate.

Days of growth of culture	Maceration time of discs (seconds)			Enzyme activity
	Sample 1	Sample 2	Mean	
3	15.0	15.8	15.40	6.49
6	11.7	12.5	12.10	8.26
9	11.7	10.8	11.25	8.89
12	11.7	12.5	12.10	8.26

Table 49c: Mean maceration time of Potato tuber discs by *Aspergillus niger* Isolate 3 filtrate and calculated enzyme activity of the culture filtrate.

Days of growth of culture	Maceration time of discs (seconds)			Enzyme activity
	Sample 1	Sample 2	Mean	
3	22.5	21.7	22.10	4.52
6	20.0	18.3	19.50	5.13
9	13.3	12.5	12.90	7.75
12	13.3	12.5	12.90	7.75

Table 49d: Mean maceration time of Potato tuber discs by *Aspergillus niger* Isolate 4 filtrate and calculated enzyme activity of the culture filtrate.

Days of growth of culture	Maceration time of discs (seconds)			Enzyme activity
	Sample 1	Sample 2	Mean	
3	16.7	16.7	16.70	5.99
6	12.5	12.5	12.50	8.00
9	11.7	10.8	11.25	8.89
12	12.5	11.7	11.10	8.26

Table 49e: Mean maceration time of Potato tuber discs by *Aspergillus niger* Isolate 5 filtrate and calculated enzyme activity of the culture filtrate.

Days of growth of culture	Maceration time of discs (seconds)			Enzyme activity
	Sample 1	Sample 2	Mean	
3	16.7	15.8	16.25	6.15
6	14.2	15.8	15.00	6.67
9	12.5	13.3	12.90	7.75
12	13.3	14.2	13.10	7.63

Although the *A. niger* Isolates were grown in 8 days in the different organic nutrients media, the mean mycelial dry weight could be reasonably compared to that of the 9 day old cultures in the Czapeck Dox medium. The mean mycelial dry weight of the Czapeck Dox medium reported earlier and those of the shallot bulb extract (Tables 54a and b) were far greater; a range of 0.298 to 0.465g (Tables 48c and 48e) than those of the other organic media (Tables 50 to 53 and Table 55). The mean dry weights of the fungi of the pale-brown shallot cultivar extracts ranged from 0.334 to 0.368g, those of the pink cultivar extracts from 0.367 to 0.411g, and those of the Czapeck Dox media ranged from 0.322 to 0.481g. In comparison the range for the rest of the media was 0.095 (Table 51) to 0.135g (Tables 50 and 53).

The initial pH's of all the media were neutral. The pH significantly drifted to the acidic side (2.67-5.68) during the growth of the cultures in the various media.

Table 50: Growth of *Aspergillus niger* Isolates in Bean Meal Extract at 32±2°C under a 12-hour day-night cycle in 8 days.

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Isolates	Initial	Final	Replicates	Mean± S.E
1	6.91	3.26	0.1336 0.1331 0.1389	0.1352±0.002 [a]
2	6.91	2.76	0.1884 0.1742 0.1789	0.1805±0.004 [b]
3	6.91	2.81	0.1142 0.1383 0.1229	0.1251±0.007 [a]
4	6.91	2.72	0.1303 0.1092 0.1276	0.1224±0.007 [a]
5	6.91	2.70	0.1372 0.1301 0.1211	0.1295±0.005 [a]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability

Table 51: Growth of *Aspergillus niger* Isolates in Cassava Dextrose Broth at 32±2^{o}C under a 12-hour day-night cycle in 8 days.

Isolates	pH		Dry weight of mycelium (g) produced in 30 ml medium	
	Initial	Final	Replicates	Mean± S.E
1	6.99	3.05	0.1077 0.1044 0.1049	0.1057±0.001 [a]
2	6.99	2.85	0.1176 0.1155 0.1136	0.1156±0.001 [b]
3	6.99	2.80	0.1054 0.1348 0.1109	0.1170±0.009 [b]
4	6.99	3.23	0.0942 0.0954 0.0978	0.0958±0.001 [c]
5	6.99	3.27	0.1129 0.1010 0.0999	0.1046±0.004 [a]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability

Table 52: Growth of *Aspergillus niger* Isolates in Oat Meal Extract at 32±2^{o}C under a 12-hour day-night cycle in 8 days

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Isolates	Initial	Final	Replicates	Mean± S.E
1	6.55	3.19	0.0949 0.0993 0.1123	0.1022±0.005 [a]
2	6.55	2.93	0.0959 0.0849 0.1231	0.1013±0.011 [a]
3	6.55	2.85	0.1153 0.1101 0.0983	0.1079±0.005 [a]
4	6.55	3.05	0.1123 0.1159 0.0895	0.1059±0.008 [a]
5	6.55	2.72	0.0932 0.1152 0.1211	0.1098±0.008 [a]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability

Table 53: Growth of *Aspergillus niger* Isolates in Potato Dextrose Broth at 32±2°C under a 12-hour day-night cycle in 8 days

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Isolates	Initial	Final	Replicates	Mean± S.E
1	6.47	3.05	0.1173 0.1493 0.1312	0.1326±0.009 [a]
2	6.47	2.67	0.2014 0.1868 0.2054	0.1312±0.037 [a]
3	6.47	2.82	0.1441 0.1395 0.1221	0.1352±0.006 [a]
4	6.47	2.73	0.1339 0.1059 0.1318	0.1239±0.009 [a]
5	6.47	2.74	0.1348 0.1233 0.1199	0.1260±0.005 [a]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability

Table 54a: Growth of *Aspergillus niger* Isolate 1 in aqueous extract of bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under a 12-hour day-night cycle in 8 days.

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Shallot cultivar	Initial	Final	Replicates	Mean± S.E
Pale-brown	6.51	3.51	0.3551 0.4060 0.3873 0.3308	0.3698±0.017 [a]
Pink	6.44	3.91	0.3913 0.3790 0.3654 0.4062	0.3855±0.009 [b]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

Table 54b: Growth of *Aspergillus niger* Isolate 2 in aqueous extract of bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under a 12-hour day-night cycle in 8 days.

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Shallot cultivar	Initial	Final	Replicates	Mean± S.E
			0.3536	
Pale-brown	6.24	2.87	0.3157	0.3674±0.021 [a]
			0.3866	
			0.4137	
			0.4064	
Pink	6.28	2.88	0.5298	0.4176±0.043 [a]
			0.3211	
			0.4134	

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

Table 54c: Growth of *Aspergillus niger* Isolate 3 in aqueous extract of bulbs of shallot (*Allium ascalonicum*) at 32±2°C under a 12-hour day-night cycle in 8 days

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Shallot cultivar	Initial	Final	Replicates	Mean± S.E
Pale-brown	6.24	5.41	0.3267 0.3241 0.3398 0.3473	0.3345±0.005 [a]
Pink	6.28	5.68	0.3820 0.3682 0.3946 0.3801	0.3812±0.005 [b]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

Table 54d: Growth of *Aspergillus niger* Isolate 4 in aqueous extract of bulbs of shallot (*Allium ascalonicum*) at 32±2°C under a 12-hour day-night cycle in 8 days

Shallot cultivar	pH		Dry weight of mycelium (g) produced in 30 ml medium	
	Initial	Final	Replicates	Mean± S.E
Pale-brown	6.24	4.49	0.3549 0.3241 0.3398 0.3473	0.35880±0.001 [a]
Pink	6.28	4.65	0.4016 0.3854 0.4216 0.3984	0.4018±0.007 [b]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

Table 54e: Growth of *Aspergillus niger* Isolate 5 in aqueous extract of bulbs of shallot (*Allium ascalonicum*) at 32±2^{o}C under a 12-hour day-night cycle in 8 days.

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Shallot cultivar	Initial	Final	Replicates	Mean± S.E
			0.3559	
Pale-brown	6.21	3.51	0.3324	0.3350±0.007 [a]
			0.3217	
			0.3299	
			0.3615	
Pink	6.24	3.40	0.3411	0.3669±0.011 [b]
			0.3924	
			0.3727	

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

Table 55: Growth of *Aspergillus niger* isolates in Sweet Potato Dextrose Broth medium at 32±2°C under a 12-hour day-night cycle in 8 days.

	pH		Dry weight of mycelium (g) produced in 30 ml medium	
Isolates	Initial	Final	Replicates	Mean± S.E
1	6.81	3.62	0.1160 0.1013 0.1129	0.1101±0.004 [a]
2	6.81	3.48	0.1314 0.1239 0.1139	0.1231±0.005 [a]
3	6.81	3.52	0.1339 0.1248 0.1312	0.1300±0.003 [a]
4	6.81	2.73	0.1129 0.1324 0.1039	0.1164±0.008 [a]
5	6.81	2.95	0.0995 0.1215 0.1198	0.1136±0.007 [a]

By the calculated Scheffe's Confidence Limit, values of Mean Mycelium Dry Weights bearing the same letters are not significantly different at 5% level of probability.

EXPERIMENT K. Mycoflora of the bulbs and the rhizosphere of shallot plants raised in soil of Anloga farms amended with Urea fertilizer.

The characteristics of soil of shallot plots in Anloga are shown in Tables 56 to 58. The main features are:

(a) The resident fungus species;

(b) Very high sand content, and

(c) Predominance of nitrogen over other chemical elements.

When Potato Dextrose Agar and Sabouraud Agar were used to isolate fungi from the same soil sample, different results were obtained. More species (9) together with Mycelia streilia were isolated with Sabouraud Agar than with Potato Dextrose Agar(6) as indicated in Tables 56 and 57. *Aspergillus* was the dominant genus in both lists of fungi and *Aspergillus niger* the dominant species. The genera of fungi encountered in the soil were *Aspergillus, Curvularia, Penicillium* and *Rhizopus*.

The soil was very sandy with 98.8 per cent sand content and very low 3.48per cent humus content (Table 58). Comparing the component chemicals, the level of nitrogen was far higher than the other chemical elements. Unexpectedly, it was followed by iron which was substantially superior to calcium, magnesium, phosphorus and potassium.

Table 56: Fungal species isolated with Potato Dextrose Agar from freshly prepared plots at Anloga for cultivation of shallots (*Allium ascalonicum*) immediately before planting.

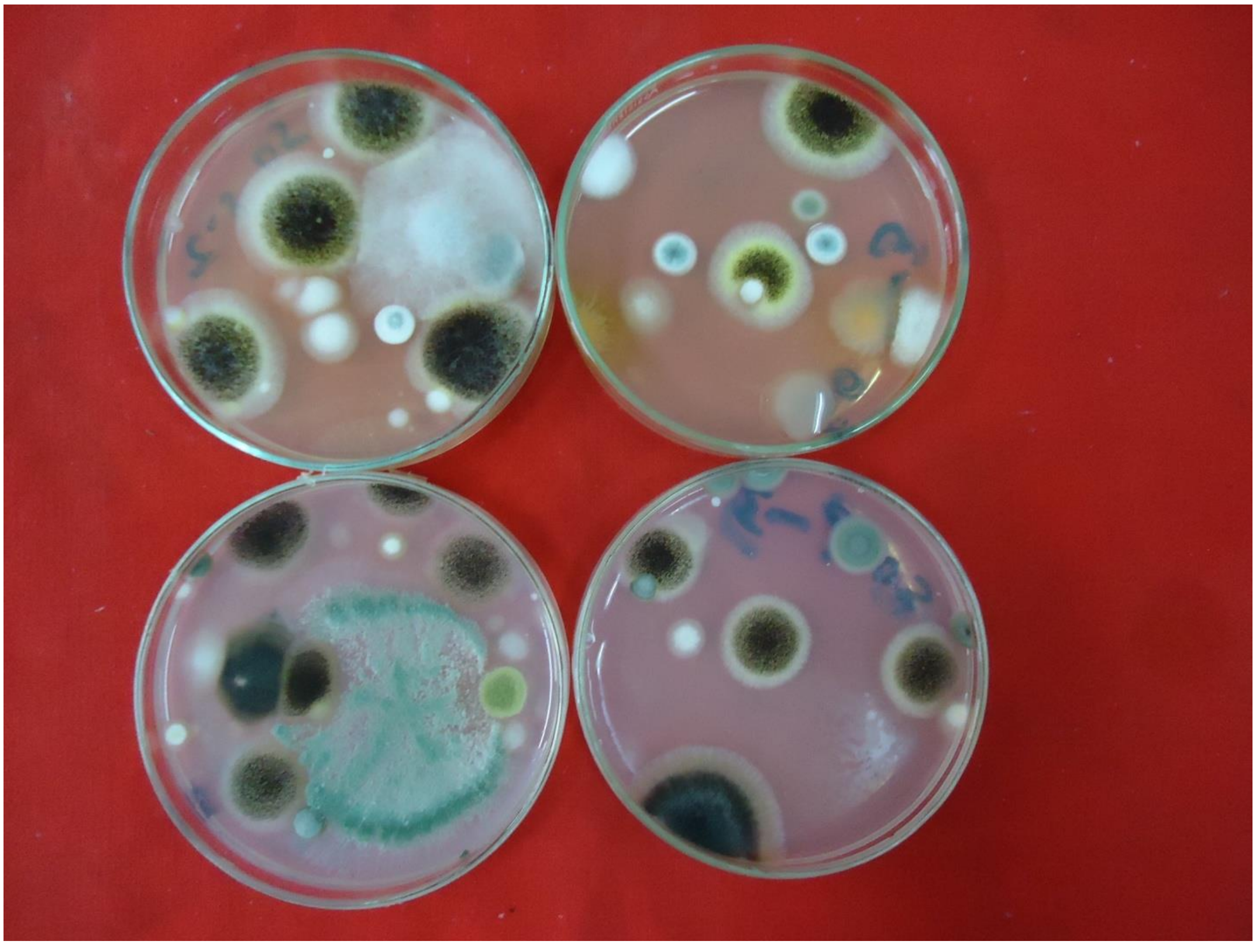
Fungus species	CFU $ml^{-1} \times 10^3$	% Frequency
Aspergillus flavus	8.0	6.4
Aspergillus niger	60.2	47.9
Curvularia lunata	15.2	12.1
Fusarium spp.	18.6	14.8
Penicillium spp.	16.0	12.7
Trichoderma viride	5.0	3.9
Mycelia sterilia	2.8	2.2

Table 57: Fungal species isolated with Sabouraud Agar from freshly prepared plots at Anloga for cultivation of shallot (*Allium ascalonicum*) immediately before planting.

Fungus species	CFU ml^{-1} x 10^3	% Frequency
Aspergillus flavus	6.0	5.1
Aspergillus fumigatus	5.0	4.2
Aspergillus niger	45.8	38.6
Curvularia lunata	8.0	6.7
Fusarium spp	15.4	13.0
Penicillium sp. 1	9.8	8.3
Penicillium sp. 2	13.0	10.9
Rhizopus spp.	3.0	2.5
Trichoderma viride	4.0	3.4
Mycelia sterilia	8.6	7.3

Table 58: Mineral and chemical composition of soil used for cultivation of shallot from Anloga.

Physico-chemical parameters		Plot 1	2	3	Mean
pH		6.98	7.00	7.01	7.00
Mineral Matter (%)	Clay	4.8481	4.7440	4.7333	4.7751
	Sand	95.1519	92.8829	92.8999	93.3116
	Silt	2.0000	2.3724	2.3667	2.2797
Organic Matter (%)	Humus	3.4401	3.5120	3.4813	3.4778
Chemical Constitu-ent (%)	Calcium	0.0046	0.0036	0.0047	0.0043
	Iron	0.0820	0.0757	0.0819	0.0799
	Magnesium	0.0076	0.0064	0.0062	0.0067
	Nitrogen	0.1008	0.1005	0.0979	0.0997
	Phosphorus	0.0160	0.0147	0.0164	0.0157
	Potassium	0.0160	0.0135	0.0154	0.0150



X 5/9

Plate 11. Photograph showing fungi isolated from freshly prepared soil of shallot farm at Anloga using Sabouraud Agar at 32±2ºC for 8 days.

Measurement of the bulbs at 20, 40 and 60 days after planting showed that both the length and width (at widest point) of the bulblets, recorded in Table 59, increased with age of both cultivars. The rate of growth of the bulblets was faster from 20 to 40 days than from 40 to 60 day after planting. Also at each measurement time, the lengths and widths of the Pale-brown cultivar were greater than those of the Pink cultivar. Therefore the Pink cultivar bulbs were comparatively smaller than bulbs of Pale-brown cultivar.

Finally, pigmentation of the scale leaves developed faster in the Pale-brown cultivar (first observed on the 40th day) than in the Pink cultivar observed on the 60th day.

The addition of Urea to the soil only slightly improved growth. The increase in the values were, however, not statistically significant at 5% level of probability. For instance, the bulbs in soils with 0.00, 1.94 and 3.88 g per 1200 g of soil measured 31.8±1.4 x 13.3±0.8, 31.8±1.8 x 14.8±0.9 and 32.4±2.0 x 15.1±1.2mm, respectively, in the Pale-brown cultivar.

Table 59: Morphology of bulblets of the two shallot cultivars developing in the greenhouse under a 12- hour day-night cycle in Anloga shallot farm soil containing different quantities of Urea fertilizer.

Concentrat-ion of Urea (g)	Days after planting	Features of bulblets	Cultivar	
			Pale-brown	Pink
0.00	20	Mean length (mm)	17.7±1.8	16.6±1.1
		Mean width (mm)	5.6±0.7	5.1±0.5
		Colour	white	white
	40	Mean length (mm)	28.8±1.5	22.4±2.3
		Mean width (mm)	10.2±0.8	6.8±0.9
		Colour	light brown	white
	60	Mean length (mm)	31.3±1.4	29.2±2.0
		Mean width (mm)	13.3±0.8	11.2±0.7
		Colour	Pale-brown	Pink
1.94	20	Mean length (mm)	20.3±1.9	18.3±2.0
		Mean width (mm)	7.2±0.3	6.8±0.7
		Colour	white	white
	40	Mean length (mm)	29.2±1.4	24.2±2.1
		Mean width (mm)	11.2±1.0	7.2±1.0
		Colour	light brown	white
	60	Mean length (mm)	31.8±1.8	28.2±1.8
		Mean width (mm)	14.8±0.9	12.2±1.0
		Colour	Pale-brown	Pink

Table 59 continued

.

Concentrat-ion of Urea (g)	Days after planting	Features of bulblets	Cultivar	
			Pale-brown	Pink
3.88	20	Mean length (mm)	17.9±2.3	17.2±1.8
		Mean width (mm)	4.7±0.7	5.2±0.5
		Colour	white	white
	40	Mean length (mm)	30.2±1.4	26.2±1.9
		Mean width (mm)	10.8±1.0	7.9±0.5
		Colour	light- brown	white
	60	Mean length (mm)	32.4±2.0	30.3±2.3
		Mean width (mm)	15.1±1.2	13.2±0.8
		Colour	Pale-brown	Pink

Tables 60 to 62 contain certain pertinent data on mycoflora of the subterranean organs of the pale-brown and pink cultivar shallot plants growing in Anloga farm soils, with or without urea fertilizer. There were members of four genera. The few species isolated from the plants reflected the species of the Anloga farm soil indicated in Tables 56 and 57. It is unusual that a single species, in this investigation *Aspergillus niger*, would so consistently predominate the fungal flora of the rhizosphere. Since other species appeared so tardily and in very low numbers, *A. niger* alone could justifiably be used to define the effect of either bulb age or urea treatment or specific plant organ.

(a) Far greater fungal populations were present in the phyllosphere and rhizosphere than in the non-rhizosphere soil.

(b) Clearly greater fungal population was associated with the bulb than with the roots.

(c) In the majority of cases the population of mycoflora was higher on the Pale-brown plants than on the Pink cultivars

(d) Colonization of the bulbs and the roots proceeded so quickly in the first 20 days of growth that very little change occurred thereafter. By the 40^{th} day a stable dense community had been established as can be deduced by the crowded dilution of Plates 12 and 13.

