Nutritional Content and Bio-Degradation of Sweet Corn Leaves Using Edible Fungi (*Pleurotus pulmonarius*)

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Certification

This is to certify that Adenike Abiola Shosanya with matriculation number LCU/PG/002607 carried out this research work titled "Nutritional content and Bio-degradation of Sweet Corn Leaves Using Edible Fungi (*Pleurotus pulmonarius*)" in the department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, Nigeria for the award of Post Graduate Diploma (PGD) in Microbiology and has not been previously submitted.

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Dedication

I dedicate this work to God Almighty, my creator, my God, the source of my wisdom and inspiration.

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Abstract

The need to feed ever-increasing human population created awareness for increased agricultural production thus leading to increased agricultural waste generation. These wastes generated in many developing countries are not properly managed, utilized or under - utilized which constitute serious health threat to human and animals in the environment through environmental pollution resulting into environmental hazards. Agro wastes are rich in nutrient composition such as protein, minerals; consequently they ought to be considered as "raw material" instead of "waste". Using them as raw materials can help to recycle waste and make the environment eco – friendly through solid state fermentation (SSF) which produces secondary metabolites and essential enzymes and serves as potential substrate to produce value – added products.

Biological degradation for both economic and ecological reasons has become the popular alternative for the treatment of agro wastes .Wastes from agricultural residues are used as bioremediation agents, biofuel and bio - control agents through microbial processing which has brought tremendous benefit to agricultural management and eco - system. This study determined the phytochemicals, minerals and vitamins present in sweetcorn leaves and the effect of biodegradation on the leaves. Sweetcorn leaves were collected, dried and milled .Crude protein analysis was carried out on the milled sample using the Association of Official Analytical Chemists (AOAC) procedure. The crude protein value was 40.82% while the crude fiber was 25.1. The fungi (*Pleurotus plumonarious*) was introduced into the specimen bottle and kept in the incubator at room temperature for 7, 14, 21 and 40 days, the result was recorded

Keywords; Agro waste, Solid state fermentation, Substrate, Ecosystem, Biological degradation, Bioremediation, Metabolites.

Word count ; 248

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List of Acronyms

Abbreviation	Meanings
SSF	Solid State Fermentation
NA	Nutrient Agar
PDA	Potato Dextrose
FIIRO	Federal Institute of Industrial Research, Oshodi
GHGs	Green House Gases
PAHs	Polycyclic Aromatic Hydrocarbons
PM	Particulate Matter
AOAC	Association of Official Analytical Chemists

Chapter One

Introduction

1.1 Background to the Study

Annually, millions of tones of agro-wastes (wastes generated from agricultural activities) such as leaves, husks, stalk e.t.c. are generated worldwide which could be converted to different assets without causing destruction or draining the regular environment (ecosystem)[1]. Increase in agro waste generation which is as a result of increase in world's population from 3.7 billion in 1970 to 7.9 billion in 2021 and it is predicted to be 9 billion in 2050[2] which will become a treat to food security in years to come[3], thus to meet up the demand of teaming millions, there is rise in livestock breeding and crop production which increases the generation of agro wastes which becomes obvious and aggravated problem after harvesting of crops[4]. These agro wastes creates problems when they are not utilized or under utilised[2], keeping them on the farm could create an avenue for breeding pests and microbial pathogens that would attack new crops[5].

Agro wastes can be re-used for fertilizing the soil and suppress soil-borne disease in plants as well as supplying nitrogen, phosphorus, potassium and different supplements to plants[6]. It could also be used in producing animal feed, biofuel and catalysts accordingly, thus supplying extra income[7]. It also serves as raw material to certain industries and absorbent for the removal of contaminants[8].

Agro wastes contains soluble and insoluble components including cellulose and lignin (e.g sugar, amino acids and organic acids). Other parts incorporates minerals, lipids, oil, waxes, resin, protein, pigment[9]. The change of agro wastes into significant items, minerals extractions, agricultural upgrade and agro waste management is the consequence of microbial activities[10] which is named to be the best strategy for treating different wastes with biotechnical approach as well as eco-friendly, financially savvy and naturally supported[11].

Recently, there is a resurgence in interest in the obsolete innovation of fungal solid state fermentation4 because of expanded comprehension of the worth of biological materials as sustainable assets[2] for the creation of energy and feed[7] as well as significant wellsprings of synthetic feedstock for the development of different chemicals. Biochemical compounds that are suitable for food, chemical and pharmaceutical industries can be produced using agro-industrial by-products like lignocellulose wastes, corn cobs, sugar cane bagasse and coffee pulp which frequently cause serious environmental issues[9].

In India, majority of their companies use agricultural products as raw materials for animal feed and manure[10,13] and can still be refined to produce useful items which can be marketed. Categories of agro-based industries are: plant based, animal based food and non-food based agro wastes.

When wastes are not properly preserved, then utilized as substrates by some fungi such as *Aspergillus* spp. it produces aflatoxins which are carcinogenic in nature[11]. Most microorganisms finds it difficult to biodegrade some structures present in plant wastes as a result of this, the wastes become environmental nuisance and endanger the lives of other inhabitants of the environment[5]. When dairy animal feed on these wastes, the metabolites produced by the microorganism using the wastes get shed via milk and some of these metabolites are poisonous, which is being taken by human in milk and has led to many life threatening disease[12]. Many livestock died as a result of ingesting infected plant waste[12].

When these wastes are dumped in the aquatic ecosystem, the waste pollute the water, rendering it unfit and un-useful to people and makes it unwelcoming to tourists which leads to loss of revenue. Open burning of agricultural wastes generates enormous amount of Green House Gases (GHGs), Polycyclic Aromatic Hydrocarbons (PAHs) and Particulate Matters (PM).

These agricultural residues are rich in bioactive compounds and can be used for the production of biogas, biofuel, mushroom and serves as raw material in various researches and industries[10]. The use of agro wastes can aid reduction in production cost[14] and reduce the pollution load from the environment.

Sweetcorn (*Zea mays convar. Saccharata var. rugosa*) generally referred to as maize or sugar corn or pole corn is a starchy food kernels, yellow in colour and some other variety assortments like orange, purple, white dark. It can be referred to as vegetable when eaten fresh and when kernels are dried, it becomes a grain. Some of the agricultural wastes from sweet corn include: the leaves, husk, stalks, cobs and left over kernels. These agro wastes becomes environmental pollutants if left untreated and unutilized or under-utilized[5].

Recently, biodegradation of environmental wastes has been subject of interest in waste management. Biodegradation is a characteristic cycle by which natural or organic matters are made into simpler products by microorganisms like bacteria and fungi[14]. It is different from composting which is a human driven process in which biodegradation occurs under a specific set of circumstances[15].

In this cycle, natural pollutants fills in as the main wellspring of carbon and energy during the development. The cycle is in three folds: first the item goes through bio-decay or deterioration which is the mechanical debilitating of its structure, the bio-fragmentation follows which is the breakdown of materials by microorganisms and lastly absorption, which is the fuse of the old materials into the new cells[16]. Biodegradation is used in relation to ecology or nature waste management and ecological remediation[17].

Fungi are significant part of decomposing micro biota on the grounds that the microorganisms (bacteria) process the breakup of organic matter and are mindful significantly for the decomposition of carbon[18]. Degradation by fungi happens on different substrates, they acquire their food through parasitic or saprophytic mode wherein enzymatic reactions on the substrate they develop on and convert the dead bodies of plants and animals to less difficult structures. Fungi possess an efficient hydrolytic system that can convert lignocellulose material to essential metabolites for growth[19].

Effective biodegradation of agro wastes is important. Agricultural residues are considered for conversion into profitable products through solid-state fermentation (SSF)[20]. Agro wastes are potential substrates to produce value added products through SSF[20]. SSF produces secondary metabolites and essential enzymes.

1.2 Aim of the Study

To determine the Nutritional Content and Biodegradation Of Sweet Corn Leaves Using Edible Fungi (*Pleurotus pulmonarius*)

1.3 Objective of the Study

1. To determine the phytochemicals available in the sweet corn leaves
2. To determine the vitamins in sweet corn leaves.
3. To carefully analyze the minerals that can be found in sweet corn leaves.
4. To determine the effect of bio-decomposition/bio-degradation of sweet corn leaves using edible fungi (*Pleurotuspulmonarius*).

1.4 Statement of the Problem

Lots of agro wastes generated are unutilized or under - utilized , the accumulation of these wastes causes serious threat to human and animal in an environment and causes environmental pollution. Studies have demonstrated the way that different agricultural wastes could be dealt with and reused as raw materials in manufacturing industries and animal feed industries rather than dumping them into stream ,canals blocking water ways , roadside and burning them which could add up to the effect of global warming.

Accordingly, this research work has started the need to comprehend that agricultural wastes are underutilized and can be used ideally and the constructive outcome of its use which incorporates rebuilding of soil wellbeing and nutritional security (food safety).

1.5 Significance of the Study

Increase in agro waste generation may be attributed to increase in human population in an environment. These generated wastes causes environmental pollution as a result of lack of proper utilization and could cause serious health hazard to human and animals in the environment.

More work or research should be done to proffer solution to the problem of indiscriminate handling of agro wastes.This study determined how agro wastes could be converted to useful products like animal feed through biodegradation by edible fungi.

Endnotes

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Chapter Two

Literature Review

2.1 Maize (*Zea mays*)

Maize is also known as corn (North American and Australian English) is a cereal grain first found in by Mexico about 10,000 years ago[1,2]. It is now domesticated in every continent except Antarctica (the cold continent), it is only fair.

Since the introduction of corn into Europe by *Christopher Columbus* and other explorers and colonizers, corn has spread 'its tentacles' to all areas and regions of the world that favours its cultivation. Corn is used as livestock feed, as source of food for humans, as biofuel, as ornamental instrument, and as raw materials in industries[3,4].

Whether white red, yellow, black, Maize surely comes in different varieties[4]. However, the white and yellow varieties of corn or maize is most preferred by people depending on the region[4]. Some people think that the varieties with colours other than white and yellow are poisonous. That is not a fact. Maize consumption becomes poisonous when infested by microorganisms which in turn poses serious health conditions and can cause death[5].

Maize is tender and is a warm-season plant. Maize grows best in loose, well-drained slightly acidic soil of pH of 5.8 to 6.8[6]. The optimum temperature for maize germination according to research is 30^{o}C. At cooler temperatures, seed germination is slower and the seeds are vulnerable and very susceptible to diseases and infections[6].

The corn plant is a member of the grass family due to its "grass-like" structure[7]. It is tall, varying in height from less than one to greater than four meters (<1 - >4) m. the stem has a thickness of about three to four centimeters (3 – 4) cm. Maize is a monoecious plant – has both male and female reproductive parts on it and it is generally protandrous – The condition in which the male reproductive organ known as *stamens* of a flower mature before the female reproductive organ known as *carpels*[7]. The main shoot ends in a staminate tassel. The large narrow leaves have wavy margins and are spaced alternatively on opposite sides of the stem. Varieties of yellow and white corn are the most popular as food although there are other

varieties with red, blue, pink and black kernels. Each ear is enclosed by modified leaves called *shucks* or *husks*[7].

