In vitro antagonistic activity of Trichoderma longibrachiatum against aspergillus flavus towards management of mycotoxins

BY

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DECLARATION

I NANDUTU BRENDA hereby declare that this research report is my real and genuine work and to the best of my knowledge, no plagiarism has been done, where such a case of similar work, proper citations have been conducted

Signature..... Date 4th May 2026

APPROVAL

This research on invitro antagonistic activity of Trichoderma longibrachiatum against aspergillus flavus towards management of mycotoxins has been submitted for examination with approval of my supervisor.

Dr Andima Moses

Signed..... Date.....4/5/2026.....

DEDICATION

This report is dedicated to my beloved aunt, Muyama Judith Sylvia who has tirelessly been there to see me through school since an early age.

I also dedicate it to my lecturers who have inspired me throughout campus, to become the best version of me and be the best citizen I can be and guided me towards the accomplishment of my research within the stipulated time

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LIST OF ABBREVIATIONS

Abbreviation	Full Term
AFs	Aflatoxins
CRD	Completely Randomized Design
IARC	International Agency for Research on Cancer
LC-MS	Liquid Chromatography-Mass Spectrometry
LSD	Least Significant Difference
m/z	Mass-to-charge ratio
NIST	National Institute of Standards and Technology
PDA	Potato Dextrose Agar
PIRG	Percentage Inhibition of Radial Growth
rt	Retention Time
WHO	World Health Organization

DEFINITION OF KEY TERMS

Aflatoxins: Potent carcinogenic mycotoxins produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate agricultural commodities.

Antagonism: A biological interaction where the presence of one organism (the antagonist) inhibits the growth or activity of another (the pathogen).

Antibiosis: A type of antagonism where an organism produces secondary metabolites or antibiotics that are toxic to other microorganisms.

Biocontrol Agent: A living organism used to suppress the population or impact of a specific pest or pathogen.

Dual Culture Technique: A laboratory method where two different microorganisms are grown on the same agar plate to observe their interactions and growth inhibition.

In vitro: Latin for "within the glass"; referring to studies performed in a controlled environment outside of a living organism, such as in a Petri dish.

Mycoparasitism: A process where one fungus acts as a parasite on another fungus, often by physically wrapping around the host hyphae and secreting cell-wall-degrading enzymes.

ABSTRACT

Mycotoxin contamination, particularly aflatoxins produced by *Aspergillus flavus*, poses a significant threat to global food security and public health. This study investigated the *in vitro* antagonistic potential of *Trichoderma longibrachiatum* as a biological control agent against *A. flavus*. The research was conducted using a Completely Randomized Design (CRD). The antagonistic activity was evaluated using the dual culture technique on Potato Dextrose Agar (PDA). Results indicated that *T. longibrachiatum* significantly suppressed the radial growth of *A. flavus*, achieving a Percentage Inhibition of Radial Growth (PIRG) of 45.56% within 72 hours of incubation.

To determine the underlying mechanism of inhibition, the culture filtrate of *T. longibrachiatum* was analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS). Metabolic profiling identified over 100 bioactive compounds, including potent antifungal cyclic dipeptides such as *Cyclo(L-Val-L-Pro)* and *Cyclo(L-Trp-L-Pro)*, alongside organic acids and terpenes like *Nerolidol*. These findings suggest that the inhibition is driven by a combination of rapid spatial competition and the secretion of a complex of secondary metabolites (antibiosis). The study concludes that *T. longibrachiatum* is a promising, sustainable alternative to chemical fungicides for managing *A. flavus* and reducing mycotoxin risks in agricultural value chains.

Keywords: *Trichoderma longibrachiatum*, *Aspergillus flavus*, Biocontrol, Aflatoxins, LC-MS, Antibiosis.

CHAPTER ONE

1.1 Background to the study

The contamination of foodstuffs by mycotoxins is one of the most significant and disturbing challenges that global food security and public health faces in the 21st century (WHO, 2023). Mycotoxins are naturally occurring, toxic secondary metabolites produced by various fungal species, including molds that proliferate on numerous crops such as cereals, dried fruits, nuts, spices, particularly under warm and humid conditions (Ahmad *et al*., 2014; WHO, 2023). These compounds are chemically stable and easily survive food processing procedures, thus ensuring their entry into the human and livestock food chain (Luo *et al*., 2021). Some mycotoxins include ochratoxin, fumonisins and aflatoxins. The adverse health consequences of mycotoxin exposure are severe, ranging from immediate acute poisoning involving vomiting, diarrhea and abdominal pain to chronic long-term effects such as immune deficiency and cancer (Omotayo *et al*., 2018). Historically, toxigenic fungi have caused major public health crises; for instance, the T-2 toxin produced by *Fusarium sporotrichoides* was responsible for Alimentary Toxic Aleukia (ATA) in Russia during the early 1940's (Pitt & David Miller, 2017). This outbreak resulted in deaths of approximately 100,000 individuals between 1942 and 1948. About 4.5 billion people of the world's population are subjected to aflatoxin contamination, in addition, 20 cases of corona virus were associated to pulmonary aspergillosis (Shabeer *et al*., 2022a). Between 550,000-600000 new cases of aflatoxin infections are reported every year and of these cases, an estimate of 420,000 people die every year worldwide from consuming contaminated food. They also affect plants in several ways by contaminating the crop seeds and causing symptoms such as boll rot, and yellow mold on the plants. Which affect the crop growth, yield, and also result in to poor market value thereby affecting the world food basket, causing world hunger, malnutrition and generally, poor standard of living. In order to meet the sustainable development goal of zero hunger in the world population,, an increased use of chemical has been observed over the past few years, where about six million tons of chemicals are used to spray crops, moreover, these chemicals have become an environmental concern as they have caused a lot of health complications to the soil as the long term use of these chemical, for example fertilizers destroy the soil structure , and also the environment because some elements are released into the environment which bring about environmental degradation, global warming, as these elements for example carbon monoxide released depletes the ozone layer. In humans it causes various health complications when these foods sprayed with a lot of chemicals are eaten. In order to minimize the effects of using these chemicals, Trichoderma based biological agents are now widely used. In the recent years Trichoderma species for example Trichoderma longibrachiatum have been reported to be an ecofriendly bio agent that is now widely used on plants to improve crop yield by

increasing nutrient uptake, thereby acting as growth promoters. In addition, it reduces the effect of other fungi on the food crops, especially while still in the garden for example aspergillus which cause boll rot and yellow mold in peanuts by acting antagonistically against aspergillus. It does this by competing for nutrients, space, and also producing metabolites which causes fungi to die out. It binds to the cell wall of the pathogen, and followed by the production of cell wall degrading enzymes and antibiotics. This Trichoderma can be applied by either spraying on the leaves, putting in the soil, or mixing with the seeds before planting. the particular method of application depends on the types plant being planted and the preference of the farmer.

1.2 Statement of problem

Mycotoxin contamination, particularly aflatoxins produced by *Aspergillus flavus*, remains a major challenge to global food safety and public health (Putri *et al*., 2024) . Aflatoxins reduce food quality, cause significant post-harvest losses, and pose serious health risks to humans and animals. The problem is further aggravated by increasing food demand and crop losses caused by fungal infections. Although synthetic fungicides are widely used to control fungal pathogens, their continued application has raised concerns due to environmental pollution, health risks, and reduced effectiveness over time. Consequently, there is growing interest in sustainable biological control strategies. *Trichoderma* species are widely recognized for their antagonistic activity against plant pathogenic fungi through competition and the production of antifungal metabolites (Mendarte-Alquisira *et al*., 2024). However, there is limited information on the specific antagonistic activity of *Trichoderma longibrachiatum* against *Aspergillus flavus*, particularly regarding its in-vitro inhibitory effects and the role of its metabolites in mycotoxin management. Therefore, this study aims to evaluate the in vitro antagonistic potential of *T. longibrachiatum* against *A. flavus* as a sustainable approach to managing aflatoxin contamination.

