SURVEY OF SOME MUSHROOM FARMS IN THE GREATER ACCRA AND CENTRAL REGIONS; THEIR MYCOLOGICAL QUALITY PROFILE AND THE PATHOGENIC ROLE OF *TRICHODERMA HARZIANUM* IN THE PRODUCTION OF *PLEUROTUS OSTREATUS*

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DECLARATION

I hereby declare that this submission is my own research work towards the M.Phil. and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree, except where due acknowledgment has been made in the text.

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ABSTRACT

The environmental and farm aerial quality as well as the mycological purity of the basic ingredients employed for mushroom production in a mushroom farm is a *sine qua non* for producing healthy sporophores and gaining financial profit from the capital investment made by the producer of the oyster mushroom. In this study, seven mushroom farms in the Greater Accra Region (Ogbojo, Nii-Boi Town, Lapaz, Adenta, Ashaley Botwe, Anyaa-Awoshie) and Kasoa in the Central Region (Kasoa) were selected to provide a baseline data to help prescribe appropriate practical scientific interventions to help curb the financial losses incurred by farmers. The seven farms were; E90 Mushroom Farm, Ogbojo; Kwesi-Babs Farm, Kasoa; Immaculate Gold Enterprise, Nii-Boi Town, Lapaz; Delabless Mushroom Farm, Adenta; 4E Mushroom Farm, Ogbojo/Ashaley Botwe; Edeyef Mushroom Farm, Anyaa-Awoshie and PCM Mushroom Farm, Ashaley Botwe. The following criteria of quality were used to assess the farms;

a. Resident mycoflora in the fruiting bodies produced on the farms using the conventional fungal population enumeration technique on three media (PDA, OGYE and DRBC)
b. Quality of the composted growth substrate (sawdust) to ascertain species diversity of resident fungi using Decimal Dilution Technique.
c. Aeromycoflora in the seven cropping rooms of the farms using the Open Plate Exposure method with the view to ascertaining the most predominant fungal species.
d. In order to assess the control method for the most ubiquitous green mould causing losses in the farm house, the causative fungus *Trichoderma harzianum* was cultured on Potato Dextrose Agar (PDA) and in Potato Dextrose Broth (PDB) amended with varying dilutions (1:1 – 1:10v/v) of the ethanol bark extract of *Anthocleista nobilis* and a copper fungicide, “Champion”.
e. Assessment of the growth yield and Biological Efficiency (BE) of *Pleurotus ostreatus* cultivated on already spawned substrate bags contaminated with *T. harzianum* and pre-spawned uncontaminated substrate bags.
f. Mineral composition and proximate analysis of fruiting bodies employing the AOAC methods.

The resident mycoflora in the mushroom fruiting body was unique for each of the selected seven (7) farms. Fungi belonging to ten (10) genera and fifteen (15) species namely *Aspergillus* (*A. alutaceus, A. candidus, A. flavus, A. fumigatus, A. terreus*), *Cladosporium* (*C. herbarum, C. macrocarpum*), *Fusarium oxysporium, Gliocladium* sp., *Penicillium* (*P. brevicompactum, P. citrinum*), *Rhizopus oryzae, Talaromyces flavus, Trichoderma harzianum, Saccharomyces* spp., and *Rhodotorula mucilaginosa* were encountered. *Aspergillus* species (6) predominated over the others followed by *Cladosporium* (2) and *Penicillium* (2). *Trichoderma harzianum* was the most ubiquitous fungus on the fruiting bodies isolated from all the seven (7) mushroom farms.The mean fungal population in the fruiting bodies were: *T. harzianum* (4.13 $\log_{10}$CFU/g); *A. flavus* (3.86 $\log_{10}$CFU/g); *A. niger* (4.10 $\log_{10}$CFU/g); *A. fumigatus* (3.60 $\log_{10}$CFU/g); A. *candidus* (3.30 $\log_{10}$CFU/g); *A. terreus* (3.20 $\log_{10}$CFU/g); *P. brevicompactum* (3.5 $\log_{10}$CFU/g); *C. herbarum* (4.20 $\log_{10}$CFU/g); *C. macrocarpum* (3.78 $\log_{10}$CFU/g); *R. oryzae* (2.53 $\log_{10}$CFU/g); *Saccharomyces* spp. (4.08 $\log_{10}$CFU/g); *F. oxysporium* (3.16 $\log_{10}$CFU/g). The fungal flora in the composted growth substrate (sawdust) belonged to eighteen (18) species spread through ten (10) genera. *Aspergillus* species (A. *candidus, A. flavus, A. fumigatus, A. niger*). *A. fumigatus* predominated. Other species isolated were *Cladosporium* (*C. herbarum, C. macrocarpum*), *Didymella* sp. *Fusarium poae, Penicillium (P. brevicompactum, P. camemberti*) *Rhizopus oryzae, Trichoderma harzianum, Rhodothorula mucilaginosa, Saccharomyces* spp. (yeast), *Verticillium fungicola* and *Mycelia sterilia.* Again, the species encountered were common and unique for each farm visited. The aeromycoflora isolated belonged to nineteen (19) species and twelve (12) genera and was predominated by *Aspergillus species* (*A. candidus, A. flavus, A. niger, A. oryzae, A. parasiticus*) followed *Penicillium* (*P. citrinum, P. brevicompactum*), *Cladosporium* (*C. herbarum, C. macrocarpum*), *Fusarium* (*F. oxysporium, F. poae*). Single species encountered were *Epicoccum nigrum, Gliocladium* sp., *Mycelia sterilia, Rhodotorula mucilaginosa, Rhizopus oryzae, Trichoderma harzianum, Verticllium fungicola* and *Saccharomyces* spp. (yeast). Radial growth of *T. harzianum* on PDA amended with different dilutions of *A. nobilis* showed that growth was commensurate lowering of depression of growth with decreasing concentration of extract. The higher the concentration, the severe the depression but the mycelium of *T. harzianum* cultured in different concentrations of the extract of *A. nobilis* approximated that of the control after 10 days. In the case of the copper fungicide, 'Champion' only the 1:5 and 1:10 v/v dilutions approximated the control after 10 days of incubation whilst the undiluted and 1:1 v/v dilutions suppressed growth by 60-70% in 10 days. Vegetative growth of the fungus in *A. nobilis* and 'Champion' fungicide followed the trend obtained on the agar. Infection of the fruiting body reduced the elemental

composition of Calcium, Copper, Iron, Potassium, Magnesium, Sodium, Phosphorus and Zinc. The proximate analysis also showed a reduction in parameters of the following: Dry matter (%), Moisture (%), Fat (%), Crude fibre (%), Crude protein (%), Total ash (%), Carbohydrate (%) and Energy (kcal/100g). The practical implication of the finding is discussed and future work suggested.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Mushroom farming is a source of stable income for individuals who engage in it. Mushrooms are cultivated for their nutritional and medicinal benefits depending on the demand and are either cultivated commercially or obtained from the wild by gatherers. Cultivated mushrooms obtained from shops, supermarkets or directly from the farm are grown by farmers using different methods and setups depending on the type of mushroom species. Cultivated mushrooms include any that can be found at the grocery store and may include species such as button mushrooms or portobello (*Agaricus bisporus*), cremini (*A. blazei*), oyster mushrooms (*Pleurotus citrinopileatus*, *P. eous, P. ostreatus, P. pulmonarius* etc.), enoki (*Flammulina velutipes*), shiitake (*Lentinus edodes*) etc. (Obodai, 1992; s, 1991).

Other types of mushrooms are obtained from the wild which are of different varieties and may only grow on the live root systems of certain species of trees or in association with other organisms and have other characteristics which make them nearly impossible to be cultivated under certain controlled environmental conditions. The most popular among the Basidiomycota (Agaricales) are *Termitomyces* whereas other wild popular mushrooms belonging to the Ascomycota include truffles (*Tuber* sp.), morels (*Morchella* sp.) and chanterelle (*Cantharellus, Craterellus, Gomphus* and *Poyozellus*) species (Obodai, 1992).

There is an estimated number of over 1,500, 000 species of fungi on earth and approximately 45,000 species of mushrooms have been described. Out of this number, 2,000 species are considered as edible whereas 46 species are cultivated for their nutritional benefit (Hawksworth, 1991); (Kirk *et al.,* 2001).

About 4,500 species of mushrooms are considered to be poisonous and 30 species as toxic and also lethal when consumed by humans because they contain certain poisonous active ingredients e.g. *Amanita* spp. (*A. muscaria, A phalloides*) (Arailde, 2013).

Some varieties of mushrooms have long been utilised mainly for their medicinal benefits. These mushrooms are not necessarily pleasant to eat, but are brewed into tea or taken enclosed capsules. Medicinal fungi have been scientifically proven to offer several benefits and applications including treatment of cancer, reducing cholesterol, as antibacterial and antifungal agents. Examples of medicinal mushrooms include *Ganoderma lucidium* (reishi), *Inonotus obliqus* (chaga), *Trametes versicolor* (Turkey Tail mushroom) whereas other mushrooms are considered as miscellaneous with less defined uses because little work has been carried out on these species e.g. *Xeromphanlina campanella, Scleroderma citrina, Macrolepiota procera* (Obodai et al., 2002).

Mushrooms contain Selenium, a mineral which can be found in the soil but yet appears naturally in water and some foods and mushrooms are also high in antioxidants. Selenium helps in combating cancer by detoxifying cancer-triggering compounds in the body. Selinium also plays a crucial role in liver enzyme function, decreases tumor growth, and prevents inflammation. In addition to Selenium, mushrooms also contain Vitamin C, fiber, and potassium which helps in maintaining cardiovascular health. Moreover, mushrooms are also rich in insoluble dietary fibers that are in high demand from people suffering from obesity, cholesterol, and coronary heart disorders (Sanchez, 2004). However, worldwide mushroom cultivation is dominated by the production of some mushroom species; *Agaricus bisporus* (champignon), *Lentinula edodes* (shiitake), and *Pleurotus ostreatus* (oyster mushroom) (Hatvani, 2008).

There are edible mushrooms that are collected in the wild in Ghana which have been documented by Obodai and Apetorgbor, 2001.

Twenty-four (24) different species of mushrooms have been documented from the Western Region of Ghana; 18 of which are edible, 6 of which are medicinal. The edible mushrooms include *Termitomyces letestui, Volvariella volvacea, Corpinus disseminates, Catharellus sp., Mycena flavescence, Schizophylum commune, Auricularia sp., Agaricus sp.* (Dhamodharan & Mirunalini (2010). All belong to the Order Agaricales, Cantharellales, Tricholomatales, Schizophyllales and Auriculariales. The medicinal mushrooms are *Schizophyllum commune, Pleurotus tuber-regium, Auricularia auriculata, Ganoderma lucidum, Clavatia sp.* and *Daldinia concentrica* (Obodai and Apertogbor, 2001, 2008; Wiafe-Kwagyan, 2014).

Mushrooms are generally known to mainly contain 90% water and 10% dry matter (Sánchez, 2004). Their chemical composition is very attractive from a nutritional point of view (Dundar *et al*., 2008; Gbolagade et al. 2006) (Table 1). The nutritional value of mushrooms in general can be realively compared to those of eggs, milk, and meat (Oei 2003). Mushrooms are also known to contain an abundance of essential amino acids as well as vitamins (Sánchez 2004).

Mushroom cultivation depends on the ability of the macrofungi to biologically convert waste from industrial and agricultural waste into food. Some fungi are cultivated for their nutritional benefits. For example, *Pleurotus, Auricularia,* and *Lentinula* species have been successfully isolated from the tree trunks of some wood species in the wild. This occurrence in nature has led to the observation and subsequent development of mushroom farming as this observation has led to the development of compost bags which are specialized in composition of nutrients for each cultivated mushroom (Tripothi and Yadar, 1992; Chiu *et al.,* 2001).

Table 1 Nutrimental value of several edible mushrooms (mg/100 g dry matter)

Edible mushroom	Lipids	Sugars	Protein	Fiber	Ash	Ca	Mg	K	Na	P	Mn	Fe	Cu	Zn
Auricularia polytricha	5.05	5.35	8.90	3.45	4.95	0.75	1.45	37.40	0.35	19.85	0.29	0.67	0.12	0.06
Lentinus subnudus	4.05	10.15	5.80	5.40	6.50	1.75	2.45	22.15	2.15	2.50	0.07	0.52	0.12	1.97
Lycoperdon pusilum	7.55	14.70	24.0	4.30	6.55	5.20	3.90	28.80	2.00	14.50	0.90	0.72	0.16	1.30
Lycoperdon giganteum	10.25	17.20	24.30	5.03	10.90	4.30	3.10	44.20	3.50	20.90	0.50	0.25	0.07	0.80
Pleurotus tuber-regium	1.70	7.70	16.30	15.60	9.20	1.90	0.80	9.51	1.50	4.10	0.15	0.32	0.02	1.91
Pleurotus florida	1.05	8.95	15.10	4.40	10.60	0.40	1.50	14.75	0.25	13.35	0.80	0.08	0.05	0.05
Psathyrella atroumbonata	5.75	7.85	17.40	11.65	9.65	1.57	4.55	43.35	6.50	15.0	0.45	0.20	0.07	0.77
Schizophyllum commune	5.50	8.75	10.30	8.30	11.60	3.90	0.55	16.80	0.65	8.10	0.30	0.10	0.08	1.20
Termitomyces microcarpus	8.75	14.15	27.70	12.80	12.90	3.50	3.65	58.75	2.00	24.20	0.72	0.70	0.07	2.82
Termitomyces globalus	6.70	14.65	32.80	10.35	15.80	5.30	6.00	48.40	1.70	31.10	0.85	0.85	0.09	3.10
Tricholoma lobayensis	4.20	7.75	13.50	8.50	9.40	1.50	0.55	18.60	2.20	9.95	0.29	0.32	0.13	0.85
Volvariella esculenta	10.95	9.85	26.05	7.0	12.65	0.80	2.45	51.45	5.90	18.75	0.44	0.42	0.11	2.50
Pleurotus sajor-caju	1.15	–	16.75	–	5.84	0.10	0.46	4.32	–	1.97	0.021	0.163	0.14	0.192

Gbolagade 2006; Dundar et al. 2008

1.1 *Pleurotus ostreatus* (Oyster mushroom)

Pleurotus species are very popular and extensively cultivated in several regions in the world including China, Thailand, Japan, South Korea and Indonesia because of the nutritive and medicinal qualities. Currently mushroom cultivation in Ghana has become an important and lucrative venture. *Pleurotus ostreatus* also known as the oyster mushroom is a common primary decomposer of agricultural and wood residues (Zadrazil and Kurtzman, 1982). Oyster mushrooms can also be found in tropical and subtropical rainforest. These species of mushroom even though can be located in the wild can be successfully cultivated under controlled conditions on the farm preferably on composted sawdust of *Triplochiton scleroxylon* (Obodai *et al*., 2000). *Pleurotus ostreatus* is generally referred to as the oyster mushroom because of its appearance, it has a shell-like cap (4 – 15cm; convex) which is broad and fan shaped and it also has a laterial stipe with gill running down the stipe which are whitish with a yellowish tinge with age. In addition to *P. ostreatus*, other species of *Pleurotus* are currently under cultivation and these are; *P. florida, P. eryngii, P. columbinus, P. sajor-caju P. cornucopiae, P. citrinopileatus, P. cystidiosus, P. sapidus, P. abellatus* (OECD, 2005).

In addition to the many general advantages of the oyster mushroom, it is reported to have a rapid growth rate and it is a very effective saprophytic colonizer. Being rich in vitamins and minerals and low in calories, sodium and cholesterol, it makes *Pleurotus* spp. a suitable source of healthy dietary food. Due to its many suitable dietary characteristic, it led to a high demand of this mushroom on the world market contributing to it being ranked as second to the cultivation of *Agaricus campestris* (OECD, 2005).

1.2 Life cycle of *Pleurotus ostreatus* (Oyster mushroom)

The schematic presentation of the life cycle of the oyster mushroom is illustrated in Figure 1. Once there is a suitable substrate and adequate environmental conditions, a basidiospore germinates to become a mass of homokaryotic mycelium each containing a single haploid nucleus. Prior to fusion, the mass of homokaryotic mycelia grows and there is a fusion between two compatible strains giving rise to a heterokaryotic mycelium. The process of growth continues and there is a division of the two nuclei in each compartment. There is a uniform distribution of the nuclear pair throughout the mycelium via clamp connections. The mycelia containing two nuclei (heterokaryotic cells) with efficient substrate colonization, environmental conditions (cooling 10ºC-21˚C, relative humidity 85-90%, and light requirement 1000-2000 lux, CO_2 < 1000 ppm) can then differentiate into fruiting bodies.

Quimio (1978) reported an optimum temperature of 28-30ºC for maximum growth of *P. flabellatus; P. ostreatus* could grow best at 25.6ºC (Block *et al*., 1958; Zadrazil, 1978) and will fruit at less than 20.0ºC. In Ghana the optimum growth of *P. ostreatus* is 30ºC (Wiafe-Kwagyan, 2014; Obodai, 1992).

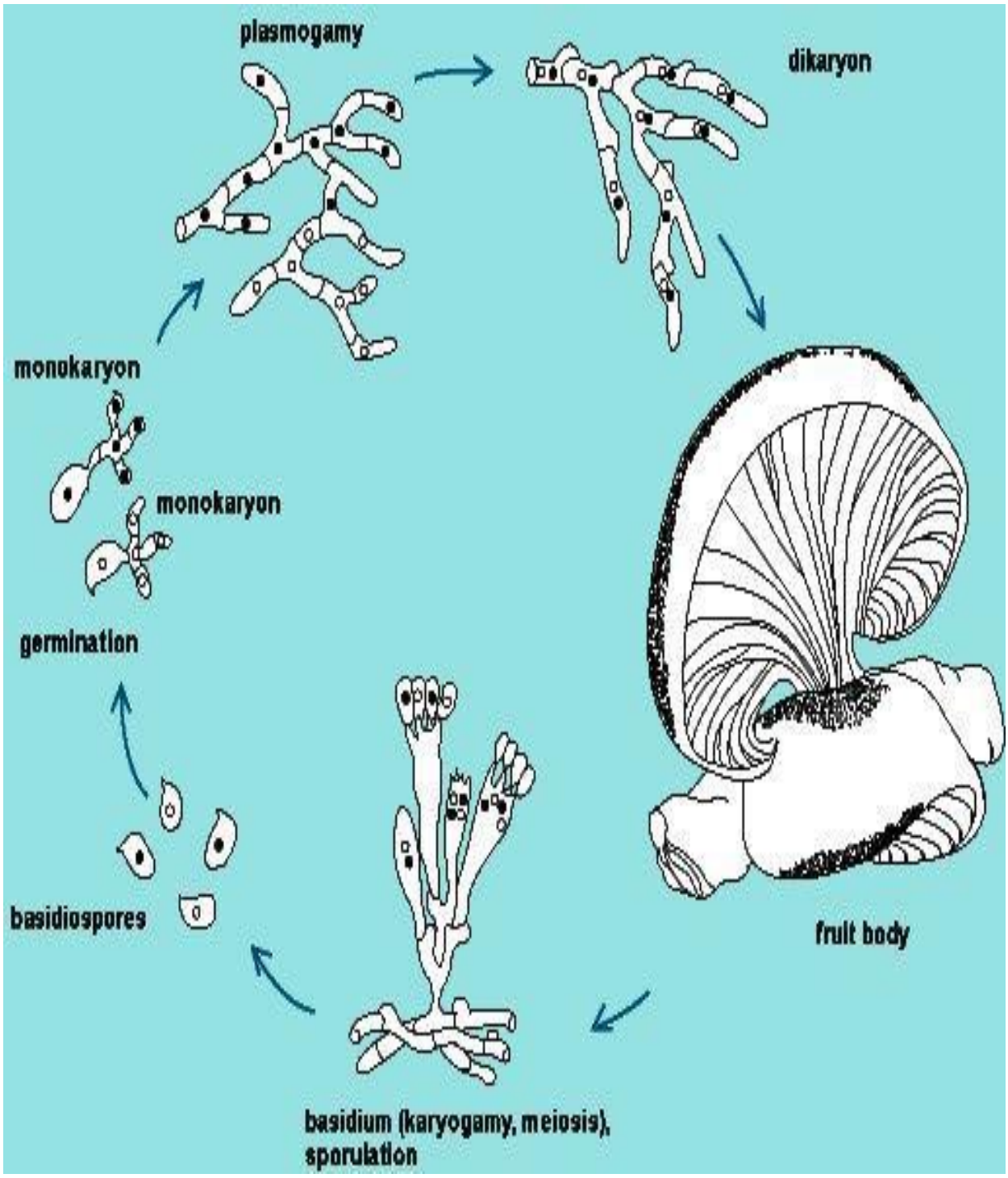

Figure 1 Life cycle of the oyster mushroom *Pleurotus ostreatus* (Martínez-Carrera, 1999).

1.3 Commercial farming *of Pleurotus ostreatus* in Ghana

Mushroom are grown on specialized substrates known as compost. To create the mushroom growing substrate, sawdust preferably from 'wawa' (*Trplochiton scleroxylon*) is used, cassava or yam peels may also be dried, milled and used. Rice wastes such as straw, husk, bran and broken rice was recently used by Wiafe-Kwagyan (2014) for the cultivation of two *Pleurotus* species (*P. eous and P. ostreatus*). Lime and rice bran with a little water are added after which the substrate mixture is heaped into a pile in order to facilitate the process of compost fermentation by some thermophilic fungi (Beffa *et al*., 1998; Obodai, 1992; Wiafe-Kwagyan, 2014).

Prior to the bagging of the substrate in heat resistant bags (polypropylene bags), water is added to the compost and evenly distributed to ensure a water distribution of about 65-70%, which provides moisture that will enable the growth of the mushroom mycelia. This moisture content is determined by performing the squeeze test. The substrate is packed into polypropylene bags, and the mouth of the bag pushed through a Polyvinyl Chloride (PVC) pipe and then fastened with a rubber band. A small opening is left at the closure and plugged with a piece of cotton wool to keep the moisture in the substrate bag. The purpose of the PVC pipe is to provide an opening for inoculation of mushroom spawn and gaseous exchange (Buswell, 1984).

The compost bags are sterilised at 100˚C in speacially designed drums. The bags are packed on the rack and the drum is then heated. The steam flows steadily from the hole in the lid till a height of 4cm is reached. Once steam starts escaping from the drum, the setup is allowed to steam for three hours. The bags are then allowed to cool and taken to a shaded area known as the inoculating room (Kortei, 2015).

Spores of mushrooms in general are difficult to impossible to handle, therefore *P. ostreatus* is not propagated by spores. Spores are also genetically different from the mature fruiting body, because of this occurrence, a pure culture of the mycelium on a substrate usually a grain (e.g. sorghum) is used as the starter and referred to as a spawn. According to Oei (1996), using spawns in the cultivation of this mushroom gives it an advantage over other fungi.

Isolation of the mycelia of the mushroom and the making of the spawn are done under sterile conditions. Grains used as substrate for the colonization of the mycelium is also sterilized to ensure proper grain colonization and eliminate competitive fungi.

The spawns are then inoculated into the substrate bags through the opening and the cotton plug is replaced on top. The bags are then sent to the incubation room and they are left until they are fully colonized. Room temperature should be maximum 30˚C to ensure adequate and timely colonization (Quimio, 1978).

After complete colonization of the substrate bags, the bag appears white, after which the bags are opened. Bags which are fully colonized by mycelia are transferred to the cropping house for the formation of fruiting bodies. The cropping house must have a relative humidity ranging from 80-90% with temperatures between 25 and 30˚C. In contact with the air, the dense mass of mycelium can now fruit into full mushrooms. Interestingly, this takes 36 hours for the mushroom to reach full maturity (Wiafe-Kwagyan, 2014).

The mushrooms which are fully developed, are detached from the substrate and are ready for eating, storage or sale. Figure 2 illustrates the general preparation process and cultivation of two edible mushrooms namely *Pleurotus* and *Agaricus*.

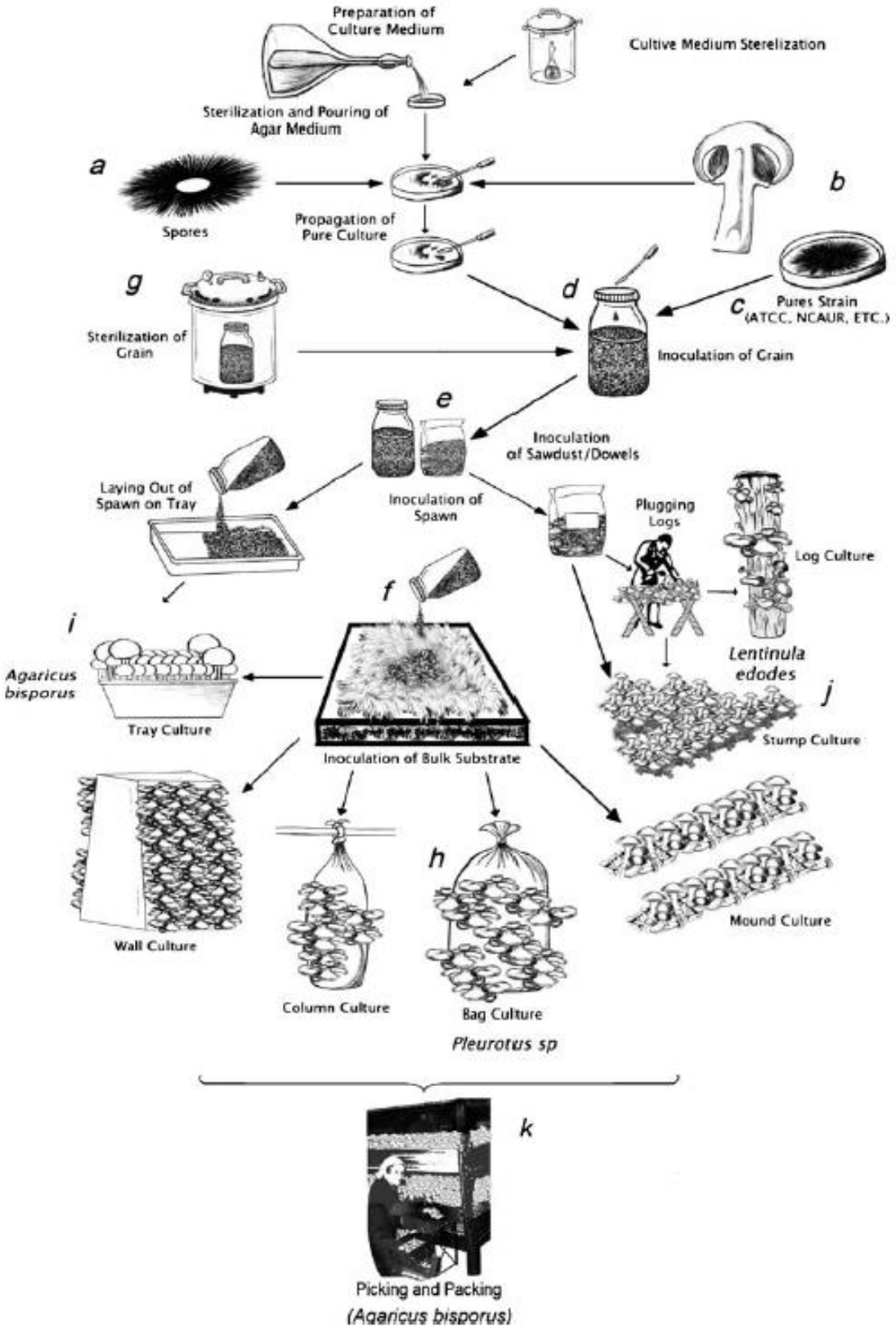

Figure 2. Cultivation and harvesting of Mushrooms, modified from Stamets (1995)

1.4 Taxonomy of *Pleurotus*

Pleurotus mushrooms are diverse and found all over different regions of the world. Oyster mushrooms belong to the genus *Pleurotus* and the division Basidiomycota. Oyster mushrooms are documented to have been initially cultivated in 1977 by Flank, Germany. The evolutionary connection between species of *Pleurotus* lack a clear connection and due to that there remains a taxonomic controversy. The genus *Pleurotus* is one of the most diverse groups of edible cultivated mushroom and they are propagated and developed by both sexual and asexual means (Cohen *et al.*, 2002).

The fruiting body morphology, mating studies between populations, biochemical analyses, microscopic observations have not provided any clear cut results in this genera (Martínez-Carrera, 1999). This confusion when it comes to the taxonomy of this genera and especially the species *Pleurotus ostreatus* is due to differences in morphology of some specimen and the similarities existing among isolates obtained from different species (Asef, 2012). To date, seventy (70) species of *Pleurotus* have been discovered with discoveries being made more frequently. The new findings upon observation are seen to be similar to previously identified species (OECD, 2005).

The genus *Pleurotus* was first recommended to be a group within the genus *Agaricus*. *Pleurotus, Lentinus* and *Panus* which were 3 genera within the group were able to be separated due the anatomical features of their sterile tissues of their hymenium bearing structures and were hence recognized as homogeneous taxonomic groups. In another study on this genus, Hilber (1982) recommended that, the crossing of monospore is fundamental to the study of this genera. *Pleurotus ostreatus* (Jacq. Ex. Fr.) Kummer is the most cultivated species among the oyster mushrooms and adopted as the type species of the genus *Pleurotus*.

The majority of mycologists worldwide use the assertion by Singer (1986) which divides the *Pleurotus* genus into six sections; *Lepiotarii* (Fr.) Pilat, *Calyptrati* Sing., *Pleurotus* Sing., *Coremiopleurotus* (Hilber), *Lentodiellum* (Murr.) Sing. and *Tuberegium* Sing. *Pleurotus ostreatus* was however placed in the *Pleurotus* section. These sections are based on the absence of a velum and a monomitic hyphal system (OECD, 2005).

Oyster mushroom are generally classified as follows:

Scientific Name: *Pleurotus* spp.

Kingdom: Fungi

Division: Basidiomycota

Class: Hymenomycetes

Order: Agaricales

Family: Pleurotaceae

Genus: *Pleurotus*

Species: *Pleurotus ostreatus*

1.5 Problem of infection in the cultivation of mushroom production in Ghana

Mushrooms are also adversely affected by a large number of biotic and abiotic agents or factors. In most cases, there is complete crop failure depending upon the stage of infection, quality of compost and environmental conditions (Sharma *et al*., 2007). *Aspergillus*, *Penicillium*, *Trichoderma*, Black moulds (*Mucor*, *Rhizopus*), *Scopulariopsis fimicola, Fusarium*, *Mycogyne perniciosa, Verticillium fungicola* are examples of some mushroom pathogenic fungi (Bahl & Chowdhary, 1980). There have been documentations of some fungi associated with the composting process and substrate and others associated with the fruiting body or both (Sandhu and Sidhu 1980).

Generally, microorganisms causing infection of mushroom can we described as being pathogenic or competing with the mushroom for nutrients and other growth requirements present in the growth substrate. The fruiting bodies of mushrooms can be attacked by pathogens such as fungi, viruses, bacteria and even insects while pathogens attacking or present in the growth substrate are mostly identified to be fungi. *Aspergillus fumigatus*, *A. terreus*, *Mucor pusillus*, *Penicillium* spp, *Rhizopus microspores*, *Trichoderma* spp were found to be associated with the composting process. *A. fumigatus* and *A. terreus* predominated over others according to a study conducted by Sandhu and Sidhu 1980.

