

**COMPARATIVE EFFECTS OF TWO MEDICINAL PLANTS AND COMMON DISINFECTANTS AGAINST AIR-BORNE FUNGI IN POULTRY HOUSE**

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**ABSTRACT**

**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 were 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 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 akabanensis* and *Fusarium solani*. The aqueous extracts of *Eupatorium odoratum* and *Vernonia amygdalina* had activity against all the isolate except *Candida akabanensis* and *Fusarium solani* and *Candida rugosa*. Common disinfectants used in this study namely Izal and Polydine showed inhibitory activity against the isolates. Ethanol extract of *Eupatorium odoratum* recorded MIC(MFC) of 100 (200) mg/ml, 100 (100) mg/ml, 200 (400) mg/ml and 100 (200) mg/ml against *A. flaus*, *F. solani*, *Candida rugosa* and *A. tubingensis* respectively. Aqueous extract of *Eupatorium odoratum* recorded MIC(MFC) of 200 (200) mg/ml, 400 (400) mg/ml and 200 (200) mg/ml for *A. flaus*, *Candida rugosa* and *A. tubingensis* respectively. The MIC and MFC of both Izal and Polydine was between 12.5% V/V and 50% V/V against all the isolates except Polydine that had MFC of 100% V/V for *Candida rugosa*.

**Conclusion:** The extracts of *Eupatorium odoratum* and *Vernonia amygdalina* has antifungal activity against all the isolates except *Candida akabanensis*. 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.**

10  
11 *Keywords: Antifungal, Minimum Fungicidal concentration, Minimum Inhibitory Concentration*  
12 *(MIC), Poultry, Sedimentary-method of isolation, Volumetric method of isolation,*

## 13 **1. INTRODUCTION**

14  
15 The air in modern poultry production systems contains a large variety of air pollutants, such  
16 as gases (ammonia and carbon dioxide), dust, microorganisms and endotoxins. These  
17 pollutants commonly known as bio-aerosols are increasingly regarded as both aggravating,  
18 environmentally harmful and major public health concern for poultry workers and visitors (1).  
19 Human exposure to airborne dust and microorganisms such as bacteria and fungi can cause  
20 diseases particularly respiratory related ailments (2). This is because a large number of fungi  
21 produce mycotoxins and volatile organic compounds that can affect human and animal  
22 health. In susceptible or highly-exposed individuals these can lead to invasive mycosis (3).  
23 exposure. Indoor exposure levels are usually much higher than outdoor levels, which not  
24 often exceed  $10^4$  spores per cubic meter (4). It has been understood that activities in these  
25 indoor places such as cleaning and feeding animals increase occupational risk of exposure  
26 to airborne microorganisms (1). Spores of some type of fungi including *Cladosporium*,  
27 *Aspergillus*, *Penicillium* and *Alternaria*, according to Eduard may carry allergens, antigens,  
28 polysaccharides, and mycotoxins and can lead to allergic respiratory disease in susceptible  
29 individuals (4). The most common poultry fungal infections, such as Aspergillosis and  
30 Candidiosis, are commonly found in the environment of birds (5). Arné and colleagues  
31 argued that since there are no treatments for infected poultry, and therefore, the only  
32 effective way to protect chickens against mycoses is prevention (6). Some of the known  
33 methods used to reduce dust and fungal spores in the air of poultry buildings are misting  
34 with water and/or aqueous solutions of essential oils (peppermint, thyme, pine and  
35 eucalyptus oils) (7). The use of biological compounds extracted from medicinal plants may  
36 offer an alternative to conventionally used disinfectants to control air-borne fungi.  
37 With respect to many reports about the impact of plant extracts against food and grain  
38 storage fungi, foliar pathogens, nematodes, soil-borne as well as air-borne fungi (8), this  
39 research was undertaken to compare the antifungal effects of Siam weed (*Eupatorium*  
40 *odoratum*) leaf extract and bitter leaf (*Vernonia amygdalina*) extracts with common  
41 disinfectants Izal and Polydine on fungi isolated from air samples of poultry houses.

