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IMPACT OF *FUSARIUM SOLANI* ON BRAIN, LIVER AND SPLEEN OF MOUSE INFECTION MODELS

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Abstract

Background: *Fusarium* species infection is on the increase leading to morbidity and mortality in immunocompromised hosts. There have been several reports of disseminated infection of *Fusarium* species in immunosuppressed patients over the last 25- 30 years.
Aim: The study aimed to determine the histopathological impact of the isolated *Fusarium solani* on the histomorphology of brain, liver and spleen tissue of albino mice.
Material and Methods: Isolates of *Fusarium solani* from humans and plants were obtained from clinical samples of patients and different plant products respectively. All the samples collected, were cultured immediately on Sabouraud Dextrose Agar slant containing 50 mg/l of chloramphenicol and 5 mg/l of gentamicin. Identification of isolates was carried out macroscopically and microscopically using standard mycological methods. Test mice were challenged with isolated conidia of *Fusarium solani* while the control mice were unexposed. After 30 days, randomly chosen surviving test mice and control were sacrificed. The brain, liver and spleen of the animals were aseptically excised for tissue burden estimation and further histological processing for light microscopical examination.
Result: *In vivo* virulence studies of *Fusarium solani* on mice brain, liver and spleen organs revealed disseminated infection of multiple organs and mortality. The presence of fungal propagules was detected in all organs, with the highest concentration found in the spleen. There were intense inflammations of the internal organs especially excretory organs compared to the control where there was no inflammation seen. Histopathological findings on the brain showed extensive oedemas with blurring of the white and grey matter junction and extensive destruction of the Purkinje cells. Splenic tissues also revealed complete destruction of the splenic bulb and presence of extensive destructive granulomas with many Langhans giant cells formed. There was

also evidence of infections in the liver tissue ranging from intense hepatocyte swelling with hyperchromatic nuclei to intense periportal mononuclear cell infiltrates arising from diffuse toxic hepatic injury.
Conclusion: This study showed strong evidence of *Fusarium solani* pathogenicity in mice from various pathological manifestations and physical signs observed

Keywords: *Fusarium*, mycosis, virulence, resistance, immunosuppressed

Introduction

Currently there is increase in wave of invasive infections caused by *Fusarium* spp in patients with underlying immunosuppression. *Fusarium* species is a common fungal mold that causes wide spectrum of opportunistic infections in man and is normally found in soil, plants, and air (ubiquitous fungus). It is also a significant plant pathogen and has been steadily documented as emerging human pathogen due to its ability to cause systemic toxicity and mortality in heavily immunocompromised individuals.[1,2] *Fusarium* spp is known to be the second common cause of invasive mold infection in immunosuppressed patients.[1,3,4] *Fusarium solani* has been recorded as the highest frequent cause of invasive disease, among *Fusarium* species, especially in patients with hematological malignancies, stem cell transplant recipients, and prolonged neutropenia. [5, 6] *Fusarium* spp is capable of producing a number of different mycotoxins, which include trichothecenes (T-2 toxin, HF-2 toxin), deoxynivalenol (Don) and nivalenol, zearalenone and fumonisms. The key function of these virulence factors is suppression of humoral and cellular immunity. Fumonisin B1, the most common, has been linked with cerebral invasion; resulting in neural axon degeneration as well as abnormal mitochondrial function.[7] Fumonisins are acutely toxic to the liver and kidney of a wide range of experimental animals

Fungal meningitis is rare globally but is a serious complication in transplant recipients. Fungal brain abscesses can develop after a fungus disseminate from any part of the body to the brain or spinal cord. *Histoplasma*, *Blastomyces*, *Cryptococcus*, *Candida* and *Coccidioides* are well known causes of fungal meningitis. Disseminated infection had been associated with cases of cerebral fusariosis and affected individual may develop single or multiple brain abscesses, endophthalmitis, meningitis, cutaneous nodules, chorioretinitis and fungemia.[8,9] The most implicated species are *F. solani*, *F. moliniforme* and *F. oxysporum* complexes [1]

Early and rapid diagnosis of *Fusarium* infection is very crucial for successful antifungal chemotherapy and survival of the patient. The organism is noted for high level of multi-drug resistance to several antifungal agent, hence combination therapy is advocated.[10,6] Decisive diagnosis is made by direct histopathological examination and identification of the organism in tissue (brain biopsy, CSF, vitreous fluid). Mycological diagnosis of fungemia can be on the basis of positive blood cultures.[6]

Fusarium infections in the patients with liver cirrhosis remain rare.[11] However, a case of disseminated *F. solani* infection with hepatic localization was reported by in a liver transplant patient[12]. The frequency of *F. solani* invasion of spleen is on the increase

among patients with haematological malignancy due to more intensive chemotherapeutic treatment of the malignancy. These patients most often present with persistent pyrexia and associated with unresponsive to broad spectrum antibiotics. The diagnosis of *Fusarium* infection is principally based on mycology and histopathology. Invasive fusariosis treatment is very challenging due to the infection severity, multi-drug resistant and profound immunosuppression of the host. The present study aimed to determine the histopathological impact of isolated *Fusarium solani* on histomorphology of brain, liver and spleen tissue of albino mice

Materials and Methods

Specimens used for this work were obtained from both humans and plants. Clinical isolates of *Fusarium solani* from humans were obtained from mycological clinical samples of patients seen at different hospitals within Enugu, Nigeria. The *F. solani* from plants were isolated from different plant products. Ethical approval for the study was obtained from the Ethical Committee of the Department of Pharmaceutics, University of Nigeria, Nsukka.

