An evaluation of anti-*Candida* and insecticidal properties of selected indigenous edible and non-edible mushrooms.

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A Dissertation submitted in fulfilment of the requirements for an award of the Master of Philosophy degree in Biotechnology

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Abstract

Many mushroom species are known to produce bioactive compounds with fungicidal, herbicidal, pesticidal and insecticidal properties, but they remain underutilized. This study examines the potential of using native mushroom species from Zimbabwe as mycopesticides and antifungal agents. The first part of the study focused on the anti-*Candida* properties of five native mushroom species from Zimbabwe, including the non-edible varieties *Russula sp*., *Amanita rhopalopus*, and *Boletus sp*., as well as the edible *Cantharellus miomboensis* and *Cantharellus symoensii*. Agar disc diffusion tests revealed varying degrees of antifungal activity, with *Cantharellus symoensii* exhibiting the most potent effect, producing a 16.6±0.4 mm zone of inhibition against the fungal pathogen *Candida albicans*. The study also found that ethanol and chloroform were more effective solvents for extracting the anti-*Candida* compounds compared to aqueous extracts.

The second part of the study investigated evaluating the insecticidal characteristics of the mushroom species *Amanita rhopalopus* against the common house fly, *Musca domestica*. Flies were exposed to various extracts of the mushroom, and the results showed that the extract with the greatest solvent extraction had 100% mortality within 5 minutes, comparable to a commercial insecticide. This suggests the mushroom could be a promising candidate for developing mycopesticides.

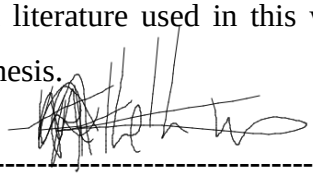
Overall, this research supports the hypothesis that mushrooms from Zimbabwe possess both insecticidal and antifungal properties, highlighting their potential for developing novel mycopesticides and antifungal medications. The findings underscore the need to further explore the underutilized bioactive potential of native mushroom species.

Dedication

In honor of my family and friends without whose support it would not have been possible.

Declaration

This thesis is the original work of, Tapiwanashe Fidelity Mutetwa (C21147639A), and has not been submitted elsewhere and neither is it intended to be submited, to any other university for the award of a degree, diploma, or certificate other than Chinhoyi University of Technology. The author has duly acknowledged all assistance received during the research work and the preparation of the thesis. Furthermore, the author certifies that all information sources and literature used in this work have been properly referenced and acknowledged within the thesis.



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Approval

I certify that Tapiwanashe Fidelity Mutetwa was under my supervision. I further certify that I have sufficiently supervised him and that he has fulfilled all requirements set before him. In my judgement, the thesis entitled, "An evaluation of *anti-Candida* properties and insecticidal properties of selected indigenous edible and non edible mushrooms", is of sufficiently high standard to meet the standards of the Master of Philosophy degree awarded by Chinhoyi University of Technology.

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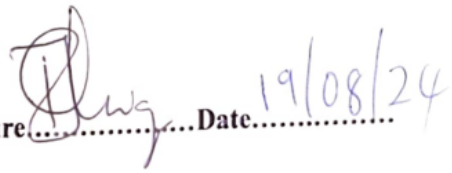
Name of Supervisor(Dr. T.J Chisango).....Signature  **.....Date**19/08/24

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CONTENTS PAGE

List of Figures and Tables

List of Figures

List of Tables

CHAPTER 1

1.1 Overview of thesis

The thesis is structured in 5 chapters. **Chapter 1** provides an overall synthesis and background on the anti-*Candida* and insecticidal properties of indigenous mushrooms in Zimbabwe. **Chapter 2** focuses on the overall literature in relation to anti-*Candida* and insecticidal nature of mushrooms. **Chapter 3** focuses on evaluating the anti-*Candida* properties of selected mushroom species, while **Chapter 4** examines the insecticidal potential of the mushroom *Amanita rhopalopus*. **Chapter 5** gives the synthesis of the overall thesis.

The research is motivated by the rich biodiversity of mushrooms in Zimbabwe and their potential for diverse applications, including therapeutic and pesticidal uses. The introduction highlights the abundance of wild edible and non-edible mushroom species in the country, with over 120 documented so far.

Chapter 3 - Evaluation of anti-*Candida* Properties:

This chapter investigates the anti-Candida potential of five indigenous Zimbabwean mushroom species - the non-edible *Russula sp., Amanita rhopalopus*, and *Boletus sp*., as well as the edible *Cantharellus miomboensis* and *Cantharellus symoensii.*

The key findings include:

Agar disc diffusion tests revealed varying degrees of antifungal activity against *Candida albicans*.

Cantharellus symoensii exhibited the most potent effect, producing a 16.6±0.4 mm zone of inhibition.

Ethanol and chloroform were more effective solvents for extracting the anti-Candida compounds compared to aqueous extracts.

Chapter 4 - Evaluation of Insecticidal Properties:

This chapter focused on investigating the insecticidal properties of the mushroom *Amanita rhopalopus* against the common housefly, *Musca domestica*.

The key findings include:

Various extracts of the *A. rhopalopus* mushroom were tested, and the extract with the greatest solvent extraction achieved 100% mortality of the flies within 5 minutes, comparable to a commercial insecticide.

This suggests the *A. rhopalopus* mushroom could be a promising candidate for developing mycopesticides.

Overall, the research supports the hypothesis that Zimbabwean mushrooms possess both insecticidal and anti-*Candida* properties, highlighting their potential for developing novel mycopesticides and antifungal medications. The findings underscore the need to further explore the underutilized bioactive potential of native mushroom species.

1.2 Back ground

This paper provides important context on the significance of studying mushrooms for their therapeutic potential, particularly against fungal infections and insect pests. Candidiasis is highlighted as a major health concern, caused by *Candida species* particularly *C. albicans.* These fungal infections can affect the skin, mucous membranes, and internal organs, leading to high morbidity and mortality, especially in immunocompromised patients. The emergence of drug-resistant *Candida* strains underscores the urgent need for new antifungal treatments (Kozak 2021).

Mushrooms are identified as a promising source of biologically active compounds, such as terpenes, that may have therapeutic application (Zhang 2016). This provides the rationale for evaluating the anti-*Candida* properties of indigenous mushroom species. Several mushroom species have been extensively studied for their antifungal properties against *Candida albicans*. For instance, research has demonstrated that extracts from *Ganoderma lucidum* exhibit significant inhibitory effects on *C. albicans,* potentially due to their bioactive compounds (Zhang, 2016). Similarly, *Pleurotus ostreatus* has shown promising antifungal activity, suggesting its potential use as a natural treatment against fungal infections (Bae, 2018). Additionally, Lentinula edodes (shiitake) has been found to contain compounds that not only inhibit the growth of *C. albicans* but also enhance immune responses (Friedman, 2016). These findings underscore the therapeutic potential of various mushroom species in combating *C. albicans* infections.

The thesis also discusses the insecticidal potential of mushrooms, using the example of *Amanita rhopalopus*. This mushroom is known to be toxic and non-edible, and was observed to kill flies during the drying process. This observation, along with precedents of other mushroom species being used in insecticides, suggests that *A. rhopalopus* may contain insecticidal compounds effective against flies like *M. domestica*.

A number of mushroom species have been investigated for their insecticidal properties, particularly against flies. For example, extracts from *Ganoderma lucidum* have shown effective larvicidal activity against *Drosophila melanogaster*, indicating potential applications in pest

management (Hossain, 2019). Similarly, studies on *Pleurotus ostreatus* have revealed its efficacy in repelling adult flies, suggesting its use as a natural insect deterrent (Sharma, 2020). Furthermore, *Lentinula edodes* has been noted for its ability to produce volatile compounds that exhibit insecticidal effects, further supporting the role of mushrooms in biocontrol strategies (Kumar, 2021). These findings highlight the potential of various mushroom species in managing fly populations through natural means.

Overally, the thesis highlights the medical and agricultural significance of studying the antifungal and insecticidal properties of mushrooms. The high unmet need for new antifungal treatments and insecticides, coupled with the rich bioactive potential of mushrooms, provides a strong justification for the research focus of the thesis.

1.3 Objectives and hypothesis

The study has 2 distinct parameters under investigation hence separate objectives and hypothesis.

1.3.1 Objectives of the study (Evaluation of anti-*Candida* properties of selected indigenous wild mushrooms)

1.3.2 Main Objective 1

To determine the anti-*Candida* properties of selected wild edible mushrooms

1.3.3 Specific Objectives of the Study

- To determine the effect of various solvents in extracting bioactive compounds from selected wild mushrooms.
- To determine the *anti-Candida* properties of wild edible mushrooms found in Zimbabwe.

1.4 Hypothesis

H_0: Mushroom extracts will not significantly inhibit the growth of *Candida albicans*

H_1: Mushroom extracts will significantly inhibit the growth of *Candida albicans*

1.5 Main objective 2 (Evaluation of the insecticidal properties of Amanita rhopalopus)

- To determine the insecticidal properties of *Amanita rhopalopus*

1.6 Specific objectives

- To evaluate the bioactive compounds in the mushroom
- To determine the best performing extraction method

- To determine Lethal concentration of the mushroom extracts

1.7 Hypothesis

- H_0 The mushroom *Amanita rhopalopus* does not have insecticidal properties
- H_1 The mushroom *Amanita rhopalopus* has insecticidal properties

CHAPTER 2

2.1 Literature review

Insecticidal and the therapeutic potential of selected indigenous mushrooms.

Introduction

Indigenous communities in Zimbabwe have long traditions of wild mushroom foraging and use. Mushrooms are an important source of food, medicine and income, especially in rural areas. Zimbabwe's diverse climate, ranging from the hot, dry Lowveld to the cool, wet eastern regions, supports a wide variety of mushroom species (Sharp, 2011). The country's Miombo woodlands, dominated by Msasa and Mnondo trees, are particularly rich in mushroom diversity (Sharp, 2011). During the rainy season (November to March), mushroom spores germinate, forming a network of fungal threads (mycelium) that eventually produce fruiting bodies (Sharp, 2011). While over 120 edible and non-edible mushroom species have been documented in Zimbabwe, it was estimated that there are hundreds more awaiting discovery (Sharp, 2011; 2014). Additionally, exploring the possibility of cultivating indigenous mushroom species could create sustainable livelihoods for local communities.

Zimbabwe's indigenous mushrooms represent a largely untapped resource with immense potential. By combining traditional knowledge with modern scientific research, we can unlock the full value of these organisms and contribute to the well-being of both people and the environment. Many edible wild mushrooms, such as *chanterelles*, *morels*, and porcini, have a rich, savory flavor that makes them highly prized by foragers and chefs (Maunder 2010). However, there are also numerous non-edible and potentially deadly species that can cause severe illness or even death if consumed. For instance, the *Amanita phalloides*, commonly known as the death cap, contains potent toxins that can lead to liver failure and death (Sexton 2015). Similarly, *Amanita muscaria*, while not typically lethal, can cause severe hallucinations and gastrointestinal distress due to its psychoactive properties (Petersen 2019). Certain mushrooms contain toxins that can cause symptoms ranging from gastrointestinal distress to organ failure like which ones. For example *Gyromitra esculenta* (false morel), which can produce gyromitrin, a toxin that may cause nausea, vomiting, and even seizures (Rätsch, 2005)

Many mushroom species produce secondary metabolites that have potent antimicrobial properties, inhibiting the growth of bacteria, fungi, and viruses. For example, *Ganoderma lucidum* has been shown to produce triterpenoids that effectively inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Zhou 2017). Similarly, *Lentinula edodes* (shiitake) contains lentinans that demonstrate antibacterial activity against *Helicobacter pylori* (Friedman 2016).

In terms of antifungal properties, extracts from *Pleurotus ostreatus* have been reported to inhibit *Candida albicans* (Bae, 2018), while *Inonotus obliquus* has shown activity against various fungal pathogens (Hajdu 2012).

Regarding antiviral effects, *Ganoderma lucidum* has also been associated with antiviral properties against the influenza virus (Kumar, 2020). Additionally, polysaccharides derived from *Lentinula edodes* have exhibited antiviral activity against the herpes simplex virus (Li, 2018). Finally, *Agaricus blazei* has demonstrated efficacy against the human immunodeficiency virus (HIV) (Sharma, 2010). These findings highlight the diverse antimicrobial potential of various mushroom species.

2.2 *Candida Albicans*.

Candida albicans is a yeast that can become pathogenic under certain conditions, leading to candidiasis, a common fungal infection that poses significant health risks, particularly in immunocompromised individuals. The increasing prevalence of drug-resistant strains has heightened the urgency for alternative therapeutic approaches. Research has shown that *C. albicans* can form biofilms, which contribute to its resistance against conventional antifungal treatments (Nobile & Mitchell, 2013). This has prompted investigations into natural sources of antifungal agents, with mushrooms emerging as promising candidates due to their rich diversity of bioactive compounds.

Several mushroom species, including *Ganoderma lucidum, Pleurotus ostreatus, and Lentinula edodes*, have demonstrated significant antifungal activity against *C. albicans*. For instance, extracts from *Ganoderma lucidum* have been shown to inhibit the growth of *C. albicans* and disrupt biofilm formation (Zhang, 2016). Similarly, *Pleurotus ostreatus* exhibits both antifungal

and immunomodulatory effects, enhancing the host's defense mechanisms against infections (Bae, 2018). *Lentinula edodes* contains polysaccharides that have been reported to enhance immune responses while directly inhibiting fungal growth (Friedman, 2016). These findings underscore the potential of mushrooms as a viable alternative or adjunct to traditional antifungal therapies, warranting further exploration into their mechanisms of action and clinical applications.

2.3 Insecticidal properties

Many fungi produce secondary metabolites with toxic properties harmful to both vertebrate and invertebrate consumers. For instance, the *Amanita* genus, particularly *Amanita phalloides* (death cap), produces potent toxins called amatoxins that can cause severe liver damage and are lethal to humans and other mammals (Sexton, 2015). Another example is *Cortinarius rubellus*, which contains orellanine, a toxin that can lead to kidney failure in humans (Erdogdu, 2020).

Invertebrates are also affected by fungal toxins. For example, *Beauveria bassiana*, a pathogenic fungus for insects, produces toxins that can kill various insect species, including pests like aphids and beetles (Inglis, 2001).

For instance, six out of eight *Amanita* species inhibited the growth of *Drosophila melanogaster* when exposed to 127 different fungi (Besl, 1987). However some *Drosophila* species like *Drosophila kuntzei* and *Drosophila phalerata* exhibited resistance to *Amanita phalloides*' amatoxins (Jaenike, 1983). The tolerance of mycophagous and non-mycophagous *Drosophila* species to amanitin, was studied and a higher tolerance in mycophagous species was found (Jaenike, 1983). Studies on ibotenic acid and its derivative, musimol, involving mycophagous and non-mycophagous *Drosophila* and *Amanita* species revealed differential survival and reproductive outcomes (Riba, 2020). While *D. bizonata* thrived on *A. pantherine* and *A. ibotengutake*, *frugivorous D. immigrans* and *D. melanogaster* typically did not. However, *D. bizonata*, *D. angularis*, and *D. brachynephros* commonly utilized various mushrooms for breeding, unlike *D. immigrans* (Nobuko, 2007).

Many mushrooms, such as those in the *Lepista* or *Cantharellus* genera, are generally avoided by insects (Bärtschi, 2016). To identify potential insecticidal compounds, toxicological screenings

were conducted on fungi from southwestern France using *Spodoptera littoralis* and *Drosophila melanogaster* as model organisms. Numerous mushroom species proved toxic to these insects when incorporated into rearing media (Mier, 1996). (Shukle, 1983).

