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Original Article

Development of a biofertilizer through microbial valorization of chicken feather waste for enhancing the growth and grain yield of maize

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ABSTRACT

The traditional physical and chemical methods of treating feather waste are high energy demanding, expensive and constitute huge treat to the environment. Hence, the use of biological mode of treatment is encouraged as it is simple, ecofriendly, and often leads to production of value-added products like enzymes and biofertilizer. In this study, chicken feather dumpsite was screened for the isolation of bacteria capable of degrading feather waste using keratin-based medium. The isolate with highest feather degrading ability was cultivated through submerged fermentation to degrade chicken feather wastes. Thereafter, plant growth-promoting effect of the resulting feather hydrolysate (FH) was evaluated on maize cultivation. The highest feather degrading isolate produced keratinolytic activity of 48.50 U/ml at 72 h of fermentation. The isolate was identified as *Bacillus licheniformis* MX through biochemical and 16S rRNA gene analyses. The FH was rich in nitrogen (12.96% w/v) and amino acids such as arginine (9.22 mg/ml), glutamic acid (8.51 mg/ml), cysteine (5.44 mg/ml), and proline (5.24 mg/ml). Foliar application of FH improved the growth and grain yield of maize in dose dependence manner with the sequence FH100% > FH75% > FH50% > FH25% > Water control. Interestingly, FH100% also performed better than the conventional NPK fertilizer treatment both on growth promotion and grain yield enhancement. Hence, this finding implies that feather degrading ability displayed by *Bacillus licheniformis* MX indicates its promising application for sustainable keratin waste management and enhancement of growth and yield of maize.

Keywords: chicken feather wastes; *Bacillus licheniformis MX;* feather hydrolysate; biofertilizer; maize

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NTRODUCTION

The steady rise in the global population indicates that it would reach about 9.7 billion people by 2050 [1]. The population tend to face the huge challenge of scanty staple and healthy foods for survival. To safeguard against the danger of food insecurity, agricultural food production currently depends largely on the use of synthetic fertilizer for the purpose of enhancing food production to feed the rapidly growing global population. However, the practice has been discouraged in modern agricultural practices due to their high production cost and environmental problems associated with their production and application. Application of chemical fertilizers in excessive amounts is not only cost intensive but also leads to their gradual entry into water systems through rainwater and seepage to contaminate groundwater, that consequently induce serious risks to human and animal health [2]. Thus, there is need to develop a better alternative to increase food production for the sustenance of the steadily rising world population in a cost-effective and environmentally benign manner. Application of eco-friendly biofertilizers or slow-release nitrogenous organic fertilizers has proven to be a safer and better substitute to boost agricultural productivity [3,4,5] for the attainment of Sustainable Development Goal 2 (SDG 2; zero hunger) of the United Nations.

Biofertilizers or slow-release nitrogenous organic fertilizers are products of microbial valorization of organic nitrogenous wastes such as feathers, hair, or other keratin-rich by-products. These wastes, which are often discarded from poultry and agro-industrial processing, are rich in proteins but highly resistant to natural degradation due to their keratin structure [4,5]. The consumption of chicken in human diet is on the increase in recent times, the practice which has led to the environmental discharge of closely 8.5 billion tons of poultry feather waste annually [6]. In Nigeria alone, about 932.5 metric tons of feather wastes are discharged annually from various poultry farms. Their disposal, utilization and management have always been a serious concern due to the presence of high recalcitrant keratin contents. Biodegradation of feathers by keratinolytic microorganisms is an efficient, cost-effective, and environmentally friendly method for bioconversion of feather wastes into nutrient-rich feather hydrolysates. Feather hydrolysates (FH) are rich in peptides, amino acids and other important products that can enhance soil microbial activity which will in turn facilitate the assimilation of nutrients by plants for better growth. They enhanced seed germination, plant growth, nutritional qualities of plant including protein, amino acids, chlorophyll content as well as phytochemical contents [4,5,7].

