STUDIES ON THE OCCURRENCE, ETHNOMYCOLOGY AND CULTIVATION OF *PLEUROTUS TUBER-REGIUM*.

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BY

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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 nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

Two hundred questionnaires were administered to people in ten selected villages in each of the Hohoe and Techiman Districts of the Volta and Brong Ahafo Regions respectively. Ten people from ten communities in each of the two districts answered the questionnaires. 52% of the respondents from the Volta Region were females with majority (30.7%) in the age group of 61-70 years while 70% of the respondents from the Brong Ahafo Region were females with majority (28.6%) in the age group of 41-50 years. Results from the questionnaires showed that the middle aged had more knowledge of *Pleurotus tuber-regium* than those from the other age groups. Respondents said ailments such as asthma, underweight in babies, boils, etc. are cured using the sclerotium of *P. tuber-regium*. Samples of *P. tuber-regium* were collected from the wild in both the Hohoe and Techiman Districts and the mycelia grown on different media and incubated at 25ºC, 30ºC and 35ºC. Potato Dextrose Agar and Wheat Dextrose Agar were the best growth media for *P. tuber-regium* at the optimum temperature of 35ºC. The result subjected to statistical analysis of variance (ANOVA) at 5% significance level showed significance differences in the mycelia growth at the different temperatures for both strains of *P. tuber-regium*. The strain from the Hohoe District grew faster than that from the Techiman District. pH 6 supported the best growth rate for both strains. Light was not essential for the mycelial growth of both strains of *P. tuber-regium*. *P. tuber-regium* was grown on various agricultural wastes (sawdust, plantain leaves, water hyacinth and millet stalk) to determine their suitability for fruitbody and sclerotium formation. Sawdust and plantain leaves produced sclerotia which gave Biological Efficiency (B.E) of 99.65% and 62.05% respectively, but only plantain leaves produced fruitbodies with B.E of 54.47%. Fruitbody induction from sclerotia in soil was carried out in both cane baskets and plastic bowls to assess formation of fruitbodies. The cane basket recorded the fastest rate of induction. Plantain leaves contained the highest concentration of crude protein followed by wawa sawdust, millet stalk and water hyacinth. Cellulose and hemicellulose were most abundant in millet stalk followed by wawa sawdust and least in water hyacinth. Lignin was most abundant in wawa sawdust and lowest in plantain leaves.

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CHAPTER ONE

INTRODUCTION

1.1 CLASSIFICATION OF MUSHROOMS

Mushrooms belong to the Kingdom of Fungi. Fungi have a distinct cellular structure like plants, but they lack chlorophyll and are therefore unable to use energy from the sun directly through chlorophyll. Thus, fungi are heterotrophic and depend on other organisms for food. They depend on the substrates on which they grow for all their nutritional requirements like carbon, water, nitrogen and minerals (Rajarathnam *et al*., 1997).

Most cultivated mushrooms belong to the Basidiomycetes, which produce spores called basidiospores. It has been estimated that our planet harbour about 1.5 million different species of mushrooms. But only 64,000 species have been described so far (Oei, 1996).

1.2 THE BASIDIOMYCOTA

The phylum basidiomycota constitute a large fungal group of many diverse forms including the rusts and smuts that cause plant diseases, the mushrooms and other large forms such as boletus, puff balls, bracket fungi and yeast-like *Cryptococcus neoformis*. The class basidiomycetes are so called because meiosis occurs in specialized structures called basidia and the resulting spores (basidiospores) are produced outside the basidium. This is in contrast to the (other major groups of fungi) Ascomycota, where meiosis occurs in an ascus, resulting in the development of ascospores (Youri, 2003).

Mushrooms can be classified into three basic ecological groups: mycorrhizal, parasitic and saprophytic. Mycorrhizal mushrooms form a mutually dependent, beneficial relationship with the roots of host plants, ranging from trees to grasses. The mycelia of these mycorrhizal mushrooms can form an exterior sheath covering the roots of plants and are called ectomycorrhizal. Those which invade the interior root cells of host plants are endomycorrhizal. In either case, both organisms benefit from this association. The resident mushroom mycelium increases the plant's ability to absorb nutrients, nitrogenous compounds and essential elements (Chen, 2005).

Edible mushrooms are the fungi which possess big fruiting bodies and can be culinary, medicinal or both. In China, there are 600 species of edible mushrooms which can be culinary and medicinal. Since 1960, Chinese scientists have researched into more than 40 types of edible mushrooms in more details e.g. *Armillariella mellea, Cordyceps sinensis, Coriolus versicolor, Ganoderma lucidum, Hericium erinaceus, Lentinus edodes* (Chen, 2005).

Mushrooms are also classified according to the type of substrate on which they grow. Those which grow purely on cellulose are referred to as cellulolytic mushrooms and they thrive mainly on cellulose-containing substrates such as rice straw, wheat straw, banana leaves, e.g. *Volvariella*. The lignocellulolytic mushrooms grow on both cellulose and lignin containing substrates. Oyster mushrooms, *Pleurotus* species belong to this category and are thus widely cultivated on a variety of agricultural wastes (Oei, 1991).

A good substrate should have the following characteristics:

i. suitable physical condition that will provide good anchorage for the mushrooms and will at the same time maintain good aeration and water holding capacity.

ii. good chemical condition that will release some nutrients from the raw materials of the compost during fermentation and pasteurization.

iii. proper condition for microbial activities that will help improve both the physical and chemical condition for mushroom growth (Chang, 1984).

Composting is a process of controlled microbial succession in the substrate. The composting material is stacked up in large piles; the micro-environmental conditions differ at different depths of the piles. During composting, a mixture of rich organic materials is converted into a stable medium which is suitable for the growth of the particular mushroom but not for competing microorganisms (Oei, 1991).

The phenology of microorganisms including bacteria, actinomyces, fungi and protozoa are different at different stages; different groups of microorganisms may dominate at different stages. The initial microflora may be mesophilic and utilize the soluble organic carbohydrates and nitrogen. This is followed by the increased growth of more thermotolerant organisms and the release of carbon dioxide, ammonia, and a considerable amount of heat. At the later stages of composting the temperature is higher and thermophilic microorganisms become dominant. Thermophilic microorganisms present throughout the compost are mainly responsible for the second stage of fermentation. Thereafter the compost becomes selective and following spawning, mushroom mycelia grow out rapidly and ramify through out the compost in a short period of time (Chang, 1984).

1.3 MUSHROOM CULTIVATION IN GHANA

Mushrooms are popular and widely consumed in most communities in Ghana. Various species found in the forest and countryside of Ghana such as *Termitomyces* (termite mushroom), *Pleurotus* (oyster mushroom), *Pholiota, Lepiota* (shaggy parasol), *Volvariella* (oilpalm mushroom) and *Coprinus* (ink cap) grow freely on anthills, tree stumps, farmlands, forest litter and other lignocellulose materials. In Ghana wild mushrooms are always abundant during the rainy season and are collected for home use and sale (Obodai, 1992).

Recently exotic tropical species of *Pleurotus* (e.g. *P. eous*, *P. cystidiosus, P. ostreatus*, *P. pulmonarius*) and *Auricularia* (e.g. *A. auricular, A. polytricha*) have been introduced into Ghana (Obodai, 1992).

1.4 ECONOMIC IMPORTANCE OF MUSHROOM PRODUCTION

Wild edible fungi are important for three main reasons: as source of food and health benefits, source of income and to maintain the health of forests. Commercial productions of mushrooms however have many more benefits.

1.4.1 Agricultural wastes recycle and animal feed

One of the economic strengths of mushroom production is that mushroom composts are made from organic agricultural waste materials. Apart from the cultivation of mushroom transforming the inedible waste to edible biomass for human consumption, the digestibility of substrates supporting growth of the mushroom is increased to the extent that the spent substrate can be used as animal feed. In order to hasten the production of mushroom fruitbodies, it is always expedient for the slow decomposing substrate to undergo a process called composting. Because mushrooms are heterotrophic organisms, they have to get all the nutritive elements from the substrate on which they grow. The compost plays a more comprehensive role in mushroom production than does soil in higher plant growth (Zhang, 2005).

Mushrooms can use many materials as nutrient for their growth. The materials are called substrates and they can be divided into two nutrient types according to their function to mushroom growth: major materials and supplementary materials (Zhang, 2005).

i. Major materials constitute the most part in the substrate and serve as a supply of carbon source. They are from various industries, such as agriculture, forestry, textile and brewery. Many kinds of agro-byproducts could be used for mushroom production. Cellulose and hemicellulose are major carbohydrates used by mushrooms. The straw or hull of many crops (e.g. rice straw and wheat straw, corn cob, cotton seed hull, peanut hull and sunflower straw), forest byproducts from the forest industry (e.g. sawdust, tree branch, stump, stub, wood scrap and barks) could be used as major materials. Industrial wastes like those from textile industries e.g. cotton waste, waste paper, brewery waste are rich in cellulose and can be used as carbon sources by mushrooms.

ii. Supplementary materials provide the nitrogen, mineral and some simple carbohydrates which are absorbed easily. Supplementary materials are not necessary for mushroom growth but they can help to get higher output. Some examples of supplementary materials include the following: wheat bran, rice bran, KH_2PO_4, $MgSO_4$, $CaCO_3$, $CaSO_4$ (Zhang, 2005).

Species of the genus *Pleurotus* are important industrially, occupying third place in worldwide production of edible mushrooms (16.3% of the total production) (Velazquez-Cederio *et al*., 2002).

Pleurotus spp. is by far the easiest and least expensive to grow. Few other mushrooms demonstrate such adaptability, aggressiveness, and productivity as *Pleurotus spp.* (preeminent wood decomposers). They grow on a wider array of forest and agricultural wastes than any other mushroom group. What is most extraordinary about oyster mushrooms is their conversion of substrate mass into mushrooms. Biological efficiencies often exceed 100%, some of the greatest, if not the greatest, in the world of cultivated mushrooms. On dry-weight basis, oyster mushrooms have a substantial protein, ranging from 15-35% and contain significant quantities of free amino acids (Stamets, 2000).

1.4.2 Production of food with specific nutritional value

Many mushrooms are known to have nutritional values and are referred to as health food. They are known to have large amount of good protein, vitamins (B1, B2, and C) and minerals and have very low fat content (Chang and Mshigeni, 2001).

14.2.1 Protein

Mushrooms are said to have a rich source of protein but actually their protein content is rather low, normally between 3-4% of their fresh weight since water content of mushroom is around 90%. The dry weight of mushrooms contains between 19-35% protein. Sawyerr (1994) observed a mushroom protein content of 19-49% while Obodai (1992) observed a protein content of 28-28.81% for *Pleurotus ostreatus* which was grown on different substrates. Mushrooms contain all the essential amino acids (Oei, 1996).

1.4.2.2 Fat

The fat content of mushrooms on dry weight basis is between 1-8%, with an average of 4%. Unsaturated fatty acids make up at least 72% of the total content mainly due to linoleic acid. The high content of linoleic acid is one of the main reasons why mushrooms are considered healthy food (Oei, 1996). Obodai (1992) reported a fat range of 1.07%-2.06% on *Pleurotus ostreatus* grown on different substrates.

1.4.2.3 Vitamins and minerals

Mushrooms are a good source of vitamins such as thiamine, riboflavin, niacine, biotine and ascorbic acids. They also contain significant amounts of phosphorous, sodium, and potassium and a small amount of calcium (Oei, 1996). Mushrooms contain larger amounts of folic acid than any other vegetable and meat (except the liver) (Sawyerr, 1994).

1.4.2.4 Carbohydrate and fibres

Fibres are part of a healthy diet. Fresh mushrooms contain relatively large amounts of fibre and carbohydrates (Oei, 1996). The cell walls of plants constitute dietary fibre and consist of cellulose, hemicellulose and lignin. Abobor (1998) reported a fibre content of 12.09% for *P. ostreatus* grown on sawdust.

1.4.3 Use of mushroom in medicine to control specific illnesses

More than 220 species of mushrooms are of medicinal value but most of them have not been utilized. Extensive study conducted in Japan and the United States show that mushrooms contain high levels of retene, a chemical substance that suppresses some cancerous growths and tumours. Extracts from some mushrooms facilitate antiviral activities against poliomyelitis and influenza (Oei, 1996).

1.4.4 Soil bioremediation

The mycelium has the unique ability to split the chemical structure of highly toxic Polychlorinated biphenols (PCBs) into non-toxic substances without enclosing toxics in their fruitbodies. This way oyster mushrooms can be used to re-cultivate industrial ground contaminated by PCB's by using principles of biotechnologies (www.greenmuseum.org, 2006).

1.4.5 Economic use of land

Mushroom farming provides the most economical way to make use of agricultural land since very little land is needed for its cultivation. This will focus on the challenges for the scientific community to fulfill the motto "Grow more food with less land". Table 1 below gives an example of the yield of dry protein/acre/year of different farming types as recorded by Sawyerr (1994).

Table 1: Yield from different farming types

FARMING TYPE	**POUNDS DRY PROTEIN/ACRE/YEAR**
Livestock	70
Fish farming	600
Soya beans	1,000
Most cereals	600
Mushroom cultivation	60,500-70,000

This high turnover is due to the relatively short time required for mushroom fruitbodies to be formed e.g. 10-14 days for oil palm mushroom (*Volvariella species)* and their efficient bio-conversion of other food sources into protein. Mushroom farming therefore has a role in the attainment of the much desired food security for a fast growing economy (Oei, 1996 and Youri, 2003).

1.4.6 Income generating

Mushroom farming can create job opportunities for the unemployed and retired people. It could also be a second income earner for those who are employed and need a second income support. It has a huge export potential since Ghana is surrounded by Francophone countries that eat lots of mushrooms, and as such could bring in foreign exchange which will in turn improve the economy for the better.

1.5 DESCRIPTION AND CLASSIFICATION OF *PLEUROTUS TUBER-REGIUM.*

Pleurotus tuber-regium had been suggested to be a *Lentinus* (*Lentinus tuber-regium* (Fr) Fries) a view further supported by Pegler (1983) primarily due to the dimitic arrangement of hypha and the overall tough flesh of the fruitbody. Recent molecular studies by Isikhuemhen *et al.* (2000) shows that this species clearly belongs to the genus *Pleurotus.* The sclerotia are globulose to ellipsoid in shape, whitish to dingy beige on sterilized or pasteurized substrates, darkening with age and dark brown to nearly black when developing in contact with soils. The initial primodia develop from the sclerotia as a spike-like proboscis, narrow at the apex and broad at the base. Once the stipe grows several inches high, a fluted cap emerges, and the stem develops a coating of darkened fibrils. The cap, centrally attached to the stem is umbilicate, with decurrent gills and with a margin, which is initially inrolled at first. The stem is scabrous with brownish fibrillose remnants. Quickly maturing, this mushroom can achieve considerable size, up to 10 inches (25cm) in diameter (Stamets, 2000).

Most research work on *P. tuber-regium* has focused on those grown in Nigeria. Although *P. tuber-regium* is traditionally known in Ghana, not much research has been carried out on it. As a result *P. tuber-regium* has not been commercially produced by mushroom farmers in Ghana. Traditionally in Ghana, it is known to possess medicinal values and have been used by herbal practitioners to cure illnesses such as under weight in children, asthma and high blood pressure among others.

Studies on medicinal mushrooms such as *P. tuber-regium* is limiting despite the fact that it is traditionally known. Ghana is predominantly an agricultural country. Most of the crops grown in the country produce a lot of wastes. The industrial processing of agricultural and forest raw materials also produce waste e.g. sawdust, cotton waste. The utilization of some of these wastes as substrates for growing edible mushrooms is very important. Apart from bringing income to the people in the communities, it will help reduce the buildup of wastes, with attendant detrimental environmental consequences.

The objective of this work was to carry out vegetative growth and cultivation studies on *P. tuber-regium* collected from two Regions in Ghana.

The specific objectives were to:

i. obtain indigenous information on *Pleurotus tuber-regium* in Ghana by administering structured questionnaires in two administrative Regions of Ghana (Volta and Brong Ahafo Regions) where the mushroom grows in the wild.

ii. determine the best medium for mycelial growth of *P. tuber-regium.*

iii. determine the optimum temperature, pH and light requirement for growth of *P. tuber-regium.*

iv. assess the yield of *P. tuber-regium* collected from the two Regions on different substrates and determine their Biological Efficiency (B.E).

v. determine the chemical composition of the substrates (agricultural and forestry) before and after cultivation of *P. tuber-regium*.

vi. determine the chemical composition of fruitbodies and sclerotia of *P. tuber-regium* from the two Regions cultivated on the different substrates.

CHAPTER TWO

LITERATURE REVIEW

2.1 EXISTENCE OF MUSHROOMS IN NATURE

Mushrooms have long been the subject of great fascination. In medieval Ireland, mushrooms were thought to be umbrellas for leprechauns; the English believed mushrooms should be gathered under a full moon to be edible and ancient Egyptians considered mushrooms as the sons of gods sent to the earth riding on bolts of thunder (Gbolagade, 2006).

Historical data indicates mushroom cultivation and consumption throughout the ancient Greek and Roman eras, and Asian civilizations have been cultivating mushrooms, specifically the shiitakes and *Volvariella* for over 2000 years. However, it was not until the 17th century in France that commercial mushroom growing began. *Agaricus* or white mushroom was the first variety to be cultivated. The first detailed record of mushroom cultivation occurred in A.D.600 during the reign of Louis XIV when Tournefort described a successful method of growing the mushroom *Agaricus bisporus* on stable manure (Gbolagade, 2006).

The original mushroom 'farms' were, and still are, located in quarry tunnels near Paris which explains why white mushrooms have long been called 'Champignons de Paris' by French chefs. Commercial cultivation of fresh mushrooms began in the U.S. near Philadelphia in the early 20th century, eventually centering the town of Kenneth Square and neighboring areas. Today, Pennsylvania leads the USA in production with California being a strong second producer. More than 20 other states in the US now add significantly to the total production of mushrooms (Stamets, 2000).

2.2 THE MUSHROOM LIFE CYCLE

Mushrooms are propagated by spores. The success of the mushroom life cycle depends upon the production of these spore-producing fruiting basidiocarps. Each spore that is released is monokaryotic, possessing haploid genetic material necessary for the propagation of the species. Upon germination, a filamentous cell called hypha extends. Hyphae continue to reproduce mitotically. Two hyphae, if compatible, come together, fuse, and become dikaryotic. The resulting mycelium is then described as being dikaryotic. After this union of genetic material, the dikaryotic mycelium characteristically grows faster than unmated mycelium arising from single spores (Stamets, 2000).

When spores germinate, several strains are produced. Incompatible strains grow away from each other, establishing their own territorial domains. When compatible spores come together and mate a much vigorous form of mycelium fans out. When spores germinate, the mycelium grows radially away from the site of origin (Fig.1). Spores taken up by wind or carried by insects and mammals are dispersed to habitats well distant from the parent mushroom. Enzymes and acids are secreted by the mushroom mycelium into the surrounding environment, breaking down lignin-cellulose complexes into simpler compounds. The mushroom mycelium absorbs these reduced organic molecules as nutrients directly through its cell walls (Stamets, 2000).

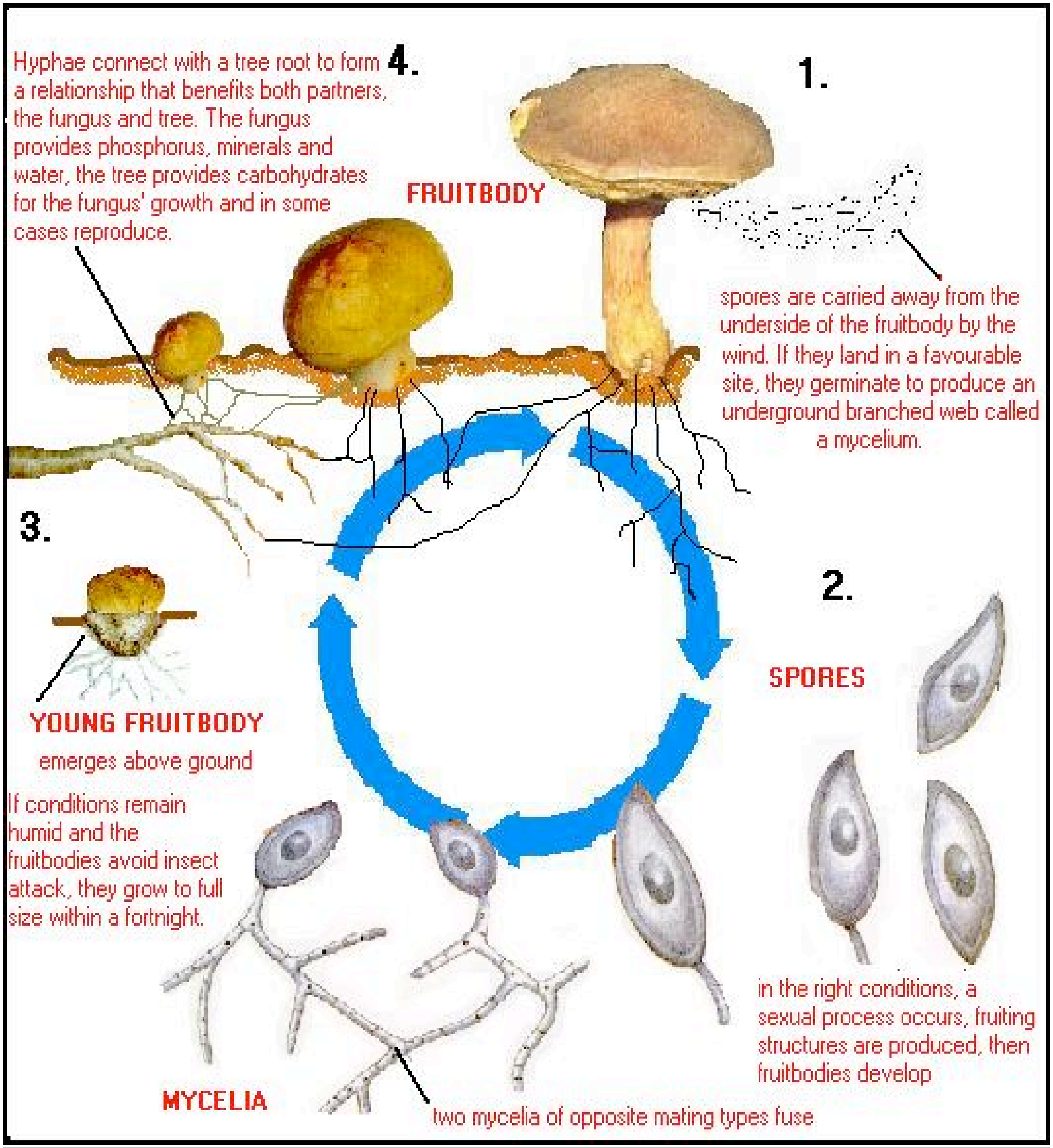

Fig. 1: A generalized life cycle of the mushroom

Source: (http://www.geocities.com/rainforest/andes/8046/)

2.2.1 Life Cycle of *Pleurotus tuber-regium*

Pleurotus tuber-regium is a white rot fungus, which derives nutrients from the degradation of lignocelullosic material (usually hardwood). In nature, dead wood is colonized either by mycelia in the soil or airborne spores discharged from individual fruitbodies. During the rainy season it is possible to see a decaying log filled with fruiting mushrooms in early years of its colonization. Once the substrate is fully colonized and has reached an advanced stage of decay, sclerotia will form at the end of the rainy season. The sclerotia survive the dry and hot season until the rains return, at which point the sclerotia will either continue to enlarge or form fruitbodies (mushrooms). Sclerotia are a compact mass of mycelia tissue that serve to store food during unfavourable conditions and are able to fruit when favourable conditions return (Isikhuemhen *et al.*, 2002).

