PROJECT TOPIC: DETERMINATION ON THE EFFICACY OF PLANT ESSENTIAL OIL ON THE CONTROL OF *Aspergillosis niger* INFECTION ON STORED MAIZE (*Zea mays*).

BY

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CHAPTER ONE

Origin of Maize

The term 'maize' seems to be derived from the word *'mahiz'* of Taino language of the Caribbean islands, which became '*maiz*' in Spanish (Oxford dictionary 2015). Based on this common name, Linnaeus included the name as species in the botanical classification of *Zea*. Maize is also popularly known as 'corn' in English-speaking countries. In some countries, 'corn' means the 'local staple', while in some others it is used for any 'cereal'. The ear of maize is unique among cereals, and morphologically similar wild progenitor of maize could not be found. Therefore, its evolution has been a great scientific challenge and of great interest for both biologists and archaeologists. Many hypotheses/theories have been proposed by different scientists to explain the origin of maize. Among them, (1) tripartite hypothesis, (2) catastrophic sexual transmutation theory, (3) *Tripsacum-Zea diploperennis* hypothesis, and, (4) teosinte hypothesis were debated and discussed in detail by different scientists.

Importance of maize

It has emerged as a crop of global importance owing to its multiple end uses as a human food and livestock feed and serves as an important component for various industrial products. Besides, maize serves as a model organism for biological research worldwide. Globally, about 1016.73 million metric tonnes of maize is produced every year the highest among major staple cereals (FAOSTAT 2013). A major portion of maize produced worldwide is used for animal consumption as it serves as a vital source of proteins and calories to billions of people in developing countries, particularly in Africa, Mesoamerica and Asia (Shiferaw *et al*. 2011). Further, it is a source of important vitamins and minerals to the human body. Along with rice and wheat, maize provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries. Maize provides over 20% of total calories in human diets in 21 countries and over 30% in 12 countries that are home to a total of more than 310 million people (Shiferaw *et al*. 2011). At present, the developed world uses more maize than the developing world, but forecasts indicated that by the year 2050, the demand for maize in the developing countries will double owing to the rapid growth in poultry industry, the biggest driver of growth in maize production (Rosegrant *et al*. 2009; Prasanna 2014). Improved maize hybrids with substantial increase in production per unit area are required to feed the ever-growing population.

Significance of *Aspergilluis niger*

Aspergillus niger is a fungal microbe of great industrial importance. This mould is used extensively in the production of citric acid and in the

production of several enzymes such as amylases, pectinases, and proteases (Godfrey and West, 1996). The 30 Mb genome of this organism has been sequenced by an industrial genome sequencing contract from the DSM N.V. (Amsterdam: DSM.ASX), to the German genomics consortium Gene Alliance (http://www.gene-alliance.com). Another proprietary sequence is available from Integrated Genomics, Chicago, IL. For the production of enzymes and other biological products, *E. coli* and *S. cerevisiae* are the two most commonly used organisms for which there is a complete genome sequence. *Aspergillus niger* is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans. In addition to its role as an opportunistic human pathogen, *A. niger* is economically important as a fermentation organism used for the production of citric acid. Industrial citric acid production by *A. niger* represents one of the most efficient, highest yield bioprocesses in use currently in the industry. The genome size of *A. niger* is estimated to be between 35.5 and 38.5 mega bases (Mb) divided among eight chromosomes/ linkage groups that vary in size from 3.5-6.6 Mb. Currently, there are three independent *A. niger* genome projects, an indication of the economic importance of this organism. The rich amount of data resulting from these multiple *A. niger* genome sequences will be used for basic and applied research programs applicable to fermentation process development, morphology and pathogenicity. (Baker SE *et al.,* 2006)

IMPORTANCE OF ALMOND ESSENTIAL OILS

Almond essential oil, extracted from the kernels of the almond tree (*Prunus dulcis*), has been prized for centuries for its versatile properties in Maize plant, health, beauty, and culinary applications. Below we will explore the significance of almond essential oil, backed by scientific research and historical usage.

Antimicrobial Properties:

Almond essential oil contains compounds such as benzaldehyde and hydrocyanic acid, which exhibit potent antimicrobial activity against a wide range of bacteria and fungi (Vázquez *et al*., 2018). These antimicrobial properties make almond essential oil an excellent candidate for inhibiting the growth of spoilage microorganisms in stored maize.

