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Master Thesis

IDENTIFICATION AND BIOLOGICAL CONTROL OF MICROSCOPIC FUNGI IN LIBRARY ENVIRONMENTS

Darbo vadovė: assoc. prof. dr. Ilona, Kerienė

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CONTENT

SUMMARY

Indoor fungal community is an important determinant of indoor air quality. In a library environment, dust accumulation on densely occupied surfaces, paper-based materials, limited ventilation, and surface moisture may support fungal persistence and pose a health risk due to prolonged exposure. Therefore, this study aimed to investigate the diversity of indoor fungi in Vilnius University Šiauliai Academy Information Centre and evaluate the antifungal efficacy of essential oils and plant extract obtained from *Origanum vulgare* (common oregano) and *Artemisia annua*. Species of *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* were found to be most abundant across gravitational sedimentation, swabbing of dust, and air samples, while no significant shift in distribution was found between spring and autumn seasons. The airborne mean fungal concentration was found to be low at 2 CFU/m^3, depicting an acceptable air quality. Essential oils exhibited stronger antifungal activity compared to plant extracts. Among antifungal agents, oregano essential oil showed the strongest activity, with the highest inhibition zone of approximately 21 mm against *Cladosporium sp.*, while *Aspergillus sp.* was the most resistant, showing only weak inhibition of approximately 7 mm at the highest tested concentration of 0.75 µL/mL. Surface treatment reduced fungal recovery, with the highest reduction observed for AEO (91.67%). However, under real surface conditions, the effects were not complete fungicidal. The study concludes that the essential oils, especially oregano essential oil, was a potent solution for reducing fungal concentration with more efficacy than alcohol based cleaning agent but their application should support, not replace, preventive management such as dust control, ventilation, humidity regulation, and regular surface monitoring.

Keywords: Indoor fungal diversity, library environment, *Origanum vulgare*, *Artemisia annua*, antifungal activity.

1. INTRODUCTION

Indoor fungal contamination is an important component of indoor environmental quality because fungal spores, hyphal fragments and surface-associated growth can affect both occupants and building materials. Enclosed institutional spaces, especially libraries and archives, are vulnerable because dust, organic debris, limited ventilation and cellulose-rich materials may support fungal persistence. Elevated fungal levels indoors are associated with respiratory irritation, allergy, asthma exacerbation and deterioration of air quality. At the same time, fungal activity can damage books, paper, furniture and other organic materials. For this reason, fungal contamination in a library should be treated as both an air-quality concern and a building-management problem.

Conventional control often depends on synthetic fungicides and chemical disinfectants. These agents may act quickly, but repeated use in enclosed spaces can increase chemical residues, environmental persistence and exposure concerns. Long-term chemical pressure may also contribute to reduced fungal susceptibility. These limitations have encouraged interest in plant-derived antifungal agents that may reduce fungal growth while lowering reliance on persistent synthetic compounds.

Essential oils and phenolic extracts from medicinal and aromatic plants are widely studied because they contain terpenoids, phenolics and other secondary metabolites with antimicrobial activity. *Origanum vulgare* is especially relevant because oregano oil commonly contains thymol and carvacrol, while *Artemisia annua* contains diverse terpenoids and phenolic compounds. Both plants therefore provide suitable candidates for evaluating plant-derived antifungal activity against fungi isolated from indoor environments.

Although plant-derived compounds have been widely tested against agricultural and food-related fungi, fewer studies have examined fungi from real institutional indoor environments. Laboratory assays also do not fully represent indoor surfaces, where fungi may interact with dust, humidity, airflow, porous materials and mixed microbial communities. This study therefore combines indoor fungal assessment with laboratory antifungal testing and preliminary field surface treatment to evaluate the practical relevance of plant-derived compounds for library fungal management.

The aim of this study was to evaluate the antifungal efficacy of essential oils and phenolic extracts obtained from *Origanum vulgare* and *Artemisia annua* against fungal genera commonly detected in a library environment. The study also assessed fungal diversity using air, sedimentation and

surface swab sampling, and considered how laboratory inhibition results relate to surface treatment under indoor environmental conditions.

1.1 Research aim and objectives

The aim of the study was to evaluate the antifungal efficacy of essential oils and plant extracts obtained from *Origanum vulgare* and *Artemisia annua* against fungal genera commonly detected in the indoor environment of a library. The objectives were:

1. To investigate fungal diversity and concentration in a library using air sampling, sedimentation and surface swab sampling.
2. To prepare antifungal agents from essential oils and plant extracts of *Origanum vulgare* and *Artemisia annua*.
3. To compare the antifungal activity of these plant-derived agents against isolated fungal genera under laboratory conditions.
4. To conduct surface treatment assessment to evaluate selected plant-derived antifungal agents under real indoor environmental conditions.

1.2 Literature review

1.2.1 Indoor fungal contamination, plant-derived antifungal strategies and sustainable environmental management. Indoor fungal contamination is shaped by the interaction between fungal sources and indoor environmental conditions. Fungal spores, hyphal fragments and other particulates may enter buildings from outdoor air or originate from indoor reservoirs such as dust, damp materials, ventilation systems and colonized surfaces. Institutional buildings often combine occupancy, limited ventilation and organic substrates, creating conditions that allow fungal particles to persist and recirculate (Oliveira et al., 2023; Loukou et al., 2024).

The health relevance of indoor fungi depends on exposure level, fungal composition and individual susceptibility. Airborne and surface-associated fungi may contribute to allergic, inflammatory and opportunistic infectious responses, particularly among sensitized or immunocompromised individuals (Oliveira et al., 2023). However, fungal exposure cannot be interpreted only through simple concentration values, because abundance and diversity vary with humidity, ventilation, season, cleaning, occupancy and outdoor influx.

Fungal contamination is also an ecological and material-preservation issue. Libraries, archives and storage facilities contain paper, wood, dust and other organic materials that can provide substrates

for fungal survival. When moisture and poor airflow are present, these materials may support colonization and recurrent aerosolization. For this reason, sustainable fungal management should address both microbial growth and the environmental conditions that maintain fungal reservoirs.

Synthetic disinfectants and fungicides can reduce fungal growth, but repeated use may leave residues, increase chemical burden and contribute to ecological toxicity or reduced susceptibility (Islam et al., 2024). Plant-derived compounds offer an alternative approach because many medicinal and aromatic plants produce secondary metabolites that inhibit fungi by affecting membranes, sporulation, germination and metabolism (Leiva-Mora et al., 2025). *Origanum vulgare* and *Artemisia annua* are relevant in this context because they contain bioactive compounds associated with antimicrobial activity (Sharifi-Rad et al., 2022; Lombrea et al., 2020).

Despite this promise, plant-derived antifungal systems should not be viewed as automatically safe or universally effective. Their activity depends on concentration, extraction method, phytochemical composition, fungal susceptibility and exposure conditions. For indoor environments, the most useful evidence comes from studies that test compounds against fungi isolated from the environment where control would be applied.

1.2.2 Fungal bioaerosols and building-specific indoor ecology. Indoor environments function as dynamic microbial ecosystems rather than passive containers of outdoor air. Fungal communities are influenced by outdoor inputs, ventilation, seasonality, moisture, building materials, dust load and occupancy (Šauliene et al., 2023; Loukou et al., 2024; Adams et al., 2013). Airborne fungal bioaerosols include spores, hyphal fragments and other fungal particles that may remain suspended, deposit onto surfaces or become resuspended after disturbance. Because many fungal propagules occur in respirable size ranges, they contribute to microbial aerosol circulation and indoor particulate exposure (Martinez-Bracero et al., 2022).

Building-specific assessment is important because the same climatic region can contain buildings with very different fungal profiles. Outdoor spores may enter through doors, windows or ventilation, but indoor filters such as humidity, airflow, dust and substrate availability determine whether those propagules persist. Regionally, Lithuanian school studies reported Cladosporium and Penicillium as dominant genera, with Alternaria, Aspergillus, Fusarium, Stachybotrys and Trichoderma occurring less frequently (Šauliene et al., 2023). Such evidence provides a useful reference, but it cannot be directly generalized to libraries.

Libraries and archives differ from ordinary classrooms or offices because they contain extensive cellulose-rich materials, including books, manuscripts, paper, wood and archival documents. These materials, together with dust and restricted airflow, may support fungal persistence and biodeterioration (El Jaddaoui et al., 2023; Hiwar et al., 2021). Paper-rich environments can also favour fungi capable of surviving on organic substrates, and genera such as *Penicillium*, *Aspergillus*, *Trichoderma* and *Stachybotrys* may persist under suitable moisture conditions (El Jaddaoui et al., 2023).

The air-surface connection is especially important in libraries. Book handling, cleaning, movement of visitors and ventilation can disturb settled dust and return spores into the air. Thus, a room may show low airborne fungal concentration at one sampling moment while still containing surface reservoirs able to release fungi later. A reliable assessment therefore requires attention to both airborne particles and surface-associated contamination.

1.2.3 Environmental determinants and health relevance of indoor fungi. Indoor fungal abundance is controlled by moisture, ventilation, temperature, occupancy, dust accumulation, surface material and cleaning practice (Loukou et al., 2024). Moisture is often the strongest determinant because it supports spore germination, hyphal growth and sporulation. Elevated relative humidity, condensation and water-damaged materials can promote fungal growth on paper, wood, textiles, dust and ventilation components. Genera such as Aspergillus and Penicillium often increase under humid conditions because moisture supports growth and reproductive activity (Loukou et al., 2024).

Ventilation affects fungal dynamics by controlling air exchange, dilution of suspended particles and moisture retention. Good ventilation can reduce the persistence of airborne propagules, whereas poor airflow may allow spores and dust to remain in enclosed areas. Ventilation systems may also become reservoirs if dust or moisture accumulates in ducts, filters or grates (Al-Rikabi et al., 2024). Within a single institutional building, fungal distribution may therefore vary between floors, rooms, archive spaces and ventilation outlets.

Settled dust is another key reservoir. Dust can contain fungal spores, hyphal fragments, pollen, skin particles, fibres and organic debris. When disturbed by movement, airflow, book handling or cleaning, these particles may become airborne again (Mihucz et al., 2022). Dust is therefore not merely passive contamination; it can preserve fungal propagules and support repeated dispersal.

In libraries, long storage periods and rarely disturbed shelves may allow this reservoir to persist over time.

Substrate availability further shapes fungal survival. Paper, cardboard, adhesives, wood, textiles and dust-associated debris can provide organic material for fungi, particularly in libraries and archives (El Jaddaoui et al., 2023). Basement rooms, compact shelving and poorly ventilated repositories may be especially vulnerable because local microclimates can develop even when average room conditions appear acceptable.

Indoor fungal exposure is relevant to health because spores and fragments may be inhaled. Fungal bioaerosols have been associated with allergic rhinitis, asthma exacerbation, hypersensitivity reactions, respiratory irritation and opportunistic infection in susceptible individuals (Oliveira et al., 2023; Belizario et al., 2021). Genera such as Aspergillus, Penicillium, Cladosporium and Alternaria are often discussed in indoor air-quality research because they are common, easily aerosolized and sometimes allergenic. Nevertheless, health-risk interpretation remains complex because exposure fluctuates and culture-based sampling may not capture non-viable fragments or metabolites.

1.2.4 Fungal biodeterioration and limitations of conventional control. Fungi naturally decompose organic material by producing extracellular enzymes able to degrade cellulose, hemicellulose, lignin, proteins and related compounds. In indoor environments this ecological role can become damaging when fungi colonize books, paper, wood, textiles, wallpaper, adhesives and dust-associated organic matter (El Jaddaoui et al., 2023). In libraries and archives, fungal biodeterioration may appear as discoloration, staining, foxing, fibre weakening, odour formation and gradual loss of document quality.

Moisture accumulation, poor ventilation and dust deposition intensify this risk (Loukou et al., 2024). Dust provides organic particles and can protect fungal propagules, while localized condensation or humidity near walls, shelves and ventilation outlets can support recurrent growth. Colonized materials may also release spores or fragments into the air when disturbed, linking material damage with bioaerosol exposure.

Conventional control commonly uses chemical disinfectants or synthetic fungicides. These may be effective in the short term, but repeated application in enclosed public environments can create concerns about residues, indoor chemical burden, toxicity to non-target organisms and reduced

fungal susceptibility (Islam et al., 2024). Some disinfectants may also be inappropriate for sensitive materials such as books, archives and historical papers. Effective management therefore requires more than surface disinfection; it must also address humidity, dust, airflow and contaminated reservoirs.