2.3 CULTIVATION OF MUSHROOM

In the commercial cultivation of mushrooms spores are not used. Their small size makes it difficult to handle. Their genetic characteristics are also a little different from that of their parent. A pure culture of mycelium on grain, sawdust, etc. called spawn (Fig. 2) is rather used to inoculate the final substrate for the commercial cultivation of mushrooms and these are done in sterile environment. Using the spawn will give the cultivated mushroom an advantage in growth compared to other fungi (Oei, 1996).

Spawn could be said to be analogues to the seeds of plants. It includes the mycelium and the substrates. Spawn production includes three (3) main steps:

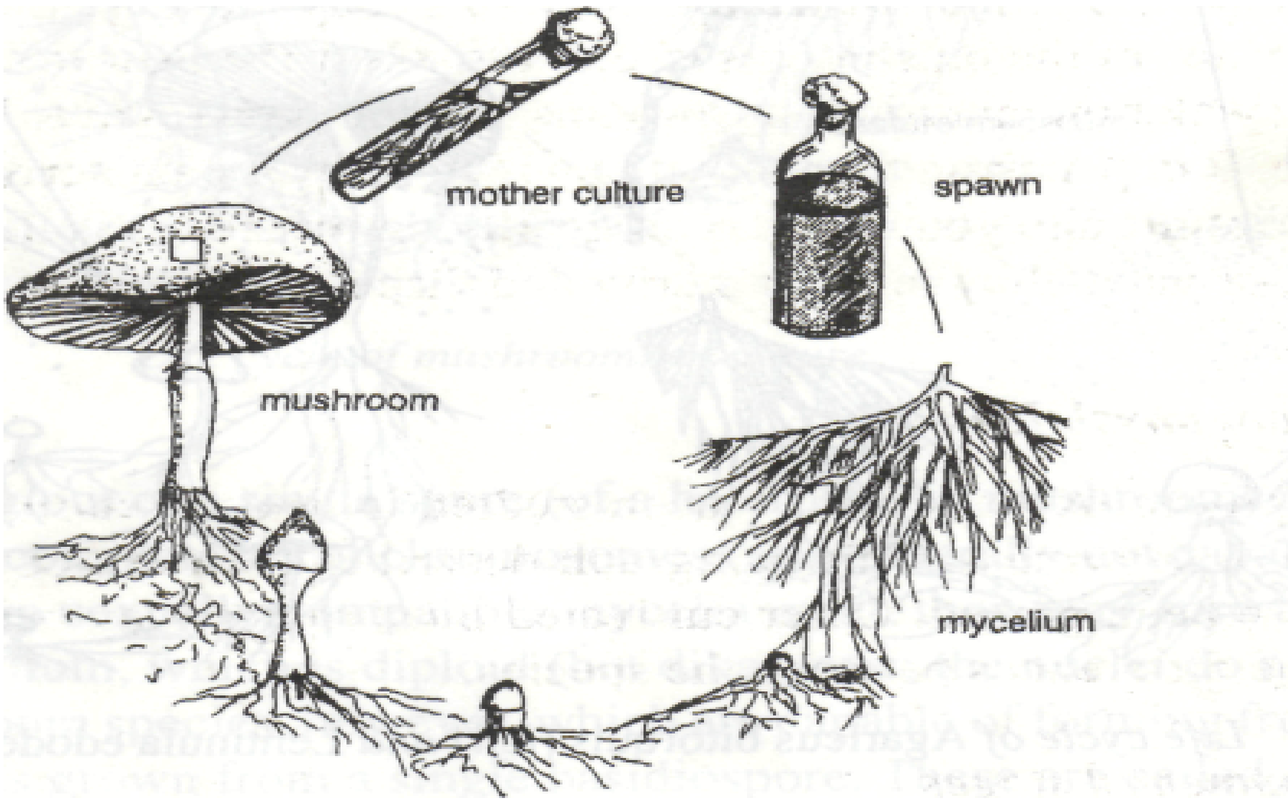

Fig. 2: Life cycle from mushroom to spawn (Oei, 1996).

2.4 CATEGORIES OF MUSHROOM

Mushrooms are divided into four main categories:

2.4.1 Edible Mushrooms: These are mushrooms which are fleshy and can be eaten e.g. *Agaricus bisporus, Pleurotus tuber-regium, Volvariella volvacea.*

2.4.2 Medicinal Mushrooms: These are mushrooms which have medicinal applications or properties e.g. *Ganoderma lucidium, Lentinula edodes.*

2.4.3 Poisonous Mushrooms: These are mushrooms which contain poisonous substances and thus not suitable to be served as food e.g. *Amanita muscaria, Amanita phalloides.*

2.4.4 Miscellaneous Mushrooms: These are mushrooms whose properties remain less defined. They are not known to have culinary uses or medicinal values. Little or no work has been carried on them (Obodai *et al.,* 2002).

Table 2.1 shows categorized numbers of species of wild edible and medicinal fungi from 110 countries.

Table 2: Numbers of species of wild edible and medicinal fungi

CATEGORY	NO. OF SPECIES
1. Edible only	1009
2. Edible and medicinal	88
3. Food only	820
4. Food and medicinal	249
5. Medicinal only	133
6. Other uses (none of above)	29
TOTAL wild useful species	**2328**
ALL edible (1+2)	1097
ALL food (3+4)	1069
ALL medicinal (2+4+5)	470

(http://www.fao.org/docrep/007/y5489e/y5489e05.htm)

2.5 NUTRITIONAL REQUIREMENTS

Most mushrooms are known to be saprophytes; as such they depend on the nutrients in the substrates on which they grow. They obtain the nutrients through the mycelium. The essential sources of nutrients include nitrogen, minerals and vitamins. Sugars and nitrogen sources are the main nutrients (Zhanzi and Zhanhua, 1997).

2.6 SPENT SUBSTRATE

Spent mushroom substrate is the material left after the cropping of mushrooms is over. It is a heterogeneous material with a wide range of particle sizes distributed through the substrate (Beattie *et al.*, 2001). Spent substrate can be used as soil fertilizers, energy source, animal fodder and as an inoculant for soil bioremediation (Oei, 1996)

2.7 DESCRIPTION OF *PLEUROTUS SPECIES*

Ecology: Saprobic; growing in shelf-like clusters on dead logs and living trees (primarily hardwoods, but sometimes on conifers); causing a white rot.

Colour of fruitbody: The colour of the fruitbody of the genus *Pleurotus* spans the rainbow: white, blue, grey, brown, golden and pink.

Cap: 4-15 cm; convex, becoming flat or somewhat depressed; kidney-shaped to fan-shaped, or nearly circular if growing on the tops of logs; somewhat greasy when young and fresh; smooth; pale brown to dark brown; margin inrolled when young, later wavy, never lined.

Gills: Running down the stem, close, whitish or with a gray tinge, sometimes yellowish in age.

Stipe: Usually rudimentary and lateral (or absent) when the mushroom is growing from the side of a log or tree. When it grows on the tops of logs and branches, or at an angle, however, it may develop a substantial and thick stalk that is dry and slightly hairy near the base.

Flesh: Thick, white.

Spore print: Whitish to grayish or lilac.

Microscopic features: Spores 8-10.5 x 3-3.5μ; smooth cylindrical to narrowly kidney-shaped (Kuo, 2005).

The genus *Pleurotus* are wild growing and can be cultivated. As gourmet mushrooms, they are preeminent decomposers which could also be used for some types of waste management, mostly agricultural waste. They thrive on most hardwoods, wood byproducts and other materials. On dry weight basis, oyster mushrooms have substantial protein, ranging from 15-35% and contain significant quantities of free amino acids. They are replete with assorted vitamins such as vitamin C (30-144mg per 100grams) and vitamin B, niacin (109mg per 100grams). The variation in the reported nutritional analysis of oyster mushrooms is due to several factors. The protein content is affected by the type of substrate and by the spawning media and rate. Strains of *Pleurotus* vary in their nutritional composition and yield performances (www.greenmuseum.org, 2006).

Pleurotus tuber-regium (Fries) Singer is an edible white-rot sclerotial Basidiomycetes that belongs to the family Pleuroteceae. It occurs in both tropical and subtropical regions of the world. It is a common mushroom in the southwest province of Cameroon, Australia, Asia and eastern and western parts of Nigeria. The fungus infects dead wood tissues but is also found between the wood and the bark or buried in the soil or grasslands. It has been found to be growing on many species of soft and hard wood like *Mangifera indica*, *Daniella oliveri*, *Treculia africana* (Ezeronye *et al.*, 2005, Isikhuemhen and Nerud., 1999, Osemwegie *et al.*, 2002).

It is capable of producing tuberous true sclerotia (23cm or more in diameter) on decaying logs towards the end of the rainy season (which falls between August and October in West Africa). From these sclerotia, fruitbodies are produced at the beginning of the following rainy season, falling between April and June (Osemwegie *et al.*, 2002).

In China *P. tuber-regium* is found mainly in southern China. In some folk recipes it is used as medicine for the treatment of coughs and asthma (Deng *et al.*, 2000).

In West Africa, *P. tuber-regium* is considered a delicacy and its fruitbodies and sclerotia serve as a valuable ingredient (soup thickener) in the preparation of various native soups and sauces. The fruitbodies and sclerotia have widespread use in the traditional way of treating anemia, stomach pains, cough, as well as malnutrition-related illnesses in children (Iwuagwu and Onyekweli, 2002; Osemwegie *et al.*, 2002).

In Nigeria *P. tuber-regium* is used as both food and medicine. The sclerotium which is hard is peeled and ground for use in vegetable soup. The sclerotium is expensive, nutritious (very rich in protein) and considered a delicacy. The tuberous sclerotium can be used as a partial replacement for melon seed (*Citrulus lanatus*) or groundnut (*Arachis hypogea*) cake in traditional preparation of sauces and soups. In many parts of tropical Africa, the sclerotium is milled with melon or groundnut seeds, seasoned and the moist mix is moulded into patties for cooking or baking. The groundnut cake patties are consumed as snacks while the fruitbodies are cooked as cheap source of protein in tropical Africa. The mushroom itself is simply chopped and used in similar soups. It may also be dried and used for future use (Isikhuemhen and Okhuoya, 1995; Nwokolo, 1987; Okhuoya and Etugo, 1993; Okhuoya and Okogbo, 1991; Oso, 1997).

Nigerian native doctors use various combinations of herbs and other ingredients in their medicine. *P. tuber-regium* is used in some of these combinations that are intended to cure headache, stomach ailments, colds and fever as well as asthma, smallpox, high blood pressure and is a tonic and treatment of coughs and malnutrition-related illnesses in children. It has also been reported that the pure culture of this fungus is able to kill and feed on nematodes (Fasidi and Olorunmaye, 1994; Oso, 1997; Osemwegie *et al*., 2002; Zoberi, 1973).

There are reports that there are many compounds such as ß-D-glucans and their non-starch polysaccharides and enzymes such as ribonuclease with potential pharmacological benefits in the sclerotium of *P. tuber-regium* (Akpaja *et al.*, 2003). An ethonomycological survey of edible mushrooms has been made in the Bia biosphere and other parts of Southern Ghana by Apetorgbor *et al*. (2006), Obodai and Apetorgbor (2001) but not much is known about *P. tuber-regium* in Ghana.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Mushroom collection and management

Pleurotus tuber-regium used for the work was collected from ten villages each in the Hohoe District of the Volta Region and the Techiman District of the Brong Ahafo Region. The communities were Aduadu, Agbonyra, Agumatsu, Dafornu, Fadome Ahor, Gbledi, Golokwati, Liati Wote, Soba, Tsebi (in the Hohoe District) and Agosa, Atakrom Atrensu, Ayeasu, Ayayo, Bonkom, Domeabra, Nkwaeso, Nkwanyeso and Twimeankwanta (in the Techiman District).

3.1.2 Maintenance of stock cultures

Stock cultures of the mushrooms collected were maintained on Potato Dextrose Agar (PDA) in 9.0cm Petri dishes. The cultures were stored in the refrigerator (Whirlpool) at 4°C and subcultured fortnightly, to make them viable.

3.1.3 Substrates used

The following substrates were used for the cultivation of *Pleurotus tuber-regium*:

i. Sawdust from 'wawa' (*Triplochiton scleroxylon)* wood: Collected from a sawmill at Ofankor in the Greater Accra Region.

ii. Water hyacinth (*Eichhornia crassipes*): Collected from a water body at Adenta in the Greater Accra Region and sun-dried.

iii. Dry millet (*Eleusine coracana*) stalk: Collected from a farm at the University for Development Studies, Nyakpala Campus.

iv. Dry plantain (*Musa sapientum*) leaves: Collected from a private farm located at the premises of the CSIR- Food Research Institute, Accra.

3.2 GENERAL METHODS

3.2.1 Sample collection areas

Samples of *Pleurotus tuber-regium* were collected from farms in the Techiman District of the Brong Ahafo Region and the Hohoe District of the Volta Region of Ghana.

The Brong Ahafo Region has a territorial size of 39,557 square kilometers, the second largest Region in the country. The Region shares boundaries with the Northern Region to the north, the Volta and Eastern Regions to the south-east, Ashanti and Western Regions to the south, and Cote d'Ivoire to the west. The Region has a tropical climate with high temperatures averaging 23.9ºC and a double maximum rainfall pattern. Rainfall ranges from an average of 1000mm in the northern parts to 1400mm in the southern parts. The Region has two main vegetation types: the moist semi-deciduous forest, mostly in the southern and southeastern parts and the guinea savannah woodland, which is predominant in the northern and northeastern parts of the Region (http://www.ghanaweb/Ghanadistricts.com).

The Volta Region has a territorial size of 20,572 square kilometers. The Region is located at the eastern part of Ghana, sharing its eastern boundary with the Republic of Togo and the western with the Volta River. The southern border is the Atlantic Ocean, while the north shares a boundary with the Northern Region. The Region has two rainfall patterns within a year, the first from March to July and the second from mid-August to October. The rainfall figures vary greatly throughout the Region and are highest in the central highland areas and in the forest zone. The maximum average annual rainfall is 2,103mm with a minimum of 1,168mm. More than half of the land area of the Region falls within the Volta River Basin, with the Volta Lake draining a substantial portion of the Region (www.ghanaweb/Ghanadistricts.com,www.touringghana.com/regions/ volta_ region.asp, www.en.wikipedia.org).

The Region's mountains form part of the Togo Range, which stretches from parts of the Ashanti and Eastern Regions into the Republic of Togo. The highest mountain in Ghana, Mount Afadzato, located in the Hohoe District is part of this range. The Region has a length of about 500 kilometers, stretching from the south to the north. It encompasses most of the vegetation zones found in the country, that is, the coastal grassland and mangrove swamps, replete with sandy beaches, the guinea savanna through moist semi-deciduous forests in the central highland areas to the undulating Sahel-savannah and the mountainous wooded savannah in the north (www.touringghana.com /regions /volta _region.asp)

The Hohoe District is in the semi-equatorial climatic zone and covers an area of 1,172 square kilometers. Temperatures are around 23ºC (www.ghanaweb/Ghanadistricts.com; www.touringghana.com/regions/volta_region.asp; www.en.wikipedia.org).

3.2.2 Preparation of culture media

a. Maize Meal Agar

Twenty grams of milled maize was heated at 60°C for 30 minutes in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

b. Millet Meal Agar

Twenty grams of milled millet was heated at 60°C for 30 minutes in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

c. Wheat Meal Agar

Twenty grams of milled wheat was heated at 60°C for 30 minutes in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

d. Sorghum Meal Agar

Twenty grams of milled sorghum was heated at 60°C for 30 minutes in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

e. Rice Meal Agar

Twenty grams of milled rice was heated at 60°C for 30 minutes in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

f. Potato Dextrose Agar

Two hundred grams of Irish potato was peeled, cut into small cubes and boiled in 500ml distilled water. It was strained and made up to 1*l*; 20g glucose and 12g agar were added.

g. Cassava Dextrose Agar

Two hundred grams of cassava was peeled, cut into small cubes and boiled in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

h. Cocoyam Dextrose Agar

Two hundred grams of cocoyam was peeled, cut into small cubes and boiled in 500ml distilled water, strained and made up to 1*l*; 20g glucose and 12g agar were added.

3.2.3 Methods of sterilization

a. Glassware: All glassware were kept clean by washing with detergent, and then rinsed with tap water. They were rinsed again with distilled water and the water allowed to drain. Petri dishes and pipettes were sterilized by heating at 160ºC for one hour in an electrically heated oven (Gallenkamp oven series).

b. Inoculation room: The floor of the inoculation room was swept clean and mopped with 5% dettol solution. The UV tube in the laminar flow cabinet was switched on for 30 minutes, while the vacuum extractor of the laminar flow cabinet was switched on 5 minutes before inoculation started.

c. Culture media: All media were sterilized in an autoclave at 1.1kg/lb steam pressure at 121ºC for 15minutes. 80mg/2ml gentamycin was added to 1liter of medium after the medium had been prepared prior to sterilization and 100mg/0.1ml streptomycin sulphate was poured into the Petri dishes before the medium was poured into them. These antibiotics were used to prevent bacterial contamination.

3.2.4 Statistical analysis

Experimental results, where necessary, were analyzed using ANOVA and results quoted at the 5% level of significance.

3.3 EXPERIMENTAL DETAILS

3.3.1. Administration of Questionnaires

3.3.1.1 Sampling

Two hundred structured household questionnaires were administered in ten villages in each of the Hohoe and Techiman districts of the Volta and Brong Ahafo Regions respectively (Plate1). The respondents were chosen randomly based on their house numbers. The choice of the study areas was based on the availability of *Pleurotus tuber-regium* in the wild in the areas. The questionnaire (Appendix A) was designed to obtain background information on respondents, types of mushrooms available, knowledge of *P. tuber-regium* such as its use, substrates and seasons in which it grows among others.

Plate 1: Student researcher administering questionnaires to a family at Twimeankwanta in the Techiman District of the Brong Ahafo Region.

3.3.2 Vegetative growth of mycelium of *Pleurotus tuber-regium* on different media at 25ºC, 30ºC and 35ºC.

20ml of various media (Maize Meal Agar, Millet Meal Agar, Wheat Meal Agar, Sorghum Meal Agar, Rice Meal Agar, Potato Dextrose Agar, Cassava Dextrose Agar, and Cocoyam Dextrose Agar) were poured in Petri dishes. There were four replicates for each medium. The plates were inoculated with 3mm discs of mycelium taken from the growing edge of 7-day-old cultures of *Pleurotus tuber-regium* grown on Potato Dextrose Agar (PDA). Three sets of the preparations were made and incubated variously at 25ºC, 30ºC and 35ºC. The vegetative growth of mycelium of the mushroom on the different media and different incubation temperatures were assessed by measuring growth of the fungus along two diameters drawn at right angles at the bottom of the Petri plates prior to inoculation. Measurements were made daily for six days.

3.3.3 Effect of temperature, pH and light on vegetative growth of *Pleurotus tuber-regium.*

3.3.3.1 Temperature

Petri dishes each containing 20ml of Potato Dextrose Agar (PDA) were inoculated with 3mm discs of 7-day-old cultures of *P. tuber-regium* and incubated at 25°C, 30°C and 35°C. There were four replicates for each temperature. Daily measurements were made of diameters of the colony along two diameters drawn at right angles at the bottom of the Petri plates prior to inoculation.

3.3.3.2 pH

To obtain the optimum pH for mycelial growth of *Pleurotus tuber- regium,* pH levels of 5, 6, 7 and 8 of Potato Dextrose Agar (PDA) were made by using 1M NaOH and 1M HCl. Four replicates were prepared for each pH value. Petri dishes, each containing 20ml of the PDA were inoculated with 3mm discs of mycelium taken from the growing edge of 7-day-old cultures of *P. tuber-regium* grown on PDA and incubated at 35ºC, the optimum temperature for growth of *P. tuber-regium.* Daily measurements of radial growth were made along two diameters drawn at right angles at the bottom of the Petri dishes prior to inoculation.

