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Original Article

Rice blast incidence and morphological variation of *Magnaporthe oryzae*, Cav. in Ebonyi State, Nigeria

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ABSTRACT

Blast disease is one of the major threats to rice production globally. This study investigates the prevalence, incidence, severity, damage index and morphological variation of blast disease in lowland rice in nine local government areas of Ebonyi State, Nigeria. Ebonyi State lies within Latitudes 6°15'57"N and 6°24'70"N, Longitudes 7°42'18"E and 8°26'20"E in the derived savannah zone of Nigeria. A survey was conducted on twenty-six (26) rice farms between October and November, 2017 when the rice had reached physiological maturity and infection of rice plants with blast was evident. Data collected from the farms included; rice variety cultivated, source of the seeds, area of land under cultivation, disease incidence, severity and damage, and control measures. Data obtained on disease incidence, severity and damage index were subjected to Analysis of Variance while significant means were separated with Tukey HSD test at p<0.05. Results on rice blast disease revealed that incidence, severity and damage index varied between fields and location due to the environmental conditions prevalent in each location. The highest mean disease incidence, severity and damage index 53.67%, 2.50 and 38.02% respectively due to blast, were recorded in Ishielu, while Ezza South had the least blast incidence

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(18.24%), Abakaliki had the least severity (1.30) and Ezza South the least damage index (6.59%). Twenty-two fungal isolates were identified and characterized from infected rice plants obtained from the field. The twenty-two isolates, identified as *Magnaporthe oryzae* (*Mo*), grown on potato dextrose agar showed various differences in their cultural and morphological characters such as colony colour, margin, pigmentation, surface texture, shapes, septate and cells. Pathogenicity study revealed the five isolates *Mo*-03, *Mo*-05, *Mo*-06, *Mo*-10 and *Mo*-17 where more virulent than others. Therefore, the present study recommends that the governments should set up a modality to educate and train rice farmers on disease symptoms and the possible eco-friendly means of control in order to increase rice production both in the study areas and in Nigeria at large.

Keywords: *Oryza sativa*, Rice Blast, *Magnaporthe oryzae,* Isolates

INTRODUCTION

Rice (*Oryza sativa*, L.) is a member of the Poaceae family and one of the most valuable cereal crops grown and consumed globally. It is a staple food in several African countries, including Nigeria, and accounts for a large portion of the diet on a regular basis (Lu et al 2018). Rice is grown on a small scale in almost all agro-ecological zones in Nigeria. However, FAO (2015) reported that Nigeria is the leading African country in rice consumption as well as one of Africa's largest rice producers and importers. Rice is an important food security crop, as well as an important cash crop for small-scale producers, who typically sell 80% of their total crop production and consume only 20%. Rice production in Nigeria increased from 3.7 million metric tons in 2017 to 4.0 million tons in 2018 (Kamai et al 2020). It is estimated that fungi alone reduce annual rice production by 14% globally (Agrios 2005). *Magnaporthe oryzae* Cav., which causes rice blast is a serious fungal disease that is threatening food security both globally and particularly in Nigeria. Blast losses are difficult to estimate due to the compounding effects of environmental factors; however, high yield losses of 40-80% are common when the pathogen finds favourable environmental conditions (Idowu et al 2013). Rice blast has received extensive research due to the importance of rice production and consumption as well as its global distribution and destructiveness. Blast can infect the aboveground tissues of rice plants at any stage of development, resulting in crop failure. Lesions on the leaves (leaf blast), leaf collars (collar blast), culms, culm nodes, panicle neck nodes (neck rot), and panicles (panicle blast) vary in colour and shape depending on varietal resistance, environmental conditions, and plant age. Despite the frequent occurrence of severe outbreaks of rice blast disease in Nigeria, there is no detailed information on the severity of the disease in Ebonyi State. In this regard, it is critical to conduct an assessment of rice disease in nine local government areas of Ebonyi State, Nigeria, as well as to identify the domains where rice blast disease may become a production constraint. As a result, the current study was carried out to determine the severity of the blast disease as well as to isolate and identify morphological characteristics of the disease in lowland rice grown in Ebonyi State, Nigeria.

