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EPIDEMIOLOGICAL SURVEY OF RICE VIRAL DISEASES AND PREVALENCE OF RYMV IN KWARA STATE, NIGERIA

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Abstract: Rice yellow mottle virus (RYMV) is a significant threat to rice production in sub-Saharan Africa, leading to yield losses and economic setbacks for farmers. A comprehensive field survey was conducted in September 2021 across 48 rice fields in Kwara State, Nigeria, to assess the incidence and severity of rice virus disease. A total of 600 leaf samples were collected from rice plants showing virus-like symptoms across four local government areas (LGAs): Ifelodun, Edu, Patigi, Ilorin South. The collected samples underwent serological and molecular analyses using Antigen-Coated Plate Enzyme-Linked Immunosorbent Assay (ACP-ELISA). Statistical analysis was carried out using the R programming language. Among the surveyed LGAs, Ifelodun recorded the highest disease incidence at 48.25%, whereas Edu had the lowest at 37.25%. The highest disease severity was observed in Ifelodun (62.25%), while Patigi recorded the lowest severity (50%). Despite the widespread occurrence of virus-like symptoms, laboratory tests confirmed the presence of RYMV only in samples collected from Belle town, Edu Local Government, highlighting the need for further epidemiological studies to determine the full extent of the virus distribution in Kwara State.

This study represents the first documented research on RYMV in Kwara State, Nigeria, and underscores the importance of continued surveillance to prevent potential outbreaks. Future research should focus on expanding the survey to other rice-growing regions in Nigeria to assess the prevalence and genetic diversity of RYMV strains.

Keywords: RYMV, Rice, ELISA, Virus symptom, Survey

1.0 INTRODUCTION

Rice (Oryza sativa) is a staple crop globally, feeding about 40% of the population (Ortiz, 2011). In West Africa, rice consumption has increased, making up over 25% of total cereal intake (Africa Focus, 2004). In Nigeria, per capita consumption is 32 kg, with demand growing by 4.7% annually, reaching 6.4 million tons in 2017 (PwC, 2018). Despite efforts to boost production, challenges such as pests and diseases continue to limit yields (Oladapo et al., 2020). Among these challenges, Rice yellow mottle virus (RYMV) is a major threat. RYMV, a singlestranded RNA virus of the genus Sobemovirus, causes yellow mottling, stunted growth, reduced tillering,

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and poor panicle exertion (Gnanamanickam, 2009). First reported in Nigeria in 1976 (Raymundo, et al., 1979), RYMV spreads through mechanical means, including farm tools, plant-to-plant contact, wind, irrigation water, and grazing animals (Abo et al., 1998; Kouassi et al., 2005). Some rice varieties, such as 'Moroberekan' and 'Bouake 189,' are highly susceptible, especially in early growth stages (Onwughalu et al., 2010).

Kwara State, located in North Central Nigeria, has a climate suitable for rice farming, with rainfall ranging from 990.3 mm to 1318 mm and humidity levels varying from 35% to 88%

(Aliyu et al., 2020). However, the presence of RYMV in this region threatens rice production, leading to potential economic losses and food insecurity. Understanding the distribution and severity of RYMV is crucial for developing effective management strategies. Since plant viruses lack chemical control, focusing on vector management and disease monitoring is essential (Alaka and Balogun, 2023).

This study aims to assess the incidence, severity, and distribution of RYMV in Kwara State. It will also involve molecular characterization of the virus to understand its spread and impact. The findings will provide crucial insights for developing effective control measures to improve rice production in Nigeria.

2.0 MATERIALS AND METHODS 2.1 Field survey

A field survey was conducted in September 2021 across four major rice-producing local government areas (LGAs) in Kwara State, Nigeria: Edu, Ifelodun, Ilorin South, and Patigi (Table 1). Farms selected for the survey had a minimum land area of 10,000 m² (Aliyu et al., 2020). Virus incidence was estimated by visually inspecting 100 randomly selected rice plants per field for symptoms such as mosaic, chlorosis, mottling, stunting, necrosis, leaf deformation, and bunching (James, 1974). Sampling was performed using a "W"-shaped pattern, with 10 plants per side, spaced 0.5 m apart (Olawale et al., 2015).

