Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.7, No.4, 2017

Pathogenicity Study of Dematiaceous Fungi Isolated from Chicken Feeds on Immunoincompetent Chickens

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Abstract
This study was carried out to investigate the pathogenicity of dematiaceous fungi isolated from chicken feeds sample in immunoincompetent chickens. A total of 40 chicken feed samples were collected from Ihiala market and screened for the presence of dematiaceous fungi using spread plate technique. The isolates obtained were characterized and identified using their microscopic and macroscopic characteristics. They were challenged orally using 0.5 ml of each of the test isolate to investigate the pathogenic potentials of each test isolate on the chickens. Dematiaceous fungi were detected in 13 chicken feed samples out of 40 chicken feed samples analysed. *Alternaria alternata, Phialophora verrucosa, Madurella grisea, Madurella mycetomatis, Exophiala jeanselmei* were the dematiaceous fungi detected from the feed sample. There was significant ($P<0.05$) decrease in the body weight of the infected immunoincompetent chickens. The total mean viable plate counts of the challenged isolates from the internal organs (heart, liver, lungs) of immunoincompetent chickens were significantly ($P<0.05$) higher. No count was recorded on the non-infected chicken. It was observed that the test isolate showed reasonable pathogenic features among immunoincompetent chickens and *Alternaria alternata* proved to be most pathogenic among the tested isolates.

INTRODUCTION

Chicken feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes (Heuser, 2014). They may also contain endogenous toxins arising principally from specific primary and secondary substances produced by fodder plants. Thus, feed toxins include compounds of both plant and microbial origin. Although these toxins are often considered separately, because of their different origins, they share several common underlying features. Thus, particular compounds within both plant and microbial toxins may exert antinutritional effects or reduce reproductive performance in farm animals (Scarbert, 2001).

Dematiceous fungi are usually defined as those that have melanin or melanin-like pigment in the wall of the hyphae and spores and can cause a variety of infections in humans known as phaeohyphomycosis (Malcolm, 2004). They are generally found in soil or associated with plants and distributed worldwide. Those causing the specific conditions of mycetoma and chromoblastomycosis in immunoincompetent patients are primarily found in tropical regions. Exposure is thought to be from inhalation or minor trauma, which may not even be noticed by the patient (Vishwakarma, 2000).

Previous studies focused on physiochemical properties and microorganisms associated with chicken feeds and used of some antimicrobial substances to control these microorganisms. Hence infections associated with Dematiaceous fungi remain one of the causes of reported periodic cases among the Immuno compromised individuals within the developing countries This shows that there is still inadequate of information on the actual species of Dematiaceous fungi associated with chicken feeds in Ihiala town and Anambra State at large, and their pathogenicity potentials that result to the reported menacing diseases among the consumers. Therefore, this study was designed to evaluate the pathogenic potentials of Dematiaceous fungi isolated from bean seeds sold in Ihiala town in Anambra State.

MATERIALS AND METHODS

Sample collection: A total of 40 samples of different types of chicken feeds (Starter, Layer, Grower, Finisher) of different brands was collected from different shops at Ihiala market using sterile polyethene bags, and kept in disinfected cooler before culture. Sampling was performed manually from different bags such that the product was collected from different parts of the bags. The sample was pooled, mixed properly and formed one cup of the feed sample, then 10g of the mixture was taken for analysis. The samples were brought to the laboratory in a cooler maintaining low temperature ($\leq 4^{\circ}C$) using ice blocks. The collected samples were processed within 6 h of its collection. The samples were collected randomly and each collected sample was marked with identification code with respect to the date and time of collection. Sampling criteria was limited to one cup of one sample and the collection criteria were not limited to any specific part.

Isolation of Fungal Organisms: According to the method of Pitt and Hocking (2002), exactly 5 g of each feed sample was added to 50 ml of sterile distilled water in a beaker mixed thoroughly with sterile spatula. The supernatant was decanted and 0.1ml of the mixture was inoculated into labeled plate containing Potatoe Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) containing 0.05% chloramphenicol and incubated at room temperature for 3-5days. Thereafter, plates were examined macroscopically for characteristic colonies according to Njobeh *et al.* (2009).

