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Comparative Effects of Two Medicinal Plants and Common Disinfectants against Air-Borne Fungi in Poultry House

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Authors' contributions

This work was carried out in collaboration among all authors. Author ISA designed the study, managed the literature searches, performed the statistical analysis and wrote the protocol. Author CUU supervised the research. Author SNE managed the analyses of the study. Author CCU wrote the first draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research was undertaken to compare the antifungal effects of *Eupatorium odoratum* leaf extract and *Vernonia amygdalina* extracts with common disinfectants on air-borne fungi in poultry houses.
Place and Duration of Study: Air in four poultry farms within Ihiala Local Government Area, Anambra State was sampled between March 2017 and October 2017.
Methodology: Poultry air of four different sites at Uli town in Ihiala local government area of Anambra state in Nigeria, were sampled using Sedimentation and Volumetric methods. Fresh leaves of *Eupatorium odoratum* and *Vernonia amygdalina* were collected from Uli town, Anambra State, air-dried, processed and extracted using Ethanol and water. Four-hundred (400) mg of the

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crude extracts were evaluated for Antifungal activity using agar diffusion method. The MIC and MFC were determined using Broth dilution methods.

Results: Five isolates namely, *Aspergillus flavus, Aspergillus tubingensis, Candida akabanensis, Candida rugosa, and Fusarium solani* were identified. Antimicrobial evaluation of the crude extracts showed that ethanol extract of *Eupatorium odoratum* had activity against all the test isolates except *Candida akabenensis* and Fusarium *solani.* The aqueous extracts of *Eupatorium odoratum and Vernonia amygladina* had activity against all the isolate except *Candida akabenensis* and *Fusarium solani and Candida rugosa*. Common disinfectants used in this study namely Izal and Polidine showed inhibitory activity against all the isolates. Ethanol extract of *Eupatorium odoratum* recorded a minimum inhibitory concentration (MIC) of 100 mg/ml against *A. flaus, F. solani,* and *A. tubingensis*, while the minimum inhibitory concentration for *Candida rugosa* is 200 mg/ml. The minimum fungicidal concentration (MFC) of Ethanol extract of *Eupatorium odoratum* against *A. flaus, F. solani, Candida rugosa* and *A. tubingensis* were 200 mg/ml, 100 mg/ml, 400 mg/ml and 200 mg/ml respectively. Aqueous extract of *Eupatorium odoratum* recorded a minimum inhibitory concentration of 200 mg/ml against *A. flaus* and *A. tubingensis,* while the minimum inhibitory concentration against *Candida rugosa* is 400 mg/ml*.* The minimum fungicidal concentration of Aqueous extract of *Eupatorium odoratum,* were 200 mg/ml, 400 mg/ml and 200 mg/ml for *A. flaus, Candida rugosa* and *A. tubingensis* respectively.

Ethanol extracts of Vernonia amygdalina leaf had lower minimum inhibitory concentrations of 100 mg/ml against *A. flavus, A. tubingensis* respectively, and 200 mg/ml against *F. solani,* while the minimum fungicidal concentrations recorded for *A. flavus*, *A. tubingensis* and *F. solani* were 200 mg/ml, 400 mg/ml and 100 mg/ml respectively. Aqueous extract of *Vernonia amygdalina* leaf had a minimum inhibitory concentration of 200 mg/ml and 400 mg/ml against *A. flavus* and *A. tubingensis* with a minimum fungicidal concentration of 400 mg/ml for both isolates only. The Minimum inhibitory concentration and minimum fungicidal concentration of both Izal and Polidine was between 12.5% V/V and 50% V/V against all the isolates except Polidine that had minimum fungicidal concentration of 100% V/V against *Candida rugosa.*

Conclusion: The extracts of *Eupatorium odoratum* and *Vernonia amygdalina* has antifungal activity against all the isolates except *Candida akabenensis*. If considered and used as a disinfectant during misting, it may decrease the cost of disinfecting poultry farms using available disinfectants in the market. These suggestion, however, need further work to validate reliability.

Keywords: Antifungal; minimum fungicidal concentration; Minimum Inhibitory Concentration (MIC); poultry; sedimentary-method of isolation; volumetric method of isolation.

