

1 **ISOLATION AND IDENTIFICATION OF MYCOTOXIGENIC ORGANISMS IN POULTRY**
2 **FEED FROM SELECTED LOCATIONS IN ABIA STATE, NIGERIA**

3 **ABSTRACT**

4 Feed contamination by fungi can lead to nutrient losses and detrimental effects on animal
5 health and production. This present study was designed to isolate and identify the mycological
6 contamination of poultry feeds in some selected parts of Abia state (farms and feed depots in
7 Umuahia north, Osioma and its environs). A total of 120 samples were collected and used for
8 the study. The samples were screened and processed using spread plate technique. The isolates
9 were identified using slide culture technique. From the samples collected, the fungi
10 contamination in feed samples from depots in Umuahia was 50%, Osioma 78% and in farms it
11 was 85%. Five fungi organisms were isolated from the feed sample which includes *Aspergillus*,
12 *Penicillium*, *Fusarium*, *Mucor* and *Yeast* which were seen in almost all the feed samples.
13 *Aspergillus* (87%) recorded the highest percentage occurrence, followed by *Penicillium* (27%),
14 *Fusarium* (24%), *Yeast* (5%) and *Mucor*(2%). The total fungi load was significant at $2.0 \times$
15 10^5 CFU/g=1 for feed samples from Umuahia North Local government Area, 7×10^5 CFU/g=1 from
16 Osioma feed depot and 1×10^6 CFU/g=1 from poultry farms thereby making the feed samples
17 unsafe for poultry consumption. Therefore, there is need for screening of feeds in these
18 locations in Abia state due to its high fungal load and percentage contamination.

19 **Keyword:** Feed, mycological agents, identification, occurrence, location

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21 **Introduction**

22 The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes and
23 nutritional quality (Cegielska-Radziejewska et al.,2013). Moulds like other microorganisms will
24 assimilate and utilize the most readily available nutrient in the material they grow upon and
25 spoilage may lead to the loss of some of the nutrients in the feed (Okoli et al.,2006).

26 Among microorganisms, fungi have important effects on the quality of feed. Fungi growth
27 sometimes leads to non-consumption of feed for poultry (Magnoli et al., 2007; Magnoli et al.,
28 2005) .Several factors may lead to the spread of fungi infections such as geographical location,
29 storage conditions, processing of various feeds and moisture. Among the mentioned factors,
30 moisture is the most important factor, hence, rendering the moisture in feed constant to lesser
31 percentage will eliminate fungal growth and aflatoxin production will be stopped (Pitt and
32 Hocking, 2009)

33 Mycotoxins are poisonous toxins/substances and secondary metabolites produced by fungi
34 (Tola and Kedebe, 2016; Lereau et al;2012; Monbaliu et al;2010). The filamentous general of
35 fungi produces secondary metabolites which have deleterious effects on human and animal
36 consumers following consumption of contaminated foods and this ultimately affects the
37 economy of the country (Mestafa et al.,2012).

38 Most toxic species belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and
39 produce mycotoxins that are of public health importance/concern such as aflatoxin, Ochratoxin
40 A, T2-toxin, fursarotoxin, furmonisins, patulin, zearalenone and deoxynivalenol (Gimemo et
41 al.,2007;Iqbal et al.,2014; Orellano.,2007). Feed contaminated with mycotoxins negatively
42 affect poultry performance and their health (Monson et al., 2014). Most mycotoxicosis of
43 poultry are caused by intake of low concentration of contaminants over a long period of time
44 resulting in the typical chronic symptoms of poor growth, poor feed efficiency and suboptimal
45 production. Ingestion of high concentration however leads to acute clinical symptoms
46 associated with specific vital organs, the immune system and other aspects of avian physiology
47 as well as mortality (Mabbet, 2004).. Fungi causes a significant loss in the poultry industry being
48 responsible for high morbidity and mortality rate especially in young birds and causes stunted
49 growth and diarrhea and fetal encephalitis (Moss, 1992). They also cause drop in egg production
50 leading to economic losses (Cast,2003). In this work, presence of potentially mycotoxigenic
51 fungi in samples of poultry feed was determined. Abia state is in Nigeria which is a tropical
52 country with a predominant hot humid environment and the environment is much favorable for
53 the propagation of fungi on feed and feed materials. To prevent economic losses in poultry,
54 isolation and identification of birds affected by fungal infection needs to be determined and such

55 studies on commercial broiler feed sample in Abia state is not well reported. This informs the
56 essence of this study in Abia state south east Nigeria.