Whole grain corn is a healthy cereal grain rich in fiber, vitamins, minerals and antioxidants[4].

2.1.1 Commercial Classifications Based Mainly on Kernel Texture

1. **Dent corn:** Dent corn, primarily grown for animal feed and food manufacturing is popularly grown in The United States of America. Dent corn has its name from the dimple-like dents on the top of the corn kernels when ready for harvesting. Dent corn can be used to make corn flakes, corn oil and "invisible corn" which is used for livestock feed. The depression in the crown of the kernel is caused by unequal drying of the hard and soft starch[8] making up the kernel. Dent corn is cultivated as a row crop grown commercially. Dent corn has high soft starch content.

2. **Flint corn:** Flint corn, also known as Calico corn or Indian corn is a type of corn whose kernel has a hard outer layer which protects the soft endosperm[8]. Due to the hard texture of its outer layer, it derives its name from *flint*, a hard sedimentary rock. Flint corn has very low water content. It is resistant to freezing. It contains little soft starch, often multicolored and has no depression. It is used ornamentally as part of thanksgiving decorations.

3. **Flour corn:** It is composed largely of soft starch and has a thin pericarp. It contains soft, mealy, easily ground kernels and is an important source of corn flour which is used to produce many dishes all around the world.

4. **Sweet corn:** Sweet corn, also known as *sugar corn* or *pole corn* is commonly sold fresh, frozen or canned as a vegetable. It has high sugar content and the plant sugar is not converted to starch as in other types of corn. It is widely used in the culinary world to make side dishes, toppings, salads and soups. It is a very good for the health and helps control type 2 diabetes even with its sweetness. This is so because the sweet corn sugar is absorbed slowly thus, stabilizing the blood sugar.

5. **Popcorn:** Is a special type of flint corn characterized by expanding and puffing up when heated. The endosperm turns into steam as the kernel is heated and continuous pressure from steam causes the rupture of the kernel's strong hull, causing the forceful expansion of the kernel to about 30 – 50 times its original size. Expansion and the forceful explosion of the kernel is because it is devoid of soft starch, causing the moisture in the cells to expand.

Upgrades in corn have resulted from hybridization, based on crossbreeding of superior inbred strains.

6. Dent corn – *Zea mays* var. indentata •Flint corn – *Zea mays* var. indurata
7. Flour corn – *Zea mays* var. amylacea
8. Sweet corn – *Zea mays* var. saccharata
9. Popcorn – *Zea mays* var. everta

2.1.2 Scientific Classification of Maize

Kingdom	Plantae

Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Clade	Commelinids
Order	Poales
Family	Poaceae
Subfamily	Panicoideae
Genus	Zea
Species	Z.mays

Maize has become a very common staple food in many parts of the world, with the total production of maize surpassing that of wheat or rice.

2.1.3 Corn Nutrition

In one ear of corn, these nutrients can obtained per serving:

10. Calories: 90g
11. Protein: 3g
12. Fat: 1g
13. Carbohydrate: 19g

14. Fiber: 2g

15. Sugars: 6g

16. Vitamin C: 7mg

Corn is composed primarily of carbs, just like any other cereal grain. Its main carb is starch, comprising 28-80% of its dry mass. Sweet corn, a type of corn has high sugar content, at 18% of the dry mass[9]. The sugar is mostly sucrose[9]. Despite its sweetness, it is not a high glycemic food. It ranks low or medium on the GI (Glycemic Index). Glycemic Index is a measure of how quickly carbs are digested and absorbed in the body. Sugar in sweet corn digests very slowly, hence its inability to cause diabetes. However, diabetic patients are advised to eat sweet corn in moderation[10].

The amount of fiber in corn is fair and the fiber content of various corn type varies. Hemicellulose, cellulose and lignin are insoluble fibers predominant in corn[10].

Corn is a reasonable source of protein. Protein ranges from 10-15% depending on the variety of corn. *Zeins* is the commonest protein in corn, attributing for 44-79% of the overall protein content but it is a poor protein because it lacks some essential amino acids. However, zeins is useful in the production of adhesives, inks, candy, nuts and pill coatings.

Fat accounts for about 5-6% of the entire corn dry mass, making it a low fat food. However, corn germ, which is a rich by-product of corn milling is plenteous in fat and used to produce corn oil, a common cooking product. Corn oil contains vitamin E, phytosterols and ubiquinone (Q10), which increases its shelf life and reduces cholesterol levels.

Corn also contains vitamins and minerals which varies with corn type. Sweet corn is rich in vitamins while popcorn is rich in minerals. Manganese, phosphorus, magnesium, zinc, and copper can be found in popcorn in varying amounts. Pantothenic acid, folate, vitamin B_6, niacin, and potassium can be found in sweet corn, also in varying amounts. Cooking corn with lime makes niacin more available for digestion and absorption.

Maize is widely cultivated all over the world and a greater weight of maize is produced every year than any other cereal. In 2020, the total production was 1.16billion tonnes, led by the United States with 31.0% of the total table[11].

Country	Production(millions of tonnes)
United States	360.3

China	260.7
Brazil	104.0
Argentina	58.4
Ukraine	30.3
India	30.2
Mexico	27.4
Indonesia	22.5
South Africa	15.3
Russia	13.9

2.1.4 Physiological Disease of Maize Plant

The maize plant is a heavy feeder. Even on well-nourished soils, additional consideration should be made in enriching the soil organically or inorganically periodically to maintain healthy crops.

The atmosphere and compost or manure are two natural sources of minerals needed by plants to flourish. Minerals are elements needed by plants in little or large amounts for healthy growth[12]. Minerals needed in little amounts are called *trace elements* or *micro elements* while those needed in large quantities are known as *macro elements*.

Every element, micro or macro has an essential effect on a maize plant and deficiency or surplus in any of these element can pose a great harm and damage to the maize plant ranging from stunted growth to yellowing of leaves to spotted leaves and so on. Below are some of the macro elements required in a maize plant, the effects of their deficiencies and excesses[13].

1. Nitrogen (N): Nitrogen is essential for lush green growth of the maize plant. It also contributes greatly to increase in yield and healthy grains. Maize plants deficient in nitrogen show sparse growth. Also, their leaves loose the lush green colouration and turn pale green[12,13]. The older leaves turn yellow and necrosis (death of cell tissues) occurs. Low or high soil pH increases the damage so does sandy soil, which is prone to leaching[14] (the condition in which soil nutrients are washed down the soil by rain or erosion, away from the plant due to large soil pores and lightness of the soil). Too much nitrogen uptake in maize plant lowers uptake of other nutrients, which reduces optimum yield, and also increases the susceptibility of maize plants to diseases.

2. Phosphorus (P): Phosphorus is a very essential nutrient needed for healthy plant growth. It is essential for triggering root, shoot and overall healthy seedling growth. Phosphorus is most important during the early stages of growth in maize plants. The lower the level of phosphorus available for use by the maize plant, the more difficult it is for growth, stress tolerance and reproduction to occur in a maize plant. Deficiency of phosphorus in maize plant is characterized by stunted growth, thinned stem, and tinges of purple on leaf margins, stems and veins, growth of dark green leaves. Reddish discolouration is visible in the early stage of the plant's life.

3. Potassium (K): Potassium is a very essential mineral in every plant, in maize plant. It is necessary for optimum growth in corn plant. It increases the crop's resistance to diseases and water stress tolerance. Optimum amount of potassium available to corn plant use makes the uptake of other nutrients by the plant easy. Dry soil conditions limits the effective uptake of potassium by the corn plant. Deficiency of potassium in corn is characterized by narrowed stems and poorly filled grains, plant peaks, and cob tips. The leaves margin also turn yellow and brown as it appears like firing or drying. The symptom advances from lower to upper leaves. Excess potassium intake limits overall plant nutrition.

4. Sulphur(S): Sulphur is a secondary macronutrient. It is essential for protein synthesis and it is required for a lot of plant functions which includes reproduction, nutrition, nitrogen fixation and chlorophyll formation. Sulphur deficiency in maize is characterized by light green colouration, stunted plant growth and delayed maturity. Also, symptoms appear on younger leaves where yellow colour stripes (interveinal chlorosis) appear.

5. Zinc (Zn): Zinc is the commonest micronutrient deficiency in corn. It is important in the production of auxins like indoleacetic acid (IAA). Zinc plays a vital role in cell elongation, carbohydrate metabolism and chlorophyll production, which in turn affects early ear development or growth and leaf size. A consistent supply of zinc is therefore needed for optimum growth and development in maize plants. Zinc deficiency is characterized by shortened internodes on the maize stalk, upper leaves show broad bands of yellow colouration and later, turn pale brown or gray known as *necrosis* (dead spots). The symptoms appear first in the middle of the leaves and progresses outward.

2.1.5 Insects That Affects Maize

6. African armyworm(*Spodoptera exempta*)
7. African sugarcane(*Eldana saccharina*)
8. Asian corn borer(*Ostinia furnacalis*)
9. Common armyworm(*Pseudoletia unipuncta*)
10. Common earwig(*Forticular auricularia*)
11. Corn delphacid(Peregrinns maidis)
12. Corn rootworms(*Diabiotica spp*)
13. Corn silkfly(*Euxesta stigmatias*)
14. Maize weevil(*Sitophilus zeamais*)
15. Southwesthern Cornborer(*Diatraea grandiosella*)
16. Stalk borer(Papaipema nebris)

Corn can get contaminated by fungi known as *Aspergillus flavus.* Aflatoxins cannot be destroyed by normal food processing such as boiling, baking, fermentation. Consumption of aflatoxin contaminated corn causes severe cellular damage and poisoning even death, in the long run. It can also cause genetic mutations and alteration of cells[15].

Aflatoxin contamination is very difficult to test for because concentrations of the toxin could be within little amounts of end products of corn rather than consistently all through the product. Aflatoxin contamination becomes worse with extreme climatic conditions.

However, to get rid of the toxin in corn, cooking the maize under high pressure, reduces the quantity of aflatoxins in corn, according to research. Normal cooking method is not capable of reducing the level of aflatoxin in corn because aflatoxins are highly thermo stable[15].

Also, mycotoxins (aflatoxins) can be removed or gotten rid of chemically using acids, bases, reducing and oxidizing agents. Studies have revealed that some of these chemicals have the ability to completely destroy or extinguish mycotoxins[16,17]. Organic acids can get rid of aflatoxins in corn[15,16].

Peanuts, also get affected by aflatoxins . Grains, plant parts and finished products affected by aflatoxins , when ingested by animals also affect animal products[17].

Corn contains anti-nutrients which are chemical compounds that prevent the human body from absorbing nutrients more than it should. However, soaking corn in water for prolonged period of time can remove many of them.

