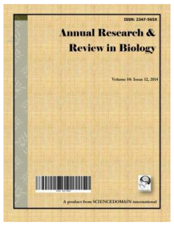

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Effect of *Rice yellow mottle virus*, *Sobemovirus* on the Contents of N P K Ca and Mg in Leaves of Infected Rice

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Authors' contributions

This work was carried out in collaboration between all authors. All authors fully contributed to the study. Author NBG conducted the study and analyzed the results in addition to the first draft that was subsequently approved by all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza* spp) is one of the most important crops produced in the world. Rice production is dampered by environmental and biological factors including mainly pathogens, such as *Rice yellow mottle virus (RYMV)*, which is the most rice devastating viral disease in Africa: whereas, These factors can be overcome by using different methods such as mineral fertilization supplying. To identify nutrients for this constraint management, a study was carried out in controlled condition in a greenhouse situated at Felix Houphouët-Boigny University station at Bingerville. The rice variety named Bouake 189 was sown in pots using local soil as substrate. Fourteen days old seedlings were inoculated mechanically with isolate of RYMV. Fertilizers N, P, K 12, 24, 18 (200 kg/ha) and

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100 kg/ha of urea (46% N) were applied respectively. The concentrations of N, P, K, Ca and Mg as well as protein content in leaves were determined likely. Chlorophyll measurement was obtained using Spad-505. The severity and the (AUSPC) of the RYMV were evaluated. The presence of the RYMV in the infected leaves was confirmed by serological analysis. The averages of the various parameters were compared by ANOVA 2 with software STATISTICA version 7.1. Results showed significant difference ($p < 0.001$) between the seedlings according to infection levels and highest concentrations of P (0.40%), K (1.10%), Ca (1.49%), Mg (0.39%) and chlorophyll (37.37) were recorded in the leaves of non-infected seedlings whereas those related to infected seedlings were of 0.39%; 0.75%; 0.67%; 0.24% and 21.23 respectively. This was contrasting with N and protein contents recording 2.41% and 15.04 for the inoculated plants and 1.75% and 10.95 for the healthy plants respectively. The average severity and viral load of *Rice yellow mottle* disease were reduced respectively from 6.22 to 3.88 and from 2.132 to 1.577 under the action of the mineral fertilization (NPK), hence improving rice growth and yield to be targeted.

Keywords: RYMV; N; P; K; Ca; Mg; proteins; fertilization.

ABBREVIATIONS

RYMV	:	*Rice Yellow Mottle Virus*
DAS-ELISA	:	*Double antibody sandwich enzyme-linked immunosorbent assay*
Das	:	*Day after sowing*
Dpi	:	*Day post inoculation*
Nt	:	*Total nitrogen*
P	:	*Phosphorus*
K	:	*Potassium*
Ca	:	*Calcium*
Mg	:	*Magnesium*
Nm	:	*10^{-9} meter*

1. INTRODUCTION

Rice (Oryza sp.) is the third cereal produced in the world. According to FAO statistical data in 2016, world production is estimated at about 741 million tons. This production is largely provided by the countries of Southeast Asia [1]. In Côte d'Ivoire, domestic production is estimated at 700 000 tons of milled rice [2] and only covers about 50% of national requirements [3], a deficit that is being filled by imports. These imports cost 235 billion CFA francs in 2009 for just over 900 000 tons of milled rice [4]. In this context, mineral fertilization, more accessible, appears as an ideal cultural practice to increase production. Indeed, the mineral fertilization is carried out according to the objectives of yield which one is fixed. This practice consists in providing the plants with reserves of assimilable mineral elements and known to give a good yield to the rice plant [5]. It is essential for the growth and development of plants. Among these mineral elements, nitrogen, phosphorus, potassium, calcium and magnesium are the major elements controlling the growth of plants and the proper development of them. However, the low production is partly due to biotic constraints, the most important in Africa being the *Rice yellow mottle virus* (RYMV). Discovered in 1966 in Kenya [6], the disease then spread to the entire African continent. Infection with RYMV also causes cellular disruption, with mainly changes in the nucleus, increased vacuole and reduced size of chloroplasts and mitochondria [7]. Infected plants show pale yellow variegation on leaves, stunting, reduced tillering, asynchronous flower formation, poor boosting of panicle, leaf discoloration and spikelet sterility [8]. Yield losses due to RYMV are enormous and vary from 10 to 100% [9,10] depending on the time of infection and the variety. Studies have reported physiological and biochemical disorders due to pathological changes in plants. Thus, the protein content of bean leaves increases with *Pseudomonas syringae* infection. *Cucumber mosaic virus* (CMV) infected leaves show a high enzymatic activity of chlorophyll and drop in chlorophyll content [11]. Infection with CMV decreases sugar content, while free amino acids and protein levels are significantly elevated in infected cucumber leaves [12,13,14] Infection with *Watermelon mosaic virus* (WMV) increases the protein content, but decreases other physiological and biochemical activities such as respiration rate and reduces the starch, sugar and total nitrogen content of diseased plants compared to healthy plants [13]. The aim of this study is to evaluate the effect of RYMV on the N P K Ca and Mg content in rice leaves infected with RYMV.

