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Fungal Pathogens Affecting the Quality of Rice (*Oryza sativa L.*) Seed in Selected Agro-ecological Zones of Liberia

Ousman Sarlia Dorley[1,*], Javan Omondi Were[2,3], Julius Onyango Ochuodho[2,3], Elmada Odeny Auma[2,3]

[1]Department of General Agricultural, University of Liberia, Capitol Hill, Liberia
[2]Department of Seed, Crop and Horticultural Sciences, School of Agriculture, University of Eldoret, Eldoret, Kenya
[3]School of Agriculture, University of Eldoret, Eldoret, Kenya
*Corresponding author: odorley2012@yahoo.com

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Abstract The study aimed to investigate the presence and characterization of fungal pathogens in rice grains collected from four major rice producing counties in Liberia. Samples were collected from rice farmers in Bong, Lofa, Montserrado, and Nimba Counties during the dry season and taken to a laboratory in Kenya for isolation and characterization of fungal colonies. The results showed that *Aspergillus* spp and *Penicillium* spp were the most abundant fungal isolates found in the rice grains. The most abundant fungal isolate was *Aspergillus niger*, followed by *A. flavus* and *Penicillium* spp. The study showed that the extensive presence of these fungi in rice grains is favored by warm and humid subtropical climates similar to Liberia, which can encourage conditions for fungal contamination. Other fungi found were *Fusarium* spp and *Pyricularia oryzae*. The findings of this study highlight the importance of controlling fungal contamination in stored rice grains to ensure food safety and security.

Keywords: *rice, fungal pathogens, seed policy, Liberia, isolates*

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1. Introduction

Cereals are the main sources of carbohydrates in many Sub-Saharan African countries. Rice is the staple crop for almost 99% of the Liberian population [1]. It had an annual per capita consumption of more than 140 kg in 2010, currently it’s about 133kg per year. The country imports nearly 300,000 metric tons yearly costing an amount of US$200 million which is the highest in Africa for the oldest independence state [2]. It plays a critical role as food and source of income for many households [3]. The crop is the main source of livelihood for about two million people either directly or indirectly [4]. The country is not however self-sufficient in the production of rice despite having huge arable land. Besides human consumption, rice is also used as animal feed (fish, poultry, and pigs) and as the source of medicinal oil extracted from the outer layer of the grain, the husk as a material for mulching among others. With such numerous benefits, the yield has been on the decline, which is alarming.

Rice farming in Liberia is mainly done by smallholder farmers scattered across the country [5]. Cultivation is dominantly rain-fed, less intensive, and characterized by low application of inputs, which do not meet the essential crop requirement in terms of agronomic, technical, and post-harvest handling strategy resulting in annual yield decline. For instance, in 2018, grain production was 257,995 tons produced from 238,090 hectares of land averaging to 1.08 tons compared to Nigeria which produced an average of 2.03 tons in the same year [6].

The main constraint facing Liberia’s rice farming is poor seed quality [3]. Studies have shown that about 50% of the crops’ yield can be attributed to the quality of seed particularly in cereals like rice [7]. Liberia’s seed industry first suffered from the 14-year-old civil war which discouraged development of higher yielding varieties [8]. Apart from inflicting damage on the infrastructural development in the seed industry, it prevented the enactment of agricultural policies that would favour the development of certified seeds. Currently, farmers mainly rely on the communal seed system which are plagued by poor quality seeds due to low genetic yield brought about by repeated usage [9]. Further, the seeds are diseased due to poor storage conditions by most farmers [10].

The diseases are mainly caused by fungal pathogens, both the field and spoilage fungi. Some of the common storage fungi like *Aspergillus* spp are known to produce aflatoxins which impair the liver and also causes liver cancer after long time of exposure. In a continent where cereals are the primary source of food, exposure to aflatoxins can be fatal. To date, nowhere else in the world does this ring true than in Kenya where about 100 people died due to aflatoxicosis in the year 2001 [11]. Therefore, the poor quality seeds due to fungal infections not only lead to low yield but may also be a health hazard. Knowledge of the fungal pathogens present in the grains is of importance both in the human and animal health and improvement of crop yield. It is in this light therefore that this study sought to characterize the fungal infections in the Liberian rice grain.

