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Production and Evaluation of a Forest Litter and *Jatropha curcas* Cake-Based Biofertilizer Developed through Anaerobic Fermentation

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Abstract

The excessive use of chemical fertilizers leads to soil degradation and water pollution. Microbial biofertilizers have gained attention for promoting sustainable agriculture while protecting the environment. Fermented Forest Litter (FFL) is an organic fertilizer made by fermenting solid agricultural residues under anaerobiosis. It contains diverse microbial communities that can enhance plant growth. This study aims to evaluate the potential of FFL supplemented with *Jatropha curcas* cake. To achieve this, FFL mixed with *Jatropha* cake was produced through solid-state fermentation and then activated by submerged fermentation. The chemical and microbiological properties of FFL and Non-Fermented Substrate (NFS) were analyzed. A germination test was conducted on tomato, okra, and maize using activated FFL (aFFL). Before fermentation, NFS had a pH of 6.38, an electrical conductivity of 2.53 mS/cm, 241 mg/kg ammonium, 672 mg/kg soluble phosphorus, 582 mg/kg nitrate, and 4662 mg/kg soluble potassium. FFL had a pH of 4.35, an electrical conductivity of 4.64 mS/cm, 1948 mg/kg ammonium, 2003 mg/kg soluble phosphorus, 66 mg/kg nitrate, and 4864 mg/kg soluble potassium. FFL showed no presence of coliforms or *Salmonella*. At a concentration of 2%, aFFL improved the germination index of okra, tomato, and maize, with respective values of 429%, 92.5%, and 127.6%. These results suggest that fermented forest litter supplemented with *Jatropha curcas* cake has promising potential as a biofertilizer.

Keywords

Biofertilizer, Solid-State Fermentation, *Jatropha curcas*, Seed Cake

1. Introduction

Since the mid-20th century, the demand for food has been steadily rising due to the exponential growth of the global population [1]. In addition, recent decades have shown an accelerated deterioration of arable land worldwide, with up to 55% of the world's arable land affected by degradation [2]. To boost agricultural yields and meet food demands, farmers implement various fertilization management practices. The primary practices used in modern agriculture rely on mineral fertilization and synthetic plant protection products [3]. Although these methods increase crop production, they unfortunately have several limitations [4]. Besides their high costs, mineral fertilization and synthetic protection pose risks of food contamination, environmental pollution, negative effects on microbial diversity, and declining soil fertility [5]. In response to these increasing threats, soil fertilization management based on microbial use has gained significant attention. Extensive research has been carried out to assess the ability of microbial biofertilizers to promote plant growth. As a result, several microbial biofertilizers have been developed and marketed globally. The main benefits of microbial biofertilizers include organic matter decomposition, molecular nitrogen fixation, protection against plant pathogens, tolerance to biotic and abiotic stresses, and the production of plant growth hormones [6]. Through these mechanisms, biofertilizers help reduce environmental pollution, combat environmentally transmitted diseases, and significantly improve the quantity and quality of agricultural products. Despite these advantages, microbial biofertilizer technologies still face challenges related to formulation, storage, application techniques, and adaptation to local environments [7]. Innovative strategies are needed to overcome these issues and foster the development of sustainable, environmentally friendly agriculture. Thanks to their interactive capabilities, microbial consortia are excellent candidates compared to microbial monocultures [8].

Fermented forest litter (FFL) is a biofertilizer obtained through the anaerobic fermentation of forest litter, agro-industrial residues, and unchlorinated water, which serves as a source of lactic acid bacteria. The FFL production process is a biotechnological method for producing microbial consortia. This technology subsequently spread to Latin America and Asia. It offers the advantage of allowing the selection of a wide variety of microorganisms, promoting the development of agroecology, and reducing farmers' dependence on chemical fertilizers. Produced in more than 50 countries and used in approximately 130 countries worldwide to date, fermented forest litter is less commonly used in several African countries, including Burkina Faso [9].

In addition, the development of the agro-industrial sector is generating biomass waste, including that from *Jatropha curcas*, which is used to produce biofuel. The process of extracting the oil generates a considerable amount of residue, including shells and cake. Indeed, one ton of *Jatropha curcas* L. seeds can produce up to 650 kg [10]. The cake has a protein content of 55% to 64% and an energy value of 19% to 48% [11]. It also contains essential amino acids like leucine, isoleucine, valine,

and threonine, making it a nutritious source suitable for microbial growth [12].

Several studies have been conducted on the chemical, biochemical, and microbiological characterization of FFL [13] [14], and some of them have reported their effects on crops [15]. However, to our knowledge, little has been done on the quality of FFL in relation to agricultural residues such as *Jatropha curcas* cake. We hypothesized that using the residue in the production of fermented forest litter could lead to the development of a quality biofertilizer, which may contribute to waste recycling and to sustainable agricultural production. This study aims to evaluate the biofertilizing potential of fermented forest litter made from agro-industrial waste, including *Jatropha curcas* cake.

