

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

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 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, 400mg/ml and 100mg/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.

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

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14 **1.0 INTRODUCTION**

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

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

24 Indoor exposure levels are usually much higher than outdoor levels, which not often exceed
25 10^4 spores per cubic meter (4). It has been understood that activities in these indoor places

26 such as cleaning and feeding animals increase occupational risk of exposure to airborne
27 microorganisms (1). Spores of some type of fungi including *Cladosporium*, *Aspergillus*,

28 *Penicillium* and *Alternaria*, according to Eduard may carry allergens, antigens,
29 polysaccharides, and mycotoxins and can lead to allergic respiratory disease in susceptible

30 individuals (4). The most common poultry fungal infections, such as Aspergillosis and
31 Candidiosis, are commonly found in the environment of birds (5). Arné and colleagues

32 argued that since there are no treatments for infected poultry, and therefore, the only
33 effective way to protect chickens against mycoses is prevention (6). Some of the known

34 methods used to reduce dust and fungal spores in the air of poultry buildings are misting
35 with water and/or aqueous solutions of essential oils (peppermint, thyme, pine and

36 eucalyptus oils) (7). The use of biological compounds extracted from medicinal plants may
37 offer an alternative to conventionally used disinfectants to control air-borne fungi.

38 With respect to many reports about the impact of plant extracts against food and grain
39 storage fungi, foliar pathogens, nematodes, soil-borne as well as air-borne fungi (8), this

40 research was undertaken to compare the antifungal effects of Siam weed (*Eupatorium*

41 *odoratum*) leaf extract and bitter leaf (*Vernonia amygdalina*) extracts with common
42 disinfectants Izal and Polidine on fungi isolated from air samples of poultry houses.

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45 **2. MATERIAL AND METHODS**

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47 **2.1 Sample Collection**

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

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

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57 **2.2 Sample Processing**

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

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63 **2.3 Isolation and Identification of Fungi**

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

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71 Fungal count in **CFU/m³** was done using the formula below:

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73 **CFU/m³**= Total colonies x 10³ / 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 Nigeria. The selection was based on the ethno medical uses for folk
78 medicine. The leaves were washed with distilled water, air-dried at room temperature
79 (30±2⁰C) for 14 days and pulverized using electronic blender (Binatone). Forty grams (40 g)
80 portion of the leaves powder was each extracted by cold maceration in 400 ml of ethanol
81 and water for 72 hours. The extracts were filtered, evaporated to dryness at 50⁰C using
82 water bath (12). The disinfectants (Izal and Polidine) were sourced from Animal Care
83 Company in Oshimili south Local Government Area, Asaba, Delta state Nigeria.

84 **2.5 Antifungal Evaluation**

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

91 Each labeled Sabouraud dextrose agar plate was uniformly inoculated with a McFarland
92 standardized test organisms. A sterile cork borer of 6 mm diameter was used to make wells

93 on the culture plates. One hundred (100) µl of various concentrations of the extracts were
94 dispensed into each agar-well, labeled with the corresponding concentrations. Fifty (50) µg
95 of ketoconazole (Ketoral) was used as positive control.

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

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101 **2.6 Determination of Minimum Inhibitory Concentration (MIC)**

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

111 **2.7. Determination of Minimum Fungicidal Concentration**

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

116 **2.8 Statistical Analysis**

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

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121 **3.0. RESULTS AND DISCUSSION**

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123 **3.1. Total Fungi count.**

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

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

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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

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130 **3.2 Identification of Fungal cells**

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

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Table 2: Cultural and Microscopic characteristics of Fungi isolates.