Management of many plant pathogenic fungi have not been successful in Liberia due to a lack of information regarding the severity and prevention of the fungi, specifically under the Liberia climatic and environmental conditions [12]. Further, the extent of the infection will also be more appreciated and therefore spur the enactment and implementation of rice seed policy in Liberia to help improve rice farming in the country. Currently, seed regulation in Liberia is carved based on ECOWAS seed laws and policies which regulates the importation and exportation of seeds [13]. The country seed policies have some challenges as the policies were carved from the Economic Community of West African States. This study will help in having well informed measures aimed at controlling the diseases.

2. Materials and Methods

The rice seeds were sampled from four (4) of the fifteen (15) counties of Liberia in the major rice agro-ecological zones Bong, Lofa, Montserrado, and Nimba Counties located in the Western, Central, Southeastern and Northern Regions of country in January to March of 2022.

Bong County covering an area of 11846.931 km² is located between 6° 24' 7" N, and 7° 25' 48" N longitude and 10° 28' 34" W and 9° 5' 38" W latitude [14]. Lofa County has two agro-ecological zones; Upper highland tropical forest and Northern savanna. Montserrado County is the smallest Liberia County covering an area of 1,912.7 square kilometers. Nimba County lies at an altitude range between 63-1730 meters above sea level and is situated at 5° 49' 26" and 7° 41' 46" N longitudes while 9° 11' 43" and 8° 16' 24" W latitudes.

2.1. Sample Collection

Samples were all collected at random from rice farmers in the 4 counties during the dry season. The rice grains were taken to the seed laboratory at the University of Eldoret, Kenya. The rice samples were sorted to separate the diseased grains to be used for the isolation experiments.