Antioxidant Activity:

The presence of antioxidants in almond essential oil, particularly vitamin E and phenolic compounds, contributed to its ability to delay lipid oxidation and preserve the quality of maize grains during storage (Sharma *et al*., 2017). By scavenging free radicals and preventing oxidative degradation, almond essential oil helped to maintain the nutritional integrity of stored maize.

Insect Repellent Effect:

Almond essential oil exhibits insecticidal and repellent properties against common pests such as maize weevils (*Sitophilus zeamais*) and grain moths (*Sitotroga cerealella*) (Tripathi *et al*., 2013). Applying almond essential oil to maize storage facilities or packaging materials can deter insect infestations, thereby reducing grain losses and ensuring food safety.

Safety and Environmental Considerations:

Almond essential oil is generally recognized as safe (GRAS) for use in food preservation and does not pose significant risks to human health or the environment when applied appropriately (European Food Safety Authority, 2008). Compared to synthetic preservatives, almond essential oil offers a natural and eco-friendly alternative for maize preservation.

Nutritional Composition:

Almond essential oil is rich in essential fatty acids, vitamins, and minerals. A study by the USDA revealed that almond oil contains significant amounts of monounsaturated fats, vitamin E, magnesium, and potassium (USDA, 2019). These nutrients contributed to its nourishing and moisturizing properties when used in skincare.

Skincare Benefits:

Almond essential oil is renowned for its emollient and moisturizing properties, making it a popular ingredient in skincare products. Research published in the Journal of Cosmetic Dermatology suggests that almond oil can improve skin barrier function and reduce transepidermal water loss, making it effective in treating dry and irritated skin conditions (Vaughn *et al.*, 2018). Additionally, its anti-inflammatory properties may help alleviate symptoms of conditions like eczema and psoriasis.

Hair Care:

The moisturizing and conditioning properties of almond essential oil extend to hair care. A study published in the International Journal of Cosmetic Science found that almond oil can penetrate the hair shaft, improving its strength and elasticity (Gavazzoni Dias, 2015). Regular use of almond oil might help to reduce hair breakage and promoted healthier, shinier hair.

Aromatherapy and Stress Relief:

Almond essential oil is valued in aromatherapy for its soothing aroma and stress-relieving properties. According to a study published in the Journal of Nursing Scholarship, inhaling almond oil vapour can induce feelings of relaxation and reduce stress levels (Bradley *et al.*, 2007). Diffusing almond oil or adding it to bathwater might help to promote relaxation and improve sleep quality.

Culinary Uses:

In addition to its skincare and aromatherapy benefits, almond essential oil is used in culinary applications for its delicate flavor and nutritional profile. Cold-pressed almond oil is commonly used in salad dressings, baking, and cooking as a healthy alternative to refined oils. Its rich, nutty flavor adds depth to dishes while providing essential nutrients.

Importance of jojoba essential oils

Jojoba essential oil, derived from the seeds of the jojoba plant (*Simmondsia chinensis*), holds significant importance in various fields, primarily plants, skincare, haircare, and aromatherapy. Here's a detailed exploration on its importance:

Natural Pest Control: Jojoba oil contains compounds with insecticidal properties, making it a potential natural insecticide for plant protection. Studies have shown that jojoba oil can effectively control certain pests, such as aphids, spider mites, and whiteflies, by disrupting their feeding behavior and causing suffocation or dehydration (Isman, 2006). Additionally, jojoba oil's emollient properties may help suffocate insect eggs and larvae, reducing pest populations without the use of synthetic chemicals.

Fungal Disease Management: Jojoba oil exhibits antifungal properties that can inhibit the growth and spread of fungal pathogens on plants. Research suggests that applying jojoba oil to plant foliage can help prevent fungal diseases such as powdery mildew, rust, and leaf spot (De Lucca *et al*., 2011). Jojoba oil's ability to disrupt fungal cell membranes and interfere with spore germination makes it a promising natural alternative to synthetic fungicides. Stress Tolerance Enhancement: Jojoba oil contains antioxidants and plant growth regulators that can help improve plant stress tolerance and resilience. Studies have demonstrated that treating plants with jojoba oil can enhance their ability to withstand environmental stressors such as drought, salinity, and extreme temperatures (De Oliveira *et al*., 2014). Jojoba oil's moisturizing properties may also help alleviate drought stress by reducing water loss through the stomata.

Root Development Promotion: Jojoba oil has been reported to stimulate root growth and development in various plant species. When applied as a soil drench or root dip, jojoba oil can promote the formation of lateral roots and increase root biomass, leading to improved nutrient uptake and overall plant growth (Singh *et al*., 2012). Jojoba oil's hormone-like compounds may play a role in regulating root growth processes, such as cell division and elongation.