1.3 Objectives of the study

1.3.1 General objectives

To determine the extent of inhibition of Trichoderma longibrachiatum on the growth rate of aspergillus flavus

1.3.2 Specific objectives

1. To compare the radial growth (colony diameter) of *Aspergillus flavus* in the presence versus absence of *Trichoderma longibrachiatum*
2. To determine the inhibitory effect of *Trichoderma longibrachiatum* on the growth of *Aspergillus flavus*
3. To determine the metabolites produced by Trichoderma longibrachiatum that bring about the inhibitory effects against Aspergillus flavus

1.4 Hypotheses for the Study

1. Null Hypothesis (H_{01}):
   There is no significant difference in the radial growth (colony diameter) of Aspergillus flavus in the presence of Trichoderma longibrachiatum compared to its growth in the absence of Trichoderma longibrachiatum.

   Alternative Hypothesis (H_{11}):
   The radial growth (colony diameter) of Aspergillus flavus is significantly reduced in the presence of Trichoderma longibrachiatum compared to its growth in the absence of Trichoderma longibrachiatum.

2. Null Hypothesis (H_{02}):
   Trichoderma longibrachiatum does not exhibit a significant inhibitory effect on the growth of Aspergillus flavus.

   Alternative Hypothesis (H_{12}):
   Trichoderma longibrachiatum significantly inhibits the growth of Aspergillus flavus.

3. Null Hypothesis (H_{03}):
   The metabolites produced by Trichoderma longibrachiatum do not significantly inhibit the growth of Aspergillus flavus.

Alternative Hypothesis (H_{13}):

The metabolites produced by Trichoderma longibrachiatum significantly inhibit the growth of Aspergillus flavus.

1.5 Justification of the study

The increasing environmental concerns associated with chemical pesticides have created a pressing need for alternative pest management strategies. Some fungi such as the Trichoderma species have demonstrated potential as biopesticides, but their interactions especially those of *T. longibrachiatum* with the target pests require further investigation. This study therefore aims to investigate these interactions, thereby informing sustainable management practices.

1.6 Scope of study

The study was carried out in Busitema University, Faculty of Science and education, Biology lab. The research was carried out in a period of about three weeks and it majorly involved the duo inoculation of the media, and the observing their antagonistic behavior.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Mycotoxin contamination poses a serious global threat to food and feed safety, with aflatoxins representing some of the most potent natural carcinogens (Shabeer *et al*., 2022) .*Aspergillus flavus* is the primary fungal pathogen responsible for this contamination, frequently colonizing staple crops such as maize, groundnuts, and other agricultural commodities, particularly in warm and humid agro-ecological zones (Ren *et al*., 2022). The economic and health implications of this pathogen are profound; aflatoxin exposure is directly linked to hepatocellular carcinoma, immunosuppression, and growth impairment in both humans and animals (Shabeer *et al*., 2022b).

Conventional management relies heavily on synthetic fungicides. However, environmental concerns and pathogen resistance have necessitated a shift toward biological control (biocontrol) (Voloshchuk *et al*., 2024a). Among the various biological control agents (BCAs), the genus *Trichoderma* has emerged as a superior antagonist due to its rapid growth, diverse metabolic profile, and aggressive mycoparasitic nature (Ren *et al*., 2022). This review focuses on the *in vitro* antagonistic activity of *Trichoderma longibrachiatum* against *A. flavus*, specifically examining the role of metabolites and comparative growth kinetics in mycotoxin management.

2.2 The Antagonist: *Trichoderma longibriachiatum*

2.2.1 Taxonomy and Classification

Trichoderma longibrachiatum belongs to the clade *Longibrachiatum*. While the genus *Trichoderma* was historically thought to contain few species, modern molecular techniques have identified hundreds of distinct species. *T. longibrachiatum* is distinct due to its thermotolerance and specific secondary metabolite profile (Schmoll, 2024).

Scientific Classification of *Trichoderma longibrachiatum*

Rank	Name
Domain	Eukaryota
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Hypocreaceae
Genus	*Trichoderma*
Species	*Trichoderma longibrachiatum*

Table 1: Scientific Classification of Trichoderma longibrachiatum

2.2.2 Morphology and Identification

Identification of *T. longibrachiatum* utilizes both morphological observation and molecular verification.

- **Macroscopic:** Colonies typically grow rapidly on Potato Dextrose Agar (PDA), initially appearing distinctively white and downy. As sporulation occurs (usually within 3–5 days), the colony turns a characteristic grayish-green to deep green, often forming concentric rings (Bissett *et al*., 2015).
- **Microscopic:** The conidiophores are branched, bearing bottle-shaped phialides that are often solitary or inflated in the middle (Samuels *et al*., 2012).
- **Molecular:** Due to morphological similarities among *Trichoderma* species, precise identification requires DNA sequence analysis, specifically comparing the Internal Transcribed Spacer (ITS) regions against the NCBI GenBank database to confirm homology (Schmoll, 2024).

2.2.3 Ecology and Growth Requirements

T. longibrachiatum is a saprophytic soil fungus found on decaying biomass, dead wood, and root surfaces. It is noted for its thermotolerance, with optimal growth occurring around 35°C, though it remains active between 20°C and 37°C. This temperature range makes it particularly suitable for controlling *A. flavus*, which also thrives in warm environments (Cavalcante *et al*., 2025).

2.3 The Pathogen: *Aspergillus flavus*

2.3.1 Classification and Morphology

Aspergillus flavus belongs to the family Aspergillaceae (formerly Trichocomaceae).
Morphologically, it produces colonies that are initially white but rapidly turn a bright yellow-green (olive) due to conidial production (González-Ramírez *et al*., 2016).

Scientific Classification of *Aspergillus flavus*

Rank	Name
Kingdom	Fungi
Phylum	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Aspergillaceae
Genus	*Aspergillus*
Species	*Aspergillus flavus*

Table 2: Scientific Classification of Aspergillus flavus

2.3.2 Mycotoxigenic Potential of *A. flavus*

The primary significance of *A. flavus* is not merely its growth, but its metabolism. It produces Aflatoxins (*B1 and B2)* which are toxic secondary metabolites. The fungus is often categorized into "S strains" (producing small sclerotia <40 μm) and "L strains" (producing large sclerotia >400 μm), with S strains typically producing higher quantities of toxins (Szonyi *et al*., 2025).

2.4 Antagonistic Activity of *Trichoderma longibrachiatum*

Trichoderma species exhibit potent antagonistic activity against various fungal phytopathogens, including *Aspergillus* species. However, this antagonism is not the result of a single mode of action but rather a complex interaction of multiple mechanisms. These mechanisms are broadly categorized into direct interactions (mycoparasitism, competition, and antibiosis) and indirect interactions (induced systemic resistance) (Guzmán-Guzmán *et al*., 2025).