Identification of Fungal Isolates: The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics of the isolates obtained from pure cultures. The characterized isolates were ascertained with the aid of fungal atlas (Watanabe, 2002).

Macroscopy: The colonies were carefully examined for fungal characteristics. The rapidity of growth, colour, and nature of the reverse side of the plate, shape, texture, consistency of the growth and other peculiar features of the colonies were observed according to the method of Watanabe, (2002).

Microscopy: This was carried out using Needle mount technique. A drop of lactophenol cotton blue solution was placed on the center of a clean grease-free slide. A fragment of the colony was placed in the drop using sterile wire loop and was covered with a cover-slip, avoiding bubbles. Excess fluid from the outside of the coverslip was wiped with cotton wool and slide was passed through the flame to warm so as to remove the remaining air bubbles and facilitate staining of the fungal element. The slide was then examined under the microscope, using low-power objective of ×10 magnifications, followed by high-power objective of × 40 magnifications to reveal the nature of the hyphae, shape, size, texture and arrangement of the conidia. The pictorial nature of the fungal organisms was confirmed using the fungal atlas (Watanabe, 2002).

Pathogenicity Study of the Test Fungi

Procurement of chickens: A total of 18 chickens (4weeks old) bred in Mrs Uche poultry farm at Ihiala market, Ihiala L.G.A, Anambra State were used for this study. They were housed in cages.

Inoculum preparation: The isolates were sub-cultured on SDA at room temperature for 10 days. Following growth, the inoculums were prepared by flooding the surface of the agar plate with sterile normal saline (0.85% NaCl), scrapping the sporulating mycelium with sterile spatula and drawing up the resultant suspension with a sterile Pasteur pipette. The suspensions were filtered through sterile gauze to remove the hyphae. The suspension was emulsified and this was done severally until the turbidity of the mixture matches with the turbidity standard of McFarland matching standard. The standard was prepared by mixing 0.6ml of 1% $BaCl_2.2H_20$ and 99.4ml of 1% ConcH_2S0_4. The turbidity was equivalent to approximately 10^6conidia/ml (Nweze and Okafor, 2010).

Inoculation into the chick via oral route: The chickens were grouped into two groups: group A and group B. Group A contained only two chickens which served as control whereas group B contained 10 chickens. The chickens in group B were starved for 24 hours and two chickens each were orally challenged with 0.5ml of each of the test isolates whereas the chickens in group A were given distilled water. All the chickens were fed normally and observed for 5 weeks. (McGinni, 2010).

Gross examination of the morphologies of the internal organs of the chicks: The chickens were selected and sacrificed at the end of the experiment, and the liver, lungs, and hearts were harvested. The morphological changes and pathological signs associated with these organs were noted and recorded (McGinni, 2010).

Re-isolation of the test organism from the infected organs: portions of the harvested organs were homogenized using sterile mortar and pestle, 1g portion was suspended into 10ml normal saline and cultured in Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05mg/ml) and incubated at room temperature for 3-5days. The number of colonies of the test organism recovered from the sample was counted and recorded (McGinni, 2010).

Statistical Analysis: The result of the data generated in this study were expressed as mean $\pm$ standard deviation ($X \pm SD$), The statistical analysis of the data generated from this pathogenic study was carried out using one-way ANOVA at 95% confidence limit (Iheukwumere and Umedum, 2013).

