**Effects of Plant Extracts and Pesticides on Rice Seed Microbial Communities in Burkina Faso.**

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ABSTRACT

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| **Aims:** As the first link in the plant production chain, seeds play a crucial role in establishing healthy, resilient crops. Contamination at this stage can reduce yields. **Objective:** This study aimed to evaluate the efficacy of two plant extracts compared to two chemical fungicides in promoting the health of three rice varieties grown in Burkina Faso, in terms of both fungi and bacteria. **Methodology:** The grains of three rice varieties were treated. Before and after treatment, the microbial community and germination rates on their seeds were assessed.**Results:** Although some varieties showed a significant reduction after treatment, the seeds retained an overall good germination capacity (>80%). Mycological analysis of the untreated seeds revealed 16 fungal species belonging to 14 genera, eight of which were rice pathogens. *Fusarium moniliforme* was the most prevalent species, particularly in the FKR62N variety. The plant extracts exhibited significant antifungal activity, achieving reduction rates of over 75% in more than 75% of cases. This result was often equivalent to or better than that of the fungicides. However, copper hydroxide sometimes stimulated the growth of undesirable fungi, such as *Aspergillus flavus* and *A. niger*. Analysis of bacterial diversity revealed 23 isolates in 10 genera, including *Enterobacter*, *Kosakonia*, *Klebsiella*, *Pseudomonas*, *Bacillus* and *Methylobacterium*. The FKR64 variety exhibited the greatest bacterial richness, with *Bacillus* and *Kosakonia* present throughout, even after treatment. Herbal treatments generally preserved greater microbial diversity than fungicides. However, mancozeb exhibited greater activity, albeit with a variable impact on bacterial communities. **Conclusion:** These results highlight the potential of *L. multiflora* and *E.* *alba* extracts as natural alternatives to chemical fungicides for improving the sanitary quality of rice seeds while preserving their beneficial microbiome. |

***Keywords: Rice, bacteria, fungi, plant extracts, chemicals pesticides, Burkina Faso***

1. INTRODUCTION

Rice (Oryza sativa L.) is a key crop in West Africa, particularly in Burkina Faso, where it plays a vital role in ensuring food security (Traoré *et al.*, 2016). However, rice production in this region is heavily impacted by various fungal diseases (Ouedraogo *et al.*, 2016; Savary *et al.*, 2019), necessitating the frequent use of chemical fungicides (Nicolopoulou-Stamati *et al.*, 2016; Popp *et al.*, 2013). Meanwhile, interest in plant extracts as an eco-friendly alternative to conventional crop protection products is growing rapidly (Zaker, 2016). It is essential to understand the impact of these treatments on the rice microbiome is essential for managing crop health sustainably (Compant *et al.*, 2019; J. Edwards *et al.*, 2015; Toju *et al.*, 2018).

The rice microbiome comprises diverse microorganisms (prokaryotes and microeukaryotes) in the rhizosphere, phyllosphere and endosphere plays a crucial role in rice growth and development (Barro *et al.*, 2022; Danso Ofori *et al.*, 2024; J. Edwards *et al.*, 2015; J. A. Edwards *et al.*, 2018; Sondo *et al.*, 2023a; Sondo *et al.*, 2023b; Turner *et al.*, 2013). It stimulates germination, growth, and pathogen resistance (Chen *et al.*, 2024; Liu *et al.*, 2024; Misu *et al.*, 2025; Zhao *et al.*, 2024). Complex interactions between the microbiome and phytosanitary treatments can affect rice's health and productivity. However, careful management of these treatments is necessary to ensure compatibility with biocontrol agents and to maintain microbial diversity, as some chemicals can reduce the effectiveness of beneficial microorganisms (Moccellin *et al.*, 2024).

Synthetic fungicides, such as copper hydroxide and mancozeb, are commonly used to control fungal diseases, such as blast and Fusarium (Kansoh *et al.*, 2000; Nguyen *et al.*, 2023). However, despite their effectiveness, these fungicides raise major environmental and health concerns. For example, copper hydroxide can accumulate in soil and water, leading to long-term ecological impacts, and mancozeb poses potential risks to human health (Kansoh *et al.*, 2000; N. Kumar *et al.*, 2023; Nguyen *et al.*, 2023). Additionally, the use of these chemicals can disrupt the balance of soil microbes, reducing the diversity of beneficial communities and favoring the emergence of pathogenic or resistant microorganisms (Fournier *et al.*, 2020; R. Meena *et al.*, 2020; Onwona-Kwakye *et al.*, 2020).