1. INTRODUCTION

The air in modern poultry production systems contains a large variety of air pollutants, such as gases (ammonia and carbon dioxide), dust, microorganisms and endotoxins. These pollutants commonly known as bio-aerosols are increasingly regarded as aggravating, environmentally harmful and major public health concern for poultry workers and visitors [1].

Human exposure to airborne dust and microorganisms such as bacteria and fungi can cause diseases particularly respiratory related ailments [2]. This is because a large number of fungi produce mycotoxins and volatile organic compounds that can affect human and animal health. In susceptible or highly-exposed individuals these can lead to invasive mycosis [3].

Indoor exposure levels are usually much higher than outdoor levels, which not often exceed 10^4 spores per cubic meter [4]. It has been understood that activities in these indoor places such as cleaning and feeding animals increase occupational risk of exposure to airborne microorganisms [1]. Spores of some type of fungi including *Cladosporium, Aspergillus, Penicillium* and *Alternaria,* according to Eduard may carry allergens, antigens, polysaccharides, and mycotoxins and can lead to allergic respiratory disease in susceptible individuals [4]. The most common poultry fungal infections, such as Aspergillosis and Candidiosis, are commonly found in the environment of birds [5]. Arné and colleagues argued that since there are no treatments for infected poultry, and therefore, the only effective way to protect chickens against mycoses is prevention [6]. Some of the known methods used to reduce dust and fungal spores in the air of poultry buildings are misting with

water and/or aqueous solutions of essential oils (peppermint, thyme, pine and eucalyptus oils) [7] The use of biological compounds extracted from medicinal plants may offer an alternative to conventionally used disinfectants to control air-borne fungi.

With respect to many reports about the impact of plant extracts against food and grain storage fungi, foliar pathogens, nematodes, soil-borne as well as air-borne fungi [8], this research was undertaken to compare the antifungal effects of Siam weed (*Eupatorium odoratum)* leaf extract and bitter leaf (*Vernonia amygdalina)* extracts with common disinfectants Izal and Polidine on fungi isolated from air samples of poultry houses.

2. MATERIALS AND METHODS

2.1 Sample Collection

Poultry air of four different sites at Uli town in Ihiala local government area of Anambra state in Nigeria, were sampled using two different methods namely; Sedimentation and Volumetric methods as previously described by [2,1]. In Sedimentation method, twenty- five sabouraud dextrose agar plates supplemented with 0.05% of chloramphenicol were exposed at different spots in each site. For volumetric method, the air samples were collected using Air Sampler cassettes exposed for 5 minutes at different spots in each site.

The samples were labelled properly and immediately transported to the laboratory for incubation and further analysis within one hour of sampling.

2.2 Sample Processing

In the laboratory, the cassettes of the air sampler were opened and the gel slides were placed on the surface of Sabouraud dextrose agar plates supplemented with 0.05% of chloramphenicol. All the culture plates were incubated at room temperature, for five [5] days as described by [9].

2.3 Isolation and Identification of Fungi

Fungi culture plates were purified by sub-culturing aseptically into new SDA media and subsequently incubated for another five [5] days at room temperature [10]. The morphological characteristics of the pure fungi culture plates were observed and recorded for seven days as previously described. [9] Fungal cells were stained using Lactophenol cotton blue and examined at a low power magnification (X40) using a light microscope. The results were compared with the descriptions in a fungal Atlas as previously reported [11].

Fungal count in CFU/m^3 was done using the formula below:

CFU/m^3= Total colonies x 10^3 / Air flow rate x collection time [2]

2.4 Collection and Preparation of Plant Materials

Fresh leaves of *Eupatorium odoratum* and *Vernonia amygdalina* were collected from Uli, Anambra State Nigeria. The selection was based on the ethno medical uses for folk medicine. The leaves were washed with distilled water, air-dried at room temperature (30±2°C) for 14 days and pulverized using electronic blender (Binatone). Forty grams (40 g) portion of the leaves powder was each extracted by cold maceration in 400 ml of ethanol and water for 72 hours. The extracts were filtered, evaporated to dryness at 50°C using water bath [12]. The disinfectants (Izal and Polidine) were sourced from Animal Care Company in Oshimili south Local Government Area, Asaba, Delta state Nigeria.