Plant-derived antifungal compounds may support more sustainable control if used carefully. Their value lies not in replacing environmental management, but in complementing it by reducing viable fungal load on selected surfaces while lowering dependence on persistent chemicals. Their application in libraries must still consider surface compatibility, odour, volatility, exposure and long-term material safety.

1.2.5 Libraries and archives as fungal reservoir systems. Libraries and archives can act as fungal reservoir systems because they combine organic substrates, settled dust and long-term storage in enclosed spaces. Books, manuscripts, paper folders, wooden shelves and textiles provide surfaces where fungal propagules may settle and survive. When humidity and airflow conditions become favourable, these reservoirs may support colonization or repeated resuspension (El Jaddaoui et al., 2023; Loukou et al., 2024).

Dust accumulation is central to this reservoir function. Dust on shelves, books, storage surfaces and ventilation grates may contain spores, hyphal fragments, pollen, fibres and organic particles. It may also protect propagules until they are disturbed by cleaning, movement, book handling or ventilation (Mihucz et al., 2022). This creates an air-dust-surface cycle in which fungal particles move between suspended, deposited and surface-associated states.

The physical structure of libraries can reinforce this cycle. Basement archives, closed rooms, compact shelving and poorly ventilated corners may develop localized moisture and weak airflow. Fungal abundance may therefore be uneven, with higher contamination near walls, shelves, ventilation grates or areas with visible dampness. Because colonized books and materials may release spores, library fungal reservoirs connect biodeterioration with indoor air quality (El Jaddaoui et al., 2023).

Common indoor and cellulose-associated genera include Penicillium, Aspergillus, Cladosporium, Trichoderma and Stachybotrys, although their dominance depends on outdoor influx and indoor environmental selection (Šauliene et al., 2023; El Jaddaoui et al., 2023). Understanding these reservoirs helps determine appropriate sampling. Active air sampling measures airborne particles

during the sampling period, sedimentation reflects deposition, and swabbing identifies persistent surface reservoirs. A multimethod strategy is therefore more informative than relying on one method alone (Martinez-Bracero et al., 2022).

This reservoir perspective also affects control. Treating visible growth alone may fail if dust reservoirs, humidity problems or poor ventilation remain. Sustainable management should combine cleaning, humidity regulation, ventilation maintenance, monitoring of high-risk areas and targeted antifungal treatment where appropriate.

1.2.6 Environmental sampling methods for indoor fungal assessment. Sampling method strongly influences the interpretation of indoor fungal contamination because different methods capture different ecological fractions. No single method fully represents suspended spores, deposited particles and surface reservoirs; therefore, complementary sampling provides a more reliable picture (Martinez-Bracero et al., 2022). This matters in libraries, where fungi may occur in air, settled dust, ventilation outlets, walls, shelves and paper-based materials.

Active air sampling collects airborne particles using a controlled airflow over a defined time. Cyclone-based samplers such as the Coriolis system concentrate bioaerosols into liquid medium and are useful for assessing airborne fungal particles near the breathing zone. Their strength is standardization across rooms or seasons. Their limitation is that they represent only the sampling interval and may miss fungi stored in dust or surfaces. Recovery can also be affected by particle size, airflow rate, morphology and viability during collection.

Passive sedimentation measures gravitational deposition of airborne particles onto exposed culture media. It is simple and useful for identifying fungi that settle onto surfaces, but it mainly captures larger particles and can underestimate smaller respirable spores. Results depend on exposure time, plate position, airflow disturbance and human activity. Sedimentation data are therefore best interpreted as evidence of deposition tendency rather than a complete measure of airborne exposure.

Surface swab sampling targets settled dust and persistent reservoirs on shelves, walls, furniture and ventilation components. It is highly relevant in libraries because dust and cellulose-rich materials may support fungal persistence, and disturbance may return particles to the air (Mihucz et al., 2022). However, swab recovery depends on surface texture, pressure, sampled area and dust

load. Results may also reflect both recent and historical deposition. For this reason, swab data should be interpreted together with air and sedimentation data.

Together, these methods provide a stronger ecological interpretation. Active air sampling reflects current airborne particles, sedimentation indicates settling propagules, and swabbing reveals surface-associated reservoirs. This combined approach helps distinguish temporary airborne contamination from persistent reservoirs that require targeted management.

1.2.7 Seasonal variation and laboratory-to-field translation. Indoor fungal communities vary over time because they are influenced by outdoor spore load, humidity, ventilation, temperature, occupancy and cleaning (Šauliene et al., 2023; Loukou et al., 2024; Adams et al., 2013). Outdoor fungal particles enter buildings through air exchange, doors, windows and ventilation systems. Warmer or more humid periods may support higher fungal activity, but the direction and strength of seasonal patterns depend on building use and indoor regulation.

Seasonality can help distinguish temporary fluctuations from persistent contamination. A short-term increase in airborne fungi may reflect outdoor influx or dust disturbance, whereas repeated detection of the same genera across seasons may indicate stable indoor reservoirs. In libraries, fungi may persist in dust, books, paper substrates, basement archives and poorly ventilated rooms even when airborne concentrations fluctuate.

Seasonal conditions also affect antifungal interpretation. Laboratory assays such as MIC determination, disk diffusion and percentage inhibition of diameter growth are useful for controlled comparisons, but they often overestimate field performance because they use stable temperature, humidity, nutrient supply and single-species cultures. Real indoor surfaces are more complex, with dust, variable moisture, porous substrates, airflow and mixed microbial communities.

This gap is especially relevant for essential oils and other volatile phytochemicals. Evaporation, diffusion, adsorption to dust or cellulose and ventilation may alter exposure concentration and persistence. Fungi on surfaces may also occur in stressed or biofilm-like communities, making them less susceptible than actively growing laboratory cultures (Basak and Guha, 2018). Consequently, field or semi-field surface assessment is needed to judge whether laboratory inhibition has practical relevance in a library environment.

1.2.8 Plant-derived antifungal compounds and their ecological relevance. Plant-derived antifungal compounds are attractive because they originate from renewable biological sources and may reduce reliance on persistent synthetic chemicals. Medicinal and aromatic plants produce phenolics, terpenoids, flavonoids, alkaloids and essential oils as defence metabolites, many of which have demonstrated antifungal activity (Znini et al., 2011; Leiva-Mora et al., 2025). Their relevance to indoor management lies in both antimicrobial potential and possible compatibility with sustainability-oriented control strategies.

Many plant-derived compounds act through multiple pathways rather than a single target. They may affect membrane stability, spore germination, sporulation, enzymatic activity, oxidative balance and metabolism (Leiva-Mora et al., 2025). This broad activity may be useful against mixed indoor fungal communities, but claims about reduced resistance should remain cautious because long-term evidence under real indoor conditions is limited.

Phenolic compounds include phenolic acids, flavonoids, tannins and related aromatic compounds. Their antifungal effects are associated with changes in membrane permeability, enzyme inhibition, oxidative imbalance and suppression of germination or sporulation (Carvalho et al., 2011; Ashok and Upadhyaya, 2013; Leiva-Mora et al., 2025). In plant extracts, activity depends strongly on extraction solvent, compound concentration, chemical structure and fungal species.

Essential oils are mixtures of volatile lipophilic compounds, mainly terpenes, terpenoids and aromatic molecules produced in glandular tissues of plants (Bounar et al., 2020). Their volatility is relevant indoors because fungal propagules may encounter vapour-phase compounds as well as direct contact. Lipophilic constituents can destabilize fungal membranes, increase permeability and disrupt cellular metabolism (Chen et al., 2026). Common antifungal constituents include thymol, carvacrol, eugenol, p-cymene and related terpenoids, with reported activity against genera such as Aspergillus, Penicillium and Fusarium (Soltani et al., 2021; Tian et al., 2022).

Origanum vulgare and *Artemisia annua* are suitable candidates because they contain volatile terpenoids and phenolic metabolites associated with antimicrobial activity (Lombrea et al., 2020; Sharifi-Rad et al., 2022). However, plant-derived antifungal activity varies with genotype, growth conditions, harvesting stage, extraction method, storage stability, composition and fungal susceptibility (Bolouri et al., 2022, Makhuvele et al., 2020). Testing these compounds against fungi

isolated from real indoor environments is therefore more informative than relying only on studies with agricultural or reference strains.

1.2.9 Selection and antifungal relevance of *Origanum vulgare* and *Artemisia annua*. The selection of *Origanum vulgare* and *Artemisia annua* was based on regional availability, phytochemical potential and published antimicrobial relevance. Both plants occur in temperate regions and are recognized as medicinal or aromatic species (Gudzinskas, 1999; Alekseeva et al., 2020; Ekiert et al., 2022). Their use also fits a sustainability perspective because locally available plant resources may reduce dependence on imported chemical agents for preliminary antifungal assessment.

Origanum vulgare L. is a perennial aromatic herb in the family Lamiaceae. Its leaves and flowering parts contain glandular structures that produce volatile oils and secondary metabolites (Skoufogianni et al., 2019; Maithani et al., 2023). Oregano essential oil is commonly rich in thymol and carvacrol, compounds associated with disruption of fungal membrane integrity and permeability (Lombrea et al., 2020; Chen et al., 2026; Pizzale et al., 2002). Other constituents, including p-cymene, gamma-terpinene, linalool, phenolic acids, flavonoids and rosmarinic acid, may also contribute to antimicrobial and antioxidant activity.

Previous studies have reported inhibitory effects of oregano essential oil against environmentally relevant fungi, including Aspergillus, Penicillium and Fusarium (Bouddine et al., 2012; Bounar et al., 2020; Soltani et al., 2021). Oregano oil is often more active than crude extracts because concentrated volatile terpenoids can interact efficiently with fungal membranes and may also act through vapour phase. Nevertheless, many oregano studies use agricultural or food-associated fungi, so results cannot be assumed to apply directly to indoor library isolates.

Artemisia annua L., or sweet wormwood, is an aromatic annual species in the family Asteraceae. It contains terpenoids, flavonoids, phenolic compounds and volatile metabolites associated with ecological defence and antimicrobial activity (Sharifi-Rad et al., 2022). Although it is best known for artemisinin, its wider phytochemical profile makes it relevant for antifungal evaluation. Compared with oregano, fewer studies have focused on *Artemisia annua* against airborne indoor fungi, making its inclusion useful for comparison.

Comparing *Origanum vulgare* and *Artemisia annua* allows evaluation of two plant sources with different phytochemical profiles and different levels of published antifungal evidence. Oregano

represents a better-established essential-oil-rich species, whereas *Artemisia annua* provides a less extensively studied but chemically diverse candidate. Testing both against library-derived isolates links local plant resources with the ecological context of indoor fungal management.

1.2.10 Antifungal mechanisms of plant-derived compounds. The antifungal activity of plant-derived compounds is mainly associated with effects on fungal membranes, metabolism, reproduction and stress responses. Essential oils are particularly important because their volatile and lipophilic constituents can interact with fungal membranes. In oregano oil, thymol and carvacrol are widely discussed as major contributors to antifungal potency (Chen et al., 2026; Kowalczyk et al., 2020).

A central mechanism is disruption of membrane integrity. Lipophilic compounds such as thymol and carvacrol can interact with ergosterol-rich fungal membranes and alter permeability (Jalendiran et al., 2026). This may cause leakage of ions, proteins, nucleic acids and metabolites, impairing osmotic regulation, nutrient transport, respiration and enzyme activity (Mani-López et al., 2021; Silva-Beltran et al., 2023). Structural changes such as membrane deformation, cytoplasmic shrinkage and hyphal collapse have also been reported after essential oil exposure (Basak and Guha, 2018).

Plant-derived compounds may also inhibit fungal development by affecting metabolism and reproduction. Phenolic terpenoids may contribute to oxidative stress, while essential oil constituents can reduce spore germination, sporulation and conidial formation (Mani-López et al., 2021; Leiva-Mora et al., 2025). This is relevant indoors because spores drive dispersal, dust contamination and recurrent colonization.

Plant extracts contain more polar phenolic compounds, including flavonoids, phenolic acids and tannins. These compounds may have lower membrane penetration than lipophilic oils, but they can influence fungal physiology through enzyme inhibition, energy disruption, oxidative imbalance, metal ion complexation and interference with germination (Carvalho et al., 2011; Ashok and Upadhyaya, 2013; Silva-Beltran et al., 2023). Consequently, extracts may show weaker or slower effects than essential oils but still contribute useful antifungal activity.