Some fungi through these studies were reported as very harmful to the cultivation of *Pleurotus* among these is *Trichoderma* sp causing what is known in mushroom production as the Green mould disease. Some species of fungi such as *Sclerotinum rolfsii* were recorded to inhibit the growth of *Pleurotus flabellatus* (Rajarathnam *et al*., 1997; 1992) while others like *Penicillium cyclopium* present in the substrate affected formation of fruiting bodies of *Pleurotus sajor-caju* right after emergence reducing yield by 50-75% (Cailleux and Diop, 1978).

During the composting process, there are some beneficial microorganisms which aid in the fermentation process and the effective breakdown of the substrate making essential nutrients and other growth requirements available for the successful growth of the mushroom under cultivation. Due to less effective sterilisation processes and handling of the sterile compost, some microorganisms may remain in the substrate, thereby competing with the growth of mushrooms and even reducing yield leading to high production costs. The prevailing atmospheric conditions present in the cropping room, mainly the aeromycoflora or air-spora may also play a crucial role when it comes to contamination in mushroom production (Kortei, 2015).

Using sawdust from the 'wawa' wood, (*Triplochiton scleroxylon*), Obodai (1992) identified the species of fungi associated with the composting process and their phenology which also depended on the *Pleurotus* species utilising the growth substrate. These fungal species belonged to thirteen fungal species belonging to eight genera *Aspergillus, Cladosporium, Mucor, Neurospora, Paecilomyces, Penicillium, Rhizopus* and *Trichoderma*. Conducting a survey of the effectiveness of compost sterilisation of some mushroom farmers, Kortei (2015) reported the dissatisfaction of 64% of Ghanaian farmers of *Pleurotus ostreatus* with the sterilisation process that makes use of the steam oil drum method. These farmers raised concerns about the effectiveness of this method in adequately sterilising substrate and grains used in the production of spawns. Issues of increased rate of contamination due to sterilisation methods were raised by many mushroom farmers.

This presents a challenge considering the fact that *Pleurotus ostreatus* is the most preferred cultivated mushroom in Ghana after a more common wild termite mushroom (*Termitomyces sp*.) usually sold on our open markets in Ghana.

The composting process of mushroom farming is mainly meant to increase nutrirent availability and utilization by mushroom production yet under vulnerable conditions as reported earlier several opportunistic microorganisms tend to contaminate farm compost and fruiting bodies such as summarized in Table 2.

Table 2. Some diseases that attack *Pleurotus ostreatus* and *Agaricus bisporus* cultivation, modified from Sánchez (2009)

Name of mushroom species	Infestation (organism)	Type of organism	Diseases	Symptoms
Agaricus bisporus	*Cladobotryum dendroides*	Fungi	Cobweb, mildew	white to pink cobweb-like fluffy mould
Agaricus bisporus	*Mycogone* sp.	Fungi	Wet bubble/white Mould	dense white growth on gills
Agaricus bisporus	*Mycogone perniciosa*	Fungi		wet bubble of fungi
Agaricus bisporus *Pleurotus ostreatus*	*Trichoderma* sp.	Fungi	Green mould	dark green mould patches on casing spreading to lesions on stipe
Agaricus bisporus	*Verticillium fungicola*	Fungi	Dry bubble/brown spot	brown irregular pitted areas on stems and caps. distortion and splitting. Severe rotting, blotch, and necrosis of caps and stem
Agaricus bisporus	*Lycoriella sp., L. ingenua*	Insect	Sciarid flies	destroy pins of developing mushrooms, and burrow or tunnel into the stems and caps of maturing mushrooms. Causes less mushroom damage than
Agaricus bisporus	*Megasellia halterata*	Insect	Phorid flies	cause less mushroom damage than sciarid flies
Agaricus bisporus	*Aphelenchoides composticola*	Nematode	Eelworms, Cephalothecium disease	degeneration of the mushroom mycelium in the compost
Agaricus bisporus,	*Pseudomonas Tolaasii*		Brown blotch	sunken, dark brown lesions
Pleurotus ostreatus, and other mushrooms	*Pseudomonas reactants* *Pseudomonas gingeri*	Bacteria	Blotch disease Ginger blotch	mild dark purple to light brown discoloration and slight surface depression that becomes darker with age Pale yellowish red discoloration that develops into a reddish ginger-coloured pale discoloration
Agaricus bisporus	Putative virus X	Virus	Virus X	slightly imperfectly formed cap structure
Agaricus bisporus	Bacilliform viruses	Virus		severe rotting, blotch and necrosis of caps and stems

The effects of some parasitic fungi can be very destructive to the growth of mushrooms. In the case of the green mould infection of these edible basiodiomycets, the mould colonises the substrate of the cultivated mushroom and the yield can be drastically reduced as young fruiting bodies are infected at emergence. In the most cases, there may not be any appearance of fruiting bodies and the compost or substrate bags would have to be discarded (Hatvani, 2008). The green mould disease of the mushroom compost has been known to limit the growth and subsequent yield of *Agaricus bisporus*. This followed the occurrence of the green mould epidemic in Northern Ireland in the year 1985. This major devastating occurrence was immediately followed by outbreaks in Ireland (1986), England and Scotland (1987), the Netherlands (1994), France (1997), Spain (1998) and Hungary (2004).

Significant production losses of *P. ostreatus* happens as a result of attacks by pests and microorganisms with the effect of *Trichoderma* species associated with the growth compost known to limit yield. Production loss of *P. ostreatus* caused by the green mould disease was first reported in South Korea (2002). Subsequent severe infections were later recorded in Italy (2004), Hungary (2004) and Romania (2004) (Hatvani, 2008).

Trichoderma aggressivum is known as the causative agent of the green mould disease of the mushroom *Agaricus bisporus*, however, this differs from the species of *Trichoderma* that causes the green mould in the cultivation of the oyster mushroom. The difference was ascertained by observation of the morphological features and by the use of DNA sequencing. As a result, these species of *Trichoderma* causing the green mould disease of the oyster mushroom have been identified as the two species; *T. pleurotum* and *T. pleuroticola*.

From Table 2, pathogenic green mould is noticed to colonise the growth substrate or on the surface of emerging mushrooms (pin heads or primordia head formation). Symptoms are usually seen between 10-15 days following spawning and the initial onset of mycelial growth.

At the early stages of infection, it is easy to miss the growth of the green mould as the *Trichoderma* species produces white mycelia. Subsequent development leads to the observance of green patches on mushroom bags as the pathogens sporulates more rapidly. Morris *et al.*, (1995), describes the symptoms of the green mould disease as the occurrence of green fungal patches as a result of sporulation and discovered on the growth substrate between 2-5 weeks of a cropping season. Recent studies by Wiafe-Kwagyan *et al*. have shown the possible antibiosis effect of *Aspergillus flavus, A. niger* and *Trichoderma harzianum* and the vegetative growth of *Pleurotus* sp.

1.6 Possible prevention and control of the incidence of pathogenic and competitive fungi to mushroom farming

In order to prevent the incident of pathogens on the mushroom farms and increase farm output and commensurate yield and make profit certain, chemicals have been successfully used in the control of mushroom pathogenic fungi. Among these are benomyl, Environ and benximidazole fungicide. According to Abosriwil and Clancy (2003) the use of fungicides in spawn treatment before spawning greatly reduced the microbial load and *Trichoderma* colonization than when these same fungicides were spread throughout the compost. However, there is a concern associated with the use of these synthetically formulated antifungal treatments as application may coincidentally reduce mycelial growth of the mushroom. Due to this reason, many chemicals are not recommended when it comes to mushroom farming and this has led to the adoption of other alternatives preventive measures as well as biological control.

Certain organisms such as *Bacillus* sp. have been identified to be antagonistic to some pathogenic fungi in the soil, including *Trichoderma* species. Győrfi and Geösel (2008) studied the antagonism of *Bacillus* sp. on three strains of *T. aggressivum* under mushroom growing conditions. Antagonistic bacteria naturally occurring in casing mixtures have been isolated and tested against *T. aggressivum* affecting *A. bisporus in vitro* and *in vivo* on mushroom beds, this

resulted in high production output as it effectively controlled the pathogenic fungi, (Bhatt and Singh, 2002).

Objectives

The overall aim of this study was to identify and characterise mushroom pathogens in cultivated *Pleurotus ostreatus* strain EM-1 in some mushroom farms in Accra and Kasoa, Ghana.

Specific Objectives were:

1. To determine the occurrence and distribution of mycoflora and fungal disease associated with oyster mushroom (*Pleurotus ostreatus*) production and substrate bags in various farms in Greater Accra and Central Region (Kasoa).
2. To identify and determine the pathogenicity of *Trichoderma* species in oyster mushroom commercial farms in Greater Accra and Kasoa, Ghana.
3. To determine the prevalent aeromycoflora in the various cropping rooms of the mushroom farm sites.
4. Make preliminary trials at control by biological and chemical methods against *Trichoderma* sp. causing green mould in oyster mushroom *in vitro*.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Sample collection

Samples were collected from mushroom farms located in Accra using a dedicated list of mushroom farmers who cultivate, *Pleurotus ostreatus* strain EM-1. The list was obtained from the Food Research Institute (FRI) - Council for Scientific and Industrial Research (CSIR), Ghana. The samples of mushrooms and compost were collected in sterile collecting containers and were immediately transported to the laboratory for microbial analysis. Seven most active mushroom farms were sampled. Samples of infected fruiting bodies and the mushroom growing substrate were collected into sterile containers by the use of a pair of sterile forceps. These forceps were sterilized using Milton (1% sodium hypochlorite + 16.5 % sodium chloride), 70% ethanol and flamed over a spirit flame.

The seven mushroom farms were; E90 Mushroom Farm, Ogbojo; Kwesi-Babs Farm, Kasoa; Immaculate Gold Enterprise, Nii-Boi Town, Lapaz; Delabless Mushroom Farm, Adenta; 4E Mushroom Farm, Ogbojo/Ashaley Botwe; Edeyef Mushroom Farm, Anyaa-Awoshie and PCM Mushroom Farm, Ashaley Botwe.

Plate 1a. *Pleurotus ostreatus* EM- 1 farming sites visited in Accra and Kasoa (Mag X 0.05)

Top Left: Kwesi Babs Farm, Kasoa

Top Right: E90 Mushroom Farm, Ogbojo

Bottom Left: PCM Mushroom Farm, Ashaley Botwe

Bottom Left: Edeyef Mushroom Farm, Anyaa-Awoshie

Plate 1b. *Pleurotus ostreatus* EM- 1 farming sites visited in Accra

Top Left: Delabless Mushroom Farm, Adenta

Top Right: 4E Mushroom Farm, Ogbojo/Ashaley Botwe

Bottom: PCM Mushroom Farm, Ashaley Botwe (Mag X 0.05).

2.2 Microbial enumeration

This was done according to the method described by ISO (Odamtten *et al*., 2018). Fungi resident in collected mushroom fruiting body and growth substrate samples were determined as prescribed using the Decimal Serial Dilution technique up to $1:10^3$.

One millilitre aliquots of the serially diluted samples were placed in either 20ml of Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol agar (DRBC, CM 727). The plates were incubated at 28˚C for 7 days. Colonies which appeared were counted and calculated as $\log_{10}$ CFU/g of sample.

2.3 Analyses of aeromycoflora from mushroom farms

To assess air contamination in the cropping rooms of the different farm sites, the Solid Plate Exposure method was employed. Petri plates containing respective media (i.e. PDA, OGYE and DRBC) were exposed for five minutes in the various cropping rooms of the mushroom farms. The fungal culture plates were incubated at 28°C for 7 days after which the colonies appearing were counted and converted to $\log_{10}$CFU/g sample. Identification of species was done by using their colour, cultural and morphological characteristics. Standard identification manuals were used to assist in the authentication of species.

2.4 Statistical analysis

Where necessary, data was analysed using T-Test and ANOVA and results quoted at the 5% level of significance ($P \leq 0.05$).

2.5 Selection of the most prominent and devastating mushroom pathogen used in inhibitory and pathogenicity tests

The most prevalent and abundant pathogenic fungus of importance when it comes to oyster mushroom cultivation, *Trichoderma* species, was isolated in all the seven (7) sampling farms and the species was being subsequently sub cultured to obtain a pure culture. It was selected because of its significance in mushroom farming as it is considered the causative organism for the green mould disease.

2.6 Maintenance of stock cultures

Stock cultures of relevant mushroom pathogenic, *Trichoderma harzianum* were maintained on slants of Potato Dextrose Agar (PDA) in McCartney tubes and on 9mm Petri dishes and kept in the refrigerator at a temperature of 8˚C. Media used were sterilized at 121˚C for 15 minutes and at 1.05 kg/cm^3 pressure.

2.8 Culture media

i. Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) was prepared by boiling 200g sliced potatoes until fully cooked. The cooked potato was strained using a muslin cloth into a beaker; 10g dextrose, a capsule of Chloramphenicol and 15g of Agar were added. The mixture was heated until the Agar dissolved and topped up to 1 litre with distilled water making a 1L media preparation.

ii. Potato Dextrose Broth (PDB)

Potato Dextrose Broth (PDB) was prepared in the same way as PDA without adding agar.

iii. Oxytetracycline-Glucose-Yeast Extract Agar (OGYE)

Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) was prepared by dissolving 5g of yeast, 20g of dextrose and 12g of Agar. The mixture was dissolved in 1 litre of distilled water to prepare the medium.

iv. Dichloran Rose Bengal Chloramphenicol Agar (DRBC)

Dichloran Rose Bengal Chloramphenicol Agar (DRBC) was prepared by dissolving 31.6g of an already formulated powder in 1L of distilled water at a pH of 5.6 and at 25˚C. Components of the formulation are listed below.

Ingredient	g/L
Peptone	5
Dextrose	10
Potassium dihydrogen phosphate	1
Magnesium sulphate	0.5
Rose Bengal	0.025
Chloramphenicol	0.1
Dichloran	0.002
Agar	15

2.9 Preparation of plant extract and formulation of fungicide used for antimicrobial test on *Trichoderma harzianum*

i. Ethanol extract of *Anthocleista nobilis* (cabbage tree) bark

This was prepared by emulsifying 200g of the dried and powdered leaves of the cabbage tree in 500ml of ethanol and agitated on the shaker for 4 days at 25˚C and at 4 revolutions per minute (rpm).

ii. Conventional copper fungicide ("Champion" Wettable Powder by Chemico Ltd.).

The antifungal media was prepared by dissolving 10g in 1 lire of distilled water.

Active Ingredient

Cupric Hydroxide (77%)

Inert Ingredient (23%)

[Metallic Copper Equivalent (50%)]

2.10 Determination of the effect of plant extract and copper fungicide on growth of *Trichoderma harzianum*

This was determined by employing two (2) major methods.

i. Radial/Diameter Growth of Assessment of *T. harzianum* on Potato Dextrose Agar (PDA) amended with selected phytoextract and copper fungicide at various concentrations

An all-purpose media, PDA was amended with various amounts of the phytoextract and antifungal chemical (1:1, 1:2, 1:5 and 1:10 v/v dilutions).

Working under the laminar hood chamber, a number of culture discs were punched in the pure culture of *Trichoderma harzianum* using a 3mm cork borer. Different amounts of the media and phytoextract and copper fungicide were measured into a sterile Petri dish and allowed to solidify at room temperature. The cultural discs of *Trichoderma harzianum* were placed at the centre of the amended media and radial growth along two diameters at the bottom of the plate observed. Cultures were incubated at 28˚C and radial growth of *Trichoderma harzianum* discs measured at intervals of 2, 5, 7 and 10 days.

ii. Determination of the Vegetative Growth of mycelium on Potato Dextrose Broth (PDB) amended with different concentrations of either phytoextract or copper fungicide.

Thirty (30) millilitres each of the different concentrations (1:1, 1:2, 1:5, 1:10 v/v dilutions) of the amended growth media were measured into eighteen (18) Erlenmeyer flasks and autoclaved to ensure sterility. Three millimeter discs of the mushroom pathogenic fungi (*T. harzianum*) were used to inoculate each Erlenmeyer flasks containing amended growth media and incubated at 28-30˚C for seven (7) days. Previously dried and weighed filter papers were used in harvesting the various cultures after the specified period. The oven (Memmert, model 4) was used to dry the filter papers at a temperature of 75˚C for 48 hours

Funnels were lined with filter papers and the funnels were placed over clean Erlenmeyer flasks and the cultures were poured into the funnels lined with the pre-dried filter paper in order to obtain the mycelium. To determine the mycelia dry weight, the harvested mycelia were dried at 75˚C for 48 hours after which the dry weight was obtained.

2.11 Pathogenicity test

A total of ten substrate bags were obtained from Kwesi-Babs Mushroom farm located at Kasoa. This farm also serves as a supplier of already inoculated substrate bags to most mushrooms farmers in Accra. Five growth substrates were inoculated with 3mm discs of *Trichoderma harzianum* and the remaining five bags left uninoculated as a control. The bags recorded growth of the test fungus in a form of green sporulation on the surface of the substrate. The bags were monitored for the appearance of pinhead formation and fruiting body formation. Parameters such as yield, Biological Efficiency (B.E), number of fruiting bodies, weight, stipe length and width and cap/pileus diameter were recorded.

The compost bags were also inoculated with a 3mm disc of *Trichoderma harzianum* and its effect on growth yield was observed as above

2.12 Assessment of total yield of fruiting bodies

Several parameters such as total number of pinheads formed, total number of fruiting bodies and fresh weight of fruiting bodies were taken. The weight was assessed using an electric balance and the Biological Efficiency (BE) was determined using the methods of Pathmashini et al., (2008) and Patra and Pani (1995). The biological efficiency (BE) value was computed using the mathematical expression below:

$$\text{B.E} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \text{ x } 100\ \%$$

2.12 Harvesting technique of mature fruiting bodies

To ascertain the above parameters; number of pinheads (primordia) formed, total number of fruiting bodies, fresh weight (g) of fruiting bodies, stipe length (mm), stipe width (mm), cap/pileus diameter (mm) and percentage conversion of pinheads to fruiting bodies (i.e. abortion of pinheads) were recorded. Matured mushrooms were identified by curl margin of the cap and were harvested by twisting to uproot from the base. In cases where it was difficult to uproot the fruiting bodies from the base the mushrooms were harvested with a sharp scalpel and put into transparent polypropylene bags. Generally, mushroom matured 48 hours (2 days) after the appearing of primordia. The biological efficiency (BE) per yield was calculated using the expression above.

2.13 Determination of moisture content

The moisture content was determined using the gravimetric method (Black, 1965). One gramme of the dried powdred mushroom sample was measured separately into previously weighed moisture cans. It was then dried in the oven at 105°C for 6hrs, cooled in a desiccator and re-weighed. The cooled sample was returned to the oven for further drying. Drying, cooling and weighing were repeated at 1hr intervals until a constant weight was obtained. The weight of moisture loss was determined and expressed as a percentage of the sample analyzed.

2.14 Determination of crude protein. AOAC, 2005 and James (1995)

About 1g of dried ground sample was weighed (W) and transferred into a digestion tube through a 1.0mm screen mesh. About 3g of a catalyst mixture of $CuSO_4$ and K_2SO_4 was added to the sample. Twenty milliliters of concentrated H_2SO_4 was added to the mixture and the tube was then placed in a digester at 380°C for 5hrs. After 5hrs the tube was removed from the digester block and was allowed to cool in a hood. At the completion of digestion when the solution was light blue or colourless, 30ml of distilled water was carefully added. Following, 40ml of 32% NaOH solution was also added in a distillation set up (Kjeldahl apparatus).

The entire mixture was distilled and collected into a conical flask containing 50ml of 4% boric acid solution. The distillation process was allowed for 6 minutes and about 100ml of the distillate was collected. The distillate known as ammonium borate was titrated against 0.1N H_2SO_4 and using 3 drops of a mixture indicator (methyl red and bromo cresol green) was added to the solution. A colour change from steel blue to pink with the addition of few drops of the acid (0.1N H_2SO_4) indicated an end point. A reagent blank determination was also carried out. The crude fiber content was calculated using the formula below:

$$\% \text{ Crude protein} = \frac{K \times N \times (V - B)}{W} \times 100\% \times F$$

$$\% \text{ Crude protein} = \frac{0.01401 \times N \times (V - B)}{W} \times 100\% \times 6.25$$

$$\% \text{ Crude protein (DMB)} = \frac{\%\text{Crude protein}}{\%\text{DM}} \times 100\%$$

% Crude protein = % Nitrogen x 6.25; DM= dry matter

Protein content (% dry weight) = (total nitrogen - chitinous nitrogen) x 6.25

V= titre volume of acid consumed: B= blank titre volume in titration

N= normality of H2S04 used in titration: W= weight of sample taken.

K = constant = 0.01401: F = conversion factor of nitrogen in protein = 6.25

2.15 Determination of proximate composition of mushroom

Nutrient analyses of fruiting bodies were done using method of AOAC (2005). To determine the total nitrogen content in the fruiting bodies, samples were dried at 60°C and analysed by the Kjeldahl method (AOAC, 2005). The total (crude) protein was estimated from the total nitrogen content, using the correction factor 4.38 or 6.25 as stated by Breene (1990). Total carbohydrate content was thus estimated using the mathematical formula:

Total carbohydrate (%, DW) = 100% - protein content (%, DW) – lipid content (%, DW) – ash (%, DW).

Carbohydrate (%) = [100 – (moisture – total ash – fiber – protein – fat)] (Nilsen 2010).

Crude fibre, fat, and minerals were analysed by method outlined by AOAC (2005).

2.16 Determination of fat content in the mushroom sample

Two grammes of the sample was weighed (W_1) and transferred into a clean alundum thimble which was previously extracted with a porous filter paper at 60°C. The thimble with the sample was covered with defatted cotton and was placed in a soxhlet reflux flask which was previously kept in an oven at 105°C for 8 hrs. The flask was then kept in a desiccator to cool to about 25°C and weighed (W_2). The thimble was mounted in a reflux flask containing 70ml of petroleum ether. When heated the solvent condenses into the reflux flask. The sample was covered until the flask was filed up and siphoned over carrying oil (fat) extract down to the boiling flask. The process was allowed to go on repeatedly for about 4hours before the defatted sample was removed and kept for crude analysis. The solvent was recovered and the flask with its oil extract was dried in the oven at 105°C for 30 minutes, cooled in a desiccator and re-weighed to obtain the weight (W_3) of the oil extract (fat). It was then expressed as a percentage of the sample analyzed. The percentage fat content was calculated using the mathematical relationship below:

$$\% \text{ Ether extract (DMB)} = \frac{W3 - W2}{W1} \times 100\%$$

$$\% \text{ Ether extract (DMB)} = \frac{\% \text{ Ether extract}}{\%DM} \times 100\%$$

% Fat = 100 (W2-W1) x 100;

W1= weight of sample;

W2 = Initial weight of extraction flask and content

W3 = Oven dry weight of flask + oil (fat) extract.

2.17 Determination of crude fibre content in the mushroom sample

In determining the crude fibre only de-fatted sample was used. 0.5g (W_1) of sample was weighed and transferred into 600ml Berzelius beaker through a 1.0mm screen mesh. 50ml of cold (25°C) 1.25% H_2SO_4 solution was added to the sample. The beaker was placed on a reflux condenser unit and heated at 100°C for exactly 30mins. A few drops of anti-reagent such as amyl alcohol was added at this stage to minimize frothing or foaming. After 30mins of boiling the H_2SO_4 solution was washed off with hot water repeatedly and was filtered immediately using light suction. Fifty millitres (50ml) of 1.25% of NaOH was added to the washed sample and was refluxed for additional 30mins. After 30mins the NaOH solution was washed off with hot water several times and then filtered using vacuum suction through Gooch crucible. The crucible with the fibre content was dried in an oven at 105°C for 10hrs and the fibre content was weighed (W_2) to obtain the yield. Crucible content was ashed in a furnace at 510°C for 3hrs was then removed from the furnace and put in an oven at 105°C for 8hrs and then weighed (W_3).

An estimation of crude fibre content was calculated as change in weight lost and was mathematically expressed as below:

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight on ignition}}{\text{Initial weight of sample}} \text{x } 100$$

$$\text{i.e. } \% \text{ Crude fibre} = \frac{W2 - W3}{W1} \text{x } 100\%$$

$$\% \text{ Crude fibre} = \frac{\text{Crude fibre}}{\%DM} \text{x } 100\%$$

W_1= weight of sample;

W_2= oven dry weight of Gooch crucible and content (i.e. before ignition)

W_3= weight of Gooch crucible and content after ignition

2.18 Determination of minerals or heavy metal elements content in the mushroom sample

About 250mg of ground air dried sample was weighed into a beaker was then placed in ignition muffle furnace (Vectar-furnace, PS3-Sweden) for drying at 400°C for 24hrs. Five millitres of hydrochloric acid (HCl) was added to the sample and the solution was dried again; subsequently 5ml of nitric acid (HNO_3) was added. After evaporation, the sample was diluted to 50ml with water. Sodium (Na) and calcium (Ca) content of the ashed sample were determined by flame photometer and K, P, Mg, Cu, Zn, Mn, Fe, and Pb by Unicam 929 Atomic Absorption Spectrophotometer (AAS) (Model PinAAcle 900T).

2.19 Determination of ash content (Van Soest *et al*., 1991)

Ash was determined by ignition in a muffle furnace; about 2.0g of the sample was weighed (W) into a porcelain crucible of known weight (W_1). It was then burnt into ashes in the muffle furnace at 130°C for 3hrs. It was then removed and put in a desiccator for cooling, it was re-weighed (W_2) and the weight of the ash was obtained and expressed as a percentage of the weight of sample analyzed. The ash content was then estimated using the formula below:

$$\text{Ash content} = \frac{W2-W1}{W} \text{x } 100\%$$

W1 = Weight of empty crucible

W2 = Weight of crucible + ashes

W = Weight of sample

2.20 Phenology of resident mycoflora in the substrate and mushroom fruiting bodies

Conventional Decimal serial dilution technique was used to estimate population of residing fungi on all the substrates used for this study. About 1.0g of the sample was placed in a sterile 250ml Erlenmeyer flask containing 100ml of 0.1% peptone diluent distilled water. The mixture was shaken in a Gallenkamp (England) Orbital shaker for 5mins at 140rev/min. About 1ml of aliquot of the suspension in the flask was transferred into a sterile McCartney tube containing 9ml of 0.1% peptone and was serially diluted up to $1:10^3$; 1ml of an aliquot of each dilution in duplicate was poured into a sterile Petri dish containing 20ml of either PDA, OGYE and DRBC media. The plates were then incubated at 28°C up to 7days. After 7 days fungal growth was determined by counting colonies and the fungal population was estimated as $\log_{10}$CFU/g sample. Identification of mycoflora was done using morphological and culture characteristics such as colour, mycelia and spore structure and colony appearance as outlined by Sigurd (1953); George and Harold (1960); Von-Arx (1970); Barnett and Barry (1972) and Robert and Ellen (1988).

When appropriate, photographs were taken of the species under the microscope for authentication with the assistance of the principal supervisor.

Experimental precautions

Sterile containers were used for sampling and sampling was done using strict sterile means.

Glassware used for this investigation were washed thoroughly with detergent, rinsed, dried and sterilised in the autoclave before use.

Reusable glass Petri plates used for cultures were sterilised in canisters in an electrically operated Gallen Kamp oven 300 plus series at 160°C for up to 8 hours. They were kept in their canisters before use.

Nonabsorbent cotton plugs were used to plug Erlenmeyer flasks containing media for vegetative growth investigations.

Inoculating needles, forceps and cork borers used were cleaned in 70% ethanol and flamed over a spirit glass burner until they were red hot.

Media used in this investigation were sterilised in the autoclave before use.

The edges of all glassware being used in the laminar flow cabinet were flamed during experimental procedures to prevent contamination.

Hands were wiped with 70% ethanol before each procedure.

The laminar flow cabinet and all working areas were wiped with 70% absolute alcohol before use.

The laminar flow hood after cleaning was left on for 15 minutes before starting work.

The inoculating room was also sterilized with 70% ethanol.

CHAPTER THREE

3.0 EXPERIMENTAL PROCEDURE

EXPERIMENT 1

3.1 Resident mycoflora associated with mushroom fruiting bodies

Like other crops, mushrooms are also affected by some pathogenic fungi when there are suitable environmental conditions that enhance the growth and proliferation of these pathogenic fungi. The conditions especially the humidity conditions as well as handling during cultivation contributes immensely to the infection of fruiting bodies of mushroom, thereby reducing their quality and yield.

The temperature ranges for the cultivation of *Pleurotus ostreatus* (EM-1) differs from climate to climate. This particular strain of *P. ostreatus* (EM-1) cultivated in Ghana grow at an optimum temperature of about 30˚C (Obodai *et al*., 2010). *Trichoderma* spp. are also noted to have an optimum growth temperature range of 25-30˚C (Singh *et al*., 2014).

In this experiment, the resident fungi of the fruiting bodies were sampled at the seven mushroom farms were determined using the decimal serial dilution technique. Exactly 1g of sample was put in 100ml of 0.1% peptone as diluted blanks. The samples were serially diluted up to $1:10^3$. One millilitre aliquots were placed in 90mm Petri plates containing 20ml of either media Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC), the plates were incubated at 28˚C for 7 days. Method used is described in the materials and methods chapter. Results obtained are presented on Table 3.

3.1.1 Determination of fungal population on mushroom fruiting body

The total number of colonies were counted and the Colony Forming Unit per Gram (CFU/g) sample was calculated using the formula summarised in materials and method and was expressed as $\log_{10}$CFU/g. The total mycoflora from the seven farms were compared. Results obtained are shown in Figure 3.

EXPERIMENT 2

3.2 Resident mycoflora associated with compost

The compost for the cultivation of *Pleurotus ostreatus* (EM-1), consists primarily of sawdust from wood, preferably 'wawa' (*Triplochiton scleroxylon*) which prior to bagging is fermented for about 28 days and turned regularly to ensure homogeneity (Obodai *et al.,* 2003).

Mushroom cultivation exploits the natural ability of fungi, in this case *Pleurotus ostreatus* (EM-1) to biologically convert solid waste from industries and agriculture into food (Tripothi and Yadar, 1992; Chiu *et al*., 2001). This underscores the importance of compost in mushroom farming.

Before spawning, the bagged compost amended with millet or rice brand and $CaCO_3$ are steam sterilised or pasteurised by traditional oil drums. Sterilisation is meant to completely kill any other organisms which are present in the compost and are likely to compete with the mushroom mycelia for utilization of the substrate as food. Pasteurisation on the other hand refers to killing off the majority of competitive organisms. This process of sterilisation or steaming to an extent reduces the microbial load but some fungi such as *Aspergilus niger, Aspergillus flavus, Mucor racemosus, Aspergillus fumigatus, Aspergillus alutaceus, Rhizopus stolonifer,* and *Fusarium oxysporum persisted* after steam sterilization (Kortei, 2015).

