Novelty Journals

ISSN 2394-9716

International Journal of Novel Research in Interdisciplinary Studies
Vol. 13, Issue 3, pp: (1-11), Month: May – June 2026, Available at: www.noveltyjournals.com

PROXIMATE COMPOSITION OF PLANT FIBRES AND FERULIC ACID RELEASE BY FUNGAL ISOLATES DURING FIBRE DEGRADATION

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DOI: https://doi.org/10.5281/zenodo.19953370

Published Date: 01-May-2026

Abstract: **The proximate composition of different plant fibres and the free ferulic acid released by fungal species during their degradation were determined. Fibre samples included sugarcane bagasse and brans of wheat, rice, maize, and barley. Fungal isolates obtained from naturally decayed fibre materials were characterized using phenotypic and molecular techniques and screened individually for ferulic acid production using the plant fibres as substrates. The frequency of occurrence of fungal isolates was: *Aspergillus flavus* (27.3%), *A. niger* (18.2%), *A. versicolor* (9.1%), *A. oryzae* (9.1%), *A. parasiticus* (9.1%), *Bipolarisspicifera* (9.1%), *Rhizomucuspusillus* (9.1%), and *Rhizopusoryzae* (9.1%). The proximate composition of the fibres ranged (%, dry weight) as follows: ash (0.17–7.83), moisture (10.35–44.34), protein (6.17–24.94), fat (1.34–7.44), carbohydrate (2.70–25.60), neutral detergent fibre (49.56–74.79), acid detergent fibre (11.05–49.68), and crude fibre (16–46). Ferulic acid production ranged from 0.3 µg/mL by *B. spicifera* to 4.1 µg/mL by *A. oryzae*. Wheat bran supported the highest ferulic acid release, highlighting its suitability as a substrate for microbial bioconversion.**

Keywords: **Ferulic acid, proximate composition, fungal degradation, plant fibres.**

1. INTRODUCTION

Plant cell walls are composed of structural polysaccharides such as cellulose, hemicellulose, and pectins, embedded in a matrix of lignin, proteins, and phenolic compounds (Carpita & McCann, 2000). Phenolic acids, particularly ferulic acid, play an important role by strengthening cell walls through cross-linking, thereby enhancing resistance to biodegradation and pathogen invasion (Tharanathan, 2002; Lynd et al., 2002).

Ferulic acid is the predominant phenolic acid in the brans of several cereal such as wheat, rice, maize, and rye (Weidner et al., 1999; Kim et al., 2006). It exhibits antioxidant properties and has broad applications in food preservation, pharmaceuticals, and cosmetics due to its protective effects against oxidative stress, cancer, diabetes, cardiovascular disease, and UV-induced skin damage (Omar, 1992; Mathew & Abraham, 2004).

Cereal brans and sugarcane bagasse are rich in lignocellulosic material and contain significant amounts of bound ferulic acid (Azuma et al., 1990; Sun et al., 2003). Traditionally, ferulic acid has been extracted by alkaline hydrolysis, though enzymatic release via microbial fermentation offers a cleaner and more sustainable alternative (Faulds & Williamson, 1999; Ahmed & Yi-Hsuju, 2007).

This study evaluated the proximate composition of selected plant fibres and screened fungal isolates from decayed fibres for their ability to release ferulic acid, with emphasis on the relationship between substrate composition and degradation.

2. MATERIALS AND METHODS

2.1 Sample Collection

Rice, maize, wheat, and barley brans, as well as sugarcane bagasse, were obtained from Lafenwa market, Abeokuta, Ogun State, Nigeria, and transported to the laboratory for analysis.

2.2 Isolation and Identification of Fungi

Isolation was performed by the dilution plate method on Potato Dextrose Agar (PDA) (Harrigan and McCance, 1966). One gram (1g) of each fibre sample was dispensed separately in 9mls of sterile water. 1ml of this solution was transferred to a second tube containing 9mls of sterile water, resulting in a 10^{-1} dilution of the spore mass in the original material. The process was repeated to yield dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. 1ml portion from each dilution was pipetted to a petri dish and cooled molten PDA medium supplemented with 200mg/l filter sterilized chloramphenicol to prevent bacterial growth (Greben *et al*., 2007) was poured over it. Evenly spread across the plates was achieved by swirling. The plates were incubated at 27^{o}C for 3 days in the incubator (Unigold Medical: DNP-9022A). The mixed colonies observed after the incubation period were transferred aseptically onto PDA media slants separately to obtain the pure cultures (Abe *et al*., 1988; Kausa*et al*., 2010). Slants were grown at 30^{o}C for 7days, stored at 4^{o}C and subcultured fortnightly (Swetha*et al*., 2007).

