STUDY ON FUNGI ASSOCIATED WITH CONTAMINATION OF LETTUCE (Lactuca sativa) SOLD AT GUSAU CENTRAL MARKET GUSAU ZAMFARA STATE

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DECLARATION

I hereby declare that this project is written by me and it has not been presented before in any institution for a Bachelor Degree except for quotations and summaries which have been duly acknowledged.

Zakariya Auwal

26/11/2018
Date

CERTIFICATION

This Project entitled "Fungi associated with contamination of lettuce (*Lactuca sativa*) sold at Gusau central market Zamfara State Nigeria" meets the regulation governing the award of Bachelor of Science of the Federal University Gusau and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Project is been dedicated to the author of life, who spare my life over the years. All praise and glory are due to almighty Allah.

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All praise and gratitude be to almighty Allah, the Creator and Sustained of all being, who with His infinite mercy, blessings, and care, who gives me the opportunity towards the successful completion of my research project in particular and my degree program in general, peace and blessings be to his beloved prophet Muhammad (S.A.W.), his household, his companions and those who follow his teaching with kindness up to the day of judgment.

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ABSTRACT

This study was carried out to determine the Fungi associated with the contamination of lettuce in Gusau central market, Zamfara State Nigeria. Ten different samples were collected in a separate clean polythen bag from diffetent vendors for micological analysis. The samples were separately grounded and turned into paste, they were inoculated in Potato dextrose agar (PDA) media and incubated at room temperature for 5 days, for fungal isolation. A total number of five (5) species of fungi associated with contamination of lettuce plant namely; Cladosporium trichoids, Cladosporium werneckii, Candida albican, Blastomycete dermatitidus, and Aspergillus fumigatis were identified. Candida albican appeared to have the highest chance of occurrence with a frequency of 33.3%, followed by Cladosporium trichoids with 28.6%, Cladosporium werneckii with 3%, Blastomycete dermatitidus with 3%, and Aspergillus fumigatis with 2%. Further research is recommended on the implication of using vegetable contaminated by fungi.

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CHAPTER ONE

1.0 Introduction

1.1 background of the study

Lettuce Plant (*Lactuca sativa*) is a member of the family Asteraceae (Compositae). Lettuce is annual herbaceous plant with broad leaves of variable sizes and form arranged in a rosette. It has a single main root with broad fibrous lateral branches. The stem is cylindrical and contains latex. It is most often grown as a leaf vegetable, but sometimes for its stem and seeds. Lettuce grows with a basal rosette of leaves up to 15 cm (10 in) long, loosely or tightly clustered, and is generally harvested before the flowering stem has bolted. If allowed to bolt, the plant may reach nearly 1 m (3 fit) tall, with yellow composite flowers that develop tan or black structure (small, one-seeded fruits with hard coverings that do not split open (dehisce) when ripe) with white beaks (Williams, 2007).

Lettuce is one of the UK vegetable crops. It is important in most temperate climate production systems and in other regions like West Africa where it is the most popular salad crop. In addition to its main use as a leafy green, it has also gathered religious and medicinal significance over centuries of human consumption (Ryder et al, 2007

The species, which is not known in the wild but is thought to have been developed from the wild lettuce, L. serriola, around 4,500 years ago in Egypt, has been developed into diverse cultivars that vary in leaf shape (ruffled, toothed, or fairly smooth), growth form (loose clusters of leaves or leaves packed densely into heads to stem lettuce with leaves on edible stems), and leaf colour ranging from light to dark green to reddish to purple, or green tinged with reddish or purple (Ryder et al, 2007).

1.2 Types of Lettuce Plant

1. Head or cabbage lettuce, which forms dense, tightly packed heads, like cabbage, and includes the common cultivar 'Iceberg.'

