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Waste to Value Aided Fertilizer: An Alternative Cleaning Technique for Poultry Feathers Waste Disposal

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Abstract

A by-product of poultry processing, feather waste is a protein biomass rich in peptides, amino acids, and valuable micro and macro minerals. The application of feather waste as soil fertilizer represents not only a sustainable feather disposal method, but may also represent an effective strategy to tackle environmental effluence. Feather waste has been found to elevate protein (0.78± 0.001 mg/g of dry wt), total carbohydrate (0.3±0.002 mg/g of dry wt), total chlorophyll (1.52±0.08 mg/g of dry wt), and proline (0.106±0.001 mg/g of dry wt) contents of plants and wheat seeds. Such increases have been associated with considerably higher phenolic and flavonoid contents. Therefore, its application may promote and improve the agro-ecosystem, soil texture, and soil bio-ecology and enhance the production of plant or products rich in bioactive substances. In this study commercially usable feather fertilizer was developed by mixing the feather waste and fly ash, which increased its effectiveness and storage ability. Laboratory application showed significantly improved crop yields. Consequently, feather hydrolysates created through the microbial conversion of feather keratin may be utilized as a potential bioactive agricultural nitrogen input.

Keywords: Feather; Wheat plant; Proline; Agro-ecosystem; Bioactive

Introduction

Wheat is the second most common cereal crop produced globally after rice. According to United States Food and Agricultural Organization figures, 690 million metric tons of wheat were produced globally in 2013. After China, India is the second largest wheat producing nation, producing 94.9 million metric tons of wheat in 2013. Panjab, Haryana, and Utter Pradesh are the three major wheat cultivation states in India. West Bengal produced 1,005 million metric tons of wheat in 2013 (Reports from Bureau of Applied Economics and Statistics, Government of West Bengal, India) (Figure 1). Wheat is a vital dietary protein source, comprising the main protein and energy supply in most countries [1]. Wheat seed storage proteins are traditionally classified into four groups according to their solubility properties; albumins, globulins, prolamins, and glutelins. Gluten is the richest wheat endosperm protein and plays a leading role in wheat flour quality. Variation in protein content and composition significantly affect wheat quality and baking properties [2].

In addition, wheat protein content and baking quality depend greatly on genetics and environmental factors, especially nitrogen availability (soil N, rate and time of application) [3]. Nitrogen rate, type of nitrogen, and timing of its application are important for increasing wheat yield [4]. The essential nitrogen element is required for the synthesis of wheat protein, nucleic acid, chlorophyll, and other cellular contents. For this reason, chemical fertilizers are regularly utilized in agricultural production in order to ensure sufficient nitrogen levels. The use of chemical nitrogen fertilizers has increased 10 fold over the last 40 years, representing a worldwide cost of 20 billion US dollars [5]. Nitrogen accumulates in plants and is drained into ground water, streams, and rivers, causing a risk to human and animal health [6]. Industrial production of nitrogen fertilizers consumes large quantities of natural gas and releases carbon dioxide, causing environmental pollution and global warming.

For this reason, reducing the use of nitrogen fertilizers is a main challenge for those in field management. One possible solution is the recycling of organic wastes, such as nutrient-rich compost. The positive effects of organic waste on soil structure, aggregate stability, and water-holding capacity have been reported in several studies [7-9] reported that chicken feathers are a rich source of organic nitrogen and recommend the development of nitrogen-rich organic amendments

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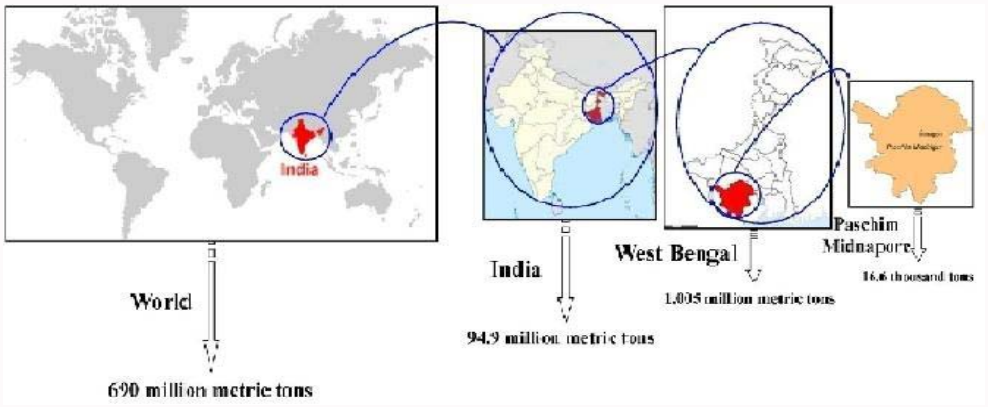


Figure 1: Schematic representation of wheat production status of the year 2012-2013 (Maps were adopted from 'GOOGLE' map).

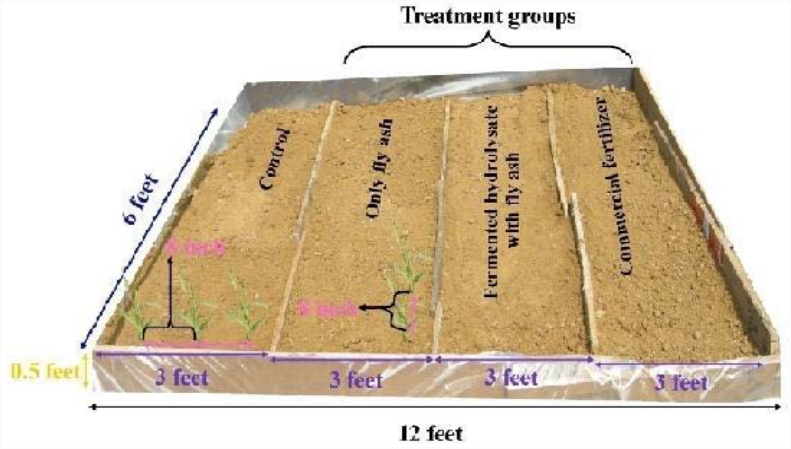


Figure 2: Geometrical representation of the wheat cultivation field area.

in order to improve plant growth and stimulate microbial activity in the soil.

