

Use of Native Inoculants in Rice (*Oryza sativa* L.) Improving Physiology and Health Under Semi-Controlled Germination Conditions

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Abstract

Original Research Article

Rice (*Oryza sativa*) is an important cereal, it represents the staple food of half of the world's population, it provides nutrients such as carbohydrates, lipids and proteins. The objective of study is to evaluate the treatment of rice seed with biologicals, determining its germination, vigor and phytosanitary. The treatments evaluated were eight using a seed inoculation with two biologicals based on *Trichoderma harzianum* (1×10^{12} CFU/g) at a rate of 0.661g/kg of seed and *Bacillus subtilis* (1.8×10^{12} CFU/ml) more *Pseudomonas fluorescens* (1.4×10^{12} CFU /ml) at 2.2 ml/kg of seed, these were: T1: Anar 2021, T2: Santa Rosa, T3: Lazarroz, T4: Irga 424, T5: Anar 2021 (without inoculant), T6: Santa Rosa (without inoculant), T7: Lazarroz (without inoculant) and T8: Irga 424 (without inoculant). The variables evaluated were germination, vigor, vigor index, foliar incidence of bacteria and seedling mortality. The study carried out demonstrated significant differences between treatments and interactions in germination, vigor, vigor index, foliar incidence of bacteria and seedling mortality. T1: Anar 2021 reached higher values of germination, vigor and vigor index; 93 %, 90 % and 2,056 % respectively; being lower values of foliar incidence of bacteria and mortality of seedlings with 4.2 % and 6.0 % respectively.

Keywords: *Bacillus subtilis*, *Pseudomonas fluorescens* y *Trichoderma harzianum*.

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I. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of half the world's population, accounting for 21 % of global caloric intake [1]. Southeast Asia cultivates and consumes 90% of rice; 60% of the world's population lives in Asia. It is grown in 115 countries around the world due to its high flavonoids, vitamin B1, phospholipids, amino acids, and lipids [2,3].

Ackaah *et al.*, [4] isolated 13 genera of seed-associated fungi from farmers in three districts, showing incidences of 72.2% and 41.96% due to grain discoloration by *Bipolaris*, *Fusarium*, *Aspergillus*, *Curvularia*, *Botryodiplodia*, and *Alternaria*. Biotic factors are linked to fungal, bacterial, and viral diseases. Continuous use of chemicals affects the environment and human health, and generates resistance in pathogens such as *Rhizoctonia solani*, *Helminthosporium oryzae*, *Sarocladium oryzae*, *Bipolaris oryzae*, *Fusarium*, *Pythium*, *Xanthomonas*, and *Pseudomonas* [5,6].

Genetic variations in rice are associated with the plant genome, with four predominant bacterial orders of the phyllosphere: *Pseudomonadales*, *Burkholderiales*, *Enterobacterales*, and *Xanthomonadales*. Being specific to distinct host loci, these bacteria include *Burkholderia glumae*, *Pseudomonas* spp., and *Xanthomonas oryzae* [7]. Biocontrol is a new control strategy, as the use of chemicals has caused environmental problems. *Trichoderma*, *Pseudomonas*, and *Bacillus subtilis* are biocontrol agents against pathogens under different ecological conditions, inhibiting disease growth through various mechanisms of action: competition, antibiosis, mycoparasitism, hyphal interaction, and enzyme secretion. *Trichoderma* interacts strongly with roots, soil, and the foliar environment, acting as a biocontrol agent against *Pyricularia oryzae* and *Rhizoctonia solani* [8,9]. Seekham *et al.*, [10] isolated 87 strains of *Trichoderma harzianum* and *Trichoderma asperellum* from rice seeds; they exhibited antagonistic activity against *Sarocladium oryzae* and *Ustilaginoidea virens*.

Yao *et al.*, [11] studied 45 bacterial isolates from the rhizosphere and phyllosphere of four wild rice varieties: *Oryza punctata*, *O. officinalis*, *O. nivara*, and *O. rufipogon*. Eighteen strains inhibited *Pyricularia oryzae*, while 33 strains enhanced N, P, K, IAA, chlorophyll availability, and plant growth. The identified beneficial bacteria of the genus *Bacillus* were *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. megaterium*, *B. cereus*, *B. firmus*, *B. velezensis*, *B. anthracis*, *B. thioparans*, *B. muralis*, *B. mycoides*, *B. pseudomycoides*, *B. indicus*, *B. methylotrophicus*, *B. oryzaecorticis*, *B. arbutinivirans*, *B. aryabhattai*, and *B. stratosphericus*.

Pandey *et al.*, [12] identified soil rhizobacteria in the rice rhizosphere through sequencing. *Bacillus subtilis*, *B. licheniformis*, and *B. vallismortis* had incidences of 41.4%, 39.8%, and 44.6%, respectively; these controlled *Ustilaginoidea virens*. Beneficial microorganisms are also located in the endosphere, with bacteria of the genera *Sphingomonas*, *Pseudomonas*, *Burkholderia cepacia*, and *Enterobacter* producing siderophores, facilitating iron uptake by the roots [13].

Seventy-nine percent of staple grains are in the hands of small- and medium-sized producers. In Nicaragua, certified rice seed accounts for 15.83% of the total 84,507 hectares cultivated. Rice husks are sometimes used as seeds, impairing germination and vigor at planting and reducing rice populations due to pathogens that affect phenological development [14].

The physiology of seed germination, seedling biomass, and growth period are rarely reported. Rice seed treatments are intentionally employed using biological, chemical, and physical approaches to improve seed vigor and emergence [15]. The objective of the present study was to evaluate the effect of treating rice (*Oryza sativa* L.) seeds with native biopesticides on physiological quality and health under semi-controlled conditions.

