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EFFECT OF THREE SELECTED AQUEOUS PLANT EXTRACTS ON THE MANAGEMENT OF RICE BLAST (MAGNAPORTHE ORYZAE) DISEASE UNDER TROPICAL FIELD CONDITION

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ABSTRACT

Field experiments were conducted during the 2018 and 2019 cropping seasons at the Teaching and Research Farm, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria, to determine the effects of foliar sprays of Morinda lucida, Piper guineense, and Xylopia aethiopica at a 7.50% w/v level of concentration on disease incidence and severity on three rice (NG/SA/DEC/07/0278, FARO-19, and FARO-21) accessions. Experiments were a 3×5 factorial arrangement fitted into RCBD with three replications. Data obtained on disease incidence, severity, and agronomic performance were subjected to analysis of variance while significant means were separated with the Tukey HSD test at p<0.05. Assessment of blast disease showed that incidence, severity, and agronomic performance varied significantly with the plant extracts. The extracts sprayed on the foliage of the rice accessions significantly (p < 0.05) reduced the intensity of blast disease development in both seasons. Disease incidence ranged from 20.98 to 29.57% compared with 41.78 and 48.90% in the unsprayed plots, while severity ranged from 1.23 to 1.55 relative to 2.93 to 3.23 in the unsprayed plots in the 2018 and 2019 cropping seasons. Qualitative analysis showed that alkaloids, cyanogenic glucosides, tannins, saponins, flavonoids, phenols, and steroids were the secondary metabolites detected from the three plant samples. However, the quantitative results showed that there was a significant variation in the amounts of secondary metabolites present in each plant sample. In conclusion, the use of resistant cultivars is the best option in the management of blast disease. However, in the absence of resistant cultivars, foliar spray of P. guineense, M. lucida, and X. aethiopica at 7.5% w/v could help in the management of rice blast disease in the field and thus be recommended as a viable option for improved rice production.

Keywords: rice, disease, blast, extract, foliar spray, management

INTRODUCTION

Rice (*Oryza sativa*, L.) is a monocot plant with two primary cultivated species, *Oryza sativa* L. and *O. glaberrima* Steud, usually known as Asian and African rice, respectively (Khush, 2013). Tanko *et al.* (2016) report that the crop is rapidly becoming

a significant staple food and cash crop in Africa. This continent produces 15 million tonnes of rice every year (Ronald *et al.*, 2014). Rice protein has the highest nutritional quality of any grain protein, despite its low protein level (Shah *et al.*, 2014). For many people in African and other developing









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nations, rice supplies the majority of calories as well as a variety of minerals, zinc, and ß-carotene (Dipti et al., 2012). Africa, particularly Nigeria, has been abundantly gifted with land and water resources that can support a massive increase in rice production (Balasubramanian et al., 2009). Rice can be grown in a variety of environments, including but not limited to dryland, rain-fed wetland, deep water and mangrove swamps, and irrigated wetland (Balasubramanian et al., 2007; Seck et al., 2012). The three rice accessions, NG/SA/ DEC/07/0278, FARO19, and FARO-21, were selected due to their level of moderate susceptibility to the blast pathogen in the previous trials. Many biotic and abiotic variables limit rice production internationally, including crop management strategies (low input and use of machinery) used by small-scale rice farmers, as well as a lack of water control techniques, soil fertility, location, pests, and diseases (Ngala, 2011; Idowu et al., 2013). Many factors contribute to low yield, but one of the biggest restrictions to production is infectious diseases such as rice blast (Magnaporthe oryzae, Cav.) (Fakir et al., 2002). Blast is one of the most serious dangers to tropical rice production. The use of resistant varieties is the most cost-effective and efficient method of controlling rice blast, particularly in resource-constrained farmer fields. Plant extracts have been known for their medicinal and antimicrobial properties since ancient times (Jabeen, 2006; Khan and Nasreen, 2010). They offer a greater scope than synthetic chemicals, as they are relatively safe, easily biodegradable, and eco-friendly (Enikuomehin, 2005; Khan and Nasreen, 2010). Some tropical aromatic plants have been shown to exert high antimicrobial activities, and since they are natural products, mostly consumed by man, there is little or no fear of poisoning even at very high concentrations (Adegoke et al., 2002). The family Annonaceae includes the widely cultivated Ethiopian pepper (Xylopia aethiopica), which is found in the West African rainforest (Burkhill, 1985). In West Africa, it is a highly valued medicinal plant that includes a number of intricate chemical components (Adegoke et al., 2003). Numerous reports on the significance of *X. aethiopica* in medicine have been published (Fleischer, 2008). Fusarium oxysporum, Aspergillus niger, and Aspergillus flavus are a few of the regularly occurring spoilage fungi that cause rot on agricultural food, and a hot water extract of the seeds of X. aethiopica has shown fungitoxic activity against them (Ngala, 2011). African black pepper (P. guineense) is a member of the Piperaceae family. In Nigeria, it goes by a variety of names, including "Uziza" in Igbo and "Iyere" in Yoruba. Ogbole et al. (2010) state that P. guineense has uses in food, medicine, cosmetics, and insecticides. They are also used to treat rheumatism, digestive disorders, and bronchitis (Mierziak et al., 2014). The plant is used for a range of uses in various forms, including whole

herbs, powders, extracts, and vapours (Khush, 2013). Anyawu and Nwosu (2014) showed the effect of the ethanol and aqueous extracts of the leaves of the plant against the bacteria S. aureus, E. coli, P. aeruginosa, and B. subtilis and the fungi C. albicans and S. cerevisiae. The brimstone tree (Morinda lucida) is referred to as Oruwo in the southwestern region of Nigeria and is a member of the Rubiaceae family. Adomi and Umukoro (2010) stated that the antibacterial action of the M. lucida leaf may be due to the presence of some metabolic poisons or substances like aromatic phenols, alkaloids, cardenolides, and saponins. According to Alma et al. (2003), aromatic phenolic compounds have been found to exhibit antibacterial activities. Karou et al. (2011) reported that the primary chemical class responsible for the antibacterial activity of plants, including Rubiaceae, is phenolic compounds. The chemical composition of aromatic phenols and their antibacterial action may be related. In-depth research was done by Ogundare and Onifade (2009) on the effect of M. lucida leaves on Escherichia coli and found that the plant had strong inhibitory activity both in vitro and in vivo. Thus, under tropical field conditions, this study examines how three plant extracts reduce the intensity of rice blast disease development in the field.