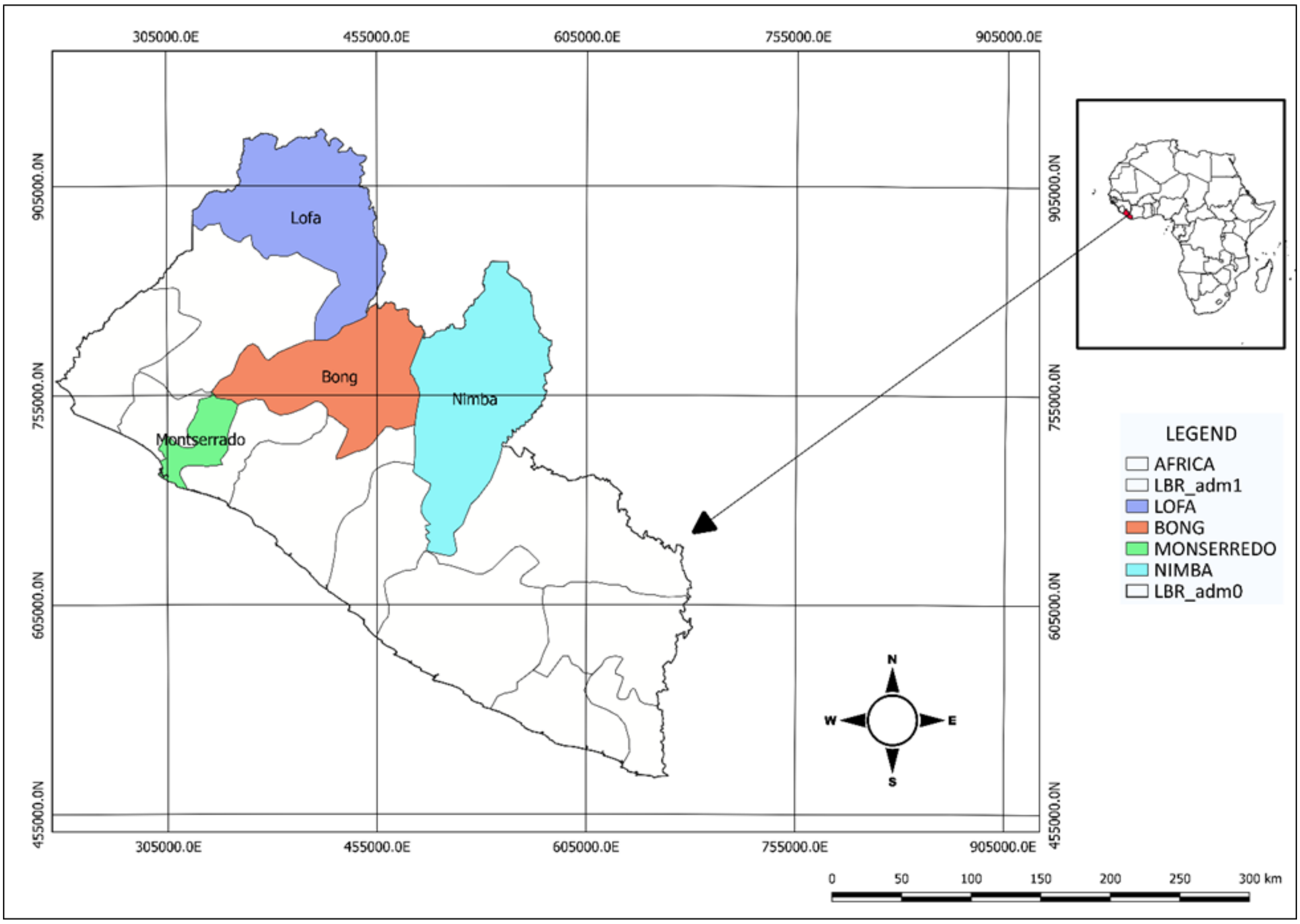

Figure 1. Map of the four (4) Major rice Producing Counties of Liberia (Dorley *et al*., 2022)

2.2. Isolation of the of Fungal Pathogens

The rice grains showing disease symptoms were surface sterilized by first washing in 2% sodium hypochlorite for 1 minute followed by washing in 70% ethanol for 30s. The grains were then washed in sterilized distilled water and placed on sterilized filter paper to remove excess moisture. The grains were then placed on Potato dextrose agar (PDA) amended with streptomycin and incubated at 27℃ for 7 days. Colonies emerging from the seeds were sub-cultured to have pure colonies. Each of the pure colonies were further multiplied to obtain several plates of pure cultures to aid in the characterization of the fungi.

2.3. Characterization of the Fungal Colonies

Pure cultures were left to grow for seven (7) days under continuous white fluorescent light (20%) for a period of seven (7) days in a Gallenkamp incubator chamber. Morphological assessment was done as prescribed by manual of [14]. The colony growth characteristics such as mycelia colour, colour at the surfaces of the media, conidia shape, textures, pores colour and size and spore description were recorded. Results will be displayed in figures.

3. Results and Discussion

Stored grains including rice are susceptible to contaminations by a variety of fungal organisms [15]. *Aspergillus* spp and *Penicillium* spp are the two genera of fungi that have greater tendency to contaminate the stored grains [16]. The current study corroborates this observation. Occurrence of these fungi in the grains is favoured by unsuitable drying environment mainly caused by warm and humid subtropical climates similar to Liberia [16,17]. The geographical location of Liberia along the West African coast would encourage conditions favouring the proliferation of these fungal contaminants. The extensive presence of these three genera of fungi has also been replicated in another study by [18]. In their study, the rice samples used were obtained from Pakistan. Like Liberia, Pakistan is also a major rice producing country and also experiences the similar climatic conditions. These two factors could be crucial contributors to this apparent uniformity.