Isolate	Macroscopy	Microscopy
<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	8.30%
<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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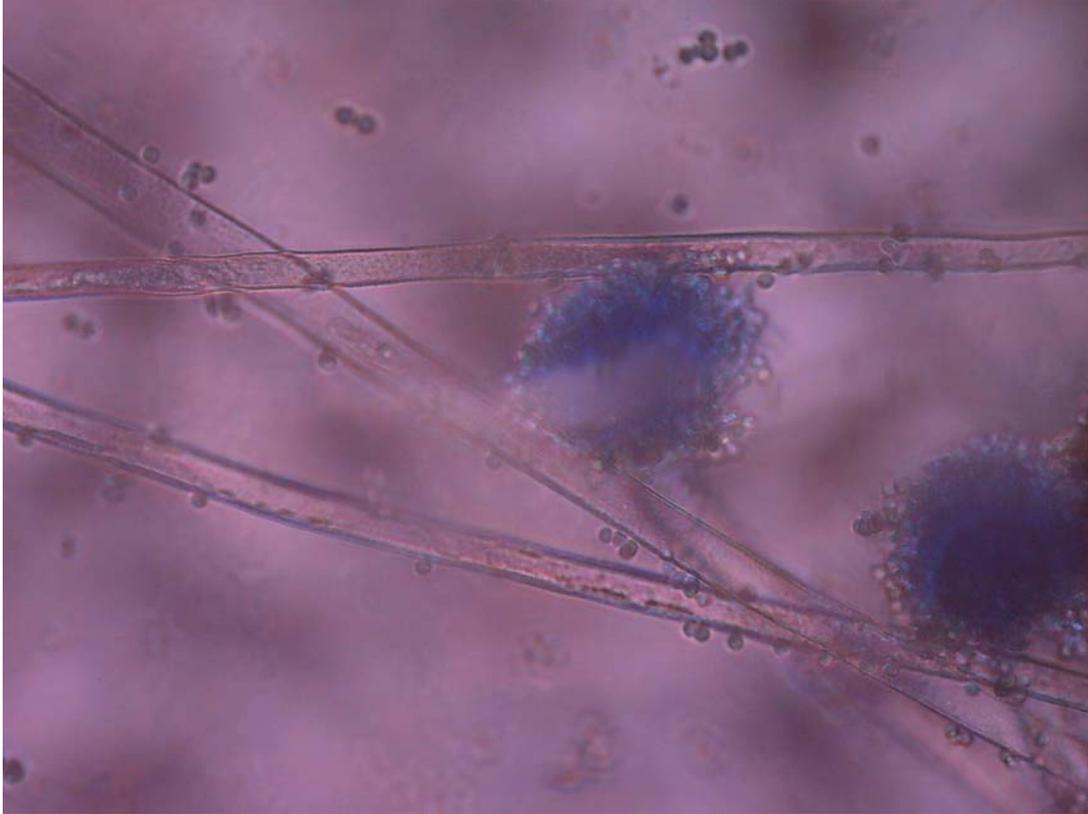
155 SFI: Sedimentary method frequency of isolation,
156 VFI: Volumetric method frequency of Isolation.

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

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

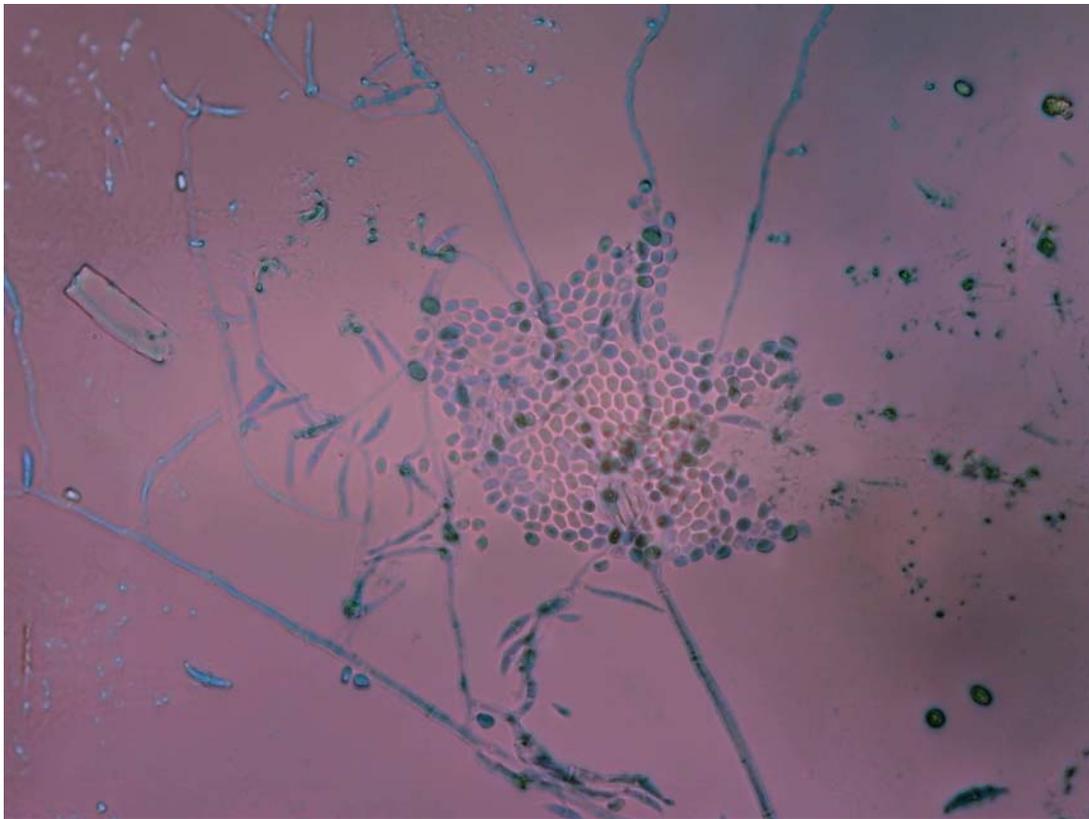
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182 Figure 3: Micrograph of *Fusarium solani* (Magnification x40)

