Improvement of Agronomic Parameters of Rice Plants Infected by Rice Yellow Mottle Virus (RYMV) using plant extracts

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ABSTRACT

Aims: The aim of this study was to assess the effect of plant extracts *Azadirachta indica*, *Piper nigrum* L, *Acacia gummifera*, *Combretum micranthum*, and *Clenodium vulgare* L on rice yellow mottle virus in rice.
Study design: The experiment was conducted in a Randomized Complete Block Design (RCBD) with five repetitions. The blocking factor was the sunshine.
Place and Duration of Study: The study was carried out at the Microbiology and Microbial Biotechnology Research Laboratory (LaboREM-Biotech) of the Faculty of Sciences and Techniques, between June and November 2020.
Methodology: The rice variety Kogoni91-1, which is highly susceptible to rice yellow mottle, was grown under greenhouse at the LaboREM-Biotech, inoculated with a highly virulent virus from the laboratory's collection and treated with plant extracts.
Results: The results showed that rice plants treated with the combination of plant extracts from *C. micranthum*, A. indica, and C. vulgare recorded higher grain and aerial biomass yields than the positive control and very close to those of the negative control. Similarly, plants treated with black pepper extracts showed a number of spikelets, a yield and an aerial biomass higher than those of the positive control and very close to those of the negative control.
Conclusion: Using plant extracts to combat rice yellow mottle could be an alternative to the application of chemical pesticides harmful to human and animal health.

Keywords: rice, rymv, plant extracts, control, agronomic parameters.

1. INTRODUCTION

Rice is an important food crop for more than 3.5 billion people (Frewer et al. 2018).**In 2020, global rice production was 503.167 million milled tons with an estimated consumption of over 501.962 million tons of milled rice. Asia continues to be on top in terms of rice consumption with 88.86% of total rice consumption (USDA, 2021).**African markets are potential outlets for Asian exporters. In 2019 alone, all African rice imports exceeded seven billion U.S. dollars (ARCADIA, 2019). Rice is one of the fastest growing food sources in sub-Saharan Africa (Sohl, 2005). Between 2012 and 2014, imports cost West Africa more than $1.7 billion, which still imports 40% of its consumption. The gap between demand and supply of rice in sub-Saharan Africa reached 891,000 tons in 2016, costing to the region approximately 282,593,283 million Euro (Hauglustaine, 2017). In 2018, rice consumption in sub-Saharan Africa was estimated at around 26.6 million tons per year (t/yr). Imports rice were about 14,600,000 tons of milled rice to cover consumption needs in the same year

(USDA, 2021). Therefore, rice plays an important role in both food security and the economy and prosperity of farmers in Sub-Saharan Africa.

Rice is the dominant commercial food crop in Mali, accounting for 12.3% of agricultural value. It contributes 5% to the Gross Domestic Product (GDP), or around 220 billion XOF (SENE KUNAFONI, 2019). National rice production was estimated at 3 million tons in 2019 (Ouedraogo et al. 2021). Rice cultivation is becoming popular for small-scale farmers mainly due to large-scale public investment, gravity-fed irrigation infrastructure and positive policy changes such as the liberalization of marketing and processing in the main production area in Mali (Office du Niger) in the late 1990s and early 2000s. the average of rice productivity in Mali is estimated at 3.79 t/ha (Ouedraogo et al. 2021). It is lower than the average in Asia which is 5.96 t/ha (USDA and IPAD, 2021).

However, Mali is an exception with 90% of its rice needs covered. Nevertheless, it imports an average of 200,000 tons per year (Fall, 2018). However, rice production in Mali faces many constraints including the use of high-performance varieties that are susceptible to insect pests and diseases such as yellow mottle (Sarra et al. 2009). This disease is caused by a virus and affects both irrigated and rainfed rice with an incidence ranging from 60 to 100% (Sarra et al. 2009). The West African Rice Development Association (WARDA) and its partners have invested a lot of time and money in researching tolerant/resistant rice varieties and other biological aspects of the disease in order to find a solution for producers in the Office du Niger (WARDA, 2000). Nowadays, rice cultivation techniques and chemical pesticides have shown their limits. The use of plant extracts as biopesticides is becoming increasingly important in crop production and protection as well as in the protection of human health and the environment (Prakash et al. 2008). Roots and leaves of some plants can reduce and inhibit the infection and spread of the virus in some cases such as tomato leaf curl, zucchini yellow mosaic, papaya circular spot, common yellow mung bean and mung bean mosaic, PVX potato and pepper mosaic virus, yellow mosaic in mung bean and urbean by inducing resistance (Chaube, et al. 2017; Awasthi, 2017; 2013; Najam et al. 2017; Sharma et al. 2017; Chaube et al. 2013; Mahmoud,2010; Satyavati, 2010; Singh, 2009; Madrigal et al. 2000). Inhibition of spinach mosaic virus was induced by *Azadirachta indica* extract (Zaidi et al. 1988). In addition, several studies have also demonstrated that the use of antagonistic endophytic bacteria reduces the effect of several rice diseases including viruses (Raupach et al. 1996; Harish et al. 2008).