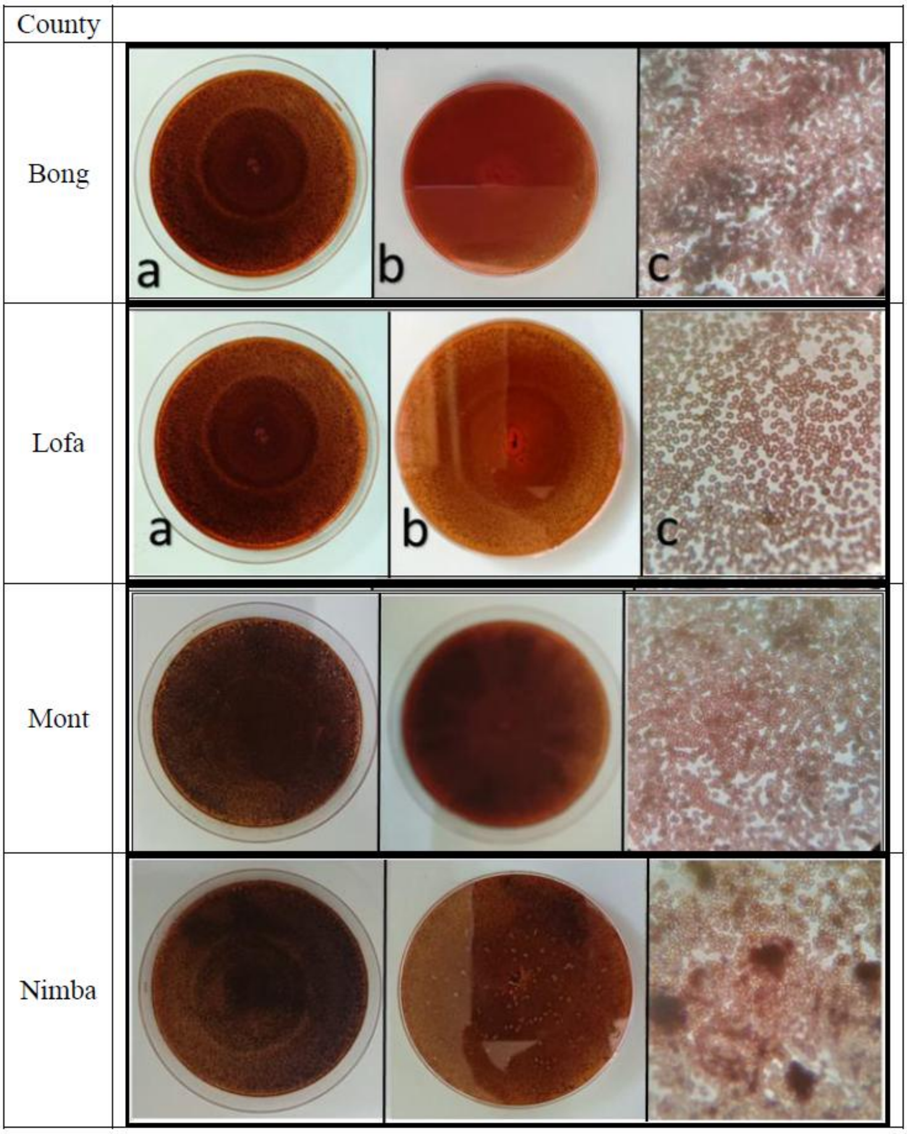

Figure 2. Colonies and conidia of *A. niger* isolated from the 4 counties. The column labeled 'a' shows the upper surface of the petri dish while those labeled 'b' shows the lower surface of the petri dishes. The isolate from Bong' County shows the dense conidia