The clinical samples (nail clippings, skin scrapings and corneal scrapings) and plants samples (pawpaw, avocado pear, carrot, plantain, water-melon, pepper, tomato seedlings, Irish potato, sweet potato, banana and palm fruit), obtained were cultured immediately in triplicate on Sabouraud Dextrose Agar (SDA) slant containing 50 mg/l of chloramphenicol and 5 mg/l of gentamicin. The cultures were incubated at 28°C for 1 week in the dark. Identification of isolates was carried out macroscopically and microscopically according to modified method of [13] with little modifications. Further characteristics for identification of the isolates was by slide cultures using SDA from the pure cultures

Animal infection

Animal experimentation was done using International Standard Procedure Guide for the Care and Use of Laboratory Animals.[14] One hundred and forty test mice and 7 controls (73 males and 74 females,(including 4 pregnant mice)); with a weight range of 32-34g obtained from the laboratory animal centre of the College of Medicine, University of Nigeria, Enugu Campus, Enugu State. After certification of the health conditions of the mice and approval by the committee for animal experiments of our institute, the mice were housed (seven per cage) in aluminum cages, with corncob bedding and free access to food and water, under standard conditions.

All isolates were first cultured on SDA for 7-10 days at 28°C. Test mice were infected by intravenous injection of a 0.2 ml of 10^6 conidia/ml suspension of *F. solani* into the lateral tail vein while the control mice were unexposed using modified method of [15]. Mice were observed for 4 weeks (30 days) post-challenge and mortality was recorded daily. The estimation of Mean survival time (MST) was done using Kaplan-Meier method in the SPSS software (version 15) and log-rank test was used for comparison among the groups.

Tissue Burden and Histopathology

After 30 days, 10 randomly chosen surviving test mice and control were sacrificed. The brains, livers and spleens of the animals were aseptically excised for further histological processing and light microscopic examination. One half of each organ was weighed and homogenized in 1 ml of sterile saline. Ten-fold serial dilutions of this homogenate were spread onto SDA plates and incubated at 28°C for 72hrs. After incubation, colonies were counted and

expressed as number of colony forming units CFU) per gram for each organ. The Log_{10} values of counted colonies were calculated and compared using the analysis of variance test. Data were analyzed with the software SPSS for windows version 15.

The remaining half of the organs were fixed in 10 % neutral buffered formaldehyde for period of 10 days, they were further treated by embedding in molten paraffin wax and automatically processed. Sections of the tissues were stained with hematoxylin-eosin for light microscopy observations. In addition, samples of spleen, brain, and liver from infected mice were homogenized, treated with KOH and stained with lactophenol cotton blue to check for fungal elements (hyphae and spores).[15]

Results

Isolation and identification of *Fusarium solani*

Fusarium solani colonies culturally grew easily and rapidly within three to five days on Sabouraud dextrose agar beginning as a white patch and quickly developed into pinkish shade. There was no pigment production on the reverse side of the tube (Figure 1). Identification was by observation of cultural and morphological characteristics, and by comparison with standard descriptions given by [16]