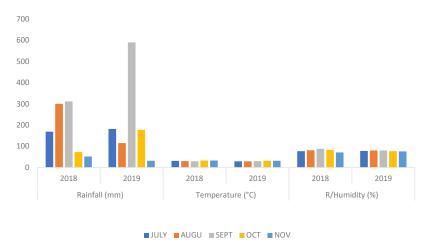
MATERIALS AND METHODS

Experimental Site

The experiments were conducted at the Teaching and Research Farm, Federal College of Agriculture, Ishiagu (Latitude 5°56"N and Longitude 7°41"E), South Eastern, Nigeria, on clay-sandy soil during the 2018 and 2019 cropping seasons. The site is located in the derived savannah agroecological zone of Nigeria. Weather data during growth periods of experimentation in 2018 and 2019 is presented in Fig. 1. The highest total amount of rainfall was recorded in the months of September 2018 and 2019, with the values of 311.6 and 589.8 mm, while the least was recorded in the month of November with the values of 0.00 and 316 mm, respectively. Rainfall recorded in September, which coincided with the vegetative-reproductive period of rice, was higher in both cropping seasons. The total amount of rainfall was higher in 2019 than in 2018 cropping seasons. This underscored mildly cooler temperatures, especially in July-September 2019 (29-30°C) than in 2018 (29-31°C) during this period. Values recorded for relative humidity during the two cropping seasons ranged between 77% and 88% compared to 77% to 80% in 2018 and 2019, respectively.

Sources of the Three Botanicals

The leaves of the Brimestone tree (*Morinda lucida*) and African black pepper (*Piper guineense*) were sourced from the premises of the Federal College



1: Weather variables for the 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

of Agriculture, Ishiagu, while the pods of Ethiopian pepper (*Xylopia aethiopica*) were sourced from the Eke market, Ishiagu, Ebonyi State.

Pre-germination of Seeds and Nursery Preparation

To improve uniform germination and early field establishment, the seeds were soaked in water for 24 hours and incubated in the laboratory for 48 hours (Mohanta *et al.*, 2003). Rice seedlings were grown in a perforated plastic bucket. Each perforated bucket with five holes at the bottom received five kilograms of sandy, loamy soil. Each pre-germinated rice seed was placed in a bucket and lightly covered with topsoil to minimise rodent and bird attack. The plant was then watered lightly every day for three weeks before transplanting.

Experimental Design

The experiments were laid out in a 3×5 factorial experiment fitted into a randomised complete block design (RCBD). The dimensions of each plot were $2\,\text{m}\times2\,\text{m}$ with an alley row of $1\,\text{m}$. The total area of land used was $352\,\text{m}^2$. Each of the three lowland rice accessions was replicated three (3) times with a plant spacing of $20\,\text{cm}$ by $20\,\text{cm}$.

Preparation of Plant Extracts

Fresh, fully expanded leaves of *M. lucida* and *P. guineense* with the pod of *X. aethiopica* were washed thoroughly with water and soaked in a 1% solution of sodium hypochlorite for 2 minutes, rinsed severely with sterile distilled water, and air-dried for about 2 weeks at room temperature. Each plant extract was prepared by separately weighing 75 g of the leaves and pod on a Mettler-Toledo AG balance, AB104, Switzerland, and blended in a Eurosonic, ES210, Great Star, Asia, blender, and 1000 ml of sterile distilled water (7.5% w/v) was added for 12 hours. The mixture was shaken thoroughly and filtered using Whatman No. 1 filter paper and was used as treatments.

Application of the Treatments

Extracts mixed with Tween 20 (polyethylene glycol sorbitan monolaurate), an emulsifier concentrate (0.01 ml/L), were sprayed on each designated plot with the aid of a knapsack sprayer at 5, 7, and 9 weeks after transplanting (WAT). Also, 1 000 ml of 0.5% broad-spectrum contact fungicide (Mancozeb) and water were also sprayed as positive and negative controls, respectively.

Disease Assessment

At 12 weeks after transplanting, the blast symptoms were scored using the method of Manandhar *et al.* (2016) (Tab. I). Percentage Disease Incidence (DI) is calculated using the formulae of Jamal *et al.* (2011).

$$DI (\%) = \frac{Number \ of \ diseased \ plant}{Total \ number \ of \ plants \ per \ plot} \times 100 \tag{1}$$

Disease Severity (DS) is calculated using the formula adopted from Gwary *et al.* (2009).

Disease severity =
$$\frac{\Sigma n \times 100}{N \times S}$$
 (2)

where

 ΣSummation,

n.....number of infected leaves,

N....number of leaves assessed, and

S..... maximum numerical grade.