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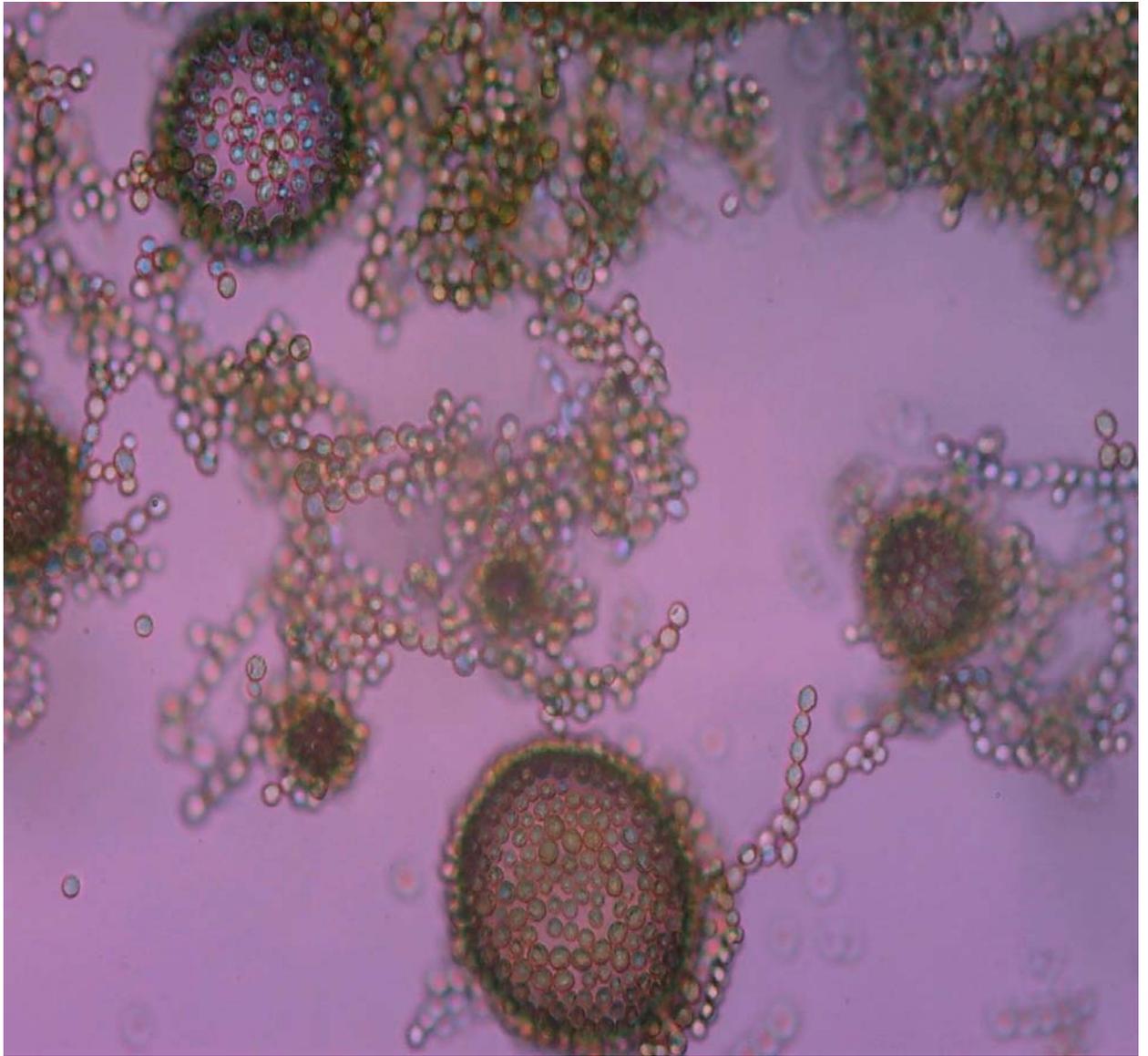
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191 Figure 4: Micrograph of *Candida rugosa* (Magnification x40)

