KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY-KUMASI GHANA.

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

STUDIES ON THE CONCENTRATION AND DIVERSITY OF AIRSPORA IN AN APIARY AT THE UNIVERSITY FOR DEVELOPMENT STUDIES- NYAMKPALA CAMPUS, NORTHERN REGION, GHANA.

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SEPTEMBER, 2011

STUDIES ON THE CONCENTRATION AND DIVERSITY OF AIRSPORA IN AN APIARY AT THE UNIVERSITY FOR DEVELOPMENT STUDIES- NYAMKPALA CAMPUS, NORTHERN REGION, GHANA.

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

BY

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SEPTEMBER, 2011

DECLARATION

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

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DEDICATION

I dedicate this work to my Mother: Madam Regina Kuora, My daughter: Eva Nuorikye, My wife: Diana Iddi and my siblings: Grace .Y. Nuorikye, Viola K. Nuorikye, Lucy T. Nuorikye and Philip C. Nuorikye.

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ABSTRACT

Fungal species were sampled from the atmosphere around beehives at an apiary at various points from the beehives at various heights above the ground at these points for eight weeks by exposing Petri dishes containing fungal growth media for five minutes. Three nutrient media were used namely: PDA, MEA and SA. Samples were taken at different times of the day to determine diurnal variation in fungal concentration.

Eleven different species of fungi were identified from the site of study. The most commonly occurring species was *Aspergillus niger* (115.31) whiles the least occurring fungi was *Trichoderma sp* (12.25).

There was significant difference between the concentration of fungi around the beehives (0meter) and the concentration distance away (100 meters). The concentration of fungi was more around the beehives than at a distance (100meters) away.

Aspergillus niger(58), *Aspergillus versicolor*(36.69), *Curvularia sp*.(24..63), *Fusarium* sp.(20.94), and Mucor(10.15) occurred more frequently at a height of 2m above ground than at 0.5m above ground whiles species such as *Aspergillus ocraceous*(10.43), *Aspergillus tamarii*(14.42), *Neurospora sp*.(31.37) , *Penicillium sp*.(22.31) , *Rhizopus*(11.69) *and Trichoderma sp*(6.15*)* were more abundant at 0.5m above ground than 2m above ground.

With regards to diurnal variation, Aspergillus niger, *Aspergillus tamari, Curvularia sp, Fusarium sp, Mucor, Rhizopus and Trichoderma sp.* occured more frequently in the evening (5.00pm or 17.00h). Species such as *Aspergillus versicolor, Aspergillus ocraceous , and Neurospora sp.* were more common in the morning (7.00am or 7.00h) .There was relatively low abundance of the various species in the afternoon (12.00noon or 12.00h). It is however observable that there

was no marked variation between the individual numbers of species for the three different times of which sampling were made.

The highest fungal counts were made in the evening, followed by morning and then noon. With regards to influence of growth media on fungal concentration, the highest numbers of fungal counts were isolated from PDA, SA and MA respectively.

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CHAPTER ONE

INTRODUCTION

1.1 **Background**

The air is seldom free of fungal spore (Lacey, 1981). Airborne fungal spores and hyphal fragments originate from contaminated animals, birds, plants, soil, manure, decaying plants and human activities (Al-Doory et al. 1980). Other possible sources of airborne fungal spores include crop plants, sites where crop products are stored, transport from field to the storage facilities, birds and insects. Apart from these sources, mowing can put numerous numbers of spores in the air (Chakraborty et al, 2003).

The composition and concentration of airborne fungal spora, are therefore largely determined by geographical location, meteorological factors, local vegetation, and human activities (Lacey, 1981), as well as by a wide range of interrelated environmental and biological factors (Lyon et al. 1984)

Concentration of fungal spores in the atmosphere at any particular moment is influenced by the processes involved in their production, release, and deposition (Lyon et al. 1984).

Much of the knowledge of the implication of presence of airborne spores comes from studies on the epidemiology of plant and animal diseases (Lacey, 1981).

The composition of the air is governed only by physical factors of the moving air and not by dry nutritional factors. Fungi in the atmosphere are mostly present in the troposphere. The aeromycoflora of a space is largely influenced by the kind of mycoflora in that particular place , even though air current sometime conveys some amount of particles; the spores of other species

from either nearby environment or distant place. *Aspergillus, Curvularia, Fusarium, Cladosporium, Penicillium, Rhizopus and Mucor* are examples of airborne fungal spores (Chakraborty et al, 2003).

Once spores are air borne, the weather, season, time of the day, geographical location can further influence the concentration and types of spores. Concentration of fungal spores and pollen are dependent on the distance from the major sources, prevailing wind direction and local topography.

The presence of fungi may be identified visually on suitable surface materials and by an unpleasant odour (mouldy smells). Fungi usually do not grow rapidly indoors or in large amounts if the environment is clean and dry. However, if the environment is damp, high levels of airborne spores can be generated. Condensation is the principal source of moisture that promotes growth of fungi on the internal surfaces of domestic dwellings. Other factors (such as daily variation of light and temperature) can affect spore formation and growth.

Fungi grow on structural materials where humidity is high or on food and stored products where there is condensation. Humidifiers, air-conditioning systems, carpeting and damp walls are the potent sources of indoor fungal allergens. Outdoor fungi such as species of *Alternaria, Aspergillus, Botrytis, Cladosporium, Curvularia, Epicoccum, Fusarium, Penicillium, Phoma, Trichoderma* etc., have been reported regularly from damp indoors. *Aspergillus, Eurotium and Penicillium* have been observed to colonize food scraps and other organic materials (Chakraborty et al, 2003).

Fungi grow outdoors wherever continuous moisture is present. Soil-borne fungi are essential for the decay of plant and animal matter. As such, compost heaps and potting composts can contain extremely high numbers of fungal spores, including *Aspergilllus fumigatus* that can cause allergy or aspergillosis. Fungi also grow in stored grain and can reach very high levels if grain moisture is above certain thresholds. Conditions are also ideal for fungal growth in many caves and underground pipes.

Dispersal of fungal spores by wind is by far the most common method for terrestrial fungi. Most air-borne spores are small, often about 1-8 μm in diameter, with a rate of fall, in still air, of less than 10 mm/sec.

The airspora is heavily influenced by the seasonal weather. A dry airspora and a wet airspora have been recognized. In dry weather the air is rich in spores of moulds, rusts, downy and powdery mildews. Heavy rain soon scrubs spores out of the air, thereafter a short-lived wet airspora develops dominated by ascospores discharged from Ascomycetes in which spore discharge depends on wetting (Gover, 1999).

There are many examples of fungi that are dispersed by insects, small animals and humankind. A common adaptation is the production of a sticky, sugar-rich spore droplet to attract the insect vector. *Ceratocystus ulmi* (Ascomycotina), the fungus that cause Dutch elm disease is vectored by beetles of the genus *Scolytus*. The spores are dispersed when the sticky sporing structures of C. *ulmi* bearing very small spores in a droplet of mucilage project into the brood galleries (carved by the hatched grubs), and contaminate the new generation of emerging beetles. The wasp, *Sirex noctilis* utilises a fungus as a means of providing a suitable food source for larvae (Upadhyay, 1981).

Exposure to fungi associated with bird or bat droppings, or soil contaminated by bird droppings, (e.g. *Histoplasma capsulatum* and *Cryptococcus neoformans*) can lead to flu-like illnesses in healthy persons or more severe effects in immuno-suppressed persons (Abbott, 2000)

As already stated fungal spores constitute a significant fraction of airborne bioparticles (Ebner and Haselwandter, 1992; Takahasi, 1997) and they are often 100–1000 times more numerous than other airborne bioparticles like pollen grains (Lacey, 1981; Lehrer et al., 1983). On the average, man inhales approximately 10 m^3 of air per day (Lynch and Poole, 1979), containing among others a large number of fungal spores, probably the largest constituents of microorganisms in bioaerosol, ranging from 3 to 30 µm in size (Stetzenbach 1998). Fungal spores of greater than 10 µm sizes are deposited in the nasopharynx and are associated with nasal and ocular symptoms, which are generally called hay fever (Luo, 1991). Spores less than 10 Am size, especially those of less than 6 µm in size, can be transported to the lower airways and lungs where allergic reactions sometimes manifest as asthma (Pepys, 1965). Higher frequencies of airborne fungal spores were recorded by many workers from occupational environments (Lacey and Crook, 1988; Vittal and Glory, 1985; Singh and Singh, 1996) including a few studies from dairy barns (Hanhela et al., 1995).

The interaction of fungal spores with lung structures may be important for the development of respiratory allergies induced by air borne fungal allergens (Geiser et al., 2000). Apart from their direct allergenic effect, fungi may carry mycotoxins in their spores or produce volatile metabolites (Miller, 1998). Inhalation of mycotoxins such as aflatoxins, secalonic acid, zearalenone and tricothecenes produced by *Aspergillus*, *Penicillium* and *Fusarium* spp, may affect the immunological response of the lung tissues or cause other hazards to human health (Gerberick, 1984).

In other instances, the prevalence of airborne fungal spores such as *Cladosporium* and *Altenaria* in a particular area is remarkably noted to be strongly associated with some respiratory and allergic diseases such as asthma, rhinitis, and hay fever among others.

Due to the presence of allergens on spores it appears that all moulds have the potential to cause an allergic reaction in susceptible humans. Mould growth in buildings is associated with an increased prevalence of respiratory symptoms and diseases, such as asthma and bronchitis. The symptoms most commonly observed are: sore throat, runny nose, hoarseness, cough, headache, fever, hay fever, fatigue and hypersensitivity pneumonitis (HP).

Many airborne fungi, Actinomycetes and Bacteria are therefore capable of causing diseases in man and animals by direct ingestion, by toxicosis (ingestion of toxic metabolites of microbes) or by allergy (sensitivity to microbial proteins and polysaccharides).

There are no exposure limits for exposure to air-borne fungi. Because of the variation in susceptibility of individuals to fungi and the diverse nature of fungi and their health effects, there are no "safe" levels of exposure. (D' Andrea, 2002).

The main health risk from occupational exposure is from inhalation of organic dusts containing fungal spores that can cause respiratory symptoms. Therefore, workers handling fungi contaminated materials must take safety measures to prevent risk of exposure to fungi. Occupations with the highest risk of exposure are: farm workers, horticulture workers/Landscape, Gardeners, Indoor worker and animal workers (D'Andrea, 2002).

Workers on composting sites may be at high risk of considerable exposure to bioaerosols depending on their work task, their proximity to the bioaerosol source and the control measures

put in place. In addition because the work is largely done outdoors, there is the potential for bioaerosols generated to disperse some distance from the point source. Consequently, there is concern that people living and working at composting sites may also be exposed to bioaerosol. (Stephen et al, 2010).

In some cases, thermotolerant fungal species associated with composting materials are recognized as being the causative agents of allergic respiratory diseases such as extrinsic allergic alveolities (e.g. Farmers lung disease, Mushroom workers lung disease), allergic rhinitis and occupational asthma, following excessive exposure (Swan et al, 2003). In addition the thermotolerant fungus *Aspergillus fumigates* is recognized as an opportunistic respiratory pathogen of immunocompromised persons (Stephen et al,2010).

Similarly work done by Gauvreau (1995) to determine fungal spore concentration over wintering beekeeping facilities recorded high concentration of fungi on these facilities. Since there are several known health risks associated with inhalation exposure to toxigenic species of mould spores, Beekeepers are at risk during the activities studied and should take precautions to prevent inhalation exposure to spores (Gauvreau and Mckenna, 1995).

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982;

Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation.

Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Davis, 1995; Diba et al., 2007; Zain et al., 2009). Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996).

1.2. **Problem statement**

The existence of Poverty particularly in the northern region of Ghana has attracted a number of Non-governmental Organizations (NGOs). Some of the NGOs including the Association of Church Development Project (ACDEP) are encouraging people to go into beekeeping as a source of employment to improve their welfare.

However beekeeping could have associated health implications due to possible worker exposure to fungal spores. Research work done by Batra et al (1973) shows that in addition to pollen, honey bees can transfer fungal spores and bacteria among flowers of different species.

Furthermore the ability of bees to vector microbes has been harnessed for biological control purposes by using honey bees and bumble bees to transfer inoculums of fungi, bacteria and viruses from hive to flowers (Kevan et al, 2003).