2. MATERIALS AND METHODS

The study was conducted under controlled conditions in greenhouse in Bingerville at the Pole Scientifique et d'Innovation of Félix

Houphouët-Boigny University. The relative humidity and mean temperature of the greenhouse during the test period were 83.5% and 27.4°C, respectively. RYMV-susceptible Bouake 189 was used in this study. The RYMV isolate collected under natural infestation conditions in Gagnoa (06°07N, 05°54W, 213 m) southwestern region Côte d'Ivoire was used to inoculate rice plants.

2.1 Propagation of the Isolate

The isolate was grown under greenhouse to obtain fresh material needed for the study. The multiplication consisted in mechanical inoculation of the isolate on the Bouaké 189 sensitive rice variety. Leaves of young plants exhibiting the characteristic symptoms of RYMV were then collected and analyzed by the Double Antibody Sandwich Enzyme Linked Immuno-Sorbent Assay (DAS ELISA).

2.2 Sampling and Physicochemical Composition of the Soil

The soil was taken at Bingerville. After drying in the open air, it was ground in a mortar. The root debris has been entirely removed from the soil and homogenized manually. This mixture was sterilized in an oven at 150°C for 3 hours before determining the physicochemical composition.

2.3 Experimental Design

The experiment was conducted according to a randomized complete block device with 3 repetitions. Six rice grains of the Bouaké 189 variety were sown at a depth of 2 cm in 5 kg of soil initially placed in plastic pots of 5 L volume and 24 cm diameter at the opening. Mating at 3 plants per pot was performed 7 days after sowing (das). The inoculated plants were separated from the 1 cm controls in each block. The chemical fertilizer NPK (12, 24 and 18) was applied as manure at the rate of 200kg/ha soil. Nitrogen was supplied as urea (46% N) at the rate of 100 kg/ha, a third of which was added to tillering, and the remaining two-thirds were applied to panicle initiation. The plants were watered regularly for the duration of the test.

2.4 Inoculum Production and Inoculation

The infected leaves samples collected from the multiplication were crushed in a mortar at a rate of 10 g of leaves per 100 ml of phosphate buffer pH 7.2. Carborundum (600 mesh, 5 mg / ml) was added to the crude extract to promote virus infiltration [15]. The tested rice plants were mechanically inoculated by manual friction. During inoculation, the gloved fingers were soaked in the inoculum, and then the leaves of the rice plants were rubbed slightly from bottom to top. The inoculated plants were then rinsed with distilled water three minutes after inoculation. Negative controls (healthy plants) were rubbed with distilled water. Inoculation of the plants was carried out 15 days after sowing (Das).

2.5 Data Collection

2.5.1 Chlorophyll content

Leaves chlorophyll levels were measured at 28 and 60 days post-inoculation (dpi) using a SPAD-502 chlorophyll meter [16,17]. SPAD-502 determines the relative amount of chlorophyll present in the plant by measuring the absorbance of the leaf at two wave lengths (about 650 and 950 nm).

2.5.2 Area under the symptom progression curve (AUSPC)

The area under the symptom progression curve (AUSPC) of infected plants was evaluated according to the method of [18].

$$AUSPC = \Sigma [(Y i + 1 + Y i) / 2] [X i + 1 - Xi]$$

With Yi: Severity at the time of observation, Xi: Time (days) at the time of observation.

2.5.3 Serological analysis

The presence of RYMV in infected rice leaf samples was confirmed using the DAS-ELISA technique as described by [19]. A first reading of the optical densities at 405 nm was carried out at 30 minutes after incubation and then a second, 1 hour later. An extract was considered positive if the absorbance's obtained are greater than the average of the absorbance of the negative control plus three times the standard deviation [20]

2.5.4 Average height of plants at tillering and flowering

The heights of healthy and inoculated plants were measured 28 and 60 days post- inoculation (dpi) at tillering and flowering, using a tape measure, starting from the neck to the end of the leaf higher.

