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Bio-Augmentation for Reducing the Traditional Panchagavya Production Time

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KEYWORDS

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ABSTRACT:

Panchagavya, a traditional Indian biofertilizer made from five cow-derived products (dung, urine, milk, curd, and ghee), has long been used in Hindu rituals and Ayurveda. Recently, its role in organic agriculture has gained attention due to its benefits for plant growth, soil health, and pest control. Traditional preparation takes 45 days, limiting large-scale application. This study aimed to reduce fermentation time and improve product quality using an accelerated method. By introducing specific microbial cultures, the fermentation period was shortened by 18 days without compromising efficacy. Enhanced microbial activity maintained the bio-stimulant properties of the product. Additionally, bio-coagulants from *Colocasia esculenta* and *Strychnos potatorum* were incorporated to reduce turbidity and improve clarity by aggregating suspended particles. Microbial profiling of the optimized Panchagavya revealed beneficial strains like *Pseudomonas*, *Bacillus*, and *Lactobacillus*, along with unidentified lipolytic and proteolytic organisms, indicating areas for future research. The study concludes that accelerated fermentation combined with natural bio-coagulants improves production efficiency and quality, making Panchagavya more viable for commercial agriculture. This approach supports sustainable practices and could significantly scale up biofertilizer production.

1. INTRODUCTION

Bioaugmentation is the supplementation or addition of bios, biologicals, microorganisms and biomaterials to facilitate better functions in the system. The classical example being the addition of bacterial culture to make curds. In the industrial process, yeast is cultured in the fermenter and added to molasses for alcohol production (1). Bioaugmentation is extensively used in soil remediation process to absorb, degrade, transform, and eliminate toxic and harmful substances. present in contaminated soils (2). Bengal gram powder and Liqurozymal (a bio extract) were found to enhance alcohol production by yeast. Panchagavya, is mixture of five products of the cow" (Sanskrit) a complex bio-fertilizer and bio-stimulant derived from bovine products. Traditionally it is used in Hindu rituals, medicines, health care and agriculture. It has gained significant interest in organic farming and also as plant growth promoter. Pancha (five) gavya (cow products) are: Cow dung, Cow urine, Cow Milk, Cow Curd and Cow Ghee.

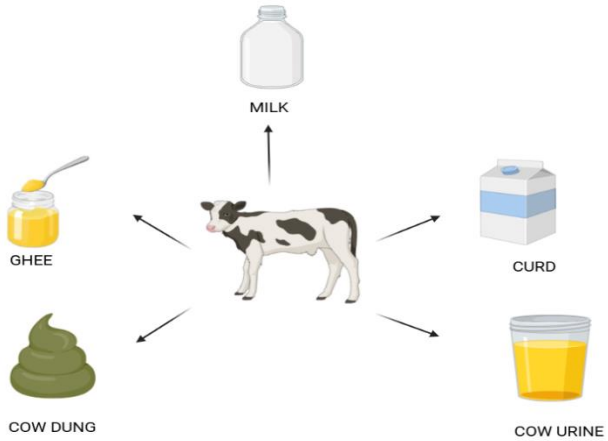

Figure 1. Five Ingredients derived from cow are mixed and fermented to produce Panchagavya.

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Panchgavya have numerous beneficial properties for both plants and soil in agriculture. It is used as a natural fertilizer and pesticide, enhancing soil fertility, improving plant growth, and protecting plants from pests and diseases (3). In Ayurvedic medicine, Panchagavya is considered to have therapeutic properties and is used for various treatments, .and requires extensive trials and validation. The preparation of Panchagavya involves a specific process to ensure proper fermentation and effectiveness. It is often combined with other natural ingredients like banana, tender coconut water, and jaggery (unrefined cane sugar) to enhance its properties (4).

Bio-coagulants are increasingly recognized as a viable alternative to chemical coagulants in water and wastewater treatment, thanks to their effectiveness in reducing pollutants such as turbidity, suspended solids, color, and organic compounds. Plant-based bio-coagulants, in particular, stand out due to their availability and consistent performance (5). Natural coagulants have been used for domestic purposes in tropical rural areas since ancient times. Among these natural ingredients, proteins and tannins can serve as primary components in bio-coagulants in *Colocasia esculenta* (6) seeds are used for purification of water (7). *Strychnos potatarum L*. and the *Colacasia* extracts were used in the recovery process of Panchagavya. The preparation of Panchagavya involves several fermentation processes using a consortium of beneficial bacteria. In these bio-formulations, cow dung plays a crucial role as a source of bacterial inoculum (8). This study presents a comparative study of the effect of natural consortia vs isolated cultures and an integrated approach for optimizing Panchagavya production by introducing a consortium of bacteria as bioaugmentation technique (9). This method enhances the plant-growth-promoting substances in Panchagavya and achieves results in less time than traditional methods. Additionally, product recovery is improved using plant-based bio-coagulants.

