

**FUNGAL LOADS OF PEPPER STORED IN AN AIRY PLACE AND AT
ALTERNATE 15°C AND ROOM TEMPERATURE FOR 14 DAYS**

By

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT
BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCE IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD
OF BACHELOR OF SCIENCE (HONOURS) DEGREE (Bsc) IN PLANT
BIOLOGY AND BIOTECHNOLOGY**

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Certification

We certify that this research work was carried out by Okoro-Lucky Sonia of the Department of Plant Biology and Biotechnology, Faculty of Life science University of Benin, Benin City, Nigeria.

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DEDICATION

I dedicate this work to God Almighty for His grace, mercy, wisdom and direction.

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I would like to use this opportunity to express my deepest gratitude to all those who have contributed to the successful completion of this project. Without their unending love, guidance and support, this project would not have been possible.

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ABSTRACT

Fungi plays a significant role in the deterioration of pepper. Pepper is a widely used and valued spice and is susceptible to fungal contamination, factors such as temperature, and storage conditions can influence the fungal load of stored pepper. This study shows the fungal dynamics of pepper (*Capsicum annum* and *Capsicum chinense*) when stored in room temperature, 15°C and alternations between 15°C and room temperature. In this research, healthy pepper samples (cayenne pepper and scotch bonnet pepper) were purchased from the market and stored in room temperature which was properly ventilated, they were also stored in at 15°C, and also alternated in 15°C and room temperature. They were monitored for 14 days and the observations recorded. Results revealed that lower temperature (15°C) had a higher amount of unaffected peppers and room temperature also had a lesser impact of spoilage than alternating temperatures. It highlights the importance of maintaining consistent temperature condition for long-term food preservation, it emphasizes the significance of implementing effective storage strategies to minimize fungal contamination in pepper. Ultimately, this study aids in enhancing optimal temperatures for maintaining the quality of stored pepper.

INTRODUCTION

CHAPTER ONE

1.1 PEPPER

Pepper, *Capsicum spp* which belongs to the family Solanaceae, which is commonly referred to as the nightshade family. The genus is native to the Americas and its fruits are grown worldwide for culinary, medicinal and spice purposes. The history of pepper cultivation dates back to ancient Mesoamerica, where the plant was domesticated over 6000 years ago. Bosland and Votava, (2012). Bell peppers were an important part of the diet of indigenous peoples of Central and South America and were also used in traditional medicine. When Christopher Columbus arrived in the Americas in the late 15th century, he was introduced to capsicum peppers, which he called "peppers" because of their spicy taste, which reminded him of the black pepper he knew from Europe. Columbus brought capsicum peppers back to Europe, where they quickly became popular as a spice and food ingredient. There are many varieties of peppers today, from sweet peppers to very hot varieties like the Carolina Reaper. The most commonly cultivated species include *Capsicum annuum*, *Capsicum chinense* and *Capsicum frutescens*. Bell peppers are grown in many parts of the world, with the largest producers being China, Mexico, Turkey and Indonesia. Bosland and Votava, (2012). It is usually grown as an annual, although some species can become perennials in warmer climates. Peppers are relatively easy to grow and can be grown in a wide variety of soils and climates, but they require warm temperatures and plenty of sunlight. In addition to its culinary uses, pepper is also used in traditional medicine to treat a variety of conditions, including pain, inflammation, and digestive problems.

1.2 DIFFERENT SPECIES OF PEPPER

There are various varieties of pepper and some common species include:

- i. Black pepper (*Piper nigrum*).
- ii. Habanero pepper (*Capsicum chinense*)
- iii. Sichuan pepper (*Zanthoxylum simulans*)
- iv. Scotch bonnet pepper (*Capsicum chinense*)
- v. Cayenne pepper (*Capsicum annum*)

Each of these pepper varieties its own unique flavor, heat level and culinary uses.

1.3 HEALTH BENEFITS OF PEPPER

The antioxidants and vitamins present in peppers can contribute to healthy skin. Vitamin C promotes collagen synthesis, which helps maintain skin elasticity and reduce the signs of aging. Additionally, the antioxidant properties of peppers may help protect the skin from damage caused by environmental factors (Pullar et al., 2017). Capsaicin, found in chili peppers, has been studied for its pain-relieving properties. When applied topically,

capsaicin can help reduce pain by desensitizing nerve receptors and inhibiting pain signals. It is commonly used in creams and patches for conditions like arthritis, neuropathy, and post-herpetic neuralgia (Derry et al., 2017). They are a good source of dietary fiber, which plays a crucial role in supporting a healthy digestive system. Fiber helps promote regular bowel movements, supports the growth of beneficial gut bacteria, and may contribute to a reduced risk of conditions like constipation, diverticulosis, and colorectal cancer (Slavin, 2013). The antioxidants and fiber in capsicum peppers contribute to cardiovascular health by reducing oxidative stress and supporting healthy blood vessels, including them in your diet may help lower the risk of heart disease. Grosso, *et al* (2016). Piperine in black pepper has been found to help improve insulin sensitivity and reduce blood sugar levels, Chen *et al.*, (2015). Pepper has been found to have protective effects on the liver, Liu *et al.*, (2014). Some studies have indicated that capsaicin and other compounds in peppers may have anticancer properties and could be beneficial in preventing certain types of cancer (Surh, 2016). Piperine in black pepper has been found to help with weight loss by increasing metabolism and reducing appetite (Whiting et al., 2014). Capsaicin in pepper has been found to improve cognitive function and memory in elderly people with vascular cognitive impairment (Yoon *et al.*, 2018)

1.3.1 NUTRITIONAL COMPOSITION OF PEPPER

Pepper is also rich in various vitamins and minerals including:

- i. Vitamin K: Peppers provide vitamin K, important for blood clotting and bone health. (Sánchez-Moreno et al.,2011).
- ii. Vitamin A: Bell peppers, especially light-colored peppers, contain large amounts of provitamin A carotenoids, which contribute to vitamin A absorption (Smith and Johnson, 2018).
- iii. Vitamin B6: Pepper is a source of vitamin B6, which is important for various metabolic processes (Smith and Johnson, 2018).
- iv. Potassium: Pepper contains potassium, which plays a role in maintaining healthy blood pressure (Smith and Johnson, 2018).
- v. Iron: Although not as rich in iron as other foods, peppers contain small amounts of this essential mineral (Smith and Johnson, 2018).
- vi. Magnesium: Magnesium, which is important for muscle and nerve function, is found in green peppers (Smith and Johnson, 2018).

- vii. Phytonutrients: Bell peppers are rich in various phytonutrients, including flavonoids and carotenoids that have antioxidant properties and potential health benefits (Rodriguez-Amaya, 2019).
- viii. Zinc: Peppers contain zinc, which is necessary for immune function and wound healing. (Bae et al., 2016)

1.2 BOTANY OF *Capsicum annuum* L.

- i. Growth habits: *Capsicum annuum* is a flowering plant that can grow as an annual or as a perennial, depending on climate and growing conditions. It usually reaches 0.5 to 1.5 meters in height, with a bushy, branched growth.
- ii. Leaves: The leaves are usually medium to dark green in color. They are simple, alternate, and oval or lance-shaped. The leaf margins are smooth or slightly serrated and the leaves alternate along the stem. (Smith and Bosland., (2012).
- iii. Flowers: The flowers are small and are usually white or yellow. These flowers have a five-lobed structure and grow singly or in clusters (inflorescences) in the leaf axils. Flowers hermaphroditic, containing both male and female reproductive organs. (Smith and Bosland., (2012).

- iv. Fruit: The fruit of *Capsicum annuum* is a famous chili pepper that varies in size, shape, color and flavor. They can be small, like peppers, or larger, like bell peppers. The fruit shape can be oblong, oblong or bell-shaped. Mature colors range from green to yellow, orange, red or even purple, depending on the specific variety. (Bosland and Votava, 2012).
- v. Seeds: Inside the fruits are many small seeds. They are usually flat, ovoid or kidney-shaped. They are a means of reproduction and dispersal when the fruit is eaten or rotted. (Bosland and Baral., 2007).