2.5 Antifungal Evaluation

Cup- plate agar diffusion using Sabouraud dextrose agar was employed. A stock concentration (400 mg/ml) of the plant extracts were made by dissolving 800 mg of the leaf powder in 2 ml of Dimethylsulfoxide (DMSO). The stock concentrations were serially diluted to obtain 100 mg/ml, 500 mg/ml, 25 mg/ml and 12.5 mg/ml. For the Common disinfectants, izal and Polidine, a double fold serial dilution was made from the stock of 100% v/v, to 50% v/v, 25% v/v, 12.5% v/v.

Each labeled Sabouraud dextrose agar plate was uniformly inoculated with a McFarland standardized test organisms. A sterile cork borer of 6 mm diameter was used to make wells on the culture plates. One hundred (100) µl of various concentrations of the extracts were dispensed into each agar-well, labeled with the corresponding concentrations. Fifty (50) µg of ketoconazole (Ketoral) was used as positive control.

The culture plates were incubated for 48 hours at 30±2°C. Antifungal activity were determined by measuring the inhibition zone diameter (in mm) produced after 48 hrs of incubation [13].

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Various concentration of the stock solution was made by double fold serial dilution to obtain, 200 mg/ml, 100 mg/ml and 50 mg/ml for the plant extracts. From the stock solution (Izal and Polidine (100% V/V), 50%, 25% and 12.5%, 6.25% V/V concentration were made. Each dilution in a test-tube was inoculated with 0.02 ml of the broth culture diluted to 0.5 McFarland standards. A positive control test tubes were inoculated with the test organisms in the absence of the test agents, while the negative control test tubes has the test agents without the test organisms. All the tubes were incubated at 30±2°C for 72 h. the lowest concentration showing no visible growth was recorded as the minimum inhibitory concentration (MIC) for each organism [14].

2.7 Determination of Minimum Fungicidal Concentration

From each negative tube in MIC assay, 1 ml was transferred onto the surface of freshly prepared Sabouraud Dextrose Agar plates (without antibiotics or extracts) and the plates were incubated at 30±2°C for 72 h for The lowest concentration showing no visible growth on SDA was recorded as minimum fungicidal concentration (MFC) for each organism [14].

2.8 Statistical Analysis

The data collected and generated in this study were organised and presented using SPSS version 20 and Microsoft Excel version 2007. The antimicrobial evaluation studies were done in triplicates. The inhibition zone diameter was reported in Mean±Standard deviation.

3. RESULTS AND DISCUSSION

3.1 Total Fungi Count

The total fungi count across the sample sites are shown in Table 1. The result revealed that the sedimentary method of sample collection had the highest number of fungal count than that of volumetric method.

3.2 Identification of Fungal cells

Three species ascribed to five fungal genera were isolated and identified from the poultry house investigated. The results of the macroscopic and microscopic observations made on the individual isolates are shown in Table 2. These Isolates were observed to be *Aspergillus flavus, Aspergillus tubingensis, Candida akabanensis, Candida rugosa, and Fusarium solani.*

In Table 3, the sedimentation method of isolation revealed that *Aspergillus flavus, Aspergillus tubingensis, Candida akabanensis, Candida rugosa, and Fusarium solani* had 32%, 24%, 8%, 12%, and 24% frequency of occurrence respectively while Volumetric method of isolation recorded a 33.3%, 25%, 8.3%, 16.7% and 16.7% frequency of occurrence respectively.

3.3 Antifungal Activity

Antimicrobial evaluation of the crude extracts showed that ethanol extract of *Eupatorium odoratum* had activity against all the test isolates except *Candida akabenensis* and *Fusarium solani.* The aqueous extracts of *Eupatorium odoratum and Candida akabenensis* had less activity than the ethanol extracts (Table 4). Common disinfectants used in this study namely Izal and Polidine showed inhibitory activity against the isolates as revealed in Table 5.