II. MATERIALS AND METHODS

A. Location and Description of the Site

The experiment was conducted in September 2023 under semi-controlled conditions at San Nicolás Farm, Malacatoya, Granada, Nicaragua. Four rice varieties were treated with native seed inoculants. The farm is located at 12°11'21" N latitude and 85°55'48" W longitude, at an altitude of 50 meters above sea level.

B. Genetic Materials

Four improved rice varieties were collected from two rice-growing areas of the country, Malacatoya and Sébaco, specifically from the farms El Plantel, San Nicolás, and JAGA Group (Table I). Several seed samples from these varieties were sent to the laboratories of the Institute for Agricultural Protection and Health (IPSA) and the National Agrarian University (UNA) for the identification of fungal and bacterial phytopathogens associated with rice seeds.

Table I: Rice Varieties Used in the Experiment

Rice varieties	Category	Farms	Locality	Collection year
Anar 2021	Registered	San Nicolas	Malacatoya	2023
Santa Rosa	Registered	El Plantel	Sébaco	2023
Lazarroz	Registered	JAGA Group	Malacatoya	2023
Irga 424	Registered	El Plantel	Sébaco	2023

C. Treatments Evaluated

Table II: Treatments Evaluated for Four Rice Varieties

Treatments	Description
T1 Anar 2021	Inoculated seed
T2 Santa Rosa	Inoculated seed
T3 Lazarroz	Inoculated seed
T4 Irga 424	Inoculated seed
T5 Anar 2021	Without treatment
T6 Santa Rosa	Without treatment
T7 Lazarroz	Without treatment
T8 Irga 424	Without treatment

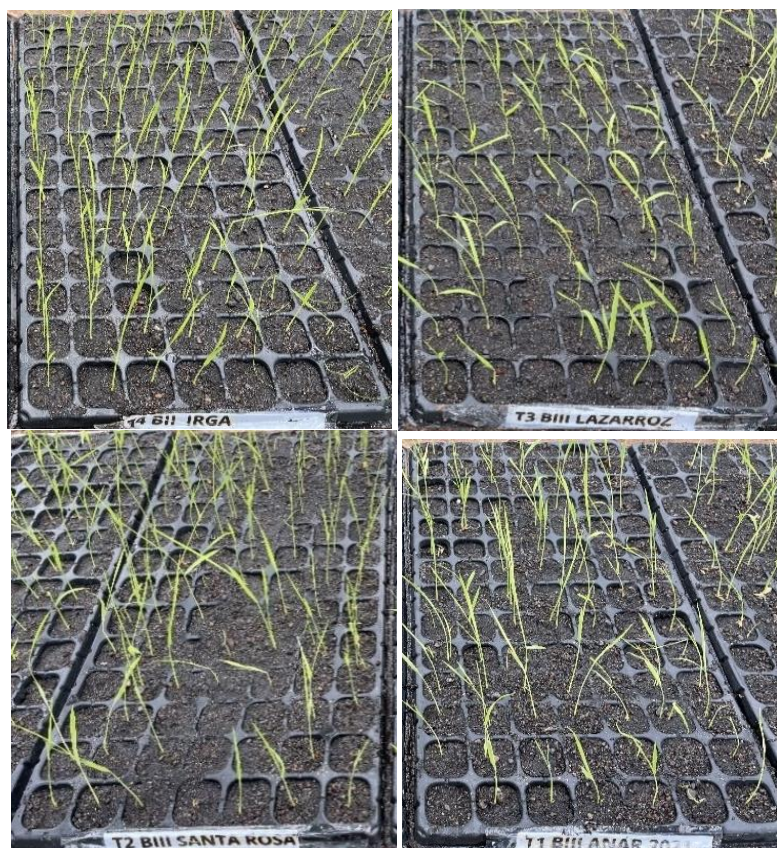


Fig. 1: Four rice varieties inoculated with biopesticides: T1 Anar 2021, T2 Santa Rosa, T3 Lazarroz and T4 Irga 424

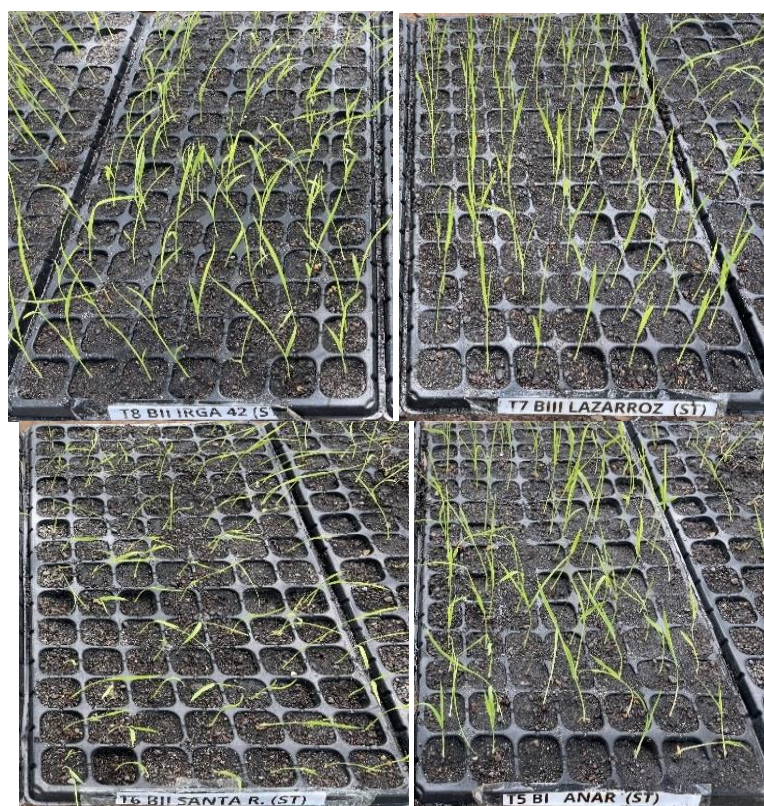


Fig. 2: Four rice varieties without inoculated treatment: T5 Anar 2021, T6 Santa Rosa, T7 Lazarroz and T8 Irga 424

Eight treatments were evaluated using four varieties of rice, seed treatments with native inoculants

based on *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum*; established in 32 germination

trays of 128 cavities (66.40 cm long. x 33.5 cm x 7.7 cm high) made of polystyrene, sowing 100 seeds per tray, the substrate used was sand as inert material (Table II).