A total of 30 farmers were interviewed using a structured questionnaire designed to capture biodata and farm management practices. Socio-economic characteristics assessed included the farmer's age, gender, occupation, educational background, rice varieties grown, seed sources, years of experience in rice farming, farm size, presence of pests and diseases, preferred rice variety, sources of labor, and production constraints. An interpreter assisted in translating questions into local languages when needed. Field surveys covered 20 farms in Edu LGA, 16 in Ifelodun LGA, 8 in Patigi LGA, and 4 in Ilorin South LGA. For each surveyed farm, data were recorded on rice variety, crop age, farm size, and field coordinates (latitude, longitude, and altitude) using a Global Positioning System (GPS) device.

Leaf samples were collected from rice plants displaying virus-like symptoms. Samples were taken from the top, middle, and lower portions of each plant to ensure comprehensive representation. A total of 600 leaf samples were collected, tagged, and stored in sealed containers with ice packs to maintain sample integrity before laboratory testing for virus detection.

Table1: Surveyed area field locations, latitude, longitude and elevation in Kwara State, Nigeria.

State	***LGAs***	***Town/Village***	***Latitude N***	***Longitude E***	***Elevation***

Kwara	Edu	Bacita Belle	9^{0}5’46.00”	4^{0}51’34.00”	90m
			9^{0}5’48.00”	4^{0}56’19.00”	80m
	Ifelodun	Agborogun	9^{0}2’10.00”	4^{0}52’5.00”	100m
		Osin	9^{0}2’5.00”	4^{0}52’34.00”	100m
	Ilorin	Sango	8^{0}31’20.00”	4^{0}34’26.00”	270m
	South	Akerebiata	8^{0}44’22.00”	5^{0}45’12.00”	120m
	Pategi	Pategi			

Diseases Incidence

The incidence of rice virus disease in the surveyed fields was determined by counting the number of plants exhibiting virus-like symptoms, following the method described by Odedara et al. (2008). Each observed symptom was recorded and expressed as a percentage of the total number of plants assessed (100 plants per field). The disease incidence was calculated using the formula:

Incidence (%) = (No. of Symptomatic Plants/ Total No. of Plant Samples) x 100

Diseases Severity

Disease severity was assessed visually using a modified version of the Standard Evaluation System (SES) developed by the International Rice Research Institute (IRRI, 2002) and adapted by Oladapo *et al.* (2020). Severity was rated on a scale of 1 to 9 based on symptom intensity: 1 – No symptoms observed, 3 – Green leaves with sparse stripes, covering less than 5% of the leaf area, 5 – Green or pale green leaves with crinkling, affecting, 6 – 25% of the leaf area, 7 – Pale yellow leaves with 26–75% of the leaf area showing symptoms, 9 – Yellow or orange leaves with more than 75% of the leaf area affected, with some plants showing signs of wilting or death. The disease severity was calculated using the formula:

Severity (%) =

(Sum of all disease ratings / No. of Plant Assessed) x Maximum Score (100)

Serological Detection

Serological detection of Rice Yellow Mottle Virus (RYMV) was carried out using the AntigenCoated Plate Enzyme-Linked Immunosorbent Assay (ACP-ELISA) protocol, following the method described by Afolabi et al. (2009). A total of 400 leaf samples were collected from the field and tested for RYMV.

For sample preparation, 0.1 g of each leaf sample was weighed and ground in a sterile mortar and pestle with 1 mL of extraction buffer. The extraction buffer contained 8 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.15 g dibasic sodium phosphate, 0.2 g potassium chloride, and 0.2 g sodium azide, all dissolved in 900 mL of distilled water, adjusted to pH 7.4 with HCl, and made up to 1 L. Additionally, 0.5 mL Tween-20 per liter and 2% polyvinylpyrrolidone (PVP) were added.

The ELISA procedure began with plate coating. A 96-well polystyrene microtiter plate was labeled and coated with 100 µL of antigen (1:10 dilution in coating buffer with 1% Dieca). The plate was covered and incubated at 37°C for 1 hour. After incubation, the plate was washed three times with PBS-Tween at 3-minute intervals and tap-dried. Blocking was performed using 200 µL per well of 3% dried skimmed milk in PBS-Tween to trap the

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virus, followed by incubation at 37°C for 30 minutes to allow binding. After incubation, the plate was washed again as described.