2. MATERIAL AND METHODS

2.1 Biological material

Extracts of local plants such as Neem (*A. indica*), black pepper (*Piper nigrum* L), tannin (*Acacia gummifera*), *Combretum micranthum*, and Calamentclinopode (*Clenodium vulgare* L) were used in this study. The variety Kogoni91-1, tested by Doumbia et al. (2020) as very susceptible to rymv among the rice varieties grown in the Niger Office area, was inoculated with the virus and treated with plant extracts. Its seeds were provided by the Institute of Rural Economy (IER). The type A virus was chosen for inoculation as a pathogen. It was isolated from the rice plant in the Niger Office area, characterized by RT-PCR and tested as the most virulent by theLaboREM-Biotech collection (Doumbia et al. 2020).

2.2 Growing Kogoni91-1 variety

The quality of the seeds was first tested using the sorting method in distilled water, which consists of soaking them and recovering the good qualities from the water. The seeds were then incubated in a GRANT GD 120 water bath at 53°C for 30 minutes to disinfect them. The seeds were sown in 10 kg pots, filled with potting soil and placed in a greenhouse according to Randomized Complete Block Design (RCBD) with five repetitions at the LaboREM-Biotech with sunlight as a blocking factor. Each block consisted of a repeat of treatments T0 (Negative Control), T1 (Positive Control), T2 (CAC: *Combretum micranthum*, *A. indica* and *C. vulgare* L.), T3 (Black Pepper), T4 (Tannin), and T5 (Pepper/Tannin). Plants were inoculated 14 days after transplanting into pots (Doumbia et al. 2020).

2.3 Identification of plant extracts effective against rymv

2.3.1 Preparation of spray plant extract solutions

2.3.1.1 Aqueous decoction of black pepper (*P. nigrum* L)

Ten grams of black pepper seeds were boiled in 100 ml of sterile distilled water on a hot plate for 15 minutes. The container was hermetically sealed, removed from the heat and cooled for a few minutes.After cooling, the contents of the container were filtered and used to spray the plants.Tween 80 was added to 0.1% of the solution (Chaudhry et al. 2006) (Fig. 1).

Fig. 1. : Black pepper (*P. nigrum* L) seed

2.3.1.2 Aqueous leaf solution

Ten grams of leaves of each species (*C. vulgare* L, *C. micranthum*, and *A. indica*) were ground in the mortar. The substrate was then filtered twice to obtain a pure and homogeneous solution for spraying. Tween 80 was added at 0.1% to the solution (Chaudhry et al. 2006) (Fig. 2).

Fig. 2.: Photo of leaves of different plant species

2.3.1.3 Tannin solution*(A. gummifera)*

Ten grams of the dried tannin fruit were weighed and ground in the mortar. The resulting powder was dissolved in 100 ml of water. The solution was filtered twice through a sieve before being used for spraying. Tween 80 was used at 0.1% in the solution (Chaudhry et al. 2006) (Fig. 3).

Fig. 3.: Fruit of tannin (*A. gummifera*)

2.3.2 Infection of rice plants with rymv

2.3.2.1 Preparation of viral inoculum

One gram of infected leaves was weighed using a precision balance (PIONEER TM OHAUS) and disinfected in 10% sodium hypochlorite solution for 30s, then in 70% ethanol for 30s and rinsed twice with sterile distilled water.The treated leaf was dried on blotting paper for 10 min and then ground in 10 ml of 0.01 M PBS. The crushed material was filtered and the filtrate obtained was mixed with 0.2 g of carborundum and kept on ice.