Soil Conditioning and Bioremediation: Jojoba plants have deep root systems that can help improve soil structure, increase organic matter content, and enhance nutrient cycling. By planting jojoba as a cover crop or intercrop, soil health can be improved, leading to better plant growth and productivity in subsequent crops (Montenegro *et al*., 2018). Additionally, jojoba plants have been shown to accumulate heavy metals from contaminated soils, offering potential for soil remediation purposes.

Skin Moisturization and Hydration: Jojoba oil is structurally similar to human sebum, the skin's natural oil. This resemblance allows it to penetrate deeply into the skin without leaving a greasy residue, effectively moisturizing and hydrating the skin. Research published in the Journal of Cosmetic Science highlights jojoba oil's ability to enhance the skin's barrier function, thereby reducing transepidermal water loss (TEWL) and maintaining optimal hydration levels (Pazyar *et al*., 2013).

Anti-inflammatory Properties: Studies suggest that jojoba oil possesses anti-inflammatory properties, making it beneficial for soothing various skin conditions, including acne, eczema, and psoriasis. Research published in the Journal of Ethnopharmacology indicates that jojoba oil exhibits antiinflammatory effects in experimental models, which can help alleviate skin irritation and inflammation (Habashy *et al*., 2005).

Skin Conditioning and Protection: Jojoba oil contains natural tocopherols (vitamin E) and antioxidants, which help protect the skin from environmental damage, such as UV radiation and pollution. Its emollient properties soften the skin, leaving it smooth and supple. Additionally, jojoba oil's noncomedogenic nature makes it suitable for all skin types, including sensitive and acne-prone skin.

Hair Nourishment and Scalp Health: Jojoba oil is a popular ingredient in haircare products due to its moisturizing and conditioning properties. It helps hydrate the hair shaft, reduce frizz, and improve manageability. Massaging jojoba oil into the scalp can also promote scalp health by moisturizing dry skin, reducing dandruff, and balancing sebum production. Aromatherapy Benefits: In addition to its skincare and haircare benefits, jojoba essential oil is used in aromatherapy for its soothing and calming effects. When used in massage oils or diffused in the air, its mild, nutty aroma can help alleviate stress, promote relaxation, and enhance overall well-being.

Importance of Teatree oils

Tea tree essential oil, derived from the Australian native *Melaleuca alternifolia* plant, boasts a plethora of benefits and applications supported by scientific research and anecdotal evidence.

Antimicrobial Properties: Tea tree oil exhibits potent antimicrobial activity against a wide range of microorganisms, including bacteria, viruses, and fungi (Carson *et al*., 2006). Its effectiveness against common pathogens like *Staphylococcus aureus* and *Candida albicans* makes it valuable for treating skin infections, acne, and even fungal nail infections (Hammer *et al*., 2006). Anti-inflammatory Effects: Studies suggest that tea tree oil possesses antiinflammatory properties, attributed mainly to its major component, terpinen-4-ol (Hart *et* *al*., 2000). This makes it useful in soothing skin irritations, reducing redness, and calming inflamed acne lesions.

Acne Treatment: Due to its antimicrobial and anti-inflammatory properties, tea tree oil is a popular natural remedy for acne. Research indicates that it can be as effective as benzoyl peroxide, a common acne medication, but with fewer side effects like dryness and irritation (Enshaieh *et al*., 2007).

Antifungal Activity: Tea tree oil is renowned for its antifungal properties, making it effective against different fungal infections, such as athlete's foot and toenail fungus. Studies have shown its efficacy in inhibiting the growth of dermatophytes and yeasts responsible for these infections (Tong *et al*., 2007).

Natural Deodorant: The antimicrobial properties of tea tree oil make it an excellent natural deodorant. It can help combat the bacteria that cause body odour, offering a chemical-free alternative to conventional deodorants and antiperspirants.

Household Cleaner: Tea tree oil's antimicrobial properties extend also to household cleaning applications. Adding it to homemade cleaning solutions can help disinfect surfaces, eliminate mould and mildew, and freshen the air, providing a safer alternative to harsh chemical cleaners.

Hair Care: Tea tree oil is commonly used in hair care products due to its ability to combat dandruff and soothe scalp irritation. It helped to maintain a healthy scalp environment, promote hair growth, and leave hair feeling refreshed and revitalized.

Insect Repellent: Tea tree oil can act as a natural insect repellent, deterring pests like mosquitoes, flies, and ants. Its strong aroma and antimicrobial properties make it an effective and environmentally friendly alternative to synthetic repellents.