A central question arises: how do plant extracts, such as those from *Lippia multiflora* and *Eclipta alba*, affect the diversity and composition of the rice microbiome compared to chemical fungicides? These plants contain active compounds, including thymol, carvacrol, geraniol, and p-cymene in *Lippia multiflora* and linalool, caryophyllene, eucalyptol, and alpha-pinene in *Eclipta alba*. These compounds have demonstrated interesting antifungal properties (Balboné *et al.*, 2022; Bassole *et al.*, 2003; S. Kumar *et al.*, 2019). Using these extracts could provide an ecological alternative to chemical fungicides by maintaining or improving rice's microbial diversity while reducing the environmental and health impacts of chemicals. This study aims to examine the impact of plant extracts on the diversity and efficacy of the rice seed microbiome compared to the use of chemical fungicides.

This study compares the effects of plant extracts and chemical fungicides on rice seed microbiome composition, growth and pathogen resistance. The study aims to shed light on the potential of natural solutions for the sustainable management of crop health while preserving the environment.

2. material and methods

**2.1 Rice seeds**

The plant material used in this study consisted of three (03) rice varieties commonly grown in in Burkina Faso rice fields and produced by INERA in the Karfiguela rice plain (Table 1). All varieties are adapted to irrigated lowlands.

**Table 1. Characteristics of rice varieties**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Varietal type** | **Ecology** | **Time of maturity** | **Yield (t/ha)** |
| **FKR 19** | Japonica | Lowland | 95 | 4-6 |
| **FKR 62 N** | Nerica L | Lowlands /irrigated | 116 | 6-7 |
| **FKR 64/****TS2** | Indica | Lowlands /irrigated | 120 | 6,50-7 |

**2.2. Plant extracts and synthetic fungicides**

In this study, the impact of two aqueous plant extracts on seed germination and associated microorganisms was assessed. The first extract came from *Eclipta alba* L. Hassk. (Asteraceae), an annual herbaceous plant that primarily grows in humid environments, particularly alongside irrigation canals (Uddin *et al.*, 2010). The second extract came from *Lippia multiflora* Moldenke (Verbenaceae), an aromatic shrub with angular, branched stems (Owolabi *et al.*, 2009). The aqueous extracts of *E. alba* and *L. multiflora* were colleted in Gampela (Burkina Faso). They were prepared by macerating 5 g of shade-dried , ground leaves in 100 mL of sterile distilled water for 24 hours, followed by filtration to obtain a 5% aqueous solution.

In parallel, two synthetic fungicides that are frequently used in Burkina Faso were selected for their ability to inhibit fungal spore germination. These were a broad-spectrum contact fungicide-bactericide based on copper hydroxide and a mancozeb-based fungicide.

**2.3. Germination evaluation**

The germination rate was evaluated by placing four batches of 25 grains, for a total of 100 grains per rice variety, on moistened blotting paper in Petri dishes. The dishes were then incubated at room temperature (approximately 25 °C) for five (05) days. After this period, the number of grains that produced a radicle at least 2 mm long was counted to determine germination. The germination rate was calculated using the following formula :

Germination Rate (%) = (Number of Germinated Grains / Total Number of Grains Tested) × 100

**2.4. Antifungal treatment of seeds**

Two treatment methods were employed. In the treatment involving plant extracts, 400 rice grains were immersed in 20 mL of an aqueous solution. The grains were then incubated for 24 hours at 28 °C before undergoing incubation using the modified blotting paper method. This method involved testing 200 seeds per variety, with 25 seeds placed on moistened blotting in each 90-mm Petri dish.

For the fungicide treatment, 50 g of seeds were coated with 0.1 g of fungicide (mancozeb or copper hydroxide) and incubated at room temperature for 24 hours. As a control batch, 400 seeds were immersed in sterile distilled water. After treatment, 200 seeds were incubated using the modified blotting paper method. The incubation process took place at 25 °C under a 12-hour cycle of UV light and darkness for seven (07) days. The first observations were made on day 4.

