Studies on Modified Jeevamrutha Bio-fertilizer Formulations for Organic Farming Application in Assam

Thesis submitted in partial

fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

by

Udaratta Bhattacharjee

Centre for the Environment

Indian Institute of Technology Guwahati

Guwahati – 781039, India

August 2023

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Dedicated to

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"Studies on Modified Jeevamrutha Bio-fertilizer Formulations for Organic Farming Application in Assam"** (submitted by Ms. Udaratta Bhattacharjee) was carried out under my supervision at the Centre for the Environment, Indian Institute of Technology Guwahati.

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DISCLAIMER

The experimental, modelling and characterization related data presented in this Ph.D. thesis was carried out by me and is reported after due verification. To the best of my knowledge, the work summarized in this Ph.D. thesis has not been submitted elsewhere for the award of any Degree or Diploma.

Udaratta Bhattacharjee

(Ms. Udaratta Bhattacharjee)

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The Ph.D. thesis targets to develop a nutrient-rich Jeevamrutha bio-fertilizer for its wider applicability in rural sectors of Assam that are characterized by highly diverse summer and winter climatic conditions. Jeevamrutha bio-fertilizer consists of cow dung, cow urine, jaggery, gram flour, soil and water. The optimization of these raw ingredients using statistical tools shall be an interesting proposition for improved soil nutrient content and plant growth. To do so, a time-dependent study needs to be conducted for the screening and optimization of the compositional set for the Jeevamrutha bio-fertilizer. Additionally, to address the intense demand for crop systems in India, it is necessary to incorporate natural and nutrient-rich substrates such as vermicompost, neem cake, tea waste and water hyacinth into the optimized Jeevamrutha bio-fertilizer. Accordingly, improvement in the soil texture and crop yield can be achieved. Thus, further optimization of the compositional set shall be targeted by considering the inclusion of other natural resources for the improvement of nutrient characteristics. On the other hand, jaggery, a carbon substrate, can be replaced with other cheap and readily available precursors such as starch rice water to investigate its efficacy in terms of nitrogen, phosphate, potassium and micro-nutrients. Further, an integrated farming technique with vermicompost and solid Jeevamrutha bio-fertilizer was deployed to achieve higher growth characteristics in *Phaseolus vulgaris*, its nutritional content and the presence of a good microbial population. In summary, this work suggests an alternate organic amendment that prompts human health benefits through organic farming with improved soil fertility factors. It is worth mentioning that the evaluation of total Kjeldahl nitrogen (TKN) in the conventional approach was replaced with ammonium nitrogen (AN) due to its rigorous and quicker assessment in the entire Ph.D. thesis work.

The first objective of the Ph.D. thesis targets the screening study with Plackett-Burman Design statistical tool. The tool has been proven to be an effective framework (91 and 92% R^2 for AN and P respectively and p-value <0.05) for the screening and identification of independent variables such as jaggery, gram flour, water and soil in the Jeevamrutha bio-fertilizer. An interesting point of this study is that these bio-formulations have been prepared with the minimal constitution of cow urine (0.02 L) in comparison with Palekar's Jeevamrutha (0.05 L) formulation. This is due to the fact that cow urine has several medicinal properties and is not readily available in the rural community. During the PBD analysis, the identified screened factors for the Jeevamrutha bio-fertilizer were jaggery, gram flour and soil. Further, using CCD statistical tool, the optimized compositions of modified Jeevamrutha formulations referred to 0.03 kg for jaggery and gram flour and 0.35-0.36 kg soil during the winter and summer seasons in Assam respectively. For this study, %TKN, AN and %P varied as 0.28-0.67%, 135.86-427.56 mg/L and 0.10-0.14%. Microbial and phytotoxicity assessments have also been addressed to further enhance the maturity of the prepared bio-fertilizer product. Hence, among the considered factors, jaggery, gram flour and soil were identified to be the critical factors that influenced the nutrient yield of the solid Jeevamrutha bio-fertilizer formulations.

Thereafter, the optimal Jeevamrutha bio-fertilizer was further modified with commercial and rural substrates such as vermicompost, neem cake, tea waste and water hyacinth. The precursors were varied as 1:1-1:6 (Jeevamrutha:Precursors) and the optimized ratio was evaluated for its %TKN, AN, P along with its physicochemical, macro- and micro-nutrients constituents and phytotoxicity assessment. The findings from this objective affirm that tea waste and water hyacinth could be targeted as alternate rural resources during the winter season. This is due to the reason that the winter in Assam witnesses a significant reduction in the AN and %TKN nutrient trends. In summary, tea waste and water hyacinth-based modified Jeevamrutha affirmed better performance in terms of %TKN, AN and %P along with its associated cost. Accordingly, these precursors can be used as nutrient-rich additives in the bio-fertilizer to enrich its applicability for field applications.

The third objective of the Ph.D. thesis targets to replace the carbon source i.e., jaggery with sugarcane juice or starch rice water to eventually improvise the nutrient yield of the modified Jeevamrutha bio-fertilizer and thereby minimize the conceptual cost indices. The findings of this work affirm that starch rice water could be utilized as a low-cost substrate for further investigation. This is due to its excellent enactment with the bio-formulation in terms of the enhanced nutritional characteristics such as %TKN (0.54-0.82%) and P (0.27-0.35%) in comparison with jaggery-based optimal Jeevamrutha (%TKN, 0.28-0.67% and %P, 0.10-0.14%). The conceptual cost analysis for starch rice water-based bio-formulation has affirmed the lowest expenditure (Rs. 20.15 per kg) in comparison with jaggery-based modified Jeevamrutha (Rs. 22.49 per kg). Thus, jaggery can be replaced with starch rice water in the modified Jeevamrutha and the replacement could serve as a relevant substrate to address the nutrient decline observed during the winter seasons.

Finally, the utilization perspectives of the integrated farming system (IFS with Jeevamrutha-Vermicompost System) have been targeted to address the detrimental nutrient decline in soil and crop productivity during winter seasons in Assam climatic conditions. A model plant *Phaseolus vulgaris* was considered for its growth profile study to characterize its morphological features with various treatments. After 45 days of seed germination, the nutritional content of the fruit and the root health of the plant were evaluated. Additionally, for each treatment, the soil was further characterized for its physicochemical constitution and microbial characteristics. Thus, the findings of the study can widen the scope of livestock-based fertilizer systems in terms of the value-added exploration of the available resources for better crop cultivation.

In summary, these findings of the Ph.D. thesis further substantiate the need to further research the scale-up, design and formulation research of the modified solid Jeevamrutha bio-fertilizer. The thesis especially emphasizes the possible scope for natural agricultural practices in terms of low-cost nutrient enhancement technology exploration and rapid crop growth and addressing the nutrient decline during the winter season in Assam.

The subjective novelty of the Ph.D. thesis is as follows:

- Developed robust analytical procedures by targeting the replacement of TKN with AN.
- Screening and optimization of solid Jeevamrutha bio-fertilizer in Assam's summer and winter seasons.
- Evaluated the efficacy of tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer in terms of its nutrient yield and cost-effectiveness.
- Corroborating phytotoxicity assay outcome with the findings of the nutrient content.
- Evaluated the efficacy of starch rice water-based Jeevamrutha bio-fertilizer in terms of nutrient yield and low cost.

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Abbreviations

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
CCD	Central Composite Design
FRCBD	Factorial Randomized Complete Block Design
HPLC-RID	High-Performance Liquid Chromatography-Refractive Index Detectors
NE	North-east
PBD	Plackett-Burman Design
RSM	Response Surface Methodology
UV-Vis	Ultraviolet Visible

Nomenclature

AN	Ammonium Nitrogen
CFU	Colony Forming Units
DCPIP	2,6-Dichlorophenolindophenol
EC	Electrical Conductivity
GI	Germination Index
IFS	Integrated Farming System
J	Jeevamrutha
K	Potassium
MC	Moisture Content
N-fixers	Nitrogen fixers
OC	Organic Carbon
P	Phosphate

P-solubilizers	Phosphate solubilizers
SE	Seed Germination
TKN	Total Kjeldahl Nitrogen
VC	Vermicompost

Notations

S_L	Lower Parametric Range
S_H	Higher Parametric Range
T1	Treatment with Jeevamrutha:Soil:Vermicompost
T2	Treatment with Jeevamrutha:soil
T3	Treatment with Vermicompost:soil
T4	Treatment with soil
W_1	Weight of fresh sample (g)
W_2	Weight of dried sample (g)

Chapter 1:
Introduction and Literature Review

Introduction and Literature Review

In this chapter, the first section delineates upon the need for organic farming and the importance of bio-fertilizers to minimize the intensive utility of chemical fertilizers and thereby enhance the sustainability of the agricultural system. In a nutshell, the thesis work emphasizes cow-excreta-based Jeevamrutha bio-fertilizer formulation, its modification and understanding of associated physicochemical mechanisms for the improvement of plant growth parameters. This bio-fertilizer is prepared with inclusive inexpensive substrates that are readily available in rural India and can hence address the utilization of available resources for the promotion of their role in the long-term economic security of the rural community and eventually the nation. Section 1.2 addresses the available prior art for the preparation, application and nutrient yield of Jeevamrutha bio-fertilizer formulations in terms of ammonium nitrogen (AN), phosphate (P) and Total Kjeldahl Nitrogen (TKN) along with their physicochemical characteristics that critically influence crop productivity and soil quality. Thereafter, the scope for further research highlighting its significance towards statistical design-based compositional fitness of the bio-formulation and associated parametric synergy with other additives on the modified formulation has been discussed in section 1.3. Finally, section 1.4 conveys the broad objectives of the thesis followed by thesis organization details in section 1.5.

