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Investigation on the Population, Diversity, and Antifungal Resistance Genes of Aeroterrestrial Microfungi and Soil Quality at the Main Gate of an Academic Environment

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ABSTRACT

Aeroterrestrial fungi play diverse roles in ecological niches, especially in the soil, contributing to the decomposition of organic matter, nutrient cycling, and suppression of plant pathogens and have implications in public health. This study investigated the population, diversity, and antifungal resistance genes of aeroterrestrial microfungi and soil quality at the Main Gate of Rivers State University, Port Harcourt as to assess the impact of microfungi at the university main entrance. Seventy two (72) air and soil samples were collected over a period of twelve months and analyzed using standard mycological techniques. Findings revealed that the highest, lowest, and mean fungal counts in the soil were 6.6 Log_{10} CFU, 4.6 Log_{10} CFU and 5.6 Log_{10} CFU respectively, while fungal counts of air were 3.8 Log_{10} CFU/min-M^2, 3.1 Log_{10} CFU/min-M^2 and 3.5 Log_{10} CFU/min-M^2 respectively. Seasonal counts of air for dry and rainy seasons were 3.7 Log_{10} CFU/min-M^2 and 3.4 Log_{10} CFU/min-M^2 respectively, while those of soil were 5.8 Log_{10} CFU/g and 5.3 Log_{10} CFU/g respectively. Fungi isolated were *Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Aspergillus oryzae; Collectotrichum fructicola*, *Fusarium proliferatum, Fusarium solani Microsporium canis, Mucor ramosissimus,* and *Penicillum chrysogenum*. They belong to physiological categories of dermatophytic, pathogenic, and saprophytic fungi which are mostly implicated in human health conditions. Antifungal susceptibility tests revealed that some isolates were resistant against four categories of fungal drugs namely; nystatin, fluconazole, ketoconazole and griseofulvin. PCR amplification and molecular detection of genes revealed the presence of ERG3, CDR1 and CDR2 resistance genes. There is therefore a need to monitor aeroterrestrial quality of the area in order to ensure safety of inhabitants, and persons transiting into the university.

Keywords: Air, soil, dermatophyes, pathogens, *Alternaria*, PCR, resistance genes, antifungal drugs.

Introduction

Microfungi are diverse groups of eukaryotic organisms deficient in chlorophyll and obtain their carbon from other sources. They include yeasts, rusts, smuts, mildews, and molds. Microfungi can be cultured in mycological media, where they display a wide range of coloration and textures. They are found in all types of habitats and are often overlooked. Some microfungi are beneficial, while others are harmful to human health, both indoors and outdoors (Khan and Karuppayil, 2012). According to Jacoby *et al*. (2017), soil is a particularly rich medium and habitat for the growth and proliferation of fungi and other organisms. It is also notable for its physiological and nutritional diversity, supporting the growth, development, and fruiting of terrestrial flora.

Since microfungi ar about the most common and widespread microorganisms in soil, it acts as a true reservoir for fungi (Rane and Gandhe, 2006; Dellagi *et al*., 2020).

Aerosols, dusts, spores, and other inorganic particles, including fungal spores, are also present in the air. Most infectious diseases suffered by plants, animals and humans are caused by airborne fungi. A significant source of fungal contamination and infections in these related environments is outdoor air, which has a significant impact on the prevalence of fungal spore levels in indoor air in places such as classrooms, offices, laboratories, hospitals, conference rooms, greenhouses, and auditoriums (Masoomeh *et al*., 2014).

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Various human activities in the sampling area, combined with poor upkeep, building designs, and erratic automobile traffic, can lead to unfavorable negative repercussions such as epidemiological problems (Manisalidis *et al*., 2020). Microfungi are beneficial in many fields, including industry, medicine, and agriculture, and they also play a role in biogeochemical recycling, biotransformation, bioremediation, and the removal of pollutants and wastes from the environment, all of which contribute to sustainable development (Vasishta *et al*., 2016; Obire *et al*., 2008; Ataikiru *et al*., 2018). However, fungal spores often produced in dusty surface soils can also float in the air and cause a number of fungus-related human diseases and illnesses. Fungal infections pose an escalating environmental and public health threat, necessitating contextual interventions to curb or halt their spread (Kohler *et al*., 2015; Manisalidis *et al*., 2020).

The aim of the study was to carry out an investigation on the population, diversity, antifungal drug resistance genes of aeroterrestrial microfungi and soil quality at the Main Gate of the Rivers State University**.** The objectives included enumeration, isolation, classification, and identification of fungi from samples of the air and soil in this area using standard mycological techniques, as well as determining antifungal drug resistance fungi and related genes, physicochemical properties of the soils (such as the availability of nutrients) and their relationship to fungal populations.

Materials and Methods

Study Area

The study location was the Nkpolu-Oruworukwo Main Gate of Rivers State University, Port Harcourt, Nigeria. The Main Campus of Rivers State University is situated in the Niger Delta region of Southern Nigeria, falling within the Port Harcourt City Local Government Area of Rivers State. Geographically, its coordinates are 4.7965° N and 6.9806° E. This location shares comparable ecological and environmental characteristics with Port Harcourt, the capital of Rivers State. The surrounding area includes Ikwerre Road, Agip Oil Company, Eagle Island, and the Mile 2 Diobu axis in Port Harcourt, Nigeria.

The surrounding area of the Nkpolu-Oruworukwo Main Gate of Rivers State University features sandy soil thinly covered in grass. It experiences consistent maintenance, including grooming, and sees a high volume of vehicle and pedestrian traffic. The university campus itself has a large population due to the concentration of academic, administrative, commercial, and religious activities on-site.

Sample Collection and Media Preparation

Soil samples were collected with the aid of a sterile hand held auger from a depth of 10 to 15 cm, from six (6) designated substations in the Main Gate environment at monthly intervals for a period of twelve (12) calendar months, from July 2021 to June 2022, covering both the wet and dry seasons. The samples were collected into sterile plastic zip-lock bags and transported the laboratory for analysis. Samples from the 6 substations were properly mixed together to form a composite samples. A total of 72 composite soil samples (each weighing about 50 grams) were using for analysis. Air samples were collected using sedimentation plate method using six (6) mycological media plates, totaling 72 plates for a period of 12 months.

All media were prepared according to manufacturer's instructions and sterilized in an autoclave at 121^{o}C for 15 minutes at 15 pounds per square inch (psi). Pipettes and other glassware were sterilized in a hot-air oven at 160^{o}C for 30 minutes. Drying of prepared culture media plates were dried in hot air oven at 70^{o}C for 10 minutes. About 75% alcohol was used to sterilize laboratory benches.

Mycological Analysis of Soil and Air Samples

Mycological analysis was carried out in the Microbiology Laboratory of the Department of Microbiology, Rivers State University, Port Harcourt. Media used for mycological analysis were Sabouraud Dextrose agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). Each medium was prepared according to manufacturer's instruction.

Soil-dilution and spread plate method was used for cultivation, enumeration and isolation of fungi from soil. Ten grams (10g) (mass) of soil samples was taken in a 250 ml Erlenmeyer flask. To this was added 100 ml (volume) of sterile normal saline and the flask was votexed for few minutes to obtain soil-water mixture.

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This represented 1:10 (m/v) or 1/10 (m/v) or 10^{-1} dilution or concentration of 1 gramme in 10 ml. Subsequently, additional two steps ten-fold serial dilutions in test tubes were prepared from the stock of 10^{-1} dilution, by adding 1ml from stock preparation into 9 ml of normal saline, yielding 10^{-1}, 10^{-2}, 10^{-3}.

Cultivation, Enumeration, Isolation, and Identification of Fungi from Air Samples

Sedimentation (Settle plates) using sterile Petri dishes were prepared and labeled appropriately. Six plates (two plates per medium - PDA, SDA, and MEA) were used for each monthly sampling. Each plate was labeled indicating media type, sample type, date, time, sampling station and plate number. A total of 72 settle plates were used for the monthly sampling for the duration of 12 months sampling period.

During sample collection the agar plates were placed face up on flat sampling rack-tray at the sampling stations at 1.5 metres from ground and at least 6 metres away from side walls or physical obstructions. The lids of sterile agar plates were removed and kept face down alongside the settle plates, while the plates were exposed for 10 minutes in upside positions. After exposure, the lids were then replaced with caution without touching the surface of the agar. An easy-to remove non dust sticky tapes were used to seal the lid of each plate. The cultured plates were incubated inversely at ambient temperature (30^0C) for 2 to 5 days. After incubation, the discrete colonies that developed for direct sedimentation on the mycological agar plates were enumerated and recorded as total fungal populations (Richter *et al.*, 2024), using the formula of Disegha and Nrior (2021).

The fungal colonies from air were isolated into pure cultures and identified by studying their colonial characteristics (macroscopy) and microscopy, and with molecular technique using ITS2 region genetic identification (Zieliński *et al*., 2020; Al-Shaarani *et al.*, 2023).

Cultivation, Enumeration, Isolation, and Identification of Fungi from Soil Samples

Aliquot of zero point one milliliter (0.1ml) of 10^{-3} dilutions from each soil sample was aseptically pipetted and separately spread-plated with flame-sterilized and cooled glass spreader on duplicate mycological plates, fortified with Ampicillin antibiotic to inhibit growth of bacterial contaminants. The cultured plates were incubated inversely at ambient temperature (30^0C) for 2 to 5 days. After incubation, the discrete colonies developed on the mycological plates were enumerated and recorded as of total fungi populations, according to the formula of Pepper and Gerba (2005).

Preparation of Fungal Isolates for Identification

Distinct fungal colonies from air and soil were isolated subcultured to obtain pure cultures, and identified by observing their colonial characteristics by macroscopy and microscopy. Examination by macroscopy included observing morphological features such as surface topography, surface texture, pigmentation, and type of mycelium, medium of growth and pace of growth. Microscopy included observing distinctive microscopic structures after staining with lactophenol blue and viewing under light microscope using 40x objective magnification (Alsohaili and Bani-Hasan, 2018). The isolates were identified using. Koneman's colour atlas and textbook of diagnotistic microbiology (Koneman *et al*., 1997) and were verified by using reverse image (yandex.com).

Antifungal Susceptibility Testing (AFST) and Identification of Resistant Fungi

Antifungal susceptibility testing was performed following the method of Aleruchi *et al.* (2019), with some modifications. Antifungal agents used for the AFST, were Ketoconazole (K), Nystatin (N), Fluconazole (F), and Greseofulvin (G). AFST was carried out to ascertain phenotypic expression of fungal resistance against the antifungal agents on culture plates. Disk diffusion assay was used. The disk variously contained Nystatin (100 units), Fluconazole (10 µg/ml), Griseofulvin (10 µg/ml), and Ketoconazole (10 µg/ml). Sizes of zones of inhibition were measured in millimeters (mm). The results were interpreted based on National Committee for Clinical Laboratory Standards (NCCLS, 2002) zone interpretation criteria for Resistant (R), Intermediate (I) and Susceptible (S) respectively.