2.5.5 N, P, K, Ca, and Mg content in leaf samples

Rice leaves (young and old, mixed) with RYMV symptoms and healthy controls were used for the analyzes. The samples were dried in an oven (*Memmert,* model 100 14L UNB) at 60°C for 48 hours until a constant weight was reached. The dried samples were ground with a mortar and pestle and stored in polyethylene bottles. The nitrogen content of the samples was determined according to the Kjeldahl method [21]. Phosphorus, potassium, calcium, and magnesium content in plant tissues of leaf samples was obtained according to [22].

2.5.6 Protein content

The total protein content in the infected and control leaves consisted a total nitrogen dosing according to Kjeldahl method and then multiplying it by a conventional factor of 6.25 (Nt x6.25). This factor corresponds to a mean nitrogen content of 16% [21]

2.5.7 Grain production

Grains from the harvest of the infected and healthy controls were dried in an oven (*Memmert* UN30) at 70°C for 24 hours and then weighed with precision balance Sartorius, model MSE 10202S.

2.6 Statistical Analysis

The averages of the different parameters were compared by ANOVA 2 with the STATISTICA version 7.1 software. The Fischer and Newman-Keuls test at the 5% threshold was used for averages classification.

3. RESULTS

3.1 Physical and Chemical Composition of Experimental Soil

Table 1 shows the physical and chemical composition of the soil used for the realization of the study.

3.2 Effect of the RYMV on the Chlorophyll Content

Analysis of the results showed a significant effect of RYMV on the chlorophyll content of the rice plants (Table 2). Thus, based on the RYMV data, healthy plants reported at 28 days post-inoculation (dpi) a mean leaf spot intensity of 28.48 while chlorophyll levels observed in infected plants were low (19.72). Similarly, at 60 dpi, chlorophyll content was higher in healthy plants (37.37) than inoculated plants (21.23).

Table 1. Physical and chemical composition of experimental soil

Chemical physico composition	Content
Clay (%)	32
Sand (%)	41
Silt (%)	27
C (%)	7.72
NT (%)	0.82
P (mg/Kg)	24.48
Ca (cmolkg $^{-1}$)	1.432
Mg (cmolkg $^{-1}$)	1.343
K (mg/Kg)	315.07
Na (cmolkg $^{-1}$)	0.135
CEC (cmolkg $^{-1}$)	6.56
Conductivity (µS)	278
pH water	6.67

3.3 Effect of the RYMV on the Rice Plants Height

The general finding is that the height of healthy plants is higher than that of infected plants. The height of the plants varied significantly according to the status of the plants. Thus, the average heights of healthy plants recorded at 28 and 60 JAI were 50.33 and 78.61 cm, respectively, while those observed in the inoculated plants obtained mean values of 27.94 and 46.94 cm (Table 2).

3.4 Effect of the Mineral Fertilization on the Chlorophyll Content

A significant difference in mineral fertilization on the chlorophyll content of rice plants was noted from the analysis of variance. The application of NPK and urea induced a high chlorophyll content in the fertilized plants when the non-fertilizer controls had the lowest chlorophyll content. Observations recorded 42 and 75 days after sowing (das) in fertilized plants had a higher average chlorophyll content than that observed in control plants lacking mineral fertilizers (Table 3).

3.5 Effect of the Mineral Fertilization on the Rice Plants Height

Mineral fertilization has shown a significant effect on height growth of rice plants. Indeed, the height of the plants without fertilizer, recorded at

42 and 75 days after sowing (das) was respectively 32.89 and 50.28 cm while the size of the plants resulting from the fertilizer supply presented respective average values 45.39 and 75.28 cm (Table 3).

3.6 Effect of the RYMV on N. P. K. Ca and Mg Content in the Rice Leaves

The amounts of N,P,K Ca and Mg in inoculated rice leaves samples and in healthy controls are shown in Table 4. The protein content was 10.95 for healthy plants and 15.04 for plants infected with RYMV, respectively. P, K, Ca and Mg levels in healthy leaves were higher, while those for the amount of Nt in infected leaves were higher (Table 4).