1.1 Background

1.1.1 Importance of Natural Farming and its Associated Benefits

The injudicious utility of chemical fertilizers, pesticides, herbicides and insecticides in agriculture has mitigated the numerous constitution and roles of beneficial microbes that exist in the soil (Ranjit, Sanjiv, et al. 2019). Thereby, this approach is detrimental to the microbial biodiversity of the ecosystem and its integrity with agriculture. All these translate into a detrimental influence on the desired incentives such as sustainable plant health and life, lower input cost for desired crop yield and high farm income (Ranjit, Sanjiv, et al. 2019). Hence, it is necessary to adopt robust remediation measures for soil enrichment and easy accessibility of the vital nutrients that facilitate good plant growth characteristics (Radha and Rao 2014).

For general consideration, the nitrogen-phosphate-potassium (NPK) ratio of chemical fertilizers and organic fertilizers with respect to N varies from 20-60% and 14% respectively. However, the presence of inorganic materials in chemical fertilizers such as urea, ammonium phosphate and ammonium sulphate stunt the growth of beneficial microbes in the soil. On the other hand, organic fertilizers improve soil structure and consistent release of nutrients for a longer duration of time. In this regard, natural or organic farming is becoming popular due to its diverse agro-ecological-based attributes that integrate livestock manures with available cheap precursors and thereby achieve a better crop yield (Rosset and Martínez-Torres 2012). In India, zero-budget natural farming (ZBNF) proposed by Shri Subhash Palekar, an Indian agriculturist, has been opined to be a holistic farming practice for its symbiotic association of soil and plant (Palekar 2006). These practices are widely adopted in different parts of India viz., Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh and Tamil Nadu (Gore and Sreenivasa 2011, Boraiah, Devakumar et al. 2017, Singh and Lal 2018, Sornalatha, Tamilarasi et al. 2018, Kulkarni and Gargelwar 2019). However, their formalization in North-East (NE) India, Assam, and a rich bio-diversity hotspot needs considerable attention.

Customized bio-products provide excellent growth-promoting factors and useful microorganisms for plant growth and development. One such bio-product prepared from an organic source is a bio-fertilizer that contains viable microbial cells that prompt the enhancement in the growth and availability of macro- and micro-nutrients in the plant upon its addition to the plant surface, soil or seed. It also aids in colonizing the rhizosphere of the host plant (Mrkovacki and Milic 2001). Since bio-fertilizers are a significant component of nutrient management techniques, they can be supplemented or complemented with chemical fertilizers to promote the health benefits of the soil and reduce its detrimental effect to achieve sustainable agricultural profits in the upcoming years (Chen 2006). The addition of green and animal manure compost along with waste deposits provides all necessary and essential soil nutrients and thereby improves the fertility status of the soil (Anandan, Priya, et al. 2016).

Commercial bio-fertilizers can be classified as bacterial, fungal, algal and organic bio-fertilizers. Apart from the microbial bio-fertilizers, organic bio-fertilizers such as vermicompost and cow-excreta-based bio-fertilizers are significant. They are rich in microbial diversity and possess an enormous amount of nutrients such as nitrogen (N), phosphate (P), organic carbon (C), potassium (K), sulphur (S), vitamins, growth hormones, enzymes and antibiotics. These augment soil's water retention capacity, texture and crop productivity (Mahdi, Hassan, et al. 2010).

1.1.2 Significance of Cow-excreta-based Organic Bio-fertilizers

Cow-excreta-based bio-products are rich in minerals such as N, P, K, S, magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co) and calcium (Ca) along with cellulose, hemicellulose and lignin. In terms of microbial diversity, these products include protozoa, yeast and several bacterial isolates essential for plant life and soil fertility (Garg and Mudgal 2007, Randhawa and Kullar 2011). Hence, its applicability in agriculture as a pest repellent, bio-fertilizer and bio-pesticide is evident, given its socio-economic prominence in the village bio-economy (Dhama, Chauhan, et al. 2005). Further, they include the easily accessible integral formulation of various liquid manures and biodynamic preparations commonly used in agriculture.

Radha and Rao (2014) addressed different biodynamic preparations from horn manure, Panchagavya and cow pat pit. The mixture consists of 500 g ghee and 5 kg cow dung that was kept in a container for 4 days at room temperature. This mixture was stirred twice a day and 3 L of cow urine, 2 L of curd, 2 L of milk, 500 g of jaggery, 12 ripened bananas, 3 L sugarcane juice, 3 L coconut water and 100 g of brewer's yeast were added and was kept for another 15 days. The mixture was stirred twice a day to improve the aeration rate. The biodynamic preparation exhibited a higher count of bacteria such as *Bacillus safensis*, *B. aureas*, *B.subtilis*, *B. licheniformis* and yeasts that utilize dextrose, sucrose and trehalose as a carbon source. The strains produced ammonia and indole acetic acid (a plant hormone). Phosphate solubilization was observed in this case. However, no nitrogen fixation was achieved. Some of these strains have demonstrated antagonist mechanisms for a plant pathogen namely, *Rhizoctonia bataticola*. The authors reported that the bacterial populations present in the biodynamic formulation enhanced the plant growth by 21%, chiefly maize. Hence, this study provided useful insights into cow-excreta-based organic farming technologies for the production of bio-fertilizer and bio-control agents and thereby customize the vital existence of dynamic microbial consortiums (Radha and Rao 2014).

Another survey investigated various bacterial and fungal strains such as *B. pumilus*, *B. laterosporus*, *B. macereans*, *Enterobacter aerogene*, *Micrococcus varians*, *Aspergillus rapens*, *A. niger*, *A. fumigates*, *A. flavus* and *Fusarium* species for the composting process. Cow dung, plantain peel, bean husk and cassava peels were used as substrate materials. These materials were thoroughly mixed in altered ratios (1:1, 1:2 and 1:3) along with a control (without cow dung) in the formulation. The control group confirmed low N content (0.83%) in comparison with the modified compost (2.21%). It could be interpreted that the quality of the compost improved with the beneficial microbes that exist in the cow dung along with other physicochemical factors such as ambient temperature, aeration, moisture content and chemical composition. Hence, cow dung addition to the compost enriched its nutrient characteristics and maturity process (Adegunloye, Adetuyi, et al. 2007).

Similarly, another study developed a bio-fertilizer with altered ratios of cow dung, left-over cooked rice and timber sawdust. The optimal formulation possessed an N, P and K content of 19670 mg/L, 6630 mg/L and 1403.2 mg/L respectively. The total organic carbon (TOC) and heavy metals (Mg, Zn and Fe) were also analyzed. These values were lower in comparison with the NPK of chemical fertilizers (10000 mg/L, 20000 mg/L, and 10000 mg/L respectively). However, the preparation process was sustainable (Oladejo et al. 2015). Cow dung as an efficient nitrogen source was added to sawdust to enhance the nutrient availability of the composting process. This study facilitated an understanding of the associated mechanism for compost material improvement through the addition of waste for a quicker seedling and growth process. Thereby, crop yield can be enhanced. Organic bio-fertilizers namely, Panchagavya, Jeevamrutha, Sanjibani, Beejamrutha, Amritpani and Kunapajola prepared from cow-excreta-based have been widely explored in rural sectors of India but not in Assam. These bio-formulations have different preparation methods and alternate raw materials that possess excellent application in crop productivity. *Oryza sativa* paddy was considered as a model plant for respective investigations with these organic bio-fertilizers (Palekar 2006, Anandan, Priya et al. 2016).

The composition of various formulations and their physicochemical characteristics has been illustrated in Table 1.1. These bio-formulations are capable to promote plant growth and protect them against certain pathogenic attacks. In this regard, Jeevamrutha bio-fertilizer has been primarily used for the enrichment of the soil for its better microbial diversity and faster decomposition of the organic constituents. Another interesting feature of the

Jeevamrutha is that it is only required for the first 3 years of the transition in crops and eventually leads to the self-sustainability of the system after its particular dose (Amareswari and Sujathamma 2014). Also, compared to other bio-fertilizers in Table 1.1, Jeevamrutha is not expensive as it does not utilize expensive rural precursors such as banana, curd, honey and ghee. The adopted rural ingredients in the bio-fertilizer are jaggery and gram flour along with cow dung and readily available cow urine. Both jaggery and gram flour are relevant resources in India and are indigenously available.

Further, it shall be noted that cow urine has commercial application in the pharmacological sector due to its antifungal and anticancer agents (Dhama, Chauhan et al. 2005, Chand and Tiwari 2012, Pradhan, Verma et al. 2018). In other words, cow urine shall not be regarded as an inexpensive rural resource for bio-fertilizer development. Also, compared to cow dung, cow urine production quantity from the animal is not significant. Hence, either reduced cow urine or no cow urine-based formulations are to be as well addressed. In summary, the generous utility of cow urine in traditional agricultural practices needs to be minimized or even omitted, given the low population of such animals in Assam.

Also, some of these organic bio-formulations have been integrated with conventional resources such as vermicompost to improvise its %NPK content and earthworm recovery (Veeresh and Narayana 2013). However, unconventional resources such as neem cake, pongamia cake, tea waste, and vegetable or fruit peel waste which are known for their higher nutritional properties have not been well-explored in the prior art and for their influence on crop production. Hence, in this study, emphasis has been laid upon Jeevamrutha bio-fertilizer formulation and its NPK enrichment using conventional and unconventional resources. Thereby, an easy and affordable preparation process has been targeted for the rural communities of Assam.

Table 1.1 summarizes various organic manures with their constituent materials along with their nutrient status. From the table, it can be stated that Panchagavya, Amritpani and Kunapajola have been the most expensive bio-formulations in comparison with Sanjibani, Beejamrutha and Jeevamrutha. This is due to the inclusion of expensive ingredients such as banana, curd, honey and ghee. Since Sanjibani and Jeevamrutha are less expensive in comparison with other bio-formulations, the Jeevamrutha bio-fertilizer formulation has been targeted in this work. The relevant justification for not considering Sanjibani is as follows.