However, four treatments of the varieties used were left without inoculants to be compared with controls.

Table III: Biopesticides Used in Rice Seed

Products	Active ingredients	Concentration (CFU/ml)	Doses (kg/s)
Super Green Plus	<i>Bacillus subtilis</i>	1.8×10^{12}	2.2 ml
	<i>Pseudomonas fluorescens</i>	1.4×10^{12}	
Tricho Blue	<i>Trichoderma harzianum</i>	1×10^{12}	0.661 g

D. Germplasm disinfection with biological inoculants

Before inoculation, the seeds were immersed in water for 24 hours to inhibit dormancy, then drained for two hours. Subsequently, treatments were applied using two biological products antagonistic to phytopathogenic fungi and bacteria affecting rice seeds. The recommended dosage per kilogram of seed was used: 2.2 ml of the fungicide-bactericide *Super Green Plus* and 0.66 g of the biological fungicide *Tricho Blue*, diluted in 1.0 L of water per kilogram of seed. A manual sprayer was employed to apply the treatments.

E. Variables Evaluated

The variables evaluated were germination, vigor, plant height, root length, vigor index, foliar bacterial incidence in seedlings, seedling mortality, and relative frequency percentage.

F. Germination of Germplasm

Once seed inoculation was completed, sowing was carried out by placing 100 seeds per tray, with four trays per treatment: 16 trays with treated seeds and 16 trays with untreated seeds. Irrigation was applied daily to

ensure effective seed germination. Germination was evaluated seven days after sowing in all 32 trays, using the formula proposed by James [16]:

$$\text{Germination (\%)} = \frac{\text{Total number of germination seeds on 7th day}}{\text{Total number of inoculated seeds}} \times 100$$

G. Germplasm Vigor

Once seed inoculation was completed, sowing was carried out by placing 100 seeds per tray, with four trays per treatment: 16 trays with treated seeds and 16 trays with untreated seeds. Irrigation was applied daily to ensure adequate seed vigor. Seed germination was evaluated seven days after sowing in all 32 trays, using the formula proposed by James [16]:

$$\text{Vigor (\%)} = \frac{\text{Total number of seeds with vigor on 7th day}}{\text{Total number of inoculated seeds}} \times 100$$

H. Plant height and Root length

These growth variables were measured by selecting 10 plants per treatment per treatment at each evaluation interval. Plant height and root length were recorded at seven days of growth. Plant height was measured from the stem base to the tip of the tallest leaf, while root length was measured from the stem base to the tip of the longest root.

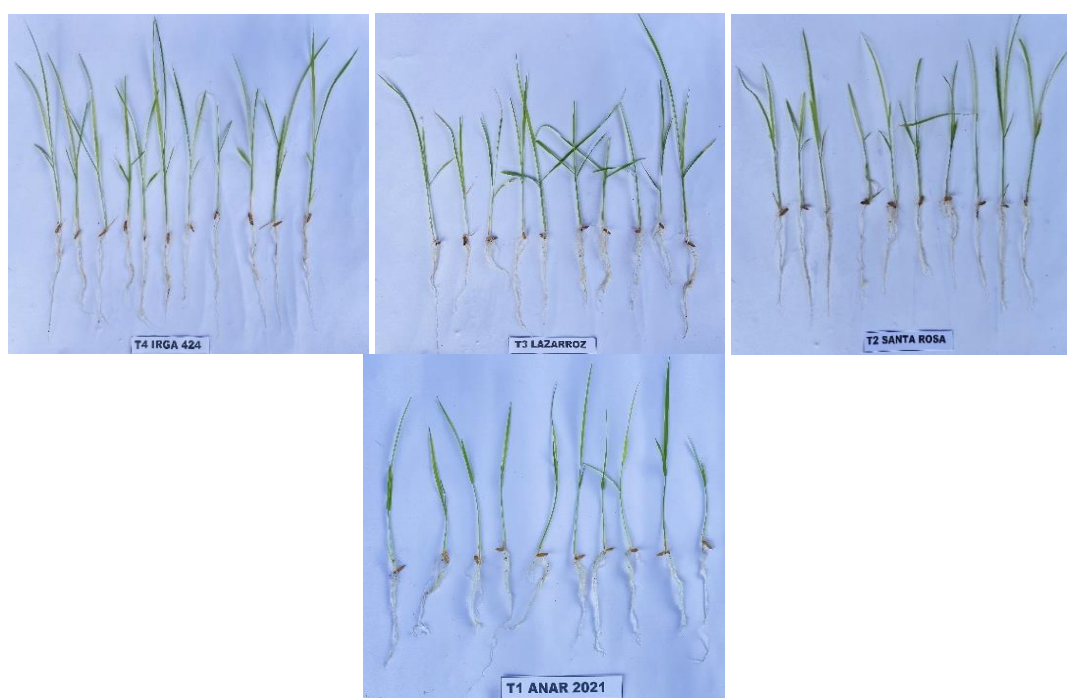


Fig. 3: Seedling height and root length: T1 Anar 2021, T2 Santa Rosa, T3 Lazarroz, T4 Irga 424 at 22 days after inoculation