Chicken feathers are waste products generated from poultry processing. Globally, 8.5 billion tons of feathers are generated annually, with approximately 350 million tons generated in India alone [10]. The large number of feather waste causes environmental problems and contaminates the air, water, and soil. However, chicken feathers can be fermented with bacterial cells to produce an organic nitrogen supplement for the cultivation of fields.

In recent years many researchers have studied and developed fermented feather hydrolysate for cultivation of different plants [8,9,11]. However, such studies have not focused on the mode of application, marketability, or stability of the feather hydrolysate fertilizer. In the current study fly ash was used as the carrier or supporting matrix for field application of feather hydrolysate. Fly ash is a waste material generated from different industries, mainly thermal plants and rice mills. India generates approximately 18,500 tons of fly ash a year [12]. Only 8 to 10 percent is utilized for the production of cement and other building materials while the largest amount is deposited for land filling, causing serious health problems to humans, animals, and plants. In addition, fly ash plays an important role in the ecosystem and its repeated exposure can cause irritation to the eyes, skin, nose, throat, and respiratory tract and result in ash poisoning [13]. This solid hazardous waste however may serve as a useful material in the production of fertilizers and pesticides for the improvement of crop production and soil fertility. In addition, fly ash can improve crop productivity and soil properties such as water holding capacity, pH level, porosity, and bulk density. In the current study we used fly ash as a carrier matrix for fermented feather hydrolysate. We aimed to examine the effectiveness of the fly ash-carrier feather hydrolysate as an organic nitrogen supplement for wheat cultivation. This paper not only highlights how the fermented materials can be used as alternative compost to commercial fertilizers, but also analyzes soil properties after application.

Materials and Methods

Chemicals and raw materials

All solvents, chemicals, and reagents used in this study were of analytical grade and purchased from local suppliers. Chicken feathers were the waste byproduct generated from the poultry shouter house in Midnapore, West Bengal, India. Raw feathers were washed under tap water repeatedly, air dried, and stored for future use. Fly ash was collected from a local rice mill in Midnapore, India. Wheat seeds (sonalika) were manufactured and certified by the National Seed Corporation, New Delhi, India and purchased from local suppliers.

Biodegradation of chicken feathers and production of fermented hydrolysate

Paenibacillus woosongensis TKB2 (GenBank- JQ248575.1), a feather-degrading, keratinase-producing bacterium was used in this study. Feather degradation was carried out in a flask containing the

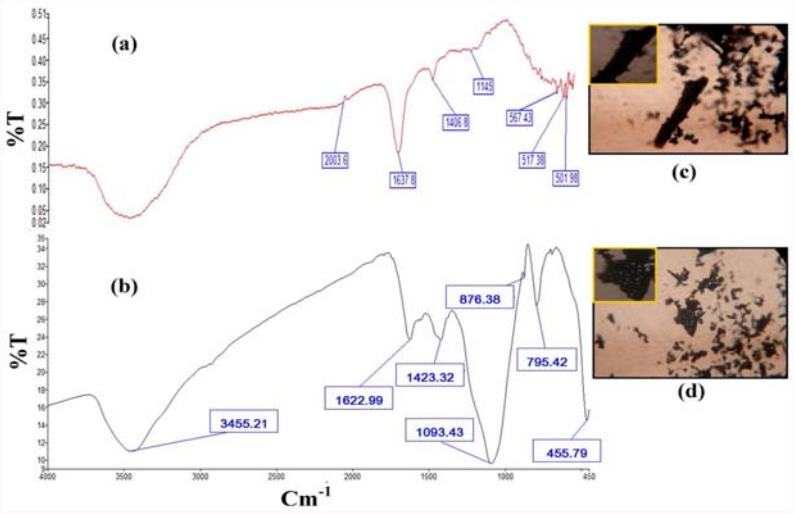


Figure 3: FTIR spectrum of fly ash mixed fermented hydrolysate (a) and the fly ash (b). Photomicrograph of fly ash mixed with fermented hydrolysate (c) and fly ash (d).

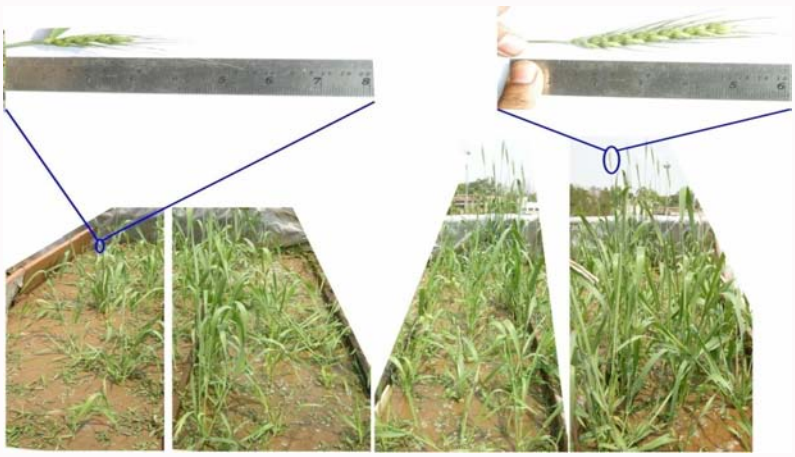


Figure 4: Cultivation areas of different treatment wheat plants and comparison of wheat seedlings length with CBFH treated and controlled plants. In the right side of the figure the wheat seedlings produced from control plants and in left side of the figure the wheat seedlings produced from CBFH treated plants.

feathers (0.75%, w/v) and a basal salt medium [KH_2PO_4 (0.05), $MgSO_4$ (0.025), $CaCO_3$ (0.02), NaCl (5.00), and $FeSO_4$ (0.015) %, w/v]. The 2-ml seed culture of *P. woosongensis* TKB2 [cell density 36×10^{10} CFU/ml] was transferred to the feather basal salt medium on a rotary shaker with 140 rpm at 30° C for 48 h [8]. The degraded broth was centrifuged then subjected to membrane filtration and pasteurized for use in subsequent studies.