Molecular Identification of Fungi Species Resistant to Antifungal Agents

Pure isolates of fungi most resistant to the antifungal agents in the susceptibility tests were further purified by resubculturing onto PDA plates. Thereafter, isolates were identified by molecular technique using ITS2 region genetic identification (Zieliński *et al*., 2020; Al-Shaarani *et al.*, 2023).

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When the cultures were fully developed, they were processed in a molecular laboratory at Ibadan (Bioinformatic Services, 24 Elizabeth Road, Mokola, Ibadan, Oyo State, Nigeria) for molecular analysis. The specific objectives were to detect functional genes responsible for resistance against the antifungal agents. The molecular process started by the extraction of fungal DNA.

Fungal DNA Extraction Using ZR Fungal DNA Miniprep (Manufactured By Zymo Research)

The fungal DNA extraction process was carried out according the manufacturer's instruction (Zymo Research in Irvine, United States. The process involved four key steps: breaking open the cells (lysis), removing proteins, getting rid of RNA, and concentrating the DNA.

Internal Transcribed Spacer (ITS) Gene Amplification of Fungal Isolates

The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (ITS 1: TCC GTA GGT GAA CCT GCG G) and Reverse primer (ITS4 TCCTCCGCTTATTGATATGS); 2µL of DNA template and then made up with 8.5µL Nuclease free water.

Primers used for the functional gene detection were as follows:

Azole (Nyst):	F: GCCACCACCCCTATAGAAACA R: AGGGTTGGATTGAGGGTTGG
Fluco	F: CATCGCCTCCTCCAGCAA R: CCAGGAACAGCACAACAAGC
Gri	F:TGTTCTCATCGTTCTTAACTTTCTC R: GGCCTAGCGCCATTACGAT
Ket	F: CTGCAGATCAACCGGAAGAA R: TCCCATTGAGTGAGTTGGCA

Cycling Conditions for the Internal Transcribed Spacer (ITS) gene were as follows: Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 50˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10˚C forever (summarized in Table 3.6). The PCR product was resolved on a 1% agarose gel apparatus at 120 V for 15 minutes and visualized on a blue light transiluminator as described above. The thermocycler programme settings and configurations for DNA amplification with loaded amplicons are given.

Functional Gene Detection: Separate PCR reactions were used to target specific genes linked to resistance against different antifungal agents.

Nucleotide Sequencing: The amplified fragments of DNA nucleotides were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit (Agi *et al.*, 2017).

Bioinformatic Analysis: Molecular sequences obtained were opened and edited in BioEdit. Sequence comparison was performed by BLASTing (BLASTN) using National Centre for Biotechnology Information (NCBI) domain. Genotypic identities, strains identities, Accession number, E-values, and percentage Relatedness were noted and recorded.

Determination of Soil Quality

Soil quality was determined by estimating physicochemical parameters including temperature, pH, moisture content, water holding capacity, electrical conductivity, total organic carbon, soil organic matter, available nitrogen, available phosphorus, available potassium, magnesium, calcium, sulfate, and zinc were measured. Soil samples were air-dried, ground to fine particles, sieved through a 2mm mesh sieve, and subjected to physical and chemical analysis using standardized methods (Carter and Gregorich, 2006).

Statistical analysis

Statistical analysis was performed on the data obtained from the study using GraphPad Prism Version 8; and data entry and structuring was performed using MS Excel for Windows Version 2010. Statistical analysis using single sample t-test was employed to reveal significant difference at $P \leq 0.05$. Mean separation of fungal populations in Post Hoc analysis with multiple comparisons was performed using Turkey test for quarterly means.

Results

Results of enumeration of fungi obtained from the air (CFU/min-M^2) during the study period are presented in Table 1. The Tables shows transformed populations in $\log_{10}$, minimum and maximum populations, ranges, as well as Mean±SEM values.

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In the air samples (Table 1), the annual mean air fungal population was 4.63 x 10^2 CFU/min-M^2. The mean monthly populations ranged from 1.20 x 10^2 to 7.04 x 10^2 CFU/min-M^2 with highest population observed in January followed by count (4.87 x 10^2) in February, while the lowest (1.20 x 10^2) was observed in March followed by 1.30 x 10^2 recorded in July.

The higher populations were observed in the months within dry season, while lower populations were recorded within months in the wet season. There were fluctuations in populations of air fungi from July to June in the succeeding year, with a slight plateau at the Logarithmic scale between December and March. Then a slight decline was observed from March to May, and then a second plateau from May to June. Statistical analysis with one sample t-test showed significance at $P \leq 0.05$.

Table 2 presents the mean monthly populations (Log_{10} CFU/g) of soil fungi during the sampling period. The highest count (2.15 x 10^6) of the soil samples was observed in May followed by count (1.87 x 10^6) observed in June, while the lowest count (8.17 x 10^4) was in July, followed by count (2.19 x 10^5) observed in October. There was slightly increase fluctuation in soil fungal populations throughout the sampling period. Statistical analysis using single sample t-test revealed a significance difference at $P \leq 0.05$.

Table 1: Mean Monthly Populations (Log_{10} CFU/min.M^2) of Air Fungi during the Sampling Period

Month	Count	Log10	Minimum	Maximum	Range	Mean±$S_{\bar{x}}$
Jul	1.30 x 10^2	2.1139	2.01703	2.88986	0.87283	2.66586±2.20
Aug	3.25 x 10^2	2.5118	2.44716	2.46090	0.01374	2.37291±1.41
Sep	1.90 x 10^2	2.2787	2.27875	2.66276	0.38400	2.50745±1.94
Oct	4.60 x 10^2	2.6627	2.20952	2.68753	0.47801	2.53491±1.95
Nov	3.61 x 10^2	2.5575	2.25527	2.80414	0.54887	2.64836±1.98
Dec	3.67 x 10^2	2.5646	2.51983	2.84757	0.32774	2.70697±2.06
Jan	7.04 x 10^2	2.8475	2.48714	2.86923	0.38209	2.71374±2.08
Feb	4.87 x 10^2	2.6875	2.51188	2.55509	0.04321	2.35083±1.85
Mar	1.20 x 10^2	2.1105	2.07918	2.95521	0.87603	2.73344±2.26
Apr	3.07 x 10^2	2.4871	2.48714	2.77452	0.28738	2.68332±1.90
May	3.31 x 10^2	2.5198	2.51983	2.96755	0.44772	2.73376±2.31
Jun	2.46 x 10^2	2.3909	2.39094	2.88986	0.49893	2.66586±2.31

Key: Count – Mean populations of fungi in Log_{10} CFU/min.M^2; CFU – Colony forming unit; /min-M^2- per minute per meter squared; $S_{\bar{x}}$ - Standard error of the mean

Table 2: Mean Monthly Populations (Log_{10} CFU/g) of Soil Fungi during the Sampling Period

Month	**Count**	**Log10**	**Minimum**	**Maximum**	**Range**	**Mean±SEM**
Jul	8.17 x 10^4	4.9120	2.0170	2.4472	0.4301	2.1072±1.12
Aug	5.44 x 10^5	5.7359	2.4609	2.6628	0.2019	2.6659±2.20
Sep	3.02 x 10^5	5.4800	2.2788	2.6875	0.4088	2.3729±1.41
Oct	2.19 x 10^5	5.3404	2.2095	2.8041	0.5946	2.5075±1.94
Nov	3.17 x 10^4	5.5006	2.2553	2.8476	0.5923	2.5349±1.95
Dec	7.04 x 10^5	5.8478	2.5198	2.8692	0.3494	2.6484±1.98
Jan	1.27 x 10^6	6.1021	2.4871	2.5551	0.0680	2.7070±2.05
Feb	3.16 x 10^5	5.4992	2.5119	2.9552	0.4433	2.7137±2.08
Mar	2.15 x 10^6	6.3323	2.0792	2.7745	0.6953	2.3508±1.85
Apr	8.97 x 10^5	5.9521	2.4871	2.9675	0.4804	2.7334±2.26
May	8.97 x 10^5	5.9241	2.4472	2.5198	0.0727	2.6833±1.90
Jun	1.87 x 10^6	6.2711	2.3909	2.6628	0.2718	2.7338±2.31

Key: Count – Mean populations of fungi in Log_{10} CFU/g; CFU – Colony forming unit; SEM – Standard error of the mean

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The populations of air fungi at Main Gate in Log_{10} CFU/min.M^2 are presented in Figure 1. The values of mean monthly fungal populations at Main Gate ranged from 3.1 to 3.9 with a mean annual population of 3.5.

The highest and lowest populations were observed in January (3.9) and March (3.1), respectively. Variation in fungal population was observed from July 2021 to January in the following year, with a steady decrease in populations to March before experiencing a rise in April and May.

The populations of soil fungi at Main Gate in Log10 CFU/g are presented in Figures 2.

The values of mean monthly fungal populations at Main Gate ranged from 4.6 to 6.6 Log_{10} CFU/g with a mean annual population of 5.5 Log_{10} CFU/g. The highest and lowest populations were observed in March and June 2022 respectively.

There was a sudden rise in soil fungal population from July 2021 (4.8 Log_{10} CFU/g, to 6.3 Log10 CFU/g in August, then declined to 5.2 Log_{10} CFU/g in September, before an observation of steady fluctuation in populations till February 2022. Overall, the lowest soil fungal population (4.6 Log_{10} CFU/g) was recorded in June 2022, the last month of sampling period.