Sanjibani can be prepared in avenues with abundant produces of cow dung and cow urine. However, there will be avenues with lesser production of these resources. For them, Jeevamrutha will be the best among the mentioned bio-formulations. Given the affirmed urbanization in even rural communities, the abundance of cow dung and cow urine is questionable in developing rural communities. For these reasons, possible avenues exist with reduced quantities of cow dung and cow urine and especially cow urine. Therefore, it is very likely that significantly urbanized rural avenues may have greater relevance for Jeevamrutha but not Sanjibani. Hence, the research devotes to the solid Jeevamrutha bio-fertilizer formulation. The solid form also offers the benefits of storage, the convenience of transportation and application. The composition, possible characterization and scope of solid Jeevamrutha have been depicted in Fig. 1.1. In the context of the Ph.D. thesis, it would be an interesting theme to evaluate the effect of Jeevamrutha bio-fertilizer on plant health and fruit yield for the case that does not include cow urine as an ingredient in the Jeevamrutha bio-formulation.

Table 1.1: A summary of prominent organic bio-fertilizers and their nutrient yield.

S. No.	Name of the Bio-formulation	Constituents	Fermentation time (days/hours)	Total Nitrogen (ppm)	Total Phosphate (ppm)	References
1.	Panchagavya	7 kg fresh cow dung, 10 L cow urine, 1 kg cow ghee, 3 L cow milk, 2 L cow milk curd, 3 kg jaggery, 2 kg ripened banana	25 days	423.32	198.58	(Sangeetha and Thevanathan 2010, Amareswari and Sujathamma 2014, Anandan, Priya et al. 2016)
2.	Jeevamrutha	10 kg cow dung, 10 L cow urine, 2 kg jaggery, 2 kg gram flour, Handful of soil, 200 L tap water	5-7 days	320.45	177.11	(Palekar 2006, Anandan, Priya et al. 2016)
3.	Sanjibani	1 kg cow-dung, 1 L cow urine, 2 L water	9 days	401.86	173.65	(Ali, Ghatak et al. 2011, Anandan, Priya et al. 2016)
4.	Beejamrutha	5 kg cow dung, 5 L cow urine, 50 g lime, 20 L water, Handful of soil	12 h	40.00	155.30	(Gore and Sreenivasa 2011)
5.	Amritpani	10 kg cow dung, 250 g cow ghee, 500 g honey, 200 L water	2-6 day	445.35	187.56	(Sangeetha and Thevanathan 2010, Anandan, Priya et al. 2016)
6	Kunapajola	10 kg Bombay duck fish, 4 kg sesame oil cake, 4 kg rice husk, 4 kg molasses, 30 L fresh cow urine	60 days	246.55	131.11	(Sarkar, Kundu et al. 2014, Anandan, Priya et al. 2016)

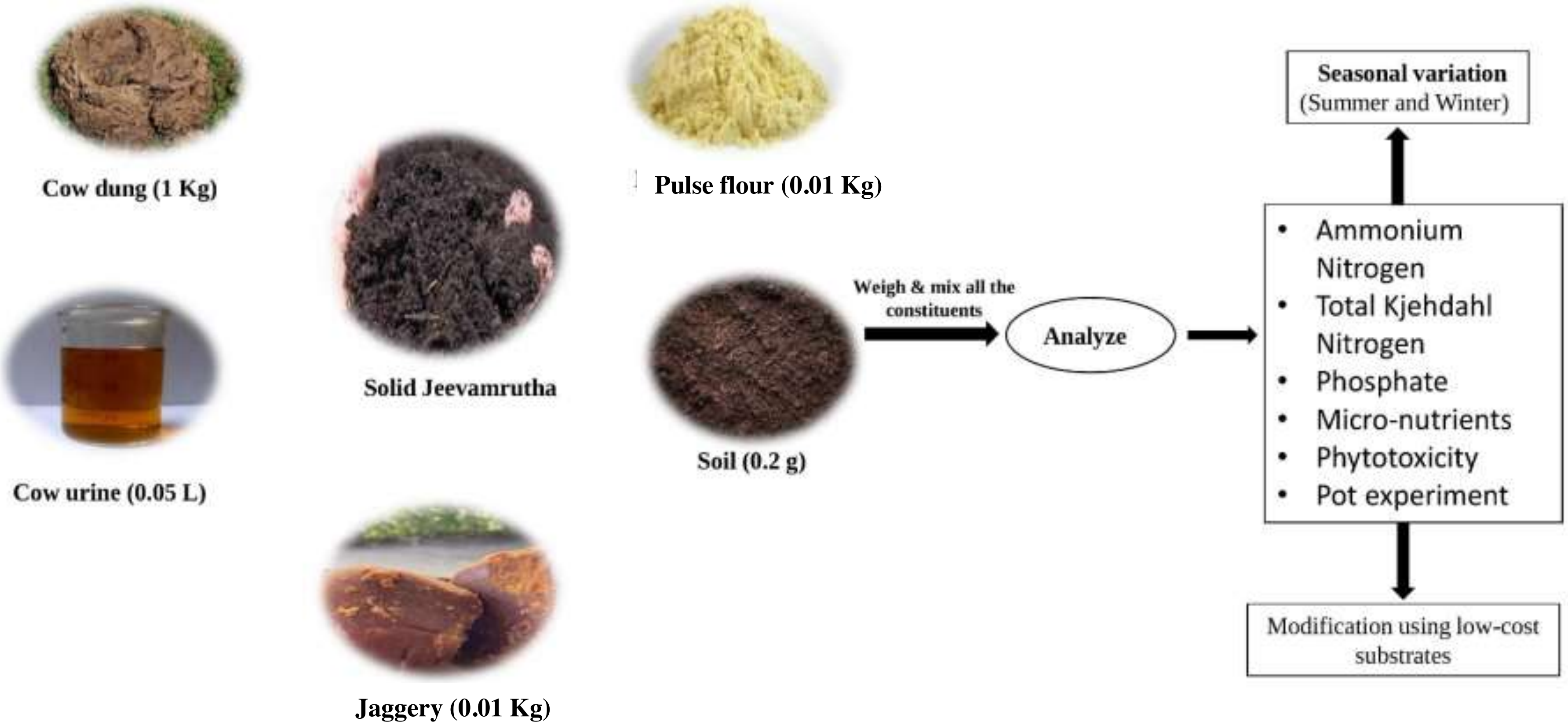


Fig. 1.1: A schematic of the solid Jeevamrutha bio-fertilizer and its characterization.

1.1.3 Preparation and Applications of Jeevamrutha Bio-fertilizer Formulations

The prevalent indigenous microbes in the Jeevamrutha provide essential nutrients for the mineralization of the soil. Jeevamrutha exists in two forms namely in a liquid and solid form. The liquid Jeevamrutha bio-formulation is prepared with 10 kg cow dung, 10 L cow urine, 2 kg jaggery, 2 kg besan flour, a handful of garden soil without any chemicals and 100 L of water (Palekar 2006). These ingredients were mixed well in mentioned proportions. Thereafter, the mixture was left to ferment for 5 to 7 days and stirred thrice a day using a wooden stick. The formulation prepared at a minimal cost boosts plant growth and yields provides resistance against insects and pests and enhances the organic carbon content in the soil. It is applied to the soil by sprinkling or by application through irrigation water. The first dose (spray) is given one month after seed sowing or transplantation. Thereafter, the second and third doses are given after 21 days and 42 days respectively. The solid Jeevamrutha bio-formulation is often achieved with 100 kg cow dung, 5 L cow urine, 1 kg of jaggery and besan flour along with a handful of soil but without any water. The mixture should be dried under sunlight and can be stored in jute bags. However, the addition of water will activate the microbes in the formulation. Two hands of solid Jeevamrutha (200 kg per acre) have been suggested to be enough for field application (Kaur, 2020). Usually, three doses of Jeevamrutha are required for field application for any conventional seed and this has been conveyed previously. This refers to one dose before seed sowing, the second after 20 days of sowing and the third dose after 45 days of sowing (Upperi, Lokesh, et al. 2009). During field application, Jeevamrutha has been opined to enhance micro-nutrient availability, better earthworm count and foster a higher constitution of microbial consortium (Kaur, 2020).

1.1.4 Summary

As outlined, the available literature has been scanty in terms of the altered nutritional compositions of Jeevamrutha bio-formulations. In summary, emerging technologies need further investigations to achieve desired results that facilitate better nutrient yield. Conventionally, compositional fitness of the bio-formulation necessitates the soil fertility and crop yield of plants. The following sections provide useful insight into the customized validation of Jeevamrutha. The prior art devotes to Jeevamrutha's statistical compositional evaluations, field applications, influence on microbial population, macro- and micro-nutrient content and phytotoxicity assessment respectively. These perspectives have been anticipated to widen the scope of concurrent research in the precise theme of the development of cost-effective, sustainable and readily available bio-fertilizer with good nitrogen and phosphate content. Thereby, the agriculture sector can be well served in developing nations.

1.2 Prior art

Based on various conceptualized and above summarized research perspectives, the Ph.D. thesis targets the development of low-cost and nutrient-rich bio-fertilizer formulations using rurally available substrates in Assam. Thereby, the thesis aims to uplift rural economic conditions and reduce detrimental consequences that arise with the enhanced utility of harmful chemical fertilizers. To date, organic solid Jeevamrutha bio-fertilizers have not been widely explored for field applications. Considering all these, the available prior art has been classified into the following themes:

a) Overview of Jeevamrutha bio-fertilizer preparation, application, and characterization.

b) Statistical assessment for the evaluation of Jeevamrutha in terms of nutrient yield.

c) Jeevamrutha integrated organic fertilizer and its influence on nutrient content.

d) Modification and nutritional improvement of Jeevamrutha bio-formulations in terms of nitrogen-phosphate-potassium (N-P-K) content.

e) Time-dependent growth study of Jeevamrutha integrated organic fertilizers to assess crop yields.

A brief overview of the available literature on the above themes has been presented in the following sub-sections.