Bio-Blast is a pesticide that was developed using chemical compounds found in mushrooms. The compounds in the fungus *Metarhizium anisopliae*, a well-known natural termite predator, were used to synthesize it. Pest Control Operators (PCOs) can now acquire this fungus as Bio-Blast® Biological Termiticide from Paragon Professional Products. The active fungus in Bio-Blast, *Metarhizium anisopliae*, was identified in 1879 by the scientist Eli Metchnikoff (U.S. Patent No. 6,165,455:). The fungus appears greenish when grown on media (Goettel, 1997).

The green fungus develops spontaneously in soil all around the world. At least six different species of the fungus have been found to infect more than 200 insect species from seven different insect groups. *Reticulitermes* spp., *Heterotermes* spp., *Formosan* termites (*Coptotermes formosanus*), and other subterranean termites are all susceptible to the material, which research. Cite it. The combination of 20 infected *Nasutitermes exitiosus* termite workers and 200 healthy workers, however, caused 100% mortality in just 14 days, according to laboratory testing (Hanel 1983). This discovery demonstrates that the fungus is transferred horizontally throughout the colony, despite the possibility that some termite species are more susceptible to it than others (Technology, 1997).

The magic mushroom, a fungus supposed to be a hallucinogenic insect repellent, was the subject of another inquiry (Gartz, 1994). Magic mushrooms are well recognized to have hallucinatory effects. It is not known exactly show the psilocybin-producing chemical affects the real mushrooms. The most common theory is that by analysing the neurochemistry of insects, psilocybin may act as a psychedelic repellent (Hoffman, 1973).

Various filamentous fungus strains are employed to provide plants with biological protection. *Trichoderma* is one of these strains that is frequently employed as a mycofungicide. It consists of a collection of imperfect saprophytic filamentous fungi that have antifungal properties against a number of diseases that are found in the rhizosphere and phyllosphere (Harman, 2004).

Several plant species benefit from the proliferation of *Trichoderma* (Calvacante, 2008). The species *T. harzianum* is regarded as this genus' most potent biological control agent. (Dodd, 2013). For instance, *T. harzianum* T35 manages *Fusarium oxysporum* by vying for resources and rhizosphere colonization (Rajesh, 2016), Inducing systemic resistance in maize against *Fusarium verticillioides* is *T. harzianum* T22. The strains SC1 and USPP-T1 of *Trichoderma atroviride* have demonstrated good efficacy for the prevention of grapevine pruning wounds. (Mondello, 2018). One of the few fungal products with antagonistic actions against powdery mildew is *pseudozyma flocculosa* (Laur, 2018). The three principal greenhouse crops affected by this disease are the tomato (*Oidium neolycopersici*), cucumber (*Sphaerotheca fulginea*), and roses (*Sphaerotheca pannosa*) (Hajloui, 1992. Other fungus, including *Coniothyrium minitans*, exhibit bio fungicidal action. This fungus has the ability to parasitize the devastating pathogen *Sclerotinia sclerotiorum*, which affects a variety of plant hosts (Bennet, 2003).

One of the most extensively researched nematicides in biological control is the fungus *Paecilomyces lilacinus*. Chitinases and proteases, among other lytic enzymes, are secreted by *P. lilacinus* to enter nematode eggs (Abass, 2016). Insecticide alternatives *Beauveria bassiana* and *Metarhizium anisopliae* target *Tetranychus cinnabarinus*, cockroaches, termites, and mosquitoes such as *Anopheles arabiensis* and *Culex quinquefasciatus* (Abass, 2016) and numerous more nuisance insects that harm crops. Other fungi, including *Muscodor albus*, have an impact on fumigation. This fungus may produce a variety of volatile compounds, including alcohols, esters, ketones, acids, and lipids, which can destroy pathogens like bacteria and mold. (Mitchell, 2010).

2.4 Antifungal activity

A significant number of mushroom species (at least 52) have since been shown to possess antifungal properties, with a majority (44) belonging to edible varieties (Reagile, 2011). While edible mushrooms have received more research attention, non-edible species also hold promise as sources of valuable antifungal compounds. Wild mushrooms, in particular, offer a rich pool of diverse species for exploration (Manzi, 1999). This could be due to the fact that a number of mushrooms have not been domesticated yet.

Studies have investigated the antifungal potential of mushroom extracts. For instance, several *Agaricus species*, including *A. bisporus*, *A. bitorquis*, and *A. essettei*, exhibited antifungal

activity against *Candida albicans* and *C. tropicalis*, with *A. bitorquis* showing the strongest effect (Öztürk, 2011). However, the antifungal efficacy of *A. bisporus* against *C. albicans* remains inconsistent (Barros, 2008).

Besides *Agaricus*, other mushrooms like *Ganoderma lucidum, Agrocybe perfecta, Climadocon pulcherrimus, Oudemansiella canarii, Pycnoporus sanguineus*, and *Armillaria mellea* have shown varying degrees of antifungal activity against different *Candida species* (Helano, 2013; Rosa, 2003; Kalyoncu, 2008).

Amanita rhopalopus is a lesser-known mushroom species that has garnered interest for its potential insecticidal properties. This fungus, belonging to the *Amanita* genus, produces various bioactive compounds that may deter insect herbivory. Research suggests that certain metabolites found in *Amanita rhopalopus* can disrupt the nervous systems of insects, leading to reduced survival rates and impaired reproduction (Rätsch, 2005). The presence of these insecticidal compounds highlights the ecological role of this mushroom in regulating pest populations in its natural habitat. Further studies are needed to isolate and characterize these compounds, which could have implications for developing natural pest control agents in agriculture, providing an alternative to chemical pesticides (Friedman, 2016).

While mushrooms show promise as antifungal agents, their potency generally falls short of conventional antifungal antibiotics. However, there is potential to enhance their effectiveness through chemical modifications (Gilardoni, 2007). Moreover, given their often superior activity against plant pathogens compared to human fungal pathogens, these compounds may find greater utility in the food industry than in clinical settings (Gebreyohannes, 2019).

2.5 *Musca domestica*

Musca domestica, commonly known as the housefly, is a ubiquitous insect found in close association with human habitats. It is easily recognizable by its greyish body, large compound eyes, and rapid flight. Houseflies are known for their role in transmitting various pathogens, including bacteria and viruses, which can lead to diseases such as gastroenteritis and food poisoning (Wang, 2019). They thrive in environments where food is present, particularly in waste and decaying organic matter.

Chemical methods for controlling *Musca domestica* (housefly) primarily involve the use of insecticides such as organophosphates, pyrethroids, and insect growth regulators (IGRs). Organophosphates, like malathion and chlorpyrifos, inhibit acetylcholinesterase, disrupting nerve function and effectively reducing fly populations (Wang, 2019). Pyrethroids, including permethrin and cypermethrin, provide rapid knockdown effects but can lead to resistance in fly populations (Graham, 2013). IGRs, such as methoprene and pyriproxyfen, target the developmental stages of houseflies, preventing larvae from maturing into adults (Huang, 2018). While these chemical methods can be effective, their use should be part of an integrated pest management strategy to minimize environmental impacts and reduce resistance risks.

Traditional pest management strategies have predominantly relied on chemical insecticides; however, these approaches often raise concerns regarding environmental impact and pesticide resistance. As a result, there has been a growing interest in alternative control methods that are more sustainable and environmentally friendly.

Current methods for managing *M. domestica* include the use of microbiological control agents, such as entomopathogenic fungi and bacteria. For instance, the application of *Beauveria bassiana* has shown effectiveness in infecting and killing fruit flies, offering a biological solution to pest management (Inglis, 2001). Additionally, the use of *Bacillus thuringiensis* (Bt) has gained traction, as it produces toxins that are specifically harmful to larval stages of many insects, including fruit flies (Gonzalez, 2010). These microbiological methods not only reduce reliance on chemical pesticides but also promote a more integrated pest management approach, enhancing the sustainability of agricultural practices

2.6 Bioactive compounds with antifungal and insecticidal properties.

The following information highlights some of the compounds that are found to have been effective against either *C. albicans* or to possess insecticidal properties and other medicinal benefits. These include amatoxins, ergosterol, polysaccharides beauvericin and muscarine.

2.6.1 Amatoxins

Amatoxins are cyclic peptides primarily found in certain species of the Amanita genus, particularly *Amanita phalloides* (death cap). The most studied amatoxins include alpha-amanitin and beta-amanitin. These compounds are highly toxic and function by inhibiting RNA polymerase II, an essential enzyme for mRNA synthesis. This inhibition leads to cell death, particularly affecting the liver, which can result in severe toxicity and death in humans and animals. Despite their toxicity, amatoxins have been explored for potential applications in targeted cancer therapies, as they can selectively kill rapidly dividing cells (Käfer, 2020).

2.6.2 Ergosterol

Ergosterol is a sterol found in the cell membranes of fungi, analogous to cholesterol in animal cells. It plays a crucial role in maintaining membrane integrity and fluidity. Ergosterol has been studied for its antifungal properties, particularly its ability to disrupt the cell membranes of fungi like *Candida albicans* (Friedman, 2016). Additionally, ergosterol and its derivatives have shown potential in pharmaceutical applications, including anti-inflammatory and anticancer activities.

2.6.3 Polysaccharides

Polysaccharides, such as beta-glucans, are abundant in many mushrooms, including *Lentinula edodes* (shiitake) and *Ganoderma lucidum* (reishi). These compounds are known for their immunomodulatory effects, enhancing the immune response against pathogens, including fungi and bacteria (Zhang, 2016). Beta-glucans can stimulate macrophage activity and enhance the production of cytokines, contributing to their therapeutic potential in cancer treatment and immune disorders. Some studies have also demonstrated their antifungal activity against *C. albicans*.

2.6.4 Beauvericin

Beauvericin is a secondary metabolite produced by the entomopathogenic fungus *Beauveria bassiana*. It exhibits insecticidal properties by disrupting cellular functions and promoting apoptosis in insect pests (Sánchez, 2017). Beauvericin's mechanism involves interference with mitochondrial function and ion transport, making it a candidate for biopesticide formulations. Its

effectiveness against a range of insect species highlights its potential in integrated pest management strategies.

2.6.5 Muscarine

Muscarine is an alkaloid found in *Amanita muscaria* (fly agaric). It acts primarily as a muscarinic acetylcholine receptor agonist, influencing neurobiology and causing a range of effects, from salivation to respiratory distress. While muscarine is not typically studied for antifungal or insecticidal properties, its neuroactive effects have been explored in pharmacological research (Rätsch, 2005).

Table 1: **Mushroom compounds and their applications**

Compound	**Source**	**Properties**	**Applications**
Amatoxins	*Amanita phalloides*	Inhibits RNA polymerase II; highly toxic	Potential use in targeted cancer therapies
Ergosterol	Various fungi (e.g., *Agaricus bisporus)*	Disrupts fungal cell membranes	Antifungal properties, anti-inflammatory activities
Polysaccharides	*Lentinula edodes, Ganoderma lucidum*	Immunomodulatory effects; enhances immune response	Cancer treatment, antifungal activity against C. albicans
Beauvericin	*Beauveria bassiana*	Disrupts cellular functions in insects	Biopesticide against various insect pests
Muscarine	*Amanita muscaria*	Neuroactive; muscarinic acetylcholine receptor agonist	Neuropharmacological research
Other Compounds	Various mushrooms	Antimicrobial, antioxidant, anti-inflammatory	Health benefits, potential therapeutic applications

2.7 Advances in treatment of fungal infections and managing pests

These include innovative approaches such as fungal nanobiotechnology, fungal derived metabolites, CRISPR and biopesticides. These methods aim to enhance effectiveness while minimizing environmental impact.

2.7.1 Fungal Nanobiotechnology

Fungal nanobiotechnology involves the use of nanoparticles synthesized from fungal metabolites or mycelium to combat fungal infections and pests. For example, silver nanoparticles produced from fungi have demonstrated potent antifungal activity against various pathogens, including *Candida albicans* and *Aspergillus* species (Gardea-Torresdey, 2020). These nanoparticles can disrupt the cell membranes of fungi, leading to cell death.

2.7.2 Applicability: The use of fungal-derived nanoparticles offers a novel approach to enhance antifungal treatments while potentially reducing toxicity to human cells. Additionally, these nanoparticles can be engineered for targeted delivery, improving therapeutic outcomes (Bafakeeh, 2020)

2.7.3 Limitations: Challenges include the scalability of nanoparticle production, potential toxicity to non-target organisms, and the need for further research to understand long-term effects and mechanisms of action (Rai, 2015).

2.7.4 Fungal-derived Metabolites

Research has focused on isolating bioactive compounds from fungi that exhibit antifungal properties, leading to the development of new therapeutic options (Friedman, 2016).

2.7.5 CRISPR and Genetic Engineering

Advances in genetic engineering, including CRISPR technology, are being explored to develop fungi with enhanced pest resistance or improved production of beneficial metabolites (Yin, 2021.

2.7.6 Biopesticides

Biopesticides, derived from natural materials such as plants, microorganisms, and fungi, are becoming increasingly popular in pest management. For instance, entomopathogenic fungi like *Beauveria bassiana* and *Metarhizium anisopliae* are being utilized to control insect pests by infecting and killing them (Sánchez, 2017). Additionally, fungal metabolites such as beauvericin and other mycotoxins exhibit insecticidal properties.

2.7.7 Applicability: Biopesticides offer a sustainable alternative to chemical pesticides, reducing the risk of resistance development and minimizing environmental impact. They can be integrated into existing pest management systems, enhancing overall efficacy (Shah, 2018).

2.7.8 Limitations: The effectiveness of biopesticides can be influenced by environmental conditions, such as humidity and temperature, which may affect fungal viability and efficacy. Additionally, regulatory hurdles and public perception can impact their adoption.

While recent advances in fungal nano-biotechnology and biological control methods offer promising alternatives for treating fungal infections and managing pests, challenges remain regarding regulatory approval, production costs, and variability in efficacy. Continued research and development are essential to overcome these limitations and fully realize the potential of these innovative approaches in medicine and agriculture.

2.8 Research Gaps

There are significant research gaps that are present in terms of fungal therapy and insecticidal effect of mushrooms. These include the following;

2.8.1 Mechanisms of Action

Research has identified insecticidal compounds in mushrooms, but detailed studies on their specific mechanisms of action are lacking. Understanding these pathways could enhance their application as natural insecticides (Khan, 2021).

2.8.2 Synergistic Effects

There is limited exploration of the synergistic effects of mushroom extracts when combined with other natural or synthetic insecticides. Investigating these combinations could lead to more effective pest management strategies (Friedman, 2016).

2.8.3 Broader Range of Species

Most studies focus on a narrow range of mushroom species. Expanding research to include diverse fungi could uncover new insecticidal compounds and broaden efficacy against various pest species (Zhang, 2016).

2.8.4 Field Studies

Much of the existing research is conducted in controlled laboratory settings, which may not accurately reflect field conditions. Conducting field studies to assess the real-world effectiveness of mushroom extracts against insect pests is essential (Bennett, 2010).

2.8.5 Resistance Mechanisms

As with antifungal agents, investigating potential resistance mechanisms in insects against mushroom-derived insecticides is critical for sustainable use (Zhao, 2017).

2.8.6 Impact on Non-Target Organisms

Limited research exists on the effects of mushroom extracts on non-target organisms, such as beneficial insects and pollinators. Evaluating the ecological impact of these natural insecticides is vital for sustainable agricultural practices (Marr, 2015).

2.8.7 Antifungal Activity Against Emerging Strains

Research has explored some Candida species, but emerging resistant strains pose significant challenges. Focusing on the antifungal properties of various mushroom extracts against these strains could provide important therapeutic options (Sanglard, 2002).

2.8.8 Standardization of Extracts

There is a need for standardized protocols for extracting and testing the bioactivity of mushroom compounds. Establishing consistent methodologies would improve comparability across studies (Kullberg, 2015).

2.8.9 Pharmacokinetics and Toxicology

Limited data are available on the pharmacokinetics and potential toxicity of mushroom extracts used medicinally. Further studies are needed to evaluate safety profiles and optimal dosing for both insecticidal and antifungal applications (Khan, 2021).