(e) Notably, *Aspergillus terreus* was isolated from the non-rhizosphere soil on many occasions but not from the phyllosphere soil (Tables 60-62)

Table 60: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in unamended Anloga shallot farm soil for 60 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3/g soil						
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
20	Pale-brown	BS	-	27.3	-	-	1.0	0.3	0.3
		RH	-	13.0	1.0	-	0.7	-	0.7
		NR	-	4.0	1.3	-	0.3	-	0.3
	Pink	BS	0.7	30.0	1.0	-	0.7	-	-
		RH	0.7	11.0	-	-	-	-	1.3
		NR	0.7	4.3	0.7	-	1.0	-	0.3
40	Pale-brown	BS	1.0	35.0	-	-	2.7	1.7	2.0
		RH	1.0	16.0	1.7	-	2.7	-	0.3
		NR	1.3	11.0	0.3	-	1.7	-	0.7
	Pink	BS	1.0	37.3	-	-	1.3	1.0	1.0
		RH	1.0	14.7	1.3	-	2.0	-	1.0
		NR	1.3	11.7	1.0	-	2.3	-	0.7

Table 60 continued

Days after planting	Shallot cultivar	Source of fungal species	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
			C.F.U's x 10^3 /g soil						
60	Pale-brown	BS	1.3	30.0	1.0	-	1.3	1.0	0.3
		RH	1.0	14.3	-	1.0	1.7	-	0.3
		NR	0.7	10.0	0.3	1.0	0.7	-	0.3
	Pink	BS	1.0	25.0	1.0	1.3	1.7	1.3	1.0
		RH	1.0	13.3	0.7	1.0	1.0	-	0.3
		NR	1.0	11.7	0.3	0.7	1.0	-	0.7

Table 61: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in Anloga shallot farm soil treated with 1.94g of Urea for 60 days.

Days after planting	Shalllot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil						
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
20	Pale-brown	BS	-	36.3	-	-	4.0	-	2.0
		RH	0.3	6.7	1.3	-	1.0	-	1.0
		NR	-	3.3	0.7	-	0.7	-	0.3
	Pink	BS	0.3	40.0	1.7	-	1.7	-	-
		RH	0.7	7.3	-	0.7	-	-	0.3
		NR	0.3	3.0	0.7	1.0	0.3	-	1.0
40	Pale-brown	BS	1.3	40.0	-	-	2.7	1.7	0.7
		RH	1.0	19.3	1.3	-	2.3	-	0.3
		NR	1.0	15.3	0.3	-	2.3	-	1.0
	Pink	BS	1.0	36.7	-	-	1.3	1.3	0.3
		RH	1.0	21.7	1.0	-	1.7	-	0.3
		NR	1.0	13.7	1.0	-	2.3	-	1.0

Table 61 continued

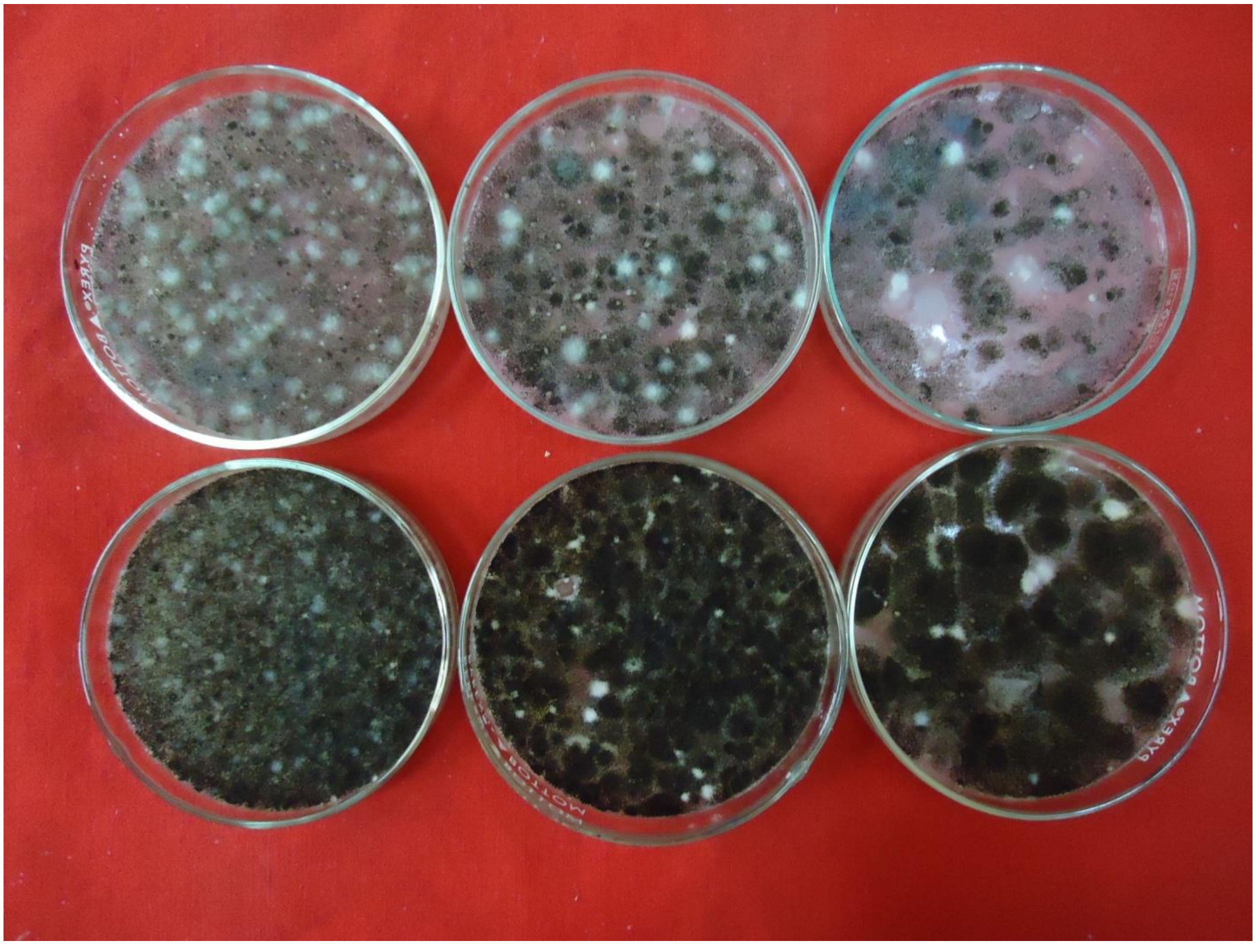
Days after Planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil						
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
60	Pale-brown	BS	1.0	31.0	1.0	-	1.3	-	-
		RH	1.0	16.7	-	1.3	1.3	-	-
		NR	0.3	13.3	0.3	1.0	1.0	-	1.0
	Pink	BS	1.0	26.7	-	1.0	2.0	-	0.3
		RH	1.0	15.0	-	1.0	1.3	-	0.3
		NR	0.3	12.0	0.3	1.0	1.0	-	1.0

Table 62: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two Shallot cultivars grown in Anloga shallot farm soil treated with 3.88 g of Urea for 60 days.

Days after planting	Shalllot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil						
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
20	Pale-brown	BS	-	42.7	-	-	3.3	-	0.7
		RH	-	15.0	1.7	-	1.3	-	0.7
		NR	0.7	3.3	1.3	-	-	-	0.3
	Pink	BS	0.7	46.0	1.0	-	1.7	-	1.0
		RH	1.0	13.3	-	0.7	-	-	0.3
		NR	-	3.0	0.7	-	1.0	-	1.0
40	Pale-brown	BS	1.3	44.0	-	-	2.7	1.7	0.3
		RH	1.3	16.7	1.3	-	3.3	-	1.3
		NR	1.3	13.3	1.3	-	3.3	-	0.7
	Pink	BS	1.0	40.0	-	-	1.3	-	1.3
		RH	1.3	20.0	1.0	-	2.0	-	1.0
		NR	1.0	11.0	1.0	-	2.0	-	0.3

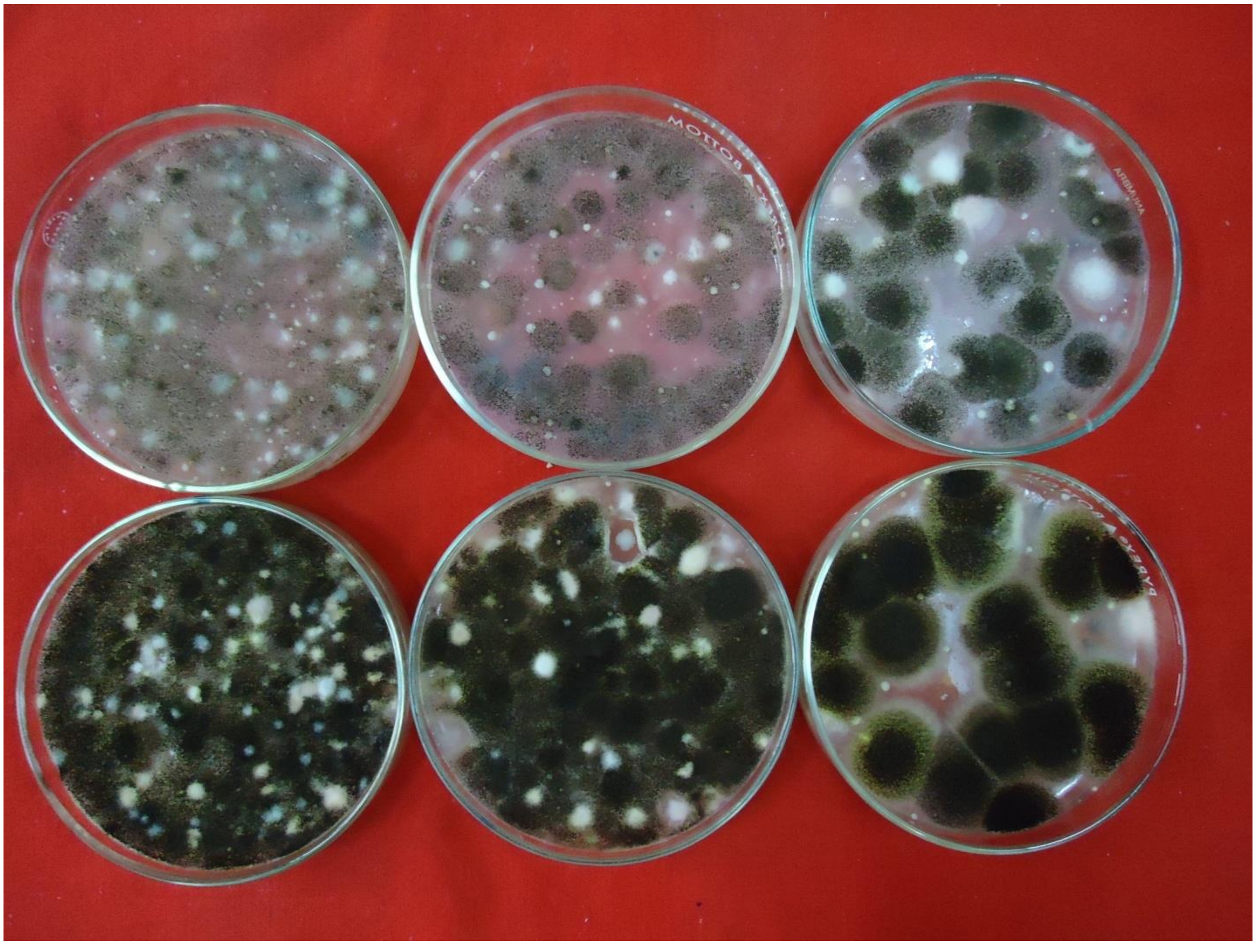
Table 62 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil						
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	*Rhizopus* spp.	Mycelia sterilia
60	Pale-brown	BS	1.0	33.3	1.0	-	1.3	1.0	1.0
		RH	1.3	12.3	-	1.3	1.3	-	0.3
		NR	0.3	11.3	-	0.7	0.7	-	03
	Pink	BS	0.7	35.3	-	1.0	1.7	-	-
		RH	0.3	11.0	-	-	1.3	-	0.7
		NR	0.7	10.0	0.3	0.7	1.0	-	0.3



X 5/9

Plate 12. Photograph showing fungi isolated from the 60 days old bulb surface of Pale-brown cultivar of shallot (*Allium ascalonicum*) using Sabouraud agar (BOTTOM) and Potato Dextrose Agar (TOP). Note carbon-black colonies of *A. niger*.



X 5/9

Plate 13. Photograph showing fungi isolated from the 60 days old bulb surface of Pink cultivar of shallot (*Allium ascalonicum*) using Sabouraud agar (BOTTOM) and Potato Dextrose Agar (TOP).

Note carbon-black colonies of *A. niger*.

EXPERIMENT L. Response of bulbs of the two shallot cultivars formed in Anloga farm soils amended with Urea fertilizer to *Aspergillus niger* inoculation using *A. niger* Isolates 1 and 2.

Urea was meant to enrich the soil. It was assumed that plants benefiting from the enhanced robust growth would be less susceptible to *A. niger* attack. The bulbs of shallot plants which received urea fertilizer in the preceding experiment (Experiment K) were, indeed, bigger than bulbs of the control plants as shown in Table 59. However, the data in Tables 63a and b indicate differently. Plants in soils amended with 1.94 g/l urea fertilizer were either rotted at the same rate as the control bulbs or at even a greater rate. The rate, however, declined with the higher urea concentration of 3.88 g/l as shown in Table 63c.

Bulbs of all treatments were invaded after experimental inoculation at 50 to 100% R.H. And as observed in the earlier tests in Experiment I and recorded in Tables 38 to 47, the diameter of the rotted areas of the bulbs increased with increasing storage humidity. Secondly, the Pale-brown cultivar was uniformly more susceptible to *A. niger* attack than the Pink cultivar at all relative humidities. Thirdly, *A. niger* Isolate 1 was consistently more virulent than Isolate 2.

Table 63a: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in unamended soil, wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	15.0±1.0 [a]	14.1±1.1 [a]	13.9±1.0 [a]	12.3±0.9 [a]
90	14.3±1.1 [a]	13.0±1.0 [a]	13.5±0.9 [a]	12.0±0.9 [a]
80	13.6±0.9 [a]	12.0±1.0 [a]	12.9±0.7 [a]	11.0±1.0 [a]
70	12.5±0.7 [a]	11.1±0.6 [a]	11.3±0.8 [a]	11.3±0.6 [a]
60	11.1±0.8 [a]	10.0±0.7 [a]	10.6±0.6 [a]	10.1±0.5 [a]
50	10.6±0.5 [a]	9.6±0.7 [a]	10.3±0.6 [a]	9.0±0.6 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 63b: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended with, 1.94 g of Urea fertilizer, wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	15.9±1.3 [a]	14.3±0.9 [a]	14.6±1.0 [a]	14.0±0.9 [a]
90	14.5±1.3 [a]	13.5±0.9 [a]	13.9±0.9 [a]	13.1±0.8 [a]
80	14.0±1.1 [a]	13.0±1.0 [a]	13.6±1.0 [a]	12.6±0.8 [a]
70	13.1±0.9 [a]	12.1±0.7 [a]	12.8±0.8 [a]	11.1±0.9 [a]
60	12.3±0.9 [a]	11.0±0.8 [a]	11.7±0.8 [a]	10.3±0.5 [a]
50	11.2±0.6 [a]	10.0±0.6 [a]	10.3±0.8 [a]	10.0±0.7 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 63c: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended with 3.88g of Urea fertilizer wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2oC for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	14.4±1.5 [a]	13.0±1.2 [a]	13.0±0.9 [a]	11.8±0.9 [a]
90	14.0±0.9 [a]	12.1±1.0 [a]	12.0±0.7 [a]	11.0±0.9 [a]
80	13.1±0.9 [a]	11.2±1.0 [a]	11.1±0.8 [a]	10.1±0.7 [a]
70	11.3±0.6 [a]	10.5±0.7 [a]	10.2±0.5 [a]	9.3±0.6 [a]
60	10.5±0.6 [a]	9.3±0.8 [a]	9.1±0.7 [a]	8.8±0.6 [a]
50	9.3±0.7 [a]	9.0±0.7 [a]	9.0±0.6 [a]	8.3±0.7 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

.

EXPERIMENT M: Mycoflora of the bulbs and the rhizosphere of shallot plants raised in soil of Anloga farms amended with Potassium fertilizer.

The data in Tables 64 to 69 highlight the effect of Potassium, a component of the indispensable plant nutrient complex, Nitrogen-Phosphorus-Potassium (NPK), on growth of the two shallot cultivars and on shallot-fungus association in soil. In addition, on pathogenicity of *Aspergillus niger* on the bulbs through its influence on permeability of plant membranes which would be a key objective of tests in the next exercise.

The results in Tables 64 show clearly that over the range of potassium concentration used, 6.67 g/l was the optimum. All parameters assessed peaked at this concentration and then declined thereafter. The sizes of the Pale-brown bulbs at 60 days in soils with 0.0, 3.33. 6.67, 10.0 and 13.33 g/l were 33.3±2.0 x 19.8±1.2, 37.5±3.1 x 22.4±1.8, 41.5±1.5x 25.3±0.9, 38.9±1.1x 27.3±1.1 and 32.8±1.1 x 21.3±0.9mm, respectively. Values for the Pink cultivar followed the same trend. The Pale-brown cultivar bulbs at 20, 40 and 60 days were bigger than their counterpart Pink cultivar bulbs.

Presence of potassium in greater amounts produced a unique shallot plant-fungus association. Although *A. niger* again crowded the shallot plants, the bulb was a more favourable organ than the roots. Some other aspects were notable.

The rhizosphere of *A. niger* population in many instances was only feebly superior as can be seen in Table 65. *A. flavus* and *A. terreus* were not as eminent as in the urea tests.

Table 64: Morphology of bulblets of the two shallot cultivars developing in the greenhouse under a 12- hour day-night cycle in Anloga shallot farm soil containing different quantities of Potassium fertilizer.

Concentration of Potassium (g/L)	Days after planting	Characteristic Features of bulblets	Cultivar	
			Pale-brown	Pink
0.00	20	Mean length (mm)	25.8±2.3	24.3±2.1
		Mean width (mm)	7.8±0.3	6.6±0.5
		Colour	White	White
	40	Mean length (mm)	30.2±2.6	28.2±1.9
		Mean width (mm)	14.5±1.1	13.0±0.6
		Colour	Pale-brown	White
	60	Mean length (mm)	33.3±2.0	32.0±2.6
		Mean width (mm)	19.8±1.2	18.3±1.6
		Colour	Pale-brown	Pink
3.33	20	Mean length (mm)	29.5±2.1	26.5±2.5
		Mean width (mm)	10.3±0.6	9.5±0.4
		Colour	White	White
	40	Mean length (mm)	33.5±3.0	29.0±2.3
		Mean width (mm)	18.5±1.0	15.3±0.9
		Colour	Pale-brown	White
	60	Mean length (mm)	37.5±3.1	34.2±2.2
		Mean width (mm)	22.4±1.8	17.2±1.1
		Colour	Pale-brown	Pink

Table 64continued

.