3.7 Effect of the RYMV and the Fertilization on the Grain Production

Fig. 1 shows the effect of RYMV disease on grain yield of plants. Thus grain production varied significantly depending on the status of the plants. The infected plants were less productive with an average of 24.85 g unlike the healthy controls which have an average production of 33.95 g. As for fertilization, it induced a high average production in plants that received mineral fertilizer (37.32 g) while untreated controls showed low average production (21.48 g).

3.8 Effect of the Mineral Fertilization on RYMV Severity

The severity and area under symptom progression curve (AUSPC) of the RYMV varied significantly with mineral fertilization. The average severity of seedlings inoculated with a mineral fertilizer was 6.22 while recorded in seedlings with mineral fertilizer averaged 3.88. The AUSPC average observed in inoculated plants with N,P,K addition (138.38) was lower than that observed in infected plants without N,PK addition (276.77) as shown in Table 5.

3.9 Serological Analysis

It is assumed that the optical density measured is proportional to the viral concentration. All inoculated leaves samples tested gave an

Table 2. Effect of the RYMV on the chlorophyll content and on the rice plants height

Status of plants	Chlorophyll content		Height (cm)	
	28 dpi	60 dpi	28 dpi	60 dpi
Infected plants	19.72 b	21.23 b	27.94 b	46.94 b
Healthy plants	28.48 a	37.37 a	50.33 a	78.61 a
Average	24.10	29.3	39.14	62.77
P-value	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	22.49	36.79	34.83	32.72

**Averages followed by the same subscripts in the same row are not significantly different (Fisher test, P=0.05)*

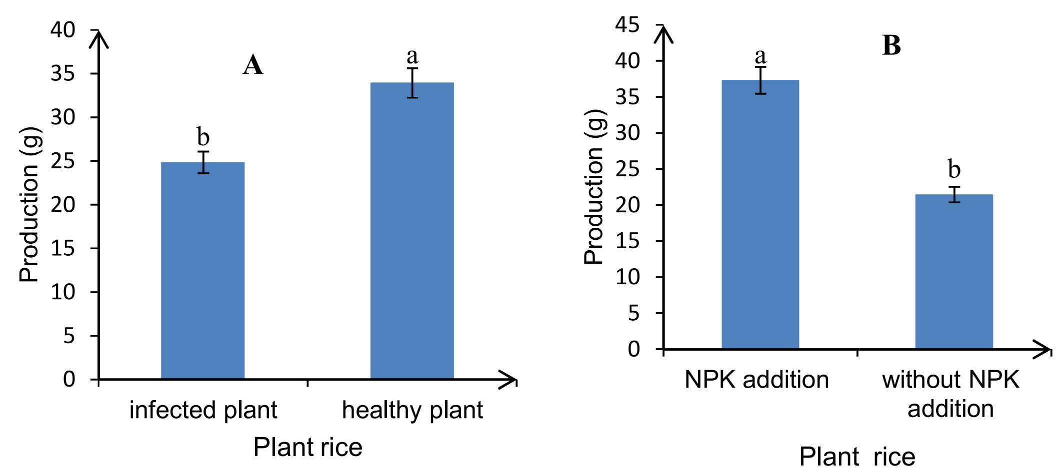

Fig. 1. Effect of the RYMV (A) and the fertilization (B) on the grain production

Table 3. Effect of the mineral fertilization on the chlorophyll content and on the rice plants height

Fertilizers	Chlorophyll content		Height (cm)	
	28 dpi	60 dpi	28 dpi	60 dpi
With NPK addition	27.04 a	36.04 a	45.39 a	75.28 a
Without NPK addition	21.15 b	22.56 b	32.89 b	50.28 b
Average	24.1	29.3	39.14	62.77
P-value	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	22.49	36.79	34.83	32.72

**Average followed by the same subscripts in the same row are not significantly different (Fisher test, P=0.05)*

Table 4. Nutrients content in infected with RYMV and healthy rice plants

Status of plants	Proteins	Nt	P	K	Ca	Mg
Infected plants	15.04 a	2.41 a	0.39 b	0.75 b	0.67 b	0.24 b
Healthy plants	10.95 b	1.75 b	0.40 a	1.10 a	1.49 a	0.39 a
Average	12.99	2.07	0.39	0.92	1.08	0.31
P-value	< 0.001	< 0.001	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	26.02	26.8	2.5	31.81	53.7	25.8

**Averages followed by the same subscripts in the same row are not significantly different (Fisher test, P=0.05)*

Table 5. Effect of the mineral fertilization on severity and AUSPC of RYMV disease

Mineral fertilization	RYMV severity	AUSPC
Inoculated plants with NPK	3.88 b	138.38 b
Inoculated plants without NPK addition	6.22 a	276.77 a
Average	5.05	207.58
P-value	< 0.0001	< 0.0001
Coefficient of variation (%)	30.49	44.48

**Averages followed by the same subscripts in the same row are not significantly different (Fisher test, P=0.05)*

absorbance at 405 nm above the detection limit. Leaves extracts inoculated with NPK provided the lowest average absorbance (1.577) while unfertilized infected plants reported the highest average absorbance (2.132) as shown in Fig. 2.