MATERIALS AND METHODS

A survey of rice leaf blast infected fields in twenty-six (26) rice farms was conducted within the following local government areas where rice is known to be cultivated: Ivo, Afikpo south, Afikpo north, Onicha, Ikwo, Abakaliki, Ohaukwu, Ezza south and Ishielu in Ebonyi State (Figure 1). In each local government area, the number of farms visited varied from Afikpo north, Onicha, Ezza south and Ohaukwu with two (2) farms to Afikpo south with five (5) farms. The survey was done between October and November, 2017 during which time rice had reached physiological maturity and infection of the rice plants with blast was evident.

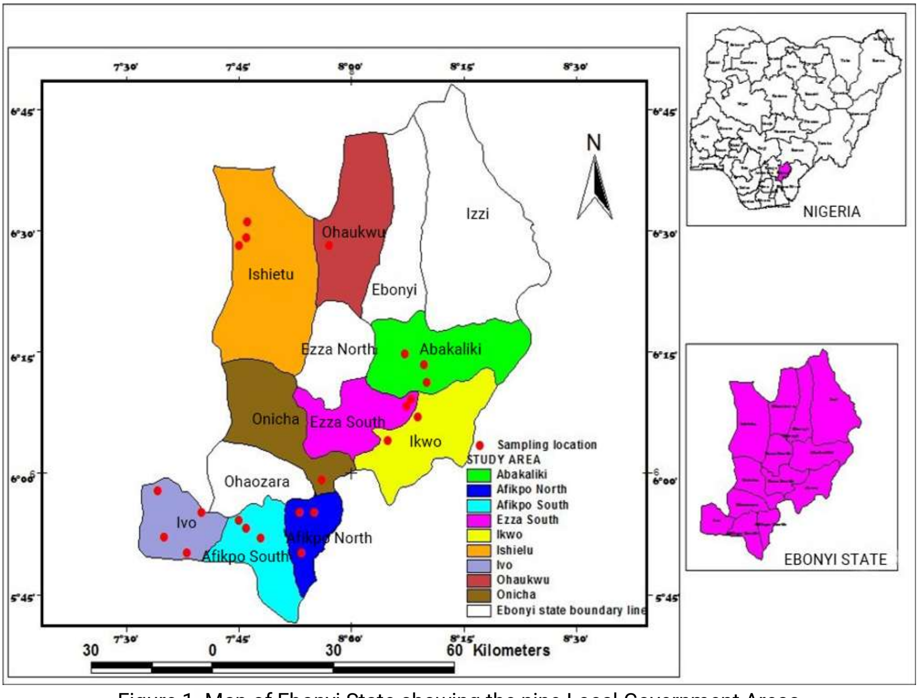

Figure 1. Map of Ebonyi State showing the nine Local Government Areas

Disease Assessment

Rice plants in each field were observed at different locations or sites using a Z-shaped method (Figure 2a and b). Ten spots with thirty observations were made per location. At each spot, three observations were made: one to the left, one to the right, and one straight-ahead of 10 plants (Manandhar et al 2016). The ten rice plants were rated for disease severity on a 0 to 4 scale (Table 1). The growth stage of the crop which was determine based on the physiological stage, coordinates and altitude were taken using a GPS instrument. In the case where the size of the farm was less than a hectare, five spots with a total of 15 observations were made. The visual scores for the rice blast incidence and severity were recorded using the method of Manandhar et al (2016). Diseased rice leaf samples were collected from each site, preserved in sample envelopes and taken to the Plant Protection

Laboratory, Federal College of Agriculture, Ishiagu, Ebonyi State for the isolation and identification of fungal organisms.

$$PDP = \frac{\text{Number of fields affected by the disease}}{\text{Total number of fields assessed}} x100$$

Percentage Disease Prevalence (PDP) was calculated according to the formulae by Mebratu et al (2015).

Percentage Disease Incidence (DI) which refers to the proportion or percentage of diseased plants (entities) within a sample population (Mebratu et al 2015). It was calculated using the formulae of Jamal et al (2011).

$$DI(\%) = \frac{\text{Number of diseased plant}}{\text{Total number of plants per plot}} x100$$

Disease Severity (DS), recorded on the scale 0-4, refers to the proportion of area or amount of plant tissue that is diseased within a sample population (Schoonoven and Pastor-Corrales 1987). It was calculated using the formula adopted from Gwary et al (2009) (Table 1).