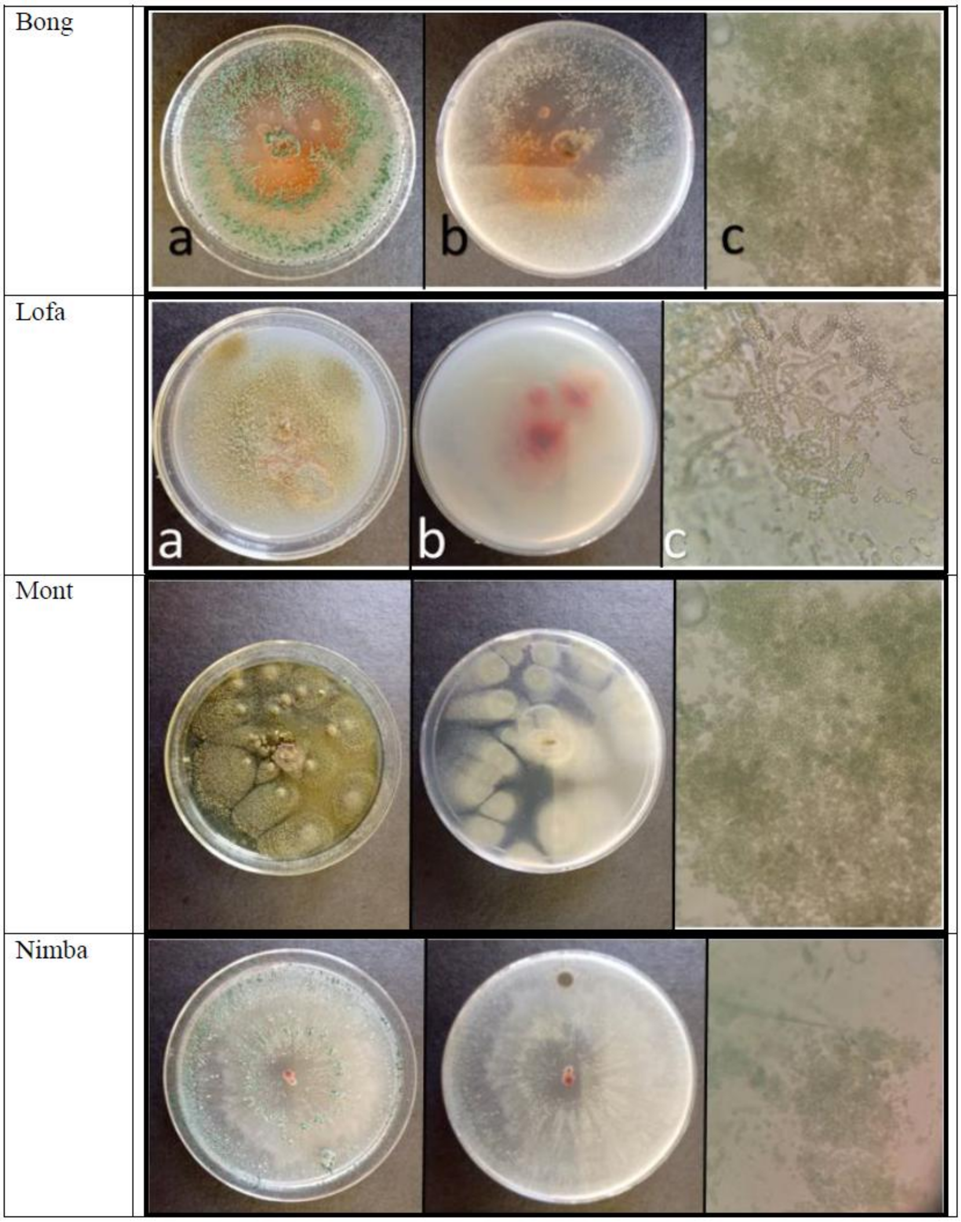

Figure 3. *Aspergillus flavus* from all the counties. From left to right the pictures show the green surface of the *A. flavus* pure culture, the rear view of the same plate, and micrographs of the dense spherical conidia. The surface of the plates appears green as well as the spore. The rear in all these were cream in colour

The *A. niger* spores from the Bong' isolates were denser than those from Lofa County. Further, *A. niger* isolated from Montserrado County had conidia with rough surface. For the *A. flavus*, there was no any evident of marked morphological variation among the counties (Figure 3). *A. niger* may contaminate grains at different stages of processing, that is pre-harvesting, drying and storage and during transportation [19]. The abundance therefore can be pinned to some or all of the stages. At the pre-harvest stage, contamination mainly comes from the environment and can be fueled by climatic conditions. The rest of the stages of contamination has an element of cross-contamination. *Aspergillus* spp, not being a field fungus, develops during drying [20]. This fungus is also more tolerant to dry conditions.

Penicillium spp isolated from the rice grains in Bong and Lofa Counties were similar in many respects. Conidia were of the same size in both regions (2 µm) but fewer aggregate numbers could be observed from the Lofa samples (Figure 4). *Penicillium* spp isolated from Montserrado and Bong counties were similar in colony morphology and greatly differed from those of the other two counties. Due to their morphological similarities, grouping *Penicillium* into different species based on morphological features alone comes with many challenges [21,22]. Further, intraspecific differences are displayed by many species [23]. The variation therefore of the *Penicillium* spp observed during the current study may less be affected by different locations as this is an inherent characteristic of the fungus. However, environmental and physiological aspects also influence to the variation of *Penicillium* spp [24,25]. Since all the isolates were cultured in the same growth medium under uniform conditions, it is highly unlikely that environmental and physiological conditions are the determinants of the disparities observed. In this case therefore, the fungus' innate variation potential only explains the differences in the features that were assessed for homogeneity.

