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# Endophytic Bacteria-based Biostimulant Improved Rice (*Oryzae sativa* L.) Growth and Production in Mali

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Rice (*Oryza sativa* L.) is usually the staple food of more than half of the world's population, and in particular that of Mali. Mali is among the largest producers of rice in West Africa. But also given the in-population growth and in consumption, Mali is forced to resort to imports to cover its rice needs, thus 45% of the rice sold on the national market comes from imports. For self-sufficiency in rice, the aim of this research is to formulate a biostimulant with high capacity to improve rice growth and production in Mali.

**Study Design:** This experimental research involving a Malian local rice cultivar named Kogoni and biostimulant endophyte bacteria isolated from rice in Mali.

**Pace and Duration of Study:** This research was carried out in greenhouse at LaboREM-Biotech and in the field at the Office of irrigated perimeters of Baguineda (OPIB) for 3 years.

To achieve this objective: endophyte bacteria with high plant growth promotion activities were selected from bacterial collection of LaboREM, biostimulants composed with selected endophytes were formulated, formulated biostimulants were tested in greenhouses and fields to prove their capacities to improve rice growth and production.

**Results:** A total of five isolates, including 3 from *Xoo* and 2 from *Xoc*, were isolated in Niono from two varieties of rice: Kogoni91-1 and Adny11. No isolate characteristic of *Xanthomonas oryzae* was observed on samples from Baguineda. The three selected rice endophytes were tested *in vitro* to assess their effectiveness in controlling *Xoo* and *Xoc*. Following this test, 100% of the endophytes showed significant antimicrobial activity against *Xoo* and *Xoc* with an inhibition diameter varying between 6 and 28.5 mm. endo Ad9 was selected for its strong ability to inhibit the growth of both pathogens at the same time.


*Keywords: Rice; Xanthomonas oryzae pv oryzae; Xanthomonas oryzae pv orizycola; endophytes; growth inhibition.*

## 1. INTRODUCTION

Rice is one of the fastest growing food sources in sub-Saharan Africa [1] It is a staple food crop for more than half of the population in the region. The gap between demand and supply of rice in sub-Saharan Africa reached 10 million tons of milled rice in 2008, costing the region an estimated $3.6 billion US dollars in rice imports (www.irri.org). Rice plays an important role both in food security and economy prosperity of farmers of sub-Saharan Africa (SSA). As for the region, rice is the dominant commercial food crop in Mali, accounting for 12.3% of agricultural value. The rice cultivation is getting popular among the small holder farmers in Mali mainly due to public-led investments in large-scale, gravity-fed irrigation infrastructure and some positive policy shifts, such as the liberalization of marketing and processing in the main production zone of the Office du Niger during the late 1990s and early 2000s. Malian rice production is competitive and can be profitable, benefiting from higher global prices and an increase in the demand for local rice [2]. However, the average rice productivity in Mali (3.38 t/ha) is below world average (4.40 t/ha) and accounts one third of the rice productivity in Australia [2]. Low rice yields are attributed mainly to soils deficiencies and diseases [3-5] Soil P and N deficiencies was observed as the main constraints in yield decreases in Africa, mainly in Mali [6,4]. To increase production in a sustainable way, one of the biological approaches of achieving this objective is through the use of P-solubilizing bacteria (PSB) [6-8]. However, most of the research on phosphate solubilizing microorganisms has focused their attention on plant responses to inoculation with phosphate solubilizing rhizobacteria (Ref) and very few studies have been undertaken on endophytes. Consequently, the effectiveness of phosphate solubilizing endophytes in increasing plant P uptake and the variables governing it are not clearly understood. [9] in recent research activities showed the capacities of bacterial endophytes to increase plant mineral-uptake and crop growth. Several other studies have demonstrated the control of rice diseases and the induction of resistance in rice plants by endophytic bacteria [4,10,11], using in a greenhouse experiment, the bacterium *Lysobacter antibioticus* (strain 13-1) showed that this strain can suppress bacterial blight of rice with an efficiency up to 69.7%. In three field trials, the same strain reduced the incidence of the bacterial blight of rice by 73.5%, 78.3%, and 59.1%, respectively. Similar results were obtained by [11] who showed that a DAPGproducing bacteria suppress the growth of *Xanthomonas oryzae* pv. *oryza*ae causal agent of the bacterial blight of rice with an efficiency up to 59%-64% in greenhouse and in field experiments. The study conducted by [12] demonstrated that bacterial-treated rice plants were resistant to bacterial leaf blight. This study demonstrates the potential of endophytic bacterial inoculum for t eco-friendly biocontrol strategies of rice bacterial leaf blight. In this respect exploration and use of native and naturally occurring microbes in diseases management and enhancing crop fitness against other biotic and abiotic stresses has great potential to improve rice yield.