Maize is an important staple food for more than 1.2 billion people in sub-Saharan Africa (SSA) and Latin America [8]. It is a versatile crop that is not just consumed as staple food, the crop is used substantially by confectionery and animal feed producers, bakery, brewery and flour mill industries [9]. The global production of maize is averagely 785 million tons with United States of America been regarded as the largest producer (42%). Africa produces 6.5% and imports 28% from outside the continent. Furthermore, Africa consumes more than 30% of global maize consumption. [8]. In Nigeria, maize accounts for between 60 and 65% of

poultry feed constituents, and about 6.5% is used by brewing companies while 13% is used for the manufacturing of industrial flours, cornflakes and other confectionery [8]. It very unfortunate that Nigeria export capacity for maize is abysmally low compared to South Africa that accounts for nearly two-third of maize export in the continent. Maize yield in Nigeria is less than 2 tonnes per hectare relative to 4.9 tonnes per hectare and 4.2 tonnes per hectare in South Africa and Ethiopia, respectively. The low maize yield in Nigeria has been attributed to factors like soil infertility, drought, pests, and diseases. Therefore, enhancement of maize production using a nutrient-rich bio-based product in a sustainable and ecofriendly approach become imperative. This study aimed to produce feather hydrolysate through the biodegradation of chicken feather wastes by bacterial isolate and to apply the feather hydrolysate as biofertilizer for the enhancement of maize growth and grain yield.

MATERIALS AND METHODS

Study area

This research study was conducted at the Microbiology Department Laboratory as well as Teaching and Research Garden of School of Life Sciences, Federal University of Technology, Minna, Niger State. Minna lies within Latitudes 9°31'15"N and 9°32'30"N and Longitudes 6°26'15"E and 6°28'00"E and covers an area of about 10 km^2. It has a temperature range of 38 to 42 °C, with lowest temperature in the month of August and highest in March. The total annual rainfall in this area is between 1086 mm and 1309 mm, spread over the months of April to November. The highest amount of rainfall is recorded in the month of August. Minna lies within the Southern Guinea Savannah Ecological Zone of Nigeria [10].

Media preparation

Chicken feather wastes and soil samples were obtained from feather dump site at the Ultra-Modern Kure Market, Minna, Niger State, Nigeria. The soil sample collected in a clean polythene bag was stored under ambient conditions until further use. Feathers were washed using tap water to remove debris, and the clean feathers obtained were dried in hot air oven at 80°C. The dried feathers were chopped into pieces before milling to powder. The minimal medium for the isolation of keratinolytic bacteria was compounded using the feather powder as follows: NaCl, 2 g/l; KH_2PO_4, 2 g/l; $MgSO_4$, 0.05 g/l; $FeSO_4.7H_2O$, 0.1 g/l; $CaCO_3$, 0.1 g/l; keratin substrate, 20 g/l; and agar–agar, 20 g/l. The medium was sterilized at 121 °C for 15 min and then fortified with 0.05 g/l of sterile nystatin to inhibit fungal growth [11].

Isolation of keratinolytic bacteria and determination of keratinolytic activity

Keratinolytic bacterial strains were isolated from soil sample obtained at the same site where feather wastes were collected. Soil sample of 1 g was dispersed in 9 ml of sterile distilled water, and the suspension was serially diluted to thin out the microbial load. Aliquot of 0.5 ml was inoculated into the feather based medium for the selective growth of keratinolytic bacterial using pour plate method. The plates were incubated at 37 °C for up to 3 days. Afterwards, distinct colonies observed through morphological features were sub-cultured on nutrient agar plates to obtain pure isolates. They were further

screened to obtain the isolate with best keratinolytic activity. Activity of keratinase produced by the bacterial isolates was determined by the modified method of [12]. The isolates were cultivated in feather based medium devoid of agar and nystatin for 4–5 days using 5% inoculum size at 37°C, and 100 rpm. At 24 hour-interval, content of fermentation flask was collected, centrifuged at 5000 rpm for 20 mins and the collected supernatant served as crude keratinase. The crude keratinase (1 ml) was incubated with1.5 g of feather powder suspended in 2 ml phosphate buffer (pH 7.5, 40 mM). The control experiment consisted of buffer and crude keratinase only. The reaction mixture was held at 40 °C for 3 h at 100 rpm. At the end of incubation, thereaction was stopped by adding 2 ml of 10% trichloroaceticacid (TCA). Centrifugation for15 min at 5000 rpm and 30 °C was used to remove undegraded substrate. The increase in absorbance at 280 nm of the filtrate of the test sample relative to that of the control is a measure of release of protein which is converted into keratinase units (1 U = 0.01 absorbance increase for 1 h reaction time). All the tests were performed in replicates [11].