3.10 Effect of the Studied Factors (RYMV and Fertilization) on the Mineral Content

The results of the analysis of variance recorded in Table 6 showed that the interactions identified had a significant effect on the mineral content in the leaves samples analyzed. In the RYMV-Fertilizer interaction plants inoculated with NPK showed higher Ca and Mg levels than plants inoculated without NPK. The N and P contents were lower in the inoculated plants without fertilizer (Table 6). However, the content of N, P, K, Ca and Mg recorded in uninfected plants with application of NPK fertilizer was higher than that observed in healthy plants without NPK input.

3.11 Effect of the Factors Studied (RYMV and Fertilization) on Height, Grain Yield and Chlorophyll Content of the Plants

A highly significant effect of the RYMV-Fertilizer interaction was noted on height, grain yield and chlorophyll content of the plants (Table 7).

4. DISCUSSION

Evaluation of the effect of *Rice yellow mottle virus* on N, P, K, Ca and Mg content in infected rice leaves revealed variation in mineral content, chlorophyll content, and the height and grain production of rice plants. Serological testing is a method of detecting *Rice yellow mottle virus* effectively and rapidly. Although considered unreliable, because of the weak reaction of some anti-RYMV polyclonal antibodies with some isolates. The serological method remains essential in the detection of *Rice yellow mottle virus* disease [23].

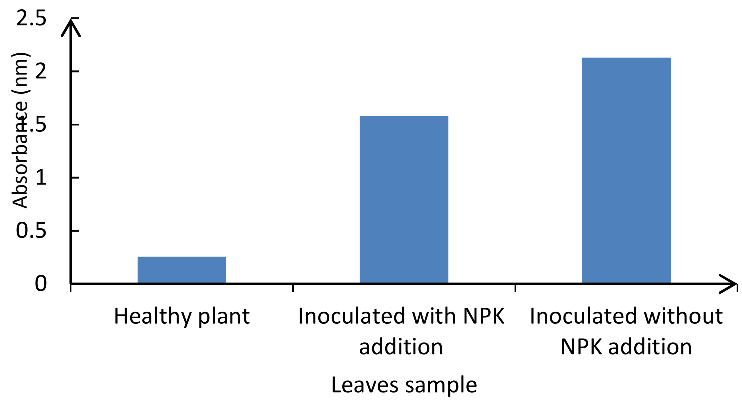

Fig. 2. Comparison of RYMV virus concentration in infected plants fertilized with NPK and without NPK (OD recorded at 405 nm)

Table 6. RYMV-fertilization interaction on the N P K Ca and Mg content in rice leaves infected

RYMV-Fertilisant	Nt	P	K	Ca	Mg
Infected plant with NPK addition	2.32 b	0.39 c	0.88 b	0.82 c	0.26 c
Infected plant without NPK addition	2.48 a	0.40 b	0.62 d	0.52 d	0.21 d
Healthy plant with NPK addition	2.31 c	0.41 a	1.38 a	2.00 a	0.43 a
Healthy plant without NPK addition	1.18 d	0.39 c	0.80 c	0.97 b	0.35 b
Average	2.070	0.390	0.920	1.080	0.310
P-value	< 0.001	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	26.8	2.5	31.81	53.7	25.8

**Averages followed by the same subscripts in the same row are not significantly different (Newman-Keuls test, P=0.05)*

Table 7. RYMV-Fertilization interaction on height, grain yield and chlorophyll content of the plants

RYMV- Fertilizer	Chlorophyll content	Height (cm)	Grain production (g)
Infected plant with NPK addition	26.16 c	60.66 c	32.33 b
Infected plant without NPK addition	16.29 d	33.22 d	17.37 d
Healthy plant with NPK addition	45.91 a	89.88 a	42.31 a
Healthy plant without NPK addition	28.82 b	67.33 b	25.60 c
Average	29.3	62.77	29.4
P-value	< 0.001	< 0.001	< 0.0001
The coefficient of variation (%)	36.79	32.72	31.56