To date, technology to biologically improve plant mineral nutrition and control pathogens are lacking in sub–Saharan Africa, particularly in Mali. That's why, theaimof this study is to improve food security in Mali by increasing locally produced rice by at least 20% through the provision of efficient, low cost and easy to use endophytic bacteria-based biopesticides.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Microorganisms

Biocontrol endophytes isolated from rice (Nie, Adny8, Adny9, REM2, REM4, DK3, DK4 and KITA) and fungal pathogen strains used in this study were isolated and maintained in the of collection of microorganisms of the LaboREM-Biotech at the Faculty of Sciences, Techniques and Technology of Bamako at the University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali.

#### 2.1.2 Rice seed

The rice variety Adny 11 used in this assay was kindly provided by the seed growers of the ″Institut d'Économie Rurale (IER)″ of Mali. The choice of Adny 11 in this assay is mainly based on the high use of this rice variety by farmers and its high demand by consumers.

### 2.2 Methods

#### 2.2.1 Selection of endophytes with high plant growth promotion activities

**Selection, in the Laboratory, of the Best Plant Growth Promoting Endophytes (PGPE):** *In vitro* germination was performed to select PGPEs that could give vigor to rice seedlings. Laboratory test was done using the napkin paper methods. Seeds of Kogoni 91-1, a highly cultivated rice variety was disinfected using a modified method of [13]. Seeds were placed in 70% ethyl alcohol for 2 min, in 10% bleach for 15 minutes and finally washed 4 times with sterile water. The treated seeds were coated according to the modified method of [14] using a mixture of an adhesive solution of arabic gum at 2% containing the PGPE inoculum to be tested. The coated seeds were left to dry under a hood to avoid contamination. The method described by [15] modified was used to determine the effect of PGPE on rice seedling vigor. Twenty (20) seeds were used for each treatment with three replicates, in a completely randomized design. After 7 days the number of germinated seeds was counted. The length of the roots and leaves of each young plant was measured to determine the vigor index using the following formula

Vigor Index = (average root length + average leaf length) ×% germination.

**Determination of the Effect of the Selected Plant Growth Promoting Endophytes on the Growth and Yield of Irrigated Rice:** The efficiency of the plant growth promoting endophytes (PGPE) isolates, selected in the laboratory test, on rice growth was evaluated under greenhouse conditions by seed bacterization as described by [16]. Bacterization of surface sterilized rice seeds was performed by imbibing the seeds in each PGPE isolate cell suspension (A600=0.5) for 6 h at 60 rpm. Rice seeds treated with sterile distilled water alone were considered as control. Seeds inoculated with bacteria or untreated were sown in plastic pots (100 mm w × 75 mm d × 85 mm h) filled with approximately 2.50 kg of air-dried soil of rice test site. The pots, maintained under greenhouse conditions and watered regularly, were held on racks in a complete randomized block design (CRD) with 4 blocks. The blocks were the dose of nitrogen (0, 25, 50 and 100 kg/ha) applied as blocking factor. Nine (9) treatments composed with the PGPE isolates and a control (RM4, Adny9, Nie1, Adny8, DK3, DK4, RM2, Kita and non-treated control) were used in this experiment. Each treatment was replicated three times. For the entire experimental design, we had a total of one hundred eight (108) experimental units. Growth parameters such as shoot length and root length were recorded 45 days after planting. Tiller number, root dry weight, 1000 seed weight and rice yield after 75 days of growth.

### 2.2.2 Field experiment

*2.2.2.1 Experimental design*

In this field experiment, we used a complete randomized bloc design (CBRD). Three blocks were set up according to the degree from the rainwater runoff slope. Endophytes selected in greenhouse test (DK4 and Adny9), their combination (DK4+Adny9) and the non-inoculated (Control) represented the four treatments studied. Each treatment was replicated three times. Seeds of rice variety Kogoni 91-1 were coated with each treatment and seeded in rice nursery. The roots of rice seedlings from nurseries were place in corresponding bacterial inoculum for one hour before transplantation in field. The test field was fertilized with 50% of the recommended nitrogen fertilizer (75kg/ha of urea) that gave the best results in the greenhouse test and normal application of phosphate fertilizer (DAP, used).