1.2.1 DISTRIBUTION OF *Capsicum annuum* IN THE WORLD

- i. America: *Capsicum annuum* is native to America, especially Central and South America. Its origin is believed to be in Mexico. The plant is still cultivated throughout the region, including countries such as Mexico, Peru, Brazil and Colombia. Various types of peppers, peppers and other peppers are grown in these regions.
- ii. North America: This pepper is widely grown in North America, especially in the United States and Mexico. Various states in the United States such as California, New Mexico, and Texas are large producers of chili peppers. Mexico, the country of origin, has a wide variety of chili peppers.

- iii. Europe: Cultivated throughout Europe for both commercial and home gardening purposes. Spain, Italy, Holland and Hungary are among the leading European countries in chili pepper cultivation. Spain in particular is known as a production center of pepper.

- iv. Asia: Widely cultivated in Asia. India, China, Thailand, and Vietnam are major producers of chili peppers, including various species of hot peppers. Chili peppers are important both culturally and culinary in India, with different varieties grown throughout the country.

- v. Oceania: Chili peppers, including the *Capsicum annuum* variety, are grown in Oceania, particularly in countries such as Australia and New Zealand. Various varieties of pepper have been adapted and cultivated in these regions for both edible and ornamental purposes.

1.2.2 DISTRIBUTION OF *Capsicum annuum* IN AFRICA

- i. North Africa: In North Africa, capsicum annum is cultivated in countries such as Morocco, Algeria, Tunisia and Egypt. They are used in traditional North African cooking to add spice and flavor to dishes such as harissa, couscous and tagines.

- ii. Ethiopia: They are grown in Ethiopia and used in sauces such as spicy stews and berbere, a traditional spice mix.

- iii. South Africa: Chili peppers, including sweet peppers, are grown in South Africa. They are used in a variety of dishes and traditional dishes to add flavor and spiciness to meals

- iv. Kenya: Cayenne peppers are grown in Kenya and used in local dishes and dishes such as pilau, nyama choma and stews.

- v. Other Regions: They are also grown in other African countries such as Ghana, Tanzania, Uganda, Cameroon, Senegal and Mali. Local varieties are grown and used in traditional cooking as they provide both spiciness and flavor.

1.2.3 DISTRIBUTION OF *Capsicum annum* IN NIGERIA

- i. Northern Nigeria: Cultivation of these peppers is particularly thriving in the northern region of Nigeria. States such as Kano, Kaduna, Katsina, Sokoto and Jigawa have a long tradition of chili cultivation. The region's climate is favorable for pepper production, and many local varieties are grown.

- ii. South West Nigeria: The cultivation is also widespread in the South West Nigeria states such as Lagos, Oyo, Ogun and Ekiti. Farmers in these areas grow different varieties for both local consumption and commercial purposes.

- iii. Southeast and South South Nigeria: Chili peppers are grown in Southeast and South South Nigeria. States such as Enugu, Anambra, Imo, Delta, Rivers and Cross Rivers grow chilies to meet local demand and supply neighboring communities.

- iv. Middle Belt Region: They are also grown in the Middle Belt Region of Nigeria, including states such as Benue, Plateau, Nasarawa and Taraba. The region's favorable climate and agricultural practices contribute to pepper production.

- v. Urban areas: In addition to rural areas, they are also cultivated in Nigerian urban areas such as Lagos, Abuja and Port Harcourt. Urban farmers and gardeners grow peppers in pots and small gardens for home consumption and as a source of fresh produce.

Capsicum annum are grown in different regions of Nigeria. Major regions known for growing this pepper include Kaduna and Plateau states in the north and Oyo states

in the southwest. These regions have favorable agroclimatic conditions and a tradition of agricultural expertise that contribute significantly to the country's pepper production.

In addition, other states such as Benue, Kano and Ogun are also growing hot peppers. These specie of pepper are widely cultivated in Nigeria, providing a stable supply of this important crop for both domestic consumption and commercial purposes.

1.2.4 ECONOMIC VALUE OF *Capsicum annum*

- i. Commercial cultivation: *Capsicum annum* is widely grown by farmers around the world. Its economic value lies in its high demand and marketability. Farmers grow different varieties to cater to different culinary tastes for domestic and export markets. Commercial cultivation of these peppers provides income for the farmers, agricultural cooperatives and agribusinesses involved in the production, processing and distribution of these peppers.

- ii. Culinary and Food Industry: It is an integral part of many different cuisines around the world and contributes to its economic value. Both chillies and bell peppers are commonly used in culinary preparations, ranging from everyday dishes to gourmet and specialty products. The culinary industry, including restaurants, food manufacturers, and spice companies, use this pepper to add flavor, spiciness, and visual appeal to a variety of foods. Demand for *Capsicum*

annuum peppers drives economic activity in the food industry and creates employment opportunities.

- iii. Export Trade: These varieties of pepper are traded internationally and contribute to the export earnings of many countries. Regions with favorable agro-climatic conditions for the production of *Capsicum annuum* can benefit economically by exporting surplus peppers to foreign markets. Countries such as Mexico, Spain, the Netherlands, and the United States are the top exporters of the pepper. International trade in these peppers stimulates economic growth, increases foreign exchange earnings and strengthens trade ties.
- iv. Value-Added Products: These pepper are not limited to fresh consumption. These are processed into various value-added products such as chili sauces, pickles, spice mixes, dried peppers and extracts. These products have their own market and contribute to the economic value of these peppers. A food processing industry that specializes in *Capsicum annuum*-based products creates additional economic opportunities, including job creation and revenue generation.



Plate 1: Cayenne pepper (*Capsicum annum*)

Source: Rainbowscotch

1.4 BOTANY OF *Capsicum chinense* Jacq.

- i. Growth Habit: It is a perennial shrub, usually reaching 1-1.5 meters in height. It has a tendency to grow thick and can be grown as an annual in cool regions.
- ii. Leaves: The leaves are dark green and lanceolate in shape. It has a glossy appearance.
- iii. Flowers: It produces small, usually white or cream colored flowers. These flowers are star-shaped and have a unique beauty. They are bisexual and contain both male and female reproductive structures.
- iv. Fruits: Capsicum fruits are characteristic of this species and come in various shapes, sizes and colors. The shape of the fruit is oblong or bell-shaped depending on the variety. When ripe, the fruit exhibits bright colors such as red, orange, yellow and even chocolate brown. (Andrews, 2015).
- v. Seeds: The fruit has many small seeds. These seeds are usually white or brightly colored and are an important part of the plant's reproductive cycle. (Guzman and Bosland 2017).

1.3.1 DISTRIBUTION OF *Capsicum chinense* IN THE WORLD

- i. Central and South America: *Capsicum chinense* is native to Central and South America, where it has a long history of cultivation. Countries such as Mexico, Peru, Brazil, Colombia, and the Caribbean islands are known for their cultivation and consumption of these peppers. The region showcases a rich diversity of these varieties, including well-known chili peppers like the habanero, Scotch bonnet, and Rocoto.
- ii. Southern Asia: Like Thailand, Vietnam, Cambodia, and Laos have incorporated different varieties into their cuisines. Peppers such as the Thai bird's eye chili and the Bhut Jolokia (ghost pepper) are examples of *Capsicum chinense* varieties that are widely used in Southeast Asian cuisine.
- iii. Africa: It has made its way to various parts of Africa through trade and cultural exchanges. Several African countries, including Nigeria, Ghana, Cameroon, and Senegal, cultivate and consume these peppers. Local varieties of *Capsicum chinense* have been adapted to suit regional tastes and are important ingredients in African cuisine.
- iv. Europe, North America, and beyond: This variety of pepper has been introduced to other parts of the world through trade and cultural exchange. They are cultivated and consumed in regions such as the United States, Mexico, the Caribbean islands, India, Australia, and parts of Europe. These regions often incorporate this pepper variety into their local cuisines and appreciate the unique flavors and heat levels they offer.