2.4.1 Direct Mechanisms of Antagonism

1. Competition for Resources

Trichoderma species are aggressive colonizers that outcompete pathogenic fungi for essential nutrients and space. By effectively capturing and utilizing available carbon and nitrogen sources in the soil or growth medium, *T. longibrachiatum* renders the environment nutrient-deficient for *Aspergillus flavus*, thereby limiting the pathogen's ability to grow and thrive (Panchalingam *et al*., 2022).

2. Mycoparasitism

This mechanism involves the direct physical attack of the pathogen. Trichoderma recognizes the pathogen, coils around its hyphae, and produces lytic enzymes to degrade the cell wall of the pathogen. This process allows Trichoderma to penetrate the hyphae and absorb nutrients from the target fungus, effectively killing it (Panchalingam *et al*., 2022).

3. Antibiosis and Metabolite-Driven Inhibition of *T. longibrachiatum*

Antibiosis is central to the antagonistic ability of *T. longibrachiatum* and represents a form of "chemical warfare." The fungus produces a vast array of secondary metabolites that inhibit *A. flavus* without requiring physical contact. Research indicates that *T. longibrachiatum* releases both **Volatile Organic Compounds (VOCs) and non-volatile substances** that diffuse through the growth medium to suppress pathogen growth (A.P. *et al*., 2020; Ramasamy *et al*., 2023)

Peptaibols: *T. longibrachiatum* is a known producer of peptaibols, specifically **tricholongins** and **longibrachins**. These are short-chain, helical peptides that insert themselves into the cell membrane of the pathogen (*A. flavus*). This insertion creates transmembrane pores, leading to the leakage of cytoplasmic contents, loss of membrane integrity, and eventual cell death (A.P. *et al*., 2020).

Hydrolytic Enzymes (CWDEs): To facilitate antibiosis and mycoparasitism, *T. longibrachiatum* secretes Cell Wall Degrading Enzymes (CWDEs) such as chitinases, $\beta - 1{,}3 -$glucanases, and **proteases**. These enzymes digest the chitin and glucan matrix that makes up the cell wall of *A. flavus*,

essentially turning the pathogen's protective barrier into a nutrient source for the antagonist (Mendarte-Alquisira *et al*., 2024). Specific antibiotic compounds such as harzianolide and trichodermin have also been associated with the genus, contributing to this inhibitory effect (A.P. *et al*., 2020).

2.4.2 Indirect Mechanisms

1. Induced Resistance

Beyond direct attack, Trichoderma can interact with plants to stimulate their innate defense mechanisms, making them more resistant to fungal pathogens. Furthermore, Trichoderma promotes plant growth and root development by altering the soil environment to be more favorable for the plant and less favorable for pathogens. This dual action of growth promotion and immune stimulation indirectly reduces the impact of A. flavus infection (Yao *et al*., 2023) .

2.5 Growth Kinetics and Competition

The physical interaction between the two fungi is defined by competition for space and nutrients.

2.5.1 Competitive Exclusion

Trichoderma species are generally characterized by a higher specific growth rate (r) compared to *Aspergillus* species. In a dual culture environment, *T. longibrachiatum* rapidly colonizes the substrate, depriving *A. flavus* of essential carbon and nitrogen sources. This "starvation" approach effectively halts the spread of the pathogen (Yao *et al*., 2023)

2.5.2 Comparative Growth Rates

When *A. flavus* is grown in monoculture (absence of antagonist), it displays a characteristic radial growth pattern that follows a sigmoid curve until the substrate is exhausted. However, in the presence of *T. Longibrachiatum, the growth rate of A. flavus is significantly suppressed* and also significant deviations are observed, such as:

- **Contact Inhibition:** Upon contact between the mycelia of the two fungi, *A. flavus* growth usually arrests.
- **Overgrowth:** Studies by da Silva *et al*. (2019) demonstrated that *T. longibrachiatum* can overgrow the colonies of *A. flavus*, effectively sporulating over the pathogen and reducing its metabolic activity.
- **Percentage Inhibition:** Depending on the strain, *T. longibrachiatum* has been reported to inhibit the radial growth of *A. flavus* by 60% to 85% in dual culture assays (Imtiaj & Lee, 2008).

This suppression is quantifiable using the **Percentage Inhibition of Radial Growth (PIRG)** formula given by Vincent (1947):

$$PIRG = \frac{R_1 - R_2}{R_1} \times 100$$

Where:

- R_1 is the radial growth of *A. flavus* in the control plate (absence of antagonist).
- $R2$ is the radial growth of *A. flavus* in the dual culture plate (presence of antagonist)

Previous studies have shown that *T. longibrachiatum* can inhibit the mycelial growth of *A. flavus* by 60% to 85% depending on the strain and environmental conditions (Gwa & Awa, 2019).

2.5.3 Comparative Effectiveness of *T. longibrachiatum* with Other Biocontrol Agents

Among Trichoderma species, T. longibrachiatum demonstrates competitive antagonistic potential against A. flavus. While *T. harzianum* is more extensively studied and commercialized, *T. longibrachiatum* has shown comparable or superior antagonistic activity in specific applications. Studies comparing multiple Trichoderma species against A. flavus have identified *T. longibrachiatum* among the most effective candidates, particularly when considering both fungal growth inhibition and aflatoxin reduction.

CHAPTER THREE

METHODS AND MATERIALS

3.1 Study Area

The study was conducted at the Microbiology Laboratory, Faculty of Science and Education, Busitema University (Nagongera Campus), located in Nagongera Sub-County, Tororo District. This research was majorly lab-based and therefore all of the experiments were carried out in the Busitema University laboratory.

3.2 Experimental Design

The experiment followed a *Completely Randomized Design* (CRD). All treatments were performed in triplicates (***n=3***) to ensure statistical validity. The experimental treatments included:

1. **Control 1:** *Aspergillus flavus* monoculture (Pathogen only).
2. **Control 2:** *Trichoderma longibrachiatum* monoculture (Antagonist only).
3. **Interaction:** Dual culture of *T. longibrachiatum* against *A. flavus*.

3.3 Source of Microbial Cultures

3.3.1 Isolation of *Trichoderma longibrachiatum*

Soil samples were collected from the agricultural gardens surrounding the Nagongera campus. The isolation of *T. longibrachiatum* was performed using the serial dilution plate technique: 1g of soil was suspended in 10ml of sterile distilled water and vortexed. Serial dilutions were prepared up to $\mathbf{10^{-4}}$. Then, 1ml of the suspension was plated onto Potato Dextrose Agar (PDA) amended with chloramphenicol ($\mathbf{250}\ mg/L$) to inhibit bacterial growth. Plates were subsequently incubated at $\mathbf{25\pm2}\ ^{\circ}\boldsymbol{C}$ for 5 days. Emerging colonies were then identified morphologically and sub-cultured to obtain pure isolates.

3.3.2 Source of *Aspergillus flavus*

Aspergillus flavus was isolated from infected maize kernels obtained from local markets in *Nagongera trading center*, Tororo. The kernels were surface sterilized with 1% sodium hypochlorite, rinsed, and plated on PDA. Identification was confirmed via microscopic observation of conidial heads and spores.

3.4 Preparation of Culture Media

Potato Dextrose Agar (PDA) was used as the basal medium for all assays. In the preparation, 39g of commercial PDA powder was suspended in 1000ml of distilled water. The mixture was heated to

boiling with constant stirring to ensure complete dissolution. The medium was then autoclaved at 121 ℃ and 15 *psi* pressure for 15 minutes. And finally, after cooling to approx. **45 ℃**, the media was poured into sterile Petri dishes (approx. 20ml per plate).