CHAPTER 3

3.1 Abstract

The study examined the anti-*Candida* properties of four native Zimbabwean mushroom species against *Candida albicans*, a fungal pathogen. The research focused on determining the effectiveness of mushroom extracts (methanol, ethanol, chloroform and water) in inhibiting the growth of this organism. Agar disc diffusion was employed to assess antifungal activity. Maybe also include the concentrations of the crude extract used. The results demonstrated varying degrees of antifungal activity across the tested mushrooms. *Cantharellus symoensii* exhibited the most potent antifungal effect, with its methanolic extract producing a 16.6±0.4 mm zone of inhibition against *C. albicans*. Other mushrooms tested included the non-edible varieties, *Russula sp, Amanita rhopalopus*, *Boletus sp* as well as the edible variety, *Cantharellus miomboensis* also showed anti-*Candida* activity. Aqueous extracts lacked any antifungal activity. The study utilized different solvent for the extraction process. This revealed that ethanol and chloroform were superior solvents for extracting antifungal compounds from *Cantharellus symoensii* and *Cantharellus miomboensis*, respectively. Overall, the findings support the hypothesis that Zimbabwean mushrooms possess anti-*Candida* properties, suggesting their potential for developing anti-*Candida* medications.

3.2 Background

The anti-Candida potential of selected indigenous mushrooms found in Zimbabwe.

Candidiasis, a fungal infection caused by *Candida* species, poses significant health risks, affecting the skin, mucous membranes, and internal organs. These infections are associated with high morbidity and mortality rates, particularly in hospitalized, immune-compromised, or critically ill patients (Maiken, 2017). Notably, *Candida* is a leading cause of delayed infections in neonatal intensive care units (Lovero, 2016), with *C. albicans* being the most prevalent species (Khan 2019).

While over 100 *Candida* species exist, *C. albicans* remains the primary concern. The escalating drug resistance of *C. albicans*, including the emergence of multi-drug resistant strains (Khedri, 2018), underscores the urgent need for novel antifungal treatments. Traditional antifungal therapies have proven inadequate, necessitating the exploration of alternative approaches, such as natural products.

Mushrooms have garnered attention in pharmacological research due to their rich source of biologically active compounds with potential therapeutic applications. Terpenes, a class of natural substances derived from plants, are of interest. These compounds are composed of isoprene units (C5H8) and have shown promise in various medicinal contexts (Bakkali, 2008). Several mushrooms are known to produce these compounds, notably *Ganoderma lucidum* (reishi), which contains triterpenoids linked to anti-inflammatory and anticancer effects (Yang 2019). *Lentinula edodes* (shiitake) is another source, with bioactive terpenoids that enhance immune function (Zhan, 2020). *Agaricus bisporus* (button mushroom) also contains beneficial terpenoids contributing to its flavour and health benefits (Friedman, 2016). Additionally, *Pleurotus ostreatus* (oyster mushroom) produces various terpenoids with potential health-promoting properties (Lee, 2018).

Apart from terpenes other mushroom compounds contain antifungal properties. Key among these are polysaccharides, such as beta-glucans, found in species like *Ganoderma lucidum* (reishi) and *Lentinula edodes m*ushrooms produce a variety of bioactive compounds that contribute to their antifungal properties. Key compounds include polysaccharides, such as beta-glucans, found

in species like *Ganoderma lucidum* (reishi) and *Lentinula edodes* (shiitake), which enhance immune responses and exhibit direct antifungal effects against pathogens like *Candida albicans* (Zhang 2016). Triterpenoids, present in *Ganoderma lucidum*, disrupt fungal cell membranes and demonstrate significant antifungal activity (Yang 2019). Additionally, phenolic compounds, which possess antioxidant properties, can inhibit the growth of various fungi (Friedman, 2016). Ergosterol, a sterol found in fungal membranes, can be converted into antifungal agents like amphotericin B, and its own antifungal effects have been noted (Friedman, 2016). Other compounds, such as alkaloids found in certain *Amanita* species and saponins in various mushrooms, also exhibit antifungal activity by disrupting fungal membranes (Khan, 2021). Overall, these compounds highlight the potential of mushrooms in developing natural antifungal therapies.

3.3 *Candida albicans*

It is one of the most prevalent fungal pathogens globally, particularly among immunocompromised individuals, with estimates suggesting it accounted for approximately 50% of all candidemia cases (Pappas, 2009). The prevalence of *C. albicans* infections has been exacerbated by factors such as the increasing use of broad-spectrum antibiotics, which disrupt normal flora, and the rise of invasive medical procedures (Kullberg & Arendrup, 2015). Notably, resistance to antifungal medications, particularly azoles, has been documented, with strains demonstrating reduced susceptibility affecting treatment outcomes (Sanglard & Odds, 2002). This growing resistance poses significant challenges for effective management and highlights the need for ongoing surveillance and research into novel antifungal therapies.

Current compounds used as antifungal agents against *Candida* species, particularly *Candida albicans*, encompass several classes of medications. Azoles are widely utilized, with fluconazole being effective for oropharyngeal and esophageal candidiasis, and itraconazole used for various *Candida* infections, especially in immune-compromised patients (Sanglard & Odds, 2002). Echinocandins, including caspofungin and micafungin, are effective against *Candida* species and are commonly used for treating invasive candidiasis and as prophylaxis in high-risk patients (Pappas, 2009). Polyene antifungals, notably amphotericin B, serve as broad-spectrum agents against serious systemic *Candida* infections, although they can

have significant side effects (Barchiesi, 2011). Additionally, allylamines like terbinafine are primarily used for dermatophyte infections but possess some efficacy against certain *Candida* species (Khan, 2021). Nystatin, another polyene antifungal, is typically applied topically for cutaneous and mucosal candidiasis. Flucytosine is often combined with amphotericin B for severe infections, particularly when resistance to other agents is a concern (Bennett 2010). Newer agents such as isavuconazole have emerged, showing efficacy against *Candida* species and providing alternatives for resistant infections (Marr, 2015). These compounds represent the current primary treatments for *Candida* infections, with ongoing research aimed at developing new agents and strategies to combat resistance.

There has been noted drawbacks associated with the use of these compounds that are used against *Candida* infections. Azoles, such as fluconazole, can lead to resistance over time, particularly among *Candida* species, which limits their effectiveness (Sanglard, 2003). Additionally, they may cause liver toxicity and have potential drug interactions due to their metabolism in the liver (Kullberg & Arendrup, 2015). Echinocandins, while effective against *Candida*, have a limited spectrum of activity and may not be effective against non-*Candida* species like *Cryptococcus* (Pappas, 2009). Some *Candida* species can develop resistance to echinocandins, although this is relatively rare (Koh 2017). Polyene antifungals, particularly amphotericin B, are associated with significant nephrotoxicity and can cause infusion-related reactions such as fever and chills (Barchiesi, 2011; Marr, 2015). Allylamines like terbinafine are mostly effective against dermatophytes and have limited use in systemic *Candida* infections (Khan, 2021). Nystatin is primarily used topically or orally for mucosal infections, restricting its application in systemic cases (Bennett, 2010). Flucytosine can result in rapid resistance development when used alone and may cause bone marrow suppression, necessitating careful blood monitoring (Bennett, 2010). Lastly, newer agents like isavuconazole have less long-term data available compared to older antifungals, and their long-term safety and efficacy are still under investigation (Marr, 2015). These limitations highlight the need for careful consideration in clinical use and ongoing research to improve treatment options and address resistance issues.

3.4 Problem statement and justification

Increasing emergence of infectious diseases caused by pathogenic bacteria, fungi, and viruses represent major threats to human health (Shridhar, 2015). *Candida* infections remain an important cause of morbidity and mortality, especially in hospitalized and immune-compromised (Maiken, 2017) *C. albicans* is an opportunist pathogen that lives commensally within the human body; it is the leading cause of human fungal infections. *C. albicans* infection usually develops as a consequence of host immune response alterations. This fungi has however developed multi-drug resistance in particular to azole drugs and is therefore increasingly becoming difficult to treat.

3.5 Justification

Mushrooms are a rich source of diverse chemical compounds with potential medicinal applications (Prasad, 2015). While there is growing recognition of their therapeutic value globally, the potential of African mushrooms, including those found in Zimbabwe, remains largely untapped. Despite Zimbabwe's rich fungal biodiversity, the nutritional, chemical, and medicinal properties of its native mushrooms are understudied.

This research aims to explore the antifungal properties of Zimbabwean mushrooms. Enhancing the understanding their potential health benefits, we can promote their wider use in disease prevention and treatment. The findings will contribute to a broader understanding of the antimicrobial capabilities of these fungi and inform the development of innovative solutions, such as antifungal creams, to address the escalating challenge of drug-resistant *Candida albicans* infections. This aligns with the principles of Education 5.0, which emphasizes the application of knowledge to real-world problems.

3.5 Materials and methods

3.6.1 Collection and identification of mushrooms and extraction of their contents.

A B

C

Figure 1 Different mushroom (5) species awaiting to be dried. *Boletus sp*, *Cantharellus miomboensis*, *Cantharellus symoensii*, *Amanita rhopalopus, Russula sp* were collected in Mutare, Zimbabwe.(-19.16667,32.61667)

The mushrooms were collected in the rainy season in December 2021/2/3. They were collected from Marondera, Rusape and Mutare. This is because some mushrooms species were only found as far as Mutare. The mushrooms were collected by hand using the help of local individuals.

They also helped in identifying the in edible mushrooms which the required gloves to be handled. After collection the mushrooms were packed in a cooler box and transported to Chinhoyi University of Technology where they were then dried.

3.6.2 Drying

The fresh mushrooms were sliced into thin strips and sun dried for seven days. The dried mushrooms were then ground to powder using an electrical blender. Mushroom extraction was carried out using chloroform, 70% ethanol, methanol and hot water solvents. The table below was used to determine the type of solvent used. A 100g of powdered mushroom were mixed with 0.5L of each solvent in a conical flask at 25 ∘C and were shaken using an incubator shaker at 150 rpm for 72h. The extracts were centrifuged at 3000 rpm for 15 min, filtered with Whatman No.1 filter paper, and evaporated and dried using a rotary evaporator at 50∘C. The extracts were kept in -80∘C deep freezer and freeze-dried by a freeze-dryer. Finally, they were stored in the 4∘C refrigerator in an amber coloured bottle for further analyses. The protocol was adopted from Zhang, 2016.

3.6.3 Preparation of Test Organisms.

C albicans ATCC 66027 were used in this study as test organism. The microorganisms were provided by the Parirenyatwa Group of hospitals microbiology laboratory. The yeast test organisms were grown in Sabouraud dextrose agar.

3.6.4 Determination of antifungal activities of extracts of wild Mushrooms.

The antifungal assay was performed by agar disc diffusion methods (Bauer, 1966). The surface of Sabouraud Dextrose Agar (SDA) plate was inoculated with 100 µl (1 × 108 cfu/ml) of the standardized pure culture suspension to obtain a lawn culture. Circular paper discs measuring 7.0 mm were cut from Whatman No. 1 filter paper using a paper perforator and sterilized in an autoclave. The disc (7 mm) was saturated with each of the reconstituted mushroom extracts,

allowed to dry and was placed firmly (with the use of sterile forceps) on the surface of the seeded agar plate. The plates were incubated for 24 - 48 h at 37°C. Antifungal activities were determined by measuring the diameter (in millimetre) of the zone of inhibition. For each of the test organism, control was determined by using fluconazole and distilled water. All experiments were performed in triplicates and the mean value was recorded. The results obtained were compared with the standard antifungal agents. The protocol was standadized by Bauer in 1966 and has since been used as the standard procedure.

3.6.5 Statistical Analysis.

Zones of inhibition in mm are given as means ± standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Differences at $P \leq 0.05$

3.6.6 Bioactive compound analysis

The following compounds were tested for using their respective procedures, tannins, saponins, flavanoids, glycosides, terpenoids and steroids.(Imaobong, 2020)

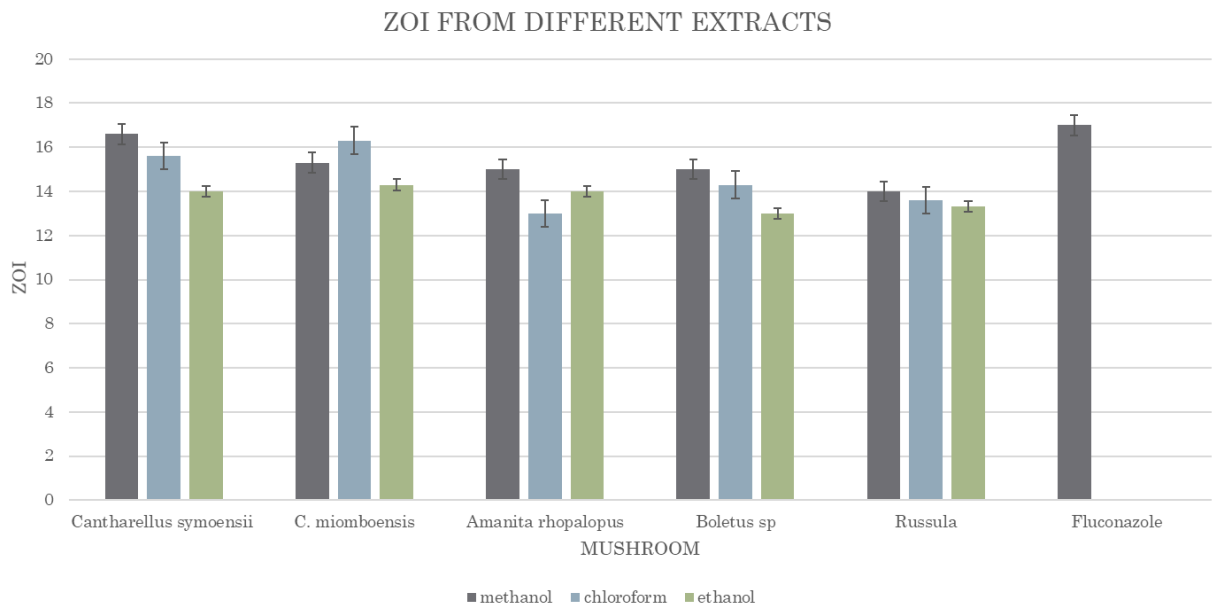
3.7 Results

Circular paper discs (7 mm in diameter) were saturated with methanol, ethanol, chloroform, hot water extracts from the mushrooms being studied, or with the antifungal drug fluconazole as a positive control. These discs were then placed onto agar plates previously seeded with *Candida albicans* ATCC 66027. The inoculated plates were incubated at a low temperature (4°C) for two hours, followed by incubation at 37°C for 18 hours. The diameter of the zones where fungal growth was inhibited around each disc was measured. The absence of inhibition was denoted by a negative sign (-). Data were presented as mean ± standard deviation (SD) from three replicate experiments. Statistical significance was determined using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.

Table 2: Antifungal activities of different extracts of mushrooms on test organism

Mushroom	Zone of inhibition diameter(mm) on *Candida albicans*			
	methanol	chloroform	ethanol	Hot water
Cantharellus symoensii	16.6±0.4	15.6±0.45	14±0.012	-
C. miomboensis	15.3±0.74	16.3±0.563	14.3±0.78	-
Amanita rhopalopus	15±0.054	13±0.0	14±0.0	-
Boletus sp	15±0.0	14.3±1.23	13±0.0	-
Russula	14±0.032	13.6±0.41	13.3±0.72	-

The standard Fluconazole showed an average of 17mm zone of inhibition.



This study was undertaken to determine the antifungal activity of mushrooms against *Candida albicans* ATCC 66027 using hot water, ethanol, methanol and chloroform as solvents. It revealed that all mushroom species exhibit moderate to antifungal potential as shown in Table 2. The different mushroom extracts exhibited various antifungal activity with a range of 12mm - 17mm in diameter. The highest antifungal activity 17mm was shown by methanol and chloroform extracts of *Cantharellus symoensii* against *C albicans*. The results in Table 2 showed that all mushrooms exhibited inhibitory activities against *C albicans*, as shown by the clear zone of inhibition measurements around the test mushroom extracts.