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MATERIALS AND METHODS

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STUDY AREA

61 Samples were collected between April – June from 2 local government areas of Abia state.

62 (Umuahia and Osisioma)

63 Abia state in Nigeria is located in a tropical rainforest between latitude 543N and longitude

64 752E. The average annual temperature and rainfall are 26.9⁰C and 2193mm respectively (Kottek

65 *et al.*,2006).

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SAMPLE COLLECTION

67 Poultry feeds were sampled from farms and different feed depots in 2 different local government

68 areas (Umuahia North and Osisioma). The total feed samples collected were one hundred and

69 twenty (120) in number (which includes Top feeds, Vital, Animal care, and Apex feed) was used

70 to isolate and identify the presence of mycotoxigenic fungi. Forty (40) feed samples were

71 collected from each of the locations. Also 40 feed samples were collected randomly from

72 poultry farms within the 2 localities. The representative samples were collected batch by batch

73 using simple random sampling technique. The sampling plan was carried out according to Food

74 and Agriculture Organization (FAO, 1993). Take 10grams from each batch and mix them

75 together. Samples were collected two weeks intervals and collection lasted three months (April-

76 June). Fungal contamination and fungal count determination was carried out in each sample to

77 determine the fungal genera and the total fungal population in the Department of Veterinary

78 Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike

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Fungal Isolation and identification

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LABOURATORY PROCEDURE

82 Sabouraud dextrose agar medium was used for the isolation of fungi in the feed samples. The

83 medium was prepared aseptically following the manufacturer's description. After autoclaving, a

84 calculated amount of penicillium and streptomycin was mixed with the medium to help inhibit

85 the growth of bacteria. Therefore, the medium was dispensed into sterile petri dishes in aseptic
86 environment.

87 Serial dilution plate technique (Omenka and Anyasor, 2010) was used for fungal isolation and
88 general fungi counts. One gram of each of the representative samples was mixed with 9ml of
89 sterile distilled water on a horizontal position and shake for 30mins to form uniform suspension.
90 For each feed sample, five dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} 10^{-5} were made from each dilution,
91 0.1ml of dilution was aseptically inoculated on Sabouraud dextrose agar supplemented with
92 penicillin and streptomycin (Vesna et al., 2010). A surface spread plate technique was used to
93 achieve uniform distribution of the spores. Inoculated plates were incubated at 25°C for 5-7days
94 for isolation of the fungi and overall quantitative enumeration of fungal colonies per gram of the
95 feed sample, isolates were identified based on colonial and microscopic morphologies (Andersen
96 *et al.*,2003; Pitt and Hocking1997). Microscopic examination of the isolate was done using wet
97 mount and slide culture technique (Leck,1999). The relative occurrence of fungal genera was
98 calculated in percentage using the following

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$$100 \text{ Percentage occurrence of fungal genus: } \frac{\text{Number of isolates} \times 100}{\text{Total Number of Fungi}}$$

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$$103 \text{ Total fungal load CFU/g: } \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume used}}$$

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107 **4.1 RESULTS**

108 Plate 1 and Plate 2 shows the morphological presentation of the colonies of *Aspergillus specie*
109 and *Fusarium species* which appears in form of an emulsion as brownish and whitish
110 colouration. Plate 3 show colonies typical of *Penicillium specie* marked with remarkable in-
111 folding while Plate 4 reveals different colouration consistent with colonies of mixed fungi
112 infection.