2. Materials and Methods

2.1. Collection of the Components Used for the Production of Fermented Forest Litter

The study was conducted in the Kadiogo region of Burkina Faso. This area is characterized by a Sudano-Sahelian climate, savanna vegetation, a dry season from November to May, and a rainy season from June to October with an average annual rainfall of 935 mm [16]. *Jatropha curcas* cake was collected from the Belwet industrial unit in Ouagadougou (12˚22'33.8"N - 1˚32'31.0"W), forest litter from the classified forest of Gonsé (12˚26'20"N, 1˚19'40"W), and millet bran. Water was collected from the Loumbila dam (12˚29'14"N, 1˚24'28"W), and the source of lactic acid bacteria was from the "La Vache Enchantée" dairy (12˚23'5"N - 1˚31'30"W).

The collection sites were chosen based on the absence of risk of chemical contamination that could inhibit microbial growth during fermentation. The Gonsé forest is classified and is characterized by an absence of human activities likely to generate chemical contamination. The Belwet unit extracts oil from *J. curcas* seeds using a pressing technique that does not involve the use of chemicals. The Faso cereal processing unit specializes in the processing of agricultural products without the use of chemicals. The "La Vache Enchantée" dairy specializes in the collection and sale of cow's milk in the city of Ouagadougou. The Loumbila dam is a source of surface water used for drinking water production in the town of Ouagadougou, outside the influence of human and agricultural activities.

2.2. Formulation of the Mixture for the Production of FFL

Forest litter is the basic material used in the production of FFL [13]. *Jatropha curcas* cake was used as a carbon source due to its high protein content. Fresh milk was used as a source of lactic acid bacteria to ensure the natural quality of the lactic acid bacteria source. Millet bran was used as a source of starch.

The process of fermented forest litter preparation was carried out in two successive fermentation stages [13]. The first stage is based on an anaerobic batch fermentation, and the second stage consists of activating the solid fermented mixture obtained from the first step by fermentation in a liquid medium. To this end,

the various components were mixed in appropriate proportions (Table 1).

Table 1. FFL production substrates and their proportions.

Constituent	Quantity
Jatropha seed cake (g/L)	270
Forest litter (g/L)	200
Millet bran (g/L)	27
Fresh milk (mL/L)	67
Dam water (mL/L)	370

Following a specific order and in a container disinfected with 70% alcohol, 200 g/L of forest litter was mixed with 270 g/L of *Jatropha* seed cake and 67 g/L of millet bran before adding a mixture of 67 ml/L of fresh milk and 370 ml/L of dam water (Figure 1).

For the activation step, the substrates used are solid LFF and sugarcane molasses, each at a concentration of 25 g/L, and dam water. Thus, to prepare 250 mL of activated LFF, 6.25 g of sugarcane molasses was mixed with 100 mL of dam water before adding 6.25 g of solid LFF. The reaction medium volume was composed of dam water. After homogenization, the bottle was tightly sealed and placed at room temperature for a one-week fermentation period. In accordance with the activation period, solid FFL was activated in seven (07) 250 mL bottles. Each day, one bottle was used to measure pH and electrical conductivity.

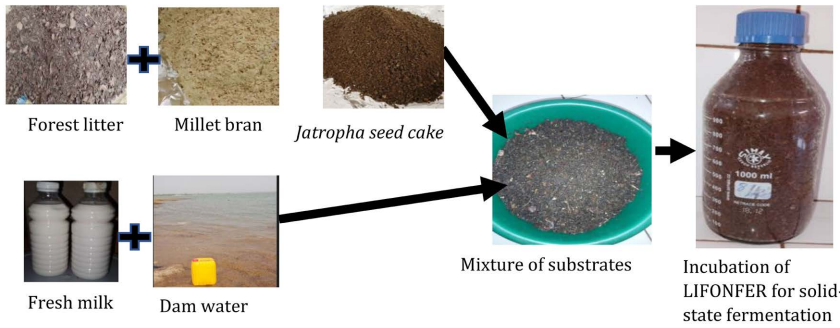

Figure 1. Order of mixing substrates for FFL production.

2.3. Assessment of the Quality of FFL

2.3.1. Sampling

Samples of NFS, the solid FFL, and the activated FFL were collected for analysis. For the microbiological analysis, 100 g of each sample was collected in a sterile bag and stored at 4˚C. The samples for chemical analysis (100 g) were taken and stored at −20˚C to avoid any biochemical activity that could modify the chemical parameters. For the activated FFL, 50 mL was collected in a sterile glass bottle and stored at 4˚C for microbiological analysis, and in a polyethylene bottle stored at −20˚C to determine the chemical parameters.

2.3.2. Chemical Analysis

The pH and the electrical conductivity of the samples were determined using the method described by Marois *et al.* [13]. Changes in pH and electrical conductivity were assessed during the seven-day FFL activation period. A sample/demineralized water suspension in a ratio of 1/5 (m/v) was analyzed using a portable pH/EC/TDS/temperature meter (Hanna instrument, Romania).

The organic carbon was determined using the organic matter incineration method [17].