The organic solvent, methanol, was the most effective in extracting antifungal compounds as shown by its highest zone of inhibition 17mm, followed by chloroform and ethanol. Hot water extracts did not show any activity against *Candida albicans*. Of the 5 tested mushrooms, two of them, namely, *Cantharellus symoensii*, and *C. miomboensis* which are edible mushrooms, have shown promising antifungal activities against *Candida albicans* ATCC 66027. Non edible mushrooms which are *Russula sp*, *Boletus sp* and *Amanita rhopalopus* also showed antifungal activity against *Candida albicans* ATCC 66027. Zones of inhibition diameter was statistically different against mushrooms and between solvents

3.8 Discussion

All tested mushroom extracts exhibited antifungal properties against *Candida albicans,* as evidenced by clear inhibition zones (Table 1). The observed variation in antifungal activity among mushroom species can be attributed to differing concentrations of bioactive compounds (Akyuz, 2010; Padmavathy, 2014). Methanol extract from *Cantharellus symoensii* demonstrated the strongest antifungal effect (17 ± SD mm inhibition zone) which was a significant difference.

Cantharellus symoensii and *C. miomboensis* extracts showed promising antifungal activity. However, the efficacy of their extracts varied across different solvents (methanol, chloroform, and ethanol), suggesting the presence of diverse bioactive compounds with varying mechanisms of action. The observed differences in antifungal activity between the two *Cantharellus* species might be due to variations in secondary metabolite composition (Gebreyohannes, 2019).

Polar solvents, such as methanol, were generally more effective in extracting bioactive compounds compared to non-polar solvents like chloroform (Gebreyohannes, 2019). The weak antifungal activity of chloroform and ethanol extracts could be attributed to the absence or degradation of bioactive compounds during extraction.

The antifungal potential of mushroom extracts is supported by previous research (Reid, 2019). Studies on *Trametes elegans* and other mushrooms have highlighted the role of diverse secondary metabolites in their antimicrobial properties (Barros, 2014). *Cantharellus symoensii* and *C. miomboensis* extracts, in particular, show promise for developing antifungal medicinal products (Alves, 2012).

While this study focused on *Candida albicans,* mushrooms have also demonstrated antimicrobial activity against bacteria (Rahi, 2016). Species like *C. heinemannianus, C. symoensii*, and *G. lucidum* exhibited particularly strong antibacterial effects.

The observed lack of antifungal activity in hot water extracts suggests that the bioactive compounds responsible for inhibiting fungal growth are more soluble in organic solvents. This aligns with previous findings (Kamra and Bhatt, 2012; Tiwari 2011).

The broad-spectrum antifungal activity exhibited by ethanol, chloroform, and methanol extracts against *Candida albicans* is significant given the increasing prevalence of antibiotic resistance in bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi*, and *Escherichia coli* (Blossom 2006; Okonko 2009; Rowe 1997; Sangeeth 2014; Stanely 2013). These findings underscore the potential of mushroom-derived compounds as alternative therapeutic agents.

The study acknowledges that the research was limited by the lack of access to more sophisticated techniques, which could have significantly enhanced the study's outcomes. Unfortunately, the laboratory facilities available were not equipped for advanced methodologies such as high-performance liquid chromatography (HPLC) or mass spectrometry, which are essential for detailed compound analysis. Moreover, similar constraints were observed in other university laboratories within the region, which also lacked the necessary equipment and resources. This limitation hindered the ability to conduct more comprehensive and nuanced investigations into the compounds extracted from the mushrooms, ultimately impacting the depth of the research findings. The study recognizes that employing these advanced techniques could have provided more accurate data and insights into the bioactive properties of the mushroom extracts.

3.9 Conclusion and Recommendation

This study investigated the antifungal properties of five different mushroom species against *Candida albicans*. The findings indicate that these local mushrooms, both edible and non-edible, possess compounds with antifungal activity. Notably, methanol extract from *Cantharellus symoensii* demonstrated the strongest antifungal effect.

Mushrooms have been recognized as a rich source of bioactive compounds with potential health benefits beyond their nutritional value. This study supports the idea that incorporating mushrooms into the diet could offer additional health advantages. To fully harness the potential of these fungi, efficient methods for mushroom cultivation and bioactive compound extraction are essential.

The results of this study provide a foundation for future research aimed at identifying and isolating the specific antifungal compounds in these mushrooms. Further investigations into the mechanisms of action of these compounds will be crucial for optimizing their therapeutic potential. By expanding our understanding of these natural compounds, we can contribute to the development of novel antifungal treatments.

CHAPTER 4

4.1 Evaluation of the insecticidal properties of *Amanita rhopalopus*, a local non-edible mushroom

4.2 Abstract

Many mushroom species are toxic to insects and have the potential to be used as mycopesticides. However, they remain underutilized, despite having bioactive compounds with fungicidal, herbicidal, pesticidal and insecticidal properties. There is potential to use mushrooms in producing mycopesticides which are living preparations that use fungal cells, such as spores and hyphae, as active ingredients to combat insects such as *Musca domestica* flies. In this study, *Musca domestica* flies were captured in a fly trap, treated to mushroom extracts of the species *Amanita rhopalopus,* and examined for mortality and repellence. In this experiment, insect mortality was determined by inserting flies in petri dishes with filter papers soaked in various mushroom extracts, with a commercial insecticide serving as the control and water serving as the negative control. The mushroom extract with the greatest solvents used for extraction had 100% mortality in the first 5 minutes, which was comparable to the commercial insecticide. The lethal concentration of the mushroom ranged from 40% in the best solvent extract to 80% in the aqueous extract, suggesting that when more components were extracted, the mushroom behaved better. The mushroom contains insecticidal characteristics and could be an ideal candidate for a mycopesticide.

Key Words: *Amanita, Musca domestica,* mycopesticide, mortality, repellence

4.3 Background of study

Non edible mushrooms are prevalent in Zimbabwe. Most cases of mushroom posoning are as a result of mushroom misidentification (Chikuni, 2019). The mushroom *Amanita rhopalopus* has been noted since time immemorial by natives from the place that it grows naturally (Manicaland) to be toxic and non-edible. The ingestion of this wild mushroom after misidentification of this toxic mushroom as an edible species *Amanita zambiana* leads to mushroom poisoning. The most common reason for this misidentification is the close resemblance in terms of colour and morphology. the symptoms of ingesting this mushroom include abdominal cramps, nausea, vomiting and severe watery diarrhoea. This is constant to what was reported in the manicapost a local newspaper in 2023 (Manicapost 2023).

When the mushroom was being dried for other purposes, it was noted that dead flies (*M. domestica*) were found around it from day one. This therefore led to the hypothesis that it may contain insecticidal properties. Literature shows that other species like the non-edible *Zambiana muscaria* were used to make active ingredients for fly traps (Mateja, 2016). This therefore sets a precedence that mushrooms may indeed be effective against insects in particular flies. There is therefore a need to conduct scientific research on the effects of the extracts of *Amanita rhopalopus* against the fly, *M. domestica*.

4.4 Introduction

The houseflies,*Musca domestica*, are a problem worldwide (Gerden, 2021). They are prevalent in many households and spread a multitude of pathogenic diseases that lead to death in human beings (Bahrndorff, 2017). They are responsible for bacterial (Shigellosis, Salmonellosis, and Cholera), protozoan (amoebic dysentery), helminthic (round worms, hookworms, pinworms, and tapeworms) and more than 65 intestinal disorders in humans and animals (Sasaki, 2000). Synthetic pesticides are often utilized in housefly control efforts, and their widespread usage has resulted in the development of pesticide resistance (Malik, 2007). Furthermore, synthetic fungicides, herbicides, nematicides or insecticides are very unspecific and possess a high general toxicity so that they have been or will soon be banned by various governments (Gayane, 2016).

Mushrooms represent a valuable natural source of biologically active compounds with fungicidal, herbicidal, pesticidal and insecticidal properties (Gayane, 2016). *Amanita muscaria*, commonly known as the fly agaric or fly amanita, is a basidiomycete of the genus *Amanita* used in traditional recipes from Slovenia to make fly traps that kill flies (Lumpert, 2016). It has been shown in some studies that species like the non-edible *Amanita muscaria* and *Zambiana muscaria* were used to make active ingredients for fly traps (Mateja, 2016). In another study, the fly *Drosophila melanogaster (Meigen)* was subjected to 127 different varieties of fungi, and it was discovered that six of the eight *Amanita (Agaricales, Amanitaceae)* species inhibited the fly's growth (BESL, 1987). On the other hand, the mycophagous species *Drosophila kuntzei* and *Drosophila phalerata* were impervious to the amatoxins made by *Amanita phalloides*. Jaenike, (1983) looked at how well mycophagous and non-mycophagous species of *Drosophila* tolerated -amanitin, a suspected RNA polymerase II inhibitor, and discovered that mycophagous species were tolerant while non-mycophagous species were intolerant. Mycophagous (*Drosophila bizonata, Drosophila angularis,* and *Drosophila brachynephros*) and non-mycophagous (*Amanita pantherine, Amanita ibotengutake,* and *Amanita muscaria*) species were studied for their effects on the survival and functionality of ibotenic acid and its derivative, musimol (*Drosophila immigrans* and *D). D. bizonata* was discovered to often multiply on *A. pantherina* and *A. ibotengutake*. *D. immigrans* and *D. melanogaster* are frugivorous, but the three mycophagous species, with the exception of *D. immigrans*, which rarely consumes and breeds on mushrooms (Nobuko, 2007), employ a range of mushroom species for breeding. Similarily a range of filamentous fungal strains protect plants, with *Trichoderma* being one of the most widely used mycofungicides (Omran, 2020).

Mushrooms produce a variety of defensive compounds, including amatoxins, phallotoxins, ibotenic acid, L-DOPA, and clitocine. These compounds can be toxic to insects, and some have been used to develop mycopesticides. For example, the fungus *Metarhizium anisopliae* is used to produce Bio-Blast, a biological termiticide (Jaenike, 1983). Another fungus, *Psilocybe*, produces psilocybin, a compound that is thought to act as a hallucinogenic insect repellent. This is supported by the fact that fungal-eating insects typically reside in areas where hallucinogenic mushrooms grow. In addition to these compounds, some fungi also produce proteins that have

insecticidal properties. For example, lectins, protease inhibitors, and lipoxygenases have all been isolated from mushrooms. These defensive compounds help mushrooms to protect themselves from being eaten by insects. They also have the potential to be used to develop new pesticides that are more environmentally friendly than traditional pesticides. The compounds produced by mushrooms can be toxic to insects in a variety of ways. Some compounds interfere with the insect's metabolism, while others damage the insect's nervous system.

Another widely available biopesticide made from *Beauveria bassiana*, affects a variety of insects including aphids, whiteflies, thrips, fire ants, and bedbugs. The fungus organisms that attach to the insect's exoskeleton gradually fill a hole in its body. The fungus' penetration, development, and spore production cause the insect's internal organs to liquefy. Because of its slow activity, the target insect has time to collect fungal spores and spread them throughout the colony. Another fungal biopesticide, *Phythium oligandrum*, protects crops and turf from approximately 20 soil-borne illnesses. As an alternative to the fumigant methyl bromide, there is a fungus-based biopesticide called *muscodor albus*. Food products are shielded against post-harvest deterioration using this technique (Anderson, 2020)It would seem that such mushrooms have extensive potential to be used as mycopesticides by taking advantage of the fungicidal, herbicidal, nematicidal, pesticidal and insecticidal properties of the bioactive compounds in them.

Mycopesticides are living preparations that use fungal cells, such as spores and hyphae, as active ingredients (Jiang, 2023). Mycopesticides can be used as biological pesticides, or biopesticides, that target specific Insect pests. The US Environmental Protection Agency (EPA) defines biopesticides as naturally occurring compounds (biochemical pesticides), microorganisms (microbial pesticides), and pesticidal substances produced by plants with genetically modified organisms (plant-incorporated protectants, or PIPs) (US EPA, 2016). Biopesticides that target specific pests often have an impact solely on the target pest and closely related creatures, safeguarding other organisms that share the same habitat (Villaverde, 2009). The main advantage of using mycopesticides is that it does not affect non-target organisms. This makes it safe for other organisms that might come into contact with it and does not disrupt the ecosystem’s balance.

The growth of the biopesticide market, including these fungal-based controls, comes in response to the increasing demand for more natural pest control tools. Biopesticides can complement conventional chemical pesticides and are cost effective and eco-friendly. They can be incorporated into any Integrated Pest Management program, contributing to sustainable pest control and healthy environment. Mycopesticides could put many chemical pesticide companies out of business simply because it eradicates the many dangers associated with chemicals, such as ozone layer depletion, as it complements nature and contributes to an ecosystem's sustainability. In addition to this, chemical-based insecticides are dangerous to people when ingested, and also to the environment as most contain chlorofluorocarbons that damage the ozone layer (Ayilara, 2023). The complex chemical make-up that constitute the pesticides that are not naturally degraded without harmful effects, nullifies the benefits of chemical-based insecticides. Most of these are also not target specific and that may lead to the disruption of an ecosystem's natural balance which may result in even more problems.

The use of fungal compounds to develop pesticides is a relatively new field of research. However, there is already some evidence that these compounds can be effective in controlling insect pests (Ayaz, 2023). The mushroom *Amanita rhopalopus,* kuneguva in the vernacular shona language, has been noted since time immemorial by natives from the place that it grows naturally to be non-edible and toxic to houseflies *Drosophila melanogaster* thus showing great potential to be utilized in the biotechnological application for the preparation of a mycopesticide. With current trends in domestication of indigenous mushroom, *A. rhopalopus* might be a candidate. Also, the requirements of Zimbabwe's education 5.0 state that it is of utmost importance to produce a product that may be beneficial for the economy. It is generally more advantageous to switch to natural pest control methods that sustain ecosystems, are target specific, and are cost effective in the long run. Also, this particular mycopesticide is cheaper to produce as the mushroom is found nationwide. It does not disrupt the human food chain as it is inedible. Thus the main objective of this study was to determine the insecticidal properties of *Amanita rhopalopus,* determine the best performing extraction method and evaluate the bioactive compounds in the mushroom. Furthermore, the lethal concentration of the mushroom extracts will be determined.

4.5 Materials and Methods

4.6.1 Collection

The mushroom samples for *Amanita rhopalopus* (see figure 1) were collected from Mutare (Zimunya, latitude -19.03419863074863, Longitude 32.640985693904824. Primary identification was done via the use of traditional knowledge possessed by the local individuals. Due to morphological analysis the mushroom closely resembles the *Amanita spp*.

Figure 2 *A. rhopalopus* collected from the wild in Mutare for use in the development of the mycopesticide.

4.6.2 Identification

Primary identification was done with the assistance of local individuals that were knowledgeable in the diverse mushrooms that grow in these areas. It was also done using online tools.

4.6.3 *M. domestica* collection

A fly trap was purchased from TM supermarket Chinhoyi and was modified. Modifications included adding a net to the trap so that the flies would not die. The net was inserted as shown in figure 2. Different kinds of house flies were collected and morphologically identified.

Figure 3 Modified fly trap for the collection of *M. domestica.* (Picture taken researcher 2022)

4.6.4 Experimental design

A simple randomized block design was used because a single factor design was used. Only a single factor (extract) varied at a time while everything else remained constant. The treatments were the mushroom extracts. Every experimental unit received the same treatments randomly.

The response variables under study were the mortality and repellence of M. *domestica* and were done separately.

4.6.5 Bioactive compound extraction

Extraction was done using 4 different solvents, ethanol, water, methanol and chloroform. This includes both polar and non-polar solvents. Maceration was done, where the samples were left in the solvent for 72 hours with constant shaking. A rotary evaporator was used to evaporate the solvents. 100g of each mushroom was used.