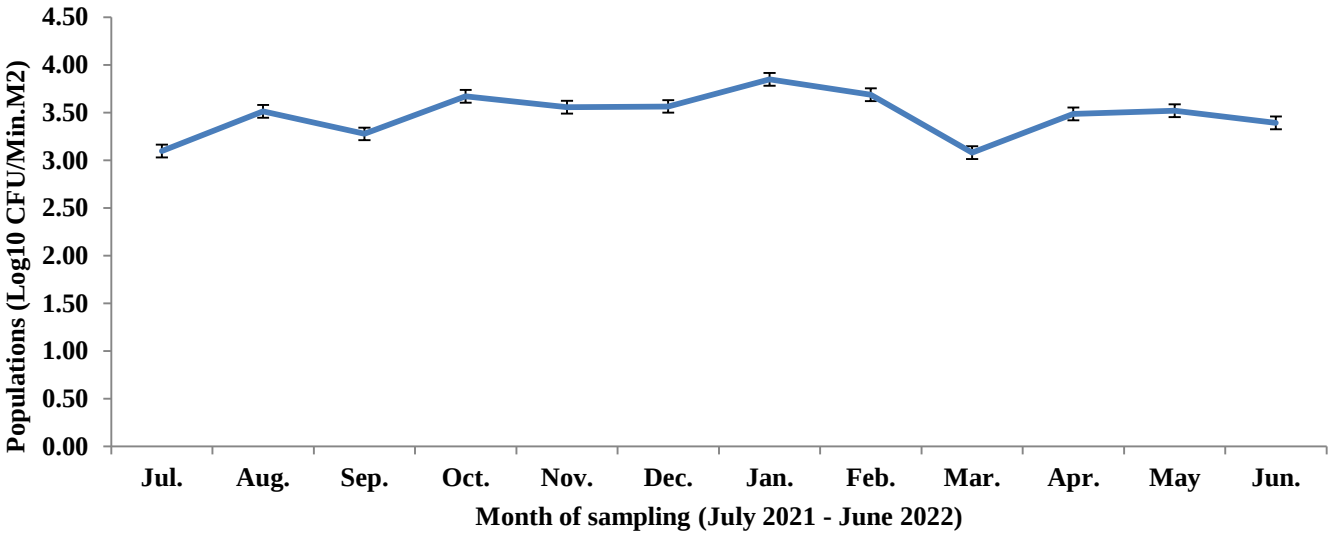

Fig. 1: Mean Values of Monthly Populations of Air Fungi at the Main Gate during the Study

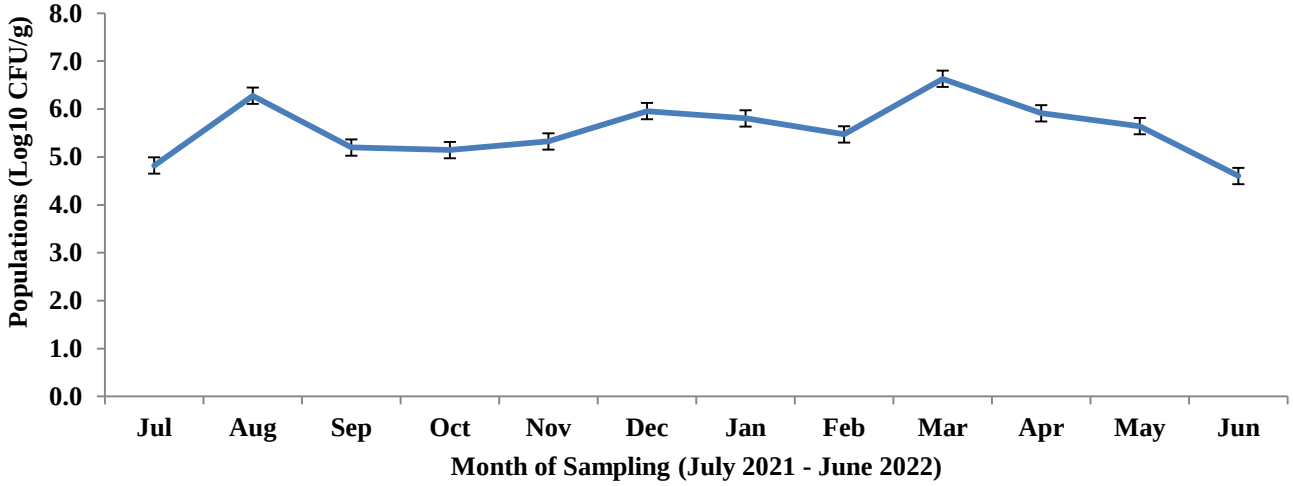

Fig. 2: Mean Values of Monthly Populations of Soil Fungi at the Main Gate during the Study

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The mean seasonal fungal populations of air (Log_{10} CFU/min-M^2) and soil (Log_{10} CFU/g) are illustrated in Figures 3 and 4 respectively. In air samples, dry season recorded higher mean log seasonal populations (Log 3.54) than the wet season (Log 3.38). While in the soil samples, Dry season recorded mean log seasonal population of Log 5.9 higher than that of wet season (Log 5.4) (Figure 4). Statistical analysis revealed a significant difference between the fungal populations of air samples during the dry and wet seasons, but there was no significant difference observed for dry and wet seasons for the soil samples.

Table 3 presents the species of fungi isolated from all samples analyzed and their respective physiological categories. Fourteen species of fungi belonging to ten genera were isolated and identified, and are placed into several physiological categories.

Aspergillus which belongs to more spectra of physiological categories, followed by *Fusarium* and *Penicillium*, were the most prevalent genera than other genera such as *Mucor*, *Microsporium*, *Pythium* and *Syncephalastrum*.

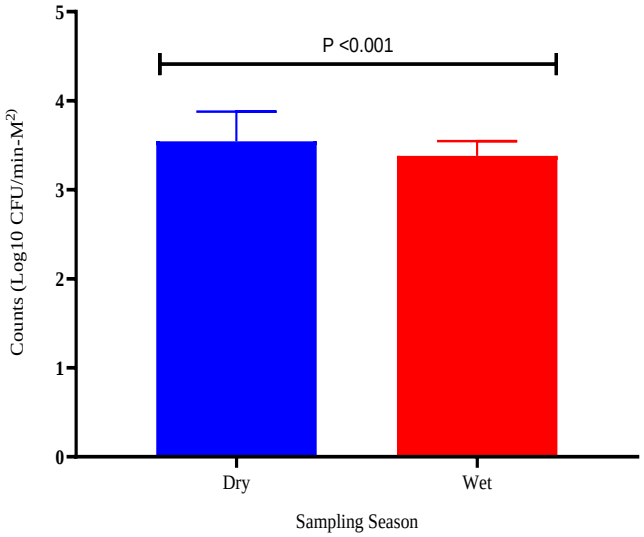

Fig. 3: Mean Seasonal Populations of Air Fungi (Log_{10} CFU/min-M^2) within the Sampling Period
Key: P ≤ 0.05 (significant)

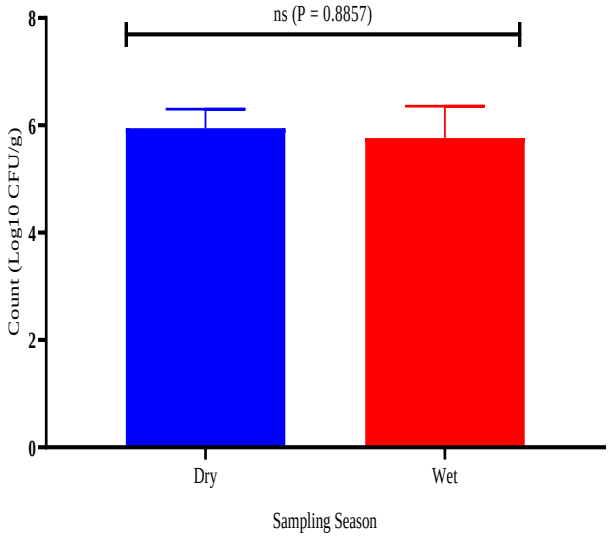

Fig. 4: Mean Seasonal Populations of Soil fungi (CFU/g) within the Sampling Period
Key: ns – not significant; P ≤ 0.05 (significant)

Table 3: Physiological Categories of Aeroterrestrial Fungi Isolated from the Main Gate

S/N	Fungi	Physiological Category
1	*Alternaria alternata*	Saprophytic, Pathogenic, phytopathogenic, mycotoxic
2	*Aphanoascus flavascens*	Enthomopathogenic, saprophytic
3	*Aspergillus fumigatus*	Saprophytic, Toxigenic
4	*Aspergillus niger*	Pathogenic, opportunistic,
5	*Aspergillus oryzae;*	Enthomopathogenic
6	*Collectotrichum fructicola*	Phytopathogenic
7	*Fusarium proliferatum*	Phytopathogenic
8	*Fusarium solani*	Saprophytic
9	*Microsporium canis*	Pathogenic, Dermatophytes
10	*Mucor ramosissimus*	Pathogenic
11	*Penicillium vanluykii*	Antibiotic producer,
12	*Penicillum chrysogenum*	Antibiotic producing
13	*Pythium myriotylum*	Phytopathogenic
14	*Syncephalastrum racemosum*	Saprophytic

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Figure 5 presents a multiple comparison of quarterly populations of air fungi during sampling period at the Main Gate. Results of mean separation of air fungal populations in Post Hoc analysis using Turkey test for quarterly means shows that there was differences in the populations of air fungi amongst the quarters, but the differences were not significant at $P < 0.05$. However, the P-values vary from quarter to quarter.

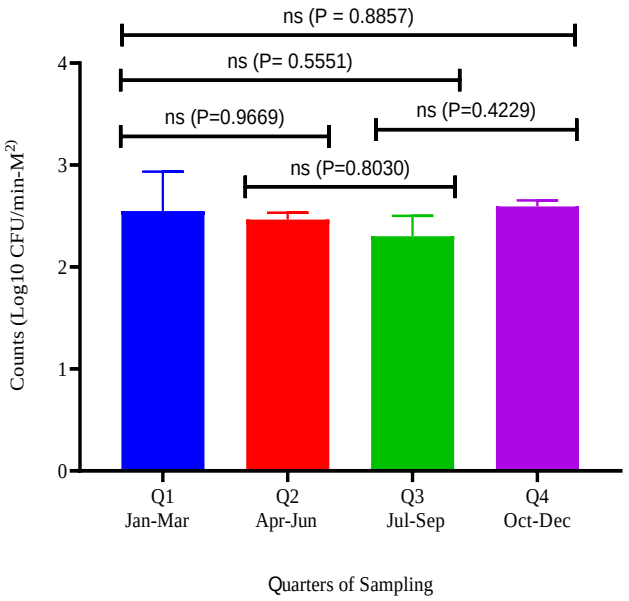

Fig. 5: Quarterly values of Air Fungal Populations at the Main Gate

Key: ns (not significant); P≤0.05 (significant)

Results of mean separation of soil fungal populations in Post Hoc analysis with multiple comparisons was performed. Using Tukey test result of quarterly means during the sampling seasons is presented in Figure 6. The result shows that there were differences in mean soil fungal populations as indicated by differences in P values within Quarters 1 to 4, but the differences were not significant at $P < 0.05$.