2.3.2.2 Inoculation of rice plants by manual rubbing

Rice plants were inoculated on the 21st day after sowing (14 days after transplanting) with virus isolates identified by RT-PCR. Inoculation was carried out by inserting two fingers (thumb and forefinger) into the inoculum (a mixture of grindings and carborundum) and pinching a leaf of the plant to be inoculated with the fingers, sliding them up and down its length (Doumbia et al. 2020). The carborundum creates wounds on the leaf to promote virus penetration (Fig. 4).

Fig. 4.: Inoculation of leaves by manual rubbing

2.3.3 Application of extracts to infested plants

Solutions obtained from plant extracts at a concentration of 1% were used directly to spray leaf surfaces as soon as symptoms appeared after inoculation. It was carried out once (01) a week until panicle initiation (Fig. 5).

Fig. 5. : Spraying rice plants infected with rymv virus using plant extracts.

The evaluation of the rymv reduction effect using plant extracts on agronomic parameters began 15 days after inoculation (IRRI, 2013). The parameters measured and their observation stage are recorded in Table 1.

Table 1. : Agronomic parameters and their observation stage

N°	Agronomic parameters	Stage
1	Number of tillers	60 days after sowing
2	Number of spikelets	After harvest
3	Height of plants	After heading
4	Yield	After harvest
5	Weight above-ground biomass	After harvest
6	Weight of dry roots	After harvest

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2.4 Data analysis

The data obtained from the plant extract application were tested for homogeneity of variance of means. Non-homogeneous data were transformed before statistical analysis. The transformed data were subjected to an analysis of variance (ANOVA) using SAS software version 9.2 (SAS, 1999). Whenever the calculated F was significant, the means were compared using Fisher's protected smallest significant difference test.

3. RESULTS

Analysis of variance showed different effects of plant extracts on the agronomic parameters measured. The effect of the extracts was most significant on yield and dry root weight compared with the control. The results of the analysis of variance of the parameters measured on the rice plants treated with the plant extracts are in Table 2.

Table 2.: Variability among plant extracts based rice parameters recorded

Sources of variation	Degree of Freedom	Fisher value					
		Plant Height	Number of tillers	Number of spikelets	Yield	Weight of dry roots	Weight above-ground biomass
Treatments	5	3,83*	2,10NS	5,15*	6,81**	6,16**	3,48*

Blocks	4	2,87 NS	1,50 NS	2,04 NS	0,67 NS	0,30 NS	0,92 NS

Not significant: NS ; *: significant f P<0,05 ; ** : very significant P< 0,01

Table 3.: Average values for height, number of tillers, number of spikelets, yield, weight of above-ground biomass and weight of dry roots according to plant extract treatments.

Treatments	Plant Height	Number of tillers	Number of spikelets	Grain yield	Weight of dry roots	Weight above-ground biomass
CAC (T2)	1,69bac	1,55a	2,27bac	1,13a	1,14a	1,35a
Black Pepper (T3)	1,73ba	1,63a	2,44a	1,33a	1,15a	1,41a
Tannin (T4)	1,55c	1,40a	1,83d	0,23b	0,65b	0,84c
Black Pepper /Tannin (T5)	1,55c	1,46a	2,02bdc	0,36b	0,88ba	1,19ba
Positive Control (T1)	1,59bc	1,32a	1,97dc	0,35b	0,79ba	0,99bc
Negative Control (T0)	1,79a	1,40a	2,30ba	1,60a	1,14a	1,33a

Means followed by the same letter are not statistically different for the LDS test at the 5% threshold. **CAC** : (*C. micranthum*, *A. indica* et *C. vulgare L.*)

Plants treated with CAC (Fig. 6) scored the highest grain and aerial biomass yields than the positive control and very similar to the negative control. Similarly, plants treated with black pepper extracts (Fig. 7) recorded the highest number of spikelets, yield and above-ground biomass than the positive control and very similar to the negative control (Table 3). All the plants treated with these two plant extracts were very closed to those of the negative control for all the parameters measured. Black pepper and CAC had the same level of protection of the plants against the rice yellow mottle virus. However, tannin extracts and the combination of tannin extracts and black pepper also had the same effects on the agronomic parameters measured, with the exception of above-ground biomass, where the combination had the highest biomass value. The CAC and black pepper treatments significantly protected yield compared with the positive control treatment. They prevented yield loss by 52.7 and 58.3% respectively.