## 42 43 44 **2. MATERIAL AND METHODS**

### 45 46 **2.1 Sample Collection**

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

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

### 55 56 **2.2 Sample Processing**

57 In the laboratory, the cassettes of the air sampler were opened and the gel slides were  
58 placed on the surface of Sabouraud dextrose agar plates supplemented with 0.05% of

59 chloramphenicol. All the culture plates were incubated at room temperature, for five (5) days  
60 as described by (9).

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### 62 **2.3 Isolation and Identification of Fungi**

63 Fungi culture plates were purified by sub-culturing aseptically into new SDA media and  
64 subsequently incubated for another five (5) days at room temperature (10). The  
65 morphological characteristics of the pure fungi culture plates were observed and recorded  
66 for seven (7) days as described by Ezekwueche *et al.* (9). Fungal cells were stained using  
67 Lactophenol cotton blue and examined at a low power magnification (X40) using a light  
68 microscope. The results were compared with the descriptions in a fungal Atlas as previously  
69 reported by Adegunloye and Adejumo (11).

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71 Fungal count in **CFU/m<sup>3</sup>** was done using the formular below:

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73 **CFU/m<sup>3</sup>**= Total colonies x 10<sup>3</sup> / Air flow rate x collection time (2)

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### 75 **2.4 Collection and preparation of plant materials**

76 Fresh leaves of *Eupatorium odoratum* and *Vernonia amygdalina* were collected from Uli,  
77 Anambra State. The selection was based on the ethno medical uses for folk medicine. The  
78 leaves were washed with distilled water, air-dried at room temperature (30±2<sup>0</sup>C) for 14 days  
79 and pulverized using electronic blender (Binatone). Forty grams (40 g) portion of the leaves  
80 powder was each extracted by cold maceration in 400 ml of ethanol and water for 72 hours.  
81 The extracts were filtered, evaporated to dryness at 50<sup>0</sup>C using water bath as described by  
82 Grillo and Lawal (12)

### 83 **2.5 Antifungal Evaluation**

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

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

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96 The culture plates were incubated for 48 hours at 30±2<sup>0</sup>C. Antifungal activity were  
97 determined by measuring the inhibition zone diameter (in mm) produced after 48 hrs of  
98 incubation (13).

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### 100 **2.6 Determination of Minimum Inhibitory Concentration (MIC)**

101 Various concentration of the stock solution was made by double fold serial dilution to obtain,  
102 200 mg/ml, 100 mg/ml and 50mg/ml for the plant extracts. From the stock solution (Izal and  
103 Polydine (100% V/V), 50 %, 25 % and 12.5 %, 6.25 % V/V concentration were made. Each  
104 dilution in a test-tube was inoculated with 0.02 ml of the broth culture diluted to 0.5  
105 McFarland standards. A positive control test tubes were inoculated with the test organisms in  
106 the absence of the test agents, while the negative control test tubes has the test agents  
107 without the test organisms. All the tubes were incubated at 30±2<sup>0</sup>C for 72 h. the lowest  
108 concentration showing no visible growth was recorded as the minimum inhibitory  
109 concentration (MIC) for each organism (14)

## 110 2.7. Determination of Minimum Fungicidal Concentration

111 From each negative tube in MIC assay, 1 ml was transferred onto the surface of freshly  
112 prepared Sabouraud Dextrose Agar plates (without antibiotics or extracts) and the plates  
113 were incubated at  $30\pm 2^{\circ}\text{C}$  for 72 h for The lowest concentration showing no visible growth  
114 on SDA was recorded as minimum fungicidal concentration (MFC) for each organism (14)

## 115 2.8 Statistical Analysis

116 The data collected and generated in this study were organised and presented using SPSS  
117 version 20 and Microsoft Excel version 2007.

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## 119 3. RESULTS AND DISCUSSION

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### 121 3.1. Total Fungi count.