Fungi and bacteria pollute the air and cause many human diseases (Gregory, 1973). Spores of fungi present in the air also cause a number of allergic reactions (Al-Doory and Domoson, 1984 and Sanches et al, 1999). Ebner et al, (1992) reported that for effective treatment of allergies, knowledge of the airborne fungal spores is of great importance. Vander Werff (1955) reported that each country has its own fungal groups which cause allergy. Airspora also has a direct influence to food spoilage and hence food borne diseases.

Knowledge of the concentration and diversity of fungal spores in and around apiaries is therefore important to the health worker, the beekeeper, neighbourhood around bee hives in controlling, managing and treating fungal infections if not avoid health issues.

1.3.0 **Main objective**

Determine the concentration and diversity of fungal airspora in an apiary and their immediate environs.

1.3.1 **Specific objectives:**

1. Determine the concentration and diversity of fungal spores in the air around selected bee hives.

2. Compare airspora concentration of sites near and further away from bee hives.

3. Compare fungal counts at different heights.

4. Compare fungal counts at different times of day.

5. Compare fungal counts on the three different fungal growth media used.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. COMMON FUNGI IN THE ATMOSPHERE

Sharma (2010) recorded *that Aspergillus niger, Aspergillus fumigatus, Cladosporium oxysporum, Alternaria alternate* were most frequent fungi on the leaf surface mycoflora of *Oscimum sanctum.* Similarly *Mucor* species *Aspergillus nidulence, Fusarium oxysporum, Nigrospora sphaerica* were the frequent fungi. Jadhav (1996) reported maximum fungal types during winter over a rice field. Tiwari (1999) also observed maximum fungal types during winter from Raipur. Singh (2006) over Spinach, Tiwari and Sharma (2008) for leaf surface of *Ocimum sanctum,*Tiwari and Saluja (2009) in *Catharanthus roseus* have also reported highest fungal incidence during winter season, moderate during rainy season and minimum number of fungal types in summer season.

Tiwari et al. (2006) observed that *Cladosporum, Nigrospora, Alternaria, Curvularia* and *Phaeotrichoconis* spores where the most frequent fungal types in the atmosphere of Raipur. Uddin (2004) reported that *Penicillium* and *Aspergillus* were most dominant saprophytes in the atmosphere.

Afzal and Mehdi (2002) reported that the species of *Aspergillus* were the most prevalent fungi in the atmosphere of Kerachi City. Many others indicated that the dominant fungi were *Clasdosporium, Alternaria, Penicillum* and *Aspergillus* in the atmosphere and their concentration differed from place to place because of local environmental variables, fungal substrates and human activities.

Recent studies by Fang et al, (2005) showed that the dominant airspora in Beijing was *Cladosporium* and the highest fungal concentration was noted in summer, because of the suitable condition for fungal growth such as air, temperature and moisture. Toqeer et al (2009) reported that 10 fungal species were recorded in the atmospheric air of Kerachi city. These fungal species were *Alternaria solani, Aspergillus candidus,A. flavu*s, *A. fumigatus*, *A. nige*r, *A. terreus*, *A. wentii, Curvularia clavata, Drechslera dematioidea* and *Penicillium notatum.*

Khattab and Levetin, (2008) stated that *Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium, Penicillium,* smut spores, and *Trichoderma* are some of the common allergenic fungi.

Adhikari et al (2003) reported that *Aspergillus* and *Penicillium* dominated the fungal airspora taken at two cattle sheds in India. Other dominant spore types recorded from the two sections of the cattle shed were *Cladosporium, Nigrospora, Periconia, Choanephora, Corynespora, Curvularia, Drechslera, Memnoniella, Torula and smut spores (Ustilaginales).*

Okten et al (2005) recorded Seven fungal genera in the atmoshphere of Edirne city (Turkey), among them, *Cladosporium* and *Alternaria* genera were generally found to be the most predominant fungi followed by *Penicillium* and *Trichoderma* .

Chadeganipour, et al. (2010) studied airborne fungi in Isfahan. In this study, 250 samples from air in Isfahan were taken and 828 colonies were isolated. The genera of isolated airborne fungi, depending upon their frequency in number of colony counts were classified as predominant and less frequent isolates. The dominant species were members of the genera *Pénicillium spp., Cladosporium spp., Aspergillus spp., Alternaria spp.* and yeasts and yeast like *(Candida spp., Geotrichum spp. and Trichosporon spp.)* and the minor components or less prevalent were *Rhizopus spp., Ulocladium spp., Curvularia spp. and Fusarium spp.*

2.2 INSECTS AND OTHER ARTHROPODS IN THE DISPERSAL OF FUNGAL SPORES

Spores are the reproductive propagules of fungi, and the various modes of spore liberation and dissemination that are prevalent among different groups of fungi are critical for the success and survival of fungal species (Ingold 1953, 1965). Aerial (or anemophilous) dissemination of fungal spores is the primary means of dispersal for many fungi. Spores of many Ascomycetes (ascospores) and Basidiomycetes (basidiospores) are forcibly ejected from the fertile tissue of the fruiting bodies to reach the air currents.

Insect-vectored spore dispersal is recognized in many groups of fungi, including Ascomycetes, Basidiomycetes,Imperfect fungi and Zygomycetes (Ingold 1953, Kendrick 1985), as well as in the myxomycetes or slime molds (Stephenson and Stempen 1994)

A general distinction has been made between the dispersal methods of dry versus sticky or slimy spores. Dry spores are dispersed by air, while sticky or slimy spores rely on water or vector dispersal (Webster, 1980).

Several types of fungal fruiting bodies produce sticky masses of spores in a thick, slimy layer. The most prominent of these are the stinkhorns (e.g., *Phallus, Mutinus*). A strong fetid odor is produced to attract flies to land on the glebal surface to feed. Many spores adhere to the legs and bodies of the flies, and the insects may remove the entire slime layer, filled with basidiospores, within a few hours. The spores are dispersed to nearby sites visited by the flies and are excreted, relatively unaltered, by the insects (Ingold 1965). In general, fungi adapted to dispersal by flies tend to offer minute, smooth-walled spores in a sugary slime coating (Ingold 1953).

Beetles and other insects may also disperse macrofungi. The fruiting bodies of the wood-inhabiting polypore, *Crytoporus volvatus,* have a membrane covering most of the fertile, spore-producing layer. Although some spores reach the air for anemophilous dispersal, the majority of

spores accumulates on the inner surface of the sheath and is disseminated by beetles, which forage within the fruiting body (Ingold 1953).

A common adaptation among the hyphomycetes (filamentous microfungi or molds) is the production of asexual spores, or conidia, in slimy droplets. These sticky masses of spores adhere to the legs and bodies of a variety of arthropods as they move across a mold-contaminated surface. Some molds will produce a large slimy droplet, often on the top of an elongated stalk. Molds such as *Graphium, Leptographium, Pesotum, Stilbella*, *Stachybotrys, Gliocladium,* and *Myrothecium* have complex conidiophores and employ this mode of dispersal (Seifert 1985, Wingfield et al. 1993, Abbott 2000,). These large, complex structures arise vertically from the substratum, and are tall enough to contact large insects migrating over the surface. Other molds, including *Trichoderma, Acremonium, Gliomastix, Fusarium,* and *Verticillium,* produce large numbers of small droplets at the apex of simple conidiophores (Carmichael et al. 1980, Ingold 1953). The conidiophores are produced in various orientations throughout the mycelium, and are particularly effective in contacting small insects moving through a mycelial mass. Spores of these molds typically have a slimy or mucilaginous coat, enabling them to adhere together in masses. Additionally, the spores are often surrounded by sugary secretions with attractive odors to entice insects (Webster 1980). The consistency and solubility of the slimy spore masses varies considerably among the various species, and ranges from extremely viscous to watery forms (Seifert 1985, Wingfield et al. 1993). Various arthropods have been implicated in dispersal of different fungi. Bark beetles (e.g., *Dendroctonus, Ips*) are well known for their involvement with dispersal of sap-stain fungi, both the ascospores and the conidia of the imperfect stages (e.g., *Leptographium, Pesotum*)(Upadhyay 1981, Wingfield et al. 1993). Other insects include flies, springtails and larval stages of various groups. Mites are also frequent colonizers of mold-

contaminated substrata. Fungus mites (e.g., *Tyrophagus*) feed on the mycelium and spores. Faecal material, or frass, of insects and mites is often packed with fungal spores, often appearing intact and unaffected by passage through the arthropods (Abbott, 2000).

Ascomycetes that rely upon insects for spore dispersal often exhibit a similar suite of features. Characters include loss of forcible spore discharge and evanescent asci, sticky ascospores, and long-necked perithecia (Cassar and Blackwell 1996). In many of these genera, the ascospores are extruded from the ascocarp neck in a droplet or sticky mass. The spore masses, called cirrhi, of genera such as *Microascus, Petriella,* and *Chaetomium* are long, sticky columns, resembling a squeezed tube of toothpaste. In these examples, dispersal is achieved in the same manner as described above for the sticky spored molds; i.e., adherence to arthropods moving throughout the fungus colonized substratum (Arx et al. 1986, Abbott 2000). In the sap-stain fungi or lumber molds (Ophiostomatales), the fungi frequently colonize the wood in the galleries of bark beetles. Ascospores are formed in wet droplets at the flared apex of the perithecial necks in *Ophiostoma, Ceratocytis,* and *Sphaeronaemella*. The long necked ascocarps and long stalked conidiophores project into the insect passageways and effectively force the insects into contact with the spore masses as they pass through the restricted spaces (Upadhyay 1981, Wingfield et al. 1993). Other ascomycetes employ insects in different ways. In some of the Onygenales (e.g., *Myxotrichum, Auxarthron, Gymnoascus*), the ascocarp peridium is composed of a mesh-like arrangement of thick-walled hyphae, and the structure is often ornamented with hooks and spines (Currah 1985). These attach to insects and other animals in much the same manner as plant burrs adhere to animal hair. As the insects move about, the spores are shaken out of the meshwork of the peridium and effectively disperse the ascospores to new substrata. In *Chaetomium*, the elaborately undulate, branched, hooked and coiled setae on the ascocarps may serve a similar

function. The ascospores adhere in mass to the setae, and are dispersed by beetles, ants, mites and other animals (Arx et al. 1986).

Some dry-spored molds may make use of insects in addition to air currents as a means of dispersal. The genus *Cephalotrichum* produces its spores in a dry head at the apex of a complex conidiophore or synnema. These synnemata are often up to a millimeter in height and are produced at right angles to the substratum surface (i.e., erect). The fused hyphae of the synnematal stalk provide resilience, and have been demonstrated to spring back into the upright condition when gently manipulated in the laboratory (Abbott 2000). As the insects move through the miniature forest of a sporulating colony and brush against the synnemata, small clouds of spores are released, effectively dusting the insect with spores. . A similar strategy may be employed by some myxomycetes (slime molds). The dry spores of their reproductive stage may be produced in similar stalked structures (e.g., *Stemonitis*) and are dispersed by tiny slime mold beetles (e.g., *Anisotoma, Agathidium*), which live and feed in the fructifications (Stephenson and Stempen 1994).

2.2.1 Bees and the dispersal of fungal spores

In addition to pollen, honey bees can transfer fungal spores and bacteria among flowers of different plant species (Batra et al, 1973; Harrison et al., 1980; Sandhu and Waraich, 1985). The ability of bees to vector microbes has been harnessed for biological control purposes, by using honey bees and bumblebees to transfer inoculum of fungi, bacteria and viruses from the hive to flowers (Kevan et al, 2003). In practice, a dispenser (or insert) is attached to the hive and loaded with a powder formulation of the desired agent. The intent is for foragers exiting the hive to become dusted with the agent and to deliver it to the target crop. The technique has been applied

to control fire blight (Erwinia amylovora) in orchards (Thomson et al., 1992; Johnson et al., 1993; Matthews, 1997; Cornish et al., 1998) and gray mold (Botrytis cinerea) in strawberry and raspberry (Peng et al., 1992; Sutton, 1995; Yu and Sutton, 1997; Maccagnani et al., 1999; McCandless, 1999; Kovach et al., 2000).