2.1.6 Common Diseases of Corn

1. Corn smut (*Ustilago maydis*): Is a fungal disease.
2. Northern Corn leaf blight
3. Maize downy mildew(*Peronosclerospora spp*)
4. Maize dwarf mosaic virus
5. Grey leaf spot
6. Stalk rot
7. Goss' wilt

2.1.7 Health Benefits of Corn

8. The fiber in corn helps to prevent colon (small intestine) cancer, regulates bowel movements, and maintains blood sugar level.
9. Popcorn helps prevent pouches in the walls of the colon (diverticulitis).
10. Corn is rich in Vitamin C, an antioxidant that helps protect the cells from damage and wards off diseases like cancer and heart diseases.
11. Corn is a good source of lutein, a carotenoid that is similar to vitamin A, commonly known for good eye health.
12. Corn is also rich in Vitamin B, E, K and minerals like Magnesium, Potassium, and Zinc, which are very necessary for overall human health.
13. It promotes healthy brain function.
14. *Quercetin*, an antioxidant found in corn plays a very vital role in treating *prostatitis* (Inflammation of the prostrate), a disease common among many men.

2.2 Sweet Corn

Sweet corn is also known as *sugar corn*. It is highly nutritional and healthy, cultivated all over the world. According to Mine *et al.*(2018), it is grown for human consumption and a raw or processed product in the food industry worldwide.

Sweet corn can be obtained in canned, fresh and frozen forms in the market. The awesome sweetness of sweet corn is as a result of the naturally occurring recessive mutation in the corn genes which controls the conversion of more sugar compared to starch inside the endosperm of

the corn kernel[17]. Sweet corn is harvested when still in the milk stage and is either prepared or eaten as a vegetable rather than field corn which is harvested when the kernels are dry and mature. The process of maturation in corn involves converting sugar to starch but in sweet corn, starch is stored poorly and must be eaten fresh, canned or frozen to avoid the kernels becoming tough and starchy.

2.2.1 Anatomy of Sweet Corn

The fruit of the sweet corn plant is called the corn kernel which is also known as *Caryopsis*. The accumulation of kernels on the cob is known as *ear*. Being a monocot, there is always an even number of rows of kernels. The husk is a tightlywrapped leave-like structure around the ear. Silk is the hair-like structure, of the pistillate flowers which emerge from the husk. The husk, silk, kernel and ear are diagrammatically explained in Figure 1 above. The silk and husk can be handremoved before processing.

2.2.2 Nutritional Value of Sweet Corn

The nutritional composition of sweet corn (100gm) is diagrammatically explained below;

1. Calories: 86kcal
2. Carbohydrate: 18.70g
3. Protein: 3.27g
4. Fat: 1.35g
5. Dietary fiber : 2g

Sweet corn is moderately rich in dietary fiber which is important for our health as it helps regulate blood sugar levels, decrease the risk of high cholesterol and prevent constipation. For weigh loss or weight maintenance, pairing sweet corn with other vegetables in a soup or salad increases the fiber level. Dietary fiber aids digestion, reduces the risk of heart disease, type 2 diabetes and bowel cancer. Fiber also helps one to stay fuller for longer period of time.

2.2.3 Similarities and differences between Sweet Corn and Field Corn

The similarities between sweet corn and field corn are;

6. Field corn and sweet corn are either called maize or corn and both contain starch.
7. Both have the same appearance and shape.
8. They are both sources of food rich in health boosting nutrients.
9. Both are used in making variety of delicacies.

The differences between sweet corn and field corn are;

10. They have different tastes. Sweet corn tastes better and sweeter.
11. Field corn plant is taller and bears more leaves.
12. Harvesting sweet corn is done more carefully to preserve its flavor and sweetness.
13. Field corn is mostly eaten by animals while sweet corn is mostly eaten by humans.

2.2.4 Sweet Corn Leaves

Sweet corn leaves are the blades that sprout from alternate sides of the corn stalk. Maize plants have numerous leaves on the stalk. The leaves are lance-shaped or obovate and typically grow up slightly before curving downwards.

The management for field corn, sweet corn and seed corn plant diseases is achieved through a combined approach of best management practices and use of foliar fungicides and fungicide seed treatments. Many corn diseases including those caused by fungi, bacteria and malnutrition manifest in the leaves. The following below are the most common diseases of sweet corn leaves;

14. Gray leaf spot
15. Anthracnose
16. Eye spot
17. Tar spot
18. Northern corn leaf blight
19. Southern corn leaf blight.

2.2.5 Uses of Sweet Corn Leaves

The following below are the uses of sweet corn leaves;

20. Ground corn leaves is used to make herbal tea.
21. It is used as beer flavor.
22. It is used to make cornmeal pancakes and dried corn pudding.
23. It is used as heating fuel.

24. It can be dried and ground into flour to make porridge, bread.

25. It can be used as food for animals.

2.2.6 Phytochemicals in Sweet Corn Leaves

Cereals contain a lot range of phytochemicals showing health benefits of lowering the risk of the occurrence of chronic diseases. As a commonly consumed grain, corn has a very special profile of nutrients and phytochemicals compared to other whole grains[18]. Because of its high nutritional quality, corn is a global crop used to fulfil the nutritional requirement of humans, cattle and other animals.

Phytochemicals are non-wholesome bioactive mixtures tracked down in different pieces of a plant. These mixtures safeguard plants from hunters and cruel natural circumstances. They are fundamental in the drug and clinical world because of their cancer prevention agent, antimicrobial and other natural properties. The phytochemicals found in sweet corn leaves are recorded and made sense of below;

26. **Flavonoid**: is the bioactive phytochemical compounds which make the plant impervious to the assault of microbes and insects and furthermore shield creatures from different sicknesses. It has solid cancer prevention agent movement, free radical-scavenging limit and restrains protein glycation.

27. **Tannins**: Are polyphenolic intensifies which show a few biological activities like calming, hostile to oxidant, free-radical scavenging and against mutagenic activities. They are normally found in eatable and unpalatable plants including tree rind, leaves,

flavors, nuts, seeds, leafy foods. Plants produce them as normal safeguard against bothers, it additionally variety and flavor to establish food sources.

28. **Saponin**: Are severe tasting typically harmful plant-determined natural synthetics that have a frothy quality when unsettled in water. They are fat and water dissolvable which gives them their typical lathery properties. They are utilized for synthesis of steroids and in carbonated refreshments. They safeguard sound plants structure bugs, contagious and bacterial microbes subsequently ingesting food that contain saponin can cause poisonousness in the human body nonetheless, serious harming is uncommon.

29. **Alkaloids**: Is a class of normally happening natural nitrogen containing compounds habitually tracked down in the plant kingdom. They safeguard plants from predators, control their development, stockpiling repositories of nitrogen and substitutes for minerals in plants.

30. **Oxalate**: Is an organic acid found in plants that regulates tissue calcium, protect it.

31. **Phenol**: Is comprehensively disseminated in plants and has strong cancer prevention agent properties. Plant phenolics are by and large engaged with guard against ultraviolet radiation or hostility by pathogenic microorganisms, parasites and predators. It additionally adds to establish plant colours and bitterness.

32. **Phytate**: Is the significant stockpiling type of phosphorus in plants while growing starts. Animals can't digest it since they don't secrete phytase, the enzyme expected to breakdown phytate and discharge phosphorus. They are normally occurring compounds tracked down in cereals and vegetables.

All pieces of corn plant are great wellsprings of phytochemical compounds which have antioxidants (substances which can forestall the oxidation response in living and non-living system and have hydrogen giving capacity) possibilities. The presence of phytochemicals in corn makes it a restorative plant which can be utilized for the treatment of numerous sicknesses.

2.2.7 Micro and Macro Nutrients in Sweet Corn

Each 100g of corn gives 365calories and each 100g of sweet corn gives 86calories. Carbohydrate and water are the super compound substances in corn. The starch content in corn is near 75% while in sweet corn, it records to around 18%. The water content in corn is around 10% while in sweet corn, it records to around 75%.

Corn and sweet corn give assortment of nutrients. The wholesome profiles of corn and sweet corn are comparable except for L-ascorbic acid (Vitamin C) which is tracked down just in sweet corn. L-ascorbic acid is a cancer prevention agent that assists with shielding the body cells from harm and avoids illnesses like malignant growth and heart sicknesses[19,20].

1.Vitamins

1. **Thiamin (Vitamin B_1):** Is found in food varieties like yeast, oat, grains, beans, nuts and meat. It helps convert food into energy to keep the sensory system sound. It is utilized to treat low thiamine, beriberi, and certain nerve infections.
2. **Niacin (Vitamin B_3):** Is a water dissolvable nutrient tracked down normally in certain food sources and sold as supplement. It helps convert nutrients into energy. In plants, particularly mature cereal grains like corn and wheat, niacin might will undoubtedly

sugar atoms in type of glycosides which fundamentally decline its bio-accessibility. The presence of niacin in grains enhances its processed items.

3. **Pantothenic acid (Vitamin B_5):** Is utilized to make co-catalyst A, a chemical compound that helps enzymes fabricate and separate unsaturated fats as well as carry out other metabolic roles and acyl transporter protein which is likewise engaged with building fats. It is found in a wide assortment of food including grains. It is likewise a water solvent vitamin.

4. **Riboflavin (Vitamin B_2):** Is one of the nutrients added to improve grain items like advanced flour. It is engaged with many body processes for instance; appropriate advancement of the skin, lining of the gastrointestinal system, platelets and legitimate cerebrum capability.

2. Minerals

1. **Selenium**: This is a helpful element for higher plants that upgrades cell reinforcement digestion, photosynthesis, secondary metabolites and carbs in plants. It assists with inhibiting the harm brought about by environmental change like dry season, saltiness, weighty metals and outrageous temperature. It safeguards the chlorophyll, which is a photosynthetic pigment.

2. **Potassium**: this is related with the movement of water, supplements and sugars in plant tissues. It is associated with catalyst enactment inside the plant, which influences protein, starch and adenosine triphosphate (ATP) creation (ATP directs the pace of photosynthesis). It likewise guarantees ideal plant development particularly in fruiting and flowering. Potassium lacking plants tend to wither on dry, bright days while a lot of

it in plants can be undesirable since it influences the manner in which the soil retains other basic supplements.

3. **Magnesium**: This is the central core of the chlorophyll molecule in plant tissue just like Iron (Fe) is in the haemoglobin molecule. Chlorophyll has the same molecular structure as haemoglobin, the molecule in red blood cells. Like any other mineral, inadequate quantity of magnesium in a plant can be fatal to it. Inadequate magnesium in a plant is characterized by poor and stunted growth. It is present in all green plants and is necessary to conduct photosynthesis. It also acts as an enzyme and helps in plant respiration. Without magnesium, it is impossible for a plant to live.

4. **Phosphorus**: Phosphorus in plants is the way to catching, putting away and changing over the sun powered energy into biomolecules like Adenosine triphosphate (ATP) that determines biochemical responses for example photosynthesis from germination through the arrangement of grain to development. It is significant in cell division and improvement of new tissues. The lack of phosphorus in plants forestalls shoot development while an excessive amount of phosphorus in the dirt can cause lacks in zinc and iron in the dirt as they become inaccessible for plant use.

5. **Iron**: It is likewise expected for photosynthesis and chlorophyll synthesis in plants. The accessibility of iron in the soil directs the dispersion of plant species in normal environment and cutoff points yield and nourishing nature of harvests. Non-haem iron is found in plant food varieties like whole grains, nuts, seeds, vegetables and salad greens.