2.3 Proximate analysis

2.3.1 Determination of Total Carbohydrate content

Five milliliters (5mL) of 72% H_2SO_4 was added to 1g of rice bran. The mixture was shaken and filtered. 1mL of the filtrate was pipette into a conical flask and 5mL of Anthrone reagent was added. 0.5ml of the solution was transferred into another conical flask (to reduce the concentration) and 30mL of Anthrone reagent was added again. It was heated for 10 minutes and cooled to room temperature. The absorbent was measured at 620nm. The same procedure was repeated for the remaining samples (barley bran, wheat bran, maize bran and sugarcane bagasse).(AOAC, 2000).

2.3.2 Determination of Protein content

One gram (1g) of rice bran was weighed into a digestion tube and two kjeltabs were added. 12mls of concentrated H_2SO_4 was carefully added and the mixture shaken gently. The exhaust system was attached to the digestion tubes in the rack and water aspirator was set to full effect. Then, the rack with exhaust was loaded into a preheated digestion block (420^{o}C). After 5minutes, the water aspirator was turned down. The digestion was continued until all the samples were clear. The rack of the tubes was removed and put in the stand to cool for 20minutes. Eighty milliliters (80mls) of deionized water was carefully added to the tubes. The steam valve was opened and distillation was carried out for 4 minutes. The distillate was titrated against standardized HCl until the blue end point was achieved. The volume of acid consumed in the titration was recorded. The blank was determined by adding 2 kjeltabs to 12mls of H_2SO_4. This was digested and subsequently treated exactly as the sample.

% protein = N x F
N = Normality of acid to 4 places of decimal

F = Conversion factor for Nitrogen to protein

14.007 = molecular weight of nitrogen (AOAC, 2000)

2.3.3 Determination of Acid Detergent Fibre (ADF)

Pre-dried crucibles were placed on a weighing balance and the weight was recorded as W_0. One gram (1g) of each sample was weighed separately into the crucibles and the weight was noted as W_1. The stand of the set of crucibles was hooked on the front of the hot extraction unit. The crucibles were transferred into the unit and were attached by pressing the lever down. The valves were placed in a closed position and 50mls of acid detergent solution was added. Two (2) drops of octanol

was also added to prevent foaming and was then heated to boiling. It was allowed to boil for 1 hour. The heater was turned off and the valve was placed in vacuum position. The cold water was open for the water suction pump and the samples were filtered. One sample was filtered at a time. Each sample was washed three times with 30mls of hot deionized water and it was sucked dry as possible using the vacuum pump (XB-IB).

With the aid of the crucible holder, the crucibles were moved from the hot extraction unit to the cold extraction unit. The valves were closed and each crucible containing the sample was filled with 25mls of acetone and then filtered by placing the valve in vacuum position. This was repeated three times for each sample. The crucibles were removed and transferred to a crucible stand at room temperature in a ventilated chamber until all the acetone was gone. The crucibles were dried at 130°C for 2 hours. They were then cooled to room temperature in a desiccator and weighed as (W_2) (AOAC, 2000).

$$\%ADF = \frac{W_2 - W_0}{W_1} \times 100$$

W_1 = Sample weight

W_2 = crucible + residue

W_0 = weight of empty crucible

2.3.4 Determination of Neutral Detergent Fiber (NDF)

Pre-dried crucibles were placed on a weighing balance. One gram (1g) of each sample was weighed seperately into the crucibles and the weight was noted as W_1. The stand of the set of crucibles was hooked on the front of the Hot Extraction unit. The crucibles were transferred into the unit and were attached by pressing the lever down. The valves were placed in a closed position and 50mls of neutral detergent solution was added with 0.25g of sodium sulfite. 2 drops of octanol was also added to prevent foaming and was then heated to boiling. It was allowed to boil for 1 hour. The heater was turned off and the valve was placed in vacuum position. The cold water was open for the water suction pump and the samples were filtered. One sample was filtered at a time. Each sample was washed three times with 30mls of hot deionized water and it was sucked as dry as possible in the vacuum pump (XZ-IB) .