Table 1. Fungal count and conversion to colony forming unit

Sample site	No. of isolates by sedimentary method	CFU/m^3	No. of isolates by volumetric method	CFU/m^3
A	58	$0.77x10^3$	12	$0.12x10^3$
B	55	$0.73x10^3$	9	$0.09x10^3$
C	49	$0.65x10^3$	11	$0.01x10^3$
D	35	$0.47x10^3$	8	$0.08x10^3$

Table 2. Cultural and Microscopic characteristics of fungi isolates

Isolate	Macroscopy	Microscopy
Aspergillus flavus	Surface was greenish – yellow to olive and have a white border. Texture was velvety to woolly.	It has uniseriate and biseriate phialides, radiating conidial head. Rough walled conidiophores. Round and rough walled conidia in chain.
Candida akabanensis	White to cream, soft, smooth to wrinkled colonies	Pseudohyphae and true hyphae with blastoconidia are present.
Fusarium solani	The surface of the colony was wooly to cottony and white creamy with dark brown zonation in colour.	It is long and branched monophialides.
Candida rugosa	The surface of the colony was white to cream colored smooth, glabrous, yeast like.	It has ellipsoidal to elongated budding blastoconidia. It has short pseudohyphae.
Aspergillus tubingensis	The surface color of the colony was black. The colony diameter was 2-7 cm.	It has branched septate hyphae. It has bunch of spores arrangement and the spore shape was round.

Table 3. Frequency of isolation of Fungi from poultry air

Isolate	SFI	SFI%	VFI	VFI%
Aspergillus flavus	8	32%	4	33.3%
Candida akabanensis	2	8%	1	8.30%
Fusarium solani	6	24%	2	16.7%
Candida rugosa	3	12%	2	16.7%
Aspergillus tubingensis	6	24%	3	25%
Total	25	100%	12	100%

SFI: Sedimentary method frequency of isolation; VFI: Volumetric method frequency of Isolation

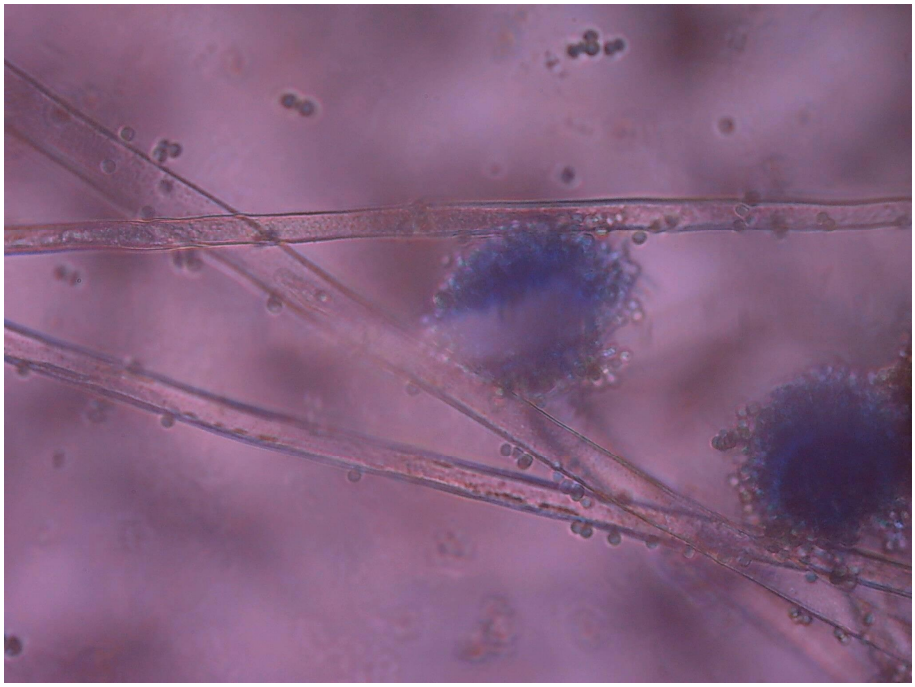

Fig. 1. Micrograph of *Aspergillus flavus* (Magnification x40)

The result of the evaluation also revealed that Izal is more effective than Polidine. Comparatively, ethanol extracts of Eupatorium odoratum and Vernonia amygdalina leaf had lower minimum inhibitory concentration and minimum fungicidal concentration against the fungal isolates than the aqueous extract of the same plant being evaluated.

