MAKERERE UNIVERSITY

COLLEGE OF NATURAL SCIENCES

SCHOOL OF BIOSCIENCES

DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY, AND BIOTECHNOLOGY

EVALUATION OF THE FUNGICIDAL ACTIVITIES OF *SOLANUM ANGUIVI* LAM. AND *EUPHORBIA HETEROPHYLLA* LINN. EXTRACTS ON THE GROWTH OF *RHIZOPUS STOLONIFER* AND *MUCOR SPP*.

BY

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19/U/6161/PS

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A RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY AND BIOTECHNOLOGY, COLLEGE OF NATURAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

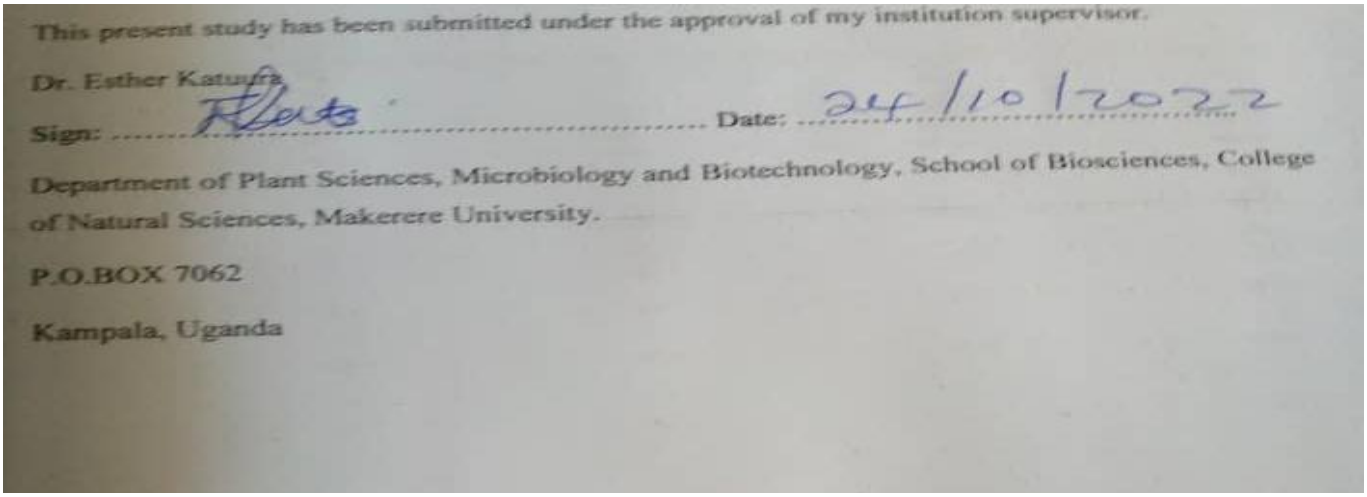
OCTOBER 2022

DECLARATION

I NTAMBI JIL BIYINZIKA, declare that this is my original work and it has not been submitted for any award in any institution of higher learning.

Signature..... Date 21st/10/22

APPROVAL



This present study has been submitted under the approval of my institution supervisor.

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ACKNOWLEDGEMENT

Thanks to the Almighty God, my supervisor, my parents, colleagues and friends who stood with me from the very beginning till the end of this study. May God bless you all for your kindness.

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LIST OF ABBREVIATIONS

PDA: Potato Dextrose Agar

MIC: Minimum Inhibition Concentration

USD: United States Dollar

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

MFC: Minimum Fungicidal Concentration

FDA: Food and Drug Authority

CDC: Centers for Disease Control and Prevention

EOs: Essential oils

WHO: World Health Organization

ABSTRACT

Food spoilage and contamination is a serious public health concern that results in food borne diseases and food insecurity that affect many people annually. *Rhizopus stolonifer* and *Mucor spp* are some of the major fungal contaminants and spoilers of food in Uganda. An in-vitro experiment was carried to evaluate the fungicidal activity of the ethanolic extracts of *Solanum anguivi* Lam fruits and *Euphorbia heterophylla* Linn leaves against the growth of *R. stolonifer* and *Mucor spp*. This study was conducted at Makerere University Department of Plant Sciences, Microbiology and Biotechnology mycology Laboratory. The fungi were isolated from soil picked at the department, purified and then identified based on colony characteristics, microscopy and a bread test. The *S. anguivi* fruits and *E. heterophylla* leaves were collected from Kyaliwajjala-Namugongo and extraction was done using maceration with ethanol as the extraction solvent. The crude ethanolic extracts were then tested against *R. stolonifer* and *Mucor spp* using the Agar well diffusion method and the minimum inhibition concentration determined. Amphotericin B was used as the positive control, both plant extracts showed no effect against *R. stolonifer* but showed inhibition against *Mucor. spp.*