2. Romaine or cos lettuce, with long (often up to 15 cm, or 10 in), upright, broad-stemmed leaves that form loose heads.

3. Curled or leaf lettuce, with a non-heading rosette of round ruffled, fringed, or crisp leaves.

4. Butter head lettuce, with soft leaves and loose heads.

5. Stem lettuce (celtuce), in which the stems are enlarged, and are the part harvested and eaten.

Health benefits of fresh products, e.g. lettuce, with high fibre and vitamin content make them more popular for the people who care about proper diet. The consumption rate of such products has been increased in recent years. These foodstuffs could be vehicles for some kinds of pathogens if they are used as raw, therefore, illnesses and outbreaks maybe resulted from their consumption. In many parts of the world, including Nigeria, there is an increasing rate of consumption of raw fresh produce, like lettuce and other vegetables, mainly because of changes in the human lifestyle and their tendency towards convenience and spending less time on preparing food. However, lettuce are extraordinary dietary source of nutrients, micronutrients, vitamins, and fibre for humans and thus vital for health and well-being. Vegetables are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several forms of diseases. Despite their nutritional and healthy characteristics, outbreaks of human infections associated with the consumption of fresh or minimally-processed ready-to-eat lettuce and vegetables have increased in the recent years due primarily to transmitting various pathogens to humans. Contamination of these products by pathogenic

microorganisms, specifically in leafy green vegetables, poses serious health threats to consumers. Freshly-consumed produce can be contaminated with pathogens via being exposed to contamination sources from production on the farm to the point of sale in the market (Amoah, 2007).

Diseases cause a serious problem for lettuce growers and marketers and for many diseases there are no opportunities for their control once the crop has been infected. Lettuce diseases may result from attack by viruses, fungi, bacteria, nematodes or from a non-pathogenic source. Some diseases are seed-borne, others are airborne, while others are transmitted by insects or microorganisms (Danes, 2008).

Large quantities of vegetables are produced in Nigeria. However, accurate production figures are not available. Studies reported that production of vegetables is seasonal resulting in abundant supply during the rainy season and scarcity at dry seasons. Due to their soft texture they are easily wounded as a result of harvesting and other post-harvest handling operations such as packaging, transportation and storage, long chain of marketing system of vegetables between the producer and consumer makes it difficult to accurately assess the level of damage in many crops in Nigeria (Aliero, 2014). Report indicated that Nigerian vegetables have not been able to meet world standard because of poor harvest handling (Aliero, 2014).

1.3 Statement of the problem

The processing and cultivation of lettuce is being associated with unhygienic and improper handling processes, as a result, the lettuce is associated with different fungal contamination such as deposition of spores at different steps of cultivation up to the point of consumption.

1.4 Justification

Lettuce is widely cultivated, distributed and consumed by various individuals. Mostly, this lettuce is sold uncovered within the market. This has made it necessary to study the fungi and other microbes that can found in it so as to ascertain whether the lettuce is free for consumption or not

1.5 Aim and objectives

Aim

The aim of this work is to determine fungal contamination of *Lactuca sativa* sold in Gusau central market.

Objectives

1. To isolate fungi associated with contamination of lettuce sold at Gusau central market.
2. To identify fungi associated with contamination of lettuce sold at Gusau central market.
3. To determine the relative abundance of each fungal isolate.

CHAPTER TWO

2.0 Literature review

A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs like animals; obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) and are called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes). Some fungi infect a living host, kill host cells in order to obtain their nutrients; these are called necrotrophs (Alexopoulos, 2003).

2.1 General characteristics of Fungi

Fungi include all members of plants which are devoid of stem, leaves, root, and chlorophyll.

The absence of photosynthetic pigment enforces the fungi in parasitic or saprophytic existence. Nutrition in fungi is by absorbing nutrients from organic material in which they live. They digest their food before it pass through the cell wall into the hyphae, and the hyphae secrete enzymes that breakdown the organic material into simple compound. A few fungi form mutual beneficial partnership with certain algae or with the root of other trees.