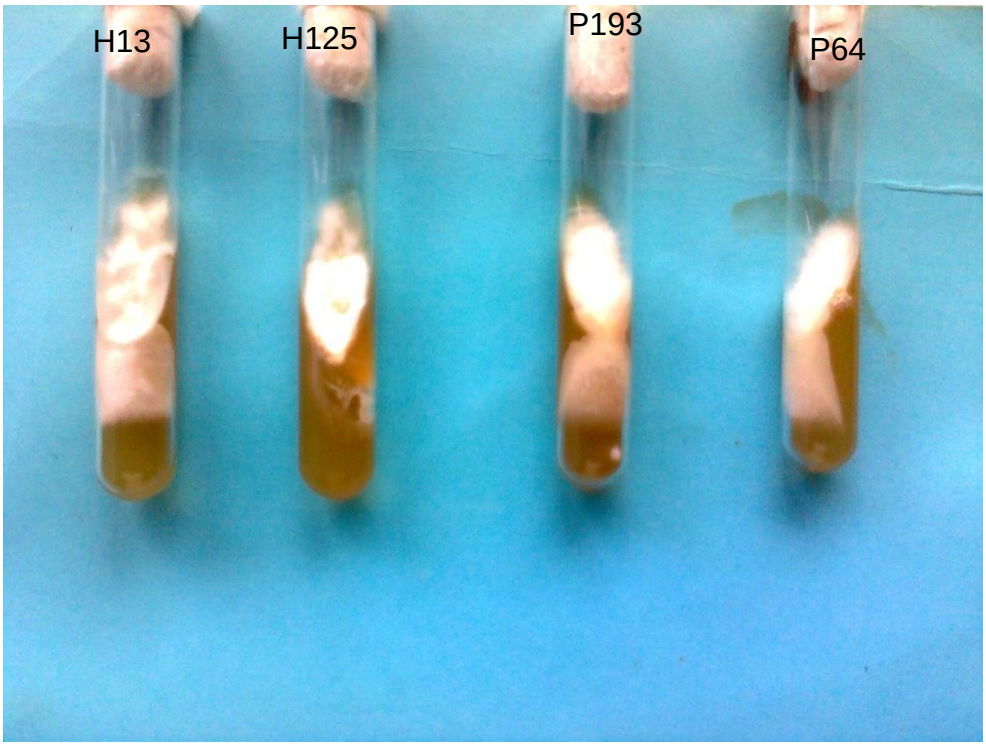

Fig. 1: Colonial morphology of *Fusarium solani* isolated from humans and plants
H- *Fusarium solani* isolates from humans
P – *Fusarium solani* isolates from plant products

Macroscopic Examination (Colonial Morphology)

Fusarium solani colonies culturally grew easily and rapidly within three to five days on Sabouraud dextrose agar beginning as a white patch and quickly developed into pinkish shade. There was no pigment production on the reverse side of the tube (Figure 1). Identification was by observation of cultural and morphological characteristics, and by comparison with standard descriptions given by [16]

Pathology

We observed that mice that received 0.2 ml of 10^6 conidia / ml conidia suspension of *F. solani* through intravenous injection into the lateral tail vein developed rapid systemic infection, with all mice becoming severely ill, very dull, malnourished, and not feeding well for three days, after which, they picked up again. They developed pinkish ring -like lesions on the upper, undersurfaces of their bodies, towards the waist in some of the mice and on the tails in some others. Generally, there were slight weight loss and the signs of the infections became intense after each inoculation. Some mice developed solid granulomas which were very large (as large as the stomach), from the neck up to the thorax and upper abdomen on the right (Fig.2). The granulomas were about 7.5cm in their longest diameter. Generally, all the infected mice become severely ill (very dull and unable to feed well for three days), after which some picked up again.

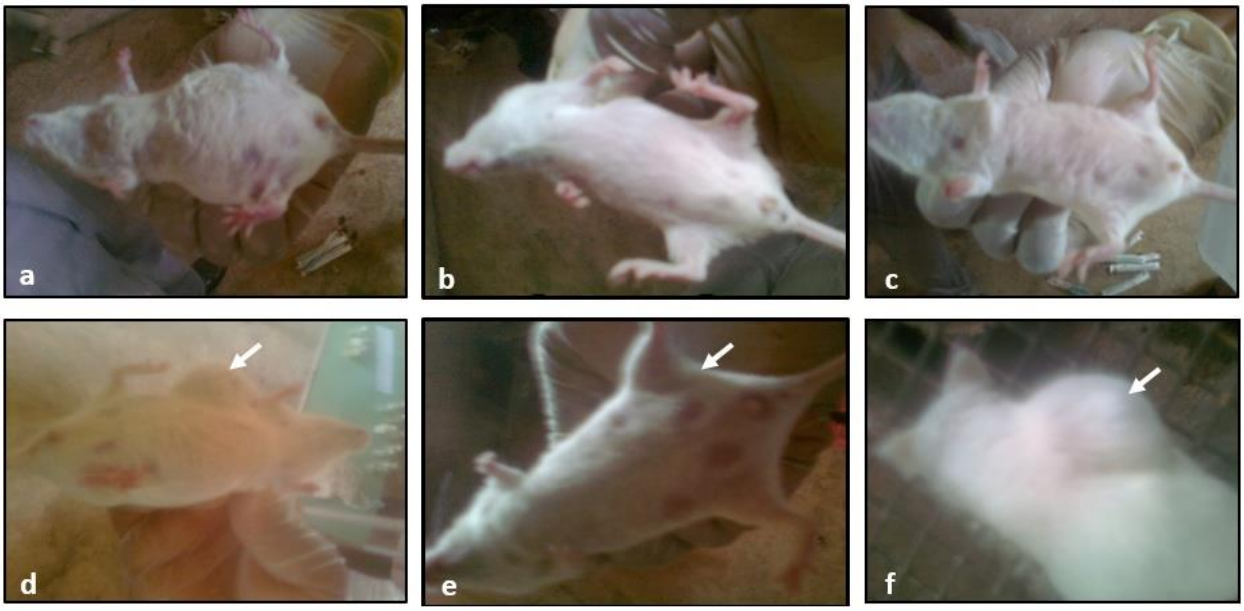

Fig.2: *Mouse infection models.* Signs of infection are noted in all mice (a – f). Mice a, b, c and d were infected with *Fusarium* conidia (P189, P196, H7, P193 and H133 strains respectively). Hair loss is observed in (a). Large granuloma is noted in (d); and (f) is a male mouse with very large solid granuloma after infection with *Fusarium solani* conidia from human.