With the aid of the crucible holder, the crucibles were moved from the hot extraction unit to the cold extraction unit. The valves were closed and each crucible containing the sample was filled with 25mls of acetone and then filtered by placing the valve in vacuum position. This was repeated three times for each sample. The crucibles were removed and transferred to a crucible stand at room temperature in a ventilated chamber until all the acetone was gone. The crucibles were dried at 130°C for 2 hours. They were then cooled to room temperature in a dessicator and weighed as (W_2). The samples were ashed in the crucible at 500°C for 3hours and cooled down to below 250°C before removing from the furnace. The crucibles were cooled to room temperature and their weights recorded as W_3 (AOAC, 2000).

$$\% NDF = \frac{W_2 - W_3}{W1} \times 100$$

W_1 = Sample weight

W_2 = crucible + residue

W_3 = crucible + ashed residue

2.3.5 Determination of crude fibre content

Pre-dried crucibles were placed on a weighing balance. One gram (1g) of each sample was weighed seperately into the crucibles and the weight was noted as W_1. The stand of the set of crucibles was hooked on the front of the hot extraction unit. The crucibles were transferred into the unit and were attached by pressing the lever down. The valves were placed in a closed position and 150mls of hot 0.128M sulphuric acid was added. Three (3) drops of octanol was also added to prevent foaming and was then heated to boiling. It was allowed to boil for 30minutes. The heater was turned off and the valve was placed in vacuum position.

The cold water was opened for the water suction pump and the samples were filtered. One sample was filtered at a time. Each sample was washed three times with 30mLs of hot deionized water and it was sucked as dry as possible in the vacuum pump (XZ-IB). 150mls of 0.223M potassium hydroxide was added to each sample with 2 drops of octanol and the samples were heated to boiling again for 30min. The heater was turned off and the valve was placed in vacuum position. The samples were then filtered. Each sample was washed three times with 30mls of hot deionized water and it was sucked as dry as possible in the vacuum pump (XZ-IB).

With the aid of the crucible holder, the crucibles were moved from the hot extraction unit to the cold extraction unit. The valves were closed and each crucible containing the sample was filled with 25mls of acetone and then filtered by placing the valve in vacuum position. This was repeated three times for each sample. The crucibles were removed and transferred to a crucible stand at room temperature in a ventilated chamber until all the acetone was gone. The crucibles were dried at 130^{o}C for 2 hours. They were then cooled to room temperature in a dessicator and weighed as (W_2). The samples were ashed in the crucible at 500^{o}C for 3hours and cooled down to below 250^{o}C before removing from the furnace. The crucibles were cooled to room temperature and their weights recorded as W_3 (AOAC, 2000).

$$\% \text{ Crude Fibre} = \frac{W_2 - W_3}{W1} \times 100$$

W_1 = Sample weight

W_2 = crucible + residue

W_3 = crucible + ashed residue

2.3.6 Moisture content determination

A clean, flat dish was dried in an oven for 1hour and was allowed to cool after drying in a dessicator. The cooled empty dish was weighed and the weight recorded as (W_1). Two grams (2g) of the sample was introduced into the dish and weighed. The weight also recorded as W_2. The dish and its content was transferred into the air oven to dry the contents for 3 hours at 105^{o}C and later transferred into dessicator to cool. The new weight of the dish and its dried content was measured and the weight recorded as W_3. (AOAC, 2000).

$$\% \text{ moisture} = \frac{(W_2 - W_3)}{W_2 - W_1} \times 100$$

2.3.7 Ash Content Determination

The dish was weighed and recorded as W_1, 2g of sample was weighed into the dish and the weight was recorded as W_2. The dried sample was charred over a bunsen flame in a fume cupboard until no more soot is given off. The dish was transferred using a pair of tong into a muffle furnace set at 550^{o}C. Ashing was completed when the charred sample was completely white in color. With the aid of a pair of tong, the dish and its content were transferred into the dessicator to cool (AOAC, 2000). The dish containing the contents were weighed and recorded as W_3.