4.7.1 Bioactive compound analysis

4.7.2 Preliminary screening

The following standard qualitative tests were done to evaluate the presence of the compounds found in these mushrooms. Tests were done according to Imaobong, 2020.

4.7.3 Test for Tannins

10 ml of bromine water was added to the 0.5 g aqueous extract. Discoloration of bromine water shows the presence of tannins.

4.7.4 Test for Saponins

5.0 ml of distilled water is mixed with aqueous crude extract in a test tube and mixed vigorously. The frothing is mixed with few drops of olive oil and mixed vigorously and the foam appearance shows the presence of saponins.

4.7.5 Tests for Flavonoids

Alkaline Reagent Test. 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color is produced, which became colorless when drops of diluted acid are added to mixture. This result shows the presence of flavonoids.

4.7.6 Tests for Glycosides

Liebermann's Test. 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous crude extract. The mixture is then cooled and H_2SO_4 concentrated added. Green color showed the entity of aglycone, steroidal part of glycosides.

Keller-Kilianin Test. A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture is mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers which shows the entity of cardiac steroidal glycosides.

4.7.7 Test for Terpenoids

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which shows the entity of terpenoids.

4.7.8 Test for Steroids

2 ml of chloroform and concentrated H_2SO_4 was added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appears that indicates the presence of steroids

4.7.9 Test for glycoside

0.2 g of the test material was extracted with 5 ml of each dilute sulfuric acid and water by boiling/warming on a water bath. Then, the acid extract was filtered and neutralized with 5% solution of sodium hydroxide.

4.8.1 Bioassays

4.8.2 Insect Mortality

To determine the mortality effect of the studied extracts against *M. domestica,* contact effect was evaluated on filter paper discs through treatment of a Whatman No.1, 8 cm diameter, area = 54.4 cm^2) (Wee L, 2000). The filter paper discs were treated with 0.5 mL of extract solutions of the mushroom. In the control treatment, the filter paper disc was treated with an equal volume of the control. The three replicates for each treatment (mushroom extract) from different extractions solutions were tested corresponding to their doses (2, 4, and 6 mg/cm^2). Mushroom extractions were compared with positive control (Mortein®) treatment. Treated and control filter paper halves were air-dried for 1 h for solvent evaporation. Then, 5 flies were released in the filter paper centre, and the lid was then sealed with parafilm. The experiment was run in the dark at 25 ± 2 ◦C. The dead insects were counted at 2 minutes, 5 minutes, 15 minutes, 30 minutes, and 60 minutes post-application. Insects without response to the gentle touch of a small probe were considered dead and data were corrected using Abbott's formula. $Efficacy=(C-T)/C\times100$

Where:

C = Growth control (the number of organisms in the control group without treatment)

T= Test (the number of organisms in the treatment group)

4.8.3 Insect Repellence (Filter Paper Disc Bioassay)

A bioassay system was used according to Wee 2020 to evaluate the activity of the studied extracts using the area preference method. A filter paper disk (Whatman No. 1, 8 cm diameter, area = 54.4 cm^2) was divided into 2 halves, one of which was then treated with 0.5 mL of solutions of the extracts. The control half was treated with an equal volume of control. The three replicates from extracts solutions were tested corresponding to the doses of 0.25, 0.5, and 0.75

mg/cm2 compared with the positive control treatment. The treated and control (positive fluconazole and negative distilled water) filter paper half were air-dried for about 60 min, allowing solvent evaporation. After that, the paper disc was joined and fixed on the bottom of a Petri dish. Five Adult flies were then released in the center of both halves. The experiment was maintained at 25 ± 2 ◦C in a dark place to eliminate any effect from light. Repellence percentage was recorded at 1 minute, 2 minutes, 3 minutes, 5 minutes, and 30 minutes depending on the number of insects noticed on both treated and untreated halves.

Percentage repellence was calculated by the following equation: Repellence (%) = (Nc − Nt)/(Nc + Nt) × 100, (1) where Nc = the number of insects on the untreated half, and Nt = the number of insects on the treated half, after the time exposure.

4.8.4 Evaluation of lethal concentration (LC50)

LC50 is the concentration at which half of the exposed animals die. This was determined by varying the percentage of extract and determining LC50 values.

4.9 Results and Discussion

4.9.1 Qualitative determination of the mushroom bioactive compounds

Table 3 Qualitative analysis of bioactive compounds in *Amanita rhopalopus*

Bioactive compound	**Preliminary screening**
Anthroquinones	Positive
Flavonoids	Positive
Glycosides	Positive
Terpernoids	Positive
Saponins	Positive

Mushroom extracts contain many bioactive compounds such as terpenoids, phenolic compounds, flavonoids, alkaloids, glycoproteins, and steroids that possess anticancer, antibacterial, antiviral and insecticidal properties (Cheung, 2008; Patel et al., 2021). In this study, the bioactive compounds from the preliminary qualitative analysis of *Amanita rhopalopus* are shown in table 1. Terpenes are often responsible for the insecticidal or insect-repellent properties of plants and fungi. Many terpenes have been found to have various insecticidal, repellent, and deterrent effects against a wide range of insect pests. Certain terpenes, such as monoterpenes and sesquiterpenes, can exhibit toxic effects on insects, leading to mortality or disruption of their physiological processes. For example, compounds like limonene, linalool, and carvacrol have been shown to possess insecticidal activity against various pests, including mosquitoes, cockroaches, and stored-product insects.

Many terpenes can act as insect repellents, deterring insects from approaching or landing on the source of the terpenes. Compounds like citronella, eucalyptol, and geraniol are commonly used as natural insect repellents, often found in insect-repelling products.

Certain flavonoids have been found to have toxic effects on various insect pests, leading to mortality or disruption of their normal physiological processes. For example, flavonoids like

quercetin, kaempferol, and luteolin have been shown to have insecticidal activity against insects such as mosquitoes, aphids, and stored-product pests.

Flavonoids can also act as insect repellents, deterring insects from approaching or landing on the source of the flavonoids. The insect-repellent properties of flavonoids are often attributed to their ability to interfere with the insect's sensory perception, making it difficult for them to locate and recognize their target.

Certain flavonoids, such as quercetin and apigenin, have demonstrated insect-repellent activity against mosquitoes, ticks, and other arthropod pests.

Many glycosides are known to have insecticidal properties. Glycosides can exhibit a range of biological activities, including insecticidal, antimicrobial, and anti-feedant effects, depending on the specific chemical structure and the targeted insect species. For example cardenolide glycosides which are a class of steroids containing a sugar moiety, found in various plants. Cardenolide glycosides have been shown to have insecticidal and antifeedant effects against a variety of insect pests, including aphids, caterpillars, and beetles. Digitoxin, a cardenolide glycoside found in Digitalis plants, has been reported to have insecticidal activity against insects such as the cotton bollworm (*Helicoverpa armigera*).

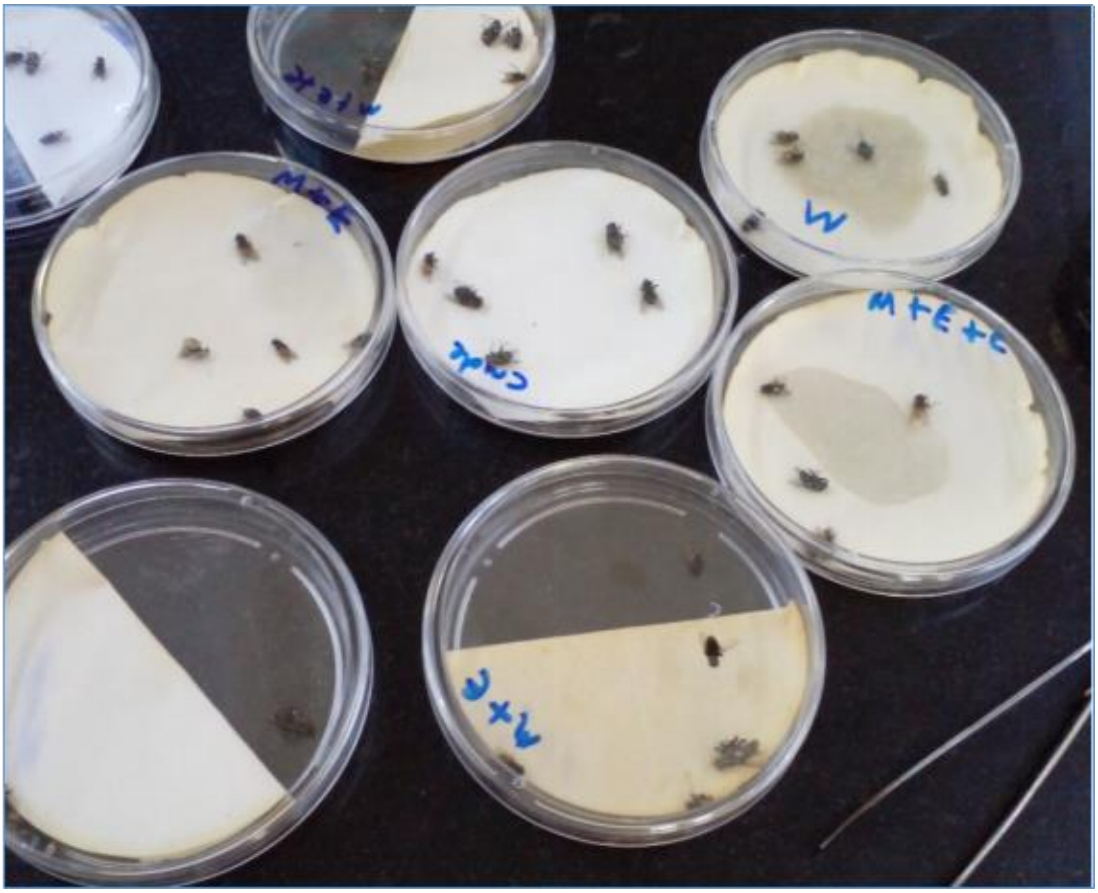
Saponins are a diverse group of glycosides with a soap-like property, characterized by their ability to form stable, foam-like solutions in aqueous systems. Many saponins have been found to possess insecticidal, anti-feedant, and growth-regulating effects against a variety of insect pests. Examples of insecticidal saponins include aescin (from horse chestnut), cucurbitacins (from cucurbitaceous plants), and ginsenosides (from ginseng).

Anthraquinones are a class of aromatic organic compounds containing a quinone structure fused to an aromatic ring. Many anthraquinone-containing plants and fungi have been reported to exhibit insecticidal, antifeedant, and insect growth-regulating properties. Examples of insecticidal anthraquinones include emodin (from rhubarb and other plants) and chrysophanol (from various fungi and plants).

4.9.2 Toxicological tests on *M. domestica*

The housefly, *M. domestica,* a wild-type strain was used as a model organism for assessing the insecticidal activity of *A. rhopalopus* mushroom extracts, the data obtained of which is shown in figure 3 and figure 4 in terms of insect mortality. The negative control results showed that house flies were still alive by day two. It was a significantly long time when compared to the positive control and treatments that showed a significant difference. Hence these results were not highlighted as they only served to prove that the control and the treatment were responsible for the mortality of the flies.

4.9.3 Insect mortality



A

B

Figure 4 A and B: The effect of different mushroom extracts concentrations on insect mortality. NB: C = Positive control of a commercial insect killer (Mortein); M = *A. rhopalopus* Methanol extracts; M+E = *A. rhopalopus* Methanol + ethanol extracts; M+E + *C* = *A. rhopalopus* Methanol + ethanol + chloroform extracts, and *A. rhopalopus* aqueus extracts after 1 hour.

The positive control for the commercial insect killer showed the fastest mortality with flies dying within the first 2 minutes. Extract obtained from the combination of the solvents, methanol, ethanol and chloroform also showed high mortality followed by the combination of methanol and ethanol and then methanol and lastly the aqueous extracts.

Triterpenes isolated from mushrooms like *Ganoderma lucidum* have been shown to exhibit insecticidal and acaricidal (mite-killing) activities. These compounds can disrupt the growth, development, and survival of various insect pests. (e.g., ganoderic acids), optimal extraction solvents: Methanol, ethanol, or chloroform. A study by Hirotani (1986) reported the isolation and structural elucidation of various ganoderic acid triterpenes from *G. lucidum.*

Toxic compounds (e.g., muscimol, ibotenic acid), may be extracted using water, methanol, or acidified aqueous solutions. While these compounds are primarily known for their toxicity to mammals, some may also exhibit insecticidal properties. For instance, muscimol from *Amanita muscaria* mushrooms has been reported to have insecticidal activity against certain crop pests. In 2003 Michelot discusses the primary toxic compounds in *A. muscaria*, such as muscimol and ibotenic acid, and their potential insecticidal activities.

The choice of solvent is critical in extracting bioactive compounds from natural sources like mushrooms. Different solvents possess varying polarities, which influence their ability to solubilize different types of compounds.

Polar solvents like methanol, ethanol, and water are effective in extracting polar compounds such as alkaloids, flavonoids, and glycosides. Non-polar solvents like chloroform are better at extracting non-polar compounds such as terpenes and lipids.

The results indicate that the combination of methanol, ethanol, and chloroform yielded the highest mortality rate, followed by combinations involving methanol and ethanol. This suggests that a combination of polar and non-polar solvents was most effective in extracting compounds with insecticidal properties from the mushroom.

Methanol and ethanol: These polar solvents likely extracted a wide range of polar bioactive compounds, including those responsible for the observed insecticidal activity.

Chloroform: As a non-polar solvent, chloroform probably extracted additional compounds, such as terpenes or lipids, which contributed to the enhanced insecticidal effect when combined with the polar solvents.

In comparison to water extraction the aqueous extract showed lower mortality rates, indicating that water was less efficient in extracting the bioactive compounds responsible for insecticidal activity compared to the organic solvents. This is consistent with the general understanding that water is a less effective solvent for extracting many bioactive compounds from plant and fungal sources.

Numerous studies have demonstrated the superior efficacy of organic solvents over water in extracting bioactive compounds from mushrooms. For instance, research on mushroom polysaccharides has shown that methanol and ethanol are effective in extracting these compounds, while water extraction often yields lower polysaccharide content. Similarly, studies on mushroom terpenoids have reported higher extraction yields using solvents like chloroform and ethyl acetate compared to water.

Negative control results that contained distilled water showed a significant difference when compared to the chemical control and the treatments, indicating that the negative control had no effect on the mortality of the flies.

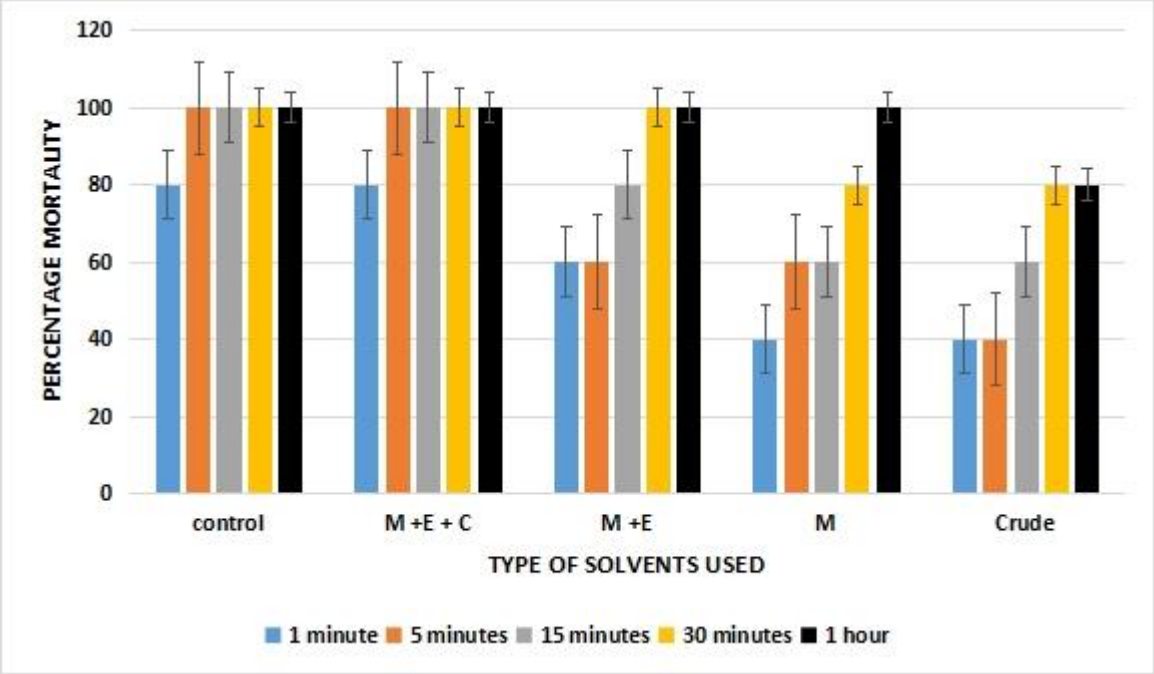


Figure 5 Mortality rate for the *M. domestica* housefly when tested against *A. rhopalopus* mushroom extracts.