3.5 Dual Culture Assay

To determine the growth rate and inhibition percentage, the Dual Culture Technique (Morton & Stroube, 1955) was employed. A 5mm mycelial plug was cut from the edge of an actively growing (5-day old) *A. flavus* culture and placed 1cm from the edge of a fresh PDA plate. A similar 5mm plug of *T. longibrachiatum* was placed on the opposite side of the plate, exactly 60mm apart from the pathogen. Plates inoculated only with *A. flavus* or *T. longibrachiatum* served as controls. These plates were then subsequently sealed with Parafilm and incubated at 25 ± 2 ℃ for 5 days**.**

3.6 Assay for Non-Volatile Metabolites

To determine if metabolites alone (without the living fungus) cause inhibition, the Culture Filtrate Techniqu**e** was used. *T. longibrachiatum* was grown in Potato Dextrose Broth (PDB) for 10 days after which the liquid culture was filtered through Whatman No. 1 filter paper and then passed through a 0.22 μm membrane filter to remove all spores/hyphae, leaving only the metabolites in the liquid. This cell-free filtrate was mixed with molten PDA (at varying concentrations) and poured into plates. A 5mm plug of *A. flavus* was placed in the center. Growth inhibition was compared to plates without the filtrate.

3.7 Extraction and Profiling of Secondary Metabolites

Some of the cell-free culture filtrate obtained from the 10-day-old culture of *T. longibrachiatum* was subjected to solvent extraction using Methanol. The organic phase was evaporated to dryness and reconstituted for analysis.

3.7.1 LC-MS Analysis

The metabolic profile of the extract from *T. longibrachiatum* was determined using Liquid Chromatography-Mass Spectrometry (LC-MS). Chromatographic separation was achieved using a reverse-phase column. The mass spectrometric analysis was performed in positive ionization mode ([M+H]+). Compounds were identified by comparing their exact mass-to-charge *(m/z)* ratios and retention times *(rt)* against established databases, including the NIST14 and MassBank libraries.

3.8 Data Collection and Analysis

3.8.1 Measurement of Radial Growth

Radial growth (diameter) of the fungal colonies was measured daily using a ruler along two perpendicular axes. Measurements were taken after 3 days and 5 days.

3.8.2 Calculation of Percentage Inhibition

The ***Percentage Inhibition of Radial Growth (PIRG)*** was calculated using the formula by Vincent (1947):

$$\boldsymbol{PIRG} = \frac{\boldsymbol{C} - \boldsymbol{T}}{\boldsymbol{C}} \times \mathbf{100}$$

Where;

C= Radial growth of *A. flavus* in the Control plate.

T = Radial growth of *A. flavus* in the Dual Culture (Treatment) plate.

CHAPTER FOUR

RESULTS

4.1 Radial Growth of *Aspergillus flavus*

The comparative growth of *Aspergillus flavus* was monitored over a period of 72 hours (3 days). The data revealed a distinct suppression of the pathogen when cultured in the presence of *Trichoderma longibrachiatum*.

Mean Radial Growth of A. flavus in Control vs. Dual Culture

Values represent the mean diameter (mm) of three replicates ($n = 3$).

Treatment	**Day 1 Mean (mm)**	**Day 2 Mean (mm)**	**Day 3 Mean (mm)**
Control (*T. longibrachiatum)*	0.33	1.57	**3.0**
Control (*A. flavus* monoculture)	0.45	1.4	**2.7**
Dual Culture (*A. flavus* + *T. longibrachiatum*)	0.53	1.03	**1.47**
Difference (*A flavus mono culture vs Dual culture*)	0.08	-0.37	**-1.23**

Table 3: Mean Radial Growth of A. flavus in Control vs. Dual Culture

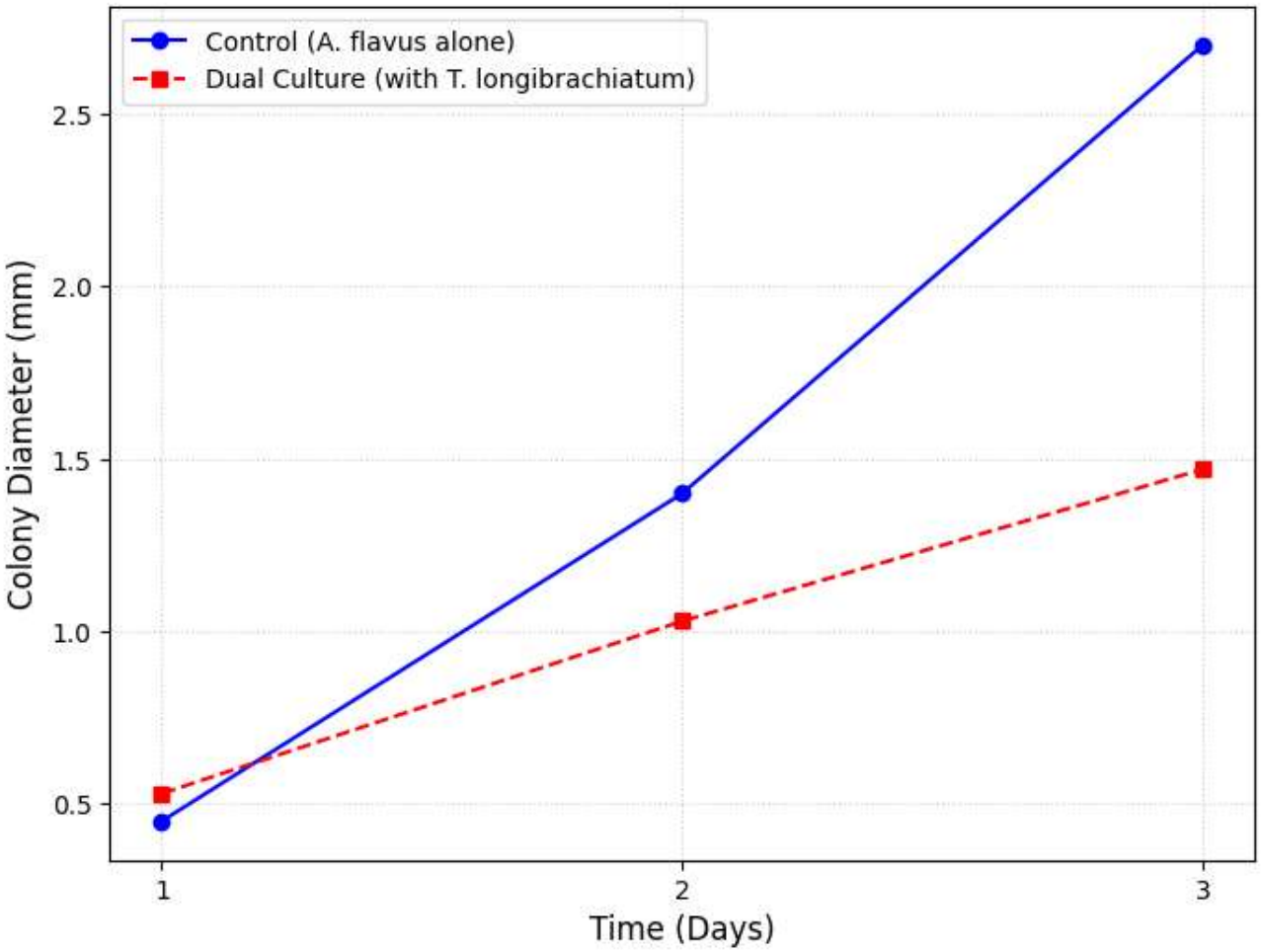

Figure 1: Growth of A. flavus in dual vs monoculture

In the control experiment, *A. flavus* exhibited steady exponential growth, expanding from a mean diameter of 0.45 mm on Day 1 to **2.70 mm** by day 3. In contrast, under dual culture conditions, the growth of *A. flavus* was observed to be restricted. While the pathogen initially established itself on day 1 (0.53 mm), its expansion slowed significantly to 1.47 mm by day 3, which is much lower than 2.70 mm of the control.