Characterization of the keratinolytic isolate

Among the isolates that were obtained, isolates with high keratinolytic activity was preliminarily characterized by morphological and biochemical methods [13] followed by molecular characterization using the 16S rRNA analysis. Genomic DNA was isolated from pure culture of the bacterial isolate using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005).The 16S rRNA region was amplified through polymerase chain reaction using 16S-27F (5´-AGA GTT TGATCMTGG CTC AG-3´) and 16S-1492R (5´-CGG TTA CCT TGT TAC GAC TT-3´) as the forward and reverse primer, respectively.The amplification was carried out under the following thermocyclic conditions: 95 °C for 5 min, followed by 25 cycles of 95 °C for 1 min, 50 °C for 30 s and 72 °C for 1.5 min, and finally 72 °C for 5 min. The amplicons were separated using agarose gel electrophoresis and were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequence Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No, D4050). The purified fragment was analyzed on the ABI 3500Xi GeneticAnalyzer (Applied Biosystems, ThermoFisher Scientific) and the sequences obtained were browsed in the database of the National Centre for Biotechnological Information (NCBI) (http:// blast. Ncbi. Nlm. Nih. Gov) via the blastn option for possible matches [4].

Bioconversion of chicken feather waste into feather hydrolysate

The keratinolytic bacterial isolate was used for inoculum development by inoculating into a medium consisting of 1% keratin powder, 0.2% yeast extract, pH 7.5 and incubated at 37 °C at 100 rpm for 24 h. Fermentation was carried out by inoculating 1 ml of inoculum into 19 ml of feather-based medium devoid of agar and nystatin in 100-ml flasks [4]. The incubation was held at 37 °C at 100 rpm for up to 7 days. Content of each flask was collected and centrifuged at 5000 rpm for 20 min and the supernatant was heated at 60 °C for 30 min to kill the bacterial culture. The heated supernatant served as

feather hydrolysate which was preserved at 4 °C for further use.

Determination of nitrogen and amino acid composition of feather hydrolysates

Composition of amino acid in the feather hydrolysates was determined using high-performance thin-layer chromatography (Model Hitachi L-8900 analysis) (Gurav *et al.,* 2020), while the amount of nitrogen was determined using a total organic carbon analyzer (TOC-L) equipped with a total nitrogen measurement (TNM-L) unit [14].

Evaluation of feather hydrolysate as biofertilizer for maize cultivation

Maize seeds were cultivated in the Teaching and Research Garden of School of Life Sciences, Federal University of Technology, Minna using a modified method of Afanador-Barajas *et al* [15]. Four maize seeds were planted per hole at depth of 2 cm in 40 L capacity sack bags filled with 20 kg of soil. Six treatments were considered, namely: 25 %, 50 %, 75 %, 100 % feather hydrolysate (FH), negative control (water only) and positive control (NPK fertilizer). Each bag was moistened with 1 L of FH concentrations immediately after sowing while equal volume of well water was used to treat both positive and negative control bags. Also, each positive control bag was treated with 3.5 g of NPK fertilizer (15:15:15) *via* broadcasting method 5 days before planting. Two weeks after germination, maize plants were thinned and 2 stands were maintained per bag. Furthermore, booster dose (1 L) of each FH concentration was appropriately applied using foliar spray technique 30 days after planting while 3.5 g NPK fertilizer was equally applied per bag using a localized method. Parameters like plant height, leaf number, and leaf area were determined at interval of 30 days. At 90 days, maize crops were harvested and grain size was estimated immediately after harvest. The experiment was a randomized complete block design with four replications.

Data analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS Version 20.0). Data were presented as mean of three replicates $\pm$ standard error. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Characteristics of Feather Degrading *B. licheniformis*

Among the bacterial isolates recovered from the chicken feather dumpsite, isolate MX exhibited the most pronounced feather-degrading capacity. The isolate was characterized as a Gram-positive, rod-shaped, motile, spore-forming, and facultatively anaerobic bacterium. It demonstrated positive reactions for lactose fermentation, citrate utilization, and urease production. Molecular identification based on 16S rRNA gene sequencing confirmed the isolate as *Bacillus licheniformis* MX (Fig. 1).