**Data Collection:** Yield, total grain and 1000 grain weight data were collected at harvest for each block and each treatment.

**Statistical Analysis:** Before analysis, collected data were tested for homogeneity of the means variance. Data with non-homogeneous variance was log-transformed before analysis. Analysis of variance was performed for each parameter (Blocks and endophytes) according to the General Linear Model (GLM) procedures using SAS software (version 9.2). Whenever the Fisher test indicates a significant effect at a probability of 0.05, Fisher's Protected Least Significant Difference (LSD) test was used to compare the means.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Effect of seed inoculation with bacterial endophytes on rice seed germination rate and seedlings' growth and vigor index *in vitro*

Results of statistical analysis for germination (%), root length (cm), shoot length (cm), vigor index (VI) of tomato seedlings, recorded seven days after sowing are presented in Table 1.

Analysis of data in Table 1, showed that all bacterial endophytes significantly increased shoot length and vigor index of rice seedlings, but did not have on significant effect on root length and germination rate. Also, no effect of repetitions on the bacterial endophytes effect were observed (Table 1).

Data on shoot length, root length and vigor index; of rice seedlings inoculated and non-inoculated with bacterial endophytes are presented in Table 2.

**Table 1. Summary of analyzes of variance (ANOVA) for germination (%), root length (cm), shoot length (cm) and vigor index (VI) of tomato seedlings**

| Sources of Variation | DDL | Fisher' F | | | |
|---|---|---|---|---|---|
| | | LP | LR | TG | IV |
| **Plant growth promoting endophytes (PGPE)** | 8 | 5.74* | 0.28NS | 0,0NS | 5.72* |
| **Repetitions** | 2 | 0,20NS | 0.35NS | 0,0NS | 0,20NS |

**Significatif à P<0.05, NS : Non significatif statistiquement. DDL: Degrés de liberté ; LP : longueur des pousses ; LR : longueur des racinaires ; TG : Taux de germination ; IV Indice de vigueur*

**Table 2. Effect of bacterial endophytes tested, on shoot length (SL), root part length (RL) an vigor idex (IV) of rice of rice seedlings**

| Isolats | Longueur Pousses | Longueur Racines | Indice de Vigueur |
|---|---|---|---|
| **RM4** | 15,50a | 10.66ba | 15.60a |
| **Adny9** | 13,66a | 11.83ba | 13.78a |
| **Nie1** | 13,66a | 12,16a | 13.78a |
| **Adny8** | 13,16a | 12,16ba | 13,29a |
| **DK3** | 12,16ba | 10,16ba | 12,26ba |
| **DK4** | 9,20bdc | 11,46ba | 9,31bdc |
| **RM2** | 8,70dc | 13,86a | 8,83dc |
| **KITA** | 6,66d | 8,16b | 6,74d |
| **T** | 11,16b | 9.00b | 11.25bac |

*NB : Les moyennes de la même colonne suivie de lettres différentes sont différentes au seuil de 5%*

Analysis of the data reported in Table 2 indicates that young plants inoculated with endophytes are mostly more vigorous than those not inoculated (control T). Young plants inoculated with the endophyte RM4 after 14 days were the most vigorous. They are followed by those inoculated with the endophytes Adny9, Nie1, Adny8 and DK3. The endophyte RM4 was able to increase seedling vigor to over 28% compared to the uninoculated control.

### 3.1.2 Greenhouse selection

Already on the 45th day after germination, rice plants inoculated with plant growth promoting endophytes, in presence and absence of nitrogen fertilization, showed great differences in aerial growth compared to non-inoculated controls (Fig. 1).

Data from the Analysis of Variance on agronomic parameters measured are shown in Table 3.

Analysis of data in Table 3, showed that all bacterial endophytes significantly increased the number of tillers, root length, grain yield and one thousand grain weights (1000 GW). Even if no effect on the number of tillers were observed, root length, total grain weight and 1000 GW varies significantly with fertilization. Contrary, no significant effect of replicates on the effect of PGPE on the measured parameters were observed (Table 3). Rice seeds inoculation with the endophyte Adny8 increases the number of tillers by 6.6%. Increases in root dry weight by 27.8% and 24,4% were observed with the endophytes Adny 8 and DK4, respectively (Table 4).

The highest increases in total grain yields were observed with DK4 (19%) and REM2 (22%). Fig. 2, shows the results obtained with REM2.

The non-application and the application of fertilizer at 50% (F50) gave the best results for all parameters measured, except for the one thousand grain weight (1000 GW) compared to the non-application of nitrogen fertilizer (Table 5).