Results from Gauvreau et al (1996) on the assessment of worker exposure over wintering beekeeping facilities showed that airborne fungi in honeybee overwintering and equipment cleaning facilities were enumerated and identified to determine worker exposure during cleaning and routine beekeeping operations. Testing was prompted by observations of extensive mold growth on dead bees and associated material and by results of a preliminary study at one Alberta beekeeping facility that showed very high numbers of mold colonies on air samples taken during worker activity. To evaluate whether high mold counts were indicative of a problem at a single site or were industry wide, approximately 120 air samples were collected with a Reuter centrifugal sampler inside 10 overwintering facilities before and during routine beekeeping activity during fall, winter, and spring periods. A set of 30 samples was collected from 15 sites used for annual equipment cleaning. This study showed that average spore counts per overwintering site ranged from 238 to 1442 colony-forming units (CFU)/m^3 prior to disturbance by workers and from 2200 to 13,931 CFU/m^3 while workers swept up dead bees. Levels of airborne molds recovered during annual cleaning of beekeeping equipment ranged from 300 to 54,700 CFU/m^3 with an average of 16,083 CFU/m^3. Potentially toxigenic, pathogenic, or allergenic molds were recovered at all sites.

2.3 EFFECT OF SAMPLING HEIGHT ON THE CONCENTRATION OF AIRBORNE FUNGAL SPORES.

Human exposure to aeroallergens usually occurs close to ground level at approximately 1.5 m. By contrast, airborne fungal spores and pollen are commonly collected from the outdoor air by fixed spore trap samplers on the roof of high buildings (often 10 to 30 m or more above ground). Sampling airborne fungal spores at these heights may underestimate spore concentrations of some important aeroallergens. Thus, it may be necessary to have other sites at the ground or human respiration level (1.5 m above the ground) to detect the concentrations of pollen and fungal spores at this level (Khattab and Levetin, 2008).

The atmosphere is characterized as being layered. It is known that atmospheric properties, such as barometric pressure, density of the air, and temperature decrease with increasing height above the ground level. These changes may affect the bioaerosols. The troposphere region is the lower layer of the atmosphere that extends from the ground up to a height of approximately 10 km. The temperature decreases as the height increases in the troposphere, and theoretically, in stable conditions near the ground level, spore concentrations decrease logarithmically with increasing height. Selection of sampler location and height is important in studying bioaerosols. There is a general agreement for using rooftops for sampling outdoor bioaerosols, because the registration is considered to be representative of bioaerosols in the region and away from the effect of local sources and possible sources of air pollution. In addition, it is high enough to avoid vandalism and bothering neighbors with sampler noise. However, a standard sampling height has not been documented. On the other hand, the issue of the suitable height of air samplers has been studied. Differences in pollen concentrations at different heights have been observed. Some studies showed that the sampling height affected pollen count, and high concentrations of some taxa

were found at lower sampling heights, depending on the source and the size of pollen grains. Several researchers interested in studying fungal aeroallergens in the indoor or outdoor air collected samples using various sampling methods from ground level to human breathing height. However, few studies have compared the concentration of outdoor airborne fungal spores at different heights (Khattab and Levetin, 2008).

Previous studies (Khattab and Levetin, unpublished data, 2005 contained in Khattab and Levetin, 2008) compared outdoor airborne fungal spore concentrations of some taxa in Tulsa and other sampling sites in northeast Oklahoma. The sampling heights were rooftop level (height of 12 m) in Tulsa and human breathing level (height of 1.5 m) in open fields in the other cities. Significantly higher concentrations were found of some spore types at 1.5 m than rooftop level.

Results of work carried out by Gilbert and Reynolds (2005) on airborne spores in the Canopy and Understory of a Tropical Rain Forest at Panama showed that spore density was consistently much greater in the understory than in the canopy. For all canopy–understory sample pairs taken within 10 min (N = 60 pairs), there was a mean of 6.06, standard deviation (SD) 0.51 log10 (spores)/m^3 in the understory and 4.95, SD of 1.02 log10 (spores)/m^3 in the canopy (paired t = 11.9, $P \leq 0.0001$). On average, there were 52-fold more spores in the understory than in the canopy (range 1.3–12times). They stated that greater spore abundance near the ground may reflect a greater abundance of fungi in the understory, the contribution of fungi active in litter decomposition, as well as better environmental conditions for spore production.

More recently, on Barro Colorado Island in Panama, Gilbert (2002) and Arnold and Herre (2003) exposed sterile Petri dishes with fungal growth media to the understory air for short periods and counted the number of colonies that grew. Gilbert (2002) estimated that 9.3 colony-forming units (CFU) /cm^2/h in the understory air. Arnold and Herre (2003) found similar spore densities, and

noted that sporefall was five-fold greater in the forest understory (about 15 CFU/cm^2/h) than in a forest clearing (3 CFU cm^2/h). The difference between understory and forest clearing, as well as the present finding of strong differences between the canopy and understory separated by less than 30 m, indicate strong gradients in spore abundance and suggest that fungi dispersal are likely to be limited in tropical forests. Studies of individual fungal species are needed to evaluate the scale of dispersal limitation for fungi with different spore dispersal mechanisms

Concentrations of bacteria and fungi were measured by Fulton (1966a) at heights of up to 3127m. He found that at low altitude, the concentrations of bacteria and fungi were similar but at the maximum altitude bacteria formed 90% of the bioaerosol. In further flights Fulton (1966b) measured concentrations of bacteria and fungi at heights of up to 2500m through frontal zones. Precipitation reduced concentrations, while high levels of turbulence increased concentrations especially when dust was raised. When dust was present the concentration of bacteria exceeded that of fungi at all altitudes. When the ground was wet the concentration of fungi exceeded that of bacteria.

In unstable air, fungal spore concentrations were found by Hirst et al (1967) to decrease roughly logarithmically with height, but stable layers of air were associated with abrupt changes of concentration. When the air had travelled away from land over sea the concentration profiles were found to be 'eroded' near the surface (Hirst et al 1967).

2.4 Diurnal variation in fungal concentration

A number of authors report measurements of airborne bioaerogens over periods of less that 24 hours. Short period measurements allow the effects of diurnal changes in the meteorological conditions on concentrations to be considered in addition to the effects of particular

meteorological events. These diurnal changes can frequently be greater than the day to day changes in meteorological conditions. A predictive model for the concentrations of *Cladosporium spp*. spores and basidiospores in the atmosphere was developed by Stephen et al, (1990). They found that most of the variability was diurnal. After removing the diurnal effect, including temperature and rainfall as inputs to the model did not improve predictions.

Results of a work carried out by Carinanos et al (1999) on diurnal variation of biological and non-biological particles in the atmosphere of Cordoba, Spain showed that with regards to diurnal pattern of fungal spores, the maximum peaks were achieved in the afternoon, although at different hours from year to year; 19.00h in 1990, 22.00h in 1991, and 16.00h in1992.They also recorded that both pollen grains and spores showed their minimum early in the morning, from 5.00h to 7.00h. Other authors have found out a single peak of fungal spores throughout the day (Carinanos et al, 1999).Gregory (1973) reported that increase in fungal spores detected in the morning, between 8.00-9.00h, may be due to the release of spores after night interruption.

Results from Gilbert and Reynolds (2005) on airborne spores in the Canopy and Undrestory of a Tropical Rain Forest at Panama, showed that within each forest stratum, spore density showed strong daily patterns, increasing sharply around sunset, remaining high throughout the night, and then declining sharply shortly after sunrise. In the understory, there was an average of 6.36, Standard deviation (SD) of 0.23 log10 (spores)/m^3 at night (1800–0600 h) compared to only 5.6, SD of 0.8 log10 (spores)/m^3 (unequal variance *t*-test, $t = 8.21$, $P \leq 0.0001$). In the canopy, there was an average of 5.91, SD of 0.34 log10 (spores)/m^3 during at night compared to 4.37, SD of 0.73 log10 (spores)/m^3 during the day ($t = 14.3$, $P \leq 0.0001$). These differences correspond to a 5.7-fold greater abundance of spores at night in the understory and a 34.7-fold greater abundance in the canopy. In agricultural systems, many pathogenic fungi have been shown to have periodic

spore release. For a number of species, few spores are found in the air at night, with a sharp increase in spore release in the early morning after a moist night during which spores are produced (Langenberg et al. 1977, Couture and Sutton 1978). Other plant pathogenic fungal species release most spores later in the day (Leach et al. 1977, Raynal 1990, Carisse and Philion 2002), in the late afternoon (Hock et al. 1995), or at night (Warner and Braun 1992, Stensvand et al. 1998, Fernando et al. 2000). In studies over sugar cane fields, Bhagawan and Pande (1988) found that fungal species differed in whether they produced spores primarily at night or in the early morning. Variation in periodicity likely reflects how different fungal species respond to environmental conditions for spore production, different mechanisms of spore release, and different environmental regimes among habitat types.

The spatial and temporal patterns observed in this tropical forest are consistent with fungal spores being produced and dispersed under those conditions generally most adequate for spore germination and plant infection. In particular, extended periods of free water on leaf surfaces from dew formation or remaining following rains is often essential for successful germination and leaf penetration (Bradley et al. 2003).

In seasonally moist tropical forest at Ft. Sherman, Panama, Hutton and Rasmussen (1970) measured air spore abundance in the forest understory or above the canopy, at different times of the day (1200, 1600, and 1900 h), and in wet or dry seasons. They placed sterile Petri plates inside horizontal 13cm diameter tubes and exposed them for one hour, overlaid the exposed plates with growth media, and counted developing fungal colonies. They used the same methods for a single mid-day sample in the canopy and understory in the forest at El Verde, Puerto Rico and made the following findings; In Panama, in both wet and dry seasons they found 1.03- to 23.7-fold more fungal spores in understory air than above the canopy. In Puerto Rico, they

captured 2-fold more spores in the understory than in the canopy. During the wet season they also found about 1.7-fold more spores at night (1900 h) than during the day (1200 h).

2.5 DISTANCE TRAVELLED BY FUNGAL SPORES

Sesartic and Dallafar (2011) stated that for transport behavior of fungal spores, most of the spores do not travel very long distances. As calculations by Gregory (1962) have shown only a fraction of about 10% of all released fungal spores is transported farther away than 100m.This fraction is called the "escape fraction". The measured concentrations are a blend of local emissions and advected spores. It is difficult to distinguish between those two groups. A possible distinction criterion might be the size or shape since larger particles are deposited more easily than smaller ones. However, Heald and Spracklen (2009) noted that the larger size fraction is less well investigated due to measurement device constraints. The farther away the sampling device is from the spore source, the more is the measured concentration influenced by deposition and other processes. This can lead to devices in immediate proximity to the ground measuring the actually emitted spore numbers, whereas other devices on higher levels might measure the escape fraction only. As most observational data was taken further away from the ground, we assume our estimate to represent the escape fraction.

Wind speed, temperature, atmospheric pressure or precipitations are important conditions determining transport and deposition of the dispersed aerosols (Hirst et al., 1967). There is evidence that fungal spores can also be transported over long distances (Griffin et al., 2006, 2001; Prospero et al., 2005) before they are deposited either due to gravity, washout by rain or impaction (Gregory, 1967). Among others, Prospero et al. (2005) found fungal spores originating

from the African desert to influence the prevailing fungal spore concentrations on the Virgin Islands in the Caribbean.

According to Gover (1999), the concentration of spores in air may be thought of as being inversely proportional to the distance from the source of production due to the effects of dilution. For many air-borne fungi the horizontal concentration of spores in air normally approaches zero at about 100 m to 200 m from the source of liberation. The vertical concentration of spore in air for some species of fungi has been found to decrease logarithmically with height above the ground. Despite most fungal spores being deposited within about 200m from their point of liberation, there is considerable evidence that some spores travel long distances (eg. spores of *Puccinia gramminis,* the wheat stem rust fungus, from Australia to New Zealand)

Stephen et al (2010) analyzing bioaerosol emissions from waste composting sites at different locations in the United Kingdom recorded the following results:

At site A, during shredding activity, concentrations of fungi were lower upwind compared to downwind, but when no shredding was taking place the upwind concentration of fungi was similar to the maximum downwind, 229 cfu/m^3 of air compared to 324 cfu/m^3 of air.

The upwind value when no activity was taking place, 229 cfu fungi /m^3 of air, was greater than the upwind value of 96 cfu fungi /m^3 of air measured during shredding, showing the variable nature even of a background value.

Fungal counts during shredding activity were greater at a distance of 125m compared to 50m from the waste being handled, with mesophilic fungi and *A. fumigatus* at concentrations up to 2,095 and 2,119 cfu/m^3 of air respectively at the 125m sampling site, compared to 158 and 45 cfu/m3 of air respectively at the 50m sampling site.