Same procedure was employed as when mushrooms were sampled. Fungi were identified on Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC).

Results obtained for this experiments are presented on Tables 4.

3.2.1 Estimation of fungal population resident in the mushroom substrate

To estimate the fungal population resident in the mushroom substrate ("wawa" sawdust), fungal population was counted and calculated as $\log_{10}$CFU/g sample after 7 days of incubation. Mycoflora population isolated from the growth substrate were compared.

Figure 4 shows a graphical representations of results.

EXPERIMENT 3

3.3 Aeromycoflora profile of mushroom farm

Fungi are ubiquitous and can easily be trapped on any suitable medium when the plates are exposed for brief periods of time. The longer the period of exposure, the more spores can be trapped and identified after incubation at the appropriate temperature. Information on the prevalent mycoflora in the cropping room also supplements knowledge on the occurrence of certain mycoflora identified on the surface of infected mushroom fruiting bodies and even on the surface of exposed substrate.

To ascertain microbial quality of the seven mushroom farms, the air quality was assessed using the conventional settle plate technique. Agar plates containing either Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) or Dichloran Rose Bengal Chloramphenicol Agar (DRBC) was exposed for 5 minutes. Results of the pooled data are presented on Table 5.

EXPERIMENT 4

3.4 Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with varying dilutions of phytoextract of *Anthocleita nobilis* at 28˚C for 10 days

The method of radial growth on the various amendments of PDA was employed to determine the rate at which the proliferation of the mycelia of the selected pathogenic fungi was inhibited.

In this experiment, the inhibitory effects of the phytoextract from the cabbage tree was tested *in vitro* at various concentrations (1:1 – 1:10 v/v dilution).

The 3mm discs of the *T. harzianum* was inoculated at the centre of the plate at the transect of two diameter lines drawn at right angles at the bottom of the petri dishes. All triplicates ranging for each of the dilutions were inoculated with 3mm discs of *T. harzianum.*

Growth of culture along two diameters was measured at the following day intervals; 2, 5, 7 and after 10 days. Results are shown in Figure 5.

EXPERIMENT 5

3.5 Radial growth assessment of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with aqueous preparation of copper fungicide "Champion" at 28˚C for 10 days

Radial growth of *Trichoderma harzianum* were assessed on the various amendments of PDA to determine the rate at which the proliferation of the mycelia of the fungus would be inhibited.

In this experiment, the inhibitory effects of the copper fungicide were tested *in vitro* at various concentrations (undiluted, 1:1 – 1:10, full strength v/v dilution).

Trichoderma harzianum was used as the pathogenic fungus in this experiment. The experiment was performed as explained in the material and methods section.

The 3mm discs of the pathogenic fungi was inoculated at the centre of the plate at the transect of two diameter lines drawn at right angles at the bottom of the petri dishes. All triplicates for the various dilutions 1:1-1:10v/v dilutions were inoculated with 3mm discs of *T. harzianum.* Growth of culture along two diameters was measured at days intervals of 2, 5, 7 and 10. Results obtained are presented in Figure 6.

EXPERIMENT 6

3.6 Vegetative growth assessment of *Trichoderma harzianum* in Potato Dextrose Broth (PDB) amended with different concentrations of phytoextract of *Anthocleita nobilis* at 28˚C c for 7 days

In some instances, the growth of a fungus on agar medium is different from what exists in a liquid medium. This is due to differences in aeration and osmotic differences created by the nature of the medium (Wiafe-Kwagyan, 2014).

This experiment was performed in the same way as Experiment 4, except in this case, no agar was included in any of the amendments. This was done to determine the rate of inhibition posed by the phytoextracts to the specific pathogenic fungi inoculated.

The 250ml Erlenmeyer flasks were plugged with cotton wool before sterilization in the autoclave. 3mm discs of *T. harzianum* were taken and inoculated into each triplicate amounting to eighteen 250ml Erlenmeyer flasks containing the amended PDB media as described in the material and method after cooling.

The dry weight accumulation over a week at 28-30˚C was assessed by the oven dry weight method (Materials and General Methods Section). Results are presented in Figure 7.

EXPERIMENT 7

3.7 Vegetative growth assessment of *Trichoderma harzianum* in Potato Dextrose Broth (PDB) amended with different concentrations of aqeous preparation of copper fungicide "Champion" at 28˚c for 7 days

This experiment was performed in the same way as Experiment 4 and 5, except in this case, no agar was included in any of the amendment. The rate and extent of inhibition posed by the antifungal chemical to *T. harzianum* was determined.

The 250ml Erlenmeyer flasks were plugged with cotton wool before sterilization in the autoclave. 3mm discs of *T. harzianum* were taken and inoculated into each triplicate amounting to eighteen 250ml Erlenmeyer flasks containing the amended PDB media as described in the material and method after cooling.

The dry weight accumulation over a week at 28-30˚C was assessed by the oven dry weight method (Materials and General Methods Section). Results obtained are represented in Figure 8.

EXPERIMENT 8

3.8 Growth and yield performance of *Pleurotus ostreatus* strain EM-1 on substrate bags contaminated with *Trichoderma harzianum* and non-contaminated substrate bags

Already spawned substrate bags were inoculated at the centre with 3mm discs of *Trichoderma harzianum* to determine their effect on pinhead formation and growth performance judging from the following parameters; Biological Efficiency, number of fruiting body, weight of fruiting body, pinhead formation, stipe length, stipe width and cap/pileus diameter.

It is well known that air contains bacterial and fungal spores sequel to this experiment, the aeromycoflora was determined. The purpose of this experiment was to investigate the effect of mushroom pathogens such as *Trichoderma* on the growth yield and performance of *P. ostreatus.*

Ten (10) already spawned bags were obtained for this experiment, 5 were inoculated on the surface of the substrate with *T. harzianum*, and the remaining 5 left as the control (uncontaminated). Growth yield was observed and recorded. Pinning, fruiting, fresh weight, stipe width and length were all recorded for the various setups. This setup was repeated three different times and results recorded after the harvest of each flush. Results are summarised in Tables 6-11.

CHAPTER FOUR

4.0 RESULTS

EXPERIMENT 1

4.1 Resident mycoflora associated with mushroom fruiting bodies

Resident mycoflora on the sampled mushroom fruiting bodies in all seven (7) farms were isolated and identified using specialized methods described in material and methods. Fifteen (15) fungi belonging to ten (10) genera, *Aspergillus* sp. (*A. niger, A. fumigatus, A. candidus, A. flavus*), *Cladosporium* sp (*C. macrocarpum, C. herbarum*), *Fusarium oxysporum, Gliocladium* sp., *Penicillium* sp. (*P. brevicompactum, P. citrinum) Rhizopus oryzae, Talaromyces flavus, Trichoderma harzianum, Rhodotorula muscilaginosa* and *Saccharomyces* (yeast) spp. were encountered. *Trichoderma harzianum* was the most abundant and relevant pathogen when it comes to *P. ostreatus* cultivation. Table 3, Plate 2 show mycoflora isolated with the fruiting bodies and their corresponding farms. Mycotoxigenic species isolated include *A. flavus, A. fumigatus*, *Fusarium* spp., and *Penicillium* spp whereas potential pathogenic species included *Cladosporium* spp. and *Trichoderma harzianum.*

Using the specialized isolation procedures as stated in the Materials and Methods section, distinctive fungal colonies were isolated on the 3 different media (PDA, OGYE and DRBC) from mushroom fruiting bodies obtained from E90 Mushroom Farm. Mycoflora identified were; *Asperigillus* species (*A. niger, A. fumigatus*), *Cladosporium herbarum, Rhizopus oryzae, Trichoderma harzianum* and yeast sp. *Trchoderma harzianum* was isolated on each of the 3 media used in this investigation. *T. harzianum* predomibated fungal species in this farm with *R. oryzae* being the least dominant.

Mushrooms from Kwesi-Babs mushroom farm showed an increased number of fungal load as experienced from the preceding farm. Mycoflora identified were *Aspergillus* species (*A. niger, A. candidus, A. flavus, A. fumigatus*), *Rhizopus oryzae, Rhodotorula mucilanoginosa, Penicillium citrinum, Trichoderma harzianum* and *Talaromyces flavus*. *A. niger* was the dominant species in this farm with *R. oryzae* being the least dominant.

The fungal profile of Immaculate Gold Ent. Mushroom farm showed less diversity as seen in other farms on all 3 media used (PDA, OGYE and DRBC). Mycoflora identified for this particular farm include *Aspergillus candidus, Cladosporium herbarum, Penicillium brevicompactum, Trichoderma harzianum* and *Saccharomyces* sp. As expected, the fungus which is the main cause of the green mould disease was isolated along with other ubiquitous fungi such as *Aspergillus niger* and *Cladosprium herbarium.* There was however the occurrence of *P. brevicompactum* which is known to be a mycotoxigenic fungus was the predominant species followed by *C. herbarum*. The least isolated from the fruiting body in this farm was *A. candidus.*

The fungal profile of the mushrooms cultivated in Delabless mushroom farm included the mushroom pathogenic fungus, *Trichoderma hariznum.* Other fungi isolated from the mushroom fruiting bodies include *Aspergillus* (*A. flavus, A. fumigatus*), *Cladosporium herbarum, Cladosporium macrocarpum, Rhodotorula mucilaginosa* and Yeast sp. On this farm, *A. flavus* predominated followed by *T. harzianum*. The least abundant was *Saccharomyces* sp.(yeast).

Mycoflora isolated from 4E Fresh mushroom farm were; *Aspergillus flavus, Cladosporium macrocarpum, Fusarium oxysporium, Rhodotorula mucilaginosa, Trichoderma harzianum* and *Gliocladium* sp. *Gliocladium* sp. isolated on one medium. There was however the occurrence of *Fusarium oxysporium* which is known to be pathogenic to some plants. It is isolated for the first time on mushrooms in this study. *T. harzianum* was the predominant fungus on this farm with the least dominant being *Gliocladium*.

A large variety of mycoflora were isolated on each media for Edeyef mushroom farm. Fungi identified were; *Aspergillus* (*A. niger, A. flavus*), *Cladosporium* (*C. herbarun, C, macrocarpum*), *Rhizopus oryzae, Rhodotorula mucilaginosa, Penicillium* (*P. brevicompactum, P. rouquefortii*) and *Trichoderma harzianum. P. brevicompactum* was the dominant species followed by *T. harzianum* (Table 3, Plate 2). The least encountered fungi resident on the mushroom fruiting bodies was *R. oryzae*.

Mycoflora profile from PCM mushroom farms were; *Aspergillus* (*A. terreus, A. ochra*ceus = A. *alutaceus*), *Cladosporium* (*C. herbarum, C. macrocarpum*), *Fusarium* (*F. oxysporium, F. poae*), *Rhodotorula mucilaginosa Trichoderma harzianum* and Saccharomyces sp. OGYE was fully colonized by *T. harzianum* which prevented the growth of any other fungi on the growth medium recording the highest population in this farm. *A. terreus* was the least mushroom resident population.

Table 3. Fungal species isolated from mushroom samples collected from seven (7) mushroom farms in Accra and Kasoa.

- *Aspergillus alutaceous* Wilhelm [7P]
- *Aspergillus candidus* Link [3D]
- *Aspergillus flavus* Link [4PO, 5P, 6POD]
- *Aspergillus fumigatus* Fresineus [1P, 2D, 4O]
- *Aspergillus niger* van Tieghem [1PD, 2POD, 6OD]
- *Aspergillus terreus* Thom [7P]
- *Cladosporium herbarum* (Pers.) Link [1O, 3O, 4P, 5D, 6PO, 7P]
- *Cladosporium macrocarpum* Preuss [4OD, 6PO, 7P]
- *Fusarium oxysporium* Schlecht. Emend. Snyder & Hansen [5P, 7D]
- *Fusarium poae* (Peck) Wr. [7D]
- *Gliocladium* sp. (Link) Schroers [5D]
- *Penicillium brevicompactum* Dierckx [3O, 5P, 6PO]
- *Penicillium roqueforti* Thom [6D]
- *Rhizopus oryzae* Went and Prinsen Geerling [1P, 2POD, 6D]
- *Rhodothorola mucilaginosa* H. C Harrison [4D, 5D, 6D, 7D]
- *Talaromyces flavus* (P. A. Dang.) C. R. Benj. [1D]
- *Trichoderma harzianum* Rifai [1POD, 2PO, 3P, 4O, 5PO, 6PO, 7PO]
- *Saccharomyces* sp. Meyen [1O, 2POD, 3POD, 4POD, 5PO, 6O, 7PD, 7D]

KEY:

1 – E90 MUSHROOM FARM, OGBOJO

2 – KWESI BABS FARM, KASOA

3 – IMMACULATE GOLD ENTERPRISE, NII-BOI TOWN, LAPAZ

4 – DELABLESS MUSHROOM FARM, ADENTA

5 – 4E MUSHROOM FARM, OGBOJO/ASHALEY BOTWE

6 – EDEYEF MUSHROOM FARM, ANYAA-AWOSHIE

7 - PCM MUSHROOM FARM, ASHALEY BOTWE

P – ISOLATED ON PDA

O – ISOLATED ON OGYE AGAR

D – ISOLATED ON DRBC AGAR

4.1.1 Estimation of total fungal population resident on mushroom fruiting bodies isolated on three different media; Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol agar (DRBC) from seven mushroom farms in Accra and Kasoa.

The use of three media enabled the detection of a wider spectrum of fungi. PCM mushroom farms located at Ashaley Botwe had the highest microbial load resident on mushrooms. There was a total fungal population of 5.32 $\log_{10}$CFU/g recorded on PDA which was 0.02 log cycles lower than the population recorded on OGYE. Delabless mushroom farm closely followed PCM mushroom farms recording the second highest then Kwesi-Babs mushroom farm and finally E90 mushroom farms. The lowest microbial load resident on the mushroom fruiting body was recorded by Immaculate Gold Ent. (2.59 $\log_{10}$CFU/g on PDA. 3.22 $\log_{10}$CFU/g on OGYE and 2.59 $\log_{10}$CFU/g on DRBC) 4E mushroom farms and Edeyef mushroom farm (Appendix 15). Results represented in Figure 3.

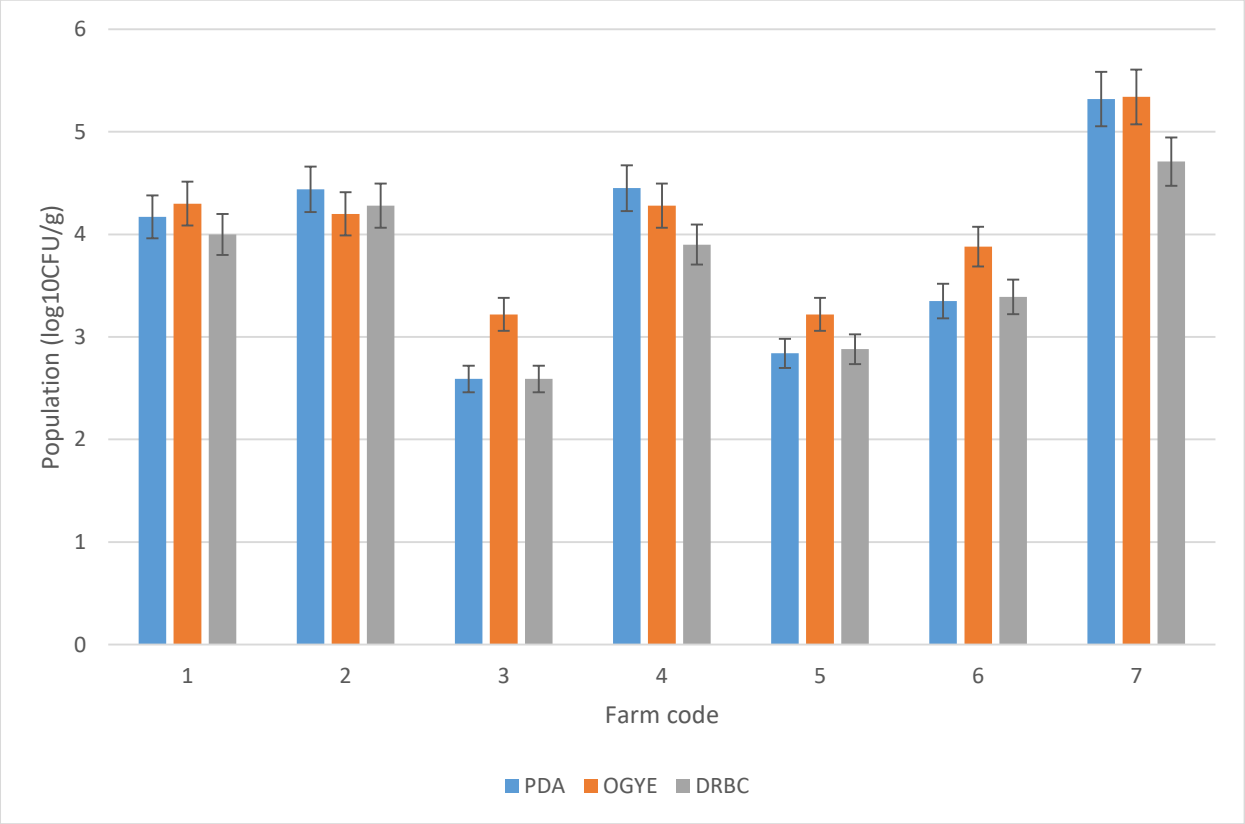

Figure 3. Comparison of the total fungal populations isolated from mushroom fruiting bodies obtained from the seven mushroom farms.

FARM CODE:

1 – E90 MUSHROOM FARM, OGBOJO

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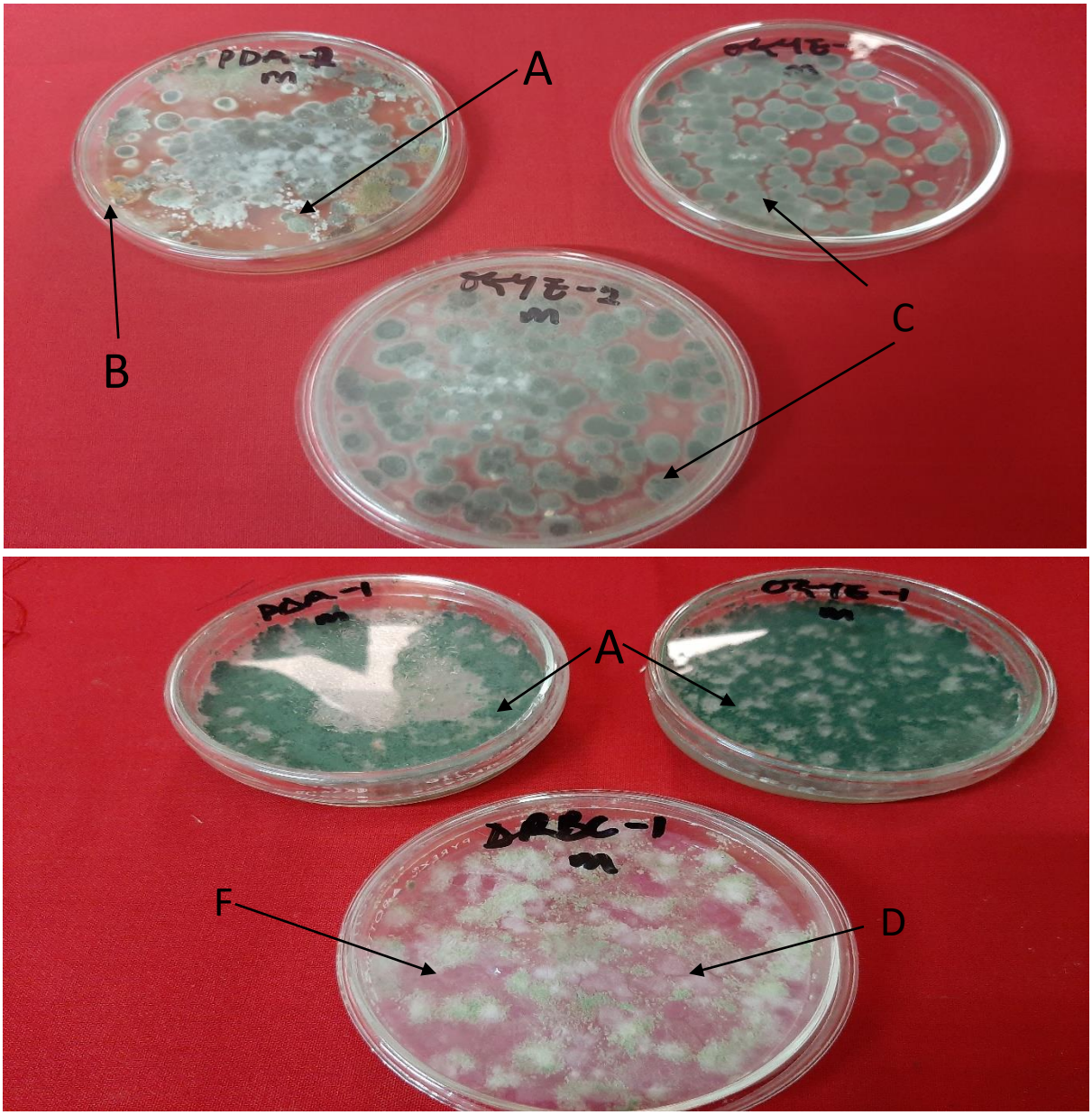

Plate 2. Representative fungal cultures of mycoflora isolated from mushroom fruiting bodies on PDA, OGYE and DRBC.

A- *Trichoderma harzianum*

B- *Taloromyces flavus*

C- *Cladosporium* sp.

D- *Aspergillus flavus*

E- *Fusarium oxysporium*

(Mag X 0.5)

EXPERIMENT 2

4.2 Resident mycoflora associated with growth substrate isolated from seven farms in Accra and Kasoa

Substrate samples were taken from the various mushroom farms visited. Samples plated from the serial dilutions on the different media; PDA, OGYE and DRBC showed different types of colonies of mycoflora which were identified as described in the materials and methods chapter.

Seventeen (18) fungi belonging to ten (10) genera were identified in this experiment; *Aspergillus* (*A. niger, A. flavus, A. candidus, A. fumigatus*), *Didymella, Rhizopus oryzae, Trichoderma harzianum, Saccharomyces* (yeast) spp, *Rhodotorula muscilaginosa, Fusarium poae, Cladosporium* (*C. macrocarpum, C. herbarum*), *Verticillium fungicola, Penicillium* (*P. brevicompactum, P. camemberti*) and *Mycelia sterilia* (Tables 4, Plate 3).

Some mycoflora associated with compost during the composting period and these are dominated by *Aspergillus flavus, Penicillium* species *and Trichoderma harzianum.* Due to the sterilization process, using steam in oil drums, which is not as effective as irradiation, some of these fungi were even identified post processing. *Cladosporium* spp., *Verticllium* spp. and *Trichoderma* spp are known to compete and restrict mycelial growth of *P. ostreatus.*

Mycoflora were isolated on three different media as used in Experiment 1. Mycoflora identified from E90 mushroom farms were; *Aspergillus* (*A. flavus, A. niger*), *Mycelia sterilia, Rhizopus oryzae, Trichoderma harzianum* and yeast sp. *A. niger* was the predominant fungal species found resident in the growth substrate followed by *T. harzianum*. *Aspergillus oryzae* recorded the least fungal population.

Fungal species isolated from the growth substrate of Kwesi-Babs mushroom farm were found to be similar to species sampled and isolated. *Aspergillus* (*A. candidus, A. niger*), *Mycelia sterilia, Rhizopus oryzae, Rhodotorula muscilaginosa* and *Trichoderma harzianum* were the

resident mycoflora in the sawdust substrate. *T. harzianum* predominated other fungal species followed by *A. niger*. The least encountered species was *R. oryzae*.

Fungi isolated from Immaculate Gold Enterprise though fewer on each media contained the primary fungal species which is a major culprit when it comes to the green mould disease. Mycoflora identified were *Aspergilus niger, Fusarium poae, Rhodotorula mucilaginosa, Trichoderma harzianum* and yeast spp. The pathogenic fungus, *T. harzianum* was the dominant species with *Sacchormyces* sp. being the least dominant.

A substantial number of mycoflora was successfully isolated from Delabless mushroom farm. There was however the isolation of another fungal species which is relevant to mushroom production and also causes the cobweb disease in *Agaricus, Verticillium fungicola.* Other fungal species isolated were; *Aspergillus* (*A. flavus, A. fumigatus, A. oryzae*), *Cladosporium* (*C. herbarum, C. macrocarpum*), *Penicillium brevicompactum, Rhodotorula mucilagginosa, Trichoderma harzianum* and yeast sp. *C. herbarum* was the most dominant species followed by *T. harzianum* and *C. macrocarpum*. *Rhizopus oryzae* was the least isolated.

4E Fresh mushroom farm had a mycoflora profile of; *Aspergillus* (*A. flavus, A. fumigatus*), *Cladosporium macrocarpum, Penicillium* (*P. camemberti, P. brevicompactum*), *Rhodotorula mucilaginosa, Trichoderma harzianum* and yeast sp. *C. macrocarpum* was the predominant species followed by *A. fumigatus* and *P. brevicompactum*. The least encountered in this farm however was *T. harzianum* and *A. flavus.*

Fungal species isolated from the growth substrate of Edeyef mushroom farm include *Aspergillus* (*A. flavus, A. niger*), *Cladosporium* (*C. herbarum, C. macrocarpum*), *Penicillium brevicompactum, Rhodotorula mucilaginosa, Trichoderma harzianum* and *Vertillium fungicola*. *T. harzianum* and *V. fungicola* which are pathogens of mushrooms in general were

also isolated from this farm. *C. macrocarpum* was the predominant species. Least in dominance resident on the growth substrate in this farm was yeast spp.

Fungal species isolated from PCM Mushroom farm were *Aspergillus* (*A. fumigatus, A. penicillioides, A. terreus*), *Cladosporium macrocarpum*, *Didymella* sp., *Fusarium* (*F. oxysporium, F. solani*), *Trichoderma harzianum* and *Verticillium* sp. There was however the isolation of a *Didymella* sp which is usually found in the soil and a pathogen affecting humans. *T. harzianum* predominated other fungal species in the growth substrate followed by *C. herbarum*. The least isolated were *A. terreus* and *Didymella* sp.

Table 4. Fungal species resident in growth substrate samples collected from seven (7) mushroom farms in Accra and Kasoa.

- *Aspergillus candidus* Link [2D]
- *Aspergillus flavus* Link [1P, 4POD, 5P, 6POD]
- *Aspergillus fumigatus* Fresineus [4O, 5D, 7PD]
- *Aspergillus niger* van Tieghem [1POD, 2OD, 3P, 4O, 6POD]
- *Aspergillus oryzae* (Ahlburg) E. Cohn [4O]
- *Aspergillus penicillioides* Speg. [7D]
- *Aspergillus terreus* Thom [7P]
- *Cladosporium herbarum* (Pers.) Link [4POD, 5P, 6PD, 7POD]
- *Cladosporium macrocarpum* Preuss [4PD, 5PD, 6POD]
- *Didymella* sp. (Fuckel) Rehm [7D]
- *Fusarium oxysporium* Schlecht. Emend. Snyder & Hansen [4P, 7D]
- *Fusarium poae* (Peck) Wr. [3P, 7P]
- *Fusarium solani* (Mart.) Sacc. [7OD]
- *Mycelia sterilia* [1O, 2O]
- *Penicillium brevicompactum* Dierckx [4PO, 5PD, 6POD, 7P]
- *Penicillium camemberti* Thom [5D]
- *Rhizopus oryzae* Went and Prinsen Geerling [1P, 2PD, 4D]
- *Rhodothorola mucilaginosa* H. C Harrison [2D, 3D, 4D, 5D]
- *Trichoderma harzianum* Rifai [1OD, 2PO, 3O, 4POD, 5P, 6OD, 7POD]
- *Verticillium fungicola* (Preuss) Zare & W. Gams [4D, 6P, 7PO]
- *Saccharomyces* sp. Meyen [1OD, 2PO, 3POD, 4P, 5PO, 6PO, 7O]

KEY*:*

1 – E90 MUSHROOM FARM, OGBOJO

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7 - PCM MUSHROOM FARM, ASHALEY BOTWE

P – ISOLATED ON PDA

O – ISOLATED ON OGYE AGAR

D – ISOLATED ON DRBC AGAR

4.2.1 Estimation of total fungal population resident in the sawdust growth substrate isolated on three different media; Potato Dextrose Agar (PDA), Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol agar (DRBC) from seven mushroom farms in Accra and Kasoa.

E90 mushroom farm had the most microbial load resident in the substarte. The highest readings were recorded on PDA (5.09 $\log_{10}$CFU/g), OGYE (5.24 $\log_{10}$CFU/g) and DRBC (4 $\log_{10}$CFU/g). Delabless mushroom farms had the second highest microbial load isolated in the substrate (4.5 $\log_{10}$CFU/g on PDA, 4.56 $\log_{10}$CFU/g on OGYE and 4.72 $\log_{10}$CFU/g on DRBC). Edeyef mushroom farms was the mushroom farm habouring the third highest microbial load. 4E mushroom farms had the lowest microbial load isolated from the growth substrate (2.88 $\log_{10}$CFU/g, 2.289 $\log_{10}$CFU/g and 2.93 $\log_{10}$CFU/g) isolated on PDA, OGYE and DRBC respectively. Kwesi-Babs mushroom farms and Immacukate Gold Ent. also had a relatively lower fungal population present in the growth substrate (Appendix 16, Table 4).

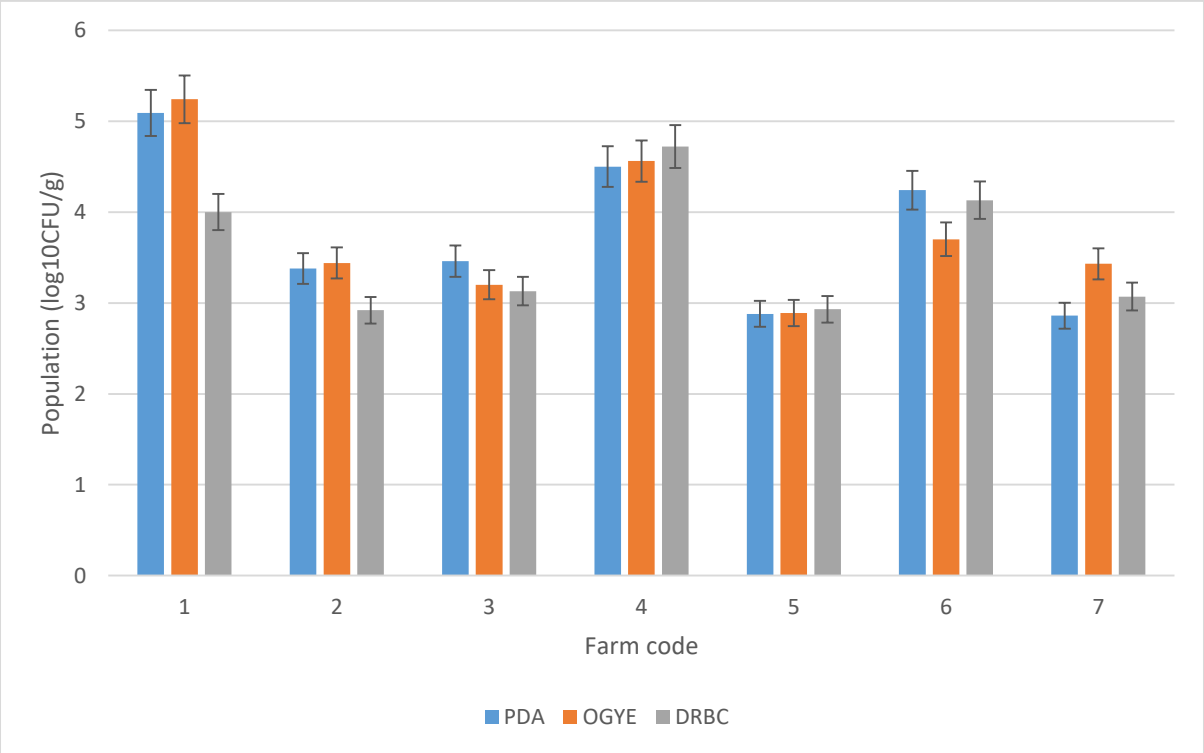

Figure 4. Comparison of the total fungal populations isolated from the growth substrate obtained from the seven mushroom farms.