1.2.1 Preparation, Characterization and Application of Jeevamrutha Bio-fertilizer System

Liquid Jeevamrutha bio-fertilizer formulation constitutes a ratio of 1 kg: 1 L: 0.2 kg: 0.2 kg: 20 L for cow dung, cow urine, gram flour, jaggery and water respectively. A little soil is added at times to the formulation. Similarly, solid Jeevamrutha refers to a ratio of 1 kg: 0.05 L: 0.01 kg: 0.01 kg for cow dung, cow urine, gram flour, jaggery and a handful of soil respectively. Table 1.2 elucidates the prior art composition for the Jeevamrutha liquid formulations. The integral microbial diversity such as bacteria, actinomycetes and yeasts in the cow dung provides favorable conditions and pH (6.5-7.8) for the plants to grow and provide all essential nutrients for soil fertility (Kulkarni and Gargelwar 2019). Similarly, cow urine enhances plant resistance against pathogens such as fungi, nematodes, certain bacteria, viruses and mycoplasma that cause plant diseases. In this regard, with a rich amino acid content, cow urine increases nitrogen percentage in the bio-fertilizer (Kulkarni and Gargelwar 2019). Gram flour and jaggery serve respectively as nitrogen and carbon sources and thereby enhance the metabolic activity of the microorganisms. This is due to the fact that gram flour contains a good amount of amino acids (Arab, Helmy et al. 2010, Sameen, Mushtaq, et al. 2021). The liquid form of the Jeevamrutha bio-fertilizer can be applied through either irrigation water or sprinkling. The solid formulation is often sun-dried and applied as 200 kg per acre during the seed germination phase (Palekar 2006).

The physicochemical characterization and plant growth analyses were carried out by Anandan et al. (2016) using standard methods and with *Oryza sativa* ADT43 as the model plant (James and Natalie 2014). Thereby, the characteristics evaluated were pH (7.03), electrical conductivity (7.9 dS/m), N (320.45 ppm), P (177.11 ppm), K (132.44 ppm), Mg (0.21%), Fe (98.33 ppm), Ca (0.09%), Zn (13.92 ppm), Gibberellic Acid (GA) (3.2 ppm), Indole Acetic acid (IAA) (6.4 ppm) and organic carbon (C) (13.92%). Using standard microbial techniques, the microbial analysis confirmed the existence of N-fixers, P-solubilizers, fungi, actinomycetes and bacteria (James and Natalie 2014). Gore et al. (2010) reported similar physicochemical characteristics of Jeevamrutha and for pH (7.07), EC (3.4 dS/m), N (770 ppm), P (166 ppm), K (126 ppm), Zn (4.29 ppm), Fe (282 ppm), Mn (10.7 ppm) and Cu (1.58 ppm). The microbial population of N-fixers, P-solubilizers, bacteria, fungi and actinomycetes were 4.6×10^{2} mL^{-1}, 4.2×10^{2} mL^{-1}, 19.7×10^{5} mL^{-1}, 13.4×10^{3} mL^{-1} and 3.5×10^{3} mL^{-1} (CFU/mL) respectively.

The application of Jeevamrutha with chemical fertilizer did confirm better tomato plant growth (plant height, 130.13 cm; root length, 17.15 cm and dry matter, 7.34 g/plant) in comparison with only chemical fertilizer system (plant height, 124.76 cm; root length, 14.69 cm and dry matter, 5.43 g/plant). On the other hand, Devakumar et al. (2014) reported significantly higher grain yield i.e. 4367 kg/ha with 1000 L/ha Jeevamrutha utilization in comparison with the case that did not involve Jeevamrutha (3519 kg/ha). The authors also validated the outcome in terms of plant yield attributes such as the number of tillers per hill (12.42) and plant grain weight (48.38 g).

Table 1.2: A summary of literature reported Jeevamrutha bio-fertilizer formulations and their fermentation time.

S. no.	Cow dung	Cow urine	Jaggery	Flour	Soil	Water	Fermentation Time	References
1.	10 kg	10 L	2 kg	2 kg	Handful	200 L	3 days	(Palekar 2006)
2.	125 g	125 ml	250 g	25 g	Handful	2.5 L	5 days	(Veeresh, Narayana et al. 2010)
3.	10 Kg	5 L	2 kg	2 kg	Handful	200 L	3 days	(Amareswari and Sujathamma 2014)
4.	10 kg	10 L	2 kg	2 kg	Handful	200 L	20 days	(Devakumar, Shubha et al. 2014)
5.	10 kg	10 L	2 kg	2 kg	Handful	200 L	-	(Anandan, Priya et al. 2016)
6.	10 kg	10 L	2 kg	2 kg	Handful	200 L	10 days	(Reshma 2016)
7.	3 kg	3 L	0.5 kg	0.5 kg	5 g	13 L	7 days	(Ukale, Bhagwat et al. 2016)
8.	10 kg	10 L	2 kg	2 kg	Handful	200 L	5-7 days	(Singh and Lal 2018) (Singh, Ramteke et al. 2018)

Table 1.3: A summary of literature reported liquid Jeevamrutha bio-fertilizer formulations and their nutrient yield.

S. No.	Cow dung (Kg)	Cow urine (L)	Jaggery (Kg)	Gram Flour (kg)	Soil (kg)	Moisture content (L)	pH	P (mg/L)	TKN (%)	Location	References
1.	1	0.1	0.02	0.02	Handful	2	7	166	0.07	Karnataka	Gore and Sreenivasa 2011
2.	1	0.1	0.02	0.02	Handful	2	4.9	1730	1.96	Karnataka	Devakumar et al. 2014
3.	1	1	0.2	0.2	0.1	20	7	177.1	0.03	Tamil Nadu	Anandan et al. 2016

Due to the presence of rich microflora and %NPK content along with other micro-nutrients in the formulation, the Jeevamrutha proved to have a better impact on the growth and yield of crop productivity in the relevant prior art (Devakumar, Subha et al. 2014). Table 1.3 elucidates the literature summary for the liquid Jeevamrutha bio-fertilizer formulations in terms of %TKN and P content.

1.2.2 Statistical Evaluation-based Nutrient Yield of Jeevamrutha Bio-fertilizer System

The available prior art is very limited in terms of statistical evaluation for the nutrient yield of the Jeevamrutha bio-fertilizer system and a detailed discussion has not been delineated. Only three prior art articles outlined the utility of statistical assessment of Jeevamrutha bio-fertilizer for grain yield, dosage, germination rate and microbial growth. These are as follows. A research group deployed the factorial randomized complete block design (FRCBD) statistical tool for the evaluation of the growth and yield of capsicum with liquid organic formulations such as Jeevamrutha, Panchgavya and cow urine. In the conducted studies, 12 treatments were carried out with various levels of Jeevamrutha (J_2) (2 levels with J_1 and J_0), cow urine (C_2) (2 levels with C_1 and C_0) and Panchagavya (P_3) spray (3 levels with P_1, P_2 and P_0). Among considered alternate fertilizer formulations, the Jeevamrutha bio-fertilizer ensured the best combinations of fruit yield (121.20 q/ha after 90 days of treatment (DAT), N-fixers (25.03×10^3 mL^{-1}) and P-solubilizers (31.50×10^3 mL^{-1})). Also, the best N-fixation and P-solubilization were obtained during the monsoon and summer seasons (Boraiah, Devakumar, et al. 2017) (Table 1.4). Further, enhanced N-fixation and P-solubilization have been achieved with the application of cow urine. Thereby, the indispensability of cow urine in organic liquid formulations has been proven.

In another investigation, various growth factors such as plant height, shoot and root length and the number of leaves, branches and flowers in Luffa acutangula were analyzed with a one-way ANOVA approach. The data were represented as Mean±SE and level of significance at 1%. The results revealed better germination (95%), seed vigor and seedling length for Jeevamrutha treated samples concerning the control sample (82.5% seed germination) (Sornalatha, Tamilarasi, et al. 2018). Similarly, FRCBD was applied to study the growth attributes of cowpea for higher grain production. For the case, twelve treatments were replicated thrice with Panchagavya and Jeevamrutha as two factors. Accordingly, the samples were tried at four and three levels respectively. The treatments were either applied directly to the soil or used as a foliar spray. The collected experimental data were subjected to statistical analysis with Fisher's ANOVA method. Accordingly, critical differences were estimated by considering the F-value significance at the 5% level. The findings of the authors reveal that a higher grain yield was achieved for the combination of Panchagavya and Jeevamrutha (1478 kg/ha) in comparison with the control case (601 kg/ha).

Table 1.4: Statistical tools deployed for the evaluation of plant growth factors, grain yield and microbial count of prominent bio-fertilizers and precursors.

S. No.	Bio-fertilizer/ Precursors used	Statistical Tool	Evaluated Parameters	References
1.	Panchagavya and Jeevamrutha	FRCBD	Higher grain yield (1478 kg/ha)	Reshma 2016
2.	Jeevamrutha, Panchagavya and cow urine	FRCBD	121.20 q/ha after 90 days of treatment (DAT), N-fixers (25.03×10^3 mL^{-1}) and P-solubilizers (31.50×10^3 mL^{-1})	Boraiah, Devakumar et al. 2017
3.	Jeevamrutha	One-way ANOVA	*Luffa acutangula* growth patterns (Higher germination, 93%)	Sornalatha, Tamilarasi, et al. 2018

Hence, statistical assessment complements the synergistic effects of Jeevamrutha which favors better grain yield (Reshma 2016). Nevertheless, microbial characterization has been well-established in prior art. However, the effect of nutrient concentration and its statistical evaluation for the plant growth attributes and crop yield is missing and henceforth requires much-needed investigations.