Total microbial counts measured by direct microscopy also increased with distance to125m.

At site B, concentrations of micro-organisms from the fixed point Partisol samplers (PM10) and Andersen samplers yielded concentrations of airborne fungi as high as 44,132 cfu/m^3 of air 10m downwind of waste shredding activities and 36,615 cfu/m^3 at 50m downwind, declining to a maximum of 7,361 cfu/m^3 250m downwind. *A. fumigatus* concentrations were as high as 43,818 cfu/m^3 at 50m downwind but showed a particularly high count of 222,048 cfu/m^3 of air at the 140/150m sampling point with the Partisol sampler, reflected but at a smaller count with the Andersen sampler. This suggested a sustained elevated bioaerosol detected with the sampler operating for a longer period also picked up to some extent by the short sampling period of the Andersen sampler.

At site C, concentrations of micro-organisms from the fixed point Partisol samplers (PM10) yielded similar concentrations of airborne fungi (1875 and 1887 cfu/m^3 of air) 10m downwind of the waste screening site whether work was being done or not. By comparison, upwind yields were 758 cfu/m^3 of air when screening was being done but 1083 cfu/m^3 with no activity. Airborne fungal concentrations declined to 1663 cfu/m^3 at 50m downwind and 1119 cfu/m^3 of air at 150m downwind when activities were taking place. With no activity, fungal concentration declined to 1589 cfu/m^3 of air at 50m and 1341 cfu/m^3 at 150m downwind. The higher overall levels with no activities taking place indicated a continuing bioaerosol source from other site activities.

At site D, concentrations of micro-organisms from the fixed point Partisol samplers (PM10) and Andersen samplers yielded concentrations of airborne fungi as high as 21,591 cfu/m^3 of air 10m downwind of compost turning activities, declining to 5,546 cfu/m^3 250m downwind compared to 2,418 cfu/m^3 upwind. *A. fumigatus* concentrations were as high as 5,000 cfu/m^3 at 10m

downwind, declining rapidly to 58 cfu/m^3 of air at 250m downwind compared to 12 cfu/m^3 upwind. Degbor (2000) recorded more fungal spores in the immediate surrounding of an apiary at Tema, Ghana than at a distance 100m away.

2.6 HEALTH EFFECTS OF FUNGI

Air pollution is an important environmental issue, which seriously affects the human health, and airborne fungi are most significant natural pollutants and pathogenic under specific circumstance (Abdel Hafez, 1984, Shelton et al, 2002). Elevated level of particle air pollution are associated with a decreased lung function, increased respiratory symptoms as coughing, shortness of breath wheezing and asthma attacks as well as chronic obstructive pulmonary disease. Cardiovascular disease, systemic mycosis in predisposed persons and lung cancer (Halonen et al, 1997, Hargreaves et al, 2003).

Researchers believed that, more than 80 genera of fungi are associated with symptoms of respiratory track allergies (Homer et al, 1995). Over 100 species of fungi were involved with serious human and animal infection whereas many other species caused serious plant diseases (Cvetnic et al, 1997). *Alternaria, Clasdosporium Aspergillus, Penicillium* and *Fusarium* were among the most common allergenic genera, for instance, elevated concentration of *Cladosporium* were usually associated with respiratory symptom (Su et al, 1992). Higher concentration of *Cladosporium* and *Penicillium* indoor may cause allergy (Li et al, 1995). Reponen et al, (1996) reported that the deposition of fungal spores in lungs and their effects on human health not only depended on their composition and concentration but also size.

First finding by Chartz Blacky showed that fungi spores have related asthma and allergic disease that cause by *Chaetomium* and *Penicillium* (Rippon, 1988). Larger spores (>10µm) were deposited in the upper airway like nose, pharynx and might result in high fever symptoms. While smaller spores (<5µm) could penetrate the lower airways and might lead to asthma and allergens (Homer et al, 1995).

Gregory, (1973) reported that fungi and bacteria contaminate water and pollute air and cause many human diseases. Spores of fungi present in the air also cause a number of allergic reactions (Al-Doory 1984 and Sanches et al., 1999). Ebner et al, (1992) reported that for effective treatment of allergies, knowledge of the airborne fungal spores is of great importance.

Geiser et al., 2000 reported that the interaction of fungal spores with lung structures may be important for the development of respiratory allergies induced by air borne fungal allergens Apart from their direct allergenic effect; fungi may carry mycotoxins in their spores or produce volatile metabolites (Miller, 1998). Inhalation of mycotoxins such as aflatoxins, secalonic acid, zearalenone and tricothecenes produced by *Aspergillus*, *Penicillium* and *Fusarium* spp, may affect the immunological response of the lung tissues or cause other hazards to human health (Gerberick, 1984). Fungi grow on structural materials where humidity is high or on food and stored products where there is condensation. Humidifiers, air-conditioning systems, carpeting and damp walls are the potent sources of indoor fungal allergens. Outdoor fungi such as species of *Alternaria, Aspergillus, Botrytis, Cladosporium, Curvularia, Epicoccum, Fusarium, Penicillium, Phoma, Trichoderma* etc., have been reported regularly from damp indoors. *Aspergillus, Eurotium and Penicillium* have been observed to colonize food scraps and other organic materials (Toqeer et al, 2009)

Many fungi (e.g., species of *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Memnoniella*) in addition to SC can produce potent mycotoxins, some of which are identical to compounds produced by SC. Mycotoxins are fungal metabolites that have been identified as toxic agents. For this reason, SC cannot be treated as uniquely toxic in indoor environments. People performing renovations/cleaning of widespread fungal contamination may be at risk for developing Organic Dust Toxic Syndrome (ODTS) or Hypersensitivity Pneumonitis (HP). ODTS may occur after a *single heavy* exposure to dust contaminated with fungi and produces flu-like symptoms. It differs from HP in that it is not an immune-mediated disease and does not require repeated exposures to the same causative agent. A variety of biological agents may cause ODTS including common species of fungi. HP may occur after repeated exposures to an allergen and can result in permanent lung damage. Fungi can cause allergic reactions. The most common symptoms are runny nose, eye irritation, cough, congestion, and aggravation of asthma.

Fungi are present almost everywhere in indoor and outdoor environments. The most common symptoms of fungal exposure are runny nose, eye irritation, cough, congestion, and aggravation of asthma. Although there is evidence documenting severe health effects of fungi in humans, most of this evidence is derived from ingestion of contaminated foods (i.e., grain and peanut products) or occupational exposures in agricultural settings where inhalation exposures were very high. With the possible exception of remediation to very heavily contaminated indoor environments, such high-level exposures are not expected to occur while performing remedial work (D' Andrea, 2002).

Inhalation of fungal spores, fragments (parts), or metabolites (e.g., mycotoxins and volatile organic compounds) from a wide variety of fungi may lead to or exacerbate immunologic

(allergic) reactions, cause toxic effects, or cause infections. There are only a limited number of documented cases of health problems from indoor exposure to fungi. The intensity of exposure and health effects seen in studies of fungal exposure in the indoor environment was typically much less severe than those that were experienced by agricultural workers but were of a long-term duration. Illnesses can result from both high level, short-term exposures and lower level, long term exposures. The most common symptoms reported from exposures in indoor environments are runny nose, eye irritation, cough, congestion, aggravation of asthma, headache, and fatigue. The presence of fungi on building materials as identified by a visual assessment or by bulk/surface sampling results does not necessitate that people will be exposed or exhibit health effects. In order for humans to be exposed indoors, fungal spores, fragments, or metabolites must be released into the air and inhaled, physically contacted (dermal exposure), or ingested. Whether or not symptoms develop in people exposed to fungi depends on the nature of the fungal material (e.g., allergenic, toxic, or infectious), the amount of exposure, and the susceptibility of exposed persons. Susceptibility varies with the genetic predisposition (e.g., allergic reactions do not always occur in all individuals), age, state of health, and concurrent exposures. For these reasons, and because measurements of exposure are not standardized and biological markers of exposure to fungi are largely unknown, it is not possible to determine "safe" or "unsafe" levels of exposure for people in general. (D' Andrea, 2002).

2.6.1 Immunological Effects

Immunological reactions include asthma, HP, and allergic rhinitis. Contact with fungi may also lead to dermatitis. It is thought that these conditions are caused by an immune response to fungal agents. The most common symptoms associated with allergic reactions are runny nose, eye

irritation, cough, congestion, and aggravation of asthma.11, 12 HP may occur after repeated exposures to an allergen and can result in permanent lung damage. HP has typically been associated with repeated heavy exposures in agricultural settings but has also been reported in office settings. Exposure to fungi through renovation work may also lead to initiation or exacerbation of allergic or respiratory symptoms. (D' Andrea, 2002).

2.6.2 Toxic Effects

A wide variety of symptoms have been attributed to the toxic effects of fungi. Symptoms, such as fatigue, nausea, and headaches, and respiratory and eye irritation have been reported. Some of the symptoms related to fungal exposure are non-specific, such as discomfort, inability to concentrate, and fatigue.Severe illnesses such as ODTS and pulmonary hemosiderosis have also been attributed to fungal exposures. ODTS describes the abrupt onset of fever, flu-like symptoms, and respiratory symptoms in the hours following a *single, heavy* exposure to dust containing organic material including fungi. It differs from HP in that it is not an immune-mediated disease and does not require repeated exposures to the same causative agent. ODTS may be caused by a variety of biological agents including common species of fungi (e.g., species of *Aspergillus* and *Penicillium*). ODTS has been documented in farm workers handling contaminated material but is also of concern to workers performing renovation work on building materials contaminated with fungi.Some studies have suggested an association between SC and pulmonary hemorrhage/hemosiderosis in infants, generally those less than six months old. Pulmonary hemosiderosis is an uncommon condition that results from bleeding in the lungs. The cause of this condition is unknown, but may result from a combination of environmental contaminants and conditions (e.g., smoking, fungal contaminants and other bioaerosols, and

water-damaged homes), and currently its association with SC is unproven. (D' Andrea, 2002).

2.6.3 Infectious Disease

Only a small group of fungi have been associated with infectious disease. Aspergillosis is an infectious disease that can occur in immunosuppressed persons. Health effects in this population can be severe. Several species of *Aspergillus* are known to cause aspergillosis. The most common is *Aspergillus fumigatus*. Exposure to this common mold, even to high concentrations, is unlikely to cause infection in a healthy person. Exposure to fungi associated with bird and bat droppings (e.g., *Histoplasma capsulatum* and *Cryptococcus neoformans*) can lead to health effects, usually transient flu-like illnesses, in healthy individuals. Severe health effects are primarily encountered in immunocompromised persons (D' Andrea, 2002).

2.7 THE INFLUENCE OF CULTURE MEDIA ON GROWTH OF FUNGI

Sharma and Pandey (2010) undertook a study to observe the influence of three different culture media namely Potato Dextrose Agar (PDA), Czapek's Dox + Yeast Extract Agar(CYA) and Lignocellulose Agar (LCA) on the mycelial growth, colony characters and sporulation patterns of ten dominant fungi isolated from decaying vegetable wastes.

They recorded that all three culture media supported the growth of test fungi to various degrees. Out of them, six fungi showed maximum mycelial growth on LCA after 7 days of incubation period, while *Penicillium sp.* (32.0±2.6 mm) and *Aspergillus* (26.3±2.3 mm) exhibited higher colony growth on PDA. *Chaetomuim funicola* (58.3±1.2 mm) and *Fusarium oxysporum* (85.7±0.3 mm) showed maximum growth on CYA medium. High growth rate of *F. oxysporum*

(85 mm) in Czapek's Dox agar, after incubation period, has also been observed by Farooq et al, (2005).

Sharma and Sharma (2011) studied the effect of culture media on some test fungi. They used eight broth media (Hi media, Bombay) namely; Sabouraud dextrose medium (SDM), Yeast extract medium (YEM), Richards medium (RM) Czapek medium (CzM), Glucose phosphate medium(GPM), Mannitol Browth medium(MBM) and ehight agar media namely(Hi media, Bombay) namely; Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA), Corn meal agar (CMA), brain heart infusion agar (BHIA), Potato Carrot agar (PCA), Mannitol salt agar (MSA), Czepex agar (CDA) and Chocolate agar (CA). They made the following conclusions; that among all the broth media studied, Sabourauds Dextrose agar medium (SDM) was found to be the more suitable for maximum growth and sporulation of soil isolates of fungi. *Trichophyton mentagrophytes* (0.452m) and *Microsporum gypseum* (0.230gm) showed maximum growth and sporulation on Sabouraud Dextrose medium followed by Potato Dextrose medium.