113 Figure1 shows the presence of *Aspergillus spp* at magnification of $\times 100$, the marked evidence of
114 oval dark hypha measuring about 0.5mm can be comparable to similar findings of *Aspergillus*
115 occurrence. Figure 2 presents a remarkable cauliflower with distinctive dark hypha and unique
116 long conidiospore characteristic of *Aspergillus*. Fig3 shows marked long conidiospore with
117 many branches about (6-10) with long dark hypha and diameter of about 0.5mm. Fig 5: indicates
118 aggregates of fungi hypha called mycelium.

119 From the study, the fungi species isolated and identified down to genus level are *Aspergillus*,
120 *Penicillium*, *Fusarium*, *Yeast* and *Mucor*.

121 Table 1 show that *Aspergillus penicillium* and *fusarium* contamination was recorded in the 3
122 locations, yeast was absent in samples collected from Umuahia while *mucor* was present only in
123 farms. Table2: shows the total samples collected from each location and the positive numbers,
124 85% were positive from farms, 78% were positive from Osisoma and 50% were positive from
125 Umuahia.

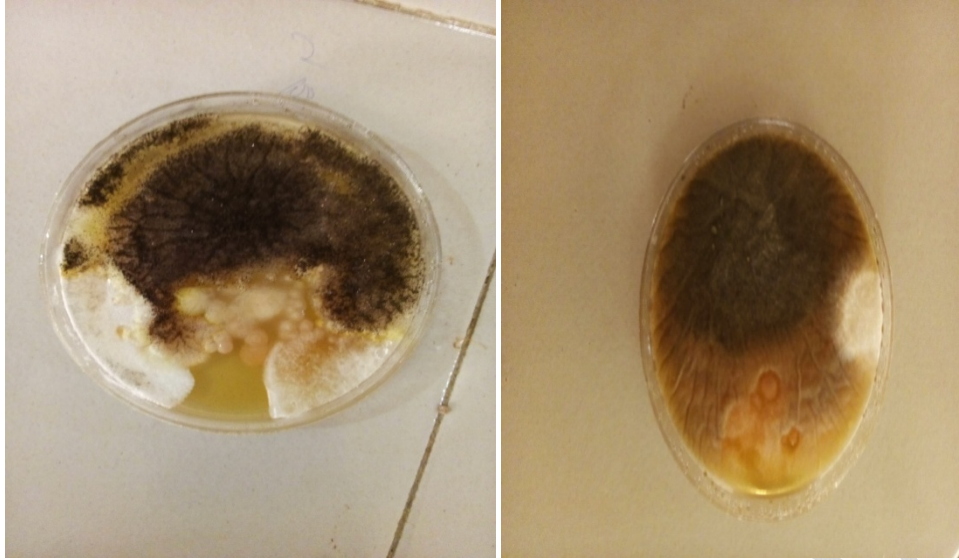
126 From the above study, the genus *aspergillus* had the highest frequency of isolate at 85% followed
127 by *penicillium* (27%), *fusarium* (25%), yeast (5%) and *mucor* (@%) as shown in figure 6. Table
128 3 shows that feed sampled from farms has the highest fungal load followed by samples from
129 Osisoma then samples from Umuahia feed distributors and depot. Table 3: suggests that the feed
130 sampled from poultry farms and Osisoma feed distributors and depots have much fungal load of
131 about 1×10^6 and 7×10^5 compared to that from Umuahia which have fungal load of about 2.0×10^5

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137 Plate:1 Colonies of *Aspergillus* and *fusarium*; Plate:2 colonies of *Aspergillus* Morphological
138 view

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147 Plate 3: Colonies of *Penicillium spp* Plate 4: colonies of mixed fungi infection (morphological
148 view)

