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Effect of Rice Blast Pathogen (*Pyricularia oryzae*) on the Nutritional Profile of Rice in Nigeria's Northern Guinea Savannah Ecological Zone

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ABSTRACT

A study was conducted to assess the effect of rice blast pathogen on the nutritional contents of rice in the Guinea Savannah Ecological Zone of Nigeria. Rice blast has significantly affected the yield of rice in the study area, prompting the investigation to determine the incidence and severity of the rice blast disease, isolate and identify the fungi associated with the blast, and evaluate the effect of the fungal pathogen on the nutritional content of the plant. All experiments were conducted following standard procedures, and data were analyzed at a 5% probability level of significance. The fungal pathogen was successfully isolated and identified from diseased rice plants as *Pyricularia oryzae*, and its pathogenicity was confirmed through induced blast symptoms on rice plants. Results of the proximate composition of the fungal-infected and apparently healthy rice showed that there was an increase in moisture (7.99%), ash extract (0.98%), and fiber (16.31%) content in the fungal-infected rice compared to the apparently healthy ones, which had 7.96%, 0.26%, and 13.58% moisture, ash extract, and fiber content, respectively. Conversely, the protein (1.20%), fat (7.68%), and carbohydrate (71.91%) content of the apparently healthy rice were relatively higher than those of the fungal-infected rice, which had 0.88%, 6.13%, and 75.12% protein, fat, and carbohydrate content, respectively. Although proximate analysis results showed differences in the nutritional contents of the diseased and healthy rice samples, statistical analysis showed no significant difference between the two samples in terms of their ash and moisture contents. Overall, this study provides insights into the nutritional changes associated with blast infection in rice plants. Such findings could contribute to the development of effective strategies for managing rice blast disease and improving rice production in Nigeria.

Keywords: Nutritional contents, *Pyricularia oryzae*, Rice, Rice blast

INTRODUCTION

Rice blast, also known as rotten neck disease, stands as one of the most destructive diseases of rice on a global scale. This pathogen inflicts considerable damage to crops, sometimes resulting in lost of up to 100 %. It is caused by *Pyricularia grisea* and has been reported in numerous countries. In Philippines, yield lost ranging from 50-85% have been documented (Kapil and Rabin, 2022). In Korea and China, losses exceeding 8% and 14 % have been reported, while India has experienced comparatively lower losses, with only 5-10% of the crop affected by blast (Kapil and Rabin, 2022). Losses attributed to this disease have been escalating since 2000. Although it does not occur annually, when it does, it wreaks havoc. A combination of preventive measures and foliar fungicides applied during the late boot stage and again when the rice is 80 to 90 percent headed can help control the disease (Allen and Sweets, 2021).

Hundreds of millions of people worldwide rely on rice as a staple food. Any failure in the

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crop, for whatever reason, poses a genuine threat of starvation. Rice blast, caused by a fungus, leads to the formation of lesions on leaves, stems, peduncles, panicles, seeds, and even roots. The potential threat of crop failure from this disease is so significant that it has been ranked among the most crucial plant diseases (Kapil and Rabin, 2022).

In Nigeria, the invasion of rice farms by blast disease is causing panic among rice farmers, as significant financial losses are being recorded (Mohammed et al., 2016). This development also poses a setback to the country's goal of achieving self-sufficiency in rice production. The disease, caused by the Ascomycete fungus *Magnaporthe oryzae*, is generally considered the most deadly disease of rice worldwide due to its extensive distribution and destructiveness under favorable conditions (David *et al*., 2011). It is known to cause approximately 60 to 100 percent yield losses (Gbenga, 2019).

Given the aforementioned problems and the need for data specific to the savannah region of Nigeria, where rice production is prominent, this research aimed to study the effect of the blast pathogen on the nutritional content of rice in that region.