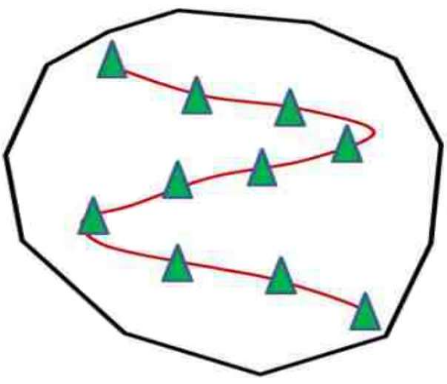

Figure 2a: Z-shaped method.

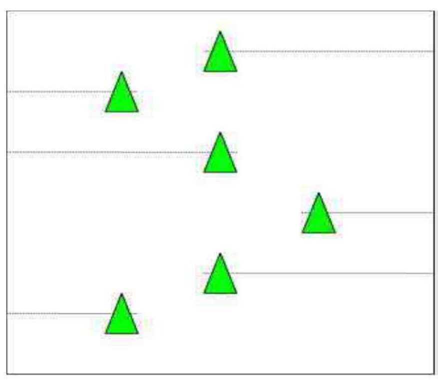

Figure 2b: Z-shaped method.

Source: Manandhar et al (2016)

$$\textit{Disease severity} = \frac{\Sigma n \text{ x } 100}{\text{N x S}}$$

Where

Σ = Summation
n = number of infected leaves
N = Number of leaves assessed
S = Maximum numerical grade

Percentage Damage Index (PDI) refers to the degree of injury from visual assessment (Nihoul et al 1991). It was calculated using the formulae by Mebratu et al (2015).

$$PDI = \frac{\text{Incidence x Severity}}{\text{Highest Severity}} x100$$

Table 1. Disease Severity rating scale

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 affected	Susceptible (S)
4	>50% of the leaves or panicles with lesions covering completely around the node.	51-100% of the leaves affected	Highly Susceptible (HS)

Source: IRRI (2002), Manandhar et al (2016)

Isolation, Purification and Identification of Rice Leaf Blast Isolates

The collected rice leaf samples showing symptoms were placed in sample bags and returned to the laboratory prior to isolation. Symptomatic rice cultivar leaves were cut into small pieces (less than 2mm in size) and surface sterilized with 1% sodium hypochlorite for 1min, followed by three washes with sterile distilled water. The leaf pieces were then placed in Petri dishes containing Potato Dextrose Agar (PDA) amended with streptomycin to inhibit bacterial growth and incubated for 24h at 25°C to encourage sporulation. After incubation, the Petri dishes were examined under a stereo-dissecting microscope for mycelial growth. Grey, dense and bushy conidial growth appearance was observed around the leaf pieces on the cultured media. A sterile moistened needle was used to extract some conidia by brushing the needle across the sporulating lesion. The conidia were placed on PDA media plates containing streptomycin (WARDA 2004). Plates were incubated at 25°C for about 7 days, with 12h of darkness and 12h of light. The identity of *M. oryzae* was confirmed by examining the conidia under a light microscope (WARDA 2004). According to the cultural and morphological characteristics of the pathogen, identification was carried out as described by Agrawal et al (1989) and Mew and Gonzales (2002). The blast isolates were designated as *Mo*-01 where Mo denoted *Magnaporthe oryzae* and the number denoted the sites according to Meena (2005).

Cultural and Morphological Characterization of the Rice Leaf Blast Isolates

The cultural and morphological characterization of different *M. oryzae* was determined by culturing the pathogen on PDA (Meena 2005). Mycelia discs 6mm in diameter from 5 days old cultures of different *M. oryzae* isolates were inoculated on the middle of Petri plates containing media. Three replications were made and kept at 30°C. The colony diameter of each isolate's growth was measured after the 10th day of incubation, and the growth was calculated in mm using a scale defined by Mebratu et al (2015) as Good=75-90mm, Moderate=56-75mm, Low/poor=56mm. Different colony characteristics such as pigmentation, mycelia color, surface texture, margin, size, shape, and septation of conidia were observed visually and microscopically (Meena 2005).

Pathogenicity Tests

The pathogenicity test was important to confirm the real cause of rice leaf blast disease according to Koch's postulates. Pathogenicity of *M. oryzae* was confirmed using the method of Chevalier et al (1991) under screen house conditions. Five seedlings of a local rice variety known as 'local foreign' were transplanted to one perforated plastic bucket (6cm diameter) containing field soil (sandy loam) and placed under screen house condition. Completely randomized design (CRD) was used and replicated three times.