Agronomic Data

The agronomic data, such as plant height (cm), number of tillers, leaf area (cm²), 100 grain weight (g), and grain yields per plant (g), were collected from five tagged plants in each plot at 12 weeks after transplanting on the following parameters in accordance with International Rice Research Institute (IRRI, 2002) procedures.

| I: Disease Severity rating sca |
|--------------------------------|
|--------------------------------|

| Scale | Reaction | IRRI Scale Equivalent | Host Response |
|-------|--|--------------------------------------|-----------------------------|
| 0 | No symptoms | No symptoms | Highly Resistant(HR) |
| 1 | 1–5% of the leaves or panicles with lesions covering completely around the node. | 1–5 % leaves area affected | Resistant (R) |
| 2 | 6–25% of the leaves or panicles with lesions covering completely around the node. | 6–15 % leaves area affected | Moderately Resistant (MR) |
| 3 | 26–50% of the leaves or panicles with lesions covering completely around the node. | 16–50 % of the leaves area affected | Moderately Susceptible (MS) |
| 4 | > 50% of the leaves or panicles with lesions covering completely around the node. | 51–100 % of the leaves area affected | Susceptible (S) |

Source: IRRI, 2002; Manandhar et al., 2016

Phytochemical Screening of the Plant Extracts

The extracts were screened for the presence of alkaloids, steroids, tannins, saponins, flavonoids, phenols, and cyanogenic glycosides.

Determination of Tannins, Steroids, Saponins, and Phenol Content

The percentage composition of tannin, steroids, saponin, and phenol content in the tested sample was determined using the AOAC methods (2010) with some modifications.

Determination of Alkaloid

Total alkaloid content in the studied plants will be measured using the 1,10-phenanthroline method described by Singh *et al.* (2004). The total alkaloid content will be expressed as mg colchicine equivalent per 100 grams of dried plant material.

Determination of Flavonoid

Flavonoid content of different plant parts extracts will be measured using the aluminium chloride colorimetric assay developed by Chang *et al.* (2002). The total flavonoid content will be expressed as mg catechin equivalent per 100 grams of air-dried plant material.

Determination of Cyanogenic Glucosides

The alkaline picrate method (Onwuka, 2005) was employed for the determination of cyanogenic glycosides.

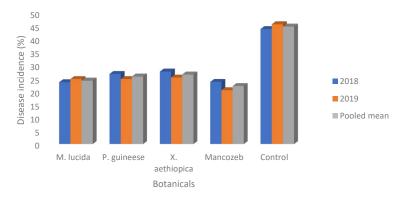
Statistical Analysis of Data

Data collected were subjected to one factorial analysis of variance (ANOVA) using Minitab software version 17. The significant means were separated using the Tukey test at P < 0.05.

RESULTS

Effect of the Botanicals on Disease Incidence of Rice Blast Leaf Disease During the 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

In 2018, the disease incidence showed a significant difference (P<0.05) among the botanicals (Fig. 2). The untreated plot had the highest (43.77%) disease incidence, followed by X. aethiopica (27.56%), while M. lucida had the least (23.50%). The result of the 2019 cropping season revealed that the untreated plot had the highest (45.56%) disease incidence, followed by X. aethiopica (25.32%), while fungicide (Mancozeb) had the least (20.46%). However, the pooled mean result showed that the disease incidence ranges from fungicide (Mancozeb) to untreated plot with the value of 22.03% to 44.66%, respectively.



2: Effect of the botanicals on disease incidence of rice blast leaf disease during 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

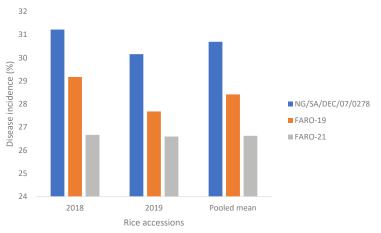
Disease Incidence of Rice Leaf Blast Disease on Rice Accessions During the 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

The disease incidence on the three rice accessions showed a significant difference among the rice tested (Fig. 3). The result showed that in the 2018 cropping season, NG/SA/DEC/07/0278 had the highest (31.22%) disease incidence while FARO-21 had the least (26.67%). The response of the disease

incidence in the 2019 cropping season and pooled mean follows the same patterns.

Interaction of Rice Accessions and Botanicals on Incidence of Rice Blast Disease During 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

The interaction between three rice accessions and five botanicals had a significant effect on blast disease incidence in both seasons (Tab. II).



3: Disease incidence of rice leaf blast disease on rice accessions during 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

II: Interaction of rice accessions and botanicals on incidence of rice blast disease during the 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

| Disease Incidence (%) | | | | | | |
|-----------------------------------|----------|---------|-------------|--|--|--|
| Interaction | 2018 | 2019 | Pooled mean | | | |
| M. lucida × NG/SA/DEC/07/0278 | 21.45de | 23.93bc | 22.56b-e | | | |
| M. lucida × FARO-19 | 29.18cd | 28.33b | 28.76bc | | | |
| M. lucida × FARO-21 | 21.19de | 21.81bc | 20.98de | | | |
| P. guineense × NG/SA/DEC/07/0278 | 27.88cde | 28.54b | 28.21bcd | | | |
| P. guineense × FARO-19 | 26.67cde | 21.76bc | 24.27b-e | | | |
| P. guineense × FARO-21 | 25.40cde | 23.78bc | 24.59b-e | | | |
| X. aethiopica × NG/SA/DEC/07/0278 | 31.10c | 28.04b | 29.57b | | | |
| X. aethiopica × FARO-19 | 26.81cde | 24.05bc | 25.43b-e | | | |
| X. aethiopica × FARO-21 | 24.77cde | 23.87bc | 24.32b-e | | | |
| Mancozeb × NG/SA/DEC/07/0278 | 26.75cde | 21.62bc | 24.19b-e | | | |
| Mancozeb × FARO-19 | 22.59de | 21.33bc | 21.96de | | | |
| Mancozeb × FARO-21 | 20.14e | 18.44c | 19.94e | | | |
| Control × NG/SA/DEC/07/0278 | 49.14a | 48.66a | 48.90a | | | |
| Control × FARO-19 | 40.59b | 42.92a | 41.76a | | | |
| Control × FARO-21 | 41.59ab | 45.09a | 43.34a | | | |
| MEANS | 29.02 | 28.15 | 28.58 | | | |
| SE | 1.28 | 1.44 | 1.33 | | | |
| CV (%) | 29.51 | 34.24 | 31.25 | | | |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey. SE means Standard Error while CV means Coefficient of Variation