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

125 Table 1: Fungal count and conversion to colony forming unit

126

Sample site	No. of isolates by Sedimentary method	CFU/m <sup>3</sup>	No. of isolates by Volumetric method	CFU/m <sup>3</sup>
A	58	$0.77\times 10^3$	12	$0.12\times 10^3$
B	55	$0.73\times 10^3$	9	$0.09\times 10^3$
C	49	$0.65\times 10^3$	11	$0.01\times 10^3$
D	35	$0.47\times 10^3$	8	$0.08\times 10^3$

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### 128 3.2 Identification of Fungal cells

129 Three species ascribed to five fungal genera were isolated and identified from the poultry  
130 house investigated. The results of the macroscopic and microscopic observations made on  
131 the individual isolates are shown in table 2. These Isolates were observed to be *Aspergillus*  
132 *flavus*, *Aspergillus tubingensis*, *Candida akabanensis*, *Candida rugosa*, and *Fusarium solani*.  
133 In table 3, the sedimentation method of isolation revealed that *Aspergillus flavus*, *Aspergillus*  
134 *tubingensis*, *Candida akabanensis*, *Candida rugosa*, and *Fusarium solani* had 32 %, 24 %, 8  
135 %, 12 %, and 24 % frequency of occurrence respectively while Volumetric method of  
136 isolation recorded a 33.3 %, 25 %, 8.3 %, 16.7 % and 16.7 % frequency of occurrence  
137 respectively.

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**Table 2: Cultural and Microscopic characteristics of Fungi isolates.**

<b>Isolate</b>	<b>Macroscopy</b>	<b>Microscopy</b>
<i>Aspergillus flavus</i>	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.
<i>Candida akabanensis</i>	White to cream, soft, smooth to wrinkled colonies	Pseudohyphae and true hyphae with blastoconidia are present.
<i>Fusarium solani</i>	The surface of the colony was wooly to cottony and white creamy with dark brown zonation in colour.	It is long and branched monophialides.
<i>Candida rugosa</i>	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.
<i>Aspergillus tubingensis</i>	The surface color of the colony was black. The colony diameter was 2-7cm.	It has branched septate hyphae. It has bunch of spores arrangement and the spore shape was round.

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Table 3: Frequency of isolation of Fungi from poultry air

Isolate	SFI	SFI%	VFI	VFI%
<i>Aspergillus flavus</i>	8	32%	4	33.3%
<i>Candida akabanensis</i>	2	8%	1	8.3%
<i>Fusarium solani</i>	6	24%	2	16.7%
<i>Candida rugosa</i>	3	12%	2	16.7%
<i>Aspergillus tubingensis</i>	6	24%	3	25%
Total	25	100%	12	100%