Analysis of degraded end products

Total free soluble protein and amino acids were estimated using the Lowry method [14] and ninhydrin methods [15] respectively. Total nitrogen (N) was determined using the Kjeldahl method and phosphorus (P), and potassium (K), using flame photometry The presence of the metals (Cu), iron (Fe), calcium (Ca), manganese (Mn), magnesium (Mg), and zinc (Zn) were determined using atomic absorption spectroscopy (Shimazu AA7000, USA), [16].

Fermented end product used as bioorganic fertilizers

Experimental site and soil characteristics: The field experiment was conducted on silty loam soil in a constructed greenhouse located at the Department of Microbiology, Vidyasagar University during wheat cultivation season (November-February). Soil was collected from a field which had been harvested with wheat for 5 continuous years and had a pH value of 6.32. The organic matter content is provided in Table 1. The field (12×6×0.5 ft) was prepared and used for wheat cultivation. Plantation was performed on 2 November 2014 in the selected plot. Four plant groups were planted in 3×6×0.5 ft areas (Figure 2); the control group (without any treatment), the fly ash treated group, the fly ash carrier base feather hydrolysate group, and the commercial fertilizer group. Wheat plants were rooted in a rows and columns consisting of 3 and 9 plantlets for each treatment at a spacing of 8×8 inches between plants in the selected plot and in rows of 27 plantlets for each treatment.

Carrier preparation for bioorganic fertilizers: Collected fly ash was dried in an oven at 60 to 70° C for 3 days. The fermented hydrolysate was mixed with dried fly ash in a ratio of 1:1 (w/v). After proper hand mixing the organic mixture was dried in a hot air oven at 50° C overnight. The mixed bio-organic fertilizer powder was applied as a basal application in the wheat field.

Physical and chemical properties of fly ash and carrier based bioorganic fertilizers: As physical and chemical characteristics are essential for better understanding the bonding characteristics

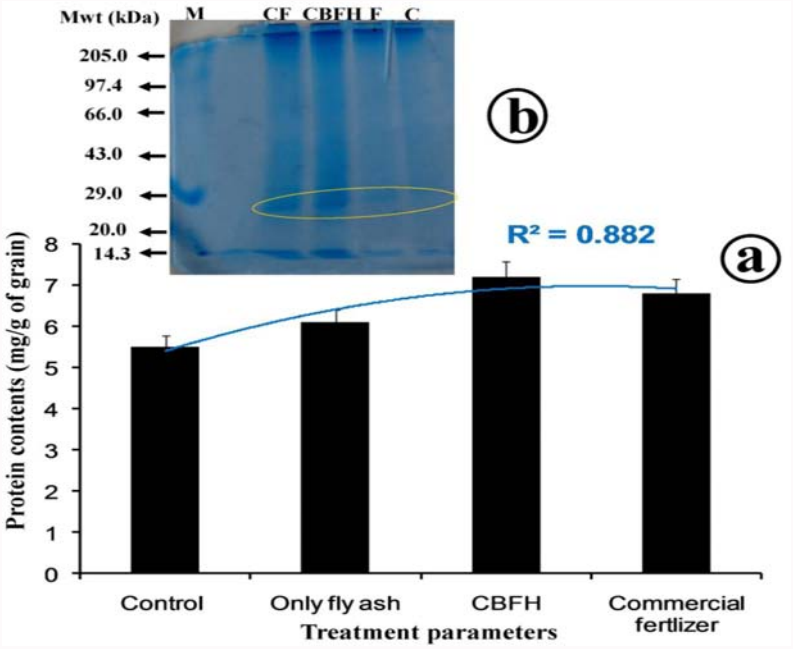


Figure 5: Effect of different treatments on wheat seeds water soluble proteins (a). Water soluble protein banding patterns of wheat in SDS-PAGE. Lane M-Marker Protein, CF- Seed protein from chemical fertilizer treated plants, CBFH- Seed protein from CBFH treated plants, F- Seed protein from Fly ash treated plants and C- Seed protein from control plants. Yellow cercal indicates probably the presence of gluten protein.

of fly ash with fermented hydrolysate, its surface properties were studied using a light microscope and bulk properties and hydraulic conductivity were studied. The bonding patterns of fly ash and fly ash mixed with fermented hydrolysate were studied using Fourier transform infrared spectroscopy (FTIR) (Parkin Elmer, USA).

Chemical properties were mainly studied to investigate the presence of macro and micro essential elements which influence the growth of plants. Valuable macro elements such as P, K, Ca, Mg, and S and micronutrients such as Fe, Mn, Zn, Cu, Co, B, and Mo were estimated using an atomic absorption spectrophotometer (Shimazu AA7000, USA).

Fertilizers stability study: As the storage salability of any marketable compound is essential for its commercialization, the organic bio-fertilizer produced from the chicken feather hydrolysate was tested for its storage stability by measuring its protein content after certain time intervals. Protein estimation was performed according to method described by [14] using bovine serum albumin (Sigma Aldrich, Japan).

Experimental design for plantation: Wheat (*Triticum aestivum*) seeds were washed thoroughly under tap water and surface sterilized using 0.1% mercuric chloride followed by repeated washing with sterilized distilled water [8] and soaked in the distilled water overnight at room temperature. Four experimental sets were prepared according to treatment type; without any treatment (Group 1, control group), fly ash (Group 2, fly ash group), fly ash Carrier Based Fermented Feather Hydrolysate (CBFH) (Group 3, CBFH or feather hydrolysate group), and commercial chemical fertilizers group (Group 4, commercial or chemical fertilizer group). Weeding and plugging was required routinely for cultivation.