*Averages *followed by the same subscripts in the same row are not significantly different (Newman-Keuls test, P=0.05)*

The results obtained showed that the protein content of healthy plants was 10.95% whereas the protein content observed in the infected plants was 15.04%. P, K, and Mg levels in healthy leaves were higher than those observed in infected plants. The amount of total N recorded in the infected leaves was 2.41% when that of healthy leaves was 1.75%. The results obtained during this study are in agreement with those of [14]. Indeed, these authors showed that the P, K and Mg content in healthy leaves was higher than that observed in infected leaves. The authors also revealed that the levels of Nt and protein recorded in the infected leaves were higher than the amounts of N and protein observed in healthy leaves. These results could be due to possible adverse effects and alterations in plant metabolism and cell integrity induced by viral infections. N, P and K are mobile elements in plants and are actively transported to young tissues when disease resistance and plant growth needed can be negatively affected by their deficiency. Calcium is a non-mobile element; it makes it possible to create links between the cell walls. It, therefore, maintains the structure between the cells by cementing them to each other. A lack of calcium implies a loss of cohesion between the cells; young leaves and fruits are therefore more sensitive to calcium deficiency. Magnesium deficiency during growth reduces the energy production required for defence functions and the inactivation of the pathogen metabolites. Indeed, plants have performed physical and chemical defences [24,25] and active defences produced after infection [26], that requires both energy and photosynthetic substrates involving Mg as a component of the chlorophyll molecule or as a cofactor for the different physiological processes. In previous studies, the virus infected plants were reported to contain more N than control plants by analytic calculations [14]. Indeed, [27] observed in the infected leaves that their own protein level decreased, but the virus specific protein increased. Therefore, the established higher total protein content was more likely to be due to the increased level of viral proteins in the plant [14]. The Authors have confirmed a general reduction in photosynthesis of infected plants by a pathogen; and a positive correlation between these effects and the chlorophyll content was noted. The reduction of chlorophyll content due to microbial pathogens is almost universal in most plants. However, [28] reported that the structure and chlorophyll content in annual and perennial foliar-infected crops depend on the type of disease and the organism in question. However, [29] reported photosynthetic capacity as a function of chlorophyll content in rice leaves. Similarly [30] found a positive correlation between leaf chlorophyll content and rice yield. Ninety percent of the grain yield of rice comes from the photosynthetic activity of the leaves [31]. As is, reducing the rate of photosynthesis would have a negative effect on rice productivity, resulting in significant yield losses. The results of this study confirm the conclusions of [32] who reported a reduction in chlorophyll levels in rust-infected beans, angular leaf spot and anthracnose. This observation is consistent with the results of [33] who reported decreased photosynthesis in coconut palms infected with lethal yellowing due to reduced chlorophyll in the leaves. Similarly [34] showed that *potato virus Y* (PVY) negatively affected photosynthesis in leaves of *Nicotiana tabacum* L. attributing this decrease to a reduction in chlorophyll content. When the effects of N, P and K fertilizer were on RYMV-induced leaves chlorosis, analysis of variance showed a significant effect on leaf chlorosis intensity after the application of the second urea fraction. This regression of symptoms coupled with a clearly low viral load (optical densities), observed in plant extracts inoculated with N,P and K addition suggested the implementation of a resistance mechanism induced by the application of urea. When the rice variety Bouake 189 was infected with *Rice yellow mottle virus*, [35] showed that the viral load was high in the first days of infection and then dropped to a lower value at 42 dpi. This level of viral load associated with nitrogen intake would explain the regression of the leaf symptom. The results of this study are in favour of effectivity of nitrogen on the foliar symptoms caused by the the *Rice yellow mottle virus*. Fertilizers (N, P and K) contribution increases the resistance of the susceptible cultivars to the *Rice yellow mottle virus*.

5. CONCLUSION

The study showed that rice leaves infected by Rice *yellow mottle virus* (RYMV) have high levels of nitrogen and protein, unlike phosphorus, to potassium, calcium and magnesium levels are higher in healthy plants. The application of N, P and K causes RYMV-induced leaf chlorosis to drop in infected plants and increases the grain yield of healthy plants. Therefore, a significant supply of N, P and K fertilizer can reduce losses due to RYMV. In addition, nutritional value of infected leaves is improved due to the high protein content and could be used for animal feed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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