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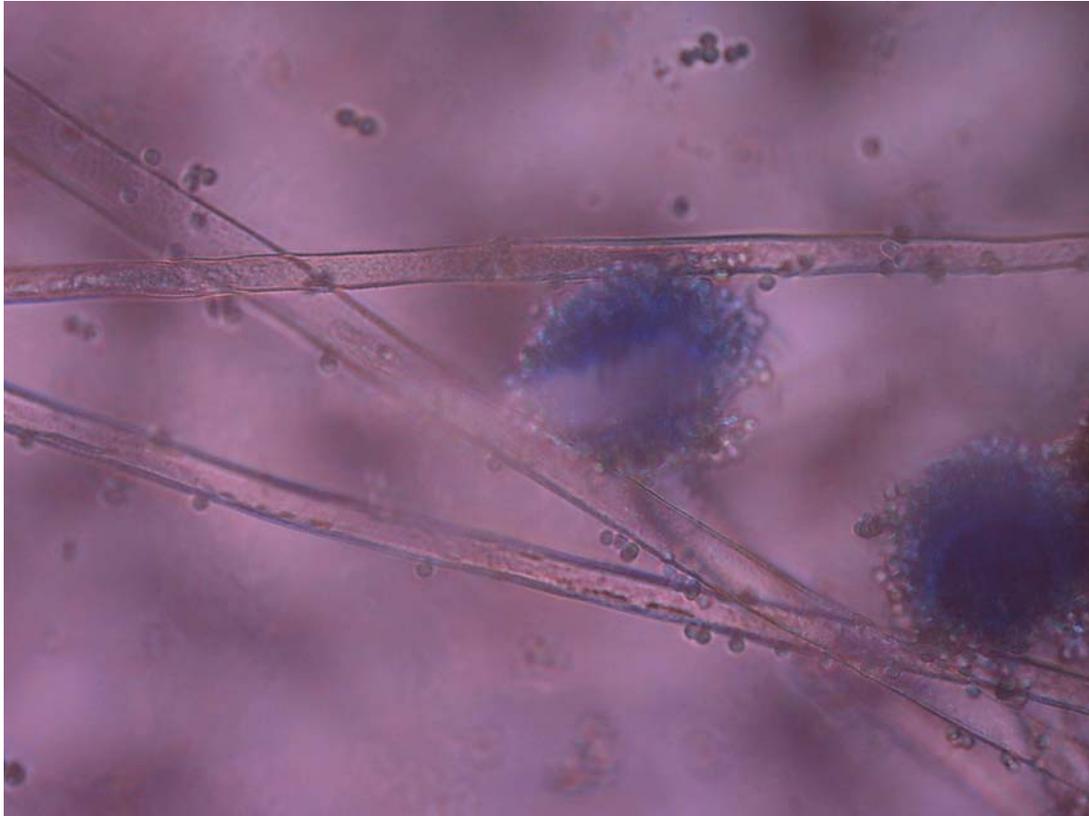
152 SFI: Sedimentary method frequency of isolation,  
153 VFI: Volumetric method frequency of Isolation.

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160 Figure 1: Micrograph of *Aspergillus flavus* (Magnification x40)

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172 Figure 2: Micrograph of *Aspergillus tubingensis* (Magnification x40)

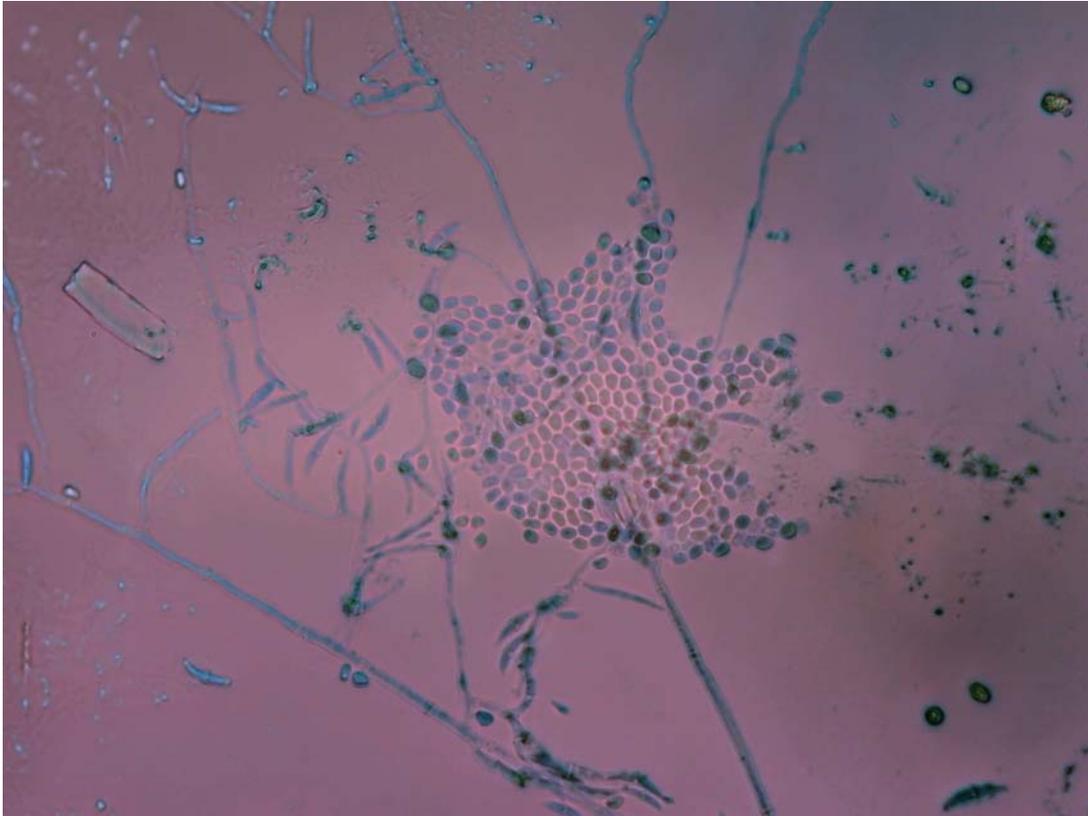
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179 Figure 3: Micrograph of *Fusarium solani* (Magnification x40)