1.3.2 DISTRIBUTION OF *Capsicum chinense* IN AFRICA

- i. West Africa: These peppers are widely cultivated and consumed in West African countries. Nigeria, in particular, is known for its cultivation of *Capsicum chinense* varieties such as the habanero pepper. Ghana, Cameroon, Senegal, and Ivory Coast also cultivate and use this pepper in their traditional cuisines.
- ii. Central Africa: They are grown and consumed in countries of Central Africa, including the Democratic Republic of Congo, Angola, and Gabon. Local varieties of *Capsicum chinense* are utilized in various regional dishes, adding spiciness and flavor to the cuisine.
- iii. Southern Africa: These peppers have also found their way to Southern Africa. Countries like Malawi, Mozambique, Zambia, and Zimbabwe cultivate and incorporate these varieties into their culinary traditions. The peppers are used in traditional stews, sauces, and condiments to enhance the flavors of local dishes.
- iv. East Africa: While *Capsicum chinense* is not as commonly cultivated as other pepper varieties in East Africa, there are still instances of its cultivation in countries like Kenya, Tanzania, and Uganda. It is often grown by farmers and used in local dishes, providing a fiery kick to the cuisine.

1.3.3 DISTRIBUTION OF *Capsicum chinense* IN NIGERIA

- i. Cross River: Is located in the southern part of Nigeria. The state's favorable agro-climatic conditions, including a tropical rainforest climate, make it suitable for cultivation.
- ii. Akwa Ibom: Situated in the southern part of Nigeria, is another notable region for its production of these varieties. The state's climate and fertile soil provide favorable conditions for growing *Capsicum chinense* peppers. Farmers in this region cultivate this pepper to cater to local consumption and commercial purposes.
- iii. Rivers: Located in the Niger Delta region of Nigeria is known for its agricultural productivity, including the cultivation of these peppers. The state's suitable climate and agricultural practices support the growth of the pepper.
- iv. Other Regions: some other regions where it is cultivated are Edo, Delta, Enugu, Imo, and Anambra, among others.

Capsicum chinense, is cultivated in various regions across Nigeria. Notable areas for its production include Cross River State, Akwa Ibom State, and Rivers State. These regions offer favorable agro-climatic conditions and fertile soils that support growth for the peppers.

Other regions such as Edo, Delta, Enugu, Imo, and Anambra also contribute to its cultivation in Nigeria. The widespread production of the pepper ensures their availability for culinary use and contributes to the vibrant spice industry in the country.

1.3.4 ECONOMIC VALUES OF *Capsicum chinense*

- i. **Culinary and Food Industry:** It is highly valued in the culinary industry for their intense heat and unique flavors. They are widely used in various cuisines, including Asian, Caribbean, and Latin American dishes. The demand for spicy and flavorful foods has created a market for the pepper and products derived from them, such as hot sauces, spice blends, and marinades. This contributes to its economic value as it drives the growth of the food industry, including restaurants, food manufacturers, and specialty spice producers.
- ii. **Export Trade:** It has a global market demand, which has led to their significant export trade. Countries which cultivate them, including China, Mexico, Thailand, and the Caribbean nations, export these peppers to various parts of the world. The export of the pepper contributes to the agricultural economy of producing countries, creating employment opportunities, generating foreign exchange earnings, and strengthening trade relationships.
- iii. **Medicinal and Pharmaceutical Applications:** These peppers contain a compound called capsaicin, which is known for its medicinal properties. Capsaicin is used in the

production of topical pain-relieving creams and patches, as it can provide relief for muscle aches, arthritis, and nerve pain. The pharmaceutical industry also utilizes these peppers in the production of certain medications. The medicinal and pharmaceutical applications of the pepper contribute to its economic value by expanding its market beyond culinary use.

- iv. Value-Added Products: They are not solely consumed as fresh produce but are also processed into value-added products. These include dried peppers, chili powders, chili flakes, and chili oils.



Plate 2: Scotch bonnet pepper (*Capsicum chinense*)

Source: Rainbowscotch

1.5 EFFECTS OF SPOILAGE ON PEPPER CONTENT

- i. **Nutrient Degradation:** When pepper spoils, its nutrient content can degrade. Essential vitamins, minerals, and other beneficial compounds present in fresh pepper may break down or diminish in concentration over time. This can result in a decrease in the nutritional value of the spoiled pepper.
- ii. **Flavor and Aroma Changes:** Spoilage can alter the flavor and aroma of pepper. The breakdown of compounds responsible for the characteristic taste and scent of fresh pepper can lead to off-flavors and unpleasant odors in spoiled pepper. The pungency, spiciness, and complexity of the flavor profile may be compromised.
- iii. **Loss of Active Compounds:** Pepper contains various active compounds, such as capsaicinoids in chili peppers, which contribute to their unique properties. Spoilage can cause a decrease in the concentration or degradation of these active compounds, resulting in diminished sensory and potential health benefits.
- iv. **Increased Oxidation:** When pepper is exposed to air or moisture during spoilage, it is susceptible to oxidation. Oxidation reactions can lead to the degradation of certain compounds, including pigments and antioxidants. As a result, the color of the pepper may change, and the antioxidant capacity may decrease.

- v. **Microbial Contamination:** Spoilage often involves the growth of microorganisms, such as bacteria, yeasts, and molds, on the pepper. These microorganisms can compete with the natural components of the pepper and produce metabolic byproducts, which can further alter the content and composition of the pepper.

- vi. **Presence of Toxins:** In some cases, spoilage can lead to the production of toxins by certain microorganisms. For example, molds can produce mycotoxins, which can be harmful if consumed. These toxins can pose a risk to human health and contribute to the deterioration of the pepper's content.

1.5.1 EFFECT OF SPOILAGE ON PEPPER VALUE

- i. **Decreased Market Demand:** When pepper spoils, its quality, taste, and appearance are compromised. Consumers seek fresh and high-quality products, and the presence of spoilage can significantly decrease the market demand for the affected pepper. This can lead to decreased sales and lower prices for the spoiled pepper.

- ii. **Loss of Product Shelf Life:** Spoiled pepper has a significantly reduced shelf life compared to fresh pepper. The shorter shelf life limits the time window for selling and consuming the product. As a result, the value of the spoiled pepper decreases as it becomes unsellable or its consumption is discouraged.

- iii. **Financial Losses:** The presence of spoilage can result in financial losses for various parties involved in the pepper supply chain, including producers, distributors, and retailers. Spoiled pepper cannot be sold or may need to be discarded, leading to wasted resources, time, and money.

- iv. **Brand Reputation:** If a brand or supplier consistently delivers spoiled pepper, it can damage their reputation in the market. Consumers expect reliable and high-quality products, and the presence of spoilage can undermine trust and negatively impact the brand's reputation. This can have long-term effects on the overall value and perception of the brand.

- v. **Inventory Management Challenges:** Managing inventory becomes more complex when spoilage occurs. Producers and retailers need to carefully monitor their stock, identify and remove spoiled pepper from their inventory, and adjust their supply chain accordingly. This adds extra costs and logistical challenges to the process.

- vi. **Consumer Perception:** The presence of spoiled pepper can lead to negative consumer perception of the overall quality and reliability of the product. This perception can extend beyond the specific batch or brand, affecting the reputation of peppers in general. It may take time and effort to rebuild consumer trust and restore the value of the product.

Fungi, plays a significant role in the spoilage and deterioration of food products, including spices like pepper. Pepper being a widely used and highly valued spice, is susceptible to fungal contamination during storage. Factors such as temperature, humidity, and storage conditions can influence the fungal load and subsequent quality of stored pepper.

Fungal load refers to the amount of fungal contamination present in a sample. In the case of pepper, fungal contamination can lead to spoilage, loss of quality, and even pose health risks to consumers. The conditions under which the pepper is stored can significantly impact fungal growth and proliferation.

Temperature is a critical factor in fungal growth, with different fungi having specific temperature ranges in which they thrive. Therefore, alternating temperatures may have different effects on fungal growth and metabolism.

Understanding the impact of storage conditions on fungal load is essential for implementing environment and alternating temperatures may provide insights into the dynamics of fungal growth and proliferation during storage. By simulating these conditions, we can assess the changes in fungal load over a defined period as in the case of this study which is in a period of 14 days.