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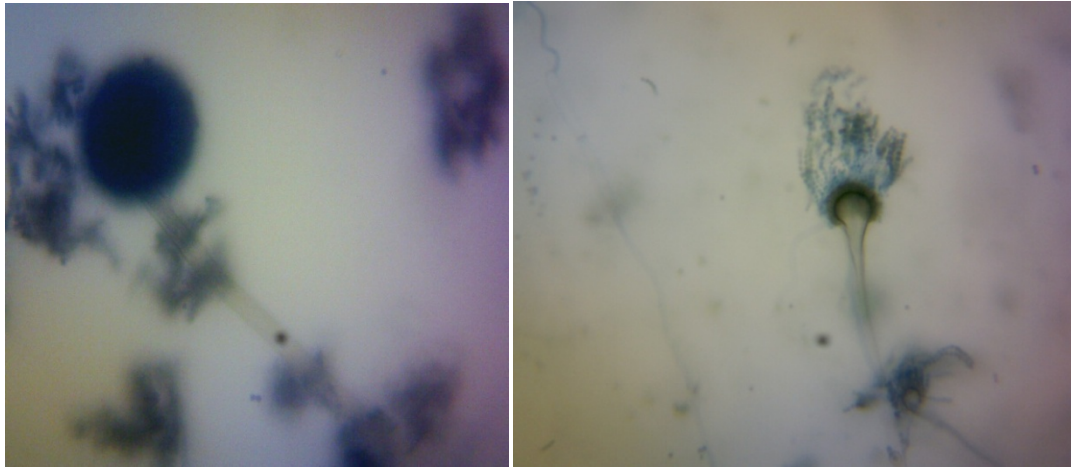
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163 Fig: 1 *Aspergillus* ×100MG Fig 2: *Aspergillus* view ×100MG

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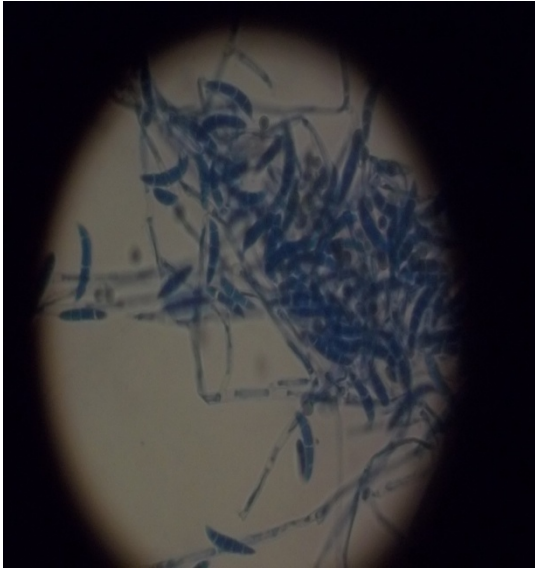
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180 Fig3 *Fusarium spp* x100MG

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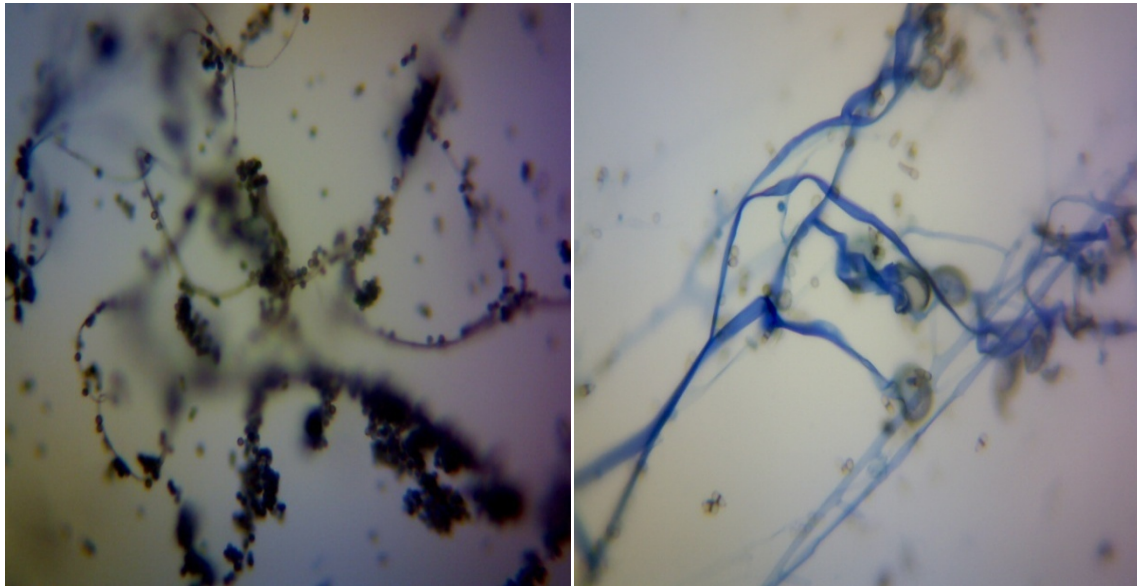
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194 Fig4: *Penicillium spp* x100MG