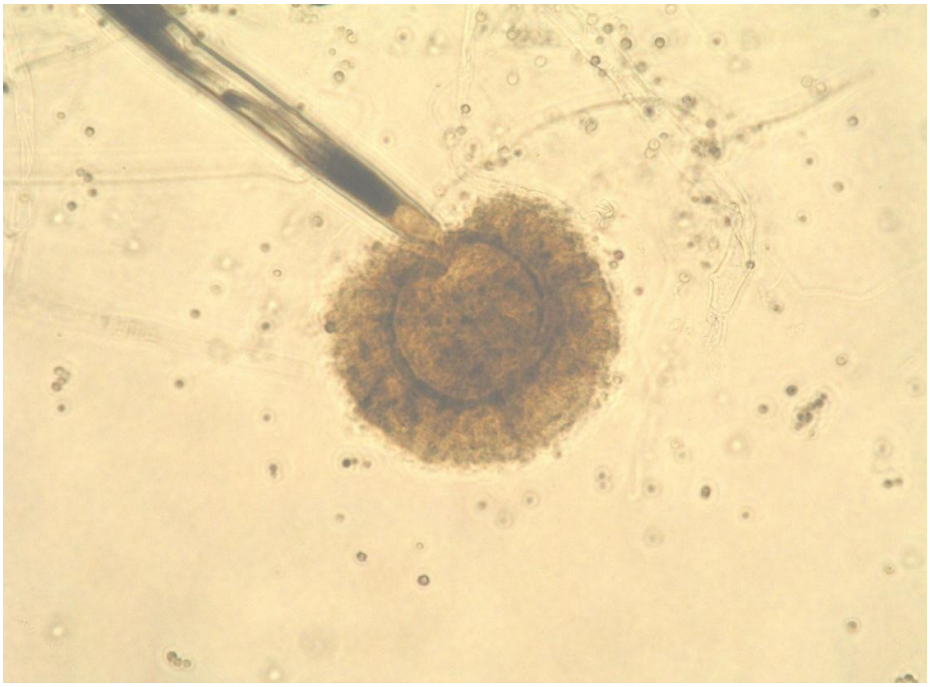

Fig. 2. Micrograph of *Aspergillus tubingensis* (Magnification x40)

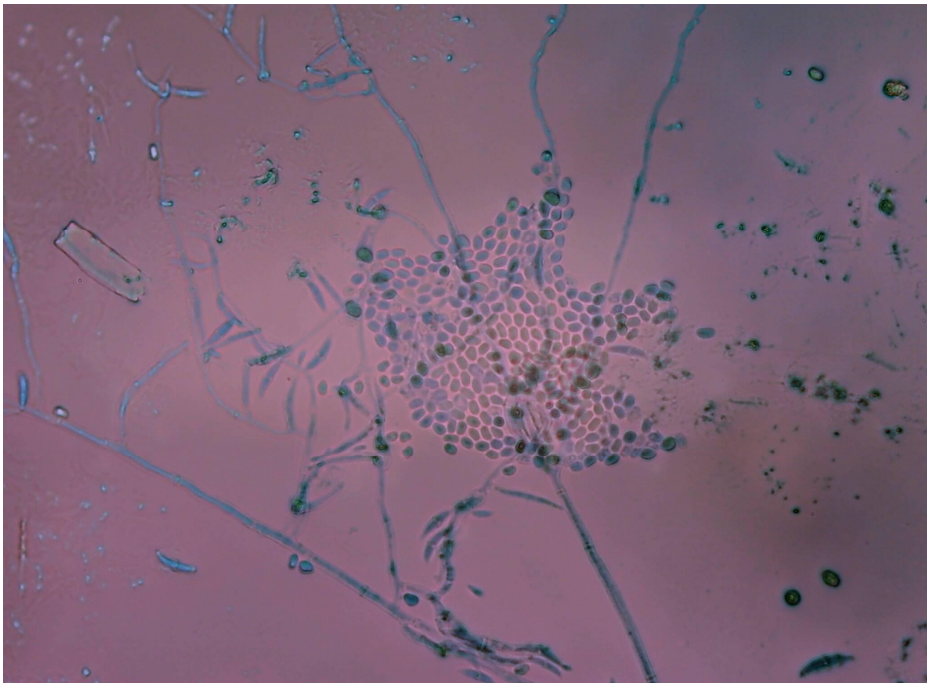

Fig. 3. Micrograph of *Fusarium solani* (Magnification x40)