1.4 LITERATURE REVIEW

There are several studies available in the literature on the impact of temperature and storage conditions on the fungal load of various food products, including peppers. A literature review shows that the effect of temperature on fungal growth in peppers is dependent on several factors, including the type of fungi present, storage conditions, and the initial fungal load.

One study by Adebowale et al. (2015) investigated the effect of storage temperature on fungal load and quality of pepper powder. The study found that fungal load increased with increasing storage temperature, with the highest fungal load observed at 35°C. The study also found that the storage temperature had a significant impact on the color and moisture content of the pepper powder.

Another study by Akinyele and Omemu (2016) investigated the effect of storage conditions on fungal growth and aflatoxin production in pepper samples. The study found that storage at 25°C and 85% relative humidity resulted in the highest fungal load and aflatoxin production, while storage at 4°C and 50% relative humidity resulted in the lowest fungal load and aflatoxin production.

A study by Lianfu et al. (2021) investigated the effect of storage temperature on the fungal community composition and diversity of pepper samples. The study found that storage at

30°C resulted in the highest fungal diversity, while storage at 4°C resulted in the lowest fungal diversity.

1.6 AIM OF STUDY

To determine the effect of temperature on the fungal load and shelf life of pepper.

1.6.1 OBJECTIVES

- i. To isolate and identify fungi associated with stored pepper.
- ii. To determine how storage temperature affect shelf life of the pepper.
- iii. To determine how alternate low-high temperature affect fungal load of the pepper.
- iv. To recommend best practices in storage of pepper to minimize spoilage.

CHAPTER TWO

MATERIALS AND METHODS

2.1 COLLECTION OF MATERIALS

The plants (*Capsicum chinense* and *Capsicum annum*) were purchased from New Benin market, Oredo Local Government Area, Edo state.

2.2 OTHER MATERIALS

Methylated spirit, lactophenol cotton blue, Potato Dextrose Agar (PDA), cotton blue, slides and cover slips, petri dish, latex gloves and aluminum foil were purchased at a public laboratory store in Ugbowo, Benin city, Edo state. Autoclave, weighing balance, beakers, glass rod and spatula were also used in the laboratory.

2.3 PREPARATION OF PEPPER SAMPLES

The pepper samples were washed thoroughly with distilled water, healthy and clean pepper was selected from each species. The pepper samples were labeled accordingly in three temperatures namely; 15°C, room temperature, alternate (which is the alternation of 15°C and room temperature). The pepper samples were placed in a petri dish on a laboratory bench in the varying temperatures in three set ups each.



Plate 3: Thoroughly washed cayenne pepper



Plate 4: Thoroughly washed scotch bonnet pepper



Plate 5: Set up of the pepper samples in various temperatures.

2.4 OBSERVATION OF PEPPER SAMPLES.

After the pepper samples were stored in their various temperatures, they were observed for any fungal growth such as, discoloration, molds, changes in texture. The presence or absence of any growth was recorded. Each sample was regularly examined every 24 hours for the period of 14 days.

2.5 PREPERATION OF POTATO DEXTROSE AGAR (PDA)

A measured amount of 9.75g of powdered PDA was transferred into a 250ml conical flask, 250ml of distilled water was measured and the 9.75g of powdered PDA was dissolved into 250ml distilled water, it was properly stirred with a glass rod for homogeneity. The neck of the conical flask was plugged with cotton wool covered with aluminum foil. The medium was then sterilized in an autoclave at 121°C for 15Ibs for fifteen (15) minutes as holding time. The sterile petri dishes were placed on a sterile bench while allowing the already sterilized medium to cool down. After cooling down, the medium was dispensed aseptically into the sterile petri dishes before inoculation was carried out.

2.6 CULTURING

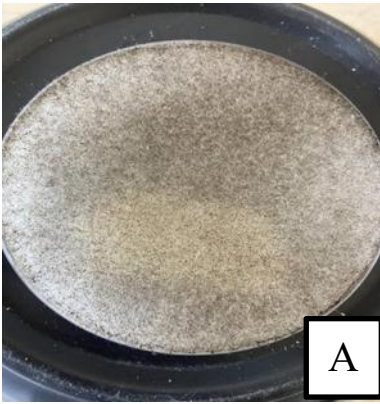
After observing the pepper samples and had seen how they had deteriorated overtime some of the pepper was selected from varying temperatures.

Potato dextrose agar (PDA) was prepared and sterilized in an autoclave at 121°C at 15Ibs for 15 minutes as holding time and poured in sterile petri dishes and allowed to solidify. A sterile cotton swab was prepared for collecting the fungal samples from the various pepper species. The cotton

swab was moisten with distilled water and used to gently rub the surface of the pepper to collect fungal material. The collected material was then used to streak the surface of the agar plate. This process was repeated for each pepper sample, and labeled in the various temperatures for identification. The petri dishes were sealed with masking tapes to allow them incubate for a period of 3 days.

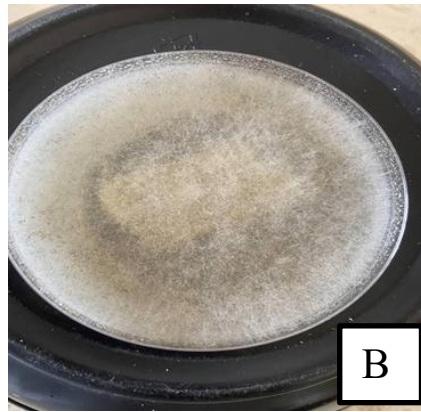
2.7 ISOLATION

After incubation, the plates were observed for any visible growth, individual colonies of fungi were observed on the surface of the agar. Potato dextrose agar (PDA) was prepared and sterilized in an autoclave at 121°C at 15lbs for 15 minutes as holding time and poured in sterile petri dishes and allowed to solidify. A single well isolated colony was inoculated on the prepared agar medium (PDA). These plates were allowed to incubate for a period of 3 days.



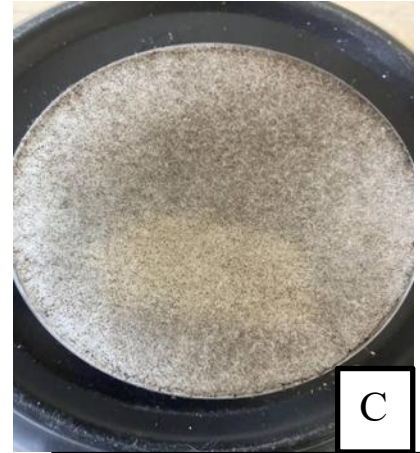
A

Plate 6: ISOLATE WHITE
ACP



B

Plate 7: ISOLATE GREEN
ACP



C

Plate 8: ISOLATE SB

2.8 FUNGAL COUNT

After isolating the micro organisms, 9ml of distilled water was dispensed aseptically into three sets of mcartney bottles (20ml), a loop of the pure cultures isolated was collected and dissolved in the bottles.

One milliliter of the stalk was then transferred into the first dilution and this was done serially to the fifth dilution (10^5), and this process was repeated for each isolated culture.

After serial dilution 1ml of each dilution from the three isolated cultures was dispensed into sterile petri dishes representing all the dilution. The molten agar medium was then dispensed into all the petri dishes accordingly and was rocked for even distribution. This was allowed to solidify and then incubated for 3days on an prepared agar medium (PDA).

After incubation, the visible colonies for each microorganisms on the agar plates were counted and recorded



Plate 9: serial dilution of isolated microorganisms.



Plate 10: Plating of isolated microorganisms.