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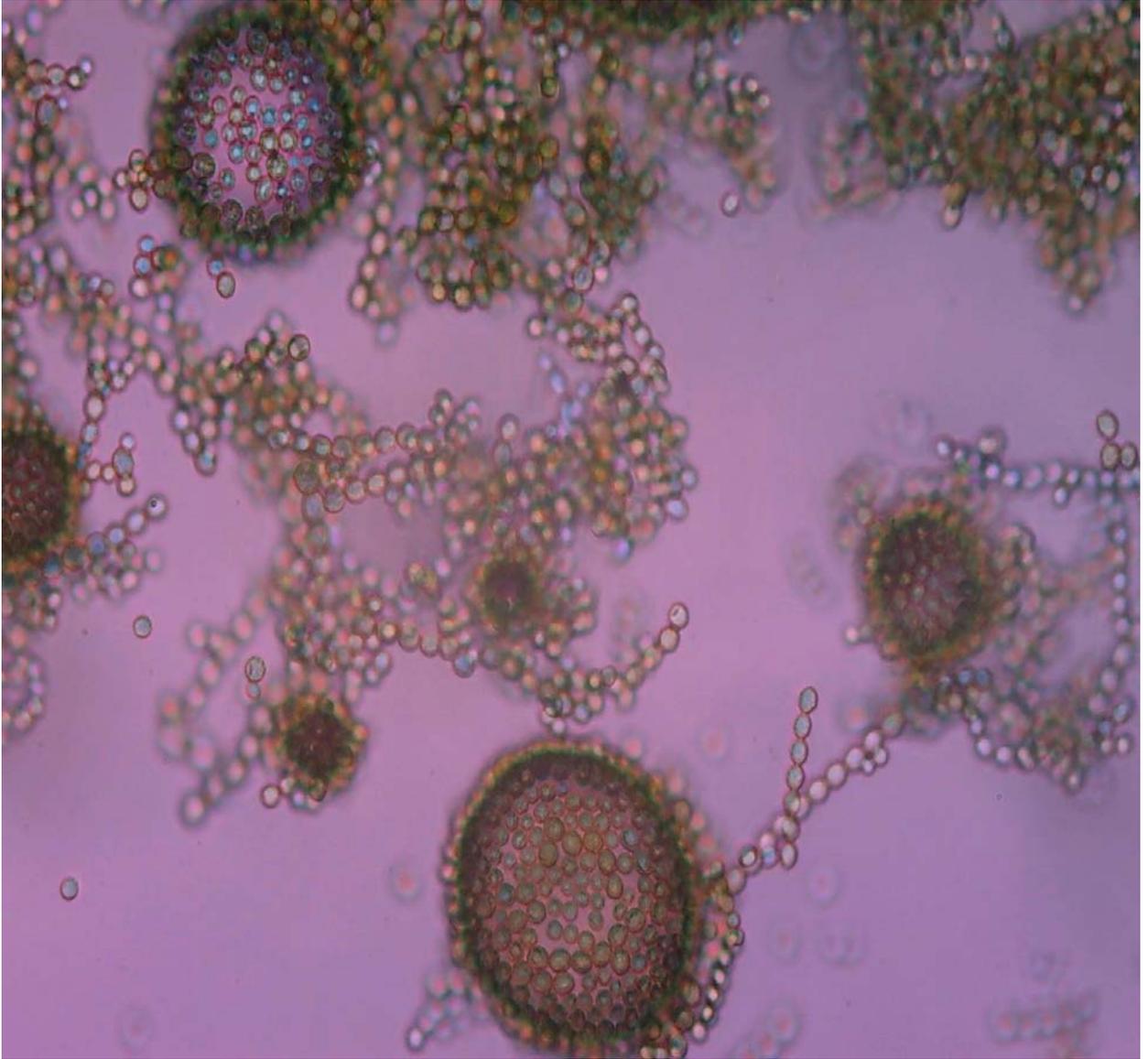
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188 Figure 4: Micrograph of *Candida rugosa* (Magnification x40)