3.3.3.3 Light

Four Petri dishes containing PDA were inoculated with 3mm discs of 7-day-old cultures of *Pleurotus tuber-regium* on PDA. They were covered with transparent polythene bags and exposed to continuous light of 350-500Lux for 10 days. Another set of four inoculated Petri dishes were covered with black polythene bags for 10 days. In order to obtain alternating treatments of light and darkness, the following techniques were adopted for other sets of inoculated Petri dishes:

a. Plates were exposed to alternating 4 hr light/ 4 hr darkness for 10 days;

b. Plates were exposed to alternating 6 hr light/ 6 hr darkness for 10 days;

c. Plates were exposed to alternating 12 hr light/ 12 hr darkness for 10 days.

The radial growth was measured daily along two diameters for each instance.

3.3.4 Cultivation of *Pleurotus tuber-regium*.

3.3.4.1 Preparation of pure culture

This involves two methods: the use of tissue or spore cultures. In tissue culture a freshly grown mushroom with membrane covering the gills is used and a pair of forceps is used to pick a piece of the mushroom and this is used to inoculate slants containing Potato Dextrose Agar (PDA) or other suitable medium under sterile environment. The slants are then kept in an incubator at 23ºC for seven days. In spore culture, spore prints are collected from a well developed mushroom. These spores are then inoculated on plates containing Potato Dextrose Agar or other suitable medium under sterile conditions and kept in an incubator at 23ºC for seven days.

3.3.4.2 Preparation of mother culture

Good quality sorghum grains are selected, free of moulds and weevils. The grains are washed and then soaked in water overnight. They are boiled for 20 minutes and spread on mesh to cool and drain out excess water. Two percent calcium carbonate (quicklime) is added to the grain to adjust the pH. The grain is mixed thoroughly and 200g of grains is put into 350ml bottles which have been previously washed and cleaned. The mouth of the bottles are covered with non-absorbent cotton wool and covered with clean sheets of paper and fastened with rubber bands. The bottles are then sterilized in an autoclave at a temperature of 121ºC and pressure of 151bs for 45 minutes. After cooling the bottles, 3mm discs of mycelium taken from the advancing edge of the culture on PDA plate are used to inoculate the grains in the bottles. They are stored at 23ºC in an incubator for 16 days for full colonization (http://www.icargoa.res.in/mushroomspawn.htm).

3.3.4.3 Spawn preparation

Two hundred grams of sorghum grains were washed with clean water (spoilt grains were removed before weighing) and soaked in clean water for eight hours. The grains were steamed for 30 minutes at 1.1kg steam pressure at 121ºC in an autoclave. They were then removed from the autoclave and spread on a cheesecloth. 100g of grains were put into four transparent 300ml glass bottles which were plugged with cotton wool, covered with paper and held in place with rubber bands. The bottles containing the grains were sterilized for one hour at 1.1kg steam pressure at a temperature of 121°C and allowed to cool to room temperature. The bottles were shaken to loosen the grains which had become swollen and stuck together during sterilization. The grains were then inoculated with 7-day-old mycelium of cultures of *P. tuber-regium* from the Volta and Brong Ahafo Regions. They were incubated at room temperature for 10 days at which time the mycelia had fully colonized the substrate.

3.3.4.4 Substrate preparation

a. *Triplochiton scleroxylon* (wawa) sawdust compost

Seventy kilograms of 'wawa' sawdust was mixed with 1% N.P.K fertilizer (25: 15: 5), 10% rice bran and 1% lime (based on dry weight of sawdust). The mixture was then moistened with clean water till the moisture content was about 70%. When this moisture content was attained and a sample was squeezed in the hand no water oozed through the fingers and only some dampness was felt. The mixture was then composted in a pyramidal block heap for a period of 28 days. During the composting (fermentation) the heap of sawdust was turned every four days to avoid anaerobic fermentation in the core section of the heap and to obtain uniform composting.

3.3.4.3 Preparation of other substrates.

i. Plantain (*Musa sapientum*) leaves: Dry plantain leaves hanging down the plant were picked and cut with a cutlass into sizes of approximately 3cm long. The samples were soaked in clean water containing 0.5% $CaCO_3$ for two hours after which they were removed and the excess water allowed to drain off.

ii. Water hyacinth (*Eichhornia crassipes*): Leaves of water hyacinth were harvested and sun dried for 7 days. They were then cut into 3cm long pieces and soaked in clean water containing 0.5% $CaCO_3$ for 2 hours. The excess water was allowed to drain off.

iii. Millet (*Eleusine coracana*) stalk: Dried millet stalks were chopped into 3cm long pieces and soaked in clean water containing 0.5% $CaCO_3$ for 24 hours (due to its toughness). The excess water was allowed to drain off.

3.3.4.4 Bagging of substrates

All prepared substrates were put into heat resistant polypropylene bags of dimension 18cm x 33cm and 0.8mm thickness, and filled with 1kg of substrate (Plate 2). The substrates were then compacted and the mouth of bags pushed through a plastic bottle neck (made of 2cm wide PVC pipe cut into 2.5cm lengths), pulled down and fastened in place with rubber band. Prior to bagging the ‘wawa’ sawdust was mixed with 1% NPK fertilizer (25:15:5) and 0.5% lime (dry weight) .The mixture was then moistened with clean water till the moisture content was about 70%. This moisture level was found to be optimum for *Pleurotus spp*. production (Obodai *et al.,* 2002).

3.3.4.5 Sterilization of substrates

All bagged substrates were sterilized by steaming for three hours in a 200-litre metal drum using an L.P.G gas burner (Plate 3). A hole was made in the centre of the lid with a 10cm nail; this allowed steam out of the drum. A wooden rack of diameter 49cm and 15cm high was placed inside the drum and the drum was filled with water to a level of 13cm. The bags were placed on top of each other except for the last layer which was placed horizontally with a space in the middle; this was to prevent vapour from dripping from the lid into the bag. The water was heated till a steady steam jetted out of the hole in the lid to a height of 10cm. At this point timing was started and heating continued for three hours. After three hours the bags were allowed to cool and later transferred to the inoculation room.

Plate 2: Photograph showing the process of weighing and bagging of substrate (in the foreground).

Plate 3: Setup with the red gas cylinder in the foreground and sterilizing units (green barrels) in the background.

3.3.4.6 Inoculation of substrates

To inoculate the bagged substrates, the spawn bottle was shaken to disperse the spawn grains; the paper and rubber band were removed. The cotton wool plug of the spawn bottle and that of the bags were then removed and approximately twenty grains of sorghum spawn were introduced into each bag under sterile conditions. The cotton wool plugs of the bags and spawn bottles were placed back after the introduction of spawn into the bags. The bags were then shaken to disperse the spawn grains on the substrate.

3.3.4.7 Incubation of inoculated bags

Inoculated bags were placed in an incubation room vertically on wooden shelves where the mycelia were allowed to grow and colonize the bags. The ambient temperature was maintained at 30±2ºC. Measurement of linear growth rate along the sides of the bag (mycelium growth rates) in each substrate was done weekly using a ruler to measure the least and highest mycelial growth level from the top simultaneously and the mean calculated. The number of days the mycelium took to colonize the bag was noted. After complete colonization (spawn run), the bags were left for another 14 days to induce formation of fruitbodies which was noticed by thickening of the mycelial strands.

3.3.4.8 Cropping

After complete colonization, the bags were moved to the cropping house with a temperature range of 30-34ºC and a relative humidity of 70-80%. The bags were arranged on wooden shelves horizontally in the cropping house. A knife was used to cut open the neck of the bags and slits made underneath the neck to allow for easy drainage of excess water. High humidity was maintained by sprinkling water onto the bags from the top row and this in turn got to the bags on the bottom row. Water was also poured on the floor of the cropping house to maintain high relative humidity. Watering of the bags was done twice daily.

3.3.4.9 Harvesting

Mature fruitbodies were identified by their caps rolling up and inwards like the shape of a funnel. They were harvested at this stage by removing them from the sclerotia on which they were attached and subsequently the weights were determined as well as the cap and stipe sizes. The sclerotia were also harvested and their weights recorded.

3.3.4.10 Fruitbody induction from sclerotia

This was done by burying the sclerotia in garden soil. Stimulation of fruitbodies was done by weighing 12kg each of garden soil into three empty plastic bowls of diameter 47cm and 27cm deep and three empty cane baskets of diameter 43cm and 22cm deep. The three bowls and baskets were labeled A, B and C. The garden soil in the bowls and baskets were moistened by mixing each of them with 200ml of tap water. Six pieces of sclerotia with a total weight of 402g were buried at a depth of 6cm below the soil in each of the bowls as well as in the baskets. They were kept in an incubation room at a temperature between 28ºC and 35ºC and watered with 200ml of water twice daily (in the morning and evening).

3.3.4.11 Yield

The yield of mushrooms and sclerotia is influenced not only by environmental conditions but also by the nature and nutrient composition of the substrate used in their cultivation. The total number of fruitbodies and sclerotia produced per bag, bowl or basket and their weights were measured. The Biological Efficiency (B.E) was calculated using the dry matter content of each substrate at bagging with the formula:

$$\textbf{B.E} = \frac{\textbf{Fresh weight of mushroom}}{\textbf{Dry weight of substrate at bagging}} \textbf{ X 100\%}$$

(Chang, 1984).

3.3.5 Chemical analyses of mushroom substrates and fruitbodies and sclerotia.

3.3.5.1 Chemical analyses of substrates

Chemical analysis of all the substrates (sawdust, water hyacinth, millet stalks and plantain leaves) used in the growth of *P. tuber-regium* before and after use was carried out quantitatively by estimation of ash, moisture, protein, cellulose, hemicellulose, lignin, crude fibre, carbohydrate, pH, and organic matter.

3.3.5.1.1 Ash determination

Ash content was estimated by the method outlined by AOAC 923.03 (2000). 5g of the sample was weighed into a pre-heated crucible (at 550 ± 10°C for 20 minutes and cooled in a desiccator to room temperature) and ignited in a muffle furnace at 550º ±10°C for seven hours. The furnace temperature was then allowed to drop to 250°C, and the crucible was transferred into a desiccator and weighed soon after reaching room temperature. The ash content was calculated as follows:

$$\textbf{\% Ash} = \frac{\textbf{(Weight of crucible+Ash)-(Weight of empty crucible)}}{\textbf{(Weight of crucible + Sample) – (Weight of empty crucible)}} \textbf{ x 100}$$

3.3.5.1.2 Moisture determination

Moisture was determined by the air oven method in accordance with AOAC 925.10 (1990). 5g samples were weighed into pre-heated metal dishes. The dishes with their contents were dried for four hours in an oven and maintained at 103ºC (timing began when oven temperature was actually 103ºC). The dishes were covered while still in the oven and then transferred into a desiccator to cool. The weights were taken at room temperature.

$$\textbf{Moisture(\%w/w)} = \frac{\textbf{\{(Weight of dish+Fresh sample)-(Weight of dish+Dried sample)\}}}{\textbf{\{(Weight of dish+Fresh sample)-(Weight of dish)\}}}$$

3.3.5.1.3 Protein determination

The Kjedahl method was used in the determination of protein. The samples were prepared in duplicates. They were ground to a homogenous component by using a laboratory mill. 0.5g of the homogenous samples were weighed onto pieces of filter paper and placed into 250ml digestion tubes. 0.1g ammonium sulphate was put into each digestion tube and 80ml of water added for Tecator Keltec systems. For the control samples 0.2g lysine dihydrochloride was put into weighing boats and transferred into each digestion tube. To blanks, control samples and test samples, one kjeltab Cu 3.5 and 15ml concentrated tetraoxosulphate (vi) acid were added and shaken gently to 'wet' the samples with the acid. When they were digested and turned greenish-blue, 25ml receiver solution (4% Boric acid) was added to the receiver flasks. Three drops of screened methyl indicator were added to the receiver solution. The cooled digest was diluted with 80ml of water; 80ml 0.1M NaOH was added to the diluted digest and the reaction was allowed to settle. The distillate was titrated with 0.1M HCl until a pink end-point was achieved and the volume of acid noted. The protein content was determined as follows:

$$\textbf{\% Protein} = \frac{\textbf{(T-B) x N x 14.007}}{\textbf{10 x Weight of sample}}$$

where T = Titration volume of sample

B = Titration volume of blank

N = Normality of acid (to 4 places of decimal).

3.3.5.1.4 Crude fibre determination

Three grams of the sample was transferred to an extraction apparatus and extracted with petroleum spirit. The extracted sample was air dried and transferred into a dry 1000ml conical flask. 200ml of 0.255N sulphuric acid was added to the sample in the conical flask and it was brought to boiling point for 30 minutes, rotating the flask every two minutes to mix the contents and remove particles from the sides. A Buchner funnel was then fitted with a perforated plate by adjusting a filter paper to cover the holes in the plate serving as a support for the filter paper. Boiling water was poured into the funnel and drained by suction. At the end of 30 minutes of boiling, the acid was allowed to stand for one minute and then poured into a shallow layer of hot water and under gentle suction. The insoluble matter was washed with boiling water into the original flask by means of a wash bottle containing 200ml of 0.313N sodium hydroxide solution and brought to boiling point. After boiling for 30 minutes it was allowed to stand for one minute and filtered through a filter paper. The insoluble matter was transferred to a filter paper and dried at 100ºC to a constant weight. The paper and content were incinerated with a dull red heat.

Crude fibre = (Weight of paper + Insoluble material) - Weight of ash.

3.3.5.1.5 Cellulose, hemicellulose and lignin determination

Cellulose and lignin were determined by the Acid Detergent Fibre (ADF) method of Van Soest and Robertson (1985); 5g of the sample was weighed in duplicate into a 600ml Berzelius beaker for refluxing. 100ml of cold acid detergent solution was then added. The solution was gently heated to prevent it from foaming. Refluxing lasted for 60 minutes. A crucible was weighed and the solution was filtered on it using light suction, washed several times with hot water of 100ºC. It was then washed by acetone, sucked with light suction and dried in an oven for 12 hours at 170ºC. The sample was then cooled in a desiccator and the weight recorded. The sample in the crucible was covered with cooled 72% sulphuric acid and stirred to break up lumps; this was left for 3 hours. It was stirred hourly as the acid drained away. In 3 hours time it was filtered using the light suction and washed with hot water until it was free from acid. The contents were left to dry, cooled in a desiccator and the weight taken. The crucible was ignited in a furnace at 550ºC for 3 hours after which it was cooled in a desiccator and the weight taken.

$$\%\text{Cellulose} = \frac{\text{Weight of residue\&crucible} - \text{weight of ash\&crucible}}{\text{Sample weight}} \times 100$$

(Faithful, 2002)

Neutral Detergent solution was used for hemicellulose determination. This procedure was carried out as in the Acid Detergent method. The difference between the neutral detergent fibre and acid detergent fibre gave the hemicellulose content of the sample:

Hemicellulose = (NDF)-(ADF)

Lignin= (ADF)-(CRUDE FIBRE)

Where NDF = Neutral detergent fibre

ADF = Acid detergent fibre

3.3.5.1.6 Carbohydrate determination

Carbohydrate content was estimated by using the relationship:

100 - (5% Moisture + Ash +% Crude protein +% Fat +% Crude fibre)

3.3.5.1.7 pH determination

Five grams of each substrate was added to equal volume of distilled water and homogenized. An electric pH meter (pH M.92) was used to determine the pH.

3.3.5.1.8 Organic matter determination

Organic matter was determined by the 'loss on ignition' procedure, where everything that is combustible is considered as organic matter.

% Organic matter = 100 - % Ash content

3.3.5.2 Chemical Analysis of Fruitbody and Sclerotium

Moisture, ash, crude protein and carbohydrate content of the fruitbody and sclerotium were determined using the same methods as those used for the substrates in 3.3.5.1. The determination of fat is described below.

3.3.5.2.1 Fat determination

Analysis was performed in accordance with AOAC 920.39C (2000), in duplicates and a variance of ± 0.2% allowed. 5g sample was extracted on a small paper in a funnel with 20ml portion of distilled water prior to drying for ether extraction. 5g of the homogenous sample was put onto a filter paper. The filter paper was folded and placed into a thimble and stuffed with grease-free cotton. Grease-free flask and Sohxlet apparatus were used for the extraction. The empty boiling flask was weighed and the weight noted. The thimble was placed in the extractor and 240ml petroleum ether was added to the flask. The solvent was distilled and evaporated off the flask containing extracted fat. The fat was dried in the oven at 103°C for one hour. It was then cooled in a desiccator and weighed. A blank was carried out using the same procedure as in the fat determination.

$$\textbf{\% Fat} = \frac{\textbf{(Weight of flask+Fat - Weight of empty flask)-Blank}}{\textbf{Weight of sample}} \times 100$$

Blank = Weight of flask without sample after extraction – Weight of empty flask

CHAPTER FOUR

RESULTS

4.1 RESPONSES FROM QUESTIONNAIRES

4.1.1 Background of respondents

4.1.1.1 Gender Distribution.

Exactly 95.2% and 99% of respondents from the Brong Ahafo Region and Volta Region respectively had knowledge of *Pleurotus tuber-regium.*

In the Brong Ahafo Region 36% who had knowledge of *P. tuber-regium* were males whilst 64% were females. Forty nine per cent of the respondents from the Volta Region who had knowledge of *P. tuber-regium* were males while 51% were females.

4.1.1.2 Age of respondents with respect to knowledge and uses of *Pleurotus tuber-regium.*

The relationship between age of respondents and knowledge of *P. tuber-regium* in the Brong Ahafo and Volta Regions is presented in Fig.3. There is no clear relationship between the age of respondents and knowledge of *P. tuber-regium.* While the highest number of respondents in the Brong Ahafo Region who have knowledge of *P. tuber-regium* (29%) were in the 21-30 age group, the highest number of respondents with knowledge of it in the Volta Region were in the 61-70 age bracket. The age group with the least knowledge of *P. tuber-regium* in the Brong Ahafo Region (2.5%) was the 70+ age group.

The results show that with the respondents from the Brong Ahafo Region, knowledge about Pleurotus tuber-regium decreases with increase in age except with 61-70 years whereas the opposite is true for the respondents from the Volta Region.

With regards to knowledge on the uses of *P. tuber-regium* and age of respondents, those with the highest knowledge on the uses of *P. tuber-regium* in the Brong Ahafo Region (24%) were in the 21-30 age group, while those with the highest knowledge on its uses in the Volta Region (27%) were in the 61-70 age group (Fig.4). Those with the least knowledge on the uses of *P. tuber-regium* in the Brong Ahafo Region (5%) were in the 70+ age group, while those with the least knowledge in the Volta Region (4%) were from the 21-30 age groups.

There is a correlation between the knowledge and the use of *Pleurotus tuber –regium*. Those who have knowledge about it, use it more than those with little or no knowledge.

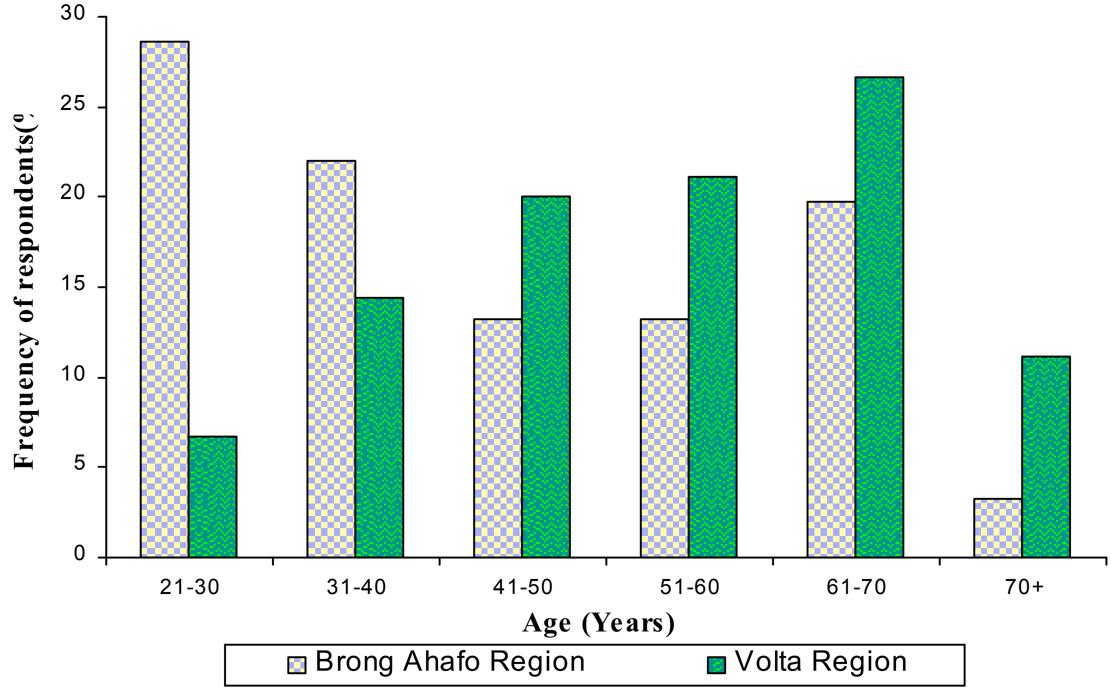

Fig. 3 Comparative age structure of respondents to questionnaire from the Brong Ahafo and the Volta Regions.