$$\%\text{Ash} = \frac{(W_3 - W_1)}{(W2 - W1)} \times 100$$

2.3.8 Determination of Fats and Oils

Three grams (3g) each of grounded samples were weighed into extraction thimbles (W_1). The samples were covered with a thin film of defatted cotton wool and the thimble adapters were attached. The extraction cups with boiling chips were weighed and recorded as (W_2). Fifty milliliters (50ml) of hexane was added to each and the thimbles were attached to the extraction unit. The extraction cups containing the solvent were inserted and they were boiled at 120^{o}C for 15min. They were rinsed with solvent for 30min and the solvent was collected. The air valves were opened and vacuum was applied to remove the last traces of the solvent. The extraction cups which now contained the fats, oils and chips were removed and the cups were dried for 30min at 100^{o}C. They were cooled in a dessicator. The dried cups containing the extracted oil were weighed and recorded as W_3 (AOAC, 2000)

$$\%fat = \frac{W_3 - W_2}{W_1} \times 100$$

W_1 = weight of samples

W_2 = weight of cup and chips

W_3 = weight of cup and extraction oil and chips

2.4 Preparation of Inoculum

Spore suspension was prepared by adding 10mls of sterile distilled water to the 7 day-old culture slants and the spores were dislodged using an inoculation needle under sterile conditions (Swetha*et al*., 2007). The entire content was filtered into a clean sterile MacCartney bottle using a muslin in a clean, sterile funnel. The filtrates stand as the Inoculum.

2.5 Preparation of the Production medium

The medium used was a Chemically Defined Medium which comprised 30g each of all the fibre materials separately prepared and a basal medium comprising of 0.1% (w/v) KH_2PO_4, 0.03% (w/v) $CaCl_2$, 0.03% (w/v) $MgSO_4.7H_2O$, 1.56% (w/v) $Na_2HPO_4.2H_2O$, 0.152% (w/v) NaH_2PO_4 and 1.0%(w/v) ammonium tartarate (Shin and Chen, 2005). The carbon sources (rice bran, wheat bran, maize bran, barley bran and sugarcane bagasse) were sterilized separately at 121^{o}C for 25mins (Ou*et al*., 2011) from the basal medium (mixture of 1.8g KH_2PO_4; 0.54g $CaCl_2$; 0.54g $MgSO_4.\&H_2O$; 28.08g $Na_2HPO_4.2H_2O$; 2.7g $NaH_2PO_4.2H_2O$ and 36g ammonium tartarate. All were dissolved in 1.8 Litres of distilled water) at 121^{o}C for 15mins (Shin and Chen, 2006).

2.6 Production of crude enzyme by each of the isolated fungal strain

Sixty milliliters (60mls) each of the sterile basal medium was aseptically dispensed separately into the sterilized 30g of carbon sources in 250ml conical flasks. 1mL of each fungal spore suspension was aseptically inoculated into each of the chemically defined medium. This procedure was carried out with all the isolated fungi using all the carbon sources with each isolate. The conical flasks were incubated at 30^{o}C for 5 days (Ganapati, 2009).

2.7 Extraction of crude enzyme

After the incubation periods, the crude enzyme was extracted. The fermented substrates were treated with 100mls of 0.05M citrate buffer, pH 5.0 by shaking (150rpm) at room temperature for 20mins to form a semi-solid mixture. Solids were then separated from the solution by filtering with filter paper (Whatman No 1). The clarified supernatant (crude enzyme) obtained was kept at 4^{o}C for further studies (Ou *et al*., 2011).

2.8 Release of Ferulic acid

The reaction mixture contained 0.9ml of substrate solution and 0.1ml of crude enzyme solution. Substrate solution was prepared by mixing 5mmol/L of methyl ferulate in dilute methanol and 0.05mol/L Citrate buffer (pH 5.0) to get the final methanol concentration of 4%. Incubation was conducted at a temperature of 40^{o}C for 20min. Enzymatic reaction was subsequently stopped by mixing samples with methanol at 1:1 (v/v) ratio and cooling down the mixture (Trzcinska *et al*., 2005).