NB: C = Positive control of a commercial insect killer; M = *A. rhopalopus* Methanol extracts; M+E = *A. rhopalopus* Methanol + ethanol extracts; M+E + *C* = *A. rhopalopus* Methanol + ethanol + chloroform extracts, and *A. rhopalopus* Aqueous extracts after 1 hour.

4.9.4 Repellence tests on *M*. domestica

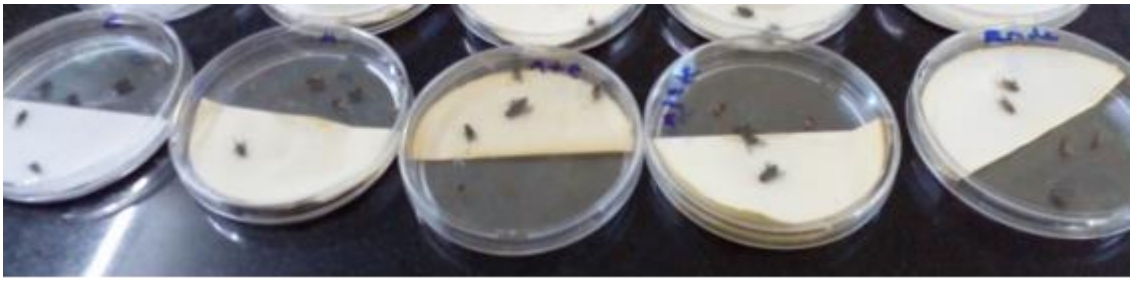


Figure 6 Analysis of the *A. rhopalopus* mushroom extracts as a potential insect repellent.

NB: C = Positive control of a commercial insect killer; M = *A. rhopalopus* Methanol extracts; M+E = *A. rhopalopus* Methanol + ethanol extracts; M+E + *C* = *A. rhopalopus* Methanol + ethanol + chloroform extracts, and *A. rhopalopus* crude extracts after 1 hour.

Further tests were done using the different *A. rhopalopus* mushroom extracts to test their repellent potential on the housefly *M domestica* (Figure 5). Percentage repellence was calculated by the following equation: Repellence (%) = (Nc − Nt)/(Nc + Nt) × 100, (1) where Nc = the number of insects on the untreated half, and Nt = the number of insects on the treated half, after the time exposure. All the flies immediately moved away from the area with the control and the extracts. Some of the flies were found dead at the inoculated areas. Most of the plates showed repellence. However, Crude extracts seemed to be the most repellent.

Methanol and methanol + ethanol + chloroform extracts had the highest repellence of 60%. While the remainder of the extracts had a repellence of 20%.

In one study, The toxicity, antifeedant activity and repellency of the crude methanol extract of the wild mushroom *Amanita muscaria* on *Sitophilus zeamais* in stored maize grains was determined by assessments, carried out between the extracts concentration of 0.05 and 0.5% w/w. Non treated and treated grains with 2% Actellic gold TM 2% dust (0.05% w/w) were used as negative and positive controls, respectively. The extract demonstrated a pest repellency of up to 96.7% after 24 hours of exposure. The findings were promising for use of *A. muscaria* as a biopesticide for maize grains storage towards supporting the ongoing IPM strategies. (Masota 2017)In another study *Chlorophyllum neomastoidea*, poisonous mushroom, was used as an insect repellent according to Ko in 2013.

There are some plants known to repel insects for example garlic (*Allium sativum*) is known for its pungent odor, garlic repels insects and some small animals. Another such plant is mint (*Mentha species*). The strong aroma of mint is disliked by many insects, including mosquitoes and ants (Arunabh, 2019). Citronella (*Cymbopogon nardus*) is a common ingredient in insect repellents, citronella oil effectively repels mosquitoes (Kaur, 2021).

4.9.5 Evaluation of lethal concentration 50 (LC50) of Amanita rhopalopus extracts

The concentrations of the extracts made were 10%, 20%, 40%, 50%, 70% and 80%. The mortality of the insects was noted as in figure 3A by placing flies in petri dishes and noting the lowest concentration that produced death in at least 50% of the flies for the LC50. The results obtained are highlighted in table 2 below.

Table 2 Evaluation of LC50 for the *Amanita rhopalopus* extracts.

Solvent	LC50 Concentration
Control	40
Methanol + Ethanol + chloroform	40
Methanol + Ethanol	70
Methanol	70
Aqueous	80

Control (LC50 = 40) represents the baseline LC50 value for the untreated or control condition.

Methanol + Ethanol + Chloroform (LC50 = 40). The LC50 value of 40 for this mixed solvent extract is the same as the control, suggesting it has a similar level of toxicity or lethality.

Methanol + Ethanol (LC50 = 70), the higher LC50 value of 70 for this mixed solvent extract indicates it has a lower level of toxicity compared to the control and the methanol + ethanol + chloroform extract.

Methanol (LC50 = 70), the methanol-only extract also has an LC50 of 70, the same as the methanol + ethanol extract. This suggests the methanol component is the primary contributor to the reduced toxicity compared to the control.

Aqueous (LC50 = 80), the aqueous extract has the highest LC50 value of 80, indicating it is the least toxic or lethal of the tested extracts.

The mixed solvent extracts (methanol + ethanol + chloroform, and methanol + ethanol) have higher LC50 values, meaning they are less toxic or lethal compared to the control. The methanol-only extract has a similar LC50 to the methanol + ethanol mixture, suggesting the methanol component is the main driver of the reduced toxicity. The aqueous extract has the highest LC50, making it the least toxic or lethal of the tested extracts.

These findings indicate that the composition and choice of solvent can significantly impact the toxicity or lethal potential of the resulting extracts. The higher LC50 values suggest the mixed solvent and aqueous extracts may be less potent or detrimental than the control, at least in the context of the specific test system or organism being studied.

4. 9.6 Conclusion

The mushroom *Amanita ropalopus* proved to be effective in killing flies as well as repel them to an extent as shown in Figures 1 to 5 and tables 1-2 when compared with the positive control, which is an already established commercial pesticide. The lethal concentration in the dry mushrooms was on average slightly high and ranged from 40%, in the best performing extract, to 80%, in the crude extract, showing that as more compounds were extracted the mushroom performed better. Thus *A. ropalopus* has insecticidal properties with the potential to be applied in various biotechnological applications.

4.9.7 Recommendations

Due to limitation in the laboratory not all bioactive compounds were qualitatively screened but some were found to be present in the mushroom. There is need to collect more mushroom samples, conduct qualitative and quantitative analysis of the bioactive compounds and produce an insecticidal prototype, as lack of enough samples hindered this step. The biochemicals responsible for the mortality and repellence need to be positively identified and isolated for use in the production of an insecticide. Other parts of the mushroom maybe explored such as mycelia

and spores. The mushroom maybe tested against other pests such as cockroaches. The mode of action of these bioactive compounds also needs to be evaluated and established as well as the possible side effects should a commercial biopesticide be produced. Fresh mushroom samples should be obtained for ease of identification and to further evaluate the mushroom's insecticidal properties.

CHAPTER 5

Synthesis

Mushrooms are known to produce a wide range of bioactive compounds, some of which possess antifungal, insecticidal, and pesticidal properties. Despite their potential, these compounds remain largely underutilized, particularly in the Zimbabwean context. The present study sought to evaluate the antifungal and insecticidal properties of selected indigenous mushroom species to explore their potential applications in medicine and agriculture.

The first experiment assessed the antifungal activity of five indigenous mushroom species against *Candida albicans*. Using the agar disc diffusion method, the study revealed that *Cantharellus symoensii* exhibited the most potent antifungal activity, with a 16.6±0.4 mm inhibition zone observed in methanolic extracts. Other mushrooms, including *Russula sp., Amanita rhopalopus, Boletus* sp., and the edible *Cantharellus miomboensis*, demonstrated varying degrees of anti-*Candida* activity. Notably, aqueous extracts exhibited no antifungal activity, whereas ethanol and chloroform were found to be the most effective solvents for extracting antifungal compounds from *Cantharellus symoensii* and *Cantharellus miomboensis*, respectively.

These findings align with previous studies that have demonstrated the antifungal properties of mushrooms. Research by Wasser, 2010 highlights that secondary metabolites produced by mushrooms, including phenolic compounds, terpenoids, and polysaccharides, exhibit antifungal activities. Similarly, studies on *Ganoderma lucidum* and *Lentinula edodes* have reported strong inhibitory effects on pathogenic fungi, including Candida species (Yuan, 2018). The effectiveness of ethanol and chloroform extractions in this study is consistent with findings from Rahi, 2021, who noted that non-polar solvents often extract higher concentrations of bioactive compounds compared to aqueous solutions.

The second experiment investigated the insecticidal effects of *Amanita rhopalopus* against the common house fly (*Musca domestica*). Various solvent extracts of the mushroom were tested, and results indicated that the most potent extract induced 100% mortality within five minutes, comparable to commercial insecticides. The lethal concentration ranged from 40% in the most

effective extract to 80% in the aqueous extract, demonstrating that extraction efficiency directly impacts bioactivity.

The insecticidal properties of mushrooms have been documented in multiple studies. For instance, *Amanita* species, including *Amanita muscaria* and *Amanita pantherina*, have been reported to produce potent neurotoxins that can impact insects and small vertebrates (Benjamin, 2012). Furthermore, previous research by Zimmermann, 2016 demonstrated that mycotoxins from *Lepista* and *Cantharellus* species can effectively inhibit insect growth and development, similar to the findings observed in this study.

Several hypotheses have been proposed regarding the insecticidal mechanisms of mushrooms. Some researchers suggest that the production of toxic metabolites serves as a defense mechanism against insects. Studies on *Beauveria bassiana*, an entomopathogenic fungus, have revealed its effectiveness in controlling *M. domestica* populations (Sharma, 2020). The results from this study suggest that *Amanita rhopalopus* may act similarly, potentially providing an alternative to synthetic chemical insecticides.

The results of this study contribute to the growing body of evidence supporting the use of mushrooms as natural antifungal and insecticidal agents. The antifungal properties of *Cantharellus symoensii* and *Cantharellus miomboensis* suggest their potential in developing natural antifungal medications. Given the increasing resistance of *Candida albicans* to conventional antifungal drugs (Patterson, 2017), exploring alternative sources of antifungal compounds is crucial.

Similarly, the insecticidal properties of *Amanita rhopalopus* highlight its potential as a natural pesticide. Synthetic insecticides are associated with environmental pollution and insect resistance, making biological alternatives an attractive option (Isman, 2020). Mycopesticides, which utilize fungal spores and secondary metabolites to target insect pests, are gaining interest as sustainable pest management solutions (Lovett, 2017). The effectiveness of *Amanita rhopalopus* in this study suggests that indigenous mushrooms could be developed into eco-friendly pest control solutions suitable for Zimbabwean agriculture.

The findings of this study open several avenues for further research. Future studies could focus on isolating and characterizing the specific bioactive compounds responsible for antifungal and insecticidal activities. Additionally, expanding the scope to test these mushrooms against other fungal pathogens and agricultural pests, such as mosquitoes, cockroaches, and crop-damaging insects, could further validate their utility.

Moreover, this research highlights the importance of conserving Zimbabwe's fungal biodiversity. Many mushroom species remain undocumented, and their bioactive properties are largely unexplored. Establishing a database of medicinal and pesticidal mushrooms from Zimbabwe could contribute to pharmaceutical and agricultural innovations, benefiting both local and global communities.

This study demonstrates that Zimbabwean mushrooms possess significant antifungal and insecticidal properties. The antifungal activity of *Cantharellus symoensii* and *Cantharellus miomboensis* against *Candida albicans* and the potent insecticidal effects of *Amanita rhopalopus* against *Musca domestica* suggest that indigenous mushrooms could serve as valuable sources of natural antifungal agents and mycopesticides. These findings align with existing literature on fungal bioactive compounds and underscore the potential of mushrooms as alternatives to synthetic pharmaceuticals and pesticides. Further exploration of Zimbabwe’s mycological resources could lead to novel biotechnological applications, enhancing both healthcare and sustainable agriculture.

Appendix

Multiple Comparisons

Dependent Variable: Dead Flies

Tukey HSD

Table 4 Turkey HSD values

(I) EXTRACTS	(J) EXTRACTS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.33333	.42164	.928	-1.0543	1.7210
	3.00	.66667	.42164	.539	-.7210	2.0543
	4.00	2.33333*	.42164	.002	.9457	3.7210
	5.00	2.33333*	.42164	.002	.9457	3.7210
2.00	1.00	-.33333	.42164	.928	-1.7210	1.0543
	3.00	.33333	.42164	.928	-1.0543	1.7210
	4.00	2.00000*	.42164	.005	.6124	3.3876
	5.00	2.00000*	.42164	.005	.6124	3.3876
3.00	1.00	-.66667	.42164	.539	-2.0543	.7210
	2.00	-.33333	.42164	.928	-1.7210	1.0543
	4.00	1.66667*	.42164	.018	.2790	3.0543
	5.00	1.66667*	.42164	.018	.2790	3.0543
4.00	1.00	-2.33333*	.42164	.002	-3.7210	-.9457
	2.00	-2.00000*	.42164	.005	-3.3876	-.6124

	3.00	-1.66667*	.42164	.018	-3.0543	-.2790
	5.00	.00000	.42164	1.000	-1.3876	1.3876
	1.00	-2.33333*	.42164	.002	-3.7210	-.9457
	2.00	-2.00000*	.42164	.005	-3.3876	-.6124
5.00	3.00	-1.66667*	.42164	.018	-3.0543	-.2790
	4.00	.00000	.42164	1.000	-1.3876	1.3876

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Dependent Variable: Dead Flies

Tukey HSD

Table 4

(I) EXTRACTS	(J) EXTRACTS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	2.00	.33333	.51640	.964	-1.3662	2.0328
	3.00	1.66667	.51640	.055	-.0328	3.3662
1.00	4.00	2.00000*	.51640	.020	.3005	3.6995
	5.00	2.66667*	.51640	.003	.9672	4.3662
	1.00	-.33333	.51640	.964	-2.0328	1.3662
2.00	3.00	1.33333	.51640	.148	-.3662	3.0328

	4.00	1.66667	.51640	.055	-.0328	3.3662
	5.00	2.33333*	.51640	.008	.6338	4.0328
	1.00	-1.66667	.51640	.055	-3.3662	.0328
	2.00	-1.33333	.51640	.148	-3.0328	.3662
3.00	4.00	.33333	.51640	.964	-1.3662	2.0328
	5.00	1.00000	.51640	.359	-.6995	2.6995
	1.00	-2.00000*	.51640	.020	-3.6995	-.3005
	2.00	-1.66667	.51640	.055	-3.3662	.0328
4.00	3.00	-.33333	.51640	.964	-2.0328	1.3662
	5.00	.66667	.51640	.702	-1.0328	2.3662
	1.00	-2.66667*	.51640	.003	-4.3662	-.9672
	2.00	-2.33333*	.51640	.008	-4.0328	-.6338
5.00	3.00	-1.00000	.51640	.359	-2.6995	.6995
	4.00	-.66667	.51640	.702	-2.3662	1.0328

*. The mean difference is significant at the 0.05 level.