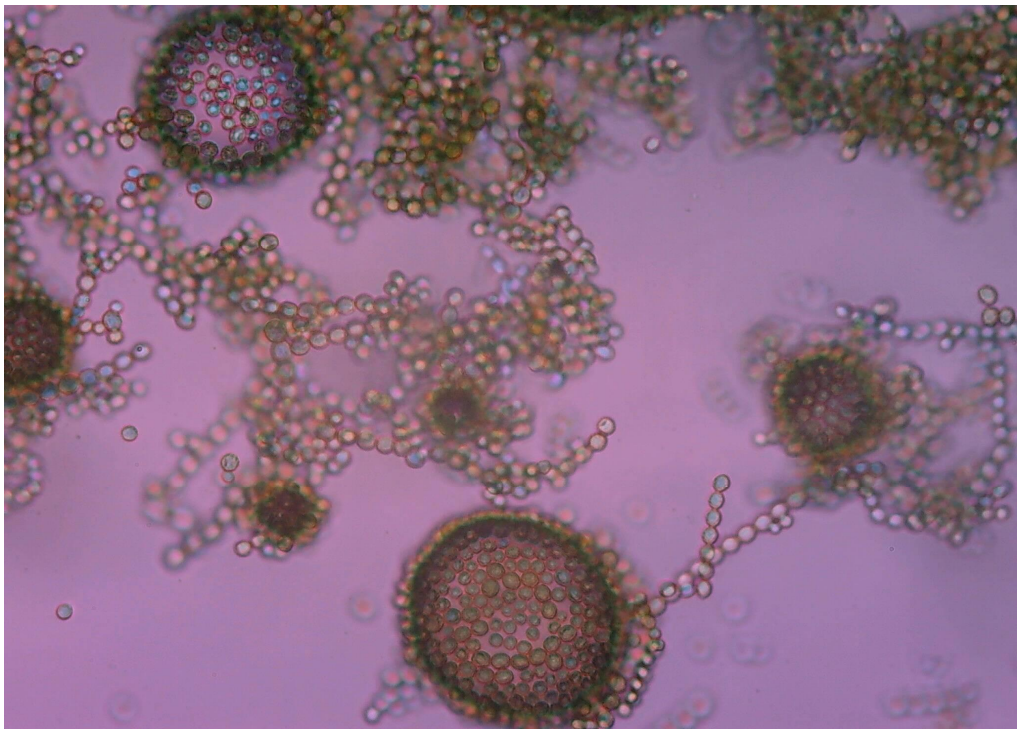

Fig. 4. Micrograph of *Candida rugosa (*Magnification x40)