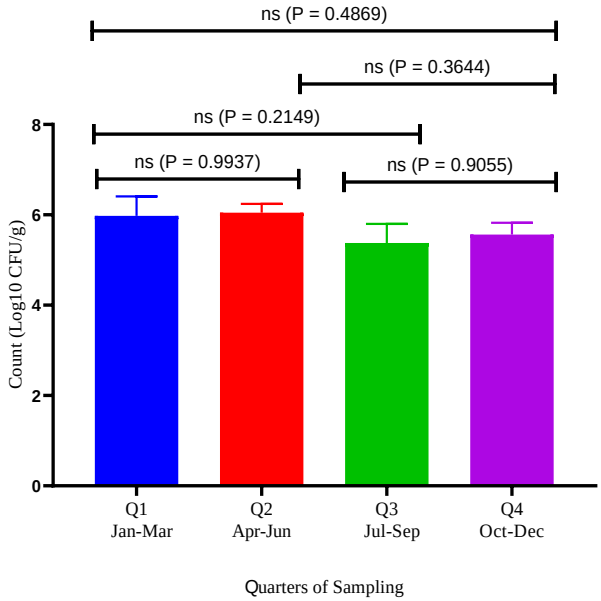

Fig. 6: Quarterly Values of Soil Fungal Populations at the Main Gate

Key: ns (Not significant); p ≤ 0.05 (significant)

Table 4 shows the mean monthly detection of soil quality at the sampling station during the sampling from July to June of the following year. The mean monthly soil parameters their ranges (Min – Max) during the sampling period are given as follows: soil temperature (°C): 32 (27.7 -37.8); pH : 6.9 (6.2 - 7.6); Moisture Content (%)- 15.04 (3.3 – 27.5); electrical conductivity (µS cm-1):102.18 (26 – 181).

Soil organic matter (%) range was 9.4 (0.69 – 24.3, available nitrogen (mg kg-1): 101.5(10 – 100); available phosphorus (mg kg-1): 26.08(10 – 50); available potassium (mg kg-1): 85.6 (20 – 160Zinc (mg kg-1): 0.04(0.02 – 0.08) and sulfate (mg kg-1): 22.27(8.5 – 42.7). The findings show irregular fluctuations of soil parameters during the sampling period, but statistically reveal no significant differences from month to month.

Table 5 presents a correlation matrix that explains the relationships between soil fungal population (SFP) and various soil quality parameters. The results showed that the soil fungal population has a strong positive correlation with Atmospheric Temperature (ATT, r =0.48), Soil Temperature (SLT, r = 0.44), Potassium (K, r = 0.49), and Zinc (Zn, r = 0.33). This suggests that higher soil temperatures and availability of certain nutrients are conducive to the growth and proliferation of soil fungi.

Soil fungal population has a moderate negative correlation with soil pH (-0.38), indicating that acidic soil conditions favor the abundance of soil fungi. The soil fungal population shows a strong negative correlation with Magnesium (Mg, r = - 0.55), suggesting that higher Magnesium levels in the soil may inhibit the growth of soil fungi.

Citation: Disegha *et al*. (2024). Investigation on the population, diversity, and antifungal resistance genes of aeroterrestrial microfungi and soil quality at the main gate of an academic environment. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 7 – 25.

Table 4: Mean Monthly Detection of Soil Quality at the Sampling Station

Parameter	Soil quality during the Months of sampling (July 2021 – June 2022)											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Temperature (^oC)	27.7	28.2	29.7	27.7	32.6	35.4	32.2	34.8	32.9	33.1	34.3	37.8
pH	6.4	6.9	7.6	6.7	7.4	7	7.5	6.9	7	6.2	6.7	6.5
Moisture content (% W/W)	17	13.5	10	16.7	27.5	15	7.5	3.3	18	10	20	22
Elec. conductivity (µS cm^{-1}).		102	98	90	163	93	118	95	26	49	181	109
Organic matter (%)	0.69	9.3	12.8	1.2	17.4	4.1	17.1	24.3	1	12.8	4.5	7.9
Available nitrogen (mg Kg^{-1})	10	50	100	150	200	125	50	40	30	150	155	158
Available phosphorus (mg Kg^{-1})	10	20	15	10	20	20	20	35	50	50	35	28
Available potassium (mg Kg^{-1})	160	120	70	20	20	70	120	85	50	80	120	110
Zinc (Zn) (mg Kg^{-1})	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.05	0.08	0.08	0.05	0.04
Sulphate (SO^{3-}) (mg Kg^{-1})	8.5	17.1	12.8	8.5	17.1	17.1	17.1	29.9	42.7	42.7	29.9	23.9

Table 5: Table 5: Correlation Matrix of Soil Fungi Population and Soil Quality Parameters

Parameter	Fungal popn	ATT (°C)	ST (°C)	pH	MoiC (% w/w)	WHC (%)	EC (µS cm^{-1})	TOC (%)	SOM (%)	N (mg Kg^{-1})	P(mg Kg^{-1})	K(mg Kg^{-1})	Ca [mg Kg^{-1})	Mg (mg Kg^{-1})	Zn (mg Kg^{-1})
Fungal Popn	1.00	0.48	0.44	-0.38	-0.14	0.18	-0.09	-0.09	-0.12	0.31	0.49	-0.31	-0.55	0.33	0.31
ATT (ºC)	0.48	1.00	0.91	-0.03	-0.12	-0.08	0.42	0.42	0.28	0.60	-0.16	-0.12	-0.54	0.53	0.60
ST (ºC)	0.44	0.91	1.00	-0.08	0.10	0.06	0.26	0.26	0.35	0.54	-0.02	-0.12	-0.59	0.45	0.54
pH	-0.38	-0.03	-0.08	1.00	-0.10	-0.53	0.41	0.41	-0.06	-0.31	-0.32	0.20	0.31	-0.37	-0.31
MoiC (% w/w)	-0.14	-0.12	0.10	-0.10	1.00	0.29	-0.46	-0.46	0.55	-0.12	-0.24	-0.11	-0.19	-0.10	-0.12
WHC (%)	0.18	-0.08	0.06	-0.53	0.29	1.00	-0.34	-0.33	0.61	-0.09	-0.01	0.31	0.24	-0.05	-0.09
EC (µS cm-1).	-0.09	0.42	0.26	0.41	-0.46	-0.34	1.00	1.00	0.03	0.12	-0.08	-0.25	-0.15	0.04	0.12
TOC (%)	-0.09	0.42	0.26	0.41	-0.46	-0.33	1.00	1.00	0.03	0.12	-0.08	-0.25	-0.15	0.04	0.12
SOM (%)	-0.12	0.28	0.35	-0.06	0.55	0.61	0.03	0.03	1.00	0.01	-0.50	0.26	0.11	-0.02	0.01
N (mg Kg-1)	0.31	0.60	0.54	-0.31	-0.12	-0.09	0.12	0.12	0.01	1.00	-0.08	-0.46	-0.54	0.99	1.00
P(mg Kg-1)	0.49	-0.16	-0.02	-0.32	-0.24	-0.01	-0.08	-0.08	-0.50	-0.08	1.00	-0.43	-0.44	-0.10	-0.08
K(mg Kg-1)	-0.31	-0.12	-0.12	0.20	-0.11	0.31	-0.25	-0.25	0.26	-0.46	-0.43	1.00	0.79	-0.46	-0.46
Ca [mg Kg-1)	-0.55	-0.54	-0.59	0.31	-0.19	0.24	-0.15	-0.15	0.11	-0.54	-0.44	0.79	1.00	-0.50	-0.54
Mg (mg Kg-1)	0.33	0.53	0.45	-0.37	-0.10	-0.05	0.04	0.04	-0.02	0.99	-0.10	-0.46	-0.50	1.00	0.99
Zn (mg Kg-1)	0.31	0.60	0.54	-0.31	-0.12	-0.09	0.12	0.12	0.01	1.00	-0.08	-0.46	-0.54	0.99	1.00

Key: Popn-Population; ATT; Atmospheric temperature; ST – Soil Temperature; pH-Hydorgen ion index; MoiC – Moisture Content; WHC – Water Holding Capacity; TOC – Total Organic Carbon; EC – Electrical Conductivity; SOM – Soil Organic Matter; N – Nitrogen; K – Potassium; P – Phosphorus; Ca – Calcium, Mg- Magnesium; Z-Zink; SO^{3-} Sulphate.

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Several soil nutrient parameters, such as Phosphorus (P, $r = 0.31$), Sulfate (SO_4, $r = 0.31$), and Calcium (Ca, $r = -0.31$), exhibit moderate correlations with soil fungal populations, indicating their potential influence on the soil fungal community. The high positive correlations between Atmospheric Temperature (ATT) and Soil Temperature (SLT, $r = 0.91$) and between Total Organic Carbon (TOC) and Soil Organic Matter (SOM, $r = 1.00$) suggest strong relationships between these parameters.

The result of the susceptibility pattern of the fungal isolates to selected antifungal agents (Ketoconazole, Nystatin, Fluconazole, and Griseofulvin) is as presented in Table 6. Results showed that *Aspergillus niger* was resistant to all the antifungal agents and was the only isolate that was resistant to Ketoconazole. While the other isolates were susceptible, *Aspergillus fumigatus* exhibited an intermediate response to Ketoconazole.

Table 6: Susceptibility Pattern of Fungi to Selected Antifungal Agents

Fungus	Ketoconazole (10 µg/ml)	Nystatin (100 units)	Fluconazole (25 µg/ml)	Griseofulvin (10 µg/ml)
Alternaria alternata	S	R	I	S
Aphanoascus flavascens	S	R	R	I
Aspergillus fumigatus	I	R	R	I
Aspergillus niger	R	R	R	R
Aspergillus oryzae	S	R	R	I
Collectotrichum fructicola	S	R	I	S
Fusarium proliferatum	S	R	R	I
Fusarium solani	S	R	R	I
Microsporium canis	S	R	R	I
Penicillium vanluykii	S	R	R	I
Penicillum chrysogenum	S	R	R	I
Pythium myriotylum	S	R	R	I
Syncephalastrum racemosum	S	R	R	I

Key: R- Resistant; S – Sensitive; I – Intermediate

Percentage distribution of resistance, sensitivity, and intermediate response the antifungal agents among fungi isolated from air and soil are shown on Table 7. It shows that 100% and 84.61% of the isolates were resistant to Nystatin and Fluconazole respectively while 84.61% were susceptible to Ketoconazole. The majority of the fungi exhibited multidrug resistance (MDR) since 84.61% of the isolates were resistant to two (2) or more antifungal agents. The fungal isolates recorded the highest percentage (76.92%) of intermediate response with Griseofulvin.