FARM CODE:

1 – E90 MUSHROOM FARM, OGBOJO

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7 - PCM MUSHROOM FARM, ASHALEY BOTWE

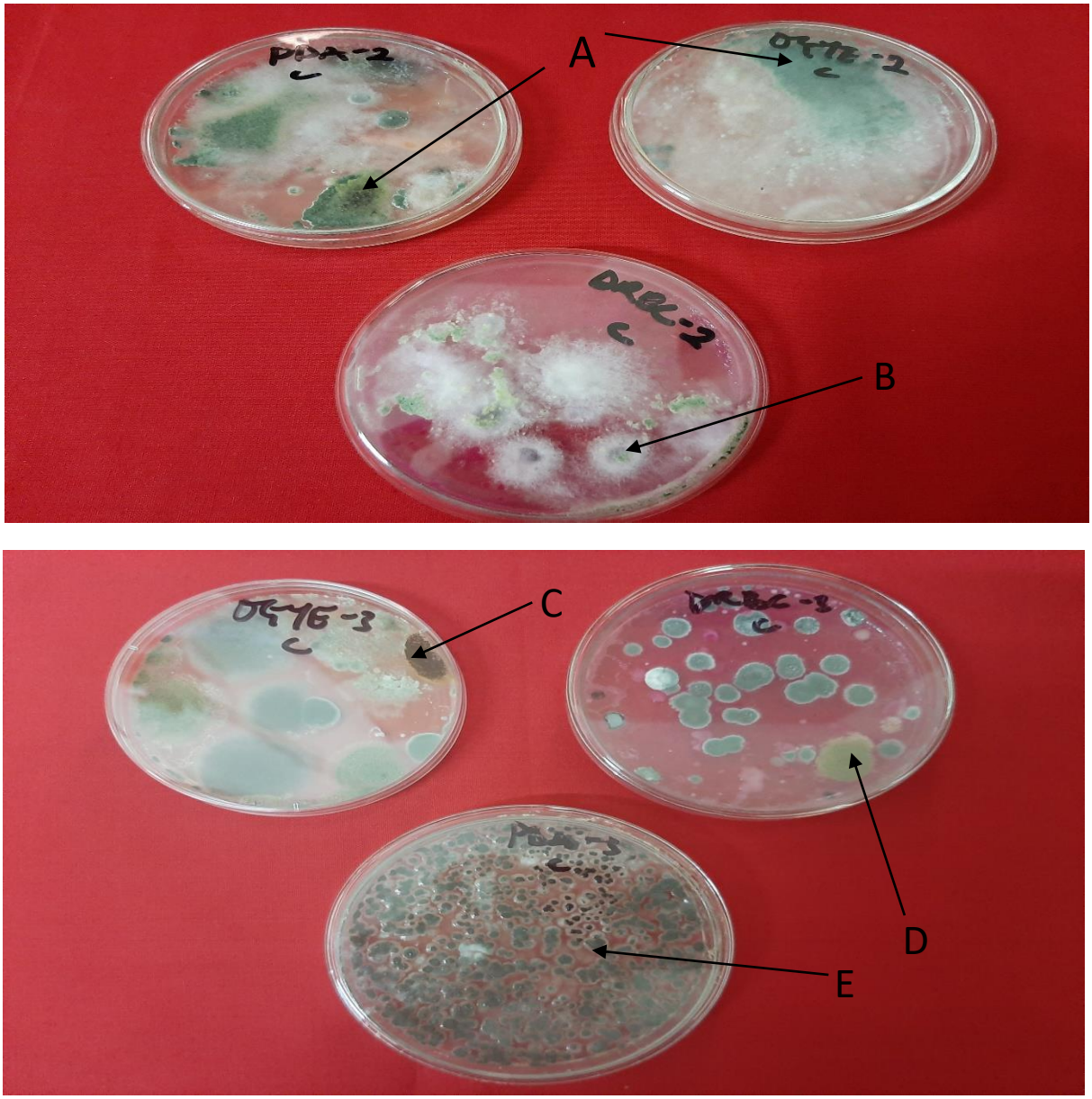


Plate 3. Representative mycoflora isolated from "wawa" growth substrate on PDA, OGYE and DRBC

A- *Trichoderma harzianum*

B- *Fusarium poae*

C- *Aspergillus niger*

D- *Aspergillus flavus*

E- *Cladosporium* sp. (Mag X 0.5)

EXPERIMENT 3

4.3 Aeromycoflora prevalent in the cropping rooms of the seven mushroom farms

The settle plate method was used to ascertain the population and aeromycoflora of the seven farms visited. The media used were; Potato Dextrose Agar (PDA) and Oxytetracycline-Glucose-Yeast Extract Agar (OGYE) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC).

Fifteen (15) species belonging to nine (9) genera were identified; *Aspergillus* spp. (*A. niger, A. parasiticus, A. flavus, A. candidus*), *Rhizopus oryzae, Trichoderma harzianum, Cladosporium* spp. (*C. macrocarpum, C. hebarum*), *Saccharomyces* spp., *Fusarium* spp. (*F. poae, F. oxysporum*) *Penicillium* spp. (*P. citrinum, P. brevicompactum*), *Epicoccum nigrum* and *Gliocladium* sp. (Table 5, Plate 4).

A. niger, R. oryzae and *T. harzianum* were identified amongst mycoflora sampled from the air in the cropping room of E90 mushroom farm located at Ogbojo. *A. niger* was most isolated with a 93% abundance. This was followed by *T. harzianum* which was 50% dominant in the air-spora sampled in the cropping room. The least encountered species in the cropping room of this farm was *R. oryzae*.

Kwesi-Babs farms located at Koasoa recorded *C. macrocarpum* being the most dominant fungus in the cropping room air-spora profile with a percentage dominance of 36.62%. This was followed by *A. niger* with the least encountered species being *R. oryzae.*

C. macrocarpum had the highest occurrence in the cropping room of Immaculate Gold located at Nii-Boi Town, Lapaz with a percentage occurrence of 41%. The least encountered fungus in this particular cropping room was *F. oxysporium* and the various yeast species.

T. harzianum predominated the air-spora profile in the cropping room of Delabless mushroom farm located at Adenta, Madina with a percentage occurrence of 72.34%. The least encountered species was *R. oryzae* with a percentage occurrence of 3%.

F. oxysporium predominated the fungal species isolated with a percentage occurrence of 65% sampled from the air in the cropping room of 4E Fresh mushroom farm. There was however the isolation of *Gliocladium* sp. which was only found the cropping room of this farm during this study. *Aspergillus candidus* and *Mycelia sterilia* were the least encountered species.

In Edeyef mushroom farm located at Anyaa-Awoshie, *C. herbarum* recorded the highest abundance in the air of the cropping room with the least fungal species being *Aspergillus niger*.

In the last mushroom farm, PCM mushroom farm, *C. herbarum* recorded the highest occurrence 94%. The least encountered in the farm were species of yeast.

Trichoderma harzianum which is the usual culprit when it comes to the devastating green mould disease in the oyster mushroom was not identified in the aeromycoflora profile of Immaculate Gold, Edeyef and PCM mushroom farm at the time of sampling.

Aspergillus niger, *Cladosporium* spp. (*C. macrocarpum, C. hebarum*) and *Trichoderma harzianum* were the fungal species which recorded the highest frequency across all seven farms with the least sampled being *Gliocladium* and *Mycelia sterilia*. *Cladosporium* spp. (*C. macrocarpum, C. hebarum*) were isolated from five out of seven mushroom farms followed by *Aspergillus* (*A. flavus, A. niger*) which were isolated from 4 out of 7 farms each.

The *Aspergillus* species are known to be mycotoxegenic with *Cladosporium* and *Trichoderma* being potentially pathogenic, however, fungi belonging to the genera *Cladosporium* are not relevant when it comes to oyster mushroom farming. Table 5 presents a pooled data of the air-spora determined from the seven mushroom farms for this study.

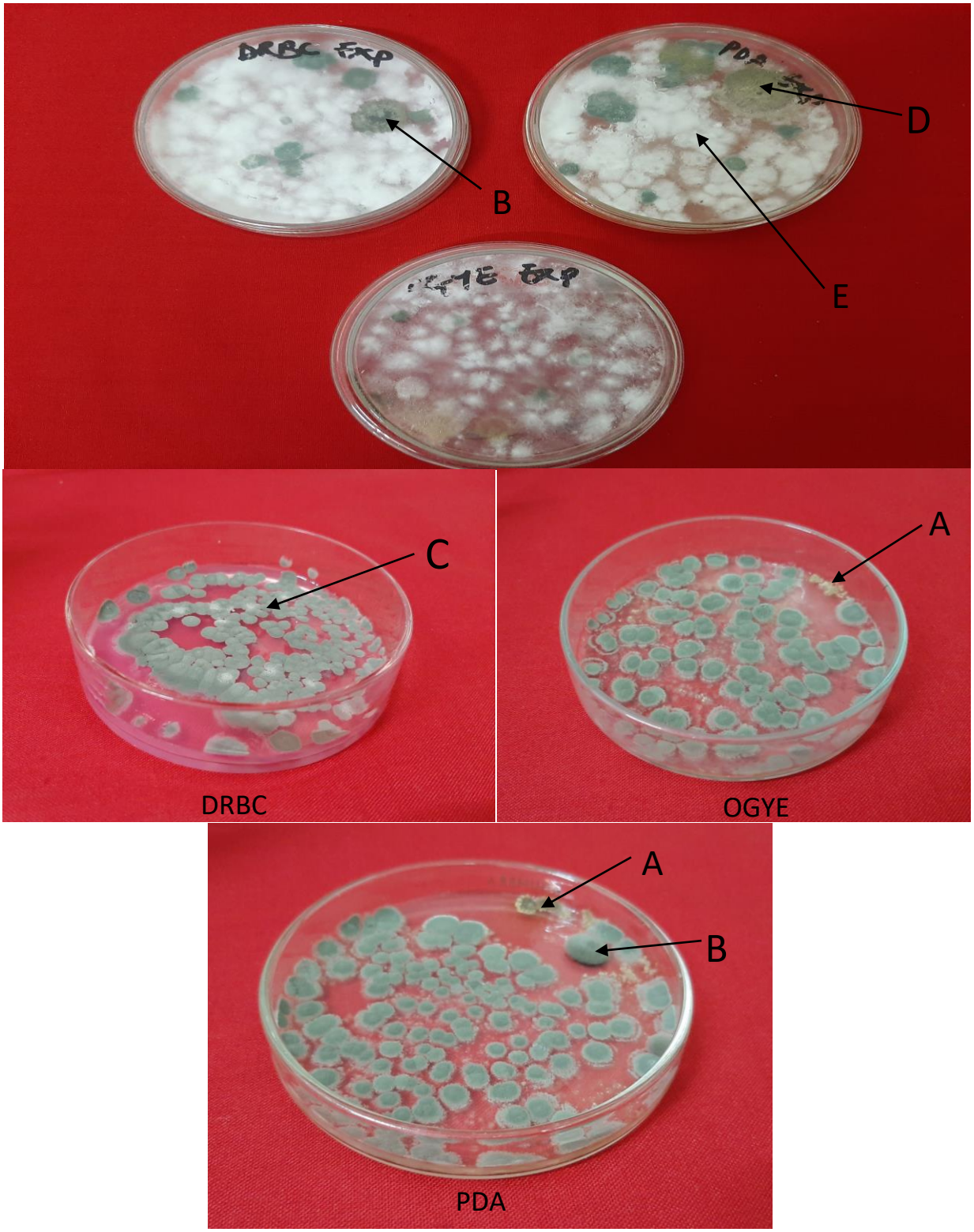


Plate 4. Representative mycoflora isolated on OGYE, DRBC and PDA using the settle plate method.

A- *Trichoderma harzianum* B- *Penicillium brevicompactum* C- *Cladosporium* sp. D- *Aspergillus flavus* E- *Verticillium fungicola* (Mag X 0.5).

Table 5. Pooled data on the aeromycoflora prevalent in the cropping rooms of the seven mushroom farms isolated on three media plates (PDA, OGYE, DRBC)

- *Aspergillus candidus* Link [4D, 5P]
- *Aspergillus flavus* Link [3PD, 4PD, 5OD, 7O]
- *Aspergillus niger* van Tieghem [1D, 2POD, 4POD, 6O]
- *Aspergillus oryzae* (Ahlburg) E. Cohn [4D]
- *Aspergillus parasiticus* Speare [2O]
- *Cladosporium herbarum* (Pers.) Link [3PD, 4POD, 5D, 6AO, 7POD]
- *Cladosporium macrocarpum* Preuss [2POD, 3POD, 4PD, 5O, 6O]
- *Epicoccum nigrum* Link [3P]
- *Fusarium oxysporium* Schlecht. Emend. Snyder & Hansen [3D, 5OD]
- *Fusarium poae* (Peck) Wr. [2OD, 4D, 5O]
- *Gliocladium* sp. (Link) Schroers [5D]
- *Mycelia sterilia* [5D]
- *Penicillium brevicompactum* Dierckx [3OD, 4D, 5PD, 7P]
- *Penicillium citrinum* Thom C. [2D]
- *Rhizopus oryzae* Went and Prinsen Geerling [1PD, 2POD, 4O]
- *Rhodothorola mucilaginosa* H. C Harrison [2D, 3D]
- *Trichoderma harzianum* Rifai [1OD, 2POD, 4PO, 5P]
- *Verticillium fungicola* (Preuss) Zare & W. Gams [7O]
- *Saccharomyces* sp. Meyen [2O, 3POD, 5PO, 7PO]

KEY*:*

1 – E90 MUSHROOM FARM, OGBOJO

2 – KWESI BABS FARM, KASOA

3 – IMMACULATE GOLD ENTERPRISE, NII-BOI TOWN, LAPAZ

4 – DELABLESS MUSHROOM FARM, ADENTA

5 – 4E MUSHROOM FARM, OGBOJO/ASHALEY BOTWE

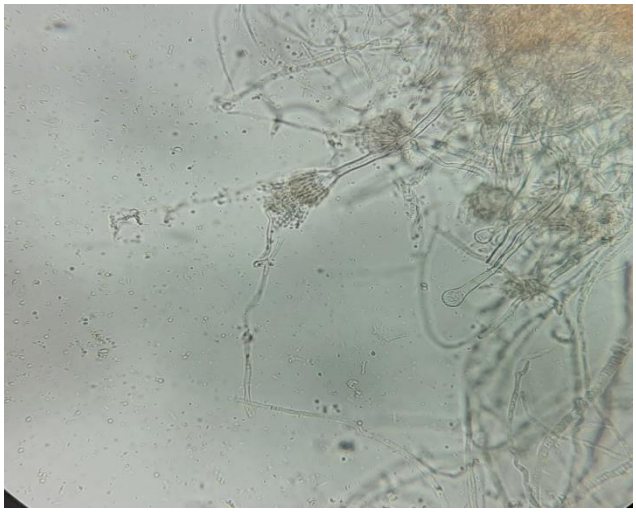
6 – EDEYEF MUSHROOM FARM, ANYAA-AWOSHIE

7 - PCM MUSHROOM FARM, ASHALEY BOTWE

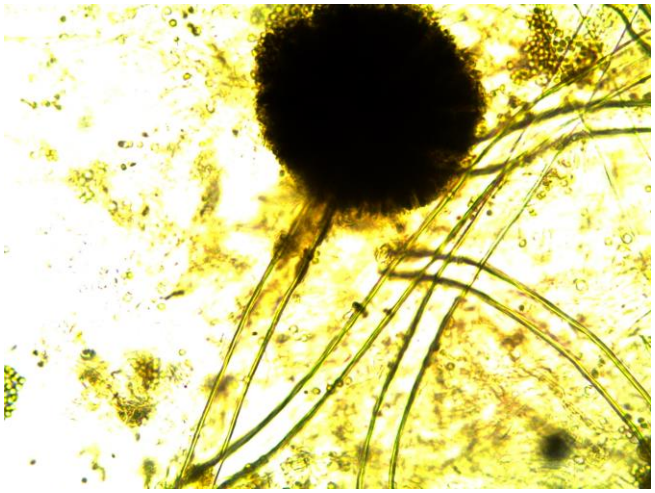
P – ISOLATED ON PDA

O – ISOLATED ON OGYE AGAR

D – ISOLATED ON DRBC AGAR

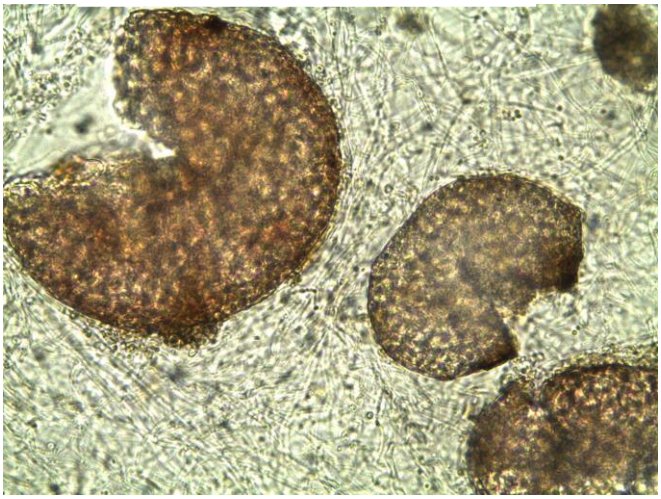


Aspergillus fumigatus

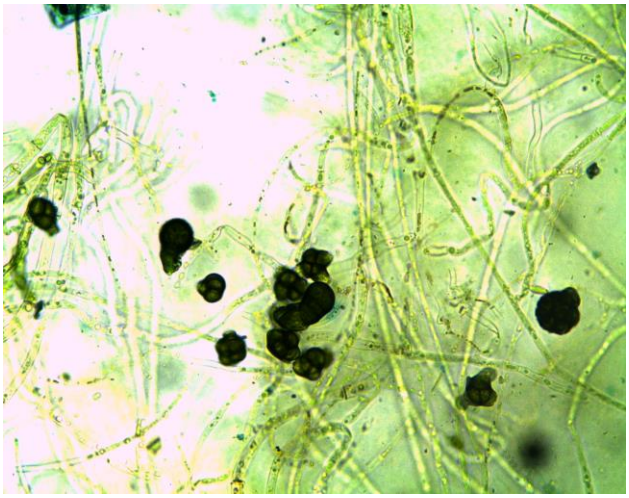


Aspergillus niger

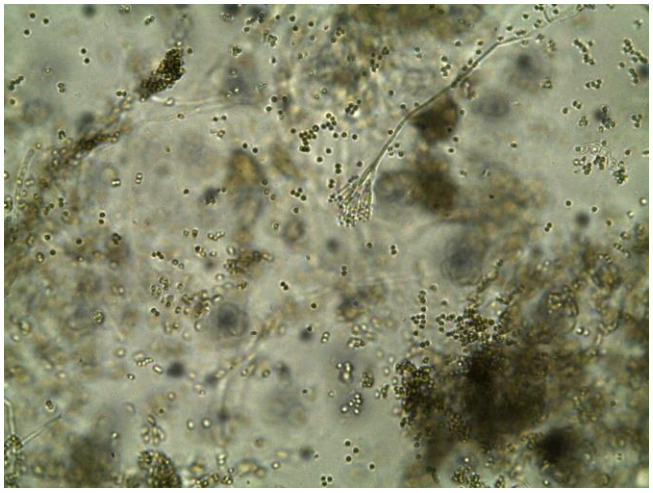
Cladosporium herbarum



Didymella sp.

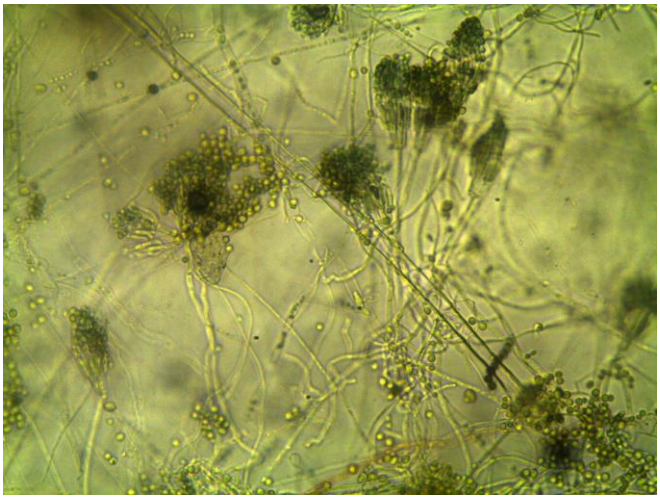


Epicoccum nigrum

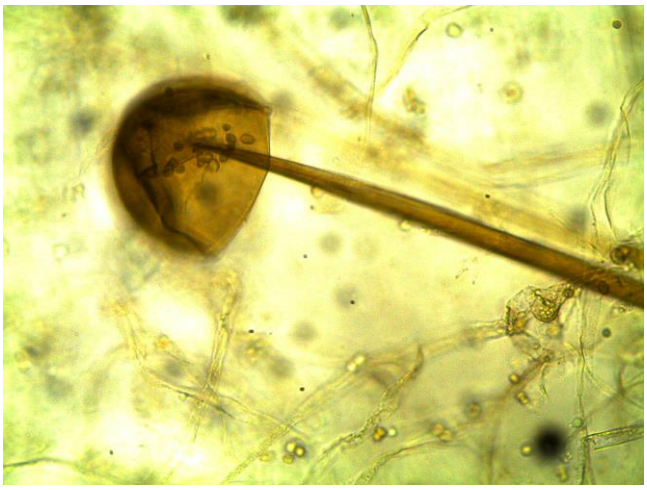


Penicillium brevicompactum

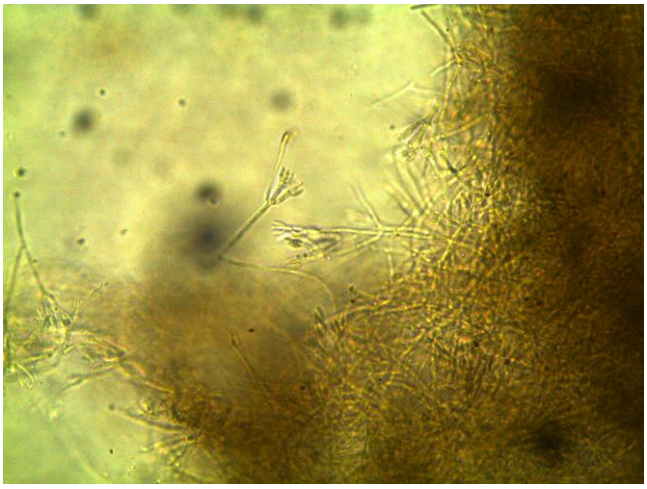
Plate 5a. Representative genera of fungi encountered and isolated (x400)



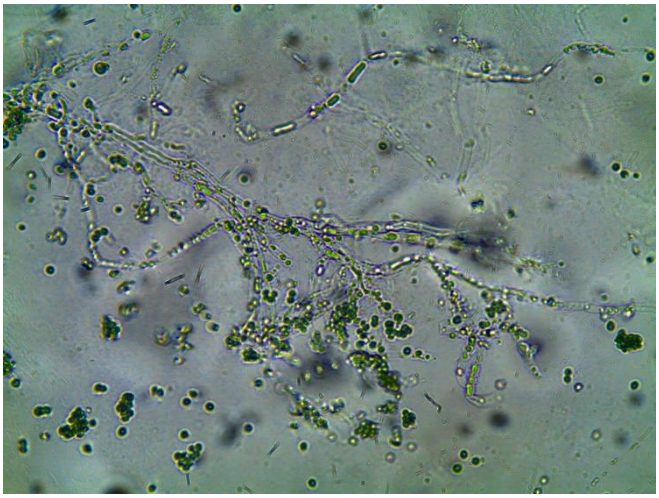
Penicillium citrinum



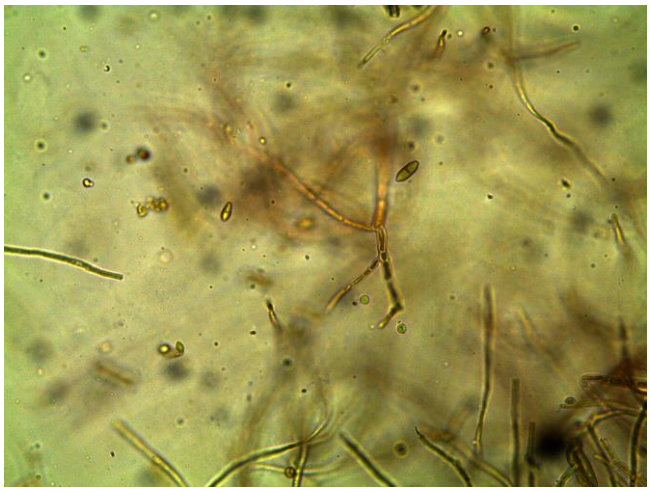
Rhizopus oryzae



Talaromyces flavus



Trichoderma harzianum



Verticillium fungicola

Plate 5b. Representative genera of fungi encountered and isolated

(x400)

EXPERIMENT 4

4.4 Radial growth assessment of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with different dilutions of bark extract of *Anthocleita nobilis* at 28˚C for 10 days

The test plant extract was used in amending PDA at concentrations of 1:1, 1:2, 1:5, 1:10 and undiluted v/v. The undiluted ethanol extract of *Anthocleita nobilis* decreased the vegetative growth of *T. harzianum* after 2 days of incubation (Plate 6a). The biotoxin present in the plant extract began to decrease in potency after 5 days. *T. harzianum* colonized the entire after 10 days (Plate 6b). Other dilutions (1:1 – 1:10 v/v) also showed a substantial inhibition of the growth of *T. harzianum* in the first 2 days with some inhibition observed at day 5 for concentrations (1:1 – 1:5 v/v). There was no statistical significance ($p>0.05$) between the various concentrations. Results of this experiment are summarized in Figure 5.

After 2 days, growth of *T. harzianum* on the agar was observed as white masses of mycelia across all dilutions without the usual green colour as observed on a full mature culture of the *T. harzianum.* There was an extensive growth on the control agar plate measuring 63mm as compared to the full strength concentration which recorded a growth of 33mm after 2 days.

Sporulation increased across all v/v dilutions after 5 days with the appearance of green patches characteristic of *T. harzianum* cultures. However, there was no significant increase in sporulation on the 1:1 v/v dilution as culture appeared white with no green patches. Growth observed on the control plate increased to 89mm whereas the full strength culture recorded a growth of 42mm showing a white fluffy mass at the growth borders.

After 10 days, there was a noticeable colonization of the entire agar plates across all v/v dilutions by *T. harzianum*. There was still inhibited sporulation observed in 1:1 v/v dilution as the mycelia mass lacked green patches even after 10 days.

The control appeared mostly of a white mass of mycelia with green patches whereas the full strength culture showed the coverage of the entire agar plate by a white mass of *T. harzianum* with very little green patches.

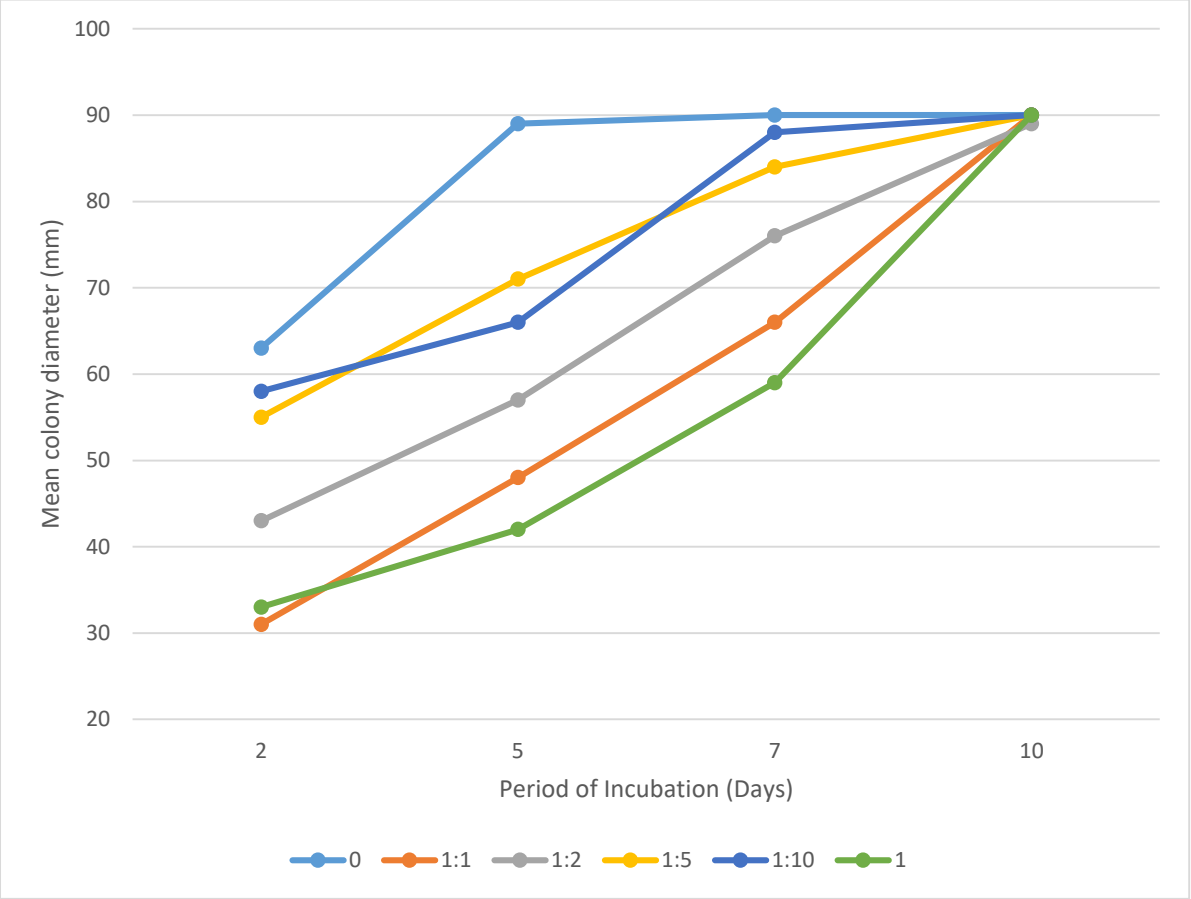


Figure 5. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with varying dilutions of ethanol extract of *Anthocleista nobilis* (Cabbage tree) at 28˚C for 10 days

KEY:

0- Control

1- Full strength of extract

Plate 6a. Influence of the extract of *A. nobilis* on the radial growth of *T. harzianum* on PDA amended with the indicated dilutions at 28˚C after 10 days.