Justification

The project topic, "Determination on the efficacy of plant essential oils on the control of *Aspergillus niger* infection on stored maize," is crucial due to its potential impact on food safety, security, and sustainability. *Aspergillus niger* is a common fungal pathogen that infects stored grains like maize, leading to quality degradation and contamination with mycotoxins harmful to humans and animals (Battilani *et al*., 2016). Therefore, finding effective control measures was a paramount measure instead of synthesis

Plant essential oils have emerged as promising alternatives to synthetic fungicides due to their natural origin, low toxicity, and potential for sustainable agriculture (Pandey *et al*., 2017). Their antimicrobial properties have been well-documented in various studies (Bakkali *et al*., 2008). However, their efficacy against specific pathogens like *A. niger* on stored maize remains to be fully explored.

This project aims to address this gap by scientifically evaluating the efficacy of plant essential oils in controlling *A. niger* infection on stored maize. Such research is essential as it can provide evidence-based solutions to mitigate fungal contamination in grain storage facilities.

Interdisciplinary research is necessary for this project, involving experts from fields such as plant pathology, agronomy, chemistry, and food science. By integrating knowledge from these diverse disciplines, for us to gain a comprehensive understanding of the mechanisms underlying the antifungal activity of plant essential oils and optimize their application methods.

The outcomes of this research could have practical implications for farmers, food processors, and policymakers. Evidence-based recommendations on the safe and effective use of plant essential oils as biocontrol in grain storage facilities which can enhance food security and safety while promoting sustainable agricultural practices.

Research Questions;

1) How do plant essential oils impact the shelf-life and quality of stored maize?

2) What are the economic implications of adopting plant essential oil-based strategies for *A. niger* control compared to conventional methods?

3 What is the effectiveness of plant essential oils in controlling *A. niger* contamination

4. How feasible is the practical implementation of plant essential oil-based treatments in grain storage facilities?

Objectives

The following objectives were considered for this study;

1)To evaluate the antifungal activity of different plant essential oils against *Aspergillus niger*

2)To assess the effect of plant essential oil concentration and exposure time on the inhibition of *A. niger* growth on maize grains.

3)To determine the efficacy of plant essential oil treatments in reducing *A. niger* contamination on stored maize

These objectives aim to comprehensively address various aspects of the efficacy, practicality, and implications of using plant essential oils for controlling *A. niger* infection on stored maize grain.

CHAPTER TWO

Literature review

The Efficacy of Composite Plant essential oils (Eos) against *Aflatoxigenic fungus Aspergillus flavus* in Maize (Fangzhi Xian *et al.,* 2010) the potency of plant essential oils in inhibiting mycelial growth and aflatoxin production in *A. flavus* was investigated. Plant essential oils can effectively control fungal contamination in food, with advantages such as being ecofriendly, along with high efficiency and low drug resistance. Eleven EOs that showed high antifungal activity in previous studies were investigated for their antifungal activity against *A. flavus* NRRL, a dominant AFB1-producing strain. Among these, mugwort, peppermint and rosemary EOs were previously proposed as potential preservatives, with the ability to extend the shelf life of food (Fangzhi Xian *et al.,* 2010). However, in this current study, these three Eos (Teatree, Jojoba, Almond) had no visible antifungal effect on *A. niger*. Moreover, in the previous work, (Fangzhi Xian *et al.,* 2010) the antifungal effects of cinnamon, oregano and lemongrass EOs were found to be the highest among all EOs tested. In particular, cinnamon EO showed remarkable antifungal activity. Previous studies have reported that cinnamon EO can inhibit fungal growth and mycotoxin production by *A. flavus*, (Elhidar *et al., 2019)* with its major antifungal ingredient being cinnamaldehyde. In our study, three EOs (jojoba, tea free, Almond) was be used in different concentrations to determine the efficacy of their control of *Aspergillus niger* infection on stored maize.

Many studies have reported that the antifungal activity of EOs can be increased by combining two or more plant essential oils to create a synergistic antifungal effect of EO vapours. For example, an oregano and thyme EO mixture was shown to have synergistic effects against moulds (*A. niger*, *A. flavus*, *A. parasiticus*, and *P. chrysogenum*) [Hossain *et al.,* 2016]. The combination of Almond, jojoba and Teatree EO vapors had little synergistic inhibitory effects on *Aspergillus niger*. The essential oil from Almond has shown remarkable antimicrobial activity. It was also found that jojoba and Teatree EO had varying effects on the growth of *A. niger*. Marín (Marin *et al.,* 2003) mentioned that a mixture of cinnamon, lemongrass and oregano EOs showed lower antifungal ability. However, we found out in my project that Teatree, Almond and jojoba CEO with a volume ratio of 1:0.1:0.0.1(*v*:*v*:*v*) showed a synergistic effect against *A. niger*. In our study, the combined formulation of these plant essential oils may result in synergistic effects and contribute to the observed antifungal activity of Almond, jojoba and Teatree plant essential oils against *A. niger*.