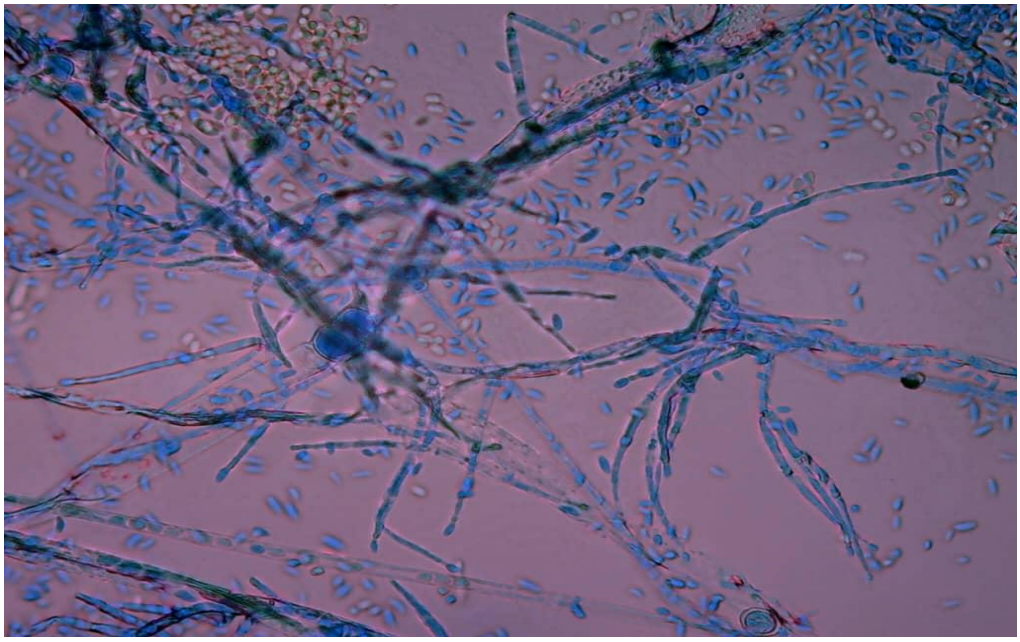

Fig. 5. Micrograph of *Candida akabenensis (*Magnification x40)

Among the disinfectants, Izal proved to be more effective against the fungal isolates with lower MIC and MFC compared to the MIC and MFC recorded for Polidine (Table 6).

3.4 Discussion

This study revealed the presence of airborne fungal organisms in poultry farms. Phenotypic

observation of the pure culture of the isolates and microscopic examination of the fungal cells revealed that the organisms isolated were *Aspergillus flavus, Candida akabenensis, Fusarium solani, Candida rugosa* and *Aspergillus tubingensis.* This finding corresponds with the findings of Jo and Kang [15] that the fungal aerosol in breeding building often contains mold from the genera *Aspergillus, Penicillium, Cladosporium, Rhizopus* and *Alterneria*.

Five species ascribed to three fungal genera were isolated and identified from the poultry house investigated. Species from the genera of Aspergillus, Candida and Fusarium made up a vast majority of the identified isolates.

Overall two species belonging to the genus Aspergillus were isolated and identified, as Aspergillus flavus and Aspergillus tubingensis. These two prevailed and made up 33.3% and 25 % respectively of all the identified isolates. The other isolates Fusarium solani, Candida akabenensis and Candida rugosa recorded an isolation frequency of 16.7%, 8.35 and 16.7% respectively of the total fungi isolated. This report is similar to other observations that Aspergillus species were the most frequent fungi in most poultry rooms [2,16].

Fungal concentrations across the four sites under study ranges from 0.01x103 cfu/m3 - 0.77x103 cfu/m3. The fungal concentrations reported inside poultry farms in this study were considerably higher than fungal concentrations reported in literature. Previous works and other studies revealed aerial contamination in the range of 3.1–6.4 log10 cfu/m3 in broiler houses, 4.5–7.6 log10 cfu/m3 in turkey houses, and4.7–8.3 log10 cfu/m3 in laying hen houses.

Table 4. Antifungal activities of *Eupatorium odoratum* and *Vernonia amygdalina* leaf extract (400 mg/ml)

Isolates	Mean inhibition zone diameter(mm) ±standard deviation				
	AEO	AVA	EEO	EVA	KET(50 µg/ml)
Aspergillus flavus	6.00± 0.770	6.30± 0.470	12.70 ± 0.940	13.7 ± 0.620	20.0 ± 1.41
Candida akabenensis	-	-	-	-	15.6 ± 0.750
Fusarium solani	-	-	8.70 ± 0.940	7.50 ± 0.600	17.0 ± 0.690
Candida rugosa	6.70± 0.940	-	8.70 ± 0.940	-	23.0 ± 0.710
Aspergillus tubingensis	5.30± 0.470	8.00± 0.41	17.0 ± 0.770	18.8 ± 0.240	19.3 ± 0.470

Key: AEO- aqueous extract of Eupatorium odoratum, EEO- ethanol extract of Eupatorium odoratum, AVA- aqueous extract of Vernonia amygdalina, EVA- ethanol extract of Vernonia amygdalina KET- ketoconazole

Table 5. Antifungal activity of common disinfectants (100 %v/v)