KEY: Top: Plates after 2 days of incubation

Bottom: Plates after 5 days of incubation.

From left: Control; 1:1; 1:2, 1:5, 1:10 and Undiluted (Full Strength) (Mag X 0.5)

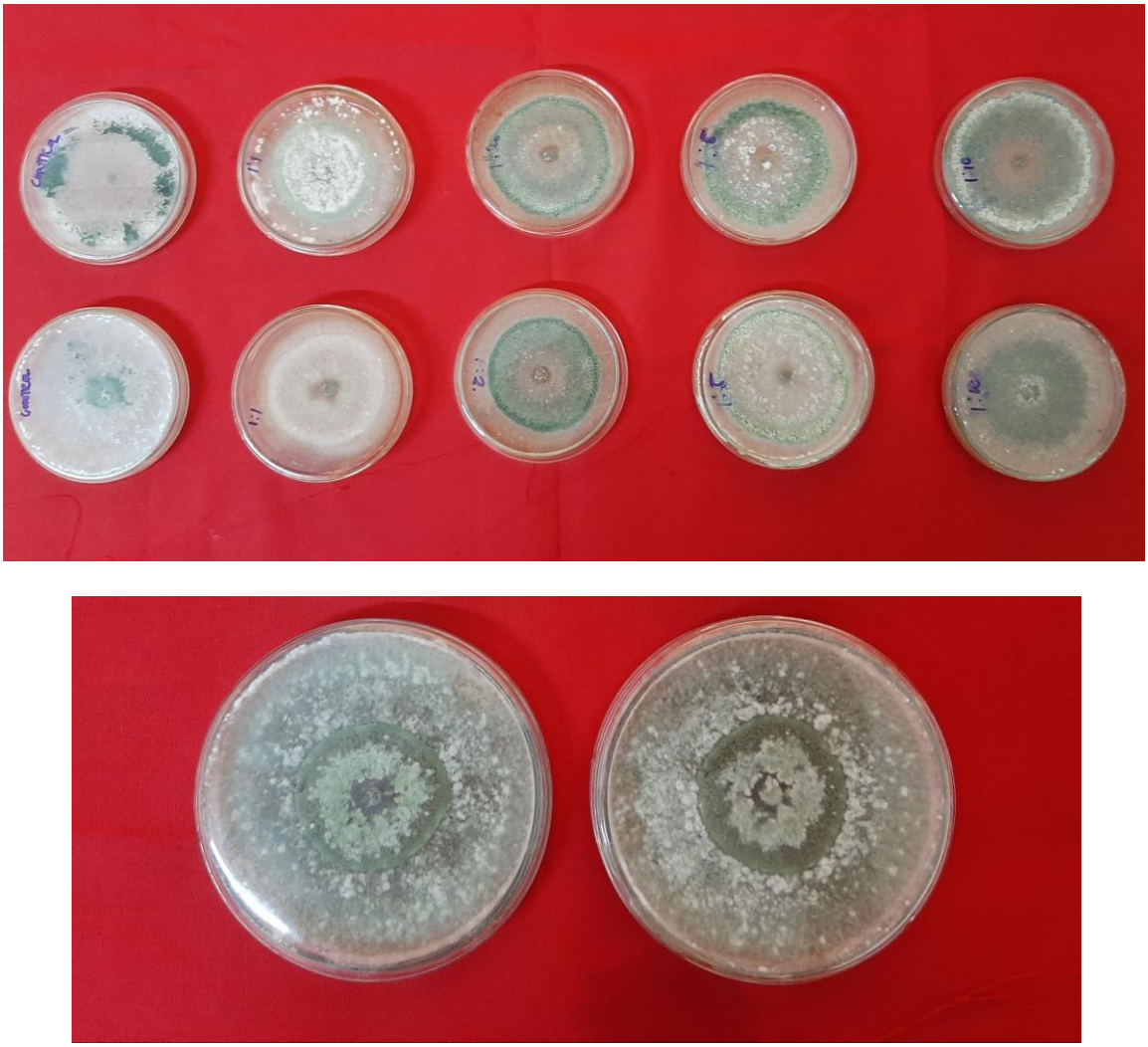


Plate 6b. Influence of the extract of *A. nobilis* on the radial growth of *T. harzianum* on PDA amended with the indicated dilutions at 28˚C after 10 days.

KEY:

Top: Plates after 10 days of incubation

From left: Control; 1:1; 1:2, 1:5, 1:10

Bottom: Plates of Undiluted (Full Strength) after 10 days.

(Mag X 0.5)

EXPERIMENT 5

4.5 Radial growth assessment of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with different dilutions of a copper fungicide "Champion" at 28˚C for 10 days

This experiment was similar to that in Experiment 4. In this experiment copper fungicide (Champion) was used in amending the PDA medium. Full strength dilutions of the antifungal and v/v dilutions 1:1 – 1:2 totally inhibited growth of the fungus on day 2, but there was noticeable growth recorded for v/v 1:5 – 1:10. There was the growth of a less fluffy white mass as observed on Experiment 4. There was however the growth of a fluffy white mass of mycelia on the control PDA whereas no growth was recorded on the full strength agar after 2 days.

The growth of *T. harzianum* after 5 days was seen to marginally increase 1:5 and 1:10 v/v dilutions. There was a slight sporulation recorded after 5 days much visible to what was recorded in the case of *A. nobilis*. *T. harzianum* recorded a diameter of 7mm whereas the control recorded a diameter of 84mm with an observable characteristic green sporulation.

After 10 days, growth on the full strength preparation of the copper fungicide agar recorded an increase to 34mm as compared to the control which fully colonized the agar plate. Other concentrations such as 1:5 and 1:10 v/v dilutions recorded an increase of growth diameter from 51mm and 61mm to 87mm and 90mm respectively.

The antifungal agent therefore, suppressed growth of the pathogenic fungi (Figure 6, Plate 7a, Plate 7b) and inhibition is significant ($P<0.05$) across all concentrations.

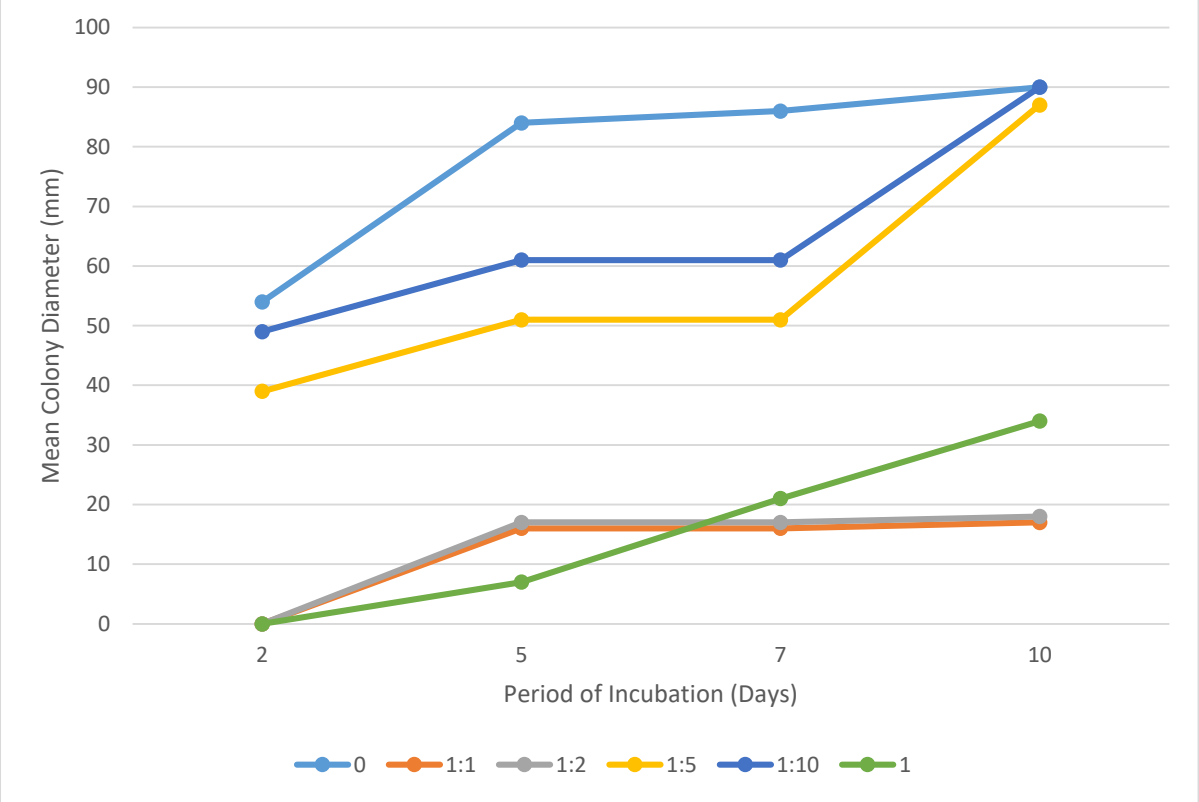


Figure 6. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with varying dilutions of a copper fungicide "Champion" at 28˚C for 10 days

KEY:

0- Control

1- Full strength of extract

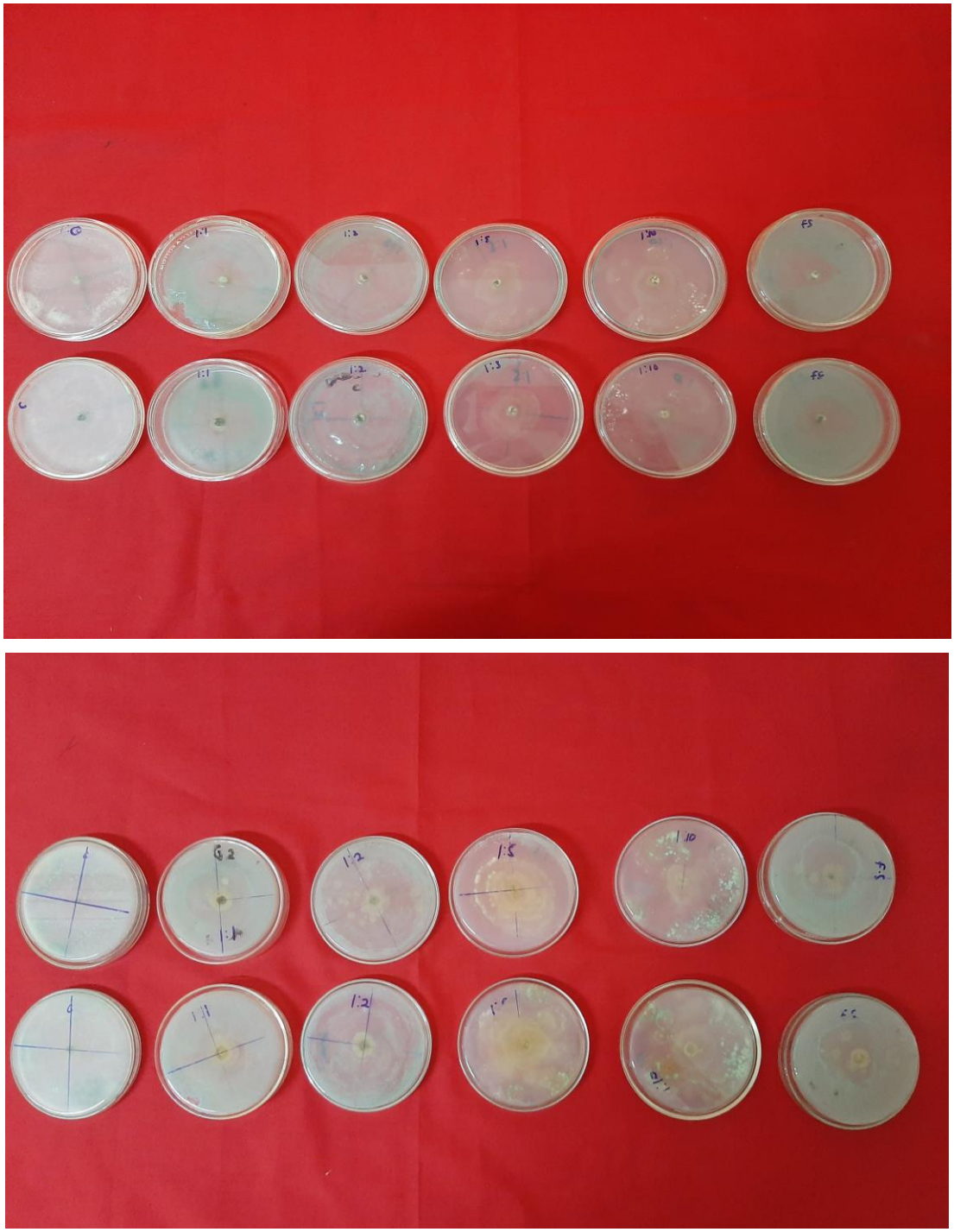


Plate 7a. Influence of copper fungicide "Champion" on growth of *T. harzianum* on PDA amended with the indicated dilutions at 28˚C after 10 days.

(Mag X 0.5). KEY: Top: Plates after 2 days of incubation

Bottom: Plates after 5 days of incubation.

From left: Control; 1:1; 1:2, 1:5, 1:10 and Undiluted (Full Strength)

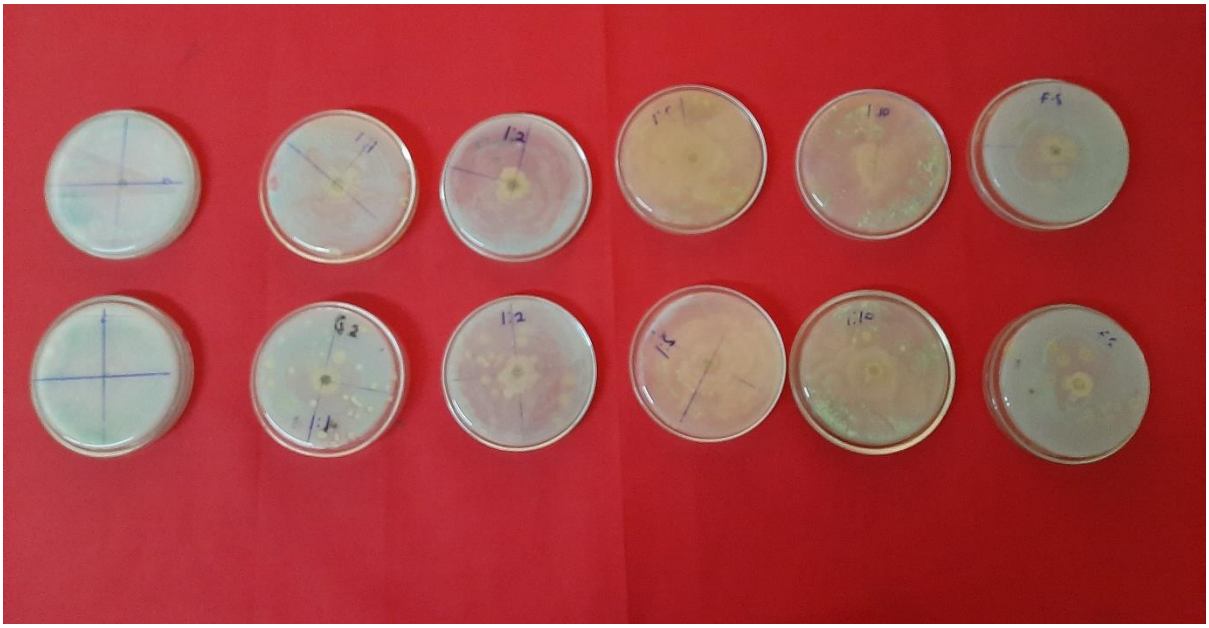


Plate 7b. Influence of copper fungicide on the radial growth of *T. harzianum* on PDA amended with the indicated dilutions at 28˚C after 10 days. Plates after 10 days of incubation.

(Mag X 0.5).

KEY:

From left: Control; 1:1; 1:2, 1:5, 1:10 and Undiluted (Full Strength)

EXPERIMENT 6

4.6 Vegetative growth of *Trichoderma harzianum* Potato Dextrose Broth (PDB) amended with different concentrations of bark extracts from *Anthocleita nobilis* at 28˚C for 7 days

The biotoxin in the ethanol bark extract of *A. nobilis* was seen to significantly suppress vegetative growth of *T. harzianum* (Plate 8a, Plate 8b).

Statistical analysis shows that the inhibitory effect was significant ($P \leq 0.05$) in the undiluted and 1:1v/v dilution of extract as compared to the control. There was therefore a substantial difference in the effect of the extract on growth in the liquid medium and an agar (solid medium). Results are summarized in Figure 7.

The undiluted (full strength) media preparation recorded the lowest mycelial growth mass of 70mg whereas the highest mycelium growth mass was recorded by the control and 1:10 v/v dilutions (160mg). This therefore indicates the efficiency of the full strength preparation of *A nobilis* in significantly inhibiting the growth of *T. harzianum* as compared the control media preparation.

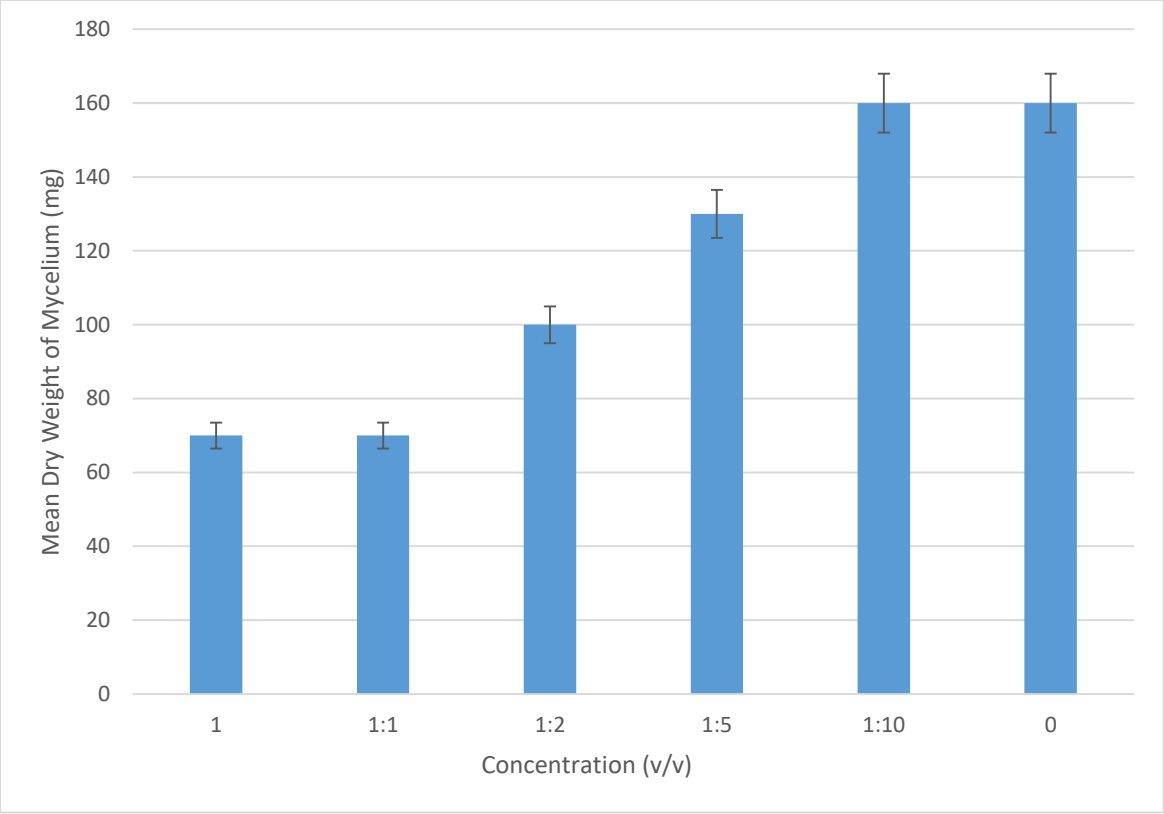


Figure 7. Effect of varying dilutions of ethanol extract of *Anthocleista nobilis* on the dry weight accumulation of *Trichoderma harzianum* in Potato Dextrose Broth (PDB) after 7 days

KEY:

0- Control

1- Undiluted (full strenght)

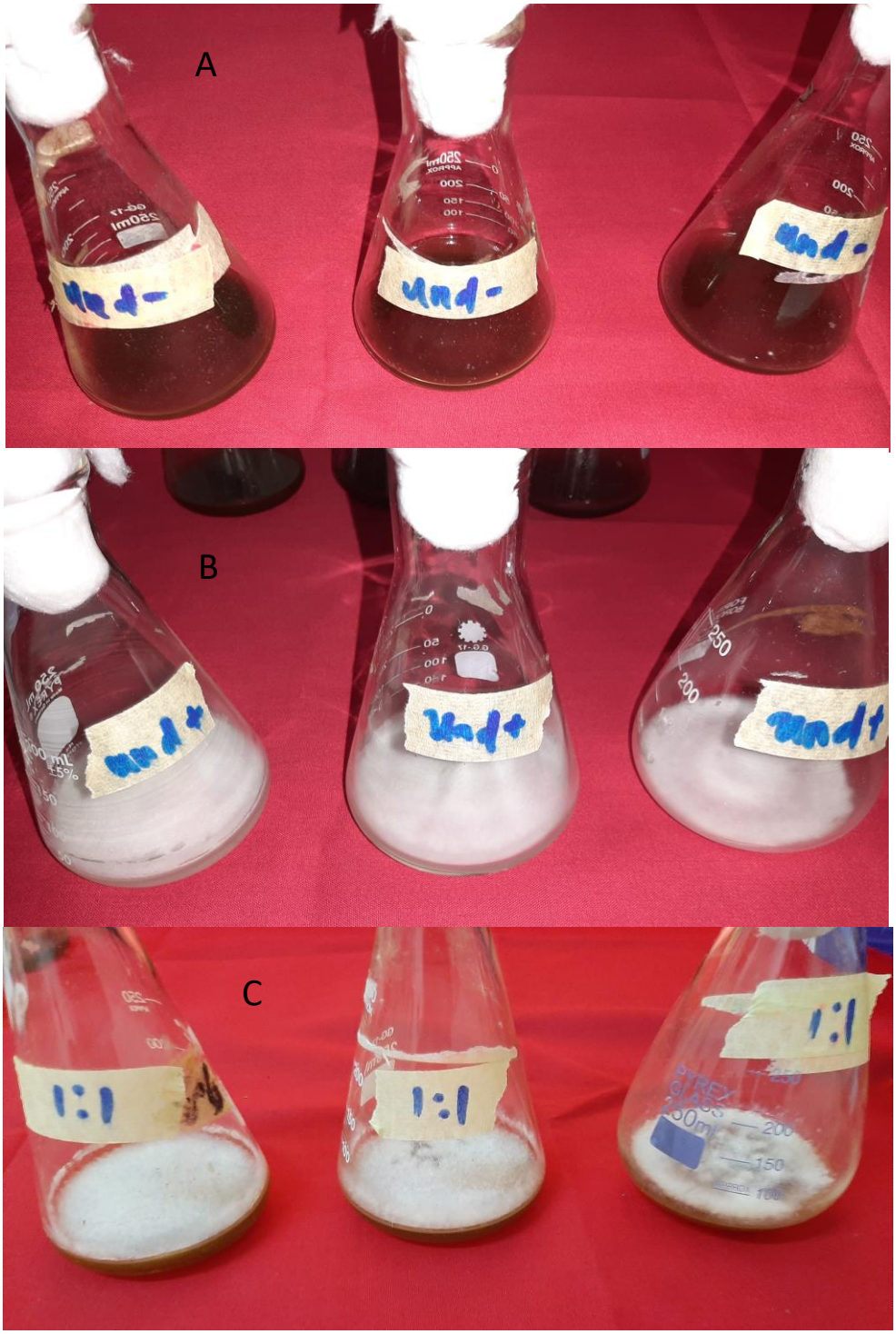


Plate 8a. Influence of ethanol bark extract of *A. nobilis* on the vegetative growth of *T. harzianum* in Potato Dextrose Broth amended with varying concentrations (full strength, control and 1:1) v/v dilutions at 28˚C for 7 days.

(Mag X 0.5)

KEY: A- Undiluted (Full Strength) B- Control C- 1:1 v/v dilution

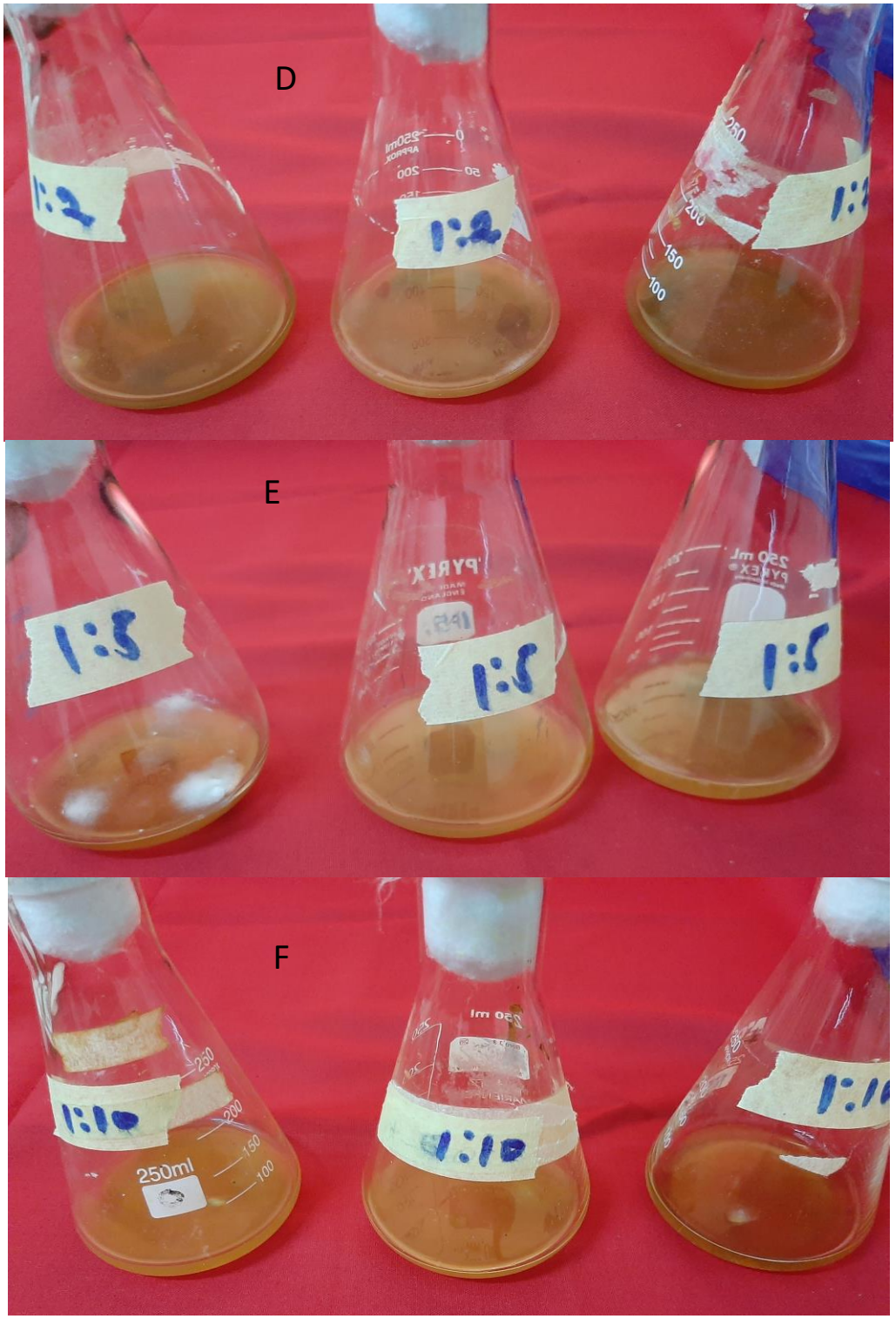


Plate 8b. Influence of ethanol bark extract of *A. nobilis* on the vegetative growth of *T. harzianum* in Potato Dextrose Broth amended with varying concentrations (1:2; 1:5; 1:10) v/v dilutions t 28˚C for 7 days

(Mag X 0.5)

KEY: D- 1:2 v/v dilution E- 1:5 v/v dilution F- 1:10 v/v dilution

EXPERIMENT 7

4.7 Vegetative growth assessment of *Trichoderma harzianum* in Potato Dextrose Broth amended with varying concentrations of a copper fungicide preparation "Champion" at 28 ˚C for 7 days

The copper fungicide effectively suppressed vegetative growth of *T. harzianum* as observed in the undiluted media formulation. (Plate 9). The control which consisted of just PDB yielded a mycelia growth of 160mg as compared to the full strength formulation of the copper fungicide which yielded a mycelium mass of 70mg.

The 160 mg mycelium growth was the lowest recorded mycelial mass yielded by the full strength formulation of the copper fungicide. 1:1 v/v dilution after 7 days also recorded a mean mycelium mass of 70mg same as the undiluted v/v dilution. The inhibitory effect of the antifungal was significant ($P \leq 0.05$) in the undiluted and 1:1v/v dilution. Results are summarized in Figure 8.