1.0 INTRODUCTION

1.1 Background

The global burden of food borne diseases is comparable to that of major illnesses, malaria, and tuberculosis (Havelaar, Torgerson, & Gibb, 2015). The burden is highest in Asia and sub-Saharan Africa. Food borne diseases in low- and middle-income countries cost about 110 billion United States dollars (USD) a year, with sub-Saharan Africa accounting for USD 16.7 billion (Jaffee, Henson, & Unnevehr, 2019). Food can be contaminated majorly by fungi among other contaminants along the value chain from production to consumption.

In Uganda, cases of food spoilage and contamination by fungi most commonly *Aspergillus flavus*, *Rhizopus stolonifer and Mucor .spp* are rapidly increasing in processed foods like bakery products e.g. bread (Musomba, 2019) and crop plants e.g. sweet potatoes annually and this has resulted into shortage and wastage of food in various parts of the country. *Rhizopus stolonifer* is responsible for causing rot in food crops such sweetpotato tubers (Scruggs & Quesada-Ocampo, 2016) and cassava which are common source of food to many people particularly in Uganda. *Mucor* is mainly associated with bread spoilage and food contamination. These fungi also produce mycotoxins when they grow on food (Amadi & Adeniyi, 2009). The mycotoxins have been proven to cause diseases like mucormycosis and adverse health effects e.g. acute poisoning and immune deficiency when ingested.

To solve this issue, people have resorted to the use of synthetic fungicides like thiabendazole, azoxystrobin and calcium propionate which is associated with various health issues like cancer, heart diseases and many problems in humans. Sanitizers like chlorine, sodium hypochlorite, calcium hypochlorite, ozone and peracetic acid have also been used to control fungi (Bautista-Baños, Bosquez-Molina, & Barrera-Necha, 2014). Continued and excessive use of these chemicals has raised concerns such as increasing public concern regarding contamination of perishables with fungicidal residues, and proliferation of resistance in the pathogen populations according to Tripathy and Dubey (2004).

Conversely, use of organic natural based preservatives to prevent fungal contamination has been done but on a small scale using plants e.g. cloves, lemongrass, ginger, and cinnamon among others

which risk becoming endangered due to overuse since they have many other roles. Discovery of other plant species with the potential to prevent fungal damages remains of great advantage to the country in both aspects one being to reduce the pressure put on other plant resources and the other is to promote human health through use of natural fungicides. This study showed that *Solanum anguivi* and *Euphorbia heterophylla* have antifungal potential against *Mucor spp*.

1.2 Problem statement

Aflatoxins remain a scourge in the country, unprecedentedly reducing the nutritional and economic value of agricultural foods (Omara et al., 2020). Fungal pathogens are proved to be a common and popular contaminant of agro-ecosystem that approximately causes 70–80% of total microbial crop loss as stated by Santra and Banerjee (2020) which has resulted into increased cases of food insecurity. Chemical fungicides on market have proved to harmful to people and the environment.

There is a lack of sufficient knowledge on the antifungal properties of *Solanum anguivi* and *Euphorbia heterophylla*.

1.3 Objectives

1.3.1 General objective

To evaluate the potential of *Solanum anguivi* and *Euphorbia heterophylla* extracts as possible bio-preservatives.

1.3.2 Specific objectives

I. To determine the diameters of the zones of inhibition of ethanolic extracts from *S. anguivi* and *E. heterophylla* against *R. stolonifer* and *Mucor spp*.
II. To determine the minimum inhibition concentration of the ethanolic extracts from *S. anguivi* and *E. heterophylla* against *R. stolonifer* and *Mucor spp*.

1.4 Hypotheses

Null: There is no significant inhibition effect on fungal growth.

Alternative: There is a significant inhibition effect on fungal growth.

1.5 Research questions

I. What are the diameters of the zones of inhibition of the plant extracts against *Rhizopus* and *Mucor* over time?

II. Which plant ethanolic extract has a lower minimum inhibition concentration?

1.6 Significance

This study is aimed at determining the antifungal potential of *S. anguivi* and *E. heterophylla* which will add more information to the existing body of knowledge about the bioactivity of these plants. This will guide formulation of policies to regulate usage and manufacture of harmful chemicals.