The fungal load of each isolated microorganisms is recorded below:

Table 2.1 ISOLATE WHITE ACP

DILUTION	REPLICATE 1	REPLICATE 2	REPLICATE 3	CFU/g
10 ¹	27	26	28	2.7×10 ²
10 ²	18	20	16	1.8×10 ²
10 ³	16	17	15	1.6×10 ²
10 ⁴	9	8	10	9 ×10 ¹
10 ⁵	3	2	4	3×10 ¹

Table 2.2 ISOLATE GREEN ACP

DILUTION	REPLICATE 1	REPLICATE 2	REPLICATE 3	CFU/g
10 ¹	57	55	59	5.7×10 ²
10 ²	39	40	38	3.9×10 ²
10 ³	36	42	30	3.6×10 ²
10 ⁴	21	24	18	2.1×10 ²
10 ⁵	18	19	17	1.8×10 ²

Table 2.3 ISOLATE SB

DILUTION	REPLICATE 1	REPLICATE 2	REPLICATE 3	CFU/g
10 ¹	43	56	30	4.3×10 ²
10 ²	28	26	30	2.8×10 ²
10 ³	18	19	17	1.8×10 ²
10 ⁴	16	18	14	1.6×10 ²
10 ⁵	11	10	12	1.1×10 ²

2.8 MICROSCOPIC EXAMINATION

Stained slides of the fungal isolates was prepared, using the lactophenol cotton blue stain, the fungal structures, including spores, hyphae, and reproductive structures were observed under a microscope. The results obtained from microscopic examination, were used for proper identification of the fungi responsible for the spoilage of both pepper species.

2.10 STATISTICAL ANALYSIS

These results was collected using frequency counts and histogram.

CAYENNE PEPPER (15°C)

Total number =126

Number of unaffected pepper = 96

Number of affected pepper = 30

CAYENNE PEPPER (ROOM TEMPERATURE)

Total number = 126

Number of unaffected pepper = 62

Number of affected pepper = 64

CAYENNE PEPPER (ALTERNATE)

Total number = 126

Number of unaffected pepper = 58

Number of affected pepper = 68

SCOTCH BONNET PEPPER (15°C)

Total number = 126

Number of unaffected pepper = 92

Number of affected pepper = 34

SCOTCH BONNET PEPPER (ROOM TEMPERATURE)

Number of unaffected pepper = 61

Number of affected pepper = 65

SCOTCH BONNET PEPPER (ALTERNATE)

Total number = 126

Number of unaffected pepper = 56

Number of affected pepper = 70

CHAPTER THREE

RESULTS

Figure 1: Illustrates the outcomes of the experiment conducted with cayenne pepper at a temperature of 15°C. In this condition, I recorded 96 peppers that remained unaffected and 30 peppers that showed signs of being affected, after 14 days.

Figure 2: Represents the results of the cayenne pepper experiment conducted at room temperature. In this case, there were 62 unaffected peppers and 64 affected peppers.

Figure 3: Shows the result on the cayenne pepper experiment conducted at alternating temperatures. I observed 58 unaffected peppers and 68 affected peppers.

Figure 4: Shows a comparative analysis of the three different temperature conditions for cayenne pepper. It indicates that 15°C had the highest number of unaffected peppers, totaling 96.

Figure 5: Displays the findings from the analysis conducted on Scotch bonnet peppers exposed to a temperature of 15°C. This condition yielded the highest count of unaffected peppers, with a total of 92, while 34 peppers were found to be affected.

Figure 6: This result shows the outcomes of the Scotch bonnet pepper analysis performed at room temperature. The number of unaffected peppers decreased to 61, while 65 peppers were observed to be affected under these conditions.

Figure 7: Shows the results of the Scotch bonnet pepper analysis conducted at alternating temperatures. In this case, 56 peppers remained unaffected, while 70 peppers were affected.

Figure 8: Shows a comparative overview of the three distinct temperature settings used for the Scotch bonnet pepper analysis. It highlights that the 15°C environment produced the highest count of unaffected peppers, with a total of 92.



Plate 11: Cayenne pepper and scotch bonnet at 15°C. A; Day 1 B; Day 7 C; Day 14



Plate 12: Cayenne pepper and scotch bonnet at Room temperature, A; Day 1 B; Day 7 C; Day 14



Plate 13: Cayenne pepper and scotch bonnet in Alternate temperatures, A; Day 1 B; Day 7 C; Day 14

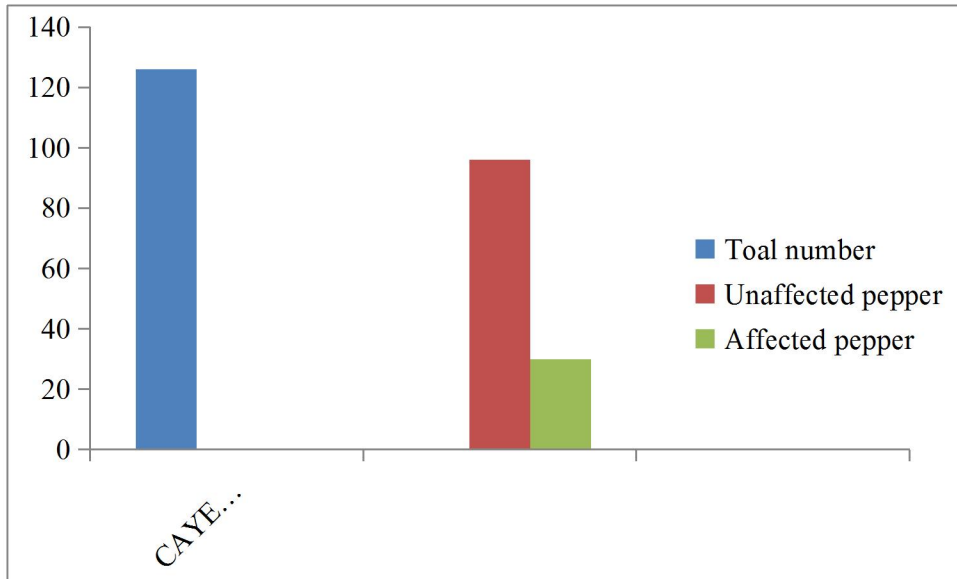


Fig 3.1: Effect of 15°C on cayenne pepper. The result shows that at 15°C the pepper was least affected, it shows that this temperature is suitable for prolonging the shelf life of the pepper.

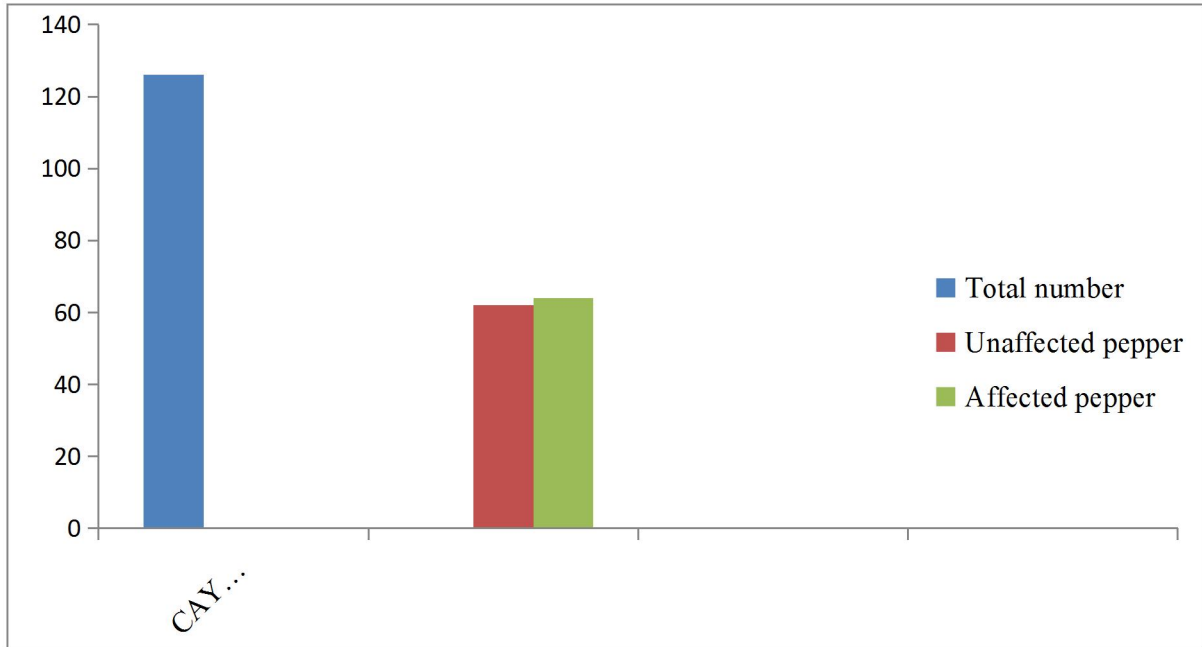


Fig 3.2: Effects of room temperature on cayenne pepper. This result shows the effect of room temperature on cayenne pepper, also prolonged the shelf life of the pepper.