2. MATERIALS AND METHODS

2.1. Raw materials required:

Panchagavya consists of nine products viz. cow dung, cow urine, milk, curd, jaggery, ghee, banana, Tender coconut and water (4).

- Cow dung - 7 kg of fresh cow dung was collected and used as the primary substrate.
- Cow ghee - 1 kg
- Cow Urine - 20 litres
- Water - 20 litres
- Cow milk - 3 litres
- Cow curd - 2 litres
- Tender coconut water - 3 litres
- Jaggery - 3 kg
- Well ripened poovan banana – 12 nos.
- Isolated bacterial cultural broth
- *Strchnos potatarum L*. and the *Colacasia* extracts

2.2. Methods of Panchagavya production:

2.2. A. Traditional Method

Step 1: 7 kg of cow dung and 1kg of cow ghee were mixed thoroughly both in morning and evening hours and kept for 3 days.

Step 2: 10 litres of fresh cow urine were added to enhance microbial activity and fermentation along with 10 litres of water. After 3 days cow urine and water were added to the mixture and kept for 15 days with regular mixing both in morning and evening hours. After 15 days the following ingredients will be mixed to the previous mixture and stirred well.

Step 3: 3 litres of cow milk, 2 litres of Cow curd, 3 litres of tender coconut water, 3 kilograms of Jaggery and around 12 numbers well ripened poovan banana are added. After adding and mixing these ingredients, the setup is maintained with mixing twice every day for 30 more days. The 45th day the reaction mixture has a fruity odour and it is filtered and used as Panchagavya.

2.2.B. Accelerated/Bioaugmentation Method:

Step 1: 7 kg of cow dung and 1kg of cow ghee were mixed thoroughly both in morning and evening hours. 5ml. isolated *Pseudomonas sp*., culture was added

Step 2: On the second day, 10 litres of fresh cow urine were added to enhance microbial activity and fermentation along with 10 litres of water, ingredients

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such as jaggery, ghee, milk, and curd were added on the second day. 5ml of each of 3 isolated cultures were added and mixed thoroughly both in morning and evening hours every day. Panchagavya was filtered and sludge was separated on 10th and 18th days.

2.3. Bacterial cultures:

Four different cultures were isolated, propagated and used in the accelerated method. They are named with codes as CC1, CC2, CC3 and CC4

2.4. Media used:

For the isolation of microbes, a variety of Hi-Media Pvt. Ltd. readymade culture media were employed to ensure the growth and differentiation of specific microbial groups. Nutrient agar was used as a general-purpose medium, supporting the growth of a wide range of non-fastidious organisms. Skimmed milk agar facilitated the detection of proteolytic activity by identifying microbes capable of breaking down casein, the primary protein in milk. Lipolytic agar was utilized to isolate and identify organisms capable of degrading lipids, indicating lipase production. Additionally, MRS agar was chosen for the selective isolation of lactic acid bacteria, promoting their growth under appropriate conditions. For further enrichment and subculturing, Nutrient broth was used to maintain and propagate the isolated microbial strains in a liquid medium. These media collectively supported a comprehensive approach to isolating and studying various microbial species.

2.5 Isolation and culturing of bacteria:

The Panchagavya sample was subjected to serial dilution to reduce the microbial load and achieve isolated colonies. Dilutions of 10-7 and 10-6 were selected based on preliminary assessments for inoculation onto various selective and differential media, including Nutrient agar, MRS agar, Lipolytic agar, and Skimmed milk agar. Nutrient agar was used as a general-purpose medium to support the growth of a broad range of non-fastidious microorganisms. MRS agar (de Man, Rogosa, and Sharpe agar) was chosen for the selective isolation of lactic acid bacteria, promoting their growth under optimal conditions. Lipolytic agar was used to screen for microorganisms with the ability to degrade lipids, thus detecting lipase-producing bacteria. Skimmed milk agar facilitated the identification of proteolytic microorganisms capable of breaking down casein, the main protein in milk.