MATERIALS AND METHODS

Study Area

The research was conducted at the laboratory of the Department of Plant Science, Modibbo Adama University Yola, situated in Yola, Nigeria. Modibbo Adama University, located in Girei Local Government Area, falls within the Northern Guinea Savannah Zone of Nigeria, located on latitude 9°18'00"N to 9°21'40"N and the longitude of 12°28'30"E of the Greenwich Meridian. This region experiences arid and semi-arid climates, characterized by distinct dry and rainy seasons. The dry season typically spans from late November to May, accompanied by the influence of the harmattan wind originating from the Sahara Desert (Adebayo, 2020). Monthly mean temperatures during the dry season range between 31°C to 40°C for maximum temperatures and 15°C to 23°C for minimum temperatures. April typically registers the highest temperatures, representing the peak of the dry season, while December and January record the lowest mean monthly temperatures, corresponding to the winter period in the region (Adebayo, 2020). The climate data utilized in this study were obtained from the Geography Department of Modibbo Adama University, Yola.

Collection of Disease Samples

During the sample collection, the approach of Nazifa *et al*. (2021) was adopted. Five villages known for rice farming were randomly sampled from the total number of villages in Girei: Gogora, Batari, Jawjaw, Bagalce, and Udawa in Girei Local Government Area, Adamawa State, Nigeria. Three farms were inspected for rice blast infection in each location, making a total of fifteen farms. In each farm, 10 rice stands with disease symptoms were assessed, totaling 150 rice stands. Three samples of diseased rice plants from each farm were collected and transported to the laboratory for investigation.

Preparation of Potato Dextrose Agar

Potato Dextrose Agar (PDA) was used to isolate the fungi. The medium was prepared by suspending 39 grams of the PDA powder in 1000 ml of distilled water. This mixture was boiled to dissolve the suspension completely. It was then sterilized by autoclaving at a temperature of 121°C for 15 minutes. The medium was then poured into a sterile petri dish under sterile conditions and allowed to solidify, according to Adebola *et al*. (2016).

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Isolation of Fungal Pathogen

The fungus was isolated from the brown spot-infected leaf samples. The infected leaf samples were washed with sterile water to remove dirt from the surface. The infected portion of the leaf, along with the green portion, was cut into small pieces. These pieces were then immersed in 1% sodium hypochlorite for 1 minute, followed by washing with sterile distilled water three times to remove excess sodium hypochlorite. The leaf bits were air-dried on blotter paper to remove excess water from the leaf surface. Then, the leaf bits were transferred into sterile petri plates containing Potato Dextrose Agar (PDA) medium amended with streptomycin sulfate to prevent bacterial contamination under aseptic conditions. The petri plates were incubated at 28 ± 2°C for 3 days, and the actively growing mycelium was sub-cultured. The isolated fungi were purified by the single spore method, and pure cultures were maintained on PDA slants at 4°C in McCartney bottles for further use,

Identification of the Pathogen

After obtaining the pure culture, the pathogen was identified using the cultural characteristics like colony color and morphological characteristics such as the shape and size of macro and micro conidia under the microscope using manuals of soil fungi (Adebola and Amadi, 2010). Stock culture of the isolate was maintained in McCartney bottle slants and stored at 4 °C in refrigerator for subsequent use (Adebola and Amadi, 2010).

Proximate Analysis

Proximate analysis comprising moisture, ash, crude fat, crude proteins, and carbohydrates contents was determined by their respective formulation of raw material using standard procedures as described below;

Moisture content

The moisture content of both healthy and diseased rice were analyzed using the drying method. Five grams of healthy and diseased rice were dried in a conventional oven (Thermo Fisher Scientific, U.S.) at 105°C for 24 hours. Then, the sample was cooled in a desiccator for 20 minutes and weighed to calculate the percentage weight loss of the sample using Equation 1 (AOAC, 2000:

$$\text{Moisture (\%)} = \frac{\text{(Weight of sample used – Dry weight of sample)} \times 100}{\text{Weight of sample used}}$$

Ash contents

The ash content of healthy and diseased rice were determined using the dry ashing method. Five grams of healthy and diseased rice samples were placed into the Muffle furnace (Carbolite, England) at 550°C for approximately 12 hours until they turned to ash. The ash content was determined using Equation 2 (AOAC, 2000:

$$\text{Ash (\%)} = \frac{\text{(Weight of +weight of crucibles) -(Weight of crucible)} \times 100}{\text{Weight of sample}}$$

Crude fat

The analysis of crude fat in healthy and diseased rice was carried out using the Soxhlet method. Five grams of the sample were transferred into a Soxhlet extraction flask (Fat Extractor E-500). This was followed by pouring; 200 ml of petroleum ether (Merck, Germany) was poured into the boiling flask attached to the Soxhlet extraction flask. The apparatus was heated in a boiling water bath For 8 hours to reflux the solvent. Then, the sample was cooled down by rotary