Plate 1 shows agarose gel electrophoresis of the internal transcribed spacer (ITS) regions of the fungal isolates' DNA. The bands visualized higher molecular weight DNA compared to the marker. Bands in Lanes originated from: B (*Aspergillus niger*), C (*Rhodotorula mucilaginosa*), H (*Rhizopus delemar*), L (*Aspergillus fumigatus*), O (*Candida tropicalis*), and S (*Trichosporon asahii*). Lane C exhibits the highest band intensity, suggesting a higher molecular weight than both the marker and other isolates.

Plate 2 depicts the amplification of the ITS region of the rRNA gene in the fungal isolates. Lanes B, C, H, O, L, and S of the gel image display the ITS rRNA gene bands at approximately 200 bp. Lane M in each gel image throughout all figures represents a 50 bp molecular marker (ladder).

Table 7: Percentage (%) Distribution of Antifungal Sensitivity among Fungi Isolated from Air and Soil Samples

Fungal Sensitivity	Ketoconazole (10 µg/ml)	Nystatin (100 units)	Fluconazole (25 µg/ml)	Griseofulvin (10 µg/ml)
Resistance	7.69	100	84.61	7.69
Susceptible	84.61	0	0	15.38
Intermediate	7.69	0	15.38	76.92

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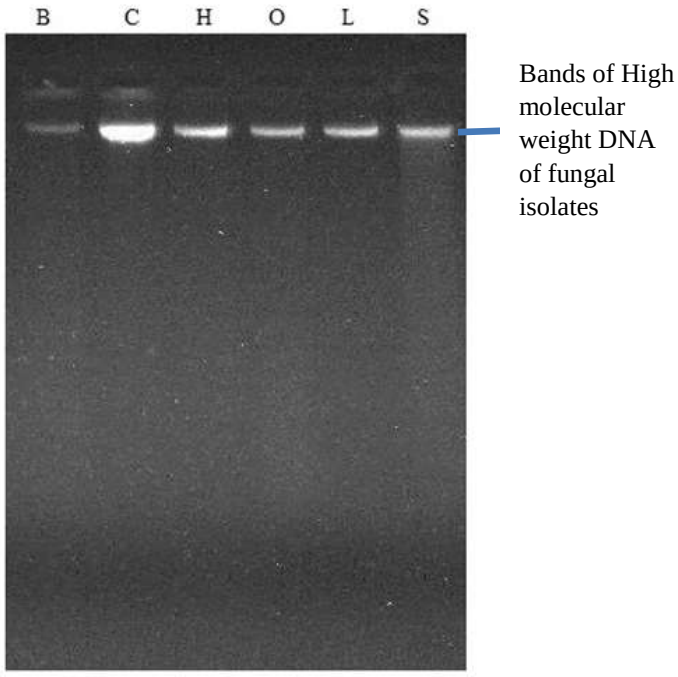

Plate 1: Agarose Gel Electrophoresis of the Fungal Isolates. Lanes B (*Aspergillus niger*), C (*Rhodotorula mucilaginosa*), H (*Rhizopus delenar*), O (*Candida tropicalis*), L (*Aspergillus fumigatus*), and S (*Trichosporon asahii*) of the Gel Image shows very high Molecular Weight DNA extracted from the Fungal Isolates; no Molecular Marker used

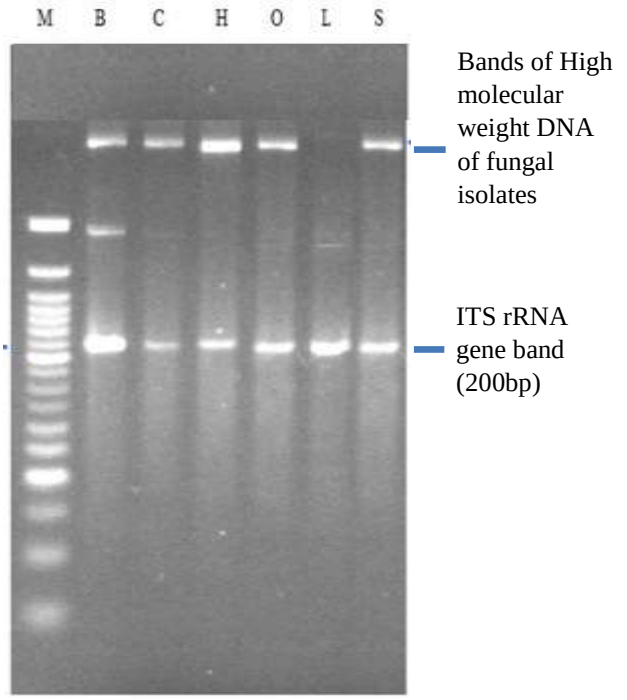

Plate 2: Agarose gel Electrophoresis Image of the Amplification of the ITS Region of the rRNA gene of fungi isolates. Lanes B (*Aspergillus niger*), C (*Rhodotorula mucilaginosa*), H (*Rhizopus delenar*), O (*Candida tropicalis*), L (*Aspergillus fumigatus*), and S (*Trichosporon asahii*) of the Gel Image shows the ITS (Internal Transcribed Spacer) rRNA Gene Bands (200bp). Lane M represents the 50bp DNA molecular marker.

Plate 3 shows the gel electrophoresis indicating the presence of genes associated with resistance to four antifungal drugs: Ketoconazole, Nystatin, Fluconazole, and Griseofulvin, in various fungal isolates through the Amplification of the ERG3 gene, associated with azole resistance, was detected only in *Rhodotorula mucilaginosa* (Lane C) and *Aspergillus fumigatus* (Lane L). This suggests that these two isolates may possess intrinsic resistance to azole antifungal drugs. Conversely, isolates in Lanes B (*Aspergillus niger*), H (*Rhizopus delemar*), O (*Candida tropicalis*), and S (*Trichosporon asahii*) lacked the ERG3 gene, suggesting potential susceptibility to azole antifungals.

Plate 4 shows the gel electrophoresis of DNA amplification of the fungal isolates. The gel image shows very high molecular weight DNA extracted from the fungal isolates.

The ITS region of the rRNA gene was successfully amplified in all fungal isolates (Plates 5, 6, and 7). This region served as a control to ensure the presence of fungal DNA in each lane. Additionally, amplification of the ITS region was used indirectly to assess the presence of genes associated with Ketoconazole (CYP51A1) and Nystatin (likely CYP51A1) resistance (Plates 5 and 6).

All isolates except *Trichocetium roseum* (Lane 8, Plate 5) showed bands corresponding to the ITS region, suggesting the presence of the Ketoconazole resistance gene (CYP51A1). Similarly, all isolates except *Fusarium proliferatum* (Lane 12, Plate 6) displayed bands for the ITS region, indicating the potential presence of the Nystatin resistance gene (likely CYP51A1).

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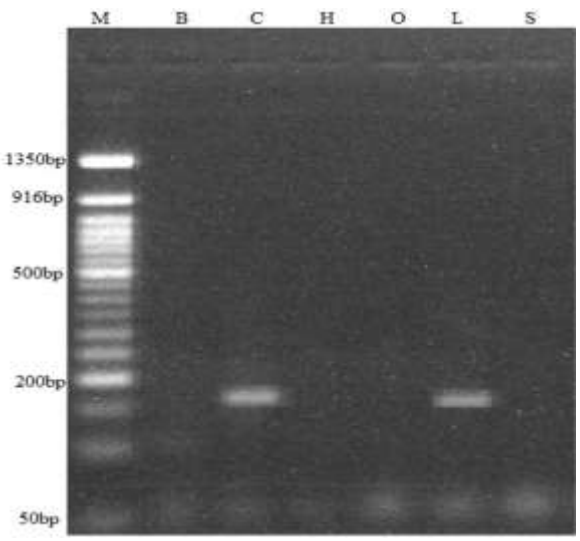

Plate 3: Gel Electrophoresis of Amplification of the ERG3 Gene at 180bp. Only fungal isolates in Lanes C (*Rhodotorula mucilaginosa*) and L (*Aspergillus fumigatus*) demonstrate the possession of resistance gene - EGR3. M is a 50bp DNA Molecular Marker. Fungal isolates in Lanes B (*Aspergillus niger*), H (*Rhizopus delemar*), O (*Candida tropicalis*) and S (*Trichosporon asahii*) do not demonstrate possession of resistance gene EGR3). M is 50bp DNA Molecular Marker

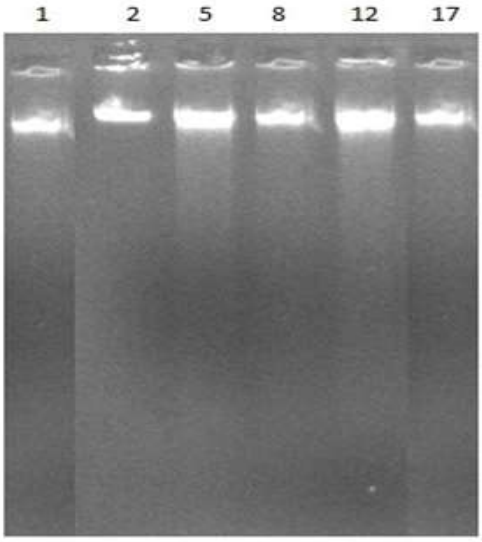

Plate 4: Agarose gel Electrophoresis of the Fungal Isolates. Lanes 1 (*Mucor ramossissimus*), 2 (*Tricophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*). The gel image shows very high molecular weight DNA extracted from the fungal isolates. No DNA Molecular Marked used

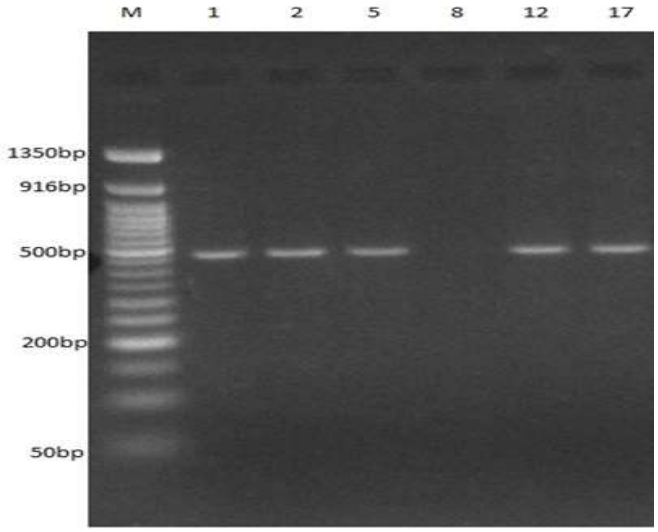

Plate 5: Agarose Gel Electrophoresis Image of the Amplification of the ITS (Internal Transcribed spacer) Region of the rRNA Ketoconazole Resistance Gene (CYP51A1) of Fungi Isolates. Fungal Isolates in lanes 1 (*Mucor ramossissimus*), 2 (*Tricophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*) of the Gel Image shows the ITS rRNA Gene Bands (501bp). All isolates except 8 (*Trichocetium roseum*) Demonstrated Possession of Ketoconazole Resistance Gene. Lane M represents the 50bp Molecular Marker (Ladder)