After inoculation, the plates were incubated at 37°C for 24 hours to allow for the development of microbial colonies. Post-incubation, well-isolated colonies from each medium were carefully selected based on distinct morphological characteristics. These colonies were subsequently picked and transferred to appropriate storage media or conditions, such as cryopreservation or refrigeration, to maintain their viability for further identification and characterization. This systematic approach ensures the accurate recovery of diverse microorganisms from the Panchagavya sample for future studies, including biochemical and molecular identification.

2.6 Parameters analysed for Panchagavya product:

The Panchagavya produced in traditional method was subjected to different parameters to study its physico-chemical properties like indole acetic acid (IAA) production, total dissolved solids (TDS), hydrogen ion concentration (pH) and total microbial count10.

2.6.1 Total Microbial count:

In the ongoing investigation of microbial communities within panchagavya, a diverse array of microorganisms has been identified, contributing to the fermentation and efficacy of this traditional formulation. The study aimed to profile the microbial flora to better understand their roles and impacts on the product. Isolation of microorganisms from a Panchagavya sample involves a series of standard methodical steps (11). First, panchagavya sample is collected in a sterile container, and if immediate processing is not possible, it is stored at 4°C. The sample is then homogenized and diluted by adding 1 ml of Panchagavya to 9 ml of sterile distilled water, with further serial dilutions prepared as needed. Using a sterile pipette, 0.1 ml of the diluted sample is transferred onto nutrient agar plates and other selective media like skimmed milk agar and potato dextrose agar to target specific microorganisms. These plates are incubated at 37°C for 24-48 hours, or at 25-30°C for up to 3 days for fungi. After incubation, microbial growth is observed, and distinct colonies are picked with a sterile loop or swab for streaking onto fresh nutrient agar plates to obtain pure cultures, followed by another round of incubation (12).

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2.6.2 Indole Acetic Acid:

Panchagavya contains macro and micro nutrients, along with various amino acids, growth regulators, vitamins, and beneficial microorganisms. These components promote plant growth and enhance the plant's immune system (13). To determine the amounts of IAA produced by each isolate, a colorimetric technique using the Van Urk Salkowski reagent was employed, following the Salkowski method. The isolates were cultured in nutrient broth or Luria Bertani broth (Himedia, India) and incubated at 35 °C for 24 hours. After incubation, the broth was centrifuged, and the supernatant was collected. A 1 ml aliquot of the supernatant was mixed with 2 ml of Salkowski's reagent (2% 0.5 FeCl3 in 35% HClO4 solution) and allowed to react in the dark. The optical density (OD) was measured at 530 nm after 30 minutes and 120 minutes (14). The preparation process, including the sources of the cultures and the final volume, to ensure reproducibility and effective application was documented.

2.7 Bio coagulation:

2.7.1 Bio coagulants:

A. *Colocasia esculenta*: Extracted from the vegetable commonly known as taro, this biocoagulant was chosen for its natural coagulating properties.

B. *Strychnos potatorum*: Seeds from this plant, also known as the clearing nut tree, were utilized for their proven efficacy in enhancing the clarification process.

2.7.2. Extraction of Bio-coagulants

The extraction of *Colocasia*, involved washing, peeling, and crushing the vegetable to obtain the juice, which was then filtered to isolate the active coagulating compounds 6. The seeds of *Strychnos potatorum* were cleaned, dried, and ground into a fine powder. This powder was then mixed with water to form a solution that could be easily incorporated into the panchagavya mixture (7).

2.7.3. Dosage of Biocoagulants:

Both biocogulants (5- 10ml/Litre @ 20% concentration) were added to the panchagayya, stirred well and allowed to settle for 60 minutes and filtered. Volume of the filtrate is estimated.

2.7.4. Addition to Panchagavya:

On the 18th day of the fast-track production process, the prepared bio-coagulants from *Colocasia esculenta* and *Strychnos potatorum* were added to the fermented panchagavya mixture. The bio-coagulants were thoroughly mixed into the solution to ensure even distribution and effective coagulation.

2.7.5 Recovery of Panchagavya:

The mixture was allowed to sit undisturbed for a specified period, during which the bio-coagulants facilitated the aggregation of fine particles and impurities (15). This process led to the formation of a clear supernatant and a settled coagulum at the bottom, effectively separating the liquid and solid phases of the panchagavya.

3. RESULTS

3.A Total Analysis of Panchagavya in Traditional Process

Panchagavya results indicates the process carried out is in line with the standard operational practices. The process takes 35 days to obtain Panchagavya of good quality as shown in Table 1.