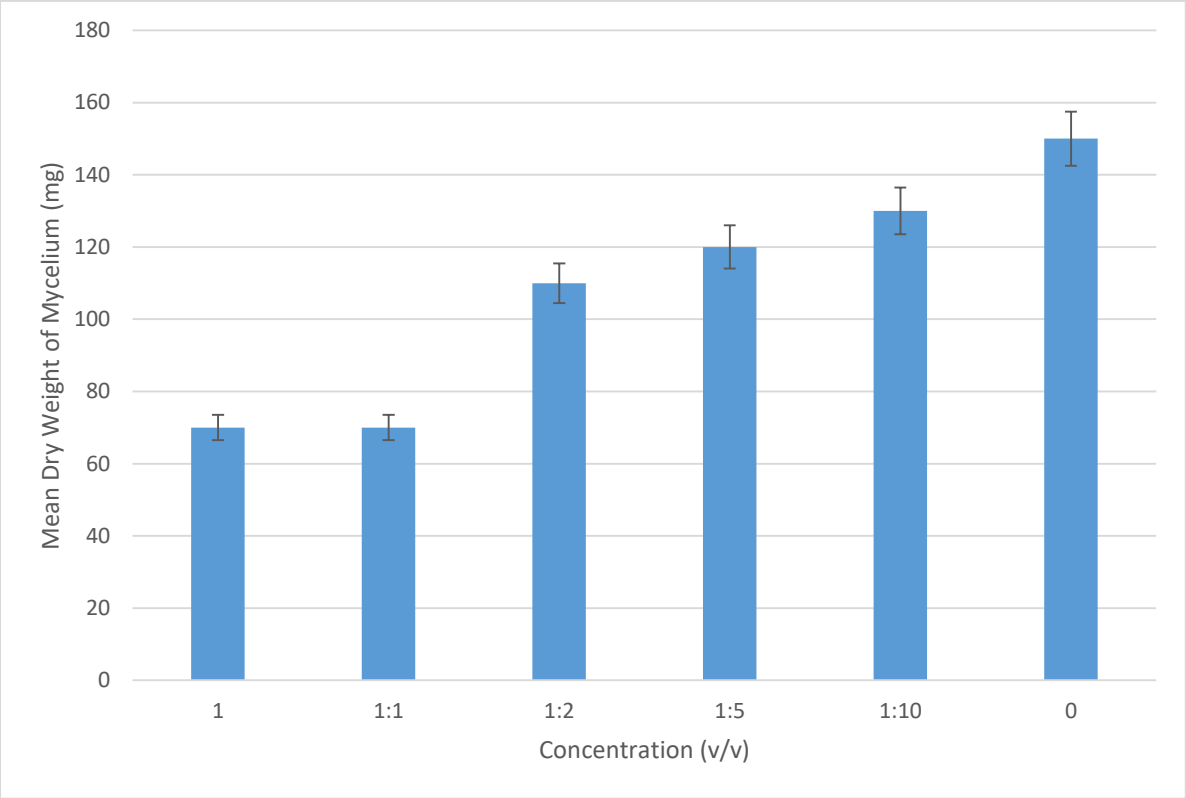


Figure 8. Influence of copper fungicide "Champion" on the mycelium dry weight of *T. harzianum* in Potato Dextrose Broth amended with indicated concentrations at 28˚C after 7 days.

KEY:

0- Control

1- Undiluted (Full Strength)

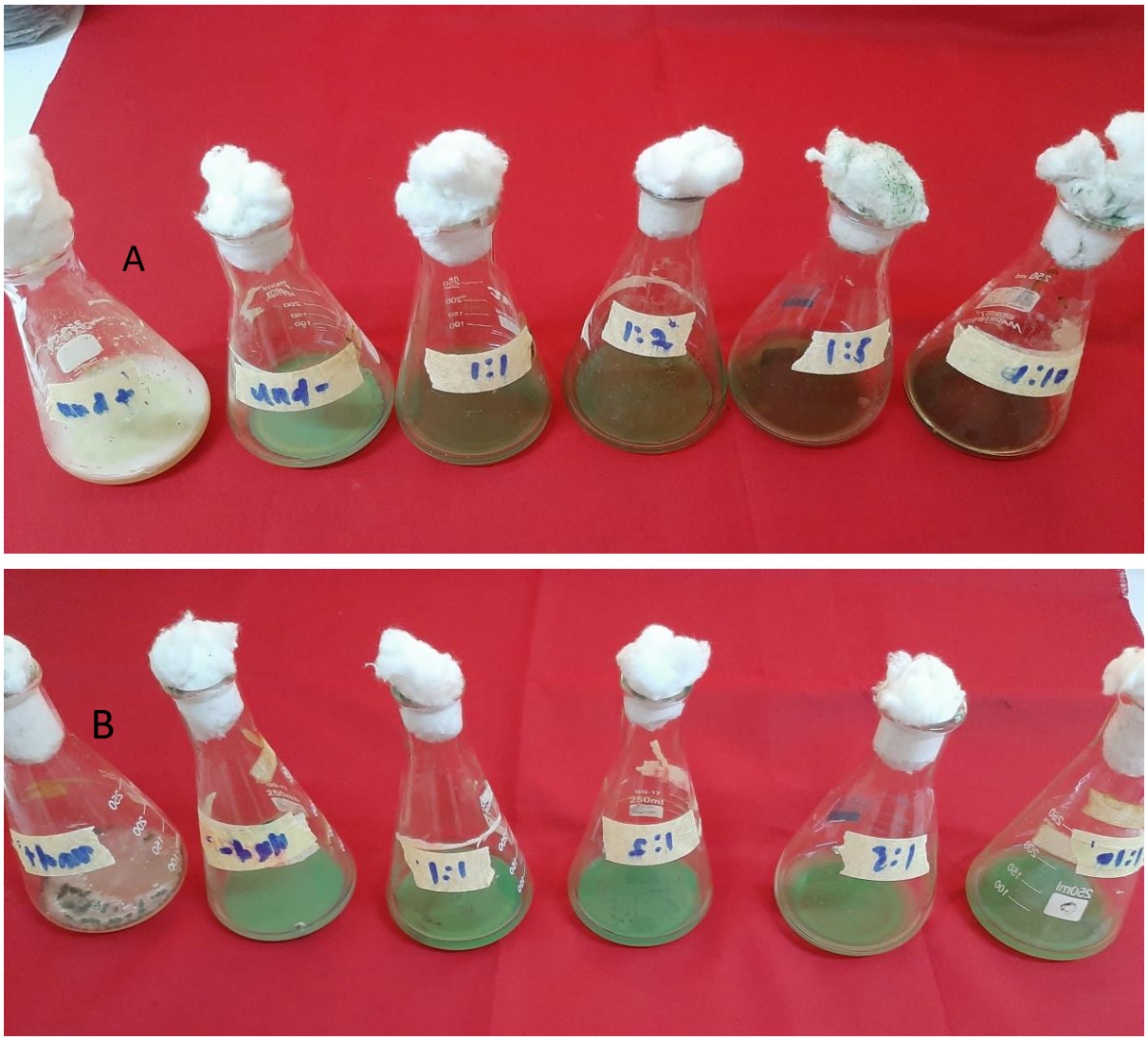


Plate 9. Vegetative growth of *T. harzianum* in Potato Dextrose Broth amended with varying concentrations of a copper fungicide "Champion" after 7 days.

(Mag X 0.5)

KEY:

A- First setup (from left: Control; full strength; 1:1; 1:2; 1:5; 1:10)

B- Second setup (from left: Control; full strength; 1:1; 1:2; 1:5; 1:10)

EXPERIMENT 8

4.8 Assessment of growth yield and biological efficiency of *Pleurotus ostreatus* strain EM-1 on pre-inoculated substrate bags contaminated with *Trichoderma harzianum* and uncontaminated bags at 28˚C for 3 weeks.

In this experiment already spawns bags were inoculated with *T. harzianum*, a common mushroom pathogen. The uninoculated spawn bags served as the control. the bags were opened to assess the following parameters; pinhead formation, number of pinheads, number of fruiting bodies, date of harvest, total yield, percentage (%) conversion of pinheads to fruiting bodies, weight of fruiting bodies, stipe length, pileus width/diameter and number of flushes per bag per treatment.

Already spawned mushroom bags were opened after full colonization of the compost bags. The bags were opened and was inoculated with *T. harzianum* on the same day.

Overall, five bags set as control were recorded to have more fruiting bodies than the bags inoculated with *T. harzianum*, except for the second flash where 3 bags for each setup (five contaminated and five uncontaminated bags) showed fruiting bodies. This resulted in an overall higher rate of conversion of pinhead to fruiting bodies in the control setup as opposed to the contaminated substrate bags with *T. harzianum* showing less conversion of pinheads to fruiting bodies.

Fruiting bodies of mushrooms harvested from the control setup showed significant maturation as compared to the contaminated bag setup. Results are summarized in Tables 6-11. Plate 10a shows an infected bag and the apparent effect of the green mould on mushroom emergence as well as the growth of a normal mushroom fruiting body.

4.8.1 Assessment of growth yield and biological efficiency of first flush of *Pleurotus ostreatus* strain EM-1 on pre-inoculated bags contaminated with Trichoderma harzianum and uncontaminated bags at 28˚C for 3 weeks.

Results are summarised in Table 6 and Table 7.

There was the formation of fruiting bodies three days after the opening of fully colonized substrate bags. Having five bags each for each of the two setups (contaminated and uncontaminated with *T. harzianum*, Plate 10a and 10b), the uncontaminated substrate bags recorded fruiting in 4 bags as compared to the appearance of fruiting in 3 bags that were contaminated with the pathogenic fungus. There was however a slight dip in the number of pinheads formed in the substrates contaminated with the pathogenic fungi as compared to the uncontaminated substrates. There was a higher conversion rate of pinheads to fruiting bodies on the contaminated substrate as compared to the uncontaminated bags.

Higher fruiting body weight were recorded for uncontaminated substrate bags 1, 2, 3 whereas bag 5 of the *T. harzianum* contaminated bag recorded a mushroom fresh weight of 61.70g as opposed to 46.80g fresh weight of the uncontaminated bag 5. Other growth parameters such as the stipe length, stipe width and cap diameter of fruiting bodies from the uncontaminated substrate were averagely and relatively higher than readings from bags contaminated from *T. harzianum.*

The highest mushroom fresh weight among the uncontaminated substrate was recorded by bag 1 having a fresh weight of 90.86g with a 37.5% pinhead conversion to fruiting bodies with the lowest fresh weight being that from bag 5 with a fresh weight of 46.80g with a 37.5% pinhead conversion to fruiting bodies. The contaminated substrates had a highest fresh weight from bag 2, 73.08g with a 79.16% pinhead conversion to fruiting bodies. 48.11g was the lowest fresh weight for this setup with a 40.91% pinhead conversion to fruiting bodies.

Table 6. First flush growth yield and biological efficiency of *P. ostreatus* strain EM-1 on uncontaminated wawa sawdust spawn bag.

Number of bags	**No. of Pinheads (Fruiting Bodies)**	**% Pinhead Conversion to Fruiting Bodies**	**Weight of Fruiting Bodies (g)**	**Stipe Length (mm)**	**Stipe Width (mm)**	**Cap Diameter (mm)**
1	16(6)	37.5	90.86	73.5±12	9±1	75±0
2	24(10)	41.66	78.03	59.7±5	10±3	60.5±3
3	32(19)	59.37	55.79	50±0	7±3	34±6
4	-	-	-	-	-	-
5	24(9)	37.5	46.80	58.5±5	12±4	55±13

Table 7. First flush growth yield and biological efficiency of *P. ostreatus* strain EM-1 on wawa sawdust spawn bag inoculated with *Trichoderma harzianum* mushroom pathogen.

Number of Bags	**No. of Pinheads (Fruiting Bodies)**	**% Pinhead Conversion to Fruiting Bodies**	**Weight of Fruiting Bodies (g)**	**Stipe Length (mm)**	**Stipe Width (mm)**	**Cap Diameter (mm)**
1	-	-	-	-	-	-
2	24(19)	79.16	73.08	52.5±1	12±4	39.5±6
3	22(9)	40.91	48.11	61±1	9±1	42.5±3
4	-	-	-	-	-	-
5	34(12)	35.29	61.70	40±6	8.5±2	42±0

4.8.2 Assessment of growth yield and biological efficiency of second flush of *Pleurotus ostreatus* strain EM-1 on pre-inoculated bags contaminated with *Trichoderma harzianum* and uncontaminated bags at 28˚C for 3 weeks.

For the second flush, there was fruiting observed on 3 substrate bags each on the day of harvesting. There was a reduction in mushroom fresh weight for both uncontaminated substrate bags and substrate bags contaminated with *T. harzianum*. The highest weight of fruiting body was recorded for the uncontaminated setup was 36.63g with an 84.61% pinhead conversion to fruiting bodies. The lowest fresh weight was 29.32g with a corresponding 77.27% pinhead conversion to fruiting bodies.

Relatively lower fresh weights were recorded from the fruiting bodies harvested from substrate bags contaminated with *T. harzianum* with the lowest mushroom fresh weight in this category being 16.19g having a 54.16% pinhead to fruiting bodies conversion. The highest mushroom fresh weight was recorded from bag 3 with a mushroom fresh weight of 26.78g with a 69.23% pinhead conversion to fruiting bodies (Table 8 and Table 9).

Table 8. Second flush growth yield and biological efficiency of *P. ostreatus* strain EM-1 on uncontaminated wawa sawdust spawn bag.

Number of Bag	**No. of Pinheads (Fruiting Bodies)**	**% Pinhead Conversion to Fruiting Bodies**	**Weight of Fruiting Bodies (g)**	**Stipe Length (mm)**	**Stipe Width (mm)**	**Cap Diameter (mm)**
1	22(17)	77.27	29.32	37.5±6	7±1	50±7
2	26(22)	84.61	36.63	41±5	8.5±2	48±4
3	-	-	-	-	-	-
4	20(13)	65	31.45	44.5±1	12±3	57±3
5	-	-	-	-	-	-

Table 9. Second flush growth yield and biological efficiency of *P. ostreatus* strain em-1 on wawa sawdust spawn bag inoculated with *Trichoderma harzianum* mushroom pathogen

Number of Bags	**No. Of Pinheads (Fruiting Bodies)**	**% Pinhead Conversion To Fruiting Bodies**	**Weight of Fruiting Bodies (g)**	**Stipe Length (mm)**	**Stipe Width (mm)**	**Cap Diameter (mm)**
1	21(18)	85.71	23.53	39±1	6±0	36±13
2	-	-	-	-	-	-
3	26(18)	69.23	26.78	48±3	5.5±1	31.5±5
4	24(13)	54.16	16.19	4.1±0.8	0.5±0	35.5±13
5	-	-	-	-	-	-

4.8.3 Assessment of growth yield and biological efficiency of third flush of *Pleurotus ostreatus* strain EM-1 on pre-inoculated bags contaminated with *Trichoderma harzianum* and uncontaminated bags at 28˚C for 3 weeks.

For the third flush, fruiting was recorded on all bags which were uncontaminated by *T. harzianum.* There was an increase in mushroom fresh weight as compared to mushroom fruiting bodies from the second flush. Amongst the uncontaminated substrate bags, the highest fresh mushroom weight was recorded by bag 3 and bag 5 which were 53.94g with a 40.62% pinhead conversion to fruiting bodies and 53.84g with a 35.71% pinhead conversion to fruiting bodies respectively.

Fruiting bodies from substrate bags contaminated with the mushroom pathogenic fungus were recorded on only two substrate bags out of the total five. The highest mushroom fresh weight was recorded from fruiting bodies from bag 3 which had a weight of 41.38g with a 73.33% pinhead conversion to fruiting bodies. The lowest was from bag 2 weighing 13.6g and had a 55.5% conversion of pinheads to fruiting bodies (Table 10 and Table 11).

Table 10. Third flush growth yield and biological efficiency of *P. ostreatus* strain EM-1 on uncontaminated wawa sawdust spawn bag.

Number of Bag	No. of Pinheads (Fruiting Bodies)	% Pinhead Conversion to Fruiting Bodies	Weight of Fruiting Bodies (g)	Stipe Length (mm)	Stipe Width (mm)	Cap Diameter (mm)
1	34(19)	55.88	19.67	50±8	9±1	39.5±10
2	23(17)	73.91	49.48	58.5±2	15.5±1	80.5±10
3	32(13)	40.62	53.94	47.5±3	9.5±1	57±10
4	40(10)	25	31.36	41±1	9±0	51.5±11
5	45(15)	35.71	53.84	50.5±8	9±4	61±2

Table 11. Third flush growth yield and biological efficiency of *P. ostreatus* strain EM-1 on wawa sawdust spawn bag inoculated with *Trichoderma harzianum* mushroom pathogen

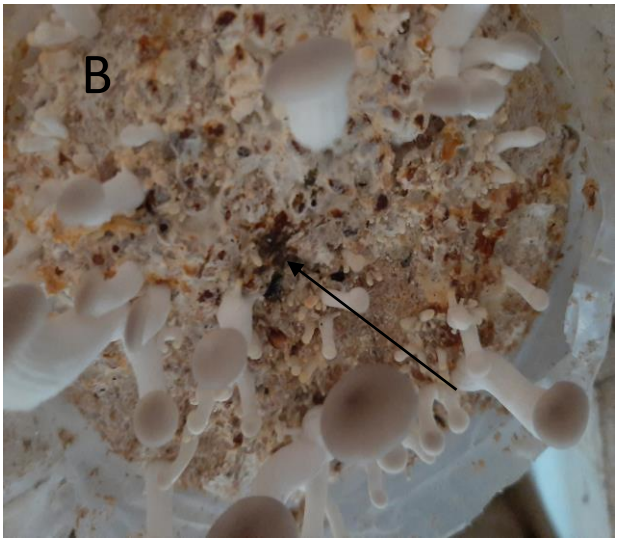
Number of Bag	No. of Pinheads (Fruiting Bodies)	% Pinhead Conversion To Fruiting Bodies	Weight of Fruiting Bodies (g)	Stipe Length (mm)	Stipe Width (mm)	Cap Diameter (mm)
1	-	-	-	-	-	-
2	18(10)	55.55	13.6	61±6	6.5±1	17±4
3	15(11)	73.33	41.38	49±11	9±1	62.5±3
4	-	-	-	-	-	-
5	-	-	-	-	-	-

4.8.4 Growth yield and Biological Efficiency (BE) of *Pleurotus ostreatus* EM-1 on uncontaminated and *Trichoderma harzianum* contaminated "wawa" sawdust growth substrate.

The highest yield (271.48g) was recorded on the first flush harvested on the uncontaminated growth substrate, followed by 208.29g harvested from the third flush. The lowest yield (66.50g) was on the third flush on the growth substrate contaminated with *Trichoderma harzianum.* Total yield for the uncontaminated substrate for the three flushes was 576.85g with the total yield recorded for the 3 flashes for the *T. harzianum* contaminated substrate being 304.37g. Biological Efficiency (B.E) was highest on the uncontaminated growth substarte (164.91%) whereas relatively lower on the *T. harzianum* contaminated substrate (87.02%). Results are summarized on Table 12.

Table 12. Total Yield and Biological Efficiency (BE) per flush on *Pleurotus ostreatus* strain EM-1 on uncontaminated and *Trichoderma harzainum* contaminated sawdust growth substrate

Substrate	**Yield/Flush (g)**			**Total yield (g)**	**Biological Efficiency (%)**
	1st flush	**2nd flush**	**3rd flush**		
Control (uncontaminated substrate)	271.48	97.08	208.29	576.85	164.91
Contaminated substrate	182.89	66.50	54.98	304.37	87.02



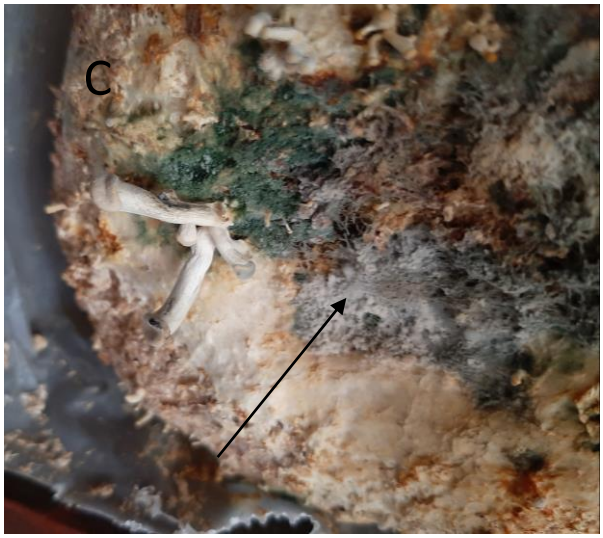


Plate 10a. Substrate bags contaminated with *Trichoderma harzianum*, note the progression and appearance of the green mould fungus on the growth substrate

(from A to C)

(Mag X0.05).

Plate 10b. Uncontaminated substrate bags with the appearance of fruiting bodies at maturation

(Top and Bottom) (Mag X0.05).

4.9 Chemical and mineral compostion of *Pleurotus ostreatus* (EM-1) cultivated on uncontaminated and *Trichoderma harzianum* contaminated growth substrates of "wawa" sawdust.

Chemical and mineral analysis were carried out on the mushroom fruiting bodies of *P. ostreatus* (EM-1) harvested on the first, second and third flushes accumulatively on uncontaminated and *T. harzianum* contaminated growth substrates.

4.9.1 Mineral compostion of *Pleurotus ostreatus* EM-1 mushroom fruiting bodies harvested from uncontaminated growth substrate.

The mineral component (Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Zinc (Zn)) of *Pleurotus ostreatus* EM-1 cultivated on a sawdust "wawa" substrate is summarized in Table 13. The fruiting bodies cultivated on the uncontaminated growth substrate contained minerals such as Calcium, Copper, Iron, Potassium, Magnesium, Sodium, Phosphorus and Zinc in amounts of 4.43, 0.04, 1.13, 16.66, 3.56, 0,01, 12.40 and 0.03mg/kg dry weight of fruiting bodies respectively.

Table 13. Total mineral content and heavy metals detection of *Pleurotus ostreatus* (Strain EM-1) grown on uncontaminated growth substrate.

Mineral content (mg/kg)							
Ca	**Cu**	**Fe**	**K**	**Mg**	**Na**	**P**	**Zn**
4.43±0.00	0.04±0.00	1.13±0.00	16.66±0.00	3.56±0.00	0.01±0.00	12.40±0.00	0.03±0.00

4.9.2 Mineral compostion of *Pleurotus ostreatus* EM-1 mushroom fruiting bodies harvested from *Trichoderma harzianum* contaminated growth substrate.

The mineral component (Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Zinc (Zn)) of *Pleurotus ostreatus* EM-1 cultivated on a sawdust "wawa" substrate contaminated with *Trichoderma harzianum* is summarized in Table 10b. Amounts of 0.99, 0.04, 0.22, 16.00, 2.61, 0.00, 12.80 and 0.00 mg/kg dry weight of fruiting bodies was detected for Calcium, Copper, Iron, Potassium, Magnesium, Sodium, Phosphorus and Zinc respectively. There was no statistical difference (P>0.05) in mineral amount detected for mushroom fruiting bodies harvested from *Trichoderma harzianum* growth substrate and the control setup (Table 13); Calcium (4.43 to 0.99mg/Kg), Iron (1.13 to 0.22mg/Kg), Magnessium (3.56 to 2.61mg/Kg) with slight drops observed in Potassium (16.66 to 16.00mg/Kg), Sodium (0.01 to 0.00mg/Kg) and Zinc (0.03 to 0.00mg/Kg) dry weight of fruiting bodies (Table 14).

Table 14. Total mineral content and heavy metals detection of *Pleurotus ostreatus* (Strain EM-1) grown on *Trichoderma harzianum* contaminated growth substrate.

Mineral contenet (mg/kg)							
Ca	**Cu**	**Fe**	**K**	**Mg**	**Na**	**P**	**Zn**
0.99±0.00	0.04±0.00	0.22±0.00	16.00±0.10	2.61±0.00	0.00±0.00	12.80±0.00	0.00±0.00

4.9.3 Proximate analyses on dry matter basis of the fruiting bodies of *Pleurotus ostreatus* EM-1 grown on uncontaminated sawdust "wawa" growth substrate.

Dry matter, moisture, fat, crude fibre, crude protein, total ash, carbohydrate all expressed in terms of percentage and energy (kcal/100g) were evaluated for Pleurotus ostreatus EM-1 cultivated on an uncontamited sawdust "wawa" growth substrate. There was a determination of dry matter, moisture, fat, crude fibre, crude protein, total ash and carbohydrate of 52.56, 13.08, 1.19, 91.13, 15.90, 8.87 and 72.86% respectively, with an energy of 334.97kcal/100g. Results are summarized in Table 15.

Table 15. Proximate Analyses of *Pleurotus ostreatus* strain EM-1 grown on uncontaminated growth substrate

Dry matter	**% Moisture**	**% Fat**	**% Crude fibre**	**% Crude protein**	**% Total ash**	**% Carbohydrate**	**Energy (kcal/100g)**
52.56±0.00	13.08±0.02	1.19±0.00	91.13±0.00	15.90±0.00	8.87±0.00	72.86±0.01	334.97±0.03

4.9.4 Proximate analyses on dry matter basis of the fruiting bodies of *Pleurotus ostreatus* EM-1 grown on sawdust "wawa" growth substrate caontaminated with *Trichoderma harzianum*.

There was a determination of dry matter, moisture, fat, crude fibre, crude protein, total ash and carbohydrate of 52.58, 12.45, 0.75, 83.67, 14.35, 16.33 and 67.81% respectively, with an energy of 273.107kcal/100g. There was a noticeable drop in energy from 334.97kcal/100g in the control (unconataminated, Table 15) to 273.10kcal/100g in the *T. harzianum* contaminated setup. Moisture 13.08 to 12.45%, fat droped from 1.19 to 0.75%, crude fibre 91.13 to 83.67%, crude protein 15.90 to 14.35% and carbohydrates 72.86 to 67.81%. There was however an increase in total ash from 8.87% to 16.33%. Dry matter remained contant. There was therefore no statistical difference (P>0.05) between values obtained for the control (fruiting bodies from uncontaminated growth substrate, Table 15) and fruiting bodies harvested from *T. harzianum* contaminated substrate. Results are summarized in Table 16.

Table 16. Proximate Analyses of *Pleurotus ostreatus* strain EM-1 grown on *Trichoderma harzianum* contaminated growth substrate

Dry matter	**% Moisture**	**% Fat**	**% Crude fibre**	**% Crude protein**	**% Total ash**	**% Carbohydrate**	**Energy (kcal/100g)**
52.58±0.00	12.45±0.00	0.75±0.00	83.67±0.00	14.35±0.01	16.33±0.00	67.81±0.02	273.10±0.04

CHAPTER FIVE

5.0 GENERAL DISCUSSION

The nutritional composition coupled with some therapeutic properties of mushrooms have led to its high demand and subsequent investments made in mushroom farming. Worldwide cultivation of mushrooms is dominated by the production of *Agaricus bisporus* (champignon), *Lentinula edodes* (shiitake), and *Pleurotus ostreatus* (oyster mushroom) (Hatvani, 2008). Ghana is no exception when it comes to the consumption and cultivation of mushrooms. Over the years, studies have been conducted into the cultivation of the edible mushroom, *Pleurotus ostreatus* and the pertinent literature in replete with technique of the various aspects of its cultivation. The most common species of cultivated mushroom cultivated here in Ghana is the Oyster mushroom (*P. ostreatus*) Strain EM-1. The cultivation of the oyster mushroom is however plagued with a number of problems such as the incidence of pathogens which usually lead to huge farm losses and high cost of production. A notable problem with the cultivation of *P. ostreatus* worldwide is the incidence of the green mould disease caused by species of *Trichoderma* (Hatvani *et al*., 2008).

The pre-treatment and post-treatment as well as several processes of the cultivation stages and the knowledge of the mushroom farmer also play an important role when it comes to mushroom cultivation. An example of essential processes involved in the cultivation of mushrooms is the sterilisation process which if not effective, encourages the incidence of devastating crop losses infection and low yield (Kortei, 2015).

In this study, the resident mycoflora on mushroom growth substrate and on the mushrooms fruiting bodies were isolated from the following seven mushroom farms; E90 Mushroom Farm, Ogbojo; Kwesi-Babs Farm, Kasoa; Immaculate Gold Enterprise, Nii-Boi Town, Lapaz; Delabless Mushroom Farm, Adenta; 4E Mushroom Farm, Ogbojo/Ashaley Botwe; Edeyef Mushroom Farm, Anyaa-Awoshie and PCM Mushroom Farm, Ashaley Botwe. Mycoflora isolated from the growth substrate belonged to the genera; *Aspergillus* sp. (*A. candidus, A. flavus, A. fumigatus, A. niger*), *Cladosporium* sp (*C. herbarum, C. macrocarpum,*), *Didymella* sp., *Fusarium (F. oxysporum, F. poae*), *Mycelia sterilia*, *Rhizopus oryzae, Rhothorula muscilaginosa, Trichoderma harzianum, Penicillium* sp. (*P. brevicompactum, P. camemberti*), *Verticillium fungicola* and *Saccharomyces* spp. (yeast) (Table 4, Plate 3).

The fungal species identified to be the most persistant in the substrate bags of E90 mushroom farm located at Ogbojo was *A. niger* isolated on all media followed by *T. harzianum*. The least encountered in the substrate bags found in this farm was *Rhizopus oryzae*. *T. harzianum* predominated over other mycoflora isolated from substrate bags in Kwesi-Babs mushroom farm located at Kasoa, Accra; with the least dominant being *R. oryzae*. Immaculate Gold Ent. mushroom farm, Nii-Boi Town, Lapaz recorded a high fungal population of *T. harzianum* which almost prevented the proliferation of other fungi with the exception of some yeast sp. *R. mucilaginosa* was the lowest in fungal population in the substrate bags located in this farm.

C. herbarum predominated other mycoflora in the substrate bags of Delabless mushroom farm located at Adenta, Madina. *T. harzianum* was the second predominant species identified to be resident in the substrate bags of this farm. *Rhizopus oryzae* was estimated to be the least persistant in substrate bags of Delabless mushroom farms.

4E Fresh mushroom farm located at Ogbojo/Ashaley Botwe haboured another *C. macrocarpum* being the most persistant species in the compost bags sampled, *A. fumigatus* was the second most persistant fungal species resident in the substrate in this farm. The least dominant or persistant species found resident in the growth substrate of 4E Fresh mushroom farm was *T. harzianum* and *A. flavus*. Similar to the most persistent fungal species isolated from the growth substrate of the previous farm, Edeyef mushroom farm in Anyaa-Awoshie also has *C. macrocarpum* as dominant in the growth substrate with the less dominat being *Saccharomyces* spp. (yeast) species. PCM mushroom farm recorded a dominance of *T. harzianum* among other mycoflora resident in the growth substrate. *A. terreus* and *Didymella* sp. which was only isolated in the substrate of PCM mushroom farm was the least dominant. Some species of *Aspergillus, Cladosporium, Penicillium, Rhizopus and Trichoderma* may have persisted in the growth subsrate from the process of composting (Obodai, 1992) and persited through ineffective sterilization. (Kortei, 2015).

For all the mushroom farms in this study, *A. niger* was identified to be the most dominant species followed by *T. harzianum*. E90 mushroom farms located Ogbojo had the highest microbial load with the mean total population of 4.78 $\log_{10}$CFU/g. 4E mushroom farms had the least microbial load with a total mean population of 2.9 $\log_{10}$CFU/g (Figure 4). Although *A. niger* is a major contaminant of food such as onion and cowpea, its effect on the cultivation of *P. ostreatus* is less severe than the pathogenic fungus *T. harzianum*. *A. niger*, however produces some toxins such as nigerone and oxalic acid.

Mycoflora persistent in the substrate bag mostly contaminate emerging fruiting bodies causing some cases of fruiting body contamination.