The maximum growth of *Microsporum gypseum* was observed on Sabouraud Dextrose agar while the least growth was observed on Potato Dextrose agar. The two media Potato carrot agar and Chocolate agar showed similar growth and both are found to be less suitable for fungal growth.

Ogunlana (1974) studied fungal air spora at Ibadan, Nigeria using two culture media namely; Sabouraud dextrose agar (SDA) and malt agar (MA) to collect the samples. He observed that there were no marked differences with the total monthly numbers of isolated colonies between the two media but observed a particularly marked difference with the fungus *Pithomyces*, which was better isolated with MA than SA.

CHAPTER THREE

MATERIALS AND METHODS

3.0 PROFILE OF THE STUDY AREA

The University for Development studies is located in the Tolon/Kumbungu District.

The Tolon/Kumbungu District Assembly is one of the 45 districts created by the erstwhile Provisional National Defense Council (PNDC) Law 207 in 1988 with Tolon as its Capital.

The District covers an area of about 2,741 square kilometers and forms about 3.9% of the total landmass of the Northern Region.

The district lies between latitudes 9^0 15` and$10^0$ 02`North and longitudes 0^0 53`and$1^0$ 25` West. It shares boundaries with West Mamprusi District to the North, West Gonja District to the South-West, Savelgu-Nanton District to the East and Tamale Metropolis to the South- East.

The indigenous people are Dagombas who constitute more than 80% of the District population. Though one can still find other tribes like Gonjas, and Ewes who do fishing along the White Volta.

The vegetative cover is basically Guinea Savanna interspersed with short drought resistant trees and grassland. The land is generally undulating with a number of scattered depressions. The soils are generally of the sandy loam type except in the low lands where alluvial deposits are found. Major tree species include the Shea nut, Dawadawa, Mango, which are economic trees and form an integral part of livelihood of its people.

The total population, according to the 2000 Population and Housing Census stands at 161,160 with the growth rate of 3%. Population density is approximately around 50 inhabitants per Kilometer Square. The current population stands at about 249,691 according to (GWEP TKDA, 2009).

The population density varies from place to place within the District with the Northern part that is across the White Volta sparsely populated. The Southern part is however, densely populated around major towns or settlements like Kumbungu, Tolon, Nyankpala, Lungbunga, Dalun, Karsulyili and Yoggu.

The relatively moderate growth rate creates a strong potential for development since there will be calls for gender consideration in the formulation of development policies in the District especially those that affect women and children.

Nationwide the Northern regions are classified as the poorest regions in Ghana. Tolon-Kumbungu is one of the poorest districts as majority of the people are Peasant and Subsistence farmers who farm on low basis making it difficult to even offer some for sale hence from "farm to the household."

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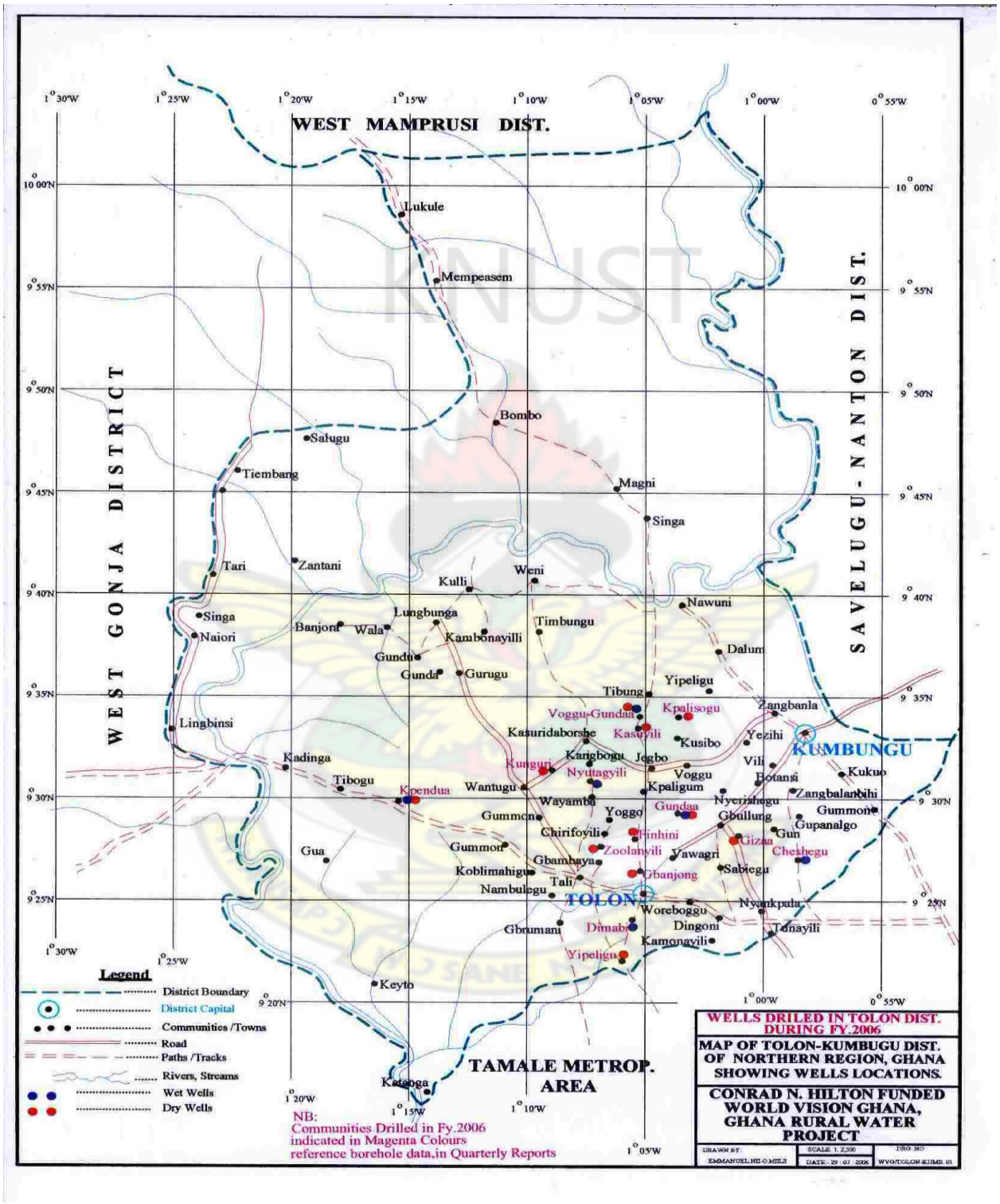

Fig 1. MAP OF TOLON KUMBUNGU DISTRICT

3.1.0 MATERIALS

Potato tubers were purchased from a grocery shop, Dextrose, Agar- Agar, Dehydrated Malt extract agar and Sabouraud agar where obtained from the Microbiology Laboratory of KNUST and some purchased from chemical shops. Distilled water was obtained from the Savanna Agricultural Research Institute (SARI) Laboratory and sometimes purchased from the Ghana Water Company Laboratory-Tamale.

Chloramphinicol added to the media prepared were also purchased from a local pharmacy shop.

3.1.1 GENERAL METHODS

3.1.1.1 Culture media

Three different culture media namely; Potato Dextrose agar (PDA), Malt extract agar (MEA) and Sabouraud agar (SA) were used for sampling. The preparations of the media were as follows:

3.1.1.2 Preparation of Potato Dextrose agar (PDA) (Amsworth and Bisby, 1971)

300g of peeled potato tubers, 10g of dextrose, and 15g of Agar-Agar were weighed and also one (1) liter of distilled water obtained. The peeled potato were cut into pieces and boiled in 400ml water until they started to break up. The suspension was strained with muslin cloth and the extract collected into a one liter measuring cylinder. The extract was then transferred into a two liter Erlenmeyer flask and the agar- agar added. The mixture was then stirred and heated in a water bath to melt the dextrose and agar-agar before the medium was dispensed into 250ml Erlenmeyer flasks. The flasks were plugged with a non- absorbent cotton wool and the plugs were covered with aluminium foil before autoclaving to prevent the entry of water vapour. Autoclaving was done at 121°C at 1.1kg per cm^2 steam pressure for 15 minutes.

3.1.1.3 Preparation of dehydrated Sabouraud agar (SA).

62g of dehydrated Sabouraud agar powder was weighed and emptied into a conical flask.

One liter of distilled water was measured using a measuring cylinder and poured into the conical flask containing the Sabouraud agar. The mixture was then stirred and heated in a water bath to melt the Sabouraud agar before the medium was dispensed into 250ml Erlenmeyer flasks. The flasks were then plugged with a non- absorbent cotton wool and the plugs covered with aluminium foil before autoclaving at 121°C at 1.1kg per cm^2 steam pressure for 15 minutes.

3.1.1.4 Preparation of dehydrated Malt extract agar (MEA).

50g of dehydrated Malt extract agar powder was weighed and emptied into a conical flask. One liter of distilled water was measured using a measuring cylinder and poured into the conical flask containing the Malt extract agar. The mixture was then stirred and heated in a water bath to melt the Malt extract agar before the medium was dispensed into 250ml Erlenmeyer flasks. The flasks were then plugged with a non- absorbent cotton wool and the plugs covered with aluminium foil before autoclaving at 121°C at 1.1kg per cm^2 steam pressure for 15 minute.

100mg of chloramphinicol was added to each of the medium swirled to mix before pouring into Petri plates to prevent bacteria growth.

3.1.2. Methods of Sterilization

All Petri dishes were sterilized at 160°C in an electrically heated oven for 6 hours.

However, sometimes Petri dishes are autoclaved at 121°C at 1.1 kg per cm^2 steam pressure for 15 minutes.

The Laminar flow in the inoculation room was switched on for at least 45 minutes and the inoculating room sprayed with 5% aqueous dettol solution before use.

The culture media were autoclaved at 121°C at 1.1 kg per cm^2 steam pressure for 15 minutes.

3.1.3. Preparation of Petri Plates

Conical flasks with molten PDA, MEA and SA and sterile Petri dishes were taken to the Inoculation Chamber where the media were poured into the Petri dishes. The Petri dishes were then left for the media to solidify. The Petri dishes were packed and enveloped with an aluminium foil to prevent contamination. They were then kept in a refrigerator for at least 24 hours after which they were observed for microbial contaminations.

The media were then put in an ice chest sterilized by cleaning with 70% ethanol and then conveyed to the sampling site.

3.1.4. Method of sampling the airspora

The Petri plate exposure method of sampling was used. In this method Petri dishes containing nutrient media are exposed for a period and then closed. Three (3) Petri dishes each containing one of the three media: Potato Dextrose Agar, Malt Extract Agar and Sabouraud Agar were placed on the hive, another set of three Petri dishes each containing one of the media were placed 100m away from the hive and at different heights: (0.5m) and (2m) above the ground at the sampling points. The media were exposed by taking off their lids for five minutes after which they were covered. These were replicated three times.

Sampling was done weekly every Saturday at three different periods, morning (7.00h), noon (12.00h) and evening (17.00h) at the apiary from March to April 2011.

The Petri plates after sampling were covered with an aluminium foil. They were then packed into ice chest sterilized by cleaning with 70% ethanol and then conveyed to the laboratory (SARI).

3.1.5. Incubation

The Petri plates used for the trapping fungal spores at the Apiary were incubated at room temperature (28°C) for 3days. The colonies were counted and the plates were left for further incubation with daily colony counts made for up to 7days. Counting was done by holding the plates against a source of illumination and point counting with a marker was done to avoid double counting.

3.1.6. Method of Identification of Fungal Colonies

After the 3 to 7 days of incubation, the Petri dishes are repacked into an ice chest sterilized by cleaning with 70% ethanol and sent to the Kwame Nkrumah University of Science and Technology (KNUST) campus for the fungal colonies to be identified. Identification was done at the Pathology Laboratory of the Department of crop and soil science and also confirmed by Mr. J.L. Terlabie (supervisor). The identification of the fungal colonies was done to the generic level and where possible to the species level.

Results obtained were subjected to Analysis of variance (ANOVA) using the Statistix Version 9.0 statistical package and treatment means were compared at P= 0.05 probability levels.