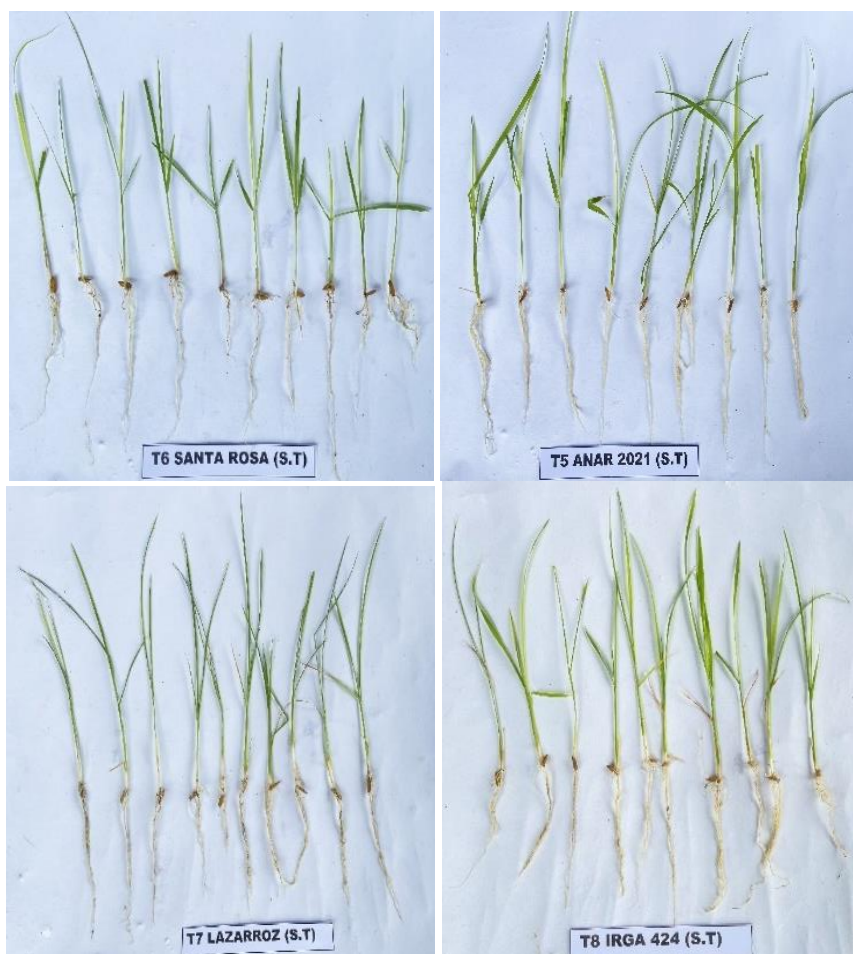


Fig. 4: Seedling height and root length: T5 Anar 2021, T6 Santa Rosa, T7 Lázaro and T8 Irga 424 at 22 days after sowing

I. Seedling vigor index

This variable was evaluated by measuring plant vigor at seven days after sowing, based on plant height and root length recorded from four replicates. Both variables were measured in centimeters, then added together and multiplied by the germination percentage, according to the formula proposed by Abdul-Baki & Anderson [17]:

P.V. I = Percentage Vigor Index.

$$\text{I.V.P (\%)} = [\text{Root length} + \text{Plant height}] \times \text{germination (\%)}$$

J. Seedling mortality

This variable was evaluated at each interval by counting the number of dead seedlings in each tray, considering four replicates across all treatments. Data was expressed as percentages, with evaluations conducted at 7, 12, 18, and 22 days after application. The formula applied was proposed by James [16]:

$$\text{Seedling mortality (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of plants evaluated}} \times 100$$

K. Foliar bacterial incidence (%)

This variable was evaluated at each interval by counting the number of seedlings with infected leaves in each tray, considering four replicates across all

treatments. Data was expressed as percentages, with evaluations conducted at 7, 12, 18, and 22 days after application. Incidence was calculated using the formula proposed by James [16]:

$$\text{Foliar incidence of bacteria (\%)} = \frac{\text{Number of plants with foliar symptoms}}{\text{Total number of plants evaluated}} \times 100$$



Fig. 5: Rice seedlings with foliar incidence of bacteria such as *Burkholderia* and *Xanthomonas*



Fig. 6: Dead plants caused by bacteria *Burkholderia* and *Pseudomonas* versus healthy plants at 22 days after application

L. Identification of pathogens found in rice seeds

Two hundred-gram samples of rice seeds from each studied variety were sent to the IPSA and UNA laboratories for the identification of fungi and bacteria associated with the seeds.

M. Statistical Analysis

The experimental design was a Completely Randomized Block Design with a factorial arrangement, consisting of eight treatments and four replicates, for a total of 32 experimental units (trays measuring 66.4 cm × 33.5 cm × 7.7 cm). Data recorded for germination, vigor, plant height, root length, vigor index, seedling mortality, foliar bacterial incidence, and relative frequency percentage were subjected to a Shapiro-Wilk test for normality and an analysis of variance. Mean separation was performed using Tukey's test at $P < 0.05$. All analyses were conducted using SAS 9.1 for Windows.

III.RESULTS AND DISCUSSION

The analysis of variance for the germination variable showed significant differences ($P < 0.05$) in the variety × germination interaction. The coefficient of determination ($R^2 = 0.75$) indicated that the model was adequate and provided a good fit between treatments. The coefficient of variation ($CV = 5.8\%$) demonstrated that the data was homogeneous. Seed treatment with *Pseudomonas fluorescens* improved germination, reaching values between 90.0% and 100.0%, whereas the control achieved only 34.0%. The varieties Bossome Mmienu, Agra Wiawso, and Mercy Juaboso reached germination rates of 94.6%, 93.3%, and 91.3%, respectively [4,18]. Similar results were reported for T1: Anar (93.0%), Irga 424 (88.0%), and the control T7: Lazarroz (75.0%).