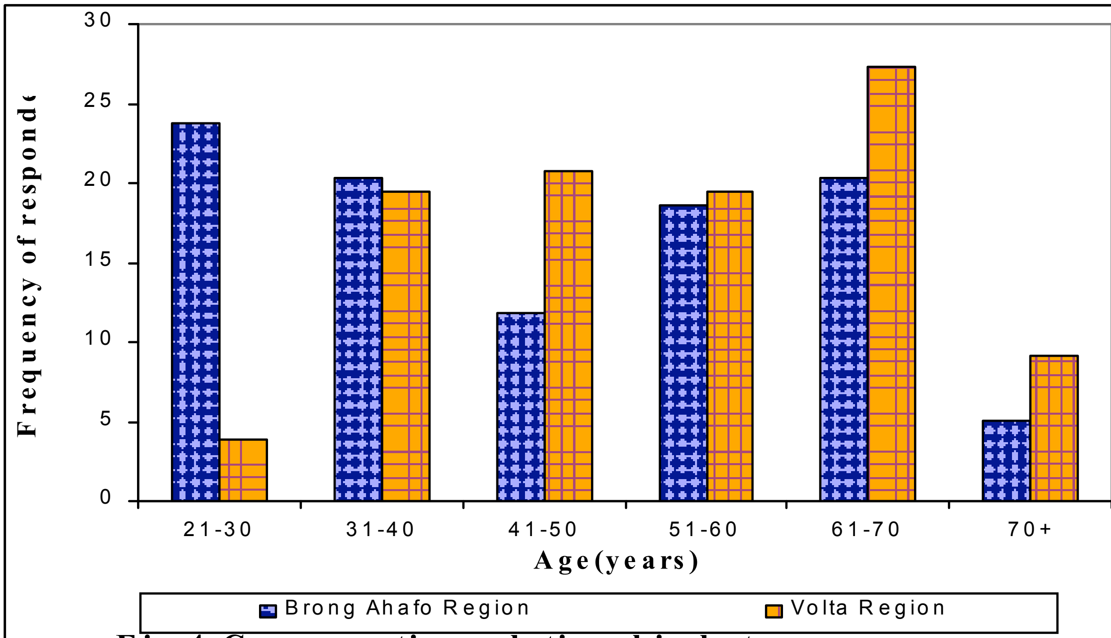

Fig.4 Comparative relationship between age and uses of *Pleurotus tuber-regium* in the Brong Ahafo and Volta Regions of Ghana

4.1.1.3 Educational background of respondents.

Figs.5a and 5b present the educational background of respondents in the Volta and Brong Ahafo Regions respectively. Ten percent of the respondents in the Volta Region did not have any formal education (illiterates) (Fig.5a). The majority of respondents in the Region (78%) had either Primary or JSS education. Six per cent had Secondary education; 3% had Post secondary education and 3% had Tertiary education. In the Brong Ahafo Region, 47% of the respondents did not have any formal education (illiterates). Forty seven per cent had Primary or JSS education, 3% had Secondary education, 1% had Post secondary education and 2% had Tertiary education (Fig.5b)

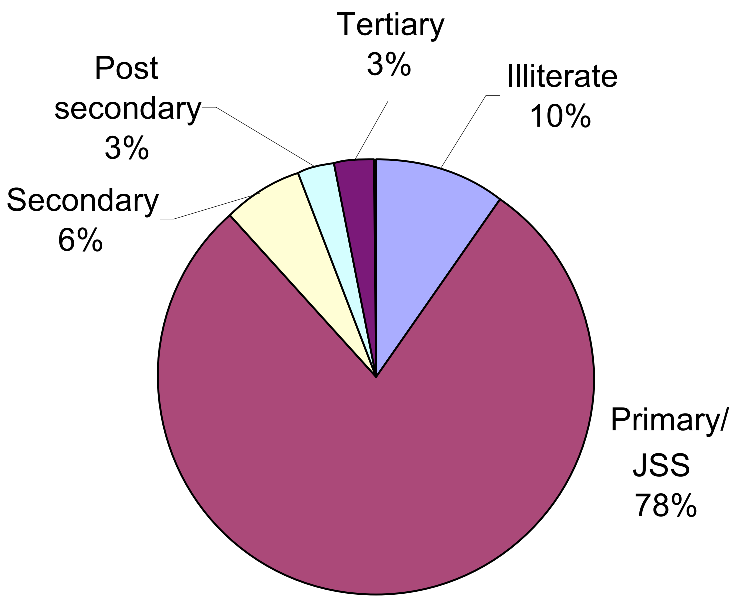

Fig.5a Educational level of respondents in the Volta Region

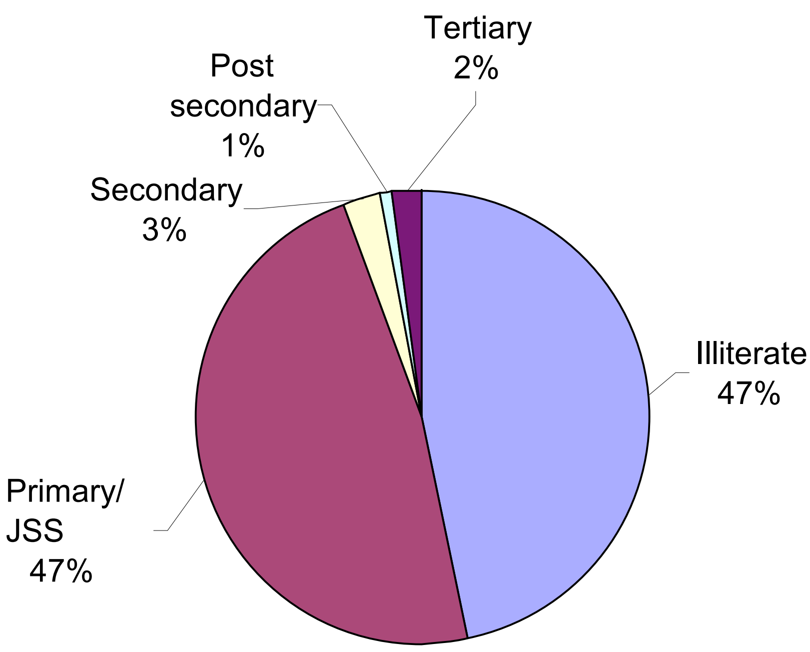

Fig.5b Educational level of respondents in the Brong Ahafo Region

4.1.2 Relationship between gender, age, educational background, origin of respondents and their knowledge of *Pleurotus tuber-regium.*

Figs.6a and 6b show relationships between educational background of respondents and knowledge of *P. tuber-regium* in the Brong Ahafo and Volta Regions respectively. The results showed that the frequency of respondents from the BAR who had knowledge about *P. tuber-regium* decrease as the level of education of the respondents rise. The highest frequency was therefore recorded with the illiterates with 50%, followed by primary/JSS with 47% and then 2% for those with secondary education and finally those with tertiary education recording 1% in that order.

The trend was similar for the respondents from the Volta Region except that here the highest was the primary/JSS with 83% followed by illiterate with 7%, 5% for Secondary; 3% for post secondary and 2% for the tertiary. The results therefore show that in both regions the respondents who were either illiterates or had education up to the JSS had better knowledge of *Pleurotus tuber-regium* than the others.

In both Regions natives had knowledge of *P. tuber-regium* than settlers. Seventy eight percent of respondents from the Volta Region who knew about *P. tuber-regium* were native and 25% were settlers; in the Brong Ahafo Region, 61% were natives while 39% were settlers.

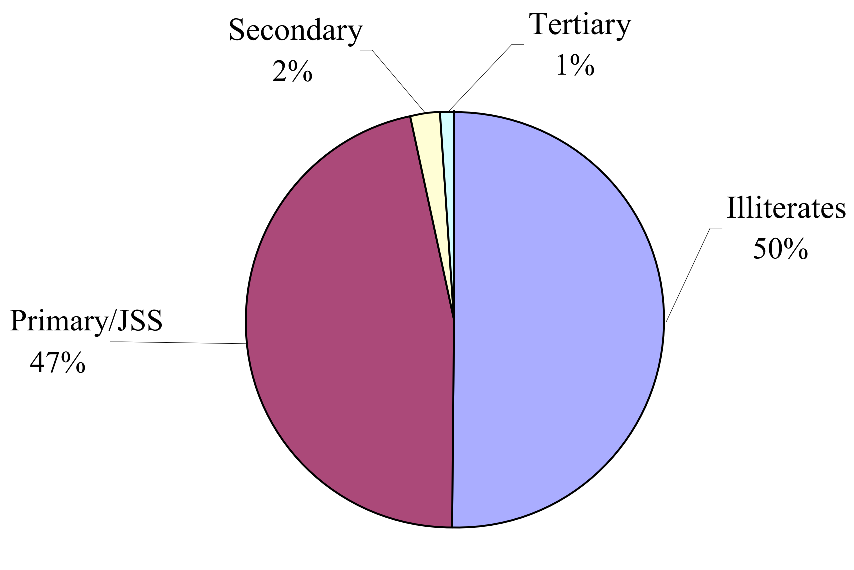

Fig. 6a Relationship between educational background of respondents and knowledge of *Pleurotus tuber-regium* in the Brong Ahafo Region.

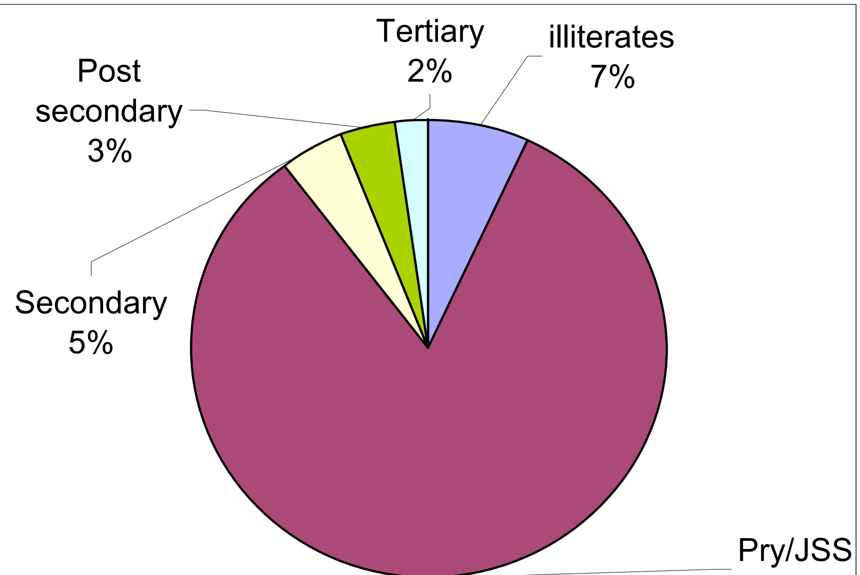

Fig. 6b Relationship between educational background of respondents and knowledge of *Pleurotus tuber-regium* in the Volta Region

4.1.3 Diversity of mushrooms found in the Volta and Brong Ahafo Regions.

The different types of mushrooms known to be available in the Volta and Brong Ahafo Regions (apart from *Pleurotus tuber-regium)* according to respondents are shown in Tables 3a and 3b. A total of 10 different mushrooms (apart from *P. tuber-regium)* are known to the respondents from the Hohoe District of the Volta Region, while a total of 19 are known to the respondents from the Techiman District of the Brong Ahafo Region. Data obtained indicate that there is a wider diversity of mushrooms in the Brong Ahafo Region than in the Volta Region.

Table 4 shows the local names given to *P. tuber-regium* in the Volta and Brong Ahafo Regions. The local names are descriptive of the shape, habitat, use, etc. of the fruitbody or sclerotia. For example the local name "anyigbadzi" given to *P. tuber-regium* in the Volta Region means the earth's heart, while "Dimitigyamegya" given to it in the Brong Ahafo Region means eat my head and leave my stalk. *P. tuber-regium* is known by six different names in the Volta Region and by only two in the Brong Ahafo Region.

Table 3a: List of other mushrooms known in the Volta Region

LOCAL NAME	ENGLISH/SCIENTIFIC NAME
Nyihlo	*Termitomyces sp.*
Kohlo	*Termitomyces sp.*
Akayi	Not known
Denkpe	*Volvariella volvacea*
Tokpetokpe/ Konumadu	*Coprinus sp.*
Atsikpatsake	*Ganoderma sp.*
Aforti	*Termitomyces sp.*
Wudzaxe	Not known
Asiandehlo	Not known
Atinhlo	*Ganoderma sp.*

Table 3b: List of other mushrooms known in the Brong Ahafo Region

LOCAL NAME	ENGLISH/SCIENTIFIC NAME
Nkankuma	*Termitomyces robustus*
Sibre	*Termitomyces schimperi*
Asasee	*Coprinus disseminatus*
Mpanpinaa	Not Known
Nsasua	*Termitomyces globulus*
Tsitsiritsi	*Termtitomyces clypeatus*
Yirekyi	Not known
Ediabronsa	Not Known
Kapowa	Not known
Bewiawi	*Cantharellus aurantiacus* (*Hygrophoropsis aurantiacus*) (False cantharelle)
Elitsi	Not known
Potrogoma	Not known
Asuntokro	*Auricularia auricular- judae*
Asuadine	Not known
Trotrowa	Not Known
Onu	*Termitomyces schimperi*
Ahinmre	*Volvariella bombycina*
Miretumtum	*Coprinus disseminatus*
Ketim	Not known

Table 4: Local names given to *Pleurotus tuber-regium* in the Volta and Brong Ahafo Regions.

VOLTA REGION	BRONG AHAFO REGION
1. Anyigbadodo	1. Fuo
2. Anyigbadzi	2. Dimitigyamegya
3. Anyigbatu	
4. Anyigbakor	
5. Dzigbe	
6. Anyigbahlo	

4.1.4 Source of knowledge on *Pleurotus tuber-regium* among respondents in the Volta and Brong Ahafo Regions

Figs.7a and 7b represent the source of knowledge on *Pleurotus tuber-regium* as indicated by respondents of the Volta and Brong Ahafo Regions. Ninety one per cent of the respondents from the Volta Region (Fig.7a) had knowledge on *P. tuber-regium* passed on to them by their parents; one per cent had the knowledge through herbalists and 8% had the knowledge on *P. tuber-regium* through other sources they could not remember. Sixty seven per cent of the respondents from the Brong Ahafo Region (Fig.7b) intimated that the knowledge was passed on to them by their parents. One per cent had the knowledge passed on to them by herbalists; 2% indicated that they had the knowledge through friends while 30% intimated that they had the knowledge through other sources they could not remember.

In both the Volta and Brong Ahafo Regions the highest percentage of respondents (91% and 67% respectively) had knowledge on *P. tuber-regium* passed on to them by their parents.

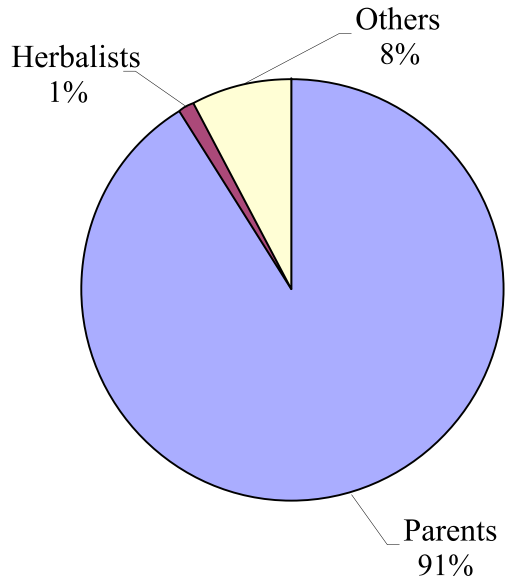

Fig.7a Source of knowledge on *Pleurotus tuber-regium* as indicated by respondents of the Volta Region.

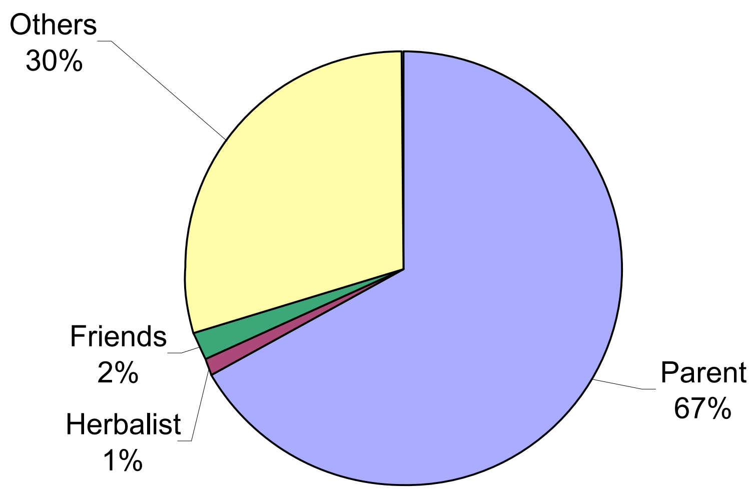

Fig.7b Source of knowledge on *Pleurotus tuber-regium* as indicated by respondents of the Brong Ahafo Region.

4.1.4.1 Knowledge on the medicinal and nutritional values of *Pleurotus tuber-regium* among respondents in the Volta and Brong Ahafo Regions.

Ninety eight per cent of the respondents from the Volta Region had knowledge only on the medicinal values of *Pleurotus tuber-regium*; 1% had knowledge on its nutritional values only while another 1% had knowledge on both its medicinal and nutritional values. Eighty one per cent of the respondents from the Brong Ahafo Region had knowledge on the medicinal values only of *P. tuber-regium*; 14% had knowledge on the nutritional values only and 5% had knowledge on both its medicinal and nutritional values. Respondents who claimed the sclerotia of *P. tuber-regium* had nutritional value said the human body derived energy from it when eaten. It was however noted that they did not eat the fruitbody.

4.1.4.2 Knowledge on specific ailments *Pleurotus tuber-regium* could treat.

Forty three per cent of the respondents from the Volta Region did not know of any ailment which *Pleurotus tuber-regium* could cure. Twenty per cent of the respondents said it was used to cure heartburn; 1% said it was used for the cure of stomach ulcer. Two per cent said it cured waist pain; 1% claimed it is used for curing skin infection. Another 1% of the respondents said it was for the cure of asthma; 4% stated it was used to cure underweight in babies, while 12% said it was for the cure of swollen body and 16% knew *P. tuber-regium* could cure more than one ailment (Fig.8a).

In the Brong Ahafo Region, 55% had no knowledge of the ailments *P. tuber-regium* could cure; 1% said it was used for the cure of heartburn, whilst 6% indicated that it was used for the cure of swollen body (Fig.8b). Another 5% said *P. tuber-regium* was used to cure underweight in babies; 1% indicated it was for the cure of asthma, 17% intimated it was used to cure waist pain, 4% said it was for the cure of high blood pressure. The remaining 11% said it could cure more than one of the above listed ailments.

Tables 5a and 5b show the various ailments *P. tuber-regium* could cure and the mode of applications of *P. tuber-regium* as gathered from respondents from both the Volta and Brong Ahafo Regions. It was observed that despite the fact that both Regions might use the sclerotia of *P. tuber-regium* for the same ailments, their mode of application was different; for example in the case of convulsion, people in the Brong Ahafo Region grind the sclerotium and put in soup or stew to be eaten or it is just smeared on the body. In the Volta Region however, the sclerotium is just ground and smeared on the body of the individual for the treatment of convulsion. Also in the Brong Ahafo Region *P. tuber-regium* is used in the treatment of breast cancer; this information was not available in the Volta Region.

Table 5a: Therapeutic applications of *Pleurotus tuber-regium* for herbal treatment in the Volta Region.

Ailment	Application of sclerotia of *Pleurotus tuber-regium*
1. Heart pains	Grind, mix with honey and drink/grind and put in alcohol and drink/cut into pieces and chew with ginger.
2. Boils	Grind and smear on the affected part/ grind and mix with palm oil and place on boil.
3. Measles	Grind and smear on the body alone or mixed with shear butter.
4. Stomach ulcer	Grind and put in alcohol before drinking.
5. High blood pressure	Grind and mix with salt before drinking.
6. Sore throat	Chew.
7. Waist pain	Grind and insert in anus.
8. Skin infection	Grind and smear on body/ cut into pieces, boil in water and drink.
9. Asthma	Grind and mix with honey/ cook with crab and drink.
10. Underweight in children	Grind and smear on body.
11. Convulsion	Grind and smear on the body.

Table 5b: Therapeutic applications of *Pleurotus tuber-regium* for herbal treatment in the Brong Ahafo Region.

Ailment	Application of sclerotia of *Pleurotus tuber-regium.*
1. Stomach ulcer	Grind with herbs, put in water and drink.
2. Piles	Grind together with cocoyam leaves and insert in anus.
3. Headaches	Mix with other herbs in water and drink.
4. Skin disease	Grind and mash with water and smear on body.
5. Breast cancer	Grind and smear all over breast.
6. Elephantiasis	Grind and mix with other herbs, then smear on affected parts.
7. Rheumatic pain	Grind, mash with water and smear at joints.
8. Loose stool in children	Grind, mash with salt and water and drink.
9. Under weight in babies	Grind and mix with white clay and smear all over body.
10. Convulsion	Grind and use in soups or stew/grind in water and smear on body.
11. Lack of vigour and strength	Put whole in bathing water.
12. Hypertension	Grind and mix with shear butter and smear over body.

Note: This information was given by most of the elderly respondents.

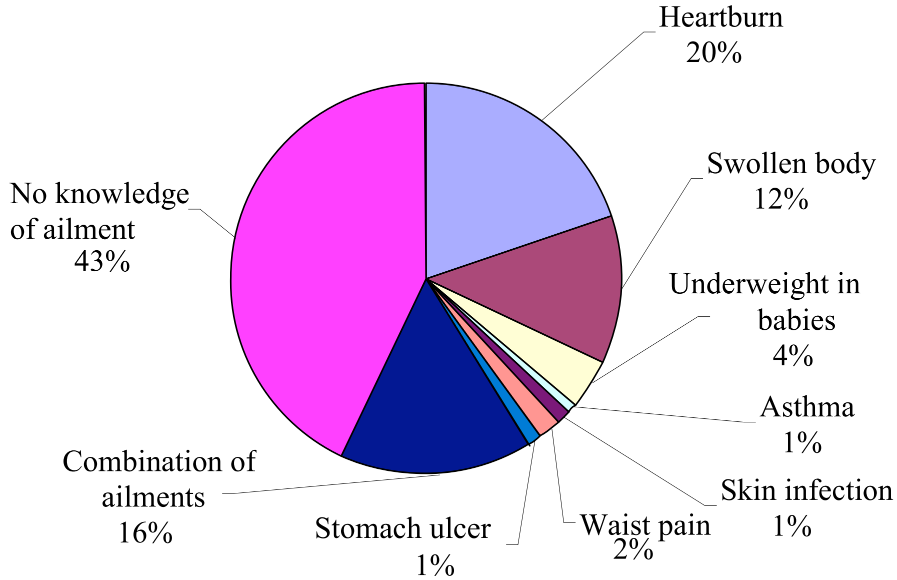

Fig.8a Respondents' knowledge of specific ailments treated by *Pleurotus tuber-regium* in the Volta Region.