Inhibition of *Aspergillus niger* production by plant essential oils is related to the down regulation of *Aspergillus* biosynthesis genes .In our present study, these genes were found to be down regulated by Concentrations levels of Almonds, Teatree and Jojoba EOs, which was consistent with the observation that Aspergillus production was reduced in all samples grown under the three essential oil (Teatree, jojoba, Almond)vapor. Furthermore, down regulation of these genes may be in response to reduced oxidative stress caused by the plant essential oils. Previous studies have shown that oxidative stress, due to the accumulation of intracellular reactive oxygen species can enhance the production of *Aspergillus niger*. Moreover, several investigations have also indicated that antioxidants can significantly reduce Aspergillosis production, whereas oxidants enhance *Aspergillus* production (Kim *et al.,* 2008).

A maize grains infection assay demonstrated that Almond has high inhibitory activity towards *A. niger* on maize grains. whereas Teatree and Jojoba has an aromatic odour. These three plant essential oils, when combined, maximize consumer satisfaction. The application of Almond, Teatree and jojoba may be a useful strategy for future grain and food packaging and storage.

Another study in relation to our study is in the previous study, where five plant essential oils were shown to be effective against the associated seedborne fungi when applied to maize crop that was naturally infected.

Their result showed that grains of maize crop treated with tested plant essential oils were significantly affected the percentage of germinated grains. While the experiment we carried out, was show the efficacy of Plant essential oils on the control of *Aspergillus niger* infection on stored maize seed, in previous study. The use of EOs had an impact on grain germination, entirely inhibiting it or greatly reducing germination rate. The frequency of associated fungi isolated from maize grains or the frequency of all fungal isolates, on the other hand, is greatly influenced by EO concentrations. These findings agreed with those reported by Karaca *et al*. (2017) which revealed that the oils had different impacts on the germination rates of wheat seeds. The most effective oils were clove and oregano, which completely prevented seed germination (Karaca *et al*., 2017). But in our study the plant essential oils were not to check the inhibition or enhancement on germination rather than on stored /post harvested maize. They significantly and unacceptably decreased the germination of treated kernels (<60%) (Karaca *et al*., 2017). Van der Wolf *et al*. (2008) demonstrated that thyme, oregano, cinnamon, and clove plant essential oils were significantly reduced the growth of fungi on cabbage seeds. Here, all the treated maize crop's grains exhibited a typical germination rate of 70–100%. It demonstrated that none of these five plant essential oils are phytotoxic to the germination of maize grains. Additionally, (Orzali *et al*. 2020) revealed that the germination rates of tomato seeds were unaffected by an essential oil of *Origanum vulgare*. Additionally, while the plant essential oils considerably reduced the contamination of the grains in this instance. It did not significantly decrease in germination. For these reasons, this plant essential oil was tested for the germination of the maize crop grains and the disease incidence for maize crop in the last phases of the previous study (Al-Ansary *et al*., 2022).

Clove was the most significant treatment as it reduced the frequency of fungal isolates to in both trials along the storage period. Wang *et al*. (2019) revealed that the complex plant essential oils (0.02%) significantly inhibited the total fungal counts and against fungi in stored maize. The oils had different impacts on the fungal load of wheat seeds. The most effective oil was clove which completely prevented fungal growth on wheat seeds, In my study serial dilution on experience materials, fungi analysis and inoculation, incubation of Ager plates, identification of isolates pure culture, data collection and data analysis will be carried out to determine the efficacy of plant essential oils on the control of *Aspergillus niger* on stored maize and procedures and results would be discussed in further chapters.

CHAPTER THREE

MATERIALS AND METHODS

EXPERIMENTAL SITE

The Experiment was carried out at University of Benin, Faculty of Agriculture, Soil Science Laboratory. It is situated approximately at Latitude 6.400269^0 and Longitude 5.624111^0.