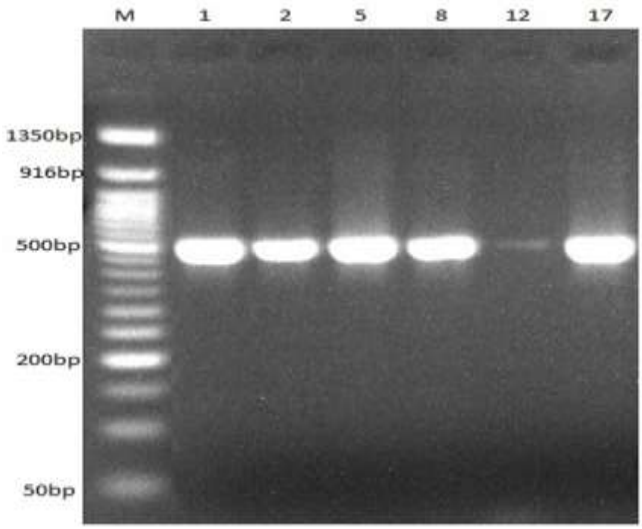

Plate 6: Agarose Gel Electrophoresis Image of the Amplification of the ITS (Internal Transcribed Spacer) Region of the rRNA Nystatin Resistance Gene (CYP51A1) of Fungi Isolates. Lanes 1 (*Mucor ramossissimus*), 2 (*Tricophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*) of the gel image shows the ITS rRNA gene bands (501bp). All Isolates, except Isolate in Lane 12 (*Fusarium proliferatum*) Demonstrated Possession of Nystatin Resistance Gene. Lane M Represents the 50bp DNA Molecular Marker

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Analysis of the Griseofulvin resistance gene (AZR1) also employed the ITS region for indirect assessment (Plate 7). All lanes, except possibly Lane 2 (*Tricophyton rubrum*) which may have degraded DNA, displayed bands corresponding to the ITS region, suggesting the presence of the AZR1 gene and potential resistance to Griseofulvin in these isolates.

Plate 8 shows an agarose gel electrophoresis image of the amplification of the Fluconazole resistance gene (ERG11) at 464 bp.

Lanes 1 (*Mucor ramosissimus*), 2 (*Trichophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*) of the gel image display the ITS rRNA gene bands at approximately 438 bp.

All isolates, except lane 5 (*Rhizopus stolonifer*) which exhibits a highly concentrated band, demonstrated the presence of the Fluconazole resistance gene. Lane M represents the 50 bp DNA molecular marker.

Molecular studies revealed very high similarity matches of isolates during ITS sequence alignment using BLAST on the NCBI open domain.

Details regarding isolate similarity matches, including strain identifications, accession numbers, e-values, and percentage (%) identity, are provided in Table 8. E-values indicate the likelihood that a given match occurred randomly; lower e-values signify better matches. The identification score reflects the degree of similarity between the query sequence and hits, with higher scores indicating better matches.

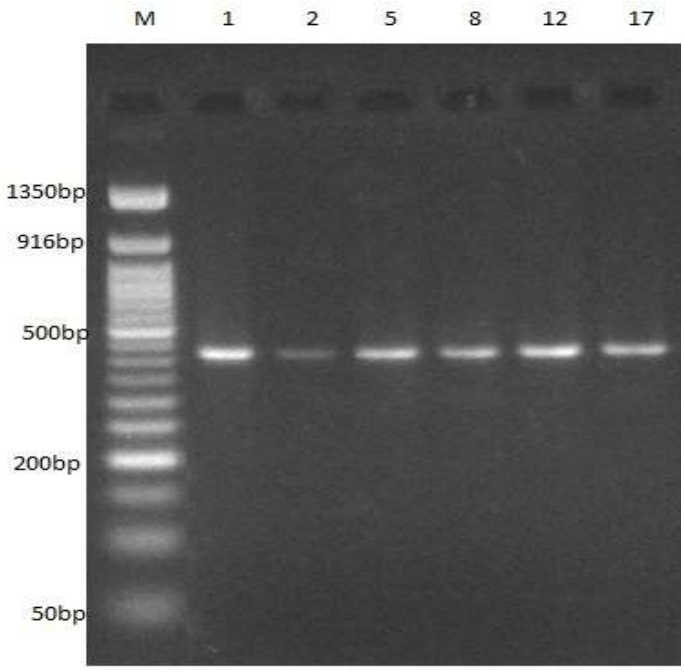

Plate 7: Agarose Gel Electrophoresis Image of the Amplification of the ITS (Internal Transcribed Spacer) Region of the rRNA Griseofulvin Resistance Gene (AZR1) of Fungi Isolates. Lanes 1 (*Mucor ramossissimus*), 2 (*Tricophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*) of the Gel Image shows the ITS rRNA Gene Bands (501bp). All Isolates (except 2, which may be highly Degraded) Demonstrated Possession of Griseofulvin Resistance Gene. Lane M Represents the 50bp Molecular Marker (Ladder)

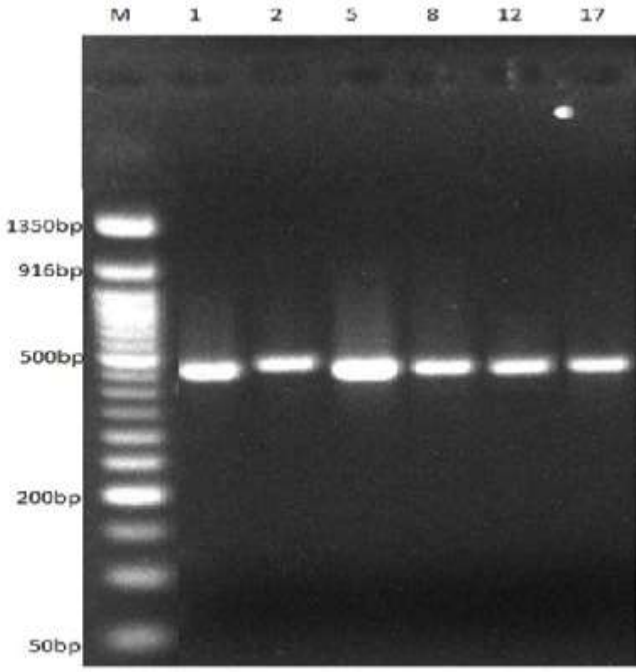

Plate 8: Gel image showing Amplification of Fluconazole Resistance Gene ERG11 at 464bp. Lanes 1 (*Mucor ramossissimus*), 2 (*Tricophyton rubrum*), 5 (*Rhizopus stolonifer*), 8 (*Trichocetium roseum*), 12 (*Fusarium proliferatum*), and 17 (*Triposporium cycadicola*) of the gel image shows the ITS rRNA Gene bands (438bp) of Fungal Isolates. All Isolates (with 5 (*Rhizopus stolonifer*), which is highly concentrated) Demonstrated Possession of Fluconazole Resistance Gene. Lane M represents the 50bp Molecular Marker

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Detection of genes using molecular techniques was performed for selected fungal isolates. Table 8 summarizes information about selected fungal isolates, including their identification (isolate code, species name, and NCBI accession number), detected genes, and physiological categories. Some isolates and their respective detected antifungal resistant genes in parentheses were *Aspergillus niger* (EGR11 and CDR1); *Aspergillus fumigatus* (CDR1, EGR3, and ERG11); *Mucor ramosissimus* (ERG11, FKS1, CYP51A1, and AZR1); *Fusarium proliferatum* (ERG11, MDR1, and AZR1). These isolates belong to several physiological categories**,** namely Pathogenic**:** Capable of causing disease in humans or other animals; Toxigenic**:** Able to produce toxins, Saprophytic**:** Decomposes dead organic matter for nutrition and Phytopathogenic**:** Causes diseases in plants.

Table 8: Isolate with Respect to Detected Genes and Physiological Categories

Isolate Code	Phenotypic Species	Isolate	Strains ID	NCBI Accession number	E Value	% Relatedness	Genes	Category
B-GD	*Aspergillus* sp	*Aspergillus niger*	DTO 399-I7	MT316340	0	98.53%	EGR11, CDR1	Pathogenic
L-GD	*Aspergillus* sp	*Aspergillus fumigatus*	DTO 402-H1	MT316338	0	99.63%	CDR1, EGR3, ERG11	Toxigenic
DC-1	*Mucor* sp	*Mucor ramosissimus*	90264	MF49528.1	0	89.11%	ERG11, FKS1, CYP51A1, AZR1	Saprophytic, rare pathogen
DC-12	*Fusarium* sp	*Fusarium proliferatum*	INVT_163	OR424637.1	5e-151	97.67%	ERG11, MDR1, AZR1	Phytopathogenic

Discussion

This study investigated aeroterrestrial microfungi and soil quality from the Main Gate of the Rivers State University, Port Harcourt. Mean monthly fungal populations of air fungi during the sampling period gave some unique results. Monthly fluctuations were observed throughout the period. There was a significant increase in the mean monthly populations of air fungi from July to December, followed by a decrease from January to June. This could be due to the following factors: Increased humidity and moderation in temperature during the months of the rainy season, which are favorable conditions for the growth of fungi. These findings are consistent with the studies of Talley *et al*., (2002), which reported that abundance of fungal spores correlates with factors outlined above, and reported that fungi spores were quantitatively high during the rainy season.

The decrease in mean monthly populations from January to March could be due to the following factors: Decreased humidity and temperature during the dry months, which are less favorable conditions for the growth of fungi. Increased ventilation and outdoor activities during the dry season Months could reduce the concentration of aerial fungal spores.

This is consistent with report of Ibrahim *et al.* (2011), which have shown a correlation of temperature and relative humidity on the growth of fungi (Odebode, 2020). The mean monthly count of soil fungi ranged from 5.4 to 6.7 Log_{10} CFU/g, with the highest mean being in March (6.7) and the lowest mean being in June (4.6). The standard error of the mean ranged from 0.03 to 0.13, indicating that the mean values are relatively precise. The minimum and maximum soil fungal populations for each month also showed some variation. For example, the minimum count in July was 5.193 Log_{10} CFU/g, while the maximum count was 5.59 Log_{10} CFU/g. This suggests that there is a relatively wide range of fungal activity in the soil throughout the sampling period.