Mortality

Mortality recorded daily for 30 days showed that a total of 42 mice died out of 140 test mice used. The mortality rate per week is as shown in Figures 2a and 2b. The ability of the *Fusarium solani* isolated from humans and plants to survive and reproduce in mice, colonize multiple organs and finally lead to the death of the host suggests that *Fusarium solani*possess pathogenicity determinants needed to cause disease in mammalian hosts.

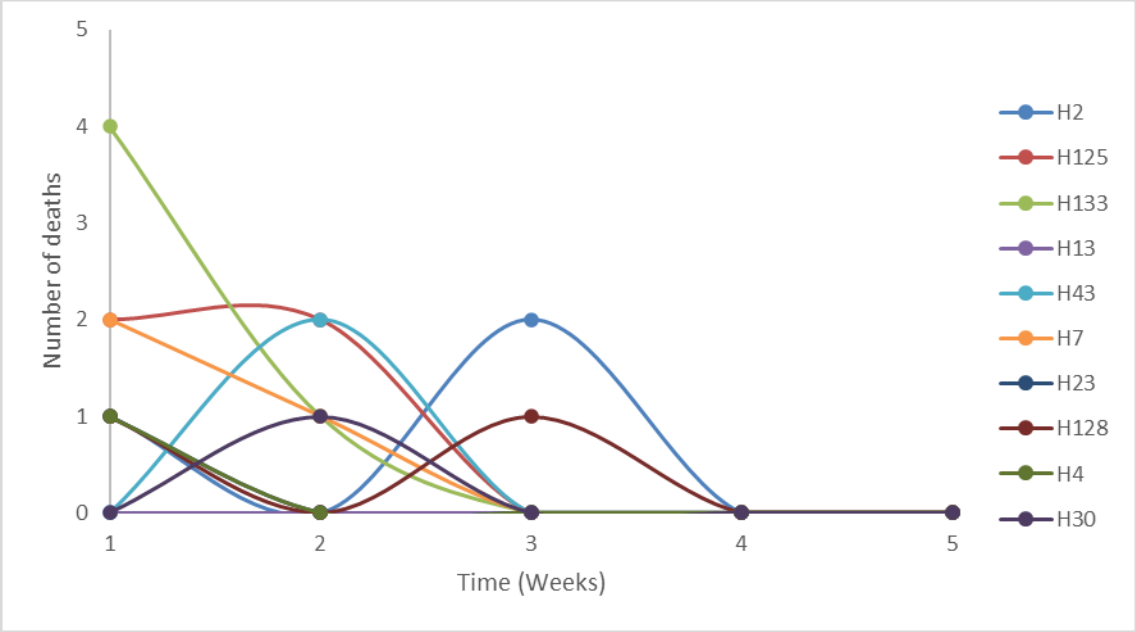

Fig 3a: Mortality Rate of Mice Inoculated with *Fusarium* Conidia from Humans, within a 30-day Period

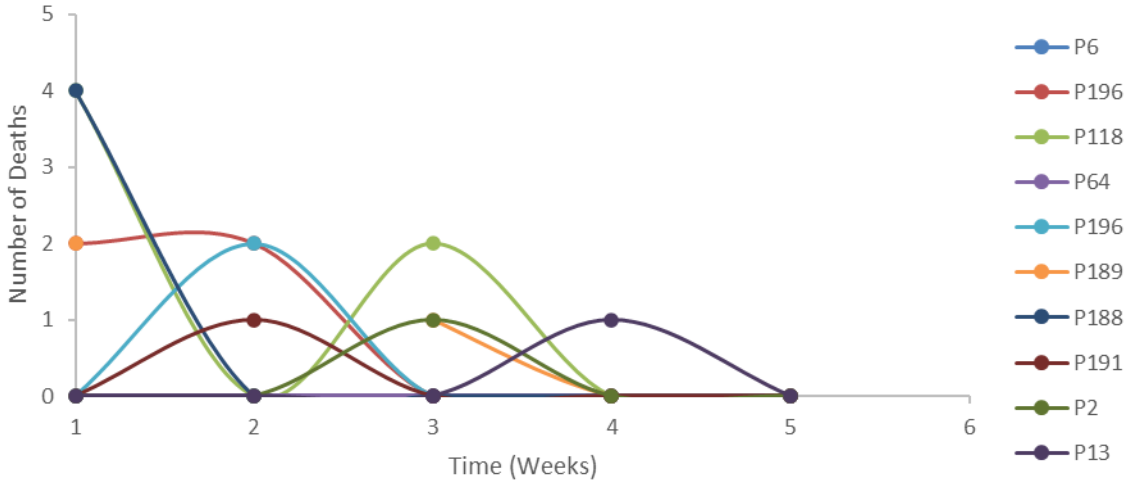

Fig 3b: Mortality Rate of Mice Inoculated with *Fusarium* Conidia from Plants, within a 30-day Period