2.8.1 Determination of the ferulic acid content

The released ferulic acid was determined by High Performance Liquid Chromatography (HPLC) (Agilent/HP 1100 series HPLC- DAD system) using an Alltima C18 column (5µm, 250mm x 4.6mm) with a suitable guard column (C18, 5µm, 7.5mm x 4.6mm). The mobile phase consisted of 1.0% acetic acid in water (A) and acetonitrite (B). The flow rate was 1.0ml/min and column temperature was maintained at 30^{o}C DAD detector was set at 320nm for acquiring the chromatogram. (Lu *et al*., 2005).

The stock solution of ferulic acid was prepared at a concentration of 500mg/L in methanol -2% $NaHCO_3$ in water (95:5). Calibration standard solutions were prepared in the concentration range of 5 – 45mg/l with methanol -2% $NaHCO_3$ in water (95: 5). The calibration curve was constructed by plotting the peak areas of the analyte against the concentration of ferulic acid (Lu *et al*., 2005). The ferulic acid released was then read on the standard calibration curve.

Novelty Journals

ISSN 2394-9716

International Journal of Novel Research in Interdisciplinary Studies
Vol. 13, Issue 3, pp: (1-11), Month: May – June 2026, Available at: www.noveltyjournals.com

3. RESULTS

Table 1. Proximate composition of selected plant fibres (%, dry weight basis)

Fibre type	Ash	Moisture	Protein	Fat	Carbohydrate	NDF	ADF	Crude fibre
Wheat bran	5.35	10.35	13.65	4.85	25.6	47.68	11.05	18.0
Rice bran	7.83	11.61	13.46	7.44	17.76	57.06	14.56	25.0
Maize bran	1.77	12.48	12.06	5.32	21.2	55.7	25.09	22.0
Barley bran	3.9	10.52	24.94	5.11	20.53	58.35	30.0	28.0
Sugarcane bagasse	0.17	44.34	6.17	1.34	2.7	74.79	49.68	46.0

Table 2. Ferulic acid released (µg/mL) by fungal isolates using different plant fibres as substrates

Fungal isolate	Rice bran	Maize bran	Wheat bran	Barley bran	Sugarcane bagasse
Aspergillus niger AN1	1.8	2.2	3.9	1.8	1.4
Aspergillus flavus AF1	0.5	2.4	3.7	2.5	2.8
Aspergillus versicolor AV	0.6	2.7	1.5	2.4	1.4
Bipolaris spicifera BS	1.3	1.8	2.6	0.3	2.3
Aspergillus oryzae AO	1.5	1.5	4.1	2.3	0.6
Rhizomucus pusillus RP	0.5	2.2	2.5	1.3	2.6
Aspergillus flavus AF2	0.7	1.4	2.2	2.6	1.3
Aspergillus parasiticus AP	0.5	1.9	1.8	1.4	2.5
Aspergillus niger AN2	1.4	2.4	2.2	1.8	2.0
Aspergillus flavus AF3	0.7	1.2	2.8	1.4	1.8
Rhizopus oryzae RO	0.5	1.8	2.5	1.4	1.9

Figures 1–6: Ferulic Acid Release by Fungal Isolates

Figure 1. Ferulic acid released by fungal isolates during degradation of rice bran. Values represent mean concentrations (µg/mL) obtained from replicate fermentations.

Figure 2. Ferulic acid released by fungal isolates during degradation of maize bran. Distinct variations in yield among *Aspergillus* species and non-*Aspergillus* fungi are evident.

Figure 3. Ferulic acid released by fungal isolates during degradation of wheat bran. Wheat bran supported the highest overall production, with *Aspergillus oryzae* and *A. niger* showing the greatest yields.

Figure 4. Ferulic acid released by fungal isolates during degradation of barley bran. *Bipolarisspicifera* showed the lowest yield across all substrates (0.3 µg/mL).

Figure 5. Ferulic acid released by fungal isolates during degradation of sugarcane bagasse. Moderate yields were observed, reflecting the higher fibre content and lower protein/fat availability of bagasse compared to cereal brans.

Figure 6. Comparative summary of ferulic acid release by fungal isolates across all substrates. Wheat bran consistently supported the highest yields, where as rice bran supported the lowest.