Similar mycoflora profile were isolated from mushroom fruiting bodies from the seven farms; (*A. candidus, A. flavus, A. fumigatus, A. niger, A. terreus*), *Cladosporium* sp (*C. herbarum, C. macrocarpum,*), *Epicoccum nigrum, Fusarium (F. oxysporum, F. poae*) *Gliocladium* sp., *Mycelia sterilia, Rhizopus oryzae, Rhothorula muscilaginosa, Talaromyces flavus, Trichoderma harzianum, Penicillium* sp. (*P. brevicompactum, P. citrinum, P. rouqueforti*), *Verticillium fungicola* and *Saccharomyces* spp. (yeast). *Trichoderma harzianum* is responsible for the green mould disease in mushrooms and was isolated in the growth substrate of each of the seven mushroom farms, and they were also found resident on mushroom fruiting bodies from each farm (Table 3). *T. harzianum* which was the second dominant in the substrate bags of E90 mushroom farm was found to be the most dominant resident on mushroom fruiting bodies in all farms. This poses a fundamental problem for the industry and needs to be addressed.

The isolation of *Talaromyces flavus* from the fruiting bodies sampled from Kewesi-Babs mushroom farm was unique and only resident in this location and associated with the mushroom fruiting bodies. *A. niger* predominated the mycoflora profile resident on sampled mushrooms from this farm. *R. oryzae* was again the least isolated species. Immaculate Gold Ent. mushroom farm recorded *P. brevicompactum*, a pathogenic fungus as the most dominant species followed by *C. herbarum. A. candidus* was the least dominant species resident on the mushroom fruiting bodies sampled from this farm. There was a predominance of *A. flavus* resident on the mushroom fruiting bodies as compared to yeast species which were the least dominant of mushrooms sampled from Delabless mushroom farm in Madina. The prepondance of *A. flavus* in harvested mushrooms is a health hazard as it produces aflatoxins (B1, B2, G1, G2) which has been recorded as the most potent carcinogen by WHO and Codex Alimentarius Commission.

4E Fresh mushroom farms in Ogbojo/Ashaley Botwe recorded *T. harzianumn* as the most dominant mushroom species resident on sampled mushroom fruiting bodies with *Gliocladium* sp. being the least dominant. *P. brevicompactum* and *T. harzianum* were the dominant fungal species resident on mushroom fruiting bodies with *R. oryzae* being the least dominant of mushroom samples taken from Edeyef mushroom farm located at Anyaa-Awoshie. *T. harzianum* was the dominant species resident on mushroom fruiting bodies sampled from PCM mushroom farms. The least dominant in this farm was *A. terreus*.

The persistent mycoflora resident on fruiting bodies was *T. harzianum*. The least encountered fungus on this media were *A. candidus A. terreus, Fusarium poae, Gliocladium* sp., *P. brevicompactum* and *Talaromyces flavus.* PCM mushroom farms had the most microbial load with Immaculate Gold Ent. having the least microbial load isolated from the mushroom fruiting bodies (Figure 3).

In addition to the resident mycoflora isolated from the growth compost and mushroom fruiting bodies, the aeromycoflora in the various cropping rooms were also sampled. Fungal spores are ubiquitous and play an important role in plant aetilogy. The Settle Plate Exposure method was used to detect the fungal profile present in each cropping room. There is paucity of information on the air-spora in these most active and major mushroom farms in the Greater Accra region and Kasoa. Data from this present study provides the first record of the aeromycoflora prevalent in these seven mushroom farms.

Aeromycoflora data obtained for this study showed the occurrence of *Cladosporium* spores (*Cladosporium herbarum, Cladosporium macrocarpum*) in five farms, which was the highest count per farm followed by spores of *Trichoderma harzianum* and *Penicillium brevicompactum* sampled from four farms.

The least frequent spores however, were spores belonging to *Verticillium fungicola*, *Penicillium citrinum*, *Mycelia sterilia*, *Gliocladium roseum*, *Epicoccum nigrum*, *Aspergillus parasiticus* and *Aspergillus oryzae* (Table 5, Plate 4). The conidia of *Cladosporium* species are the most abundant spores especially present in temperate regions and commonly distributed in tropical and subtropical regions. In some areas, the hourly concentration of *Cladopsorium* can exceed 100,000 spores/m^3 (Ingold, 1971; Lacey, 1996; Lacey & Venette, 1995). This finding clearly explains the common occurrence of this particular air dispersed fungal spore in 5 out of the 7 farms sampled in this study. They are also reported to sporulate more even after the rains which can be likened to the ground wetting practices employed by most oyster mushroom farmers to maintain an optimum cropping room temperature. This fungus is pathogenic to humans and not mushrooms and are not known to produce any mycotoxins of concern (Lacey, 1996).

Immaculate Gold Ent. mushroom farm recorded a 93% abundance of *C. macrocarpum* from the air in the cropping room. The lowest occurrence (3%) was recorded by *R. oryzae.* Other higher *Cladosporium* spore concentration were sampled from the cropping rooms of Edeyef mushroom farm and PCM mushroom farm, species being *C. herbarium*. This confirms the general assertion that spores of *Cladsporium* are abundant mostly in tropical and subtropical regions (Ingold, 1971). 4E Fresh mushroom farms recorded *F. oxysporium,* a prominent soil fungus as having the highest concentration in the air (65%). The type and amount of fungal spores present in the atmosphere are largely influenced by the temperature, relative humidity, wind speed and time of the day (Chakraborty *et al*., 2003).

Handling during harvest can be another vehicle by which these pathogenic species successfully infect cropping room reducing expected yield.

It is instructive to encourage mushroom farmers to wash the fruiting bodies thouroughly before packing for sale on the market to avoid growth of resident fungi during storage. Plate 5a and Plate 5b show representative photographs of fungi encountered in this study.

Trichoderma spp. (*T. aggressivum, T. asperellum, T. atroviride, T. citrinoviride, T. hazianum, T. longibrachiatum, T. pleurotum, T. pleuroticola* and *T. virens*) are well known to be the cause of the green mould disease in the production of mushrooms such as Champignon (*Agaricus bisporous*), Shitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus* sp.).

In this study, the particular species of *Trichoderma* affecting the cultivation of oyster mushroom in the farms visited was *T. harzianum*. *Trichoderma* species are asexual, filamentous and soil inhabiting fungi which has teleomorphs belongs to *Hypocrea* (Ascomycota, Hypocreales, Hypocreaceae, Pyrenomycetes) (Bellettini *et al*., 2017).

The green mould disease caused by the *Trichoderma* species has been known for long and has the appearance of a green sporulation on the growth medium or substrate as seen in Plate 10a. In severe cases, depending on the stage of growth of the pathogen, fruiting of the mushroom might be curtailed altogether which is a huge loss to mushroom cultivation industry (Park *et al.*, 2005; Sinden & Hauser, 1953). Usually, the green mould disease is transmitted to the fruiting bodies from the growth compost. According to Komoń *et al.*, 2007, *Trichoderma* species can be observed at the initial stage of substrate preparation but are later killed by the process of pasteurization or sterilization. Sandhu and Sidhu (1980); Obodai (1992), identified certain fungi associated with the composting process which includes *Trichoderma*, and described it as pathogenic with some fungi such as *Monilia*, *Fusarium*, *Penicillium* and *Sclerotinum rolfsii* as competitors in the cultivation of *Pleurotus*.

Considering the role of the adverse effect of resident fungi on mushroom production, the study of fungal pathogens has been inadequate (Kim *et al.*, 2013). Undoubtedly, the incidence of *Trichoderma* species in the cultivation of mushrooms causes much crop loss to the farmer (Cailleux and Diop, 1978).

Though the causative pathogen of the green mould is killed by the process of pasteurization, they tend to reappear even after this process which can be due to ineffective sterilization as reported by Kortei, 2015. It was reported that the oil drum steaming method of pasteurization in most cases tend to be ineffective making most pathogenic and competing species of fungi persist. Even in some instances where effective sterilisation is achieved, these pathogens including *Trichoderma* can reappear through the process known as spawning.

Ineffective sterilization techniques also used in the sterilization of the grains used in spawn preparation can also be the alternative source of contamination causing the fungal load contributing to compost infection. During incubation of an already spawned bag, the temperature of the compost increases to 30˚C due to the mycelial activity of *P. osteatus*. In the case where the pH of the compost is acidic to neutral pH (5-7) and relative humidity at a maximum of 80%, the green mould was seen to proliferate the substrate more (Bellettini *et al*., 2017).

Komon-Zelazowska *et al.* (2007) suggested the process of alkalization of the growth substrate by the application of calcium hydroxide (lime) in order to increase the pH of the growth substrate to pH (8-9), effectively slows the proliferation of the green mould. The process of liming may be the reason why the green mould disease in not as devastating in the United States as it is in other countries since it has been incorporated into cultural practices of mushroom farming.

However, this method is seen not to be effective on *T. pleuroticola* and hence must not be regarded as the ultimate solution to green mould disease prevention or control (Komon-Zelazowska *et al.*, 2007). The pasteurization of the growth substrate and the grains used in the making of the spawn at 60˚C for 10 hours is also known to inhibit growth of *Trichoderma* species. Findings from this study confirms the prominent presence of pathogenic *Trichoderma harzianum*.

The incidence of pathogenic and competitive mycoflora could be responsible for low yield, low farm output at the end of a cultivation season and even the lack of interest to continue cultivation expressed by most farmer with frequent disease incidence (Komon-Zelazowska *et al.*, 2007).

Farmers interviewed from the seven farms expressed concerns mainly about the issue of contamination. Many made references to disease incidence by physical identification on the field by colours. The fruiting mushroom bodies showing the yellow colour did so because of the superficial yellow lesions usually caused by *Pseudomonas* spp. (Bellettini *et al*., 2017). In some cases, brown-reddish blotches on *P. ostreatus* observed on the field was due to the bacteria, *P. tolaasii*. (Lo Cantore and Iacobellis, 2014). There were other instances where there was excessive yellowing of the pileus and cap which curtailed the growth of the already emerged pinhead (primodium). Another main complaint was about the green colouration both on the growth compost and on freshly emerged fruiting bodies. Isolation in our laboratory indicated the presence of *Trichoderma harzianum* which was obviously causing the green mould disease as described in most pertinent literature.

The issue with contamination in some cases caused a 100% abortion rate of pinheads and subsequent fruiting bodies formation and thousands of bags had to be discarded by the mushroom farms. This contributes a major financial loss.

The best way to prevent the yield losses associated with the incidence of pathogenic disease such as the green mould is prevention of contamination. However, if there has already been presence of infection, control measures can be employed to effectively prevent it from escalating. Chemical treatments are known to be the most effective treatments for the control of the green mould disease, but despite the increase in commercial farming of mushrooms, only a few chemicals have been accepted for the treatment of growth substrate and spawns (Abosriwil and Clancy 2003).

In the past, spawns were treated with benzimidazole fungicides and were producing good results until some pathogens such as *Trichoderma* spp. became resistant to the chemical treatment (Grogan, 2008; Romaine *et al*., 2005).

Other chemical treatments such as benomyl and carbendazim are known to undergo degradation by microbes curtailing their effectiveness to control infection. Environ (a commercial disinfectant), Prochloraz, prochloraz + carbendazim and Thiabendazol were reported to be most effective in reducing compost colonization by species of *Trichoderma* (Abosriwil and Clancy, 2002; 2003).

Data from practical application in the field concluded that the best way of control is by treatment of the spawn with the appropriate chemical rather than treatment of the compost when trying to prevent the growth of *Trichoderma* spp. In this study, a wettable powder composed of Cupric Hydroxide (77%), Inert Ingredient (23%) and Metallic Copper Equivalent (50%) product name “Champion Antifungal” was tried. There was a significant ($P<0.05$) reduction in poliferation of growth of *Trichoderma harzianum in vitro* when grown on the Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) amended with different concentrations of the copper fungicide (Champion).

This finding agrees with the report of Abosriwil and Clancy (2003) (Plate 7a and 7b, Figure 6), that chemical treatments are the best control against the proliferation of pathogenic fungi such as *Trichoderma* spp. resulting in the devastating green mould disease.

In this present work, vegetative inhibitory test in PDB media amended with the fungicide indicated further the ability of this particular chemical treatment "Champion" in preventing or curtailing the growth of *T. harzianum* (Plate 9, Figure 8). Vegetative growth of the test fungus variably reduced as the concentration of the chemical reduced in the descending order: Undiluted < 1:1v/v <1:2v/v <1:5v/v <1:10v/v.

There have, however, been some concerns about possible heavy metal contamination of the mushroom fruiting bodies as well as the possibility of these chemical treatments interfering with the growth of the mycelia of the cultivated mushroom. In some cases, vigour of the spawns was reduced as a result of the chemical treatment. Recently farmers have resorted to prevention based entirely on the use of biological controls such as plant extract to curb disease incidence in their mushroom farms (Grogan, 2008).

In some mushroom farms visited in this present work, farmers were somehow informed about the mushroom pathogens and they employed the use of plant extracts such as the neem seed and leaf extract to keep the green mould and other pests and diseases under control.

In the recent past, some angiosperm plants with fungitoxic active substances against mushroom pathogens but which are rather harmless to the mushrooms and humans have been used as control measures. Several of these plants have been reported to possess active ingredients which prevent proliferation of pathogens and happily have rather harmless side effects as compared to synthetic chemicals (Ushamalini, *et.al*., 1997). In this study, the ethanol extract of the bark of *Anthocleista nobilis* was tested *in vitro* against the growth of *Trichoderma harzianum.*

A. nobilis is known to contain certain secondary metabolites which enables it prevent microorganisms as well as insect pests. *A. nobilis* contains secondary metabolites such as xanthones and secoiridods such as anthocleistol. The bark has been found to contain quinolone alkaloid brucine and monoterpene glycoside loganoside or loganine. Secoiridoid glycosides and xanthones are known to have antibactical and antifungal properties (Siler *et al*., 2010).

Data from this present study shows that there was no statistical significance ($P>0.05$) differences between the vegetative and radial growth in the medium amended in the extracts of *A. nobilis* (Figure 5) Growth of the test fungus was severly depressed in the undiluted extract and the inhibitory effect was gradually removed by further dilution. Prolonged incubation removed the inhibitor principle such that growth in the medium amended with the biotoxin finally approximated that in the control. This suggests that the biotoxins present in the plant extract was depleted quickly during the course of the 10-day incubation (Plate 6a and Plate 6b). Regular application of the plant extract preferably after every three days, will be more effective in meeting its purpose of prevention and control of the green mould fungus (*T. harzianum*).

There is always a difference in growth of fungi cultured on solid and liquid cultures (Figure 5, Figure 7). Vegetative growth was effectively inhibited in the undiluted full strength preparation in the liquid medium further confirming the ability of the plant extract to be used as a biocontrol agent of the green mould fungus (*T. harzianum*) by local mushroom farmers. PDB amended with varying concentration of ethanol extratct of the bark of *A. nobilis* showed increasing inhibition as the concentration of the phytoextract was increased.

Tables 6-7 summarise the fruiting bodies recorded in substrate bags inoculated or contaminated with *Trichoderma harzianum* and uninoculated healthy bags allowed to form fruiting bodies without inoculation of *T. harzianum* on the growth substrate for the first flush.

There was a relatively lower rate of fruiting bodies formation from bags inoculated with *T. harzianum* than the uninoculated substrate bags. Records of the parameters of growth such as the stipe length, stipe width and cap diameter of mushrooms formed in the contaminated substrate bags was also lower compared to the control.

Contaminated substrate bags showed a profuse sporulation of the green mould which in some cases prevented fruiting or very little fruiting of the mushroom. Presumably, the green mould compete for space and nutrients more effectively than the mushroom and can produce secondary toxic metabolite, extracellular enzymes as well as various volatile organic compounds which can substantially lower or even entirely block commercial production (Hatvani *et al*., 2008).

Fruiting was recorded in 4 out of 5 bags in the first flush of susbstrate bags (Plate 10b). This came with a corresponding yield ranging from 46.00 to the highest yield which is 90.86g (Table 6). *T. harzianum* contaminated substrate bags recorded fruiting in 3 out 5 bags with yield ranging from 48.11 to 73.08g (Table 7). The inoculated fungus was seen to be undergoing proliferation even though there were fruting of *P. ostreatus* from these compost bags (Plate 10a). The second flush recorded the least yield yet as there was fruiting in only 3 out of 5 bags ranging from 29.32-36.63g (Table 8, Table 9). The inoculated bags as seen in the first setup recorded 3 fruiting bags out of five. The third flush recorded fruiting in five out of 5 substrate bags from uncontaminated bags with just 2 fruiting observed out of 5 bags in the contaminated substrate (Table 10, Table 11). The restriction in fruiting in most cases observed among substrate bags contaminated with *T. harzianum* substantially reducing yield can obviously be attributed to *T. harzianum* as reported also by Jayalal and Adikaram (2007).

An accumulation of the yield over three flushes for both substrates contaminated with *T. harzianum* and uncontaminated substrate bags (Table 12) showed a significant difference in the total yield and biological efficiency (BE), with the uncontaminated substrate bags producing a total yield of 576.86g and a corresponding BE of 164.91%. Contaminated substrate bags had a total yield of 304.37g with a BE of 87.02%.

This data agrees with the findings of Obodai 1992 that *Trichoderma* produces metabolites that are antagonistic to *P. ostreatus*.

Mineral analyses were carried out on dried mushroom samples of mushrooms from the uncontaminated (Table 13) and contaminated bags (Table 14). Some heavy metals such as Copper (Cu), Iron (Fe), Manganese (Mn), and Zinc (Zn) were detected in the fruiting bodies of *P. ostreatus* strain EM-1. This indicates the ability of mushrooms to effectivel take up some heavy metals from the environment (Zhu *et al*., 2010) due to their dense mycelial network that extends throughout the growth substrate (Garcia *et al*., 1998 and 2005).

Heavy metals like iron (Fe), copper (Cu), and Zinc (Zn) found in mushroom are essential metals since they play an important role in biological systems (Unak *et al*., 2007). Iron (Fe) consumption in the right amounts prevents anaemia, with Copper (Cu) and Zinc being an important part of enzymes involved in physiological processes such metabolism and protein synthesis (Senesse *et al*., 2004; Silvestre *et al*., 2000; Ma and Betts, 2000). Values (mg/kg) of Ca, Fe, K, Mg, Na and Zn in the uncontaminated susbstrates bag decreased from 4.43 to 0.99, 1.13 to 0.22, 16.66 to 16.00, 3.56 to 2.61, 0.01 to 0.00, 0.03 to 0.00mg/kg respectively. Copper (Cu) concentration of mushrooms cultivated from contaminated and uncontaminated growth substrate remained unchanged (0.04mg/kg) whereas there was an almost negligible increase in Phosphorus values from mushroom cultivated from *T. harzianum* contaminated substrate to uncontaminated substrate (12.80 to 12.40mg/kg). Data from this work cannot fully explain this.

Future work will ascertain this. There was no statistical difference (P>0.05) (Appendix 25) in values for tested elements; Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Zinc (Zn) in the contaminated and uncontaminated bags. However, values obtained from both setups confirm the study by Wiafe-Kwagyan (2014), indicating the serious extent of inhibition of the growth and depletion of nutrients of *P. ostreatus* and *P. oeus* by *T. harzianum*.

Wiafe-Kwagyan (2014) stated that, there is a practical adverse implication on yield when *T. harzianum* is allowed to reach epidemic populations on substrates as seen in Plate 10a.

Proximate analyses for mushrooms cultivated on uncontaminated substrate and *T. harzianum* contaminated substrate and are presented in Table 15 and Table 16. There was a reduction of energy in dried mushroom samples of mushroom from uncontaminated to contaminated growth substrates (from 334.97 to 273.10 kcal/100g), probably used up by the contaminant in growth. There was no statistical (P>0.05) difference between values obtained for dry matter, moisture, fat, crude fibre, crude protein, ash content, carbohydrate and energy between mushroom samples obtained from both *T. harzianum* contaminated substrate and uncontaminated substrates (Appendix 26). Dry matter content was almost the same for mushrooms from both setups, i.e uncomatinated substrate to contaminated substrate (52.56 to 52.58%). There was a reduction in fat, crude fibre, crude protein and carbohydrate values (1.19 to 0.75, 91.13 to 83.67, 15.90 to 14.35, 72.86 to 67.81% respectively). Pressumably due to metabolic activity of the contaminant, there was an increase in total ash in the contaminated bags (16.33%) as compared to uncontaminated bags (8.87%). Presumably contributed by the contaminated mycelium, moisture content in mushrooms of the contaminated setup was 12.45% with 13.08 moisture content in mushrooms from the uncontaminated setup. The difference in nutrient values obtained in this work shows the effect of the activity of *T. harzianum* in reducing the quality of harvested mushrooms as reported by Cailleux and Diop (1978).

In addition to the issue with contamination which is a major concern to farmers, the cost of setting up and maintaining a mushroom farm seems to be one major factor contributing to a relatively less number of mushroom cultivation farms in Accra. Most farmers have to invest over 10,000 cedis for commercial farming which is not very favourable to prospects seeking to engage in this venture since most farmers self-fund their mushroom ventures. The revenue accumulated at the end of the growing season differs from farm to farm with some farmers making up to 1,200 cedis. Most obtain low profit due to low yield, lack of ready market and short shelf life of mushrooms.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

This study has shown that cultivation of mushroom can be a profitable business if the microbiological quality assurance principles of Good Manufacturing Practices (GMP) and Hazard Analysis at Critical Control Points (HAACP) are rigorously applied. It was clear from the data collected that the substrates in the seven (7) farms were not well sterilized and contained a miscellary of contaminants predominated by *T. harzianum* and *A. niger* not excepting *Penicillium.*

The fruiting bodies were also consequently laden with the same range of fungi as in the compost. Majority of the aeromycoflora isolated from the room of the farms were found in the final product of the sporophores. Cleanliness of the environment of the production rooms is key to obtaining good yield of fruiting bodies. The matter is accentuated by the mushroom farmers moisterising the floors of the cropping room to raise the relative humidity prescribed for sustained production. *T. harzianum* has therefore become a perennial problem for the industry. The following recommendations are being made:

1. The survey of the quality of the mushroom farms should continue in order to alert farmers on the economic consequences of neglect.
2. The sterilisation process of the substrate for spawning and cropping bags should not be compromised with.
3. Farmers should invest in purchasing small scale humidifiers which can be applied to increase moisture in the cropping room.
4. Routine cleaning of cropping room, preparation room and implements used in farm work will be worthwhile.

5. Fumigation of the facilities after a cropping season would be ideal in curtailing contamination by fungal spores.
6. Farmers should consider the use of solar dryers to dehydrate fruiting bodies that could not be sold for preservation and marketing.

REFERENCES

Abosriwil SO, Clancy KJ. (2002). A protocol for evaluation of the role of disinfectants in limiting pathogens and weed moulds in commercial mushroom production. *Pest Management Science*. 58:282-289.

AOAC (2005). Official methods of analysis of AOAC International, 18th Edition, AOAC International, Gaitherburg, Maryland USA p 69-80.

Abosriwil SO, Clancy KJ. (2003). A mini-bag technique for evaluation of fungicide effects on *Trichoderma* spp. in mushroom compost. *Pest Management Science*. 60:350-358.

Arailde F.U. (2013). General notions of mushrooms: In Training course on edible and medicinal mushrooms. *Embrapa - Genetic Resources and Biotechnology*.

Asef M. R. (2012). Intersterility groups of *Pleurotus ostreatus* complex in Iran. *Mycology* 3(2):147-152.

Bahl, N and Chowdhary, PN. 1980. *Podospora faurelii*, a new competitor in the mushroom cultivation (*Volvariella volvacea*) *Curr. Sci*. 50: 37.

Barnett, H. L. and Barry. B.H. (1972). Illustrated genera of imperfect fungi. (3rd Ed.) Burgess publishing company USA; SBN: 8087-02661.

Beffa, T., Staib, T. and Lott Fischer, J. (1998). Mycological control and surveillance of biological waste and compost. *Journal of Medical Mycology*. 36: 137-145.

Bellettini MB, Bellettini S, Vítola FMD, Fiorda FA, Maccari Júnior A, Soccol CR. (2017). Residual compost from the production of *Bactris gasipaes* Kunth and *Pleurotus ostreatus* as soil conditioners for Lactuca sativa 'Veronica'. *Semin-Cienc Agrar*. 38:581---94.

Bhatt N, Singh RP. (2002). Casing soil bacteria as biocontrol agents against the mycoparasitic fungi of *Agaricus bisporus*. Proceedings of the 4th International Conference on Mushroom Biology and Mushroom Products. 1-9.

Black C.A. (1965). "Methods of Soil Analysis: Part I Physical and mineralogical properties". American Society of Agronomy, Madison, Wisconsin, USA.

Buswell, J.A. (1984) Potentials of spent mushroom substrates for bioremediation purposes. Compost 2: 31-35.

Cailleux, R. and Diop, A. (1978). Recherches preliminaries sur la fructification du *Pleurotus eryngii* en conditions de culture non steriles et es incidences pratiques. Rev. *Mycol*. 42: 1-11.

Cailleux, R. and Diop, A. (1978). Recherches preliminaries sur la fructification du *Pleurotus eryngii* en conditions de culture non steriles et es incidences pratiques. *Rev. Mycol*. 42: 1-11.

Chakraborty P, Bhattacharya SG. & Chanda S. (2003). Aeromycoflora of an agricultural farm in West Bengal, India: A five-year study (1994–1999), Grana, 42:4, 248-254, DOI: 10.1080/00173130310016941.

Chang, S.T. (1999). Global impact of edible and medicinal mushrooms on human welfare in the 21st century: non-green revolution. *International journal of medicinal mushrooms* 1(1): 1-7.

Chiu, S. W., Law, S. C., Ching, M. L., Cheung, K. W. and Chen, M. J. (2001). Themes for mushroom exploitation in the 21th century: sustainability, waste management and conservation. *The Journal of General and Applied Microbiology* 46: 269-282.

Cohen, R., Persky, L. and Hadar, Y. (2002). Biotechnological application and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl, *Microbiol Biotechnol*. 58: 582-544.

Dhamodharan, G., & Mirunalini, S. (2010). A Novel Medicinal Characterization of *Agaricus Bisporus* (White Button Mushroom) Pharmacologyonline 2: 456-463 (2010) Newsletter Dhamodharan and Mirunalini. Pharmacologyonline Newsletter Dhamodharan and Mirunalini, 2, 456–463. Retrieved from http://pharmacologyonline.silae.it/files/newsletter/2010/vol2/55.Mirunalini.pdf.

Dundar A, Acay H, Yildiz A (2008) Yield performances andcnutritional contents of three oyster mushroom species cultivated on wheat stalk. *Afr J Biotechnol* 7:3497–350.

eds.), pp. 291-304.

Garcia, M. A., Alonso J., Fernandez G, Melgar, M.A. (1998). Lead concentration in edible Wild mushrooms in northwest Spain as an indicator of environmental contamination. Archives of Environmental Contamination and Toxicology. 34(4):330-335.

García, M. A., Alonso, J., and Melgar, M. J. (2005). Agaricus macrospourus as Potential Bioremediation Agent in Compost Material Contaminated with Heavy Metals. J. *Chem. Tech. Biotech.* 80: (3)-325-330.

Gbolagade J, Ajayi A, Oku I, Wankasi D (2006) Nutritive value of common wild edible mushrooms from Southern Nigeria. *Global J Biotechnol Biochem* 1:16–21.

Gbolagade J. S. (2006). Bacteria associated with compost used for cultivation of Nigerian edible mushrooms *Pleurotus tuber-regium* (Fr.) Singer, and *Lentinus squarrosulus* (Berk.). *Afr J Biotechnol* 5:338–342.

George, S. and Harold, R. (1960). *An introduction to industrial mycology* (5th Ed.). Published by Butler and Tanner Ltd., Frome and London. Great Britain.

Grogan H. (2008). Challenges facing mushroom disease control in the 21st century. *Proceedings of the 6th International Conference on Mushroom Biology and Mushroom Products*. 120-127.

Győrfi J, Geösel A. (2008). Biologicai control against *Trichoderma* species in *Agaricus* cultivation. Proceedings of the 6th International Conference on Mushroom Biology and Mushroom Products. 158-164.

Hatvani L. (2008). Mushroom pathogenic Trichoderma species: occurrence, biodiversity, diagnosis and extracellular enzyme production. Ph.D. *School of Biology Department of Microbiology Faculty of Science and Informatics*, University of Szeged.

Hatvani, L., Kocsubé, S., Manczinger, L., Antal, Z., Szekeres, A., Druzhinina, I. S., Komo Zelazowska, M., Kubicek, C. P., Nagy, A., Vágvölgyi, C. & Kredics, L. (2008): The green mould disease global threat to the cultivation of oyster mushroom (*Pleurotus ostreatus*): a review. *Mushroom Sci*. 17: 485-495.

Hawksworth, D. L., (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research* 95: 641-655.

Hilber O. (1982). Die Gattung Pleurotus. Biblioth. Mycol. 87: 448. In: Advances in Mushroom Biology and Production (R.D. Rai, B.L. Dhar and R.N. Verma,

Ingold, C.T. (1971). Fungal Spores: Their Liberation and Dispersal, *Clarendon Press*, Oxford, 1971.

Jayalal, R.G.U. and Adikaram, N. K. B. (2007). Influence of Trichoderma harzianum metabolites on the development of green mould disease in the oyster mushroom. *Cey. J. Sci. (Bio.Sci)* 36 (1): 53-60

Ma. J and Betts N. M. (2000). Zinc and Copper Intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII). *The Journal of Nutrition*. Vol 130 Issue 11. 2838-2843.

Kim SW., Kim S., Lee HJ., Park JW., and Ro HS. (2013). Isolation of Fungal Pathogens to an Edible Mushroom, *Pleurotus eryngii*, and Development of Specific ITS Primers. *Department of Microbiology and Research Institute of Life Sciences*, Gyeongsang National University, Jinju 660-771, Korea.

Kirk, P. M., Cannon, P. F, David, J .C, and Stalpers, J. A. (eds) (2001). Ainsworth & Bisby's dictionary of the fungi. 9th edition. *CABI Publishing*, Wallingford.

Komo´n-Zelazowska M, Bissett J, Zafari D, Hatvani L, Manczinger L, Woo S, Lorito M, Kredics L, Kubicek C. P, Druzhinina I. S. (2007). Genetically closely related but phenotypically divergent *Trichoderma* species cause world-wide green mould disease in oyster mushroom farms. *Appl Microbiol Biotechnol*. 73:7415-26.

Kortei J. N. K. (2015). Comparative Effect of Steam and Gamma Irradiation Sterilization of Sawdust Compost on the Yield, Nutrient and Shelf-Life of Pleurotus Ostreatus (Jacq.Ex. Fr) Kummer Stored in Two Different Packaging Materials. Ph. D Thesis. *Department of Nuclear Agriculture and Radiation Processinguniversity of Ghana*. Pp 7-17.

Kurtzman, R. H. and Zadrazil, F. (1982). Physiological and taxonomic considerations for cultivation of Pleurotus mushrooms. In Chang, S. T. and Quimio, T. H. (Eds). Tropical Mushrooms; Biological Nature and Cultivation Methods, p. 299-306. Hong Kong: *The Chinese University Press.*

Lacey, J. (1996). Spore dispersal - its role in ecology and disease: The British contribution to fungal aerobiology. *Mycol Res*. 100: 641-660.

Lo Cantore P & Iacobellis NS. (2014). Characterization of fluorescent pseudomonads responsible for the yellowing of oyster mushroom (*Pleurotus ostreatus*). *Phytopathol Mediterr*. 53:54-65.