Once found effective in inhibiting fungal growth, these plants can be used by people as possible biological controls for the common food borne fungi as an alternative to synthetic fungicides.

1.7 Justification

This study will unveil the potential of *S. anguivi* and *E. heterophylla* in inhibiting the growth of *Rhizopus stolonifer* and *Mucor spp* that are among the major contaminants of food.

2.0 LITERATURE REVIEW

Plants produce many organic bioactive compounds called secondary metabolites that do not play a direct role in the photosynthesis, growth and development of the plant but are crucial in ensuring survival of the plant by performing many important functions like protection from herbivores. These compounds are also known to have antioxidant, antifungal, antibiotic and antiviral activity, hence greatly contributing to the plant's defense system against any kind of pathogens (Tegegne, Abiyu, & Libesu, 2021).

2.1 *Solanum anguivi* Lam.

Solanum anguivi L. locally known as Kantukuma, eshiga and entakara in Uganda is a plant species under the family Solanaceae native to and found in most non arid parts of Africa (Bukenya-Ziraba, 2004). *Solanum anguivi* is widely distributed on the African continent and its neighboring islands. It has been recorded from West Africa, as well as Central Africa, East Africa, southern Africa and Madagascar, but it probably occurs in all non-arid regions throughout tropical Africa. *S. anguivi* mainly grows in the wild, though sometimes, e.g. in Uganda and Côte d'Ivoire, it is a semi-cultivated vegetable (Bukenya-Ziraba, 2004). The fruits are commonly sold in the local markets though data on production and trade are scarce or not available.

Solanum anguivi Lam. is a non-tuberous and widely distributed plant that possesses various medicinal properties. In most cases, the plant prefers to grow in humid temperature and commonly found as weed in gardens (Elekofehinti, Kamdem, & Kade, 2013). It grows up to 3 metres tall and the stems are often prickly, the fruits are used fresh, or dried and ground to a powder, as medicine against high blood pressure whereas some are chewed as a remedy for coughs and chest pains. The roots are used to treat toothache in patients (Tropical Plants Database, 2022).

Studies about the bioactive potential of *Solanum anguivi* L. plants have been done by many people though very few if any have been carried out to determine its antifungal potential and there is hardly any work documented on its antifungal properties, the majority are mainly on anti-diabetic and antimicrobial properties. Various researchers have reported the presence of various phytochemicals in *Solanum anguivi* L. which include saponins, flavonoids, coumarins, vitamin C, phenolics and alkaloids that are responsible for its bioactivity (Nakitto, Muyonga, & Byaruhanga, 2021). The phenolic compounds in *Solanum anguivi* L. include gallic acid, chlorogenic acid and caffeic acid (Elekofehinti et al., 2013). According to Ripperger & Himmelreich (1994), steroid

alkaloid glycosides that include solamargine, anguivine and isoanguivine have been isolated from the roots of *Solanum anguivi* L. Former studies have reported that *S. anguivi* fruit extracts possess anti-oxidant properties such as reducing properties.

There is a controversy about whether *Solanum indicum* Linn. is the same as *Solanum anguivi* Lam. *S. indicum* has been reported as a synonym of *S. anguivi* by some researchers whereas others have described them as different species though both have similar phytochemicals (Nakitto, et al. 2021).

Reports have also been made on the antifungal properties of *Solanum nigrum* leaf extracts against five different mycotoxin-producing fungi, a plant under the same genus as *S. anguivi*. The results of the tests showed that the extract inhibited the growth of two out of five tested mycotoxin producing fungi, this was attributed to phytochemical compounds found in the extract which include alkaloids, saponins, flavonoids, tannins and others (Musto, 2015). Therefore the similarities in the phytochemicals present in *S. anguivi*, *S. indicum* and *S. nigrum* may indicate similarities in their antifungal properties (Nakitto, et al. 2021).

2.2 *Euphorbia heterophylla* L.

Euphorbia heterophylla L. is a plant species under the family Euphorbiaceace. It is commonly known as the milk weed (wild poinsettia) and locally as Kisanda/kisandasanda (Nabukenya, Rubaire-Akiiki, & Olila, 2014). It occurs throughout most of tropical Africa and the Indian Ocean islands, as well as in the Mediterranean region and South Africa (Mosango, 2008). *E. heterophylla* grows in disturbed localities as a weed of cultivation, in gardens and along roadsides and it is propagated by seed which germinates under tropical conditions and remains dormant under temperate circumstances (Mosango, 2008).