The total nitrogen, total phosphorus, and total potassium of the samples were also determined using the Kjeldahl method [18]. Total nitrogen and phosphorus contents were determined by colorimetry using the SKALAR flow analyzer (segment flow analyzer, model SANplus 4000-02, Skalar Holland) [19]. Total potassium content was determined using a flame spectrophotometer (Jenway PFP7 flame photometer) [20].

The water-soluble nutrients were determined from a distilled water extract of NFS and FFL samples in a 1:20 (m/v) ratio [21]. From the extracts, nitrates were measured using a spectrophotometer at 410 nm [22]. Soluble phosphorus and ammonium were measured using the colorimetric method with the SKALAR automatic analyzer, and the total potassium was measured using a flame spectrophotometer.

2.3.3. Microbiological Analysis

The microbiological quality of NFS and FFL was assessed through the determination of total coliforms, thermotolerant coliforms, and *Salmonella* spp. Total and thermotolerant coliforms were determined using Chromocult Coliform agar [23]. *Salmonella* spp. were evaluated in accordance with AFNOR standard NF 08.052.

In addition, daily monitoring of lactic acid bacteria, yeasts, and molds was carried out during the activation of FFL.

2.4. Seed Germination Tests Using the Activated FFL

To assess the phytotoxicity of activated FFL, crops commonly grown in Burkina Faso, such as maize (*Zea mays*), tomatoes (*Solanum lycopersicum* L.), and okra (*Abelmoschus esculentus*) were used [24].

The activated FFL is filtered using a 0.5 mm sieve, and the filtrate was diluted to concentrations of 2% and 5%, the concentrations commonly used in agricultural practices [9], for the germination test. A randomized complete block design was adopted and carried out under laboratory, day, and night temperature and light conditions based on three treatments with two (02) replicates each: activated FFL at 2%, activated FFL at 5%, and a sterile distilled water solution (control) [25]. Ten (10) seeds from each crop were placed in a 90 mm diameter Petri dish containing a Whatman paper disc. The dishes were watered every two (02) days with 5 ml of each irrigation solution for 07 days.

Germination rate (GR), radicle elongation (RE), and germination index (GI) were determined according to the following formulas.

$$\mathrm{GR}(\%) = \frac{\textit{Number of seeds germinated in fermented substrates}}{\textit{total number of seeds sown}} \times 100 \quad (1)$$

$$\mathrm{RE}(\%) = \left(\frac{\textit{average root elongation in the treatment}}{\textit{average root elongation in the control group}} \right) \times 100 \quad (2)$$

$$\mathrm{GI}(\%) = \frac{\text{Germination rates}(\%) \times \text{radicle elongation}(\%)}{100} \quad (3)$$

2.5. Statistical Analysis of Data

A paired t-test, with a significance threshold of $p < 0.05$, was performed on Jamovi software to compare the means of the chemical parameters before and after fermentation. The relationship between the different parameters was established according to Pearson's correlation. An ANOVA was performed using XLSTAT software version 2016.02.27444 at a significance level of $p < 0.05$ to compare the mean values of the germination indices and root elongation for the different dilutions of activated FFL for each species.

3. Results

3.1. Chemical Characteristics of Raw and Fermented Substrates

The chemical characteristics of the non-fermented substrate (NFS) and fermented forest litter (FFL) are shown in Table 2. The average electrical conductivity of NFS and FFL is 2.53 to 4.64 mS/cm and 6.38 to 4.36 for the pH, respectively. The paired t-test indicated that the variation in pH was not statistically significant ($p = 0.072 > 0.05$). At the same time, the variation in EC is statistically significant ($p = 0.018 < 0.05$). The paired t-test showed that these variations are not statistically significant.

The average organic matter and carbon content, as well as the average C/N ratio, changed from 766.229 to 736.644 (g/kg), from 445.482 to 439.277 (g/kg), and from 16.03 to 14.94. According to the paired t-test, only the variation in organic matter content was statistically significant ($p = 0.042$).

The average contents of ammonium, nitrate, soluble phosphorus, and soluble potassium ranged from 241.17 to 1948.48 (mg/kg), from 672.60 to 2003.25 (mg/kg), and from 4662.13 to 4864.83 (mg/kg). The t-test showed that the increase in ammonium and soluble potassium content was statistically significant ($p = 0.004$ for NH_4^+ and $p < 0.001$ for K). The average nitrate content varied from 582.0 to 66.2 (mg/kg). The t-test showed that this decrease was statistically significant ($p = 0.023$).

The relationships between the different parameters are shown in Table 3. Positive and statistically significant correlations were found between ammonium content and EC (Pearson's $r = 0.994$ and $p = 0.004$), nitrate content and pH (Pearson's $r = 0.996$ and $p = 0.004$), and soluble phosphorus and ammonium contents (Pearson's $r = 0.965$ and $p = 0.035$). The relationship test also showed a negative correlation between pH and EC (Pearson's $r = -0.996$ and $p = 0.004$), pH and ammo-

nium content (Pearson's r = −0.990 and p = 0.010), between nitrate content and EC, and nitrate content and ammonium content (with Pearson's r = −0.998 and p = 0.002), between soluble phosphorus content and pH, and soluble phosphorus content and nitrate content (Pearson's r = −0.956 and −0.960 and p = 0.044 and 0.040, respectively).