CHAPTER FOUR

RESULTS

The tables of results show the mean values of the various parameters taken at four (3) hives for eight weeks of study.

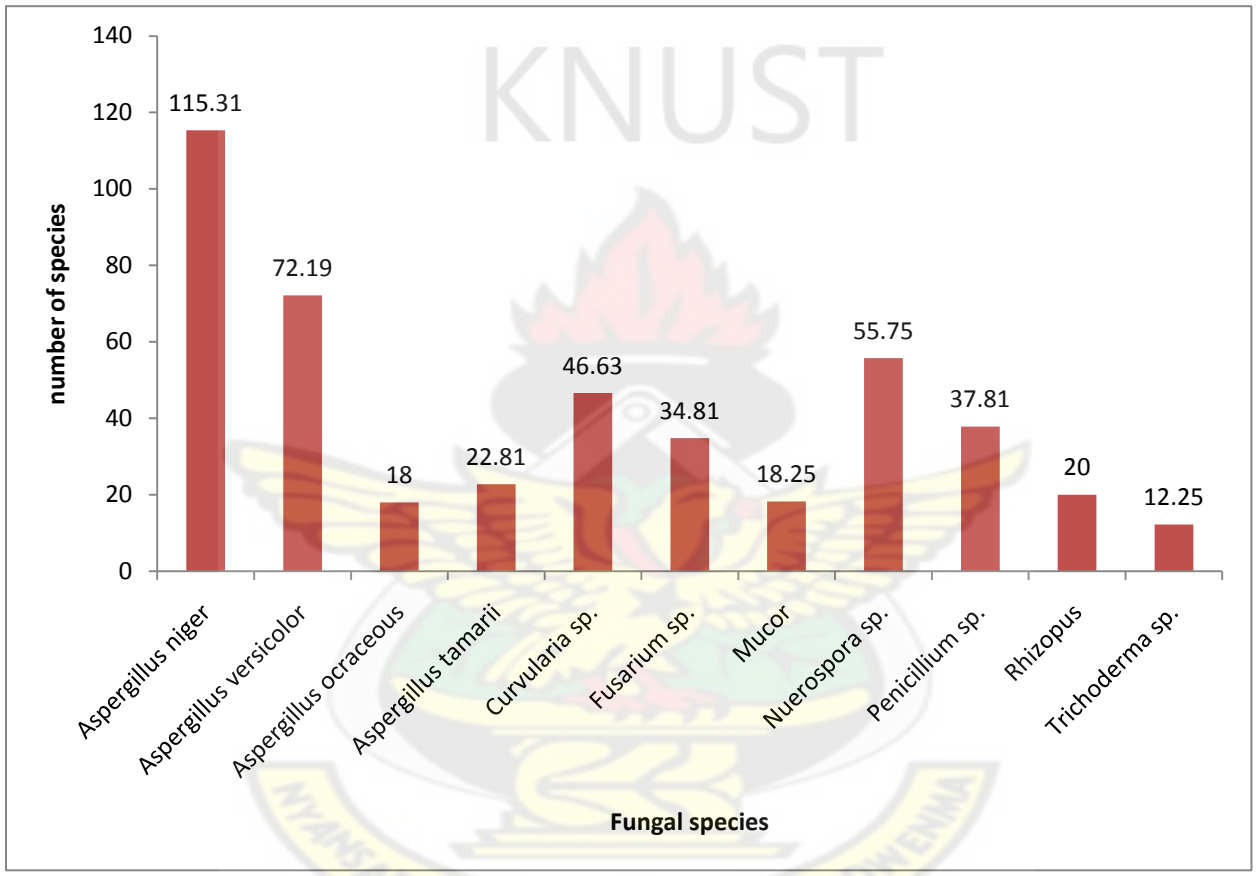

Fig. 2. Mean numbers of fungal species isolated by all three media used for eight weeks

Fig. 2. Above provide information on the different species of fungi isolated and their respective abundances for the eight weeks period of sampling. A total of eleven fungal species were identified at the site of study.

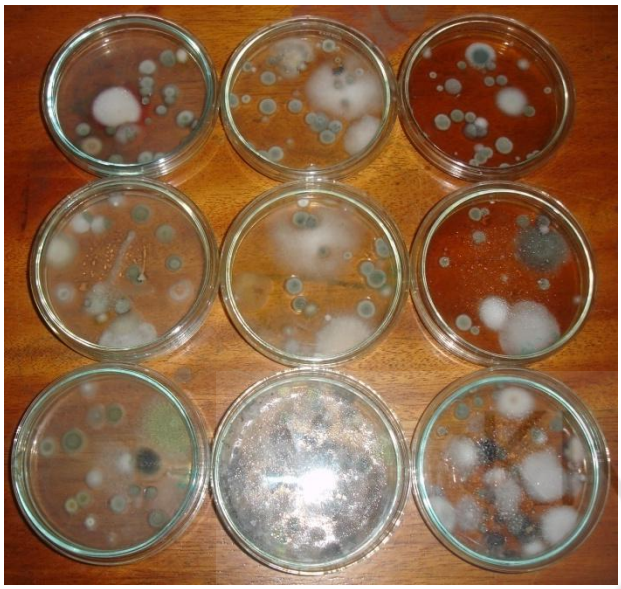

Plate 1. Photogragh of nutient media showing fungi trapped from the atmosphere , 0meter from the apiary. 05/03/2011.

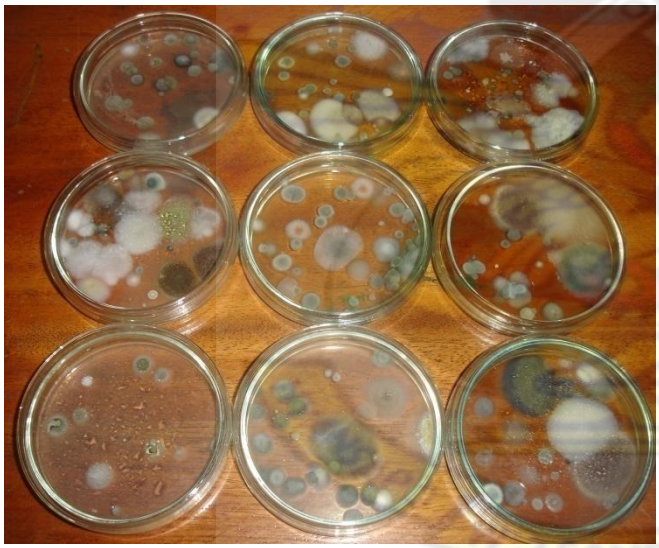

Plate 2. Photogragh of nutient media showing fungi trapped from the atmosphere , 100meters from the apiary. 05/03/2011.

The most abundant species was *Aspergillus niger* (115.31). Other commonly occurring species were, *Aspergillus versicolor*(72.19), *Neurospora sp*(55.75)., *Curvularia sp*(46.63)., *and Penicillium sp*(37.81) . The least abundant species was *Trichoderma sp*(12.25). Other less occurring species were *Aspergillus tamarii*(22.81*), Rhizopus(20), Mucor*(18.25)*, and Aspergillus ocraceous*(18).

Table 1. Mean concentration of fungal colonies isolated in the immediate surrounding (0m) of the apiary and 100m away for eight weeks

Sampling Period	Distance from hives	
	On hive (0m)	100m
Wk 1	261.00	196
Wk2	233.00	180.50
Wk3	220.50	148.50
Wk4	230.50	169.50
Wk5	306.00	277.00
Wk6	305.00	218.50
Wk7	264.50	227.00
Wk8	287.00	199.50

Wk represent week.

The results indicated on Table 1 shows that, there were high concentrations of fungi at zero meters from the hive than at 100m away from the hives for all the periods of study. With the exception of week two ($P>0.05$), all the remaining weeks of the study showed significant difference between the mean concentrations of fungi ($P<0.05$).

Table 2. Mean concentration of fungi captured at 0.5m and 2m above the ground.

Sampling Period	Height above ground	
	Low (0.5m)	High(2m)
Wk1	241.00	216.00
Wk2	212.00	201.50
Wk3	176.00	193.00
Wk4	176.00	203.50
Wk5	196.50	256.50
Wk6	283.00	241.50
Wk7	275.50	214.00
Wk8	240.00	244.50

The data in Table 2 above shows that, the mean fungal concentrations were higher for weeks 3, 4, and 8 for height of 2m above ground whiles higher mean concentration were recorded at a height of 0.5m above ground for weeks 1, 2, 5, 6and 7.The difference in the concentration of fungi at the two heights for each week of sampling were however, not significant ($P>0.05$) except for week 5($P<0.05$).

Table 3. The mean number of different fungal species found at the different heights above ground.

Fungal species	Height above ground	
	Low(0.5m)	High(2m)
Aspergillus niger	57.31	58.00
Aspergillus versicolor	35.50	36.69
Aspergillus ocraceous	10.43	7.57
Aspergillus tamarii	14.42	8.39
Curvularia sp.	22.00	24.63
Fusarium sp.	13.87	20.94
Mucor	8.10	10.15
Neurospora sp.	31.37	24.38
Penicillium sp.	22.31	15.50
Rhizopus	11.69	8.13
Trichoderma sp.	6.15	6.10

The results of the mean count of species captured at the heights of 0.5m and 2.0m above the ground are shown on Table 3 above. The data showed that, *Aspergillus niger*(58), *Aspergillus versicolor*(36.69), *Curvularia sp*.(24..63), *Fusarium* sp.(20.94), and Mucor(10.15) occurred more frequently at a height of 2m above ground than at 0.5m above ground whiles species such as *Aspergillus ocraceous*(10.43), *Aspergillus tamarii*(14.42), *Neurospora sp*.(31.37) , *Penicillium sp*.(22.31) , *Rhizopus*(11.69) *and Trichoderma sp*(6.15*)*. Were more abundant at 0.5m above ground than 2m above ground.

Table 4. The mean concentration of fungal counts at different times of the day for eight weeks.

Sampling Period	Time of day		
	Morning(7.00h)	Noon(12.00h)	Evening(17.00h)
Wk1	140.50	161.00	149.50
Wk2	137.50	132.50	143.50
Wk3	134.00	112.50	122.50
Wk4	130.00	124.50	145.50
Wk5	169.00	167.00	188.50
Wk6	185.00	183.50	163.50
Wk7	144.00	163.50	142.50
Wk8	161.50	148.00	175.00

The results of the mean concentration of the mean fungi isolated for the eight weeks of sampling are shown on Table 4 above. The data shows that, the mean concentrations of fungal spores were highest in the evening for weeks 2, 4, 5, and 8. For weeks 3 and 6, mean concentration of fungal spores were highest in the morning whiles in week 1 and 7 mean concentration of fungal spores were highest frequency in the afternoon. The differences in the mean concentration of the fungi for week 1 to week 7 were not significant ($P>0.05$). However, week 8 showed significant difference ($P<0.05$) between the fungal counts.

Table 5. The mean concentration of fungal species isolated at the different times of the day.

Fungal species	Time of day		
	Morning(7.00h)	Noon(12.00h)	Evening(17.00h)
Aspergillus niger	33.66	38.79	42.86
Aspergillus versicolor	26.75	26.53	19.62
Aspergillus ocraceous	6.88	6.06	5.06
Aspergillus tamarii	7.64	7.39	7.78
Curvularia sp.	15.66	14.48	16.49
Fusarium sp.	9.88	10.74	14.19
Mucor	5.87	5.75	6.63
Neurospora sp.	21.91	16.72	17.07
Penicillium sp.	12.13	13.25	12.43
Rhizopus	7.31	4.56	8.13
Trichoderma sp.	4.00	3.58	4.67

The results on Table 5 above shows that, Aspergillus niger, *Aspergillus tamari, Curvularia sp, Fusarium sp, Mucor, Rhizopus and Trichoderma sp.* occured more frequently in the evening (5.00pm or 17.00h). Species such as *Aspergillus versicolor, Aspergillus ocraceous , and Neurospora sp.* were more common in the morning (7.00am or 7.00h) .There was relatively low abundance of the various species in the afternoon (12.00noon or 12.00h). It is however observable that there was no marked variation between the individual numbers of species for the three different times of which sampling were made.