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193 **3.3. Antifungal Activity**

194 Antimicrobial evaluation of the crude extracts showed that ethanol extract of *Eupatorium*  
 195 *odoratum* had activity against all the test isolates except *Candida akabenensis* and  
 196 *Fusarium solani*. The aqueous extracts of *Eupatorium odoratum* and *Candida akabenensis*  
 197 had less activity than the ethanol extracts (table 4). Common disinfectants used in this study  
 198 namely izal and polydine showed inhibitory activity against the isolates as revealed in table  
 199 5. The result of the evaluation also revealed that Izal is more effective than Polydine.  
 200 Comparatively, ethanol extracts of *Eupatorium odoratum* and *Vernonia amygdalina* leaf had  
 201 lower minimum inhibitory concentration and minimum fungicidal concentration against the  
 202 fungal isolates than the aqueous extract of the same plant being evaluated.

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

205 Table 4: Antifungal activities of *Eupatorium odoratum* and *Vernonia amygdalina* leaf extract  
 206 (400mg/ml)

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Isolates	mean inhibition zone diameter(mm) ±standard deviation				
	AEO	AVA	EEO	EVA	KET(50µg/ml)
<i>Aspergillus flavus</i>	6±0.77	6.3±0.47	12.7±0.94	13.7±0.62	20±1.41
<i>Candida akabenensis</i>	-	-	-	-	15.6±0.75
<i>Fusarium solani</i>	-	-	8.7±0.94	7.5±0.60	17±0.69
<i>Candida rugosa</i>	6.7±0.94	-	8.7±0.94	-	23±0.71
<i>Aspergillus tubingensis</i>	5.3±0.47	8±0.41	17±0.77	18.8±0.24	19.3±0.47

208 Key: AEO- aqueous extract of *Eupatorium odoratum*, EEO- ethanol extract of *Eupatorium*  
 209 *odoratum*, AVA- aqueous extract of *Vernonia amygdalina*, EVA- ethanol extract of *Vernonia*  
 210 *amygdalina* KET- ketoconazole  
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213 Table 5: Antifungal activity of common disinfectants (100 %v/v)

Isolates	mean inhibition zone diameter(mm) ±standard deviation		
	IZ	PD	KET(50µg/ml)
<i>Aspergillus flavus</i>	19±0.41	16.3±0.43	20±1.41
<i>Candida akabenensis</i>	20±0.50	14±0.51	15.6±0.75
<i>Fusarium solani</i>	22±0.71	13±0.41	17±0.69
<i>Candida rugosa</i>	18±0.71	21±0.71	23±0.71
<i>Aspergillus tubingensis</i>	21.3±0.47	14.3±0.47	19.3±0.47

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215 Key: IZ- Izal, PD- Polydine

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

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

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### 238 3.3 Discussion

239 This study revealed the presence of airborne fungal organism of poultry farms screened with  
 240 volumetric and sedimentation sampling method. Phenotypic observation of the pure culture  
 241 of the isolates and microscopic examination fungal cells revealed that the organisms isolated  
 242 were *Aspergillus flavus*, *Candida akabensis*, *Fusarium solani*, *Candida rugosa* and  
 243 *Aspergillus tubingensis* which corresponded with the findings of Jo and Kang (15) that the  
 244 fungal aerosol in breeding building often contains mold from the genera *Aspergillus*,  
 245 *Penicillium*, *Cladosporium*, *Rhizopus* and *Alternaria*.