**2.5. Analysis of fungal flora**

The effect of the treatments on the fungal community was assessed by identifying the fungi present on the treated and untreated grains.

The fungi were identified first with a binocular magnifying glass and then with an optical microscope based on their morphological characteristics (e.g., conidia, mycelium, and chlamydospores) according to the criteria of Mathur and Kongsdal (2003). Fungal frequency was calculated using the following formula :

Fungal frequency (%) = (Number of seeds infested by a fungus/Total number of seeds incubated) × 100.

The rate at which the treatments reduced the fungus was also calculated.

Reduction rate = [fungus abundance on untreated control - fungus abundance on treated sample] / fungus abundance on untreated control x 100.

**2.6. Analysis of bacterial microflora**

The bacterial community present before and after treatment was assessed using the Liquid Essay method (Agarwal & Sinclair, 1996). Three batches of 400 grains of each variety/treatment were ground and suspended in 20 mL of sterile distilled water for 24 hours. After centrifugation, serial dilutions were spread on Nutrient Agar (NA) medium. Bacterial colonies representative of diversity were collected and purified based on color and morphology.

**2.7. Sequencing of bacteria**

Total bacterial DNA was extracted from the isolates using the rapid proteinase K method described by Wilson (2001). The 16S rRNA gene was amplified by PCR using the primers FGPS1509 (AAGGAGGATCCAGCCGCA) and FGPS6 (GGAGTTAGATCTGGCTCAG), following the recommendations of Normand *et al.* (1992). The PCR reaction followed a standard protocol comprising an initial denaturation step at 94 °C for two minutes, followed by 35 cycles of 30-second denaturation at 94 °C, 30-second hybridization at 55 °C, and one-minute extension at 72 °C, followed by a final extension step at 72 °C for 10 minutes. The integrity and quality of the amplified DNA were verified by agarose gel electrophoresis targeting a fragment of around 1500 bp. Sequencing was performed by Genoscreen using the Sanger method. High-quality PCR products were sequenced using the internal primer 16S-1080.r (GGGACTTAACCCAACATCT) in accordance with Moulin *et al.* (2001). The obtained sequences were aligned and compared with the NCBI database using the BLAST tool to identify bacterial species.

**2.8. Statistical Analysis of Data**

Statistical analyses were carried out using R 3.6.0 software. Following a Shapiro-Wilk normality test, a non-parametric Kruskal-Wallis test was performed to compare the effects of treatments on germination rate and microbial diversity at the 5% threshold.

3. results and discussion

**3.1 Results**

**3.1.1. Impact of treatments on germination rate**

The seeds retained a high germination capacity of above 80% before and after treatment. However, statistical analysis revealed a significant reduction in the germination rate of the treated seeds compared to the untreated control, except for the *Eclipta alba* and *Lippia multiflora* treatments for the FKR19 and FKR64 varieties, respectively (Figure 1). No significant difference was observed among the different treatments for FKR62N.



**Fig. 1.** **Effect of treatments on the germination capacity of rice seeds**

**3.1.2. Diversity of fungal microorganisms on rice seeds**

The aim of this analysis was to evaluate the frequency and pathogenic or saprophytic nature of the fungi present. The mycological analysis of untreated rice seeds revealed that the fungal diversity varied by variety.

A total of 16 fungal species belonging to 14 different genera were identified. Only two of the genera had two species each ; the rest were represented by a single species.

Thus, 12 species were recorded on FKR64, 10 on FKR62N and 11 on FKR19 (see Table 2). Eight of these species were identified as pathogenic to rice.

Overall, the infection rate by the main pathogenic fungi remained low. However, *Fusarium moniliforme* was the most frequently found species on all three varieties studied. The highest incidence of this fungus was observed in the FKR62N variety (1.81%).

Several saprophytic fungi were also detected. Although that they are not pathogenic to the plant, they are likely to alter the sanitary and physiological quality of the seeds (Table 2).