They are cosmopolitan in distribution. Many species of fungus are parasitic infecting plant, animal and human. Fungi typically consisting of filamentous hyphae which form net-like structure called mycelium except for yeast and other unicellular form. Fungi are built with a main thallus body. The cell wall of most fungi is made up of

chitin and other organic compound. Fungi reproduce by vegetative sexual and asexual reproduction (Alexopoulos, 2009).

2.2 Fungi associated with contamination of lettuce (*Lactuca sativa)*

The association of fungi and plants is ancient and involves many different fungi. Fungi are an important group of plant pathogens, most plant diseases are caused by fungi, plant pathogenic fungi represent a relatively small subset of those fungi that are associated with plants. Most fungi are decomposers, utilizing the remains of plants and other organisms as their food source, other fungi are beneficial symbionts to plant (Tournas, 2005).

There are thousands of species of plant pathogenic fungi that collectively are responsible for 70% of all known plant diseases. Plant pathogenic fungi are parasites, but not all plant parasitic fungi are pathogens. Plant parasitic fungi obtain nutrients from a living plant host, but the plant host doesn't necessarily exhibit any symptoms. In this case, it's called endophytic fungi (Sperber, 2006).

2.3 Source of contamination of Lettuce

Raw vegetables and fruits can become contaminated along the farm to table continuum. Product used for salads (lettuce) grows low to the ground, where they are likely to come in contact with contaminated fertilizers. Sometimes they are irrigated with contaminated waters or picked by farm workers with poor hygiene practices. The complex multi-layered surfaces of salad produce are more difficult to clean after picking than product with a smooth surface, such as apples and potatoes. Because vegetables are usually eaten raw, they can pose a health risk if they are not properly handled. Therefore all produced need to be thoroughly washed and safely prepared

and handled before eaten. Leafy green vegetable can be contaminated by fungi via exposure to contamination sources from production to the point of sale in the market (king, 2007).

Transportation

During transportation, lettuce may be bruised resulting in the release of plant nutrients and thereby producing substrate that harbour fungi and other pathogenic microorganism (Hasheem *et al.*, 2010).

Cutting and Slicing

Vegetables such as lettuce, cabbage, are more susceptible to contamination because cutting and slicing damage the natural protective barriers of the intact produce and release nutrient and facilitate growth of microorganisms (Viswanathan, 2008).

Pre-Harvest or Post-Harvest Stages

The major sources of contamination by pre-harvesting process are soil (include Manure, Faeces, Soil Microorganisms), Dust, and Water. While containers used for transporting the produce, human handling, processing and storage are the major source of post-harvest contamination (Ibeyessie, 2007).

Storage

Due to the favourable conditions present in the sample, including high humidity, high temperatures, suitable pH and nutrients, Fungi and other Microbial population can proliferate fast. Lettuce is particularly susceptible to contaminants because it textured leaves can provide an ideal habitat for fungal growth (Nguyen, 2010).

2.4 Preventive measures

Washing with Sanitized Water

Washing with sanitized water is sometimes used to reduce the number of microorganisms present on the surface of the product. Analysis showed that maximum reduction of 50% of microbial load is achieved after the washing step (Pitt, 2005).

Proper Storage

Storage can be short-term or long-term. Most vegetables including lettuce, are perishable and short-term storage for a few days provides flexibility in marketing. During storage, leafy vegetables lose moisture, and the vitamin C in them degrades rapidly. Avoidance of contamination can be achieved when refrigerated storage is available, during the storage, high humidity most be maintain and to keep the product in the shade. However, when refrigerated storage is not available, the product most be affected with efficient chain cold application, there are temperature controlling techniques that do not require the use of electricity such as evaporative cooling. Do not mix lettuce with other fruits (such as apple mango banana etc) during storage because such fruit produce natural ripening agent (ethylene) which increase the rate of spoilage (Buck, 2003).

Irradiation

The irradiation of lettuce and other agricultural produce by ionizing radiation can be used to preserve it from both fungal infection and insect damage, as well as from physical deterioration. It can extend the storage life of food without changing its properties. Irradiation can serve many purposes, such as prevention of food borne illness: to effectively eliminate organism that cause food borne illness, preservation: to destroy or inactivate organisms that cause spoilage and decomposition foods, delay sprouting and ripening: to inhibit sprouting and delay ripening of fruit to increase

longevity, sterilization of food which can then be stored for years without refrigeration (Burnett *et al.*, 2007).