Inoculum Production and Inoculations

After germination, at the stage of 3-4 leaves (3 weeks), the plants were inoculated. The seedlings in each pot were sprayed with 50mL of spore suspension adjusted to 10^5 spores/mL using a haemocytometer (Takan et al 2004). The conidial suspensions were sprayed on the rice seedlings until runoff while the control pot was left unsprayed. Atomizer sprayers were washed with sterile distilled water after being rinsed with 95% ethanol (Hans et al 2003). The plants were covered with polythene bags for 4h to enhance disease development. Emerging symptoms on the inoculated plants were observed and scored according to Manandhar et al (2016) and compared with symptoms described on the naturally infected plants from the field. Periodical observations were made for the development of symptoms on the leaves from 7 days after inoculation. The virulence nature of twenty-two *M. oryzae* isolates was determined based on rice seedling foliage symptoms which subsequently led to disease incidence and severity of the blast as previously stated. The fungi were re-isolated from the artificially inoculated rice seedling leaves showing typical *M. oryzae* symptoms and the culture obtained was compared with the original culture.

Statistical Analysis of Data

The collected data were subjected to ANOVA using Minitab software version 17, and the significant means were separated using the Tukey test at P 0.05. $p<0.05$.

RESULTS

Survey

The survey results revealed that farmers in the study areas were mainly cultivating FARO-43, FARO-44, FARO-45, FARO-55, Iron rice and Local foreign rice varieties. The size of each farm varied from farm to farm and ranged from half a hectare to five hectares per farm in each local government area. The rice was planted as a mono-crop and most of the farmers did not recognize blast symptoms as a form of disease. Therefore, no disease management practices except weeding and fertilizer applications were applied. The major means of land preparation by the farmers was manual with the use of herbicides. Farmers sourced their seeds from the Agricultural Development Project (ADP) within each local government area, local markets, friends and relatives, previous season's harvest and state government agencies as well as from their various local governments. No disease control measures were taken by the farmers.

Prevalence, Incidence, Severity and Percentage Damage Index of Rice Leaf Blast Disease in the Major Rice Producing Area of Ebonyi State

Table 2 shows the prevalence, incidence, severity and percentage damage index of rice leaf blast disease in the major rice producing areas of Ebonyi State. The rice blast disease was observed on all the rice inspected at variable disease levels. The incidence of rice blast in the nine different local government area varied from 18.24-53.67%. The highest mean incidence of rice blast was recorded in Ishielu (53.67%) followed by Onicha (48.62%), while the least was recorded in Ezza South (18.24%). The overall mean incidence for the nine local government areas in Ebonyi state was 31.26%. The highest severity was recorded in Ishielu with a range of 1.81-3.14 and mean severity values of 2.50. This was followed by Onicha, with a range of 1.53-2.12 and mean severity of 1.80 while the least severity was recorded in Abakaliki with a range of 1.10-1.50 and mean severity value of 1.30. The overall mean severity for the nine local governments in Ebonyi State, Nigeria was 1.68.

Rice leaf blast percentage damage index also showed a similar pattern as that of incidence and severity within the nine local governments. The highest damage index was recorded in Ishielu with range of 5.10-70.92% and mean damage index values of 38.02%. This was followed by Onicha, with a range of 10.71-29.72% and mean damage index of 20.23%. While the least damage index was recorded in Ezza South with a range of 5.16-8.02% and mean damage index of 6.59%. The overall mean damage index for the nine local governments in Ebonyi state was 14.49%.

Disease Incidence, Severity and Damage Index Across the Locations

The study revealed similar symptom types occurred on all farms surveyed although leaf blast disease incidence varied. The rice blast disease severity at a variable level was observed on all the rice cultivated inspected fields across the locations (Table 3). The field in Nkalagu within Ishielu local government showed a moderately susceptible cultivar while other surveyed locations within the state showed either resistant or moderately resistant cultivars. The results of the assessment revealed that incidence, severity and damage index of the disease varied from location to locations across the altitude and the entire nine local government areas surveyed. Nkalagu had the highest (90.01%) disease incidence, followed by Ogobui (56.07%) while Achoinoidigu had the least (15.71%) disease incidence. The disease severity varied from Umuta to Nkalagu with the value of 1.32-3.14 respectively (Table 2). Similarly, Nkalagu had the highest (70.93%) percentage damage index, followed by Ogobui (29.72%) while Edema had the least (5.07%) percentage damage index.