In the 2018 cropping season, the Control \times NG/SA/DEC/07/0278 had the highest (49.14%) disease incidence, followed by Control \times FARO-21 (41.59%), while $M.\ lucida \times$ FARO-21 had the least (20.14%). In the 2019 cropping season, disease incidence also follows the same pattern. Disease incidence was also significantly higher in Control \times NG/SA/DEC/07/0278 (48.66%), followed by Control \times FARO-21 (43.34%), while fungicide (Mancozeb) \times FARO-21 had the least (19.94%). However, the pooled mean showed that disease incidence was significantly higher in Control \times NG/SA/DEC/07/0278 (48.90%), followed by Control \times FARO-21 (45.09%), while fungicide (Mancozeb) \times FARO-21 had the least (18.44%).

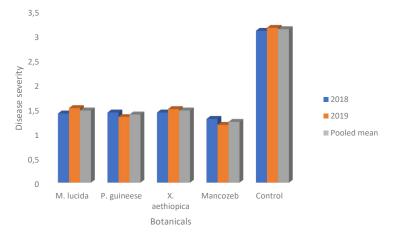
Effect of Botanicals on the Disease Severity of Rice Leaf Blast Disease During the 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

In 2018, the disease severity showed a significant difference (P < 0.05) among the botanicals (Fig. 4). The untreated plot had the highest (3.08) disease severity, followed by *X. aethiopica* and *P. guineense*

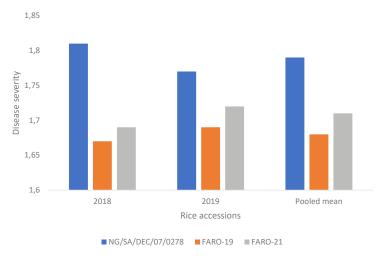
with 1.42 apiece, while fungicide (Mancozeb) had the least (1.29). In the 2019 cropping season, a similar trend was also recorded in disease severity with a significant difference. The untreated plot had the highest (3.14), followed by *M. lucida* (1.51), while fungicide (Mancozeb) had the least (1.17). However, the pooled mean result showed that the disease severity ranged from 1.23 to 3.11 in fungicide (Mancozeb) to untreated plot respectively.

Disease Severity of Rice Blast Disease on Rice Accessions During the 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

The disease severity on the three rice accessions showed a significant difference among the rice tested (Fig. 5). The result showed that in the 2018 cropping season, NG/SA/DEC/07/0278 had the highest (1.81), while FARO-19 (1.67) had the least. In the 2019 cropping season, disease severity ranged from 1.69 to 1.77. The pooled mean severity shows that NG/SA/DEC/07/0278 had the highest (1.79) while FARO-19 had the least (1.68).



4: Effect of the botanicals on disease severity of rice blast disease during the 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State



5: Disease severity of rice blast disease on rice accessions during the 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

Interaction of Rice Accessions and Botanicals on the Severity of Rice Blast Disease During the 2018 and 2019 Cropping Seasons in Ishiagu, Ebonyi State

The interaction between the rice accessions and botanicals had a significant effect on blast disease severity in both seasons (Tab. III). In 2018, Control × NG/SA/DEC/07/0278 had the highest (3.33), followed by Control × FARO-21 (3.10), while fungicide (Mancozeb) × FARO-19 had the least (1.20). Similarly, the severity of the 2019 cropping season shows that Control × NG/SA/DEC/07/0278 had the highest (3.20), followed by Control × FARO-21 (3.17), while fungicide (Mancozeb) × FARO-19 had the least (1.20). However,

the pooled means followed the same trend, and the severity ranges from 1.20 for Control \times FARO-21 to 3.23 for fungicide (Mancozeb) \times FARO-19.

Effect of Botanicals on the Agronomic Performance of Rice Tested During the 2018 Cropping Season in Ishiagu, Ebonyi State

Agronomic performance of the three rice accessions tested during the 2018 cropping season in Ishiagu is presented in Table IV. The plant height was not significantly different (P>0.05) among the treatments. The plot treated with *P. guineense* had the highest (93.23 cm), followed by *X. aethiopica* (92.20 cm) while the untreated plot had the least

III: Interaction of rice accessions and botanicals on severity of rice blast disease during the 2018 and 2019 cropping seasons in Ishiagu, Ebonyi State

| Disease severity | | | | | | |
|-----------------------------------|--------|-------|-------------|--|--|--|
| Interaction | 2018 | 2019 | Pooled mean | | | |
| M. lucida × NG/SA/DEC/07/0278 | 1.53c | 1.57b | 1.55b | | | |
| M. lucida × FARO-19 | 1.43c | 1.50b | 1.47b | | | |
| M. lucida × FARO-21 | 1.23c | 1.47b | 1.35b | | | |
| P. guineense × NG/SA/DEC/07/0278 | 1.37c | 1.40b | 1.38b | | | |
| P. guineense × FARO-19 | 1.53c | 1.23b | 1.38b | | | |
| P. guineense × FARO-21 | 1.37c | 1.37b | 1.37b | | | |
| X. aethiopica × NG/SA/DEC/07/0278 | 1.50c | 1.53b | 1.52b | | | |
| X. aethiopica × FARO-19 | 1.37c | 1.47b | 1.42b | | | |
| X. aethiopica × FARO-21 | 1.40c | 1.47b | 1.43b | | | |
| Mancozeb × NG/SA/DEC/07/0278 | 1.30c | 1.17b | 1.23b | | | |
| Mancozeb × FARO-19 | 1.20c | 1.20b | 1.20b | | | |
| Mancozeb × FARO-21 | 1.37c | 1.13b | 1.25b | | | |
| Control × NG/SA/DEC/07/0278 | 3.33a | 3.20a | 3.23a | | | |
| Control × FARO-19 | 2.80b | 3.07a | 2.93a | | | |
| Control × FARO-21 | 3.10ab | 3.17a | 3.13a | | | |
| MEANS | 1.722 | 1.729 | 1.726 | | | |
| SE | 0.105 | 0.110 | 0.107 | | | |
| CV (%) | 41.04 | 42.84 | 41.58 | | | |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey. SE means Standard Error while CV means Coefficient of Variation