6. **Zinc**: Zinc is a significant part of different compounds that is liable for driving numerous metabolic responses in all plants. Development and improvement will stop if specific

enzymes are absent in plant tissue. It is a micronutrient fundamental for plant development. Without zinc, the plant will encounter hindered development, appearance of earthy colored spots on plant leaves and development of contorted leaves.

7. **Copper**: it is one of the eight fundamental plant micronutrients expected for the majority enzymatic exercises. It is required additionally for chlorophyll and seed creation. It is realized that a high sum or level of substances, even the main ones can be exceptionally unsafe and poisonous in living creatures thus, it is not out of the question that an elevated degree of copper in plants can be harmful to plants and its lack can prompt expanded weakness to illnesses which can cause critical yield misfortune in little grains. Likewise, the synthesis of vitamin A is firmly weaved to the presence of copper in plants.

2.3 Agricultural Waste

Agricultural wastes are otherwise called agro wastes. They are the remainders, deposits and left-overs from agrarian activities from cultivation of the land to handling of agricultural produce from the land, raising of animals to animal products gathering and handling. Agricultural wastes are the non-item result of agrarian/agricultural creation and items which might contain components and substances that can be helpful to man with monetary qualities that are not exactly the expense of assortment, transportation and handling for gainful use[21].

Agricultural wastes contain animal wastes (excrement, animal remains), food handling waste (only 20% of maize is canned and 80% is a waste), crop waste (corn stalk, sugar cane bagasse) and risky and harmful agricultural waste (pesticides, insecticides, herbicides). The increment and extension of agricultural production has normally brought about the increment of agrarian wastes[22]. The wastes created rely upon the kinds of agricultural exercises completed.

Practically all farming exercises create wastes even in huge amounts when done for an enormous scope. These wastes comprise disturbance to the climate, present serious wellbeing dangers to people and even to animals through ecological contamination[21]. Taking care of them might bring about colossal financial misfortune and a danger to food security.

2.3.1 Types of Agro Wastes

Farm residue is an illustration of agricultural waste which can be additionally named field deposits/residues and processing residues.

Field deposits are the remaining plant parts in the field after crop harvesting which incorporates; leaves, stalks, stem, seed units, while processing residues are leftovers after the harvest has been handled/processed into one more valuable product for instance; husks, leaves, stalks, shell, roots and other homestead remainders handled to take care of for creatures, composts, soil improvement and different activities[23].

Agricultural wastes can likewise be ordered in light of their accessibility as well as characteristics that separate them from other solid fuels like charcoal, wood and singe briquettes.

Industrial deposits: Food processing organizations like juice, meat, chips, and confectionary organizations make gigantic measure of organic residues and related effluents. The organic left overs can be utilized to produce an assortment of fuel source. Wastes from food enterprises contains a critical centralization of BOD, COD and other suspended materials which were left unutilized or untreated which adversely affects the climate, human and animal wellbeing.

2.3.2 Agricultural Waste Generation

The waste produced relies upon the kind of agricultural exercises completed[21].

1. **Waste from farmland development activities:** During the time spent developing insects and weeds on ranch land, there is need for pesticides and herbicides, subsequent to utilizing them, farmers could toss their containers into fields, lakes causing erratic natural outcome. For example, food contamination, risky food cleanliness and contaminated farm land. Likewise, during extreme utilization of manures, some of it goes into lakes, some held in the dirt which will bring about the contamination of land and water surface, some will dissipate and will become denitrated causing air contamination.

2. **Livestock production:** these incorporate solid wastes, for example, excrement and organic materials in the butcher house, waste water, for example, urine, cage wash water, waste water from washing animals and cleaning of butcher house, air poisons like H_2S, CH_4 and odours. These untreated wastes can produce ozone harming substances, adversely affect soil fertility and cause water contamination. It can likewise spread illnesses to humans and animals and affects the climate.

3. **Waste from aquaculture:** how much feed utilized in aquaculture will decide the amount of waste created. One significant waste in aquaculture is the metabolic waste, which can be suspended or broken up. The temperature around animals influences their feeding. Rise in temperature expands their food consumption hence increments in measure of waste created.

4. **Waste utilization course:** the agro waste created should be utilized quickly or stored for additional utilization in a condition that won't permit its deterioration or render it

unacceptable for processing into the ideal finished result. There are different application to which these wastes can be utilized;

1. Fertilizer application.
2. Absorbents in the elimination of heavy metals.
3. Pyrolysis (heating of agro waste to a temperature of 400-600^{o}C in the absence of oxygen to vapourize a portion of the material leaving a char behind.
4. Animal feed.

2.3.3 Influence of Agricultural Solid Wastes on Human Health and the Environment

1. Improper treatment of agro solid wastes impacts change in environment which hampers food creation.
2. Gas discharge from greenhouse if not decreased will be danger to the plant and its occupants.
3. Unpredictable burning of wastes produces environment related outflows.
4. Obstructing of streams by solid wastes can result into flooding.

2.3.4 Effective Management of Agro Solid Wastes

5. Fertilizing the soil: Kitchen agro wastes could be utilized as animal feed due to their high organic matter constituent and supplements.
6. Substitute for mushroom development: numerous agro wastes like leaves could act as substrate on which fungi (mushroom) can be developed[21].

7. Non-customary feed fixings: non-customary feed fixing *mycomeat* has been created from agro solid wastes which fill in as substrate, the wastes filled in as substrate and a combination of the substrate and the developed fungi (mushroom) was fed to broiler chicks as feed.

8. Conventional soap making: some agro wastes like cocoa pod core are used to make black soap.

9. Elective energy source and bio-fuel creation

10. Creation of silica: agro strong wastes like corn cob, rice husk are expected wellspring of silica.

2.4 Fungi

Fungus, plural fungi are among the most widely distributed organism on the earth. Yeasts and mushrooms have been used by humans for way so long a time yet, the biology of fungi has been poorly understood until recently. Fungi had been classified as plants because like plants, they are firmly rooted to the ground, they possess root-like and stem-like structures. However, after a lot of research, it has been proven that fungi are more of animals than plants. They share characteristics rather than share a single common ancestor.

Fungi are eukaryotic organisms bearing complex cellular arrangement. They possess double membrane-bound nucleus whose DNA is wrapped around histone proteins just like in other eukaryotic cells. In contrast to plants, fungi do not possess chlorophyll or chloroplast neither do they undergo photosynthesis. They are heterotrophs and obtain all nutrient necessary for growth from their diet. Unlike a lot of animals which ingest food to gain nutrients (digestion), fungi

carries out feeding in the reverse order. Digestion first, then ingestion. Food nutrients are digested, broken down by enzymes secreted through the hyphae into the environment followed by the ingestion of broken down food particles due to the exo-digestion process through the large surface area of the mycelium.

Fungi are mostly saprophytic in nature i.e. organisms that feed on and derive nutrients from dead and decaying organic matter, especially plant material. The digestive enzyme secreted by fungi is able to break down complex polysaccharide such as lignin and cellulose. Complex chemical structures that cannot be broken down by humans, animals or even plants. Fungi is a very important organism in the ecosystem.

They reproduce sexually and/or asexually. Asexually through a lot of processes which includes; fragmentation (new colonies emerge from fragments of hyphae), budding (a mitotic division of cell to produce buds, new organisms) and sporulation, also mitotic involves the release of spores onto the environment to induce the growth of young organisms. Fungal spores are so much lighter that plant pores. Perfect mushrooms reproduce sexually and asexually and the imperfect ones reproduce asexually through a process called mitosis.

Fungi thrive well in moist and slightly acidic environments. They can grow with or without light and require oxygen to survive. They are of great economic importance as they can be harmful and useful to the environment. Many of them possess very bright colours due to cellular pigments. The cellular pigments helps protect them from ultraviolet radiation and some of them are poisonous. Fungi are of great medicinal and environmental importance.

Examples of fungi are; yeasts, rusts, smuts, mildews, moulds, mushrooms and so on.

2.4.1 Structure of Fungi

The following points explain the structure of fungi:

1. Fungi possesses a dense and clear nucleus which is surrounded by a protein known as histone.
2. Fungi possesses thread-like structure which are long called hyphae. A cluster of hyphae in mesh-like order forms a structure called mycelium.
3. The cell wall of fungi is made of polysaccharides and chitin. They also possess pigments which may be poisonous but help shield them from ultraviolet rays.
4. The cell wall comprises a protoplast, which is differentiated into other cell parts such as cell organelles and nuclei.
5. Almost all the fungi possess filamentous structure except the yeast cell.
6. Fungi are eukaryotic cells (organisms or cells which contain a nucleus and other membrane bound organelles).

2.4.2 Characteristics of Fungi

7. Fungi possess very small nuclei.
8. Fungi store food in form of starch.
9. Biosynthesis of the chitin occurs in fungi.
10. Fungi has no embryonic stage, some develop from the spores.
11. They lack chlorophyll hence cannot undergo photosynthesis.
12. Some fungi reproduce by means of spores. The process is called sporulation.

13. They exhibit the phenomenon of alternation of generation.

14. They are eukaryotic, non-vascular, non-motile and heterotrophic organism.

15. Their mode of reproduction is sexual or asexual.

16. They are parasitic and can infect the host.

2.4.3 Classification of Fungi

1. Based on nutrition;

1. **Saprophytic nutrition:** Fungi feeds on dead and decaying organic substances through exo-digestion. For example Rhizopus. Penicillum, Aspergillus.

2. **Parasitic**: Fungi lives on other living organisms (plant or animals) and absorb nutrients from their hosts. This is a lot more harmful to the host (organism a parasite is attached to). Absorption of nutrients from the host leaves the host malnourished and can lead to the death of the host. For example: Puccinia, Taphrina.

3. **Symbiotic nutrition:** Fungi lives by having independent relationship with other species in which they both mutually benefit. There is no organism harmed in a symbiotic relationship. It is a mutual one. For example; Lichen, Mycorrhiza.

2. Based on spore formation

1. **Zygomecytes:** This is formed by the fusion of two different cells. The sexual spores are known as *zygosperes* while the asexual are known as *sporangiosphores.* For example; mucor.

2. **Ascomycetes**: They are known as *sac fungi*. They can be decomposers, parasitic or saprophytic. The sexual spores are called *ascospores* and asexual reproduction occurs by spores called *condiospores.* For example; saccharomyces.

3. **Bacidomycetes**: These fungi are the commonest bacidomycetes and they live as parasites. Sexual reproduction occurs by *bacidiospores* and asexually by *conidia* also by budding in fragmentation. For example; Agaricus.

4. **Deuteromycetes**: These fungi are also known as *imperfect fungi* because they do not follow the regular reproduction cycle as other fungi. They do not undergo sexual reproduction but reproduce asexually by *conidia.* For example; Trichoderma.

2.4.4 Reproduction in Fungi

Reproduction in fungi is both sexual and asexual. The sexual mode of reproduction is known as teleomorph and the asexual mode of reproduction is known as anamorph.

1. **Vegetative reproduction:** this takes place by budding, fission and fragmentation. It involves the reproduction of new fungal organisms using the vegetative/ somatic part of the fungi. It is mitotic in nature.