Concentrat-ion of Potassium (g/L)	Days after planting	Characteristic Features of bulblets	Cultivar	
			Pale-brown	Pink
6.67	20	Mean length (mm)	31.2±1.3	27.7±1.1
		Mean width (mm)	10.5±0.5	10.0±0.6
		Colour	White	White
	40	Mean length (mm)	37.5±1.3	25.3±1.0
		Mean width (mm)	21.5±1.0	16.3±0.6
		Colour	Pale-brown	White
	60	Mean length (mm)	41.5±1.5	32.3±1.4
		Mean width (mm)	25.3±0.9	20.2±1.1
		Colour	Pale-brown	Pink
10.00	20	Mean length (mm)	29.2±1.1	24.0±0.9
		Mean width (mm)	9.5±0.7	7.6±0.4
		Colour	White	White
	40	Mean length (mm)	34.3±1.3	31.3±1.2
		Mean width (mm)	22.5±1.0	17.3±0.8
		Colour	Pale-brown	White
	60	Mean length (mm)	38.9±1.1	36.2±1.3
		Mean width (mm)	27.3±1.1	23.5±1.0
		Colour	Pale-brown	Pink

Table 64 continued

Concentration of Potassium (g/L)	Days after planting	Characteristic Features of bulblets	Cultivar	
			Pale-brown	Pink
	20	Mean length (mm)	23.5±0.9	17.5±0.9
		Mean width (mm)	8.9±0.2	7.2±0.5
		Colour	White	White
	40	Mean length (mm)	30.5±1.4	27.0±1.1
13.33		Mean width (mm)	18.4±0.7	16.8±0.9
		Colour	White	White
	60	Mean length (mm)	32.8±1.1	29.3±1.0
		Mean width (mm)	21.3±0.9	20.8±0.8
		Colour	Pale-brown	Pink

Table 65: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in unamended Anloga shallot farm soil for 60 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	Mycelia sterilia
20	Pale-brown	BS	4.3	50.3	-	-	-	0.3
		RH	-	13.3	-	-	2.3	0.7
		NR	2.0	13.7	1.0	-	-	0.7
	Pink	BS	5.0	47.3	-	-	2.7	0.7
		RH	1.3	15.7	-	-	4.0	0.3
		NR	1.7	16.7	1.3	-	2.7	0.3
40	Pale-brown	BS	-	38.3	-	1.3	1.7	1.0
		RH	-	11.7	1.7	-	1.3	1.0
		NR	-	26.7	-	-	2.7	0.7
	Pink	BS	-	33.0	1.7	-	2.7	1.3
		RH	0.7	13.3	-	-	1.0	2.0
		NR	-	28.0	-	-	2.3	1.0

Table 65 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3/g soil *Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
60	Pale-brown	BS	0.3	36.7	-	0.7	0.3	0.3
		RH	0.7	17.7	0.3	-	0.3	2.0
		NR	-	24.0	-	0.3	1.0	1.0
	Pink	BS	-	28.3	-	-	1.0	1.3
		RH	-	14.3	0.7	-	0.7	1.7
		NR	-	23.3	-	0.3	0.7	1.3

Table 66: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in Anloga shallot farm soil treated with 3.33g of Potassium for 60 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
20	Pale-brown	BS	4.0	54.3	-	-	6.0	1.3
		RH	2.7	40.0	-	-	3.3	1.0
		NR	3.0	16.0	1.7	-	-	-
	Pink	BS	-	50.7	-	-	8.0	1.0
		RH	-	38.7	0.7	-	4.5	1.0
		NR	1.7	19.3	-	-	4.0	0.7
40	Pale-brown	BS	-	46.7	-	1.7	1.3	0.3
		RH	-	14.3	-	-	0.7	1.0
		NR	-	23.3	-	-	1.0	-
	Pink	BS	-	44.3	0.7	-	2.7	0.7
		RH	-	26.7	1.0	-	1.0	1.0
		NR	-	30.7	-	-	1.0	1.0

Table 66 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3/g soil *Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
60	Pale-brown	BS	0.3	40.3	-	0.7	1.7	0.3
		RH	2.3	20.0	-	-	1.0	1.0
		NR	-	22.7	-	-	0.7	0.3
	Pink	BS	-	37.7	-	1.0	1.3	0.7
		RH	-	16.0	1.0	-	1.0	0.3
		NR	-	25.0	-	-	1.0	0.3

Table 67: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in Anloga shallot farm soil treated with 6.67g/l of Potassium for 60 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
20	Pale-brown	BS	-	76.7	-	-	4.3	2.0
		RH	2.7	57.0	-	-	1.7	0.7
		NR	-	49.3	-	-	10.0	2.0
	Pink	BS	-	72.3	-	-	11.0	1.3
		RH	1.0	51.3	-	-	2.3	-
		NR	-	51.7	-	-	7.3	` 1.0
40	Pale-brown	BS	-	66.7	2.0	0.7	-	0.3
		RH	0.7	37.3	1.0	-	1.3	0.3
		NR	-	40.3	-	2.0	2.7	0.3
	Pink	BS	-	46.0	0.7	-	2.7	0.3
		RH	-	32.7	-	-	0.7	1.0
		NR	-	33.3	0.7	-	1.0	-

Table 67 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
60	Pale-brown	BS	-	56.7	-	0.7	0.7	0.3
		RH	-	30.0	-	-	1.0	0.7
		NR	-	36.0	-	1.3	1.3	0.3
	Pink	BS	1.0	43.3	-	1.7	1.0	-
		RH	-	34.3	0.3	-	0.7	0.3
		NR	-	30.0	-	-	2.7	0.3

Table 68: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in Anloga shallot farm soil treated with 10.00 g/l of Potassium for 60 days.

Days after planting	Shalllot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	Mycelia sterilia
20	Pale-brown	BS	-	70.0	-	-	5.0	1.0
		RH	1.7	47.7	-	-	1.7	0.3
		NR	1.0	30.0	-	-	3.7	1.0
	Pink	BS	-	7.5	-	-	6.0	1.0
		RH	-	45.0	-	-	3.3	0.3
		NR	2.7	33.3	0.7	-	3.3	0.3
40	Pale-brown	BS	-	62.7	-	1.0	1.7	0.3
		RH	0.7	20.2	-	-	1.3	0.3
		NR	-	19.3	-	-	1.7	0.7
	Pink	BS	-	59.3	1.0	-	1.3	0.3
		RH	0.7	19.0	-	1.0	-	-
		NR	-	16.3	1.0	-	0.7	0.3

Table 68 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3/g soil *Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	Mycelia sterilia
60	Pale-brown	BS	-	50.0	-	0.3	0.7	1.3
		RH	1.0	23.0	0.7	-	1.7	1.3
		NR	-	21.7	-	1.0	1.0	1.0
	Pink	BS	1.0	51.0	-	1.7	0.7	2.0
		RH	-	23.3	-	-	0.3	1.0
		NR	-	18.3	-	1.7	0.7	1.0

Table 69: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in Anloga shallot farm soil treated with 13.33 g/l of Potassium for 60 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
20	Pale-brown	BS	-	66.7	-	-	6.7	2.0
		RH	1.7	40.7	-	-	2.0	0.3
		NR	1.0	29.0	-	-	5.3	-
	Pink	BS	-	67.3	-	-	8.3	2.0
		RH	-	43.3	-	-	6.7	1.3
		NR	1.7	26.7	-	-	-	0.3
40	Pale-brown	BS	-	47.0	-	0.7	1.7	-
		RH	1.0	14.0	-	-	-	0.3
		NR	-	20.0	-	-	3.0	0.3
	Pink	BS	-	50.0	0.3	-	1.0	1.0
		RH	0.7	16.7	-	-	0.3	0.7
		NR	-	21.3	-	-	1.7	1.3

Table 69 continued

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil					
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp	*Penicillium* spp	Mycelia sterilia
60	Pale-brown	BS	-	33.3	-	0.3	0.7	0.3
		RH	-	16.3	0.3	-	1.3	0.3
		NR	-	20.0	-	1.0	0.7	1.0
	Pink	BS	0.7	38.3	-	1.3	-	0.3
		RH	-	17.7	-	1.3	-	1.0
		NR	-	17.0	-	0.7	0.3	0.3

EXPERIMENT N. Response of bulbs of the two shallot cultivars formed in Anloga soils amended with Potassium fertilizer to inoculation with *Aspergillus niger* using *A. niger* Isolates 1 and 2

In the earlier tests described in Experiment I (Tables 38-42) and L (Table 63), the rate of rotting following wound inoculation was slowest at 50% R.H. The rate increased with increasing humidity to achieve the fastest rate at 100% R.H. Secondly, the pale-brown cultivar bulbs were invaded at a faster rate by *Aspergillus niger* than bulbs of the Pink cultivar at all storage humidities. That was also what happened in this experiment when shallot bulbs formed by plants grown in the Anloga farm soil amended with potassium fertilizer were wound-inoculated with *Aspergillus niger* Isolates 1 and 2 according to data in Tables 70a-e.

In addition, the rate of rotting was affected by the potassium fertilizer. It predisposed the bulbs formed to *A. niger* attack. Bulbs formed in soils treated with 6.67 g/l potassium fertilizer were invaded fastest, and the rate decreased as the potassium concentration decreased as the potassium concentration decreased or increased. For example at 100% R.H. the mean diameters of the rotted areas of bulbs of the pale-brown cultivar which developed in soils amended with potassium fertilizer of 0.0, 3.33, 6.67, 10.00 and 13.33 g/l and inoculated with *A. niger* Isolate 1 were 15.3±0.8, 16.2±1.0, 17.0±1.9, 16.5±1.3 and 16.0±1.3 mm, respectively.

Table 70a: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in unamended soil, wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	15.3±0.8 [a]	13.4±0.9 [a]	14.1±1.3 [a]	12.8±1.2 [a]
90	14.7±0.5 [a]	12.8±0.8 [a]	13.8±1.3 [a]	11.5±0.9 [a]
80	13.3±0.6 [a]	12.5±0.7 [a]	13.0±1.0 [a]	11.0±1.0 [a]
70	11.5±0.6 [a]	10.0±0.4 [a]	12.0±0.9 [a]	10.7±0.6 [a]
60	10.8±0.4 [a]	9.5±0.2 [b]	11.5±0.6 [a]	10.0±0.8 [a]
50	10.0±0.7 [a]	9.0±0.5 [a]	10.2±0.8 [a]	9.2±0.5 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 70b: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended with, 3.33 g/l of Potassium fertilizer wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	16.2±1.0 [a]	15.3±1.3 [a]	15.1±1.3 [a]	13.7±1.5 [a]
90	15.2±1.2 [a]	14.8±1.0 [a]	14.3±0.9 [a]	12.6±1.1 [a]
80	14.3±0.8 [a]	13.0±1.0 [a]	13.3±0.8 [a]	12.0±0.8 [a]
70	13.2±0.6 [a]	12.3±0.8 [a]	12.5±1.0 [a]	10.8±0.8 [a]
60	12.8±0.4 [a]	12.0±0.7 [a]	12.0±1.0 [a]	10.1±0.5 [a]
50	10.2±0.4 [a]	9.5±0.7 [a]	10.0±0.8 [a]	9.0±0.6 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 70 c: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended with 6.67 g/l of Potassium fertilizer wound-inoculated with mycelium of *A. niger* Isolates 1and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	17.0±1.9 [a]	16.5±1.2 [a]	14.9±1.2 [a]	15.0±1.1 [a]
90	16.0±1.5 [a]	16.1±1.3 [a]	14.1±1.2 [a]	14.3±0.9 [a]
80	15.5±1.0 [a]	15.1±0.9 [a]	13.0±0.8 [a]	13.1±1.1 [a]
70	14.8±1.2 [a]	14.0±0.9 [a]	12.7±0.9 [a]	12.3±0.8 [a]
60	13.2±0.9 [a]	13.0±0.8 [a]	12.5±0.6 [a]	11.1±0.6 [a]
50	12.0±0.8 [a]	11.2±0.5 [a]	11.3±0.6 [a]	10.8±0.7 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 70d: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended with, 10.00 g/l of Potassium fertilizer wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	16.5±1.3 [a]	15.8±2.1 [a]	15.5±0.9 [a]	14.1±1.0 [a]
90	15.2±1.1 [a]	14.8±1.3 [a]	15.1±1.0 [a]	13.8±0.8 [a]
80	14.0±1.1 [a]	13.3±1.1 [a]	14.3±0.8 [a]	13.0±0.9 [a]
70	13.2±0.7 [a]	12.5±0.9 [a]	13.3±0.7 [a]	12.3±0.7 [a]
60	12.5±0.8 [a]	12.0±0.9 [a]	12.5±0.5 [a]	11.5±0.7 [a]
50	11.3±0.5 [a]	11.0±1.0 [a]	11.8±0.5 [a]	11.0±0.5 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

Table 70e: Diameter of rot of shallot bulbs (*Allium ascalonicum*) grown in soil amended 13.33 g/l of Potassium fertilizer wound-inoculated with mycelium of *A. niger* Isolates 1 and 2 and incubated at 50-100% relative humidity at 32±2^{o}C for 3 days.

	Mean diameter of rot (mm) in shallot cultivars caused by *A. niger*			
	Isolate 1		Isolate 2	
Storage Humidity (%)	Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
100	16.0±1.8 [a]	14.8±1.5 [a]	14.3±1.1 [a]	13.2±0.9 [a]
90	15.3±1.2 [a]	13.9±1.0 [a]	13.8±0.7 [a]	13.0±0.8 [a]
80	14.0±1.1 [a]	12.5±1.1 [a]	13.2±0.9 [a]	12.2±0.5 [a]
70	12.0±0.9 [a]	11.0±1.0 [a]	11.5±0.8 [a]	11.5±0.7 [a]
60	11.5±1.0 [a]	10.8±0.7 [a]	11.0±0.5 [a]	10.7±0.5 [a]
50	11.2±0.8 [a]	10.0±0.8 [a]	11.0±0.6 [a]	10.2±0.6 [a]

By the calculated Scheffe's Confidence Limit, pairs of Values for each *A. niger* Isolate in horizontal rows bearing the same letters are not significantly different at 5% level of probability.

EXPERIMENT O. Mycoflora of the bulbs and rhizosphere of plants of the two shallots cultivars grown in soils of farms of non-alliaceous crops

The bulb is the propagation body for raising shallots. It was clear from the data in Experiment C (Table 9) that most of the planting material might have been inoculated in the soil with *Aspergillus niger.* The repeated planting of the plots in Anloga region could have probably built a reservoir of *A. niger,* putting the shallots in danger. The data in Tables 71 to 74 revealed that the non-rhizosphere soils of cassava, groundnut and maize carried low levels of *A. niger* populations. Nevertheless the young shallot bulbs of both cultivars secreted exudates that stimulated growth of *A. niger* in their vicinity. Although the bulbs developed at apparently the same rate into practically the same size in 20 days as indicated in Table 71; *A. niger* in the maize farm was stimulated to a higher degree than those in the other two soils.

Remarkably, the roots were less supportive and almost the same *A. niger* population was uniformly recorded in the rhizosphere and non-rhizosphere soils.

Table 71: Some morphological and colour characteristics of 20-day old bulblets of the two shallot cultivars developing in soils of indicated non-alliaceous crop farms in the greenhouse under 12-hour day-night cycle.

Source of Soil	Features of bulblets	Cultivar	
		Pale-brown	Pink
Cassava (*Manihot esculenta*) farm	Mean length (mm)	25.5±2.0	24.6±3.1
	Mean width (mm)	7.0±0.3	7.1±0.3
	Colour	White	White
Groundnut (*Arachis hypogea*) farm	Mean length (mm)	26.7±1.9	25.3±1.6
	Mean width (mm)	7.9±0.4	7.7±0.3
	Colour	White	White
Maize (*Zea mays*) farm	Mean length (mm)	25.9±2.3	25.4±1.5
	Mean width (mm)	7.9±0.9	7.6±0.5
	Colour	White	White

Table 72: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in cassava (*Manihot esculenta*) farm soil for 20 days

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil							
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus tamarii*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	*Rhzopus* spp.	Mycelia sterilia
20	Pale-brown	BS	-	43.3	-	-	2.3	2.7	-	-
		RH	1.0	5.7	-	2.3	-	-	1.0	0.3
		NR	-	4.7	2.0	-	-	2.3	1.0	-
	Pink	BS	2.3	40.7	1.0	-	1.0	1.0	-	-
		RH	1.0	5.3	-	-	2.3	-	-	-
		NR	-	4.0	-	-	1.0	2.3	-	0.3

Table 73: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in groundnut (*Arachis hypogea*) farm soils for 20 days.

Days after planting	Shallot cultivar	Source of fungal species	C.F.U's x 10^3 /g soil							
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus tamarii*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus* spp.	Mycelia sterilia
20	Pale-brown	BS	-	33.3	-	-	0.7	1.0	-	1.0
		RH	1.7	2.0	-	1.0	-	-	-	0.3
		NR	1.0	3.3	-	0.7	-	-	-	0.3
	Pink	BS	-	36.3	1.0	0.7	0.7	2.3	-	0.7
		RH	0.3	2.0	-	0.3	-	-	1.0	0.3
		NR	-	3.0	1.0	1.0	1.0	-	-	-

Table 74: Fungal species, isolated with Sabouraud Agar from non-rhizosphere (NR) and rhizosphere (RH) soils, and from soil particles adhering to bulbs (BS) of the two shallot cultivars grown in maize (*Zea mays*) farm soils for 20 days.

		Source of	C.F.U's x 10^3 /g soil					
Days after planting	Shallot cultivar	fungal species	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus terreus*	*Fusarium* spp.	*Penicillium* spp.	Mycelia sterilia
20	Pale-brown	BS	-	56.7	-	0.7	1.0	0.3
		RH	1.0	6.7	2.7	-	1.7	0.3
		NR	0.7	5.3	0.7	-	1.7	1.0
	Pink	BS	-	54.3	-	2.3	2.3	1.0
		RH	1.0	6.3	1.0	-	1.0	-
		NR	2.0	5.6	1.0	-	-	0.3

EXPERIMENT P. Conidiation of *Aspergillus niger* Isolates 1, 3 and 5 on ground bulb tissue of the two shallot cultivars and the germination capacity of the conidia.

The blended bulb tissue of shallot supported vigorous vegetative growth of the three test *A. niger* isolates according to the data in Tables 75 to 77. The isolates, however, showed variable relationship with the substrates. Isolate 3 grew better on the Pink cultivar tissue than on the Pale-brown cultivar tissue. Thus, the mean diameters of the Isolate 3 on the Pink cultivar tissue in Table 76 were on each day statistically significantly higher at 5 percent level of probability. Cultures of Isolates 1 (Table 75) and 5 (Table 77), on the other hand grew faster in the first three days of incubation on the pale-brown cultivar tissue and lagged behind thereafter.

The results in Table 78, however, showed that sporulation on the two types of tissues by each *A. niger* isolate was practically the same.

A. niger Isolates 1, 3 and 5 produced equally healthy conidia on bulb tissue of both shallot cultivars, which germinated to the same degree in the different organic nutrients (Tables 79 to 81). There was 100 percent germination in undiluted Potato Dextrose Broth and Sweet Potato Broth by all isolates. Percentage germination was slightly lower in the undiluted Bean Meal Broth of 90.8 to 94.8 percent (Tables 79 and 80). Percentage germination then declined with increasing dilution of the media to eventually zero percent germination in the 1: 30 dilution media. Mean germ-tube length followed the same pattern of response to medium concentration.

Potato Dextrose broth was the most suitable conidium germination medium.

Table 75: Growth of *Aspergillus niger* Isolate 1 on blended bulbs of the two cultivars of shallot (*Allium ascalonicum*) under a 12-hour day-night cycle at 32±2^{o}C.

	Mean diameter of culture (mm) ±S.E on	
Day after Inoculation	Pale-brown cv. Medium	Pink cv. Medium
1	18.5±2.0 ^{a}	20.0±1.6 ^{a}
2	22.3±3.7 ^{a}	21.3±2.0 ^{a}
3	34.0±3.4 ^{a}	33.3±2.4 ^{a}
4	42.7±2.7 ^{a}	43.0±2.5 ^{a}
5	49.8±3.0 ^{a}	59.8±1.4 ^{b}
6	58.3±2.2 ^{a}	63.5±3.1 ^{a}
7	67.0±1.9 ^{a}	73.2±2.1 ^{a}

By calculated Scheffe's Confidence Limits, values of Mean Culture Diameters in the same horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 76: Growth of *Aspergillus niger* Isolate 3 on blended bulbs of the two cultivars of shallot (*Allium ascalonicum*) under a 12-hour day-night cycle at 32±2^{o}C.

.

Day after Inoculation	Mean diameter of culture (mm) ±S.E on	
	Pale-brown cv. Medium	Pink cv. Medium
1	25.5±2.6 [a]	30.0±0.6 [a]
2	32.3±3.7 [a]	41.3±2.3 [a]
3	48.0±3.8 [a]	53.3±2.4 [a]
4	52.7±3.7 [a]	63.0±2.9 [a]
5	59.8±4.0 [a]	69.8±2.4 [a]
6	70.3±4.2 [a]	76.5±3.1 [a]
7	77.0±4.9 [a]	83.2±3.1 [a]

By calculated Scheffe's Confidence Limits, values of Mean Culture Diameters in the same horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 77: Growth of *Aspergillus niger* Isolate 5 on blended bulbs of the two cultivars of shallot (*Allium ascalonicum*) under a 12-hour day-night cycle at 32±2^{o}C.

Day after Inoculation	Mean diameter of culture (mm) ±S.E on	
	Pale-brown cv. Medium	Pink cv. Medium
1	22.7±1.3 [a]	21.5 ±0.0 [a]
2	26.3±0.2 [a]	25.6±0.1 [a]
3	31.5±0.2 [a]	30.8±0.1 [a]
4	47.7±0.2 [a]	45.8±0.1 [a]
5	65.8±0.2 [a]	69.8±0.1 [b]
6	77.3±0.3 [a]	78.5±3.1 [a]
7	84.3±0.2 [a]	88.0±0.0 [b]

By calculated Scheffe's Confidence Limits, values of Mean Culture Diameters in the same horizontal row bearing the same letters are not significantly different at 5% level of probability.

Table 78: Degree of sporulation of *Aspergillus niger* Isolates 1, 3 and 5 grown on blended tissue of bulbs of the two shallot cultivars under a 12-hour day-night cycle at 32±2ºC for 7 days.