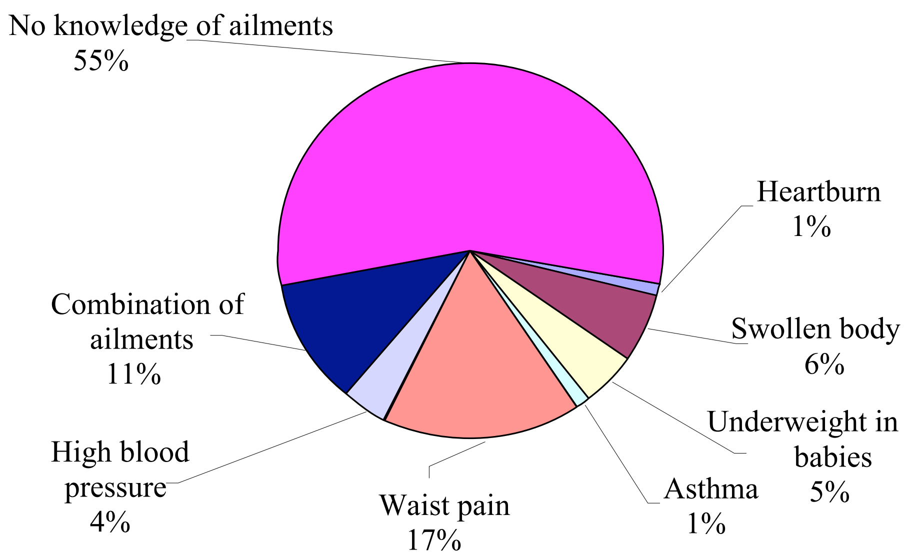

Fig.8b Respondents' knowledge of specific ailments treated by *Pleurotus tuber- regium* in the Brong Ahafo Region.

4.1.4.3 Knowledge on cultivation and preservation methods of *Pleurotus tuber-regium.*

Respondents from both the Volta and Brong Ahafo Regions claimed they do not have any knowledge on the commercial cultivation of *Pleurotus tuber-regium* by man in their regions.

Respondents from both Regions said sclerotia of *P. tuber-regium* were preserved by the following methods: Sun drying the whole sclerotium, peeling off the outer covering and sun drying, covering the sclerotium in polyethylene bags and storing in cool dry place in a room or on trees on the farm.

4.1.4.4 Localities, substrates and seasons in which *Pleurotus tuber-regium* is found growing in the wild.

Figs.9a and 9b show the localities where *Pleurotus tuber-regium* was found in the Volta and Brong Ahafo Regions. Seventy six per cent of the respondents in the Volta Region (Fig.9a) found *P. tuber-regium* growing in abandoned farmlands. Six per cent said they found it in forests, and 18% could not remember exactly where it was found. Among the respondents from the Brong Ahafo Region (Fig.9b), 56% found *P. tuber-regium* in abandoned farmlands. Eighteen per cent found it in forests, while two per cent found it in open fields; 24% said they could not remember where it was found.

Majority of respondents in the Volta Region (96%) found *P. tuber-regium* growing on soil in the wild, while four per cent of them found it growing on either soil or decaying tree stumps. In the Brong Ahafo Region, 81% of the respondents found *P. tuber-regium* growing on soil in the wild; Four per cent found it growing on decaying tree stumps and 15% found the fungus growing on either soil or decaying tree stumps.

Majority (96%) of the respondents in the Volta Region found *P. tuber-regium* growing during the rainy season while four per cent said this mushroom was found both in the rainy and dry seasons. In the Brong Ahafo Region, 96% of the respondents found *P. tuber-regium* during the rainy season; two per cent found it during the dry season while another two per cent said the fungus was found either during the rainy or dry season.

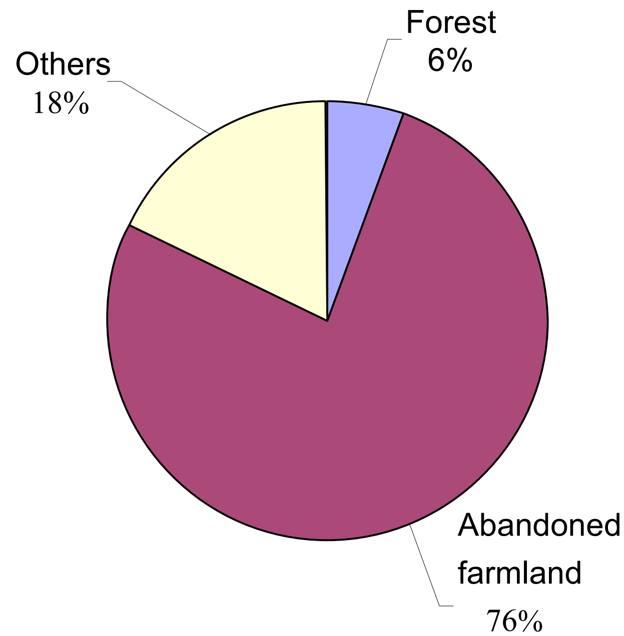

Fig.9a Localities where *Pleurotus tuber-regium* was found growing in the Volta Region.

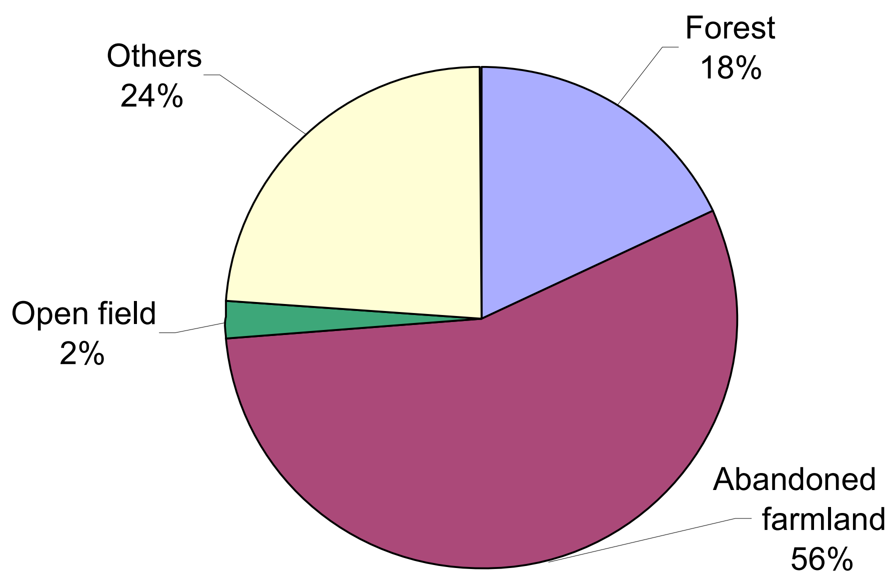

Fig.9b Localities where *Pleurotus tuber-regium* was found growing in the Brong Ahafo Region.

4.2 VEGETATIVE GROWTH OF MYCELIUM OF *PLEUROTUS TUBER-REGIUM* ON SIX DIFFERENT MEDIA.

4.2.1 Mycelia growth of *Pleurotus tuber-regium* from the Brong Ahafo and Volta Regions on six different media (Potato Dextrose Agar, Millet Dextrose Agar, Sorghum Dextrose Agar, Wheat Dextrose Agar, Rice Dextrose Agar and Carrot Dextrose Agar) was assessed at 25ºC, 30ºC and 35ºC.

The best growth rate of mycelia of *P. tuber-regium* from the Brong Ahafo Region was similar on Wheat Dextrose Agar (WDA) and Potato Dextrose Agar (PDA). On the Wheat Dextrose Agar (WDA) the mycelium covered a diameter of 3.2cm on day one; growth continued to increase steadily and fully covered the medium (9.0cm) in six days. For the PDA, growth on the first day covered a diameter of 3.0cm and continued to increase till the sixth day when the mycelium covered the media (9.0cm) (Fig.10a). With *P. tuber-regium* from the Brong Ahafo Region, the media preference was WDA or PDA, followed by Millet Dextrose Agar (MDA), Sorghum Dextrose Agar (SDA), and Rice Dextrose Agar (RDA) (Figs.10a and 10b).

P. tuber-regium from the Volta Region grew best on WDA followed by PDA, MDA, RDA or SDA. CDA supported the poorest growth.

Growth of *P. tuber-regium* from the Volta and Brong Ahafo Regions on different media at 30ºC are presented in Figs.11a and 11b respectively. *P. tuber-regium* from the Volta Region grew best on WDA followed by PDA; CDA and RDA supported the poorest growth. At 35ºC *P. tuber-regium* from both the Volta and Brong Ahafo Regions grew fastest on WDA and PDA (Fig.12a and 12b). This was followed by MDA, SDA in that order. Again CDA and RDA gave the poorest growth.

Single factor analysis of variance of the mycelia growth on the different agar media and the growth rate on the different media at 95% confidence level for both strains showed a significant difference in the results obtained for the rate of radial growth of mycelia on Wheat Dextrose Agar and Potato Dextrose Agar from the other media. This showed a P-value of 1.83E-35. The best medium at 30ºC was Wheat Dextrose Agar, whilst the medium that supported the least growth was Carrot Dextrose Agar. Both strains showed a significant difference in the results obtained and had a P-value 8.72E-26. Mycelial growth at 35ºC showed a significant difference with a P-value of 3.31E-25. Figs13a and 13b compares the mycelia growth of *P. tuber-regium* from Brong Ahafo and Volta Regions on Potato Dextrose Agar (PDA).

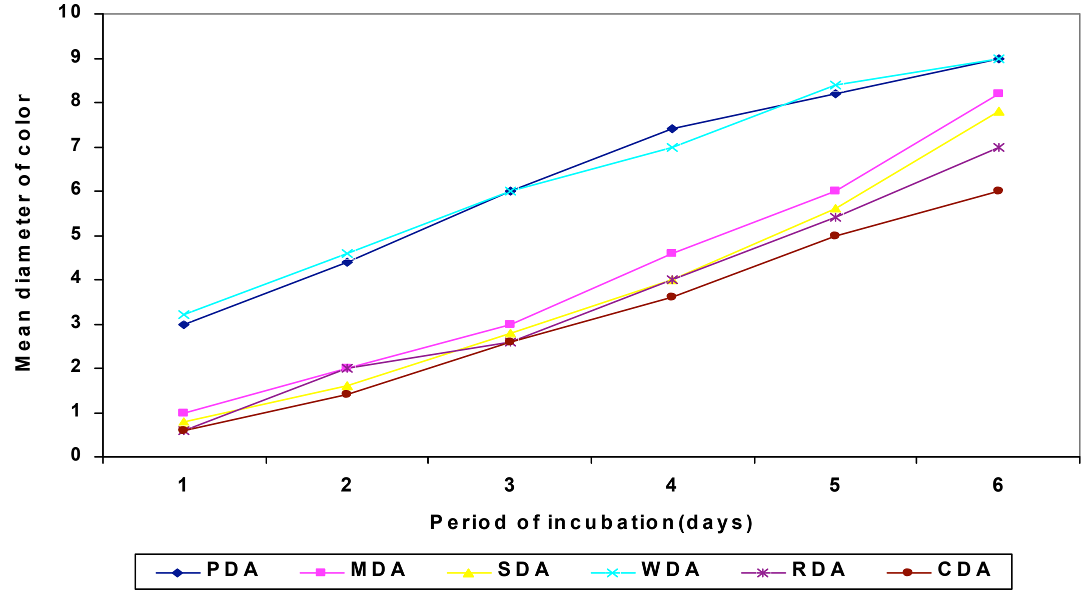

Fig.10a Radial growth of *Pleurotus tuber-regium* from Brong Ahafo Region on different media incubated at 25°C

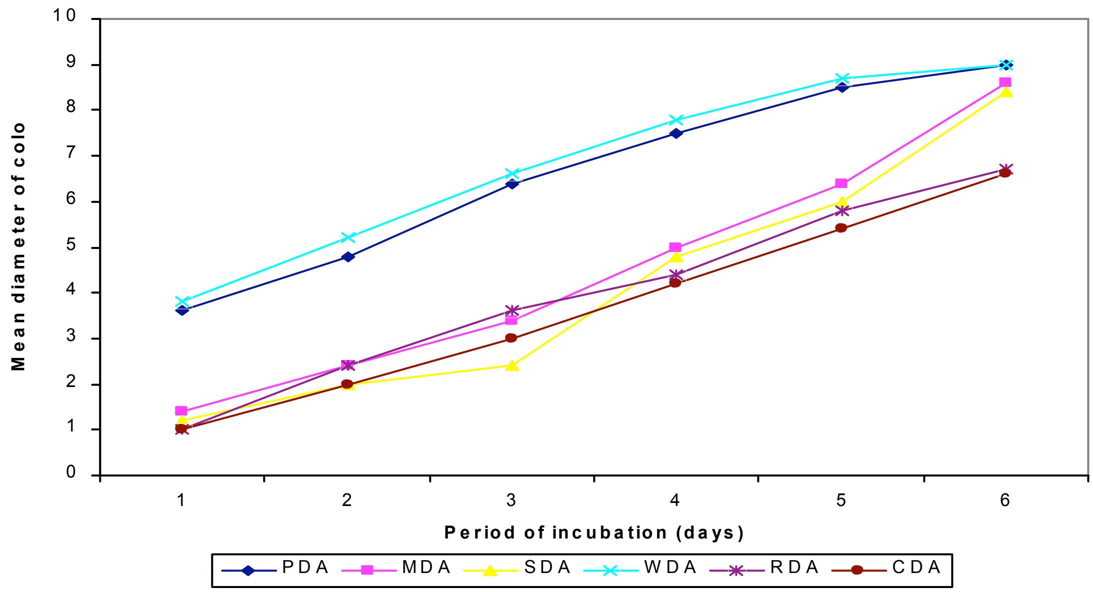

Fig. 10b Radial growth of *Pleurotus tuber-regium* from Volta Region on different media incubated at 25°C

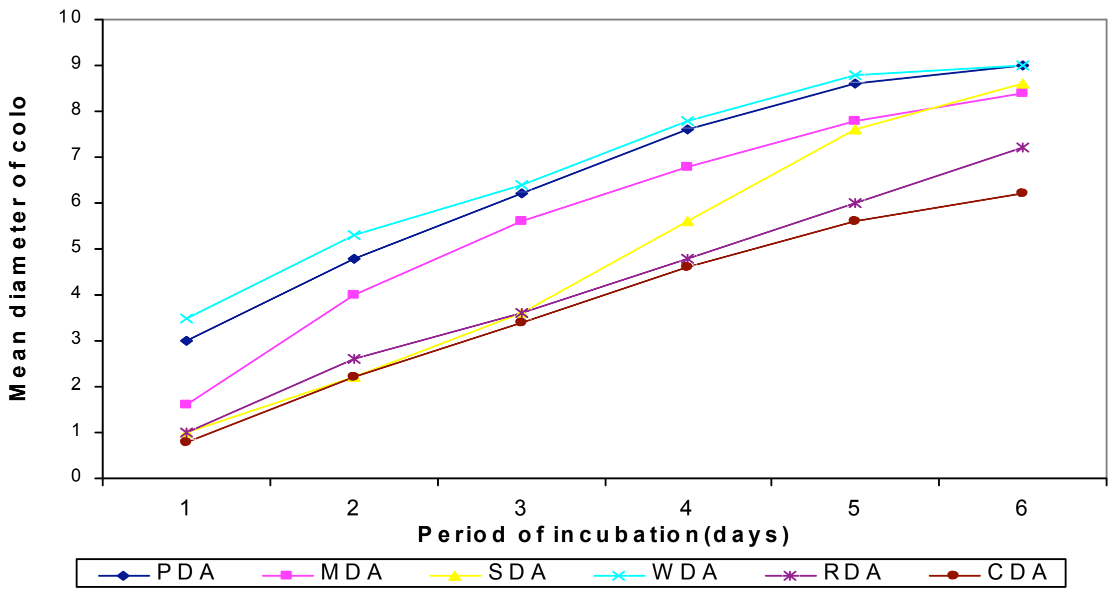

Fig.11a Radial growth of *Pleurotus tuber-regium* from Brong Ahafo Region on different media incubated at 30°C

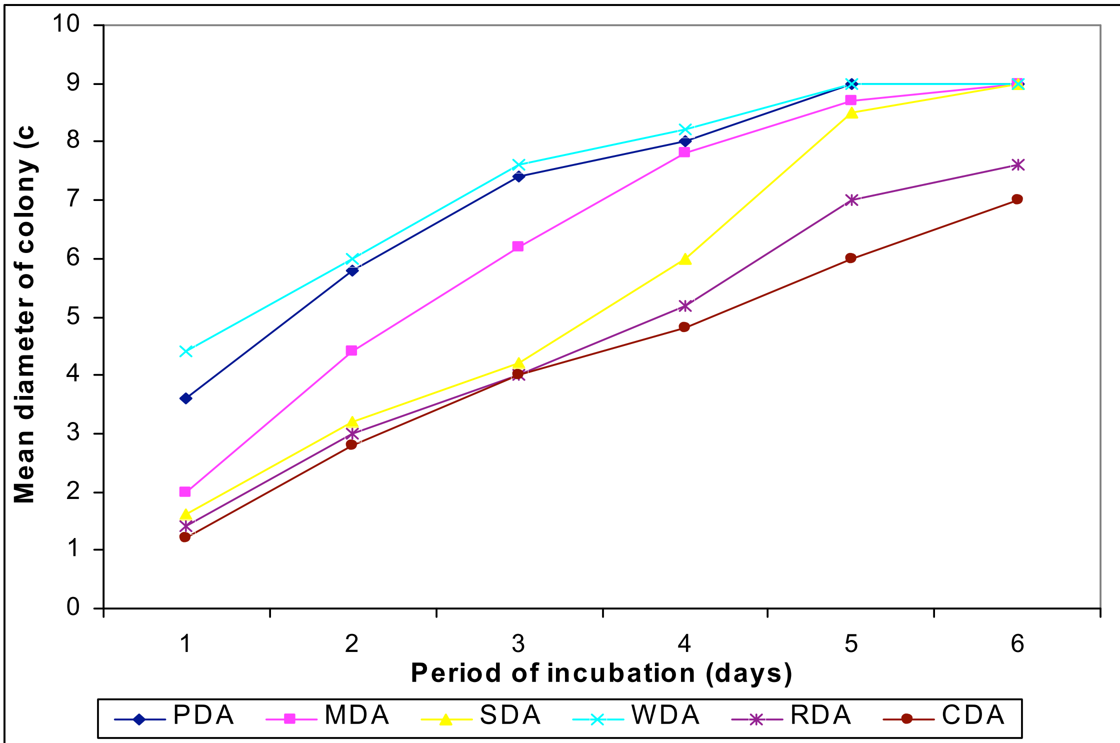

Fig.11b Radial growth of *Pleurotus tuber-regium* from the Volta Region on different media incubated at 30°C

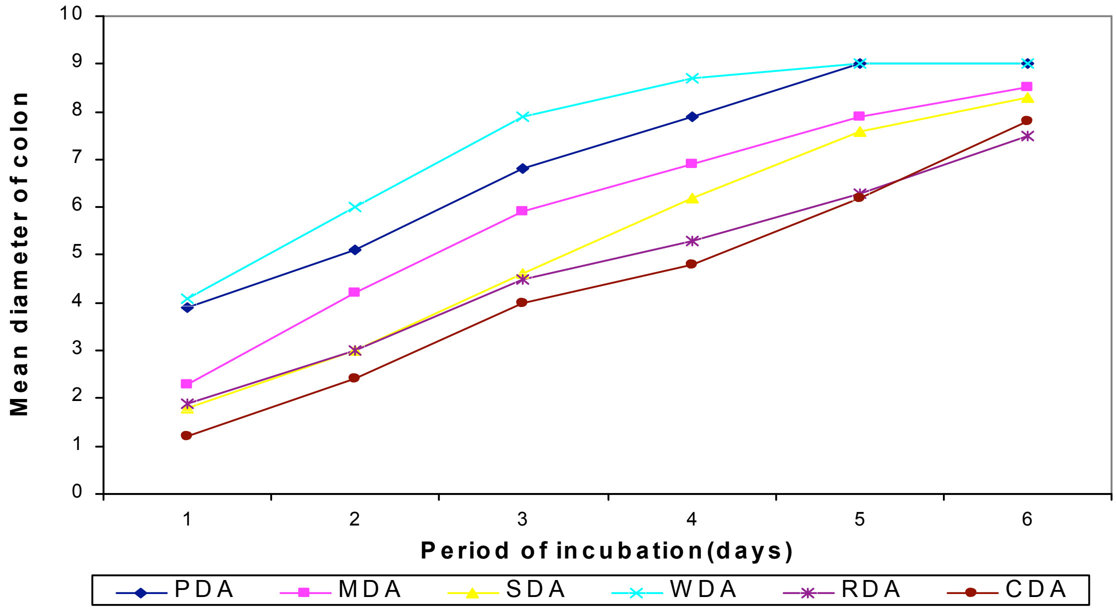

Fig.12a Radial growth of *Pleurotus tuber-regium* from Brong Ahafo Region on different media incubated at 35ºC

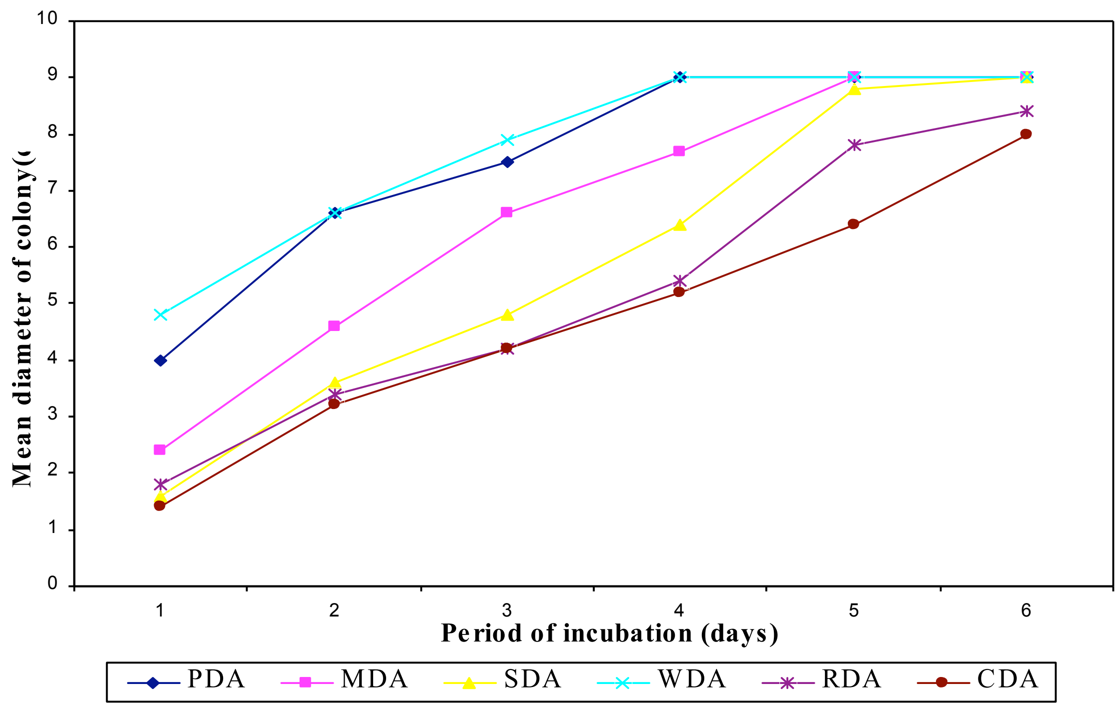

Fig.12b Radial growth of *Pleurotus tuber-regium* from the Volta Region on different media incubated at 35ºC

4.2.2 Mycelia growth of *Pleurotus tuber-regium* from the Volta and Brong Ahafo Regions on Potato Dextrose Agar at 25ºC, 30ºC, 35ºC.