2. **Asexual reproduction:** this takes place with the help of spores called conidia or zoospores or sporangiospores.

3. **Sexual reproduction:** this reproduction occurs by ascospores, basidiospores and oospores.

2.4.5 Uses of Fungi

Fungi are very important organisms in the ecosystem. Without them, the ecosystem food chain will be useless and incomplete. They are also of great importance to animals and humans. The following points below are some of the uses of fungi in the environment;

4. **Reusing**: Fungi help to reuse/recycle dead and rotten matter.
5. **Source of food:** Mushrooms (a fungi) which are refined or eatable are utilized as food by humans.
6. **Prescriptions/medicine:** Fungi are utilized in the development of antitoxins and to control illnesses in human and animals for instance; Penicillin from Penicillium.
7. **Bio-control specialists:** Fungi are associated with taking advantage of bugs, little worms and help in controlling pests. Spores of organisms are utilized as spray on crops.
8. **Food waste:** Fungi help in reusing organic materials and are additionally answerable for significant deterioration and financial misfortunes of put away food.

2.5 Edible mushroom

They are palatable meaty specie of macro fungi (organisms which bear fruiting structures that are sufficiently enormous to be seen with the unaided eye). They can show up above or beneath the ground. Edibility might be characterized as a measures that remembers the shortfall of

noxious impacts for human. Eatable mushrooms are consumed for their dietary and culinary worth. They incorporate numerous fungal species that are either collected wild or developed.

Mushrooms are cholesterol free and contain modest quantity of fundamental amino acids and vitamin B. The consumable mushroom should be distinguished appropriately prior to eating since certain mushrooms are profoundly noxious.

2.5.1 Nutritional Content of a Mushroom

Nutrient	Amount of Nutrient(In one cup of mushroom)
Calories (Energy)	21.1
Protein(g)	3.0
Carbohydrate(g)	3.1 including 1.9g of sugar
Calcium(mg)	2.9
Iron(mg)	8.6
Phosphorus(mg)	82.6
Potassium(mg)	305
Sodium (mg)	4.8
Zinc(mg)	0.5
Copper(mcg)	305
Selenium(mcg)	8.9
Chlorine(mg)	16.6
Folate(mcg DFE)	16.3

Mushroom also contains vitamin B_5 including Thiamine, Riboflavin, B_6 and B_{12}, also vitamin C and D.

Red brown mushrooms are:

1. Water – 92%
2. Carbohydrate – 4%
3. Protein – 2%
4. Fat – Less than 1%

2.5.2 Health Benefits of Mushroom

5. Mushroom contains protein, vitamins, minerals and cell reinforcements (synthetic/chemical substances that assist the body with wiping out free radicals). Every one of these have different medical advantages. Oxidants in mushroom are; Selenium, L-ascorbic acid, Chlorine.
6. The cell reinforcement/antioxidant content in mushroom forestalls lung, prostrate, breast and different kinds of malignant growth, as per The National Cancer Institute.
7. Mushroom contains vitamin D which forestalls and treats a few sorts of harmful and cancerous infections.
8. Potassium in mushrooms assists with directing blood pressure and decreases hazard of hypertension and cardiovascular sicknesses.

9. It gives foliate or folic acid which is an imperative enhancement/supplement for pregnant women to help fetal wellbeing

10. Mushrooms can be utilized for drying fleece and other natural fibers. Before the approach of engineered colors (synthetic colours), mushrooms were the wellspring of numerous textile colors.

11. A few mushrooms are utilized as fire starters. They are otherwise called *tinder fungi*.

12. White button mushrooms can be utilized or eaten instead of red meat to accomplish solid weight reduction.

13. The consumption of mushroom upgrades generally nourishment and diet quality.

14. Mushrooms enhances immune system because of the long chain polysaccharide particularly the alpha (α) and beta (β) glycan particles present in them.

2.6 Biodegradation

Fungi are achlorophyllous creatures/organisms and rely upon other living organic entities for their food either being a parasite or saprophyte. Saprophyte fungi are great bio-degraders. Biodegradation is the naturally catalyzed decrease in the intricacy of chemical compounds. It is the most common way of breaking natural substances into smaller compounds by living microbial organisms.

Mineralization: when biodegradation is finished, the interaction is called *mineralization*.

Mycodegradation: This is the course of biodegradation by fungi.

2.6.1 Mechanism of Fungal Degradation

Degradation by fungi happens on different substrates. They acquire their food through parasitic or saprophytic mode wherein enzymatic reactions on the substrate they develop on and convert the dead bodies of plants and animals to less difficult structures.

Eatable mushroom grown is an intervened fungal degradation wherein nonconsumable buildup are changed over into wellspring of nourishment for organisms. Cultivation of mushroom can be named as a viable method for using the buildup in agricultural products to create important items for humankind.

The cultivation of mushroom of any sort is appropriate in microbial science, environmental engineering and solid-state fermentation in the transformation of domestic, agricultural, industrial, forestry wastes into nourishment for humans. Agricultural wastes that can be utilized as substrate incorporates; wheat straw, maize leaf, saw dust, paddy straw, corn cob, groundnut shell, sugarcane bagasse.

2.7 The Edible Mushroom - *Pleurotus pulmonarius*

Pulmonarius, a consumable fungi is generally known as The Indian Oyster, The Italian Oyster, Phoenix Mushroom or The Lung Oyster. It is boundless in temperate and subtropical forests all through the world. In the Eastern US, this specie can be found commonly on hard wood while in the West, it very well may be normally tracked down on conifers.

*Pleurotus pulmonarius*is the most cultivated oyster mushroom specie in Europe and North America. Its development is especially like the development of other *Pleurotus* species like *Pleurotus ostreatus*, done by moving the mycelium from a petridish onto grain andafterward,

moving the grain produce after the mycelium colonizes it to substrate of straw, wood chips, sawdust, cardboard, coffee beans and other cellulose-based substrates.

2.7.1 Scientific Classification of *Pleurotus pulmonarius*

Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	Pleurotaceae
Genus	Pleucotus
Species	*Pleurotuspulmonarius*

2.7.2 Health Benefits of *Pleurotuspulmonarius*

1. *Pleurotuspulmonarius* has pharmacological properties. Its extracts might have conceivable restorative applications for a large number of conditions.
2. A polysaccharide called β-D-Glucan from *Pleurotuspulmonarius* decreases aversion to torment in mice.
3. It shows strong mitigating and pain relieving properties.

4. Methanol extract from *Pleurotuspulmonarius* showed anti-inflammatory and against growth (anti-tumor) action practically identical to the standard reference drugs Diclofenac and Cisplatin.

5. A recent report reasoned that concentrates of P. *pulmonarius* may slow multiplication of malignant growth cells with high galectin-3 levels.

6. It is successful in the treatment of hay fever restraining the release of histamine.

7. It is helpful in treatment of Colitis clinically.

8. Its extract hinders colon malignant growth development

9. It has antimicrobial properties and displays cell antioxidant exercises in vitro.

2.7.3 Biodegradation Using Edible Fungi (*Pleurotuspulmonarius*)

Pleurotus is an eatable mushroom that can be grown on an assortment of substrates and conditions. It is exceptionally simple to cultivate on agricultural residues which are accessible on farmland to build its yield. It produces enzyme framework which degrades the lignocellulosic parts of the substrates and empowers the mushroom to involve it for their digestion which makes the mushroom a rich wellspring of protein, dietary fiber, nutrients and minerals.

Yearly, wastes are created on the farmland which are being disposed of into water bodies, causing natural/environmental irritation or burning them which causes ecological contamination and depletes the ozone layer. The utilization of fungi specie to debase these wastes changes them over into helpful items.

Agro wastes substrates include;

1. Animal production solid wastes: these are solid wastes that are delivered from the development of animals. Examples include; animal remains, damaged feeders.

2. Food and meat handling solid wastes: these solid wastes are created because of handling crop or animal items for human use. Examples include; bones of animals, feathers, and banana strips, and so on.

3. Food and meat handling solid wastes: these solid wastes are created because of handling crop or animal items for human use. Examples include; bones of animals, feathers, and banana strips, and so on.

4. Crop creation solid wastes: these are produced from agricultural exercises because of crop production. Examples are; husks, leaves, stalks, and so forth.

5. On-farm clinical solid waste: these kind of solid wastes are created from the utilization of insect sprays, herbicides, immunizations utilized on animals. Examples are; needles, containers of insect sprays, chemical wrappers.

6. Horticultural production solid wastes: these wastes are produced because of cultivation and keeping up with horticultural plants and beautification of the scene. Examples are; pruning, trimmed flowers, and so forth.

7. Solid wastes from industries: these wastes are from the utilization of agricultural produce to deliver other helpful items which probably won't bring about agricultural solid wastes Examples are; paper creation utilizing agricultural items as raw materials which delivers some agricultural wastes.

8. Synthetic wastes: these are produced from the utilization of insect sprays, herbicides and pesticides on the farm or storage facility which is being taken care of by illiterate farmers coming about in ecological hazards. Examples are; disposing of chemical containers into lakes which might result into the food contamination, prompting end of lives in the water and people polishing off them.

Substrates are supplements expected for development, digestion, and action of microbial cells. It is the surface on which creatures e.g. plants, parasite or animals live on. It is likewise the surface on which enzymes follow up on.

Organisms can grow on different growth substrate which gives them adaptable metabolic activities. All microorganism needs carbon as substrate for developing cell parts.

Impacts of Agro solid wastes on the Soundness of Humans and the Climate

9. Unloading of waste to obstruct streams and ways, including building houses to impede streams and ways which can result to flooding which annihilates lives and properties.

10. Increment in greenhouse gas outflow results to increment in ecological temperature which makes serious danger humans and the earth.

11. The consuming of agrarian solid waste depletes the ozone layer which might bring about an unnatural weather change.

12. Expansion in human populace increases agrarian wastes through expanded farming activities coming about to food insecurity.

How to Manage Agro Solid Wastes

Agro solid wastes can be managed by reusing them to create different items that are valuable and can be accomplished through;

13. Composting: Li et.al (26) suggested that kitchen wastes (solid wastes) gotten from wasted food could be utilized to take care of animals through disinfection, manures, through composting and bio-energy, through anaerobic digestion. The organic constituent of the wastes and their supplements in the waste decide their value as manure.

    Substrate for cultivating fungi (mushroom): different strong wastes have been utilized to culture mushroom as substrates.

14. Animals feed: Farming solid wastes, because of reusing, has been fed to animals to diminish the expense of feed and to expand its protein content.

15. Creation of local soap: a portion of the agro solid wastes are turned into valuable items an example is the cocoa pod which can be utilized to deliver the local black soap or can be permitted to disintegrate and add supplements to the soil.

16. Wellspring of elective energy and biofuel creation: green energy could be produced from agrarian solid wastes through anaerobic assimilation/digestion.

2.8 Solid State Fermentation (SSF)

Solid State Fermentation is a fermentation cycle utilized in delivering catalysts/enzymes, it happens by developing microorganisms on a strong substrate which has an exceptionally low dampness content.