Host organs invasion

The fungal propagules were detected in multiple organs, with the highest concentration found in the spleen followed by liver, while the brain had the least (Table 1). The internal organs of most of the sacrificed mice showed intense inflammations. There were no statistically significant (P = 1.000) differences in weight among organs and between cages

The number of colony forming units (CFUs) of the conidia per gram, calculated from the spleen was significantly higher (P = 0.013) than that from the brain and liver but there was no significant difference between the colony count in the liver and that in the brain (P = 0.753).

However, the brain had the lowest colony count. The *F. solani* inoculated into the different mice were recovered again from the various organs of the sacrificed mice

Table 1: Colony counts of *Fusarium solani* recovered from different mice organs

TREATMENT MICE GROUPS	BRAIN			LIVER			SPLEEN		
	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})
1	1	3	0.4771	708	545	2.7364	600	1200	3.0792
2	300	1000	3.0000	200	133	2.1239	500	1000	3.0000
3	10	33	1.5185	400	267	2.4265	400	1333	3.1248
4	16	53	1.7243	1000	1000	3.0000	12	40	1.6021
5	11	37	1.5682	600	600	2.7782	490	2450	3.3892
6	1	3	0.4771	300	375	2.5778	58	193	2.2856
7	8	40	1.60206	400	400	2.6021	48	160	2.2041
8	18	45	1.6532	290	290	2.4624	390	780	2.8921
9	2	7	0.8451	201	201	2.3032	38	190	2.2788
10	5	17	1.2304	560	373	2.5717	659	2197	3.3418

Histopathology of the Mice Organs

In vivo virulence studies of *Fusarium solani* on mice brain, liver and spleen organs resulted in disseminated infection on the organs and mortality.

Brain: Brain of mice infected with microconidia of *F*. solani were equally damaged, with some mice showing diffuse encephalitis with total destruction of the Purkinje cells and fibrosis extending to the white matter and no demarcation between the grey and white matter. In others, there were extensive brain oedemas or widening of both white matter and Purkinje cells, with blurring of the white and grey matter junction and extensive destruction of the Purkinje cells. Severe encephalitis with balloon degeneration of the Purkinje cells was also noticed in others. Some strains were more toxic to the brain than others, showing gliosis (fibroid tissues) with heavy loss of brain tissue (Loss of Purkinje cells) and extensive destruction of the neurons. Fig.4a showed normal brain of control mice while Fig4b -4j revealed specific damage to the brain by *F. solani* isolated from both humans and plants.