Mycelial radial growth rate of *P. tuber-regium* increased with increasing temperature on PDA is shown on Figs.13a and 13b. At 35ºC *P. tuber-regium* from both Brong Ahafo and Volta Regions grew faster than at 25ºC and 30ºC. Also the strain from the Volta Region colonized the media in a shorter incubation period of four days at 35ºC than that from the Brong Ahafo Region which took five days to colonize the same medium at 35ºC.

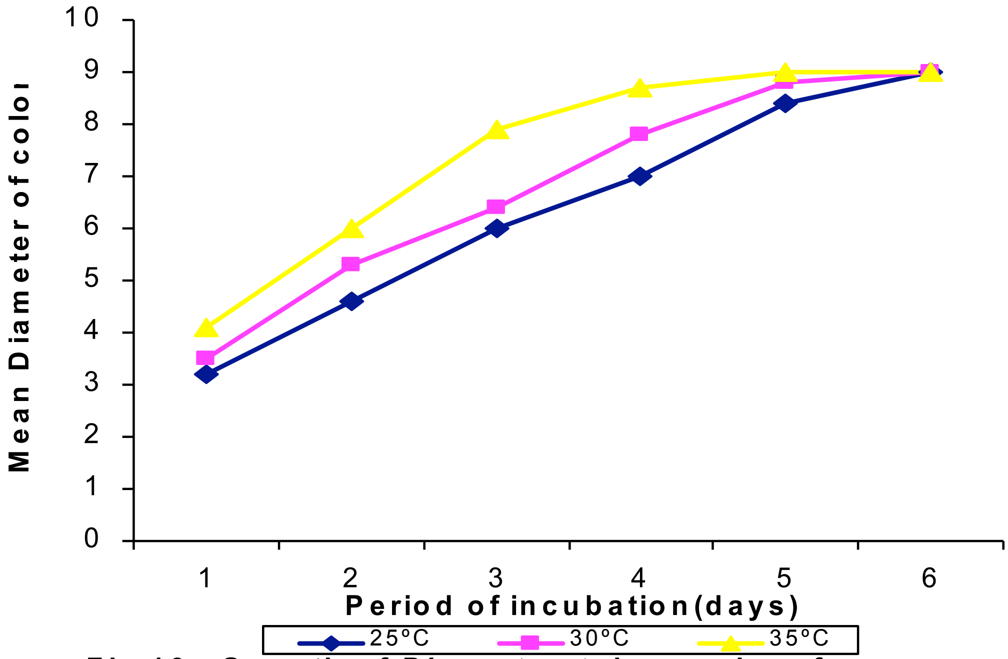

Fig.13a Growth of *Pleurotus tuber-regium* from Brong Ahafo Region on Potato Dextrose Agar (PDA)

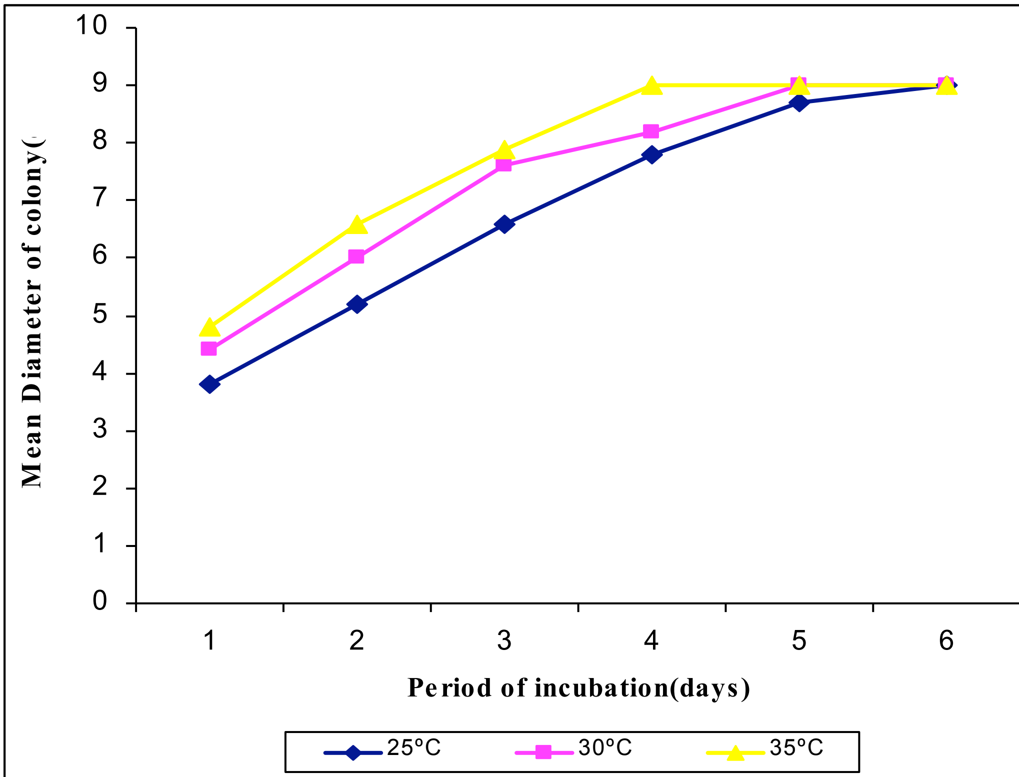

Fig.13b Growth of *Pleurotus tuber-regium* from Volta Region on Potato Dextrose Agar (PDA)

4.2.3 Effect of pH on the mycelial growth of *Pleurotus tuber-regium* from Volta and Brong Ahafo Regions at 35ºC.

The effects of pH on growth of *Pleurotus tuber-regium* from the Volta and Brong Ahafo Regions on PDA at 35ºC are presented in Tables 6a and 6b respectively. The effect of pH on the mycelia growth rate was similar with the two strains of *P. tuber-regium*. *P. tuber-regium* from the Volta and Brong Ahafo Regions grew best at pH 6, followed by pH 7 and 8 in decreasing order. Growth was least at pH 5. In all cases growth was faster with *P. tuber-regium* from the Volta Region. The Petri plates were completely covered at pH 6, 7 and 8 in 4, 6 and 7 days. On the other hand, *P. tuber-regium* from Brong Ahafo Region fully colonized the same medium in 6, 7 and 8 days at the same respective pH's.

Table 6a: Mycelial growth of *Pleurotus tuber-regium* from the Volta Region at 35ºC on Potato Dextrose Agar amended with the indicated pH's.

Period of incubation (Days)	Mean Diameter of mycelia (cm) at pH			
	5.0	6.0	7.0	8.0
1	2.7±0.12	4.0±0.13	2.7±0.11	2.3±0.16
2	4.5±0.15	6.2±0.09	5.0±0.19	4.3±0.13
3	5.2±0.07	7.5±0.14	5.4±0.12	5.0±0.12
4	5.8±0.09	9.0±0.05	6.5±0.11	6.1±0.13
5	6.3±0.03	-	7.8±0.08	7.0±0.06
6	7.6±0.11	-	9.0±0.11	8.2±0.07
7	8.7±0.07	-	-	9.0±0.04
8	9.0±0.04	-	-	-

- → overgrown the Petri plate.

Table 6b: Mycelial growth of *Pleurotus tuber-regium* from the Brong Ahafo Region at 35ºC on Potato Dextrose Agar amended with the indicated pH's.

Period of Incubation (Days)	Mean Diameter of mycelia (cm) at pH			
	5.0	6.0	7.0	8.0
1	1.4±0.08	3.7±0.14	2.2±0.09	1.8±0.09
2	2.3±0.11	4.5±0.09	3.4±0.10	2.9±0.11
3	3.1±0.08	6.0±0.11	4.7±0.06	3.7±0.06
4	3.9±0.13	7.2±0.10	5.9±0.12	4.9±0.05
5	4.5±0.12	8.3±0.09	7.0±0.08	5.7±0.04
6	5.4±0.12	9.0±0.11	8.2±0.04	7.0±0.04
7	6.3±0.06	-	9.0±0.02	8.2±0.09
8	6.7±0.11	-	-	9.0±0.09
9	7.0±0.05	-	-	-
10	8.2±0.09	-	-	-
11	9.0±0.05	-	-	-

- → overgrown the Petri plate.

4.2.4 Growth of *Pleurotus tuber-regium* on Potato Dextrose Agar in different light durations.

The effect of different light regimes on the growth of *Pleurotus tuber-regium* is shown in Tables 7a and 7b. *P. tuber-regium* from the Volta Region exposed to continuous light intensity colonized the medium in nine days. In continuous darkness, growth was very fast and totally covered the medium in five days. In alternating 4hr light/4hr darkness, the mycelia covered 2.5cm of the medium on the first day; growth increased steadily and it colonized the entire medium in nine days. Growth of the mycelium in alternating 6hr light/6hr darkness was similar to that of the 4hr light/4hr darkness. In 12hr light/12hr darkness only 2.4cm of the medium was covered after one day. Growth increased steadily until the entire plate was covered in 7 days. Thus growth in continuous darkness supported the fastest growth rate whilst continuous light exposure supported the slowest growth rate.

Pleurotus tuber-regium from the Brong Ahafo Region (Table 7b) grew slowest in continuous light and colonized the medium in nine days. Growth in continuous darkness on the other hand supported the best and fastest rate and the mycelium colonized the medium in six days. Growth was similar in all alternating light/dark conditions, the mycelia in the different light/dark regimes colonizing the media in nine days (Table 7b).

Table 7a: Growth of *Pleurotus tuber-regium* from the Volta Region on PDA at different light duration regimes at 35ºC.

Period of incubation (Days)	Mean Diameter of colony (cm) in				
	Continuous light	Continuous darkness	4Hrs light/ 4Hr darkness	6Hrs light/ 6Hrs darkness	12Hrs light/ 12Hrs darkness
1	1.9±0.03	3.7±0.07	2.5±0.07	2.3±0.08	2.4±0.09
2	2.7±0.05	4.9±0.12	3.2±0.07	3.0±0.09	3.2±0.08
3	3.6±0.05	5.9±0.07	3.9±0.09	3.9±0.11	4.0±0.11
4	4.4±0.09	7.8±0.07	4.6±0.11	4.4±0.10	4.9±0.10
5	5.1±0.06	9.0±0.09	5.3±0.12	5.0±0.11	6.5±0.08
6	6.0±0.05	-	6.1±0.10	6.2±0.14	7.8±0.11
7	7.0±0.07	-	6.9±0.07	7.5±0.05	9.0±0.12
8	8.2±0.08	-	8.6±0.09	8.4±0.08	-
9	9.0±0.09	-	9.0±0.09	9.0±0.12	-

- ⟶ over grown the media

Table 7b: Growth of *Pleurotus tuber-regium* from the Brong Ahafo Region on PDA at different light duration regimes at 35ºC.

Period of incubation (Days)	Mean Diameter of colony (cm) in				
	Continuous light	Continuous darkness	4Hrs light/ 4Hrs darkness	6Hrs light/ 6Hrs darkness	12Hrs light/ 12 Hrs darkness
1	1.0±0.10	2.2±0.05	2.1±0.07	2.0±0.12	2.0±0.14
2	2.2±0.12	3.4±0.08	2.9±0.07	2.8±0.14	2.7±0.12
3	3.1±0.10	4.6±0.08	3.5±0.08	3.7±0.11	3.2±0.11
4	3.9±0.11	6.0±0.07	4.1±0.10	4.2±0.11	4.1±0.09
5	4.0±0.09	8.6±0.04	4.7±0.11	5.1±0.06	4.8±0.10
6	5.6±0.06	9.0±0.06	5.0±0.12	6.0±0.06	5.8±0.12
7	6.9±0.05	-	6.4±0.09	7.4±0.07	6.6±0.12
8	7.4±0.04	-	7.5±0.08	8.2±0.07	7.9±0.10
9	9.0±0.04	-	9.0±0.05	9.0±0.08	9.0±0.12

- ⟶ over grown the media

4.3 CULTIVATION OF *PLEUROTUS TUBER-REGIUM* ON DIFFERENT SUBSTRATES.

The mycelia of *Pleurotus tuber-regium* from the Volta Region growing on the water hyacinth grew fastest and colonized the entire bag in two weeks (Fig.14a). Water hyacinth (WH) supported fast growth rate in first week and the mycelia colonized 71% of the substrate after 1 week; the remaining 29% of the bag was covered by the mycelium in the second week. The mycelium on plantain leaves (PL) substrate on the other hand covered 37% of the bag in the first week, a further 31% of the bag in the second week, and finally covered the remaining 32% of the substrate in the third week (Plate 4). Mycelium on millet stalk (MS) colonized 31% of the bag in the first week. After the second week the mycelium colonized another 35% of the substrate; in the third week it colonized the remaining 34%.

Mycelial growth on sawdust (SD) was the slowest. The mycelia colonized 27% of the sawdust in the first week, then another 38% of the bag in the second week and the final 32% of the bag in the third week.

P. tuber-regium from the Brong Ahafo Region grew fastest in the first week of incubation on water hyacinth (WH) (Fig.14b). It colonized 74% of the substrate in the first week and the other 26% in the second week, thus colonizing the whole substrate in two weeks. The plantain leaves (PL) was the next in line. Thirty six percent of the substrate was colonized in first week one. In the second week the mycelia colonized another 35% of the bag. Mycelia colonization continued to the third week, colonizing the remaining 29% of the substrate.

Irrespective of the types of substrates used the mycelium of *P. tuber-regium* from the Brong Ahafo and Volta Regions covered the entire bag after three weeks (Fig 14a and 14b).

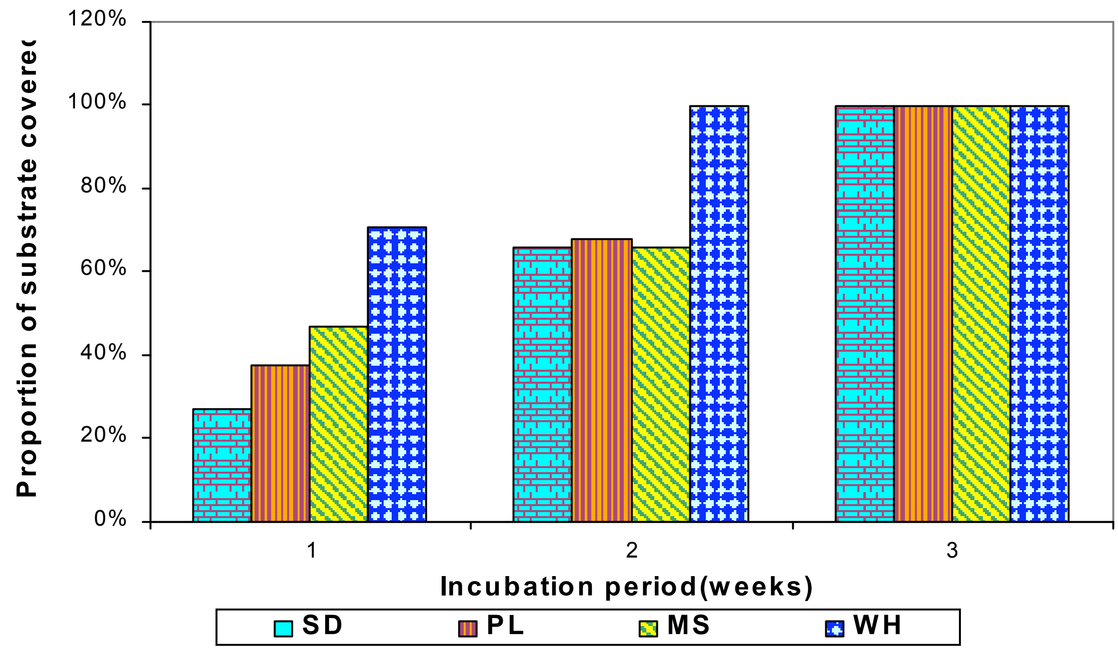

Fig.14a Growth of mycelia of *Pleurotus tuber-regium* from Volta Region on bagged substrates

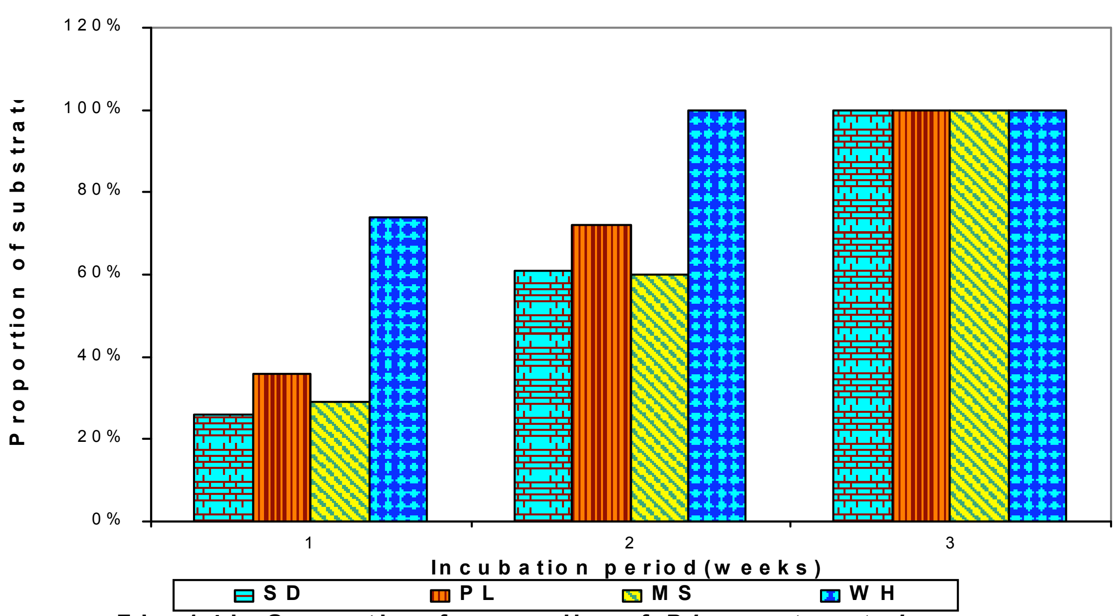

Fig.14b Growth of mycelia of *Pleurotus tuber-regium* from Brong Ahafo Region on bagged substrates

Legend: SD- Sawdust, PL- Plantain leaves, MS- Millet stalk, WH- Water hyacinth

Plate 4: Mycelium growth on bagged plantain leaves incubated at 30±2ºC for 16 days (x $^{1}/_{4}$).

4.4. YIELD OF FRUITBODIES AND SCLEROTIA FORMATION FROM THE CULTIVATED *PLEUROTUS TUBER-REGIUM*.

4.4.1. Fruitbodies and Sclerotia.

When *Pleurotus tuber-regium* was cultivated on bagged substrates, only the bagged plantain leaves produced fruitbodies (Plates 5a and 5b). A total of seven flushes (1089.4g of fruitbodies) was recorded (Table 8). The mean cap diameter ranged from 9.9 to 13.0cm and the stipe diameter ranged from 4.4 to 5.3cm. (Table 8)

The total weight of the fruitbodies increased from 124.5g in the first flush to 198.0g in the fourth flush and then decreased gradually to 133.7g in the seventh flush. In the same way the number of fruitbodies increased from eight in the first flush to 15 in the fourth, and then decreased to 10 in the seventh.

The mean cap and stipe diameters increased from 9.9cm and 4.9cm respectively in the first flush to 13.0cm and 5.0cm respectively in the fourth flush and then decreased to 9.9cm and 4.4cm respectively in the seventh flush.