Keratinolytic Activity and Bioconversion of Feathers into Hydrolysate by *Bacillus licheniformis*

Bacillus licheniformis MX produces keratinase, an extracellular enzyme responsible for the breakdown of keratin-rich feather substrates. During fermentation, the organism extensively degraded the feathers, resulting in a

blackish, turbid fermentation broth accompanied by a characteristic pungent odour (Fig. 2). Also, the keratinolytic activity (KA) of *Bacillus lincheniformis* MX when in the feather medium is as shown in Fig. 2. It produced KA in the range of 14.6–48.5 U/ml. The maximum KA (48.5 U/ml) was produced at 72 h of fermentation. The course of pH during the degradation of feathers by *Bacillus lincheniformis* MX rose from the initial value of 6.0 to reach maximum value of 8.5 during 120 h of fermentation (Fig. 3)

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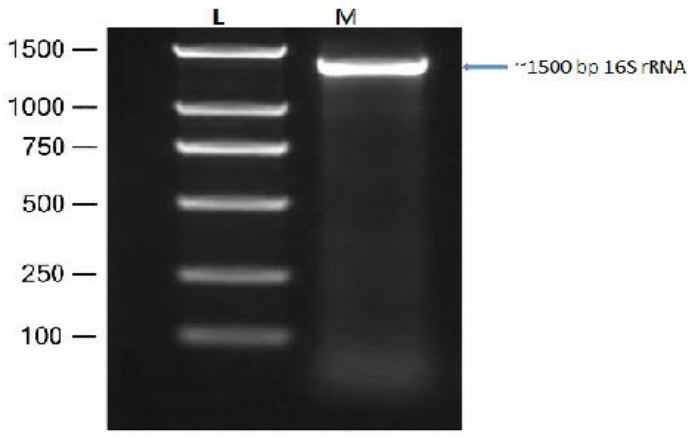

Fig 1: Gel electrophoresis image of 16S rRNA amplicon of *Bacillus licheniformis*. L, DNA ladder; M, 16S rRNA amplicon of *Bacillus licheniformis*

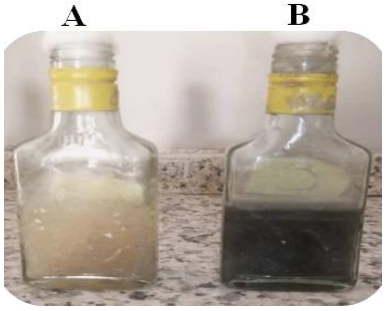

Fig. 2: Bioconversion of chicken feather waste into feather hydrolysate. A (control, undegraded chicken feather after 7 days of fermentation), B (Degraded chicken feather waste by *B. licheniformis* after 7 days of fermentation)

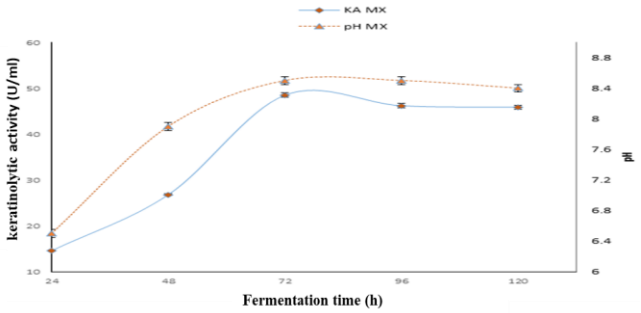

Fig. 3: Keratinolytic activity and pH change during the growth of *B. licheniformis* MX in feather-based medium

Amino acid and nitrogen composition of feather hydrolysate

The amino acids constituents of the FH obtained feather degradation by *B. licheniformis* MX are shown in Table 1. The likes of arginine (9.22 mg/ml), glutamic acid (8.51 mg/ml), cysteine (5.44 mg/ml), and proline (5.24 mg/ml) were found predominant while tyrosine (0.81 mg/ml), histidine (0.74 mg/ml), methionine (0.49 mg/ml), and tryptophan (0.36 mg/ml) were small in quantity. The nitrogen in the FH was estimated to be 12.96% (w/v). This reflects the proteinaceous nature of the FH and also indicates the hydrolysis of feather substrate B *licheniformis* MX 12.60 ± 0.15% (w/v),

