SAYAM Vol-I, Issue-II (December, 2023), Page No-01-06

Journal Homepage: https://sayamjournal.com/

Article

Efficiency Assessment of Carrier-Based Application of Amino Acid Produced From Bio-Degradation of Chicken Feather in Plant Growth and Regulation

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ARTICLEINFO

Keywords:
Fermented Feather Hydrolysate,
carrier material,
Foliage spray

Received : 06/11/2023
Accepted : 20/12/2023
Date of Publication: 30/12/2023

ABSTRACT

Purpose: Fermented feather hydrolysate has a growth growth-promoting effect on plants due to its higher plant-available nitrogen contents. In this study, we want to investigate the outcome of carrier materials along with fermented feather hydrolysate.
Methods: Utilizing Bacillus wiedmanni SAB10, a nitrogen-rich liquid fertilizer was created. In 72 hours at pH 10, the cell-free hydrolysate was produced by submerged fermentation of waste poultry birds litter (1.25%, w/v) as single media supplemented with white poultry feather (1%, w/v). To create a blended bio-organic fertilizer that was applied to the leaves of Moong plants, the fermented hydrolysate was combined with rice water and corn starch in a 1:1 weight /volume ratio. Plant physiological and biochemical characteristics were measured.
Results: A substantial amount of protein (4.8 mg/L), oligopeptides, and total amino acids are present in fermented hydrolysate, which affects the development and production of moong (*Vigna radiata*) plants in clay pot trials. Compared to other treated and control groups, rice starch, when used as a carrier ingredient with feather hydrolysate, produced a higher yield and better growth metrics.
Conclusion: carrier-based fermented feather hydrolysate enhances the quality of plants and helps them grow more. For the growing of moong beans, fermented poultry feather degraded material is a valuable fertilizer for the foliage. Soon, it will be a wise liquid fertilizer due to its marketable chunks and economical, eco-friendly nature.

Introduction

Agriculture output is continuous process by proper fertilization management in addition practices. Organic fertilizers are honored as a synthetic-based alternative that could also contribute to soil quality and together with farming and eco-friendly perspectives, several other biological wastes have been explored as fertilizers and also examined possible methods for recycling and trash control (Sobucki et al. 2018). The chicken agribusiness produces a large amount of organic waste on a daily basis. One of the main by-products is chicken waste, such as feathers and litter, which is made up of bedding material, excrement, and spilled feed (Tamreihao et al. 2018). The US Department of Agriculture forecasts that more than 100.5 million tons of meat were formed in 2020 based on survey data. More than 4.7 million tonnes of poultry feathers were consequently produced globally, of which over 350 million tonnes came from India (Wilkens et al., 2020). Over dumping of this material may lead to increased bacterial or viral disease growth in lakes and other bodies of water, nitrate leaching into groundwater, and phosphorus discharge into neighboring bodies of water (Bhari et al. 2021).

90 % of the keratin in chicken feathers is a good source of minerals and amino acids. It is an undoable, generally stable structure that is firmly packed into a supercoiled polypeptide form as a-helix (alpha keratin) and b-sheets (beta keratin), and it is cross-linked by disulfide, hydrophobic, and hydrogen bonds (Paul et al. 2013). Traditional degradation processes like feather breakdown need bear energy inputs and also reduce the content of amino acids and net protein utilization values. Biological treatment of chicken feathers with one type of protease known as keratinase by keratinolytic microbes (Brandelli 2008). Keratinase is produced by some bacterial and fungal species as a feasible and environmentally favorable preference for the expression fhydrolysate that can be used as bioactive peptides, biofertilizers, detergent, leather, and pharmaceutical diligence, etc. (Paul et al. 2013). Over the once decades, numerous advancements have been made to the agronomic use of organic waste increased usage of composted animal waste will lower the costs of market available manures with high-intensity product procedures. This composted fertilizer waste is also eco-friendly.