Mechanistic interpretation must remain linked to environmental context. Laboratory cultures on nutrient media are more accessible to antifungal compounds than fungi established in dust, porous surfaces, paper materials or mixed surface communities. Accordingly, mechanisms observed in

vitro help explain potential activity, but they do not guarantee the same response under fluctuating humidity, airflow and surface complexity typical of institutional buildings.

1.2.11 Methodological approaches and limitations in antifungal evaluation. Standardized antifungal testing is necessary for comparing plant-derived compounds because essential oils and extracts differ in volatility, polarity, diffusion and composition. Common indicators include disk diffusion, minimum inhibitory concentration and percentage inhibition of diameter growth. Each method captures a different aspect of activity, and none alone is sufficient for predicting environmental performance.

Disk diffusion is widely used as an initial screening method. It measures the inhibition zone around treated disks and allows direct comparison among fungal isolates and treatments. However, zone size reflects not only antifungal potency but also diffusibility, volatility, medium composition, fungal growth rate and incubation conditions (Mani-López et al., 2021). This is especially important for essential oils, which may act through both direct contact and vapour-phase effects.

MIC determination identifies the lowest concentration that visibly inhibits fungal growth under defined laboratory conditions (Van de Vel et al., 2019). It is useful for comparing relative potency and selecting concentrations for further testing, but results depend on inoculum density, fungal growth stage, medium, solvent, incubation time and endpoint interpretation. MIC should therefore be viewed as a laboratory threshold rather than a direct field application dose.

Percentage inhibition of diameter growth measures reduction in radial colony expansion relative to untreated controls (Kadium et al., 2023). This is particularly useful for filamentous fungi because it captures partial growth suppression as well as complete inhibition. Together, disk diffusion, MIC and growth-inhibition measurements provide complementary evidence of antifungal response.

Laboratory assays remain limited because they usually use stable temperature, humidity, nutrient media and single-species cultures. Indoor fungi may instead persist on dust, ventilation surfaces, paper, walls and mixed microbial communities. Such surface-associated or biofilm-like growth may be less susceptible than actively growing cultures (Basak and Guha, 2018). Plant-derived compounds may also behave differently on indoor substrates because oils can evaporate or adsorb to surfaces, while extracts may penetrate poorly into porous or dust-covered materials.

1.2.12 Laboratory and field indicators of antifungal performance. Laboratory and field indicators should be interpreted together because they measure different dimensions of antifungal performance. MIC values identify inhibitory thresholds under controlled conditions (Van de Vel et al., 2019), while disk diffusion indicates comparative susceptibility and compound spread through agar (Mani-López et al., 2021). Percentage inhibition of diameter growth provides information on partial mycelial suppression and concentration-dependent response (Kadium et al., 2023).

Field surface treatment provides a different type of evidence. Real indoor surfaces contain dust, uneven texture, variable moisture, mixed microbial communities and established fungal colonies. These conditions influence how a compound spreads, persists, evaporates or contacts fungal structures. A compound that produces a large inhibition zone in vitro may not fully suppress growth on a porous wall or dust-covered surface. Conversely, a treatment with modest laboratory inhibition may reduce culturable fungal load if it remains stable on the treated surface.

This distinction is important for libraries because fungi may persist on walls, shelves, ventilation grates, books and dust reservoirs rather than as uniform laboratory colonies. Surface-associated fungi may be more tolerant due to limited nutrients, environmental stress and mixed-community interactions. Reduction in CFU after treatment indicates reduced culturable abundance, but it does not prove complete removal or permanent prevention of recolonization. Surviving spores, residual hyphae, untreated dust and persistent moisture may allow regrowth.

For indoor fungal management, field treatment should therefore be considered one component of a broader strategy. Laboratory assays identify promising agents, while surface assessment shows whether the activity remains useful under environmental conditions. The most defensible approach combines controlled testing with practical measures such as dust control, ventilation maintenance, humidity regulation and periodic monitoring.

1.2.13 Culture-based fungal identification and its limitations. Culture-based identification remains useful in indoor fungal studies because it allows viable propagules to grow on nutrient media for enumeration, observation, isolation and antifungal testing. This is especially important when the study aims not only to detect fungi but also to obtain living isolates for disk diffusion, MIC determination and growth-inhibition assays. Common indoor genera such as *Penicillium*, *Aspergillus, Cladosporium, Alternaria* and *Trichoderma* can often be recognized at genus level using colony colour, texture, growth pattern, hyphal morphology, conidiophore structure and spore arrangement (Šauliene et al., 2023; Oliveira et al., 2023).

The main strength of culture-based methods is that they link detection with biological activity. Viable isolates are directly relevant to colonization potential and antifungal susceptibility. In libraries, culturable fungi are important because viable propagules may recolonize dust, walls, shelves, ventilation outlets and paper-based materials. Culture-based sampling also enables comparison across locations and seasons if procedures are standardized.

However, culture-based methods do not represent the complete fungal community. Not all fungal propagules are viable, and not all viable fungi grow under the selected medium, temperature or incubation period. Fast-growing fungi may overgrow slower taxa, while dormant or nutritionally specialized fungi may remain undetected. Thus, culture-based results usually represent the dominant culturable fraction rather than total fungal diversity (King et al., 2020; Cox et al., 2020).

Quantitative interpretation also requires caution. A colony may originate from one spore, a cluster of spores or a hyphal fragment. CFU values should therefore be interpreted as indicators of culturable abundance rather than exact counts of all fungal particles. Non-viable spores and fragments may still contribute to bioaerosol exposure, meaning that culture-based methods can underestimate total fungal material (Martinez-Bracero et al., 2022; Naik and Zabłocka-Godlewska, 2026).

Morphological identification has limited taxonomic resolution. Many fungi can be identified to genus level, but species-level identification may be uncertain without molecular methods. Molecular approaches can detect non-culturable fungi and provide finer resolution, but they do not automatically provide viable isolates for antifungal testing. In the context of this study, culture-based and microscopic methods are appropriate because viable isolates were required, but the

findings should be interpreted as representing dominant culturable and microscopically detected fungi rather than the full fungal community.

1.2.14 Research gap and thesis positioning. The reviewed literature shows that indoor fungal contamination is an ecological and environmental concern linked to airborne particles, surface reservoirs, material biodeterioration and indoor environmental quality. Libraries and academic information centres are specialized indoor ecosystems because they contain cellulose-rich materials, dust reservoirs, restricted ventilation zones and possible microclimatic variation. These factors can support fungal persistence and deterioration of paper-based materials (El Jaddaoui et al., 2023; Loukou et al., 2024).

Plant-derived antifungal compounds, especially essential oils and phenolic-rich extracts, may offer an alternative to persistent synthetic disinfectants because they act through mechanisms such as membrane disruption, metabolic interference, oxidative stress and inhibition of sporulation or germination (Leiva-Mora et al., 2025; Gupta et al., 2023). Their practical effectiveness should not be assumed, however, because it depends on concentration, composition, extraction method, fungal susceptibility, exposure conditions and surface context.

Four gaps are particularly relevant. First, many indoor fungal studies focus on residential buildings, schools, healthcare environments or moisture-damaged structures rather than academic libraries. Second, many antifungal studies use agricultural, food-associated or reference strains rather than fungi isolated from real indoor environments. Third, essential oils and crude extracts are often studied separately rather than compared against the same isolates. Fourth, laboratory inhibition results are rarely connected with realistic surface treatment under indoor conditions.

This thesis addresses these gaps by investigating fungal diversity in a library environment and evaluating *Origanum vulgare* and *Artemisia annua* essential oils and extracts against indoor fungal isolates. By combining air, sedimentation and swab sampling with laboratory antifungal assays and field surface assessment, the study is positioned at the intersection of indoor fungal ecology, library environmental management and sustainable plant-derived fungal control.

1. METHODOLOGY

2.1 Selection of the study area and sampling criteria

The selected study area was the indoor area of Vilnius University Šiauliai Academy Information Centre (Figure 1), Vytauto gatve 84, Šiauliai Municipality, 76351, Lithuania (N 55˚55’47.41”, E 23˚18’48.715”). It represented an enclosed institutional indoor environment where fungal spores may accumulate in air and on surfaces. The library environment contained large amounts of organic and cellulose-based materials, such as books, paper, shelves, furniture, and surfaces for dust deposition, which might support fungal persistence under favorable environmental conditions. In addition, regular human movement, limited ventilation, and long-term storage of materials might influence the distribution of airborne and surface-associated fungi. Therefore, the library provided a suitable environment for investigating indoor fungal diversity, fungal concentration, and the potential use of plant-derived antifungal agents under environmentally relevant conditions.

Figure 1. Vilnius University Siauliai Academy Information Centre

Three sampling methods were chosen for a comprehensive assessment of fungal genera, conducted in two seasons in 2025: spring and autumn. Indoor fungi might occur in different forms, including airborne spores suspended in the air, spores that settled onto surfaces over time, and fungal propagules present on indoor surfaces. As an active sampling method, air samples were taken through Coriolis Cyclone Air Trap for sampling (n=18) the airborne biological elements in the air at a inhalation height. Secondly, fungal deposition was collected by sedimentation (n=45) on nutrition media plates as a passive gravitational method. And thirdly, fungal deposition in dust samples (n=57) was collected by swabbing to determine surface-associated fungal contamination.

Locations were chosen considering places with most human traffic, surfaces prone to dust accumulation, air filtration system and objects with visible mold growth (Figure 2). Total sample number was 120 in each season. During the spring sampling, the artificial heating and ventilation system was running (although it was a practice to open a number of windows to let in additional air flow); and in autumn, natural ventilation prevailed. The temperature was maintained at 19˚C, while relative humidity was kept at 35% on average. The cleaning solution used by the library was ethanol-based.

Figure 2. Sampling locations from indoor environment

2.1.1 Coriolis Cyclone Air Trap. Collection of indoor air samples: Coriolis Cyclone Air Trap (A) was used for drawing air at a high flow rate of 250 litres per minute for a standardized pumping duration of 10 minutes. A conical bottle (B) filled with 15 ml of sterile distilled water was used for each sampling where bio-particles would be collected. The bottles were installed on the trap before running. The air trap was placed on about 1.5 metres height from the floor at approximately human breathing level. After sample collection, each solution was filtered using vacuum filtration equipment (C) with a 0.45 μm cellulose nitrate membrane filter (D). Then each filter containing particles was mounted on a microscope slide (E) and cover with a coverslip (F) for stability. Two slides were prepared. The workstations and equipment should be disinfected before and after handling to prevent contamination. Light microscope was used to identify and count colony-forming units (CFU) of fungal spores or bioaerosols. For the identification of microscopic fungal spores, the Color Atlas of Fungal Spores (2023) was used as a reference. The estimation of bioaerosol concentration was done by the number of colonies formed per cubic metre of air, CFU/ m3. For the calculation, the formula of Carvalho et al. (2008) was applied. Bioaerosol concentration, CFU/ m^3 = K / FV Where:

K = number of microscopic fungi on the filter in units

F = pumping time, min

V = intake of air volume, m^3/min [1 litre = 0.001 m3]

2.1.2 Sedimentation on nutrient media: Collecting sedimentation from indoor air samples were done by sedimentation on nutrient medium, incubating and analyzing the colonies grown by light microscopy. Potato Dextrose Agar (PDA) medium was prepared in petri dishes where sedimentation would occur to support colony growth. For preparation, 18 g of PDA was mixed with 1 litre of distilled water and then it was sterilized by autoclaving for 2.5 hours. 3 ml of 20 % citric acid was added to the cooled medium. The medium was put in the petri dishes to ready them for sampling. Prepared petri dishes were kept on the flat surface on chosen spots. The lids were open and uncovered petri dishes were exposed to air for 10 min. After the exposure for 10 min, the petri dish lids were closed and taped with parafilm. Then they were put inside a thermostat at 24±1 °C for incubation. Wait time was 5 days for the incubation. After 5 days, the petri dishes

were taken out to observe if molds grew on the surface of the nutrient medium. The number of molds were recorded under a light microscope. The genera of the microscopic fungi was determined with the species composition. The concentration of fungal spores in the air was calculated. A maximum of 150 CFU of fungi should be counted in a Petri dish. If more colonies are present, the result should be reported as " >150 CFU/plate".

2.1.3 Dust deposition swabbing from surfaces. The general procedure consists of using a sterile cotton swab dipped in distilled water to collect the dust from a 10 cm^2 portion of the selected site, delimited by a paper stencil, and subsequently depositing the material on a Petri dish with a Potato Dextrose Agar medium. The Petri dishes were marked according to the site sampled, type of procedure, and initials of the researchers. The Petri dishes were then wrapped in parafilm and deposited in a thermostat at the temperature of 24 degrees Celsius for incubation, for a period of 5 days. The cultures were then analyzed under a microscope in order to identify the fungal diversity present in the air of the selected study sites, and results were recorded in an Excel table. The number of viable particles on the sedimentation surface of a sediment sample is expressed as the number of microorganisms deposited per unit time, 1 dm^2 /hour (1 dm^2 = 100 cm^2).