All parts of *Euphorbia heterophylla* contain milky latex: leaves 0.42%, stems 0.11%, roots 0.06% and whole plant up to 0.77% (Mosango, 2008). Studies on *E. heterophylla* latex have revealed the presence of medically active metabolites possessing antifungal and antibacterial properties that make the latex extracts more potent than the standard drugs like fluconazole and tetracycline which were used against both the bacterial and fungal strains such as *Aspergillus niger, Fusarium oxyporum* and *Penicillium spp*. (Pruthvi, Mahesh & Sahaya, 2020). The latex is irritant to the skin and eyes and may be employed as a rubefacient and to remove warts and corns.

In East Africa, *E. heterophylla* is used for the treatment of gonorrhea and to accelerate wound healing. It is also used as a purgative and lactogenic agent, as a cure for a migraine (Apiamu, Evuen & Ivy, 2013). Villagers have traditionally used *E. heterophylla* parts to treat a variety of ailments (Omale & Emmanuel, 2010). These include treatment of constipation, bronchitis and asthma (Falodun, Okunrobo, & Uzoamaka, 2016). The medicinal properties of *E. heterophylla* are attributed to its bioactive compounds such as alkaloids, tannins, flavonoids, steroids, glycosides and saponins that have antimicrobial and antifungal properties (Vani, Rahaman, & Rani, 2017).

Elshamy, Abd-EL Gawad and EL Gendy (2019) claim that the essential oils (EOs) of *Euphorbia heterophylla* are poorly studied. Therefore, there is limited information documented about the antifungal potential of *E. heterophylla* against various fungal strains like *Rhizopus* and *Mucor*. Furthermore, *E. heterophylla* is claimed to be toxic when used in high doses as herb though not much is known about the toxic effect of the herb as stated by Okolie et al. (2015).

2.3 The fungal strains

Rhizopus stolonifer also known as the common bread mold is considered the most important species in the genus *Rhizopus*. It is a fast-growing fungus developing on a broad range of temperatures and relative humidities (Hernández-Lauzardo, Bautista-Baños, & Velázquez-del Valle, 2006). *R. stolonifer* spores are airborne, found in many places such as orchards and packing houses.

Disease symptoms that characterize *R. stolonifer* infection are watery areas quickly covered by coarse, gray hairy mycelia forming a mass of black sporangia at their tips. Infection usually occurs during harvest and handling.

Due to the wide array of hosts that *R. stolonifer* can infect and its fast penetration and colonization, it has become an important target to control (Bautista-Baños et al., 2014). This fungus is known to cause Rhizopus soft rot which is one of the most devastating post-harvest diseases of many crops such as sweetpotatoes, cassava and berries (Park, Park & Kim, 2020).

Results from a study to determine mould incidence and aflatoxin contamination of maize kernels carried out among dealers (traders) in the three agro-ecological zones of Uganda indicate that Aflatoxin levels increased with storage time such that maize samples from the Mid-Altitude (dry

and moist) stored for more than six months had mean levels greater than the 20 ppb FDA/WHO regulatory limits (Kaaya & Kyamuhangire, 2006), *R. stolonifer* was one of the fungal species identified responsible for the increase in aflatoxin levels. This shows that maize consumers in Uganda are facing a risk of aflatoxin poisoning from this fungus.

Mucor spp. is grouped under the mucor genus. The *Mucor* genus is a polyphyletic group pertaining to early diverging lineages of fungi and includes a high number of ubiquitous species. Certain pathogenic *Mucor spp* have been reported as a threat for animal and human health and identified more frequently as mycosis causative agents, especially in immuno-compromised patients (Morin-Sardin, Nodet & Coton, 2017). Furthermore, Centers for Disease Control and Prevention (2021) reported that *Rhizopus* and *Mucor* are the most common types of fungi that cause mucormycosis, these should therefore be controlled to protect people from exposure to their aflatoxins.

Saito, Michailides and Xiao (2016) also identified *Mucor spp*. as the fungus responsible for causing a plant disease called mucor rot in mandarin fruits.