The average monthly counts of soil fungi were relatively high, indicating a suitable environment for fungal growth in the soil. This is reflected in a previous report by Peter *et al*. (2021), which points to correlations between soil vegetation type and the community composition of the total fungal populations. Also, the fact that there is a rather wide range of fungal activity all year-round suggests that the soil is not under any significant environmental pressures that might inhibit or disrupt fungal growth.

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In general, the result during the sampling period provides valuable insights into the dynamics of soil fungal populations.

The mean seasonal air fungal count in the dry season (3.54) is significantly higher than in the rainy season (3.38) at $p \leq 0.05$. This is consistent with findings from other studies, which have also shown that fungal populations are higher in the dry season (Chubuike *et al*. 2023).There are a number of possible explanations for fungal populations being higher in the dry season. One possibility is that the warmer and drier conditions of the dry season are more favorable for fungal growth. Another possibility is that the wind, which is often stronger in the dry season, can help to disperse fungal spores more widely, alongside the dispersal of pollen grains by insects. The higher fungal populations in the dry season could have a number of implications for human health. For example, people who are allergic to fungi may experience more symptoms during the dry season. Additionally, people who are immunocompromised or have other health conditions may be at increased risk of fungal infections during the dry season (Baxi *et al*., 2016; Low and Rotshein).

In this study, a variety of air and soil fungi physiological categories were isolated and identified, some of which are pathogenic, saprophytic, and some entomopathogenic. For example, *Alternaria alternata* is a common allergen that can cause respiratory problems and plant diseases. *Aspergillus fumigatus* is a common cause of opportunistic infections in people with weakened immune systems, as well as allergic reactions. Others such as *Mucor ramosissimus* can cause a variety of infections, including mucormycosis, a serious infection that can affect the sinuses, lungs, and brain.

The implications of the presence of pathogenic fungi at the Main Gate are a potential health concern, especially for persons, and especially students, with weakened immune systems. It is important to take steps to reduce exposure to these fungi, such as avoiding contact with decaying matter and wearing gloves when handling plants. The presence of saprophytic and entomopathogenic fungi at the Main Gate is not necessarily a health concern. However, these fungi can be beneficial in some cases.

For example, saprophytic fungi help to break down dead matter, and entomopathogenic fungi can help to control insect populations as biocontrol agents.

The study on soil quality and nutrients yielded intriguing findings with fluctuations observed across various parameters during the sampling period. Soil temperature ranged from 27.7°C to 37.8°C, typical for a tropical climate in agreement with (Onwuka *et al*., 2018). The pH levels remained within a favorable range of 6.2 to 7.6 for most plants. Moisture content was highest in the rainy season and lowest in the dry season, aligning with seasonal variations. Water holding capacity was relatively high, indicating good water retention crucial for plant growth.

Electrical conductivity was highest during the rainy season, attributed to salt leaching by rainwater. Soil organic matter content was relatively high, a vital factor for plant nutrition and soil structure improvement. Nitrogen levels peaked during the rainy season and dipped during the dry season due to leaching and volatilization. Phosphorus levels remained fairly consistent throughout the year. Potassium levels were highest in the dry season and lowest during the rainy season, with it being prone to leaching. Zinc levels were relatively stable year-round. Sulfate levels followed a similar pattern to nitrogen, being highest during the rainy season and lowest in the dry season, owing to its mobility in soil.

In general, the soil quality was relatively good, with a suitable pH range, high water-holding capacity, and soil organic matter. However, improvements could be made in nitrogen, potassium, and sulfate levels, all essential for plant growth. These findings provide valuable insights into optimizing soil health for agricultural purposes.

Correlation of soil fungi populations/counts (SFCs) with soil quality parameters are clearly observed in this study. The correlation matrix provided insights into the complex interplay between soil fungal population and various soil physicochemical properties, highlighting the importance of understanding these relationships for effective soil management and ecosystem functioning at the Main Gate area. There is a very weak correlation between Soil fungal populations and soil quality indicators. This suggests that these soil parameters have little or no effect on the abundance of SFCs.

The findings of this study have several implications for understanding the ecology of Soil fungal populations and their role in the environment in agreement with Isong *et al*. (2022).

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The positive correlation between Soil fungal populations and K and SO^{3-} suggests that these nutrients may be important factors in controlling soil fungal abundance. This could be because K and SO^{3-} are essential nutrients for fungal growth and reproduction. Alternatively, it is possible that K and SO^{3-} are simply indicators of other environmental factors that are more directly responsible for influencing soil fungal abundance, conforming to the report by Haro and Benito (2019).

The weak correlation between Soil fungal populations and soil pH, N, P, Ca, Mg, and Zn suggests that these soil parameters have little or no direct effect on high soil fungal count abundance. This somehow contradicts the reports of Adamo *et al*. (2021), which state that "soil physico-chemical properties have a greater effect on soil fungi". While findings of this study do suggest insignificance of soil fertility parameters in relation to soil fungal populations, it does suggest that they are not the primary factors controlling fungal abundance in the environment.

Generally, the results of this study suggest that soil fungal abundance is influenced by a complex interplay of environmental factors. K and SO^{3-} appear to be important nutrients for soil fungal counts, while other soil parameters seemed to suppress soil fungal counts. More research is needed to better understand the mechanisms underlying these relationships and to determine the full range of factors that influence abundance of soil fungal populations in the environment.

In this study, all of the fungi tested for antifungal susceptibility were susceptible to Ketoconazole (K), except *Aspergillus fumigatus*, which was intermediate. All of the fungi tested were resistant to Nystatin (N) and Griseofulvin (G). Fluconazole (F) was effective against some of the fungi tested, but not all. For example, *Aspergillus niger*, *Fusarium proliferatum*, *Mucor ramosissimus*, *Aspergillus oryzae*, and *Aphanoascus flavascens* were all resistant to Fluconazole.

The findings suggest that Ketoconazole (K) is the most effective antifungal agent against the fungi tested in this study. Fluconazole (F) is also effective against some of the fungi tested. Nystatin (N) and Griseofulvin (G) were not effective against any of the fungi tested in this study, except for *Alternaria alternate* which was susceptible to the treatment of Griseofulvin (Ghannoum *et al.*, 1999). The findings of this study showed that Ketoconazole (K) is the most effective antifungal agent against the fungi tested in this study. Fluconazole (F) is also effective against some of the fungi tested, but it is important to note that some fungi are resistant to Fluconazole. Nystatin (N) and Griseofulvin (G) were not effective against any of the fungi tested in this study (Johnson and Perfect, 2010).

Percentage distribution of antifungal sensitivity among the fungi isolates showed that 100% and 84.61% of the isolates were resistant to Nystatin and Fluconazole respectively with Ketoconazole being the most effective antifungal agent as 84.61% of the isolates was susceptible to Ketoconazole. The majority of the fungi exhibited multidrug resistance (MDR) since 84.61% of the isolates were resistant to two (2) or more antifungal agents. The fungal isolates recorded the highest percentage (76.92%) of intermediate response with Griseofulvin. These findings suggest that the aeroterrestrial fungi isolated are generally resistant to the antifungal agents tested in this study. This is a concern, as it means that these fungi may be more difficult to treat if they cause an infection.

This study also detected genes possessed by the fungal isolates and revealed that, ERG11 gene appears to be prevalent across all listed fungi, regardless of category. It suggests ERG11 might play a fundamental role in the baseline function of many fungal species. The function of the ERG11 gene is to enable the conversion of lanosterol into ergosterol, thereby contributing to the synthesis of a key component of fungal cell membranes (Ghannoum and Rice, 1999).

The study also detected genes which are associated with pathogenicity and toxigenicity in *Aspergillus niger* (pathogenic) and *Aspergillus fumigatus* (toxigenic). Both isolates possess CDR1 and ERG11 genes which suggest potential overlap in resistance mechanisms between these categories. More research is needed to determine if these shared genes contribute similarly to both pathogenicity and toxin production.
According to Prasad *et al*. (2015) its main function is to provide resistance against multiple drugs, thereby diminishing their efficacy in treating *Candida* infections. The primary role of the CDR1 gene is to enhance the survival and drug resistance of *Candida* species by effectively removing drugs from within the cell (Prasad *et al*., 2015).

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Mucor ramosissimus, a rare pathogen exhibited the most diverse set of resistance genes (ERG11, FKS1, CYP51A1, and AZR1). The function of the FKS1 gene is to contribute to the synthesis of beta-glucan, an essential component of the fungal cell wall necessary for maintaining cell shape and protection against environmental stresses (Bulone, 2009). The function of the CYP51A1 gene is to enable the conversion of lanosterol into downstream sterols, thereby contributing to the synthesis of essential molecules necessary for cellular structure and function (Majdic *et al*., 2000). The function of the AZR1 gene is to confer resistance to azole antifungal agents, thereby promoting the survival and persistence of fungal pathogens when exposed to these drugs (Cowen *et al*., 2014).

Phytopathogenic fungi such as *Fusarium proliferatum* expressed ERG11, MDR1, and AZR1 genes. The presence of MDR1 (multidrug resistance) genes suggests this fungus might have broader resistance capabilities beyond the specific antifungal drugs tested. Studying the functionality of MDR1 in this context could be crucial for developing effective antifungal treatments for plant diseases. The main role of the MDR1 gene is to enhance cell survival and promote drug resistance by actively expelling drugs from the cell, ultimately reducing their effectiveness (Rockwell, 2013).

Specificity of categories was observed: while some genes (ERG11) seem widespread, others appear more specific to certain categories. For example, EGR3 is only found in *Aspergillus fumigatus* (toxigenic), suggesting a potential link to toxin production and resistance. Further research could explore the role of these category-specific genes in fungal virulence and develop targeted antifungal strategies. The EGR3 gene produces a protein called Early Growth Response Protein 3, which is part of the Early Growth Response (EGR) family (Pérez *et al*., 2011).

In conclusion, the findings of this study revealed seasonal variations in fungal populations, with higher levels in the dry season. The study also identified a diversity of fungi, including potentially pathogenic and toxigenic species which could pose a health risk to people who are exposed to them. The saprophytic saprophytic fungi help to break down dead matter and in nutrient recycling, and entomopathogenic fungi can help to control insect populations as biocontrol agents.

The soil quality was generally good, but there was room for improvement in nitrogen, potassium, and sulfate levels. The study found a weak correlation between fungal abundance and most soil quality parameters, except for potassium and sulfate.