1.2.3 Influence of Jeevamrutha and Other Organic Fertilizers on Plant Growth and Nutrient Content

In addition to the critically enhancing crop yield, Jeevamrutha biodynamic microbial consortiums have been found to improve earthworm density and henceforth vermicompost recovery (95%) (Veeresh, Narayana, et al. 2010). Maximum density and growth rate of earthworms were achieved with Jeevamrutha treated sample (24±2.65 and 0.8±0.00) in comparison with those treated without the bio-fertilizer-based microbial community (19.33±2.89 and 0.64±0.04). Also, higher net biomass (38.67 mg) of *Eudrilus eugeniae* was obtained after 21 days of inoculation with Jeevamrutha-treated samples in comparison to the untreated sample (30.67 mg). In another study, the combination of organic bio-formulations such as Jeevamrutha and Beejamrutha exhibited outstanding characteristics in terms of plant growth and the number of leaves (177.8 cm and 40.6 in 50 days) in comparison with the untreated sample (105.9 cm). Also, the shoot length of the plant was higher (127.8 cm) for the Jeevamrutha+Beejamrutha case (20:2%) in comparison with the other combination case comprising of Jeevamrutha+Beejamrutha+Panchagavya (15:1.5:2%) (122.9 cm) (Sornalatha, Tamilarasi, et al. 2018). The integrated combination of organic biodynamic formulations resulted in higher yield quality, crop productivity and plant disease management strategies due to the presence of several bio-active compounds (Mohanty, Senapati, et al. 2014). Plant growth characteristics such as the number of leaves, leaf index, plant height, number of pods per plant, seed weight per plant and pod length significantly improved with the application of 7.5% Panchagavya and 1000 L/ha of Jeevamrutha (28.60, 1.87, 65.60 cm, 22.16, 9.98, 21.31 cm) (Reshma 2016).

The higher plant growth profile achieved with the application of additional organic bio-formulations could be due to the complementary effect exhibited by rhizobacteria that translocate carbohydrates to develop root nodules per plant and also enhance root volume (Velmurugan and Mahendran 2015, Adak and Kibritci 2016). Also, no pest attacks or diseases were observed in the crop yield (Reshma 2016).

Table 1.5: Literature reported plant growth and nutrient yield data of organic bio-fertilizers.

S. No.	Bio-fertilizer/Precursors	Evaluated parameters	References
1.	Jeevamrutha (500 L/ha), Beejamrutha (200 L/ha) and Panchagavya (500 L/ha)	N:3.36, P:0.35 and K:0.87	Gore and Sreenivasa 2011
2.	7.5% Panchagavya and 1000 L/ha of Jeevamrutha	No. of leaves, 28.60; leaf index, 1.87; plant height, 65.60 cm; no. of pods per plant, 22.16; seed weight per plant, 9.98. No pest attack	Reshma 2016
3.	Jeevamrutha and Beejamrutha	Higher shoot length (127.8 cm) than the untreated group (105.9 cm)	Sornalatha, Tamilarasi, et al. 2018).

Another study was conducted with the combined application of organic bio-formulations such as Jeevamrutha (500 L/ha), Beejamrutha (200 L/ha) and Panchagavya (500

L/ha), organic bio-formulations with chemical fertilizer (150N:100P:60K) and only organic bio-formulations for the evaluation of the NPK content and tomato crop yield. It has been observed that %NPK was maximum for a chemical-treated sample with organic formulations (4.38, 0.43 and 1.03 respectively) followed by the combination of organic bio-formulations (3.36, 0.35 and 0.87 respectively). Thereby, the stimulated response of the growth regulators is due to the presence of available nutrients provided by the biodynamic organic formulations (Gore and Sreenivasa 2011) (Table 1.5).

1.2.4 Nutritional Improvement of Jeevamrutha Bio-Fertilizer with Unconventional Fertilizers

To the best of the knowledge of the thesis author, no work has been addressed to date for the %NPK improvement in Jeevamrutha bio-fertilizer using unconventional resources such as neem cake, pongamia cake, tea waste, water hyacinth and vegetable or fruit peel waste. Hence, the prior art has been presented for the other available similar formulations.

Natural fertilizer such as fruit peel waste has profound nutritional benefits due to the pertinent microbial diversity of the system. These peels contain potassium, manganese, sodium, calcium, iron, carbohydrate, proteins and sugars that contribute to the overall growth and development of the crops as well as improving the soil texture (Arapoglou, Varzakas, et al. 2010, Mohapatra, Mishra, et al. 2010). One such study included fruit peels such as pomegranate, orange, sweet lime and banana peels for in vitro propagation of the fenugreek plant. The nutritive values provided by the fruit peel formulations were better in terms of NPK in mg/g (4.7, 3.5 and 2.1) in comparison with the soil sample (1, 2.3 and 1.3) and were suitable for plant growth (Mercy, Mubsira et al. 2014). Similar work has been carried out using sweet lime and banana peel to analyze the growth characteristics of coriander and mint plants along with alterations in the soil composition. N, K and %C for the peel powder were 28, 299 and 0.97 mg/kg in comparison with the control sample (6.72, 70 and 0.54 mg/kg respectively). A maximum plant length of 15.3 cm was achieved with a mixture of these fruit peels in comparison to the soil sample without fruit peels (9.8 cm) (Adrija and Navni 2018).

Panwar (2015) conducted a waste management study on the physicochemical characteristics and soil fertility improvement through the addition of banana peel to the soil. The authors found that the nutrition of the soil can be increased in terms of phosphate (2.34 µg/mL), ammonia (3.7 µg/ml) and sulphate content (11.9 µg/ml). All these were evaluated for the mentioned parameters with a spectrophotometric method (Panwar, 2015). Similarly, a mixture of fruit peels such as orange (pH 5.8), sweet lime (pH 5.4), pomegranate (pH 4.8) and banana (pH 7.9) peels was investigated as a fertilizer for better N content. The acidic peel powder (9.1 mg/g) performed better than the alkaline peel powder (banana 5.5 mg/g) to enhance N content (Jariwala and Syed 2017). Khairnair and Nair (2019) studied the efficiency of fruit peel and eggshell on the nutritional content of the fertilizer. The N and C content for the banana peel, sweet lime peel and eggshell powder were 0.79 and 36.2%, 1.19 and 38% and 0.39 and 12.6% respectively (Khairnar and Nair 2019). Hence, fruit peel waste is a good alternative for fertilizer development with no additional cost.

Another study incorporated compost, vermicompost and neem cake with bio-fertilizer (Azotobacter) and applied the mixture in the field at a rate of 25 kg/ha. The maximum %N, %C and %ash were achieved for neem cake (1.96, 43.46 and 74.93 respectively) in comparison to the control group (0.5, 21.17 and 36.5 respectively). Hence, neem cake could be used as a low-cost resource to improve nutrient biodynamic formulations (Bhalshakar 2020). Milk tea waste along with NPK fertilizer was also studied to observe the growth patterns in wheat and sandy soil biochemical characteristics. A higher %NPK uptake was witnessed for a combination of tea waste and chemical fertilizer. For the case, the root and shoot enhanced by 65.7, 34.3 and 47.4% and 68.9, 58.3 and 57.1% respectively in comparison with the control group without any amendment. Also, soil enzymatic activities such as ß-glucosidase (81.6%), urease (95.2%)

and dehydrogenase (97.2%) improved (Sial, Liu, et al. 2019) (Table 1.6). In another brief study, waste tea powder was analyzed as compost and in terms of its physicochemical parameters. These referred to pH (7), EC (7.8 dS/m), sulphate (0.5%), chloride (1%), P (0.05%), Ca (1.3%), organic matter (45%), and Mg (0.58%). Also, the plants exhibited rapid growth patterns in terms of germination rate (90%), leaf index (4 cm^2), leaf density (120) and plant height (18 cm) (Gurav and Sinalkar 2013). Thereby, tea waste has been proven to be a potentially cheap substrate for the improvement of soil nutrition characteristics.

Table 1.6: Literature nutrient data summary of commercial and rural precursors.

S.No.	Precursors	TKN (%)	AN (mg/L)	P (%)	References
1.	Vermicompost (VC) (1:4)	1.5	-	0.3	Rosi and Thatheyus, 2018
2.	Water hyacinth (WH) (200 kg/ha)	0.00016 (Nitrate)	-	0.003	Andika at al. 2016
3.	Neem seed cake (NC) (60 g soil+15%urea and 6mg neem cake)	5.5 *Enhanced N-fixers*	-	0.55	Elnasikh et al. 2011
4.	Milk Tea waste (TW) (5%)	3.7	-	0.42	Sial et al. 2019
5.	Sugarcane juice and Panchagavya	-	-	(Enhanced microbial count)	Radha and Rao, 2014
6.	Rice waste, cow dung and timber sawdust	1.9	-	0.6	Oladejo and Fasan, 2015

In another study, water hyacinth, a freshwater plant, has been analyzed to be relevant for the preparation of organic liquid fertilizer. The chemical composition of water hyacinth is sulphate (56 mg/L), lead (1.66 mg/L), Cu (1.21 mg/L), Mg (220 mg/L), Ca (310 mg/L), nitrate (2.06 mg/L), phosphate (58 mg/L) and chloride (44 mg/L). Thereby, leaf area, the number of leaves and plant height improved with the formulation. Hence, the production of low-cost rurally available precursors is necessary to enhance their utilization as a raw resource for the improvement in the nutritional composition of the organic bio-fertilizers (Andika, Ogada, et al. 2016).

On the other hand, the organic liquid bio-formulation Panchagvaya was modified with sugarcane juice extracts. Thereby, growth-promoting bacteria in the soil were evaluated. The identified strains such as *Bacillus safensis, B. subtilis, B. cereus and B. licheniformis* were capable to produce ammonia and indole acetic acid. Therefore, sugarcane juice is an efficient precursor to improve the microbial strength of the bio-formulations (Radha and Rao 2014). Also, the utility of rice waste, sawdust and cow dung as a fertilizing resource has been well-explored in another prior art (Oladejo et al. 2015). Henceforth, the incorporation of starch water (a waste from cooked rice avenues) into the bio-fertilizer to promote the nutrient benefits of the soil will be a novel and interesting proposition.