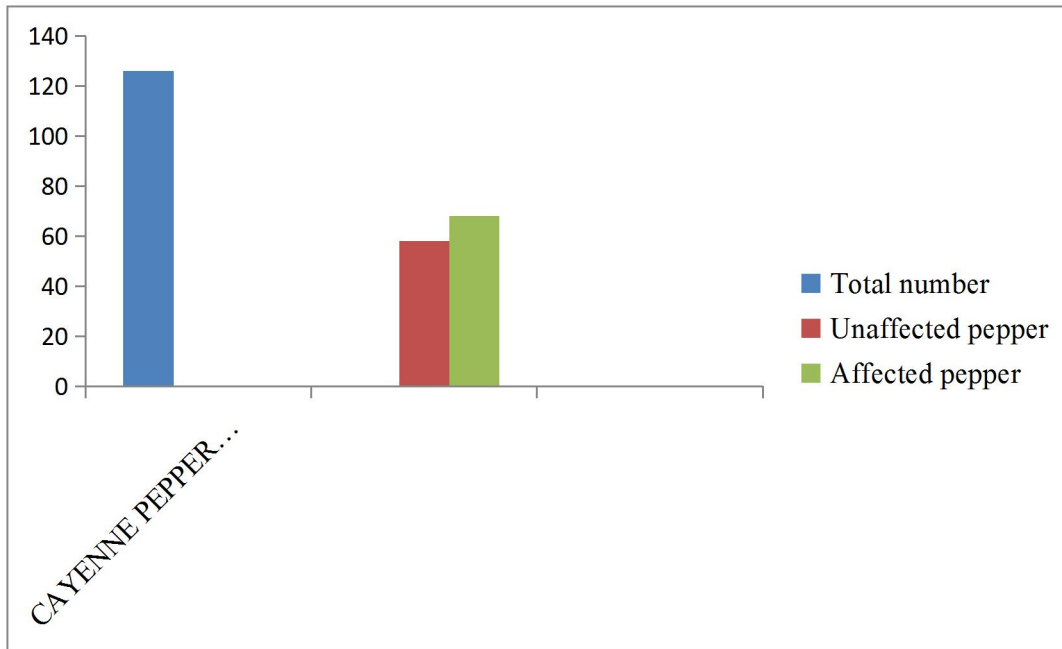


Fig 3.3: Effects of alternating temperatures on cayenne pepper, The result shows that alternating the temperatures had the highest amount of affected pepper thereby reducing the shelf life of the pepper.

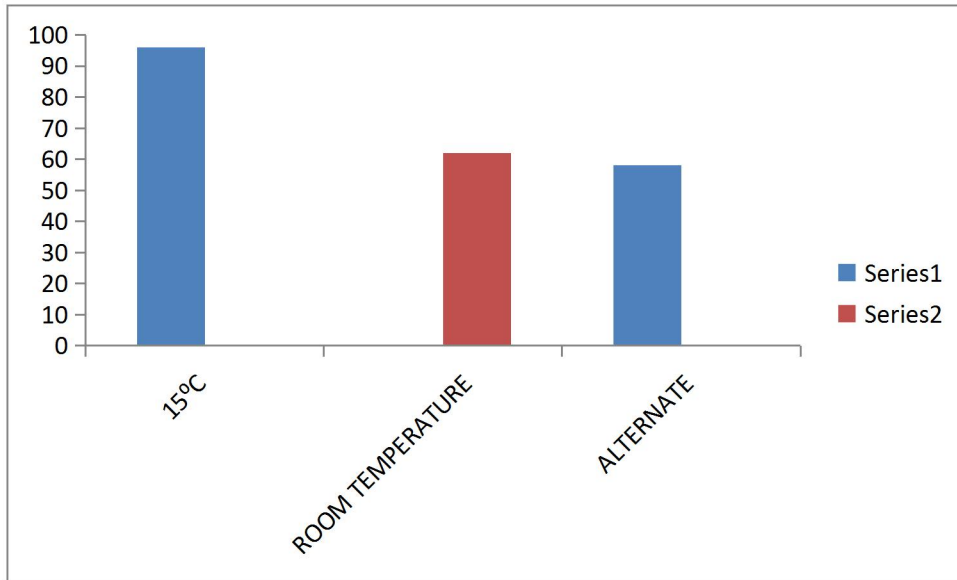


Fig 3.4: The comparison between the effect of, 15°C, room temperature and the alternate (alternation of 15°C and room temperature) on cayenne pepper. Totally the result shows that 15°C is more effective in prolonging the shelf life of scotch bonnet pepper, followed by room temperature.

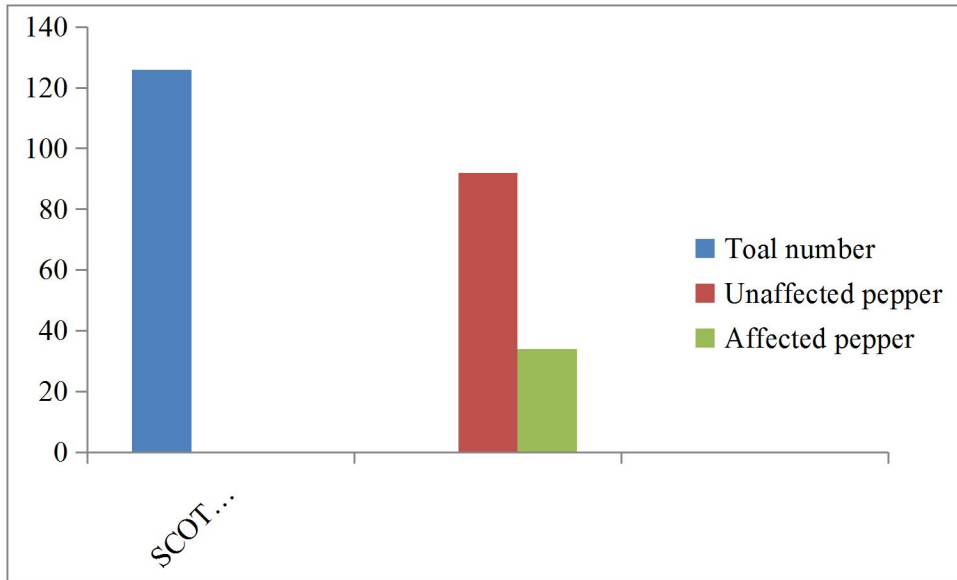


Fig 3.5: Effects of 15°C on scotch bonnet pepper. The result shows that at 15° C the pepper had the highest number of unaffected pepper and has more effect in prolonging the shelf life the pepper,