Table 2. Prevalence, incidence, severity and damage index of blast disease of rice in the major rice producing area of Ebonyi State

S/N	LGA	No. of fields inspected	Infected fields	Prevalence (%)	Incidence (%)		Severity		Damage Index (%)	
					Range	Mean	Range	Mean	Range	Mean
1	Ivo	4	4	100	19.17-46.67	32.92	1.40-1.99	1.70	8.13-26.35	17.24
2	Ishielu	3	3	100	17.33-90.00	53.67	1.80-3.14	2.50	5.10-70.93	38.02
3	Afikpo South	5	5	100	26.93-31.54	29.24	1.44-1.72	1.60	10.20-11.02	10.61
4	Afikpo North	2	2	100	27.14-36.92	32.03	1.55-1.76	1.70	9.34-12.40	10.87
5	Onicha	2	2	100	41.23-56.00	48.62	1.53-2.12	1.80	10.71-29.72	20.23
6	Ikwo	3	3	100	21.54-22.31	21.93	1.30-1.45	1.40	6.87-8.06	7.47
7	Ezza Sourth	2	2	100	15.71-20.77	18.24	1.38-1.61	1.50	5.16-8.02	6.59
8	Abakaliki	3	3	100	16.15-23.85	20.00	1.10-1.50	1.30	5.35-7.98	6.67
9	Ohaukwu	2	2	100	16.92-32.45	24.69	1.46-1.68	1.60	6.06-19.3	12.68
Total		26	26	900		281.34		15.10		130.38
Mean				100		31.26		1.68		14.49

Table 3. Disease incidence, severity and damage index across the locations

LGA	Locations	Longitude	Latitude	Altitude (m)	DI (%)	DS	PDI (%)
Ishielu	Edema	7.7499	6.4660	59	17.33q	1.81b-d	5.07r
Ivo	Ngwogwo	7.5679	5.9603	65	30.04g	1.40g	10.74i
Ivo	Ike	7.6340	5.8334	52	46.65c	1.98bc	26.34c
Ivo	Amagu	7.5832	5.8662	62	25.71k	1.76c-e	11.13g
Ezza south	Amorie	8.1339	6.1496	73	20.76o	1.61d-g	8.01l
Ishielu	Nkalagu	8.1336	6.1163	56	90.01a	3.14a	70.92a
Ishielu	Onuankwo	7.7666	6.4827	49	40.66d	1.97bc	18.18d
Ikwo	Eka	7.7670	6.5152	57	22.31m	1.45e-g	8.05kl
Onicha	Ogobui	7.9333	5.9828	27	56.07b	2.11b	29.71b
Ivo	Akaeze	7.6663	5.9163	51	18.84p	1.62d-g	8.12k
Afikpo south	Osso-Eda	7.7972	5.8645	44	26.91j	1.71c-f	11.01h
Abakaliki	EBSU	8.1195	6.2443	83	16.14s	1.36g	5.56o
Abakaliki	Mbabo	8.1663	6.1669	79	18.45p	1.37g	5.34p
Ikwo	Umuta	8.0660	6.0663	37	21.53n	1.32g	6.86m
Abakaliki	Barrack	8.1610	6.2218	77	23.84l	1.52d-g	7.98l
Ezza south	Achoinoidigu	8.1222	6.1361	72	15.71t	1.38g	5.16q
Ohaukwu	Ngbo	7.9501	6.4665	54	16.93r	1.46e-g	6.05n
Afikpo north	Amangwu	7.8866	5.8338	34	36.91e	1.55d-g	12.40e
Afikpo north	Amaser	7.8835	5.9167	45	31.54f	1.48e-g	11.18g
Afikpo north	Ozaraukwu	7.9165	5.9156	36	29.21h	1.43fg	11.96f
Afikpo south	Igboro	7.7665	5.8835	46	27.13j	1.55d-g	10.07j
Afikpo south	Ndigbe	7.7503	5.9001	40	27.86i	1.57d-g	11.11g
Means					30.02	1.6606	13.68
SE					2.05	0.0490	1.73
CV (%)					55.44	23.96	102.75

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. LGA=Local government area, DI=Disease incidence, DS=Disease severity and PDI=Percentage damage index