RESULTS

A total of 40 maize seed samples were collected from Ihiala market, Uli centre market. Out of the 40 Chicken feed samples, 113(32.50%) samples where positive to dematiaceous fungi where as the remaining 27(67.50%) samples were negative to dematiceous fungi (Table1). A total of 1(10.00%) chicken feed sample from Sample A, 4(40.00%) chicken feed samples from Sample B , 2(20.00%) chicken feed samples from Sample C and 6(60.00%) chicken feed samples were positive to dematiaceous fungi. The result revealed that the samples that were positive to dematiaceous fungi were seen mostly among Chicken feeds samples from Sample D while Chicken feed samples from Sample A showed least occurrence of dematiceous fungi. The prevalence of Dematiaceous fungi in the studied Chicken feed samples is shown in Table 2. The result revealed that *Madurella grisea* (30.77%) was mostly encountered in the studied Chicken feed samples whereas *Madurella mycetomatis*(11.54%) recorded the least occurrence. The occurrence *of Alternaria alternata, Phialophora*

verrucosa, Maurella grisea, Madurella mycetomatis and Exophiala jeanselmei were significantly (P<0.05) most in the chicken feed samples collected from Sample D and least in Sample A and C. The occurrence of *Exophiala jeanselmei* was non-significantly (P≥ 0.05) higher in the samples collected from Samples A and C than samples collected from Sample B. *Alternaria alternata* and *Madurella mycetomatis* were not detected in Sample A whereas Phialophora verrucosa and Madurella mycetomatis were not detected in Sample C.

The obvious pathological signs of the challenged dematiceous fungi isolated on the .infected chickens are shown in Tables 3, 4 and 5. Anorexia, Weakness,Weight loss and diarrhoea were common in chickens infected by Phialophora verrucosa, Alternaria alternata, Madurella grisea Madurella mycetomatis and Exophiala jeanselmei,. Cough was not significantly (P>0.05) observed mostly on those chickens infected by Phialophora verrucosa, Madurella mycetomatis and Madurella grisea. Bloody diarrheoa and death were only observed in chickens infected with Alternaria alternata, Exophiala jeanselmei and Madurella grisea. The gross pathology of the internal organs and intestines of the infected chickens are shown in Table 4. Air sacculitis,`lung haemorrhage, odema of lungs, congestion of heart and congestion of lungs, were only seen in those chickens infected by *Alternaria alternata, Exophiala jeanselmei and Madurella grisea*, and they were significantly (P<0.05) decreased as the age of the chickens increased. Perihepatitis, pericarditis, liver congestion and odema of liver were only seen in those chicks infected by *Phialophora verrucosa, Alternaria alternata, Exophiala jeanselmei, Madurella grisea* and *Madurella mycetomatis* . The liver haemorrhage was non-significantly higher in *Alternaria alternata* while the occurrence of heart haemorrhage and oedema of heart were not observed in those chicks infected with *Phialophora verrucosa, Madurella mycetomatis, Madurella grisea, Alternaria alternate and Exophiala jeanselmei.* The total mean viable plate counts of the challenge dematiaceous fungi of infected immune-incompetent chickens are shown in Table 5. The result revealed that the total mean viable plate counts of *Alternaria alternata* was the highest while *Madurella mycetomatis* was the least. The total mean viable plate counts of infected immune-incompetent chickens was significantly (P<0.05) higher than the control immuno-incompetent chickens

Table 1: Chicken feeds samples that was positive to dematiaceous fungi

Sample	Positive sample (%)	Negative sample (%)	Total (%)
A	1(10.00%)	9(90.00%)	10(25.00%)
B	4(40.00%)	6(60.00%)	10(25.00%
C	2(20.00%)	8(80.00%)	10(25.00%)
D	6(60.00%)	4(40.00%	10(25.00%)
Total (%)	13(32.50)	27(67.50%	40(100.00%)

Table 2: Prevalence of dematiaceous fungi in the studied chicken feed samples

Isolate	A (%)	B (%)	C (%)	D (%)	Total (%)
Alternaria alternate	0(0.00)	2(3.85)	1(1.92)	7(13.46)	10(19.23)
Phialophora verrucosa	2(3.85)	1(1.92)	0(0.00)	5(9.62)	8(15.38)
Madurella grisea	1(1.92)	3(5.77)	3(5.77)	9(17.31)	16(30.77)
Exophiala jeanselmei	2(3.85)	4(7.69)	1(1.92)	5(9.62)	12(23.08)
Madurella mycetomatis	0(0.00)	2(3.85)	0(0.00)	4(7.69)	6(11.54)
Total	5(9.62)	12(23.08)	5(9.62)	30(57.69)	52(100.00)