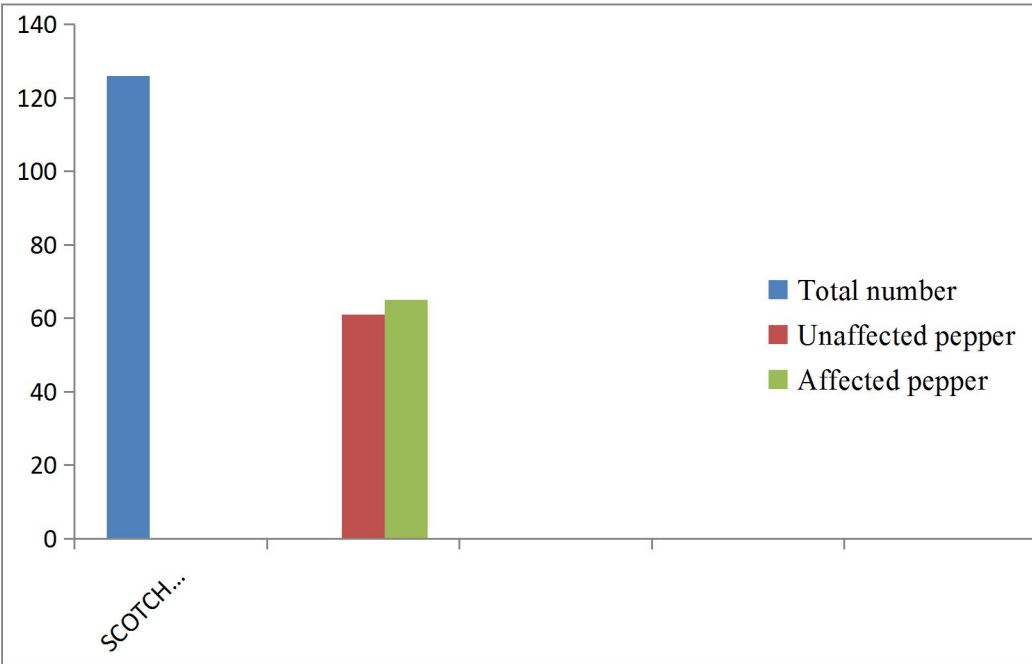


Fig 3.6: Effect of room temperature on scotch bonnet pepper. The result shows that at room temperature the pepper had a high number of unaffected pepper, and is also suitable for prolonging the shelf life of scotch bonnet pepper.

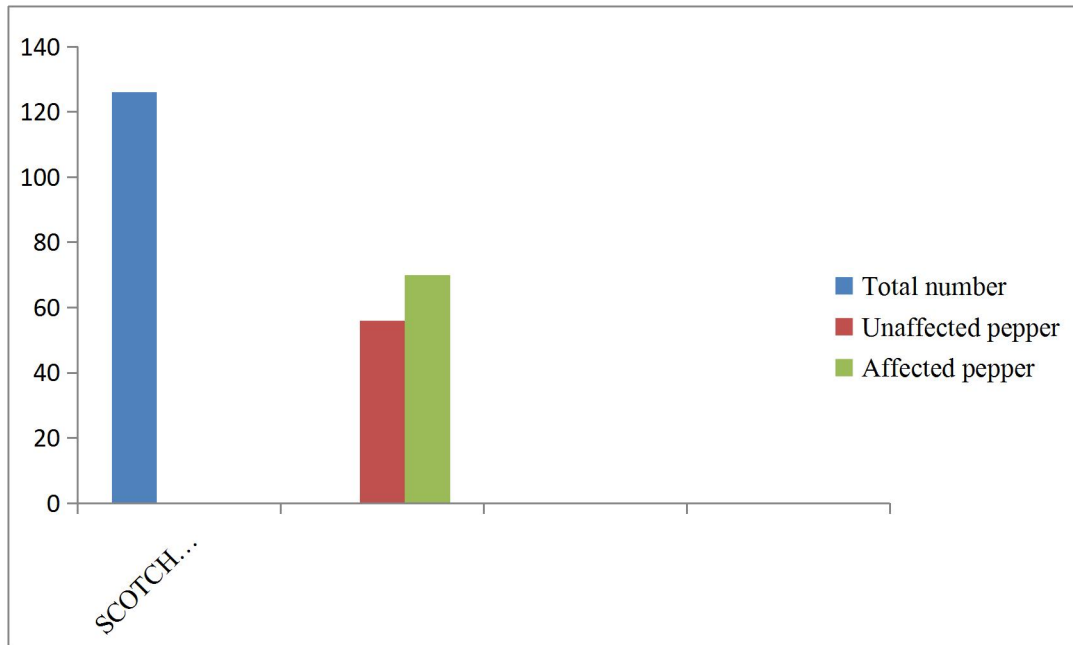


Fig 3.7: Effect of alternating temperature on scotch bonnet pepper. The result shows that alternate temperature has the highest amount of affected pepper thereby reducing the shelf life of scotch bonnet pepper.

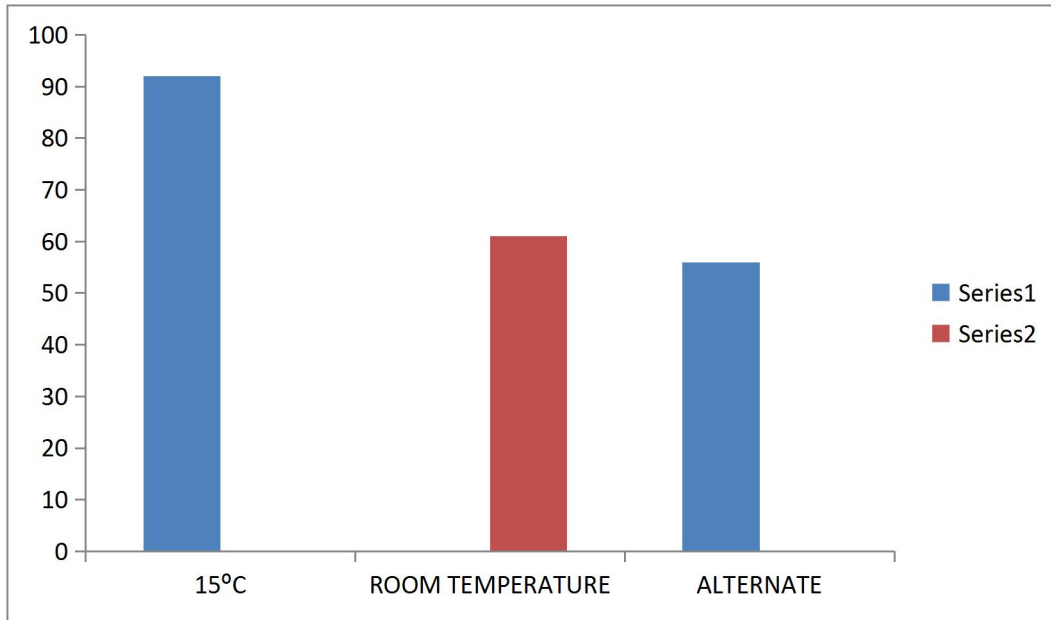


Fig 3.8: The comparison between the effect of, 15°C, room temperature and the alternate (alternation of 15°C and room temperature) on scotch bonnet pepper. The result shows that 15°C was the best temperature, followed by room temperature compared to the alternations.

The organisms found in the isolated cultures were *Rhizopus* and *Mucor*.

3.1 Morphological characteristics of *Rhizopus*

- i. Colony appearance: *Rhizopus* appeared as fast-growing, black and white fluffy mass on the growth media.
- ii. Rhizoids: Root-like structures.
- iii. Hyphae: Long, thread-like filaments.
- iv. Sporangia: Spherical structure, large and contains sporangiospores.
- v. Sporangiospores: Small single-celled spores.

3.2 Microscopic view of *Rhizopus*



Plate 14: *Rhizopus* found in ISOLATE GREEN ACP

3.3 Morphological characteristics of *Mucor*

- i. Colony appearance: *Mucor* grew rapidly over the substrate forming a fluffy, white mass.
- ii. Rhizoids: Root-like structures.
- iii. Sporangia: Spherical or oval shaped and are filled with spores.
- iv. Sporangiospores: Erect unbranched hypae.
- v. Hyphae: Long branched, and formed a complex network called mycelium.