Finally, two species, *Verticillium* sp. and *Phoma lingam*, were classified as nonpathogenic in the context of this crop because they are primarily saprophytic and rarely associated with pathological symptoms in rice.

**Table 2. Fungi associated with three rice varieties**

|  |  |  |
| --- | --- | --- |
|  | **Fungi** | **Frequency of fungi (%)** |
| **FKR19** | **FKR62N** | **FKR64/TS2** |
| **Rice pathogens**  | *Curvularia lunata* *Fusarium moniliforme**Bipolaris oryzae**Pyricularia oryzae* *Nigrospora oryzae**Alternaria padwickii* *Rhizoctoria solani* | 0,561,690,310,06000 | 0,51,810,380,06000,13 | 0,561,560,190,060,060,250,19 |
|  | *Colletitrichum dematium* | 0 | 0 | 0,06 |
| **Deteriorates seed quality** | *Phoma sorghina**Cladosporium sphaerospermum* *Aspergillus niger**Aspergillus flavus**Strachybotrys chatarum**Rhizopus spp.* | 0,060,190,190,130,130 | 00,130,060,0600,13 | 0,190,380,060,0600,19 |
| **Contaminants** | *Verticillum sp.* | 0,06 | 0 | 0 |
|  | *Phoma lingam* | 0,31 | 0,06 | 0 |

**3.1.3. The impact of treatments on the frequency of fungi associated with seeds**

We compared the antifungal efficacy of two plant extracts (*Lippia multiflora* and *Eclipta alba*) to that of two chemical fungicides (copper hydroxide and mancozeb) on three varieties of rice seed.

The results show that the plant extracts exhibit strong antifungal activity against of the majority identified fungal species, achieving a reduction rates of 100% in over 75% of cases (see Table 3). Their efficacy is often comparable to or superior to that of the chemical fungicides.

Important pathogens, such as *Curvularia lunata*, *Bipolaris oryzae* and *Fusarium moniliforme*, showed variable sensitivities depending on the variety and treatment. *F. moniliforme* was reduced by 37.04 % with *L. multiflora* on FKR19, by 68.97% on FKR62N, and by 24% with *E. alba* on FKR64 (see Table 3). *B. oryzae* was effectively controlled by the plant extracts (Table 3). *C. lunata* was reduced by 100 % under certain conditions (e.g., FKR19 with copper and mancozeb), but its efficacy was reduced under others conditions (50 % with *E. alba* on FKR62N). Other rice pathogens, such as *Alternaria padwickii*, *Colletotrichum dematium*, *Nigrospora oryzae* and *Rhizoctonia solani*, were completely eliminated by all treatments when present (Table 3).

*Fusarium moniliforme* and *Aspergillus niger* exhibited greater resistance, with reductions varying by rice variety and treatment (Table 3). In some cases, copper hydroxide stimulated the growth of fungi, such as *A. flavus* and *A. niger* (Table 3).

None of the plant extracts tested promoted fungal growth on seeds. Among the treatments tested, mancozeb proved to be the most effective. Extracts of *Lippia multiflora* and *Eclipta alba* exhibited variable efficacy depending on the fungus (Table 3).

These results highlight the potential of *L. multiflora* and *E. alba* extracts as natural alternatives to conventional chemical fungicides for controlling rice fungal diseases.