Sclerotia were formed in the bagged 'wawa' sawdust and plantain leaves (Plate 6a), but not in the bagged millet stalks and water hyacinth. Table 9a shows the numbers and weight of the three harvests of sclerotia (Plate 6b) from the cultivation of *P. tuber-regium* on 'wawa' sawdust. The first harvest had a total of 12 sclerotia which weighed 1,144g. The second had a total of 12 sclerotia weighing 698g, while the third gave six sclerotia weighing 151g. As growth progressed the number of sclerotia harvested as well as their weights decreased. Thus the three harvests provided mean sclerotium weights of 95.3g, 58.2g and 25.2g respectively.

Table 9b shows the numbers and weights of the three harvests of sclerotia from the cultivation of *P. tuber-regium* on plantain leaves (Plate 6c). A total of 10 sclerotia were harvested at the first harvest and this gave a total of 814g. The second gave eight sclerotia weighing 298g; the third gave eight pieces of sclerotia weighing 129g. The three harvests had mean sclerotium weight of 81.4g, 37.3g and 16.1g respectively. During the period of cultivation, the total sclerotium yield by weight from the sawdust (1,993g) was higher than the yield from the plantain leaves (1,241g).

Table 8: Flushes of fruitbodies, weight and dimension by *P. tuber-regium* raised on plantain leaves.

Flush Number	Appearance of fruitbodies after inoculation (days)	Interval between flushes (days)	Number of fruitbodies	Total Weight of fruitbodies (g)	Mean Cap diameter (cm)	Mean Stipe diameter (cm)
1	60	-	8	124.5	9.9±0.16	4.9±0.09
2	78	18	11	160.0	12.6±0.10	4.6±0.12
3	95	17	7	158.2	10.6±0.12	4.7±0.11
4	109	14	15	198.0	13.0±0.12	5.0±0.14
5	123	14	11	159.9	11.3±0.11	4.4±0.15
6	141	18	8	155.1	10.6±0.09	5.3±0.17
7	169	28	10	133.7	9.9±0.11	4.4±0.16
TOTAL WEIGHT				1,089.4g		

Plate 5a: Appearance of first fruitbodies of *P. tuber-regium* on bagged plantain leaves 60 days after incubation at 30±2ºC (x $^{1}/_{4}$).

Plate 5b: Fruitbodies of *P. tuber-regium* on plantain leaves substrate 80 days after incubation at 30±2ºC.

Table 9a: Sclerotia harvested from 'wawa' sawdust

Harvest	Sclerotium number and weight (g)												Total weight of sclerotia in harvest (g)	Mean weight of Sclerotium in harvest (g)
	1	2	3	4	5	6	7	8	9	10	11	12		
1st	97	93	93	95	94	112	78	101	85	97	96	103	1,144	95.3
2nd	70	47	92	76	70	57	67	56	43	44	41	38	698	58.2
3rd	24	28	18	23	42	16	-	-	-	-	-	-	151	25.2
							TOTAL WEIGHT						1,993	

Table 9b: Sclerotia harvested from plantain leaves

Harvest	Sclerotium number and weight (g)										Total weight of sclerotia in harvest (g)	Mean weight of Sclerotium in harvest (g)
	1	2	3	4	5	6	7	8	9	10		
1st	88	82	91	76	88	94	92	70	71	62	814	81.4
2nd	43	46	58	39	32	31	25	24	-	-	298	37.3
3rd	24	21	24	19	14	16	7	4	-	-	129	16.1
							TOTAL WEIGHT				1,241	

Plate 6a: Photograph showing sclerotia of *Pleurotus tuber-regium* protruding in bagged 'wawa' sawdust (right) ($x^{1}/_{4}$).

Plate 6b: Photograph showing sclerotia of *Pleurotus tuber-regium* harvested from bagged 'wawa' sawdust. (Note the different shapes and sizes of the sclerotia) ($x^{1}/_{2}$).

Plate 7c: Photograph showing the external morphology of the fruitbodies (cap) emerging from sclerotia of *Pleurotus tuber-regium* buried in soil placed in plastic bowl for 5days ($x^{1}/_{2}$).

Table 10: Formation of fruitbodies of *Pleurotus tuber-regium* induced from sclerotia buried in plastic bowls and cane baskets containing soil for 149 and 105 days respectively at 30±2ºC.

Type of container	Flush	Appearance of fruitbodies after burial (days)	Number of fruitbodies	Total weight of fruitbodies (g)	Mean cap Diameter (cm)	Mean stipe length (cm)	Mean stipe circumference (cm)
a. PLASTIC BASKET	1	61	6	140	4.9±0.12	6.1±0.05	2.9±0.09
	2	83	10	170	5.7±0.09	5.8±0.05	3.9±0.10
	3	101	5	270	5.9±0.11	6.1±0.06	3.9±0.05
	4	120	4	150	3.6±0.10	4.2±0.10	2.8±0.05
	5	149	7	160	4.4±0.04	4.9±0.11	2.5±0.07
b. CANE BASKET	1	49	13	250	6.1±0.09	6.9±0.11	3.4±0.04
	2	52	2	190	5.5±0.11	6.3±0.121	3.0±0.07
	3	60	9	290	6.7±0.05	7.6±0.21	5.2±0.15
	4	79	2	110	3.1±0.07	4.5±0.13	2.4±0.12
	5	105	1	100	2.8±0.12	2.8±0.15	1.5±0.11

4.4.3 Biological Efficiency (B.E)

The Biological efficiency (B.E) of the fruitbodies and sclerotia harvested from the various substrates is shown in Table 11. The bagged plantain leaves yielded fruitbodies with a B.E of 54.5% while the sclerotia gave a B.E of 62.1%. The 'wawa' sawdust did not produce any fruitbody but yielded sclerotia which gave the highest B.E of 99.7%. The plastic bowl method of producing fruitbody in soil gave a B.E of 89.0% whilst the cane basket gave a B.E of 94.0%. The two soil induction methods thus obtained high biological efficiencies with that of the cane basket being superior.

Table 11: Biological efficiencies of fruitbodies and sclerotia.

SUBSTRATE	B.E (%)
Bagged plantain leaves (fruitbody)	54.5
Bagged plantain leaves (Sclerotia)	62.1
Bagged 'wawa' sawdust (Fruitbody)	0.0
Bagged 'wawa' sawdust (Sclerotia)	99.7
Soil in plastic bowl (fruitbody)	89.0
Soil in cane basket (fruitbody)	94.0

4.5 CHEMICAL ANALYSES

4.5.1 Chemical constituents of fresh and spent substrates of *Pleurotus tuber-regium*.

The ash, crude protein, cellulose, hemicellulose, lignin, crude fibre, organic matter content and pH of the fresh substrates (plantain leaves, water hyacinth, millet stalk and 'wawa' sawdust) used in growth of *P. tuber-regium* as well as those of the spent substrates are presented in Table 12. The ash content of water hyacinth (13.07g/100g) was the highest, followed by that of plantain leaves (10.50g/100g), 'wawa' sawdust (9.80g/100g) and least in millet stalk (6.02g/100g). Crude protein content was highest in plantain leaves (15.92g/100g), followed by that in the 'wawa' sawdust (12.50g/100g), millet stalks (6.73g/100g) and water hyacinth (1.34g/100g).

Cellulose was abundant in 'wawa' sawdust (34.04%), followed by that in millet stalk (25.05%) and plantain leaves (22.68%); it was least in water hyacinth (20.23%). Hemicellulose was more abundant in millet stalk (21.41%) than in plantain leaves (20.67%) and least in water hyacinth and 'wawa' sawdust (15.71% each). Lignin was most abundant in 'wawa' sawdust (20.43%) and lowest in plantain leaves (14.45%); millet stalk had 13.53% while water hyacinth had 12.22%.

The pH value of 'wawa' sawdust (7.01) and plantain leaves (6.70) was slightly neutral to acidic; acidic in water hyacinth (5.97) and millet stalk (5.49).

The spent substrates recorded lower values for most of its constituents. The ash values ranged from 2.10 to 10.90%. Crude protein value ranged from 1.12 to 18.49%, with plantain leaves recording the highest, followed by 'wawa' sawdust, millet stalk and water hyacinth in that order. Cellulose was highest in ‘wawa’ sawdust (25.91%); this was followed by millet stalk (19.11%), then plantain leaves (13.00%) and least in water hyacinth (5.17%). Hemicellulose value ranged from 6.97 to 13.18%, lignin ranged from 8.00 to 10.76%, crude fibre from 5.41 to 20.16% while organic matter value ranged from 80.12 to 85.37%. The pH values recorded ranged from 6.34 to 8.27 and were higher than in the corresponding substrates.

Table 12: Proximate composition of fresh and spent substrates (in parenthesis) used in the cultivation of *Pleurotus tuber-regium*.

SUBSTRATE		PERCENTAGE COMPOSITION OF							
Common name	Scientific name	Ash (g/100g)	Crude protein (g/100g)	Cellulose (g/100g)	Hemicellulose (%)	Lignin (%)	Crude fibre (%)	Organic matter (%)	pH
Plantain Leaves	*Musa sapientum*	10.50±0.03	15.92±0.06	22.68±0.10	20.67±0.03	14.45±0.04	25.69±0.14	86.81±0.13	6.70±0.05
		(6.11±0.13)	(18.49±0.01)	(13.0±0.15)	(10.25±0.21)	(8.29±0.10)	(12.12±0.11)	(83.81±0.13)	(7.91±0.07)
Water Hyacinth	*Eichhornia crassipes*	13.07±0.05	1.34±0.11	20.23±0.06	15.71±0.09	12.22±0.05	12.53±0.22	84.12±0.17	5.97±0.05
		(10.90±0.15)	(1.12±0.14)	(5.17±0.10)	(6.97±0.15)	(9.16±0.05)	(5.41±0.22)	(80.12±0.17)	(6.60±0.12)
Millet Stalk	*Eleusine coracana*	6.02±0.09	6.73±0.04	25.05±0.05	21.41±0.05	13.53±0.03	32.11±0.17	90.37±0.11	5.49±0.07
		(2.10±0.03)	(5.50±0.07)	(19.11±0.05)	(11.27±0.05)	(8.00±0.13)	(10.09±0.09)	(85.37±0.11)	(6.34±0.09)
'Wawa' Sawdust	*Triplochiton scleroxylon*	9.80±0.11	12.5±0.15	34.04±0.09	15.71±0.10	20.43±0.04	40.01±0.15	88.02±0.17	7.01±0.09
		(4.59±0.10)	(11.98±0.03)	(25.91±0.17)	(13.18±0.13)	(10.76±0.22)	(20.16±0.07)	(84.02±0.17)	(8.27±0.04)

4.5.2 Nutrient constituents of fruitbodies and sclerotia of *Pleurotus tuber-regium* harvested from the Hohoe District of the Volta Region and Techiman District of the Brong Ahafo Region.

4.5.2.1 Nutrient constituent of fruitbodies of *P. tuber-regium*

The amounts of moisture, ash, fat, crude protein, carbohydrate and energy in the fruitbodies of *Pleurotus tuber-regium* from Volta and Brong Ahafo Regions are shown in Table 13a. Moisture content in the cap of *P. tuber-regium* from Volta Region was slightly higher (56.5g/100g) than that from the Brong Ahafo Region (52.3g/100g). Moisture content of the stipe of *P. tuber-regium* from Volta Region was 63.3g/100g and that from Brong Ahafo Region was 60.1g/100g. The moisture content of the stipe was more than that of the cap. Ash content of the cap of *P. tuber-regium* from the Volta Region was 2.1g/100g while that from the Brong Ahafo Region was 2.0g/100g. The ash content of the stipe of *P. tuber-regium* from the Volta and the Brong Ahafo Regions were the same (0.4g/100g). The fat content of the cap of *P. tuber-regium* from the Volta Region (0.3g/100g) was less than that from the Brong Ahafo Region (0.5g/100g). The fat content of the stipe of *P. tuber-regium* from the Volta Region (0.1g/100g) was less than that from the Brong Ahafo Region (0.6g/100g). Crude protein content of the cap of *P. tuber-regium* from the Volta Region (9.3g/100g) was less than that from the Brong Ahafo Region (9.9g/100g). Crude protein of the stipe of *P. tuber-regium* from the Volta Region (2.1g/100g) was less than that from the Brong Ahafo Region (2.4g/100g). The carbohydrate content of the cap of *P. tuber-regium* from the Volta Region (31.8g/100g) was less than that from the Brong Ahafo Region (39.5g/100g) and that for the stipe from the Volta Region (34.1g/100g) was less than that from the Brong Ahafo Region (38.1g/100g).

Table 13a: Nutrient constituents of fruitbodies of *Pleurotus tuber-regium* from Hohoe District of the Volta Region and Techiman District of the Brong Ahafo Region.

PARAMETER	PROXIMATE COMPOSITION (g/100g) IN FRUITBODY FROM			
	VOLTA REGION		BRONG AHAFO REGION	
	Cap	Stipe	Cap	Stipe
Moisture	56.5±0.03	63.3±0.06	52.3±0.09	60.1±0.09
Ash	2.1±0.07	0.4±0.03	2.0±0.05	0.4±0.02
Fat	0.3±0.02	0.1±0.16	0.5±0.09	0.6±0.11
Crude protein	9.3±0.11	2.1±0.05	9.9±0.06	2.4±0.06
Carbohydrate	31.8±0.05	34.1±0.07	39.5±0.09	38.1±0.10

4.5.2.2 Nutrient constituents of sclerotia of *Pleurotus tuber-regium*

The nutrient constituents of sclerotia of *P. tuber-regium* from the Volta and the Brong Ahafo Regions are shown in Table 13b. The moisture content of the sclerotia from the Brong Ahafo Region (41.03g/100g) was slightly higher than that from the Volta Region (40.76g/100g).The ash content (5.90g/100g) on the other hand was higher in the sclerotia from the Volta Region (5.60g/100g). The fat content of the sclerotia was low but higher in sample from the Brong Ahafo Region (1.03g/100g) than in the one from the Volta Region (0.93g/100g).

Sclerotia from the Volta Region recorded almost the same crude protein (10.8g/100g) as that from the Brong Ahafo Region (10.55g/100g) but carbohydrate in sclerotia from the Volta Region (41.13g/100g) recorded a much less value than that from the Brong Ahafo Region (43.13g/100g).

Table 13b: Nutrient constituents of sclerotia of *Pleurotus tuber-regium* from Hohoe District of the Volta Region and Techiman District of the Brong Ahafo Region.

PARAMETER	PROXIMATE COMPOSITION OF CONSTITUENT (g/100g) IN SCLEROTIA FROM	
	VOLTA REGION	BRONG AHAFO REGION
Moisture	40.76±0.71	41.03±0.85
Ash	5.90±0.41	5.6±0.64
Fat	0.93±0.23	1.03±0.08
Crude protein	10.8±0.90	10.55±0.32
Carbohydrate	41.13±0.21	43.13±0.73

CHAPTER FIVE

DISCUSSION

A substantial number of questionnaire respondents from the Hohoe District (184) as well as those from the Techiman District (176) (representing 92% and 88% respectively) knew about *P. tuber-regium*, although majority of them who responded in the affirmative were between the ages of 21 and 70 years. Forty nine per cent of the total respondents from the Hohoe District who said they knew about *P. tuber-regium* were males while 51% were females. In the case of respondents from the Techiman District who said they knew about *P. tuber-regium,* 36% of the total respondents were males whilst 64% were females. The larger number of female respondents with knowledge of *P. tuber-regium* agrees with the findings of Akpaja *et al*. (2003) who stated that mushroom hunting seemed to be gender related and men do not make deliberate efforts to go looking for mushrooms. Therefore mushroom collection remains the preserve of women and children. According to studies carried out in Southern Ghana by Apetorgbor *et al*. (2006), it was observed that women were especially involved in mushroom collection and has extensive knowledge as well as deriving incomes from selling mushrooms.

Interestingly results from both Regions showed that fewer respondents within the 71+ age group have less knowledge of *P. tuber-regium.* It could be said that perhaps at this age the respondents must have lost knowledge on it due to dementia associated with old age.

It was observed that more Illiterates and semi-illiterates (97% from the Brong Ahafo Region and 90% from the Volta Region) had knowledge of *P. tuber-regium* and many other mushrooms since they were directly involved in farming, and other field activities, which agrees with the findings of Apetorgbor *et al*. (2006).

It was noted that *P. tuber-regium* was rare to find these days and could be categorized as endangered since they are only collected from the wild (farmlands, abandoned farmlands and forests). Their scarcity could be due to the rampant bush fires observed at the sampling areas and bad farming practices. Isikhuemhen and Nerud (1999) observed that there had been a drastic fall in sclerotium collection from the wild, as a result of rapid deforestation.

Apart from *P. tuber-regium* 10 other different types of mushrooms were reported to be familiar among respondents from the Hohoe District of the Volta Region, whilst 19 types are known among respondents of the Techiman District of the Brong Ahafo Region. In each of the two Regions *P. tuber-regium* is given different local names with different meanings. For example, in the Volta Region it is called Anyigbakor (the earth's mound), Anyigbadzi (the earth's heart), Anyigbahlo (the earth's mushroom), and in the Brong Ahafo Region it is called Fuo and Dimitigyamegya (eat my head and leave my stalk). Other mushrooms mentioned included *Auricularia auricular-judae, Coprinus disseminatus, Termitomyces globulus, T. robustus*, *Volvariella volvacea* and *V. bombycina.* These edible mushrooms among others were encountered in earlier reports by Apetorgbor *et al*. (2006), Obodai and Apetorgbor (2001). Most of the locally known mushrooms in the Brong Ahafo Region were similar to those in the Bia Biosphere Reserve (Obodai and Apetorgbor, 2001).

Respondents claimed that knowledge on the medicinal values of the *P. tuber-regium* was passed down to them mostly by their parents and in some cases by herbalists. Respondents in the two Regions have similar uses for *P. tuber-regium* but their mode of formulation and application were different, probably due to differences in their cultural practices. An example is in the case of convulsion where respondents from the Hohoe District said they blended the sclerotium of *P. tuber-regium* and smeared it on the body of the individual and for the same ailment respondents from the Techiman District said they blended the sclerotium for use in soups or stews. According to Akpaja *et al*. (2003), and Fasidi and Olorunmaye (1994) *P. tuber-regium* has been used in combination with various herbs to cure asthma, small pox, high blood pressure, cough and obesity in Nigeria. Some respondents claimed they do not use the fruitbody or the sclerotium of *P. tuber-regium* for the preparation of meals, but only use the sclerotium to treat certain ailments (as stated in Tables 5a and 5b).

Respondents who claimed they knew that the sclerotium had nutritional values and were used in the preparation of meals could not explain how it was applied. In Nigeria, Akpaja *et al*. (2003) and Okhuoya and Etugo (1993) reported that the tuber was highly nutritive and very rich in proteins, very expensive and considered a delicacy. The fruitbody and the sclerotium are used as thickeners in the preparation of native stews and soups as well as in the treatment of ailments such as asthma, cough and obesity. The fruitbodies and sclerotia were also known to be used in traditional treatment of anaemia, stomach pains, cough as well as malnutrition-related illnesses in children (Iwuagwu and Onyekweli, 2002; Okhuoya and Okogbo, 1991; Osemwegie *et al*., 2002).

Most of the respondents from the Hohoe District said they found *P. tuber-regium* growing wild in the soil, while some said they found it either growing in soil or on decaying tree stump. In the Techiman District 81% said *P. tuber-regium* was found in the wild growing on soil, four per cent said it grew on decaying tree stump and 15% said it grows either on soil or decaying tree stump. *P. tuber-regium* was known to appear mostly in the rainy season but sometimes also in the dry season. Osemwegie *et al*. (2002) and Iwuagwu and Onyekweli (2002) reported that *P. tuber-regium* is found growing on dead decaying logs and on grassland. It has also been reported to be growing on dead decaying logs of hard and soft woods like *Mangifera indica, Daniella oliveri and Treculia africana* and the underground tuber is produced initially embedded within the remains of most decayed prostrate log or between the wood and bark (Isikhuemhen and Okhuoya, 1995; Okhuoya and Okogbo, 1991; Oso, 1977).

According to the respondents, sclerotia of *P. tuber-regium* are preserved by peeling off the outer covering and sun drying. They are then wrapped in polythene and stored in a cool dry room or on trees. Akpaja *et al*. (2003) noted the major methods of preserving mushrooms as sundrying and smoking.

All the test media supported the mycelia growth of *P. tuber-regium* to some extent. Wheat Dextrose Agar supported the fastest mycelia growth and this was followed by

Potato Dextrose Agar. This may be due to the large amount of hemicellulose and cellulose content in both potato and wheat. Chandra and Purkayastha (1977) reported that most carbohydrates support growth of *P. tuber-regium* mycelium. Most media having high polysaccharides support good mycelia growth (Binding, 1972). Carrot Dextrose Agar, on the other hand supported the least mycelia growth of both strains of *P. tuber-regium.* This may also be due to the low content of hemicellulose and cellulose in carrot.

Growth rates of the two strains of *P. tuber-regium* were fastest at 35ºC on all the media used, whilst 25ºC supported the least growth rate of the mycelia. This agrees with the findings of Jandaik and Kapoor (1976) that the mycelium of tropical species of *Pleurotus* may do well and even better when incubated at temperatures above 30ºC. Poppe and Hofte (1995) reported that most enzymes have their optimum range of activity and that ranges below or above the optimum may render the enzymes inactive. Sawyerr (2000) also reported that activities of enzymes liberated by the mushroom hyphae are high at temperatures above 28ºC but enzyme denaturing occurs at 40ºC or temperatures just below 40ºC.

The pH value 6.0 supported the best growth rate for mycelia growth whilst the pH value of 5.0 recorded the least growth rate of the two strains of *P. tuber-regium* (Tables 6a and 6b). This could be due to the fact that extracellular enzymes that are extruded by the mushroom for degrading the complex macromolecules i.e. cellulose and hemicellulose into simple soluble compounds to be taken up by the mushroom are very active at a pH of 6.0 and subsequently inactive at a pH of 5.0. This supports the findings of Poppe and

Hofte (1995) that most enzymes have their various optimal range of activity, and ranges below and above the optimal value might render them inactive or slow their activities.