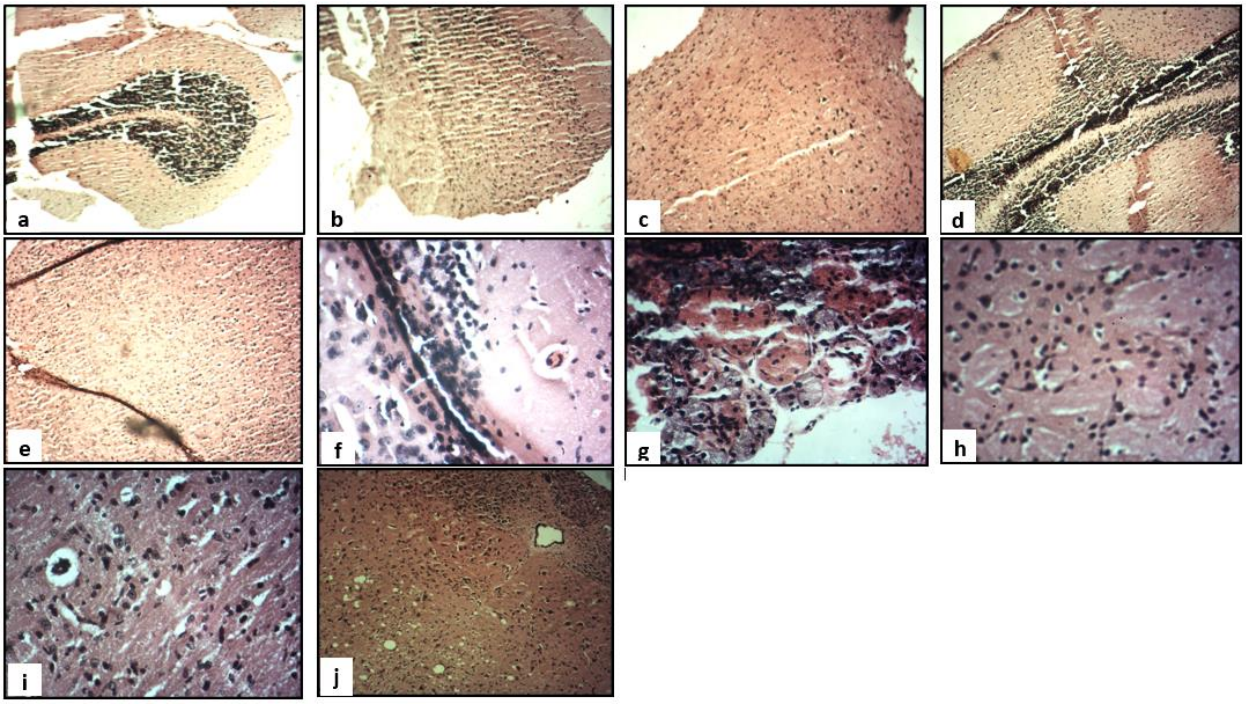

Fig.4: *Photomicrographs of Brain sections of control and Fusarium species infection models;* (a) **Normal control:** Section shows outer white matter and inside grey matter (Purkinje cells) devoid of inflammation with distinct and sharp borders. (b**) H2 strain:** Extensive brain oedema or widening of both white matter and Purkinje cells, with blurring of the white and grey matter junction. Extensive destruction of the Purkinje cells is also observed. (c) **H125 strain:** Complete destruction of the Purkinje cells and fibrosis extending to the white matter with no demarcation between the grey and white matter. Strain is more toxic than strain H1. (d) **P193 strain:** Almost normal brain with slight reduction in the number of the Purkinje cells and extensive oedema of the white matter. (e) **H133 strain:** Diffuse encephalitis with complete destruction of the Purkinje cells. (f) **H13 strain:** Moderate loss of Purkinje cells, Oedema of the white matter and presence of giant cell. (g) **H132 strain:** Severe encephalitis with ballooning degeneration of the Purkinje cells. (h) **H43 strain**: Gliosis (pink fibroid tissues) with heavy loss of brain tissue (Purkinje cells) and extensive destruction of the neurons. (i) **P64 strain:** Near complete loss of Purkinje cells with encephalitis (with cyst). (j) **P6 strain:** Moderate loss of Purkinje cells with severe (gliosis) (Fibrosis with cyst).

Liver: Mice livers infected with conidia of *F*. solani isolated from humans and plants also showed evidence of infections, ranging from intense hepatocyte swelling with hyperchromatic nuclei to intense periportal mononuclear cell infiltrates from diffuse toxic hepatic injury. In some mice, complete loss of hepatocytes was noticed while others showed ballooning degeneration of the hepatocytes with intra cytoplasmic eosinophilic accumulations (microconidia) with severe oedema and hepatitis (inflammation of the liver), as well as fused and hazy cells. The presence of microconidia in the cytoplasm was also noticed. Fig.5a shows the control liver while Fig.5b- 5k revealed various damages caused by the *F. solani* to the liver of infected mice.