Solid State Fermentation utilizes results of agro industries like wheat grain, maize husk, sugar beet mash as substrates which are extremely modest and can without much of a stretch be gotten.

The filamentous fungi is the appropriate microorganism for Solid State Fermentation and a few microbes (bacteria), yeast and other fungi.

Advantages of Solid State Fermentation

1. There is no requirement for pre-treatment of the substrate compound to that of the fluid media.
2. The medium is extremely modest, simple to stop by and straightforward.
3. Since it misses the mark on presence of free fluid, there is low or diminished tainting/contamination in SSF.
4. It has high volumetric efficiency.
5. The equipment utilized in SSF are basic.
6. The downstream cycle and the waste disposed is simple and decreased.

Disadvantages of Solid State Fermentation

7. There is no requirement for pre-treatment of the substrate compound to that of the fluid media.
8. The medium is extremely modest, simple to stop by and basic.
9. Since it misses the mark on presence of free fluid, there is low or diminished defilement in SSF.
10. It has high volumetric efficiency.
11. The gear utilized in SSF are straightforward.

12. The downstream cycle and the waste disposed of is simple and decreased.

Application of Solid State Fermentation

13. Production of metabolites such as antibiotics, organic acids, enzymes.

14. Production of lipids, biofuels

15. Production of aromas and flavours for food industries.

16. Extraction of bioactive compounds.

Factors Affecting Microbial Degradation

The proficiency of microorganism in degradation relies upon different variables: the physiochemical boundaries of the climate, type and convergence of contaminators, their capacity to get to the microorganisms. The kind of nourishment (biological elements) or the environment of the living beings are the variables that impacts the rate at which microorganisms could debase/degrade substrates. These elements incorporate; temperature, dampness content, pH, presence of different substrates, redox climate/environment, the reaction of the microorganisms to the environment.

Endnotes

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Chapter Three

Material and Methods

3.1 Collection and Preparation of Samples

Dried sample of sweet corn leaves were collected from *Agricwas farm, Aboke village, Lagelu local government, Ibadan, Oyo state.*

The samples were taken to the laboratory at *Lead City University* for wilting overnight. The next day, the samples were chopped into smaller portions before oven drying at 105^{o}C for 3days until a constant weight was obtained for dry matter determination in the laboratory. It was milled thereafter and stored inside a covered jar.

Serial dilution

150ml of sterilized distilled water.

5 agar prepared according to manufacturer's specification.

1. *Salmonella slugella* agar: 9.453g
2. Potato dextrose agar: 5.85g
3. EMB: 5.394g
4. Nutrient agar: 4.2g

5. MacConkey agar: 7.275g

150ml of the distilled water was added to each of the agars in the conical flasks.

2g of Agar-agar was added as a solidifying agent.

Homogenize for at least 1hour in the water bath.

Sterilize in the autoclave for 30minutes.

9ml of distilled water was dispensed into 10 sterilized beakers.

1g of the milled sweetcorn leaf sample into distilled water.

3.2 Isolation of Bacteria

1g of sweetcorn leaf sample was dispensed into 100ml of distilled water.

For serial dilution;

1ml of the mixture of sweetcorn leaf was dispensed into one of the prepared 9ml of beakers containing distilled water.

Four plates of agar were prepared and one plate serving as control.

For PDA which is for fungi growth;

1.5ml of streptomycin injection solution was added to dilute the agar (PDA). The diluted agar was poured into plates containing the serial diluted sweetcorn leaf solution.

After cooling, the plates were wrapped with foil paper and incubated for 48hours to check for the organisms present in it.

3.3 Materials and Apparatus

1. **Materials**

The materials used for this study include: petri dishes, incubator, aluminum foil, spirit lamp, needle and syringe, beakers, cotton wool, lighter, hand gloves, marker, paper tape, facemasks, favor bottles, weighing balance, water bath, autoclave, measuring cylinder, inoculating loop, microscope, refrigerator.

2. **Reagents and consumables**

These includes; Ethanol, distilled water, Streptomycin injection, injection water, nutrient agar, *Salmonella slugella* agar, EMB, Potato dextrose agar, MacConkey agar.

3. **Sterilization**

All the media used were sterilized in the autoclave at 121^{o}C for 15minutes. The glass wares were washed with detergent, rinsed with clean water allowed to dry and placed in an oven for 2-3hours. Work bench was always disinfected with 95% alcohol before and after experiment. Inoculating loop would be sterilized by flaming using the spirit lamp.

3.4 Sample Processing

The diluted leaf sample would be inoculated as various agar: PDA, nutrient, EMB, MacConkey, *Salmonella shigella* and incubated aerobically at 37^{o}C for 48hours.

Preliminary Identification

After incubation, the plates would be observed for growth and the isolates would be identified based on their colonial morphology. Gram staining technique would be used to identify the organisms present.

3.5 Gram Staining

The staining technique was discovered by *Christian Gram* in 1884 to differentiate between Gram positive and Gram negative bacteria.

Principle: Gram positive cells possess thick peptidoglycan cell wall that is able to retain crystal violet iodine complex that occurs during staining while Gram negative cells have a thin peptidoglycan layer, thus Gram positive cells do not decolorize with ethanol which Gram negative cell do. This allows the negative cells accept the counter stain safranin and appear pink to red while the Gram positive cells will remain blue to purple due to the retention of the crystal violet color.

Procedure:

1. Smear made on a grease-free slide and heat fixed.
2. Flooded with crystal violet (primary stain) for 30-60 seconds. Rinsed with flowing tap water.
3. Lugol's iodine (mordant) was added in drops and left for abort 10-60 seconds and then was shed off with distilled water.
4. Decolourizer (ethanol) was added in drops for 5 seconds and washed off immediately with distilled water.

5. Safranin (secondary stain) was added and allowed to stay for about 40-60 seconds after which it was rinsed off with distilled water and blotted dry.

6. A drop of immersion oil was used to cover the stain and viewed under light microscope under the x100 magnification.

3.6 Biodegradation of sweetcorn leaves by *Pleurotus pulmonarius*

*Pleurotus pulmonarius*was collected from Federal Institute of Industrial Research, Oshodi (FIIRO). The specimen bottles used were thoroughly washed, dried for 20minutes at 100^{o}C.

25g of the milled sample was weighed into each specimen bottles and 70ml of distilled water was added. The bottles were tightly covered and sterilized in the autoclave for 30 minutes at 121^{o}C and allowed to cool down. Each of the treatment was replicated.

3.6.1 Inoculation

The milled sweetcorn served as the substrate. The *Pleurotus pulmonarius* was inoculated at the center of the substrate and covered immediately. The inoculated substrate were kept in the incubator at 30^{o}C and 100% relative humidity. The growth monitored on the 7th day, 14th day, 21st day and 40th day.

After 40 days of inoculation, the experimental bottles were harvested by autoclaving again to terminate the mycelia growth. Samples of the biodegraded samples were oven dried to constant weight for chemical analysis.

3.6.2 Crude protein determination [(AOAC official method) (988.05)]

The crude protein in the sample were determined by the routine semi-micro kjeldahl procedure/technique. This consists of three techniques of analysis namely digestion, distillation and titration.

3.6.3 Digestion

0.2g of the milled sweetcorn sample was weighed carefully into two Kjeldahl digestion tubes and ensure that all the sample materials got to the bottom of the tubes. Two Kjeldahl catalyst tablet was added into it then 15ml of concentrated H_2SO_4.

The bottles were set into the appropriate hole of the digestion block heaters in the fume cupboard. The digestion was left for 2 days after which a clear colorless solution was left in the tube.

The digest was cooled and carefully transferred into 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

3.6.4 Distillation

The distillation was done with Markhan Distillation Apparatus which allows volatile substances such as Ammonia to be steam distilled with complete collection of the distillate.

The apparatus was steamed out for about 10 minutes, the steam generator is then removed from the heat source to all the developing vacuum to remove condensed water. The steam generator is then placed on the heat source (i.e. heating mantle) and each component of the apparatus was fixed up appropriately.

3.6.5 Determination

5ml portion of the digest above was pipetted into the body of the apparatus via the small funnel aperture. To this was added 5ml of 40% (w/v) NaOH through the same opening with the 5ml pipette. The mixture was steam-distilled for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric acid plus mixed indicator solution placed at the receiving tip of the condenser. The Boric acid plus indicator solution changes color from red to green showing that all the ammonia liberated have been trapped.

3.6.6 Titration

The green color solution obtained was then titrated against 0.01N HCl contained in a 50ml Burette, At the end point or equivalent point, the green color turns to wine color which indicate that alltheNitrogen trapped as Ammonium Borate[$(NH_4)_2BO_3$] have been removed as Ammonium chloride(NH_4Cl).

The percentage nitrogen in this analysis was calculated using the formula;

%N =Titre value x Atomic mass of Nitrogen x Normality of HCl used x 4

OR

%N = (Titre value x Normality/Molarity of HCl used x Atomic mass of Nitrogen x Volume of flask containing the digest) x 100/1

Weight of sample digested in milligram x volume of digest for steam distillation.

The crude protein content is determined by multiplying percentage nitrogen by a constant factor of 6.25 i.e. %CP = %N x 6.25

3.7 Fiber determination

Apparatus: Heating mantle, crucibles, furnace, sieve cloth, fibre flask, funnel, analytical weighing balance, and desiccator.

Reagents: 0.255NH_2SO_4, 0.313N NaOH and acetone.

Determination: 2.0gm of the sample was accurately measured into the fibre flask and 100ml of 0.255 NH_2SO_4 was added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fiber sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fiber flask to which 100ml of (0.313N NaOH) was added and heated under reflux for another 1 hour. The mixture was filtered through a fiber sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible and the residue were oven-dried at 150^oC overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_1. The crucible with the weight W_1 was transferred to the muffle furnace for ashing at 550^oC for 4hours.

The crucible containing white or gray ash (free of carbonaceous materials) was cooled in the desiccator and weighed to obtain W_2. The difference W_1-W_2 gives the weight of fibre. The percentage of fibre is obtained by the formula; %fibre = (W_1 - W_2/ Weight of sample) x 100

Nitrogen-free extract was determined by difference. This was done by subtracting sum of (moisture% + %crude protein + %ether extract + %crude fibre + %ash) from 100 i.e [100-(%M + %CP + %EE + %CF + %ash)

3.8 Procedure for Phytochemicals, Vitamins and Mineral Analysis in Sweet Corn Leaves

3.8.1 Procedure for phytochemical analysis of sweetcorn leaves.

1. Collection of sweetcorn leaves.
2. Cleaning and drying in the oven.
3. Powdering: Grinding/milling into powder.

1. Quantitative test for Tannin

1g of the sample was weighed and extracted with 25ml of the solvent mixture of 80.20 Acetone. 10% Glacial Acetic acid for 5hours.

Filter and measure the absorbance at 500nM.

Also, measure the absorbance of the reagent black.

A standard graph was made with 10, 20, 30, 40, 50mg/100mg of Tannic acid. The concentration of Tannin was read off taking into consideration any dilution factor.