Table 2. Chemical characteristics of the unfermented mixture and FFL.

Parameter	NFS	FFL	p-value
pH	6.38 ± 0.22	4.35 ± 0.015	0.072
E.C. (mS/cm)	2.53 ± 0.16	4.64 ± 0.1	0.018
Total nitrogen (g/kg)	30.30 ± 0.12034	30.21 ± 0.00611	0.609
Total phosphorus (g/kg)	5.05 ± 0	4.73 ± 0.105	0.205
Total potassium (g/kg)	14.60 ± 2.04055	15.54 ± 0.78483	0.590
O.C. (g/kg)	445.31 ± 9.22487	439.28 ± 0.022.98	0.642
C/N	16.02 ± 0.13	14.70 ± 0.4	0.153
O.M. (g/kg)	766.23 ± 16.89877	736.44 ± 18.87292	0.042
NH_4^+ (mg/kg)	241.17 ± 7.83	1948.48 ± 19.52	0.004
Soluble P (mg/kg)	672.60 ± 32.6	2003.26 ± 237.25	0.097
Soluble K (mg/kg)	4662.13 ± 608.1	4864.83 ± 608.1	0.001
NO_3^- (mg/kg)	582.00 ± 25	66.15 ± 6.16	0.023

Legend: NFS (Non-Fermented Substrate), FFL (fermented forest litter), E.C. (electrical conductivity), O.C. (organic carbon), C/N (carbon/nitrogen ratio), and O.M. (organic matter). NH_4^+ (ammonium), P (phosphorus), NO_3^- (nitrate), K (potassium).

Table 3. Correlation matrix between the chemical parameters of solid FFL.

		pH	E.C.	NH_4^+	NO_3^-	Soluble P	Soluble K	O.M.
E.C.	Pearson's r	−0.996**	—					
	ddl	2	—					
	p-value	0.004	—					
NH_4^+	Pearson's r	−0.990*	0.994**	—				
	ddl	2	2	—				
	p-value	0.010	0.006	—				
Nitrate	Pearson's r	0.996**	−0.998**	−0.998**	—			
	ddl	2	2	2	—			
	p-value	0.004	0.002	0.002	—			
Soluble phorus	Pearson's r	−0.956*	0.942	0.965*	−0.960*	—		
	ddl	2	2	2	2	—		
	p-value	0.044	0.058	0.035	0.040	—		

Continued

Soluble potassium	Pearson's r	−0.259	0.284	0.180	−0.223	−0.035	—	
	ddl	2	2	2	2	2	—	
	p-value	0.741	0.716	0.820	0.777	0.965	—	
Organic matter	Pearson's r	0.700	−0.725	−0.649	0.680	−0.459	−0.864	—
	ddl	2	2	2	2	2	2	—
	p-value	0.300	0.275	0.351	0.320	0.541	0.136	—
Organic carbon	Pearson's r	0.539	−0.502	−0.414	0.469	−0.365	−0.702	0.728
	ddl	2	2	2	2	2	2	2
	p-value	0.461	0.498	0.586	0.531	0.635	0.298	0.272

3.2. Evolution of Chemical Characteristics during the FFL Activation Process

During the activation of FFL, the pH gradually decreased from 5.09 to 3.98 (Figure 2). The electrical conductivity increased slightly, from 3.52 to 3.77 mS/cm.

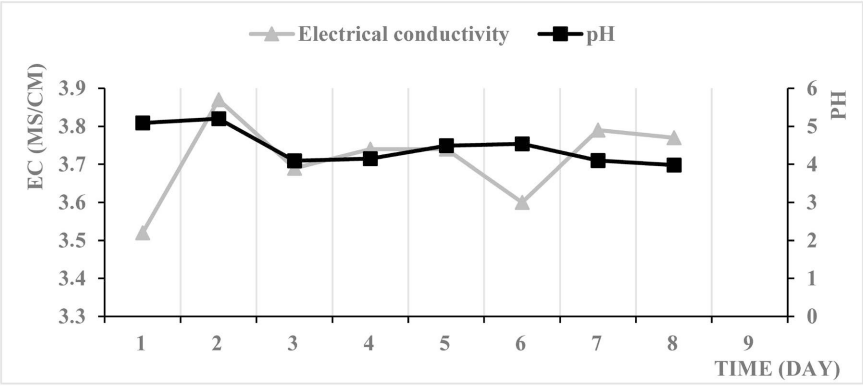

Figure 2. Evolution of pH and electrical conductivity during the process of FFL activation.