This phenomenon is stirring us to induce the present study to investigate the efficiency of carrier materials along with liquid feather hydrolysate produced by *Bacillus wiedmanni* SAB10 as fertilizer for the increase manufacture of moong beans in pot trial condition.

Materials and Methods

Raw materials

The analytical grade solvents, substances, and reagents used in this investigation were all acquired from nearby vendors. In Jhargram, West Bengal, India, a poultry shouter house provided feathers and litter for the birds. Raw feathers were regularly cleaned with tap water, allowed to air dry, and then preserved for further use.

Degradation of chicken feathers and construction of fermented hydrolysate

In this work, a keratinase-producing, feather-degrading bacteria called *Bacillus wiedmanni* SAB10 was employed. The process of decomposing feathers was done in a beaker with chicken litter (1.25 percentage, w/v) and feathers (1percentage, w/v) at pH 10. A three percent inoculation of *Bacillus wiedmanni* SAB10 was added to the feather medium and shaken for seven and a half hours at a speed of 72 rpm and 40° C. The making of total amino acids during fermentation was made the most of by optimizing the fermentation situations. The broken-down broth was centrifuged, exposed to membrane filtration, and then decontaminated for use in later research.

Examination of degraded finish products

Total free soluble proteins and amino acids were assessed using the Lowry method and Ninhydrin methods. Total amino acid measured by UV spectrophotometer at 620 nm (Moore and Stein 1954).

Fermented end products used as organic fertilizers on selected plants for experiment

Moong beans (*Vigna radiata L*) are common pulse cultured allover India and used in this study as an experimental plant. On the selected plot, the plantation was carried out on 2 February 2023. In this study, forty 8′ inch clay pots were used. The two kg of ordinary garden soil, which had an appropriate moisture value of 70% w/v, was present in each container. There were 10 pots in each of the experimental groups.

Carrier preparation for bio-organic fertilizers

Here two types of carriers were used along with feather hydrolysate fertilizers. One was the corn starch and the other was the rice water. The rice water was prepared byboilingricefor45minwith water. And corn starch was mixed with the water and heated for 2 minutes. Corn starch was mixed with fermented feather hydrolysate in a ratio of 1:1 (w/v). Also, fermented end product was mixed with rice water in a ratio of 1:1 (w/v). the mixed biological fertilizer was applied as a foliar use in Moong plants.

Experimental design for plantation

Physiological and phenotypic characteristics of Moong plants and Moong

After three months of cultivation, moong plants were carefully removed from the ground and their roots extracted. The length of the seed leaves, the branches of the seedlings, the width, height, and weight of the stems and roots, as well as the Moong weight of the plants that were subjected to various treatments, were all measured. The uprooted plants were given a 10-minute soak in a phosphate buffer (pH 7) after being cleaned with regular saline. A light microscope was used to examine the severed root tips, which were 2.5 cm from the end of the root tip, at a 10X magnification. Using a microscope, the physiological features of the xylem and phloem in the stem and roots were also investigated. Thin sections of the roots were produced and stained with Lactophenol Cotton Blue in order to evaluate the colonization of bacterial cells in the roots. The dyed roots were examined under a 10X light microscope. Chemical examination of moong vegetation and moong seeds.

Assessment of chlorophyll contents

After a month of planting, fresh leaves from every plant were gathered. After a gentle wash, the leaves were blotted dry. After one gram of leaf tissue parts was crushed in eighty percent (v/v) acetone, the amount was finally reduced to twenty milliliters, and the combination was refrigerated for the entire night. A spectrophotometer was used to estimate the absorbance of the supernatant at 645 nm after it had been collected (Arnon 1949).

Assessment of available proteins, amino acids, and whole carbohydrates

One gram of freshly harvested moong seeds was mashed in 70 % ethanol and 100 mM phosphate buffer (pH 7; for protein and carbs). The extracts were centrifuged for 10 minutes at 10,000 rpm, and the protein content was estimated using the supernatant (Lowry 1951).