Physiological and phenotypic characteristics of wheat plants and wheat: Wheat plants were cultivated for 3 months then carefully uprooted from the soil and the roots removed. Physical characteristics such as height, width, and weight of the steams and roots, length of seed foliage, branches of seedlings, and wheat weight of the differently treated plants were measured. The uprooted plants were washed with normal saline followed by a phosphate buffer (pH 7) for 10 minutes. Root tips (2.5 cm from the end of the root) were cut and observed under light microscope (Olympus C1002) at 40 X magnification. The physiological properties of xylem and phloem present in the stem and roots were also examined under microscopic study. To investigate the root colonization of bacterial cells, thin sections of the roots were prepared and stained with safranin. The stained roots were observed under light microscope at 40 X magnification.

Chemical analysis of wheat plants and wheat:

Estimation of chlorophyll contents: Fresh leaves of all plants were collected after 1 month of plantation. Leaves were washed gently, and blotted to dry. Leaf tissue (1 g) was crushed in 80% (v/v) acetone and the finally the volume was adjusted to 20 ml and after that the mixture was stored overnight in refrigerator. The tissue extract was filtered using glass wool fitted into a funnel, followed by centrifugation at 10, 000 rpm for 10 min. the supernatant was collected and its absorbance was measured at 645 nm using a spectrophotometer (UV-2300, Japan) [17].

Estimation of free protein, amino acids and total carbohydrates: Fresh cultivated wheat seeds (1 g) was mashed in a 100 mM phosphate buffer (pH 7; for protein and carbohydrates) and in 80% ethanol (for amino acids). Extracts were centrifuged at 10,000 rpm for 10 min and the supernatant was used for the estimation of free protein [14]. The amino acids were estimated according to [15]. The carbohydrate content was measured using the Anthrone method [18] by measuring the absorbance at 630 nm under a UV-VIS spectrophotometer (UV-2300, Japan).

Estimation of free proline: The free proline present in the vegetative tissue was estimated according to the method proposed

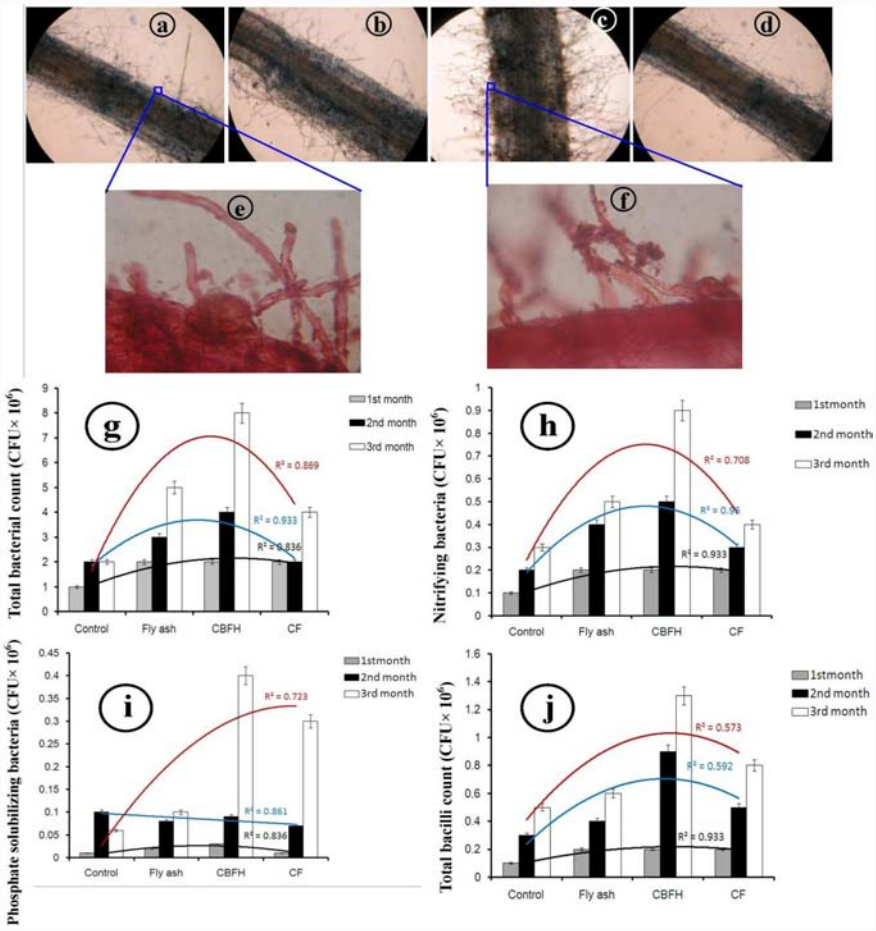


Figure 6: Microscopic view of root hair formation from cortical cell of wheat plants. (a) control, (b) plants treated with fly ash, (c) plants treated with CBFH and (d) plants treated with chemical fertilizers. Photo micrograph of microbial colonization on root hair presence on control plants roots (e) and CBFH treated plant roots (f). Total number of microorganisms (g) and main microbial populations [(h) nitrogen fixing bacteria, (i) phosphate solubilizing bacteria and (j) Bacilli] in soil samples treatment with different conditions. The number of microorganisms was expressed as colony-forming units per g of dry soil (C.F.U.10^6 g^{-1} soil) ($P<0.05$).

by [19]. Stem tissue (1 g) was homogenized in 3% (w/v) aqueous 5-sulfosalicylic acid followed by centrifugation. An equal amount of acid ninhydrin and glacial acetic acid were added to the supernatant. After 1 hour of incubation, the mixture was extracted with toluene. The extract was aspirated and the optical density was measured at 520 nm using a UV-Vis spectrophotometer (UV-2300, Japan) using toluene as a control. Proline contents (mg/g fresh weight) were determined using standard L-proline.