1.2.5 Time-dependent Morphological Characteristics of Crops Tested with Jeevamrutha Integrated Organic Fertilizers

Rameeza et al. (2016) studied the influence of aging on organic liquid formulations. The authors evaluated the shelf life of the formulation and the aging effect on the biochemical characteristics of the system. To do so, 24 weeks old Jeevamrutha was stored and alterations in color, pH and odor were observed. It was found that with increasing storage time, the preparation became a dark-colored alkaline system with a foul odor. The EC varied from 0.23-2.25 dS/m for the 20 weeks' formulation. For this time frame, N, P and K content was in the range of 0.3-0.8%, 0.03-0.26%, and 0.05-0.1% respectively. Micro-nutrients such as Ca, Mg, S, Fe, Zn and Mn varied from 24.1-66.4 ppm, 50.33-62.83 ppm, 81.62-280.11 ppm, 53.56-104.16 ppm, 0.4-7.5 ppm and 1.7-26.9 ppm respectively. The highest microbial population of bacteria, actinomycetes and fungi in CFU/mL were analyzed to vary as 243.33-123.33×10^6 mL^-

^{1}, 32.66-102×10^{5} mL^{-1} and 1.33-44×10^{4} mL^{-1} respectively. Also, coconut water was used as an additional precursor to modify the bio-formulation. Nevertheless, a time-dependent study for Jeevamrutha liquid formulation has been assessed. However, the impact of macro- and micro-nutrients on the plant growth profile and microbial growth patterns has not been targeted by the authors (Rameeza 2014).

Another study explored the influence of liquid bio-fertilizer such as Jeevamrutha, Beejamrutha and Panchagavya on the qualitative and quantitative analysis of bottle gourd. Morphological characteristics of the plant were evaluated in terms of flower count, plant height and root and shoot length. The plant height, shoot and root length and the number of leaves, branches and flowers were highest (162.1 cm, 122.9 and 56.3, 52.8, 10.8 and 49.6 respectively) for the combination of the liquid formulations case (1.5% Panchagavya, 1% Beejamrutha and 10% Jeevamrutha) in comparison with the control sample without fertilizer (76.1 cm, 50.8 and 25.3, 25.8, 2.4 and 16.4 respectively). Similarly, yield parameters such as fruit weight, length and width were analyzed to be better outcomes for the combination of the liquid fertilizers (2.57 kg, 27.1 and 23.1 respectively) in comparison with the control sample (1.01 kg, 11.1 and 10 respectively). Phytonutrient characterization revealed that these formulations have been resourceful in terms of vitamin C (4.12 mg/100g), antioxidant properties (354.12 mg/g), ß-carotene (3.58 mg/100g) and phenol content (85.73 mg/100g). Further investigations are needed to emphasize the agricultural practices that focus on the ability of organic farming techniques to improve the nutritional value of the crops and soil health (Sakthivel, Dhanapal, et al. 2022). Table 1.7 summarizes the literature comparison of different integrated fertilizer systems and their role in influencing plant growth and nutritional properties.

The integration of bio-fertilizer and vermicompost on the growth of tomato plants with the pot experiment-based methodology was addressed. The results affirmed considerable improvement in mineral composition in the plants through N-fixation and fruit productivity.

The plant height, length of roots and the number of leaves and branches were significantly higher for the integrated system (119 cm, 50.3 cm, 17.3 and 5 respectively) in comparison to the control-based treatment case (60.3 cm, 20 cm, 7.6 and 2 respectively) (Gopinathan and Prakash 2014).

Shozeb et al. (2013) investigated the growth and productivity of *Glycine max* and *Vigna Mungo* plants using chemical fertilizer, vermicompost and bio-fertilizer. The pot culture was evaluated for each treatment case and a combination of these formulations. To do so, vermicompost and bio-fertilizer were mixed (1:4 ratio) and the combination was thereafter altered to achieve a ratio of 0.5:1:1:4 for chemical fertilizer, vermicompost and bio-fertilizer respectively. The results show that for *Glycine max*, carbohydrate content (0.73 mg/g) was higher for the case of the integrated formulation (chemical fertilizer, vermicompost and bio-fertilizer). On the other hand, phenol (0.95 mg/g) and protein (3.1 µg/mL) content were maximum in the bio-fertilizer-integrated-vermicompost treated soil. However, a higher seed germination rate (82.85% and 88% respectively) in bio-fertilizer and vermicompost-treated soil were observed for *Glycine max* and *Vigna Mungo* respectively (Shozeb and Aruna 2013). Hence, it can be well depicted that the high nutritional content of grains can be achieved with an integrated organic farming system. This system customizes to minimize the application of chemical fertilizers that have an adverse effect on soil health and fertility rate. In short, the integrated systems ascertain the strong application potential of these organic bio-formulations in terms of improved cost analytics and economic feasibility.

Table 1.7: Literature data summary of nutrient and morphological characteristics of alternate integrated fertilizer systems.

Model Plant	Farming System	Nutritional Characteristics	Morphological/Yield factors	References
Phaseolus vulgaris	Azotobacter, Phosphate solubilizing bacteria, Rhizobium, Azospirillum and Vesicular-arbuscular mycorrhizae	Moisture (%)-12 Total sugar (%)-6.35 Protein (%)-18.9	Number of pods-23 Pod length (cm)-11.22 Pod weight(g)-4.64	Meena et al., 2018
Phaseolus vulgaris	NPK+ Rhizobium+ Micro-nutrients	-	Pod length (cm)-3.42 Pod weight (g)-6.7	Jena et al., 2020
Phaseolus vulgaris	Farm Yard Manure, Vermicompost, poultry manure and neem seed cake	Protein content (%)-20.10	Plant height (cm)-33.63 Number of branches/plant-5.40 Dry matter/plant (g)-16.29 Number of pods/plant-15.27	Sondawale et al., 2023

1.3 Research Gaps

1.3.1 Seasonal Influence on the Compositional Optimality and Nutrient Yield of Jeevamrutha Bio-fertilizer Formulations

Seasonal aspects for field application-based investigation were carried out in capsicum cultivation during the summer and monsoon seasons. It was found that maximum microbial enumeration existed during monsoon season for N-fixers and P-solubilizers (24.49×10^3 mL^{-1} and 30.50×10^3 mL^{-1} (CFU/mL) respectively) (Boraiah, Devakumar, et al. 2017). However, the authors did not address the major nutritional characteristics of the bio-formulation with respect to the microbial alterations for both seasons.

Similarly, a field study involved the yield assessment of finger millet during monsoon seasons. The authors deployed 1000 L/ha Jeevamrutha bio-fertilizer formulation and achieved a 19.14% higher grain yield (Ashokh Aravind, Senthil Kumar, et al. 2020). Gore et al. (2010) conducted a field study on tomato and nutrient yield in sterilized soil during the monsoon season. However, the soil's nutritional characteristics have not been thoroughly evaluated prior to the field application. The authors did not delineate the role of microbes in enhanced crop productivity. The beneficial role of the microbes during the seasonal variations for nutrient and crop yield has not been addressed, despite considering its efficiency in terms of %NPK and other essential micro-nutrients. During the harsh winter period, the depletion of microbial count is evident with a decline in the microbial growth phase (Vigneron, Lovejoy, et al. 2019). Henceforth, a linkage between microbial count and nutrient yield shall be critically assessed.

Considering the above-mentioned important lacunae and the influence of microbial consortium during summer and winter seasons in Assam, the primary gap in the prior art can be addressed through a mature understanding of the role of the microbes in nutrient uptake and soil fertility enhancement. To date, liquid Jeevamrutha bio-fertilizer has been widely explored in terms of physicochemical constituents, microbial population and crop yields for both summer and monsoon seasons. However, the seasonal influence of solid bio-fertilizers has not been comprehensively studied to date. These lacunae can also be better addressed through time-based studies on the nutrient yield in terms of AN, P and TKN and associated microbial alterations. The influence of seasonal variations on nutrient yield and microbial alterations also depends on the region of interest. Assam has wider climatic alterations in terms of seasonal influence. The region's emphasis on organic farming prompts the influence of seasons in terms of the efficacy of bio-fertilizer formulations for optimal nutrient yield, consortium constitution and field application.

1.3.2 Screening, Optimization and Seasonal Variation of the Nutrient Yield of Jeevamrutha Bio-fertilizer Systems

From the available prior art literature, it can be analyzed that the liquid Jeevamrutha bio-fertilizer demonstrated its extensive utility for the field application of various crop yields such as tomato, finger millet and capsicum. One such field study was carried out for capsicum crops using FRCBD and three combinations of the bio-formulations. Thereby, capsicum yield, N-fixers and P-solubilizers were analyzed. The statistical analysis for these treatments confirmed enhanced microbial activity and better plant growth factors (Boraiah, Devakumar, et al. 2017). However, the authors did not target the optimality of the best compositional set and their critical response variability in terms of physicochemical constituents and nutritional factors.

Similarly, the influence of Jeevamrutha on the cowpea growth was statistically interpreted with Fisher's statistical method of ANOVA (Analysis of Variance) (Sutar, Sujith, et al. 2019). However, an optimum time-dependent study in terms of microbial alterations, physicochemical and nutrient content has not been addressed by the authors. Such investigations are needed to achieve better outcomes in terms of nutrient yield, plant productivity and soil fertility status. In this regard, screening and optimization of the associated factors for the best compositional set in the Jeevamrutha bio-fertilizer can be authenticated and converged for a suitable optimal combination of the process parameters.

A preliminary screening study is important to reduce the number of experimental suites that converge upon the influence of critical factors on nutrient yield. For such purposes, the Plackett-Burman design (PBD) as a statistical tool has been well-established to screen and identify critical responses through nominal experimental suites (Plackett and Burman 1946). The optimization with the statistical tool aids in the evaluation of the process variables for their fitness and thereby reduces energy, time and resource inputs. Also, Central Composite Design (CCD) proved to be an efficient statistical tool for the reduction in the number of experimental runs and classification of the sensitivity of the significant factors related to the optimal compositional set and chosen degrees of freedom (Chaudhari and Shirkhedkar 2020). Considering these lacunae, a second research gap has been identified as the compositional fitness or optimality of the solid Jeevamrutha bio-fertilizer formulation for their maximum nutrient responses and supplementary parameters.