CFU = (number of microscopic fungi deposited on the plate/surface area of the medium πR^2 expressed in dm^2) / exposure time, h.

1) The area formula πR^2 was used to calculate the exact area of the medium on the Petri dish in dm^2. A 90 mm Petri dish had a medium area of 86 mm, so the deposition area = $\pi R^2 = 3.14 \times (86/2)^2 = 5805.86$ $mm^2 = 0.58$ dm^2.

2) Calculated the number of spores in an area of 1 dm^2 by proportion.

3) Calculated how many spores are deposited in this area for a sedimentation time of 10 minutes. The minutes are converted into hours. The results are expressed in units.

Coriolis cyclone air trap

Sedimentation on nutrient media

Swabbing dust deposition from surfaces

Figure 3. Sampling methods

2.2 Preparation of plant-derived anti-fungal agents

2.2.1 Collection of plant material. *Artemisia annua* and *Origanum vulgare* were selected due to their abundance in the research locality. Aerial plant materials were collected from VU Šiauliai Academy Botanical Garden (N 55˚55’57.76”, E 23˚16’58.711”) at the full flowering stage (*O. vulgare* in April and *A. annua* in September). After collection, the fresh plant materials were chopped into small pieces using a knife for extraction. On the other hand, for making essential oil, fresh plant materials were oven dried.

Artemisia annua

Origanum vulgare

Figure 4. Plant materials for preparing anti-fungal agents

2.2.1.1 Extraction of essential oil. Essential oils were extracted from dried plant material of *Origanum vulgare* and *Artemisia annua* by hydro-distillation using a Clevenger-type apparatus. Approximately 80 g of dried plant material was placed in a 2000 mL round-bottom flask and mixed with sufficient water to fully immerse the plant material. The mixture was hydro-distilled for 4 to 4,5 hours. The plant and water ratio was approximately 1 : 8 w/v. After distillation, the essential oil layer was separated from the hydrosol and collected in dark glass bottles. The extracted oils were stored under refrigerated conditions until further use. They were mixed with Dimethyl Sulfoxide (DMSO) for preparing the stock solution.

2.2.1.2 Plant extract preparation and calculation of total phenolic compound. Freshly cut plant part (small pieces) was weighted 2.5 g and mixed with 25ml of 75% methanol (extractant) solution. The weight of the plant sample and the extractant ratio was 1:10 (for example, 1g: 10 mL). Samples were prepared in plastic tubes in four replicates. Tubes were shaken in an ultrasound water bath shaker at 40C temperature for 0.5 h. After that, the mixture was filtered through a 0.22 – 0.45 µm membrane filter.

A series of 05 standard solutions of Rutin in a concentration range of 1.50 mg/ml, 0.75 mg/ml, 0.375 mg/ml, 0.187 mg/ml and 0.0935 mg/ml were prepared. The Rutin reagent was dissolved in 75% methanol solution. The standard solutions were labelled. Next, 7.9 ml of distilled water was added to each flask. 100 µl of plant extract were added to each and mixed. Then, 500 µl of Folin-Ciocalteu reagent was added and mixed. The samples were kept for about 6 minutes and 1.5 ml of 20% sodium carbonate solution were added. A blank sample was also prepared without the plant extract. All samples were incubated in dark for 50-60 minutes, periodically stirring.

To measure the optical density of the samples at ~750 nm, they are poured into a cuvette with 10nm thickness of light path. The maximum absorption of light is recorded. At first, measurements of the blank sample were taken. Then, the standard solution of known concentrations was run from the lowest value. Finally, samples with plant extract were ran in the spectrophotometer and all absorbance values were recorded.

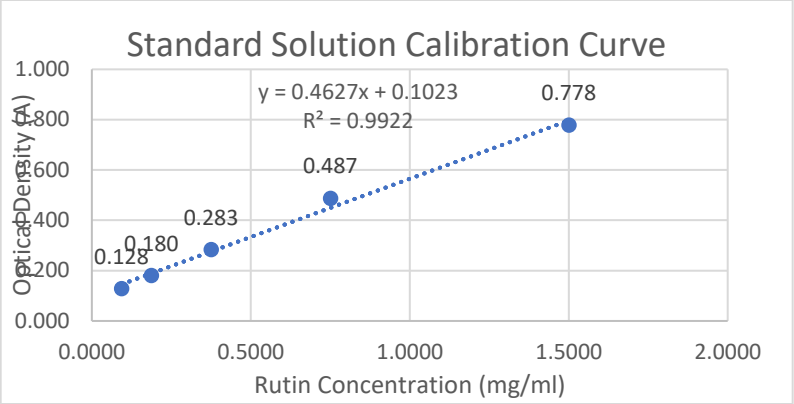


Figure 5: Calibration curve for the standard working solution

Based on the measurement results of the standard solutions, a calibration curve was drawn while obtaining the line equation, $y = kx \pm b$. The parameters of the linear equation were used to calculate the total phenolic compounds (TPC) in the plant extract, expressed in mg/ml. Knowing the amount of phenolic compounds c (mg/mL), the volume of the extract V (mL) and the mass of the sample m (g), TPC in the mass of the plant expressed in mg/g were calculated. $TPC = (c \times V)/m$ [mg/g] . From the TPC values, the absolute (%) and random errors (standard deviation, relative standard deviation) were calculated.

2.3 Anti-fungal tests

2.3.1 Fungal strains isolation and agar dilution. Both of the extracted phenolic compounds and essential oil were screened for anti-fungal activity against the fungal array found. Mycelium was separated from each identified species and grown on Potato Dextrose Agar (PDA) media for pure culture. To determine the lowest antifungal agent concentration (minimal inhibitory concentration, MIC) at which there was a visual absence of growth, the agar dilution method was used. 03 Different concentrations of essential oil (0.25, 0.50 and 0.75 µL/mL) and plant extract (25, 50 and 75 µL/mL) were prepared and incorporated in liquid PDA media. Media with Dimethyl Sulfoxide (DMSO) and 75% methanol served as the negative control for all tests. After adding the antifungal agents to the liquid PDA, the medium was mixed thoroughly and poured into sterile petri plates to solidify. 05 mm plugs of pure culture was cut and placed on the treated solid plates. They were incubated in the dark for 05 days at 24°C. Minimum inhibitory concentrations (MICs) were determined from the visible inhibition of mycelial growth.

2.3.2 Disk diffusion and measurement of inhibition zone. Conidia suspension from young culture was made in a sterile saline solution, and turbidity was adjusted to 530 nm. The suspension was made from adopting the 0.5 McFarland standard (McFarland, 1907). 100 µL of suspension was 80 spread thoroughly on each plate (03 replicates for each concentration), air-dried at 60°C until 81 evaporation of residual water, Sterile filter paper discs (05 mm diameter) were infused with 20 µL of each concentration, placed on PDA plates inoculated with fungal suspension and incubated for 05 days. For calculating the average values and standard deviations (SD) from the test repeated three times, were recorded.

2.3.3 Surface treatment with anti-fungal solutions. Areas showing fungal contamination were selected and marked using a stencil to obtain comparable treatment surfaces. For each treatment concentration, five replicate areas were prepared. The selected plant-derived antifungal solutions were sprayed uniformly to the marked wall surfaces, while control areas were maintained for comparison. The solvent control was included to distinguish the antifungal effect of the plant-derived compounds from any possible effect of the solvents used for preparation. The control solution consisted of DMSO and methanol without essential oil or plant extract respectively, for all tests in the study. Control areas were treated in the same way as the treatment areas, using the same surface area, application method, and exposure conditions. After treatment, the wall surfaces were left for 05 days under normal indoor environmental conditions. Surface samples were collected from each marked area using sterile cotton swabs and transferred for analysis. The fungal growth recovered from treated and control areas was compared to assess the antifungal performance of each concentration. Where fungal colonies were reduced after treatment, the percentage reduction was calculated using the following formula:

Reduction percentage = {(control count - treatment count) / control count}×100

2.4 Data analysis

The research used both descriptive and inferential statistics. Fungal diversity was described with the measure of mean by sampling method and seasonality. Seasonal differences between autumn and spring were tested using the Mann–Whitney U test, as the data were non-normally distributed and the two seasonal groups were independent. The test was ran separately for three sampling methods, therefore, to avoid a false positive result, the Bonferroni adjustment

of p=value was made. Results were reported using median, interquartile range, test statistic, and p-value, with Bonferroni-adjusted p-values calculated for multiple comparisons.

For fungal samples, values were aggregated at the sample level before analysis to avoid pseudo-replication. Quantitative analysis of total phenolic compounds from plants required plotting of absorbance vs. concentrations in reagent. Differences in inhibition values were analyzed for each treatment condition (i. e., mean of inhibition ±SD in different concentrations). After surface treatment, fungal unit reduction percentage (if any) was calculated. Microsoft Excel was used for data organization, preliminary calculations, and graph preparation, while RStudio was used for non-parametric statistical testing and selected visualizations. The codes for RStudio was written with AI assistant (ChatGPT, 2026) (Appendix 3)

3. RESULTS AND DISCUSSION

3.1 Airborne fungal diversity in the library

The fungal abundance and composition assessed by this study found 11 fungal genera (microscopic identification given in Appendix 1). The average units of fungi are displayed by sampling method and seasonality in Figure 1.

In both seasons, swabs taken from surface dust captured the highest diversity (09 genera identified among the autumn samples), where *Penicillium*, *Aspergillus* and *Cladosporium* were the most prevalent (concentration mean ranged from 2 - 4 CFU/Plate). *Stachybotrys* and *Rhizopus* were only found in autumn, while Trichoderma was present only in the spring samples. The cyclonic air trap captured *Acremonium*, *Alternaria*, *Botrytis* and *Aspergillus* as the most abundant (mean concentration up to 2 CFU/m^3). Gravitational deposition by sedimentation on plates had the lowest diversity and found mostly contained species of *Penicillium*, *Aspergillus*, *Alternaria* and *Acremonium*, while their mean concentration ranged up to 20 CFU/dm^2/h.

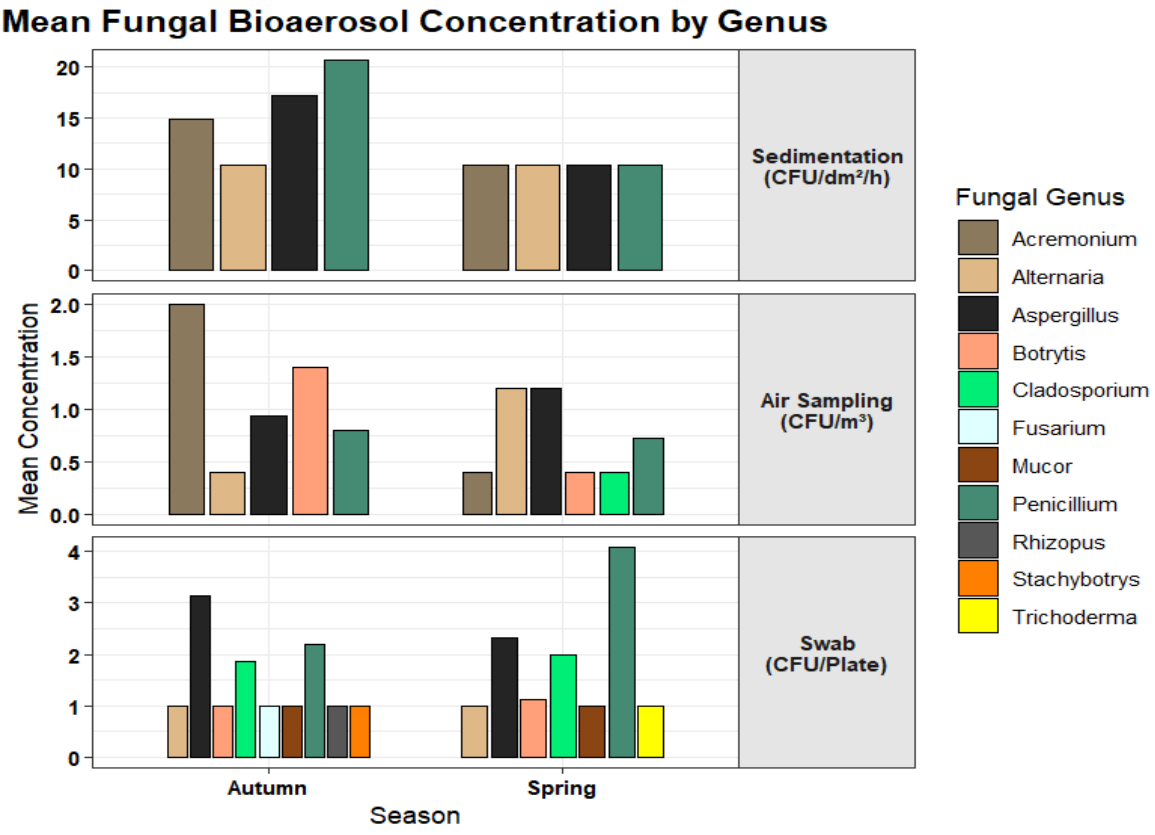


Figure 6. Fungal genera identified from indoor samples

The Mann-Whitney test compared the fungal concentration between autumn and spring and showed no significant seasonal variation after the Bonferroni correction (values given in Appendix 2; adjusted p-values ranged from 0.109 to 1.000). A Bonferroni adjustment was applied because seasonal differences were tested separately for the three sampling methods. Among the samples taken by the sedimentation method, a major portion had no detectable fungal growth. Therefore, both autumn and spring had a median of 0. The effect size, r = 0.182, was small. After Bonferroni correction, the adjusted p-value becomes 0.1392, and no shift was detected in the central tendency of fungal concentration.