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evaporation. The flask was then placed in an oven for 15 minutes at 105°C. Lastly, a desiccator was used to cool down the flask containing the sample again, and it was weighed. The crude fat was measured using Equation 3 (AOAC, 2000:

$$\text{Fat (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Crude protein

For crude protein content, the micro-Kjeldahl method (method 950.36) was used. Exactly 0.15 g of healthy and diseased rice shifted in a boiling tube. Then, 0.8 g of mixed catalysts and 2.5 ml of concentrated sulfuric acid (H_2SO_4) (Merck Germany) were added to the tube and heated. This was followed by pouring, 10 ml of 45 % sodium hydroxide (NaOH) solution (Merck, Germany) were slowly added to the distillation tube to separate the two layers of the solution. The conical flask containing 2% boric acid (Merck Germany) was placed on the distillate platform, and the distillation of ammonia was allowed to take place. The ammonium borate in the distillate was titrated with 0.05 M H_2SO_4 until the end point was reached, and the amount of titrating was recorded. The percentage of proteins was calculated using Equation below (AOAC, 2000:

$$\text{Protein (\%)} = \frac{(\text{The volume of titrating sample} - \text{Volume of titrating blank}) \times 0.05\text{N} \times 14 \times 100}{\text{Weight of sample}}$$

Crude fibre content

The crude fibre content was determined using method 962.09 of AOAC (2000). About 0.5 g of the sample was boiled in 50 ml of 0.3 M H_2SO_4 under reflux for 30 minutes, followed by filtering through a 75 mm sieve under suction pressure. The residue was washed with distilled water to remove the acid. The residue was then boiled in 100 ml of 0, 25 M sodium hydroxide under reflux for 30 minutes and filtered under suction. The insoluble portion was washed with hot distilled water to free the alkaline. this was then dried to a constant weight in the oven at 100 °C for 2 hours, and then cooled in the desiccator. The dried sample was ashed in a muffle furnace to subtract the mass of ash from the fiber, and then the percentage of fibre was determined.

Carbohydrates

The total carbohydrates content of healthy and disease rice was determined by difference using Equation 5 (AOAC, 2000):

$$\text{\% total carbohydrate} = 100 - (\text{\% moisture} + \text{\% ash} + \text{\% crude fat} + \text{\% crude proteins} + \text{\% fibre}).$$

Data Analysis

Data obtained from the proximate analysis were subjected to statistical analysis of variance (ANOVA) to determine the significant differences among means. The Least Significance Different (LSD) was used to separate the means where there are significant differences. The analysis was carried out using the Paleontological Statistics, at a 5% level of significance.

RESULTS

Incidence of Rice Blast Disease in the Study Area

Results from the survey revealed varying levels of rice blast incidence across the study sites in Girei (Table 1). The incidence of the disease varied significantly ($p = 0.05$) across the five study sites. Udawa village had the highest incidence of rice blast disease in the study area with a mean incidence of 70.0%, followed by Batare and Jawjaw with

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incidences of 56.7% and 46.7%, respectively. Gogora and Bagalce had the lowest incidence of rice blast among the villages surveyed, each with an incidence of 43.3%.

Table 1: Incidence of Rice Blast Disease in Some Villages in Girei Local Government Area of Adamawa State during the 2023 Rainy Season

Villages	Incidence (%)
Gogora	43.3
Batare	56.7
Jawjaw	46.7
Bagalce	43.3
Udawa	70.0
Mean	52.0
p-value	0.1235
LSD	9.60

Isolation and Identification of the Fungal Pathogen

The fungal pathogen responsible for rice blast was isolated on Potato Dextrose Agar (PDA) medium from different parts of diseased rice plants collected from various rice fields in Girei, Adamawa State, Nigeria. The fungal isolate was identified as *Pyricularia grisea* based on its characteristics on culture media and its morphological characteristics under a light microscope (Table 2 and Plates I A and I B).