Analysis of total phenolics and flavonoids present in wheat stem and wheat: Stem tissue and wheat of different treated plants were crushed in a mixer grinder (Bajaj Bravo, India) to produce uniform slurries. For extraction, 5 g of slurry was homogenized with different solvents (methanol, ethanol, and water) in a ratio of 1:10 and stay for overnight using an open shaker. After that the mixture was centrifuged at 10,000 rpm at 4° C in a cold centrifuged machine (REMI CPR-24, India). The supernatant was collected and used for the analysis of antioxidants, phenolics, and flavonoids.

The total phenolics content in plants were estimated using Folin–Ciocalteu's reagent [20]. An aliquot (500 µl) of Methanolic Extract (ME), Ethanolic Extract (EE), and aqueous extract (WE) of wheat stem tissue was mixed with 500 µl of distilled water and 1,800 µl of the Folin–Ciocalteu reagent, followed by the addition of 1200 µl 15% (w/v) sodium carbonate. After 90 min of incubation in the dark place, absorbance of the reaction mixture was measured at 765 nm. Control (blanks) was prepared without any extract of wheat tissue or seeds. Total phenolic content was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of fresh weight using a calibration curve.

Total flavonoid content of ME, EE, and WE of the wheat and stem tissue was determined using the colorimetric method of [21]. Plant extract (500 µl) was mixed with distilled 2,000 µl of water followed by addition of 10% (w/v) aluminum chloride (100 µl), and 1 M potassium acetate (100 µl) and incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm (UV-2300, Japan). Total flavonoids content (mg/g of fresh weight) was quantified according to the standard curve of rutin.

Evaluation of gluten content present in the wheat: Wheat seeds from different treated wheat plants were ground to a fine powder and 100 mg of powder was used separately for protein extraction. 1 ml of distilled water was added to each micro tube containing 100 mg powder and centrifuged at 13,000 g for 10 min at 4° C. The supernatant was used to determine the protein concentration and SDS-PAGE analysis. Protein samples were separated on 12% SDS-PAGE based on the method described by [22]. Staining and de-staining of the gel were carried out by the standard Coomassie brilliant blue G-250 (Mark, India) staining method.

Figure 7: Formulated CBFH fertilizer in packaged form.

Soil and plant analysis

The total nitrogen content (N) was estimated using the method described by Kjeldahl and P and K using atomic absorption spectrophotometric method (Shimazu-7000AA, Japan). The flame photometry was used to determine the content of the soils. Soil and plant samples were subjected to digestion in a mixture of acids [nitric acid/perchloric acid (3:1, v/v)] followed by filtration and the presence of Cu, Fe, Ca, Mn, Mg, and Zn was estimated using atomic absorption spectrophotometry (Shimazu-7000AA, Japan). After cultivation the soil of the four treatment groups were incubated in amounts equivalent to 20 g of dry mixture in hermetic flasks kept in a dark room at 20° C. The experiment was conducted in three replicates. Flasks with untreated soil samples and without soil samples served as controls and blanks, respectively. All Analytical Techniques Were Performed According to APHA (1998).

Influence microbial growth in rhizosphere

In order to determine the numbers of beneficial microorganism present in the rhizosphere and plant root, three plants were sampled randomly from each treatment group. The rhizosphere soil was obtained as described by [23]. The wheat plant was carefully uprooted from the soil and shaken gently to remove the bulk soil and the roots were cut into 1-cm segments. 5 g were placed into tubes containing 45 ml sterile distilled water and sonicated for 15 min to separate bacteria and fungi from the roots and rhizosphere soil. The liquid was serially diluted for enumeration of total bacteria. Pikovskaya's medium was used for phosphate solubilizers and Ashby N-free sucrose medium for N_2 fixers Dubey & Maheshwari et al. The number of phosphate solubilizing and nitrogen fixing bacteria was expressed as colony forming units (CFU) per gram of dry soil Nustorova et al.

Commercialization steps

To convert the hydrolysate into a form easily used by farmers, it hydrolysate was mixed with fly ash, a semi-porus material used for mixing fermented broth, as a carrier. Through this method, the organic fertilizer can be easily handled, packed, stored, transported, and used. The fly ash was dried in sunlight and a hot air oven to reach minimum moisture (0.1%) level and to ensure less contamination before being sterilized by autoclaving. The fermented hydrolysate was mixed with the fly ash and kept in trays covered with polythene at 22-24° C for 2 to 5 days. These organic feather fertilizers could then be used directly or packed and stored.

Statistical analysis

Data represented in the figures and tables are given as the mean of values. All experiments were performed independently in triplicates. Standard error from means were calculated. The student's t-test was used to statistically validate our results. Values of $p < 0.05$ were considered significant.

Result and Discussion

India's growing poultry industry generates approximately million tons of harmful organic solid by-products, including chicken feathers of 350 million tons. Despite high nitrogen content, chicken feathers cannot directly be applied as organic fertilizer source due to the non-available form of nitrogen [24]. Therefore, methods to recycle feather waste into renewable nutrient sources are needed [25]. It was reported that *P. woosongensis* TKB2 facilitated the biodegradation of chicken feathers within 48 hours [26].

As wheat is the common commercial cereal crop next to rice grown in tropical regions, we selected it for our study. The commercial cultivation of wheat requires a substantial amount of fertilizers, which is costly and may be hazardous when used excessively. Therefore, the development and utilization of biochemical alternatives to chemical fertilizers such as feather hydrolysate, organic nitrogen, and other important macro- and microelements are needed as organic nutrients.