**Table 3. Effect of treatments on fungi associated with rice seeds**

|  |  |
| --- | --- |
|  | **Reduction rate after treatment (%)** |
| **Variety** | **Fungi** | ***L. multiflora*** | ***E. alba*** | **C. hydroxide** | **Mancozèbe** |
| **FKR19** | *Aspergillus flavus* | 100 | 100 | 100 | 100 |
| *Aspergillus niger* | 66,67 | 100 | 100 | 66,67 |
| *Bipolaris oryzae* | 100 | 100 | 20 | -20 |
| *Cladosporium sphaerospermum* | 100 | 100 | 100 | 100 |
| *Curvularia lunata* | 77,78 | 88,89 | 100 | 100 |
| *Fusarium moniliforme* | 37,04 | 62,96 | 100 | 33,33 |
| *Phoma lingam* | 100 | 100 | 100 | 100 |
| *Phoma sorghina* | 100 | 100 | 100 | 100 |
| *Pyricularia oryzae* | 100 | 100 | 100 | 100 |
| *Strachybotrys chatarum* | 100 | 100 | 100 | 100 |
| *Verticillum cinnabanium* | 100 | 100 | 100 | 100 |
| **FKR62N** | *Aspergillus flavus* | 100 | 100 | -100 | 100 |
| *Aspergillus niger* | 100 | 100 | 0 | 100 |
| *Bipolaris oryzae* | 100 | 83,33 | -66,67 | 100 |
| *Cladosporium sphaerospermum* | 50 | 100 | 100 | 100 |
| *Curvularia lunata* | 62,50 | 50 | 100 | 100 |
| *Fusarium moniliforme* | 68,97 | 82,76 | 24,14 | 51,72 |
| *Phoma lingam* | 100 | 100 | 100 | 100 |
| *Pyricularia oryzae* | 100 | 100 | 100 | 100 |
| *Rhizoctoria solani* | 100 | 100 | 100 | 100 |
| *Rhizopus spp.* | 100 | 100 | 100 | 100 |
| **FKR64** | *Alternaria padwickii* | 50 | 100 | 100 | 100 |
| *Aspergillus flavus* | 100 | 100 | 0 | 100 |
| *Aspergillus niger* | 100 | -77,78 | -177,78 | 100 |
| *Bipolaris oryzae* | 100 | 33,33 | 33,33 | 100 |
| *Cladosporium sphaerospermum* | 83,33 | 100 | 100 | 100 |
| *Colletitrichum dematium* | 100 | 100 | 100 | 100 |
| *Curvularia lunata* | 88,89 | 77,78 | 66,67 | 100 |
| *Fusarium moniliforme* | 32 | 24 | 44 | 88 |
| *Nigrospora oryzae* | 100 | 100 | 100 | 100 |
| *Phoma sorghina* | 100 | 100 | 100 | 100 |
| *Pyricularia oryzae* | 100 | 100 | 100 | 100 |
| *Rhizoctonia solani* | 100 | 100 | 100 | 100 |

*L. multiflora* : *Lippia multiflora*, *E. alba* : *Eclipta alba*, C. hydroxide: copper hydroxide

**3.1.4. Isolation and characterization of bacteria associated with rice seeds**

Analysing the sanitary condition of the seeds enabled us to isolate a total of 23 bacterial strains, including eight from FKR19, six from FKR62N, and nine from FKR64 (see Table 4). Molecular analysis of the bacterial isolates from the different rice varieties revealed notable diversity among bacterial genera. However, some sequences were of poor quality and uninterpretable. A total of ten distinct bacterial genera were identified among the analysed strains (*Enterobacter*, *Kosakonia*, *Klebsiella*, *Pseudomonas*, *Sphingomonas*, *Stenotrophomonas*, *Methylobacterium*, *Citrobacter*, *Staphylococcus* and *Bacillus*) (see Table 4).

Five genera were identified in the FKR19 variety: *Enterobacter*, *Pseudomonas*, *Sphingomonas*, *Stenotrophomonas* and *Methylobacterium* (Table 4). Three isolates (SM-19-J, SM-19E-Bt, and SM-19L-O) exhibited poor sequence quality and could not be identified.

The FKR62N variety showed relatively lower diversity, with three genera detected. *Kosakonia* was identified in two isolates, while *Enterobacter* and *Citrobacter* were found in one isolate each. Two sequences (SM-62-B and SM-62-Bt) were unusable (Table 4).

The FKR64 variety stood out due to its greater bacterial richness. Five genera were identified: *Enterobacter* (three isolates) and *Klebsiella* (three isolates) were the most prevalent, with three isolates each. The present of Other genera, such as *Kosakonia*, *Bacillus* and *Staphylococcus*, indicates bacteria diversity that is potentially favourable to plant growth and health. No sample of this variety resulted in a poor sequence (Table 4).