3.B Total Analysis of Panchagavya in Accelerated Process

Accelerated process produced good quality of Panchagavya. Optimum time it takes 10 days of time, and maximum production was obtained at 18 days of time. Addition of bacterial cultures not only enhanced the productivity but also the time taken by 25 days as depicted in Table 2.

Figure 2: The resultant Panchagavya product at the end of traditional and accelerated process

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S. No	DAYS	TDS	pH	IAA (ppm)	Microbial Count. Per ml			
					Total Count	Lipolytic Bacterial Count	Proteolytic Bacterial Count	Fungal colony count
1	1	434	4.34	-	$6x10^{-6}$	$4x10^{-7}$	$12x10^{-7}$	$3x10^{-6}$
2	3	434	4.37	4	$7x10^{-6}$	$4x10^{-7}$	$13x10^{-7}$	$7x10^{-6}$
3	11	466	5.12	8	$6x10^{-6}$	$6x10^{-7}$	$10x10^{-7}$	$8x10^{-6}$
4	15	543	5.28	10	$9x10^{-6}$	$9x10^{-7}$	$23x10^{-7}$	$5x10^{-6}$
5	14	546	5.28	17	$8x10^{-6}$	$10x10^{-7}$	$36x10^{-7}$	$8x10^{-6}$
6	18	556	5.34	17	$8x10^{-6}$	$16x10^{-7}$	$87x10^{-7}$	$8x10^{-6}$

3.1 Physico-chemical parameters of Panchagavya:

Panchagavya was made and the values are given below: PH- 5.5, Total nitrogen-230 ppm, Total potassium-320 ppm, Total phosphate-325 ppm, Gibberellic acid-3.4 ppm, Indole acetic acid-13.1 ppm and Total bacterial-86*106 CFU/ml as per the literatures (16).

Table 1: Results of Panchagavya (Traditional Process)

S.No	Days	pH	IAA (ppm)	Microbial Count. Per ml			
				Total Count	Lipolytic Bacterial	Proteolytic Bacterial Count	Fungal Count
1	3	5.34	-	$7x10^{-6}$	$5x10^{-7}$	$11x10^{-7}$	-
2	11	5.37	-	$7x10^{-6}$	$6x10^{-7}$	$14x10^{-7}$	$3x10^{-6}$
3	15	5.42	2	$8x10^{-6}$	$6x10^{-7}$	$18x10^{-7}$	$7x10^{-6}$
4	18	5.68	2	$10x10^{-6}$	$10x10^{-7}$	$22x10^{-7}$	$6x10^{-6}$
5	25	5.88	4	$11x10^{-6}$	$11x10^{-7}$	$34x10^{-7}$	$9x10^{-6}$
6	35	6.14	6	$20x10^{-6}$	$17x10^{-7}$	$90x10^{-7}$	$6x10^{-6}$
7	40	6.24	8	$26x10^{-6}$	$20x10^{-7}$	$88x10^{-7}$	$5x10^{-6}$

Table 2: Results of Panchagavya (Accelerated Process)

3.2 Comparison of IAA of the two methods used:

On the 40th day, 8 ppm of IAA was observed. The accelerated process gave 17ppm of IAA is dropped down to 11 days from 40 days. The results indicate the productivity of the IAA is higher. The suggested optimal production has 11 days which can be extended to 18 days under extended storage period.

Table 3: IAA production in traditional & accelerated methods

DAY	Indole Acetic Acid (in PPM)	
	Traditional Method	Accelerated Method
Day 1	-	-
Day 3	-	4
Day 11	-	8
Day 15	2	10
Day 18	2	17
Day 25	4	-
Day 35	6	-
Day 40	8	-

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3.3 Microbial Composition:

The study revealed the presence of several lipolytic and proteolytic organisms. These microorganisms were observed to possess enzyme activities that break down fats (lipolysis) and proteins (proteolysis), respectively and the culture labelled as VD 1, VD 2, VD 3 and VD 4 which denotes the unidentified cultures. However, their specific identities and roles within the panchagavya fermentation process are still under investigation. Lipolytic Organisms are the organisms which contribute to the degradation of lipids, which is crucial for breaking down fatty acids present in the panchagavya ingredients. The exact species responsible for this activity are yet to be identified. Proteolytic microorganisms facilitate the breakdown of proteins into peptides and amino acids, which can be critical for the nutritional quality and functional properties of panchagavya. The specific strains involved are still to be characterized.