The findings of this study could have potential applications in a number of areas as it has provided a valuable contribution to our understanding of soil fungi ecology dynamics, soil health and public health concerns at the study location. The information from this study could be used in a number of ways to improve agricultural practices, environmental monitoring, and climate change research.

References

Adamo, I., Castaño, C., Bonet, J. A., Colinas, C., De Aragón, J. M., & Alday, J. G. (2021). Soil physico-chemical properties have a greater effect on soil fungi than host species in Mediterranean pure and mixed pine forests. *Soil Biology and Biochemistry, 160*, 108320. https://doi.org/10.1016/j.soilbio.2021.108320

Aleruchi O., Obire, O. & Williams, J.O. (2019). Antimicrobial Resistance of Fungi Isolated from Oil-Producing Vicinity. *International Journal of Research and Innovation in Applied Science (IJRIAS). 4 (12):* 145 – 147.

Al-Shaarani, A.O.A., Quanch, Z.M., Wang, X., Muah, M. H.M., & Pecoram, V.L. (2023). Analysis of airborne fungal communities on pedestrian bridges in urban environments. *Microorganisms*, *11(8)*, 2097.

Alsohaili, S.A. & Bani-Hasan, B. M. (2018). Morphological and Molecular Identification of Fungi Isolated from Different Environmental Sources in the Northern Eastern Desert of Jordan. *Jordan Journal of Biological Sciences, 11(3),* 329 – 337.

Ataikiru, T.L., Okerentugba, P.O. & Iheanacho, C.C.(2018). Bioremediation of Bonny light crude oil polluted soil by bioaugmentation using yeast isolates (*Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700). *International Research Journal of Public and Environmental Health, 5(4*), 52-61.

Baxi, S. N., Portnoy, J. M., Larenas-Linnemann, D., Phipatanakul, W., Barnes, C., Baxi, S., Grimes, C., Horner, W. E., Kennedy, K., Larenas-Linnemann, D., Levetin, E., Miller, J. D., Phipatanakul, W., Portnoy, J.

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M., Scott, J., & Williams, P. B. (2016). Exposure and health effects of fungi on humans. *Journal of Allergy and Clinical Immunology*, *4(3)*, 396–404. https://doi.org/10.1016/j.jaip.2016.01.008

Bulone, V. (2009). Biosynthetic enzymes for (1,3)-B-Glucans and (1,3;1,6)-β-Glucans in protozoans and chromistans. In *Elsevier eBooks* (pp. 233–258). https,//doi.org/10.1016/b978-0-12-373971-1.00007-8

Carter, M.R. & Gregorich, E.G. (2007). *Soil Sampling and Methods of Analysis*. 2nd Edition, CRC Press, Boca Raton. https://www.crcpress.com/

Cowen, L.E., Sanglard, D., Howard, S.J., Rogers, P.D. & Perlin, D.S. (2014). Mechanisms of antifungal drug resistance. *Cold Spring Harbor Perspectives in Medicine*, *5(7)*, 10-19. Doi.10.1101/cshperspect.a019 752.

Dellagi, A, Quillere, I, & Hirel B. (2020). Beneficial soil-borne bacteria and fungi: a promising way to improve plant nitrogen acquisition. *Journal Experimental Botany*, *71(15*), 4469-4479. doi: 10.1093/jxb/eraa112.

Disegha, G. C. & Nrior, R.R. (2021). Profiles of microorganisms and diseases associated with bioaerosols and ways of identifying them. *Journal of Biology and Applied Sciences*, *1(2)*, 70-85,

Ghannoum, M. A. & Rice, L. B. (1999). Antifungal Agents: Mode of Action, Mechanisms of Resistance, and Correlation of These Mechanisms with Bacterial Resistance. *Clinical Microbiology Reviews*, *12(4)*, 501–517. https://doi.org/10.1128/cmr.12.4.501

Haro, R, & Benito, B. (2019). The Role of Soil Fungi in K^+ Plant Nutrition. *International Journal of Molecular Science*, *20(13)*, 3169. doi: 10.3390/ijms 20133169.

Ibrahim, M., Rabah, A.B., Liman, & Ibrahim, N.T. (2011). Effect of Temperature and Relative Humidity on the Growth of *Helminthosporium fulvum*. *Nigerian Journal of Basic and Applied Science*, *19(1)*, 127- 129.

Isong, I.A., John, K., Okon, P.B., Ogban, P.I. & Afu, S.M. (2022). Soil quality estimation using environmental covariates and predictive models: an example from tropical soils of Nigeria. *Ecological Process*, *11*, 66. https://doi.org/10.1186/s13717-022-00411-y

Jacoby, R., Peukert, M., Succrurro, A., Koprivova, A. &, Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Journal of Plant Science*. https://doi.org/10.3389/fpls.2017.01617.
Johnson, M. D. & Perfect, J. R. (2010). Use of antifungal combination therapy: agents, order, and timing. *Current Fungal Infection Reports*, *4(2*), 87–95.

Khan, A.A.H. & Karuppayil, S.M. (2012). Fungal pollution of indoor environments and its management. *Saudi Journal of Biological Science*; *19 (4)*, 405–426.

Kohler, J. R., Casadevall, A., & Perfect, J. (2014). The spectrum of fungi that infects humans. *Spring Harbor Perspectives in Medicine*, *5(1*), a019273. https://doi.org/10.1101/cshperspect.a019273

Koneman, E.W., Washington, C. W., William, M. J., Schreckenberger, P., Woods, G.L., Deirdre, L. C. & Hall, G.S. (1997). *Koneman's colour atlas and textbook of diagnotistic microbiology* (6th Ed.). Baltimore: Lippincot Williams and wilkins.

Low, C., & Rotstein, C. (2011). Emerging fungal infections in immunocompromised patients. *F1000 Medicine Reports*, *3*. https://doi.org/10.3410/m3-14

Majdic, G., Parvinen, M., Bellamine, A., Harwood, H.J., Ku, W.W., Waterman, M.R., Rozman, D. (2000). Lanosterol 14alpha-demethylase (CYP51), NADPH-cytochrome P450 reductase and squalene synthase in spermatogenesis, late spermatids of the rat express proteins needed to synthesize follicular fluid meiosis activating sterol. *Journal of Endocrinology*, 166(2), 463-74. doi, 10.1677/joe.0.1660463.

Manisalidis, I., Stavropoulou, E., Stavropoulos, A., & Bezirtzoglou, E. (2020). Environmental and Health Impacts of Air Pollution: A review. *Frontiers in Public Health*, *8*. https://doi.org/10.3389/fpubh.2020. 00014.

Masoomeh, S. G., Sanaz, A G, Narges, A, & Mehdi, R. A.(2014) Investigation on distribution of airborne fungi in outdoor environment in Tehran, Iran. *Journal of Environmental Health Science and Engineering*, *12*, 54. doi: 10.1186/2052-336X-12-54.

NCCLS (2002). Performance standards for antimicrobial susceptibility testing: Twelfth Informational Supplement. National Committee for Clinical Laboratory Standards NCCLS document, M100-S12. PA, USA.

Citation: Disegha *et al*. (2024). Investigation on the population, diversity, and antifungal resistance genes of aeroterrestrial microfungi and soil quality at the main gate of an academic environment. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 7 – 25.

Obire, O., Anyanwu E.C., &. Okigbo, R.N.(2008). Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents. *Journal of Agricultural Technology, 4(2),* 81-89.

Odebode, A., Adekunle, A., Stajich, J., & Adeonipekun, P. (2020). Airborne fungi spores distribution in various locations in Lagos, Nigeria. *Environmental Monitoring and Assessment*, *192(2).* https://doi.org/10. 1007/s10661-019-8038-3

Onwuka, B. & Mang B. (2018). Effects of soil temperature on some soil properties and plant growth. *Advanced Plants Agricultural Res*earch, *(1),* 34-37. DOI: 10.15406/apar.2018.08.00288.

Pepper, I.L. & Gerba, C.P. (2005). *Environmental Microbiology*, A Laboratory Manual, 2nd Ed. Academic Press, Boston, MA.

Pérez-Cadahía, B, Drobic, B., Davie, J.R. (2011). Activation and function of immediate-early genes in the nervous system. *Journal of Biochemistry and Cell Biology*, 89, 61–73. https,//doi.org/10.1139/O10-138.

Peter, M. l., Brendan, F., Kassim,I. T., Kerri, K., Martin, R., Marie-Laure, G., Bakary, S., Aïssata, T., Gbamon, K., Nourou, S. & Yorou, A. R. (2021). Soil fungal communities of ectomycorrhizal dominated woodlands across West Africa. *Journal of Mycological Keys*, *81*, 45–68.

Prasad R, Banerjee A, Khandelwal N.K., Dhamgaye S. (2015).The ABCs of Candida albicans Multidrug Transporter CDR1. *Journal of Eukaryot Cell*, 14(12), 1154-64. doi, 10.1128/EC.00137-15.

Rane, G. & Gandhe, R. V. (2006). "Seasonal distribution of soil fungi from forest soils of Jalgon District, Aharashtra", *Zoo's Print Journal, 21 (9),* 2407-2409.

Richter, A., Blei, F., Hu, G., Schwitalla, J. W., Lozano-Andrade, C. N., Xie, J., Jarmusch, S. A., Wibowo, M., Kjeldgaard, B., Surabhi, S., Xu, X., . . . Kovács, Á. T. (2024). Enhanced surface colonisation and competition during bacterial adaptation to a fungus. *Nature Communications*, *15(1)*. https://doi.org/10.1038/s41467-024-48812-1

Rockwell, N. C. (2013). Multidrug resistance membrane proteins. In *Elsevier eBooks* (pp. 210–214). https,//doi.org/10.1016/b978-0-12-378630-2.00215-2.

Talley, S. M., Coley, P. D., & Kursar, T. A. (2002). The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecology*, *2(1),* 7. https://doi.org/10.1186 / 1472- 6785-2-7

Vashishta, B.R, Sinha, A. K. & Kumar, A. (2016). *Botany for degree students: Fungi* (*Revised Multi-colour edition)*. New Delhil: S. Chand Publishing, *p*.794.

Zieliński, B., Sroka-Oleksiak, A., Rymarczyk, D., Piekarczyk, A., & Brzychczy-Włoch, M. (2020). Deep learning approach to describe and classify fungi microscopic images. *PLOS ONE*, *15(6),* e0234806. https,//doi.org/10.1371/journal.pone.0234806.

Citation: Disegha *et al*. (2024). Investigation on the population, diversity, and antifungal resistance genes of aeroterrestrial microfungi and soil quality at the main gate of an academic environment. *International Journal of Microbiology and Applied Sciences*. *3(2)*: 7 – 25.