**Table 4. Bacteria isolated from rice seeds and their taxonomic affiliation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variety** | **Isolate code** | **Results**  | **Accessions numbers** |
| **FKR19** | SM-19-J | Mauvaise séquence |  |
| SM-19-R | *Methylobacterium* | NR\_135210.1 |
| SM-19-B1 | *Enterobacter*  | NR\_180237.1 |
| SM-19-B2 | *Pseudomonas* | NR\_040859.1  |
| SM-19E-Jt | *Sphingomonas* | NR\_113637.1 |
| SM-19E-Bt | Mauvaise séquence |  |
| SM-19E-J | *Stenotrophomonas* | NR\_118008.1 |
| SM-19L-O | Mauvaise séquence |  |
| **FKR62N** | SM-62-B | Mauvaise séquence |  |
| SM-62-J1 |  *Kosakonia* | NR\_025566.1 |
| SM-62-B | *Enterobacter* | NR\_179166.1 |
| SM-62-Bt | Mauvaise séquence |  |
| SM-62-J2 | *Kosakonia* | NR\_025566.1 |
| SM-62-J3 | *Citrobacter* | NR\_118105.1 |
| **FKR64** | SM-64-R | Staphylococcus | NR\_036903.1 |
| SM-64-J | *Enterobacter* | NR\_180237.1  |
| SM-64-Jt1 | *Kosakonia* | NR\_025566.1 |
| SM-64-Jt2 | *Klebsiella* | NR\_037084.1 |
| SM-64-J2 | *Enterobacter* | NR\_180451.1 |
| SM-64-Bt1 | *Bacillus* | NR\_180419.1 |
| SM-64-Bt2 | *Klebsiella* | NR\_134062.1 |
| SM-64-B | *Klebsiella* | NR\_114506.1 |
| SM-64-J3 | *Enterobacter* | NR\_180237.1 |

**3.1.4. Effect of treatments on bacterial diversity of rice seeds**

The effect of various treatments on rice seeds was assessed by identifying cultivable bacteria. For the FKR19 variety, three common isolates (SM-19-J, *Enterobacter*, and SM-19-O) were identified consistently across all treatments, except for those involving mancozeb, in which an additional isolate (*Methylobacterium*) was detected. This increased the total number of isolates to four (see Table 5). The number of specific isolates remained at three for plant extracts and copper hydroxide, suggesting that these treatments had little influence on apparent bacterial diversity. In contrast, mancozeb appears to have led to the emergence of an additional isolate.

The greatest diversity was observed in the FKR62N control, with five isolates identified, including *Enterobacter*, *Kosakonia*, *Citrobacter*, and SM-62-Bt. The other treatments resulted in the isolation of only two to three taxa, dominated by *Enterobacter* and *Kosakonia* dominating. Both the copper hydroxide and mancozeb treatments recovered these two genera. Plant extracts showed lower diversity, with only two isolates identified, but at least one common isolate (SM-62-O) was recovered in all treatments.

Overall, bacterial diversity was greater for FKR64. The control group had five isolates representing five genera: *Staphylococcus*, *Kosakonia*, *Klebsiella*, *Bacillus*, and *Enterobacter*. Treatment with mancozeb led to the detection of four isolates representing high diversity, including *Klebsiella*, *Kosakonia*, and *Bacillus*. Copper hydroxide led to the recovery of *Kosakonia* and *Bacillus*. *E. alba* and *L. multiflora* resulted in the isolation of three and two taxa, respectively. Bacillus was consistently present in all treatments (see Table 5). The frequent presence of *Bacillus* and *Kosakonia* in FKR64 could indicate a stable association with this variety, as well as the resilience of these genera to the applied treatments.