Chemical analysis of fermented hydrolysate and characterization of fly ash based fertilizers

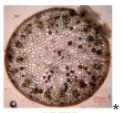
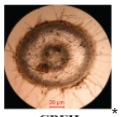
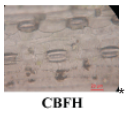
Feathers are a rich source of keratin protein (~90%) which is solubilized into short peptides and amino acids using microbial keratinase. The fermented feather hydrolysate created in this study contained an adequate quantity of amino acids (3.11 mg/ml) and soluble peptide (3.78 mg/ml). Elemental analysis of the feather hydrolysate is given in Table 2. Currently, the majority of available hydrolyzed protein fertilizers are created through a chemical hydrolytic process resulting in high levels of unwanted D-amino acids [27]. This biodegraded keratin waste contained amino acids which may have a chelating action on micronutrients [28]. A mixture of peptide and amino acid is the ideal fertilizer for plant growth and development [29]. Similarly [30] reported that absorption and assimilation of amino acids into plants from the soil. Thus, it is clear that such organic residues enhance plant growth, particularly in terms of yield and nutritional quality of crops.

Fly ash's physical properties vary greatly depending on coal type,

Table 1: Physical and chemical properties of soil (at initial stage), feather hydrolysate and fly ash.

Components and Physical properties	Feather hydrolysate	Fly ash	Soil
Total protein (mg/ml)	3.78±0.01	-	1.14±0.001
Total amino acids (mg/ml)	3.11±0.002	-	0.34±0.0002
Total nitrogen (%)	1.32±0.002	-	1.21±0.001
Bulk density	37.3± 1.12 lb/ft^3	1.1±0.001 g/cc	2.07±0.002
Water-holding capacity (%)	-	37.2±1.9	39.2±0.05
Porosity (%)	-	54.1±1.8	76.6±0.2
Cu (%)	0.012±0.0001	3.3±0.01	85.2±0.3
Fe (%)	0.102±0.002	40.3±0.5	20.1±0.02
Ca (%)	0.181±0.001	0.1±0.002	11.2±0.01
Mn (%)	0.021±0.0001	143.1±2.21	151.1±0.5
Mg (%)	0.087±0.0002	0.03±0.0003	70.2±0.3
Zn (%)	0.055±0.0002	34.6±1.12	9.83±0.01
S (%)	1.031±0.002	0.6±0.001	4.1±0.001
Co (%)	-	0.07±0.002	0.3±0.002
B (%)	-	54.3±1.21	0.02±0.0001
Mo (%)	-	0.6±0.001	1.02±0.002

Table 2: Different plant physiological changes observed with treated and untreated wheat plants.

Treatment groups	Steam height (cm)[a]	Steam width (cm)[a]	Steam weight (g)[a]	Root height (cm)[ab]	Root width (cm)[ab]	Root weight (g)[ab]	Length of seed foliage (cm)[ab]	Branches of seedlings	Wheat weight (g)[c]	Yield (Kg)[d]	Microscopic observation		
											No of xylem and Phloem in wheat stem	Size of root cortex and endodermis (µm)	No of stomata present in leaf (/100×200 µm area)
Control	63.1±0.2	4±0.02	22±0.2	18.7±0.1	7.0±0.01	25.0±0.1	5.0±0.01	2±0.001	0.052±0.0002	0.1±0.002	20±0.2	20±0.1	5±0.001
Only fly ash	71.2±0.1	4.2±0.01	28±0.2	20.1±0.2	8.1±0.02	26.2±0.2	8.3±0.02	4±0.002	0.055±0.0001	0.3±0.002	22±0.1	24±0.2	5±0.001
CBFH	80.2±0.2	5.1±0.02	34±0.1	28.1±0.2	9.3±0.01	28.7±0.2	14.2±0.03	8±0.003	0.089±0.0002	0.6±0.001	24±0.2	40±0.3	6±0.001
Commercial fertilizers	80.4±0.3	4.8±0.01	32.3±0.1	23.3±0.1	9.6±0.02	24.8±0.1	12.8±0.01	7±0.002	0.085±0.0003	0.57±0.001	24±0.3	38±0.3	6±0.002
Visual evaluation											 CBFH *	 CBFH *	 CBFH *

*Small bar indicates 20 µm; [a]-average white, weight and width of plants represented with one plant data; [b]-total foliage root system; [c]-one seed; [d]- total wheat yield from the experimental area (3×6×0.5 feet).

boiler type, the coal's ash content, the combustion method, and collection methods. The fly-ash used in the current experiment had a silt loam texture with 82% of particles with a diameter of less than 0.02 mm. Fly ash particles were of a porous irregular structure with smaller amorphous particles and crystals (plerospheres) (Figures 3c,3d). Fly-ash collected from the rice mill was of a low bulk density (1.1 g cm^{-3}), hydraulic conductivity, and specific gravity (3.0 g cm^{-3}). Feather fertilizers left a coated structure on the surface of fly ash porous particle when mixed (Figures 3c,3d). FTIR spectroscopy was used to determine the bonding pattern of the feathers' proteinaceous material with the fly ash molecule weight (Figures 3a,3b). A broad peak was observed at 3500 cm^{-1} due to the presence of -OH/-NH functionality in the fermented hydrolysate, which is probably due to intermolecular H-bonding. However, it may be considered that the peaks narrowed in the fly ash due to the partial destruction of H-bonding among these groups. The peak at 1637 cm^{-1} may have been due to stretching vibrations of the amide carbonyl in the fermented hydrolysate. The peak materialized at a lower frequency (1622 cm^{-1}) in the fly ash without the addition of feather hydrolysate, due to the interaction of the amide carbonyl with the fly ash.

Factors influencing physical properties can also be responsible for variations in fly ash's chemical properties. The chemical composition of the fly ash is given in Table 1. The fly ash contained the essential elements Ca, Fe, Mg, and K. The lime readily reacts with the acidic components of the soil, resulting in the release of other nutrients such as S, B, and Mo in the form and amount favorable to crop plants [31]. The properties of soil following fly ash application have been studied to determine the usefulness of this industrial waste as an agronomic amendment ([32]; Inam, 2007). While the physical and chemical properties of soil following fly ash amendment differ due to both original soil and fly ash properties, certain generalization can be made in most cases.