A. niger Isolate	Number of conidia per ml of suspension of the tissues of the cultivar (x 10^4)	
	Pale- brown	Pink
1	6.51	5.63
3	4.64	4.48
5	6.12	6.09

Table 79: Germination of conidia produced by 7-day old *Aspergillus niger* Isolate 1 incubated in liquid organic nutrients at 32±2^{o}C for12 hours.

Type of Medium	Medium dilution*	Percentage Germination		Mean Gem tube length ± S.E. (µm)	
		Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
Bean	Undiluted	94.8	90.8	210.3 ±7.3 [a]	190.3±4.9 [b]
Meal	1:1	74.8	69.9	173.8±6.3 [a]	150.3±4.3 [b]
Broth	1:10	23.2	18.3	59.9±3.8 [a]	48.4±2.5 [a]
(BMB)	1:20	14.8	12.4	15.9±1.7 [a]	12.3±1.2 [a]
	1:30	0.0	0.0	-	-
Potato	Undiluted	100	100	240.3±9. 2 [a]	233.4±7.8 [a]
Dextrose	1:1	86.7	83.4	220.3±7.3 [a]	210.9±10.2[a]
Broth	1:10	25.8	20.5	78.2±5.4 [a]	82.4±8.8 [a]
(PDB)	1:20	19.2	14.0	25.3±4.3 [a]	21.3±3.9 [a]
	1:30	0.0	0.0	-	-
Sweet	Undiluted	100	100	228.4±7.9 [a]	220.4±6.9 [a]
Potato	1:1	80.2	78.3	200.9±8.8 [a]	189.3±8.3 [a]
Broth	1:10	24.4	21.3	82.3±4.8 [a]	74.4±2.3 [a]
(SB)	1:20	15.8	13.2	20.2±3.4 [a]	23.8±4.9 [a]
	1:30	0.0	0.0	-	-

1. *Undiluted medium : Distilled water.
2. By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each each medium bearing the same letters are not significantly different at 5% level of probability.

Table 80: Germination of conidia produced by 7-day old *Aspergillus niger* Isolate 3 incubated in liquid organic nutrients at 32±2^{o}C for12 hours.

Types of Medium	Medium dilution*	Percentage Germination		Mean Gem-tube length ± S.E. (μm)	
		Pale-brown cv.	Pink cv.	Pale-brown cv.	Pink cv.
Bean Meal Broth (BMB)	Undiluted	95.2	94.8	198.4±6.8 [a]	200.2±7.7 [a]
	1:1	78.3	75.3	190.3±6.2 [a]	185.3±6.3 [a]
	1:10	25.3	24.2	54.8±4.4 [a]	49.3±4.8 [a]
	1:20	18.2	15.2	24.3±4.8 [a]	20.3±3.3 [a]
	1:30	0.0	0.0	-	-
Potato Dextrose Broth (PDB)	Undiluted	100	100	232.4±6.9 [a]	230.0±7.3 [a]
	1:1	92.8	90.5	218.5±5.5 [a]	200.3±4.5 [a]
	1:10	32.4	30.4	80.3±4.3 [a]	82.5±5.1 [a]
	1:20	22.3	15.3	30.2±4.0 [a]	27.3±4.8 [a]
	1:30	0.0	0.0	-	-
Sweet Potato Broth (SB)	Undiluted	100	100	235.5±9.9 [a]	231.4±8.8 [a]
	1:1	89.3	82.5	220.3±7.9 [a]	210.1±9.3 [a]
	1:10	30.4	24.3	78.5±6.3 [a]	79.3±3.9 [a]
	1:20	18.3	16.2	28.3±3.3 [a]	24.2±4.8 [a]
	1:30	0.0	0.0	-	-

1. *Undiluted medium : Distilled water.
2. By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each each medium bearing the same letters are not significantly different at 5% level of probability.

Table 81: Germination of conidia produced by 7-day old *Aspergillus niger* Isolate 5 incubated in liquid organic nutrients at 32±^{o}C for12 hours.

Types of Medium	Medium dilution*	Percentage Germination		Mean Gem tube length ± S.E. (µm)	
		Pale-brown c.v.	Pink c.v.	Pale-brown c.v.	Pink c.v.
Bean	Undiluted	92.5	90.3	195.3±6.3 [a]	195.3±7.2 [a]
Meal	1:1	80.2	75.3	183.4±4.6 [a]	177.4±6.2 [a]
Broth	1:10	23.8	21.5	60.3±3.8 [a]	58.9±6.0 [a]
(BMB)	1:20	20.5	18.7	28.3±3.4 [a]	24.3±3.3 [a]
	1:30	0.0	0.0	-	-
Potato	Undiluted	100	100	238.8±8.2 [a]	230.4±10.3 [a]
Dextrose	1:1	93.5	90.3	218.2±4.3 [a]	220.5±6.3 [a]
Broth	1:10	34.3	35.3	83.4±3.9 [a]	79.3±4.8 [a]
(PDB)	1:20	26.2	27.8	32.5±5.1 [a]	26.5±5.9 [a]
	1:30	0.0	0.0	-	-
Sweet	Undiluted	100	100	213.3±6.3 [a]	208.8±8.2 [a]
Potato	1:1	89.9	86.5	208.3±4.2 [a]	198.1±6.3 [a]
Broth	1:10	30.3	29.1	85.5±4.3 [a]	84.4±4.9 [a]
(SB)	1:20	23.2	20.2	30.4±3.9 [a]	31.8±5.5 [a]
	1:30	0.0	0.0	-	-

1. *Undiluted medium : Distilled water.
2. By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each each medium bearing the same letters are not significantly different at 5% level of probability.

EXERCISE Q. Longevity of conidia formed on the two shallot substrates by *Aspergillus niger* Isolates 1, 3 and 5 at different relative humidities

The results of the lengthy survival tests in Fig. 4 and Appendices B-V indicate that conidia of the three *A. niger* isolates (Isolate 1, 3 and 5) exhibited many similar characteristics. To summarize:

(a) The conidia retained vitality at the favourable relative humidities for 200 days

(b) The conidia remained viable for a longer time at higher relative humidities than at lower relative humidities

(c) At 100% R.H., the most favourable relative humidity for survival, at least 43.8, 42.1 and 32.3 per cent of the conidia of Isolates 1 (Appendix H), 3 (Appendix O) and 5 (Appendix V) respectively, were alive after 200 days' storage on the Pink cultivar.

(d) The higher the percentage survival, the longer the germ-tubes.

(e) Percentage viability of conidia of the three *A. niger* isolates at any particular relative humidity was relatively the same.

(f) For any *A. niger* isolate there was no significant difference between viabilities of conidia formed on bulb tissues of the Pale-brown and Pink shallot cultivars.

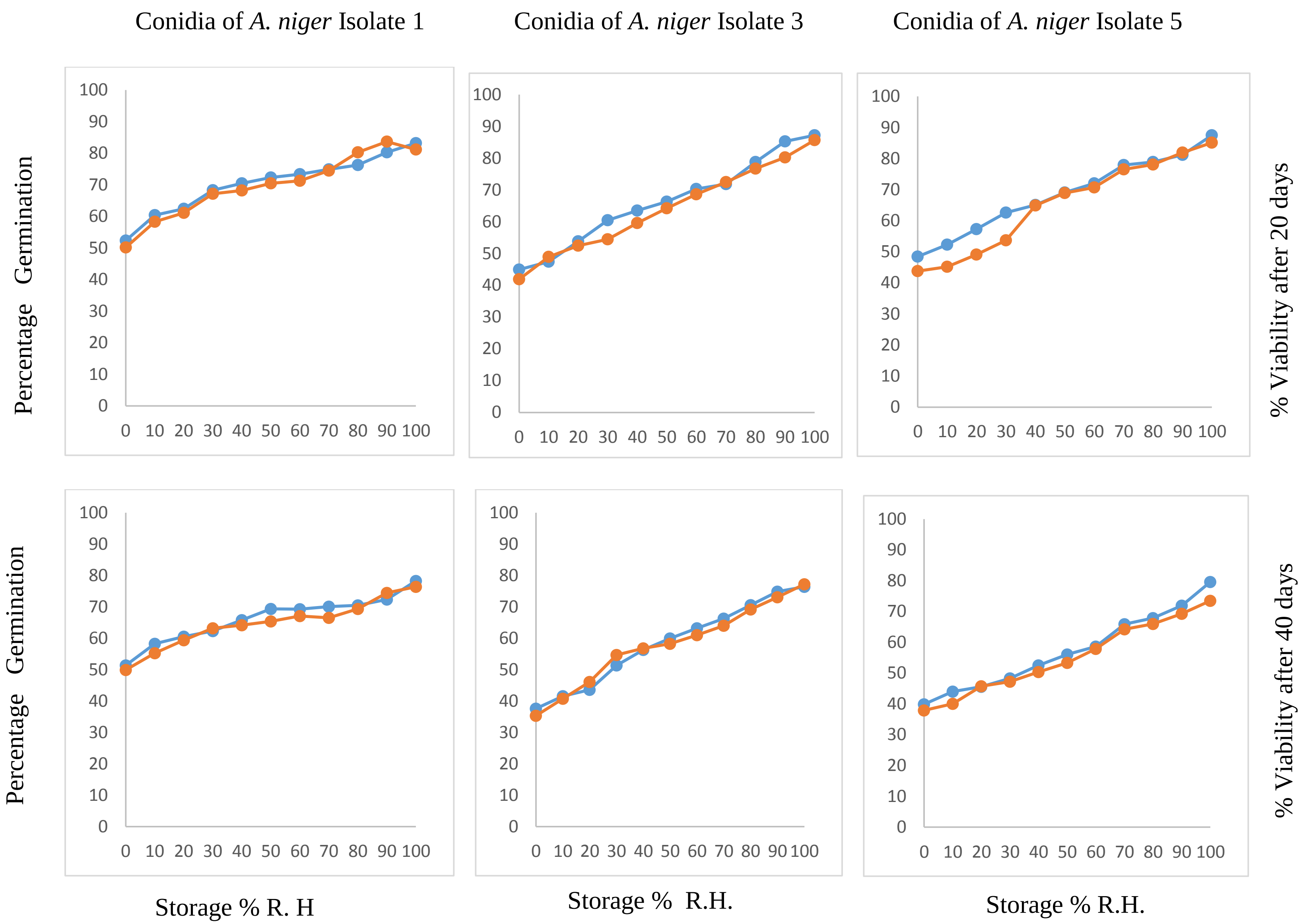


Figure 4a: Viability of conidia of three isolates of *Aspergillus niger* formed on ground Pale-brown ( ) and Pink ( ) shallot bulbs and stored at different Relative Humidities at 32±2°C under 12-hour Day-Night light cycle.

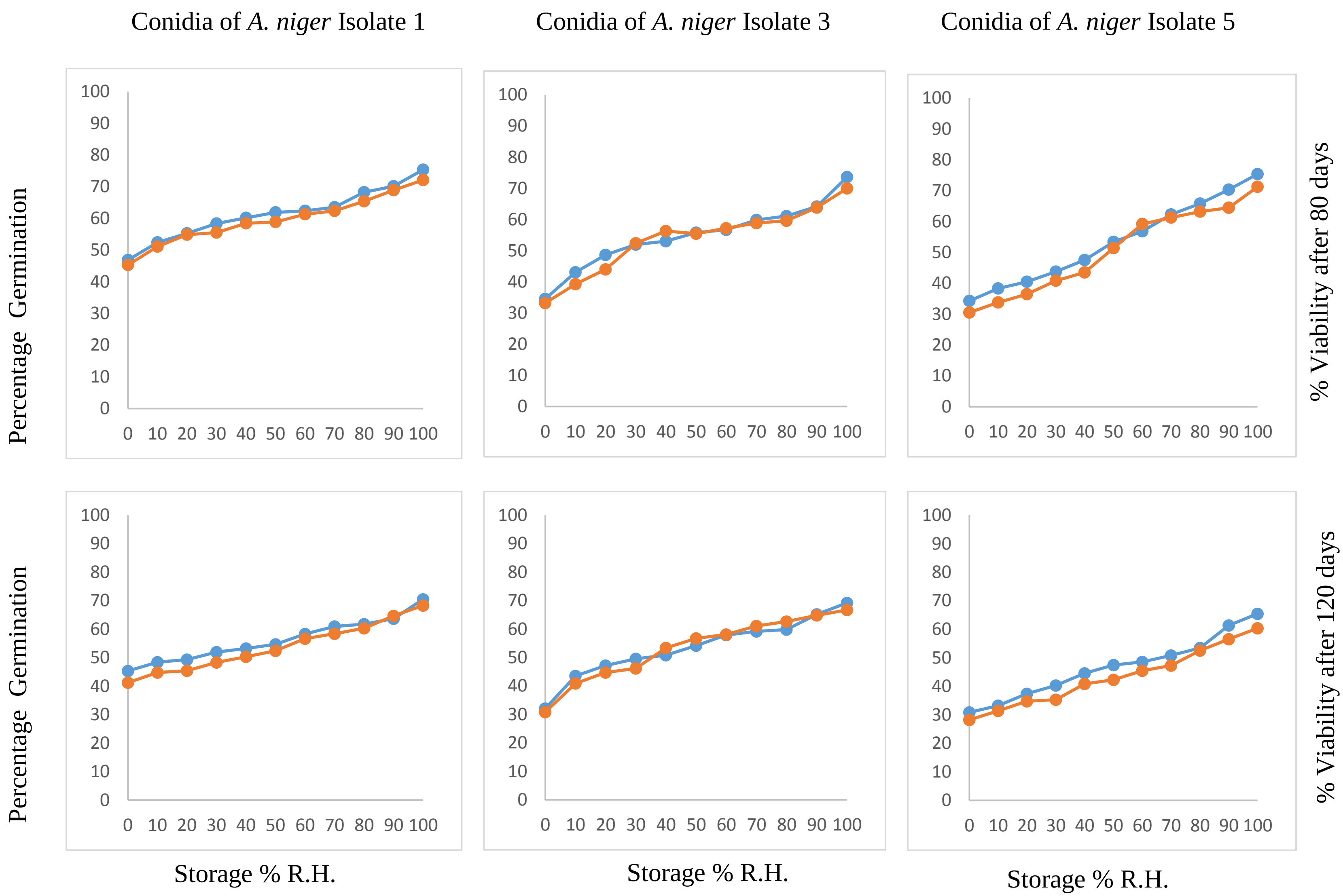


Figure 4b: Viability of conidia of three isolates of *Aspergillus niger* formed on ground Pale-brown ( ) and Pink ( ) shallot bulbs and stored at different Relative Humidities at 32±2°C under 12-hour Day-Night light cycle.

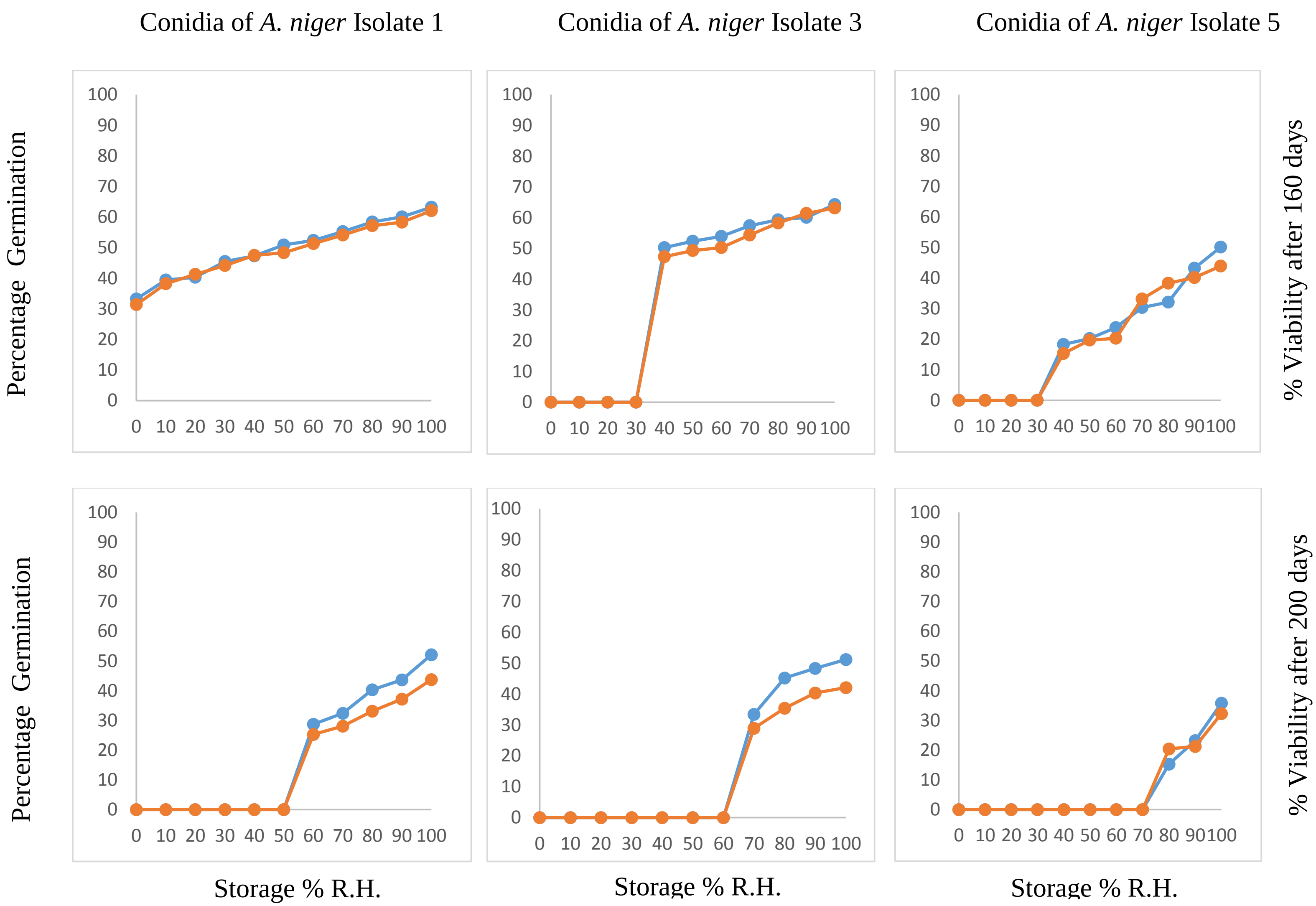


Figure 4c: Viability of conidia of three isolates of *Aspergillus niger* formed on ground Pale-brown ( ) and Pink ( ) shallot bulbs and stored at different Relative Humidities at 32±2ºC under 12-hour Day-Night cycle

V. DISCUSSION

In his book, *Useful plants of Ghana, West African use of wild and cultivated plants*, Abbiw (1990) stated that, there are about six varieties of shallots based on size of bulbs, colour and degree of scent'. The six varieties were not named. Neither did Abbiw (1990) state the source of information and further relevant particulars could, not, therefore, be obtained. During the three years of this study, persistent and wide survey for shallot cultivars in Ghana revealed only two cultivars, *viz*., Pale-brown and Pink cultivars (see Figs. 1 and 2, and Plates 5,6 and 7) exist which became the subject of the present investigation

The large onions (*Allium cepa*), have three major cultivars-Bawku, Red Creole and Texas Grano being cultivated extensively in the country (Teye, 1994). The Bawku variety is medium-sized, globular in shape and has a firm flesh and purple-coloured scale leaves. The Red Creole bulbs are ovalish, small to medium in size with a firm flesh and red scale leaves. Texas Grana bulbs are large in size, flask-shaped with elongated neck and have a comparatively softer flesh and straw-yellow scale leaves. The size of the onion market far exceeds that of shallots and this reduces the interest in shallots. Diminished interest in shallots would discourage their cultivation. It was prudent to find ways to support continuing shallot production to maintain a flourishing shallot market because of the valuable contribution of shallot to human health. One of the ways by which this can be done is by identifying the shallot cultivar that can be recommend as the preferred choice for higher productivity. This cultivar should significantly, among other attributes, be able to withstand the ubiquitous *Asperigillus niger*, the cause of the black spot of onions and shallots, and be able to able to discourage its persistence in the ecosystem.

The results of the present experiment show the significance of the Pink cultivar for shallot production in Ghana. Bulbs of both cultivars suffer from high *A. niger* natural infection, but more so by those of the pale-brown cultivar. The shallot bulb, either of a single unit or either of a major bulb and an attached mini- bulb, is firmly covered by a double –layer of pigmented scale leaves. Yet, the fungus colonizes not only the exposed parts but the covered parts as well. From the data in Table 9, the mean percentages of bulbs, bearing *A. niger* growth on the abaxial surface of the outer scale leaf , adaxial surface of the outer scale leaf, abaxial surface of the inner scale leaf, adaxial surface of the inner scale leaf, abaxial surface of the outermost fleshy leaf base and the contact surfaces of the major bulb and mini- bulb of the Pale – brown cultivar was 72 , 83, 85, 75, 66 and 75 percent, respectively, and of the Pink cultivar, lower values of64, 71, 81, 72, 65 and 67 percent, respectively.