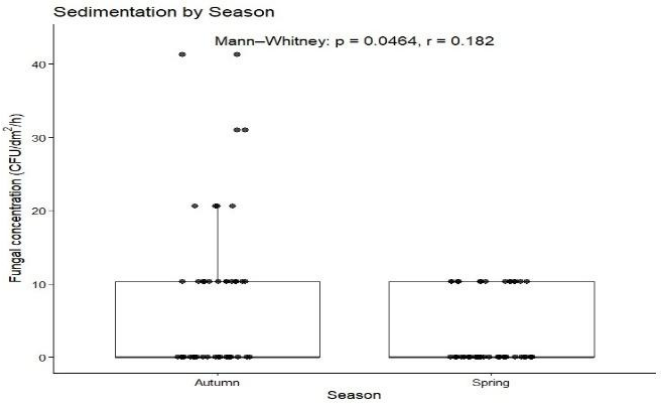


Figure 7: Fungal concentration in sedimentation samples during autumn and spring

The test showed a higher tendency for airborne fungal concentration in autumn than in spring (effect size, r = 0.34). However, it was statistically insignificant after Bonferroni correction.

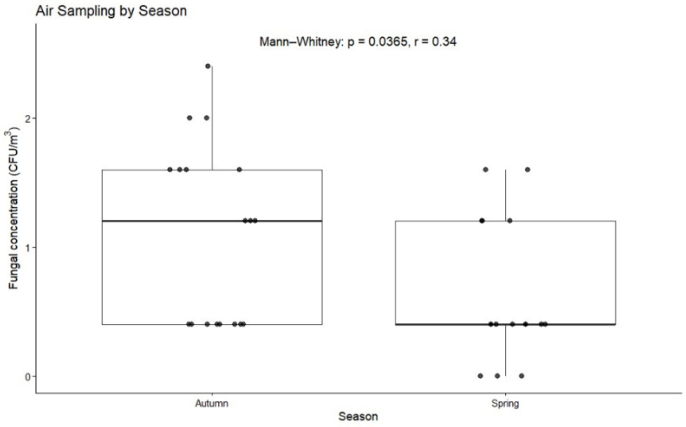


Figure 8: Fungal concentration in air samples during autumn and spring

The swab results also showed no strong evidence that surface-associated fungal counts differed between autumn and spring. Spring had a slightly higher median than autumn, but the difference was not statistically significant with a p-value of 0.4312, far above 0.05, and the effect size is negligible.

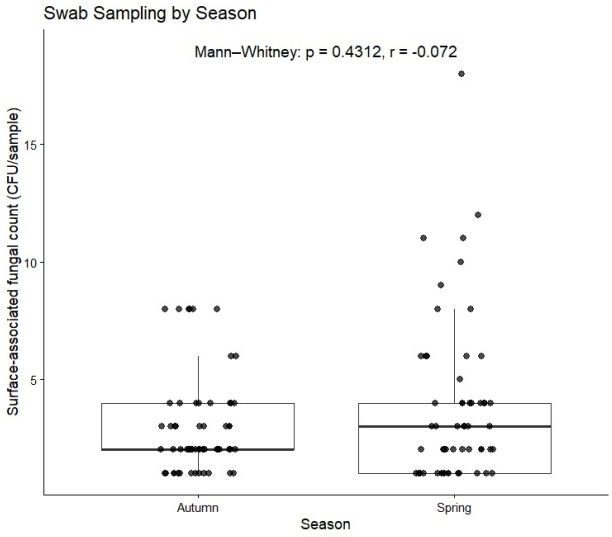


Figure 9: Fungal concentration in swab samples during autumn and spring

Overall, the results did not differ in diversity across the two seasons. The results suggested that air sampling and sedimentation showed possible seasonal tendencies (higher autumn values), but none remained statistically significant. It may be associated with comparatively lower human traffic, limited air flow, and stagnant environmental conditions in the library, where humidity and temperature were kept similar throughout the year by means of artificial heating and ventilation. Based on the results of indoor fungal diversity, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* were identified as the most prevalent and representative genera within the studied indoor environment. These genera were consistently detected across locations and seasons, indicating their potential impact on indoor air quality. Therefore, these fungal strains were selected for isolation and evaluation of the inhibitory effects of plant extracts and essential oils against these selected fungi.

3.2 Antifungal agents

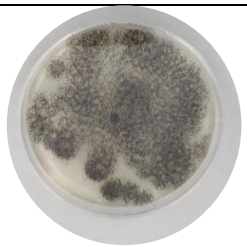
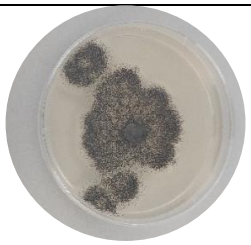
From the material of *Origanum vulgare* (common oregano) and *Artemisia annua* (sweet wormwood), plant extract (PE) and essential oil (EO) were prepared to be used against the fungal

genera. The essential oils from oregano (OEO) and wormwood (AEO) were obtained with a yield of 0.75% and 0.60% (w/w), respectively, based on the dry weight of the plants. This corresponds to approximately 133 g of dried oregano and 167 g of dried wormwood required to obtain 1 mL of essential oil, with a density of 1 g/ml. The total phenolic content (TPC) calculation showed that oregano contained a higher phenolic content compared to wormwood. In oregano extract (OPE), the average value of TPC was 118.1±5.2. Wormwood extract (APE) exhibited considerably lower TPC with an average value of 69.1±1.9 mg/g.

3.3 Determination of minimum inhibitory concentration (MIC)

Against the 04 fungal genera, MIC of the antifungal agents was determined, at which there was a visual absence of growth detected. The inhibition varied depending on the strain. The growth of *Penicillium sp.* and *Cladosporium sp.* was stopped at the lowest OEO concentration. *Alternaria sp.* needed a moderate concentration for inhibition, while *Aspergillus sp.* was inhibited at the higher concentration of 0.75 µL/mL.

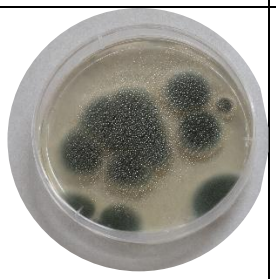
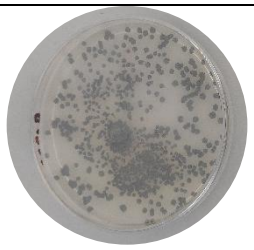
Figure 10 : Comparison of MICs using OEO against 04 fungal strains

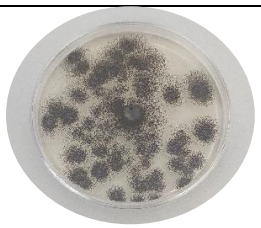
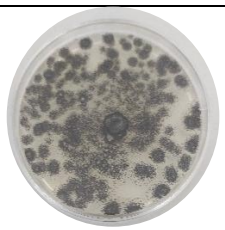
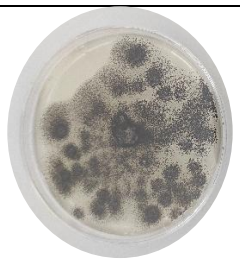
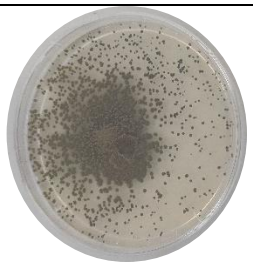
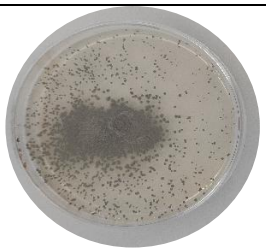
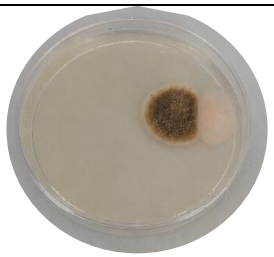
		OEO concentrations (µL/mL)		
	Control	0.25	0.50	0.75
Penicillium sp.			-	-
Aspergillus sp.				

Cladosporim sp.			-	-
Alternaria sp.				-

On the other hand, oregano plant extract (OPE) exerted no inhibitory effect at the lower concentrations of 25 µL/mL and 50 µL/mL across all tested fungal strains. The inhibitory threshold for OPE was reached only at the maximum tested concentration of 75 µL/mL for the majority of the strains. *Penicillium sp*., *Cladosporium sp*., and *Alternaria sp*. all exhibited an MIC at 75 µL/mL. However, *Aspergillus sp.* remained entirely resistant to the extract throughout the testing range, showing no inhibition even at the highest concentration.

Figure 11: Comparison of MICs using OPE against 04 fungal strains

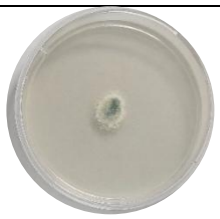
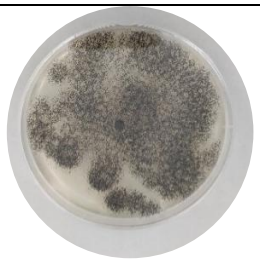
		OPE concentrations (µL/mL)		
	Control	25	50	75
Penicillium sp				

Aspergillus sp.				
Cladosporium sp.				
Alternaria sp.				

Again, broth dilution assay results for AEO at different concentrations demonstrated selective antifungal activity, with stronger inhibition against *Penicillium* sp. and *Alternaria sp.*, weaker inhibition against *Cladosporium sp.*, and no observable effect against *Aspergillus sp.*

Figure 12: Comparison of MICs using AEO against 04 fungal strains

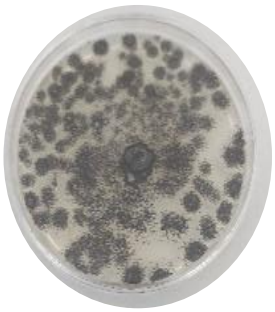
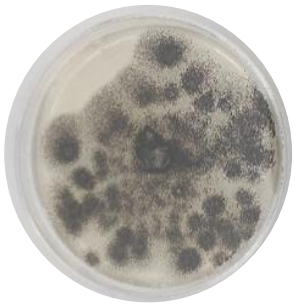
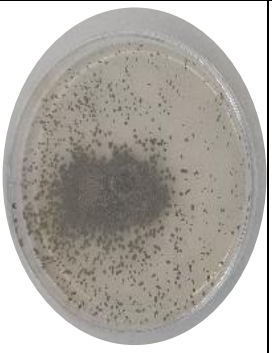
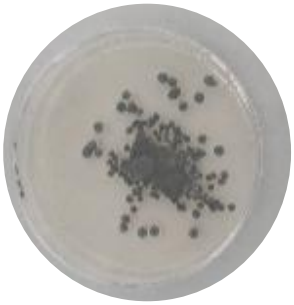
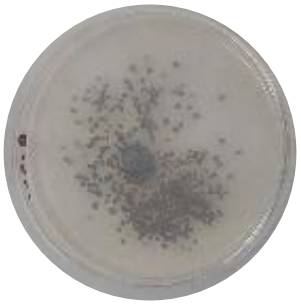
		AEO concentrations (µL/mL)		
	Control	0.25	0.50	0.75

Penicillium sp.				-
Aspergillus sp.				
Cladosporim sp.				
Alternaria sp.				-

APE displayed a more limited antifungal spectrum compared to OPE. While only *Penicillium sp.* showed sensitivity 75 µL/mL, its growth did not stop completely. The rest of the fungi had no inhibitory effect to APE at any of the tested levels (25–75 µL/mL).