Flowering, harvesting and yield

Flowering was observed 50 days after plantation in the feather hydrolysate-treated and commercial chemical fertilizer-treated groups and after 65 days in the fly ash-treated plants and control groups. Wheat plants in the feather hydrolysate and commercial fertilizer groups were harvested on 1 February 2014 and in the untreated and fly ash treated groups on 16 February 2014. Plant physiology data of all plants are provided in Table 3 and Figure 4.

Table 3: Chemical properties of wheat plants and wheat grains.

Treatment groups	Total chlorophyll (mg/g)	Proline (mg/g)	Total carbohydrates (mg/g)	Total phenolics (mg/g)						Total flavonoids (µg/g)					
				WE		ME		EE		WE		ME		EE	
				S	V	S	V	S	V	S	V	S	V	S	V
Control	0.736±0.05	0.065±0.002	0.19±0.002	0.756±0.001	0.190±0.001	0.878±0.003	0.187±0.002	0.765±0.004	0.132±0.001	71.9±0.04	20.4±0.01	77.8±0.04	21.3±0.01	73.2±0.03	20.8±0.03
Fly ash	0.801± 0.02	0.071±0.001	0.21±0.002	0.890±0.001	0.205±0.002	1.002±0.003	0.221±0.005	0.990±0.004	0.211±0.001	74.5±0.03	21.3±0.01	80.4±0.03	21.5±0.02	76.4±0.03	21.1±0.02
CBFH	1.52±0.08	0.106±0.001	0.3±0.002	1.121±0.002	0.302±0.003	1.372±0.050	0.572±0.002	1.134±0.005	0.442±0.001	78.1±0.02	20.2±0.01	86.3±0.05	22.1±0.02	80.3±0.02	21.5±0.02
Chemical fertilizers	1.11± 0.01	0.112±0.002	0.27±0.001	1.023±0.004	0.278±0.002	1.172±0.030	0.343±0.009	1.067±0.003	0.301±0.002	75.5±0.03	20.1±0.02	84.4±0.04	21.3±0.01	78.8±0.05	21.2±0.01

WE- Aqueous Extract, ME- Methanolic Extract, EE- Ehanolic Extract, S- Seeds and V-Vegetative Steam Tissues

Table 4: Metal and nutrient analysis of the soils and vegetative tissue of the wheat plants treated with different treatment conditions.

Nutritional parameters and metals	Soil				Wheat plants			
	Control	Fly ash	CBFH	CF	Control	Fly ash	CBFH	CF
N (%)	0.80±0.02	1.02±0.001	4.56±0.001	4.21±0.001	2.21±0.002	1.89±0.0003	2.25±0.002	2.03±0.002
P (%)	0.30±0.03	1.10±0.002	1.231±0.001	2.31±0.001	0.11±0.0001	0.43±0.0001	0.67±0.0002	0.55±0.0002
K (%)	1. 61±0.01	1.78±0.001	2.23±0.002	2.34±0.002	2.34±0.001	2.40±0.002	3.02±0.001	2.56±0.001
Cu (mg/kg dry wt)	90.25±0.23	100.23±0.2	107.23±0.3	102.21±0.2	3.43±0.002	2.90±0.001	4.52±0.002	4.23±0.002
Mg (mg/kg dry wt)	76.35±0.32	86.21±0.3	134.21±0.1	121.45±0.4	102.32±0.4	144.23±0.3	177.22±0.4	166.78±0.3
Mn (mg/kg dry wt)	333.18±0.34	345.53±0.5	354.45±0.6	350.78±0.6	176.51±0.6	170.07±0.4	189.92±0.2	190.23±0.2
Ca (mg/kg dry wt)	20.52±0.11	56.67±0.1	76.89±0.1	77.67±0.2	1.23±0.002	2.03±0.002	2.25±0.2	3.11±0.001
Zn (mg/kg dry wt)	25.45±0.13	45.56±0.2	81.23±0.2	68.87±0.1	22.11±0.03	26.67±0.1	32.42±0.1	37.67±0.2
Fe (mg/kg dry wt)	25.62±1.22	43.32±0.1	55.44±0.2	50.54±0.2	20.23±0.02	31.07±0.1	56.55±0.2	50.37±0.2

Wheat plant evaluation

Photosynthetic pigment: Chlorophylls are the most essential pigments for photosynthesis. Wheat plants treated with the CBFH showed higher total chlorophyll content (1.52±0.08 mgg^{-1}), followed by plants treated with chemical fertilizers (1.11± 0.01 mgg^{-1}), and plants treated with only fly ash (0.801± 0.02 mgg^{-1}). The control plants had the lowest total chlorophyll content (0.736±0.05 mgg^{-1}) (Table 4).

According to [33], chlorophyll formation is dependent on nutrients such as N, Mg, S, Ca, Fe, and Mn as well as the Zn available to the plants. The feather hydrolysate contained a sufficient level of these metals, which may have enhanced the chlorophyll content. Higher chlorophyll content is known to be a marker of physiologically healthy plants and that plants with insufficient nutritional inputs show poor growth and chlorosis [34].

Estimation of free proline: Proline accumulation in the CBFH group (0.106±0.001 mgg^{-1}) was similar to that in the chemical fertilizer group (0.112±0.002 mgg^{-1}), whereas the fly ash treated group had a proline content of 0.071±0.001 mgg^{-1} and the control group 0.065±0.002 mgg^{-1}. All results are given in Table 4.

Previous studies have reported that accumulation of proline by plants as an adaptive response to overcome the unfavorable conditions [35]. Some incidental evidence shows that accumulation of proline may also occur for development purposes in non-physiologically stressed conditions. Proline accumulation can serve an important role in flower development and enhancement as a metabolite and signal molecule or in increased demand during protein synthesis [36].

Estimation of total carbohydrate and protein content: The highest level of carbohydrates was present in the wheat fruits collected from plants supplemented with CBFH (0.3±0.002 mg/ml), followed by those treated with chemical fertilizers (0.27±0.001 mg/ml), fly ash (0.21±0.002 mg/ml), and those in the control group (0.19±0.002 mg/ml).