3.3. Microbiological Quality of NFS and FFL

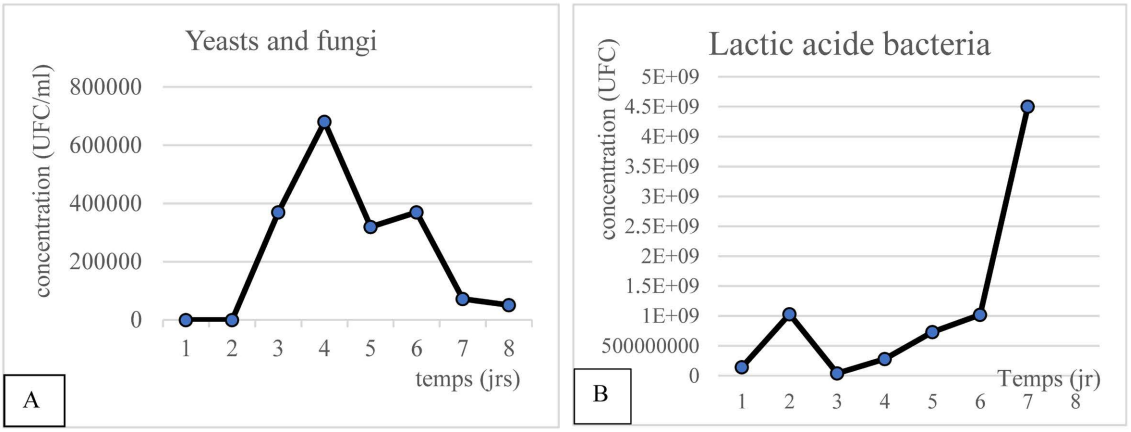

Figure 3. Evolution of pH and electrical conductivity during the process of FFL activation.

The results of microbiological analyses of NFS showed a total coliform concentration of 3.75×10^5 CFU/g of substrate and no *Salmonella*. After fermentation, the results of microbial analyses showed a total reduction in the total coliform load and no *Salmonella* in FFL.

The results of the evolution in lactic acid bacteria, yeast, and mold loads are shown in Figure 3. These results show that on the first day of activation, no growth in lactic acid bacteria, yeast, or mold was observed. From 24 hours after activation until the last day, the concentration of lactic acid bacteria increased from 1.44×10^8 to 4.5×10^9 CFU/ml, and the concentration of yeast and mold varied from 3.7×10^5 to 5.1×10^4 CFU/ml.

3.4. Relationship between the Evolution of Lactic Acid Bacteria, pH, and EC during FFL Activation

Relationships between the parameters of lactic acid bacteria load and the chemical parameters of active FFL are shown in Table 4. A negative correlation is found between the evolution of pH during activation and changes in electrical conductivity (Spearman's rho = −0.703, p = 0.039), as well as the evolution of lactic bacteria concentration (Spearman's rho = −0.750, p = 0.033). These correlations suggest that the gradual decrease in pH during activation is associated with the fermentation activity of lactic acid bacteria. Indeed, the growth of lactic acid bacteria generates lactate, which decreases the pH.

Table 4. Correlation matrix between the parameters of lactic acid bacteria load and the chemical parameters of active FFL.

		BL (UFC/ml)	pH	E.C. (Ms/cm)
BL (UFC/ml)	Spearman's rho	—		
	ddl	—		
	p-value	—		
pH	Spearman's rho	−0.750*	—	
	ddl	5	—	
	p-value	0.033	—	
E.C. (mS/cm)	Spearman's rho	0.396	−0.703*	—
	ddl	5	5	—
	p-value	0.811	0.039	—

3.5. Seed Germination Affected by FFL

The results of the germination tests are reported in Table 5. Figure 4(A) shows the radicle elongation of the germinated seeds, and Figure 4(B) shows the germination index of the seeds. At a concentration of 2%, the activated FFL showed a germination rate of 95% for okra and tomato and 100% for maize. The average root elongation was 5.25 ± 0.04 mm, 3.15 ± 0.3 mm, and 12.67 ± 0.7 mm for okra, tomato, and maize, respectively. The germination index was 429% ± 29.7% for

okra, 92.5% ± 1.6% for tomato, and 127.6% ± 8.93% for maize. At a concentration of 5%, the activated FFL showed a germination rate of 90% for okra, 95% for tomato, and 100% for maize. The root elongation was 8.2 ± 0.24 mm for maize, while no elongation was noticed for okra and tomato. The germination index was 82.24% ± 3.54% for maize.

After adjusting the acidity to the neutral pH (pH = 7), FFL diluted to 2% showed a germination rate of 85% for okra, 95% for tomato, and 100% for maize. The average root elongation was 6.4 ± 0.19 mm for okra, 5.2 ± 0.07 mm for tomato, and 16.3 ± 0.47 mm for maize. The germination index was 465% ± 0.35% for okra, 151.8% ± 13.14% for tomato, and 164.5% ± 7.09% for maize. FFL diluted to 5% showed a germination rate of 100% for okra, 95% for tomato, and 100% for maize. The average root elongation was 7.1 ± 0.47 mm for okra, 2.9 ± 0.11 mm for tomato, and 13.6 ± 0.35 mm for maize. The germination index was 612.63% ± 22.83%, 85.5% ± 8.31%, and 136.73% ± 1.61% for okra, tomato, and maize, respectively.