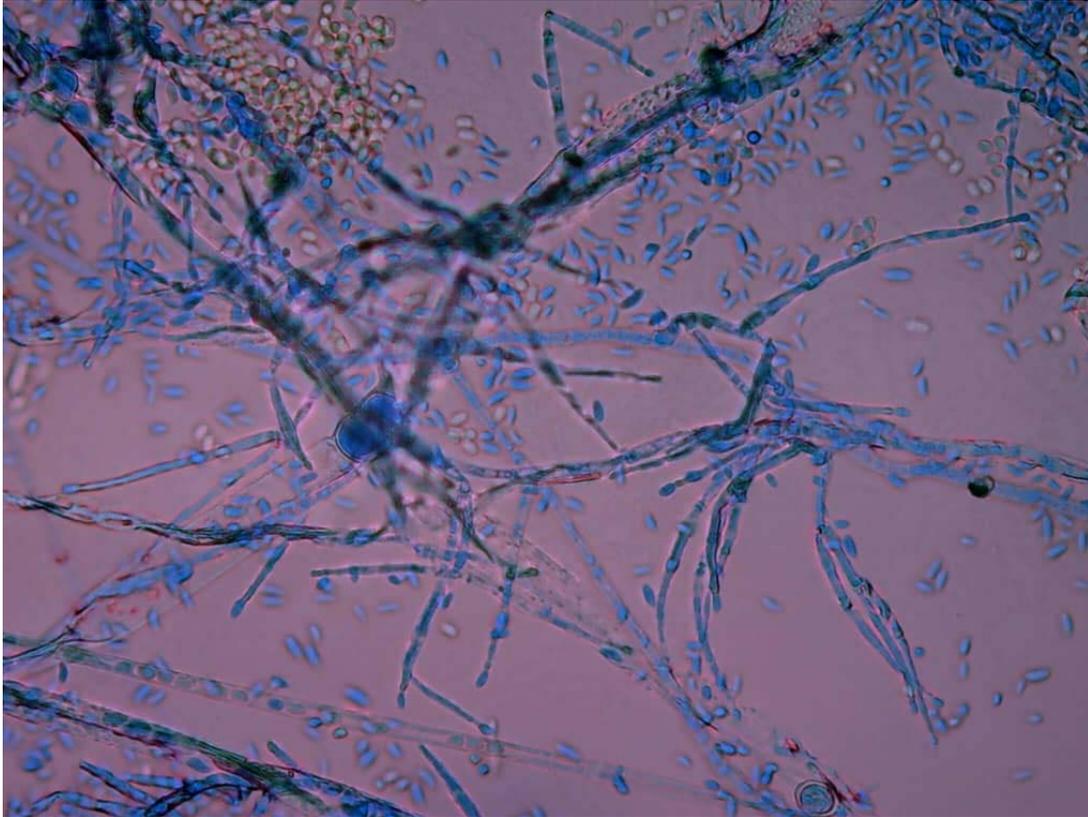
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202 Figure 5: Micrograph of *Candida akabenensis* (Magnification x40)

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3.3. Antifungal Activity

206 Antimicrobial evaluation of the crude extracts showed that ethanol extract of *Eupatorium*
207 *odoratum* had activity against all the test isolates except *Candida akabenensis* and
208 *Fusarium solani*. The aqueous extracts of *Eupatorium odoratum* and *Candida akabenensis*
209 had less activity than the ethanol extracts (table 4). Common disinfectants used in this study
210 namely IZAL and Polidine showed inhibitory activity against the isolates as revealed in table
211 5. The result of the evaluation also revealed that IZAL is more effective than Polidine.
212 Comparatively, ethanol extracts of *Eupatorium odoratum* and *Vernonia amygdalina* leaf had
213 lower minimum inhibitory concentration and minimum fungicidal concentration against the
214 fungal isolates than the aqueous extract of the same plant being evaluated.