Table IV: Analysis of Variance of Germination

Source variation	SS	DF	MS	F	P-value
Block	242.0	1	242.0	10.1	< 0.0062
Variety	610.6	3	203.5	8.5	< 0.0015
Germination	25.1	3	8.3	0.3	> 0.7898
Variety*germination	238.1	9	26.4	1.1	< 0.4147
	$R^2 = 0.75$		C.V= 5.8%		

SS= Sum of squares; DF = Degrees of freedom; MS = Mean squares; F= F-statistic; P = Probability value; R^2 = Coefficient of determination; C.V= Coefficient of variation; Significant effects ($P < 0.05$) indicate among treatments or their interaction.

Alattas *et al.*, [19] demonstrated that *Pseudomonas* spp., as a biocontrol agent, improved plant health through the production of antimicrobial compounds, nutrient competition, and increased seed germination. Kunwanlee and Plodjinda [20] evaluated seed treatment of the Jasmine Red Rice variety with *Trichoderma harzianum*, which increased the germination rate to 90.5%, thereby improving physiological quality compared to the uninoculated control. Similar results were obtained for T1: Anar 2021, with 93.0% germination, surpassing the control by 19.35% seven days after sowing.

Inoculation with native strains of *Bacillus subtilis* and *Trichoderma* improved growth, achieving rapid and synchronized germination (90.6%), greater plant length (30.6 cm), and increased vigor [21,22]. The use of inoculants in rice seed produced results similar to T1: Anar 2021, which reached 93.0% germination. *Trichoderma harzianum* enhanced seedling growth, ranging from 15.0% to 39.0%, while root length increased from 24.0% to 48.0% [23]. The Malacatoya

study reported higher values in plant height (14.6%–33.7%) and root length (39.3%–45.0%) compared to the control.

The analysis of variance for the vigor variable showed significant differences ($P < 0.05$) in the variety $\times$ vigor interaction. The coefficient of determination ($R^2 = 0.9724$) indicated that the model provided a good fit, explaining variability among treatments. The coefficient of variation ($CV = 1.9\%$) demonstrated data homogeneity and the absence of dispersion. Raj *et al.*, [24] reported greater vigor and faster germination (8–11 days), with higher growth and vigor obtained by T4: *Phosphobacteria* and T10: *Azospirillum lipoferum* + *Phosphobacteria*. Rajer *et al.*, [25] used inoculants *Bacillus velezensis*, *B. atrophaeus*, *B. inaquosorum*, *B. subtilis*, and *B. pumilus*, increasing vigor index values ranging between 78.8% and 108.7%. These findings support our results, where *T. harzianum*, *B. subtilis*, and *P. fluorescens* reached 90.0% vigor in T1: Anar 2021, surpassing T7: Lazarroz, which achieved 70.0%.

Table V: Analysis of Variance of Vigor

Source variation	SS	DF	MS	F	P-value
Block	675.2	1	675.2	279.6	< 0.0001
Variety	492.3	3	164.1	67.9	< 0.0001
Germination	34.5	3	11.5	4.7	< 0.0157
Variety*vigor	76.7	9	8.5	3.5	< 0.0152
	$R^2 = 0.9724$		C.V= 1.9 %		

SS= Sum of squares; DF = Degrees of freedom; MS = Mean squares; F= F-statistic; P = Probability value; R^2 = Coefficient of determination; C.V= Coefficient of variation; Significant effects ($P < 0.05$) indicate among treatments or their interaction.

The analysis of variance for plant height showed significant differences ($P < 0.05$) in the variety $\times$ height interaction. The coefficient of determination ($R^2 = 0.72$) indicated that the model provided a good fit between treatments and the variable. The coefficient of variation ($CV = 11.4\%$) demonstrated data homogeneity and the absence of dispersion. Inoculation with native biopesticides favored the increase in plant height.

EurekaAlert [26] reported that microbial inoculants applied by immersion or seed coating with *Bacillus subtilis* and *Trichoderma asperellum* achieved significant effects on the initial development of rice plants, reaching an average height of 116 cm compared with the control treatment, while also improving grain yield by 100%, evidencing their potential as a sustainable strategy to enhance establishment and productivity.

Table VI: Analysis of Variance of Plant Height

Source variation	SS	DF	MS	F	P-value
Block	38.9	1	38.9	20.3	< 0.0004
Variety	19.5	3	6.5	3.4	< 0.0451
Plant height	2.5	3	0.8	0.4	> 0.7213
Variety*plant height	14.9	9	1.6	0.8	< 0.5729
	$R^2 = 0.72$		C.V= 11.5 %		

SS= Sum of squares; DF = Degrees of freedom; MS = Mean squares; F= F-statistic; P = Probability value; R^2 = Coefficient of determination; C. V= Coefficient of variation; Significant effects ($P < 0.05$) indicate among treatments or their interaction.

Seekham *et al.*, [10] studied 87 strains of *Trichoderma*, of which 12 exhibited strong antagonistic properties against pathogens of the RD59 variety, being effective in promoting root length (9.7 cm) and plant height (29.0 cm). Parveen *et al.*, [27] isolated 125

endophytic microorganisms from four rice varieties, of which 100 were bacteria located in the root (48.0%), stem (35.0%) and leaves (20.0%). In addition, five bacterial and five fungal isolates were identified: *Bacillus licheniformis*, *Stenotrophomonas rhizophila*, *S.*

maltophilia, *T. afroharzianum* and *T. harzianum*. These isolates promoted plant height of 34.2 cm, 33.2 cm, 32.2 cm, 32.0 cm, 31.1 cm compared with 29.1 cm in the control. The benefits achieved in the four varieties from Sébaco and Malacatoya showed significant differences.

The analysis of variance showed significant differences ($P < 0.05$) in root length for the variety $\times$ root length interaction. Furthermore, the coefficient of determination ($R^2 = 0.9688$) indicated that the model presented a good fit, explaining the variability among varieties in root length. The coefficient of variation (11.3%) indicated that the data was homogeneous and

showed no dispersion, confirming the reliability of the model.