Fresh moong seeds (1 g) were mashed in 70 % ethanol and the amino acids were measured using the Ninhydrin method. Take two milliliters. 0.5 ml of ninhydrins solution and 0.5 acetate buffer were combined with extracts. For 30 minutes, place the sample in a water bath. After that, use a UV-VIS spectrophotometer to measure absorbance at 620 nm (Moore and, Stein 1954).

The Anthrone technique was used to calculate the amount of carbohydrates. Mix 2 ml of the sample with 2 ml of the anthrone reagent. After 10 minutes of boiling, the mixtures were allowed to cool to room temperature, and the absorbance at 630 nm was measured using a spectrophotometer (Plummer 1990).

Assessment of free proline

We homogenized stem matter (1 g) in 3 % (w/v) water 5-sulfosalicylic acid and then we centrifuged it. Acid ninhydrin in equal amounts and also glacial acetic acid were put into the supernatant. The mixture was incubated for 1 hour before extraction with toluene. Toluene extract was made and optical density at 520 nm was measured using a UV-Vis spectrophotometer as control with toluene reference. Proline amino acids (mg/g fresh weight) were resolute by standard L-proline (Liang 2013).

Study of total Phenolics and flavonoids present in Moong trunk and Moong beans

Slurries were made by crushing stem tissue in a mixer grinder. An open shaker was used to homogenize 5 g of slurry with various solvents (ethanol and water) in a 1:10 ratio and leave it overnight for extraction. Following that, the mixture was centrifuged in a cold centrifuged machine at 10,000 rpm and 4° C. The supernatant was gathered and employed in the examination of flavonoids, phenolic acids, and antioxidants (Chang et al. 2002).

Result and Discussion

In current years India's rising poultry trade generates roughly million tons of dangerous dense by-products and 350 million tons of chicken feathers. High nitrogen content is present in chicken feathers but it is difficult to use chicken feathers as an organic fertilizer because they lack available nitrogen. Soil and foliar spray of amino acids increase variables of nitrogen metabolism and productivity. Thus, methods to reclaim feather waste into reusable nutrient sources are demanding (Sobucki et al. 2019). It was reported that *Bacillus wiedmanni* SAB10 promoted the biodegradation of chicken feathers within 72 h (Sahoo et al. 2023). We chose mung beans as our experimental subject because they are a common commercial crop in tropical regions. Growing moons commercially requires significant amounts of fertilizer, which can be expensive and dangerous if used in excess.

Analysis of chemicals in fermented hydrolysate and characterization of carrier-based fertilizers

About 90 % of the keratin protein found in feathers is soluble in amino acids and short peptides thanks to the action of microbial keratinase (Adetunji et al. 2012). Under submerged fermentation conditions, the fermented poultry feather hydrolysate generated in this experiment had an appropriate amount of soluble amino acids (4.89 mg/L). The fermented chicken feather hydrolysate contained 7.95 mg/L of total oligopeptides and 10.05 mg/L of total protein. The bulk of hydrolyzed protein fertilizers on the market today are made via a chemical hydrolytic procedure that results in high concentrations of undesirable D-amino acids. Amino acids found in this biodegraded keratin debris may chelate micronutrients. For plants to grow and develop, the best fertilizer is a combination of amino acids and peptides (Bose et al. 2014).

Green plant growth and yield induction employing the fermented feather hydrolyzate pollen test