A significant feature was the colonization of hidden areas under the cover of the scale leaves in both cultivars. One possibility was that, insects and mites might have transported conidia and fragments of hyphae to those parts. In her report on *Fusarium solani* infection of shallot bulbs, Halm (1971) included photomicrographs of sections of the disc invaded by the fungus, *A. niger* and showed that invading hyphae could enter the bulb by the same route.

The colonies on the swollen leaf bases were not as wide spreading as those on the scale leaves (Plate 8). The conidiophores on the scale leaves were also longer than those on the swollen leaf bases, that is the hidden parts (Table 10). The conidiophores on both cultivars on the abaxial surface of the outer scale leaf were almost twice the length of conidiophores on the abaxial surface of the outermost fleshy leaf base. Greater development on the scale leaves was certainly due to the higher levels of oxygen in that micro-habitat. There is no information in the pertinent literature to suggest the role of substances from the different organs in the growth of the conidiophores. Conidiophores on the Pink cultivar were approximately 2/3rd the length of those on Pale-brown cultivar. Many substances are exuded by leaves through the cuticle including cations such as calcium, magnesium, potassium, sodium; amino acids; ammonia; free sugars; pectic substances; organic acids; sugar alcohols; vitamins and growth-regulating substances (gibberellin) (Preece and Dickinson, 1971). The shorter length of conidiophores on the pink cultivar suggests that the exudate of that cultivar restrained growth.

Many plant pathogenic fungi produce cutinolytic enzymes (Albersheim, *et. al.*, 1969; Brown, 1965; Van den Ende and Linskens,1974). Products of decomposed cuticle are added to the exudates augmenting both stimulatory and toxic substances in the exudates. Ensuing higher level of nutrients will promote fungal growth and higher levels of toxic compounds will inhibit growth. The ability of *A. niger* to decompose the cuticular layers of the bulbs of the pale-brown and pink cultivars should be examined in any future studies to provide information that would explain the lesser growth on the pink cultivar.

In addition, such a study will explain why *A. niger* was unable to attack the bulbs in the surface-inoculation tests (Table 37). Hashioka *et al* (1967) reported that germ tubes and appressoria of *Pyricularia oryzae* form liquid droplets, and the cutin just beneath them was dissolved. Also, the cuticle of apple fruits breaks down just at the penetration point of *Venturia inagegualis*.

Nicholson *et al*. (1972) demonstrated the presence of a membrane bound 'infections sac' in contact with the host plant cuticle at the penetration point. The present study did not extend to these details but could form the basis for future studies.

In general, the shorter the conidiophore the smaller the vesicle and the fewer the number of sterigmata that will be require to cover the vesicle, thereby reducing the productiveness of the conidiophores. The Pink cultivar under this circumstances will prove useful in limiting the amount of infective conidia that will be released into the ecosystem.

The data in Table 43 to 47 concern another aspects of pathogenesis of *A. niger* . Experimentally wound inoculated bulbs of both cultivars using the five isolates of *A. niger* and incubated at 50-100% R.H. were rotted to varying degrees depending on the storage humidity. The isolates produced conidiophores on the rotted tissues at 80 - 100 % R. H. only, no doubt determined by available moisture. The conidiophores in all tests and at corresponding relative humidities were shorter on the pink cultivar bulbs than the pale-brown cultivars.

It was not surprising that there were longer conidiophores on the intact structures of the bulb (Table 10) and shorter conidiophores on the rotted tissues (Tables 43 to 47). The probability that factors in infected tissues could reduce the quality of the substratum has been suggested by some workers. Cole and Wood (1961), for example, found that there were more compounds in apple fruits rotted by *Botrytis cinerea*, *Penicillium expansum*, *Pyrenochaeta furfuracea* and *Sclerotina fructigena* than in uninfected apples. There were also greater number of these compounds in extracts of apple rotted by *P. expansum* and *P. furfuracea* than in extracts of rots produced by *B. cinerea.*

A. niger conidia did not germinate in distilled water (Tables 12 - 31). This confirms pertinent findings of earlier workers (e.g. Amewowor 1980; Kesse, 1995). Amewowor (1980) and Kesse (1995) could not obtain *A. niger* conidium germination even in complex media which could be judged to be of very low concentration. Amewowor (1980) prepared root exudates of 5-day old seedlings of bambara groundnut (*Voandzeia subterranean* Thouars) analysed chemically to identify the component amino acid and ammonia, and determine their concentrations. The amino acids were alanine, ammonia, aspartic acid, glutamic acid, glycine, histidine, iso-leucine, leucine, lysine, serine, threonine, tryptophan and valine of the respective concentrations of 0.015, 0.004, 0.021, 0.074, 0.029, 0.013, 0.002, 0.004, 0.002, 0.036, 0.007, 0.025, and 0.004 μmols/ml.The conidia did not germinate in this complex medium.

Kesse (1995) also obtained no germination of *A. niger* conidia in solution of 0.1 per cent peptone, 1.0 percent dextrose, 1.0 per cent sucrose and exudates of grains of three maize cultivars - Mixed white, Obaatanpa and Yellow.

The two shallot cultivars will apparently not contribute to conidium germination on the bulbs under normal conditions under the influence of the exudates. If the metabolism of the plant organ is likely to be related to the composition of the exudate, this is not manifested in the shallot bulbs. The data in Tables 3 to 8 indicate significantly higher levels of the chemical substances (Calcium, Magnesium, Nitrogen, Phosphorus, Potassium and Sodium) in the Pale-brown cultivar bulbs. Citing three important examples, the mean respective percentages for the Pale-brown and Pink cultivars shown in Table 8 were 1.456 ± 0.07 and 1.192 ± 0.05 for nitrogen, 0.406 ± 0.01 and 0.367 ± 0.01 for phosphorus and 1.142 ± 0.03 and 0.979 ± 0.08 for potassium. Present data cannot explain why both did not influence conidium germination in distilled water droplets. Future studies should endeavour to identify the constituents of the exudates of the scale leaves and swollen leaf bases. The results of such investigations may also help to explain the consistent higher conidium germination and longer germ-tubes on the scale leaves.

Nutrient droplets splash on the bulbs must be of significantly high concentration in order to be able to stimulate satisfactory germination of any *A. niger* conidia on the bulbs. For, good germination occurred only in the 1:10 and 1:20 dilutions of Potato Dextrose Broth (Tables 12-16), undiluted and 1:1 dilution of Bean Meal Broth and Sweet Potato Broth (Tables 79 to 81), and 5.0 and 10.0g/l solutions of galactose, glucose and sucrose (Tables 17 to 31). Percentage conidium germination in these media were accompanied by vigorous germ-tube growth. The germination percentages recorded on the Pale-brown and Pink cultivars were closely similar, showing no recognizable trend. On some occasions, the percentage germination and mean germ-tube length were slightly higher on the Pale-brown cultivar, and on other occasions it was the reverse.

The conidia will play a significant role in infection in the market stalls if they settle in wounds, and so will the mycelium, for, the conidia germinated extremely well in extracted fluid of the bulbs (Table 32). The mycelium also failed to incite infection in bulbs which were surface-inoculated (see Table 37) while the bulbs were readily invaded by the mycelium following wound inoculation (Tables 37 to 42 and Plate 10). Conidia of *A. niger* Isolate 1, for example, also attained 100 and 93.5 per cent germination, respectively in the fluids of Pale-brown and Pink cultivars. The corresponding germination percentages by Isolates 2, 4 and 5 were 100 and 100 percent (Tables 33, 35 and 36), and for Isolate 3 were 97.2 and 87.4 per cent (Table 34). With reference to the diluted fluids, lower percentage germination was obtained with the fluids of the Pink cultivar. This is an indication that the Pink would be less vulnerable to *A. niger* attack.

Crops have their pests as well, which create the wounds that serve as entrances for pathogens. Onions and shallots have common pests. Onion trips (*Thrips tabaci* Lindeman) are the principal insect pests of onions and shallots (Hill, 1983; Hill and Waller, 1988).They are polyphagous and cosmopolitan pests of onions and shallots and many other vegetables. Both the nymphs and adults penetrate the epidermis of leaves and swollen leaf bases, and suck the sap that exudes. These insects, which measure about 1.0mm in length, hide during daytime in between the leaf bases. The cotton leaf worm, *Spodoptera littoralis* Boisd, is another polyphagous pest attacking a wide range of crops. The larvae feed on the leaves and severe attacks can cause reduction in bulb weight and size (Hill, 1983; Hill and Waller, 1988).The bulb nematode, *Ditylenchus dipsaci* invades the leaf bases of seedlings and older plants which swell up with loosening of the cells. The disease is aptly called “bloat” because of this symptom (Hill and Waller, 1988).

A. niger introduced into the wounds creaked by these invertebrates will invade bulbs under fairly wide range of relative humidity with invasion proceeding faster in the Pale-brown cultivar bulbs as the data in Tables 38 to 42. *A. niger* is a notable member of the xerophilic species which are prominent among storage fungi in “dry seeds and grains”. It was not surprising, therefore, that it invaded the bulbs at the low relatice humidity of 50% R.H. The relative humidities which permit growth of some prevailing storage fungi are 65% for *Aspergillius halophilicus*, 70-73% for *Aspergillus restrictus* and *Aspergillus repens,* 80% for *Aspergillus candidus* and *Aspergillus ochraceus*, and 85% for *Aspergillus flavus* (Christensen, 1973).

Growth of *A. niger* from 100%R.H down to 50%R.H. will inflict considerable loss on products in storage and will limit productivity. Damage by *A. niger* was accentuated at 80 to 100% R.H. where the fungus went further to sporulate and to contribute to its survival. Kesse (1995) had earlier also recorded the following mean culture diameters: 58.0±1.3, 60.7±0.4, 62.0±0.8, 63.0±0.9, 63.3±0.2 and 63.0± 0.8mm on maize meal agar plate plates in 7days at 62.6, 65.0, 73.4, 85.2, 92.8 and 100% R.H., respectively. The fungus in the rotted shallot bulb tissue in this investigation was unable to sporulate at 50 to 70% due naturally to deficient water supply. Tests were not made below 50% R.H. to establish the lower percentage relative humidity limit. This should be studied in a future research as it will be of practical value.

The seemingly limitless profligacy of fungi in the production of spores is impressive. Of important, also, is the development of mechanisms or devices that serve to provide maximum distribution of these spores. In some species of fungi special mechanisms are lacking, and hence distribution appears to be largely fortuitous. This might appear to be the case in the dispersal of *A. niger* conidia. The dispersal can be considered successful if the spore lands on a suitable substratum in a viable condition.

The conidia of *A. niger* are long-lived. In a longevity test which employed conidia of *A. niger* isolates 1, 3 and 5 formed by mycelia growing on ground bulb tissue of Pale-brown and Pink cultivars, at least 43.8% (Appendix H), 42.1% (Table Appendix O) and 32.3% (Appendix V) of the conidia were viable after storage for 200 days at the best storage humidity of 100% R.H. The conidia were stored at zero to 100% R.H. at 32±2ºC under day\night light cycle. They died quickest at zero to 30% R.H. (Appendix N 94 and U) and live longest at the higher humidities of 70 to 100% R.H.(Appendix H and O).

A. niger conidia exhibited one of the many ways in which fungal spores respond to storage humidity. Other species whose spores have been found to be killed more rapidly by low humidities include *Phytophthora infestans* (Glendenning *et. al*., 1963). *Phytopthora meadii* (Pereis and Fernado, 1966) and *Trachysphaera fructigena* (Maramba and Clerk, 1974). In this group of fungi, the desiccation of the spores may disrupt the essential minimal metabolic processes which sustain life of the spore. Gottlieb (1966) wrote, "The one general physiological characteristic that separates the spore phase from the vegetative phase of a fungus is the low metabolic activity of spores compared with that of mature hyphae. Yet, some respiration occurs even in resting spores and their weight decreases, even synthesis may occur though at a very low rate. This low-level metabolism in resting spores keeps the metabolic machinery in repair".

As the *A. niger* conidia live long, their concentration in the air rises because there is a continuous production by bulbs in the stores. The high concentration of the living conidia increases the incidence of ontomycosis of the outer ear of the workers in that environment. In order to safeguard their health, regular monitoring of the levels of the airspora and the disinfection of the premises when necessary should be adopted. Such monitoring has been done under similar circumstance. For example, Gregory *et. al*.(1953) were concerned with concentration of basidiospores of the dry rot fungus (*Merulius lacrymans*) in the air of buildings, and, Lacey and Lacey (1964) worked on spores, generated by saprophytic fungi of moulding hay, in the air of farm buildings.

A phenomenon, termed Iterative germination, in *Aspergillus niger* was described by Anderson and Smith (1971). The term is used to describe those spores that produce the sporogenous apparatus and do not produce any mycelium. This definition serves to distinguish a morphological variation from the usual microcycle conidia where hyphae, considerably reduced are produced. This phenomenon has been seen in diverse species such as, *Penicillum digitatum* (Zeidler and Margalith, 1973), *Neurospora crassa* (Cortat and Turian, 1974), *Penicillium italicum* (Van Gertel, 1983) and *Zoophthora radicans* (Van Roermund *et al*., 1984). The process is induced by a heat period and other specific cultural conditions. Iterative germination was induced in *A. niger* conidia exposed to 44^{o}C for 48 hours (Smith and Anderson, 1973). The phenomenon occurs under conditions too severe for normal vegetative growth and thereby reinforces the survival value derived from the sporulation process. This potential should be investigated in *A. niger* conidia formed on bulbs of the two shallots cultivars in future studies which may be related to the persistence of the fungus in the ecosystem.

Substrates for microbial growth in soils vary not only in kind but also in amount and this variability affects some groups of microbes more than others. Warcup (1967) pointed out that, a source of energy and nutrients sufficient for the growth and multiplication of a bacterium may be barely adequate for a germinating fungus spore and quite inadequate to sustain extensive mycelial development.

The growth of *A. niger* on so many parts of the shallot bulb, indicated in Table 9 and in Plate 8, including effectively protected areas suggested a close association that could have begun in the soil where *A. niger* is ever present. In their manual *'A manual of the Aspergilli'*, Thom and Raper (1945) stated that, *Aspergillus niger*, the black *aspergerlli* are abundant in all soils examined, and from studies which have been made by the authors and other investigators, it would appear that they are particularly abundant in soils from tropical and sub-tropical areas'. In this research work every sample of non-rhizosphere soil contained *A. niger*. It was really the level of the *A. niger* population in the non-rhizosphere by which the effects of exudates of the plants, and, applied fertilizers could be assessed. It is well known that if the exudates are stimulating, the population close to the plant will increase.

The results of the investigation indicated that, in unamended Anloga farm soils and unamended soils of non-alliaceous farms at Legon, the region around the bulb and roots contained stimulatory substances which increased growth and multiplication of *A. niger* (see Tables 60, 65, 72, 73 and 74). For example the *A. niger* population in the unamended Anloga farm soil planted with the Pale-brown shallot cultivar of 4.0 x 10^3 C.F.U's /g of soil in the non-rhizosphere soil rose to 27.3 x 10^3 C.F.U's/g of soil around the young bulb and 13.0 x 10^3 C.F.U's/ml around the roots in 20 days (Table 60). In the cassava farm soil, from 4.7 x 10^3 to 43.3 x 10^3 C.F.U's/g of soil around the bulbs and only 5.7 x 10^3 C.F.U's/g of soil around the roots (Table 72).

The response of *A. niger* to the shallot plants fall into two categories represented by the examples cited above.

a. There was stimulation of *A. niger* populations around both the bulb and the roots, but significantly higher around the bulbs.
b. Stimulation was very high around the bulb and very negligible around the roots. This event concerned both cultivars and hence influenced, similarly, colonization of the bulbs of both cultivars. The results can only be fully explained if the nature and quantity of the exudates from the bulbs and roots are known.

Table 59 shows that the plants did not seem to benefit from the urea fertilizer at the concentrations used judging from the sizes of the bulbs after 40 and 60 days. At least, the urea concentration was adequate. For in a preliminary test, urea concentration beyond 3.88g per 1200g of soil killed the plants. Woldetsadik (2003) observed that the nitrogen fertilizers, either ammonium nitrate or ammonium sulphate or urea at rates of 50-200kg /ha to rain-fed shallot plants (DZ- Sht- 78", " Dz- Sht -91" and "Fedis" cultivars) in Ethiopia, induced more vegetative growth, delayed maturing and reduced bulblet sizes. He could, however, obtain marketable bulbs per plant when he added supplemental irrigation. It sees advisable to consider nitrogen fertilization on the basis of soil fertility and moisture availability.

In contrast, potassium fertilization had a favourable effect on the shallot plants of both cultivars. Thus, at 20 days, the mean lengths of bulbs of 0.00 g/l potassium treatment of the Pale –brown and Pink cultivars were 25.5±1.3 and 24.3±2.1 mm, respectively. The corresponding values for the bulbs at the optimum potassium concentration of 6.67g/l were 31.2±1.3 and 27.7±1.1mm, respectively. The mean widths of the bulbs at 20 days for 0.00 g/l of potassium concentration were 7.8±0.3 and 6.6±2.1mm were respectively and for the 6.67g/l potassium treatment were 10.5±0.5 and 10.0±0.6mm, respectively (see Table 64).

The more important reason for applying the urea and potassium fertilizers was to determine their effect on infection of bulbs by *A. niger*. All plant parasitic diseases are the result of the interaction of a virulent pathogen, a favourable environment, genetic constitution, light, moisture, nutrients, non-nutrients chemicals, pH, and temperature which determine the growth of plants and affect their susceptibility to disease. These factors affect form and rate of growth of plants, chemical composition of the cell walls, distribution of water within the plant, rates of metabolism, and, production of metabolites.

The pertinent literature contains extensive information on the effects of nutrients on disease (e.g. Hesterberg and Jurgensen, 1972; Yarwood 1976). Heavy generalized fertilization, for example, usually increases disease, especially those caused by powdery mildews and rusts. Of the separate major nutrients, nitrogen usually increases disease incidense. Potassium may decrease it, and phosphorus is intermediate. With both fertilizers, inoculated bulbs of both shallot cultivars were rotted by two test *A. niger* isolates at 50- 100% R.H. The diameters of the rotted tissues at each incubation relative humidity were greater in bulbs of the pale-brown cultivar than the pink cultivar bulbs (Tables 64 and 71). The addition of fertilizers did not change the results of the previous experiments. The Pale-brown cultivar apparently became more susceptible with the potassium fertilization than the Pink cultivar. It must be emphasized that the fertilizers did not impose significant resistance to *A. niger*. A fact that the results suggest is that, urea, and potassium treatment especially, were good for both shallot plants (Table 64) and the fungus. From the point of view of agricultural productivity, the major objective should be predisposition to resistance with no loss in crop productivity.

In conclusion, both the Pale-brown and Pink shallot cultivars are not resistant to *A. niger* and that condition was not attained by fertilizer application. The two cultivars supported abundant sporulation by *A. niger* and the conidia produced had high germination capacity and they equally lived long. Nevertheless, it will be reasonable to suggest that because of the slightly lesser level of natural infection, depressed conidiophore development, lesser support of conidium germination, slower rate of rot of bulbs by *A. niger* growth, and lesser stimulation of *A. niger* growth in the phyllosphere and rhizosphere, the pink cultivar should be considered the preferable choice for the shallot industry, and, for future shallot programmes.

RECOMMENDATIONS

a. On the basis of the conclusion of the Discussion, the Pink cultivar should be adopted for expanded cultivation of shallot.

b. The Pink cultivar should be adopted for shallot breeding programmes to produce improved stock.

c. Crop rotation should be adopted in shallot farms to prevent building up of *A. niger* inoculum in the soils due to inevitable stimulation of *A. niger* by the exudates of the bulbs and roots.

d. The normal humidity in the shallot stalls will support sporulation of contaminant *A. niger*. The load of these long-lived spores will build up to the detriment of the health of the handlers. Regular disinfection of the atmosphere of the stores should be carried out to destroy the air-borne living conidia which cause ontomycosis.

e. The appropriate, amount of fertilizer soil-moisture level should be determined through trials to improve shallot productivity and minimize *A. niger* colonization.

f. Planting materials should be disinfected to prevent inoculation of the soil

g. There should be careful selection of crops for interplanting to avoid using alternative host plants or plants with exudates which are very favourable to *A. niger* such as bambara groundnut, groundnut and tomato

h. Monitor levels of invertebrate pests that feed on the bulbs and create wounds in both the field and store houses and spray appropriate pesticides when they reach dangerous threshold.

i. The *A. niger* attack was so pervasive and the infection of even the Pink cultivar is so high that its adoption for expanded cultivation is not likely to yield much appreciable relief. The District Assembly of Anloga should organize and manage any expansion of the cultivation of the crop and establish a shallot pickling industry to preserve the harvested product and to transform a local trade into an export enterprise.