Comparative Proximate Composition the Healthy and Diseased Rice Plants

Table 3 shows the results of the proximate composition of the fungal-infected and apparently healthy rice. The results indicate an increase in moisture (7.99%), ash extract (0.98%), and fiber (16.31%) content in the fungal-infected rice compared to the apparently healthy rice, which had 7.96%, 0.26%, and 13.58% moisture, ash extract, and fiber content, respectively. Conversely, the protein (1.20%), fat (7.68%), and carbohydrate (71.91%) content of the apparently healthy rice were relatively higher than those of the fungal-infected rice, which had 0.88%, 6.13%, and 75.12% protein, fat, and carbohydrate content, respectively indicating the influence of the pathogen om the nutrition contents of the rice.

Table 2: Characteristics of *Pyricularia grysea* isolated from Rice in Girei

Fungal pathogen	Cultural characteristics	Morphological characteristics
Pyricularia grysea	Greyish white colour, elevated mycelium with rough margin and formations of wrinkles and zonations. 7 to 14 days	Pathogen produced septate and branched mycelium and conidia were produced in clusters on long septate, slender conidiophores. The shape of conidia in all the isolates were pyriform and hyaline to pale olive, 2 septate and 3 celled.

Table 3: Comparative Proximate Composition the Healthy and Diseased Rice plants

Treatment	**Amount (%)**					
Nutrients	Fats	Moisture	Ash	Protein	Carbohydrate	Fiber
Healthy	7.68	7.96	0.26	1.20	71.91	13.58
Diseased	6.13	7.99	0.98	0.88	67.36	16.31
Stand. Dev.	1.09	0.02	0.50	0.22	3.21	1.93
p-value	0.0014	0.9567	0.0001	0.0221	0.2333	0.0310
LSD	0.22	0.59	0.05	0.10	3.67	0.95

*Data are mean of three replicates

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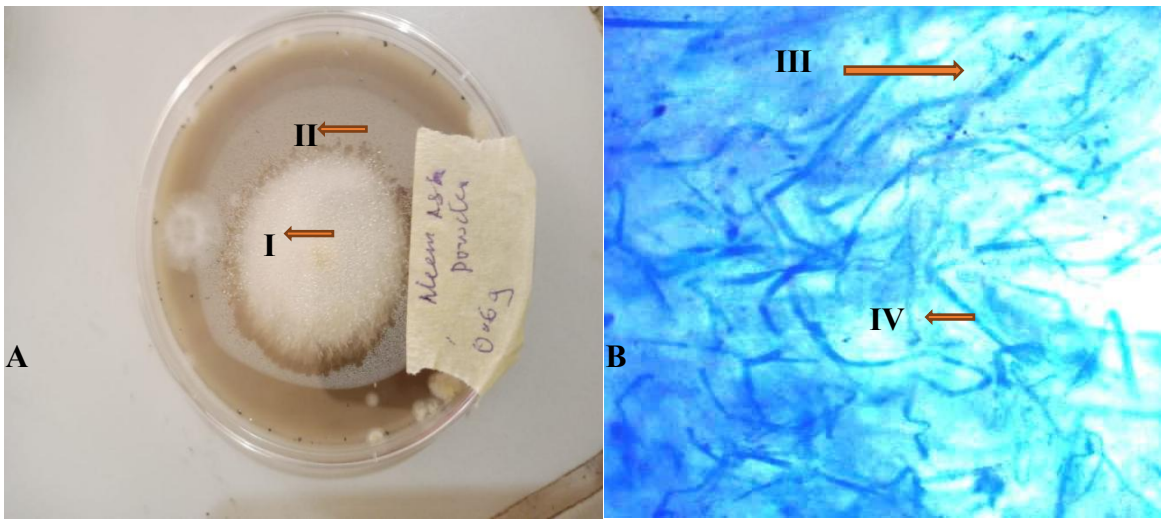

Plate I: Cultural and morphological characteristics of a seven-day-old *Pyricularia grysea* (A-front plate, I & II- greyish white old and elevated advancing mycelia, B-Conidia at x10 objective, III & IV- macroconidium and microconidium).