2. **Phenol**

2ml of the extract was mixed with 0.5ml of Folin-Ciocalteau reagent and 1.5ml Sodium Carbonate (20%)

Mix for 15 seconds and allowed to stand at 40^{o}C for 30 minutes to develop color.

Measure A_{765} Express as GAE/g Gallic acid equivalent

3. **Saponin**

1g of the sample was weighed and 5ml of 20% Ethanol and was kept in a water bath at 55^{o}C for 4 hours.

The residue was filtered and washed with 20% Ethanol twice.

The extract was reduced to about 5ml in the oven.

5ml of petroleum Ether was added to the concentrated extract inside a separating funnel and the petroleum ether layer was discarded.

3ml of Butanol was added to it and washed with 5ml of 5% Sodium Chloride. The Butanol was later poured into a weighed petri-dish and kept in an oven to evaporate to dryness and weigh the residue.

4. **Phytates**

Extract of 1g of sample was mixed with 0.2M HCl to 0.5ml of extract 1ml Fe^{3+} solution e.g. Ferric Ammonium Sulphate. The tube was heated in water bath for 30 minutes, cooled and centrifuged.

1.5ml of 2,2-Bipyridine solution was added to 1ml of supernatant. It was measured at 519nM with distilled water as blank.

5. **Oxalate**

2g of sample was boiled in 40ml of water for 30 minutes in a reflux condenser.

10ml of 20% of Na_2CO_3 was added and boiled for another 30 minutes. The liquid extract was filtered and washed with hot water until the wash water does not show any alkaline reaction.

The combined wash water and filtrate were combined to a small volume and cooled.

With constant stirring, (1.1) HCl was added drop-wise until the final acid concentration after neutralization is about 4% at which stage a heavy precipitate appears (which was allowed to flocculate)

The extract was carefully filtered into a 250ml flask and make up to mark and kept overnight and the supernatant was filtered through a dry filter paper in a dry beaker.

An aliquot of the filtrate was taken in a 400ml beaker diluted with water to 200ml and make just ammonical and re-acidify with lactic acid. I cold medium, 10ml of a 10% Calcium Chloride solution was added and stirred well to include Calcium oxalate precipitate to appear and allowed to settle overnight.

Carefully, the clean supernatant liquid was decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. The precipitate was dissolved into HCl (1:1), Oxalic acid was re-precipitated by adjusting the pH with ammonium hydroxide solution.

The content was boiled and allowed to settle overnight. The oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution.

Calculation;

1ml of 0.05N $KMnO_4$ = 0.00225 anhydrous oxalic acid

= % oxalic acid

= Titre value x 0.00225 x 100/1

= Titre value x 0.1125

6.**Alkaloids**

1g of the sample was weighed and 20ml of 10% Acetic acid was added into Ethanol, shaken and allowed to stand for 4hours. It was filtered and the filtrate was evaporated to about a quarter of its original volume. Some drops of concentrated ammonia was added, the precipitate was filtered through a weighed (W_1) filter paper and the filter paper allowed to dry in the oven at 60^{o}C.

The filter paper was weighed after drying to constant weight.

%Alkaloids = $W_2 - W_1$ x 100

3.8.2 Procedure for vitamins analysis of sweetcorn leaves

1. **Niacin (Vitamin B_3)**

5g of sample was blended and 100ml of distilled water was added to dissolve all Nicotinic acid or Niacin present. 5ml of this solution was drawn into 100ml volumetric flask and made up to mark with the distilled water. 10-50ppm of Niacin stock solution was also prepared. The absorbance of the diluted stock solution and sample extract were measured at a wavelength of 385nm on a spectrophotometer for absorbance at the specified wavelength to obtain the gradient.

Factor amount of Niacin in the sample was calculated using the formula;

Mg/100gNaicin = Absorbance x Dilution x Gradient factor stock

= factor solution/100

2. Thiamine (Vitamin B_1)

1g of sample was weighed into 100ml volumetric flask, 25ml of MH_2SO_4 was added to rinse any adhering sample particle off the flask, the flask was set in a boiling water bath to ensure a complete dissolution of the sample in the acid.

The flask was shaken frequently in the first 5 minutes and subsequently every 5 minutes for 3 minutes. 5ml of Taka-diastase in 0.5M sodium acetate solution was added and flask set in cold water to cool content below 500C, the flask was stopped and kept at 45-50 for 2 hours and thereafter made up to 100ml in ark after mixing thoroughly.

The mixture was filtered through a No.42 Whatman filter discarded the first 10 minutes and keeping the remaining. 10ml of the remaining mixture filtrate was pipetted into a 50ml and volumetric flask and 5ml of acid potassium chloride solution was added, shaking thoroughly to mix well.

Standard Thiamine solution of range 10mg/ml to 50mg/ml were prepared from 100mg/ml stock and treated same way prepared from sample above.

The absorbances of the sample as well as that of standards were read on a fluorescent UV spectronometer (Cecil A20 model) of a wavelength of 285nm.

Vitamin B_1 in mg/100g was calculated using the formula;

(Absorbance x Average gradient x dilution factor)/Weight of sample

3. **Riboflavin (Vitamin B_2)**

1g of each sample was weighed into a 250ml volumetric flask, 5ml of 5NHCl was added, followed by the addition of 5ml of dichloroethane. The mixture was shaken and 90ml of de-ionized water was added. The whole mixture was thoroughly shaken and was heated on a steam bath for 30 minutes to extract all the riboflavin. The mixture was then cooled and made up to volume with de-ionized water.

It was then filtered, discarding the first 20ml of the aliquot. 2ml of the filtrate obtained was pipetted into another 250ml volumetric flask and made up to mark with de-ionized water.

Standard solution was prepared by dissolving 0.05mg riboflavin into 100ml of distilled water. Different standard solution concentration of between 0 to 5ppm were prepared from above to obtain the equivalence. The absorbance, the standard and samples were read on the fluorescent spectrophotometer at 460nm wavelength.

Amount of B_2 in samples calculated by;

Vitamin B_2 (mg/100g) = (Meter reading x standard x dilution of sample)/Weight of sample

4. **Pantothenic acid (Vitamin B_5)**

1g of sample was weighed into a 250ml of volumetric flask and shook with 200ml distilled water for 10 minutes. It was diluted to mark to distilled water. The mixture was filtered through Whatman No.42 filter paper into a 100ml volumetric flask.

5ml of aliquot of the sample filtrate was pipetted into a 2ml beaker, 5ml potassium bromide (KBr) (12%), 10ml of $KMnO_4$ were added and mixed thoroughly with a glass. The mixture was transferred to a stopped flask put in a boiling water bath for 10 minutes. The hot solution cooled

in ice for 5 minutes and 20% freshly prepared H_2SO_3 was added drop-wise to decolourize the excess $KMnO_4$ this clear colourless solution. 10ml of 2,4dinitrophenyl mrazine (5g/1) was added and mixed thoroughly. The mixture was heated on a steam bath for 15 minutes and cooled later to room temperature. The yellow precipitate obtained was dried for 30 minutes in an oven at 100^{o}C. The dry precipitate is dissolved in hot pyridine solution and mixed thoroughly form in homogenous suspension.

The suspension was filtered through a Whatman No.42 filter paper into a 50ml volumetric flask and made up to mark with pyridine solution all aliquot of the solution above was pipetted into 200ml of flask 50ml of distilled water added followed by the addition of 5ml NaOH solution to develop the due colour.

Absorbance of sample and standard pantothenic acid solution of range 10ug/ml – 50ug/ml prepared from ug/ml stock pantothenic acid were read on a spectronic spectrophotometer at a wavelength of 570nm. Pantothenic acid was calculated in ug/ml using the formula;

Absorbance x gradient factor x dilution factor

All analysis were performed using the method reported by;

1. Association of Official Analytical Chemists (A.O.A.C 2005)
2. Official methods of Analysis of A.O.A.C International, Gaithersburg, MD, USA.
3. Methods of Vitamins Assay (8th edition), 2006. Inter-science publishers.

3.8.3 Procedure for mineral analysis of sweetcorn leaves

1. Calcium, Potassium and Sodium determination

The ash of each sample obtained was digested by adding 5ml of 2MHCl to the ash in the crucible and heat to dryness on a heating mantle. 5ml of 2MHCl was added again, heat to boil and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask.

The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentration of calcium, potassium and sodium on the Jenway Digital Flame Photometer(PFP7 model) using the filter corresponding to each mineral element.

Concentration of each of the element was calculated using the formula; %Ca/%K/%N = Meter reading (MR) x slope x dilution factor/1000

2. Phosphorus determination

Phosphorus was determined routinely by the Vanado-molybdate colourimetric or spectrophotometric method. The ash of each sample obtained was treated 2MHCl solution as described for calcium determination above. 10ml of the filtrate solution was pipetted into 50ml standard flask and 10ml of Vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development.

The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a spectronic 20 spectrophotometer or colourimeter at a wavelength of 470nm.

The percentage phosphorus was calculated from using the formula;

%Phosphorus = (Absorbance x slope x dilution factor)/10000

3. **Determination of Selenium, Magnesium, Zinc, Iron, Copper, Manganese, using Buck 200 AAS**

The digest of the ash of each sample above as obtained in calcium and potassium determination was washed into 100ml volumetric flask with de-ionized or distilled water and made up to the mark. The diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements were read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

Samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist A.O.A.C 1984, 1990, 1998. All analysis were carried out in duplicate.

Endnotes

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Chapter Four

4.1 Result

The study analyzed:

1. Various organisms growing on different agar substrate using the serial dilution of sweet corn leaves.
2. Phytochemicals present in sweet corn leaves.
3. Vitamins in sweet corn leaves. •Minerals in sweet corn leaves.
4. Nutritive value of sweet corn leaves before and after biodegradation

4.1.1 Table Showing microorganisms that can be found growing on Sweet Corn Leaves using different agar after 48hours of Incubation.