VI. SUMMARY

1. Two cultivars of shallot (*Allium ascalonicum*), namely, Pale-brown and Pink, used in this investigation are named for the pigmentation of their scale leaves.

2. Both cultivars are commercially cultivated in Anloga, a coastal district in the Volta Region, and bulbs used in the experiments were purchased periodically from Anloga.

3. The bulbs of the Pale-brown cultivar were larger, with a mean length of 3.62cm, a mean width of 2.91cm and a mean fresh weight of 8.79 g.

4. The smaller bulbs of the Pink cultivar had a mean length of 3.26cm, a mean width of 2.08cm and a mean fresh weight of 5.98g.

5. The growing plants of the Pale-brown cultivar in the field were larger, greener and more lush with longer, averagely 40.96cm long and averagely 5.38mm wide hollow cylindrical leaves.

6. The hollow cylindrical leaves of the Pink cultivar in the field were smaller in the, Pale green and averagely 38.67cm long and averagely 4.84mm wide.

7. Chemical analysis showed higher concentrations of calcium, magnesium, nitrogen, phosphorus, potassium and sodium in bulbs of the Pale-brown than in those of the Pink cultivar.

8. The abaxial and adaxial surfaces of the outer scale leaves, abaxial and adaxial surfaces of the inner scale leaves, abaxial surfaces of the outermost fleshy leaf base and the contact surfaces of the mini-bulbs of not less than 64% of bulbs of both cultivars in stores were extensively colonized by *A. niger*.

9. In bulbs of the pale-brown cultivar, *A. niger* colonies at the abaxial surface of the outer scale leaf, abaxial surface of the outermost fleshy leaf base and the contact surfaces of the mini-bulb bore 2,124. 1,855.3, 1125.8 and 1524.6 µm long conidiophores, respectively.

10. The corresponding sites of infection of bulbs of the Pink cultivar bore significantly shorter conidiophores of 1,720.5, 1,480.3, 799.8 and 1,000.8 µm, respectively.

11. Five distinctively identifiable *Aspergillus niger* isolates, designated, Isolate 1, Isolate 2, Isolate 3, Isolate 4 and Isolate 5, growing on naturally infected shallot bulbs were selected and used in all the tests of the investigation.

12. The five *A. niger* Isolates were employed to provide, in particular, a spectrum of response in the tests and not to compare the potential of the isolates.

13. The experiments were carried out, in the absence of incubators in the laboratory, at room temperature of 32±2ºC and under normal day-night light conditions.

14. *A. niger* conidia did not germinate in drops of distilled water on glass slides, and on parts of the bulbs.

15. Any exudate that might have been released by the scale leaves and swollen leaf bases of the bulbs of both shallot cultivars did not also induce germination of the conidia of the five *A. niger* isolates in drops of very dilute (1:40) Potato Dextrose Broth except conidia of Isolate 1 which germinated tardily (1.0 – 3.9 per cent).

16. Conidium percentage germination declined with increasing Potato Dextrose Broth dilution on the different parts of the bulbs. Taken together, the highest germination percentages recorded were:
    a. 92.4 and 88.4 percent in 1:10 dilution on pale-brown and pink cultivars, respectively.
    b. 73.7 and 68.8 per cent in 1:20 dilution on pale-brown and pink cultivars, respectively.
    c. 68.8 and 47.4 per cent in 1:30 dilution on pale-brown and pink cultivars, respectively.

17. Percentage germination was mostly higher in Potato Dextrose Broth suspension drops on parts of the Pale-brown cultivar bulbs than on the Pink cultivar bulbs.

18. Germ-tubes also grew longer in the Potato Dextrose Broth suspension drops on the pale-brown cultivar than in corresponding drops on the pink cultivar bulbs.

19. Germination of conidia of the five *A. niger* isolates was far better on scale leaves than on the swollen leaf bases.

20. Conidium germination on the scale leaves and swollen leaf bases of the two shallot cultivars, in suspension drops of galactose, glucose and sucrose of concentrations of 2.5, 5.0 and 10.0 g/l followed the same trend which were recorded in the Potato Dextrose media. However, the levels of percentage germination were considerably lower. A concentration of 1.25g/ l of all the three sugars did not support germination.

21. Considering all the tests together, the highest percentage of conidia of the five *A. nige*r isolates in the three series of tests were:
    a. 51.3 and 51.2 per cent in 2.5 g/l media on pale-brown and pink cultivars, respectively.
    b. 43.5 and 43.1 per cent in 5.0 g/l media on pale-brown and pink cultivars, respectively.
    c. 25.2 and 28.2 per cent in 10.0 g/l media on pale-brown and pink cultivars, respectively.

22. The different effects of the shallot cultivars on *A. niger* were also shown by conidium germination in aqueous extracts of the bulbs, tested at concentrations S (undiluted) and

at dilutions of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$. The differences in the influence of the extracts were manifested at particularly the lower concentrations. At $\frac{1}{8}$ and $\frac{1}{16}$ dilutions, germination in the extracts of the bulbs of the pink cultivar were much lower than that of the pale-brown cultivar.

Respective conidium germination percentages in the pale-brown cultivar and pink cultivar extracts by,

a. Isolate 1 at dilution-

$\frac{1}{8}$ were 73.3 and 64.2 per cent.

$\frac{1}{16}$ were 78.4 and 28.7 per cent.

b. Isolate 2 at dilution

$\frac{1}{8}$ were 100 and 93.3 per cent.

$\frac{1}{16}$ were 96.6 and 90.9 per cent.

c. Isolate 3 at dilution

$\frac{1}{8}$ were 91.2 and 89.5 per cent.

$\frac{1}{16}$ were 90.3 and 74.1 per cent.

d. Isolate 4 at dilution

$\frac{1}{8}$ were 100 and 92.3 per cent.

$\frac{1}{16}$ were 87.7 and 64.4 per cent.

e. Isolate 5 at dilution

$\frac{1}{8}$ were 100 and 96.0 per cent.

$\frac{1}{16}$ were at 77.5 and 47.7 per cent.

23. But vegetative growth response showed a reverse trend. Statistically significant mycelial dry weight was recorded in aqueous extract of bulbs of the Pink cultivars than in that of the Pale-brown cultivar in all the five *A. niger* Isolates.

24. The germinated conidia, especially those at the higher concentrations of the media, produced long germ-tubes which grew in all directions on the surface of the scale leaves and the swollen leaf bases. They did not enter the stomata and there was no direct penetration of the epidermal cells.

25. Growing *A. niger* isolates altered markedly the pH of the medium.
    a. Initial pH 6.99 of Cassava Dextrose Broth drifted to a final pH 3.04.
    b. Initial pH 6.47 of Potato Dextrose Broth drifted to a final pH 3.00.
    c. Initial pH 6.91 of Bean Meal Extract drifted to a final pH 3.93
    d. Initial pH 6.81 of Sweet Potato Dextrose Broth drifted to a final pH 3.23.
    e. Initial pH 6.55 of Oat Meal Extract drifted to a final pH 3.14

26. The mycelium of 3mm culture disc of *A. niger* placed on surface-sterilized bulbs of the two shallot cultivars and held at 100% R.H. withered and did not infect the bulbs.

27. Wound-inoculated bulbs of the two shallot cultivars with 3mm culture disc of *A. niger* were rotted by the fungus at 50-100% R.H.

28. Rate of developments of rot was greatest at 100% R.H. and decreased with decreasing storage relative humidity.

29. Humidities of 50-100% R.H. affected invasion of the bulbs of the two shallot cultivars in many specific ways.

   a. The surface diameters of the rotted tissues were greatest at 100% R.H.
   b. The bulbs were rotted at all the relative humidities.
   c. After 10 days, the rotted tissues were greatest at 100% R.H.
   d. Mycelium in the rotted tissues of bulbs at 80 to 100% R.H. produced conidiophores and conidia by the 10^{th} day after inoculation.
   e. The conidiophores were longest at 100% R.H. and shortest at 80% R.H.

30. With regard to shallot cultivar- *A. niger* relationship:

   a. The rate of rotting was faster in bulbs of the pale-brown cultivar.
   b. The mean diameters of the rots caused by *A. niger* Isolates 1, 2, 3, 4, and 5 in bulbs of the pale-brown cultivar held at 100% R.H. were 12.1, 12.3, 12.8, 10.4 and 12.6 mm, respectively, and of the pink cultivar, 10.4, 10.3, 11.0, 7.9 and 11.3, respectively.
   c. Mean lengths of the conidiophores formed on the diseased tissues at 100, 90 and 80% R.H. on the Pale-brown cultivar bulbs were 702.1, 653.4 and 610.0µm, respectively, and on the pink cultivar were 664.3, 627.8 and 601.5µm, respectively.
   d. Sporulation density at each % R.H. was the same on bulbs of both cultivars.

31. Plants of both cultivars of shallot had a definite effect on fungi in the neighbourhood of the bulbs and the roots.

32. The soil of Anloga shallot farms was very sandy and contain very low humus content of 3. 48%.

33. Fungal genera occurring in phyllosphere, rhizosphere and non- rhizosphere soils were *Aspergillus, Fusarium, Penicillium* and *Rhizopus* with *Aspergillus* as the predominant genus in all the three soil zones.

34. *Aspergillus niger* was the most abundant *Aspergillus* species in all the three soil zones. For each type of soil it constituted more than half of the population.

35. *Aspergillus* species isolated from the phyllosphere and rhizosphere of both shallot cultivars were *A. flavus, A. niger* and *A.* t*erreus*.

36. Stimulation of the fungal species in the phyllosphere and rhizosphere of both shallot cultivars was maintained over 60 days.

37. *A. niger* was also the dominant fungus species in the similar studies using cassava, groundnut and maize farm soils at Legon.

38. The shallot plants and fungi around the bulbs and roots did not respond to addition of urea (1.94 and 3.88 g) to the soil of Anloga farms.

39. Addition of Potassium (3.33, 6.67. 10.00 and 13.33 g/l) increased sizes of the shallot plants and population in all the three zones of the soil. The optimum Potassium concentration was 6.67 g/l.

40. Bulbs of Pale-brown cultivar which grew in soils with added Urea and Potassium still rotted faster after wound-inoculation with *A. niger*.

41. Conidia of three selected *A. niger* Isolates 1, 3 and 5 which earlier had proved equally viable by attaining 100 percent germination in Potato Dextrose Broth and Sweet Potato Broth, also showed similar longevity.

42. The conidia survived longest at the higher humidities of 60- 100% R.H. and lost viability quickest at lower humidities of 0.0- 50 % R.H.

43. Conidia formed by mycelium on bulb tissue of the Pale-brown cultivar showed slightly higher percentage viability on each assessment occasion than those formed on tissues of the Pink cultivar.

44. Because of the many occasions on which the Pink cultivar depressed conidiation by suppressing length of conidiophores, depressed conidium germination, depressed length

of germ-tubes produced by germinated conidia, was less rapidly rotted following wound-inoculation, and was colonized to a lesser extent in storage by *A. niger*. It was concluded that the Pink cultivar should be adopted for expansion of shallot cultivation in Ghana and be regarded as a suitable cultivar for future shallot programmes in attempts to produce improve stock that would reduce *A. niger* threat to the shallot industry.

45. Among the many salient recommendations, the highly important proposition is the call on the Anloga District Assembly to establish a shallot pickling industry that will effectively preserve the harvested bulbs and transform a local trade into an export enterprise.

VII REFERENCES

ABARCA, M.L., M.R., BRAGULAT, G., SASTELLA and F.J. CABANES. (1994). Ochratoxin a production by strains of *Aspergillus flavus* var. *niger*. APPLIED ENVIRON. MICROBIOL., 60: 2650-2652.

ABBEY, L., R. KANTON and H. BRAIMAH. (1998). Susceptibility of shallots to the timing and severity of leaf damage. JOURNAL OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY 73, 803-805.

ABBIW, D.K. (1990). *Useful plants of Ghana, West African crops* of wild and *cultivated plants.* Intermediate Technology Publications and the Royal Botanic Gardens, Kew. 337pp.

ADOMAKO, P. 1959. Onion cultivation in Kusasi. Ghana Farmer 3(4): 129-131.

AL-ABDALALL, A.H.A., (2009). Production of aflatoxins by *Aspergillus flavus* and *Aspergillus niger* strains isolated from seeds of pulses. J. FOOD AGRIC. ENVIRON., 7: 33-39.

ALBERSHEIM, P., T.M. JONES and P.D. ENGLISH. (1969). Biochemistry of the cell wall in relation to infective processes. ANN REV. PHYTOPATHOL. 7, 171-194.

ALLEN, T. (1945). Some observations on the relationship of *Alternaria tenuis* to canine eczema. J.AMER. VET MD. ASS. 106, 163-164.

AMEWOWOR, D.H.A.K. (1980). Studies on rhizosphere fungal population of Bambara groundnut (*Voandzeia subterranean* Thouars) with special relation to the *Aspergilli* flora. Ph.D. Thesis. University of Ghana.

ANDERSON, J.G. and J.E. SMITH (1971). Synchronous initiation and maturation of *Aspergillus niger* codiophores. TRANS. BR. MYCOL. SOC. 56, 8-29

AUSTWICK, P.K.C. (1966). The role of spores in the allegies and mycoses of man and animals. In the Fungus Spores (1966) ed. M.F. Madelin, 321-337. Butterworths, London

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY-AOC. (1984). Official method of analysis of the Association of Analytical Chemistry, 14 ed. Artington. 1141pp.

BALDWIN and GOODMAN. (1963). Prevalence of *Erwinia amylovora* in apple buds as detected by phage typing. PHYTOPATHOLOGY 54, 1299-1303.

BARLOW, P.W. (1975).The roof cap. In J.G. Torrey and D.T. Clarkson, eds. *The Development and Function of roots*. Academic Press, London and New York.pp.21-54.

BATEMAN, D.F. and R.L.MILLAR,(1966). Pectic enzymes in tissue degradation. ANN. REV. PHYTOPATHOL. A, 119-146.

BENNETT, J.W., 1985. Molds, Manufacturing and Molecular Genetics. In: Molecular Genetics of Filamentous Fungi, Timberlake, W.E. (Ed.). Alan R. Liss Inc., New York.

BERJAK V. and O.P. ARYA. (1970). Otomycosis due to *Aspergillus niger*. EAST AFR MED J. 47: 247-53.

BIGELLIS, R. (1991). Fungal metabolites in food processing In ARORA, D., K.G. MUKERJI and E.H. MARTH, Eds. *Handbook of applied mycology*. Vol. 3. Foods and feeds. Marcel Dekker, Inc., New York.

BRAY, R.H. and L.T. KURTZ. (1945). Determination of total, organic, and available forms of phosphorus in soils. SOIL SCIENCE, 59: 39-45

BREWSTER, J. L. (1990). Physiology of crop growth and bulbing. In *Onions and allied crops*. Vol I (ed. H. D. Rabinowitch and J. Brewster). Baca Raton, Florida, CRC Press.

BROWN. W. (1965). Toxins and cell wall dissolving enzymes in relation to plant disease. ANN. REV. PHYTOPATHOL.3, 1-18.

CHANDLER J.R. (1968). Malignant external Otitis. LARYNGOSCOPE. 78: 1257-94

CHRISTENSEN, C.M. (1957).Deterioration of stored grains by fungi. BOTANY REVIEW, 23,108-134.

CHRISTENSEN, C.M. (1973). Loss of viability in storage microflora. SEED SCI. AND TECHNOL. 1, 547-562.

Clerk, G. C. (2012). Unpublished data (personal communication)

CLERK, G.C. (1974). *Crops and their diseases in Ghana*. Ghana Publishing Corp. 144pp, Accra.

COCHRANE, V.W. (1958). Physiology of fungi. John Wiley & Sons, Inc. New York. 124 pp.

COLE, M. and R.K.S. WOOD. (1961). Pectic enzymes and phenolic substances in apples rotted by Fungi. ANNALS OF BOTANY, N.S. Vol. 25, No. 100.

COMMONWEALTH MYCOLOGICAL INSTITUTE. (1966).

CORTAT, M. and G. TURIAN. (1974). Conidiation of *Neurospora crassa* in submerged culture without mycelial phase. ARCHIV FUR MICROBIOLOGIE. 85, 305-309.

CROSSE, J.E., R.N. GOODMAN and W.H. SHAFFER Jr. (1972). Leaf damage as a predisposing factor in the infection of apple shoots by *Erwinia amylovora* PHYTOPATHOLOGY 62, 176-182.

CURRAH, I. and F.G. PROCTOR.(1990).Onions in the Tropical Regions. Bulletin 35, Natural Resources, Institute, U.K.

CURTIS, R.W. (1958). Curvatures and malformations in bean plants caused by culture filtrate of *Aspergillus niger*. PLANT PHYSIOL. 33, 17-22.

de VIRES, H., BREDEMEIJER, G. and W. HEINEN. (1967).The decay of cutin and cuticular components by soil micro-organisms in their natural environment. *ACTA BOT. NEERL.* 16: 102-10

DILLON-WESTON, W.A.R. and E.T.HALMAN. (1932). Effect of ultraviolet radiation on theurediniospore of black stem rust, *Puccinia graminis tritici*. *SCI. AGR.* 12, 81-87.

DOWLER, W.M. and D.H. PETERSON. (1967). Transmission of *Pseudomonas syringae* in peach trees by bud propagation. PLANT DISEASE REPORTER 51, 666-668

DREW, M.C. (1979). Properties of roots which influence rates of absorption. In: J.L. Harley and R.S. Russell, eds. *The soil-root interface*. Academic Press, London, New York and San Francisco. pp. 21-23,

DUECK, J. and H.A. QUAME. (1973). Fireblight in Southern Ontario in 1972. CANADIAN PLANT DISEASE SURVEY. 53, 101-104.

EDWARDS, J.H. and T.S. AL-ZUBAIDY. (1977). Medical aspects. In J. E. Smith and J. A. Pateman, (eds.), Genetics and physiology of *Aspergillus*. Academic Press, NY.

EDWIN, R., I. PALENCIA, D.M. HINTON and C.W. BACON. (2010). The black *Aspergillus* species of maize and peanuts and their potential for mycotoxin production. TOXINS, 2: 399-416.

FARR, D.F., G.F. BILLS, G.P. CHAMURIS and A.Y. ROSSMAN. (1989). Fungi on Plants and Plant Products in the United States (APS Press, St. Paul, MN).

FELIPE, M.R., J.M. POLUEZO and A.M. CINTAS. (1979). Acid phosphatase localization at the surface of young corn roots. AGROCHIMICA 23,145-151.

FOSTER, R.C. (1982).The fine structure of epidermal cell mucilages of roots. NEW PHYTOL. 11, 727-740.

FOX R.T.V, J.G. MANNERS and A. MYERS. (1971). Ultrastructure of entry and spread of *Erwinia carotovora* var *atroseptica* into potato tubers. POTATO RES. 14, 61-73.

FOX R.T.V. (1972). The ultrastructure of potato tuber infected by soft rot organism *Erwinia carotovora* var *atroseptica*. In: Proc. 3rd Conf. Plant Pathogenic Bact. 95-120. (H.P. MASS GEESTRANUS). Wageningen: Pudoc.

GASSNER, G. and E. NIEMANN. (1955). Uber die Beeinflussung der sporenkeimung des Zwergsteinbrandes durch verschiedene Chemikalien. PHYTOPATH. Z. 23, 121-140.

GATT, S. and Y. BARENHOLZ. (1973). Enzymes of complex lipid metabolism. ANN. REV. BIOCHEM. 42, 61-90.

GLENDENNING, D., J.A. MACDONALD and J. GRAIGER. (1963). Factors affecting the germination of sporangia in *Phytophthora infestans*. TRANS. BRIT. MYCOL. SOC. 46, 311-609.

GOODMAN, R.N. (1976). Physiological and Cytological Aspects of the Bacterial Infection Process. In: *Physiological Plant Pathology*. Ed. HEITEFUSS, K and P.H. WILLIAMS. 172-193pp. SPRINGER-VERLAG BERLIN HEIDELBERG, NEWYORK.