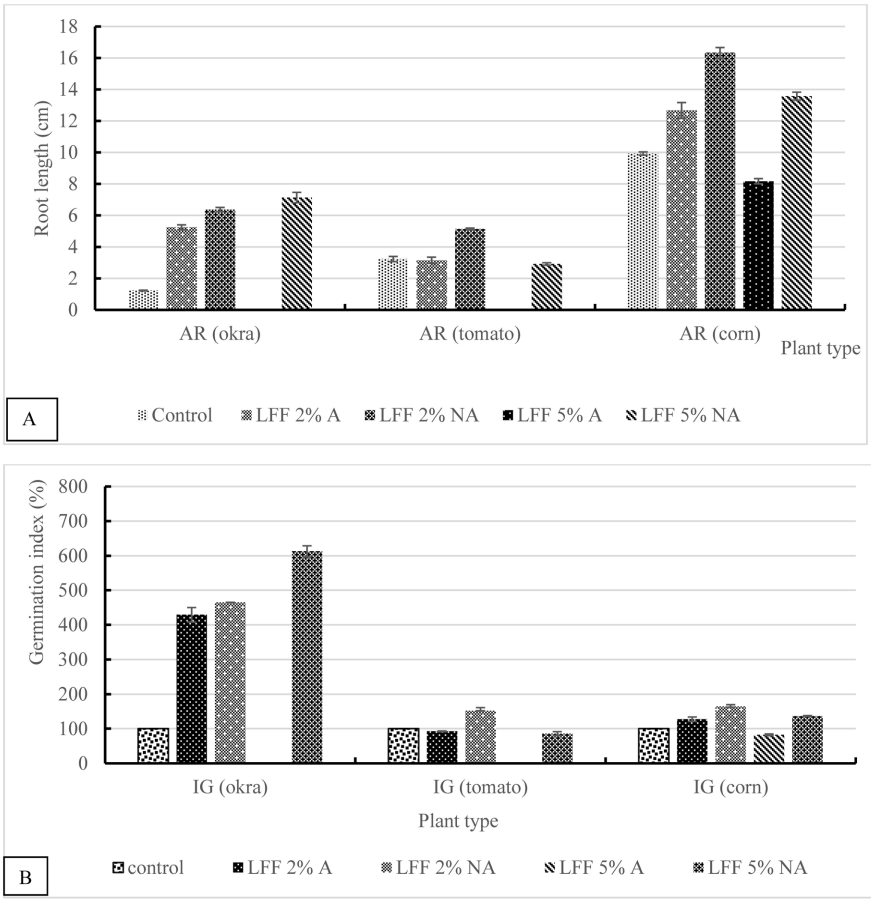

Figure 4. Root elongation of seedlings (A) and seed germination index (B).

With the control, the germination rate was 95% for okra and 100% for tomato and maize. The root elongation was 1.23 mm, 3.23 mm, and 9.93 mm for okra, tomato, and maize, respectively. The germination index was 100% for all crops.

The comparison of the germination indices showed a significant difference between the different FFL solutions and the control for okra seeds ($0.01 < p < 0.001$). For tomato, only the germination index of the 2% FFL solution at neutralized pH showed a significant difference compared to the control ($p = 0.004$). For maize, the 2% FFL solution at neutral pH and the 2% solution at acidic pH showed a significant difference compared to the control ($p = 0.019$ and $p < 0.001$). No significant difference was observed between the seeds' germination rate in the controls and the values obtained when the seeds were watered with the various activated FFL solutions (Table 6).

Table 5. Root elongation and germination index (A: acidic; NA: non-acidic).

	Treatment	Mean	Standard error
Okra GI (%)	FFL 2% A	429.00	21.0000
	FFL 2% NA	465.01	0.2485
	FFL 5% A	0.00	0.0000
	FFL 5% NA	612.63	16.1404
	control	100.00	0.0000
Tomato GI (%)	FFL 2% A	92.49	1.1085
	FFL 2% NA	151.79	9.2935
	FFL 5% A	0.00	0.0000
	FFL 5% NA	85.51	5.8768
	control	100.00	0.0000
Maize GI (%)	FFL 2% A	127.58	6.3179
	FFL 2% NA	164.48	5.0115
	FFL 5% A	82.24	2.5058
	FFL 5% NA	136.73	1.1403
	control	100.00	0.0000
Okra RE (mm)	FFL2% A	5.25	0.1500
	FFL 2% NA	6.37	0.1333
	FFL 5% A	0.00	0.0000
	FFL 5% NA	7.13	0.3333
	control	1.23	0.0250
Tomato RE (mm)	FFL 2% A	3.15	0.2000
	FFL 2% NA	5.15	0.0500
	FFL 5% A	0.00	0.0000
	FFL 5% NA	2.92	0.0750
	control	3.23	0.1667
Maize RE (mm)	FFL 2% A	12.67	0.5000
	FFL 2% NA	16.33	0.3333

Continued

	FFL 5% A	8.17	0.1667
	FFL 5% NA	13.58	0.2500
	control	9.93	0.1000

Table 6. Correlation between the parameters of lactic acid bacteria load and the chemical parameters of active FFL.