Figure 5a shows the significant effect of CBFH and chemical fertilizers on seed protein content. The greatest amount of seed protein was observed in the CBFH group (7.2 mg/g of grain). Results indicated that there were slight differences in seed gluten content (data not shown). Figure 5b demonstrates the SDS-PAGE protein profile of wheat grains under different fertilization treatments. However, during emergence of the wheat ear, the intensity of the 27 kDa molecular weight band increased in the CBFH group and also in the fertilizer group. It is therefore assumed that this 27 kDa protein may be gluten.

Total phelolice and flavonoide compounds: Polyphenolics are widely distributed in plant species and are the most potent natural antioxidants [37]. In the current study, total phenolic and flavonoid levels increased in the wheat plants supplemented with CBFH. CBFH and chemical fertilizer treated plants were most effective in enhancing the phenolic content in the wheat grains (1.372±0.050 mg/g dry wt-CBFH and 1.172±0.030 mg/g dry wt-chemical fertilizers) and vegetative parts (0.572±0.002 mg/g dry wt-CBFH and 0.343±0.009 mg/g of dry wt-chemical fertilizers). A similar trend was observed in total flavonoids; the CBFH treated plants exhibited maximum readings, i.e., (86.3±0.05 µg/g dry wt) and (22.1±0.02 µg/g dry wt) in seeds and vegetative extract, respectively. The data regarding total phenolic and flavonoid contents of fly ash, chemical fertilizers and CBFH treated and control plants are given in Table 4.

In plants, polyphenols are part of the defense mechanism against biotic and abiotic stress and contribute to plant colors. Polyphenols are ubiquitous in all plant organs and are therefore vital to the human diet by providing a supplementary dietary source of various antioxidant phytochemicals. Polyphenols' antioxidant properties may be related to their redox properties, which allow them to act

as a reducing agents or hydrogen/electron donors, to scavenge free radicals, and to terminate radical chain reactions [38]. Antioxidants have plays a beneficial role to human health and reduce the risk of cancer and cardiovascular disease. It also prevents or repair cell damage caused by reactive oxygen species [39]. Currently, natural polyphenol antioxidants from plant sources have gained attention for their protective effects against different degenerative diseases and the toxicity and carcinogenicity of the synthetic antioxidants. Previously, increases in polyphenol levels in crops treated with organic waste have been reported, suggesting that organic waste may cause changes that favor the accumulation of antioxidants [40,41] reported that organically grown spinach and Chinese cabbage contained antioxidant activity 120% and 20–50% higher, respectively, than conventionally grown variants.

Analysis of soil nutritional parameters

Organic wastes are utilized in agriculture mainly to improve the physical and chemical properties of the soil and to provide a nutrient source for growing crops. The current study investigated the potential of degraded chicken feather products as a renewable fertilizer. We found the presence of macro (N, P, K, Ca, and Mg) and micro (Fe, Mn, Zn, and Cu) nutrients in the feather hydrolysate (Table 5). Fertilizing with CBFH may have increased the mineral contents of both the soil and plants in comparison with the chemical fertilizers and other treatments. The control plants showed lower metals accumulation. Feather waste by-products of poultry processing can be directly used as slow release nitrogen fertilizer for organic farming [42]. Nevertheless, the disadvantage of this process is the extremely low bioavailability of proteinacious nitrogen to plants as feathers are difficult to degrade using common proteases. For this reason, we examined the potential of *P. woosongensis* TKB2 to solubilized the chicken feathers into small peptides and amino acids, which played extraordinary role as ideal crop bio-inducer.

Rhizosphere microbial ecology

Soil biogenity data (cfu/g soil) of the heterotrophic microbial populations showed different dynamics of total microbes, phosphate solubilizing bacteria, and nitrogen fixing bacteria (Figure 6). Greater levels of N_2 fixer (mixotrophs) and phosphate solubilizing bacteria were found in the hydrolysate treated groups than the controls. Microscopic study of the root surface of CBFH treated plants revealed a greater presence of microbes on the surface of the root epithelium in the CBFH group than in the control group (Figure 6). The fermented hydrolysate contained the micro and macro-nutrients that induced microorganism growth in the soil. Plant Growth-Promoting Rhizobacteria (PGPR) is beneficial soil bacteria that colonize plant roots and result in increased plant growth [43]. While PGPR have been identified within many different bacterial taxa, most commercially developed PGPR are species of Bacillus which form endospores that confer population stability during formulation and storage of products. In addition, rhizopheric bacteria produce antifungal metabolites and release iron-chelating siderophores that separate the iron and makes it unavailable to other organisms, thus suppressing fungal pathogen growth [44]. The addition of PGPR to commercial peat-based substrates used in vegetable farming has been reported to strengthen plants, control root disease, and increase crop yields [45].

Fertilizer commercialization steps

A moisture level 0.1% was maintained during the mixture of feather hydrolysate and fly ash [46-48]. The fly ash-based feather fertilizer was packed in polythene bags which can be distributed to farmers for use in their fields (Figure 9). The feather hydrolysed nutrient can be easily immobilized or mixed with a carrier such as fly ash for application as a biofertilizer [49]. Field trials are necessary using the developed bioorganic fertilizer using fly ash [50,51].

Conclusion

In the present study we described a novel technique for the application of feather degraded products as an organic nutrient supplement for wheat crops cultivation and development. Such products are a important approach for sustaining soil characteristics and were found to be rich in healthy compounds, induce the production of plant sources, and improve their quality. The results indicated that this method can play a dual role as a bio-enhancer reducing the use of chemical additions to agriculture and in eliminating feather waste and resultant disposal problems. In addition, the strain produced antifungal metabolites. Thus, feather hydrolysate obtained upon degradation of feathers by the TKB2 bacterial culture may be a useful nitrogenous input for agricultural soil as well as a biocontrol agent against fungal phytopathogens.

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