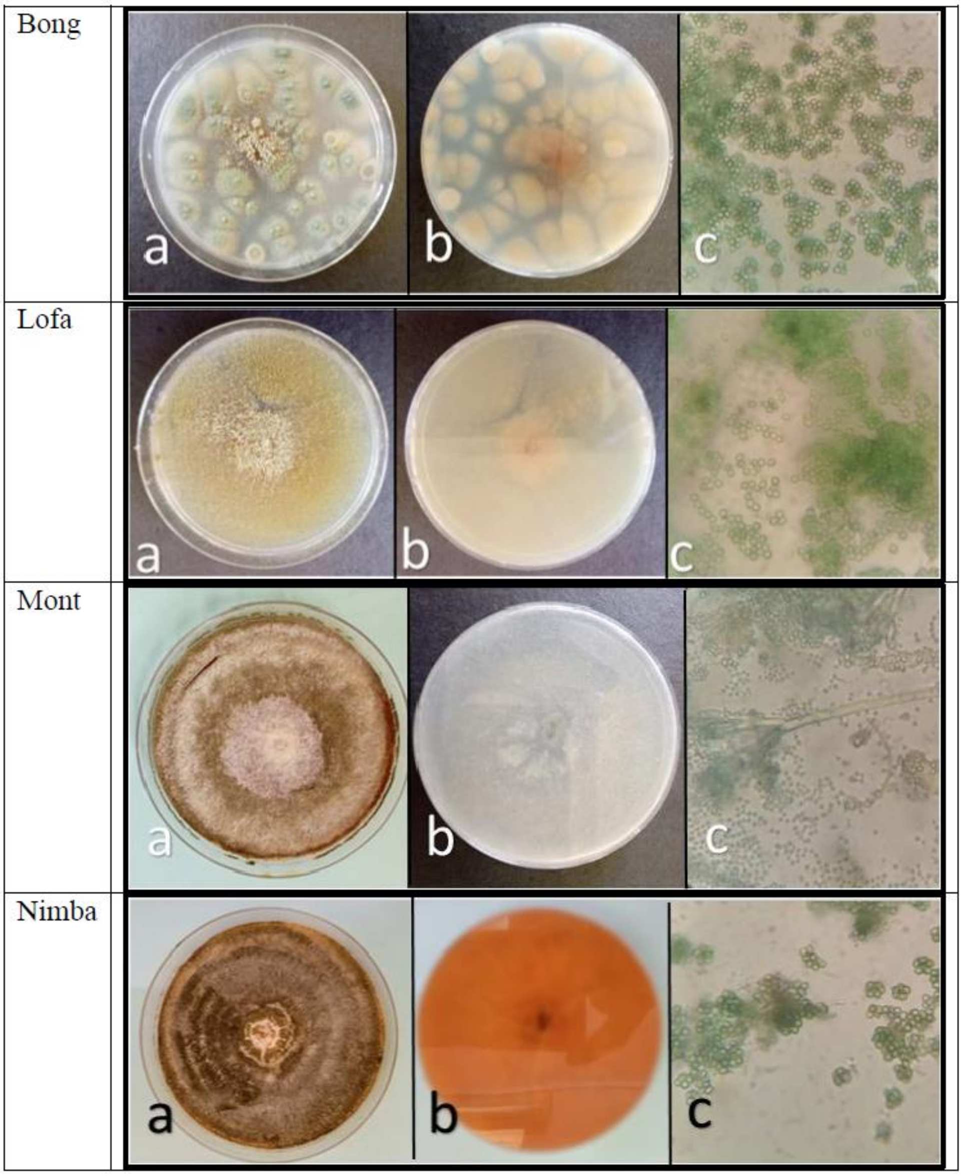

Figure 4. *Penicillium* spp isolated from all the counties. From left to right the pictures show the green surface of the *A. flavus* pure culture, the rear view of the same plate, and micrographs of spherical conidia. The colony characteristics greatly differed among the samples from the counties. The ones presented here are the most dominant form of the colony in each county

Apart from *Aspergillus* and *Penicillium* spp, *Fusarium* spp was also found to be present. *Fusarium* spp was not found in Montserrado but was present in all of the counties. Conidia were all long and slender (Figure 5). Compared to *Aspergillus* spp, *Fusarium* did not feature very prominently and this contrasts many studies done in temperate countries. In Italy, *Fusarium* was found to outnumber *Aspergillus* in most rice samples [16].

The same was replicated in Brazil (26). As mentioned earlier, contamination by *Aspergillus* spp is exacerbated by warm and humid conditions experienced in countries such as Liberia. It is not surprising therefore that in Montserrado, the county lying along the coast, has no *Fusarium* spp isolated from the rice samples collected from here. The conditions here must be too favourable for *Aspergillus* spp that perhaps another contaminant like *Fusarium* spp is edged out. And while this fungus has been found to be present in the field, its presence has been shown to reduce drastically after drying [27]. Drying in the sun followed by keeping in the kitchen attic as many farmers, would surely reduce the occurrence further [3].