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

249 Overall two species belonging to the genus *Aspergillus* were isolated and identified, as  
 250 *Aspergillus flavus* and *Aspergillus tubingensis*. These two prevailed and made up 33.3% and  
 251 25 % respectively of all the identified isolates. The other isolates *Fusarium solani*, *Candida*  
 252 *akabensis* and *Candida rugosa* recorded an isolation frequency of 16.7%, 8.35 and 16.7%  
 253 respectively of the total of isolated fungi. This finding is similar to the reports of Nichita  
 254 & Tirziu; Witkowska & Sowińska, (2, 16) who observed that the most frequent fungi in most  
 255 poultry rooms were *Aspergillus* species.

256 The Fungi concentrations reported inside poultry farms in this study (**Tables 1**) is low and  
 257 differ considerably in the literature. Previous work and other studies revealed aerial  
 258 contamination in the range of 3.1–6.4 log<sub>10</sub> cfu/m<sup>3</sup> in broiler houses, 4.5–7.6 log<sub>10</sub> cfu/m<sup>3</sup>  
 259 in turkey houses, and 4.7–8.3 log<sub>10</sub> cfu/m<sup>3</sup> in laying hen houses. Fungal concentrations in  
 260 broiler, hen, and turkey houses were determined at 4.0–5.9, 3.8–5.8, and 2.7–5.5 log<sub>10</sub>  
 261 cfu/m<sup>3</sup> respectively (16).

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

267 The invitro antifungal activity assay of leaf extracts of *Eupatorium odoratum* and *Vernonia*  
 268 *amygdalina*, on the fungal isolates from poultry farm revealed that the ethanol extract of the

269 leaves had greater activity against the isolates than that of aqueous extract. This  
270 corresponds with reports of (14). These may be attributed to the fact bioactive compound in  
271 the leaves were more extractable in ethanol than water as previously suggested by (17). The  
272 *Eupatorium odoratum* and *Vernonia amygdalina* didn't have any effect on *Candida rugosa*,  
273 the both plant appeared to be more efficacious against *Aspergillus tubingensis* and  
274 *Aspergillus flavus*. The comparison between the plant extracts and common disinfectants  
275 shows that had higher efficacy against the fungal isolates than that of plant extracts.  
276 The plant extracts compared favorably in efficacy with IZAL and Polydine, and therefore may  
277 be considered for use as a disinfectant in prevention and control of infection in the poultry  
278 farms. These promising results shows that misting poultry  
279 houses with extracts of *Eupatorium odoratum* and *Vernonia amygdalina* could be an  
280 effective prevention method against fungal aerosol in broiler houses.  
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#### 283 4. CONCLUSION

284 This research showed that solvent extracts (ethanol and aqueous) of *Eupatorium odoratum*  
285 and *Vernonia amygdalina* has antimicrobial effect on the aerial fungal isolates except for  
286 *Candida akabensis*, although *Eupatorium odoratum* extracts showed more antimicrobial  
287 activity on more number of isolates than that of *Vernonia amygdalina*. The plant extracts  
288 competed favorably with the common disinfectants with respect to antifungal activities on the  
289 isolates. The results of this research has pointed to the potentials of these plant extracts  
290 against air borne fungi isolates and therefore has paved way further research on the effect of  
291 other known medicinal plants on the air borne fungi.  
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#### 294 **COMPETING INTERESTS DISCLAIMER:**

295  
296 Authors have declared that no competing interests exist. The products used for this  
297 research are commonly and predominantly use products in our area of research and  
298 country. There is absolutely no conflict of interest between the authors and  
299 producers of the products because we do not intend to use these products as an  
300 avenue for any litigation but for the advancement of knowledge. Also, the research  
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302 of the authors.  
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