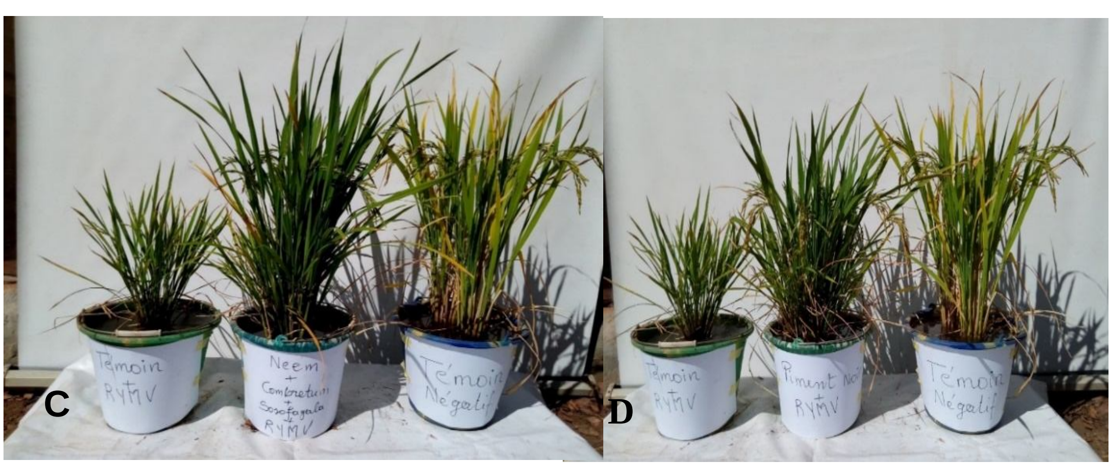

Fig. 6.: Effect of plant extracts on rice plants infected with rymv; **C**: combination of *C. micranthum*, *A. indica* and *C. vulgare* L extracts compared with positive and negative controls; **D**: *P. nigrum* extracts compared with positive and negative controls.

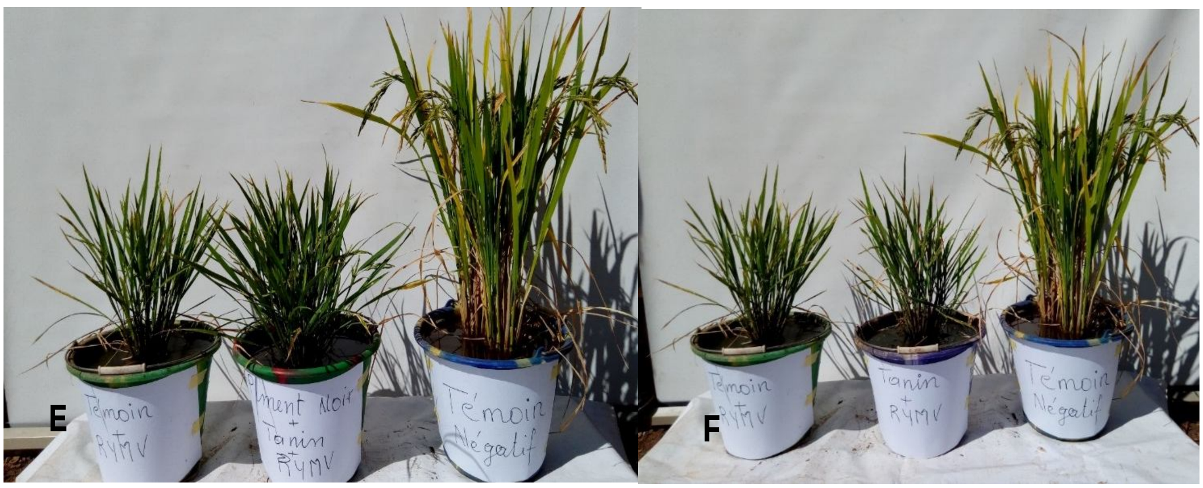

Fig. 7. : Effect of plant extracts on rice plants infected with rymv; **E**: combination of *P. nigrum* and *A. gummifera* extracts compared with positive and negative controls, **F**: *A. gummifera* extracts compared with positive and negative controls.