2.4 Biological control as an alternative to synthetic fungicides and its benefits.

Seeking solutions from nature for solving problems is the age-old practice for mankind, and natural products are proved to be the most effective way for keeping up the balance of development as well as the "healthy, wealthy, and well" condition of Mother Nature (Santra & Banerjee, 2020).

Zhang et al., (2020) claims that though chemical fungicides remain the primary treatment and control of fungal diseases, biological controls can also be a potential control method for postharvest diseases of fruits and vegetables in place of chemical fungicides.

Also Tripathy and Dubey (2004) state that the ultimate goal of current research in this area is to develop and evaluate various alternative control strategies to reduce dependency on synthetic fungicides and that natural products with biological activity have the potential to replace synthetic fungicides.

As an alternative to synthetic agents, microbial natural products have been considered as a source of diverse antifungal agents for plant disease control. Furthermore, these natural products have

been regarded as environment-friendly because their biodegradability could solve the residual problem of synthetic fungicides (Park et al., 2020).

Again, Santra and Banerjee (2020) also claim that the use of nature derived fungicidal products that minimize the event of fungal infections plays a big role in achieving a sustainable agriculture.

3.0 MATERIALS AND METHODS

3.1 Study site

The experiment was carried out at the Makerere University Department of Plant Sciences, Microbiology and Biotechnology mycology lab.

Study design

An in vitro Lab based experiment was conducted involving determining the zones of inhibition of the ethanolic extracts of *Solanum anguivi* and *Euphorbia heterophylla* against pure isolates of *Rhizopus stolonifer and Mucor spp*. The extracts were screened for their ability to inhibit fungal growth in the media dishes.

3.2 Collection and preparation of Plant material

Fresh *Solanum anguivi* fruits used for the study were bought from Kyaliwajjala market in Namugongo and a sterilized sample taken to the Makerere University Department of Plant Sciences Herbarium for identification and authentication by a plant taxonomist. The collected fruits were washed in clean tap water to remove all the dust particles and then air dried in an oven and ground into a powdery fine solid.

Fresh *Euphorbia heterophylla* leaves were randomly selected from Nsawo in Namugongo, washed in clean water and voucher samples taken to the herbarium for identification and authentication by a plant taxonomist. The leaves were dried under shade for 7 days after which they were ground into powder using a dry mill.

The powders of both plants were stored in air tight polythene bags prior to use.

3.3 Preparation of Fungal samples

Rhizopus stolonifer and *Mucor spp*. were isolated from soil samples picked from the Department of Plant sciences, Microbiology and Biotechnology. They were then purified by sub-culturing them on potato dextrose agar (PDA) media. Identification of the fungal strains was done using a microscope and morphological basis (shape, size & colour) at the year three laboratory. The *Rhizopus stolonifer* colonies formed complex rhizoids whereas *Mucor spp*. colonies lacked rhizoids (Agrios, 2001). *Rhizopus stolonifer* colonies had black specks on them and had a robust growth that filled the entire petri dish. *Mucor* colonies were cottony and greyish-white with a

relatively rapid growth. A test on bread was carried out and the results were positive for both fungi (Appendices).

3.4 Chemical extraction

This was done using maceration according to (Federico Casassa, Beaver, Mireles, & Harbertson, 2013) with modifications.

100g each of the fine powders of *Solanum anguivi* and *Euphorbia heterophylla* were weighed and placed into two well labelled glass containers. 400ml of ethanol were added to the container followed by shaking vigorously. The containers were then placed in a dark area for 3 days at room temperature with agitation. The mixture was then filtered using a filter paper and a sieve to separate the clear solution from the other particles. The liquid extracts obtained were then poured on metallic dishes and taken in an oven to evaporate off ethanol at 35ºC for 2 days. The solids that remained on the metallic dishes were collected and weighed in beakers.

3.5 Determination of the zones of inhibition

The zones of inhibition for the plant extracts *S. anguivi* and *E. heterophylla* were determined using the agar well diffusion approach (Balouiri, Sadiki, & Ibnsouda, 2016) with necessary modifications.

Procedure

The experiment begun with media preparation, 10g of PDA were measured and dissolved in 250ml of distilled water. The mixture was then sterilized by autoclaving at 15Ibs pressure and 121ºC for 15mins. The media was left to cool and then poured into petri dishes under asceptic conditions, the media was left to stand for 15mins to solidify.