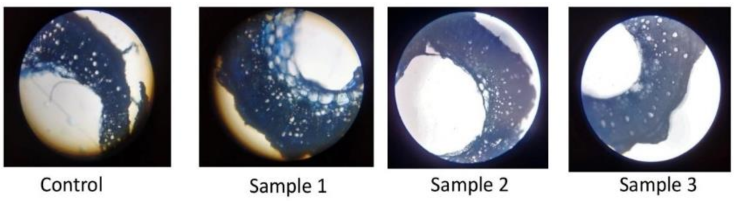
Inongoing research, spraying fermented feather hydrolyzate has significantly increased the yield of moong bean plants. It was observed that the increase in rice yield was higher when sprayed on soil mixed with corn starch and rice water than when sprayed on the soil. Therefore, foliar spraying was selected as a method of spraying feather hydrolyzate on flower pots. Previously, some researchers reported that foliar application resulted in better results in various crops (Sahoo et al., 2022). The choice of dosage andduration had to be determined through other analyzes affecting the final grain yield (both primary and secondary outbreaks) as well as the agronomic characteristics of mungbean. Very interestingly, feather hydrolyzate mixed with corn starch and rice starch used plants showed significant improvement in important agronomic parameters including root height (4.75 ± 0.02 cm and 3.9 ± 0.01 cm) and shoot height (30.1 ± 0.01 cm). It has been shown to show improvement. . ± 0.03 cm and 27.2 ± 0.02 cm). The biochemical characteristics of several plants and grains were also improved after feather hydrolysate spray (Table 2), indicating improved quality under these conditions. All details are provided below: Table 1

Table 1: Morphological assessment of moong bean

Physiological observation	**Control**	**Sample 1 (corn starch with feather hydrolysate)**	**Sample 2 (rice starch with feather hydrolysate)**	**Sample 3 (feather hydrolysate)**
Shoot height (cm)	25.2±0.01^{a}	27.2±0.02^{b}	30.1±0.03^{b}	28.5±0.02^{b}
Root height (cm)	2.8±0.01^{a}	3.9±0.01^{a}	4.75±0.02^{a}	4.1±0.03^{a}
Branches (no.)	3±0.2^{a}	4±0.01^{a}	5±0.01^{a}	4±0.001^{a}
Pod number	7±0.02^{a}	10±0.1^{a}	12±0.02^{a}	10±0.2^{a}
Average leaf diameter(sq. cm)	4.9±0.01^{a}	6.2±0.3^{a}	7.2±0.03^{a}	6.9±0.32a
No of nodule formation	12±0.1^{a}	18±0.1^{a}	20±0.01^{a}	17±0.65^{b}
Average no of grain in mature pod	5±0.1^{a}	9 ±0.01^{a}	11±0.3^{a}	10±0.021^{a}

Carrierbased feathers were found to be sprayed with the hydrolyzate twice (once before the first bloom and once before the second bloom). This type of result may be owing to the result of higher concentrations, which may trigger the production of certain plant hormones (auxins and gibberellins) that provide higher nutrient content to the leaves and help achieve greater flowering (Nurdiavati et al., 2019). The quality of dal grains was also better in sample 2 (Figure 1). After collection, root tips were cut and examined under a microscope (Figure 2).

Figure 1 Visual observation of moong bean, Sample 1- feather hydrolysate with Corn starch treated plant, Sample 2- feather hydrolysate with rice starch treated plant.



Control Sample 1 Sample 2 Sample 3

Figure 2 Vascular bundle of treated plants

Moong plant assessment for Photosynthetic pigments

Chlorophylls are the utmost vital pigments for plant photosynthesis. Total chlorophyll content of Moong plants treated with corn starch (45.91 ± 0.02 mg/g) and plants treated with rice starch (48.1 ± 0.01 mg/g). The control plants had the lowest whole chlorophyll content (12.75 ± 0.02mg/g). Chlorophyll creation is said to be reliant on nutrients like N, Ca, Mg, Fe, S, and Mn as well as the amount of Zn that the plants have access to. Feather hydrolysates contain enough amounts of these metals to increase chlorophyll content (Bose et al., 2014). It is common knowledge that plants with high chlorophyll content are physiologically healthy, while plants with low nutrient content have slow growth and lack of chlorophyll pigments.

Assessment of free proline:

Proline gathering was (0.16 ± 0.01 mg/g) in the corn starch group, - 0.12 ± 0.02 mg/g in the rice starch group, and - 0.32 ± 0.02 mg/g in the control group. All outcomes are shown in Table 2. Previous studies have shown that amino acid proline accumulation by plants is an adaptive response to comeback adverse conditions. Some anecdotal evidence suggests that proline accumulation may happen for developmental purposes under non-physiologically stressful situations.