The *Fusarium* spp isolated from each of the counties that were present, were markedly different from each other on spore shape and size and sporulation. Diversity of this kind has also been identified elsewhere [28]. In that case, differences appeared to be driven by varied agro-climatic zones. While Bong and Nimba had only one type of isolate, Lofa had two types of the fungus based on spore shape. Lofa is the food basket of Liberia, where rice is staple. Huge tracts of land used to cultivate a particular crop would increase the selective pressure of pathogens. This could explain the apparent diversity of *Fusarium* isolated from this county. Certain rice varieties such as jasmne and DT8 have also been shown to be more susceptible to attack by *Fusarium* spp [29]. There are many varieties of rice cropped in Liberia. In a rich rice growing region like Lofa, the diversity of rice varieties must be greater. The susceptible varieties hence fuel the spread of *Fusarium* spp in this county leading to the presence of more than one morphotype of the fungus.

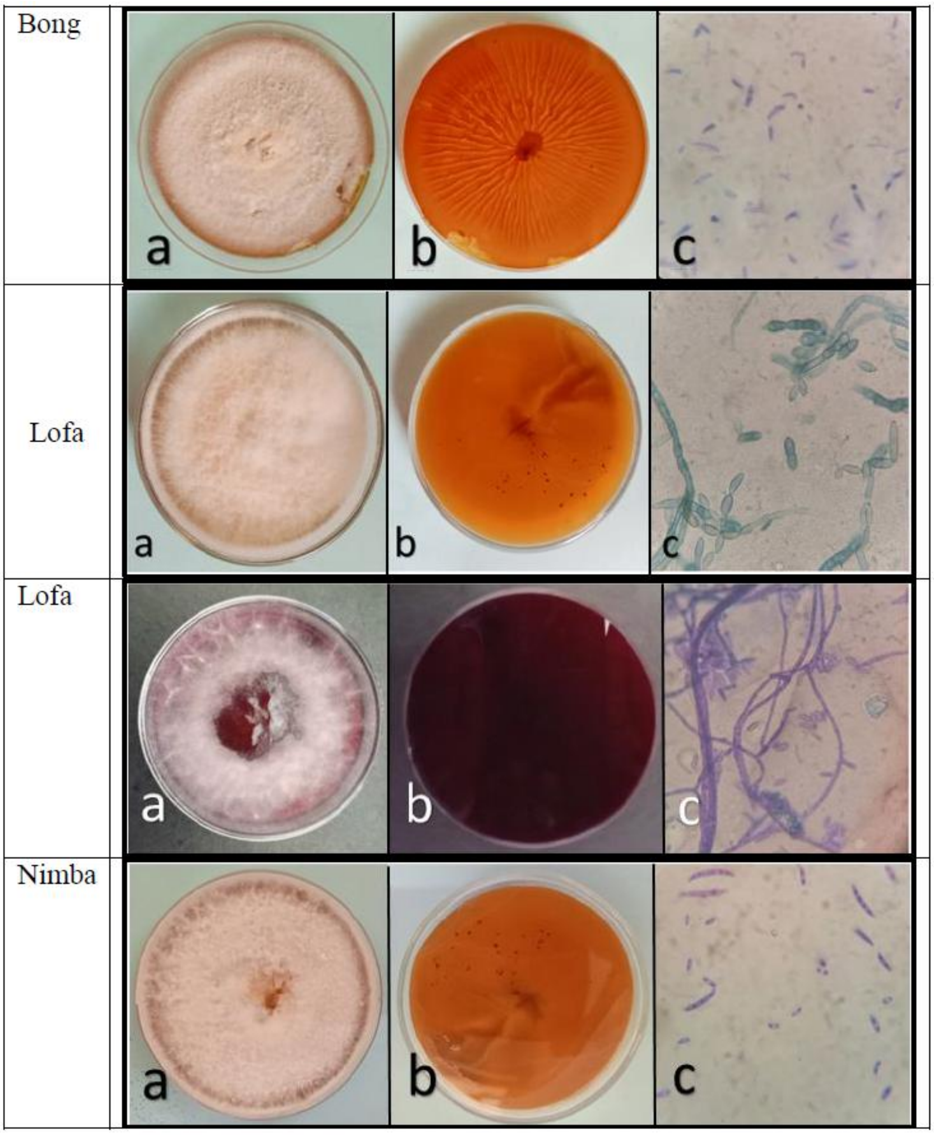

Figure 5. *Fusarium* spp isolated from all the counties except Montserrado. From left to right the pictures show the surface of the pure culture, the rear view of the same plate, and micrographs of the dhow-shaped conidia. The colony characteristics greatly differed among the samples from the counties. Two different colonies were isolated from Lofa