		Okra GI	Tomato GI	Maize GI	Okra RE	Tomato RE	Maize RE
	Pearson's	0.518	0.822	0.601	0.532	0.810	0.602
pH	ddl	3	3	3	3	3	3
	p-value	0.371	0.088	0.283	0.356	0.096	0.283

4. Discussion

4.1. Chemical Characteristics of Solid FFL

The results showed a non-significant decrease in the pH and a significant increase in the electrical conductivity following the fermentation of the substrates. Marois *et al.* [13] reported a decrease in pH and an increase in electrical conductivity after fermentation of FFL based on forest litter from two different climates. In addition, the results revealed a negative correlation between pH and conductivity. This negative correlation indicates that an acidic pH promotes the mineralization of the organic matter and the release of ions (phosphate ions, potassium ions, ammonium ions, bicarbonate ions, etc.) by the endogenous microorganisms during the fermentation process. This release of ions increases the electrical conductivity. Indeed, Ajaweed *et al.* [26] reported that a high ion concentration leads to an increase in electrical conductivity. The decomposition of organic matter by endogenous microorganisms, which can lead to increased electrical conductivity, contributes to making nutrients available to plants [27]. However, increased electrical conductivity can lead to increased salinity, which would negatively impact nutrient absorption by plants. Indeed, Nikiema *et al.* [28] reported that when it exceeds 4 dS/m, electrical conductivity can reduce plants' ability to absorb water. The EC value obtained with the FFL (4.64 mS/cm) was roughly equal to this threshold value. Adverse effects on soil physicochemical properties and soil microbial community metabolism associated with high soil salinity have been reported [29]. Diluting activated FFL before application can help mitigate the adverse effects of high salinity. The production of organic acids by lactic acid bacteria can lead to a decrease in pH in the fermentation medium. Lowering the pH has many advantages for FFL. An acidic pH during fermentation promotes the elimination of pathogenic microorganisms by weakening the cell wall and inhibiting several metabolic activities. In addition, a decrease in pH due to the production of organic acids promotes the solubilization of nutrients like inorganic phosphates by chelating metal cations [30]. The chemical composition shows that the average total

nitrogen, total phosphorus, and total potassium contents decreased from 30.30 to 30.21 (g/kg), from 5.05 to 4.73 (g/kg), and from 14.60 to 15.54 (g/kg), respectively; but these variations are not statistically significant. These results show that the fermentation did not have any significant effect on the total nitrogen, total phosphorus, and total potassium. Organic matter, organic carbon, and C/N ratio content decreased by 29.585 (g/kg), 6.205 (g/kg), and 1.09, respectively, but not statistically significant. However, the NPK content of FFL may contribute to enhancing its fertilizing capacity. The organic matter content (75.5%) and C/N ratio of the solid matter of the produced FFL show that it is of good quality for composting [26]. For the water-soluble nutrients, the fermentation significantly increased the ammonium and the soluble potassium contents, representing 1707.31 (mg/kg) and 202.70 (mg/kg), respectively. The nitrate content decreased, corresponding to a decrease of 516.0 (mg/kg). The increase in ammonium content could result from the mineralization of organic nitrogen during the fermentation process. Indeed, Chen *et al.* [31] reported that during the degradation of organic matter, certain microorganisms convert organic nitrogen (osamines and amino acids) into ammonium. The high ammonium concentration at the end of fermentation could be explained by the high protein content of the cake, coupled with the high enzymatic activity of microorganisms capable of degrading organic nitrogen. In fact, *Jatropha* seed cake has a protein content of 57.3 to 64.4% [32]. On the other hand, the loss of nitrate can be explained by denitrification during the fermentation process, a phenomenon in which nitrates are converted into molecular nitrogen under anaerobic conditions by denitrifying bacteria. Mahmoud *et al.* [33] reported a very significant loss of nitrate during anaerobic fermentation in connection with the addition of organic matter to the fermentation medium. Ammonium and nitrate are the forms of nitrogen that can be assimilated by plants. A mixed supply of ammoniacal nitrogen and nitrates is very favorable for nitrogen absorption by plants, which improves photosynthesis and carbon metabolism [34]. Ammonium, which is present in high concentrations in the developed FFL, could be converted into nitrate in the presence of oxygen, which would help regulate the NH_4^+ / NO_3^- ratio. The increase in soluble potassium content could result from the endogenous microbial activity of the biomass mixture. Microorganisms mineralize organic matter and convert the potassium present in the medium into a water-soluble form [35]. The increase in soluble phosphorus content after fermentation is not statistically significant but could be explained by the mineralization of organic phosphorus during fermentation by certain microbial groups [36]. Organic phosphorus is converted into water-soluble forms such as hydrogen phosphate (HPO_4) and phosphoric acid (H_2PO_4) by biological means so that it can be assimilated by plants. Positive correlations were observed between ammonium content and electrical conductivity, and between soluble phosphorus content and ammonium content. The positive correlation between ammonium content and electrical conductivity may indicate that as the ammonium content increases, the soluble ion content also increases. The positive correlation between ammonium content and sol-

uble phosphorus content may indicate simultaneous release of these elements following mineralization. Ammonium and soluble phosphorus contents were negatively correlated with pH. These correlations show that acidic conditions promote the availability of ammonium and soluble phosphorus. This justifies biological activities such as fermentation and the degradation of organic matter by the endogenous microbial community. The developed FFL showed better levels of soluble elements (ammonium, soluble phosphorus, and soluble potassium) after the fermentation. These elements are the main nutrients for plants. Additionally, the microorganisms responsible for the various changes during fermentation may have several properties that help plants grow. Furthermore, FFL supplemented with *Jatropha* seed cake exhibited higher electrical conductivity compared to FFL supplemented with wheat bran produced by Marois *et al.* [13]. The FFL supplemented with *Jatropha* seed cake may be richer than the FFL supplemented with wheat bran.