1.3.3 Integration of Commercial and Rural Precursors with Jeevamrutha Bio-fertilizer System

Complex agricultural fertilizer utilization practices prompt the utility of mixed fertilizer systems and integrated farming methods. Thereby, the advantage of any fertilizer to enhance soil health and plant growth can be further substantiated through mixed formulations. In rural India, mixed fertilizer formulations have been studied with bio-fertilizers integrated with chemical fertilizers, and other organic fertilizers such as vermicompost. Considering the available prior art and relevance of commercial and rural resources that can supplement Jeevamrutha bio-fertilizer in Assam, the following resources have to be considered. Firstly, the region has high tea consumption and tea waste can be generated from both eateries and tea processing industries. Thereby, tea waste can be effectively targeted for its modification with Jeevamrutha bio-fertilizer. Secondly, the region has large water bodies and inadequate water quality management in the rural environment. Thereby, water hyacinths can be abundantly found in the region. The same can be explored for modification with the bio-fertilizer. Thirdly, vermicompost can be prepared but not in large quantities in comparison to the quantities prepared as Jeevamrutha bio-fertilizer. Hence, it can be well-explored for integration as a commercial source. The same is the case for neem cake. Hence, two commercial resources namely vermicompost and neem cake and two rural resources namely tea waste and water hyacinth can be targeted for feasible modification with solid Jeevamrutha bio-fertilizer in Assam.

In the prior art, the integration of vermicompost and Jeevamrutha has been analyzed due to its excellent nutritional constituents. The Jeevamrutha-integrated-vermicompost formulation enhanced the biological and physicochemical properties of the soil in terms of their qualitative efficiency in the state of Karnataka, India (Veeresh and Narayana 2013). Hence, it would be further interesting to target the modification of solid Jeevamrutha bio-fertilizer with vermicompost and thereby evaluate the associated nutritional and microbial alterations in Assam.

Also, in an earlier investigation, neem cake was integrated with other microbial bio-fertilizers such as *Azospirillum* and *Azotobacter*. The investigations confirmed better plant growth dynamics in terms of their fresh and dry weight (Bhalshakar 2020). However, the authors did not assess the relevance of both nutritional and microbial growth characteristics for the bio-fertilizers. Hence, the integration of neem cake with Jeevamrutha can serve as a viable approach to providing essential nutrients for plant growth. Thereby, further improvement in soil fertility can be assessed and inferred.

Similarly, rurally available precursor such as tea waste compost has been assessed for their physicochemical analysis and associated crop yield alterations. The compost development process has a cost-efficacy advantage and this can have a remarkable effect in terms of its integration with any bio-fertilizer (Gurav and Sinalkar 2013). To date, no literature addressed the incorporation of tea waste with any bio-fertilizer. Hence, tea waste-modified Jeevamrutha bio-formulation can emphasize its bio-pesticidal property, betterment in soil biochemical factors and cost-effectiveness. Thereby, the conducted research can be beneficial to minimize the long-term usage of chemical fertilizers (Andika, Ogada, et al. 2016).

Also, water hyacinth, a freely available freshwater plant contains about 0.04% N and 0.06% P in its fresh form. The available state-of-the-art confirms its utility to produce liquid organic fertilizer. To date, no literature reported on the integration of Jeevamrutha and water hyacinth. Hence, it would be interesting to analyze the nutrient yield improvement in terms of

AN, TKN and P, associated physicochemical properties alterations and microbial growth patterns for the water hyacinth-modified Jeevamrutha bio-fertilizer system.

The production and incorporation of these rural resources into the Jeevamrutha bio-fertilizer development will reduce the associated cost and can facilitate biodiversity conservation along with a reduction in the effective long-term usage of chemical fertilizers. Thus, addressing such lacunae for summer and winter seasons, the production and development of integrated bio-fertilizer systems should be encouraged for effective waste management strategies and thereby foster the betterment of the economy and rural livelihood.

1.3.4 Alternate Carbon Source-based Jeevamrutha Bio-Fertilizer Formulations

Jaggery serves as one of the important components and as an energy source during the preparation of Jeevamrutha formulation (Vijayendra, Bansal, et al. 2001). In the prior art, liquid organic manure Panchagavya was modified with sugarcane juice. Thereby, beneficial effects have been confirmed in terms of microbial counts, plant growth attributes and improved maize crop yield (Radha and Rao 2014). However, matured information in terms of the physicochemical, microbial characteristics and %NPK of the bio-formulation has not been addressed for the Jeevamrutha bio-fertilizer.

With its alkaline nature, sugarcane juice instead of jaggery can foster abundant alterations in the iron, potassium and magnesium content of the bio-fertilizer. In other words, the development and modification of nutrient-rich Jeevamrutha bio-formulation can be targeted for even higher combinations of nutrient yield and soil fertility and thereby enable the reduction in inorganic fertilizer consumption in rural India. Also, it shall be noted that sugarcane juice may provide better moisture content during its preparation process. For this reason, it will activate microbial diversity in the bio-formulation to promote its nutritional properties.

On the other hand, starch rice water (water obtained after cooking rice in the traditional method) has no additional chemicals or additives and provides essential minerals, N, P and K to fertilize plants. It has high glucose content that could be utilized by plants for growth and development. Hence, starch water will be beneficial as an energy source absorbed by the plant roots and leaves. In summary, it would be an interesting topic to evaluate the scope of using sugarcane juice and starch rice water as a replacement for jaggery-based solid Jeevamrutha to achieve cost-effective, nutrient-rich and sustainable organic bio-fertilizer in Assam.

1.3.5 Plant Growth Characteristics of Jeevamrutha Integrated Farming System

Several organic bio-formulations on their own or in combination with other liquid and bulky manures ensured higher growth factors such as plant height, number of pods per plant, number of fruits and flowers, leaf area, pod number, length and weight in *Phaseolus vulgaris*. Conventional resources and vermicompost-enriched-biofertilizers exhibited remarkable growth characteristics in tomato and groundnut plants (Gopinathan and Prakash 2014, Kumar et al. 2014). Vermicompost (with 2-3% N, 1.55-2.25% P, micro-nutrients and humus) stabilizes organic constituents and improves oxygen availability, soil porosity and water retention capacity (Gore and Sreenivasa 2011). However, none of the previous literature investigated microbial diversity of the vermicompost-integrated-bio-fertilizer formulation, associated nutritional properties of the fruit and the moisture content of the soil after its application.

Such studies in the Integrated Farming System (IFS) are needed due to its higher crop productivity and budget-optimized strategies being widely accepted by farmers in rural India. Considering this, the IFS could provide superior results and a better understanding of various growth attributes during its developmental phase. Additionally, this farming system ensures an enhancement in biological and physicochemical characteristics that fosters the production of nutritional products for human well-being through the utilization of economically sustainable precursors in the agricultural zones of rural India with a sub-tropical climate.

1.4 Objectives of the Ph.D. thesis

The overall objective of the thesis is to develop a nutrient-rich Jeevamrutha bio-fertilizer for its wider applicability in rural sectors of Assam being characterized by highly diverse summer and winter climatic conditions. To obtain better shelf life of the developed bio-formulations, solid Jeevamrutha bio-fertilizer has been targeted. A pertinent time-dependent study needs to be targeted for screening and optimization of the optimal compositional set prevalent for further improvement using natural resources. Further, an integrated farming technique using vermicompost and solid Jeevamrutha bio-fertilizer shall be deployed to achieve higher growth characteristics in *Phaseolus vulgaris*, nutritional content and presence of microbial population. Also, the evaluation of TKN in a few samples will be replaced by AN owing to its rigorous analytical procedure for faster assessment. AN has a key role in plant metabolism-related features and hence, its significance is noteworthy. Considering these broad prospectives, the objectives of the thesis have been set as follows:

1. Seasonal variation of solid Jeevamrutha bio-fertilizer in terms of ammonium nitrogen, total Kjeldahl nitrogen, phosphate and microbial alterations in Assam and compare these with the reported Palekar's compositional optimality. Thereby, screen and optimize Jeevamrutha constitution in terms of ammonium nitrogen (AN), total Kjeldahl nitrogen (TKN), phosphate (P), physicochemical constituents and microbial alterations using Plackett-Burman Design (PBD) and Central Composite Design (CCD) based statistical methodologies in Assam.
2. Evaluate the influence of further modified Jeevamrutha bio-fertilizer formulation in terms of ammonium nitrogen, total Kjeldahl nitrogen, phosphate, physicochemical constituents and microbial alterations using vermicompost, neem cake, tea waste, and water hyacinth precursors for summer and winter seasons in Assam.

3. Replace jaggery with sugarcane juice and starch rice water in the further modified bio-fertilizer formulation and evaluate the altered bio-fertilizer nutritional and microbial count during the summer and winter seasons.
4. To investigate the Jeevamrutha-integrated-vermicompost farming system for its biological, physicochemical influence and plant growth characteristics.

1.5 Organization of the Thesis

As outlined in section 1.3 of the thesis, a significant scope does exist to address and conduct further research in the field of modified Jeevamrutha formulation using inexpensive and rurally available precursors. The objectives set for the Ph.D. thesis provide deeper insights into this central objective of the thesis. In this section, a summary of all subsequent chapters of the thesis is presented in the following paragraphs.

Chapter 2 details upon materials and methods that were deployed to carry out the Ph.D. thesis. These are (a) materials required to perform the preliminary thesis work, (b) procedures applied to evaluate the nutrient composition of the Jeevamrutha bio-fertilizer formulations based on spectrophotometric methods and TKN analysis, (c) methodology of the statistical tools deployed to screen and optimize the compositional fitness of the bio-fertilizer, (d) analytical methodology adopted to examine the micro-nutrient content in the bio-formulation, (e) physicochemical characterization and nutrient analyses of the fruit sample obtained from pot experiments, and (f) phytotoxicity assay of the optimized compositional set for its suitability during soil application.