Table 3: Obvious pathological signs of the test isolates on the infected immune-incompetent chickens

Parameter	PV	AA	EJ	MG	MM	Control
Weakness	3	3	3	3	3	0
Anorexia	3	3	3	3	3	0
Cough	1	3	3	2	1	0
Weight loss	3	3	3	3	3	0
Diarrhoea	3	3	3	3	3	0
Bloody diarrhoea	0	3	2	2	0	0
Death	0	3	2	1	0	0

PV = *Phialophora verrucosa,* EJ = *Exophiala jeanselmei*, AA = *Alternaria alternata*
MM =*Madurella mycetomatis*, MG =*Madurella grisea*

Table 4: pathological features of the interval organs of the infected immune-incompetent chickens

Parameter	PV	$AA^{N=3}$	EJ	MG	MM	Control
Perihepatitis	1	2	2	1	1	0
Pericarditis	1	2	2	1	1	0
Liver haemorrhage	0	1	0	0	0	0
Heart haemorrhage	0	0	0	0	0	0
Lungs haemorrhage	0	3	2	2	0	0
Liver congestion	3	3	3	3	3	0
Congestion of heart	0	2	1	1	1	0
Congestion of lungs	0	3	2	1	0	0
Oedema of liver	3	3	3	3	2	0
Oedama of heart	0	0	0	0	0	0
Oedema of lung	0	3	3	2	0	0
Air sacculitis	0	3	3	2	0	0

PV = *Phialophora verrucosa* , AA = *Alternaria alternate*, EJ = Exophiala jeanselmei
MG = *Madurella grisea,* MM = *Madurella mycetomatis*

Table 5: total mean viable plate cunt of the challenged isolates in the internal organs of immuno-incompetent chickens

Isolate	Heart	Liver	Lungs
PV	200 ± 0.00	17.00 ± 0.00	19.00 ± 0.82
AA	21.00 ± 0.71	24.00 ± 2.00	26.00 ± 1.41
EJ	18.00 ± 0.82	21.00 ± 0.82	23.00 ± 2.00
MG	6.00 ± 0.00	15.00 ± 0.00	19.00 ± 1.41
MM	4.00 ± 0.00	11.00 ± 0.71	14.00 ± 0.82
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

PV = Phialophora verrucosa, AA = Alternaria alternate, EJ = Exophiala jeanselmei
MG = Madurella grisea, MM = Madurella mycetomatis

DISCUSSION

The presence of dematiaceous fungi in the studied samples could be traced from the management practices, feed ingredients, transportation of the feeds, poor handling and sanitary conditions attributed to the feeds samples (Malcolm, 2004).

The significant pathological signs such as anorexia, weakness, diarrhea, bloody diarrhea, cough, weight loss, death and respiratory distress corroborated the findings of (Favero, 1993). The occurrence of air sacculitis, pericarditis, perihapatitis, lungs haemorrhage, liver haemorrhage among those immuno-competent chickens and immuno-incompetent chickens infected by dematiaceous fungi could be due to the organism's capability of invading the liver, lungs and hearts of the infected chickens. The wide distribution of dematiaceous fungi mostly *Alternaria alternata* in the liver, lungs and hearts of infected chickens could probably indicate concurrent extra-intestinal infections. Liver, heart, lung congestion, associated with these organisms was due to the affinity of the organisms to the liver and heart cells and their proliferative capabilities in the lungs (Barnet and Hunter, 2003).