DISCUSSION

Rice production worldwide is constrained by fungal and bacterial diseases. It has been observed that many farmers, particularly in Adamawa State of Nigeria, are not well informed about the devastating effects of these diseases. There was a high incidence of rice blast disease in the study areas. Rice blast disease has also been identified in rice fields in other parts of Nigeria (Hadiza *et al*., 2022), the Philippines and Korea (Kapil and Rabin, 2022), confirming the prevalence of this disease in rice fields globally. This finding could partly account for the low yield of rice recorded in Girei, making diseases a major threat to rice production in this area. Some of the agronomic practices adopted by farmers such as source of irrigation water, bedding pattern, source of seeds and plant spacing might be the reason for the observed high incidences of rice blast and other diseases in the area. This agrees with the findings of Asare-Bediako *et al*. (2015), who reported that agronomic practices adopted by farmers influence the incidence and severity of pests and diseases in the crops cultivated on their fields.

Pyricularia grisea was the fungus isolated from diseased rice samples collected from fields across the rice cultivation sites in Girei Local Government Area of Adamawa State. This fungus has also been reported to have been isolated from rice in different parts of the world (Scheuermann *et al*., 2012; Apinya *et al*., 2020; Amoghavarsha *et al*., 2021). Pathogenicity of the fungal isolate was conducted on rice seeds and on potted rice seedlings under laboratory and screen house conditions. In all cases, the isolate was pathogenic on the rice samples examined. There seemed to be differences in the response to this fungal pathogen on the seeds in the laboratory and on seedlings in the pots. Langner (2018) reported that during the off-season and when atmospheric moisture is reduced, the pathogenesis of *Pyricularia grisea* is difficult to recognize in the field, making it challenging to observe the characteristic symptoms of the disease.

Furthermore, latent infection occurs frequently, making visual diagnosis impossible (Agrawal *et al*., 2018). This finding also agreed with the report of Kalboush (2019), who cultured rice-infected samples with blast collected from

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different rice cultivars and locations during the 2015-2016 seasons, revealing that twenty *P. grisea* isolates were successfully isolated by the single spore technique. Similarly, Apinya *et al*. (2020) reported that rice blast disease is caused by the ascomycete fungus *Pyricularia oryzae*. The investigation adopted various fungal morphological characteristics and phylogenetic analysis, including Inter-Simple Sequence Repeat (ISSR) and Sequence-Related Amplified Polymorphism (SRAP).

Results of the proximate composition of the fungal-infected and apparently healthy rice show that there was an increase in moisture, ash extract, and fiber content of the fungal-infected rice compared to the apparently healthy ones, while the protein and carbohydrate content of the apparently healthy rice plants were relatively higher than those of the fungal-infected ones. The variation in the nutritional contents of these sets of experimental rice was explained by several scientists worldwide. Rott (2000) reported that rot-infected plant tissues often exhibit higher moisture content compared to healthy ones due to the metabolic activity associated with the infection process and water uptake by the plant tissues. Ash content, representing the inorganic mineral content, may vary between healthy and infected rice. These alterations in ash content in diseased rice may be due to changes in nutrient uptake and metabolism (Ravichandra *et al*., 2015). The diversion of nutrients towards defense mechanisms against the pathogen may result in reduced protein synthesis (Ghosh *et al*., 2017). Crude fat content in rice, like other starchy crops, is generally minimal, but infection can influence lipid metabolism in plants. These changes in crude fat content may reflect alterations in metabolic pathways induced by the disease (Vidhyasekaran *et al*., 2014).

Infected rice may exhibit alterations in crude fiber content due to structural modifications induced by fungal infection. Increased crude fiber levels can indicate cell wall thickening or lignification as a response to pathogen attack (Ravichandra *et al*., 2015). Carbohydrate metabolism is significantly affected by red rot infection, leading to changes in carbohydrate composition and content. Reduced carbohydrate levels in infected plants may result from disrupted photosynthetic activity and nutrient translocation (Vidhyasekaran *et al*., 2014).

CONCLUSION

It may be concluded from this study that *Pyricularia grisea* is a common pathogenic fungi which cause rice blast in the study area. The result from the pathogenicity test indicated that the isolated fungus is pathogenic and attributed to the cause of rice blast in the region.

It is also clear from the result that the fungus not only cause blast on rice plants but also reduces the nutritional values of rice seeds as well. Fungal infection may leads to a reduction in carbohydrate and protein contents of the rice which might have a remarkable effect on the value of the rice, especially is one of the stable food in Nigeria.

We therefore recommended timely spraying of the rice with fungicides to reduce the damaging activities of the pathogen and contamination with mycotoxins and other related fungal metabolites that might be harzadous to human health.

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