Table 2: Analysis of plant development and yield of moong bean plants treated with different combinations of additives and feather hydrolysate.

Groups	Control	Sample 1 (corn starch with feather hydrolysate)	Sample 2 (rice starch with feather hydrolysate)	Sample 3 (feather hydrolysate)
Chlorophyll content (mg/gm)	12.75 ± 0.02[a]	45.91 ± 0.02[c]	48.1 ± 0.01[c]	46 ± 0.05[c]
Proline content (mg/gm)	0.32 ± 0.002[a]	0.16 ± 0.001[a]	0.12 ± 0.002[a]	0.23 ± 0.02[a]
Total carbohydrate (mg/gm)	20.15 ± 0.002[a]	29.5 ± 0.002[b]	31.15 ± 0.001	29.87 ± 0.04[b]
Total protein (mg/gm)	12.25 ± 0.001[a]	16.87 ± 0.003[a]	18.25 ± 0.002[a]	18.21 ± 0.01[a]
Phenolic content (mg/gm)	40.52 ± 0.002[c]	46.5 ± 0.001[c]	55.25 ± 0.002[c]	50.12 ± 0.01c
Flavonoid content (mg/gm)	22.75 ± 0.002[b]	29.8 ± 0.001[b]	32.5 ± 0.003[b]	30.01 ± 0.02[b]

Assessment of total carbohydrate and protein content:

The maximum level of carbohydrates existed in the moong seed accumulated from plants treated with cornstarch (29.5 ± 0.02 mg/g), with rice starch (31.15 ± 0.01mg/g), and those in the control group (20.15 ± 0.02 mg/g). Protein was existed in the moong seed gathered from plants which are treating with corn starch (16.87 ± 0.03mg/g); with rice starch (18.25 ± 0.02mg/g) and those in the control group (12.25 ± 0.01mg/g).

Total Phenolices and flavonoid compounds:

Polyphenols are extensively distributed in plants and are the most powerful normal antioxidants in nature. In the study, the data regarding entire phenolic contents in corn starch treated moong plant 46.5 ± 0.01 mg/g, rice water treated moong plant 55.25 ± 0.02mg/g and control 40.52 ± 0.02mg/g. flavonoid contents in corn starch treated moong plant 29.81 ± 0.01 mg/g, rice water treated moong plant32.5 ± 0.03 mg/g and control 22.75 ± 0.02 mg/g. In plants, polyphenols are portion of defense mechanisms against biotic and abiotic stresses conditions and pay an important role in to plant color. Being present in all plant organisms, polyphenols are essential to the human diet because they offer an additional dietary source of different antioxidants, including photochemical ones. The redox characteristics of polyphenols, which enable them to function as reducing agents or hydrogen/electron donors, scavenge free radicals, and break the cycle of radical reactions, may be connected to their antioxidant qualities (Paul et al. 2018). Antioxidants are important to human health because they lower the risk of cardiovascular disease and cancer. Additionally, it guards against or fixes cell damage brought on by reactive oxygen species. Normal polyphenol antioxidants derived from natural plant are currently receiving more attention due to their preventive qualities against many degenerative diseases as well as the toxicity and carcinogenicity of synthetic antioxidants (Kaur et al. 2021). Increased polyphenol levels in crops treated with biological waste or organic manures have been previously reported, signifying that organic waste may cause beneficial changes and the gathering of antioxidants (Paul et al. 2018).

Conclusion

This study presents a new technology for utilizing feather decomposition products as organic nutritional supplements in the development and cultivation of lunar crops. Treatment of the carrier-based fermented feather hydrolysate which helps the growth of the plant and improved its quality.

Here we use two carriers one is cornstarch and another is rice water along with fermented hydrolysate. Rice water carrier with fermented feather hydrolysate works better in plant development than the cornstarch carrier with fermented feather hydrolysate. Results show that this technique can serve a dual role as a bio stimulant, decreasing the use of chemical additives in agriculture and eliminating poultry feather and litter waste and subsequent removal problems.

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