Figure 13: Comparison of MICs using APE against 04 fungal strains

		OPE concentrations (µL/mL)		
	Control	25	50	75

Penicillium sp				
Aspergillus sp.				
Cladosporium sp.				
Alternaria sp.				

Overall, *Aspergillus sp.* was the most resistant strain, resisting all but the highest concentration of OEO. *Cladosporium sp.* and *Alternaria sp*. showed a selective sensitivity, being more susceptible to oregano-derived solutions rather than that of wormwood. Plant extract from

both oregano and wormwood had weaker efficacy and all fungi growth remained resistant to the wormwood extract solutions.

3.4 Determination of fungal inhibition zone

The disk diffusion assay was used to evaluate antifungal activity by measuring inhibition zones around treated disks. Among the agents, OEO exhibited the highest overall potency, achieving a peak inhibition diameter of approximately 21 mm against *Cladosporium sp*. at the maximum concentration of 0.75 µL/mL, followed closely by AEO, which recorded an inhibition zone of approximately 13 mm against the same genus.

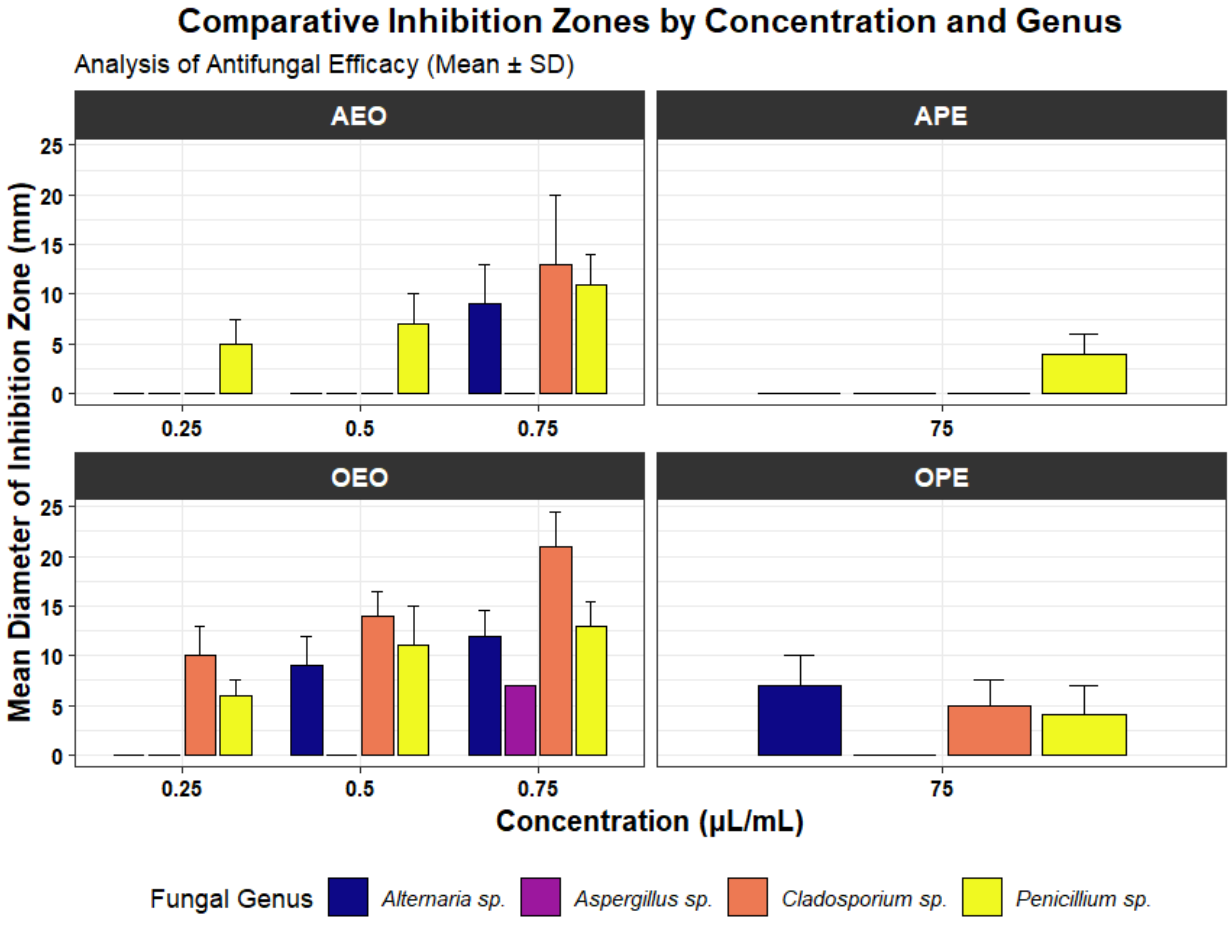


Figure 14: Diameter of growth inhibition zones of fungal strains from disk diffusion

Again, *Aspergillus sp*. appeared to be the most resistant genus across most treatments, frequently showing zero or minimal inhibition zones except at the highest concentration of OEO, where a

subtle response of approximately 7 mm was recorded. The efficacy of OPE and APE remained comparatively lower and more restricted; OPE showed a moderate effect on *Alternaria sp.* (7 mm) and *Cladosporium sp.* (5 mm), while APE demonstrated a limited inhibitory effect primarily on *Penicillium sp.* (4 mm) at its specified concentration. The standard deviation bars suggest that the consistency of inhibition differed between treatments and fungal isolates.

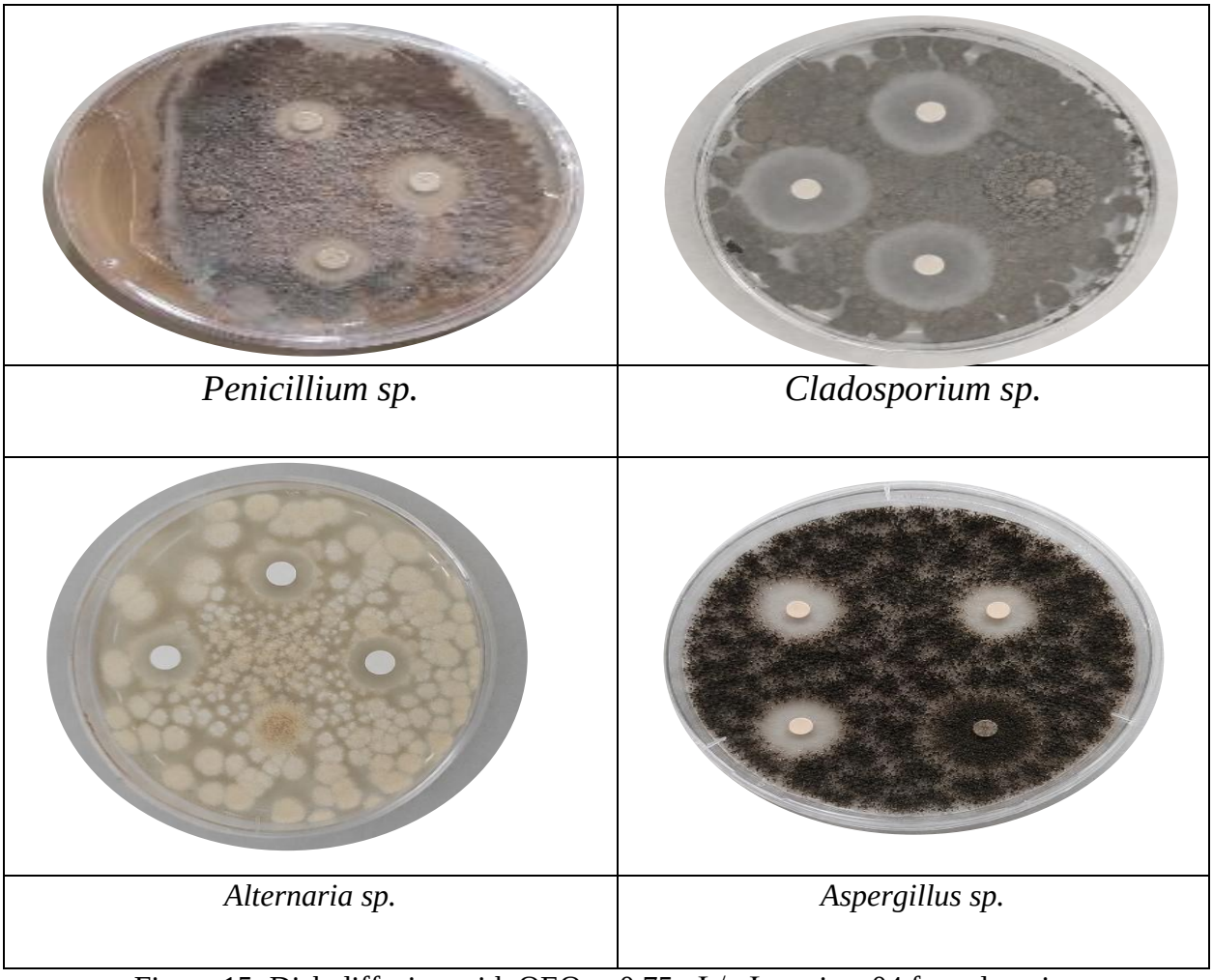


Figure 15: Disk diffusion with OEO at 0.75 µL/mL against 04 fungal strains

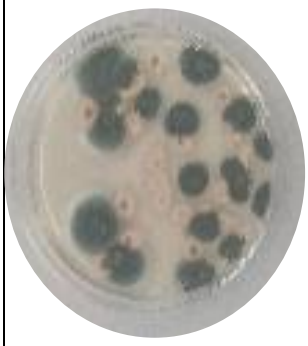
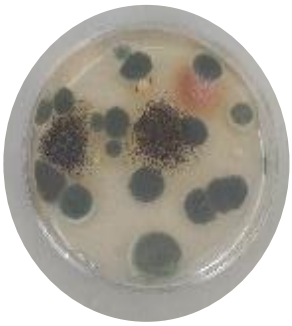
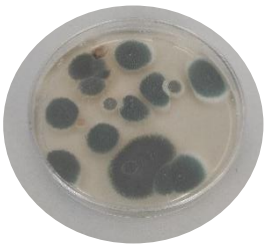
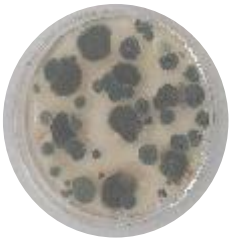
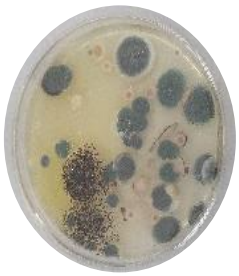
3.5 Outcome of anti-fungal treatment on indoor surfaces

Following the determination of MICs for growth inhibition, the efficacy of the antifungal agents *Origanum vulgare* essential oil (OEO), *Artemisia annua* essential oil (AEO), and their respective plant extracts (OPE and APE) was evaluated on indoor surfaces with visible fungal growth.

Table 1: **Reduction in fungal concentration (CFU/Plate) 05 days after surface treatment**

Antifungal Agent (μL/mL)	Average CFU/Plate (before treatment)	Average CFU/Plate (after treatment)	Reduction (%)
OEO 0.25	18	7	61.1
OEO 0.50	12	3	75.0
OEO 0.75	9	2	77.8
AEO 0.50	17	3	82.4
AEO 0.75	12	1	91.7
OPE 75	14	2	85.7
APE 75	25	9	64.0

Even though not fungicidal, both plant-derived essential oil solutions had a decreasing effect on surface fungal presence (average reduction percentage from 75% to 91.7%). In case of plant extract, OPE proved considerably more effective than APE, achieving an 85.7% reduction compared to 64.0%, respectively.

			
Control plate	Sample taken before treatment with OEO	Control plate	Sample taken after treatment with OEO
			

	Sample taken before treatment with AEO		Sample taken after treatment with AEO

Figure 16: Reduction in fungal load fafter surface treatment with essential oils (0.75 (µL/mL)

Overall, the results suggest that AEO 0.75 µL/mL and OPE 75 were the most effective treatments under the tested surface conditions.