Carderelli *et al.*, [28] reported that rice seed treatment with growth-promoting bacteria such as *Bacillus* spp. and *Paenibacillus yonginensis* increased root length by 26%. In the present study, a higher increase of 39.3% in root length was achieved with *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Trichoderma harzianum*, compared to the control. Varieties that did not receive any treatment showed reduced root growth and development, being significantly surpassed by the treated groups.

Table VII: Analysis of Variance of the Root Length

Source variation	SS	DF	MS	F	P-value
Block	36.3	1	36.3	47.0	< 0.0001
Variety	317.5	3	105.8	136.8	< 0.0001
Length	0.8	3	0.2	0.3	< 0.7847
Variety*Length	5.8	9	0.6	0.8	< 0.5884
	$R^2 = 0.9688$		C.V=11.3 %		

SS= Sum of squares; DF = Degrees of freedom; MS = Mean squares; F= F-statistic; P = Probability value; R^2 = Coefficient of determination; C. V= Coefficient of variation; Significant effects ($P < 0.05$) indicate among treatments or their interaction.

Significant differences ($P < 0.05$) were observed in vigor index percentages among the treatments evaluated. T4: Irga 424 treated with antagonistic biologicals reached the highest vigor index (2,553%), while the lowest was observed in T2: Santa Rosa (1,377%). Swain *et al.*, [29] evaluated six

Trichoderma strains and reported the highest vigor indices in *T. erinaceum* on the Sahabhazi dhan variety (3,270%) and *T. harzianum* (3,003.3%). The maximum vigor index obtained under semi-controlled conditions in this study was like those reported in Malacatoya (Figure 1).

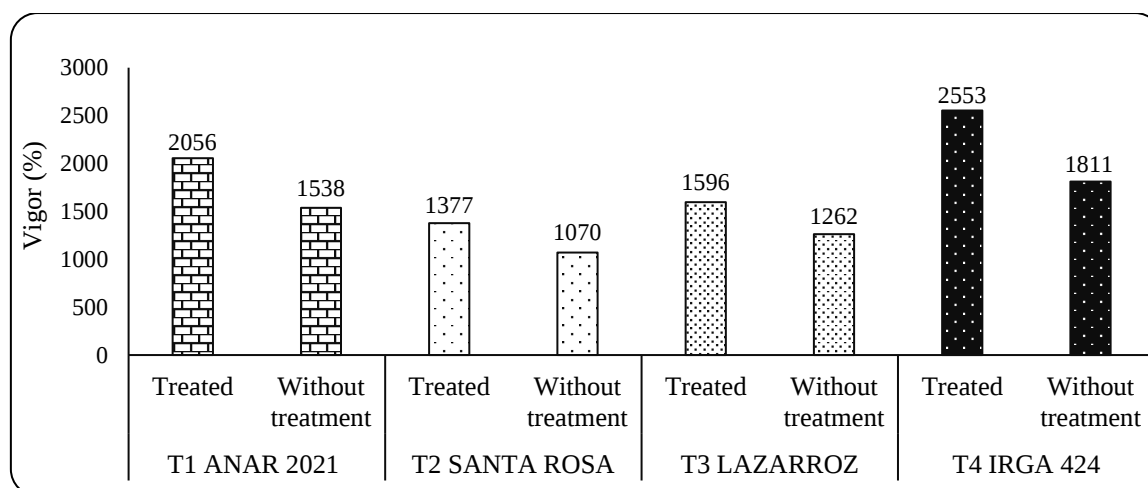


Fig. 9: Percentage of seedling vigor index at 7 days after sowing

The ANOVA showed significant differences ($P < 0.05$) in seedling mortality among treatments. The highest mortality was observed in T6: Santa Rosa, which did not receive any seed inoculant, reaching 9.2%, 17.2%, 19.2%, and 21.2% at 7, 12, 18, and 22 days after sowing (DAS), respectively. In contrast, T4: Irga 424 showed the lowest mortality values, with 1.0%, 2.0%,

2.7%, and 3.0% at 7, 12, 18, and 22 DAS, respectively (Table 8). These results indicate that treating rice seeds with biopesticide inoculants was crucial, as it reduced seedling mortality, enhanced seedling health, and preserved seedling vigor under semi-controlled conditions.

Table VIII: Percentage Mortality of Rice Seedling Under Semi-Controlled Conditions

Treatment	Seed Inoculant	7 das	12 das	18 das	22 das
T1 Anar 2021	Super Green Plus + Tricho Blue	3.5 bc	4.7 bc	5.5 bc	6.0 cde
T2 Santa Rosa	Super Green Plus + Tricho Blue	2.5 bc	3.7 bc	4.5 cd	7.2 bcd
T3 Lazarroz	Super Green Plus + Tricho Blue	1.0 c	4.0 bc	5.2 bc	6.0 cde
T4 Irga 424	Super Green Plus + Tricho Blue	1.0 c	2.0 c	2.7 d	3.0 e
T5 Anar 2021	Without inoculant	5.7 ab	6.5 b	7.2 b	8.5 bc
T6 Santa Rosa	Without inoculant	9.2 a	17.2 a	19.2 a	21.2 a
T7 Lazarroz	Without inoculant	2.0 c	5.2 bc	7.5 b	10.5 b
T8 Irga 424	Without inoculant	2.5 bc	3.7 bc	4.5 cd	4.2 de
C.V%		35.0	18.8	15.0	16.6
R ²		0.69	0.91	0.96	0.95
Tukey (P 0.0 5%)		*	*	*	*

Means with a common letter are not significantly different (P <0.05); das= days after sowing

Two bacterial species were identified at the UNA laboratory in the Santa Rosa variety: *Burkholderia* (4.0%) and *Pseudomonas* (4.0%), as well as the fungus *Aspergillus* spp. (4.0%). These bacteria were responsible for the highest seedling mortality, reaching 21.2% in the last evaluation. The Lazarroz variety showed 10.5% dead plants at 22 DAS, with the following pathogens identified: *Fusarium* (2%), *Dreschslera* (1%), *Helminthosporium sativum* (2%), *Aspergillus* (4%), *Rhizopus* (1%), *Pseudomonas* (4%), and *Bacillus* (4%). The latter two bacterial species caused damage to rice seedlings throughout all field evaluations.