4.2 Antagonistic Efficiency of *T. longibrachiatum* (percentage inhibition)

The Percentage Inhibition of Radial Growth (PIRG) was calculated based on the day 3 measurements using the formula by Vincent (1947).

Table 4.2: Efficacy of *T. longibrachiatum* against *A. flavus* (Day 3)

Parameter	**Value**
Mean Diameter of Control $(\boldsymbol{C})$	2.70 mm
Mean Diameter of Dual Culture (*T*)	1.47 mm
Reduction in Size	1.23 mm
Percentage Inhibition (PIRG)	**45.56%**

$$\boldsymbol{PIRG} = \frac{\boldsymbol{C} - \boldsymbol{T}}{\boldsymbol{C}} \times \mathbf{100}$$

$$= \frac{\mathbf{2.70 - 1.47}}{\mathbf{2.70}} \times 100$$

$$= \mathbf{45.56\%}$$

Where;

C= Radial growth of *A. flavus* in the Control plate.

T = Radial growth of *A. flavus* in the Dual Culture (Treatment) plate.

The presence of *T. longibrachiatum* significantly altered the growth kinetics of *A. flavus*. The calculated Percentage Inhibition of Radial Growth (PIRG) on Day 3 was 45.56%. This indicates that within just 72 hours, the antagonist was able to suppress nearly half of the potential growth of the pathogen.

4.3 Evaluation of metabolite activity and profiling

The antagonistic potential of *T. longibrachiatum* is largely attributed to antibiosis, where the antagonist releases secondary metabolites that inhibit the growth of the pathogen without requiring physical contact. Metabolic profiling of the *T. longibrachiatum* culture filtrate identified a diverse array of bioactive compounds, several of which are well-documented for their antifungal and anti-aflatoxigenic properties.

4.3.1 Key Bioactive Metabolites Identified

The analysis revealed over 100 compounds, including lipids, organic acids, and dipeptides. The most significant compounds identified with known antimicrobial activity are summarized in Table 4.3. The full list of the 114 metabolites can be found in *Appendix II.*

Major bioactive metabolites produced by *T. longibrachiatum*

Compound Name	**Compound Class**	**Biological Relevance**
Cyclo(L-Val-L-Pro)	Cyclic Dipeptide	Potent antifungal agent, inhibits spore germination
Cyclo(leucylprolyl)	Cyclic Dipeptide	Broad-spectrum antimicrobial activity
Cyclo (leucylprolyl)	Cyclic Dipeptide	Broad spectrum antimicrobial activity
Nerolidol	Sesquiterpene	Inhibits fungal growth and ergosterol synthesis
Caffeic Acid	Phenolic Acid	Known to inhibit aflatoxin biosynthesis in Apsergillus
Palmitamide	Fatty Acid Amide	Contributes to the overall inhibitory environment
1H-Indole-3-carboxylic acid	Indole derivative	Involved in signaling and growth inhibition

Table 4: Major bioactive metabolites produced by T. longibrachiatum

CHAPTER FIVE

5.1 DISCUSSION

The results of this study clearly demonstrate that *Trichoderma longibrachiatum* exhibits strong antagonistic activity against *Aspergillus flavus*, a major aflatoxin-producing fungus of global concern. The dual culture assay showed that the radial growth of *A. flavus* was significantly reduced in the presence of *T. longibrachiatum*, with an inhibition rate of *45.56% after three days*. This finding aligns with earlier studies reporting that *Trichoderma* species are effective antagonists of aflatoxigenic fungi due to their rapid colonization ability and diverse antifungal mechanisms (Ren *et al*., 2022)

The observed suppression of *A. flavus* growth can be attributed to several mechanisms. First, *Trichoderma* species are known to outcompete pathogens for nutrients and space, a phenomenon well documented in biocontrol literature (Ren *et al*., 2022). In this study, the reduced radial growth of *A. flavus* in dual culture suggests that *T. longibrachiatum* rapidly occupied the substrate, limiting the resources available to the pathogen. This competitive exclusion has been reported in similar studies where *Trichoderma* strains inhibited the growth of *A. flavus* and other mycotoxigenic fungi (Voloshchuk *et al*., 2024b). Second, mycoparasitism likely contributed to the antagonistic effect. *Trichoderma* species are known to recognize, coil around, and degrade the hyphae of target fungi through the secretion of cell wall-degrading enzymes such as chitinases and β-1,3-glucanases (Singh *et al*., 2024). These enzymes weaken the pathogen's cell wall, leading to structural collapse. The reduced growth rate of *A. flavus* in this study is consistent with such enzymatic activity. Third, antibiosis appears to be a major mechanism in this interaction. The metabolite profiling conducted in this study identified several bioactive compounds such as cyclic dipeptides, phenolic acids, and sesquiterpenes. These compounds have been widely reported to inhibit fungal growth, disrupt membrane integrity, and interfere with toxin biosynthesis (Catara *et al*., 2021; Ramasamy *et al*., 2023b). For example, caffeic acid is known to suppress aflatoxin biosynthesis in *Aspergillus* species, while nerolidol disrupts ergosterol synthesis, a key component of fungal cell membranes. The presence of these metabolites supports the idea that *T. longibrachiatum* can inhibit *A. flavus* even without direct physical contact, as demonstrated in other studies where culture filtrates alone significantly reduced pathogen growth (Al-Askar *et al*., 2022). This suggests that *T. longibrachiatum* could be effective in both soil and foliar applications, where direct hyphal interaction may not always occur.

The findings of this study have important implications for sustainable agriculture. Aflatoxin contamination remains a major challenge in tropical regions, including Uganda, where warm and

humid conditions favour fungal proliferation (Shabeer *et al*., 2022). Chemical fungicides, though widely used, pose environmental and health risks and may lead to resistant fungal strains. Biological control using *Trichoderma* offers an eco-friendly alternative that aligns with global efforts to reduce chemical pesticide use (Cavalcante *et al*., 2025). While the inhibition rate observed in this study (45.56%) is moderate compared to some reports showing up to 85% inhibition, it still demonstrates significant antagonistic potential (Prakasam & Sharma, 2012). Variations in inhibition rates may be due to strain differences, environmental conditions, or nutrient availability. Further studies using multiple isolates of *T. longibrachiatum* could help identify the most effective strains for field application.

The study has certain limitations. Because the experiments were carried out under controlled laboratory conditions, the findings may not fully reflect what happens in field environments where factors such as soil microorganisms, temperature changes, and moisture levels can influence the effectiveness of biocontrol agents. In addition, although metabolite profiling revealed several compounds with antifungal properties, the study did not directly measure aflatoxin levels. Future work should include aflatoxin quantification to better determine how *T. longibrachiatum* affects toxin production.