The significant mean viable plate counts of *Phialophora verrucosa, Exophiala jeanselmei, Madurella grisea, Madurella mycetomatis, Alternaria alternata* recorded from the internal organs of the infected chickens supported the reports of many researchers (Hamilton and Gomez, 2002; Revankar,2004; McGinni, 2010). The presence of these dematiaceous fungi in the liver, heart, and lungs suggest that the organs contain sufficient nutrients and favourable environment for the growth of the dematiaceous fungi. The activities of the invaded organisms on the organs might cause degradation of the nutrients, obstruction of the lumen of the organs, deterioration and deformation of the organs, thereby producing pathological lesions that can clinically manifest on the infected chickens. This is in consonance with the report of Hamilton and Gomez (2002). The highest occurrence of dematiaceous fungi most especially *A. alternata* and *E. jeanselmei* in the liver, lungs and heart of chickens suggests that these organs are important site for detection of dematiaceous fungi. Similar finding was stated by Gow *et al.,* (2002).

CONCLUSION

This study revealed the presence of *Phialophora verrucosa*, *Alternaria alternata*, *Madurella grisea*, *Madurella mycetomatis*, and *Exophiala jeanselmei* in the chicken feed samples of which *Madurella grisea* was mostly detected from the studied samples. The immuno-incompetent chickens that were orally infected by these organisms exhibited different pathological features due to septicemic infection and deaths.

REFERENCES

Farevo, J.P., Corbeau, M., Benkirane, G., Trave, J.F., Dixon, P., Aucouterier, S., Rasheed, P. and Parker, J.W. (1993). Inhibition of human immunodeficiency virus infection by the lectinjacalin and by a derived peptide showing a sequence similarly with Gp 120. *Environmental Journal of Immunology* 23: 179–185.

Gow, N.A.R., Brown, A.J.P and Odds, F.E. (2002). Fungal morphogenesis and host invasion. *Current Opinion in Microbiology* **5**(4), 366–371.

Hamilton, A.J.and Gomez, B.L. (2002).Melanins in fungal pathogens. *Journal of Medical Microbiology* **51**: 189–191.

Heuser, G.F. (2014).Feeding Poultry. Norton Greek Press. Pp. 527 –626.

Jacobson, E.S. (2000).Pathogenic roles for fungal melanins.*Clinical Microbiology Review* **13**: 708–717.

Iheukwumere, I. H. and Umedum, C. U. (2013). Effect of *Gossypium hisutum* leaf extracts on Gram negative bacteria isolated from cervix of females with unexplained infertility. *African Journal of Science* **14**:3261–3270.

Malcolm, F. (2004).The Encyclopedia of Farm Animal Nutrition, 1st Edition.CABI Publishing, Wallingford, p.68.

McGinnis, M.R. (2000). Dematiacous fungi.In E.H. Lennette. A. Balows. W. Hausler.Jr., and J.P. Traunt (ed). Dematiaceous fungi. Manual of Clinical Microbiology.3rd Edition.American Society for Microbiology, Washington D.C. p.602–610.

Njobeh, P.B., Dutton, M.F., Koch, S.H. and Chuturgoon, A. (2009).Contamination with storage fungi of human foods.*Cameroon International Journal of Food Microbiology* **135**: 193–198.

Nweze, C. A. and Okafor, J. C. (2000). Researchers switch cancer off and on in mice. *Journal of Medical Microbiology* **10**: 45–50.

Pitt, J.I. and Hocking, A.D. ().Primary keys and miscellaneous Fungi.In Fungi and food spoilage 2nd Edition. Blackie Academic and Proffesional London. Weinheim New York, pp. 57–177.

Placinta, C.M, D'Mello, J.P.F. and Macdonald, A.M.C. (2003).A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Science and Technology* **78**: 21–37.

Revanker, S.G. and Sutton, D.A. (2010).Melanized fungi in human disease. *Clinical Microbiology Review* **23**: 884–928.

Scalbert, (2001). Antimicrobial properties, Tannin phytochemistry. *African Journal of Biotechnology* **30**: 3875–3883.

Vishwarkarma, S.L., Pal, S.C., Kasture, V.S. and Kasture S.B. (2000). Neuroprotective evaluation of extract of ginger. *Journal of Phythotherapy Resources* **16**:621–626.

Watanabe, T. (2002). Pictorial atlas of soil and seed fungi. In: Morphologies of Cultured Fungi and Key to Species. Lewis Publishers, London, Washington D.C., pp. 410.