Cultural and Morphological Characteristics of Magnaporthe oryzae (Mo) Isolates from Nine Local Government of Ebonyi State, Nigeria

The cultural and morphological study revealed different variations among twenty-two isolates of *Magnaporthe oryzae* (*Mo*) observed on the culture plates. All twenty-two *Mo* isolates grown on potato dextrose agar showed varying colony colour, margin, pigmentation, surface texture, shapes, septates and cells. Isolates of *Mo* varied in colony colour from dark grey, light grey to grey. The entire margin and colony texture for the twenty-two isolates were irregular and cottony respectively. Pigmentation varied from black, dark brown to light brown while mycelial growth was from poor to moderate growth. The shape of isolates was pyriform; with isolates *Mo*-01, *Mo*-04, *Mo*-06, *Mo*-08, *Mo*-10, *Mo*-14, *Mo*-16, *Mo*-19 and *Mo*-20 having 1-septate with two cells while isolates *Mo*-02, *Mo*-03, *Mo*-05, *Mo*-07, *Mo*-09 *Mo*-11, *Mo*-12, *Mo*-13, *Mo*-15, *Mo*-17, *Mo*-18, *Mo*-21 and *Mo*-22 had 2-septates with three cells (Table 4).

Table 4. Cultural and morphological characteristics of *Magnaporthe oryzae* (*Mo*) isolates from nine Local Governments of Ebonyi State, Nigeria

Isolates	Colony colour	Margin	Pigmentation	Colony texture	Colony diameter (mm)	Mycelial growth	Shapes	Septates	Cells
Mo-01	Light grey	Irregular	Dark brown	Cottony	33.51	Poor	Pyriform	One	2-cells
Mo-02	Grey	Irregular	Black	Cottony	40.11	Poor	Pyriform	Two	3-cells
Mo-03	Light grey	Irregular	Light brown	Cottony	64.50	Moderate	Pyriform	Two	3-cells
Mo-04	Light grey	Irregular	Light brown	Cottony	41.00	Poor	Pyriform	One	2-cells
Mo-05	Grey	Irregular	Brown	Cottony	46.90	Poor	Pyriform	Two	3-cells
Mo-06	Light grey	Irregular	Dark brown	Cottony	74.25	Moderate	Pyriform	One	2-cells
Mo-07	Dark grey	Irregular	Dark brown	Cottony	42.00	Poor	Pyriform	Two	3-cells
Mo-08	Light grey	Irregular	Light brown	Cottony	28.67	Poor	Pyriform	One	2-cells
Mo-09	Grey	Irregular	Dark brown	Cottony	39.67	Poor	Pyriform	Two	3-cells
Mo-10	Grey	Irregular	Black	Cottony	32.00	Poor	Pyriform	One	2-cells
Mo-11	Light grey	Irregular	Light brown	Cottony	37.61	Moderate	Pyriform	Two	3-cells
Mo-12	Grey	Irregular	Black	Cottony	23.50	Poor	Pyriform	Two	3-cells
Mo-13	Dark grey	Irregular	Dark brown	Cottony	42.70	Poor	Pyriform	Two	3-cells
Mo-14	Grey	Irregular	Dark brown	Cottony	57.00	Moderate	Pyriform	One	2-cells
Mo-15	Grey	Irregular	Black	Cottony	38.92	Poor	Pyriform	Two	3-cells
Mo-16	Light grey	Irregular	Light brown	Cottony	58.50	Moderate	Pyriform	One	2-cells
Mo-17	Dark grey	Irregular	Black	Cottony	51.25	Moderate	Pyriform	Two	3-cells
Mo-18	Grey	Irregular	Dark brown	Cottony	34.01	Poor	Pyriform	Two	3-cells
Mo-19	Dark grey	Irregular	Black	Cottony	45.60	Poor	Pyriform	One	2-cells
Mo-20	Grey	Irregular	Dark brown	Cottony	59.50	Moderate	Pyriform	One	2-cells
Mo-21	Light grey	Irregular	Light brown	Cottony	53.21	Moderate	Pyriform	Two	3-cells
Mo-22	Grey	Irregular	Brown	Cottony	43.67	Poor	Pyriform	Two	3-cells

*Colony diameter scale: Good=75-90mm, Moderate=56-75mm, Low/poor=<56mm (Mebratu et al 2015).
**Mo-Magnaporthe oryzae*