4.2. Microbiological Quality of Solid FFL

The prepared FFL is characterized by the total absence of coliforms and *Salmonella*. Valdes *et al.* [37] reported the absence of coliforms and *Salmonella* in fermented forest litter in Cuba. The total reduction in coliform concentration may be related to the acidic pH of the medium, as well as the chemical composition of the medium. These results suggest that natural selection occurred during the fermentation. This demonstrates the safety of fermented forest litter and guarantees its sanitary quality.

4.3. Quality of the Activated FFL

The activated FFL showed a high concentration of lactic acid bacteria compared to yeasts and molds. The presence of lactic acid bacteria in high concentration, yeasts, and molds in activated FFL is a key factor in FFL's effectiveness as a biofertilizer. Thanks to their metabolic capacity, these microbial groups have many properties that promote plant growth. The mechanisms of the plant growth-promoting properties of lactic acid bacteria are based on the degradation of organic matter, the production of growth hormones, bioactive molecules, and other secondary metabolites [38].

Furthermore, the presence of these microorganisms may explain the changes observed in the physicochemical characteristics of the FFL.

4.4. Effect of FFL on Seed Germination

A comparison of the different FFL solutions and the control showed a significant difference between the germination indexes for okra. For tomato, only the germination index of the 2% FFL solution at neutral pH showed a significant difference compared to the control. For maize, the 2% FFL solutions at acidic and neutral pH showed a significant difference compared to the control. For all crops, there was no significant difference between the seed germination rate of the controls

and the germination rate of seeds watered with the various activated FFL solutions. According to Finch-Savage & Footitt [39], temperature, humidity, light, and oxygen are factors that can influence seed germination by modulating enzyme activity during the germination process. At acidic pH, only 2% FFL improved the average root elongation and the germination index compared to the control for okra and maize seedlings. At a concentration of 5%, activated FFL inhibits the development or causes root rot in seedlings, resulting in a germination index of zero. Our results show that at a concentration of 2%, active FFL improves the germination index of okra, compared to the control. For tomato and maize, at the same concentration, activated FFL only improved the germination index after adjusting the acidity of the solution to neutral pH. At a concentration of 5%, activated FFL only improved the germination index of okra after adjusting the acidity to neutral pH. Given that the germination index was determined based on the germination rate and root elongation, the active FFL solutions used had effects on root development. These effects may be related to the salinity, the pH, ammonium content, etc., of the activated FFL solutions used. However, no statistically significant correlation was observed between pH and germination index or root elongation. Milon *et al.* [40] reported that acidity can hurt root growth. This acidity alters the roots' ability to absorb nutrients and thus reduces root elongation. The effect of active FFL solutions may be mainly based on chemical characteristics other than pH. According to the work of Kong *et al.* [41], electrical conductivity, organic carbon, dissolved nitrogen, ammonium content, potassium, zinc, and copper content are factors that can negatively influence the germination index. In our study, the chemical characteristics of the FFL solutions used were not determined. In fact, laboratory germination tests have shown promising results. However, the approach used in Petri dish experiments does not necessarily reflect performance in complex soil conditions.

5. Conclusion

This study enabled the production and evaluation of the potential of forest litter fermented with *Jatropha curcas* cake. The fermented mixture had improved levels of ammonium, soluble phosphorus, and soluble potassium, as well as improved electrical conductivity. The evolution of the chemical characteristics at the end of the fermentation indicates the presence of a microbial community in FFL capable of promoting plant growth. Indeed, the FFL showed a high concentration of lactic acid bacteria. The germination tests demonstrated that the aFFL can enhance the germination index, particularly when its acidity is neutralized by dilution before application. These results demonstrate that the produced FFL increased nutrients beneficial to plants and a microbial community with plant growth-promoting properties, which can be utilized as a biofertilizer. The use of FFL supplemented with *Jatropha* cake solution diluted to 2% can improve seed germination. However, this solution could improve seed germination more effectively if the pH of the solution is adjusted with an alkaline solution before application.

Authors' Contributions

PALE, D.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; KIBA, D.I.: conception and design, analysis and interpretation of data, and drafting the article; BISSIRI S.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; COMPAORE, C. O. T.: analysis and interpretation of data, drafting the article; NIKIEMA, M.: conception and design, analysis and interpretation of data; CHRISTEN, P.: conception and design, analysis and interpretation of data, and drafting the article; and MAIGA, Y.: conception and design, analysis and interpretation of data, and drafting the article.

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Conflicts of Interest

There are no conflicts of interest.

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