Al Amin *et al.*, [30] conducted a pathogenicity test by inoculating BRRIdhan29 rice seeds from various locations in Bangladesh to demonstrate the fungal inoculum of *Fusarium moniliforme*. Control seeds were wet with distilled water only and sown in pots. Five biocontrol agents—*Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma* spp., and *Achromobacter* spp.—showed growth inhibition of 71.6%, 58.6%, 69.8%, and 73.5%, respectively, against Bakanae disease (*Fusarium moniliforme*).

The ANOVA indicated significant differences (P < 0.05) among treatments in foliar bacterial incidence. T6: Santa Rosa showed the highest incidence values, reaching 4.7%, 5.5%, 6.5%, and 9.0% at 7, 12, 18, and 22 DAS, respectively. In contrast, T1: Anar 2021 exhibited the lowest incidence values, with 1.7%, 2.0%, 4.0%, and 4.2% at 7, 12, 18, and 22 DAS, respectively. These results demonstrate that seed treatment with biopesticides was effective in reducing foliar bacterial incidence in rice seedlings. The bacteria identified in the laboratory were *Pseudomonas* spp. and *Burkholderia glumae*.

Studies conducted at the IPSA laboratory on four rice varieties identified the fungus *Fusarium* with low incidence values, ranging from 1.0% in the Irga 424 variety to 2.0% in Lazarroz. This confirms the presence of this important pathogen in rice seeds. Nurcahyanti *et al.*, [31] demonstrated that formulations composed of *Pseudomonas putida*, *Bacillus subtilis*, and *Trichoderma* spp. suppressed bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) by 21.0% and rice blast (*Pyricularia oryzae*) by 4.0%. In this study, the T6: Santa Rosa variety reached 9.0% foliar bacterial incidence, which was higher under semi-controlled conditions.

Table IX: Percentage of Foliar Incidence of Bacteria Under Semi-Controlled Conditions

Treatment	Seed inoculant	7 das	12 das	18 das	22 das
T1 Anar 2021	Super Green Plus + Tricho Blue	1.7 b	2.0 c	4.0 b	4.2 b
T2 Santa Rosa	Super Green Plus + Tricho Blue	2.5 ab	3.5 bc	4.2 ab	4.7 b
T3 Lazarroz	Super Green Plus + Tricho Blue	2.7 ab	3.7 bc	5.0 ab	5.2 b
T4 Irga 424	Super Green Plus + Tricho Blue	3.7 ab	5.5 ab	6.2 ab	6.5 ab
T5 Anar 2021	Without inoculant	3.0 ab	4.2 bc	6.0 ab	7.0 a
T6 Santa Rosa	Without inoculant	4.7 ab	5.5 ab	6.5 ab	9.0 ab
T7 Lazarroz	Without inoculant	3.2 ab	5.0 abc	5.7 ab	6.5 ab
T8 Irga 424	Without inoculant	5.0 a	7.5 a	7.2 ab	8.2 a
C.V%		46.0	29.7	25.6	17.5
R ²		0.3863	0.6255	0.4347	0.7156
Tukey (P 0.05%)		*	*	*	*

Means with a common letter are not significantly different (P <0.05); das= days after sowing

Seven fungal genera and two bacterial genera were identified as phytopathogenic agents of rice grain.

The relative frequencies of the pathogens were: *Aspergillus* spp. 25.0%, *Helminthosporium sativum*

12.5%, *Fusarium* spp. 12.5%, *Burkholderia* spp. 12.5%, *Pseudomonas* spp. 12.5%, *Nigrospora* spp. 6.2%, *Dreschlera* spp. 6.2%, *Rhizopus* spp. 6.2%, and *Penicillium* spp. 6.2% (Figure 2). According to Khamari [32], microorganisms associated with rice seed include *Bipolaris oryzae* (2.5%–8.5%), *Helminthosporium oryzae* (2.5%–8.5%), *Alternaria padwickii* (5.3%–

13.3%), *Fusarium moniliforme* (11.6%–21.6%), *Fusarium oxysporum* (1.2%–4.3%), *Curvularia lunata* (1.9%–7.5%), *Aspergillus* spp. (1.7%–6.5%), *Nigrospora oryzae*, *Penicillium* spp., *Rhizopus stolonifer*, *Dreschlera oryzae*, *Pyricularia oryzae*, *Pseudomonas fuscovaginae*, *Burkholderia glumae*, and *Xanthomonas oryzae*.