For antibody incubation, 100 µL of sap from healthy cowpea leaf samples mixed with a pool of 21 different vegetable and cowpea antibodies was added to each well, followed by incubation at 37°C for 2 hours and another round of washing. Enzyme and substrate addition followed, where 100 µL of goat anti-rabbit antibody conjugated with enzyme (diluted 1:2000) was added to each well. The plate was incubated, washed, and tap-dried before 100 µL of pnitrophenyl phosphate (pNPP) substrate (3 mg in 30 mL substrate buffer) was added to each well and left for color development.

The optical density (OD) of each well was measured after 1 hour and again after overnight incubation using an ELISA reader (Diagnostic and Medical Solutions Micro Plate Reader - ELISA Plate Analyzer) at a wavelength of 405 nm.

Statistical Analysis

Statistical data analysis was performed using R Software (PC-Windows 11 version). Symptom severity scores were analysed using analysis of variance (ANOVA).

3.0 RESULTS AND DISCUSSION

Assessment of Rice Yellow Mottle Virus Disease Phenotype

The most common symptoms of Rice Yellow Mottle Virus (RYMV) observed across all cultivated rice varieties in Kwara State included mottling/yellowing, chlorosis, leaf curling, and deformation (Fig. 1). Additional symptoms such as spikelet distortion, necrosis, sterility of flowers, stunted growth, and leaf distortion were also recorded. In farmers' fields, mottling with irregular patches of distinct light and dark areas was frequently observed.

During the field survey, symptoms induced by RYMV, including necrotic lesions, leaf yellowing, and brown blotches (brown streaking), were commonly encountered at varying levels of incidence. Among the affected plants, mottling/yellowing was the most prevalent symptom (48%), followed by leaf curling (20%), irregular patches (13%), leaf deformation (10%), and leaf necrosis (9%) (Table 2).

Table 2: Assessment of RYMVD symptom phenotypes on rice plants in Kwara State

State	***LGAs***	***Town/Village***	***Mottle/ yellowing***	***Leaf Necrosis***	***Leaf curling***	***Leaf deformation***	***Irregular patches***
Kwara	Edu	Bacita Belle	3	0	4	0	3
			30	0	7	1	2
	Ifelodun	Agborogun	5	0	3	3	2
		Osin	5	0	2	3	2
	Ilorin	Sango	2	5	3	0	2
	South	Akerebiata	3	4	1	3	2
	Patigi	Patigi					

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%Total	**48**	**9**	**20**	**10**	**13**

Figure 1: Disease symptoms observed in a rice farmer's field in Belle, Edu Local Government, Kwara State. The rice is at the flowering stage, showing; (a) chlorosis and mottle yellowing (b) necrosis.

Distribution of Incidence and Severity of Virus-Like Symptoms in Rice Observed During the Field Survey

Table 3 presents the distribution of incidence and severity of virus-like symptoms observed during the field survey conducted in Kwara State, Nigeria. In Edu Local Government, two locations were surveyed. Belle recorded the highest disease incidence at 41.25%, while Bacita had the lowest at 37.25%. In Ifelodun Local Government, Osin had the highest incidence at 48.25%, whereas Agborogun recorded 42.25%. Surveys were also conducted in Ilorin South and Patigi Local Government, where the recorded incidences were 47.87% and 38.13%, respectively.

In terms of disease severity, Bacita in Edu Local Government had the highest percentage at 55%, while Belle recorded 52.5%, though the difference was not statistically significant. In

Ifelodun Local Government, Osin had the highest severity at 62.5%, while Agborogun recorded 57.5%. In Patigi Local Government, Patigi recorded a disease severity of 50%, while in Ilorin South Local Government, Sango Akerebiata had the highest recorded severity at 65%.