Non-application (F0) and application of fertilizers at 50% (F50) increased the root dry weight by 18.7% and 10.4% compared with the application of fertilizer at 25% (F25). Non-application (F0) and application of fertilizer at 50% (F50) increased the total grain weight by 23.61% and 19.9%, respectively. But the difference between F0 and F50 was not statistically significant.

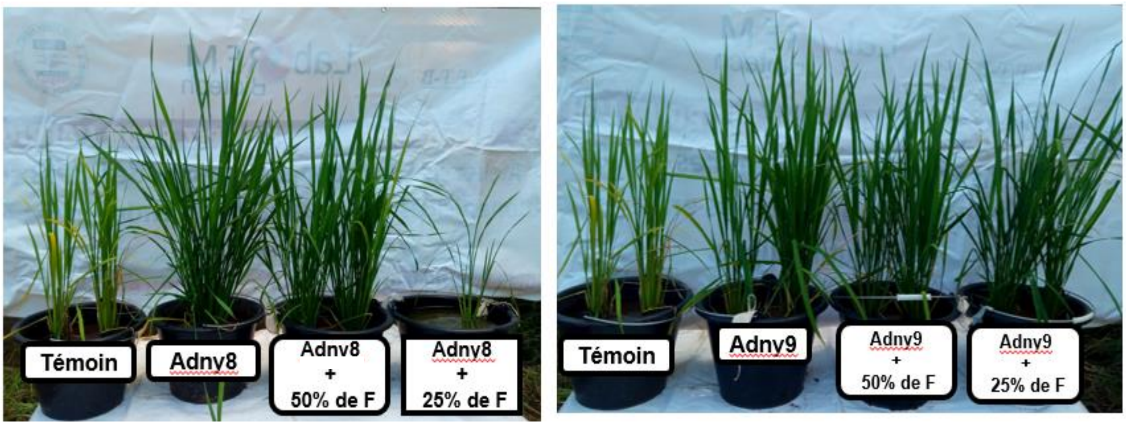


**Fig. 1. Effects of: (a) Adny 8 inoculated without N-application, or with N-application at 25% or 50%, and (b) Adny 9 inoculated without N-application, or with N-application at 25% or 50%**

**Table 3. Summary of analyzes of variance (ANOVA) for tiler number, root length, total grain weight and one thousand grain weight (W1000)**

| Sources of Variation | DF | Fisher values | | | |
|---|---|---|---|---|---|
| | | Tiller | 1000 GW | Root length | Total grain weigth |
| **Endophytes** | 9 | 6.18 *** | 14.36*** | 17.62*** | 12.80*** |
| **Fertilisation** | 3 | 2.42NS | 82.53*** | 20,87*** | 14.50*** |
| **Repetitions** | 3 | 0.33NS | 1.04NS | 0.01NS | 0.50NS |

**, **,*** Significatif à P<0.05; P<0.01 et P <0.001 respectivement, NS: Non significatif statistiquement, DDL: degre of freedon*

**Table 4. Effects of treatments (isolates) on tiller number, one thousand grain weight, root dry weight and total grain weight**

| Endophytes | Number of tillers | RDW | TGW |
|---|---|---|---|
| Nie1 | 8,00bc | 33,49abc | 28,27ab |
| REM2 | 8,66bc | 28.38c | 35,05a |
| RM4 | 9,00bc | 30,05bc | 32,02ab |
| DK3 | 7,33c | 11.62e | 15.32c |
| DK4 | 10,66b | 37,24ab | 36,86a |
| Adny8 | 15,11a | 39a | 25,78b |
| Adny9 | 10,55b | 26.55d | 28,08ab |
| KITA | 11.55b | 29.05c | 34,19ab |
| T | 14,11a | 28,14c | 28,39ab |

*The means in the same column followed by different letters are different at the 5% level*