The fungal inoculum were then spread across the media surface using a sterile swab. By using a clean cork borer, wells were drilled in each agar dish carefully. 40µl of positive control (amphotericin B(50mg)) were pipetted into one well. An empty well was used as the negative control and 200µl of the plant extracts were pipetted into two wells. Three replicates were used for each plant extract.

The set up was placed in an incubator at 25ºC and monitored for 7days. Results were recorded respectively.

Minimum inhibition concentration (MIC) determination

To determine the MIC, 4g of the solid extract were weighed and dissolved in 4ml of absolute ethanol.

Dilution was then done by transferring different volumes from the original mixture into four McCartney bottles with sterile distilled water for each extract to form different concentration ranges (75%, 50%, 40% and 25%). A cotton swab dipped in fungi solution was then inoculated into each dilution and the bottles shaken.

Incubation was done at 25ºC for 24 hours. 0.5ml from each bottle were then pour plated with agar medium and incubated at 25 ºC for 3 days. Observations were made to see which plates had fungal growth, the least dilution plate that had no fungal growth on plate was considered to be the Minimum Inhibition Concentration.

Data analysis

All data was tested for normality using the Shapiro-wilk test and subjected to one way analysis of variance followed by a Tukey's multiple comparison test using Microsoft excel software.

3.6 RESULTS

Both *Solanum anguivi* and *Euphorbia heterophylla* ethanolic extracts formed no zones of inhibition against *Rhizopus stolonifer* throughout the experiment (Appendices).

The ethanolic extracts of both plants formed zones of inhibition against *Mucor spp* on the petri-dishes (Appendices). The diameters of these zones were measured using a transparent millimeter ruler.

Analysis

Table 1: shows the diameters of zones of inhibition of Mucor spp growth at the different days

Time	**Diameter (mm) of *Solanum anguivi***			**Positive control (mm)**			**Diameter (mm) of *Euphorbia heterophylla***		
DAY 3	23	17.5	22	25	26	24	20	19	21.5
DAY 4	13	11	14	15	16	15.5	15	16	13
DAY 5	10	9	11	12	11	13	12.5	10	11

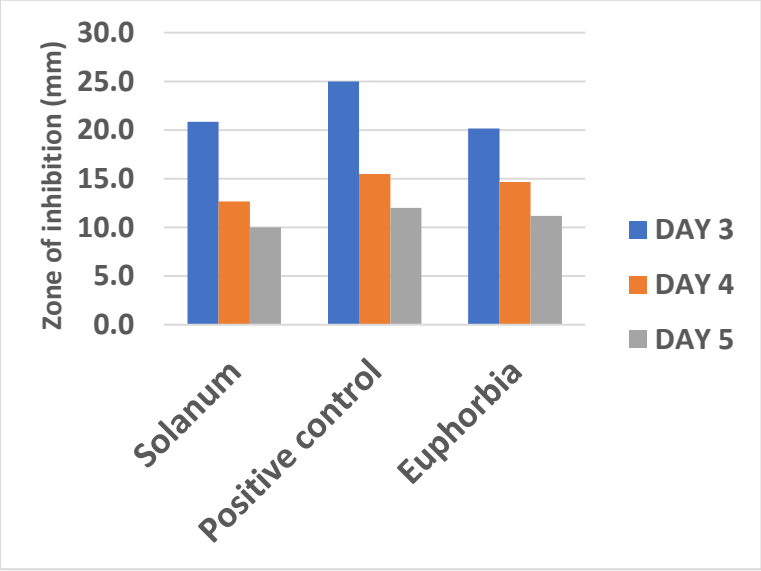

***Figure 1*:** shows the average diameter of the zones of inhibition over time

Trend: the diameter of the zones of inhibition decreases with increase in number of days

Table 2: shows the summary of the Shapiro-Wilk test results

	P-value at (n=3) & (α=0.05)		
Time (Days)	***S. anguivi***	**Positive control**	***E. heterophylla***
Day 3	0.327547	1	0.780439
Day 4	0.636886	1	0.63690
Day 5	1	1	0.780440

All obtained P-values were greater than 0.05 hence all data was normal.

Both ethanolic extracts showed a very significant inhibition effect against *Mucor spp* growth ($P<0.05$) at $p=0.05$ using one- way Anova analysis, hence (**Ho is rejected**).