It was noted that the strain of *P. tuber-regium* from the Volta Region recorded a higher growth rate at all the different pH values it was exposed to than *P. tuber-regium* from the Brong Ahafo Region (Tables 6a and 6b). The difference in time period for the mycelia of the two different strains of *P. tuber-regium* to cover the media at the different pH values must have occurred due to the differences in adaptability of the mycelia to the various pH values. Presumably, *P. tuber-regium* from the Volta Region had a better adaptability to the various pH values than *P. tuber-regium* from the Brong Ahafo Region.

Continuous darkness supported the fastest growth of *P. tuber-regium* mycelia whilst continuous light exposure of 350-500Lux supported the least growth (Tables 7a and 7b). Therefore light was not needed for the mycelial growth of both strains of *P. tuber-regium.* Wood and Smith (1987) noted that colonization by *Pleurotus spp.* occurs in a dark room. Continuous darkness is needed for good growth of the mycelia. In continuous light the mycelium grows but at a very slow rate.

All the bags were fully colonized within 21 days of inoculation. Mycelia growth in the water hyacinth was the fastest followed by that of the plantain leaves, sawdust and millet stalk. There is an inverse relationship with initial cellulose and crude fibre content. This does not agree with. Xiujin *et al*. (2001) and Platt and Hadar (1983) observed that *Pleurotus* spp. had greater capability to digest lignin and this degradation played an important role in the development of the mycelium. Sawdust yielded heavier and more sclerotia (1,993g) than the plantain leaves (1,241g). Using one of their selected strains Okhuoya *et al*. (1998) obtained 735g fresh sclerotia from one kilogram sawdust substrate with five per cent wheat bran supplementation. The differences in yield in the different substrates could be due to the nutrient status of the respective substrates. Many lignocellulosic substrates can support growth of *P. tuber-regium* (Isikhuemhen and Nerud, 1999). Sawdust substrate which has the highest cellulose value of 34.04% is more likely to be similar to that obtained in decaying logs, the natural habitat of the fungus. The fact that the sawdust did not produce fruitbody could be attributed to the fact that it was still forming mycelia and storing food reserves in the sclerotia. The food reserves stored in the sclerotia would later be used in the formation of fruitbodies. The millet stalk and water hyacinth produced neither fruit bodies nor sclerotia because the fungus was still forming mycelia or still utilizing the substrates.

Within the period of study only the plantain leaves produced fruitbodies and this could be due to the ability of the fungus to quickly utilize the nutrients for mycelium formation and food storage (sclerotium formation) and later convert them to form fruitbodies. The physiology of fruitbody morphogenesis requires rapid and steady supply of soluble nutrients such as glucose.

As the flushes progressed the number of sclerotia produced also reduced. This occurred because the nutrients in the substrate were used up to produce the earlier flushes. The fruitbodies from the second and third flushes were less because the nutrient left within the substrate was less and so this affected the yield.

Soil supplies water for the growth of the mycelium and fruitbodies, it buffers weather conditions in the growing room, protects the mycelium from drying and provides an environment suitable for the stimulation of fruitbodies and their development (Oei, 1996). The faster rate of induction of the fruitbody from sclerotia buried in the cane basket could be attributed to the structure of the cane basket. The cane basket had holes in it. This allowed exchange of gases between the garden soil, buried sclerotia and the outer environment which promoted faster growth rate. In the case of the plastic bowl, the bowl did not have holes in it and this restricted the circulation of air between the buried sclerotia and the outer environment. This delayed growth and fruitbody formation.

The plastic bowl method gave a B.E of 89.0% whilst that of the cane basket gave a B.E of 94.0%. The two methods had high B.E but that of the cane basket was slightly higher. This may be because the cane basket presumably allowed more aeration between the mycelia and its environment, thus giving better growth and yield.

The chemical constituents of substrates and spent substrates used for the cultivation of *Pleurotus tuber-regium* are shown in Table 12. The values of the chemical constituents of all the substrates used were very different. The pH varied between 5.49 and 7.01 which are within the range of pH for the best growth of *Pleurotus* spp. (pH 5.5 to 7.0). For the spent substrate, the pH increased and was within the range of 6.34 and 8.27. This could

have occurred because the mushroom produced metabolites that affected the hydrogen ion concentration (Zadrazil and Kurtzman, 1982).
Cellulose content of the substrates ranged from 20.23 to 34.04%. 'Wawa' sawdust had the highest cellulose value of 34.04%, this accounted for the high yield of sclerotia produced by the 'wawa' substrate.

Cellulose content of the spent substrates ranged from 5.17 to 25.91%. The most degraded substrate was water hyacinth whose cellulose content reduced from 20.23 to 5.17%. This implies that more cellulose was used up during the cultivation of the mushroom. This ability to degrade cellulose was attributed to the synergetic action of three different types of hydrolases called cellulases (Datta and Chakravarty, 2001).

The nutrient composition of the sclerotium and fruitbody varied depending on the part of *P. tuber-regium* used (Table 13). The ash, fat and carbohydrate compositions were higher in the sclerotium than in the fruitbody while the crude protein and moisture contents were higher in the fruitbody than in the sclerotium. The crude protein and the ash compositions were very high in the pileus compared to those in the stipe but those of the fat and carbohydrate do not differ much. These values show that the fruitbodies of *P. tuber-regium* are good sources of nutrients for man.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The response gathered from the questionnaire administration in both the Volta and the Brong Ahafo Regions, showed that *P. tuber-regium* had medicinal properties and was used to cure several ailments. The sclerotia which were collected from the wild were becoming endangered as a result of bush fires and other negative farming practices.

Wheat Dextrose Agar (WDA) supported the fastest mycelia growth at an optimum temperature of 35ºC for both strains, but the strain from the Hohoe District of the Volta Region showed a higher growth rate than that from the Techiman District of the Brong Ahafo Region.

Pleurotus tuber-regium grew best at pH 6.0 whilst pH 5.0 was inferior in supporting growth of both strains of *P. tuber-regium.* The strain from the Volta Region colonized the media faster than that from the Brong Ahafo Region.

Continuous darkness supported the fastest growth rate of the mycelia of *P. tuber-regium* while continuous light exposure supported the least growth.

Among the substrates used only plantain leaves produced fruitbodies. Sclerotia formation occurred in wawa sawdust and plantain leaves only. The sawdust yielded heavier and more sclerotia than the plantain leaves. The Biological Efficiency of the sclerotia was higher than that of the fruitbodies.

Induction of *P. tuber-regium* in cane baskets stimulated the growth of a higher and better yield of fruitbodies than induction using the plastic bowls.

The crude fibre content of the substrates used for the cultivation of *P. tuber-regium* varied from 12.53 to 40.01%. Crude protein content varied from 1.34 to 15.92%. The chemical constituents of the spent substrates were lower than those in the initial substrates.

Of the two strains of *P. tuber-regium* used, that from the Hohoe District of the Volta Region exhibited higher growth rate, higher yield and more viable than that from the Techiman District of the Brong Ahafo Region.

6.2 RECOMMENDATIONS

1. More investigation should be carried out on the medicinal use of the sclerotia and fruitbodies of *Pleurotus tuber-regium* among indigenous Ghanaians and a documentation of the various strains in the country should also be done. The general public, especially the younger ones should be educated on the existence and uses of *P. tuber-regium*.

2. Mushroom farmers and the general public should be educated on the nutritional and medicinal characteristics of *P. tuber-regium*. They should also be encouraged to grow and use *P. tuber-regium* for its nutritional and medicinal benefits and as a source of additional income.

3. It is recommended that the strain from the Volta Region be used in *P. tuber-regium* cultivation instead of that from the Brong Ahafo Region since it proved to be faster in growth.

4. The use of other agricultural and forestry wastes in the cultivation of *P. tuber-regium* should be carried out to find out if they would also support *P. tuber-regium* growth and production of sclerotia as well as the fruitbodies. This will encourage the use of these wastes in mushroom production and help in the natural recycling for food, medicine and improvement of the environment.

5. More education on bush fires and other bad farming practices should be carried out by the District Assemblies and other stakeholders to help prevent further erosion of *P. tuber-regium* in the wild.

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APPENDICES

APPENDIX A

QUESTIONNAIRE

A. Background and General Information

i. Name of respondent………………………

ii. Name of town or village…………………..

iii. District……………………………………

iv. Region…………………………………...

v. Ecological Zone …………………………..

vi. Date………………………………………

1. Sex
Male□ Female□

2. Age
< 20□21-30□31-40□41-50□51-60□ >60□

3. Educational Level
Illiterate□ Primary/middle/JSS□ Secondary/SSS□ Postsec□ Tertiary□

4. Origin
Native□ Settler□

B. Production/ Availability
5. What kinds of mushroom are available here?
Domo□ Oyster□ *Termitomyces*□ *Auricularia* (Woodear) □ *Pleurotus tuber-regium*□ others (Specify)……………………………………………………………………………

6a. Do you know about *Pleurotus tuber-regium*?
Yes□ No□

6b. What is its local name?...

6c. How did you get to know about it? Through
Parent□ Herbalist□ Friends□ Others (Specify)…………………………………………….

7. How long? 1-3yrs□ 4-6yrs□ >6 □

8. Which area is it normally found?
Forest□ Farmland□ Abandoned farmland□ Open field□ Pasture land□
Others…………………………………………………………………………………….

9a. On what do you find it growing?
Soil□ Decaying tree stump□ others (Specify)……………………………………………

9b. If on tree stump, give name of tree…………………………………………………..

10. In which month does the fruit body normally appear?..

11. What are the uses?
Medicinal□ Nutritional□ others …………………………

I. The Tuber
a. i If Medicinal what ailments are they use to treat?
....
…………………………………………………………………………………………...
…………………………………………………………………………………………...

a. ii. How are they used to treat the ailments?
………………………………………………………………………………………….
………………………………………………………………………………………….
………………………………………………………………………………………….

b. i If Nutritional what does the body gain from it?
………………………………………………………………………………………….
………………………………………………………………………………………….
………………………………………………………………………………………….

b. ii How are they used/applied?
………………………………………………………………………………………….
………………………………………………………………………………………….
………………………………………………………………………………………….

II. Fruitbody
a. i If Medicinal what ailments are they use to treat?
………………………………………………………………………………………….
………………………………………………………………………………………….
………………………………………………………………………………………….

a. ii. How are they used to treat the ailments?
…………………………………………………………………………………………………
……………………………………………………………………………………………….....
...………………………………………………………………………………………………

b. i If Nutritional what does the body gain from it?
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

b. ii How are they used/applied?
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

12. If 13a and b are medicinal, how are they used for the various ailments?
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

13a. Do you know if it can be grown by man?
Yes□ No□

13b. Can it be grown throughout the year?
Yes□ No□

13c. If no, what month/ season can they be grown?
……………………………………………………………………………………………….....

14. Do you know if it can be preserved?
Yes□ No□

15. If yes, how is this done?
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………

APPENDIX B

ANOVAS

LENGTH OF MYCELIA ON DIFFERENT BAGS (*Pleurotus tuber-regium* from Brong Ahafo Region)

Table 1a

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	4	22.8	5.7	5.26
Row 2	4	18.4	4.6	1.033333
Row 3	4	16.2	4.05	7.896667
Column 1	3	15.3	5.1	0.97
Column 2	3	15.1	5.033333	0.413333
Column 3	3	14.8	4.933333	0.863333
Column 4	3	12.2	4.066667	20.81333

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	5.646667	2	2.823333	0.418547	0.675832	5.143253
Columns	2.096667	3	0.698889	0.103607	0.954956	4.757063
Error	40.47333	6	6.745556			
Total	48.21667	11				

LENGTH OF MYCELIA ON DIFFERENT BAGS (*Pleurotus tuber-regium* from Volta Region)

Table 1b

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	4	26.8	6.7	3.666667
Row 2	4	22.9	5.725	1.8425
Row 3	4	18.2	4.55	9.283333
Column 1	3	18	6	0.91
Column 2	3	17.8	5.933333	0.343333
Column 3	3	18.8	6.266667	0.173333
Column 4	3	13.3	4.433333	22.30333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	9.271667	2	4.635833	0.728364	0.520965	5.143253
Columns	6.189167	3	2.063056	0.324139	0.808369	4.757063
Error	38.18833	6	6.364722			
Total	53.64917	11				

LENGTH OF MYCELIA ON DIFFERENT BAGS (COMBINED)

Table 1c

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	4	22.8	5.7	5.26
Row 2	4	26.8	6.7	3.666667
Row 3	4	18.4	4.6	1.033333
Row 4	4	22.9	5.725	1.8425
Row 5	4	16.2	4.05	7.896667
Row 6	4	18.2	4.55	9.283333
Column 1	6	33.3	5.55	0.995
Column 2	6	32.9	5.483333	0.545667
Column 3	6	33.6	5.6	0.948
Column 4	6	25.5	4.25	17.287

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	19.51208	5	3.902417	0.737546	0.606923	2.901295
Columns	7.58125	3	2.527083	0.477612	0.702604	3.287382
Error	79.36625	15	5.291083			
Total	106.4596	23				

APPENDIX C

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 25ºC (*Pleurotus tuber-regium from* Brong Ahafo Region)

Table 2a

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	4.6	0.766667	0.374667
Row 2	6	8	1.333333	0.518667
Row 3	6	11.4	1.9	0.736
Row 4	6	15.3	2.55	0.691
Row 5	6	19.7	3.283333	0.717667
Row 6	6	24	4	0.48
Column 1	6	19.6	3.266667	1.618667
Column 2	6	12.4	2.066667	1.794667
Column 3	6	11.3	1.883333	1.705667
Column 4	6	19.4	3.233333	1.410667
Column 5	6	10.8	1.8	1.376
Column 6	6	9.5	1.583333	1.093667

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	44.18889	5	8.837778	273.5213	5.61E-21	2.602987
Columns	16.78222	5	3.356444	103.879	6.77E-16	2.602987
Error	0.807778	25	0.032311			
Total	61.77889	35				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 25ºC (*Pleurotus tuber-regium* from Volta Region)

Table 2b

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	6	1	0.44
Row 2	6	9.4	1.566667	0.534667
Row 3	6	13.2	2.2	0.672
Row 4	6	17	2.833333	0.702667
Row 5	6	21	3.5	0.752
Row 6	6	25.3	4.216667	0.461667
Column 1	6	20.8	3.466667	1.502667
Column 2	6	13.6	2.266667	1.794667
Column 3	6	12.9	2.15	1.783
Column 4	6	21.3	3.55	1.403
Column 5	6	12.2	2.033333	1.266667
Column 6	6	11.1	1.85	1.111

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	43.51472	5	8.702944	275.3128	5.18E-21	2.602987
Columns	17.02472	5	3.404944	107.7135	4.4E-16	2.602987
Error	0.790278	25	0.031611			
Total	61.32972	35				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 25ºC (COMBINED).

Table 2c

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	4.6	0.766667	0.374667
Row 2	6	6	1	0.44
Row 3	6	8	1.333333	0.518667
Row 4	6	9.4	1.566667	0.534667
Row 5	6	11.4	1.9	0.736
Row 6	6	13.2	2.2	0.672
Row 7	6	15.3	2.55	0.691
Row 8	6	17	2.833333	0.702667
Row 9	6	19.7	3.283333	0.717667
Row 10	6	21	3.5	0.752
Row 11	6	24	4	0.48
Row 12	6	25.3	4.216667	0.461667
Column 1	12	40.4	3.366667	1.429697
Column 2	12	26	2.166667	1.642424
Column 3	12	24.2	2.016667	1.605152
Column 4	12	40.7	3.391667	1.306288
Column 5	12	23	1.916667	1.216061
Column 6	12	20.6	1.716667	1.021515

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	88.80375	11	8.073068	272.6132	8.62E-44	1.967547
Columns	33.77625	5	6.75525	228.1128	1.83E-35	2.382823
Error	1.62875	55	0.029614			
Total	124.2088	71				

APPENDIX D

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 30ºC
(*Pleurotus tuber-regium from* Brong Ahafo Region)
Table 3a

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	5.6	0.933333	0.386667
Row 2	6	7.1	1.183333	0.433667
Row 3	6	10.5	1.75	0.451
Row 4	6	12.6	2.1	0.512
Row 5	6	14.4	2.4	0.5
Row 6	6	16.7	2.783333	0.733667
Row 7	6	18.6	3.1	0.488
Row 8	6	20	3.333333	0.574667
Row 9	6	22.4	3.733333	0.478667
Row 10	6	25.5	4.25	0.651
Row 11	6	25.4	4.233333	0.558667
Row 12	6	27.3	4.55	0.495
Column 1	12	42.2	3.516667	1.412424
Column 2	12	37.4	3.116667	1.952424
Column 3	12	31.3	2.608333	2.335379
Column 4	12	44.2	3.683333	1.214242
Column 5	12	26.7	2.225	1.243864
Column 6	12	24.3	2.025	1.011136

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	97.61375	11	8.873977	150.1557	7.19E-37	1.967547
Columns	28.06458	5	5.612917	94.97564	8.72E-26	2.382823
Error	3.250417	55	0.059098			
Total	128.9288	71				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 30ºC
(*Pleurotus tuber-regium* from Volta Region)

Table 3b

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	7.1	1.183333	0.433667
Row 2	6	12.6	2.1	0.512
Row 3	6	16.7	2.783333	0.733667
Row 4	6	20	3.333333	0.574667
Row 5	6	25.5	4.25	0.651
Row 6	6	27.3	4.55	0.495
Column 1	6	22.2	3.7	1.448
Column 2	6	19.9	3.316667	2.349667
Column 3	6	17	2.833333	2.738667
Column 4	6	23.1	3.85	1.231
Column 5	6	14.1	2.35	1.411
Column 6	6	12.9	2.15	1.119

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	49.36	5	9.872	116.0502	1.81E-16	2.602987
Columns	14.87333	5	2.974667	34.96865	1.66E-10	2.602987
Error	2.126667	25	0.085067			
Total	66.36	35				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 30ºC COMBINED

Table 3c

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	5.6	0.933333	0.386667
Row 2	6	7.1	1.183333	0.433667
Row 3	6	10.5	1.75	0.451
Row 4	6	12.6	2.1	0.512
Row 5	6	14.4	2.4	0.5
Row 6	6	16.7	2.783333	0.733667
Row 7	6	18.6	3.1	0.488
Row 8	6	20	3.333333	0.574667
Row 9	6	22.4	3.733333	0.478667
Row 10	6	25.5	4.25	0.651
Row 11	6	25.4	4.233333	0.558667
Row 12	6	27.3	4.55	0.495
Column 1	12	37.4	3.116667	1.952424
Column 2	12	31.3	2.608333	2.335379
Column 3	12	44.2	3.683333	1.214242
Column 4	12	26.7	2.225	1.243864
Column 5	12	24.3	2.025	1.011136

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	97.61375	11	8.873977	150.1557	7.19E-37	1.967547
Columns	28.06458	5	5.612917	94.97564	8.72E-26	2.382823
Error	3.250417	55	0.059098			
Total	128.9288	71				

APPENDIX E

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 35ºC (*Pleurotus tuber-regium* from Brong Ahafo Region)

Table 4a

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	6.4	1.066667	0.454667
Row 2	6	12.3	2.05	0.555
Row 3	6	16.5	2.75	0.827
Row 4	6	20.1	3.35	0.683
Row 5	6	24.5	4.083333	0.549667
Row 6	6	27.4	4.566667	0.266667
Column 1	6	22.4	3.733333	1.478667
Column 2	6	19.2	3.2	2.204
Column 3	6	15.2	2.533333	2.314667
Column 4	6	23.1	3.85	1.355
Column 5	6	13.9	2.316667	1.609667
Column 6	6	13.4	2.233333	1.430667

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	50.70222	5	10.14044	201.022	2.41E-19	2.602987
Columns	15.41889	5	3.083778	61.13216	3.3E-13	2.602987
Error	1.261111	25	0.050444			
Total	67.38222	35				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA AT 35ºC
(*Pleurotus tuber-regium* from Volta Region)

Table 4b

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	7.1	1.183333	0.433667
Row 2	6	12.6	2.1	0.512
Row 3	6	16.7	2.783333	0.733667
Row 4	6	20	3.333333	0.574667
Row 5	6	25.5	4.25	0.651
Row 6	6	27.3	4.55	0.495
Column 1	6	22.2	3.7	1.448
Column 2	6	19.9	3.316667	2.349667
Column 3	6	17	2.833333	2.738667
Column 4	6	23.1	3.85	1.231
Column 5	6	14.1	2.35	1.411
Column 6	6	12.9	2.15	1.119

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	49.36	5	9.872	116.0502	1.81E-16	2.602987
Columns	14.87333	5	2.974667	34.96865	1.66E-10	2.602987
Error	2.126667	25	0.085067			
Total	66.36	35				

DIAMETER OF MYCELIA (CM) ON DIFFERENT MEDIA at 35ºC COMBINED

Table 4c

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	6.4	1.066667	0.454667
Row 2	6	12.3	2.05	0.555
Row 3	6	16.5	2.75	0.827
Row 4	6	20.1	3.35	0.683
Row 5	6	24.5	4.083333	0.549667
Row 6	6	27.4	4.566667	0.266667
Row 7	6	7.1	1.183333	0.433667
Row 8	6	12.6	2.1	0.512
Row 9	6	16.7	2.783333	0.733667
Row 10	6	20	3.333333	0.574667
Row 11	6	25.5	4.25	0.651
Row 12	6	27.3	4.55	0.495
Column 1	12	44.6	3.716667	1.330606
Column 2	12	39.1	3.258333	2.073561
Column 3	12	32.2	2.683333	2.321515
Column 4	12	46.2	3.85	1.175455
Column 5	12	28	2.333333	1.373333
Column 6	12	26.3	2.191667	1.160833

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	100.1178	11	9.101616	136.3796	9.14E-36	1.967547
Columns	30.00944	5	6.001889	89.93295	3.31E-25	2.382823
Error	3.670556	55	0.066737			
Total	133.7978	71				