The findings from this research add to the growing body of evidence supporting *T. longibrachiatum* as a useful biological control agent against *A. flavus*. Its capacity to suppress fungal growth and produce inhibitory metabolites highlights its potential role in sustainable crop protection strategies.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study demonstrated that *Trichoderma longibrachiatum* has significant in vitro antagonistic activity against *Aspergillus flavus*. The dual culture assay showed a 45.56% reduction in radial growth of *A. flavus* within three days, confirming the inhibitory effect of the antagonist. Metabolite profiling identified several bioactive compounds with antifungal properties, supporting the role of antibiosis in the antagonistic interaction.

The findings suggest that *T. longibrachiatum* can be considered a promising biological control agent for managing aflatoxin contamination. Its ability to suppress fungal growth and produce inhibitory metabolites provides a sustainable alternative to chemical fungicides.

6.2 RECOMMENDATIONS

There is need to conduct field trials as to evaluate the effectiveness of *T. longibrachiatum* under natural agricultural conditions.

Communities and agribusinesses should embrace the use of *Trichoderma-based* biocontrol agents as pesticides to reduce over-reliance on chemical fungicides.

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APPENDICES

APPENDIX I

RT Min	Adduct	Molecular Formula	Precursor	Exact Mass	Compound Name

11.91	M+H	$C_{16}H_{32}O_2$	257.248	256.24	Spectral Match to Pentadecanoic acid, 14-methyl- from NIST14
9.73	M+H	$C_{16}H_{33}NO$	256.262	255.26	Palmitamide
0.71	M+H	$C_{10}H_{13}N_5O_4$	298.09	297.09	Spectral Match to Adenosine, 5'-S-methyl-5'-thio- from NIST14
0.33	M+H	$C_{10}H_{13}N_5O_4$	268.104	267.097	ADENOSINE
9.81	M+H	$C_{44}H_{82}NO_6PS$	784.584	783.56	Spectral Match to Arachidonoylthio-PC from NIST14
9.56	M+H	$C_{20}H_{39}NO_3$	342.3	341.293	Gly-C18:0
1.33	M+H	$C_9H_7NO_2$	162.055	161.047	1H-Indole-3-carboxylic acid
3.88	M+H	$C_{11}H_{13}NO_3$	206.082	205.07	N-ACETYL-PHENYLALANINE
10.98	M+H	$C_{12}H_{27}O_4P$	267.172	266.16	Spectral Match to Tributyl phosphate from NIST14
7.37	M+H	$C_{15}H_{26}O$	223.206	222.2	Spectral Match to NEROLIDOL from NIST14
12.01	M+H	$C_{20}H_{34}O_2$	309.28	308.27	9Z,11E,13E-Octadecatrienoic acid ethyl ester
9.77	M+H	$C_{21}H_{40}O_4$	357.3	356.29	Spectral Match to Monoelaidin from NIST14
9.87	M+H	$C_{40}H_{78}NO_8P$	732.59	731.58	Spectral Match to 1-Stearoyl-2-myristoyl-sn-glycero-3-phosphocholine from NIST14
9.35	M+H	$C_{42}H_{78}NO_8P$	756.59	755.58	Spectral Match to 1-Hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine from NIST14
7.52	M+H	$C_{21}H_{38}N+$	304.3	303.29	Spectral Match to Benzyldodecyldimethylammonium from NIST14
9.88	M+H	$C_{44}H_{84}NO_8P$	786.637	785.63	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine from NIST14
1.98	M+H	$C_{18}H_{20}N_2O_3$	313.155	312.148	Spectral Match to Phe-Phe from NIST14
8.19	M+H	$C_{24}H_{50}NO_7P$	496.339	495.33	Spectral Match to Lyso-PC (16:0) from NIST14
9.61	M+H	$C_{16}H_{30}O_2$	255.232	254.22	Spectral Match to cis-9-Hexadecenoic acid from NIST14
2.39	M+H	$C_{14}H_{18}N_2O_3$	263.139	262.132	Spectral Match to Phe-Pro from NIST14
9.61	M+H	$C_{42}H_{82}NO_8P$	760.621	759.61	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14
8.4	M+H	$C_{24}H_{38}O_4$	391.284	390.277	BIS(2-ETHYLHEXYL) PHTHALATE
7.38	M+H	$C_{18}H_{39}NO_3$	300.29	317.293	Spectral Match to Phytosphingosine from NIST14
6.23	M+H	$C_{12}H_{14}O_4$	177.055	222.089	Spectral Match to Diethyl phthalate from NIST14
9.77	M+H	$C_{18}H_{34}O_2$	283.263	282.26	Spectral Match to Elaidic acid from NIST14
9.17	M+H	$C_{23}H_{48}NO_7P$	482.324	481.32	Spectral Match to 1-Pentadecanoyl-sn-glycero-3-phosphocholine from NIST14
8.7	M+H	$C_{22}H_{46}NO_7P$	468.309	467.3	Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine from NIST14
3.03	M+H	$C_{11}H_{12}Cl_2N_2O_5$	323.02	322.01	Spectral Match to Chloramphenicol from NIST14
10.88	M+H	$C_{20}H_{34}O_8$	211.144	402.225	Acetyl tributyl citrate
3.03	M+H	$C_{17}H_{20}N_4O_6$	377.146	376.139	Spectral Match to (-)-Riboflavin from NIST14
9.72	M+H	$C_{20}H_{39}NO_2$	326.269	325.26	Spectral Match to N-Oleoylethanolamine from NIST14
2.94	M+H	$C_{13}H_{14}N_2O_3$	247.108	246.1	N-ACETYL-D-TRYPTOPHAN
9.2	M+H	$C_{19}H_{38}O_4$	331.284	330.28	1-Hexadecanoyl-sn-glycerol
3.73	M+H	$C_{15}H_{22}N_2O_3$	279.171	278.163	Leu-Phe
9.38	M+H	$C_{23}H_{46}NO_7P$	480.308	479.3	1-(9Z-Octadecenoyl)-sn-glycero-3-phosphoethanolamine
9.87	M+H	$C_{16}H_{33}NO_3$	288.253	287.25	Lauryl diethanolamide
8.78	M+H	$C_{16}H_{22}O_4$	279.159	278.15	Spectral Match to Dibutyl phthalate from NIST14