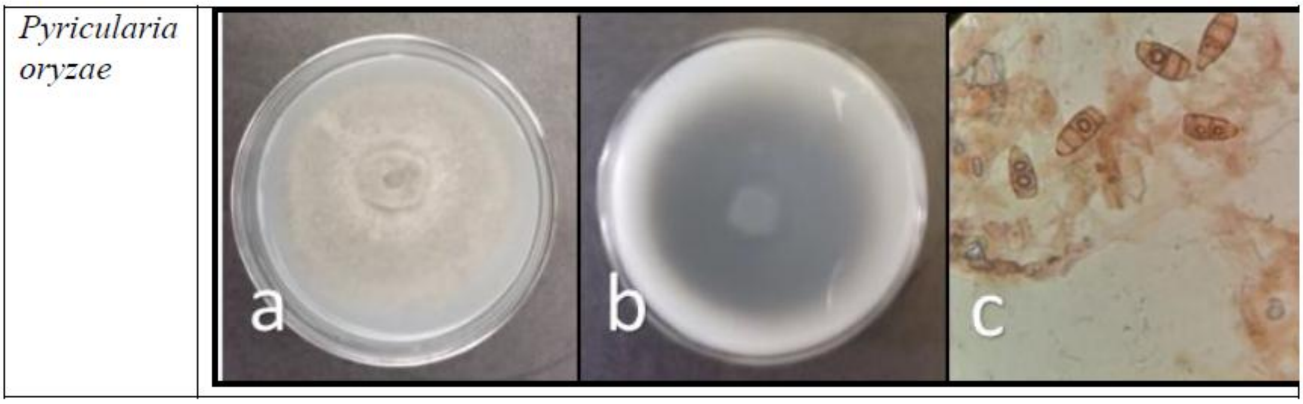

Figure 6. *Pyricularia oryzae* isolated from Lofa County. This was unique only to this region of Liberia. Septations are clearly visible in the conidia. the colony was grey-white with a dark rear

Another fungal pathogen that was only isolated from Lofa, is the *Pyricularia oryzae* (syn *Magnaporthe oryzae*). This fungus was unique to Lofa county. The colony colour appears grey white while the back appeared dark in the old sections and white in the younger areas (Figure 6). The spores were cylindrical with three septations. Sporulation was however low as only few conidia could be found as observed from the microscope field. This is a very destructive fungus causing rice blast disease and occurs in every rice growing region of the world [30]. The disease is present all through the rice growth cycle and develops in the seed, panicles, necks, collars, leaves and nodes [31]. The symptoms are majorly characterized by blast lesions on leaves and its isolation is readily carried out from these parts [32]. Lofa and Nimba, where this fungus was isolated, are the major rice growing counties of Liberia. It therefore goes that more pathogens would be isolated from such a region. There could be other reasons to explain the apparent low occurrence of the disease despite *P. oryzae* being touted as the major threat to rice. One factor driving the occurrence of the disease cultivation of susceptible rice varieties. Two rice species are cultivated world-wide, *Oryza sativa* L (Asian) and *O. glaberrima* (African) [33]. *Oryza glaberrima* is abundantly cultivated in West Africa and is known to be resistant to blast disease of rice. Excessive use of nitrogen-based fertilizers further fuels the occurrence of rice blast disease [34]. In the survey carried out during the current study, nearly all the farmers interviewed do not apply any type of fertilizer in their paddies [3]. Therefore, denial of this critical ingredient is expected to impede the development of rice blast disease in this region. Indeed, reduction in the use of nitrogen based fertilizers has been proven to presage decrease in the occurrence of leaf blast of rice [35]. Burning of straws is a cultural practice that has been shown to reduce bulk of disease inoculum passed on to the following season [36]. This is a dominant practice by the farmers from this region [3]. Whereas, its presence should inform decisions like introduction of nitrogen based fertilizers to the farmers. Such developments should be accompanied with stringent measures against *Pyricularia oryzae*.

4. Conclusions

The study showed that rice varieties grown in Liberia are susceptible to contamination by a variety of fungal organisms. *Aspergillus niger* was the most abundant fungal isolate followed by *A. flavus*, *Penicillium* spp *Fusarium* spp and *Pyricularia oryzae*. The results of this study are important for better understanding the fungal contamination of rice and the measures that can be taken to prevent or reduce it.

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