3.5 Discussion

Standards depicting a good quality indoor air varied across regions and were prescribed as total airborne microbial concentration rather than fungal only. While WHO (2009) warned that the total concentration of biological agents should not exceed 1,000 CFU/m^3, Capitelli (2009) recommended a stricter standard of 300 CFU/m^3 as the maximum limit for fungal concentration. According to this study, the fungal concentrations were found to be very low, suggesting good air quality inside the library. Indoor fungal genera varied widely depending on the use of the environment, sampling methods and locations. Baudet et al. (2021) used a Coriolis cyclonic liquid air sampler in healthcare facilities located in France, which resulted in *Alternaria, Aspergillus, Cladosporium, Eurotium, Penicillium,* and *Rhodotorula.* Lu et al. (2021) used conical inhalable samplers inside apartments and townhouses of Copenhagen and detected *Acremonium, Aspergillus, Chaetomium, Cladosporium, Penicillium, Rhizopus, Trichoderma, Ulocladium* and *Wallemia.* Šauliene, et al. (2023) studied the indoor environment of schools in Lithuania and found fungal quantity and diversity across seasons, locations, ventilation system, outdoor influence, human activity etc. Although the library fungal diversity resonated with Šauliene et al. (2023), it differed to find a significant correlation with seasons. Šauliene et al. (2023) found Acremonium (average concentration higher in spring) was found mostly in passive gravitational air samplings and Cladosporium, Penicillium (average concentration higher in autumn-winter) in swabs samplings.

Several studies reported that *Origanum vulgare* and *Artemisia annua* had strong antifungal activity, particularly their essential oils. Kocić-Tanackov et al. (2012) reported complete inhibition of *Penicillium sp.* using oregano extract at 2.50 mL/100 mL, while Rus et al. (2016) found that visible growth of *Penicillium aurantiogriseum* was inhibited at 0.5 mg/L, although a higher concentration of 5 mg/L was required for complete fungicidal effect. Similarly, Felšöciová et al.

(2020) reported MIC values of 0.125–0.75 µL/mL against *Penicillium sp.*, Hrytsyk et al. (2021) found that Artemisia vulgaris extract produced inhibition against *Aspergillus niger* with a zone of 9.92 ± 0.98 mm, but did not completely stop mycelial growth or conidial formation. Therefore, among the common fungi in the library, *Aspergillus spp.* may be difficult to fully inhibit using plant extracts or essential oils, especially while full mycelial and conidial growth.

Townsend and Levetin (2005) evaluated in vitro antifungal activity of *Melaleuca alternifolia* (tea tree) oil in various concentrations against several indoor fungal genera, including *Aspergillus niger, Aspergillus versicolor, Stachybotrys chartarum, Alternaria sp., Cladosporium sp.,* and *Penicillium sp.* The research suggested a concentration of 1.0% to exhibit total growth inhibition of all species tested. However, only with 5.0% spray application on building materials (ceiling tile and sheet rock squares) was all growth successfully inhibited. Though studies are limited, the results of indoor surface conditions indicated a significant divergence from laboratory conditions. While the in vitro agar dilution assays achieved a total visual absence of fungal growth, treatment of naturally contaminated indoor surfaces may result only in a quantitative reduction of fungal load rather than complete eradication. The presence of airborne spores in a non-sterile environment, which prevents the complete elimination of Colony Forming Units (CFUs). The results reflected the complexity of real indoor surface conditions. Laboratory assays are valuable for initial screening because they allow controlled comparison between treatments, concentrations, and fungal genera. Further research should also examine different treatment concentrations, exposure times, repeated applications, and long-term persistence of antifungal effects after treatment.

These findings suggest that antifungal efficacy determined through MIC and disk diffusion assays may not fully predict performance within complex indoor environmental systems. Laboratory assays provide important preliminary evidence regarding antifungal activity under standardized conditions; however, real indoor environments contain heterogeneous surfaces, fluctuating humidity and airflow conditions, particulate accumulation, and mixed microbial communities that may alter compound diffusion, persistence, and fungal susceptibility. In addition, fungal growth within indoor environments may occur as structured surface-associated colonies rather than actively growing planktonic cultures typically used in laboratory assays. Consequently, the reduced and variable antifungal performance observed during field treatment likely reflects the

ecological complexity of real indoor environments compared with simplified laboratory testing systems.

4. CONCLUSIONS

1. A total of 11 fungal genera were identified in the library's indoor environment, with *Penicillium sp*., *Aspergillus sp*., *Cladosporium sp*., and *Alternaria sp*. representing the most frequently detected genera across sampling locations and seasons. Surface swab sampling revealed the highest fungal diversity, indicating that dust and indoor surfaces acted as important reservoirs of fungal propagules. The fungal concentrations detected from all sampling methods suggested library air quality was generally acceptable in terms of fungal load during the study period. Seasonal variation in fungal abundance was not detected.

2. Among the tested plants, *Origanum vulgare* produced higher essential oil yield and greater total phenolic content than *Artemisia annua*. Essential oils from both plants demonstrated stronger antifungal activity than plant extracts under laboratory conditions. Oregano essential oil produced the largest inhibition zones among all tested treatments and showed comparatively strong inhibitory activity against several indoor fungal genera.

3. *Penicillium sp*. and *Cladosporium sp.* were the most susceptible to the essential oil-based treatments in controlled conditions, while *Aspergillus sp.* was the most resistant strain in mycelial growth. Antifungal potency was dependent on both the agent utilized and the inherent susceptibility of the target fungal genus.

4. In vivo performance showed a reduction of fungal concentration at surfaces. Field surface treatment oil resulted in a measurable reduction of fungal contamination on affected indoor wall surfaces, although antifungal performance under environmental conditions was lower than that observed in laboratory assays. This difference highlights the influence of environmental variability, surface complexity, and fungal growth conditions on practical antifungal effectiveness in real indoor environments. However, the efficacy was not fungicidal in a controlled environment. For the successful formulation of botanical fungicides, concentrations of plant-derived agents should be tested at indoor environment. The results indicated that antifungal activity observed against isolated fungal strains under laboratory conditions should be interpreted as preliminary evidence only. Before plant-derived compounds can be recommended for indoor fungal management, their efficacy should be validated on real contaminated surfaces under environmentally relevant conditions.

5.1 Recommendations. The findings of this study suggested that plant-derived essential oil solutions were potent for fungal management inside the library environment. Recognizing fungi as one of the biological components regulating air quality, their long-term persistence should be reduced. The traditional ethanol-based cleaning solutions can be supplemented with solutions that can reduce fungal load from surfaces. Diffusers can be tested for their efficacy in reducing airborne concentration. However, this fungal control should not be a standalone method but should be combined with dust removal from the surfaces, humidity and air flow regulation, and periodic fungal monitoring. These approaches together can reduce fungal persistence over time.

5.2 Limitations of the study. Several limitations of this study should be acknowledged. First, the analysis did not separately compare fungal diversity or concentration among individual microenvironments across floor levels, rooms, user zones or specific sampling locations in the library. Second, fungal identification was performed only to the genus level. It did not determine species-level identity and limits the health-related interpretation of the results, as different species within the same genus may differ in allergenic potential, toxin production and environmental tolerance, and susceptibility to antifungal treatments. Future studies should include location-based analysis and species-level identification using molecular or advanced morphological methods. Third, influential environmental parameters such as relative humidity, temperature, ventilation rate, air movement, and dust accumulation were not monitored throughout the study. Therefore, the study could not fully explain the environmental drivers of fungal variation.

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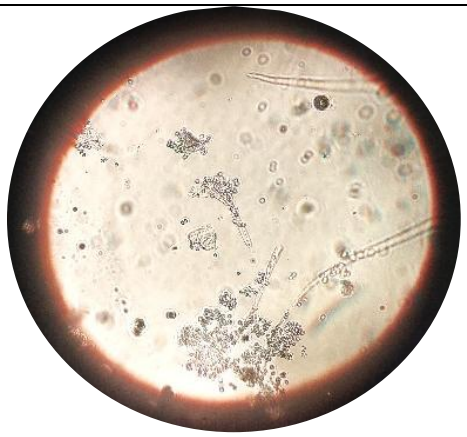
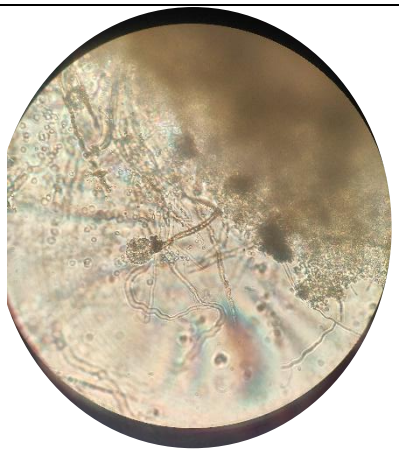
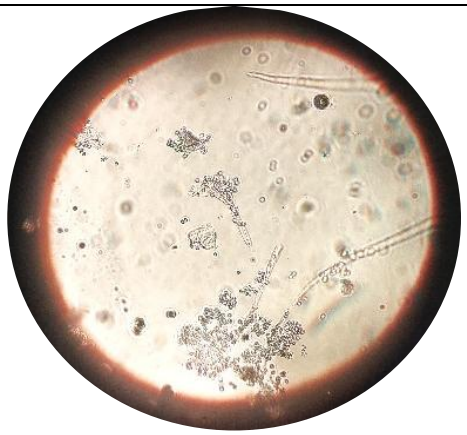
APPENDICES

Appendix 1

Microscopic identification of 11 fungal genera diversity in the library

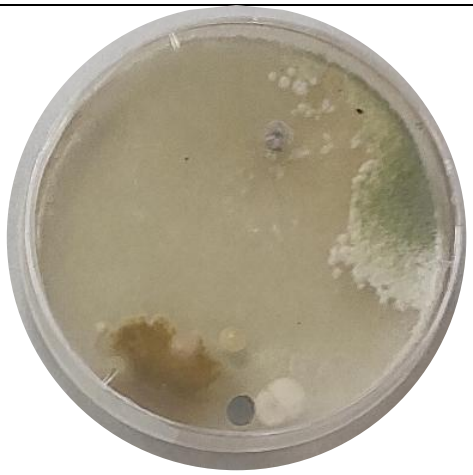
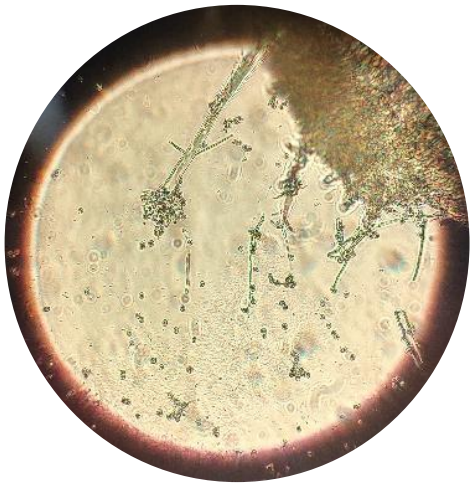
	Fungal growth	Microscopic identification
Penicillium sp.		

	Fungal growth	Microscopic identification
Aspergillus sp.		

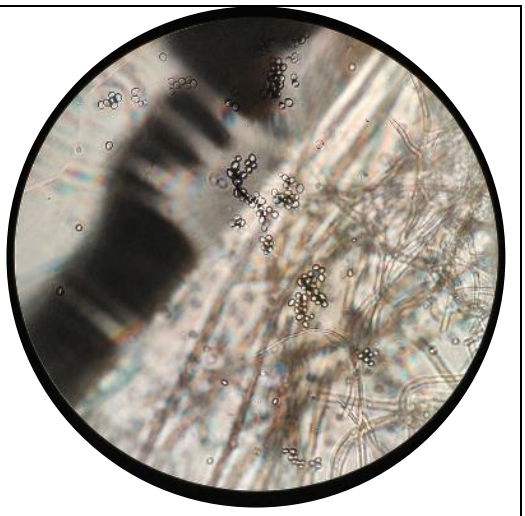
	Fungal growth	Microscopic identification
Cladosporium sp.		

	Fungal growth	Microscopic identification
Alternaria sp.		 

	Fungal growth	Microscopic identification
Rhizopus sp.		