Table 1: Amino acid composition of the feather hydrolysate

Amino acids	Concentration:(mg/ml)
Leucine	5.30 ± 0.12
Lysine	3.70 ± 0.07
Isoleucine	2.51 ± 0.10
Phenylalanine	2.64 ± 0.07
Tryptophan	0.36 ± 0.05
Valine	2.75 ± 0.09
Methionine	0.49 ± 0.08
Proline	5.24 ± 0.04
Arginine	9.22 ± 0.12
Tyrosine	0.81 ± 0.05
Histidine	0.74 ± 0.04
Cysteine	5.44 ± 0.03
Alanine	2.69 ± 0.07
Glutamic acid	8.51 ± 0.10
Threonine	1.86 ± 0.06
Serine	4.40 ± 0.11
Aspartic acid	5.36 ± 0.07

Effect of Feather Hydrolysate as Biofertilizer on maize cultivation

The effect of feather hydrolysate (FH) as a biofertilizer on maize after 90 days of cultivation is shown in Table 2. The FH application significantly enhanced maize growth compared with water control. FH100% produced the highest values for all measured parameters, recording 218.6 cm plant height, 682.4 cm^2 leaf area, 14.3 leaves per plant, 4.52 (mg/g FW) chlorophyll content, and 3.20 kg grain

weight. The performance of maize generally increased with increasing FH concentration, with the sequence FH100% > FH75% > FH50% > FH25% > Water control. Interestingly, FH100% also performed better than the conventional NPK fertilizer treatment for all parameters.

Table 2: Effect of feather hydrolysate on growth and yield-related parameters of maize

Treatment	Plant Height (cm)	Leaf Area (cm^2)	Number of Leaf	Chlorophyll Content (mg/g FW)	Grain Weight (Kg)
FH100%	218.60 ± 4.80^{a}	682.40 ± 15.70^{a}	14.30 ± 0.60^{a}	4.52 ± 1.40^{a}	3.20 ± 0.40^{a}
FH75%	207.40 ± 5.20^{ab}	641.80 ± 18.30^{ab}	13.70 ± 0.60^{ab}	4.30 ± 1.20^{ab}	3.03 ± 0.30^{ab}
FH50%	194.80 ± 4.60^{b}	596.30 ± 16.50^{b}	13.00 ± 0.50^{b}	4.06 ± 1.30^{b}	2.88 ± 0.40^{b}
FH25%	180.20 ± 5.10^{c}	548.70 ± 14.90^{c}	12.30 ± 0.60^{bc}	3.82 ± 1.50^{c}	2.70 ± 0.30^{c}
NPK	201.70 ± 4.90^{ab}	617.50 ± 17.20^{ab}	13.50 ± 0.50^{ab}	4.18 ± 1.10^{ab}	2.95 ± 0.30^{ab}
Water	151.60 ± 4.30^{d}	452.80 ± 13.60^{d}	10.80 ± 0.50^{c}	3.24 ± 1.30^{d}	2.30 ± 0.30^{d}

Values are presented as mean $\pm$ standard deviation ($n = 3$). Means within each column followed by different superscript letters are significantly different at $p < 0.05$.