Appendix

Table 5: Zones of inhibition of mushroom different extracts.

Extracts of mushrooms	Strain inhibition zone diameter (mm)		
	Candida albicans ATCC 66027		
	Replicate 1	Replicate 2	Replicate 3
Cantharellus symoensii			
Methanol	17	17	16
Chloroform	16	15	16
Ethanol	14	14	14
Hot water	0	0	0
C. miomboensis			
Methanol	16	15	15
Chloroform	17	16	16
Ethanol	14	14	15
Hot water	0	0	0
Amanita rhopalopus / kuneguva			
Methanol	15	16	14
Chloroform	13	14	13
Ethanol	14	14	14
Hot water	0	0	0
Boletus sp			
Methanol	15	15	15
Chloroform	14	13	16
Ethanol	15	13	14
Hot water	0	0	0
Russula sp			
Methanol	14	14	14
Chloroform	14	13	14
Ethanol	13	15	12

Hot water	0	0	0
Control antifungal			
fluconazole	14	14	14

References

1. Abass, J. K. (2016). Exploitation of Nematicidal Potential of Paecilomyces lilacinus against Root Knot Nematode on Eggplant. *Science*, 85-90.
2. Alves, M.J., Ferreira, I.C., Dias, J., Teixeira, V., Martins, A. and Pintado, M., 2012. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta medica, 78(16), pp.1707-1718.
3. Anderson, M. (2020, November 21). *School Integrated Pest Management*. Retrieved from Texas A&M Agrilife Extension: https://schoolipm.tamu.edu/2020/11/21/the-fungus-among-us-restoring-ecosystems-and-controlling-pests/
4. Anh, N.T.H., Cuong, T.D., Dat, N.T. (2020). Bioactive Compounds from Rhopalopus Mushrooms: Chemistry and Bioactivities. Molecules, 25(13), 3031.
5. Aram D. Stump, S. E. (2011). Distribution and Mechanism of α-Amanitin Tolerance in Mycophagous Drosophila (Diptera: Drosophilidae). *Environmental Entomology*, 1604-1612.
6. Ardigo, S., Herrmann, F.R., Moret, V., Déramé, L., Giannelli, S., Gold, G. and Pautex, S., 2016. Hypnosis can reduce pain in hospitalized older patients: a randomized controlled study. BMC geriatrics, 16(1), pp.1-8.
7. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant Candida: Epidemiology, molecular mechanisms, and treatment. Journal of Infectious Diseases, 216(S3), S445-S451.
8. Arunabh Agnihotri, S. Wazed Ali, Apurba Das, R. Alagirusamy, 11 - Insect-repellent textiles using green and sustainable approaches, 2019, Pages 307-325,
9. Ayaz M, Li CH, Ali Q, Zhao W, Chi YK, Shafiq M, Ali F, Yu XY, Yu Q, Zhao JT, Yu JW, Qi RD, Huang WK. Bacterial and Fungal Biocontrol Agents for Plant Disease Protection: Journey from Lab to Field, Current Status, Challenges, and Global Perspectives. Molecules. 2023
10. Ayilara MS, Adeleke BS, Akinola SA, Fayose CA, Adeyemi UT, Gbadegesin LA, Omole RK, Johnson RM, Uthman QO, Babalola OO. Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. Front Microbiol. 2023

11. Bae, K., et al. (2018). "Antitumor and immunomodulatory activities of mushroom polysaccharides and their derivatives." Frontiers in Pharmacology, 9, 1-10.

12. Bahrndorff S, de Jonge N, Skovgård H, Nielsen JL. Bacterial Communities Associated with Houseflies (Musca domestica L.) Sampled within and between Farms. PLoS ONE. 2017;12(1):e0169753. doi: 10.1371/journal.pone.0169753.

13. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M., 2008. Biological effects of essential oils–a review. Food and chemical toxicology, 46(2), pp.446-475.

14. Barchiesi, F., et al. (2011). "Amphotericin B: An old drug with a new future." Expert Review of Anti-Infective Therapy, 9(11), 1137-1150.

15. Barchiesi, F., et al. (2011). "Amphotericin B: An old drug with a new future." Expert Review of Anti-Infective Therapy, 9(11), 1137-1150.

16. Barros, L., Cruz, T., Baptista, P., Estevinho, L.M. and Ferreira, I.C., 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food and Chemical Toxicology, 46(8), pp.2742-2747.

17. Barros, L.; Cruz, T.; Baptista, P.; Estevinho, L.M.; Ferreira, I.C.F.R. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem. Toxicol. 2008, 46, 2742–2747.

18. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). "Antibiotic susceptibility testing by a standardized single disk method." American Journal of Clinical Pathology, 45(4), 493-496.

19. Benjamin, D. R. (2012). Mushrooms: Poisons and panaceas: A handbook for naturalists, mycologists, and physicians. WH Freeman & Co.

20. Bennet, L. W. (2003). Survival of the biocontrol agents Coniothyrium minitans and Bacillus subtilis MBI 600 introduced into pasteurised, sterilised and non-sterile soils. *Soil biology*, 1565-1573.

21. Bennett, J. E., et al. (2010). "Flucytosine: A review of its use in the treatment of fungal infections." Clinical Infectious Diseases, 50(11), 1640-1647.

22. BESL, H. K. (1987). The effect of fungal fruitbodies on larvae of Drosophila. *PubMed*, 273–285.

23. Biswas, S., Van Dijck, P. and Datta, A., 2007. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of Candida albicans. Microbiology and Molecular Biology Reviews, 71(2), pp.348-376.

24. Buruleanu, L.C., Radulescu, C., Georgescu, A.A., Danet, F.A., Olteanu, R.L., Nicolescu, C.M. and Dulama, I.D., 2018. Statistical characterization of the phytochemical characteristics of edible mushroom extracts. Analytical Letters, 51(7), pp.1039-1059.

25. C J Geden, D Nayduch, J G Scott, E R Burgess, A C Gerry, P E Kaufman, J Thomson, V Pickens, E T Machtinger, House Fly (Diptera: Muscidae): Biology, Pest Status, Current Management Prospects, and Research Needs, Journal of Integrated Pest Management, Volume 12, Issue 1, 2021, 39,

26. Calvacante, L. P. (2008). Effect of moisture on Trichoderma conidia production on corn and wheat bran by solid state fermentation. *Food Bioprocess,* 100-104.

27. Cha, M.H., Heo, J.Y., Lee, C., Lo, Y.M. and Moon, B., 2014. Quality and Sensory Characterization of White Jelly Mushroom (T remella fuciformis) as a Meat Substitute in Pork Patty Formulation. Journal of Food Processing and Preservation, 38(4), pp.2014-2019.

28. Cha, M.H., Heo, J.Y., Lee, C., Lo, Y.M. and Moon, B., 2014. Quality and Sensory Characterization of White Jelly Mushroom (T remella fuciformis) as a Meat Substitute in Pork Patty Formulation. Journal of Food Processing and Preservation, 38(4), pp.2014-2019.

29. Chang, S.T. and Miles, P.G., 2004. Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC press.

30. Chaudhary, R. and Tripathy, A., 2015. Isolation and identification of bioactive compounds from Irpex Lacteus Wild Fleshy Fungi. Journal of Pharmaceutical Sciences and Research, 7(7), p.424.

31. Cheung, P.C., 2013. Mini-review on edible mushrooms as source of dietary fiber: Preparation and health benefits. Food Science and Human Wellness, 2(3-4), pp.162-166.

32. Chikuni, A. (2019). "Mushroom poisonings in the Harare area: A case study." Journal of Environmental and Public Health.

This study reports on incidents of mushroom poisoning, analyzing the types of mushrooms involved and the clinical outcomes.

33. Chomcheon, P., Kheawkum, B., Sriwiset, P., Dulsamphan, S. and Dulsamphan, C., 2013. Antibacterial activity of crude extracts from edible mushrooms Pleurotus citrinopileatus and Tricholoma crassum Berk. Thai J. Pharm. Sci, 37, pp.107-111.

34. Chowdhury, S.P., Hartmann, A., Gao, X. and Borriss, R., 2015. Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42–a review. Frontiers in microbiology, 6, p.780.

35. Chu, K.T.; Xia, L.; Ng, T.B. Pleurostrin, an antifungal peptide from the oyster mushroom. Peptides, 2005, 26, 2098–2103.

36. Crowthar, T. (2017). Gladiator Games: In Nature's Showdowns, Biodiversity Shields Weaker Competitors. *Nature Ecology and Evolution.*

37. Dodd, L. S. (2013). Hypocrea atroviridis sp. nov., the telemorph of Trichoderma atroviridae. *Mycologia*, 27-40.

38. Duffy, S.K., O'Doherty, J.V., Rajauria, G., Clarke, L.C., Hayes, A., Dowling, K.G., O'Grady, M.N., Kerry, J.P., Jakobsen, J., Cashman, K.D. and Kelly, A.K., 2018. Vitamin D-biofortified beef: A comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source. Food chemistry, 256, pp.18-24.

39. Finimundy, T. C., G. Gambato, R. Fontana, M. Camassola, M. Salvador, S. Moura, J. Hess, J. A. P. Henriques, A. J. P. Dillon, and M. Roesch-Ely. "Aqueous extracts of Lentinula edodes and Pleurotus sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity." Nutrition Research 33, no. 1 (2013): 76-84.

40. Friedman, M. (2016). "Nutritional and health benefits of mushrooms." Nutrition Reviews, 74(8), 495-514

41. Friedman, M., et al. (2016). "Nutritional and health benefits of mushrooms." Nutrition Reviews, 74(8), 495-514.

42. Gabbatiss, J. (2017). Magic mushroom chemical may be a hallucinogenic insect repellent. *Newscientist*.

43. Garcia-Garcia, P. R. (2015). Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. *Life Science*, 274-283.

44. Gartz, J. (1994). "Mushrooms as insect repellents: Psilocybin mushrooms and their potential use." Mushroom Research, 3(1), 45-50.

45. Gayane S. Barseghyan, Avner Barazani, Solomon P. Wasser, Chapter 8 - Medicinal Mushrooms with Anti-Phytopathogenic and Insecticidal Properties, Editor(s): Marian Petre, Mushroom Biotechnology, Academic Press, 2016,
46. Gbolagade, J.S. and Fasidi, I.O., 2005. Antimicrobial activities of some selected Nigerian mushrooms. African Journal of Biomedical Research, 8(2), pp.83-87.
47. Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Investigation of Antioxidant and Antimicrobial Activities of Different Extracts of Auricularia and Termitomyces Species of Mushrooms. ScientificWorldJournal. 2019
48. Gilardoni, G.; Clericuzio, M.; Tosi, S.; Zanoni, G.; Vidari, G. Antifungal acylcyclopentenediones from fruiting bodies of Hygrophorus chrysodon. J. Nat. Prod., 2007, 70, 137-139.
49. Goettel, M. S., & Inglis, G. D. (1997). "Fungi: Hyphomycetes." In: Biological Control of Insect Pests Using Entomopathogenic Fungi. Biological Control: A Global Perspective, 1st ed. CABI Publishing.
50. Guichard, D. J. (2017). Le plan Ecophyto de réduction d'usage des pesticides en France: décryptage d'un échec et raisons d'espérer. *Agric*.
51. Hajdu, Z., et al. (2012). "Antifungal activity of Inonotus obliquus against pathogenic fungi." Phytotherapy Research, 26(8), 1190-1194.
52. Hajloui. (1992). Cytochemical analysis of the antagonistic activity of Sporothrix floculosa on rose powdery mildew, Sphaerotheca pannosa. *Phytopathology*, 583-589.
53. Hanel H, J. W. (1983). Preliminary field tests on the use of Metarhizum anisopliae for the control of Nasutitermes exitosus. *pctonline*, 305-313.
54. Harman, G. E., et al. (2004). "Overview of mechanisms and uses of Trichoderma species." Phytopathology, 94(11), 243-258.
55. Heleno, S.A.; Ferreira, I.C.F.R.; Esteves, A.P.; Ciric, A.; Glamoclija, J.; Martins, A.; Sokovic, M.; Queiroz, M.J.R.P. Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. Food Chem. Toxicol., 2013, doi: 10.1016/j.fct.2013.04.025.
56. Hirotani, M., Ino, C., Furuya, T., & Shiro, M. (1986). Ganoderic acid derivatives from the cultured mycelia of Ganoderma lucidum. Phytochemistry, 25(8), 1909-1912.

57. Hofmann, A., & Schmid, H. (1973). "Psilocybin and its analogues: Effects on the central nervous system." Psychopharmacologia, 33(4), 351-358.
58. Hossain, M. S., et al. (2019). "Larvicidal activity of Ganoderma lucidum against Drosophila melanogaster." Journal of Insect Science, 19(3), 1-7.
59. Hubbard, H. E. (2014). The biochemistry behind biopesticide efficiency. *Sustain*, 1-8.
60. Imaobong E Daniel, Ekemini I. Akpan, Edidiong C. Utam, Department of Chemistry, University of UYO, PMB 1017, UYO, Akwa Ibom state, Nigeria.
61. Isman, M. B. (2020). Botanical insecticides in the twenty-first century—fulfilling their promise? Annual Review of Entomology, 65, 233-249.
62. J, C. (2017). Global markets for biopesticides. *BCC Res*.
63. Jaenike, J. G. (1983). α-Amanitin tolerance in mycophagous Drosophila. *Science*, 165-166.
64. Jiang Y, Wang J. The Registration Situation and Use of Mycopesticides in the World. J Fungi (Basel). 2023
65. Kaewchi. Soytong, H. (2009). Mycofungicides and fungal biofertilizers. *Mycopathologia*, 81-89.
66. Kalyoncu, F.; Oskay, M.; Sağlam, H.; Erdoğan, T.F.; Tamer, A.Ü. Antimicrobial and antioxidant activities of mycelia of 10 wild mushroom species. J. Med. Food, 2010, 13, 415-419.
67. Kamaraj, C., Rahuman, A. A., Bagavan, A., Elango, G., Zahir, A. A., & Santhoshkumar, T. (2015). Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. Parasitology Research, 114(5), 1695-1705.
68. Kathiravan, M.K.; Salake, A.B.; Chothe, A.S.; Dudhe, P.B.; Watode, R.P.; Mukta, M.S.; Gadhwe, S. The biology and chemistry of antifungal agents: A review. Bioorg. Med. Chem., 2012, 20, 5678–5698.
69. Kaur, H., Bhardwaj, U., & Kaur, R. (2021). Cymbopogon nardus essential oil: a comprehensive review on its chemistry and bioactivity. Journal of Essential Oil Research, 33(3), 205–220.
70. Khan, M. I., et al. (2021). "Natural antifungal compounds from fungi: A review." Mycology, 12(1), 1-20.

71. Ko, Pyung Yeol, Kang, Shin-Ho, Song, Gwanpill, & Jeun, Yong Chull. (2013). Traditional Knowledge on Wild Mushrooms in the Surrounding Villages Hallyeo-Haesang National Park. The Korean Journal of Mycology, 41(2), 127–131.
72. Koh, H. C., et al. (2017). "Echinocandin resistance in Candida." Mycoses, 60(4), 239-246.