Agar type	Organism Suspected	Characteristics
Salmonella-shigella	Shigella suspected	Gram negative rod
Nutrient agar	Lactobacillus suspected	Gram positive bacilli with spores
EMB	No growth	No growth
Mac-conkey	No growth	No growth
PDA	*Aspergillus niger/Aspergillus fumigatus*	Filamentous fungi, gram negative bacteria Filamentous fungi, gram negative bacteria

4.1.2 Table Showing Phytochemicals Present in Sweet Corn Leaves

Phytochemicals	Replicate 1 (plant)	Replicate 2 (plant)
Saponin (mg/100mg)	63.87	64.11
Tannin (mg/100mg)	91.35	90.88
Phenol (mg/100mg)	235.20	234.99
Alkaloid (mg/100mg)	23.75	23.69
Oxalate (mg/100mg)	17.93	17.86
Phytate (mg/100mg)	125.81	126.09

4.1.3 Table Showing Vitamin Present In Sweet Corn Leaves

Vitamins	Value 1	Value 2
Thiamin(B_1) (mg/100mg)	3.89	4.02
Pantholetic (B_5) (mg100mg)	5.26	5.29
Niacin (B_3) (mg/100mg)	3.11	3.26
Riboflavin (B_2) (mg/100mg)	2.15	2.08

4.1.4 Table Showing the Result of Minerals in Sweet Corn Leaves

Minerals	Value 1	Value 2
Selenium (µg/g)	0.01	0.02
Potassium (g/kg)	13.97	14.07
Magnesium (g/kg)	2.85	2.79
Phosphorus (g/kg)	1.71	1.58
Sodium (g/kg)	0.82	0.86
Iron (mg/kg)	71.33	71.40
Manganese (mg/kg)	233.36	232.88
Zinc (mg/kg)	57.95	58.17
Copper (mg/kg)	7.73	7.69

4.2 Nutritive Value of Sweet Corn Leaves

After titration, the titre values below were obtained;

Samples (cm^3)	1st Titre	2nd Titre
Final reading	1.16	1.17
Initial reading	0.00	0.00
Total volume of acid used	1.16	1.17

Average titre value = 1st titre + 2nd titre/2

= (1.6 + 1.7)/2

= 2.33/2

= 1.165cm^3

Crude protein = %N x 6.25

To get %N;

%N = Titre value x Atomic mass of Nitrogen x Normality of HCl used x 4 So;

%N = 1.165 x 14x 0.01 x 4

= 0.653

CP = %N x 6.25

= 4.082

Parameters	Sweet Corn Leaves
Crude protein	4.08
Crude Fiber	25.1
Nitrogen Free Extract	0.653

Parameters	Sweet Corn Leaves
Crude Protein	6.04
Crude Fiber	22.72
Nitrogen free extract	33.43

Discussion

At the expiration of 48hours incubation, table 4.1 shows the result of the organisms suspected (due to their morphology) to be growing on different agar used in culturing the microorganisms.

On Salmonella-shugella agar, shigella which is a non-spore forming rods, nonmotile facultativaly anaerobic, gram negative was suspected.

On the nutrient agar plate, Lactobacillus which is a lactic acid bacteria, gram positive, rod shaped, non-motile, non-spore forming bacteria that has no catalase enzyme was suspected.

No growth was recorded on EMB and MacConkey agar plates.

Aspergillus species were suspected to be growing on the potato dextrose agar plate. It has a cottony appearance which has a white colour, then turn to yellow and finally black. It is a gram negative bacteria and a filamentous fungi commonly found in the soil.

Table 4.2 and 4.3 after 40 days of fungal degradation, proximate analysis was carried out on the experiment. As seen in the table, after the degradation by *Pleurotuspulmonarius*, the crude protein increased from 4.08 to 6.04 which may be as a result of the addition of the protein in the fungi to the leaves during the process of degradation down the fiber content thereby increasing its protein content.

Also, the crude fiber after degradation decreased from 25.1 to 22.72 this might be the ability of the fungi to produce enzymes that could breakdown the complex carbohydrate i.e. crude fiber in the waste thereby increasing the protein content of the leaves.

Table 4.4, 4.5 and 4.6 show the result of the phytochemicals, vitamins and minerals present in sweet corn after a standard analysis at biochemical lab. They reveal that sweet corn leaves are

rich in all these, but previous tables, 4.2 and 4.3 show that it is low in protein, thus if it is degraded by edible fungi, it increases its protein content.

These analysis signifies that biodegradation of sweet corn leaves by an edible fungi increases the nutritive value of the leaves and with this, the leaves can be used to feed animals instead of polluting the environment.

Endnotes

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Chapter Five

Summary, Conclusion and Recommendation

5.1 Summary

Biodegradation has essentially expanded in the nutritive worth of sweet corn leaves, the cycle will be valuable and at a diminished expense. Agro wastes are helpful items from the course of agricultural exercises which can be reused for additional utilization. Likewise, different nutrients, vitamins and phytochemicals in the sweet corn passes on add to its dietary substance.

5.2 Conclusion

Discoveries of this study shows that there is need to teach that multitude of associated with agricultural exercises to try not to dispose of agro wastes which could cause ecological contamination, infections and financial misfortune.

Additionally, it showed that the agro wastes could be switched over completely to helpful items which will create income and fill in as the natural substance for the development of other valuable items.

It additionally uncovered that there are different minerals, nutrients and phytochemicals in agro wastes i.e. maize leaves and that microorganisms for example parasites could debase agro wastes, develop and flourish there as found in the examination work. The microorganism debase the complex substances into less difficult ones which can be utilized as animal feed.

5.3 Recommendation

Generation of agricultural wastes is inescapable, subsequently founded on this exploration/research work, it is suggested that;

1. Rather than disposing of agricultural wastes such as plant leaves, husks, and so on, they can be utilized to create animal feed.

2. It is important to do more research on various fungi and their capacity to decompose agro wastes.

3. It is of great importance to figure out what could the justification be for why a few eatable fungi couldn't degrade agro waste and what should be possible or what could be added for biodegradation to happen.

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Appendices

Appendix I

Figure 1: The Morphology of Maize Plant

Appendix II

Figure 2: Dent Corn

Appendix III

Figure 3: Flint Corn

Appendix IV

Figure 4: Flour Corn

Appendix V

Figure 5: Sweet Corn

Appendix VI

Figure 6: Popcorn

Appendix VII

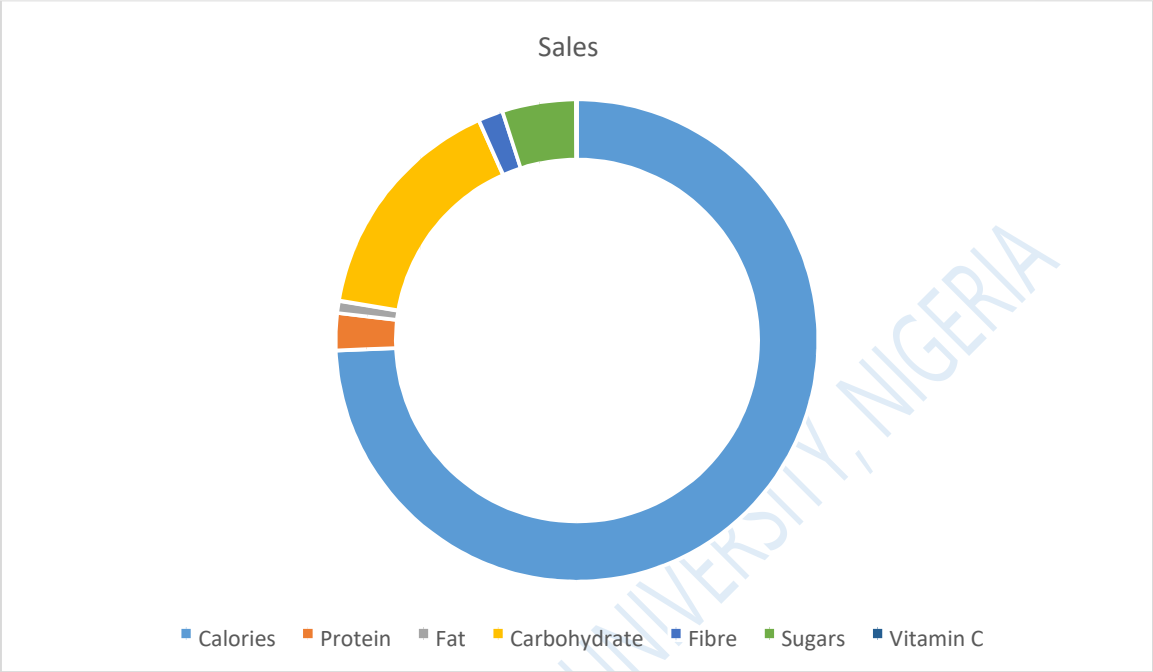

Figure 7: Pie Chart Showing the Nutritional Composition of Maize

Appendix VIII

Figure 8: Nitrogen Deficiency in Maize Plant Showing Stunted Growth and Pale Green Leaf Discolouration

Appendix IX

Figure 9: Phosphorus Deficiency in Maize Plant Showing Purple Discolouration on the Leaves

Appendix X

Figure 10: Potassium Deficiency in Maize Plant Showing Dried-Like and Yellow Coloured Leaves

Appendix XI

Figure 11: Sulphur Deficiency in Maize Plant Characterized by Interveinal Chlorosis

Appendix XII

Figure 12: Zinc Deficiency in Maize Plant Showing Necrosis

Appendix XIII

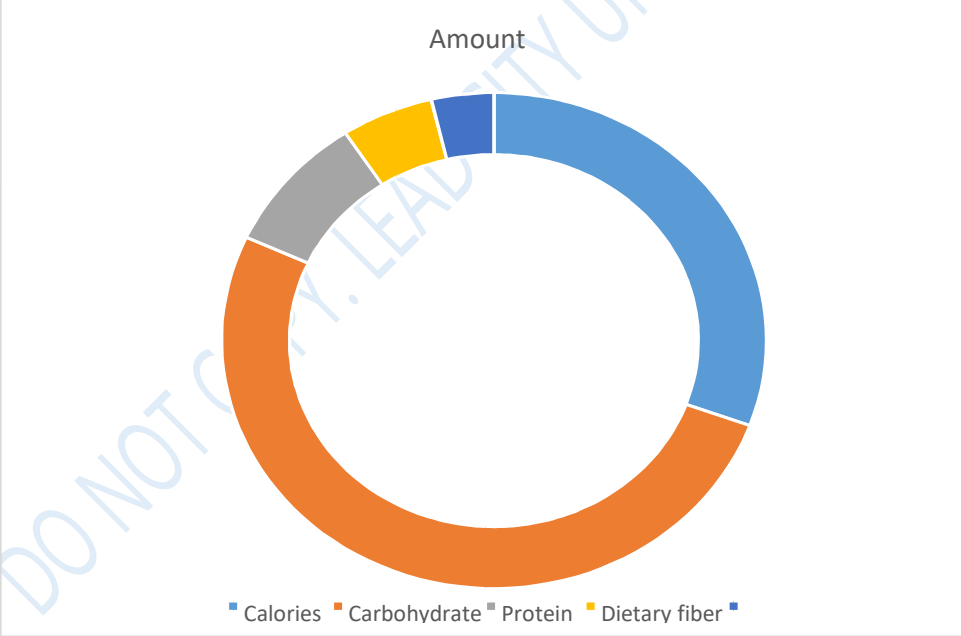

Figure 13: Pie Chart Showing the Nutritional Composition of Sweet Corn (100gm)

Appendix XIV

Figure 14: Sporulation in Mushroom

Appendix XV

Figure 15: A Well Labelled Basic Fungal Cell

Appendix XVI

Figure 16: Examples of Edible Mushroom

Appendix XVII

Figure 17: Solid State Fermentation

Appendix XVIII

Figure 18: Sample of Sweet Corn Leaves Collected

Appendix XIX

Figure 19: Agar Used for Culturing Microorganism

Appendix XX

Figure 20: Digestion Process in the Fume Cupboard

Appendix XXI

Figure 21: *Pleutorus pulmonarius* Growing on Substrate (Sweet Corn Leaves)

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University Compliance Certification

This is to certify the thesis by Shosanya, Adenike Abiola with matric number LCU /PG/002607 in the department of Biological Sciences, Facaulty of Natural and Applied Sciences , Lead City University ,Ibadan is in full compliance with the approved format and style .

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