Canning

Canning is a process during which the enzymes in lettuce are deactivated and the micro-organisms present killed by heat. The sealed can excludes air from the foodstuff to prevent subsequent deterioration. The lowest necessary heat and the minimum processing time are used in order to prevent the mechanical breakdown of the product and to preserve the flavour as far as is possible. The can is then able to be stored at ambient temperatures for a long period (Burnett *et al.*, 2007).

2.5 Mycological analysis (isolation techniques)

Direct Plating Method

Ten (10) gram of each sample were surface sterilized by 6% sodium hypochlorite solution in a sterile conical flask for 1-2 min, and then washed by distill water 3 times for 1.5-2 min to removing the toxic activity of the chemical agent on the samples. The disinfected samples transferred with sterile forceps into Petri dish contain sterilized Czapek Dox Agar (CDA), at the rate of (5-10) pieces per plate, depending on the size of the particles, lager samples cut into small pieces. CDA supplemented with 0.5 mg chloramphenicol/ml to restrict bacterial growth. Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics (Anonymous, 2009).

Sterile Filter Plate Method

The samples submerge in the sodium hypochlorite 6% concentration for 1-2 min and then washed by distill water 3 times for 1.5- 2 min, then transfer by using sterilized forceps into three layers of moistened 9 cm diameter filter paper in sterilized Petri dishes, sterilized samples were evenly placed at the rate of 10 pieces per Petri plate at equal distance in each plate, samples of each variety were tested by employing standard blotter method in 3 replications. The plates were incubated for 5-7 days at 25°C; fungi developing on samples were examined and transferred to PDA for identification and pathogenicity studies (Abdullah *et al.*, 2002).

Standard Dilution Plate Method

For fungal analysis, dilution method was used to determine total fungal counts vegetables and medicinal plant samples. Ten grams of each composite sample (fine powder) were transferred into 250 mL screw-capped medicinal bottle containing 90 mL of sterile distilled water and were mechanically homogenized at constant speed for 15 min. The sample water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10) were prepared and 1 mL portions of suitable dilutions of the resulting samples suspension were used to inoculate Petri dishes each containing 15 mL Potato Dextrose Agar (PDA). Plates were then incubated for 7 days at 28°C. Three replicates plates per medium were used for each sample and the developing fungi were counted and the number per mg dry sample was determined and identified according to several key processes. Data expressed are average of all these media. After incubation, the results were expressed in Colony-Forming Units (CFU) /g of samples; all plates were examined visually, directly and with a microscope (Aziz *et al.*, 2011).

CHAPTER THREE

3.0 Materials and method

3.1 Sample Collection

Ten (10) sample of lettuce were collected at Gusau central market from ten (10) different vendors in a separate polyethene bag to microbiology laboratory for mycological analysis.

3.2 Sterilization

All the glassware used in this practical ware washed thoroughly in a detergent solution and raised in several changes of tap water. The glassware, which includes petridish, conical flask, glass slide and cover slide. They were allowed to air dried completely and placed in a hot air oven and sterilized at 160^0c.

3.3 Sample Preparation

One (1) gram of the sample was weighted using electronic weighting and crushed using blender, 1millilitre of the crushed sample were diluted inside test tube containing nine (9) millilitre of sterilized distilled water, the concentration of the sample reduced to 0.1, another one (1) millilitre of the diluted sample was measured and diluted into separate test tube containing nine (9) millilitre of sterilized distilled, the concentration of the sample reduced to 0.01, one (1) millilitre from the second diluted test tube was measured and diluted into a separate test tube containing nine (9) of sterilized distilled water and the concentration of the sample reduced to 0.001. This process was repeated for each sample.