73. Kozak, T. K., & Chowdhury, A. (2021). "Emerging Drug Resistance in Candida: The Need for Novel Antifungal Agents." Journal of Antimicrobial Chemotherapy, 76(5), 1135-1142.
74. Kullberg, B. J., & Arendrup, M. C. (2015). "Invasive candidiasis." New England Journal of Medicine, 373(15), 1445-1456.
75. Kumar, S., et al. (2020). "Antiviral activity of Ganoderma lucidum against influenza virus." Journal of Medicinal Food, 23(1), 1-8.

76. Kumar, V., et al. (2021). "Volatile compounds from Lentinula edodes exhibit insecticidal activity." Journal of Agricultural and Food Chemistry, 69(5), 1452-1460.
77. Laur. (2018). Effectors involved in fungal–fungal interaction lead to a rare phenomenon of hyperbiotrophy in the tritrophic system biocontrol agent–powdery mildew–plant. *New Phytol*, 713-725.
78. Leahy J, M. M. (2014). Biopesticide oversight and registration at the U.S. Environmental Protection Agency. *ACS Symposium Series*, 3-18.
79. Lee, I.K., Yun, B.S., Cho, Y.S., Kang, H.W., Yu, S.H., Seo, G.S., Whang, W.K., Yoo, I.D. (2015). Antifungal Activities of Rhopalopilins A and B from the Mushroom Rhopalopus japonicus. Journal of Food Science and Technology, 52(8), 5188–5194.
80. Li, Y., et al. (2018). "Antiviral activity of polysaccharides from Lentinula edodes against herpes simplex virus." Journal of Ethnopharmacology, 217, 263-270.
81. Lindequist, U.; Niedermeyer, T.H.J.; Jülich, W.-D. The pharmacological potential of mushrooms. eCAM, 2005, 2, 285-299
82. Lindequist, U.; Niedermeyer, T.H.J.; Jülich, W.-D. The pharmacological potential of mushrooms. eCAM, 2005, 2, 285-299.
83. Lovett, B., & St. Leger, R. J. (2017). The insect pathogens. Microbiology Spectrum, 5(5), 1-17.

84. Luo, D.-Q.; Wang, F.; Bian, X.-Y.; Liu, J.-K. Rufuslactone, a new antifungal sesquiterpene from the fruiting bodies of the Basidiomycete Lactarius rufus. J. Antibiotics, 2005, 58, 456-459.
85. Maiken Cavling Arendrup, Thomas F Patterson, Multidrug-Resistant Candida: Epidemiology, Molecular Mechanisms, and Treatment, The Journal of Infectious Diseases, Volume 216, Issue suppl_3, 15 August 2017, Pages S445–S451
86. Malik, Anushree & Singh, Neena & Satya, Santosh. (2007). House fly (Musca domestica): A review of control strategies for a challenging pest. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes.
87. Manzi, P.; Gambelli, L.; Marconi, S.; Vivanti, V.; Pizzoferrato, L. Nutrients in edible mushroom: An inter-species comparative study. Food Chem., 1999, 65, 477–482.
88. Maribeng, R., 2011. Screening, isolation and characterization of lectins extracted from mushrooms indegenous to Southern Africa.
89. Marr, K. A., et al. (2015). "Isavuconazole: A new broad-spectrum antifungal agent." Clinical Infectious Diseases, 61(2), 254-257.
90. Marr, K. A., et al. (2015). "Isavuconazole: A new broad-spectrum antifungal agent." Clinical Infectious Diseases, 61(2), 254-257.

91. Masota, Nelson E. and Mihale, M. J. and Sempombe, Joseph and Henry, Leonia and Mugoyela, Veronica and Sung'hwa, Fortunatus (2017) Pesticidal Activity of Wild Mushroom *Amanita muscaria* (L) Extracts against Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) in Stored Maize Grains. Journal of Food Security, 5 (2). pp. 26-32.
92. Mateja Lumpert, S. K. (2016). Catching flies with *Amanita Muscaria*: traditional recipes from Slovenia and their efficacy in the extraction of ibotenic acid. *PMID*.
93. Maunder, A. B., et al. (2010). "Chanterelle mushrooms: A culinary treat." Mushroom Science, 22(1), 71-76.
94. Michelot, D., & Melendez-Howell, L. M. (2003). Amanita muscaria: chemistry, biology, toxicology, and ethnomycology. Mycological Research, 107(2), 131-146.
95. Ming Wang, V. T. (2002). Proteins as Active Compounds Involved in Insecticidal Activity of Mushroom Fruitbodies. *Journal of Economic Entomology*, 603-607.

96. Mitchell, S. M. (2010). Volatile antimicrobials from Muscodor crispans, a novel endophytic fungus. 270-277.

97. Mondello, L. (2018). Management of grapevine trunk diseases: knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathol*, 369-383.

98. Moukha, S., Férandon, C., Mobio, T. and Creppy, E.E., 2011, October. Safety evaluation of Agaricus subrufescens varieties and their products of therarpeutic interest or for disease prevention. In 7. International Conference on Mushroom Biology and Mushroom Products. INRA.

99. Mushroom, the delicacy that can be lethal | The ManicaPost

100. Nassiri H, Zarrin M, Veys-Behbahani R, Faramarzi S, Nasiri A. Isolation and identification of pathogenic filamentous fungi and yeasts from adult house fly (Diptera: Muscidae) captured from the hospital environments in Alivaz city, Southwestern Iran. J Med Entomol. 2015;52(6):1351–1356. doi: 10.1093/jme/tjv140.

101. Ngai, P.H.K.; Ng, T.B. A ribonuclease with antimicrobial, antimitogenic and antiproliferative activities from the edible mushroom Pleurotus sajor-caju. Peptides, 2004, 25, 11–17

102. Nicot. (2011). Classical and augmentative biological control against diseases and pests: critical status analysis and review of factors. *First Ed*.

103. Nobuko Tuno, K. H. (2007). Tolerance of Drosophila Flies to Ibotenic Acid Poisons in Mushrooms. *Journal of Chemical Ecology*, 311-317.

104. Omran Zaki, F. W. (2020). Limiting factors of mycopesticide development. *Elsevier*.

105. Öztürk, M.; Duru, M.E.; Kivrak, S.; Mercan-Doğan, N.; Türkoglu, A.; Özler, M.A. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. Food Chem. Toxicol., 2011, 49, 1353–1360.

106. P, L. (n.d.). Global markets for biopesticides. *BCC Res*.

107. Pappas, P. G., et al. (2009). "Invasive candidiasis: A global perspective." Nature Reviews Microbiology, 7(12), 789-799.

108. Pappas, P. G., et al. (2009). "Invasive candidiasis: A global perspective." Nature Reviews Microbiology, 7(12), 789-799.

109. Pfister, J.R. Isolation and bioactivity of 2-aminoquinoline from Leucopaxillus albissimus. J. Nat. Prod., 1998, 51, 969-970. [31] Wang, H.; Ng, T.B. Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom Ganoderma lucidum. Peptides, 2006, 27, 27-30.

110. Pinna, S.; Gévry, M-F.; Côté, M.; Sirois, L. Factors influencing fructification phenology of edible mushrooms in a boreal mixed forest of Eastern Canada. For. Ecol. Manag., 2010, 260, 294-301.

111. Postma J, M. M. (2003). Microbial enrichment to enhance the disease suppressive activity of compost. *Soil Biology*, 157-163.

112. Poucheret, P.; Fons, F.; Rapior, S. Biological and pharmacological activity of higher 477 fungi: 20-Year retrospective analysis. Mycologie, 2006, 27, 311-333.

113. Poucheret, P.; Fons, F.; Rapior, S. Biological and pharmacological activity of higher 477 fungi: 20-Year retrospective analysis. Mycologie, 2006, 27, 311-333.

114. Prasad, S., Rathore, H., Sharma, S. and Yadav, A.S., 2015. Medicinal mushrooms as a source of novel functional food. Int J Food Sci Nutr Diet, 4(5), pp.221-225.

115. Quang, D.N.; Bach, D.D.; Hashimoto, T.; Asakawa, Y. Chemical constituents of the Vietnamese inedible mushroom Xylaria intracolorata. Nat. Prod. Res., 2006, 20, 317-321.

116. Rahi, D. K., Malik, D., & Singh, S. (2021). Medicinal mushrooms: Potential candidates as antibacterial and antifungal agents. 3 Biotech, 11(2), 49.

117. Rajesh, R. A. (2016). Trichoderma: a significant fungus for agriculture and environment. *Agric*.

118. Rajkumar, S. B. (2018). *Mycopesticides: Fungal Based Pesticides for Sustainable Agriculture.* Springer, Singapore.

119. Ramesh, C.H. and Pattar, M.G., 2010. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India. Pharmacognosy research, 2(2), p.107.

120. Riba, M., et al. (2020). "Differential effects of ibotenic acid and muscimol on survival and reproduction in mycophagous and non-mycophagous Drosophila." Ecological Entomology,

45(3), 483-493.Goettel, M. S., & Inglis, G. D. (1997). "Fungi: Hyphomycetes." In: Biological Control of Insect Pests Using Entomopathogenic Fungi. Biological Control: A Global Perspective, 1st ed. CABI Publishing.

121. Richardson, M. and Lass-Flörl, C., 2008. Changing epidemiology of systemic fungal infections. Clinical microbiology and infection, 14, pp.5-24.

122. Rosa, L.H.; Machado, K.M.G.; Jacob, C.C.; Capelari, M.; Rosa, C.A.; Zani, C.L. Screening of Brazilian Basidiomycetes for antimicrobial activity. Memórias do Instituto Oswaldo Cruz, 2003, 98, 967-974.

123. Royse, D.J.; Sanchez-Vazquez, J.E. Influence of precipitated calcium carbonate (CaCO3) on shiitake (Lentinula edodes) yield and mushroom size.Biores. Technol., 2003, 90, 225-228.

124. Rukuni, A., & Muposhi, V. (2018). "Mushroom poisoning: A public health concern in Zimbabwe." Zimbabwe Medical Journal, 56(2), 25-28.
This paper discusses specific cases of mushroom poisoning, the demographics of affected individuals, and the health implications.

125. S. Biswas, P. van Dijck, and A. Datta, “Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of Candida albicans,” Microbiology and Molecular Biology Reviews, vol. 71, no. 2, pp. 348–376, 2007.

126. Sanglard, D., & Odds, F. C. (2002). "Resistance of Candida albicans to antifungal agents: Molecular mechanisms and clinical consequences." The Lancet Infectious Diseases, 2(2), 65-75

127. Sarwar. (2015). *Int J*, 141-147.

128. Sasaki, T., Kobayashi, M., & Agui, N. (2000). Epidemiological potential of excretion and regurgitation by Musca domestica (Diptera: Muscidae) in the dissemination of *Escherichia coli. J Med*, 945-949.

129. Sharma, A., et al. (2020). "Insecticidal potential of Pleurotus ostreatus against houseflies." Pest Management Science, 76(2), 856-861.

130. Sharma, P., Rani, J., Singh, D. K., & Gupta, R. (2020). Effectiveness of Beauveria bassiana against Musca domestica and its impact on bacterial community composition. Scientific Reports, 10(1), 11278.

131. Sharma, R. A., et al. (2010). "Antiviral activity of Agaricus blazei against HIV." Phytotherapy Research, 24(4), 591-595.

132. Shikongo, L.T., Chimwamurombe, P.M. and Lotfy, H.R., 2013. Antimicrobial screening of crude extracts from the indigenous Ganoderma lucidum mushrooms in Namibia. African Journal of Microbiology Research, 7(40), pp.4812-4816.

133. Smania, E.F.A.; Delle Monache, F.; Smania A. Jr.; Yunesc, R.A.; Cuneoa, R.S. Antifungal activity of sterols and triterpenes isolated from Ganoderma annulare. Fitoterapia, 2003, 74, 375–377.

134. Smania, E.F.A.; Monache, F.D.; Yunes, R.A.; Paulert, R.; Junior, A.S. Antimicrobial activity of methyl australate from Ganoderma australe. Rev. Bras. Farmacog., 2007, 17, 14-16.

135. Stamets, P. (2018). Mushroom-Based pesticides could make chemical pesticides obsolete.

136. Technology, P. C. (1997). Bio Blast. *pctonline*.

137. Tefera, P. (2003). Germination, radial growth, and sporulation of Beauveria bassiana to Chilo partellus (Lepidoptera: Pyralidae) at different temperatures. *Biocontrol Science*.

138. Turkoglu, A.; Duru, M.E.; Mercan, N.; Kivrak, I.; Gezer, K. Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. Food Chem., 2007, 101, 267–273.

139. US EPA, O. (2016). *What are biopesticides*. Retrieved from https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides

140. Valverde, M.E., Hernández-Pérez, T. and Paredes-López, O., 2015. Edible mushrooms: improving human health and promoting quality life. International journal of microbiology, 2015.

141. Villaverde, S.-M. S.-E.-G.-P. (2009). Biopesticides in the framework of the European Pesticide Regulation. *Pest Management Science*, 2-5.

142. Wang, Y. and Xu, B., 2014. Distribution of antioxidant activities and total phenolic contents in acetone, ethanol, water and hot water extracts from 20 edible mushrooms via sequential extraction. Austin Journal of Nutrition and Food Sciences, 2(1), p.5.

143. Wang, Y.; Bao, L.; Yang, X.; Li, L.; Li, S.; Gao, H.; Yao, X-S.; Wen, H.; Liu, HW. Bioactive sesquiterpenoids from the solid culture of the edible mushroom Flammulina velutipes growing on cooked rice. Food Chem., 2012, 132, 1346-1353.

144. Wasser, S. P. (2010). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. International Journal of Medicinal Mushrooms, 12(1), 1-16.

145. Wong, J.H.; Ng, T.B.; Cheung, R.C.F.; Ye, X.J.; Wang, H.X.; Lam, S.K.; Lin, P.; Chan, Y.S.; Fang, E.F.; Ngai, P.H.K.; Lia, L.X.; Ye, X.Y.; Jiang, Y.; Liu, F. Proteins with antifungal properties and other medicinal applications from plants and mushrooms. Appl. Microbiol. Biotechnol. 2010, 87, 1221-1235.

146. Yahia, E.M., Gutiérrez-Orozco, F. and Moreno-Pérez, M.A., 2017. Identification of phenolic compounds by liquid chromatography-mass spectrometry in seventeen species of wild mushrooms in Central Mexico and determination of their antioxidant activity and bioactive compounds. Food Chemistry, 226, pp.14-22.

147. Yamaç, M.; Bilgili, F. Antimicrobial activities of fruit bodies and/or mycelia 587 cultures of some mushroom isolates. Pharm. Biol., 2006, 44, 660–667.

148. Yamaç, M.; Bilgili, F. Antimicrobial activities of fruit bodies and/or mycelia 587 cultures of some mushroom isolates. Pharm. Biol., 2006, 44, 660–667.

149. Yoon, K.N., Lee, J.S., Lee, I.K., Yun, B.S. (2018). Antifungal and Antibacterial Activities of Ethanol Extract and Fractions from the Mushroom Rhopalopus coreanus. Brazilian Journal of Microbiology, 49(1), 54–60.

150. Yuan, H., Sun, L., Chen, M., Wang, J., & Wang, Y. (2018). Pharmacological and therapeutic effects of Ganoderma lucidum on immune function with a focus on its anti-cancer and immunomodulatory properties. Cancer Letters, 417, 168-180.

151. Zhang, Q., et al. (2016). "Bioactive compounds from mushrooms: A review." Journal of Ethnopharmacology, 194, 71-90

152. Zhou, L., et al. (2017). "Antibacterial activities of *Ganoderma lucidum* and its bioactive compounds." Natural Product Research, 31(10), 1214-1218.

153. Zimmermann, G., Piepenbring, M., & Bruckner, D. (2016). Fungal secondary metabolites as insecticides. Journal of Mycology Research, 10(2), 125-140.

154. Zorzenon, J.F. Praga dos cogumelos comestíveis. Instituto Biológico, Centro de Sanidade Vegetal, São Paulo-Brasil, 88-93.