Table 3: shows the summary of Tukey's test results

	P-value at (α=0.05)		
Time (Days)	***S. anguivi***	**Positive control**	***E. heterophylla***
Day 3 vs Day 4	0.005771	0.0000261	0.006024
Day 3 vs Day 5	0.001352	0.00000404	0.000453
Day 4 vs Day 5	0.301642	0.006175	0.044362

The Tukey's Multiple comparison test at ($p=0.05$) showed that there is a significant difference in the average diameters of the zones of inhibition of the ethanolic extracts of *S. anguivi*, *E. heterophylla* and positive control at the different days. However, there was no significant difference in the average diameters of the zones of inhibition between Day 4 and Day 5 for *S. anguivi*.

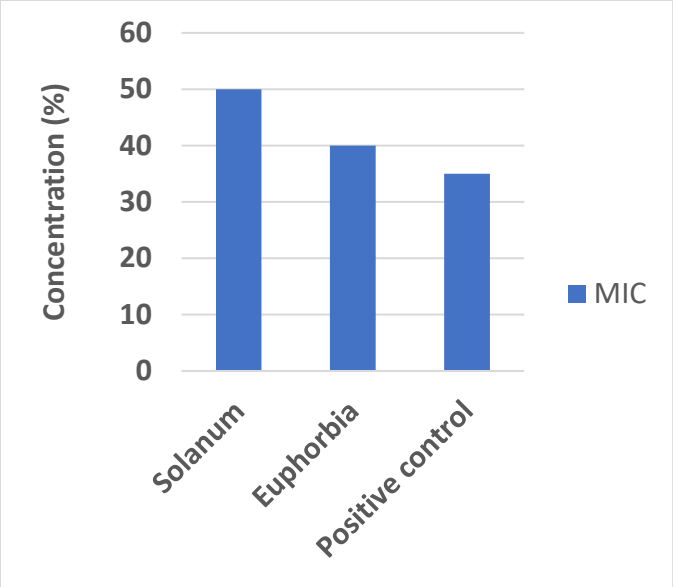

Figure 2: shows the minimum inhibition concentration of the ethanolic extracts

Discussion

There was no effect on the growth of *Rhizopus stolonifer* from ethanolic extracts of *S. anguivi* and *E. heterophylla*. Similar studies have showed that *R. stolonifer* is not susceptible to some ethanolic extracts (Mbah, Egbuonu, Omodamiro, Jeremiah, & Nwanne, 2019). This confirms that ethanol is not a good choice of extraction solvent for some plants which might be due to under extraction of the bioactive components from the powder thus lowering the concentration of those compounds below the amount required to inhibit *R. stolonifer* growth. Based on current knowledge, this is probably the first study to test the ethanolic extracts of *S. anguivi* fruits and *E. heterophylla* leaves against *R. stolonifer.*

The ethanolic extracts from both *S. anguivi* and *E. heterophylla* showed a significant inhibition effect against *Mucor spp.* growth which is in agreement with previous studies that have proven *Solanum anguivi* fruits and *E. heterophylla* leaves to have compounds such as alkaloids, triterpenoids, steroids, phenols, saponins, tannins and flavonoids that possess antifungal activity (Nakitto, et al. 2021), (Pruthvi, Mahesh & Sahaya, 2020). Therefore, based on these findings, *S. anguivi* and *E. heterophylla* possess bioactive compounds with activity against *Mucor spp.*

However, *E. heterophylla* had a lower minimum inhibition concentration (40%) than *S. anguivi* (50%) which reveals presence of more bioactive compounds in *E. heterophylla* leaves. Bosah (2015) in a similar study also found out that the ethanolic leaf extracts of *E. heterophylla* were the most effective in inhibiting fungal growth of all the extracts that were used. This is important in such studies because only a little concentration of the extract is needed to inhibit fungal growth as compared to the latter.

Reports have shown that the leaves of *E. heterophylla* contain 0.42% latex which is higher than in any other part, studies on *E. heterophylla* latex have revealed the presence of active metabolites possessing antifungal and antibacterial properties (Pruthvi, Mahesh & Sahaya, 2020). This explains the low minimum inhibition concentration of *E. heterophylla* leaf extracts.

The diameters of the zones of inhibition of *S. anguivi* and *E. heterophylla* decreased with increase in time, this indicates that *Mucor spp* became less susceptible to the ethanolic extracts over time. This is attributed to the increase in spore production and mycelial growth of the fungus over time. Very few if any or no studies have described the reason for this decrease in diameter of the

inhibition zones of ethanolic extracts from both *S. anguivi* and *E. heterophylla* over time however, one possible cause is that the fungus developed tolerance to the extracts over time hence the decrease in diameter.