MATERIALS USED

The following materials were used for the experiment:

Sodium hypochloride solution, maize seed, Mortar and pistol, Distilled water, Whatman filter paper, Funnel, Syringe Tube, Ager, Autoclave, Stirrer, Measuring scale, Conical flask Foil, Petri dishes, Measuring cylinder, Red and yellow capsule, Paper tape, Lamina-flow cabinet, Forceps, Bunsen burner, Matches, Ethanol, Beaker, Cock borer, Inoculating needle, Gloves, Face mask Six(6) universal bottle. Marker, Micropipette Surgical knife Incubator.

SOURCES OF EXPERIMENTAL MATERIALS:

The PDA (potatoe dextrose Ager),Petri dishes was sourced from a chemical shop at university of Benin, opposite the Animal Farm at maingate, the plant essential oils were procured from Pat Aza Humulitres Enterprise, Red and Yellow capsules served as antibiotics, paper tape, syringe ,face mask were sourced at a pharmacy at June 11,university of Benin, Benin City, Edo State, sodium hypochloride solution, foil paper was sourced at Ekosodin supermarket Benin city, and all other experimental materials like measuring cylinder, auto clave, universal bottle, etc were all sourced and found at the University of Benin, Benin City, Edo state Soil science laboratory.

EXPERIMENTAL DESIGN AND STATISTICS:

The Experimental design used for this experiment is 3 by 4 factional in Completely Randomized Design (CRD), each treatment has three replications plus a control.

STATISTICAL TOOLS USED FOR ANALYSIS

The Statistical tools used for my analysis is ANOVA TABLE and Data Visualization Tools: Graphical tools;histogram, that helped me to visualize my data distributions and relationships

SERIAL DILUTION PROCESS OF EXPERIMENTAL MATERIAL

Ten fold serial dilution was prepared for the different plant essential oils as treatment.

Four universal bottles were arranged and labelled properly.. We prepared the stock solution first by adding 1ml of Ethanol, 1ml of Treatment and 8ml of water to make 10ml mark altogether in the first universal bottle (the stock sample) and was stirred to allow the oil(treatment) disperse evenly. 1ml of the aliquot solution in the first universal bottle(stock solution) was transferred to the second universal bottle and stirred to give a homogenous mixture. This was done orderly till the very last universal bottle. The aliquot solution from universal bottle 2, 3, and 4 were labelled in different concentration each and pipetted in the wells created in each petri dish.

PREPARATION OF SAMPLE

The stored maize grain was extracted from maize cob, It was then soaked in 5% sodium hypochloride solution to remove contaminants, then rinsed with distilled water. The maize grain grinded using a mortar and pistol. It was then mixed with distilled water and stirred to give a homogeneous solution It was filtered ,using the whatman filter paper,into a 250ml beaker

PREPARATION OF CULTURE MEDIA

We measured 680 ml of water using 500ml,250ml conical flask and 30ml measuring cylinder.

We then measured 26.52 gram of PDA on the weighing scale and diluted 26.52 gram of PDA in 680 ml of water in a conical flask, the solution was tilted to enable a homogeneous solution. Then the solution was placed in an autoclave for the 15 minutes at 121 degree Celsius. It was removed and left to calm down to 45 degree Celsius (cheek hot). The 500gram of red and yellow capsules was diluted in 4ml of distilled water and was filled to a 12 ml mark with distilled water. It was filtered into a measuring cylinder first It was filtered a second time into another mess cylinder using whatman filter paper to give a clear solution. 1.25 ml of the red and yellow capsules was added to the cheek hot Ager solution, to avoid bacteria and confirmation for the pathogen culture using a syringe and tilted sideways. 20ml of the Ager solution was turned into 27 Petri dishes each They were placed in the laminar flow cabinet and allowed to solidify.

IDENTIFICATION OF PATHOGEN

The filtered residue was collected using a syringe and inoculated at the center of the solidified agar media and spread with a spreader and sealed with masking tape and labelled to allow the micro organisms to grow. The pathogen was incubated for six days to identify the morphological growth of the microbe which was viewed in the microscope and pictorial on the structurer of the organisms.

INOCULATION METHOD

Agar well point innoculation was employed to administer plant essential oil as treatment at 0.01%, 0.1% and 1% concentration inside the well created by cock borer with the help of micro pippette.

20ml of molten prepared potato dextrose agar (PDA) were poured into the petri dishes. Then the petri dishes were incubated at room temperature for an interval of 48-96 hours.

DETERMINATION OF MICROBIAL LOAD

The microbial load of the sample was determined visually by observing the colonies formed after 72hours.