Martínez-Carrera D (1999). Oyster mushrooms. *McGraw-Hill Yearbook of Science and Technology*. Ed.: M. D. Licker. McGraw-Hill, Inc., New York. ISBN 0-07-052625-7 (447 pp.) [http://books.mcgraw-hill.com]. pp. 242-245.

Morris E, Doyle O, Clancy KJ. (1995). A profile of *Trichoderma* species. I—Mushroom compost production. *Mushroom Science*; 14:611-618.

Nilsen, S. S. (2010). Food Analysis Laboratory Manual (htpp://www. springer.com. 978-1-4419-1462-0).

Obodai, M and Apetorgbor, M. M. (2008). Proximate composition and nutrient content of some wild and cultivated mushrooms in Ghana. *Journal of Ghana Science Association*. 10(2): 139-144.

Obodai, M and Odamtten, G. T. (2003). Fungal phenology and attendant changes in agricultural lignocelluloses waste for mushroom cultivation: Status Prospects and Applications in Food Security. Unpublished data. Pp 1-10.

Obodai, M. (1992). Comparative studies on the utilization of agricultural waste by some mushrooms (*Pleurotus* and *Volvacea* species). MPhil Thesis, *Department of Botany*, University of Ghana, Legon pp:64-65.

Obodai, M. and Apetorgbor, M. M. (2001). An ethonobotanical study of mushroom germplasm and its domestication in the Bia Biosphere Reserve. CSIR Food Research Institute. Man and the Biosphere. Final Report submitted to Environmental Protection Agency under the sponsorship of UNESCO-MAB. Pp. 14-16.

Obodai, M. and Vowotor, K. A. (2002). Performance of different strains of *Pleurotus* species under Ghanaian conditions. *Journal of Food Technology*. *Africa*. 7: 98-100.

Obodai, M., Amoa-Awua, W. and Odamtten, G. T. (2010). Physical and chemical and fungal phenology associated with the composting of wawa sawdust (*Triplochiton scleroxylon*) used in the cultivation of oyster mushrooms in Ghana. *Int. Food Res*. J., 17: 229-237.

Obodai, M., Sawyer, L.C.B. and Johnson, P.N.T. (2000). Yield of seven strains of oyster mushrooms (Pleurotus spp.) grown on composted sawdust of *Triplochiton scleroxylon. Tropical Science*. 40: 95-99.

Odamtten G. T., Nartey L. K., Wiafe Kwagyan M., Anyebuno G. & Kyei Baffuor G. (2018). "Resident microbial load, toxigenic potential and possible quality control measures of six imported seasoning powders on the Ghanaian market". J*ournal of Nutritional Health and Food Engineering.*

OECD (2005). Consensus Document on the Biology of *Pleurotus* spp. (Oyster Mushroom). Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. ENV/JM/MONO (17). *Series on Harmonisation of Regulatory Oversight in Biotechnology* No. 34.

Oei P (2003) Manual on mushroom cultivation: techniques species and opportunities for commercial application in developing countries. *Tool Publications,* Amsterdam 274.

Oei P. (1996). Mushroom Cultivation with special emphasis on appropriate techniques for developing countries. *Tool Publications*. Leiden The Netherlands pp. 141.

Oei, P. (1991). Some aspects of mushroom cultivation in developing countries. In: *Mush. Sci*. 13(2)777-780.

Park M. S, Seo G. S, Lee K. H, Bae K. S, Yu S. H., (2005). Characterization of *Trichoderma* spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of rDNA. *Plant Pathol* J. 21:229-36.

Patra, A. K, Pani, B. K. (1995). Evaluation of banana leaf as a new alternative substrate to paddy straw for oyster mushroom cultivation. J Phytol Res 8:145–148.

Pathmashini, L.; Arulnandhy, V.; Wijeratnam, R.S.W. Cultivation of oyster mushroom. (2008.) (Pleurotus ostreatus) on sawdust. Ceylon Journal of Science (Biological Science), v.37, p.177-182, DOI: 10.4038/cjsbs. v37i2.505

Quimio, T. H., (1978). Indoor cultivation of *Pleurotus ostreatus*. Philippines Agriculturist, 61: 253-262. Subramanian, T. R., 1986. Nutritive Value. Mushroom Extension bulletin. *Indian Institute of Horticulture Research*, India, 8: 36.

Rajarathnam, S., M.N. Shashirekha and Zakia Bano (1992). Inhibition of growth of the mushroom *Pleurotus flabellatus* (Berk and Br.) Sacc. on rice straw by *Sclerotium rolfsi*

Rajarathnam, S., M.N. Shashirekha, Zakia Bano and P.K. Ghosh. (1997). Renewable Lignocellullosic waste, the growth substrate for mushroom production: National strategies.

Romaine CPD, Royse DJ, (2005). Schlagnhaufer. Superpathogenic *Trichoderma* resistant to TopsinM found in Pennsylvania and Delaware. *Mushroom News*. 53:6-9.

sacc. Adv. Appl. *Microbial*. 37: 233-236.

Sanchez C. (2004). Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol. Adv. 27:185-194.

Sandhu and Sidhu, M. S. (1980). The fungal succession on decomposing sugarcane bagasse. Trans. Br. *Mycol. Soc*. 75(2): 281-286.

Sandhu and Sidhu, M. S. (1980). The fungal succession on decomposing sugarcane bagasse. *Trans. Br. Mycol. Soc*. 75(2): 281-286

Sharma, S. R., Kumar, S. and Sharma, V.P. (2007). Diseases and Competitor Moulds of mushrooms and their management. National Research Centre for Mushroom, India *Council of Agricultural Research*.

Senesse P, Meance S, Cottet, V., Faivre J, Boutron-Ruault M. C (2004). High dietary iron and copper and risk of colorectal cancer: a case –control study in Burgundy, France. *Nutr cancer*. 49:66-71.

Sigurd, F. (1953). Practical mycology. Manual for identification of fungi. Published by A.W. Brogger Boktr. Forlag, Oslo Norway. Pp 63-67.

Šiler B., Mišić D, Nestorović J, Banjanac T, Glamočlija J, Soković M, and Ćirić A. (2010). Antibacterial and Antifungal Screening of Centaurium *pulchellum* Crude Extracts and Main Secoiridoid Compounds. Institute for Biological Research "Siniša Stanković",

Silvestre MD, Lagarda MJ, Farra R, Martineze-Costa C, Brines J (2000). Copper, iron and zinc determination in human milk using FAAS with microwave digestion. Food Chem. 68:95-99.

Sinden J & Hauser E. (1953). Nature and control of three mildew diseases of mushrooms in America. *Mushroom* Sci.; 2:177-80.

Singer, R. (1986). The Agaricales in modern taxonomy. 4th Ed. Pp 174-179. *Koeltz Scientific Books*. Germany.

Singh A, Shahid M, Srivastava M, Pandey S, Sharma A. (2014). Optimal Physical Parameters for Growth of *Trichoderma* Species at Varying pH, Temperature and Agitation. *Virol Mycol* 3: 127. doi:10.4172/2161-0517.1000127.

Stamets P. (1995). The role of mushroom in nature. Retrieved from http://www.fungi.com

Tripothi, J. P. and Yadar, J. S. (1992). Optimization of solid substrate fermentation of wheat straw into animal feed by *Pleurotus ostreatus* – a pilot effort. *Animal Feed Science and Technology*, 37: 59-72. University of Belgrade, Bul. despota Stefana 142, 11060 Belgrade, Serbia

Unak, P., Lambrecht, F. Y, Biber, F. Z. and Darcan, S. (2007). Iodine measurements by isotope dilution analysis in drinking water in Western Turkey. *J. Radio analytical Nuclear Chem*. 273:649-651.

Ushamalini, C., Rajappan, K., Kausalya, Gangadharan and Gangadharan, K. (1997). Management of charcoal rot of cowpea using bio-control agents and plant products. *Indian Phytopath* 50: 504-507.

Von-Arx, J. A. (1970). The genera of fungi sporulating in pure culture. *Verlag Von. J. Cramer*. Published in Lehre. ISSN 1992-0067.

Wiafe Kwagyan M. (2014). Comparative Bioconversion of Rice Lignocellulosic Waste and Its Amendments by Two Oyster Mushrooms (*Pleurotus* Species) And The Use of the Spent Mushroom Compost as Bio-Fertilizer for The Cultivation of Tomato, Pepper and Cowpea. PhD Thesis. 4: 93-122.

Wiafe-Kwagyan M., Odamtten G. T. and Obodai M. (2015). Possible antibiosis effect of the metabolite of three fungal species resident on rice straw and husk compost on the in-vitro radial and vegetative growth of Pleurotus ostreatus P-31. *Int. T. Curr Microbial Appl.* 4(8): 525 – 538.

Zhu F, Qu L, Fan W, Qiao M, Hao H, Wang X (2010). Environmental Monitoring Assessment. DOI 10.1007/s10661.01-1728-5

APPENDICES

Appendix 1a. Occurrence of fungal species isolated from mushroom samples collected from E90 mushroom farms on two (2) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **38**	*Trichoderma harzianum* **70**	*Trichoderma harzianum* **35**
Aspergillus fumigatus **41**	*Cladosporium herbarum* **64**	*Aspergillus niger* **25**
Rhizopus oryzae **3**	Yeast spp. **65**	
Trichoderma harzianum **78**		

Appendix 1b. Occurrence of fungal diversity resident on the compost samples collected from E90 mushroom farm isolated on two media (PDA, OGYE and OGYE)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **155**	*Trichoderma harzianum* **57**	*Aspergillus niger* **20**
Rhizopus oryzae **3**	*Aspergillus niger* **59**	*Trichoderma harzianum* **44**
Aspergillus flavus **84**	*Mycelia sterilia* **44**	
	Yeast spp. (cream) **50**	

Appendix 2a. Occurrence of fungal species isolated from mushroom samples collected from Kwesi-Babs Farm located at Adam Nana, Abom Road, Kasoa on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **106**	*Trichoderma harzianum* **54**	*Rhizopus oryzae* **2**
Yeast spp. **94**	*Rhizopus oryzae* **2**	Yeast spp. **17**
Rhizopus oryzae **2**	Yeast *spp.* **61**	*Rhodotorula mucilanoginosa* **45**
Trichoderma harzianum **81**	*Aspergillus niger* **57**	*Penicillium citrinum* **9**
		Aspergillus candidus **5**
		Aspergillus flavus **28**
		Aspergillus fumigatus **36**
		Aspergillus niger **46**
		Talaromyces flavus **4**

Appendix 2b. Occurrence of fungal diversity resident on compost sampled from Kwesi-Babs Farm located at Adam Nana, Abom Road, Kasoa isolated on three (3) media (PDA, OGYE & DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Yeast spp. **5**	*Trichoderma harzianum* **72**	*Aspergillus niger* **27**
Rhizopus oryzae **3**	*Aspergillus niger* **67**	*Aspergillus candidus* **19**
Trichoderma harzianum **191**	*Mycelia sterilia* **27**	*Rhodotorula muscilaginosa* **21**
	Yeast spp. **41**	*Rhizopus oryzae* **3**

Appendix 3a. Occurrence of fungal species isolated from mushroom samples collected from Immaculate Gold Ent. mushroom farm located at Nii-Boi Town, Lapaz on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Yeast spp **5**	*Penicillium brevicompactum* **54**	Yeast spp. **12**
Trichoderma harzianum **24**	*Cladosporium herbarum* **48**	*Aspergillus Candidus* **14**
	Yeast spp. **36**	

Appendix 3b. Occurrence of fungal diversity resident on the compost samples collected from Immaculate Gold Ent. mushroom farm located at nii-boi town, lapaz isolated on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **90**	*Trichoderma harzianum* **112**	Yeast spp. **4**
Fusarium poae **84**	Yeast sp. **7**	*Rhodotorula mucilaginosa* **46**
Yeast spp. **41**		

Appendix 4a. Occurrence of fungal species isolated from the mushroom samples collected from Delabless mushroom farm at Adenta, Madina on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Yeast spp. **50**	*Trichoderma harzianum* **50**	*Cladosporium macrocarpum* **27**
Aspergillus flavus **96**	*Cladosporium macrocarpum* **46**	Yeast spp. **3**
Cladosporium herbarum **134**	Yeast spp. **19**	*Rhodothorola mucilaginosa* **26**
	Aspergillus fumigatus **49**	*Aspergillus flavus* **24**
	Aspergillus flavus **27**	

Appendix 4b. Occurrence of fungal diversity resident on the compost collected from Delabless mushroom farm at Adenta, Madina isolated on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Fusarium oxysporium **5**	*Trichoderma harzianum* **7**	*Aspergillus falvus* **6**
Trichoderma harzianum **11**	*Aspergillus niger* **6**	*Cladosporium macrocarpum* **11**
Yeast spp. **8**	*Cladosporium herbarum* **4**	*Rhodotorula muscilaginosa* **5**
Cladosporium herbarum **10**	*Penicillium brevicompactum* **3**	*Rhizopus oryzae* **1**
Aspergillus flavus **11**	*Aspergillus flavus* **3**	*Trichoderma harzianum* **8**
Penicillium brevicompactum **12**	*Aspergillus oryzae* **4**	*Verticillium fungicola* **7**
Cladosporium macrocarpum **7**	*Aspergillus fumigatus* **7**	*Cladosporium herbarum* **5**

Appendix 5a. Occurrence of fungal species isolated from mushroom samples collected from 4E Fresh mushroom farm located at Ogbojo/Ashaley Botwe on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Yeast spp. **4**	*Trichoderma harzianum* **26**	*Cladosporium macrocarpum* **15**
Aspergillus flavus **6**	Yeast spp. **12**	*Rhodothorola mucilaginosa* **13**
Trichoderma harzianum **7**		*Gliocladium* **3**
Fusarium oxysporium **8**		
Penicillium brevicompactum **9**		

Appendix 5b. Occurrence of fungal diversity resident on compost samples collected from 4E Fresh mushroom farm located at Ogbojo/Ashaley Botwe isolated on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Cladosporium macrocarpum **7**	Yeast sp. **25**	*Aspergillus fumigatus* **16**
Trichoderma harzianum **4**		*Cladosporium macrocarpum* **15**
Yeast spp. **1**		*Rhodotorula muscilaginosa* **10**
Cladosporium herbarum **5**		*Penicillium camemberti* **9**
Aspergillus flavus **4**		*Penicillium brevicompactum* **14**
Penicillium brevicompactum **3**		

Appendix 6a. Occurrence of fungal species isolated from the mushroom samples collected from Edeyef mushroom farm located at Anyaa-Awoshie on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Trichoderma harzianum **27**	*Trichoderma harzianum* **25**	*Rhizopus oryzae* **2**
Aspergillus flavus **11**	*Aspergillus niger* **27**	*Rhodothorola mucilaginosa* **42**
Trichoderma harzianum **25**	Yeast spp. **10**	*Aspergillus falvus* **35**
Cladosporium macrocarpum **24**	*Aspergillus flavus* **24**	*Aspergillus niger* **45**
Penicillium brevicompactum **26**	*Penicillium brevicompactum* **30**	*Penicillium rouqueforti* **27**
Cladosporium herbarum **12**	*Cladosporium macrocarpum* **26**	
	Cladosporium herbarum **20**	

Appendix 6b. Occurrence of fungal diversity resident on compost samples collected from Edeyef mushroom farm located at Anyaa-Awoshie isolated on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Cladosporium macrocarpum **28**	Yeast spp. (white) **2**	*Aspergillus niger* **24**
Verticillium fungicola **25**	*Cladosporium macrocarpum* **13**	*Cladosporium macrocarpum* **27**
Yeast spp. **6**	*Penicillium brevicompactum* **6**	*Rhodotorula muscilaginosa* **14**
Cladosporium herbarum **29**	*Aspergillus flavus* **7**	*Aspergillus falvus* **12**
Aspergillus flavus 26	*Aspergillus niger* **8**	*Penicillium brevicompactum* **18**
Penicillium brevicompactum **24**	*Trichoderma harzianum* **14**	*Trichoderma harzianum* **21**
Aspergillus niger **37**		*Cladosporium herbarum* **18**

Appendix 7a. Occurrence of fungal species isolated from the mushroom samples collected from PCM mushroom farm located at ashaley botwe on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Trichoderma harzianum **55**	*Trichoderma harzianum* **221**	Yeast spp. **20**
Yeast spp. **15**		*Rhodothorola mucilaginosa* **24**
Aspergillus ochraceus **30**		*Fusarium oxysporum* **27**
Cladosporium macrocarpum **49**		*Fusarium poae* **35**
Aspergillus terreus **15**		
Cladosporium herbarum **47**		

Appendix 7b. Occurrence of fungal diversity resident on compost samples collected from PCM mushroom farm located at Asahley Botwe isolated on three (3) media (PDA, OGYE and DRBC)

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Trichoderma harzianum **5**	Yeast spp. (white) **12**	*Aspergillus fumigatus* **25**
Fusarium poae **7**	*Cladosporium herbarum* **15**	*Cladosporium herbarum* **21**
Verticillium sp. **3**	*Fusarium solani* **10**	*Fusarium oxysporum* **19**
Cladosporium herbarum **3**	*Verticillium* spp. **6**	*Fusarium solani* **3**
Aspergillus fumigatus **5**	*Trichoderma harzianum* **30**	*Aspergillus penicillioides* **15**
Penicillium brevicompactum **5**		*Trichoderma harzianum* **18**
Aspergillus terreus **2**		*Didymella* sp **(3)**.

Appendix 8. Aeromycoflora prevalent in the cropping room of E90 mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **(28)**	*Aspergillus niger* **(30)**	*Trichoderma harzianum* **(14)**
Rhizopus oryzae **(2)**	*Trichoderma harzianum* **(31)**	*Rhizopus oryzae* **(2)**
		Aspergillus niger **(29)**

Appendix 9. Aeromycoflora prevalent in the cropping room of Kwesi-Babs mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Rhizopus oryzae **(2)**	*Aspergillus niger* **(15)**	*Rhodotorula mucilaginosa* **(5)**
Aspergillus niger **(25)**		*Aspergillus niger* **(11)**
Cladosporium macrocarpum **(19)**	*Rhizopus oryzae* **(1)**	*Cladosporium macrocarpum* **(9)**
Trichoderma harzianum **(12)**	Yeast spp. **(3)**	*Fusarium poae* **(5)**
	Cladosporium macrocarpum **(26)**	*Rhizopus oryzae* **(2)**
	Fusarium poae **(5)**	*Penicillium citrinum* **(2)**
	Aspergillus parasiticus **(2)**	
	Trichoderma harzianum **(19)**	

Appendix 10. Aeromycoflora prevalent in the cropping room of Immaculate Gold Ent. Mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus flavus **(5)**	Yeast spp. **(3)**	*Rhodothorola mucilaginosa* **(3)**
Cladosporium herbarum **(9)**	Yeast spp. (White) **(4)**	Yeast spp. **(2)**
Cladosporium macrocarpum **(14)**	*Penicillium brevicompactum* **(11)**	*Cladosporium herbarum* **(16)**
Yeast spp. **(3)**	*Cladosporium macrocarpum* **(13)**	*Cladosporium macrocarpum* **(24)**
Epicoccus nigrum **(4)**		*Fusarium oxysporium* **(6)**
		Penicillium brevicompactum **(9)**

Appendix 11. Aeromycoflora prevalent in the cropping room of Delabless mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus niger **(3)**	*Aspergillus niger* **(2)**	*Aspergillus flavus* **(20)**
Aspergillus falvus **(3)**	*Trichoderma harzianum* **(10)**	*Aspergillus niger* **(1)**
Trichoderma harzianum **(34)**	*Rhizopus oryzae* **(1)**	*Cladosporium macrocarpum* **(3)**
Cladosporium macrocarpum **(3)**	*Cladosporium herbarum* **(15)**	*Penicillium brevicompactum* **(4)**
Cladosporium herbarum **(4)**		*Fusarium poae* **(17)**
		Aspergillus candidus **(2)**
		Aspergillus oryzae **(2)**
		Cladosporium herbarum **(14)**

Appendix 12. Aeromycoflora prevalent in the cropping room of 4E Fresh mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Aspergillus candidus **(2)**	*Fusarium oxysporium* **(18)**	*Aspergillus flavus* **(2)**
Aspergillus falvus **(2)**	*Fusarium poae* **(24)**	*Aspergillus niger* **(3)**
Trichoderma harzianum **(9)**	Yeast spp. **(2)**	*Cladosporium herbarum* **(4)**
Yeast spp. **(4)**	*Cladosporium macrocarpum* **(4)**	*Penicillium brevicompactum* **(3)**
Penicillium brevicompactum **(13)**		*Gliocladium* **(3)**
		Mycelia sterilia **(1)**
		Fusarium oxysporium **(30)**

Appendix 13. Aeromycoflora prevalent in the cropping room of Edeyef mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Cladosprium herbarum **(4)**	*Cladosporium macrocarpum* **(3)**	-
	Cladosporium herbarium **(1)**	-
	Aspergillus niger **(2)**	
		-

Appendix 14. Aeromycoflora prevalent in the cropping room of PCM mushroom farm isolated from exposed media plates for a period of five (5) minutes and incubated for a week

Potato Dextrose Agar (PDA)	Oxytetracycline Glucose Yeast Extract (OGYE)	Dichloran Rose Bengal Chloramphenicol Agar (DRBC)
Cladosporium herbarum **(28)**	*Penicillium brevicompactum* **(7)**	*Cladosporium herbarium* **(46)**
Penicillium brevicompactum **(14)**	Yeast spp. **(3)**	*Verticillium* spp. **(3)**
Yeast spp. **(2)**	*Verticillium* spp. **(4)**	
	Aspergillus flavus **(5)**	
	Cladosporium herbarum **(10)**	

Appendix 15. Comparison of the total fungal population isolated from mushroom fruiting bodies of seven mushroom farms in Accra and Kosoa.

Farm	PDA	OGYE	DRBC
1	4.17	4.3	4
2	4.44	4.2	4.28
3	2.59	3.22	2.59
4	4.45	4.28	3.9
5	2.84	3.22	2.88
6	3.35	3.88	3.39
7	5.32	5.34	4.71

Appendix 16. Comparison of the total fungal population isolated from composted growth substrate of seven mushroom farms in Accra and Kosoa.

Farm	PDA	OGYE	DRBC
1	5.09	5.24	4
2	3.38	3.44	2.92
3	3.46	3.2	3.13
4	4.5	4.56	4.72
5	2.88	2.89	2.93
6	4.24	3.7	4.13
7	2.86	3.43	3.07

Appendix 17a. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with an ethanol extract of *Anthocleista nobilis* (cabbage tree)

	RADIAL GROWTH (mm)											
Day	2			5			7			10		
Concentration												
Control	**48**	**5**	**9**	**90**	**89**	**90**	**90**	**90**	**90**	**90**	**90**	**90**
1:1	32	33	28	42	62	41	66	62	69	90	90	90
1:2	43	41	44	53	52	67	70	73	84	89	90	90
1:5	52	56	58	63	76	74	82	80	90	90	90	90
1:10	61	60	52	62	65	72	87	90	88	90	90	90
Full strength	35	38	26	40	47	40	51	72	56	90	90	90

Appendix 17b. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with an ethanol extract of *Anthocleista nobilis* (cabbage tree)

	Radial growth (mm)			
Day	2	5	7	10
Concentration				
Control (0)	**63**	**89**	**90**	**90**
1:1	31	48	66	90
1:2	43	57	76	89
1:5	55	71	84	90
1:10	58	66	88	90
Full strength (1)	33	42	59	90

Appendix 18a. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with "champion" antifungal (10g/L)

	Radial growth (mm)											
Day	2			5			7			10		
Concentration												
Control	**50**	**52**	**60**	**83**	**80**	**90**	**90**	**90**	**90**	**90**	**90**	**90**
1:1	0	0	0	16	16	16	16	16	24	16	16	18
1:2	0	0	0	18	16	18	28	24	24	19	16	18
1:5	42	40	36	44	40	68	76	86	90	89	85	87
1:10	54	50	44	54	56	74	80	80	90	90	90	90
Full strength	0	0	0	12	0	2	25	25	12	26	36	40

Appendix 18b. Radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with "champion" antifungal (10g/L)

	RADIAL GROWTH (mm)			
Day	2	5	7	10
Concentration				
Control (0)	**54**	**84**	**86**	**90**
1:1	0	16	16	17
1:2	0	17	17	18
1:5	39	5.1	51	87
1:10	49	61	61	90
Full strength (1)	0	7	21	34

Appendix 19a. Dry weight of *Trichoderma harzianum* cultivated in Potato Dextrose Broth (PDB) amended with an ethanol extract of *Anthocleista nobilis* (cabbage tree) for seven (7) days

Concentration	Dry weight (mg)		
	1	2	3
Control	**150**	**160**	**160**
1:1	60	50	110
1:2	70	120	110
1:5	110	130	140
1:10	150	160	160
Full strenght	50	70	80

Appendix 19b. Dry weight of *Trichoderma harzianum* cultivated in Potato Dextrose Broth (PDB) amended with an ethanol extract of *Anthocleista nobilis* (cabbage tree) for seven (7) days

Concentration	Dry weight (mg)
Control	**160**
1:1	70
1:2	100
1:5	130
1:10	160
Full strength	70

Appendix 20a. Dry weight of *Trichoderma harzianum* cultivated in Potato Dextrose Broth (PDB) amended with "champion" antifungal (10g/L) for seven (7) days

Concentration	Dry weight (mg)		
	1	2	3
Control	**160**	**150**	**150**
1:1	70	80	70
1:2	100	130	90
1:5	100	120	140
1:10	130	140	120
Full strength	70	70	60

Appendix 20b. Dry weight of *Trichoderma harzianum* cultivated in Potato Dextrose Broth (PDB) amended with "champion" antifungal (10g/L) for seven (7) days

Concentration	Dry weight (mg)
Control	**150**
1:1	70
1:2	110
1:5	120
1:10	130
Full strenght	70

Appendix 21a. First flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on uncontaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	16	6	90.86	8.2	6.5	1	0.8	7.5	7.5
2	24	10	78.03	7.3	6.6	1.2	0.8	5.8	6.3
3	32	19	55.79	5	5	0.9	0.5	3.8	3
4	-	-	-	-	-	-	-	-	-
5	24	9	46.8	6.2	5.5	1.5	0.9	6.4	4.6

Appendix 21b. First flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on *T. harzianum* contaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	-	-	-	-	-	-	-	-	-
2	24	19	73.08	5.3	5.2	1.5	0.9	3.5	4.4
3	22	9	48.11	6	6.2	1	0.8	4.5	4
4	-	-	-	-	-	-	-	-	-
5	34	12	61.7	3.6	4.4	1	0.7	4.2	4.2

Appendix 22c. Second flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on uncontaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	22	17	29.32	4.2	3.3	0.6	0.8	5.5	4.5
2	26	22	36.63	4.5	3.7	1	0.7	5.1	4.5
3	-	-	-	-	-	-	-	-	-
4	20	13	31.45	5.2	3.7	1.4	1	5.5	5.9
5	-	-	-	-	-	-	-	-	-

Appendix 21d. Second flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on *T. harzianum* contaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	21	18	23.53	3.8	4	0.6	0.6	2.7	4.5
2	-	-	-	-	-	-	-	-	-
3	26	18	26.78	4.6	5	0.5	0.6	2.8	3.5
4	24	13	16.19	4.7	3.5	0.5	0.5	2.6	4.5
5	-	-	-	-	-	-	-	-	-

Appendix 21e. Third flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on uncontaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	34	19	19.67	5.6	4.4	1	0.8	3.9	4
2	23	17	49.48	6	5.7	1.6	1.5	8.6	7.5
3	32	13	53.94	5	4.5	1	0.9	6.4	5
4	40	10	31.36	4	4.2	0.9	0.9	5.9	4.4
5	45	15	53.84	5.6	4.5	0.6	1.2	5.9	6.3

Appendix 21f. Third flush growth yield and Biological Efficiency (BE) of *P. ostreatus* strain EM-1 cultivated on *T. harzianum* contaminated growth substrate.

BAG	PINHEADS	FRUITING BODIES	WEIGHT	STIPE LENGTH		STIPE WIDTH		CAP DIAMETER	
1	-	-	-	-	-	-	-	-	-
2	18	10	13.6	6.7	5.5	0.6	0.7	1.4	2
3	15	10	41.38	6	3.8	1	0.8	6	6.5
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-

Appendix 22. Statistical analyses of radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with an ethanol extract of *Anthocleista nobilis* (cabbage tree

Anova

SUMMARY

Groups	Count	Sum	Average	Variance
1	4	33.2	8.3	1.78
1:1	4	23.5	5.875	6.3825
1:2	4	26.5	6.625	4.129167
1:5	4	30	7.5	2.406667
1:10	4	30.2	7.55	2.543333
0	4	22.4	5.6	6.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25.06214	6	4.177024	0.838399	0.554254	2.572712
Within Groups	104.625	21	4.982143			
Total	129.6871	27				

Appendix 23. Statistical analyses of radial growth of *Trichoderma harzianum* on Potato Dextrose Agar (PDA) amended with a copper fungicide "Champion"

Anova

SUMMARY

Groups	Count	Sum	Average	Variance
1	4	30.3	7.575	2.349167
1:1	4	4.3	1.075	0.569167
1:2	4	4.5	1.125	0.6425
1:5	4	18.7	4.675	0.3225
1:10	4	22.8	5.7	0.32
0	4	6.2	1.55	2.283333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	152.2733	5	30.45467	28.16978	6.47E-08	2.772853
Within Groups	19.46	18	1.081111			
Total	171.7333	23				

Appendix 24. Statistical analyses of vegetative growth of *T. harzianum* in PDB amended with varying concentrations of ethanol extract *Anthocleista nobilis* in comparison to PDB amended with varying concentrations of copper fungicide "champion"

Anova

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1	2	0.31	0.155	5E-05
1:1	2	0.14	0.07	0
1:2	2	0.21	0.105	0.00005
1:5	2	0.25	0.125	5E-05
1:10	2	0.29	0.145	0.00045
0	2	0.14	0.07	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.01336 7	5	0.00267 3	26.73333	0.00049 5	4.38737 4
Within Groups	0.0006	6	0.0001			
Total	0.01396 7	11				

Appendix 25. Statistical analyses of mineral content of mushroom fruiting bodies harvested from uncontaminated growth substrate and *T. harzianum* contaminated growth substrate.

Minerals	Uncontaminated	Contaminated
Ca	4.43	0.99
Cu	0.04	0.04
Fe	1.13	0.22
K	16.66	16
Mg	3.56	2.61
Na	0.01	0
P	12.4	12.8
Zn	0.03	0

P Value = 0.415206

Appendix 26. Statistical analyses of chemical content of mushroom fruiting bodies harvested from uncontaminated growth substrate and *T. harzianum* contaminated growth substrate.

	Uncontaminated	Contaminated
Dry matter	52.56	52.51
% moisture	13.08	12.45
% fat	1.19	0.75
% Crude fibre	91.13	83.67
% Crude protein	15.9	14.35
% Total ash	8.87	16.33
% Carbohydrate	72.86	67.81
Energy (kcal/100g)	334.97	273.1

P Value = 0.432457