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

217 Table 4: Antifungal activities of *Eupatorium odoratum* and *Vernonia amygdalina* leaf extract
218 (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>	6.00 ± 0.770	6.30 ± 0.470	12.70 ± 0.940	13.7 ± 0.620	20.0 ± 1.41
<i>Candida akabenensis</i>	-	-	-	-	15.6 ± 0.750
<i>Fusarium solani</i>	-	-	8.70 ± 0.940	7.50 ± 0.600	17.0 ± 0.690
<i>Candida rugosa</i>	6.70 ± 0.940	-	8.70 ± 0.940	-	23.0 ± 0.710
<i>Aspergillus tubingensis</i>	5.30 ± 0.470	8.00 ± 0.41	17.0 ± 0.770	18.8 ± 0.240	19.3 ± 0.470

220 Key: AEO- aqueous extract of *Eupatorium odoratum*, EEO- ethanol extract of *Eupatorium*
221 *odoratum*, AVA- aqueous extract of *Vernonia amygdalina*, EVA- ethanol extract of *Vernonia*
222 *amygdalina* KET- ketoconazole
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225 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 ± 0.410	16.3 ± 0.430	20.0 ± 1.41
<i>Candida akabenensis</i>	20.0 ± 0.500	14.0 ± 0.510	15.6 ± 0.750
<i>Fusarium solani</i>	22.0 ± 0.710	13.0 ± 0.410	17.0 ± 0.690
<i>Candida rugosa</i>	18.0 ± 0.710	21.0 ± 0.710	23.0 ± 0.710
<i>Aspergillus tubingensis</i>	21.3 ± 0.470	14.3 ± 0.470	19.3 ± 0.470

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227 Key: IZ- Izal, PD- Polidine

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

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Isolates	Minimum Inhibitory concentration (Minimum fungicidal Concentration) mg/ml					
	AEO	AVA	EEO	EVA	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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249 3.3 Discussion

250 This study revealed the presence of airborne fungal organisms in poultry farms. Phenotypic
 251 observation of the pure culture of the isolates and microscopic examination of the fungal
 252 cells revealed that the organisms isolated were *Aspergillus flavus*, *Candida akabensis*,
 253 *Fusarium solani*, *Candida rugosa* and *Aspergillus tubingensis*. This finding corresponds with
 254 the findings of Jo and Kang (15) that the fungal aerosol in breeding building often contains
 255 mold from the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus* and *Alternaria*.

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

259 Overall two species belonging to the genus *Aspergillus* were isolated and identified, as
 260 *Aspergillus flavus* and *Aspergillus tubingensis*. These two prevailed and made up 33.3% and
 261 25 % respectively of all the identified isolates. The other isolates *Fusarium solani*, *Candida*
 262 *akabensis* and *Candida rugosa* recorded an isolation frequency of 16.7%, 8.35 and 16.7%
 263 respectively of the total fungi isolated. This report is similar to other observations that
 264 *Aspergillus* species were the most frequent fungi in most poultry rooms (2), (16).

265 Fungal concentrations across the four sites under study ranges from 0.01×10^3 cfu/m³
 266 -0.77×10^3 cfu/m³. The fungal concentrations reported inside poultry farms in this study were
 267 considerably higher than fungal concentrations reported in literature. Previous works and
 268 other studies revealed aerial contamination in the range of 3.1–6.4 log₁₀ cfu/m³ in broiler
 269 houses, 4.5–7.6 log₁₀ cfu/m³ in turkey houses, and 4.7–8.3 log₁₀ cfu/m³ in laying hen
 270 houses. Fungal concentrations in broiler, hen, and turkey houses were determined at 4.0–
 271 5.9, 3.8–5.8, and 2.7–5.5 log₁₀ cfu/m³ respectively (16).

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

277 The *in vitro* antifungal activity assay of leaf extracts of *Eupatorium odoratum* and *Vernonia*
 278 *amygdalina*, on the fungal isolates from poultry farm revealed that the ethanol extract of the
 279 leaves had greater activity against the isolates than that of aqueous extract. This
 280 corresponds with other reports (14). These may be attributed to the fact that bioactive
 281 compounds in leaves are more extractable in ethanol than water as previously suggested

282 (17). The *Eupatorium odoratum* and *Vernonia amygdalina* didn't have any effect on *Candida*
283 *rugosa*. Both plants showed to be more efficacious against *Aspergillus tubingensis* and
284 *Aspergillus flavus*. The comparison between the plant extracts and common disinfectants
285 showed that disinfectants had higher efficacy against the fungal isolates than the plant
286 extracts. This report is consistent with other reports that showed that chemical disinfectants
287 are more efficient than herbal agents (18).

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

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

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

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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 DISCLAIMER:

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.

Ethical: NA

Consent: NA

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