Table 6. Mean concentration of fungi isolated from the three different media used for the eight weeks

Sampling Period	Media used		
	PDA	MEA	SA
Wk1	178.00	124.00	155.00
Wk2	167.50	121.00	125.00
Wk3	142.00	116.00	111.00
Wk4	133.00	123.00	144.50
Wk5	176.50	157.00	190.50
Wk6	155.50	166.50	174.50
Wk7	156.50	140.00	154.50
Wk8	156.50	141.00	187.00
Maen Total	1265.50	1088.50	1242.00

The data in Table 6 above showed that, the highest number of fungi was isolated from Potato Dextrose Agar (PDA) for weeks 1, 2, 3, 6, and 7, whiles the highest number of fungi were isolated from Sabouraud agar (SA) for weeks 4, 5 and 8.There were comparably low fungal counts on Malt Extract Agar (MEA). The difference in the abundance of fungi isolated from the three media were significant for weeks 1, 2, 3, 5, and 8 ($P<0.05$). However weeks 4, 6, and 7 did not show significant difference ($P>0.05$) between the numbers of fungi isolated from the three different media used.

Table7. Mean concentration of fungal species isolated by the three nutrient media used.

Fungal Species	Nutrient media		
	PDA	MEA	SA
Aspergillus niger	43.46	35.58	36.27
Aspergillus versicolor	26.71	20.53	25.66
Aspergillus ocraceous	7.81	4.63	5.56
Aspergillus tamarii	7.88	7.69	7.24
Curvularia sp.	16.29	15.84	14.50
Fusarium sp.	8.65	12.55	13.61
Mucor	5.50	5.75	7.00
Neurospora sp.	19.56	17.63	18.56
Penicillium sp.	16.88	8.54	12.39
Rhizopus	7.25	3.81	8.94
Trichoderma sp.	3.19	3.31	5.75

The data in Table 7 above showed that, *Aspergillus niger, Aspergillus versicolor, Aspergillus ocraceous, Aspergillus tamarii, Curvularia sp, Neurospora sp., and Penicillium sp* showed more growth on Potato Dextrose Agar (PDA) than on Malt Extract Agar (MEA) and Sabouraud agar (SA). Species such as *Fusarium sp., Mucor, Rhizopus* and *Trichoderma sp*. showed higher growth on SA than on PDA and MEA. There were relatively moderate numbers of growth of all fungal species on all the media used.

CHAPTER FIVE

DISCUSSION

5.1 **Diversity of fungal spores at the study area**

A total of eleven fungal species were isolated on the three different media used. *Aspergillus niger, Aspergillus versicolor, Neurospora sp. and Curvularia sp*. were the most common fungi in the atmosphere at the area of study. This is partly supported by Sharma (2010) who reported that *Aspergillus niger and Aspergillus fumigatus* are some of the most frequent fungi on the leaf surface mycoflora of *Oscinum santam*. Similar observations were made by Uddin (2004) who reported that *Penicillium* and *Aspergillus,* were the most dominant saprophytes in the atmosphere. Afzal and Mehdi (2002) also reported that species of *Aspergillus* were the most prevalent fungus in the atmosphere of Karachi. This is in consonance with the findings of this study.

However, *Cladosporium* which was reported by Fang et al (2005) to be the dominant fungi in Beijing and other studies elsewhere was absent at the site of the study. Hirst and Stadman also made similar report that *Cladosporium* is the most numerous day time spores.

The least abundant species at the study area were *Trichoderma sp., Mucor, Rhizopus* and *Aspergillus tamarii*. Chadeganipour, et al. (2010) reported that the dominant species of airborne fungi in Isfahan were members of the genera *Pénicillium spp., Cladosporium spp., Aspergillus spp., Alternaria spp.* and yeasts and yeast like *Candida spp., Geotrichum spp. and Trichosporon spp.* and the minor components or less prevalent were *Rhizopus spp., Ulocladium spp., Curvularia spp. and Fusarium spp in*. The occurrences of these species are in support of this study although some of the species were not identified in this study.

Khattab and Levetin, (2008) stated that *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Fusarium*, *Penicillium*, smut spores, and *Trichoderma* are some of the common allergenic fungi. Some of these fungi were identified in this study. Apart from their direct allergenic effect, fungi may carry mycotoxins in their spores or produce volatile metabolites (Miller, 1998). According to Gerberick (1984), inhalation of mycotoxins such as aflatoxins, secalonic acid, zearalenone and tricothecenes produced by some of the species identified by this study such as *Aspergillus*, *Penicillium* and *Fusarium* spp, may affect the immunological response of the lung tissues or cause other hazards to human health.

5.2 **Concentration of fungal spores at different distances from apiary**

The total number of fungal species isolated from the beehives for the entire eight weeks were higher than the total number isolated hundred meters away from the beehives. With the exception of week 2, they were no significant difference between the total colony count zero (0) meters from beehives and also hundred meters from the beehives. This is supported by the work of Degdor (2000), who recorded higher fungal count at an apiary at Tema as compared to a distance of 100m away. According to Batra et al (1973) and Sandhu and Waraich (1985), honeybees in addition to pollen can transfer fungal spore and bacteria among flowers of different plant species.

Kevan et al (2003) reported that the ability of bees to vector microbes has been harnessed for biological control purposes, by using honeybees and bumblebees to transfer inoculums of fungi, bacteria and viruses from the hive to flowers.

According to Gregory (1962) and Glover (1999), the concentration of spores in air may be thought of as being inversely proportional to the distance from the source of production due to

the effects of dilution. For many air-borne fungi the horizontal concentration of spores in air normally approaches zero at about 100 m to 200 m from the source of liberation. These could explain the high fungal concentration around the beehives.

Gregory (1958) stated that in calm conditions only about 0.05 percent of spore would be expected to travel further than hundred meters form a source close to the ground; in dull windy weather this increases to about 10 percent. The calm condition of the site of study could therefore also account for low fungal concentration at the site hundred meters from the apiary.

5.3 **Concentration of fungal spores at different heights**

The results on Table 2, showed higher fungal counts at height of 2m above the ground than at height 0.5m above the ground. However, the differences between them for each week were not significant ($P>0.05$) except for week 5 ($P<0.05$). Moreover, species such as *Aspergillus ocraceous, Aspergillus tamarii, Neurospora sp.,* and *Penicillium sp*., were found more frequently at the height of two meters above the ground than at the height of 0.5m above the ground. Aspergillus niger, *Aspergillus versicolor, Curvularia sp., Fusarium sp* and *Mucor* were more frequent at height of 0.5m above ground than at height of two 2m above ground. It is however noticeable that the differences between the individual species at two heights were not significant ($P>0.05$). This is not supported by Khattab and Levetin (2005) who reported significant difference between species such as *Alternaria, Penicillium/Aspergillus* and smut spores at two different heights of 1.5 meters and 30 meters above ground.

Atluri et al (1988) indicated that the concentration of different spore types decreased logarithmically with increasing height (0.15 to 4.72m) above a rice crop in India. The concentration of various spore types decreased in different order at the different heights.

The results of this study were not in consonance with the above observation for some species such as *Aspergillus niger*, *Aspergillus versicolor*, *Curvularia sp.*, *Fusarium sp*. and Mucor. However, the results of this work is supported by Khattab and Levetin who made similar findings when they studied fungal spores at two different heights and recorded higher concentration of *Alternaria*, *Cladosporium*, *Drechslera*, *Nigrospora and Pithomyces* at height of 30m above ground than at 1.5m above ground.

Chakraborty et al (2003) concluded that *Alternaria, Curvularia and Drechslera* spore concentrations were highest at 1m and decreased as the sampling height increased, whereas ascospores, *Aspergillus*-type spores, basidiospores, and Cladosporium spore concentrations increased with increasing heights in all seasons. They generally noticed that the smaller spores were commonly found at the high sampling level and large spores were more common at ground level. The result of this work does not support their results for some spore and analogous for other spore types.

According to Khattab and Levetin (2005) the increase in some fungal spore types and pollen concentration at the ground level may be a result of the effect of one or a combination of several factors. These factors include the proximity to bioaerosol sources (soil and vegetation) at ground, aerodynamic characteristics, size and shape of the sampled bioaerosols, the effect of meteorological conditions or release, dispersal and deposition of fungal spores and pollen and the effect of vertical temperature gradient of the air. These factors could contribute to the results obtained in this study. They also stated that some fungal species live on decaying leaves and dead vegetation on the ground. Spore concentrations of these saprophytic species are expected to be higher near the ground than rooftop level. This may be the reason for the high concentration

of *Penicillium* and some species of *Aspergillus* which are considered as saprophytes at height close to ground.

5.4 **Diurnal variation of fungal spores**

From Table 4, the highest concentration of fungi was recorded in the evening. This is followed by higher frequency in the morning and then in the afternoon in that order.

Carinanos et al (1999) stated that in the diurnal variation pattern for fungal spores, the maximum peaks are achieved in the afternoon although at different hours from year to year, 19.00h in 1990, 22.00h in 1991, and 16.00h in 1992. Considering the time for the peak values, Carinanos et al (1999) findings are consistent with the results is this work.

Carinanos et al (1999) also reported that the increase in the fungal spores in the morning, between 8.00-9.00h may be due to the release of spores after the night interruption (Gregory 1973). This also could explain the higher frequency of fungi recorded in the morning.

The results of this work is also supported by Gilbert and Reynolds (2005) who reported that spore density showed strong daily patterns, increasing sharply around sunset, remaining high throughout the night, and then declining sharply shortly after sunrise.

Hutton and Rasmussen (1970) reported that, there were more fungal spores at night (1900 h) than during the day (1200 h). This is supported by the findings of this work.

In agricultural systems, many pathogenic fungi have been shown to have periodic spore release. For a number of species, few spores are found in the air at night, with a sharp increase in spore release in the early morning after a moist night during which spores are produced (Langenberg et al. 1977, Couture and Sutton 1978). Other plant pathogenic fungal species release most spores later in the day (Leach et al. 1977, Raynal 1990, Carisse and Philion 2002), in the late afternoon

(Hock et al. 1995), or at night (Warner and Braun 1992, Stensvand et al. 1998, Fernando *et al.* 2000). These could be the reason for the variation in the concentration of fungal species at different times of the day.

5.5 **Influence of nutrient media on fungal growth**

The variation of mean fungal counts on the three different nutrient media were significant for weeks 1, 2, 3, 5 and 8.Weeks 4, 6 and 8 did not show significant difference between the number of fungi isolated as shown on Table 6. This is not supported for some of the weeks by Ogunlana (1974) who studying the influence of media on fungal colonies isolated at Ibadan, Nigeria reported that the total number of fungal colonies isolated were not significant in respect of two media used; Sabouraud agar (SA) and Malt agar (MA) as recorded on table 7.

He also reported that certain species especially *Pithomyces sp.*, showed marked difference between the two media showing much growth on MA. In this work the highest number of fungal growth were found on PDA than on MEA and SA.

All three culture media supported the growth of different fungi to various degrees as also observed by Sharma and Pandey (2010) and Sharma and Sharma (2011). Species such *as Aspergillus niger, Aspergillus versicolor, Aspergillus ocraceous, Aspergillus tamarii, Curvularia sp, Nuerospora sp., and Penicillium sp* showed more growth on Potato Dextrose Agar (PDA) This is partly supported by the work of Sharma and Pandey (2010) who reported that *Penicillium sp.* and *Aspergillus* exhibited higher colony growth on PDA.

CONCLUSION

The concentration of honeybees could have influence on the frequency of fungi at a site.

Higher fungal counts were made at a distance zero meters from the hive than at a distance 100 meters from the bee hives.

With regards to the diversity of fungal species at the site of study, eleven fungal species were identified. *Aspergillus niger* was the most commonly occurring fungi at the site whiles *Trichoderma sp*, was the least occurring species.

In the case of effect of time of day on the concentration of fungal species (that is diurnal variation in the concentration of fungi), some fungal species recorded higher concentration in the evening than at noon and in the morning. However, fungal species were more commonly occurring mostly in the evening (17.00hrs) than at noon and morning (7.00hrs).

With regards to the effect of height above the ground, some species of fungi occurred more frequently at height of 0.5m above the ground whiles some fungal species were more frequently occurring at a height of 2m above the ground.

The media used had profound influence on the concentration as well as the diversity of the fungal species. PDA recorded the highest concentration followed by MEA and SA in that order.

RECOMMENDATIONS

Apiaries like other occupational sites such as compost site have high incidence of fungal spores. Workers and people resident in these areas would have to take measures to reduce if not completely prevent fungal infections.