PURE CULTURE

From the stock culture different colours were identified which were subcultured for pure culture to identify the new pathogen. Pathogen identified from the pure culture was *Aspergillus niger* which has dark black colouration of colonies. The pure culture was prepared and finally used for the experiment using point inoculation method.

DURATION OF EXPERIMENT

The experiment lasted for a duration of 14 days(two weeks)

DATA COLLECTION

The data collection was carried out on a daily basis for 7 days with the use of vernier calipers to measure the distance between the aspergillosis Niger and the wells containing the plant essential oils.

DATA ANALYSIS

Data was analysed using ANOVA table, means were compare with the use of least significant difference (LSD) at a probability iof 5% $p<0.05$

CHAPTER FOUR
DATA ANALYSIS USING ANOVA TABLE AND RESULT

0.01% Concentration

Table 4.0:

TEATREE	ALMOND	JOJOBA	CONTROL
0.74	0.57	0.23	1.66
2.56	1.87	1.34	2.02
0.5	2.07	2.2	1.57
0.61	1.21	1.5	1.07
1.07	2.46	1.1	1.14
1.76	2	1.66	1.55
1.71	1.59	1.6	1.75
1.56	1.07	0.25	0.75
2.06	1.21	1.57	1

ANOVA: Single factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0.74	8	11.83	1.47875	0.503698
0.57	8	13.48	1.685	0.245829
0.23	8	11.22	1.4025	0.31465
1.66	8	10.85	1.35625	0.185998

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.506762	3	0.168921	0.540471	0.658527	2.946685
Within Groups	8.751225	28	0.312544			
Total	9.257988	31				

0.1% Concentration

Table 4.1

TEATREE	ALMOND	JOJOBA	CONTROL
0.63	2.59	0.34	1.66
2.19	2.11	1.24	2.02
0.44	1.44	1.61	1.57

0.61	1.33	1.04	1.07
0.79	2.2	0.44	1.14
0.81	1.77	0.79	1.55
1.37	1.61	2.31	1.75
1	0.66	1.34	0.75
1.46	0.74	2.27	1

ANOVA: Single Factor
SUMMARY

Groups	***Count***	***Sum***	***Average***	***Variance***
Column 1	9	9.3	1.033333	0.304675
Column 2	9	14.45	1.605556	0.418828
Column 3	9	11.38	1.264444	0.504978
Column 4	9	12.51	1.39	0.173

Column 1	1.033333
Column 2	1.605556
Column 3	1.264444
Column 4	1.39

ANOVA

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1.544956	3	0.514985	1.469832	0.241261	2.90112
Within Groups	11.21184	32	0.35037			
Total	12.7568	35				

1% Concentration

Table 4.2

TEATREE	ALMOND	JOJOBA	CONTROL
1.41	2.73	0.19	1.66
2	1.81	0.73	2.02
0.73	0.43	2.16	1.57
1.19	0.79	1.2	1.07
1.37	1.99	0.31	1.14
2.16	1.57	1.29	1.55
1.3	0.3	1.54	1.75
1.6	0.41	1.2	0.75
0.33	1.43	1.49	1

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
TEATREE	9	12.09	1.343333	0.3247
ALMOND	9	11.46	1.273333	0.7092
JOJOBA	9	10.11	1.123333	0.3887
CONTROL	9	12.51	1.39	0.173

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.366075	3	0.122025	0.305904	0.820908	2.90112
Within Groups	12.7648	32	0.3989			
Total	13.13088	35				

Table 4.1: The effect of different concentration levels on the various treatments

Treatments	**0.01%**	**0.1%**	**1%**
Teatree	1.48	1.03	1.34
Almond	1.69	1.61	1.27
Jojoba	1.40	1.26	1.12
Control	1.36	1.39	1.39
LSD	0.66	0.24	0.82