Fig5: *Fungi mycelium* x100MG

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199 Table 1: Fungi Genera Isolated From Some Selected Locations in Abia State

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Fungi organisms	Umuahia North	Osioma	Farms (both)
Aspergillus	+	+	+
Penicillium	+	+	+
Fusarium	+	+	+
Yeast	-	+	+
Mucor	-	-	+

201 Keys + (positive) – (Negative)

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203 **Table2: Percentage and frequency of Fungi Contamination of The Feed Sampled From**
204 **Various Locations**

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Locations	No of samples	No of samples contamination	Level of % contamination
Farms	40	34	85
Osisioma	40	31	78
Umu North	40	20	50

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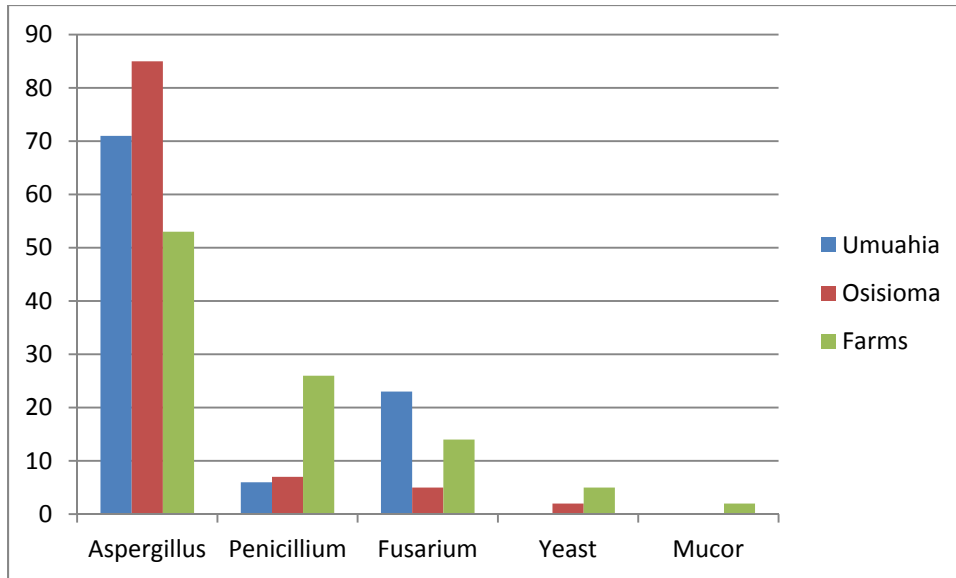
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219 **Figure6: Percentage occurrence of Fungi organisms Isolated from 3 different locations in**
 220 **Abia State**

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232 Table 3: Total fungal load of feed sampled from each location

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Locations	Total fungal count CFU/g-1
Umuahia North	2.0×10^5
Osioma	7×10^5
Farms	1×10^6