IV: Effect of botanicals on the agronomic performance of rice tested during 2018 cropping season in Ishiagu, Ebonyi State

| Treatments | Plant height at maturity (cm) | Number of tiller/plant | Leaf area (cm²) | 100 Grain weight (g) | Grain yields per plant (g) |
|---------------|-------------------------------|------------------------|--------------------|-------------------------|-------------------------------|
| M. lucida | 89.76c | 11.78a | 691.82ab | 3.50a | 39.73a |
| P. guineense | 93.23ab | 11.78a | 708.22a | 3.66a | 42.14a |
| X. aethiopica | 92.20bc | 13.22a | 681.78ab | 3.58a | 45.93a |
| Mancozeb | 96.17a | 13.44a | 744.41a | 3.66a | 50.46a |
| Control | 80.77d | 8.11b | 487.06b | 3.04b | 27.08b |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey

(80.77 cm). The number of tillers per plant revealed that the plot treated with *X. aethiopica* had the highest (13.22), while the untreated plot had the least (8.11). The leaf area ranges from 487.06 cm² for the untreated plot to 744.41 cm² for fungicide. The result of 100 grains weight showed that fungicide (Mancozeb) and *P. guineense* had the highest with 3.66 g apiece, while the untreated plot had the least (3.04). However, the result of the grain yield showed that the plot treated with *X. aethiopica* had the highest (45.93 g), while the untreated plot had the least (27.08 g).

Agronomic Performance of Rice Tested During the 2018 Cropping Season in Ishiagu, Ebonyi State

The agronomic performance of the three rice accessions tested during the 2018 cropping season showed a significant effect (Tab. V). The accession NG/SA/DEC/07/0278 had the highest (110.88 cm) plant height, while FARO-19 had the least (78.50 cm). The number of tillers per plant ranges from 10.60 to 13.40. The accession NG/SA/DEC/07/0278 had the largest (774.72 cm²) leaf area, while FARO-19 had the smallest (588.74 cm²). The result of the grain yield revealed that FARO-19 had the highest (44.81 g) grain yield while NG/SA/DEC/07/0278 had the least (38.52 g).

V: The agronomic performance of rice tested during 2018 cropping season in Ishiagu, Ebonyi State

| Accessions | Plant height at maturity (cm) | Number of tiller/plants | Leaf area (cm²) | 100 Grain weight (g) | Grain yields per plant (g) |
|-------------------|-------------------------------|----------------------------|--------------------|-------------------------|----------------------------|
| NG/SA/DEC/07/0278 | 110.88a | 10.60b | 774.72a | 3.95a | 38.52a |
| FARO-19 | 78.50c | 13.40a | 588.74b | 3.23b | 44.81a |
| FARO-21 | 81.89b | 11.00b | 624.51b | 3.28b | 39.87a |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey.

VI: Interaction of rice accessions and botanicals on the agronomic performance of rice during 2018 cropping season in Ishiagu, Ebonyi State

| Interaction | Plant height at maturity (cm) | Number of tiller/plant | Leaf area (cm²) | 100 Grain weight (g) | Grain yields per plant (g) |
|-----------------------------------|-------------------------------------|------------------------|--------------------|-------------------------|-------------------------------|
| M. lucida × NG/SA/DEC/07/0278 | 107.88cd | 11.67a-d | 647.33ab | 4.03abc | 42.27abc |
| M. lucida × FARO-19 | 79.77fg | 13.00a-d | 742.13ab | 3.08d | 40.73abc |
| M. lucida × FARO-21 | 81.63ef | 12.00a-d | 685.98ab | 3.40cd | 36.20abc |
| P. guineense × NG/SA/DEC/07/0278 | 115.17ab | 13.33a-d | 880.94ab | 4.25a | 36.92abc |
| P. guineense × FARO-19 | 80.87ef | 9.00b-d | 547.29ab | 3.32cd | 50.53abc |
| P. guineense × FARO-21 | 83.64ef | 13.00a-d | 696.43ab | 3.52bcd | 38.97abc |
| X. aethiopica × NG/SA/DEC/07/0278 | 109.53bc | 14.33a-c | 880.41ab | 3.30d | 44.50abc |
| X. aethiopica × FARO-19 | 83.20ef | 14.67ab | 672.60ab | 4.13ab | 51.33ab |
| X. aethiopica × FARO-21 | 83.87ef | 9.33b-d | 492.34ab | 3.17d | 41.97abc |
| Mancozeb × NG/SA/DEC/07/0278 | 119.53a | 11.00a-d | 924.01a | 4.28a | 42.03abc |
| Mancozeb × FARO-19 | 82.17ef | 16.67a | 554.55ab | 3.47bcd | 55.20a |
| Mancozeb × FARO-21 | 86.81e | 12.67a-d | 754.68ab | 3.27d | 54.13a |
| Control × NG/SA/DEC/07/0278 | 102.30d | 8.00d | 540.93ab | 3.08d | 26.27c |
| Control × FARO-19 | 66.50 h | 8.33cd | 427.15b | 3.00d | 26.87bc |
| Control × FARO-21 | 73.50g | 8.00d | 493.10ab | 3.04d | 28.12bc |
| MEANS | 90.42 | 11.667 | 662.7 | 3.4891 | 41.07 |
| SE | 2.36 | 0.526 | 32.4 | 0.0737 | 1.69 |
| CV (%) | 17.53 | 30.25 | 32.75 | 14.16 | 27.68 |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey. SE means Standard Error while CV means Coefficient of Variation