GOTTLIEB, D. (1950).The physiology of spore germination in fungi. BOT. REV. XVI, 229-257.

GOTTLIEB, D. (1966). Biosynthetic processes in germinating spores. In M.F. MADELIN (ed.) *The Fungus Spore*. Butterworths. London.

GREENLAND, D.J. (1979). The physics and chemistry of the soil-root interface: some comments. In: J.L. Harley and R.S. Russell, eds. *The soil-root Interface.*_Academic Press, London, New York and San Francisco. pp. 83-98.

GREGORY, P.H., J.M. HIRST and F.T. LAST. (1953). Concentrations of basidiospores of the dry rot fungus (*Merulius lacrymans*) in the air buildings. ACTA ALLEG. KBH. 6, 168-174.

GRUBBEN, G.J.H. (1994). Constraints for shallot, garlic, and welsh onion in Indonesia: a case study on the evolution of *Allium* crops in the equatorial tropics. ACTA HORTICULTURAE 358, 333-340.

HAHNE, J. (1925). Utersuchungen Uber die Keimungsbedingungen von Tilletia-Spores. KUHN-ARCH. 9,157-163.

HALM, M. (1971). *Physiology and pathogenicity of Fusarium Solani* (Mart) Sacc*. causing bulb rot of shallot (Allium ascolanicum* Linn*.) with special reference to pre-penetration_phase*. M.Sc. Thesis. University of Ghana.

HASHIOKA, Y., H. IKEGAMI, O. HORINO and T. KAMEI. (1967). Fine structure of rice blast. II. Electromicrograhs of the initial infection. RES. BULL. FAC. AGR. GIFU UNIV. 24, 78-90.

HAWKER, L.E. (1950). *Physiology of Fungi.* University of London Press Ltd. Warwick Square, London.

HAYDEN, N.J. (1989). Observations on harvesting and storing onions in northern Sudan. ONION NEWSLETT. TROP., 1:19-23.

HESTERBERG, G.A. and M.F. JURGENSEN. (1972).The relation of forest fertilization to disease resistance. FORESTRY CHRUN.

HILL, D.S. (1983). Agricultural insect pests and their control. CAMBRIDGE UNIVERSITY PRESS, CAMBRIDGE.

HILL, D.S. and J.M. WALLER. (1988). *Pests and diseases of tropical crops. Field handbook*. Longman Group UK Ltd.

HINCH, J.M. and A.E. CLARKE. (1980). Adhesion of fungal zoospores to root surface is inediated by carbohydrate determinants of the root slime, PHYSIOL. PLANT PATHOL. 16, 303-308.

HORST, W.J., A. WAGNER and H. MARSCHNER. (1982). Mucilage protects root meristems from aluminium injury. Z. PFLNZENPHYSIOL. 105,435-444.

IRVING, H. R. and B.R.GRANT. (1984). The effects of pectin and plant root surface carbohydrates on encystment and development of *Phytophthora cinnamon* zoospores. J. GEN. MICROBIAL. 130, 1015-1018.

JENNY, H. and K. A. GROSSENBACHER. (1963). Root-soil boundary zone as seen in the electron microscope. PROC. SOIL SCI. SOC. AM. 27, 273-277.

JONES, H.H. and L.K. MANN. (1963). Onions and Allies. World Crop Inter. Sci. Publishers Inc. New York. Pp.286.

KERSHAW, K.A. (1973). *Quantitative and Dynamic Plant Ecology* (2nd Ed.). ELBS and Edward Arnold (Publishers) Ltd. 308 pp.

KESSE, R.E.O. (1995). *Studies on the Mycoflora of Grains of Maize (Zea mays L.) and the survival of the contaminant Aspergillus species.* M.Phil, Thesis, University of Ghana, Legon.

KHAN M.R., M. KIHARA, and A.D. OMOLOSO (2001). Antimicrobial activity of *Cassia alata*. Fitoterapia, 75(2), pp. 561-564

KING, K.W. and M.I. VESSAL. (1969). Enzymes of the cellulose complex. In: *cellulases and their Applications*. Advances in chemistry, Series No.95 p, 7-25. (R.F. GOULD, ed.), Wash. D.C: AMER. CHEM. SOC.

LACEY, J. and LACEY, M.E. (1964). Spore concentration in the air in farm buildings. TRANS. BR. MYCOL. SOC. 47, 547-55.

LOUTHRENOO, W., Y.S. PARK, L. PHILIPPE and H.R. SCHUMACHER. (1990). Localized peripheral calcium oxalate crystal deposition caused by *Aspergillus niger* infection. J. RHEUMATO., 17: 407-412.

LUCENTE F.E. (1993). Fungal infections of the external ear. OTOLARYNGOL CLIN NORTH AM. 26: 995-1006.

MAGAN, N. and S-LANCEY. (1987). The Monogragh. British Crop Protection Council. No. 37, 43-52. In: *STORED PRODUCT PEST CONTROL*. Proceedings of a symposium held at the University of Reading, pp. 25-27.

MAMBIAR, E.K.S. (1976). The uptake of zinc-65 by oats in relation to soil water content and root growth. AUST. J. SOIL RES.14, 67-74.

MANDOUL, R., AUREL, L. and L. LANTERNCO. (1954). La maladie de la canne de Provence on Algerie. Ball. Soc. PATH. EXOT. 47, 572-577.

MARAMBA, P. and G.C. CLERK. (1974). Survival of conidia of *Trachysphaera fructigena.* TRANS. BRIT. MYCOL. SOC. 63, 391-393.

MARTIN, J.K. (1977). Factors influencing the loss of organic carbon from wheat roots. SOIL BIOL. BIOCHEM – 9, 1-7.

MATSUBARA, H. and J. FEDER. (1970). Other bacterial, mold and yeast proteases. In: *The Enzymes. Vol. III. Hydrolysis: Peptide Bonds*. 3rd.ed. p.721-795. (P. D. Boyer ed.). New York. Academic Press.

MAY, G. and T. ADAMS. (1997). The Importance of fungi to Man. GENOME. RES., 7: 1041-1044. Mc MURRAY, W.E. and W.L. MAGEE (1972). Phospholipid metabolism. ANN. REV. BIOCHEM. 41, 129-160.

MESSIAEN, C.M., H. LOT and B. DELLECOLLE. (1994). Thirty years of France's experience in the production of disease-free galic and shallot mother bulbs. ACTA HORTICULTURAE 368, 275-279.

MIKI, N.K., K.J. CLARKE and M.E. McCULY. (1980). A histological and histochemical comparison of the mucilages on the root tips of several grasses. CAN.J.BOT. 58, 2581-2595.

MISLIVEC, P. B. and S.TUITE. (1970). Temperatures and relative humidities requirements of species of *Penicillium* isolated from yellow dent corn Kernels. MYCOLOGIA 62, 75-88.

MUGLISTON T. and G. O'DONOGHUE. (1985). Otomycosis a continuing problem. J. LARYNGOL OTOL. 99: 327-33.

NABOS, J. (1971). The onion in Niger. Ford Foundation/IITA/IRAT Seminar on Vegetable Crop Research, Ibadan.

NAKAGAWA, Y., K. SHIMAZU, M. EBIHARA and K. NAKAGAWA, (1999). *Aspergillus niger* pneumonia with fatal pulmonary oxalosis. J. INFECT. CHEMOTHER 5: 97-100.

NARUMO, A. and R.W. CURTIS (1961). Chemical studies on Malformin. PLANT PHYSIOL. 36, SUPPL. X111.

NESTER, E.W., C.E. ROBERTS, NANCY N. PEARSALL and B.J. McCARTHY. (1975). *Microbiology:* Holt, Rinechart and Winston., New York.

NICHOLSON, R.L., J. KUC and E.B. WILLIAMS. (1972). Histochemical demonstration of transistory esterase activity in *Venturia inaequalis*. PHYTOPATHOLOGY 62, 1242-1247.

NOONIMABE, P., W. MAHAKARNCHANAKULB, K.F. NIELSEND, J.C. by *Aspergillus niger* in Thai coffee beans. FOOD ADDIT. CONTAM. 26: 94-100.

NORMAN, J.C. (1992). *Tropical Vegetable Crops*. Arthur Stockwell Ltd. Devon

OADDES, J.M. (1978). Musilages at the root surface. *J. SOIL SCI.* 29, 1-16.

OSGUTHORPE J.D. and D.R. NIELSON. (2006). Otitis externa: review and clinical update. AM FAM PHYSICIAN. 74: 1510-6.

PATHAK, C.S. (1994). *Allium* improvement for the tropics: problems and AVRDC strategy. ACTA HORTICULTURAE 358, 23-32.

PEREIS, S.O. and J.M. FERNANDO. (1966). Studies on the biology of *Phytophthora meadii*. TRANS. BRIT. MYCOL. SOC. 49, 311-325.

PERFECT J.R., G.M. COX, J.Y. LEE, S.W. CHAPMAN and C.A. KAUFFMAN (2009). The impact of culture isolation of *Aspergillus* species. A hospital-based survey of aspergillosis. CLINICAL INFECTIOUS DISEASE 33, 1824-1833

PERRONE, G., A. SUSCA, G.COZZI, K. EHRLICH and J. VARGES. (2001). Biodiversity of *Aspergillus* species in some important agricultural products. STUDENTS MYCOLOGY, 59, 53-66.

PITT J.I. and A.D. HOPKING. (1997). *Fungi and food spoilage*. 2nd Edn., Springer, New York, 593.

POULOSE K.O., S. AL KHALIFA, P. SHENOY and R.K. SHARMA. (1989). Mycotic infection of the ear (otomycosis): a prospective study. J. LARYNGOL OTOL. 103: 30-5.

POSTLETHWAIT, S.N. and R.W. CURTIS (1959). Histology of malformations produced in bean plants by culture filtrate of *Aspergillus niger*. AMERICAN J. BOTANY 46, 31-35.

PREECE, T.F. and C.H. DICKSON. (1971). *Ecology of Leaf Surface Micro-organism*s. London/ New York. Academic.

PREININGER, T. (1937-38). Dutch Maisbrand (*Ustilago maydis*) bedingte Dermatomykose. ARCH, DERM. SYPH.1769 109-113.

REESE, E.T. (1963). Advances in enzymatic Hydrolysis of cellulose and related Materials. New York: Pergamon Press

REID, C.P.P. and G.D. BOWEN. (1979). Effects of soil moisture on V/A mycorrhiza formation and root development in Medicago. In: J.L. Harley and R. Scott- Russell. eds. The *Soil-Root Interface*. Academic Press, London, New York and San Francisco. pp. 211-221.

ROMBOUTS, F.M. and W. PILNIK. (1972). Research on pectin depolymerases in the Sixties-a literature review. Chem. Rubb. Comp. Crit. Rev. FOOD TECHNOL.3, 1-26.

ROTEM, J. and H. J. AUST. (1991).The effect of ultraviolet and solar radiation and temperature on survival of fungal propagules. JOURNAL OF PL. PATHOLOGY. 133, 76-78.

ROUGIER, M. (1981). Secretory activity of the root cap. In: W. Tanner and F.A. Loewus, eds. *Encyclopedia of Plant Physiology. New Series. Plant Carbohydrates II* Vol.138. Springer Verlag. Berlin. pp. 542-574.

ROUGIER, M. and A. CHABOUD. (1985). Mucilages secreted by roots and their biological function. ISREAL J. BOT. 31, 129-146.

ROVIRA, A.D, G.D. BOWEN and R.C. FOSTER. (1983). The significance of Rhizophere microflora and mycorrhizas in plant nutrition. In: A. Lauchli and R.L. Bieleski,eds. *Plant Inorganic Nutrition. Encyclopedia of Plant Physiology,* New Series. Vol. 158. Springer Verlag, Berlin. pp. 61-93.

RUINEN, L. (1966). The phyllosphere IV Cuticle decomposition by micro-organisms in the phyllosphere. ANN. INST. PASTEUR 111:342-46.

SCHUSTER, E., N. DUNN-COLEMAN., J.C. FRISVAD and P.W. VAN DIJCK, (2002). On the safety of *Aspergillus niger*: A review. APPLIED MICROBIOL. BIOTECHNOL, 59: 426-435.

SINNADURAI, S. and J.F. ABU. (1977). Onion farming in Ghana. ECONOMIC BOTANY. 31, 312-314.

SITHOLEY R.V. (1971). Observations on the three dimensional structure of the leaf cuticle in certain plants. ANN. BOT. London 35: 637-39

SMALLEY, E.B. and H.N. HANSEN. (1962). *Penicillin* decay of garlic. PHYTOPATHOL. 52, 666-678.

SMITH, E.L. (1960). Peptide bond cleavage (survey). In: *The Enzymes*, vol. IV. p. 1-10 (P.D. Boyer, H. LARDY and K. Myrback, eds). New York: Academic Press.

SMITH, J.E. and J.G. ANDERSON. (1973). Differentiation in the *Aspergilli*. SYMPOSIUM SOCIETY OF GENERAL MICROBIOLOGY. 23, 295-337.

SNEH, B., L. BURPEE and A. OGOSHI. (1991). Identification of Rhizoctonia species. 1st Edn., APS Press, St. Paul, Minnesotta, USA., pp: 133.

SOITOUT, M. (1969). IRAT Research on vegetables. L'Agronomie Tropicale. Vol. XXIV (4): 1-24.

SOLOMON, M.E, (1952). Control of humidity with Potassium hydroxide, Sulphuric acid and other solutions. BULL. ENT. RES. 42, 543-544.

STAIANO, M., P. BAZZICALUPO, M. ROSSI and S. DAURIA. (2005). Glucose biosensors as models for the development of advanced protein-based biosensors. MOL. BIOSYST., 1: 354-362.

SUMIATI, E. (1994). Response of shallot and garlic to different altitudes. ACTA HORTICULTURAE 358, 395-400.

TAKAHASHI, M. and R.W. CURTIS. (1961). Isolation and characterization of malformin. PLANT PHYSIOL. 36, 30-36

TEYE, M, M. (1994). *Studies on the mycoflora of okra (Abelmoschus esculentus L.* Moerich*), Onion (Allium cepa L,* var. *cepa), pepper (Capsicum annuum L.) and Tomato (Lycopersicum esculentum* Mill*) plant and their stored products.* Ph.D. Thesis, University of Ghana, Legon.

TEYEGAGA, A. and G.C. CLERK, (1972). Germination and survival of conidia of *Cercospora canescens* Ellis et Martin. TROP. AGRIC. 46, 197-204.

THAN K.M., K.S. NAING and M. MIN. (1980). Otomycosis in Burma and its treatment. AM J TROP MED HYG .29:620-3

THOM, C. and RAPER, K.B. (1945). Manual of the *Aspergilli*. Williams and Wilkins, Baltimore.

TURCHENEK, L.W. and J.M OADES. (1976). Organo-clay particles in soils. In: W.W. Emerson and A.R. Dexter, eds. Modification of Soil Structure. Wiley, Chichester. pp.137-144.

TORTOE C. (1997). *Studies on some physiological characteristics and pathogenicity of Sclerotium rofsii Sacc causing colar rot of Cocoyam, Colocassia and Xanthosoma species in Ghana*. M. Phil Thesis. University of Ghana, Legon.

UMALII-GARCIA, M., D.H. HUBBELL, M.H. GASKINS and F.B. DAZZO. (1980). Association of *Azospirillum* with grass roots. APPL. ENVIRON. MCROBIAL.39, 219-226.

Van den ENDE, G, and H.F. LINSKENS. (1974). Cutinolytic enzymes in relation to pathogenesis. ANN. REV. PHTOPATHOL. 12, 247-258.

VAN GERTEL, J.F.E. (1983). Microcycle conidiation in *Penicillium italicum*. EXPERIMENTAL MYCOLOGY 7, 289-291.

VAN ROERMUND, H.J.W., D.R. PERRY and D. TYRREL. (1984). Influence of temperature, light, nutrients and pH in determination of the mode of conidial germination in *Zoophthora radicans*. TRANS. BRIT. MYCOL. SOC. 67, 27-31.

VAN SUMERE, C.F., C. VAN SUMERE- DE PRETER and G.A. LEDINGHAM, (1957). Cell wall-splitting enzymes of *Pucinia graminis* var. *tritici*.CANAD. J. MICROBIOL. 3, 761-770.

WALKER, J.C and M.A. STAHMANN. (1965). Chemical nature of disease resistant in plant. ANN. REV. PLANT PHYSIOL. 6, 351-366.

WALLIS, F.M., F.H.J. RUKENBERG, J. JOURBERT and M.M. MARTIN (1973). Ultrastructural histopathology of cabbage leaves infected with *Xanthosomonas campestris.* PHYSIOL. PLANT PATHOL. 3, 371-378.

WARCUP, J.H. (1967). Fungi in Soil. IN *Soil Biology*. Eds. A. BURGES and F. RAW. Academic Press. London, Ward, O.P., 1989. Fermentation Biotechnology. Prentice Hall, Englewood Cliffs, New Jersey.

WARD, O.P. (1989). Fermentation biotechnology. Prentice Hall, Englewood Cliffs, NJ.

WILLIAMSON, B. and HANDLEY (1970). Penetration and infection of orchid protocorms by *Thanatephaorus cucumeris* and other *Rhizoctonia* isolates. PHYTOPATHOLOGY, 60, 1092-1096.

WOLDETSADIK, K. (2003). Shallot (*Allium ascolanicum*) Responses to Plant Nutrients and Soil Moisture in a Sub-humid Tropical Climate. PhD Thesis. Swedish University of Agricultural Sciences. Sweden.

YANG, J., MEYERS, K.J., VAN DER HEIDE, J. and LIU, R.H. (2004). "Varietal differences in phenolic content, and antioxidant and antiproliferative activities of onions". J. AGRIC. FOOD CHEM **52** (21): 6787–6793.

YARWOOD, C.E. (1976). Modification of the Host Response-Predisposition. In, *Physiological Plant Pathology*. R. HEITEFUSS and P.H. WILLIAMS (Eds.) ENCYCLOPEDIA OF PLANT PHYSIOLOGY, NEW SERIES, VOL.4 SPRIMGER-VERLAG, BERLIN, HEIDELBERG, NEW YORK.

YEHIA, M.M., H.M. Al-Habib H. and N.M. SHEHAB. (1990). Otomycosis: a common problem in North Iraq. J LARYNGOL OTOL. 1990; 104: 387-9.

ZEILDER, G. and P. MARGALITH. (1973). Modification of the sporulation cycle in *Penicillium digitatum* (Sacc.) CANAD. J. MICROBIOL. 19, 481-483.