Beekeepers and other workers around beehives would have to put on protective clothes not only against bee stinks but also against exposure to fungi. Such protective clothes should include gloves and a good quality respirator. The pore size of the filters on the respirator must be one (1) micron or less and they should be changed on a regular basis.

The concentration of fungi is known to be diluted to about zero from its source to distances of 100m to 200m. Apiaries should therefore be sited at least 200m away from households to reduce exposure to humans.

People leaving close to apiaries should improve ventilation in their houses.

Further work should be carried out in this area using air samplers (Volumetric Spore Traps) which provide information on the density of airspora.

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APPENDIX

ANALYSIS OF VARIANCE (ANOVA) TABLES.

ANOVA Table 1. Total number of fungal species

Source	DF	SS	MS	F	P
Species	10	2415833	241583	131.41	0.0000
Error	33	60665	1838		
Total	43	2476498			

Grand Mean 330.05 CV 12.99

ANOVA Table 2. Mean number of different fungal species

Source	DF	SS	MS	F	P
Species	10	37747.4	3774.74	131.41	0.0000
Error	33	947.9	28.72		
Total	43	38695.3			

Grand Mean 41.256 CV 12.99

ANOVA Table 3.1. Mean concentration of different species for week 1

Source	DF	SS	MS	F	P
Species	10	65522.9	6552.29	126.60	0.0000
Error	33	1708.0	51.76		
Total	43	67230.9			

Grand Mean 41.545 CV 17.32

ANOVA Table 3.2. Mean concentration of different species for week 2

Source	DF	SS	MS	F	P
Species	10	36693.6	3669.36	68.45	0.0000
Error	33	1769.0	53.61		
Total	43	38462.6			

Grand Mean 37.591 CV 19.48

ANOVA Table 3.3. Mean concentration of different species for week 3

Source	DF	SS	MS	F	P
Species	10	21826.9	2182.69	77.95	0.0000
Error	33	924.0	28.00		
Total	43	22750.9			

Grand Mean 33.545 CV 15.77

ANOVA Table 3.4. Mean concentration of different species for week 4

Source	DF	SS	MS	F	P
Species	10	25608.2	2560.82	60.62	0.0000
Error	33	1394.0	42.24		
Total	43	27002.2			

Grand Mean 36.364 CV 17.87

ANOVA Table 3.5. Mean concentration of different species for week 5

Source	DF	SS	MS	F	P
Species	10	64204.5	6420.45	136.08	0.0000
Error	33	1557.0	47.18		
Total	43	65761.5			

Grand Mean 47.682 CV 14.41

ANOVA Table 3.6. Mean concentration of different species for week 6

Source	DF	SS	MS	F	P
Species	10	62964.2	6296.42	68.67	0.0000
Error	33	3026.0	91.70		
Total	43	65990.2			

Grand Mean 48.364 CV 19.80

ANOVA Table 3.7. Mean concentration of different species for week 7

Source	DF	SS	MS	F	P
Species	10	31289.6	3128.96	80.54	0.0000
Error	33	1282.0	38.85		
Total	43	32571.6			

Grand Mean 40.909 CV 15.24

ANOVA Table 3.8. Mean concentration of different species for week for week 8

Source	DF	SS	MS	F	P
Species	10	35284.9	3528.49	106.14	0.0000
Error	33	1097.0	33.24		
Total	43	36381.9			

Grand Mean 44.045 CV 13.09

ANOVA Table 4.1. Concentration of fungi at different heights for week 1

Source	DF	SS	MS	F	P
Height	1	1250.00	1250.00	1.04	0.3480
Error	6	7240.00	1206.67		

Total 7 8490.00

Grand Mean 228.50 CV 15.20

ANOVA Table 4.2.Concentration of fungi at different heights for week 2

Source	DF	SS	MS	F	P
Height	1	220.50	220.50	0.17	0.6917
Error	6	7633.00	1272.17		
Total	7	7853.50			

Grand Mean 206.75 CV 17.25

ANOVA Table 4.3. Concentration of fungi at different heights for week 3

Source	DF	SS	MS	F	P
Height	1	578.00	578.000	0.98	0.3595
Error	6	3524.00	587.333		
Total	7	4102.00			

Grand Mean 184.50 CV 13.14

ANOVA Table 4.4. Concentration of fungi at different heights for week 4

Source	DF	SS	MS	F	P
Height	1	98.00	98.00	0.08	0.7847
Error	6	7202.00	1200.33		
Total	7	7300.00			

Grand Mean 200.00 CV 17.32

ANOVA Table 4.5. Concentration of fungi at different heights for week 5

Source	DF	SS	MS	F	P
Height	1	3444.50	3444.50	11.32	0.0151
Error	6	1825.00	304.17		
Total	7	5269.50			

Grand Mean 262.25 CV 6.65

ANOVA Table 4.6. Concentration of fungi at different heights for week 6

Source	DF	SS	MS	F	P
Height	1	722.0	722.00	0.43	0.5371
Error	6	10114.0	1685.67		
Total	7	10836.0			

Grand Mean 266.00 CV 15.43

ANOVA Table 4.7. Concentration of fungi at different heights for week 7

Source	DF	SS	MS	F	P
Height	1	968.00	968.000	1.48	0.2694
Error	6	3924.00	654.000		
Total	7	4892.00			

Grand Mean 225.00 CV 11.37

ANOVA Table 4.8. Concentration of fungi at different heights for week 8

Source	DF	SS	MS	F	P
Height	1	40.50	40.500	0.12	0.7451
Error	6	2097.00	349.500		
Total	7	2137.50			

Grand Mean 242.25 CV 7.72

ANOVA Table 5.1. Concentration of fungi isolated on nutrient media for week 1

Source	DF	SS	MS	F	P
Media	2	5874.7	2937.33	5.53	0.0272
Error	9	4784.0	531.56		
Total	11	10658.7			

Grand Mean 152.33 CV 15.13

ANOVA Table 5.2. Concentration of fungi isolated on nutrient media for week 2

Source	DF	SS	MS	F	P
Media	2	5312.7	2656.33	3.79	0.0638
Error	9	6301.0	700.11		
Total	11	11613.7			

Grand Mean 137.83 CV 19.20

ANOVA Table 5.3. Concentration of fungi isolated on nutrient media for week 3

Source	DF	SS	MS	F	P
Media	2	2216.00	1108.00	3.82	0.0630
Error	9	2612.00	290.22		
Total	11	4828.00			

Grand Mean 123.00 CV 13.85

ANOVA Table 5.4. Concentration of fungi isolated on nutrient media for week 4

Source	DF	SS	MS	F	P
Media	2	926.00	463.000	0.97	0.4146
Error	9	4285.00	476.111		

Total 11 5211.00

Grand Mean 133.50 CV 16.34

ANOVA Table 5.5. Concentration of fungi isolated on nutrient media for week 5

Source	DF	SS	MS	F	P
Media	2	2264.67	1132.33	4.67	0.0406
Error	9	2182.00	242.44		
Total	11	4446.67			

Grand Mean 174.67 CV 8.91

ANOVA Table 5.6. Concentration of fungi isolated on nutrient media for week 6

Source	DF	SS	MS	F	P
Media	2	1248.67	624.333	0.70	0.5218
Error	9	8030.00	892.222		
Total	11	9278.67			

Grand Mean 177.33 CV 16.84

ANOVA Table 5.7. Concentration of fungi isolated on nutrient media for week 7

Source	DF	SS	MS	F	P
Media	2	602.00	301.000	1.00	0.4060
Error	9	2714.00	301.556		
Total	11	3316.00			

Grand Mean 150.00 CV 11.58

ANOVA Table 5.8. Concentration of fungi isolated on nutrient media for week 8

Source	DF	SS	MS	F	P
Media	2	4382.00	2191.00	13.00	0.0022
Error	9	1517.00	168.56		
Total	11	5899.00			

Grand Mean 161.50 CV 8.04

ANOVA Table 6.1. Concentration of fungi at different times of day for week 1

Source	DF	SS	MS	F	P
Time	2	844.67	422.333	0.85	0.4575
Error	9	4450.00	494.444		
Total	11	5294.67			

Grand Mean 150.33 CV 14.79

ANOVA Table 6.2. Concentration of fungi at different times of day for week 2

Source	DF	SS	MS	F	P
Time	2	242.67	121.333	0.20	0.8194
Error	9	5363.00	595.889		
Total	11	5605.67			

Grand Mean 137.83 CV 17.71

ANOVA Table 6.3. Concentration of fungi at different times of day for week 3

Source	DF	SS	MS	F	P
Time	2	926.00	463.000	1.66	0.2434
Error	9	2510.00	278.889		
Total	11	3436.00			

Grand Mean 123.00 CV 13.58

ANOVA Table 6.4. Concentration of fungi at different times of day for week 4

Source	DF	SS	MS	F	P
Time	2	948.67	474.333	0.96	0.4179
Error	9	4434.00	492.667		
Total	11	5382.67			

Grand Mean 133.33 CV 16.65

ANOVA Table 6.5. Concentration of fungi at different times of day for week 5

Source	DF	SS	MS	F	P
Time	2	1128.67	564.333	2.98	0.1017
Error	9	1705.00	189.444		
Total	11	2833.67			

Grand Mean 174.83 CV 7.87

ANOVA Table 6.6. Concentration of fungi at different times of day for week 6

Source	DF	SS	MS	F	P
Time	2	1152.67	576.333	0.61	0.5639
Error	9	8490.00	943.333		
Total	11	9642.67			

Grand Mean 177.33 CV 17.32

ANOVA Table 6.7. Concentration of fungi at different times of day for week 7

Source	DF	SS	MS	F	P
Time	2	1098.00	549.000	2.42	0.1442
Error	9	2042.00	226.889		

Total 11 3140.00

Grand Mean 150.00 CV 10.04

ANOVA Table 6.8. Concentration of fungi at different times of day for week 8

Source	DF	SS	MS	F	P
Time	2	1458.00	729.000	4.47	0.0449
Error	9	1469.00	163.222		
Total	11	2927.00			

Grand Mean 161.50 CV 7.91

ANOVA Table 7.1. Concentration of fungi at different distances for week 1

Source	DF	SS	MS	F	P
Distance	1	8450.0	8450.00	7.32	0.0353
Error	6	6928.0	1154.67		
Total	7	15378.0			

Grand Mean 228.50 CV 14.87

ANOVA Table 7.2. Concentration of fungi at different distances for week 2

Source	DF	SS	MS	F	P
Distance	1	5512.5	5512.50	4.49	0.0783
Error	6	7361.0	1226.83		
Total	7	12873.5			

Grand Mean 206.75 CV 16.94

ANOVA Table 7.3. Concentration of fungi at different distances for week 3

Source	DF	SS	MS	F	P
Distance	1	10368.0	10368.0	17.04	0.0062
Error	6	3650.0	608.3		
Total	7	14018.0			

Grand Mean 184.50 CV 13.37

ANOVA Table 7.4. Concentration of fungi at different distances for week 4

Source	DF	SS	MS	F	P
Distance	1	7442.0	7442.00	7.34	0.0351
Error	6	6082.0	1013.67		
Total	7	13524.0			

Grand Mean 200.00 CV 15.92

ANOVA Table 7.5. Concentration of fungi at different distances for week 5

Source	DF	SS	MS	F	P
Distance	1	15312.5	15312.5	41.37	0.0007
Error	6	2221.0	370.2		
Total	7	17533.5			

Grand Mean 262.25 CV 7.34

ANOVA Table 7.6. Concentration of fungi at different distances for week 6

Source	DF	SS	MS	F	P
Distance	1	12168.0	12168.0	7.13	0.0370
Error	6	10244.0	1707.3		
Total	7	22412.0			

Grand Mean 266.00 CV 15.53

ANOVA Table 7.7. Concentration of fungi at different distances for week 7

Source	DF	SS	MS	F	P
Distance	1	12482.0	12482.0	19.49	0.0045
Error	6	3842.0	640.3		
Total	7	16324.0			

Grand Mean 225.00 CV 11.25

ANOVA Table 7.8. Concentration of fungi at different distances for week 8

Source	DF	SS	MS	F	P
Distance	1	15312.5	15312.5	40.49	0.0007
Error	6	2269.0	378.2		
Total	7	17581.5			

Grand Mean 243.25 CV 7.99