Interaction of Rice Accessions and Botanicals on the Agronomic Performance of Rice During the 2018 Cropping Season in Ishiagu, Ebonyi State

Tab. VI showed the interaction of rice accessions and botanicals on the agronomic performance of rice during the 2018 cropping season in Ishiagu, Nigeria. The results of the agronomic performance revealed that plant height, number of tillers per plant, leaf area, 100 grains weight, and grains yield showed significant effects during the 2018 cropping season. The P. guineense × NG/SA/DEC/07/0278 had the largest (115.17 cm), followed by M. lucida × NG/SA/DEC/07/0278 (107.88 cm), while Control × FARO-19 had the smallest (66.50 cm). The number of tillers per plant ranges from 8.00 for Control \times FARO-21 to 14.67 for X. aethiopica \times FARO-19. The result of the leaf area revealed that Mancozeb × NG/SA/DEC/07/0278 had the largest (924.01 cm²), followed by P. guineense × NG/SA/ DEC/07/0278 (880.94 cm²), while Control × FARO-19 had the smallest (427.15 cm²). Also, the result of 100 grains weight revealed that P. guineense × NG/ SA/DEC/07/0278 was the heaviest (4.25 g), followed by X. aethiopica \times FARO-19 (4.13 g), while Control × FARO-19 was the lightest (3.00 g). However, X. aethiopica \times FARO-19 (51.33 g) had the highest grain yield, followed by P. guineense × FARO-19 $(50.53 \,\mathrm{g})$, while Control × NG/SA/DEC/07/0278 was the poorest grain yielders (26.27 g).

Effect of Botanicals on the Agronomic Performance of Rice Accessions Tested During the 2019 Cropping Season in Ishiagu, Ebonyi State

The report of the agronomic performance of the three rice accessions tested during the 2019

cropping season in Ishiagu is presented in Tab. VII. The plant height showed a non-significant difference among the treatments. The P. guineense had the highest height (92.75 cm), followed by X. aethiopica (92.02 cm), while the untreated plot produced the least (81.56 cm). The number of tillers per plant revealed that the plot treated with P. guineense had the highest number (14.56), followed by M. lucida (13.86), while the untreated plot had the lowest (9.15). The leaf area ranges from *P. guineense* from 1718.05 cm² to 2670.11 cm² untreated plot. The result of 100 grains weight showed that *P. guineense* had the highest weight (3.70 g), while the untreated plot had the lowest (3.06 g). However, the result of the grain yield showed that the plot treated with X. aethiopica had the highest grain yield (38.12 g), followed by P. guineense (37.39g), while the untreated plot had the lowest (22.86 g).

Agronomic Performance of Rice Accessions Tested During the 2019 Cropping Season in Ishiagu, Ebonyi State

The result of the agronomic performance of the three rice accessions tested during the 2019 cropping season is presented in Tab. VIII. The accession NG/SA/DEC/07/0278 had the highest plant height (111.08 cm), while FARO-21 had the lowest (80.83 cm). The number of tillers per plant ranges from 12.87 for FARO-19 to 13.33 for FARO-21. The accession NG/SA/DEC/07/0278 had the largest leaf area (2154.21 cm²), while FARO-19 had the smallest (2041.84 cm²). The result of the grain yield revealed that FARO-19 (43.18 g) had the highest grain yield while NG/SA/DEC/07/0278 (27.98 g) had the lowest grain yield.

VII: Effect of botanicals on the agronomic performance of rice accessions tested during 2019 cropping season in Ishiagu, Ebonyi State

| Treatments | Plant height at maturity (cm) | Number of tiller/plant | Leaf area (cm²) | 100 Grain weight (g) | Grain yields per plant (g) |
|---------------|----------------------------------|---------------------------|-----------------|-------------------------|-------------------------------|
| M. lucida | 90.17b | 13.86a | 2117.77ab | 3.63a | 33.90ab |
| P. guineense | 92.75ab | 14.56a | 1718.05b | 3.70a | 37.39a |
| X. aethiopica | 92.02ab | 12.96a | 2250.12ab | 3.56a | 38.12a |
| Mancozeb | 94.83a | 15.15a | 1718.76b | 3.71a | 40. 28a |
| Control | 81.56c | 9.15b | 2670.11a | 3.06b | 22.86b |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey.

VIII: The agronomic performance of rice tested during 2019 cropping season in Ishiagu, Ebonyi State

| Accessions | Plant height at maturity (cm) | Number of tiller/plant | Leaf area (cm²) | 100 Grain weight (g) | Grain yields per plant (g) |
|-------------------|-------------------------------|---------------------------|--------------------|-------------------------|----------------------------|
| NG/SA/DEC/07/0278 | 111.08a | 13.16a | 2154.21a | 3.93a | 27.98b |
| FARO-19 | 78.90b | 12.87a | 2041.84a | 3.40b | 43.18a |
| FARO-21 | 80.83b | 13.33a | 2088.83a | 3.27b | 32.36b |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey.