0.57	M+H	$C_{11}H_{22}N_2O_3$	231.17	230.163	Spectral Match to Val-Ile from NIST14
2.68	M+H	$C_{20}H_{23}N_3O_4$	426.202	425.195	Phe-Gly-Phe
10.73	M+H	$C_{44}H_{86}NO_7P$	772.587	771.58	Spectral Match to 1-(1Z-Octadecenyl)-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14
9.94	M+H	$C_{18}H_{37}NO_2$	300.29	299.28	Spectral Match to Palmitoyl ethanolamide from NIST14
7.54	M+H	$C_{10}H_{16}N_2O_2$	197.128	196.121	cyclo(L-Val-L-Pro)
5.17	M+H	$C_{16}H_{17}N_3O_2$	284.139	283.132	cyclo(L-Trp-L-Pro)
2.37	M+H	$C_{14}H_{15}N_3O_2$	258.124	257.117	Cyclo (Trp-Ala)
0.3	M+H	$C_6H_{14}N_4O_2$	175.119	174.112	ARGININE
9.94	M+H	$C_{48}H_{80}NO_8P$	810.598	809.59	Spectral Match to 1,2-Diarachidonoyl-sn-glycero-3-phosphocholine from NIST14
0.94	M+H	$C_{11}H_{20}N_2O_3$	227.176	226.168	Spectral Match to Pro-Ile from NIST14
7.95	M+H	$C_{24}H_{48}NO_7P$	494.324	493.32	Lyso PC (16:1)
7.64	M+H	$C_{11}H_{20}N_3O_3PS$	306.103	305.1	Pirimiphos-methyl
11.1	M+H	$C_{44}H_{79}N_1O_8P_1$	780.553	779.55	PC-DAG (16:0/18:3)
9.09	M+H	$C_{20}H_{22}N_2O_2$	323.212	322.2	1,4-Bis (N-isopropylamino) anthraquinone
9.94	M+H	$C_{42}H_{84}NO_7P$	746.569	745.56	Spectral Match to 1-Hexadecyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14
8.94	M+H	$C_{26}H_{54}NO_7P$	524.371	523.36	Spectral Match to 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine from NIST14
9.35	M+H	$C_{36}H_{66}NO_8P$	704.558	703.55	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14
3.42	M+H	$C_{17}H_{23}N_3O_3$	318.181	317.174	Leu-Trp
3.26	M+H	$C_{16}H_{21}N_3O_3$	304.166	303.158	Val-Trp
2.92	M+H	$C_{14}H_{20}N_2O_3$	265.155	264.148	Val-Phe
0.47	M+H	$C_{14}H_{18}N_2O_4$	279.134	278.127	Spectral Match to Tyr-Pro from NIST14
7.5	M+H	$C_{18}H_{39}NO_2$	302.305	301.3	Spectral Match to SPHINGANINE from NIST14
2.37	M+H	$C_{12}H_{24}N_2O_3$	245.186	244.179	Ile-Leu
7.78	M+H	$C_{18}H_{35}NO$	282.279	281.27	9-Octadecenamide
7.02	M+H	$C_{20}H_{16}N_2O_4$	349.118	348.111	CAMPTOTHECIN
0.31	M+H	$C_4H_9NO_3$	120.065	119.058	Thr
9.77	M+H	$C_{18}H_{28}O_2$	277.216	276.21	9,12-Octadecadiynoic Acid
8.81	M+H	$C_{16}H_{35}NO_2$	274.274	273.27	Lauryl diethanolamine
12.75	M+H	$C_{11}H_{18}N_2O_2$	211.144	210.137	Massbank:PR311142 Cyclo(leucylprolyl)
9.94	M+H	$C_{30}H_{60}N_4O_3$	525.438	524.43	Arg-C24:0
8.78	M+H	$C_{21}H_{44}NO_7P$	454.293	453.29	Spectral Match to 1-Hexadecanoyl-sn-glycero-3-phosphoethanolamine from NIST14
8.44	M+H	$C_{22}H_{46}NO_5P$	436.319	435.31	Spectral Match
10.99	M+H	$C_{18}H_{32}O_2$	281.247	280.24	Massbank: EQ331602 Linoleic acid
0.54	M+H	$C_{11}H_{12}N_2O_2$	205.097	204.09	L-Tryptophan
1.18	M+H	$C_9H_8O_4$	163.039	180.042	Caffeic acid
0.3	M+H	$C_4H_8N_2O_3$	133.061	132.053	Asn
1.24	M+H	$C_{11}H_{18}N_2O_2$	211.144	210.137	Cyclo(leucylprolyl)

9.87	M+H	$C_{41}H_{78}NO_8P$	744.59	743.58	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine from NIST14
5.58	M+H	$C_{24}H_{38}O_4$	391.284	390.28	Spectral Match to Bis(2-ethylhexyl) phthalate from NIST14
0.44	M+H	$C_{10}H_{20}N_2O_4$	233.15	232.142	Thr-Ile
0.46	M+H	$C_9H_{11}NO_3$	182.081	181.074	Tyr
8.81	M+H	$C_{12}H_{23}NO$	198.185	197.18	Spectral Match to Azacyclotridecan-2-one from NIST14
11.23	M+H	$C_{19}H_{34}O_3$	311.258	310.25	13(S)-HODE methyl ester
9.5	M+H	$C_{14}H_{26}O_2$	227.201	226.19	Spectral Match to Myristoleic acid from NIST14
9.88	M+H	$C_{12}H_{11}O_4P$	251.046	250.04	Diphenyl phosphate
2.37	M+H	$C_{11}H_{11}NO_3$	206.081	205.074	Spectral Match to DL-Indole-3-lactic acid from NIST14
5.56	M+H	$C_{18}H_{32}N_2O_4$	341.243	340.236	Asn-C14:1
9.92	M+H	$C_{40}H_{76}NO_{10}P$	762.528	761.52	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoserine from NIST14
9.2	M+H	$C_{24}H_{40}O_5$	393.299	392.29	Spectral Match to URSOCHOLIC ACID from NIST14
4.3	M+H	$C_{14}H_{19}NO_3$	250.144	249.137	Phe-C5:0
9.77	M+H	$C_{18}H_{35}NO$	282.279	281.27	9-Octadecenamide, (Z)
5.75	M+H	$C_{12}H_{21}N$	180.175	179.167	3,5-Dimethyladamantan-1-amine
9.1	M+H	$C_{15}H_{24}O_2$	219.174	236.178	Spectral Match to Curcumol from NIST14
10.88	M+H	$C_{21}H_{38}O_4$	355.284	354.28	monolinolein
10.02	M+H	$C_{16}H_{32}O_2$	257.248	256.24	Spectral Match to Palmitic acid from NIST14
1.57	M+H	$C_{17}H_{34}N_6O_4$	387.283	386.275	Val-Ile-Arg
8.72	M+H	$C_{30}H_{50}O_2$	425.378	442.381	Betulin
8.71	M+H	$C_{17}H_{26}O_4$	295.227	294.22	Massbank:NA002996 [6]-Gingerol
10.98	M+H	$C_{18}H_{36}O_2$	285.279	284.27	Spectral Match to stearic acid from NIST14
3.17	M+H	$C_9H_{18}N_2O_3$	203.14	202.132	L-Alanyl-L-norleucine
0.31	M+H	$C_{12}H_{24}N_2O_7$	309.166	308.158	Fructoselysine
9.87	M+H	$C_{22}H_{43}NO$	338.341	337.33	Spectral Match to 13-Docosenamide from NIST14
3.09	M+H	$C_{18}H_{20}N_2O_5$	345.144	344.137	Tyr-Tyr
8.92	M+H	$C_{24}H_{40}O_5$	391.284	408.288	Spectral Match to Cholic acid from NIST14
9.88	M+H	$C_{39}H_{76}NO_8P$	718.574	717.57	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine from NIST14
3.44	M+H	$C_{18}H_{20}N_2O_4$	329.15	328.142	Phe-Tyr
9.06	M+H	$C_{17}H_{28}O_3$	281.211	280.2	12(S)-Hydroxy-16-heptadecynoic acid
0.68	M+H	$C_{17}H_{22}N_2O_7$	367.15	366.142	1-beta-D-Glucopyranosyl-L-tryptophan
9.94	M+H	$C_{24}H_{46}NO_8P$	552.366	551.36	Spectral Match to 1,2-Dioctanoyl PC from NIST14
3.03	M+H	$C_{11}H_{12}Cl_2N_2O_5$	323.02	322.013	Massbank: EQ307003 Chloramphenicol
8.94	M+H	$C_{26}H_{54}NO_7P$	524.371	523.363	1-Stearoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine
4.63	M+H	$C_6H_{12}O_2$	117.091	116.084	acetic_acid_isobutyl_ester
2.94	M+H	$C_{13}H_{18}N_2O_4$	267.134	266.126	Thr-Phe
9.94	M+H	$C_{44}H_{74}NO_8P$	780.553	779.55	Spectral Match to 1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycero-3-phosphocholine from NIST14
9.87	M+H	$C_{33}H_{66}NO_8P$	636.459	635.452	1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine - 40.0 eV