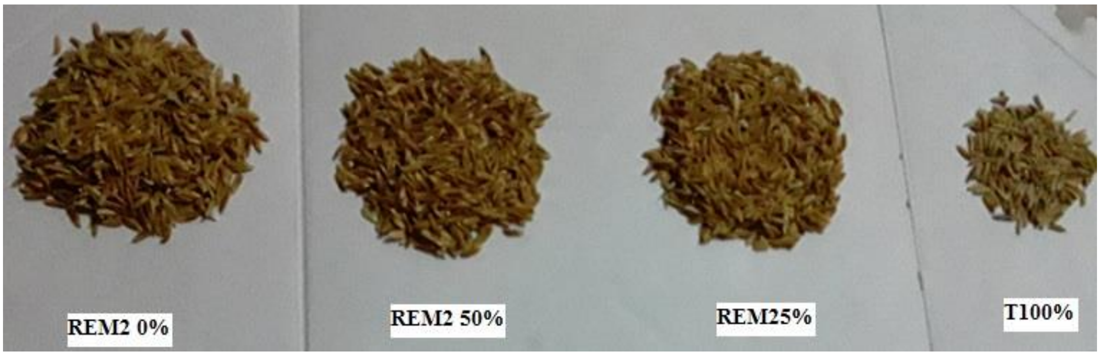


**Fig. 2. Image of paddy rice from the inoculation of the endophyte REM2 at the different nitrogen fertilizer level**

**Table 5. Effects of the application of mineral nitrogen fertilization on the tiller number, the root dry weight (RDW) and the total grain weight (TGW) and the one thousand grain weight of rice (1000 GW)**

| Nitrogen application | 1000 GW | RDW | TGW |
|---|---|---|---|
| F0 | 10,66ab | 29,24ab | 32,40a |
| F25 | 9,44b | 26,20b | 24,75b |
| F50 | 11,33a | 32,24a | 30,90a |

*The means in the same column followed by different letters are different at the 5% level. F0: inoculated with endophytes without any nitrogen fertilization; F25: endophytes with 25 nitrogen fertilization and F50: endophytes with 50 nitrogen fertilization*

#### 3.1.3 Effect of inoculation with selected endophytes on rice cultivated in field

After harvesting, 1000 grain weight, total grain weight and yield per hectare were determined. The data collected and analyzed are shown in Tables 6 and 7.

Table 6 showed significant differences for all the treatments according to the different parameters. Regarding the repetitions, unlike the total weight and the grain yield, a significant difference was not obtained for the 1000 grain weight.

The Table 7 below provides information on the effect of the endophyte DK4, Adny 9 and their combination on rice yield and one thousand rice grain weight (1000 GW).

Compared to the control, all the endophyte treatments increased the one thousand grain weight. But, Adny 9 gave the highest increase (7,35%) compared to the control. For grain yield (in ton per hectare), the best result was obtained by the endophyte Adny9 with 9.5T/ha with an increase of 28,8% compared to the control. The endophyte Adny9 was followed by the DK4 with 8.7T/ha and increase of 21,8% compared to control. The combination of the isolate Adny9 and DK4 comes after DK4 with 7.3T and increase of 6,84% compared to the control with a yield of 6.80 T/ha.

**Table 6. Results of the analysis of variance of the parameters (1000 grain weight; total grain weight and yield per hectare) after harvest**

| Sources of Variation | DF | Fisher F | | |
|---|---|---|---|---|
| | | 1000 GW | Total grain weight | Yield |
| **Endophytes** | 3 | 15,86** | 52,32*** | 52,32*** |
| **Blocks** | 2 | 0,68NS | 7,24* | 7,24* |

**Significant, **Very significant, *** highly significant and NS: Not significant; DF: Degrees of freedom, 1000GW: One thousand grains weight*

**Table 7. Effects of treatments (endophytes) on one thousand grain weight and yield**

| Endophytes | 1000 GW | Yield |
|---|---|---|
| **DK4** | 23.77b | 8,70b |
| **Adny9** | 24.35a | 9,56a |
| **Adny9 + DK4** | 23,81b | 7,30c |
| **Control** | 22.56c | 6,80c |

*The means of the same column followed by different letters are different at the 5% threshold*

## 3.2 Discussion

The *in vitro* test, using seedlings inoculated with endophytes, showed an increase of more than 38% in the vigor of rice seedlings inoculated with the endophyte REM4, compared to non-inoculated controls. Similar results were obtained by [17] who had increases in vigor of more than 30% with the inoculation of rice with a Pseudomonas fluorescens. The inoculated rice seeds were sown on two sheets of sterilized filter paper (Whatman). [18] obtained an increase of more than 100% in the vigor of rice seedlings inoculated with endophytes isolated from rice seeds, compared to non-inoculated controls. These differences may be due, not only, to the ability of the strains used to produce substances (phytohormone) promoting germination and growth of seedlings, but also, to a difference in the techniques for carrying out the tests. In this study, agar contained in test tubes was used as a germination and growth medium, while [18] used filter paper as a support for carrying out the test.