**Table 5. Bacterial strains were isolated before and after the application of seed treatments**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variety** | **Treatment** | **Isolates number** | **specific isolates identified** | **Common isolates with controls.** |
| **FKR19** | *E. alba* | 3 | SM-19-J, *Enterobacter*, SM-19-O | 2 |
| *L. multiflora* | 3 | SM-19-J, *Enterobacter*, SM-19-O | 2 |
| C. hydroxide | 3 | SM-19-J, *Enterobacter*, SM-19-O | 2 |
| Mancozèbe | 4 | SM-19-J, *Methylobacterium*, *Enterobacter*, SM-19-O | 3 |
| Témoin | 3 | SM-19-J, *Methylobacterium*, *Enterobacter* | - |
| **FKR62N** | *E. alba* | 2 | SM-62-B, SM-62-O | 1 |
| *L. multiflora* | 2 | *Enterobacter*, SM-62-O | 1 |
| C. hydroxide | 3 | *Enterobacter*, *Kosakonia*, SM-62-O | 2 |
| Mancozèbe | 3 | *Enterobacter*, *Kosakonia*, SM-62-O | 2 |
| Témoin | 5 | *Enterobacter, Kosakonia*, *Citrobacter*, SM-62-Bt | - |
| **FKR64** | *E. alba* | 3 | *Bacillus*, *Enterobacter*, SM-64-O |  |
| *L. multiflora* | 2 | *Bacillus*, SM-64-O | 1 |
| C. hydroxide | 3 | *Kosakonia*, *Bacillus*, SM-64-O | 2 |
| Mancozèbe | 4 | *Kosakonia*, *Klebsiella*, *Bacillus*, SM-64-O | 3 |
| Témoin | 5 | *Staphylococcus*, *Kosakonia*, *Klebsiella* *Bacillus*, *Enterobacter*  | - |

*L. multiflora* : *Lippia multiflora*, *E. alba* : *Eclipta alba*, C. hydroxide: copper hydroxide

**3.2. DISCUSSION**

Our results show that the rice seeds studied have a high germination capacity. This capacity exceeds 90% for the FKR19 and FKR64 varieties and is slightly lower for the FKR62 variety (87%). The absence of significant differences in germination power after treatment indicates tolerance to the applied treatments. These results suggests that the treatments did not alter seed viability, which corroborates the results of Bashyal *et al.* (2020), who demonstrated that rice seeds tolerate antifungal treatments well.

A mycological analysis revealed the presence of several major pathogenic fungi, including *Fusarium moniliforme*, *Bipolaris oryzae*, *Curvularia lunata* and *Pyricularia oryzae*. These findings corroborate the observations of Habib *et al.* (2012) and Naher *et al.* (2016), who highlighted the fungal diversity of rice seeds. Notably, *F. moniliforme* was the most prevalent species, accounting for 18% and 6% of the samples, respectively. The intervarietal differences in pathogenic fungal frequency suggest variable susceptibility to fungal infections among varieties, consistent with the work of Kumar *et al.* (2023).

The presence of saprophytic or spoilage fungi, such as *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium sphaerospermum*, and *Phoma sorghina*, raises major concerns, even at low frequencies. These fungi compromise seed germination, degrade nutrient reserves, and produce mycotoxins that are harmful to plant health and food safety (Magan *et al.*, 2011; Pitt & Hocking, 2022). For example, Aspergillus flavus, for example, is a recognized producer of aflatoxins, which are carcinogenic mycotoxins that can contaminate grain and affect crop quality (Tola & Kebede, 2016). Therefore, the presence of these fungi, even at low levels, therefore represents a significant risk, particularly in agricultural systems where storage conditions are often suboptimal.

The presence of contaminants such as *Verticillium* sp. and *Phoma* *lingam* at specific points suggests possible cross-contamination or inadequate storage condition control, which can promote fungal proliferation and reduce seed quality (Christensen, 1972). These observations highlight the importance of maintaining optimal storage and handling conditions for seeds to prevent contamination and microbiological degradation (Martín *et al.*, 2022).

Compared to the control, the applied treatments reduced the frequency of fungal pathogens, though the efficacy varied depending on the agent used. Mancozeb was the most effective, outperforming copper hydroxide and plant extracts. These results confirm the findings of Nguefack *et al.* (2007), who demonstrated that synthetic fungicides reduce fungal infections in cereal seeds. However, the efficacy of the aqueous extracts of *Eclipta alba* and *Lippia multiflora* suggests their potential as an alternative to chemicals, as Meena *et al.* (2017) also suggested in their of antifungal plant extracts. More recently, Ei *et al.* (2024) demonstrated that certain plant extracts exert antifungal activity and induce beneficial effects on rice plant growth. Their study showed that applying plant extracts stimulates the activity of antioxidant enzymes (SOD, POD, and CAT), enhances the biosynthesis of secondary metabolites, and improves plant vigor. These findings underscore the importance of developing optimized formulations based on plant extracts to enhance their efficacy, particularly in Burkina Faso, where there is a high demand for sustainable solutions that reduce the use of chemical pesticides.