9.81	M+H	$C_{40}H_{76}NO_8P$	758.606	757.6	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14
0.54	M+H	$C_8H_{18}NO_6P$	258.11	257.102	SN-GLYCERO-3-PHOSPHOCHOLINE
1.05	M+H	$C_{13}H_{18}N_2O_3$	251.139	250.132	Caffeoyl putrescin
11.75	M+H	$C_{20}H_{38}O_2$	311.295	310.29	Oleic acid ethyl ester
8.35	M+H	$C_{19}H_{38}N_2O_3$	343.332	342.325	Massbank: Lauramidopropyl betaine
0.55	M+H	$C_9H_{17}NO_5$	220.118	219.111	Massbank:LU087003 Pantothenate
8.81	M+H	$C_{18}H_{32}O_3$	297.242	296.24	9(10)-EpOME
11.91	M+H	$C_{20}H_{36}O_2$	309.279	308.27	Spectral Match to Linoleic acid ethyl ester from NIST14
2.77	M+H	$C_{20}H_{21}N_3O_3$	352.165	351.158	Spectral Match to Trp-Phe from NIST14
8.72	M+H	$C_{30}H_{50}O_2$	425.378	442.381	Betulin CollisionEnergy:102040
2.27	M+H	$C_{15}H_{20}N_2O_2$	261.16	260.152	cyclo (Phe-Leu)
3.17	M+H	$C_{12}H_{10}N_4O_2$	243.088	242.081	lumichrome CollisionEnergy:205060
9.61	M+H	$C_{19}H_{39}O_7P$	410.266	409.26	Spectral Match to 1-Hexadecanoyl-2-sn-glycero-3-phosphate from NIST14
9.94	M+NH4	$C_{41}H_{82}N_1O_{12}S_1$	812.553	812.556	SQDG (16:0/16:0); [M+NH4]+ C41H82N1O12S1
0.71	M+H	$C_7H_{19}N_3$	146.107	145.1	Spectral Match to Spermidine from NIST14
8.92	M+H	$C_{24}H_{40}O_5$	391.284	408.288	Spectral Match to Cholic acid from NIST14
9.77	M+H	$C_{20}H_{39}NO_2$	326.269	325.261	Spectral Match to N-Oleoylethanolamine from NIST14
8.78	M+H	$C_{21}H_{44}NO_7P$	454.293	453.285	Spectral Match to 1-Hexadecanoyl-sn-glycero-3-phosphoethanolamine from NIST14
2.94	M+H	$C_{13}H_{14}N_2O_3$	247.108	246.1	N-ACETYL-D-TRYPTOPHAN
9.77	M+H	$C_{18}H_{34}O_2$	283.263	282.256	Spectral Match to Elaidic acid from NIST14
8.7	M+H	$C_{22}H_{46}NO_7P$	468.309	467.301	Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine from NIST14
9.61	M+H	$C_{42}H_{82}NO_8P$	760.621	759.613	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14
9.2	M+H	$C_{19}H_{38}O_4$	331.284	330.277	1-Hexadecanoyl-sn-glycerol
8.94	M+H	$C_{26}H_{54}NO_7P$	524.371	523.363	Spectral Match to 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine from NIST14
1.33	M+H	$C_9H_7NO_2$	162.055	161.048	1H-Indole-3-carboxylic acid
9.61	M+H	$C_{16}H_{30}O_2$	255.232	254.224	Spectral Match to cis-9-Hexadecenoic acid from NIST14
3.03	M+H	$C_{11}H_{12}Cl_2N_2O_5$	323.02	322.013	Massbank:EQ307003 Chloramphenicol
9.77	M+H	$C_{21}H_{40}O_4$	357.3	356.292	Spectral Match to Monoelaidin from NIST14
9.73	M+H	$C_{16}H_{33}NO$	256.263	255.256	Palmitamide
7.38	M+H	$C_{18}H_{39}NO_3$	300.29	317.293	Spectral Match to Phytosphingosine from NIST14
8.19	M+H	$C_{24}H_{50}NO_7P$	496.339	495.332	Spectral Match to Lyso-PC (16:0) from NIST14
9.5	M+H	$C_{14}H_{26}O_2$	227.201	226.193	Spectral Match to Myristoleic acid from NIST14
10.98	M+H	$C_{12}H_{27}O_4P$	267.172	266.164	Spectral Match to Tributyl phosphate from NIST14
8.4	M+H	$C_{24}H_{38}O_4$	391.284	390.277	BIS(2-ETHYLHEXYL) PHTHALATE
7.37	M+H	$C_{15}H_{26}O$	223.206	222.198	Spectral Match to NEROLIDOL from NIST14
8.78	M+H	$C_{16}H_{22}O_4$	279.159	278.152	Spectral Match to Dibutyl phthalate from NIST14
3.03	M+H	$C_{17}H_{20}N_4O_6$	377.146	376.138	Spectral Match to (-)-Riboflavin from NIST14

0.57	M+H	$C_{11}H_{22}N_2O_3$	231.17	230.163	Spectral Match to Val-Ile from NIST14
5.58	M+H	$C_{24}H_{38}O_4$	391.284	390.277	Spectral Match to Bis(2-ethylhexyl) phthalate from NIST14
8.81	M+H	$C_{12}H_{23}NO$	198.185	197.178	Spectral Match to Azacyclotridecan-2-one from NIST14
9.94	M+H	$C_{18}H_{37}NO_2$	300.29	299.282	Spectral Match to Palmitoyl ethanolamide from NIST14
9.35	M+H	$C_{42}H_{78}NO_8P$	756.59	755.582	Spectral Match to 1-Hexadecanoyl-2-octadecadienoyl-sn-glycero-3-phosphocholine from NIST14
11.91	M+H	$C_{16}H_{32}O_2$	257.247	256.24	Spectral Match to Pentadecanoic acid, 14-methyl- from NIST14
9.56	M+H	$C_{20}H_{39}NO_3$	342.3	341.293	Gly-C18:0
10.02	M+H	$C_{16}H_{32}O_2$	257.248	256.24	Spectral Match to Palmitic acid from NIST14
9.94	M+H	$C_{42}H_{84}NO_7P$	746.569	745.561	Spectral Match to 1-Hexadecyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14
9.87	M+H	$C_{22}H_{43}NO$	338.341	337.334	Spectral Match to 13-Docosenamide from NIST14
10.98	M+H	$C_{18}H_{36}O_2$	285.279	284.272	Spectral Match to stearic acid from NIST14
6.23	M+H	$C_{12}H_{14}O_4$	177.055	222.089	Spectral Match to Diethyl phthalate from NIST14
2.37	M+H	$C_{11}H_{11}NO_3$	206.081	205.074	Spectral Match to DL-Indole-3-lactic acid from NIST14
9.81	M+H	$C_{44}H_{82}NO_6PS$	784.585	783.56	Spectral Match to Arachidonoylthio-PC from NIST14