4.DISCUSSION

Rice yellow mottle virus is the most damaging rice disease in West Africa. It causes crop losses ranking from 20 to 100% depending on the rice variety, virus strain, vegetative stage of the plant, and environment (Ouattara, 2017). Several management methods such as improving genetic resistance and cultural practices (Sy, 2014), have been used without success. For several years now, plant extracts have been used as an alternative to chemical products against phytopathogenic microorganisms. In this specific case, extracts of the plants *A. indica*, *P. nigrum* L, *A. gummifera*, *C. micranthum*, and *C. vulgare* L have been used against rice yellow mottle caused by a virus of the sobemovirus genus (Banwo et al. 2004). Extracts of the plants *C. micranthum*, *A. indica*, and *C. vulgare* were combined in a single mixture, and this combination protected rice plants against a yield loss of 52.7%. The antiviral activity of *C. micranthum* and A. indica species used in combination has been demonstrated individually by several authors. Iobbi et al. (2022) showed that the auto-oxidation products of the methanoic extract of *C. micranthum* leaves exerted antiviral activity against the tomato brown rough fruit virus. Similarly, Chaude et al. (2017; 2013), Singh et al. (2009) and Zaidi et al. (1988) reported that *A. indica* leaf extract reduced the effect of mung bean common mosaic virus and spinach common mosaic virus. Extracts of the *C. vulgare* species have rarely been tested against viruses in general, but Opalchenova and Obreshkova (1999) have demonstrated their antibacterial activity.

The results from current study are in agreement with those of Satyavati, (2010) who used foliar spraying at fortnightly intervals in the field with a combination of Neem, Baan, Neem, and soap against chilli vein band virus (CVBV) and cucumber mosaic virus (CMV) in tomato. This combination reduced the effect of the disease by improving the yield of treated plants compared with untreated positive controls by 10.90 and 4.36 tons per ha for chilli and 21.52 and 6.48 tons per ha for tomato, respectively. This difference could be explained by the experimental environment and also by the frequency of spraying, which was one week in the greenhouse and two weeks in the field. The results of this study are similar to those of Sharma et al. (2017) who used the roots of *Boerhaaviadiffusa* L., the leaves of *Clerodendrum aculeatum* L., *A. indica* L., and the bark of *Terminalia arjuna* L. The root extract of *B. diffusa* proved to be the most effective in delaying the onset of symptoms and increasing fruit number, diameter, weight and yield, followed by *A. indica* against the natural infection of watermelon virus disease in the field.

When black pepper (*P. nigrum*) and tannin (*A. gummifera*) extracts were applied individually and in combination to plants infected with yellow mottle, only black pepper extracts prevented a yield loss of 58.3% compared to the positive control. Tannin extracts and the combination of black pepper extracts and tannin did not show any effect on yellow mottle in rice. On the other hand, Kaczmarek (2020) and Buzzini et al. (2008) showed the activity of tannin extracts against certain viruses at different stages of infection and replication in the medical field such as herpes simplex virus (HSV), human immunodeficiency virus (HIV), leukaemia virus, influenza A virus, papilloma viruses and noroviruses. This difference could be linked to the use of the active ingredient (tannic acid) by the extracting authors. The use of crude extracts, as in current study, may reduce their effectiveness against the virus.

Other authors have also noticed the effectiveness of some plant species in inhibiting infection by certain viruses. This is the case of Mahamoud et al. (2010) who, by inoculating tobacco plants with a mixture of serum from the latex of *Ficus nitida*, inhibited the incidence of necrosis virus by 57.1% and reduced the number of local lesions of bean yellow mosaic by 69.32%. Similarly, Singh et al. (2004) using *Boerhaaviadiffusa* root extracts which reduced the severity and incidence of mung bean yellow mosaic disease (*Vignaradiata*) by 80-90%. The same treatment maintained the number of nodules and pods while significantly preserving bean growth and grain yield from loss. In addition, results from actual researches confirmed those of Jabar et al. (2020) who demonstrated 20% and 0.23% reduction respectively in the incidence and severity of the disease caused by tomato yellow leaf curl virus (TYCV). Plant length and leaf area were increased by 130 cm and 28 cm respectively using *Cladophora crispate* algal extract. However, Sofy et al. (2018) achieved a reduction in virus effect of 15.36% and courgette yellow mosaic virus (ZYMV) of 13.71% using Salix and pomegranate zest extracts.

5. CONCLUSION

Extracts of CAC and black pepper in combination improved agronomic parameters by controlling yellow mottle in rice. Extraction and use of the active ingredients could increase their effectiveness in inhibiting infection by the yellow mottle virus.

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COMPETING INTERESTS

The authors declared that there were no conflicts of interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors: Author BD designed the study, performed the statistical analysis, wrote the protocol, andwrote the first draft of the manuscript. Authors SD and AK managed the analyses ofthe study. Author DOmanaged the literature searches. Authors DN, SS, RF, CT, AHD, MD and AHB read and approved the final manuscript."

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