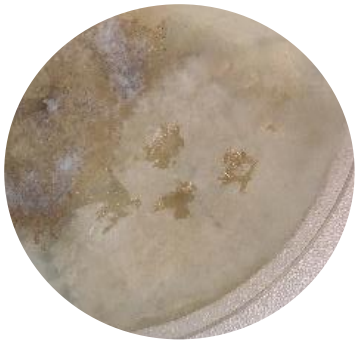
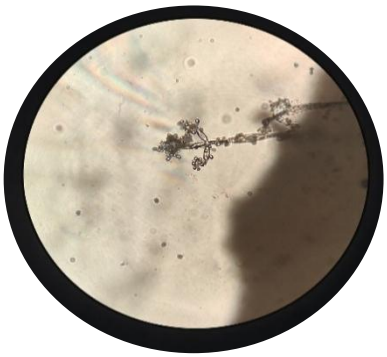
	Fungal growth	Microscopic identification
Trichoderma sp.		

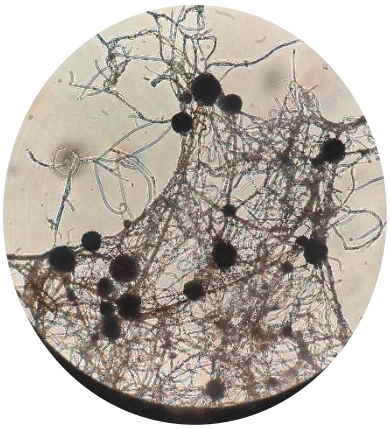
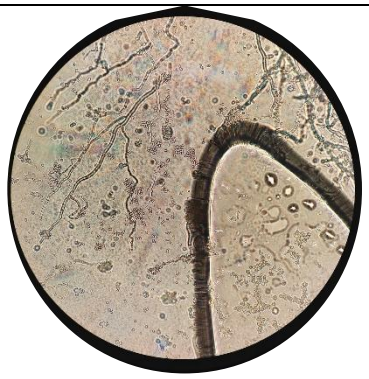
	Fungal growth	Microscopic identification

Stachybotris sp.		

	Fungal growth	Microscopic identification
Fusarium sp		

	Fungal growth	Microscopic identification

Botritis sp.		

	Fungal growth	Microscopic identification
Mucor sp.		
Acremonium sp.		

Appendix 2

Mann-Whitney test values generated by R Studio

	Method	Autumn_n	Spring_n	Autumn_Median_IQR	Spring_Median_IQR	W_statistic	Z_value	P_value	Effect_size_r	Effect_size_magnitude	Bonferroni_P	Raw_p_interpretation	Bonferroni_interpretation
1	Air Sampling	18	16	1.2 (IQR: 0.4–1.6)	0.4 (IQR: 0.4–1.2)	201.5	1.984	0.0365	0.340	moderate	0.1095	Significant before correction	Not significant after Bonferroni correction
2	Swab	57	57	2 (IQR: 2–4)	3 (IQR: 1–4)	1488.0	-0.774	0.4312	-0.072	negligible	1.0000	Not significant	Not significant after Bonferroni correction
3	Sedimentation	45	45	0 (IQR: 0–10.32)	0 (IQR: 0–10.32)	1226.0	1.723	0.0464	0.182	small	0.1392	Significant before correction	Not significant after Bonferroni correction

Appendix 3

R Studio codes written with artificial intelligence assistance

Fungal data analysis

```
library(readxl)
library(dplyr)
library(ggplot2)
library(stringr)
# ==============================
# FILE READ
# ==============================
autumn <- read_excel("C:/Users/uchac/OneDrive/Desktop/ Final_Analysis /Autumn Fungal Data 2025.xlsx")
spring <- read_excel("C:/Users/uchac/OneDrive/Desktop/ Final_Analysis /Spring Fungal Data 2025.xlsx")
# ==============================
# CLEAN FUNCTION (FINAL)
# ==============================
clean_data <- function(df){
  names(df) <- make.names(names(df), unique = TRUE)
  names(df) <- str_trim(names(df))
   num_cols <- names(df)[sapply(df, is.numeric)]
  value_col <- num_cols[which.max(sapply(df[num_cols], mean, na.rm = TRUE))]
```

```
  df <- df %>%
   rename(Value = all_of(value_col))
  if("Fungul.Genus" %in% names(df)){
   df <- df %>% rename(Fungus = Fungul.Genus)
  }
  if("Unit.of.Bioaerosol.Concentration" %in% names(df)){
   df <- df %>% rename(Unit = Unit.of.Bioaerosol.Concentration)
  }
  df <- df %>%
   mutate(
     Value = as.numeric(Value),
     Fungus = str_trim(Fungus),
         # Name corrections
     Fungus = case_when(
       Fungus %in% c("", NA, "None") ~ "Other/None",
       Fungus == "Aspergillu" ~ "Aspergillus",
       Fungus == "Bortytis" ~ "Botrytis",
       TRUE ~ Fungus
     )
   ) %>%
   filter(Fungus != "Other/None", Value > 0)
  return(df)
}
# ============================
# MERGE & FINAL DATA
# ============================
plot_data <- bind_rows(
  clean_data(autumn),
  clean_data(spring)
)
final_plot_data <- plot_data %>%
```

```
  group_by(Season, Unit, Fungus) %>%
  summarise(
    Mean = mean(Value, na.rm = TRUE),
    .groups = "drop"
  )
# ==============================
# CUSTOM LABELS
# ==============================
unit_labels <- c(
  "CFU/dm²/h" = "Sedimentation\n(CFU/dm²/h)",
  "CFU/m³"    = "Air Sampling\n(CFU/m³)",
  "CFU/Plate" = "Swab\n(CFU/Plate)"
)
# ==============================
# CUSTOM COLORS
# ==============================
fungus_colors <- c(
  "Penicillium" = "aquamarine4",
  "Aspergillus" = "gray14",
  "Cladosporium" = "springgreen2",
  "Alternaria" = "burlywood",
  "Acremonium" = "navajowhite4",
  "Botrytis" = "lightsalmon1",
  "Fusarium" = "lightcyan1",
  "Mucor" = "saddlebrown",
  "Stachybotrys" = "darkorange1",
  "Trichoderma" = "yellow"
"Rhizopus" = "gray34"
)
# ==============================
# PLOT
```

```
# ==============================
p <- ggplot(final_plot_data,
            aes(x = Season,
                y = Mean,
                fill = Fungus)) +

  geom_bar(
    stat = "identity",
    position = position_dodge(width = 0.7),
    width = 0.6,
    color = "black"
  ) +

  facet_grid(
    Unit ~ .,
    scales = "free_y",
    labeller = as_labeller(unit_labels)
  ) +

  scale_fill_manual(values = fungus_colors) +

  labs(
    title = "Mean Fungal Bioaerosol Concentration by Genus",
    x = "Season",
    y = "Mean Concentration",
    fill = "Fungal Genus"
  ) +

  theme_bw() +

  theme(
```

```
    # Facet label formatting
    strip.text.y = element_text(
      size = 9,
      face = "bold",
      angle = 0,
      hjust = 0.5
    ),

    strip.background = element_rect(fill = "gray90"),

    # Plot margins
    plot.margin = margin(10, 50, 10, 10),

    # Axis text
    axis.text = element_text(
      face = "bold",
      color = "black"
    ),

    # Plot title
    plot.title = element_text(
      hjust = 0.5,
      face = "bold",
      size = 14
    )
  )

# ==============================
# SHOW PLOT
# ==============================
```

Code for inhibition zone

```
# ============================
#  LIBRARIES
# ============================
library(readxl)
library(dplyr)
library(ggplot2)
library(stringr)

# ============================
#  FILE READ
# ============================
file_path <- "C:/Users/uchac/OneDrive/Desktop/ Final_Analysis /Inhibition Zone.xlsx"
data <- read_excel(file_path)

# ============================
#  DATA PREPARATION
# ============================
data_clean <- data %>%


  filter(!`Anti-fungal Agent` %in% c("AEO", "APE", "ApO")) %>%

  mutate(

    # X-axis
    Concentration = as.factor(`Concentration (µL/mL)`),

    # fungal species clean
    Fungal_Species = str_trim(`Fungal Species`),

    # genus extract
    Fungus = word(Fungal_Species, 1),

    # numeric conversion
    Mean_Zone = as.numeric(`Mean Inhibition Zone (mm)`),
    SD = as.numeric(`Standard Deviation (SD)`),

    # antifungal agent
    Agent = str_trim(`Anti-fungal Agent`)
  ) %>%

  mutate(
```

```
  # spelling correction
  Fungus = case_when(
    Fungus %in% c("Aspergillu", "Aspergilius", "Aspergillus") ~ "Aspergillus",
    Fungus %in% c("Bortytis", "Botrytis") ~ "Botrytis",
    TRUE ~ Fungus
  ),

  # SD missing 0
  SD = ifelse(is.na(SD), 0, SD)
) %>%

# remove NA mean values
filter(!is.na(Mean_Zone))

geom_bar(
  stat = "identity",
  position = position_dodge(width = 0.8),
  width = 0.7,
  color = "black"
) +

# Error bar
geom_errorbar(
  aes(
    ymin = Mean_Zone,
    ymax = Mean_Zone + SD
  ),
  position = position_dodge(width = 0.8),
  width = 0.25,
  color = "black"
) +

facet_wrap(~Agent, scales = "free_x") +

scale_fill_manual(
  values = fungus_colors,
  name = "Fungal Genus"
) +

labs(
  title = "Comparative Inhibition Zones by Concentration and Genus",
  subtitle = "Analysis of Antifungal Efficacy (Mean ± SD)",
  x = "Concentration (µL/mL)",
  y = "Mean Diameter of Inhibition Zone (mm)"
) +
```

```
# Sedimentation data
sed_data <- sample_totals %>%
  filter(`Sampling Method` == "Sedimentation")

# Sedimentation result
sed_result <- results %>%
  filter(Method == "Sedimentation")

# Label for plot
sed_label <- paste0(
  "Mann–Whitney: p = ", sed_result$P_value,
  ", r = ", sed_result$Effect_size_r
)

# Position of label
sed_y <- max(sed_data$total_value, na.rm = TRUE) + 2

# Plot
sed_plot <- ggplot(sed_data, aes(x = Season, y = total_value)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(width = 0.15, height = 0, alpha = 0.7, size = 2) +
  annotate("text", x = 1.5, y = sed_y, label = sed_label, size = 4) +
  labs(
    title = "Sedimentation by Season",
    x = "Season",
    y = expression("Fungal concentration (CFU/dm"^2*"/h)")
  ) +
  theme_classic()

print(sed_plot)

ggsave(
  "C:/Users/uchac/OneDrive/Desktop/ Final_Analysis /Sedimentation_by_Season.png",
  plot = sed_plot,
  width = 7,
  height = 5,
  dpi = 300
)

air_data <- sample_totals %>%
  filter(`Sampling Method` == "Air Sampling")
```

```
# Air result
air_result <- results %>%
  filter(Method == "Air Sampling")

# Label for plot
air_label <- paste0(
  "Mann–Whitney: p = ", air_result$P_value,
  ", r = ", air_result$Effect_size_r
)

# Position of label
air_y <- max(air_data$total_value, na.rm = TRUE) + 0.2

# Plot
air_plot <- ggplot(air_data, aes(x = Season, y = total_value)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(width = 0.15, height = 0, alpha = 0.7, size = 2) +
  annotate("text", x = 1.5, y = air_y, label = air_label, size = 4) +
  labs(
    title = "Air Sampling by Season",
    x = "Season",
    y = expression("Fungal concentration (CFU/m"^3*")")
  ) +
  theme_classic()

print(air_plot)

ggsave(
  "C:/Users/uchac/OneDrive/Desktop/Final_Analysis /Air_Sampling_by_Season.png",
  plot = air_plot,
  width = 7,
  height = 5,
  dpi = 300
)

# Swab data

swab_data <- sample_totals %>%
  filter(`Sampling Method` == "Swab")

# Swab result
swab_result <- results %>%
  filter(Method == "Swab")

# Label for plot
swab_label <- paste0(
```

```
  "Mann–Whitney: p = ", swab_result$P_value,
  ", r = ", swab_result$Effect_size_r
)

# Position of label
swab_y <- max(swab_data$total_value, na.rm = TRUE) + 1

# Plot
swab_plot <- ggplot(swab_data, aes(x = Season, y = total_value)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(width = 0.15, height = 0, alpha = 0.7, size = 2) +
  annotate("text", x = 1.5, y = swab_y, label = swab_label, size = 4) +
  labs(
    title = "Swab Sampling by Season",
    x = "Season",
    y = "Surface-associated fungal count (CFU/sample)"
  ) +
  theme_classic()

print(swab_plot)

ggsave(
  "C:/Users/uchac/OneDrive/Desktop/ Final_Analysis /Swab_Sampling_by_Season.png",
  plot = swab_plot,
  width = 7,
  height = 5,
  dpi = 300
)
```