Disease Incidence, Severity and Corresponding Reaction for Rice Cultivar Inoculated with different Isolates of Magnaporthe oryzae

Pathogenicity test results revealed that the disease symptoms and development of twenty-two *Mo* isolates on susceptible local cultivar (local foreign) were the same as that in the field showing disease incidence and severity (Table 5). Diamond and spindle shaped lesions with grey center and dark brown to necrotic margins were observed on all of the rice seedlings after the 7th day of inoculation. Seven days after inoculation, disease severity range (1.53-2.71) was recorded whereas five *M. oryzae* isolates (*Mo*-03, *Mo*-05, *Mo*-06, *Mo*-10 and *Mo*-17) were virulent with 2.51-2.71 disease severity while the others were less virulent having between 1.53-2.37 disease severity. There were no disease symptoms on the control plants. The disease resistant was relatively low in the screen house when compared with the disease observed on the field during survey. All the isolates were the causative agents for blast disease of rice as confirmed by the pathogenicity test carried out on *M. oryzae* isolates.

Table 5. Disease incidence, severity and corresponding reaction for rice cultivar inoculated with different Isolates of *Magnaporthe oryzae*

Isolates	Disease incidence (%)	Disease severity
Mo-01	17.67l	2.01gh
Mo-02	21.31h	2.31e
Mo-03	33.45b	2.53a-c
Mo-04	16.54m	1.53l
Mo-05	29.67d	2.51b-d
Mo-06	30.11c	2.71a
Mo-07	15.41n	1.60j-l
Mo-08	19.22j	1.82i
Mo-09	24.31f	2.34de
Mo-10	29.67d	2.60ab
Mo-11	7.67t	1.73ij
Mo-12	19.06k	2.37c-e
Mo-13	22.22g	1.87hi
Mo-14	13.37p	2.30e
Mo-15	24.56e	2.33de
Mo-16	9.96r	2.00gh
Mo-17	34.63a	2.70a
Mo-18	10.53q	2.10fg
Mo-19	21.22i	2.20ef
Mo-20	9.35s	1.71i-k
Mo-21	7.60t	1.54kl
Mo-22	14.71o	2.10fg
Control	0.00u	0.00m
Means	12.000	2.0000
SE	0.804	0.0990
CV (%)	55.68	41.12

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. *Mo- Magnaporthe oryzae*; Severity scale: 01.49- Less Virulent (LV) and 2.50–5.00= Virulent (V). Data was taken 7 days after inoculation.