Analysis of bacterial communities on three local rice varieties (FRK19, FKR62N, and FKR64) revealed 10 bacterial genera, reflecting the high diversity of microbial community associated with rice seeds (Chen *et al.*, 2024 et Hardoim *et al.*, 2008). This bacterial diversity confirms previous observations on the richness of the microbiome associated with rice in Burkina Faso. Sondo *et* *al.*, 2023a isolated over 3,000 bacterial strains from rice roots grown in western Burkina Faso. They identified several genera found in the present study, including *Pseudomonas*, *Bacillus*, *Methylobacterium*, *Enterobacter*, *Klebsiella*, and *Stenotrophomonas*. These genera are well known for their plant growth-promoting properties (PGPR) and their potential for the biological pathogens.

A complementary study by Barro *et al.*, (2022) revealed that the cropping system significantly influence the composition of the rice root microbiome. The authors observed significant variation in the diversity and structure of bacterial communities depending on the agroecological context, highlighting the effect of agricultural practices on microbial assembly.

Interestingly, *Bacillus* remained stable despite the application of treatments. This genus is well known for its biocontrol potential against plant pathogens (Kloepper *et al.*, 2004). This resilience could be exploited in developing integrated strategies that combine biocontrol and antifungal treatments. However, eliminating *Methylobacterium* with certain treatments could affect seed physiology because these bacteria stimulate plant growth (Madhaiyan *et al.*, 2004). More recently, Oeum *et al.* (2024) compared the microbiota of diseased and healthy rice plants and showed that *Methylobacterium* genera are signatures of plant health. These bacteria emerged as microbial indicators of plant health and exhibited significant biocontrol capacity against *Xanthomonas oryzae* pv. *oryzae*, a major rice pathogen.

Under experimental conditions, some strains reduced disease symptoms by up to 77%. These results suggest that unintentionally altering the beneficial microbiota, particularly by losing *Methylobacterium*, could compromise seed development and their natural defenses against pathogens (Oeum *et al.*, 2024).

Similarly, the emergence of *Enterobacter* following fungicide treatment raises questions about the indirect effects of fungicides on the balance of the microbial community, as observed by Compant *et al.* (2010). These microbial changes deserve particular attention because they could influence seed health and performance in the long term.

The results confirm that treatments influence the microbial diversity of rice seeds, including both fungi and bacteria. They underscore the importance of understanding the interactions between treatment agents and microbiota to optimize integrated protection strategies. Such an understanding is crucial to developing approaches that respect biological equilibrium while ensuring sanitary efficacy.

**4. CONCLUSION**

This study emphasizes the diversity and abundance of fungal and bacterial communities associated with rice seeds and the substantial impact of applied treatments on their composition. Mycological analysis revealed the presence of both pathogenic and saprophytic fungi, with profiles that depend on the variety. While most treatments effectively reduced their frequency, *Fusarium moniliforme* persisted at low levels, suggesting partial tolerance or resistance to certain treatments. From a bacterial perspective, the treatments induced notable changes in microbial composition, with certain groups experiencing a reduction or relative stability depending on the type of treatment. Plant extracts from *Eclipta alba* and *Lippia multiflora* were less effective than mancozeb but showed promise as interesting natural alternatives to be further explored from a biocontrol perspective. These results highlight the importance of considering the phytosanitary efficacy of treatments and their impact on seed microbiota, which is a key factor in long-term plant health. Thus, an integrated approach combining chemical and biological agents could offer sustainable protection, balancing efficacy and microbial balance preservation. However, more research is needed to test the effects of treatments on seedling health in controlled environments and in the field and to evaluate their long-term impact on growth, yield, and the cultivable seed microbiome. These investigations will improve our understanding of the microbial mechanisms involved and guide the development of biocontrol solutions adapted to local agroecological contexts. Finally, this study highlights the diversity of the microbiome associated with rice in Burkina Faso. The consistent presence of beneficial bacterial genera in both seeds and the rhizosphere suggests a microbial functional continuum that is likely to play a pivotal role in crop vitality and the sustainability of agricultural systems.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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