IX: Effects of some botanicals on the agronomic performance of rice during 2019 cropping season in Ishiagu, Ebonyi State

| Interaction | Plant height at maturity (cm) | Number of tiller/plant | | 100 Grain weight (g) | Grain yields per plant (g) |
|-----------------------------------|-------------------------------|---------------------------|-----------|----------------------|-------------------------------|
| M. lucida × NG/SA/DEC/07/0278 | 109.64b | 12.00ab | 1899.04ab | 3.89a-d | 26.06c |
| M. lucida × FARO-19 | 81.30de | 10.78ab | 2507.76ab | 3.59a-e | 38.40a-c |
| M. lucida × FARO-21 | 79.55de | 13.00ab | 1946.50ab | 3.40c-e | 37.24a-c |
| P. guineense × NG/SA/DEC/07/0278 | 115.15ab | 14.22ab | 2071.16ab | 4.12abc | 26.01c |
| P. guineense × FARO-19 | 80.38de | 16.45a | 1281.17b | 4.20ab | 57.74a |
| P. guineense × FARO-21 | 82.73d | 13.78ab | 1801.83ab | 3.52b-e | 37.08a-c |
| X. aethiopica × NG/SA/DEC/07/0278 | 111.37ab | 15.33ab | 2466.41ab | 3.46b-e | 34.84a-c |
| X. aethiopica × FARO-19 | 82.63d | 15.11ab | 2190.71ab | 3.31de | 45.24a-c |
| X. aethiopica × FARO-21 | 82.07d | 15.00ab | 2093.23ab | 3.17de | 34.29a-c |
| Mancozeb × NG/SA/DEC/07/0278 | 117.32a | 16.22a | 1965.38ab | 3.54b-e | 28.45bc |
| Mancozeb × FARO-19 | 81.54de | 12.33ab | 1475.39ab | 4.30a | 52.08ab |
| Mancozeb × FARO-21 | 85.63d | 15.11ab | 1715.50ab | 3.29de | 31.60bc |
| Control × NG/SA/DEC/07/0278 | 101.93c | 8.00b | 2369.05ab | 3.13e | 24.57c |
| Control × FARO-19 | 68.62f | 9.67ab | 2754.17a | 3.09e | 22.42c |
| Control × FARO-21 | 74.14ef | 9.78ab | 2887.10a | 2.96e | 21.60c |
| MEANS | 90.27 | 13.118 | 2095.0 | 3.5327 | 34.51 |
| SE | 2.36 | 0.531 | 95.7 | 0.0690 | 1.87 |
| CV (%) | 17.55 | 27.13 | 30.65 | 13.10 | 36.40 |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey. SE means Standard Error while CV means Coefficient of Variation

X: Qualitative and quantitative analysis of secondary metabolite present in tested leaf samples

| Phytochemical compounds | M. lucida | X. aethiopica | P. guineense |
|-----------------------------|-----------|---------------|--------------|
| Alkaloids (%) | 1.91b | 1.78c | 8.24ab |
| Cyanogenic glucoside (ug/g) | 4.55a | 4.23a | 0.11c |
| Tannins (mg/100 g) | 0.64c | 0.57d | 1.39c |
| Saponins (%) | 0.73c | 0.64d | 8.60a |
| Flavonoids (%) | 2.29b | 2.12b | 6.95b |
| Phenol (%) | 0.16c | 0.09e | 0.95c |
| Steroids (%) | 0.14c | 0.07e | 0.10c |
| MEANS | 1.489 | 1.361 | 3.761 |
| SE | 0.330 | 0.312 | 0.825 |
| CV (%) | 101.53 | 105.05 | 100.52 |

Means in the same column followed by different alphabets are significantly different (P < 0.05) using Tukey. SE means Standard Error while CV means Coefficient of Variation

Interaction of Rice Accessions and Botanicals on the Agronomic Performance of Rice During the 2019 Cropping Season in Ishiagu, Ebonyi State

Tab. IX showed the interaction of rice accessions and botanicals on the agronomic performance of rice during the 2019 cropping season in Ishiagu, Nigeria. The results revealed that plant height,

number of tillers per plant, leaf area, 100 grains weight, and grains yield showed significant effects during the 2019 cropping season. The P. guineense \times NG/SA/DEC/07/0278 had the highest plant height (115.15 cm), followed by X. aethiopica \times NG/SA/DEC/07/0278 (111.37 cm), while Control \times FARO-19 had the lowest (68.62 cm). The number of tillers per plant ranges from 8.00 for Control \times NG/SA/DEC/07/0278 to 16.45 for P. guineense \times FARO-19.

The result of the leaf area revealed that Control x FARO-21 had the largest (2887.10 cm²), followed by Control x FARO-19 (2754.17 cm²), while *P. guineense* x FARO-19 had the smallest (1281.17 cm²). Also, the result of 100 grains weight revealed that *P. guineense* × FARO-19 had the highest grain yield (4.30 g), followed by *P. guineense* × NG/SA/DEC/07/0278 (4.12 g), while Control × FARO-21 had the lowest (2.96 g). *P. guineense* × FARO-19 had the highest grain yield (57.74 g), followed by Mancozeb × FARO-19 (52.08 g), while Control × FARO-21 had the least (21.60 g).

Qualitative and Quantitative Analysis of Secondary Metabolites Present in Tested Leaf Samples

Tab. X revealed the results of qualitative and quantitative analysis of the secondary metabolite

present within M. lucida, X. aethiopica, and P. guineense tested. Qualitative results showed that alkaloids, cyanogenic glucosides, tannins, saponins, flavonoids, phenols, and steroids were the secondary metabolites detected from the three plant samples. However, the quantitative results showed that there was a significant variation in the quantity of the compounds. Alkaloids and saponins were found much higher in P. guineense, with the values of 8.24-8.60, respectively, than in other samples. Cyanogenic glucoside was also found higher in both X. aethiopica and M. lucida with the values of 4.23 and 4.55, respectively, than P. guineense with the value of 0.11. The amounts of steroids present were small across the three tested leaf samples compared with other secondary metabolites.