Isolates	Mean inhibition zone diameter(mm) ±standard deviation		
	IZ	PD	KET(50 µg/ml)
Aspergillus flavus	19.0 ± 0.410	16.3 ± 0.430	20.0 ± 1.41
Candida akabenensis	20.0 ± 0.500	14.0 ± 0.510	15.6 ± 0.750
Fusarium solani	22 .0 ± 0.710	13.0 ± 0.410	17.0 ± 0.690
Candida rugosa	18.0 ± 0.710	21.0 ± 0.710	23.0 ± 0.710
Aspergillus tubingensis	21.3 ± 0.470	14.3 ± 0.470	19.3 ± 0.470

Key: IZ- Izal, PD- Polidine

Table 6. Comparative minimum inhibitory and minimum fungicidal concentrations of Plant extracts and common disinfectants

Isolates	Minimum Inhibitory concentration (Minimum fungicidal Concentration) mg/ml					
	AEO	AVA	EEO	EVA	IZ (%v/v)	PD (%v/v)
Aspergillus flavus	200(200)	200(400)	100(200)	100(200)	12.5(25)	12.5(25)
Candida akabenensis	-	-	-	-	12.5(50)	50 (50)
Fusarium solani	-	-	100(100)	200(400)	12.5(25)	25(50)
Candida rugosa	400(400)	-	200(400)	-	25(50)	50 (100)
Aspergillus tubingensis	200(200)	400(400)	100(200)	100(100)	12.5(25)	50 (50)

Fungal concentrations in broiler, hen, and turkey houses were determined at 4.0–5.9, 3.8–5.8, and 2.7–5.5 log10 cfu/m^3 respectively [16].

In this study, the volumetric method for isolation, produced less although distinct growth, unlike the sedimentation method that produced more growth in the culture plates. Sedimentation method of isolation proved to yield more colony forming unit than the volumetric method possibly due to large surface area covered by the sedimentation method compared to surface area covered by volumetric method.

The *in vitro* antifungal activity assay of leaf extracts of *Eupatorium odoratum* and *Vernonia amygdalina*, on the fungal isolates from poultry farm revealed that the ethanol extract of the leaves had greater activity against the isolates than that of aqueous extract. This corresponds with other reports [14]. These may be attributed to the fact that bioactive compounds in leaves are more extractable in ethanol than water as previously suggested [17]. The *Eupatorium odoratum* and *Vernonia amygdalina* didn't have any effect on *Candida rugosa*. Both plants showed to be more efficacious against *Aspergillus tubingenesis* and *Aspergillus flavus*. The comparison between the plant extracts and common disinfectants showed that disinfectants had higher efficacy against the fungal isolates than the plant extracts. This report is consistent with other reports that showed that chemical disinfectants are more efficient than herbal agents [18].

After the antifungal evaluation analysis of *Eupatorium odoratum*, *Vernonia amygdalina* and disinfectants, Izal was found to be the most effective disinfectant against airborne fungi isolates. The results of this study showed that Izal will be more effective in disinfection of poultry houses followed by Polidine. Whereas, ethanolic extracts of *Eupatorium odoratum* was found to be the most effective herbal extract in disinfecting poultry houses as it had more activity against all the test isolate except *Candida akabenensis.* Aqueous extract of *Vernonia amygdalina* may not be considered effective in disinfecting poultry houses due to poor activity recorded across the test isolates.

However, the plant extracts used in this study compared favorably in efficacy with Izal and Polidine, and therefore may be considered for use as a cheap disinfectant in prevention and control of infection in the poultry farms.

These promising results shows that misting poultry houses with extracts of Eupatorium *odoratum* and *Vernonia amygdalina* could be an effective prevention method against fungal aerosol in broiler houses.

4. CONCLUSION

This research showed that solvent extracts (ethanol and aqueous) of *Eupatorium odoratum* and *Vernonia amgdalina* has antimicrobial effect on the aerial fungal isolates except for *Candida akabenensis*, although *Eupatorium odoratum* extracts showed more antimicrobial activity on more number of isolates than that of *Vernonia amgdalina*. The plant extracts competed favorably with the common disinfectants with respect to antifungal activities on the isolates. The results of this research has pointed to the potentials of these plant extracts against air borne fungi isolates and therefore has paved way further research on the effect of other known medicinal plants on the air borne fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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