Table 3: Distribution of incidence and severity of virus-like symptoms of rice observed during the field survey in the Kwara State, Nigeria

State	*LGAs*	*Village/Town*	*No. of Farms*	*% Incidence*	*% Severity*
Kwara	Edu	Bacita	10	37.25	55.00
		Belle	10	41.25	52.50
	Ifelodun	Agborogun	8	42.25	57.50
		Osin	8	48.25	62.50

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	Ilorin South	Sango/ akerebiata	4	47.87	65.00
	Patigi	Patigi	8	38.13	50.00
Mean±SE				**42.5±2.51**	**57.08±3.09**

Effect of Rice Variety on Incidence and Severity of Rice Yellow Mottle Virus

The results indicated that the rice variety Ebagbanji had the highest mean disease incidence at 63.89%, while Aroso recorded the lowest at 12% (Table 4). Similarly, disease severity was highest in Ebagbanji, with a mean percentage severity of 86.67%, whereas Aroso had the lowest severity at 24%.

Table 4: Effect of Rice Variety on Incidence and Severity of Rice Yellow Mottle Virus

S/N	*Varieties*	*% Incidence*	*% Severity*
1.	Alhajibaba	15.67 12.00 67.67	33.33 24.00 86.67
2.	Aroso	59.00	70.00
3.	Ebagbanji		
4.	Egwazanpa		
5.	Faro 44	47.62	72.65
6.	Faro 46	65.00	60.00
7.	Olam	36.1	52.00 53.33
8.	Tiakoshi	42.33	70.00
9.	Unknown	51.00	
10.	Zariaji	23.5	40.00
	SE±	**2.52**	**3.09**

Detection of RYMV by ELISA

The rice leaf samples collected from the field were tested for Rice Yellow Mottle Virus (RYMV) using ELISA. The results revealed that 2 out of 48 samples tested positive for RYMV (Figure 2). The positive samples were collected from Belle in Edu Local Government, confirming the presence of RYMV in the area. The absorbance values at a spectrophotometric wavelength of 405 nm were up to 1.5 times those of the healthy controls after both the onehour and overnight readings.

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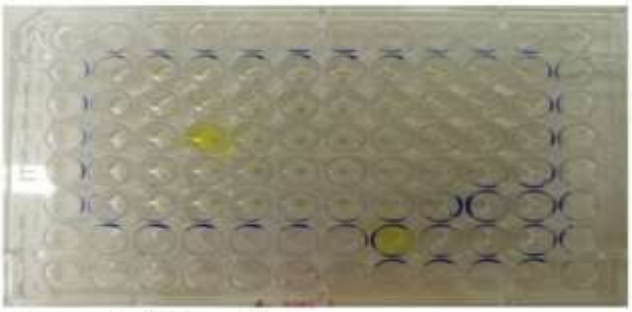
1Hour Reading



Overnight Reading

- To get the actual Titer value, the Overnight Healthy and Buffer controls values are use to find the standard deviation of the two values.
- The mean of the Overnight Healthy and Buffer control value were calculated.
- The standard deviation value calculated was added to the mean and then multiply by 2, the value will be used to compare the titer value of the samples.
- If the value calculated is greater than the titer value of the sample, that means the sample is negative.
- If the titer value of the sample is greater than the value calculated, that means the sample is positive.
- The yellow colour shows the sample is positive, while the other colour shows negative.

Sample ID	1 Hour	Overnight	Results	
1	0.075	0.121	-	
2	0.08	0.138	-	
3	0.077	0.132	-	
4	0.074	0.124	-	
5	0.075	0.129	-	
6	0.076	0.131	-	
7	0.079	0.151	-	
8	0.081	0.161	-	
9	0.083	0.17	-	
10	0.078	0.13	-	
11	0.08	0.159	-	
12	0.079	0.16	-	
13	0.082	0.186	-	
14	0.081	0.173	-	
15	0.086	0.199	-	
16	0.084	0.19	-	
17	0.09	0.199	-	
18	0.082	0.17	-	
19	0.082	0.169	-	
20	0.087	0.165	-	
21	0.076	0.15	-	
22	0.077	0.146	-	
23	2.091	4	+++	
24	0.078	0.15	-	
25	0.08	0.163	-	
26	0.078	0.153	-	
27	0.08	0.15	-	
28	0.08	0.152	-	
29	0.081	0.167	-	
30	0.078	0.153	-	
31	0.076	0.13	-	
32	0.074	0.132	-	
33	0.079	0.145	-	
34	0.078	0.153	-	
35	0.076	0.137	-	
36	0.082	0.175	-	
37	0.081	0.162	-	
38	0.077	0.136	-	
39	0.08	0.169	-	
40	0.078	0.129	-	
41	0.067	0.103	-	
42	0.077	0.15	-	
43	0.079	0.166	-	
44	0.075	0.129	-	
45	0.087	0.162	-	
46	0.079	0.156	-	
47	0.077	0.128	-	
48	0.076	0.139	-	
Disease	0.836	3.997	+++	0.000707
Healthy	0.075	0.122	-	0.1225
Buffer	0.075	0.123	-	0.246414
Buffer	0.075	0.121	-	