3.4 Microscopic view of *Mucor*

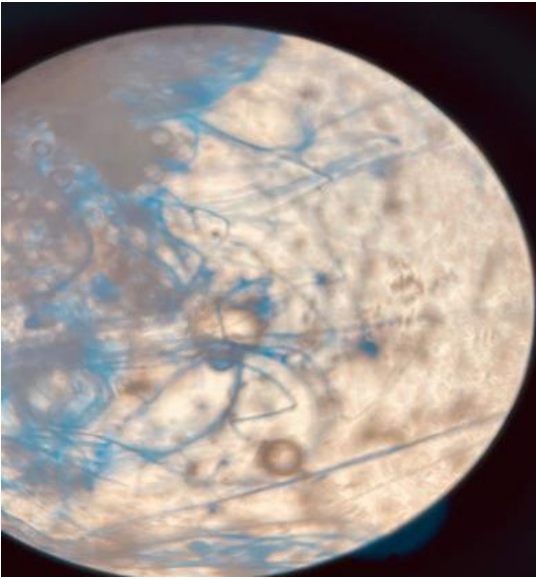


Plate 15: *Mucor* found in ISOLATE
WHITE ACP



Plate 16: *Mucor* found in ISOLATE
SB

CHAPTER FOUR

DISCUSSION

Fungal load refers to the amount of fungal contamination present on a specific substance. Fungi are ubiquitous microorganisms that can be found in the environment, including on food products. Their presence on pepper can lead to spoilage, changes in flavor, and potential health risks if pathogenic fungi are involved. Proper storage conditions are crucial in preventing the growth and proliferation of fungi. In a study by Lianfu *et al* (2016) investigated the effect of storage temperature on the fungal community composition and diversity of pepper samples. The study found that 30°C resulted in the highest fungal diversity than 4°C. In this study, 15°C was found to be optimal for storage of cayenne pepper (*Capsicum annum*) and scotch bonnet pepper (*Capsicum chinense*) as both varieties had the highest number of unaffected pepper for the period of 14 days. Storing pepper in room temperature has benefits in terms of reducing fungal load. Adequate airflow helps maintain a dry environment, as it wicks away moisture and prevent the build-up of humidity, it also claimed to have a reduced impact in the spoilage of both species of pepper than alternating. The alternating between 15°C and room temperature, can have varying effects on fungal growth. Lower temperatures, like 15°C, are generally less favorable for fungal activity, as most fungi prefer warmer conditions. This can help inhibit the growth and reduce the fungal load on the pepper. It is important to be cautious about temperature fluctuations. Sudden changes in temperature can lead to increased moisture levels. This moisture can provide an ideal environment for fungi to thrive, counteracting the benefits of the lower temperature. Therefore, it's crucial to strike

a balance and ensure that the transitions between temperatures are gradual to minimize fungal contamination, as alternating 15°C and room temperature had a crucial impact in prolonging the shelf life of cayenne pepper and scotch bonnet pepper. Storing pepper at stable and low temperatures can help reduce fungal load. Proper ventilation, and moisture control are key factors in preventing fungal contamination and maintaining the quality and preservation of stored pepper.

CONCLUSION

Fungal load of peppers stored at different temperature conditions has provided valuable insights. Maintaining peppers at a stable temperature of 15°C, emerged as the most effective strategy for minimizing fungal growth and preserving pepper quality. Room temperature storage, was the second-best in terms of fungal load. This suggests that room temperature can indeed be a suitable option for pepper storage when at 15°C is not feasible. The presence of adequate ventilation in the room temperature storage environment likely contributed to this favorable outcome. However, the alternating 15°C and room temperature conditions did not prove to be a suitable choice, as they did not significantly reduce fungal contamination. While at a stable 15°C environment remains the best choice, room temperature, especially when accompanied by good airflow, can serve as a viable alternative for prolonging the shelf life of pepper.

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Appendix 1

Table 1: Cayenne pepper (15°C)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 3
Day 1	3 unaffected	3 unaffected	3 unaffected
Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	3 unaffected	3 unaffected	3 unaffected
Day 6	3 unaffected	3 unaffected	3 unaffected
Day 7	3 unaffected	3 unaffected	3 unaffected
Day 8	3 unaffected	3 unaffected	3 unaffected
Day 9	2 affected 1 unaffected	3 unaffected	3 unaffected
Day 10	2 affected 1 unaffected	3 unaffected	2 affected 1 unaffected
Day 11	2 affected 1 unaffected	2 affected 1 unaffected	2 affected 1 unaffected
Day 12	2 affected 1 unaffected	2 affected 1 unaffected	2 affected 1 unaffected
Day 13	2 affected 1 unaffected	2 affected 1 unaffected	2 affected 1 unaffected
Day 14	2 affected 1 unaffected	2 affected 1 unaffected	2 affected 1 unaffected

Table 2: Cayenne pepper (ROOM TEMPERATURE)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 3
Day 1	3 unaffected	3 unaffected	3 unaffected
Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	3 unaffected	3 unaffected	3 unaffected
Day 6	3 unaffected	3 unaffected	3 unaffected
Day 7	2 affected 1 unaffected	2 affected, 1 unaffected	3 unaffected
Day 8	3 affected	2 affected, 1 unaffected	2 affected, 1 unaffected
Day 9	3 affected	3 affected	3 affected
Day 10	3 affected	3 affected	3 affected
Day 11	3 affected	3 affected	3 affected
Day 12	3 affected	3 affected	3 affected
Day 13	3 affected	3 affected	3 affected
Day 14	3 affected	3 affected	3 affected

Table 3: Cayenne pepper (ALTERNATE)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 1
Day 1	3 unaffected	3 unaffected	3 unaffected
Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	3 unaffected	3 unaffected	3 unaffected
Day 6	2 affected 1 unaffected	3 unaffected	3 unaffected
Day 7	3 unaffected	1 affected 2 unaffected	1 affected 2 unaffected
Day 8	3 unaffected	3 unaffected	3 unaffected
Day 9	3 unaffected	3 unaffected	3 unaffected
Day 10	3 unaffected	3 unaffected	3 unaffected
Day 11	3 unaffected	3 unaffected	3 unaffected
Day 12	3 unaffected	3 unaffected	3 unaffected
Day 13	3 unaffected	3 unaffected	3 unaffected
Day 14	3 unaffected	3 unaffected	3 unaffected

Table 4: Scotch bonnet pepper (15°C)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 3
Days 1	3 unaffected	3 unaffected	3 unaffected

Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	3 unaffected	3 unaffected	3 unaffected
Day 6	3 unaffected	3 unaffected	3 unaffected
Day 7	3 unaffected	3 unaffected	3 unaffected
Day 8	3 unaffected	3 unaffected	3 unaffected
Day 9	3 unaffected	1 affected 2 unaffected	1 affected 2 unaffected
Day 10	3 unaffected	1 affected 2 unaffected	1 affected 2 unaffected
Day 11	1 affected 2 unaffected	2 affected, 1 unaffected	1 affected 2 unaffected
Day 12	3 affected	2 affected, 1 unaffected	3 affected
Day 13	3 affected	3 affected	3 affected
Day 14	3 affected	3 affected	3 affected

Table 4: Scotch bonnet pepper (ROOM TEMPERATURE)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 3
Day 1	3 unaffected	3 unaffected	3 unaffected
Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	1 affected 2 unaffected	3 unaffected	3 unaffected
Day 6	2 affected 1 unaffected	3 unaffected	1 affected 2 unaffected
Day 7	3 affected	3 unaffected	1 affected, 2 unaffected
Day 8	3 affected	2 affected 1 unaffected	2 affected, 1 unaffected
Day 9	3 affected	2 affected, 1 unaffected	2 affected, 1 unaffected
Day 10	3 affected	2 affected, 1 unaffected	2 affected, 1 unaffected
Day 11	3 affected	3 affected	3 affected
Day 12	3 affected	3 affected	3 affected
Day 13	3 affected	3 affected	3 affected

Day 14	3 affected	3 affected	3 affected
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Table 5: Scotch bonnet pepper (ALTERNATE)

DAYS	REPLICATE 1	REPLICATE 2	REPLICATE 3
Day 1	3 unaffected	3 unaffected	3 unaffected
Day 2	3 unaffected	3 unaffected	3 unaffected
Day 3	3 unaffected	3 unaffected	3 unaffected
Day 4	3 unaffected	3 unaffected	3 unaffected
Day 5	3 unaffected	3 unaffected	3 unaffected
Day 6	3 unaffected	2 affected 1unaffected	3 unaffected
Day 7	2 affected 1 unaffected	1 affected, 2 unaffected	2 affected, 1 unaffected
Day 8	2 affected 1 unaffected	1 affected, 2 unaffected	3 affected
Day 9	3 affected	3 affected	3 affected
Day 10	3 affected	3 affected	3 affected
Day 11	3 affected	3 affected	3 affected
Day 12	3 affected	3 affected	3 affected
Day 13	3 affected	3 affected	3 affected
Day 14	3 affected	3 affected	3 affected