Previous reports confirmed the efficacy of different plant extracts against the pathogenicity of different fungi because secondary plant metabolites have a marked potential as a resource of effective antifungal agents (El-Shahir, El-Wakil, Abdel Latef, & Youssef, 2022). These support the findings of this study.

CHAPTER 4

4.1 Conclusion

The results of this study showed that ethanolic extracts of *Solanum anguivi* and *Euphorbia heterophylla* can be used as potential biological controls against *Mucor spp* since they can inhibit its growth.

4.2 Recommendation

I recommend that more research is done to find out ways in which extracts of *E. heterophylla* and *S. anguivi* can be used as effective bio-preservatives. Studies such as screening of the natural organic compounds and identification of the active agents in the different parts of these plants that can be used as precursors for synthesis of bio-preservatives.

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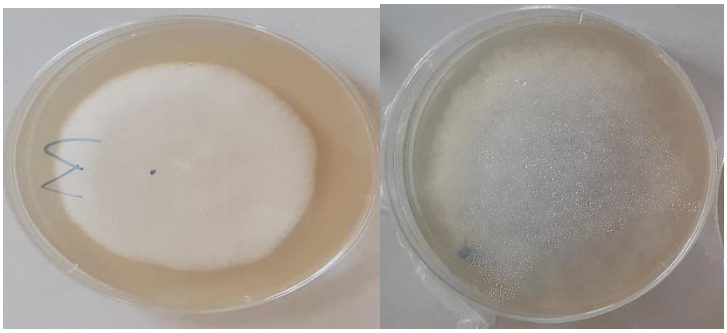
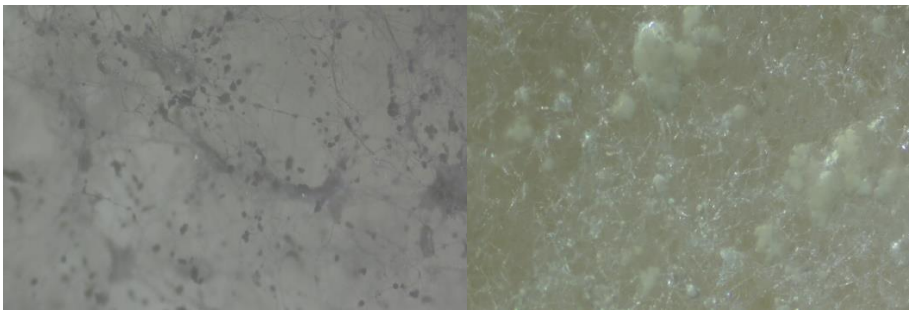
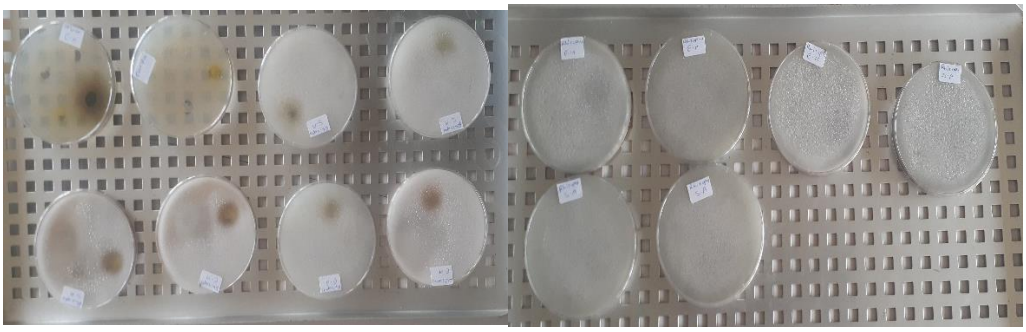
APPENDICES



Mucor spp. & *Rhizopus stolonifer* pure colonies



Rhizopus stolonifer & Mucor spp. colony surface viewed under microscope



Rhizopus stolonifer results DAY 3 & DAY 4

Mucor spp. zones of inhibition

Bread test

Two freshly baked burns without cracks were bought from a bakery and placed in an oven for 15minutes to sterilize them. Under asceptic conditions, each burn was placed in a moistened sterile polythene bag. The fungi were then inoculated into the polythene bags which were thereafter sealed and stored at room temperature in the dark for 3 days.

Mucor spp

Before & After 3 days

Rhizopus stolonifer

Before & After 3 days