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UNDER PEER REVIEW

250 DISCUSSION

251 The study established that all the poultry feeds sampled harbored one fungi organism or the
252 other. Most of these organisms found in the poultry feed are those commonly found in soil and
253 water. The fungi isolated in this study were similar to those microorganisms reported by (Makun
254 Halize *et al.*, 2010; Atehnkeng *et al.*, 2008; Kpodo *et al.*, 2010). Also from this result there is
255 indication that feeds from farms has the highest percentage of fungal contamination of about
256 85% (Table 2) and this may be due to poor sanitary measures adopted in the processing and
257 storage or due to poor environmental and personal hygiene practice in the farm as well as lack of
258 proper biosecurity. *Aspergillus* specie has the highest fungi percentage occurrence affecting most
259 of the poultry feed sampled and this can be as a result of the organisms ability to thrive in high
260 osmotic pressure and this is in agreement with (Geiser *et al.*, 2009). Also because its spore are
261 common component of aerosols and they drift on air current dispersing themselves both short
262 and long distances and when in contact with solid feeds or liquid surfaces they tend to germinate
263 if the condition of the moisture is right and this view is consistent with the findings of Gioconda
264 and Richard, (2004). From the study, *Aspergillus species* was the predominant organism isolated
265 and this findings is in agreement with (Rosa *et al.*, 2006; Oliveira *et al.*, 2006; Figueora *et al.*,
266 2009)

267 This research could not ascertain whether contamination occurred at the manufacturer level,
268 retailers or farmers, though, (Pitts and Hocking 1997; Monge *et al.*, 2013) established that
269 *Aspergillus* is predominant in cereals and other ingredient used in producing poultry feeds in the
270 tropics. Contamination of poultry feeds particularly by pathogen may occur prior to processing,
271 distribution and or storage. Other studies have similarly concluded that cereals and other
272 ingredient use in producing poultry feed may be source of product contamination. This does not
273 exclude the fact that environment/ moist surface facilitate the growth of fungi. The occurrence of
274 *Aspergillus*, *Penicillium* and *fusarium spp* could be due to absorption of moisture during
275 storage.(Gow, 2002). The stored poultry feed might have reabsorbed moisture from the
276 environment which then supported the growth of the microorganism in addition to the
277 contamination during processing.

278 The total fungal load in the analyzed finished feed samples in this study were about
279 1.9×10^6 cfu/g-1 which is higher than that reported in Slovakia, in 2003 of 1.9×10^3 cfu/g-1) as

280 reported by Magnoli, (1994). According to mycological quality criterion, good fungal count
281 should be less than 3×10^4 . (Adesokan, 2005) The fungal load of poultry in this study was found
282 to be higher than the required load, hence the sampled poultry feeds are not good for poultry
283 consumption because they could lead to aflatoxicosis which results in reduction of both
284 production rate and meat quality. Also, from this result there is indication that feeds from farms
285 has the highest fungal count of about 1×10^6 (Table 4) and this may be due to poor sanitary
286 measures adopted in the processing and storage or due to poor environmental and personal
287 hygiene practice in the farm as well as lack of proper biosecurity, followed by feed samples
288 from Osisioma which have about 7×10^5 which could be as a result of high stocking density. The
289 Feed samples from Umuahia has the least fungal load of about 2.0×10^5 which may be due to
290 good sanitary measures and low stocking density adopted by feed distributors and depots in
291 Umuahia.

292 The presence of fungi in the poultry feeds was analyzed using ANOVA of 95% confidence
293 interval and value $p < 0.05$ considered statistically significant. Also the post hoc shows that there
294 was a strong association between the presence of *aspergillus* and *fusarium*, *penicillium*, *yeast*
295 and *mucor*.

296 CONCLUSION

297 Since no vaccine exist for any of the fungal diseases of poultry therefore, the timely adoption of
298 good management practices, strict biosecurity, effective disease diagnosis and suitable
299 preventive measures along with necessary treatment like use of probiotics with appropriate
300 chemotherapeutic agents are good measures to have a check and control the fungal disease of
301 poultry apart from the fungal infection. Mycotoxins are a major concern as they are the leading
302 cause of immune suppression in birds lowering their resistant level in viral and bacterial disease
303 and increase mortality. Thus a holistic approach is required to combat the adverse effect on high
304 economic returns from the poultry production. There is need for regular surveillance and
305 monitoring of important mycotoxins with the use of conventional as well as modern diagnostic.

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