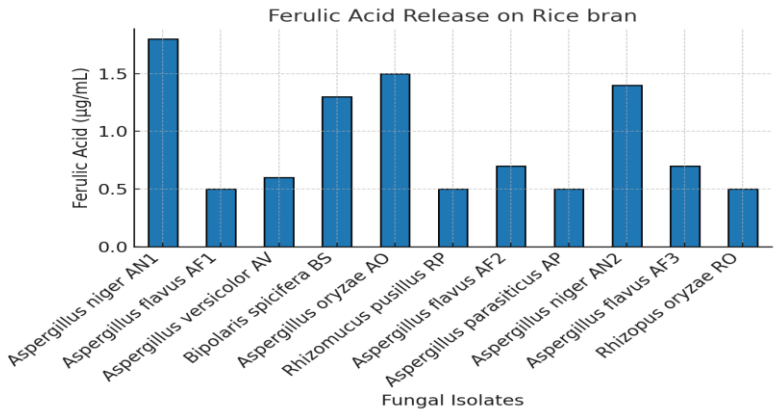

Figure 1. Ferulic acid released by fungal isolates on Rice bran.

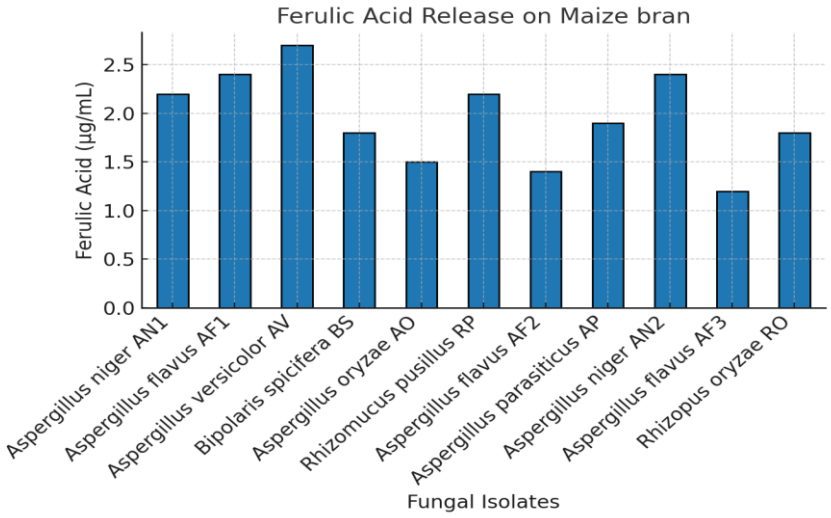

Figure 2. Ferulic acid released by fungal isolates on Maize bran.

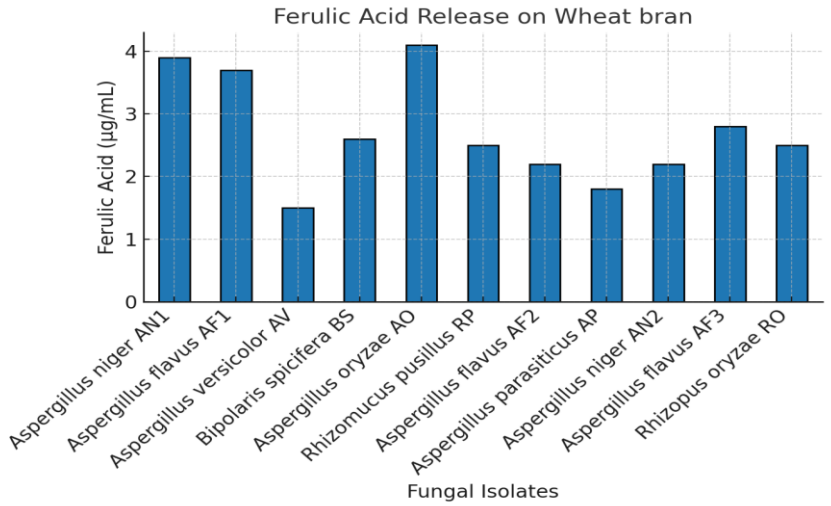

Figure 3. Ferulic acid released by fungal isolates on Wheat bran.