Endophytes selected following the *in vitro* test were tested in greenhouses. The best result was obtained with the endophyte DK4 with 30% increases compared to the control. Similar results were obtained with [19] who obtained a 30% increase with a rhizosphere bacterium (PGG2) isolated and inoculated into rice. In the same way, the endophytes DK4 and REM2 were able to increase rice grain yield by 30% and 23%, respectively. The best results have been obtained with the use of endophytes, followed by the application of 50% of the recommended dose of mineral fertilization. Inferior results were obtained by [20] who use, in rice cultivation, bacterial isolates (BRRh-4 and BRRh-5) selected on the basis of their ability to solubilize phosphorus; were able to increase the grain yield of rice compared to the non-inoculated control, but fertilized with 50% of the recommended dose of NPK. Grain yield was increased by 5% with BRRh-4 and 17% with BRRh-5. The discrepancy between their results and ours may not only be due to the difference between the rice varieties used Adny 9.1 in our trial and BRRI dhan-29 in this trial. It should also be noted that the type and characteristics of the soil can also play an important role. Similar results were found by [21] who also had their best result with a combination of 50% algae and 50% inorganic NPK fertilizers. These results are lower than those of [22] who obtained their best results with the associative use of these phyto beneficial bacteria with application of 75% NPK.

In this study, the grain yield of the control in the field without inoculation was 6.8 T/ha, which is close to the average yield (6.5T/ha) in rice cultivation in Mali for several years [23]. Compared to non-inoculated controls, the grain yield of rice plants inoculated and having received 50% of the recommended mineral fertilization (Urea + DAP) increased by 40%, 30% and 8%; respectively for Adny9, DK4 and the Adny9-DK4 combination. The effectiveness of the application of mineral fertilizers in combination with microorganisms has been proven in the field by [22] in the field. Indeed, [22] by inoculating, in the field, a medicinal and aromatic plant (Somnifera) with bacteria promoting the growth of plants while varying the doses of mineral fertilizers applied by 100%, 75%

and 50% showed that the inoculation with microorganisms plus the application of mineral fertilization at a dose of 75%; allowed not only to improve the length of the plants, the dry weight of the root of the plants, but also to increase the dry weight of the aerial part compared to the other two doses. This saving of 25% in chemical fertilizers is lower than the saving of 50% obtained in the present study. [24] obtained lower results with the use of 25% nitrogen-based fertilizer and 50% PGPR. This dose allowed them to have a 6% increase in rice grain yield. Similarly, lower increases were obtained in work by [25] used three PGPRs on two rice varieties (Jianyou G2 and ZD14) with brown rice in the field under natural conditions. Their use of PGPR increased the grain yield of the field from 4.83 to 9.16%.

After inoculation with the Adny 9 endophyte, combined with the use of 50% of the recommended dose of mineral fertilization (Urea + DAP), the P1000 seeds was increased by 7.35%. This result is superior to those of [23] who had a 5.5% increase with the use of the 50:50 combination of nitrogen-based fertilizer and PGPR on the crop. rice. This difference in performance can be explained by the difference in performance between the bacteria and also by the type of mineral fertilizer used, since [23] used nitrogen fertilizer only. Moreover, the difference may come from the variety of rice used, the type and the mineral composition of the soil on which the trial was established. [26], after inoculation of rice with Azotobacter sp. and fertilization with NPK (60:60:60), have obtained results on the overall growth and the physiological aspects of rice in the field that are far superior to those obtained in this trial. Indeed, they obtained a 20% increase in 1000 grain weight. This large difference may be due to the fertilization system and the inoculation technique where they immersed the 21-day-old plants separately in bacterial suspensions (1.2 × 108 cells/ml) for 6 hours before transplanting them. In this trial, the seeds were coated before sowing in the nursery and the seedlings pulverized after transplanting to the fields and only 25% and 50% of the recommended fertilizer dose was used.

## 4. CONCLUSION

The vigor of young rice plants increased for those inoculated by endophytes in the laboratory. Apart from the number of tillers, the inoculation of the seeds with the different endophytic bacterial isolates had a considerable impact on the young plants compared to the uninoculated controls. All the endophyte treatments fertilized with 50% of urea gave higher yields and 1000 grain weights compared to the non-inoculated control treatments which received 100% recommended fertilizers. But, the endophyte Adny 9 gave the higher increases in yield and 1000 grain weight. Thus, inoculated rice with the endophyte Adny9 in the presence of 50% of the recommended nitrogen fertilizer could be used to improve rice yield while reducing the quantity of chemical fertilizer formerly used.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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