DISCUSSION

Over the years, the use of industrial chemicals like propionic acid and ammonia against fungal diseases has attracted a lot of attention in the fight to control pathogenic fungus on agricultural fields. It has been demonstrated that these compounds are effective at reducing fungal development in the field. However, they could cause chemical poisoning, environmental toxicity, and the formation of fungi that are resistant to the chemical agent when they are concentrated on the grains. Plant-based chemical origin has been an alternative means of controlling pathogenic fungi in the field due to their fewer poisoning activities. Since they are natural compounds that are mostly consumed by humans and several tropical aromatic plants have been proven to have strong antibacterial activity, there is little to no concern for poisoning, even at very high concentrations (Adegoke et al., 2002). Most of these plant species with potential pesticidal properties are M. lucida, P. guineense, and X. aethiopica, which belong to the families of Rubiaceae, Piperaceae, and Annonaceae, respectively (Devanand and Rani, 2008). In vivo activities of these plant extracts demonstrated that rice blast disease incidence and severity were greatly reduced in the field by the spray of aqueous extracts of M. lucida, P. guineense, and X. aethiopica. This may be due to the fact that plant extracts are effective at preventing the germination of pathogen spores because secondary metabolites, which are antifungal components found in plant extracts, operate as a barrier to the development of disease. According to Stephen et al. (2016), the leaf of P. guineense contains alkaloids, tannins, saponins, flavonoids, hydrogen cyanides, and phenols. Dahunsi et al. (2020) also show the presence of tannins, alkaloids, flavonoids, terpenoids, saponins, steroids, phenols, and cardiac glycosides in various concentrations in M. lucida. Aguoru et al. (2016) reported that alkaloids, saponins, tannins, reducing sugar, anthraquinones, steroids, flavonoids, and glycosides were present in X. aethiopica. According to Mierziak et al. (2014), phenolic chemicals, notably flavonoids, have been shown to contribute to the defence of plants against pathogens through a variety of mechanisms of action. Similarly, Khoa et al. (2011) showed that sheath blight lesion length could be reduced by up to 68% under controlled and semi-controlled settings by foliar spraying and seed soaking with an aqueous extract of Chromolaena odorata. Aqueous extracts of C. odorata significantly reduced the severity of several significant rice diseases, such as bacterial blight (Xanthomonas oryzae pv. oryzae) (up to 50%), brown spot (Bipolaris oryzae), and blast (Pyricularia oryzae) (up to 45%). Amadioha (2000) further supported that the ethanol extract, cold water extract, hot water extract, and oil extract of Azadirachta indica seeds offered the best M. oryzae control due to the higher level of phenols (4.8 mg/g) and flavonoids (24.5 mg/g) compounds in A. monophylla. Parimelazhagan (2001) found that leaf extracts of A. monophylla prevent rice disease up to 82.22%, followed by Plumbago rosea at 70.57%. Foliar spraying of rice seedlings with the aqueous extract of M. lucida, P. guineense, and X. aethiopica at a concentration of 7.5% w/v significantly increased the growth and yield of the rice investigated. With the exception of the untreated plots, all of the botanicals significantly increased the plant height, number of tillers, leaf area, and grain yield in the treated plots for three rice cultivars. Harish et al. (2008) found that spraying rice plants twice with neem cake extract and Nerium oleander leaf extract in the field helped to increase the yield by 23% and 18%, respectively. The increase in growth and yield parameters observed could be due to nutrients supplied by the plant extracts and the control of the pathogens, allowing more energy to be allocated to growth and yield.

CONCLUSION

The results of this study demonstrated the *in vivo* antifungal potential of aqueous extracts of *M. lucida*, *P. guineense*, and *X. aethiopica* on rice leaf blast. It is evident that spraying rice with aqueous extracts of *M. lucida*, *P. guineense*, and *X. aethiopica* at 7.5% w/v helps to reduce the incidence and severity of leaf blast disease of rice in the field. Also, these botanicals are natural fungicides and may not control the disease completely; however, they can be cost-effective, environmentally safe, and easy to obtain by the resource-poor farmers in Nigeria. It can help in reducing the build-up of inocula in the field, which can further reduce the gravity of the disease on the incoming rice crop in the next season. Since blast disease is one of the most devastating biotic threats to rice production, the use of botanicals can help minimise yield losses and thus reduce chemical pesticide applications in rice cultivation areas in Nigeria. The differences in performance of the three rice accessions in lowland circumstances demonstrate that environmental factors have a significant influence on these rice accessions. The genetic link between agronomic variables related to blast resistance demonstrated for these rice accessions would aid plant breeders worldwide in improving rice yield, particularly in Nigeria.

Recommendations

The potential of aqueous extracts of *M. lucida, P. guineense,* and *X. aethiopica* at a 7.5% w/v level of concentration in the control of rice leaf blast disease *in vivo* is highly recommended. The study also recommends that the use of these botanicals in combinations and/or with other botanicals should be further investigated on rice blast. This would help to establish the mechanism of control of fungal pathogens in tropical agriculture where cost of commercial fungicides is prohibitive.

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Conflict of Interest

The authors confirm that there is no conflict of interest in this article.

Authors' Contributions

This work was carried out by the two authors. The two authors designed the experiment, coordinated the experiment, laboratory analysis and analysed the data. Author YBA collected the data, processed the data, perform literature searches, and wrote the draft manuscript. Author ACG prepared the final manuscript while authors PAR and ASO proof read the final manuscript. The final manuscript was read by the four authors.

This is an ORIGINAL ARTICLE.

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