LSD: Least significant difference

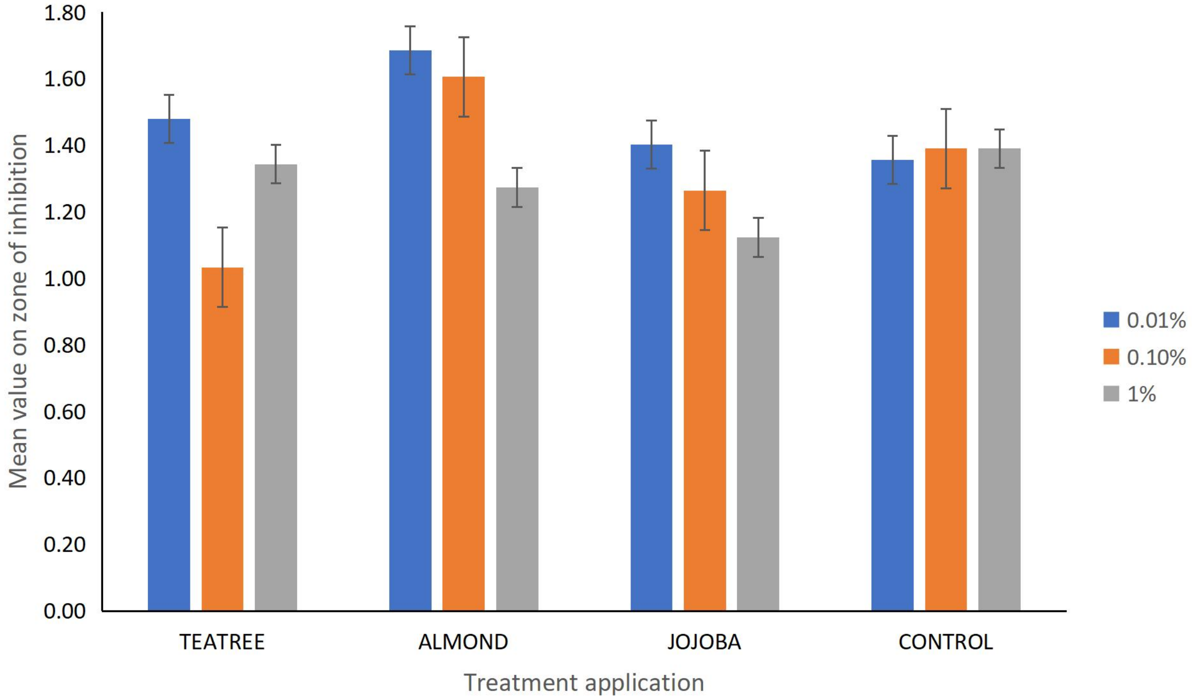

Fig 4.1: Graphical representation of the effect of different concentration levels on treatments.

CHAPTER FIVE

5.0 DISCUSSION

The data analysis of the experiment shows that LSD of all the Treatment (oils) at different concentrations is greater than 0.05(5%),which means that there is no significant difference among the treatment mean.it is important to note that the LSD follows a rule of being significant if less than 5%, and not significant if greater than 5%.

The LSD is used to Determine if the difference between the means of two or more groups is statistically significant, this significance is interpreted based on the comparison of the LSD value to the above observed difference in means (Douglas, 1976).

Also from the result represented in fig 4.1 in the charts, there was no significant difference among the three treatments, However Almond oil had significant treatment effect at 0.01% and 0.1% in supressing the tested pathogen when compared with the rest plant essential oils and the control. This confirms the previous works of (Fangzhi *et al*., 2010) who observed the potency of plant essential oils in inhibiting *Aflatoxigenic fungus Aspergillus flavus* in maize.

CONCLUSION

Based on the experiment carried out, the following conclusion can be deduced;

The LSD (Least Significant Difference) analysis indicates that there is no statistically significant difference among the treatment means for oils at different concentrations, as all LSD values exceed the 5% significance threshold.

According to Douglas C. Montgomery's "Design and Analysis of Experiments" (1976), the LSD is typically considered significant if less than 5% and not significant if greater than 5%.

Despite the lack of significant differences among treatments, the charts reveal that Almond Plant essential oils exhibit the highest efficacy against the control of the fungus *Aspergillus niger*, followed by Tea Tree plant essential oils, and then Jojoba plant essential oils at various concentration levels.

The control group shows identical outputs at 0.1% and 1% concentrations but slightly differs at 0.01%. However, it consistently exhibits the least efficacy against the control of the fungus *Aspergillus niger* on stored maize.

Overall, while no significant differences were observed among treatments, Almond Plant essential oils emerge as the most effective option against *Aspergillus niger*, indicating its potential for fungal control in stored maize.

RECOMMENDATIONS

Based on the results, discussion and conclusion adduced, Implement the use of Almond Plant essential oils as a primary method for controlling *Aspergillus niger* in stored maize. Since Almond Plant essential oils consistently exhibited the highest efficacy among the treatments tested, they are likely to provide effective fungal (*Aspergillus niger*) control while minimizing the risk of contamination in stored maize, it is also beneficial because it can serve as a biocontrol, which is less toxic and more environmentally friendly than the use of chemical control of fungi (fungicides).

Additionally, further research could explore optimizing the concentration and application methods of Almond Plant essential oils to enhance its effectiveness even further.