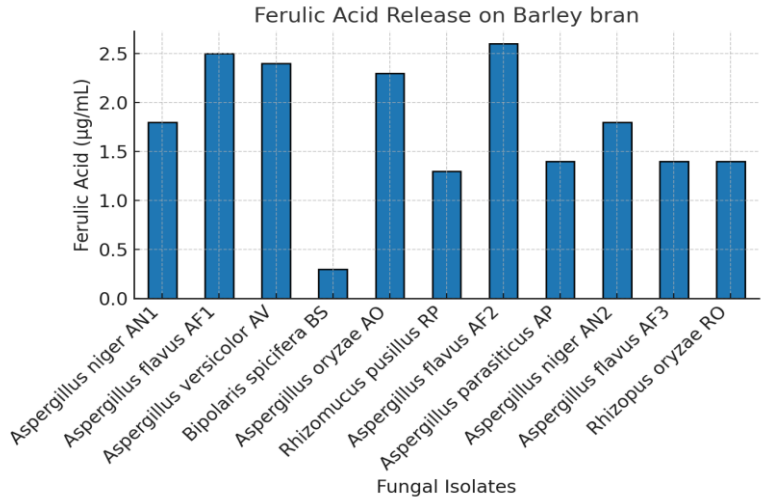

Figure 4. Ferulic acid released by fungal isolates on Barley bran.

Novelty Journals

ISSN 2394-9716

International Journal of Novel Research in Interdisciplinary Studies
Vol. 13, Issue 3, pp: (1-11), Month: May – June 2026, Available at: www.noveltyjournals.com

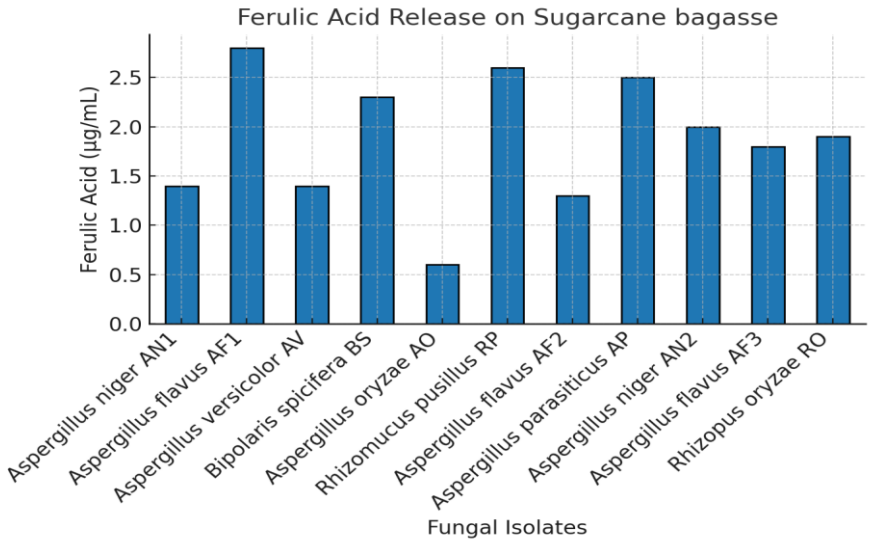

Figure 5. Ferulic acid released by fungal isolates on Sugarcane bagasse.

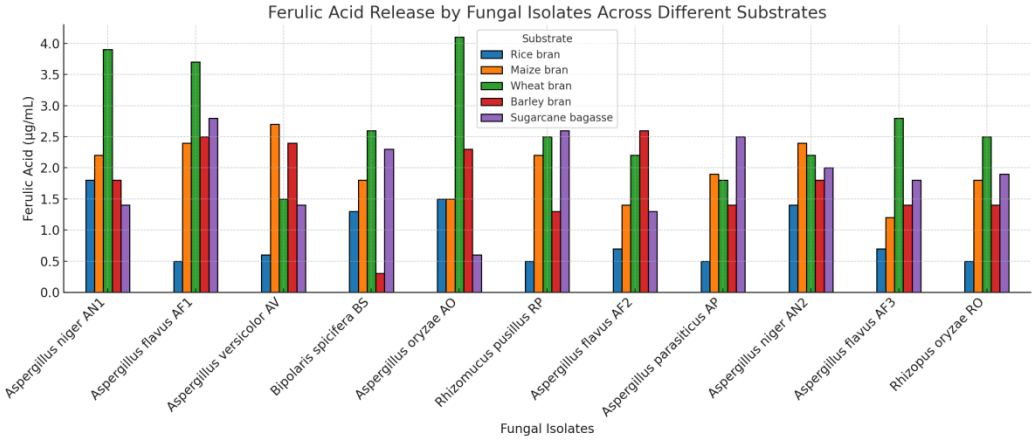

Figure 6. Comparative summary of ferulic acid release by fungal isolates across all substrates.