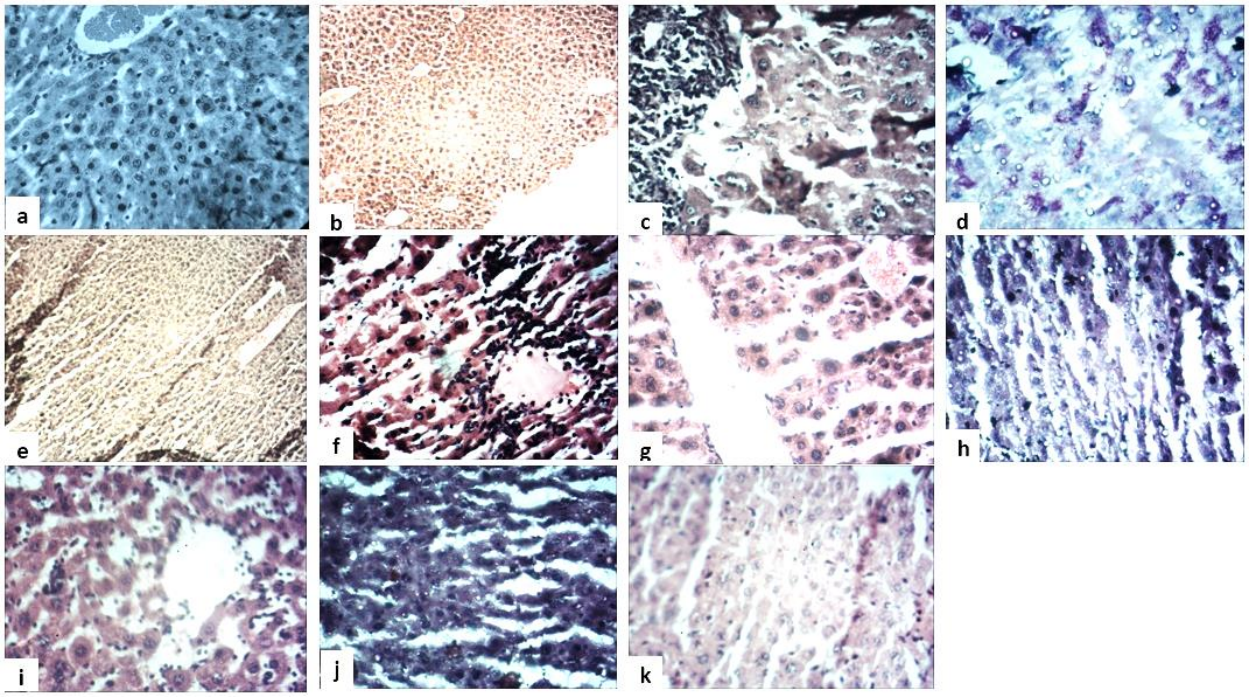

Fig. 5: *Photomicrographs of Liver sections of control and Fusarium species infection models;* (a) **Normal control:** Section shows normal central veins, portal tracts, sinusoids and hepatocytes. (b**) H2 strain:** Portal tracts are not visualized and there is loss of periportal hepatocytes; however, centrilobular hepatocytes are better preserved. (c) **H125 strain:** Intense hepatocyte swelling with hyperchromatic nuclei and intense periportal mononuclear cell infiltrates from diffuse toxic hepatic injury. Note the presence of microconidia within the cytoplasm. (d) **P189 strain:** Ballooning degeneration of the hepatocytes with intra cytoplasmic eosinophilic accumulations (microconidia), severe odema, and fused hazy cells. (e) **H193 strain:** Extensive hepatocyte toxic injury with swelling. (f) **H133 strain:** Mild swelling of hepatocytes with centrilobular inflammation. (g) **H13 strain:** Hepatocytes with granular or ground glass cytoplasm and reactive changes including binucleation. (h) **H132 strain**: Mild reactive changes in the liver section. (i) **H43 strain:** Evidence of generative liver changes and hepatitis (inflammation of the liver). (j) **P64 strain:** Liver ground glass hepatocytes with ballooning degeneration. (k) **P6 strain:** Ballooning degeneration of hepatocytes.

Spleen: The inoculated *F*. solani also disseminated to the spleen of the infected mice with evidence of damage such as extensive destructive granulomas with many Langhans giant cells formed from type IV hypersensitivity reaction. Some strains showed complete destruction of the splenic pulp with giant cell granulomas seen diffusely (Quite toxic), while others showed balloon degeneration of the splenic cells and foamy macrophages (splenitis). Fig6a showed normal spleen while plates 6b- 6j revealed damages from different *Fusarium* strains.