DISCUSSION

Previous studies have similarly documented the recovery of feather-degrading bacteria from feather-contaminated environments, with Gram-positive organisms constituting a substantial proportion of the isolates. Members of the genus *Bacillus* have been particularly prevalent in several feather dumpsites, although some Gram-negative genera, including *Pseudomonas*, *Vibrio*, and *Aquamicrobium*, have also been reported [4,11]. Quite a number of findings have shown KA of some bacterial species that are similar to the values herein. For instance, Lateef *et al* [11] reported KA of 50.4 U/ml for *B. safensis* LAU 13 isolated from a feather dump site using feather waste as keratin substrate. Similarly, KA (45.2) was also reported when a strain of *Aquamicrobium defluvii* isolated from feather dumpsite was cultivated on feather medium [4]. In a related study, Alahyaribeik *et al.* [16] reported maximum KA of 50.41 U/ml after 48 h of fermentation by a strain of *B. licheniformis* using feathers as substrate. Studies have shown that there exist a positive correlation between keratinolytic activity and keratin degradation. High KA typically results to more efficient and faster keratin degradation [17,18]. Moreso, the shift in pH during keratinase production corresponds with the report of Gupta and Ramnani [17], that environment with pH of 6 to 9 favors keratin degradation by most microorganisms. The rise in alkalinity of the fermentation medium could be ascribed to the discharge of ammonium through deamination reaction occurring during keratin hydrolysis.

Feather hydrolysate obtained after removing the undegraded feathers was rich in ammonium nitrogen content (12.95%). The nitrogen concentration falls within the range of values reported by previous authors. For instance, Sobucki *et al* [14] reported a nitrogen content of 15.36% for feather hydrolysate of *Bacillus* sp. CL18. A nitrogen content of 12% was reported for the feather protein hydrolysate of *Chryseobacterium sediminis* RCM-SSR-7 [19]. In addition, FH has been reported to contain substantial

amounts of nitrogen (13% or more) that provide a long-lasting and slow-releasing nutrient source [5].

There are evidence that support the richness of FH in amino acids. According to Adelere and Lateef [4], the FH obtained upon degradation of chicken feathers by *B safensis* LAU 13 was rich both in nitrogen and amino acids like glycine (3.86 mg/ml), alanine (3.45 mg/ml), and leucine (2.52 mg/ml). In a related study, Hendrick *et al* [20] reported amino acid contents of valorized chicken feather by *Chryseobacterium cucumeris* FHN1 as arginine (3.44%), phenylalanine (3.59%), glycine (3.68%), leucine (4.41%), histidine (4.54%), valine (4.63%), aspartic acid (4.75%), serine (5.19%), glutamic acid (5.86%), and proline (6.08%). Others included hydroxyproline (0.01%), methionine (0.43%), tryptophan (0.86%), cysteine (1.11%), and lysine (1.13%). The release of amino acids after the degradation of feathers occurs because feathers are composed of over 90% keratin, an insoluble, rigid, and structural protein. Its degradation by microorganisms releases soluble, smaller peptides fragments and amino acids. The variation in types and quantities of amino acids might be attributed to the differences in the keratin degrading ability of microorganisms. The richness in nitrogen and amino acids of keratin hydrolysates potentiates their application as alternative source of protein in animal feed formulation [21].

The results obtained herein corroborate previous findings where studies have established that keratin/feather hydrolysate is a very robust source of nitrogen, ammonia and amino acids that can be exploited as a slow-release nitrogen fertilizers [5,21,22]. Application of FH increases had been reported to boost physical, chemical and biological activities in the soil. It heightens protein, amino acids, and chlorophyll content of plant. FH can also enhance seed germination and growth performance of plants [23]. Tamreihao *et al* [24] reported a significant improvement in the growth of rice plant cultivated on the soil treated by FH obtained after feather hydrolysis by *Amycolatopsis* sp. MBRL 40 over the control rice plant cultivated on the ordinary soil. Most recently, Saleem et al [7] reported an overwhelming enhancement of spinach growth using the biofertilizer derived from chicken feather wastes valorized by keratinolytic bacterial consortium. Thus, the FH produced by *B. licheniformis* demonstrated plant growth-promoting ability of maize which adds to the existing reports on the potential application of feather hydrolysates as organic fertilizers.

CONCLUSION

In this study, *Bacillus licheniformis* MX significantly hydrolyzed chicken feathers to produce robust feather hydrolysates that were rich in nitrogen, amino acids like arginine, proline, leucine, glutamic acid, cysteine and aspartic acid. The extracellular keratinase produced exhibited overwhelming keratinolytic activity (48.5 U/ml) at 72 h of fermentation. Application of 100%FH significantly enhanced the growth and grain yield of maize than water and NPK fertilizer treatments. Hence, the FH can be deployed as organic nitrogen fertilizer to substitute synthetic NPK fertilizer for improved and sustainable production of maize.

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