APPENDICES

APPENDIX A: Lengths, diameter and weights of bulbs of shallot (*Allium ascalonicum)*

Length (cm)		Diameter (cm)		Weight (g)	
Pale- brown cv.	Pink cv.	Pale- brown cv.	Pink cv.	Pale- brown cv.	Pink cv.
3.48	2.93	2.45	2.06	10.00	6.03
3.13	3.74	2.52	1.87	7.58	8.69
3.53	2.66	2.87	2.20	9.89	4.76
3.23	3.15	3.04	2.08	7.45	7.82
3.52	3.14	2.79	2.87	9.33	5.26
3.73	3.73	2.50	2.04	9.71	5.05
3.34	3.37	2.43	2.51	10.95	4.52
3.44	3.42	2.60	3.11	6.48	8.81
4.01	3.23	2.89	2.34	9.90	4.43
3.66	2.83	2.94	2.07	7.16	5.09
3.38	2.94	2.38	1.97	9.21	5.05
3.43	3.83	2.52	1.88	9.42	4.87
3.77	2.77	3.00	2.01	8.89	6.12
4.00	3.45	2.72	1.89	7.89	7.23
3.33	3.78	2.65	1.65	6.95	6.19
3.51	2.85	2.35	1.83	8.59	4.84
3.45	3.34	2.83	2.01	7.96	6.05
3.83	3.33	2.63	1.68	10.01	5.89
3.92	3.75	3.12	1.72	9.37	5.99
4.18	2.99	3.08	1.88	8.99	6.89

APPENDIX B: Percentage of conidia of *Aspergillus niger* Isolate 1 formed on ground bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 10 days at different relative humidities at 32±2°C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (µm)	Percentage Germination	Mean germ-tube length ±S.E (µm)
0	54.9	120.1±4.6 [a]	53.6	110.3±4.2 [a]
10	62.7	122.0±3.1 [a]	60.7	118.9±3.3 [a]
20	63.4	143.4±4.2 [a]	65.6	140.0±5.7 [a]
30	69.2	152.7±6.1 [a]	71.0	158.1±4.0 [a]
40	72.4	160.3±3.7 [a]	71.7	163.8±3.7 [a]
50	75.9	175.9±5.2 [a]	74.3	160.3±6.0 [a]
60	79.4	180.2±3.0 [a]	77.6	172.0±3.6 [a]
70	81.8	187.3±3.1 [a]	78.9	177.0±4.3 [a]
80	84.4	183.4±4.6 [a]	85.6	187.2±4.3 [a]
90	88.6	190.0±3.2 [a]	88.9	185.2±3.6 [a]
100	91.6	190.3±4.3 [a]	89.6	188.3±4.2 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX C : Percentage of conidia of *Aspergillus niger* Isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 20 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (μm)	Percentage Germination	Mean germ-tube length ±S.E (μm)
0	52.4	118.4±4.2 [a]	50.2	121.4±1.7 [a]
10	60.4	124.1±3.1 [a]	58.3	123.3±1.9 [a]
20	62.4	129.3±2.1 [a]	61.1	127.4±2.8 [a]
30	68.3	138.1±6.2 [a]	67.2	130.8±4.6 [a]
40	70.5	130.3±5.2 [a]	68.2	134.3±3.4 [a]
50	72.3	133.4±4.2 [a]	70.5	148.4±4.2 [a]
60	73.4	147.4±3.2 [a]	71.3	140.2±2.3 [a]
70	74.9	148.3±2.4 [a]	74.5	143.4±6.3 [a]
80	76.3	159.8±3.1 [a]	80.3	158.3±1.3 [a]
90	80.3	173.4±2.3 [a]	83.7	163.4±3.4 [a]
100	83.2	163.2±3.1 [a]	81.2	172.4±3.4 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX D: Percentage of conidia of *Aspergillus niger* Isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 40 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle.(Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	51.4	115.4±1.8 [a]	49.9	113.4±1.9 [a]
10	58.3	120.3±3.2 [a]	55.3	120.8±2.5 [a]
20	60.5	123.5±2.4 [a]	59.4	126.4±2.8 [a]
30	62.3	130.4±6.3 [a]	63.2	132.4±4.2 [a]
40	65.8	135.4±1.7 [a]	64.2	133.7±6.3 [a]
50	69.4	141.2±2.3 [a]	65.4	143.7±4.1 [a]
60	69.3	150.3±5.1 [a]	67.1	150.1±2.3 [a]
70	70.1	156.4±3.2 [a]	66.5	149.3±4.3 [a]
80	70.5	168.3±2.4 [a]	69.4	150.3±5.2 [b]
90	72.4	170.2±2.3 [a]	74.5	152.4±2.6 [b]
100	78.3	173.4±3.1 [a]	76.4	168.2±1.3 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX E: Percentage of conidia of *Aspergillus niger* isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 80 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle.(Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)
0	46.9	103.4±4.8 [a]	45.3	100.2±4.3 [a]
10	52.4	112.5±3.2 [a]	51.1	110.4±1.6 [a]
20	55.3	116.3±2.7 [a]	54.9	112.5±1.8 [a]
30	58.4	118.5±1.8 [a]	55.5	114.3±2.4 [a]
40	60.2	120.4±3.4 [a]	58.5	121.5±2.3 [a]
50	61.9	121.1±2.8 [a]	58.9	130.4±1.9 [b]
60	62.4	136.3±3.7 [a]	61.3	133.4±5.0 [a]
70	63.5	148.2±6.2 [a]	62.4	139.8±4.2 [a]
80	68.3	147.1±5.3 [a]	65.4	144.4±5.3 [a]
90	70.1	158.2±2.4 [a]	68.9	154.3±2.2 [a]
100	75.4	163.4±1.3 [a]	72.1	158.2±1.8 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX F: Percentage of conidia of *Aspergillus niger* isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 120 days at different relative humidities at 32±2°C under 12-hour day-night cycle.(Percentage germination based on 400-500 observed conidia).

Storage Percentage Relative Humidity	Pale-brown cultivar		Pink cultivar	
	Percentage Germination	Mean germ-tube length ±S.E (μm)	Percentage Germination	Mean germ-tube length ±S.E (μm)
0	45.3	121.2±5.4 [a]	41.2	120.4±5.5 [a]
10	48.4	131.4±4.3 [a]	44.8	123.2±4.5 [a]
20	49.3	132.8±3.2 [a]	45.4	124.5±3.2 [a]
30	51.9	143.1±2.5 [a]	48.3	129.4±2.9 [b]
40	53.2	144.3±1.9 [a]	50.3	128.4±3.7 [b]
50	54.7	146.4±2.8 [a]	52.4	127.5±3.2 [b]
60	58.3	150.2±4.3 [a]	56.7	128.4±4.0 [b]
70	60.9	148.3±5.3 [a]	58.4	132.6±4.2 [a]
80	61.8	154.2±4.2 [a]	60.3	130.4±5.3 [b]
90	63.7	153.1±2.3 [a]	64.7	134.5±4.3 [b]
100	70.5	160.2±1.9 [a]	68.3	138.4±3.2 [b]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX G: Percentage of conidia of *Aspergillus niger* Isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 160 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle . (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)
0	33.2	100.8±2.8 [a]	31.4	96.1±5.8 [a]
10	39.4	110.3±5.1 [a]	38.2	99.8±4.1 [a]
20	40.3	102.4±4.1 [a]	41.3	108.4±4.2 [a]
30	45.5	117.3±4.2 [a]	44.2	110.4±3.8 [a]
40	47.3	118.5±3.7 [a]	47.5	113.1±3.3 [a]
50	50.9	124.3±2.8 [a]	48.4	130.4±2.8 [a]
60	52.4	132.4±1.9 [a]	51.3	133.5±1.8 [a]
70	55.3	143.8±3.4 [a]	54.1	139.4±4.2 [a]
80	58.4	142.1±2.4 [a]	57.2	140.4±5.2 [a]
90	60.1	144.5±6.3 [a]	58.3	150.3±3.4 [a]
100	63.2	147.2±1.2 [a]	62.1	153.4±2.1 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX H: Percentage of conidia of *Aspergillus niger* Isolate 1 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 200 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).88

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	0.0	-	0.0	-
10	0.0	-	0.0	-
20	0.0	-	0.0	-
30	0.0	-	0.0	-
40	0.0	-	0.0	-
50	0.0	-	0.0	-
60	28.7	80.2±1.8 [a]	25.3	78.2±3.2 [a]
70	32.4	88.3±3.1 [a]	28.1	82.4±1.9 [a]
80	40.3	93.4±3.2 [a]	33.1	90.2±4.3 [a]
90	43.7	103.1±4.3 [a]	37.2	98.1±2.8 [a]
100	52.1	112.1±6.3 [a]	43.8	102.3±4.1 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX I: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 10 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (µm)	Percentage Germination	Mean germ-tube length ±S.E (µm)
0	50.2	58.1±3.2 [a]	55.0	55.4±3.5 [b]
10	61.2	67.8±2.7 [a]	60.4	66.6±3.1 [b]
20	66.4	97.4±6.2 [a]	63.7	78.8±3.8 [a]
30	67.6	93.8±4.2 [a]	64.7	80.1±4.7 [a]
40	68.9	101.2±5.4 [a]	66.3	91.4±4.0 [a]
50	70.9	124.2±5.5 [a]	73.0	119.2±6.0 [a]
60	73.1	143.1±7.2 [a]	74.7	112.7±6.4 [b]
70	74.1	136.6±4.2 [a]	75.8	121.1±4.4 [a]
80	81.6	151.2±6.0 [a]	78.2	146.1±7.3 [a]
90	90.0	175.1±5.6 [a]	90.8	188.1±10.2 [a]
100	93.6	197.4±4.1 [a]	91.1	192.4±9.9 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability

APPENDIX J: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 20 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	44.9	108.8±2.6 [a]	41.9	91.4±4.0 [b]
10	47.4	112.5±3.1 [a]	48.9	91.9±4.5 [b]
20	53.8	119.7±2.7 [a]	52.5	108.8±3.0 [a]
30	60.5	120.8±3.5 [a]	54.5	113.4±3.6 [a]
40	63.5	127.1±3.0 [a]	59.6	122.5±3.1 [a]
50	66.3	127.7±3.6 [a]	64.2	119.1±2.2 [a]
60	70.3	135.0±4.5 [a]	68.6	131.1±3.4 [a]
70	71.8	136.1±4.2 [a]	72.5	134.1±4.1 [a]
80	78.8	139.8±4.1 [a]	76.7	139.5±4.9 [a]
90	85.3	140.1±3.4 [a]	80.2	143.3±4.8 [a]
100	87.2	143.0±5.2 [a]	85.7	141.3±4.7 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability

APPENDIX K: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of two cultivars of *Allium ascalonicum* viable after storage for 40 days at different relative humidities at 32±2ºC under 12-hour day-night cycle (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	37.6	87.6±3.7 [a]	35.3	86.0±3.2 [a]
10	41.6	92.3±2.5 [a]	40.8	91.7±3.3 [a]
20	43.6	93.2±3.7 [a]	46.1	93.8±2.9 [a]
30	51.4	108.6±3.1 [a]	54.7	111.1±2.4 [a]
40	56.3	115.2±3.2 [a]	56.8	117.6±3.4 [a]
50	60.0	122.7±4.0 [a]	58.3	123.8±3.5 [a]
60	63.2	128.4±3.2 [a]	61.0	127.3±4.0 [a]
70	66.3	129.2±3.1 [a]	64.0	126.8±2.8 [a]
80	70.6	130.0±4.1 [a]	69.2	127.7±3.7 [a]
90	74.9	136.0±4.7 [a]	73.1	129.9±4.0 [a]
100	76.4	131.4±3.3 [a]	77.2	130.8±2.6 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX L: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 80 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (µm)	Percentage Germination	Mean germ-tube length ±S.E (µm)
0	34.6	77.0±3.1 [a]	33.2	78.6±3.2 [a]
10	43.1	83.0±3.0 [a]	39.3	79.1±3.3 [a]
20	48.7	81.4±3.1 [a]	44.0	80.7±2.5 [a]
30	52.0	87.3±3.1 [a]	52.4	92.9±3.1 [a]
40	53.1	89.4±3.0 [a]	56.3	93.5±2.6 [a]
50	55.8	94.5±3.0 [a]	55.5	101.4±2.3 [a]
60	56.7	104.4±3.7 [a]	57.2	103.4±2.9 [a]
70	59.9	104.0±3.3 [a]	58.9	106.3±3.5 [a]
80	61.1	100.3±5.4 [a]	59.6	106.1±3.2 [a]
90	64.2	104.7±3.5 [a]	63.9	109.4±2.6 [a]
100	73.7	107.9±3.3 [a]	70.0	108.8±2.9 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability

APPENDIX M: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 120 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	32.1	70.3±5.1 [a]	30.8	65.3±2.5 [a]
10	43.5	76.1±2.3 [a]	40.9	73.3±2.3 [a]
20	47.2	80.2±4.2 [a]	44.7	83.2±4.3 [a]
30	49.5	85.3±3.3 [a]	46.2	87.3±3.2 [a]
40	50.8	93.2±1.5 [a]	53.4	90.5±2.4 [a]
50	54.2	102.8±2.8 [a]	56.7	94.3±1.9 [a]
60	57.9	100.4±4.3 [a]	58.1	98.9±2.8 [a]
70	59.2	105.3±2.9 [a]	61.1	103.5±2.3 [a]
80	59.8	118.5±4.3 [a]	62.6	107.2±5.5 [a]
90	65.2	112.3±5.5 [a]	64.8	118.3±3.4 [a]
100	69.2	123.4±2.3 [a]	66.7	120.2±2.8 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX N: Percentage of conidia of *Aspergillus niger* isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 160 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle.(Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	0.0	-	0.0	-
10	0.0	-	0.0	-
20	0.0	-	0.0	-
30	0.0	-	0.0	-
40	50.3	100.0±2.1 [a]	47.3	99.3±2.0 [a]
50	52.4	106.1±3.8 [a]	49.3	108.1±2.3 [a]
60	53.9	110.3±2.4 [a]	50.3	112.3±1.4 [a]
70	57.4	118.0±2.3 [a]	54.4	115.1±1.3 [a]
80	59.3	115.3±4.3 [a]	58.3	121.4±1.4 [a]
90	60.1	120.1±1.9 [a]	61.4	116.3±2.3 [a]
100	64.3	122.7±3.2 [a]	63.2	118.4±3.1 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX O: Percentage of conidia of *Aspergillus niger* Isolate 3 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 200 days at different relative humidities at 30oC 32±2^{o}C under 12-hour day-night cycle (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	0.0	-	0.0	-
10	0.0	-	0.0	-
20	0.0	-	0.0	-
30	0.0	-	0.0	-
40	0.0	-	0.0	-
50	0.0	-	0.0	-
60	0.0	-	0.0	-
70	33.4	95.4±2.8 [a]	28.9	92.3±3.2 [a]
80	45.2	98.3±3.2 [a]	35.4	93.8±4.1 [a]
90	48.3	103.2±1.3 [a]	40.3	90.4±2.6 [b]
100	51.2	113.4±2.1 [a]	42.1	102.4±4.3 [a]

By the calculated Scheffe’s Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX P: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 10 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (μm)	Percentage Germination	Mean germ-tube length ±S.E (μm)
0	53.8	113.7±3.5 [a]	55.6	105.5±3.0 [a]
10	59.5	127.4±4.1 [a]	57.8	118.2±3.6 [a]
20	65.5	128.7±3.8 [a]	63.4	125.9±4.2 [a]
30	69.5	130.7±3.2 [a]	64.9	135.8±4.9 [a]
40	71.9	142.2±4.6 [a]	73.5	136.1±4.1 [a]
50	77.3	146.6±4.8 [a]	77.1	137.7±4.0 [a]
60	81.0	156.3±5.2 [a]	79.1	151.2±6.4 [a]
70	84.7	159.0±4.0 [a]	85.3	165.6±4.8 [a]
80	89.7	164.6±4.9 [a]	86.4	154.1±6.2 [a]
90	91.1	184.2±3.5 [a]	87.5	175.4±6.7 [a]
100	92.3	195.2±3.4 [a]	93.1	184.2±7.8 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability

APPENDIX Q: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 20 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (µm)	Percentage Germination	Mean germ-tube length ±S.E (µm)
0	48.4	115.6±3.5 [a]	43.8	105.5±3.7 [a]
10	52.3	125.9±3.1 [a]	45.2	111.9±3.2 [b]
20	57.3	127.2±3.6 [a]	49.1	121.7±3.6 [a]
30	62.6	132.8±4.1 [a]	53.7	129.9±3.1 [a]
40	65.1	134.7±3.9 [a]	64.9	140.9±4.4 [a]
50	69.1	140.0±4.8 [a]	68.9	139.2±6.1 [a]
60	72.0	143.3±4.8 [a]	70.7	140.3±5.3 [a]
70	77.9	147.7±4.6 [a]	76.5	150.2±5.3 [a]
80	78.9	148.5±4.7 [a]	78.1	152.0±4.9 [a]
90	81.2	153.8±5.0 [a]	81.9	148.7±4.7 [a]
100	87.5	154.3±4.7 [a]	85.1	156.2±5.0 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX R: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 40 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (μm)	Percentage Germination	Mean germ-tube length ±S.E (μm)
0	39.9	85.8±3.3 ^{a}	37.9	82.7±3.0 ^{a}
10	44.0	88.8±2.9 ^{a}	40.0	90.2±3.8 ^{a}
20	45.6	102.3±3.7 ^{a}	45.7	92.0±4.0 ^{a}
30	48.3	109.8±3.6 ^{a}	47.2	104.1±2.7 ^{a}
40	52.5	117.5±3.0 ^{a}	50.4	109.2±3.0 ^{a}
50	56.1	120.3±2.5 ^{a}	53.3	111.0±2.0 ^{b}
60	58.6	120.8±4.5 ^{a}	57.9	116.7±3.6 ^{a}
70	65.9	124.5±3.2 ^{a}	64.2	116.3±4.0 ^{a}
80	67.9	125.3±3.1 ^{a}	66.0	116.1±3.4 ^{a}
90	71.9	132.2±3.4 ^{a}	69.3	126.8±3.8 ^{a}
100	79.6	130.7±3.6 ^{a}	73.5	139.5±4.5 ^{a}

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX S: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 80 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (μm)
0	34.3	73.4±5.1 [a]	30.5	62.3±3.4 [a]
10	38.3	75.3±4.2 [a]	33.8	70.4±4.3 [a]
20	40.5	83.4±3.7 [a]	36.5	79.3±1.8 [a]
30	43.8	87.8±2.3 [a]	40.8	80.7±2.5 [a]
40	47.5	85.3±1.9 [a]	43.5	83.5±3.3 [a]
50	53.4	90.8±2.7 [a]	51.4	90.3±4.2 [a]
60	56.8	94.3±2.3 [a]	59.2	95.4±5.4 [a]
70	62.3	98.5±3.8 [a]	61.3	97.8±3.3 [a]
80	65.8	103.8±4.0 [a]	63.2	94.3±2.6 [a]
90	70.3	100.4±3.7 [a]	64.5	98.5±2.9 [a]
100	75.4	105.3±4.2 [a]	71.3	101.4±1.9 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX T: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 120 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)	Percen-tage Germ-ination	Mean germ-tube length ±S.E (µm)
0	30.8	65.5±3.2 [a]	28.2	60.2±4.3 [a]
10	33.2	80.3±2.7 [a]	31.4	64.3±2.7 [a]
20	37.4	85.4±1.8 [a]	34.7	80.5±5.3 [a]
30	40.3	87.2±4.3 [a]	35.3	71.4±5.1 [a]
40	44.5	90.3±5.5 [a]	40.8	80.7±4.0 [a]
50	47.4	95.3±2.3 [a]	42.3	82.5±1.5 [b]
60	48.5	98.4±4.5 [a]	45.4	87.4±2.5 [b]
70	50.8	101.2±2.3 [a]	47.3	90.3±2.7 [b]
80	53.4	100.4±1.9 [a]	52.5	95.1±2.3 [a]
90	61.3	103.8±4.3 [a]	56.5	99.1±3.4 [a]
100	65.4	110.4±3.2 [a]	60.3	102.4±3.0 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability

APPENDIX U: Percentage of conidia of *Aspergillus niger* Isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 160 days at different relative humidities at 32±2^{o}C under 12-hour day-night cycle. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percen- tage Germ- ination	Mean germ-tube length ±S.E (µm)	Percen- tage Germ- ination	Mean germ-tube length ±S.E (µm)
0	0.0	-	0.0	-
10	0.0	-	0.0	-
20	0.0	-	0.0	-
30	0.0	-	0.0	-
40	18.3	60.5±1.3 [a]	15.3	58.5±3.2 [a]
50	20.2	72.4±5.2 [a]	19.7	63.4±2.6 [a]
60	23.8	85.5±2.3 [a]	20.3	82.8±1.7 [a]
70	30.4	90.4±1.5 [a]	33.2	83.4±2.8 [a]
80	32.1	93.2±3.2 [a]	38.3	90.8±3.0 [a]
90	43.2	100.1±1.8 [a]	40.2	93.2±2.5 [a]
100	50.1	103.4±2.4 [a]	43.9	108.3±1.3 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability.

APPENDIX V: Percentage of conidia of *Aspergillus niger* isolate 5 formed on macerated bulbs of the two cultivars of *Allium ascalonicum* viable after storage for 200 days at different relative humidities at 32±2°C under normal day/ night light regime. (Percentage germination based on 400-500 observed conidia).

	Pale-brown cultivar		Pink cultivar	
Storage Percentage Relative Humidity	Percentage Germination	Mean germ-tube length ±S.E (µm)	Percentage Germination	Mean germ-tube length ±S.E (µm)
0	0.0	-	0.0	-
10	0.0	-	0.0	-
20	0.0	-	0.0	-
30	0.0	-	0.0	-
40	0.0	-	0.0	-
50	0.0	-	0.0	-
60	0.0	-	0.0	-
70	0.0	-	0.0	-
80	15.3	53.4±1.2 [a]	20.4	58.4±3.1 [a]
90	23.2	60.2±1.3 [a]	21.2	61.3±2.8 [a]
100	35.8	78.4±3.8 [a]	32.3	73.4±5.5 [a]

By the calculated Scheffe's Confidence Limits, Values of pairs of Mean Germ-tube Lengths in each horizontal row bearing the same letters are not significantly different at 5% level of probability