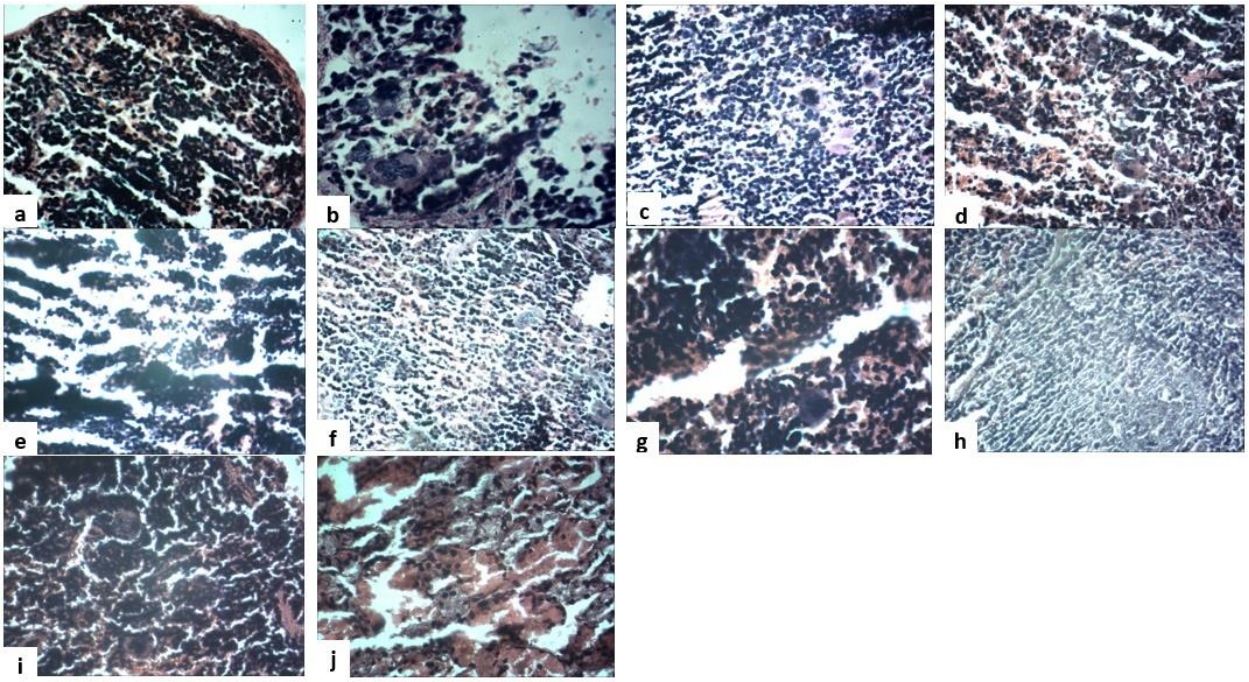

Fig. 6: *Photomicrographs of Spleen sections of control and Fusarium species infection models;* (a) **Normal control:** showing normal red pulp and sinusoids. (b**) H2 strain:** showing extensive destructive granulomas with many Langhans giant cells. Complete destruction of the splenic pulp is also observed. (c) **H125 strain:** Diffuse granulomatous inflammation in keeping with a type IV hypersensitivity reaction. Note presence of numerous Langhans giant cells. (d) **P189 strain:**showing granulomatous inflammation and presence of numerous giant cells. (e) **P193 strain:** shows no obvious pathological change. The histoarchitecture of the tissue appears normal. (f) **H133 strain:** shows granulomatous splenitis and the presence of giant cells. (g) **H13 strain:** showing mild splenic granuloma with few giant cells. (h) **H132 strain**: showing essentially normal spleen with mild splenitis but absence of giant cells. (i) **H43 strain:** Diffuse giant cell granulomas (Quite toxic). (j) **P64 strain:** Ballooning degeneration of the splenic cells and foamy macrophages (splenitis).

Discussion

Fusarium are fungi genus that commonly inhabit soil, and had been documented that they cause both plant disease [17,18] as well animal/human infections.[19,20] Tissue invasion of this organism in human with immunocompromise condition had been well reported by researchers.[21] Thus early diagnosis can help in rapid antifungal therapies which are essential for patient survival. In resource limited environment, cultural identification of this fungi still remained an essential tool. In this study, cultural characterization of one of the *Fusarium* species (*F. solani)*, were done. The identification technique involved observation of cultural and morphological characteristics, through comparing the isolates with standard fungal atlas using as stated by [16] One of the striking observations in this study was that infected mice showed poor eating and drinking habit as well as inactive behavior and weight loss. It was reported previously that animals infection do results in inactivity, weight loss and poor feed habits.[22] This had been documented as markers of severe infection.[22] Though majority of the mice became active after few

days, the cause of the death of those that could not survive could be attributed to systemic infection. Similar report was also documented by [23] that injection of *F. solani* into lateral vein of the mice tail results in death of some of the mice. In addition, the mice demonstrated some degrees of infection which includes pinkish ring-like lesions on the upper, undersurfaces of their bodies towards their inguinal region and tails. This might depict inflammation/necrosis at the site were the organism had disseminated into. [24] had stated that the *Fusarium* multiple necrotizing lesion are usually notice in the trunk and the extremities especially in human and that this can be an important and an early hint in the diagnosis.

Survival of the fungi in animal model had been linked with its ability to cause infection in immunosuppressed mice. 15 Despite the fact that immunocompetent mice were used in this study unlike other study, animal mortalities were still recorded. A previous work has stated that immunosuppression of animals prior to infection with *F. solani* affects the severity of disease in experimental animals [25]; this might explain lower death rate recorded in this study against high death rate in other study. [20]

Dissemination of *Fusarium* infection in human had been documented [26-28], and Murine model had been demonstrated. [29] Dissemination of the organism into spleen, liver and brain was observed in affected animals. This was noticed through the detection of the fungal propagules in the organs mentioned; this was in concordance with the reports of [20], who also observed that the organism germinated in the kidney of the mice while ungerminated microconidia of *F. solani* were found in liver and brain.

The internal organs of most of the sacrificed mice showed intense inflammations.

This result confirmed the work of [15], where fungal popagules were also recovered in multiple organs although their work was on *F. oxysporium*. In that study, spleen also recorded the highest concentration, followed by the liver. There were no statistically significant (P = 1.000) differences in weight among organs and between cages

The number of colony forming units (CFUs) of the conidia per gram, calculated from the spleen was significantly higher (P = 0.013) than that from the brain and liver but there was no significant difference between the colony count in the liver and that in the brain (P = 0.753). However, the brain had the lowest colony count. The *F. solani* inoculated into the different mice were recovered again from the various organs of the sacrificed mice. In human however, skin is the most frequent affected organ in fusarium dissemination infection in immunocompromised individuals. [27]

Conclusion

This result of this study expresses high occurrence of *Fusarium solani* in both humans and plants and also the ability to disseminate into various organs causing morbidity and mortality. This confirmed that it possesses very potent virulence factors which might include mycotoxin production.

Competing Interest

There exists no conflict of interests.

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