Figure 2. ACP-ELISA plate of results and table for RYMV

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Discussion

This study provides insights into the incidence and severity of *Rice Yellow Mottle Virus* (RYMV) infection in rice fields across Kwara State, Nigeria. A total of 600 symptomatic and asymptomatic leaf samples were collected from four local government areas and tested for RYMV using (ACP-ELISA) techniques. The results revealed that only samples from Belle town, Edu Local Government tested positive for RYMV, as strong bands were observed in the reactions. This finding was recorded despite farmers reporting that they had planted seeds saved from previous seasons.

The negative results obtained in other areas may be influenced by environmental factors, particularly rainfall, which has been reported to affect virus distribution in different ecosystems (Shoyinka *et al.,* 1997). Rainfall could reduce the available inoculum, limiting the spread of the virus (Eric *et al.,* 2019). Additionally, the absence of RYMV in some locations may be attributed to a lack of infection in host plants or the absence of transmission by vectors, as reported by Mohammed *et al.* (2019).

Field observations confirmed that the viral symptoms recorded were consistent with the typical signs of RYMV, including mottling and yellowing of leaves. However, variations in symptom expression were noted across different rice cultivars and growing conditions. Some plants exhibited streaking, leaf whitening, and, in severe cases, necrosis. RYMV infection often results in stunting, reduced tillering, poor panicle exertion, and sterility, with early infections leading to plant death in susceptible cultivars (Bakker, 1974; Omiat *et al.,* 2023).

The detection of RYMV in rice plants at Belle town, Edu Local Government suggests that rice crops in the surveyed areas are susceptible to viral infection, which could potentially impact yield, though the effects may be subtle and go unnoticed. Onasanya *et al.* (2011) previously reported that RYMV incidence in farmers' fields ranged between 15–70%, which aligns with the findings of this study. Disease incidence in Bacita, Edu Local Government, was 37.25%, while the highest incidence was recorded in Osin, Ifelodun Local Government, at 48.25%. These values are significantly high, indicating a persistent challenge that requires urgent attention.

The high RYMV incidence may also be linked to the presence of wild plants and volunteer weeds surrounding rice farms, which serve as reservoirs for the virus and its vectors. Continued monitoring and management strategies are necessary to mitigate the spread and impact of RYMV on rice production in the region.

CONCLUSION

Training and retraining of farmers and stakeholders in rice production are essential to promote the adoption of proper sanitary measures during planting. Implementing these practices can help reduce the incidence of *Rice Yellow Mottle Virus* (RYMV). Although many farmers reported burning leftover rice plants before replanting, this method may not completely eliminate infected plants. Under favorable conditions, particularly during the rainy season, regrowth may occur, serving as a potential source of inoculum. Additionally, many rice fields were surrounded by fallow lands with overgrown weeds, which could harbor the virus and facilitate its transmission in subsequent seasons. It is crucial to educate farmers on the importance of field sanitation to minimize virus reservoirs.

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The use of hybrid rice varieties with resistance to RYMV is strongly recommended as a preventive strategy. Resistant cultivars can significantly reduce the risk of infection and enhance rice productivity in affected areas.

This study confirmed the occurrence of RYMV infection in Olam rice plants cultivated in Belle, Edu Local Government Area of Kwara State. This finding provides critical information for developing effective disease management strategies to control RYMV and prevent its spread in rice-growing regions.

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