The study of microbial flora in panchagavya has unveiled a complex and diverse microbial community. Key bacteria such as *Pseudomonas, Bacillus,* and *Lactobacillus* play significant roles in fermentation, nutrient breakdown, and product efficacy. Additionally, the presence of unidentified lipolytic and proteolytic organisms highlights the potential for further research into their specific functions and impacts on the panchagavya formulation. Continued exploration and characterization of these microorganisms will provide deeper insights into optimizing and enhancing panchagavya production and its applications.

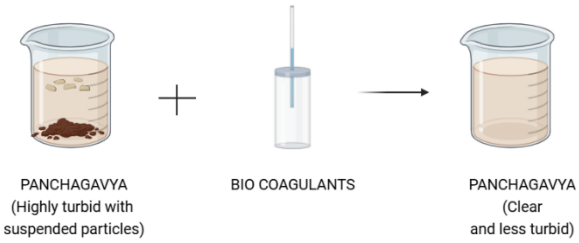
3.4 Bioaugmentation of Panchagavya:

There was a shift in the Indole Acetic Acid (IAA) levels after the addition of the culture. Comparatively, when mixed culture and *Pseudomonas sp* is added together, the IAA production levels were higher than when they are added separately in the setup. This study achieved the optimum IAA level (8ppm as per TNAU) on the day 10-15. By adopting this accelerated methodology, the production time of panchagavya was dramatically reduced from the standard 45 days to just 10 days. Despite the shorter production period, the final product retained all the desired qualities and effectiveness of traditionally produced panchagavya. The presence of active microbial cultures, essential nutrients, and beneficial compounds was confirmed through qualitative and quantitative analyses, validating the efficacy of the fast-tracked product. This innovative approach demonstrates the potential for significant improvements in traditional panchagavya production. By leveraging isolated microbial cultures and optimizing fermentation conditions, it is possible to produce high-quality panchagavya in a fraction of the time typically required. This method not only enhances production efficiency but also ensures that the end product remains effective for agricultural and other applications.

3.5 Recovery by bio-coagulants:

The introduction of bio-coagulants significantly enhanced the clarity and purity of the panchagavya. The recovered product was of high quality, with a marked reduction in turbidity and suspended particles. This clarified panchagavya retained all its beneficial properties, making it suitable for various agricultural applications. The utilization of natural bio-coagulants derived from *Colocasia esculenta* and *Strychnos potatorum* presents a promising advancement in the production of panchagavya. This method not only improves the recovery and quality of the final product but also aligns with sustainable and eco-friendly practices (17). The successful integration of these bio-coagulants into the production process highlights the potential for further innovations in the field of organic agricultural inputs.

Figure 3: An illustration showing role of bio coagulants



in recovery of Panchagavya

The starting amount of the product is shown in different volumes (10 ml, 15 ml, 20 ml, 25 ml). The sedimented volume is the portion of the product that has settled or sedimented out. For example, when 10 ml of product is processed, 7 ml sedimented in method A while 3 ml sedimented in method B. Method A generally shows a higher sedimented volume than the Method B. The supernatant volume represents the volume of liquid remaining after sedimentation, or the product that hasn't settled. For instance, with 10 ml of product, 3 ml is supernatant in method A, while 7 ml is in method B. The recovery percentage indicates the percentage of the product recovered after the process. The method B consistently shows higher recovery percentages than the traditional method, implying that method B is more efficient in recovering the product. Overall, the

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Accelerated method (B) performs better in terms of recovery, even with a lower sedimented volume, making it a more efficient approach as depicted in Table 4.

4. CONCLUSION

In traditional methods being uncontrolled, it is subjected to variations in the product quality and quantity. In the present method microbial bioaugmentation enhances the uniformity of production in terms of quality and quantity. The study indicates the Panchagavya production can be improved and a better-quality product can be obtained, further it reduces the time period of production to 18 days from 40 days. This facilitates reduced input cost of production and also it increases the capacity of the processing plant by 300%. Further, the bioaugmentation studies revealed higher percentage of recovery of the end product by significant percentage. The application of bioaugmentation can help other fermentation processes to increase their productivity.

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Table 4: Recovery of Panchagavya with bio-coagulant where A indicates product obtained by Tradition method and B indicates product obtained by Accelerated method

S. No.	Volume of product	Sedimented volume		Supernatan volume		Recovery %	
		A	B	A	B	A	B
1	10 ml	7ml	3ml	3ml	7ml	30%	70%
2	15 ml	8ml	5ml	7ml	10 ml	47%	67%
3	20 ml	10m	5ml	10m	15 ml	50%	75%
4	25 ml	10m	5ml	15m	20 ml	60%	80%

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