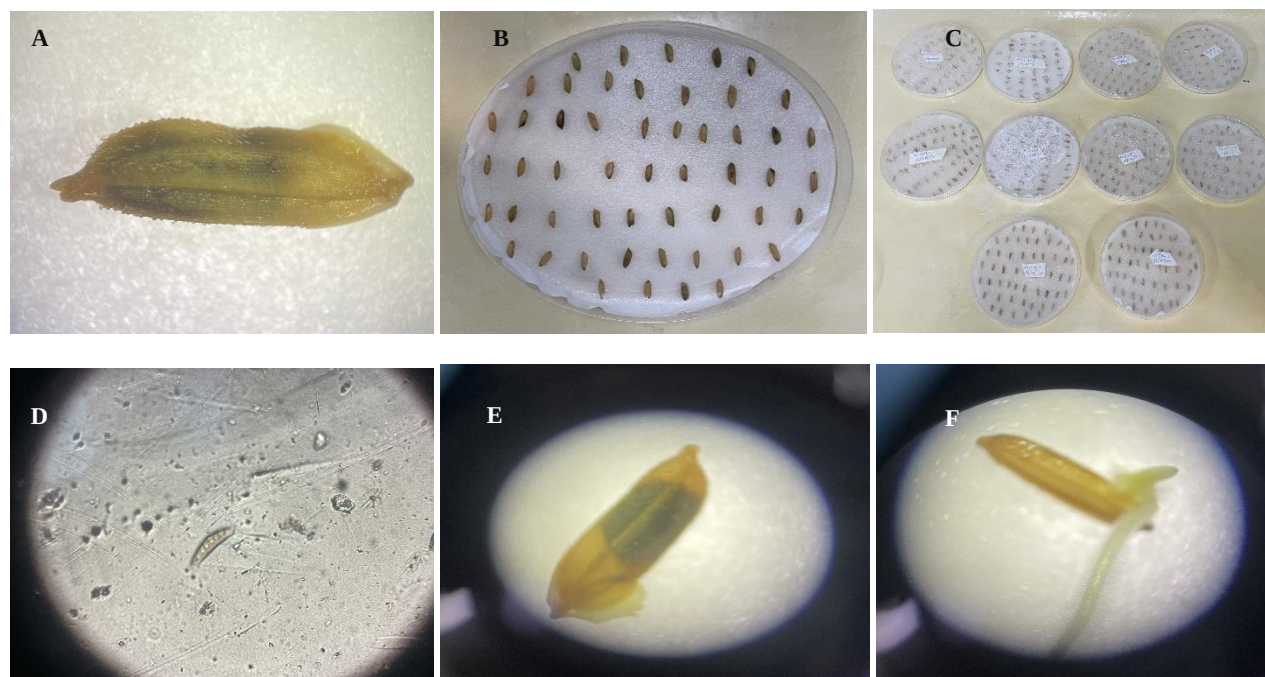


Fig. 10: A) y B) Grains subjected to laboratory analysis, C) y D) Grains severely affected by pathogens, E Healthy grain with good vigor, F) *Dreschlera* spp. Identified in the grains by the IPSA laboratory

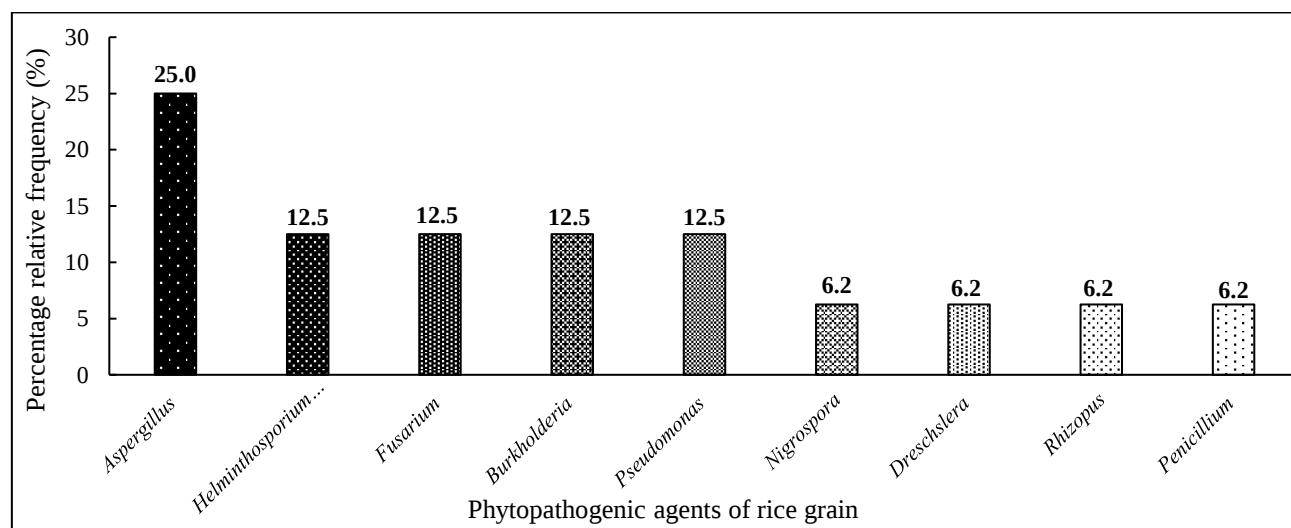


Fig. 11: Relative percentage frequency of phytopathogens affecting rice seeds

Chen *et al.*, [33] conducted an in-depth analysis of the dynamics and composition of bacterial communities associated with rice seeds, showing that genera such as *Pantoea*, *Pseudomonas*, and *Sphingomonas* play key roles in seed colonization and vertical transmission to seedlings. Fungi associated with rice grains reduces grain quality and impair seed

germination [34]. Globally, approximately 150 fungal species have been reported, with *Alternaria alternata*, *Alternaria padwickii*, *Aspergillus flavus*, *Aspergillus niger*, and *Bipolaris oryzae* being the most common. Comparable findings were observed in Malacatoya, where *Burkholderia* and *Pseudomonas*, as well as

Aspergillus, *Helminthosporium*, *Fusarium*, *Nigrospora*, *Dreschlera*, *Rhizopus*, and *Penicillium*, were detected.

CONCLUSIONS

Seed inoculation of four improved rice varieties was effective, showing significant differences in the interactions between treatments and variables. Treatment 1 (Anar 2021) achieved the highest germination (93.0%), vigor (90.0%), and vigor index (2,056%) at the final evaluation.

Treatment 1 (Anar 2021) preserved rice seedling health by reducing foliar bacterial incidence by 48.7% compared to T8. Furthermore, this treatment was superior to T6, showing 71.6% lower seedling mortality at the final evaluation.

The diseases identified in the IPSA and UNA laboratories included *Fusarium* spp., *Nigrospora* spp., *Helminthosporium sativum*, *Dreschlera* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., *Burkholderia glumae*, and *Pseudomonas* spp.

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