4. DISCUSSION

Fungal Isolation and Identification

A total of 11 fungal isolates were obtained, dominated by *Aspergillus* species (72.7%). The frequency of occurrence was highest for *A. flavus* (27.3%), followed by *A. niger* (18.2%). This dominance aligns with previous reports describing the ubiquity of *Aspergillus* in lignocellulose degradation due to their extensive enzymatic arsenal (Gao et al., 2008).

Proximate Composition of Plant Fibres

Proximate analysis (Table 1) revealed significant variation among fibres. Wheat bran contained the highest carbohydrate (25.6%) and relatively high protein (13.7%), whereas barley bran had the highest protein overall (24.9%). Sugarcane bagasse, though low in protein and fat, was rich in crude fibre (46%) and neutral detergent fibre (74.8%). Rice bran contained the highest fat (7.4%) and ash (7.8%). These findings agree with earlier reports (Ou *et al.*, 2011; Kaur *et al.*, 2011).

Ferulic Acid Release by Fungal Isolates

Ferulic acid production varied widely depending on the fungal isolate and substrate (Table 2). Wheat bran consistently supported the highest yields, with *A. oryzae* producing 4.1 µg/mL, followed by *A. niger* (3.9 µg/mL) and *A. flavus* (3.7 µg/mL). The lowest production (0.3 µg/mL) was recorded by *B. spicifera* on barley bran.

Substrate composition influenced enzyme activity and ferulic acid release. Wheat bran's loose structure and balanced nutrient profile likely facilitated fungal growth and enzymatic degradation (Babu&Satyanarayana, 1995). In contrast, rice bran supported low yields, possibly due to its higher phytic acid content, which limits nutrient availability (Eggum et al., 1981).

Overall, results confirm that fungal species differ in their ability to degrade lignocellulosic substrates and release ferulic acid, with *A. oryzae* showing the greatest potential for industrial applications.

5. CONCLUSION

This study demonstrated that fungal isolates obtained from decayed plant fibres vary significantly in their ability to release ferulic acid, with production strongly influenced by substrate composition. Wheat bran proved to be the most effective substrate, supporting the highest ferulic acid yields, particularly with *Aspergillus oryzae* and *A. niger*. In contrast, rice bran and sugarcane bagasse supported relatively lower production levels, likely due to their compositional characteristics. The findings highlight the importance of careful selection of both fungal strains and substrates in optimizing ferulic acid production. These results provide a basis for further biotechnological applications aimed at sustainable valorization of agro-industrial residues for value-added product development.

6. RECOMMENDATIONS

1. **Optimization of Substrate Use**
   - Wheat bran should be prioritized as a substrate for microbial bioconversion of ferulic acid due to its high yield potential.
   - Further studies should investigate pre-treatment methods for low-yield substrates (e.g., rice bran, sugarcane bagasse) to enhance their suitability.
2. **Selection of Potent Fungal Strains**
   - *Aspergillus oryzae* and *A. niger* demonstrated superior ferulic acid release and should be considered lead candidates for industrial-scale applications.
   - Development of fungal consortia may also be explored to combine enzymatic strengths of multiple isolates.
3. **Process Development and Scale-Up**
   - Fermentation conditions, including pH, temperature, and incubation time, should be optimized for maximum enzyme activity and ferulic acid yield.
   - Pilot-scale trials are recommended to validate laboratory findings for industrial feasibility.
4. **Biotechnological Applications**
   - The potential use of fungal-derived ferulic acid should be explored in food preservation, pharmaceuticals, and cosmetics, especially as a natural antioxidant.
   - Sustainable valorization of agricultural residues through microbial fermentation can reduce waste and generate value-added products.

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