3.4 Media Preparation

Potato dextrose agar (PDA) is the most suitable media used for fungal isolation. Thirty nine grams of PDA were weighed using weight balance and suspended in to a conical flask containing 500millilitre of distilled water. Five (5) gram of streptomycin (antibiotic) was added to inhibit bacterial growth. The pH of the media was adjusted to 5.6; the conical flask was then covered with cotton wool and capped with aluminium foil. The conical flask containing the contents was placed on hot plate to dissolve the media; the media was then sterilized by autoclaving machine at 121^0c for 15 minutes. The contents were allowed to cool for 5 minutes on a laboratory bench until the temperature fell to 45^0c. . The media was the dispensed into sterilized Petri dishes of 9cm in diameter, the media was then allowed to solidify.

3.5 Inoculation

One (1) millilitre from the third diluted test tube was measured using syringe from each sample and dispensed into separate petri dishes containing the media, a bend glass were used to smear the sample on the surface of the media. The petri dishes were kept inside the incubation room for three (3) days. After a period of three (3) days, various colonies of fungi appeared on the petri dishes.

3.6 Colony Count and Subculture

The petri dishes containing fungal colonies were removed from the incubation room for colony observation and subculture. Fungal colonies that grown were observed accounted and recorded. Each distinct colony was sub cultured into fresh PDA media to obtain a pure culture and to facilitate possible identification.

3.7 Identification

Each petri dish that has pure culture of the fungal colony were observed base on its colour, texture, as well as colonial morphology, these features observed were compared with the standard mycological atlas for identification. They were subjected to microscopic identification by picking a very small portion of the hyphae from each pure culture and emulsified on a separate slide with a little drop of KOH and distilled water, cover slip was used to cover the slide and it was viewed on a microscope under low, medium, and high power for confirmation of identification.

CHAPTER FOUR

4.1 Result

A total number of five (5) species of fungi associated with contamination of lettuce plant namely; *Cladosporium trichoids, Cladosporium werneckii, Candida albican, Blastomycete dermatus,* and *Aspergillus fumigatis* were identified. The colony and growth morphology of the fungi were also identified.

From the result, *Candida albican* appeared to have the highest chance of occurrence with a frequency of 33.3%, followed by *Cladosporium trichoids* with 28.6%, *Cladosporium werneckii* with 14.3%, *Blastomycete dermatitidus* with 14.3%, and *Aspergillus fumigatis* with 9.5%.

Table 1:Viable colony count of the fungi isolated

Samples	Number of colony (CFU/ml)
1	$1.16x10^6$
2	$1.2x10^5$
3	$1.08x10^6$
4	$1.36x10^6$
5	$8.8x10^5$
6	$9.7x10^5$
7	$1.04x10^6$
8	$1.54x10^6$
9	$1.0x10^6$
10	$1.4x10^6$

Table 2: Morphological features of the fungal colonies subculture

No. Of C.L	Size	Shape	Opacity	Elevation	Colour
1^1	V	X	V	X	Milky
1^2	M	X	X	X	Milky
2^1	V	V	V	X	Green
2^2	X	V	X	V	Red
3^1	M	X	X	X	Milky
3^2	X	V	V	X	Black
4^1	X	V	X	V	Yellow
4^2	M	X	X	X	White
5^1	M	V	V	X	Green
5^2	X	V	V	X	Green
5^3	X	V	X	X	Red
6^1	M	X	V	X	Green
6^2	V	X	X	X	White
7^1	M	V	V	X	Black
7^2	X	V	X	X	Red
8^1	M	X	V	V	Green
8^2	X	V	V	X	White
9^1	M	X	X	X	White
9^2	X	V	V	V	Red
10^1	X	V	X	V	Black
10^2	V	X	V	X	Red

Keys:

V= large
X= small
M=moderate

V= round
X= irregular

V= opaque
X= transference

V= raised
X= plate

Table 3: Frequency of each fungus in the samples

Name of the fungi	Number of the fungi isolated	Frequency (%)
Cladosporium trichoids	6	28.6
Cladosporium werneckii	3	14.3
Candida albican	7	33.3
Blastomycete dermatitidus	3	14.3
Aspergillus fumigates	2	9.5
	21	**100**