DISCUSSION

The survey and study conducted on twenty-six (26) rice farms within nine local government areas in Ebonyi State revealed the presence of blast symptoms on the leaves of rice plants which indicates that none of the varieties grown in the rice farms surveyed were either totally immune or highly resistant to the fungus. Similar blast symptoms were also reported on five rice farms surveyed in the Aninri local government area of Enugu State (Yekini et al 2019). The farmers grow mostly FARO-43, FARO-44, FARO-45, FARO-55, which are improved varieties while some farmers preferred the local cultivars: Iron rice or Local foreign (Long grains) that displayed moderate to high blast symptoms. According to Mebratu et al (2015), different cultivars in Ethiopia also showed variations in incidence and severity of blast disease. The presence of blast symptoms on the surveyed farms could be attributed to continuous cultivation of a single crop (monocrop) over a period of time which favors blast development and no form of disease management was practiced by the farmers. The assessment of blast disease revealed that the disease incidence, severity, and damage index varied from field to field and local government area to area due to differences in geographical and environmental conditions. Ishielu and Onicha had the highest mean incidence, severity, and damage index of the disease, which could be attributed to the presence of shade trees, shrubs, and the lower altitude of the rice fields. These conditions reduce light intensity and increase humidity that favors the distribution of the pathogen. On the contrary, the sampling sites in Ohaukwu, Abakaliki and Ezza South were characterized by higher altitude that did not favor the spread of the pathogen. Similarly, Srivastava and Tewari (2002) indicated that altitude influences the incidence and severity of the disease. All twenty-six farms tested within the nine local governments area had 100% blast disease prevalence. This indicates that the rice blast disease had spread throughout the selected areas. According to WARDA (2004), the incidence and severity of rice blast varies across different locations and cultivars in different years, and blast is prevalent and severe during the vegetative stages of rice plants. The assessment results in this study revealed that the disease's incidence, severity, and damage index varied from mild to moderately severe depending on the area. This also supports the findings of Nutsugah and Twumasi (2001) that incidence and severity of blast across rice growing areas in Ghana, where the disease is a serious threat to production varies from low to high blast incidence. The phenotypic characteristics of the twenty-two *M. oryzae* (*Mo)* isolates varied in colony color from dark grey, light grey to grey. These results also agreed with Gopal et al (2012) that finger millet blast isolates showed a colony color of grey to black on oat meal agar medium. The margin and colony texture for the twenty-two isolates were irregular and cottony respectively. Similarly, Meena (2005) also reported that the colony characteristics of *Mo* isolates on oat meal agar media showed greyish black and irregular colony margins, and that some isolates on potato dextrose agar medium also were irregular on the colony margins. According to Bandyopadhyay et al (2009), the oat meal agar media produced off-white, good, and regular mycelial growth. According to Vanaraj (2013), the colony color of *Mo* isolates appeared grey on potato dextrose agar medium. The pigmentation varied from black, dark brown to light brown while mycelial growth was from poor to moderate growth. The findings of this study corroborate the report of Mew and Misra (1994) that colonies of *Mo* isolate on potato dextrose agar medium had blackish pigmentation. Mew and Gonzales (2002) discovered that *M. oryzae* pathogen colonies on potato dextrose agar medium grew slowly and were black on the reverse side of the agar plates. Pathogenicity tests of the twenty-two test *M. oryzae* isolates on the susceptible local cultivar (local foreign) revealed that disease symptoms and the development of diamond and spindle shaped lesions with grey centers and dark brown to necrotic margins on all of the rice seedlings occurred on the 7th day after inoculation. The result justifies the findings of Getachew et al (2014) that similar typical symptoms of dark brown lesions developed on finger millet leaves 7 days after inoculation. The relatively low disease incidence observed in the screen house when compared with the disease on the field during the survey could be as a result of the more controlled environment. The study by Mebratu et al (2015) further indicated that six *Mo* isolates were able to cause disease development on a susceptible local cultivar after inoculation. The re-isolated *M. oryzae* isolates from inoculated seedlings exactly matched with the characteristics of the original isolates. This indicted that all isolates were the causative agents for the observed blast disease on the rice. This study also revealed a variation in aggressiveness and virulence characteristics of these isolates that had infected the local cultivar. Similarly, host range, and variations in morphology and pathogenicity of the genus *Magnaporthe* on finger millet were reported by Adipala (1989).

CONCLUSION

Rice leaf blast disease was found to prevail in most rice growing local government areas in Ebonyi State, Nigeria. However, disease incidence, severity and damage index varied significantly across the surveyed local government areas and altitudes. Over all, Ishielu had the highest blast incidence (53.67%), followed by Onicha (48.62%) while Ezza South recorded the least (18.24%) within the state. The morphological study conducted on the twenty-two *Mo* isolates grown on potato dextrose agar showed varying colony color, margin, pigmentation, surface texture, shapes, septates and cells. The pathogenicity conducted confirmed that the *M. oryzae* organisms were the causal agents of blast disease of rice in Ebonyi State, Nigeria. All the isolates of *M. oryzae* were able to infect all the rice cultivars and out of the twenty-two isolates, *Mo*-03, *Mo*-05, *Mo*-06, *Mo*-10 and *Mo*-17 were the most virulent isolates. These most virulent isolates showed the highest disease severity on a local rice variety during the in vivo test under screen-house condition.

RECOMMENDATIONS

Assessment of the incidence and severity of plant disease is important to determine the extent of its distribution and the status of the disease throughout the state in order to prioritize a control strategy. Therefore, the present study recommends that the governments should set up a modality to educate and train rice farmers on disease symptoms and the possible eco-friendly means of control in order to increase rice production both in the study areas and in Nigeria at large. Further studies could also be conducted on prevalence and damage index of rice blast on lowland rice cultivars in other rice growing zones of the country.

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YBA and OOC collected the data, processed the data, perform literature searches and wrote the draft the manuscript. Author ACG prepared the final manuscript. The final manuscript was read by the three authors.

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AVAILABILITY OF DATA AND MATERIALS

The data that support these findings are available from the corresponding authors upon reasonable request.

ETHICAL CONSIDERATIONS

This article did not include human subjects or animal studies.

COMPETING INTEREST

The authors declare that there are no conflict of interest.

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