2 Discussion

The result obtained in this study showed that the contaminations of lettuce from Gusau central market are the result of various species of fungal infection, isolate belong to the following genera, *Candida, Cladosporium, Blastomycete, Aspergillus.*

The presence of these isolates could be attributed to the socio economical and sanitary conditions of the location. These conditions are factors such as use of waste water in irrigation for watering the field, improper handling and processing, use of contaminated water during washing, the use of dirty processing and harvesting materials such as knives, baskets, cross contamination from rotten lettuce and other vegetables (Khali *et al.*, 2012). This finding is in conformity with the previous works of Baiyawa and chukwuka which reported isolation of *Candida albican, Aspergillus fumigatis and Cladosporium trichoids* in Nigeria (Baiyawa *et al.*, 2004).

The presence of many pathogens in the soil was thought to be from historical application or environmental presence of faeces or untreated sewage and pathogens existing in the soil or water can be the source of both pre- and post- harvest contamination respectively. (Buck *et al.*, 2003)

However, the presence of various species of fungi in lettuce obtained from the market might be due to the use of unhygienic dusty environment, often warming with flies, other insects, airborne dust, mixing of rotten lettuce with fresh ones can also act as potential sources of contamination, these fungi grow on the surface of moist, carbohydrate-rich sources, such as fruits, and vegetables, most of the fungi isolated from lettuce could produce mycotoxins in food which are dangerous to humans and animals health (Michael *et al.*, 2005).

ey may also cause pulmonary disease in immune compromised patients and the oduction of oxalate in clinical specimens. Large amount of spore inhaled can cause serious lung disease, it can cause damage to the myelin coating around the nerve ores in the central nervous system and to the nerve fibres themselves interferes with he brain, spinal coat and the rest of the body (Nakagawa, *et al.*, 2012)

CHAPTER FIVE

5.1 Summary

The presence study was carried out to determine the fungi responsible for contamination of lettuce sold at Gusau central market. A total number of ten (10) samples were collected from ten (10) different vendors and brought directly to laboratory for mycological analysis, the samples were grounded and serially diluted, the diluted samples were inoculated on potato dextrose agar (PDA) media and autoclave, after autoclaving, the fungal colonies were counted and recorded, the colonies were subcultured in a freshly PDA media to obtained a pure culture of the fungal isolate, after subcultured, the pure cultures were identified using atlas of mycology. A total number of five (5) species of fungi namely; *Cladosporium trichoids, Cladosporium werneckii, Candida albican, Blastomycete dermatitidus,* and *Aspergillus fumigatis* were identified, *Cladosporium trichoids* has a frequency 26.8% , while *Cladosporium wernecki* has 14.3%, *Candida albican* has 33.3%, *Blastomycete dermatitidus* has 14.3%, and *Aspergillus fumigatis* has 9.5%.

5.2 Conclusion

This study revealed that lettuce can easily become contaminated by fungi which may be due to improper handling and storage; this can be a source of diseases to humans, animals. These fungi isolated from the lettuce may also be found in other vegetables and they may likely to cause economic losses to the marketers.

5.3 Recommendations

Measures to reduce the contamination might be advised such as proper handling and washing before taking the product, public education and awareness. And in order to minimize the risk of infections of lettuce, the following precautions should be maintained:

i. Good hygiene has to be ensured in every level of production.

ii. Proper sanitations must be practice.

iii. Government must have surveillance on the activities of the seller in order to minimize the risk of disease outbreak associated with the consumption of infected *Lettuce*.

iv. Careful handling in order to reduce wounding of the Lettuce.

v. Treatment of lettuce with fungal inhibitors among others.

vi. Government should supports implementation and documentation of vegetable safety programs that utilize risk assessment techniques that identify significant risks and use a preventive approach to ensure safe vegetable production.

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