Chapter 3 addresses Assam's seasonal effect (summer and winter) on the Palekar's unmodified Jeevamrutha solid bio-fertilizer formulation in terms of nutrient yield as AN, TKN and P content. Depending on the temperature range, humidity and other physical factors, these experimental findings can be validated for the effect of microbial consortium on the overall bio-fertilizer for summer and winter seasons. Further, this study will provide an outlook towards the sensitivity of the associated precursors in the bio-formulation to identify, screen and optimize the modified Jeevamrutha bio-fertilizer with rational and scientific methodology. The relevance of significant factors identified and optimized using Plackett-Burman Design (PBD) and Central Composite Design (CCD) will contribute to the critical response variables and justify the modest precursor usage to develop a sustainable, low-cost and rapid organic bio-fertilizer with higher nutritional characteristics. Hence, the developed empirical model with the chosen reference composition could be further investigated with or without modification using low-cost natural resources for higher crop productivity and better soil texture.

Chapter 4 discusses the role of low-cost rural precursors such as neem cake, vermicompost, tea waste and water hyacinth in the further nutritional improvement of the optimized Jeevamrutha bio-fertilizer. The conducted studies reflect upon the influence of the additional substrates on microbial diversity as well as nutritional properties of the bio-fertilizer and thereby reduce the cost associated with the organic farming system and wider utility of chemical fertilizers and harmful pesticides.

Chapter 5 elaborates upon the effect of sugarcane juice and starch rice water by replacing jaggery for its efficacy on the nutrient characterization, microbial count and physicochemical properties of the developed optimized Jeevamrutha bio-fertilizer formulations during summer and winter seasons. Thereafter, a comparison study with jaggery has been conducted to observe the alterations of the readily available rural substrates. An interesting feature of the conducted work is to examine the criticality of the carbon source in terms of nutrient outcome, expenditure and possible scope for the replacement of jaggery with other inexpensive carbon sources.

Chapter 6 presents the findings of the Jeevamrutha-integrated-vermicompost farming system. The chapter describes the growth process of *Phaseolus vulgaris* (common bean) to target the benefits associated with organic cultivation. Also, the fruit samples collected after 45 days were carefully evaluated to identify the case with higher nutritional characteristics such as carbohydrate, soluble protein and vitamin C content.

Chapter 7 summarizes key conclusions being drawn from the dissertation. Thereafter, a brief overview of future research directives has been presented.

Chapter 2:
Materials and Methods

Materials and Methods

This chapter summarizes the materials adopted and methods followed in the Ph.D. thesis. These have been outlined as (a) materials necessary to carry out the thesis work, (b) procedures associated with the preparation of solid Jeevamrutha bio-fertilizer, (c) methodologies involving the screening and compositional optimization of the experimental factors using Plackett-Burman Design (PBD) and Central Composite Design (CCD), (d) procedure adopted for the inclusion of other rural precursors into the optimized Jeevamrutha formulation, (e) procedures involved in the replacement of jaggery in the optimally modified Jeevamrutha formulation with either sugarcane juice or starch rice water as the carbon source, and (f) analytical methodology deployed to evaluate the physicochemical, nutritional and biological assessment of the bio-fertilizer formulation along with the fruit yield.

2.1 Screening and Optimization of Jeevamrutha Bio-fertilizer Formulations in Assam

2.1.1 Chemicals, Reagents, Formulation Precursors and Apparatus

Several chemicals were procured to determine the nutritional characteristics of bio-fertilizer such as ammonium nitrogen (AN), phosphate (P), total Kjeldahl nitrogen (TKN) and other physicochemical characteristics. Analytical grade (> 99.9% purity) chemicals such as disodium hydrogen phosphate dihydrate, sodium-potassium tartrate, sodium salicylate, sodium nitroprusside solution, sodium hydroxide, ammonium molybdate, hydrazine sulphate, Anthrone reagent, Bradford's reagent along with nutrient agar, potato dextrose agar, starch M-protein agar, Jensen's medium and Pikovskaya's agar were obtained from HiMedia, India. Whatman filter paper no. 42 was purchased from GE Healthcare Life Sciences, UK. Sodium hypochlorite was procured from Merck, USA and hydrochloric acid, nitric acid, sulphuric acid, oxalic acid and perchloric acid were obtained from Finar, India.

Fresh cow dung and cow urine were procured from a cattle farm near Indian Institute of Technology Guwahati, Assam, India. Jaggery and gram flour were purchased from a local market near the market complex of IIT Guwahati. The soil was dug from a shady place inside the IIT Guwahati campus.

General apparatus and analytical instruments used in this study include a centrifuge, hot-air oven, desiccator, weighing balance, magnetic stirrer with hot plate, stainless steel water bath, vortex shaker, pH meter (Mettler Toledo, UK), conductivity meter (VSI Electronics, India), TOC analyzer (Multi N/C 2100S, Analytikjena, Germany), UV-Vis spectrophotometer (Shimadzu, UV-1900), Kjeldahl nitrogen analyzer (VELP SCIENTIFICA, UDK129), Flame photometer (FPM 128, Systronics, India) and High-Performance Liquid Chromatography (Shimadzu, RID-20A). The photographic images of the types of equipment used in the studies are depicted in the Appendix B section of the Ph.D. thesis.

The climatic parameters (Temperature, Rainfall, Relative Humidity and Solar Radiation) in Guwahati, Assam were collected from Climate Data Service Portal for a period of ten years (1992-2022) respectively. For summer (May-October) and winter (December-March) months, the average temperature, rainfall and relative humidity in Guwahati varies as 31.9-32.1°C and 23.9-30.5°C; 313.3-107.6 and 4.5-51.3 mm and 83-88.2% and 79.8-75.9% respectively. On the other hand, solar radiation varies from 3-6 kWh/m^2/day for both seasons in Guwahati.

2.1.2 Characterization of Jeevamrutha Bio-fertilizer System Precursors

Raw ingredients such as cow dung, cow urine, jaggery, gram flour and soil used for the preparation of Jeevamrutha bio-fertilizer were subjected to initial characterization for their pH, %TKN, %P and %K content. The %TKN was estimated using a Kjeldahl nitrogen analyzer, %P through digestion followed by UV-Vis spectrophotometric method, %K with flame photometer and pH using a pH meter (Worth 1985, Baethgen and Alley 1989, Nahm 2003, Adelowo, Oladeji et al. 2016).

2.1.3 Preparation of Jeevamrutha Bio-fertilizer System

The solid Jeevamrutha bio-fertilizer formulation was prepared as per Palekar's methodology with a fixed choice of 1 kg cow dung but with a reduced cow urine constitution (0.02 L), jaggery (0.02 kg), gram flour (0.02 kg) and soil (handful). The bio-fertilizer mixture was thoroughly monitored for 0-14 days with continuous stirring at least twice a day with a sterilized spatula. A plastic vessel was used for its storage and was covered with a muslin cloth to avoid the formation of maggots and flies.

2.1.4 Sugar and Protein Content Characterization of Jaggery and Gram Flour Precursors

The carbon and nitrogen sources such as jaggery and gram flour in Jeevamrutha bio-fertilizer were characterized for sugar and soluble protein content using HPLC-RID and Bradford's method. For HPLC-RID, 10 g of each sample was dissolved in 200 mL of distilled water and kept in a water bath at 60°C for 15 minutes. The solution was cooled and filtered. For the sample quantification, the obtained peaks were identified by comparing them with retention times (Bonjoch and Tamayo 2001, Gangola, Jaiswal et al. 2014). Aminex HPX-87C column (Bio-Rad) was used for the sugar estimation.

In the jaggery, sugars such as sucrose, glucose and fructose were quantified. The soluble protein content was determined using Bradford's method by dissolving 100 mg of gram flour with 100 mL of distilled water. 1 mL of this solution was mixed with 5 mL of

Bradford's reagent and the absorbance was measured at 595 nm with a UV-Vis spectrophotometer and BSA standard (Bradford 1976, Okonwu and Enyinnaya 2016). A calibration curve has been illustrated in the Appendix A section of the Ph.D. thesis.

2.1.5 Statistical Analysis

Plackett-Burman Design (PBD)-based screening and Central Composite Design (CCD)-based optimization studies were applied for the statistical evaluation of the associated sensitive substrates in the Jeevamrutha bio-fertilizer for their significance. Thereby, the achieved responses have been evaluated for their confidence levels and subsequent mitigation of errors beyond the acceptable range in experimental investigations was ensured.

2.1.5.1 Plackett-Burman Design (PBD)-based Screening of Precursors in Jeevamrutha Bio-fertilizer System

Based on the PBD-based experimental design, the concentration ranges of other precursors (0.02-0.04 and 0.005-0.04 kg of jaggery; 0.02-0.04 and 0.005-0.04 kg of gram flour; 0.02-0.04 and 0.005-0.04 L of moisture; 0.2-0.4 and 0.05-0.4 kg of soil and 3-6 and 3-8 pH) for lower (S_L) and higher (S_H) parametric range were chosen. The alternate parametric range was chosen to understand the pertinent sensitivities of the parametric range. Thus, the best-screened significant factors identified by the PBD-based study were further accounted for CCD-based statistical optimization tool.

Thus, in due course of PBD-based modified formulations being adopted in our work, a constitution of cow dung (1 kg), cow urine (0.02 L), pH (8) and moisture content (0.04 L) was chosen as the fixed choice of formulation parameters. The bio-fertilizer mixture for each sample was thoroughly monitored for 9-12 days with continuous stirring at least twice a day. The vessel was covered with a muslin cloth to avoid the formation of maggots.

2.1.5.2 Evaluation of Ammonium Nitrogen (AN) and Phosphate (P)

Conventionally, the TKN method involving three hierarchical steps of digestion, distillation and titration is time-consuming and laborious for a vast number of runs targeted in the thesis. As an alternative, the analytical method for the AN estimation is rigorous and involves digestion followed by spectrophotometric analysis. Nonetheless, for a few samples, TKN estimation using a total Kjeldahl nitrogen analyzer has been considered in this work due to its high relevance in the nutrient characterization of the bio-fertilizers. Similarly, phosphate estimation using UV-VIS spectrophotometric method is fairly easy due to the elimination of time-consuming extraction processes and sophisticated instrumentation.

For the determination of AN nutrient yield, the fresh cow-dung sample was first digested at 150°C for 2 h with a hot plate. Thereafter, the 3 mL of digested sample was cooled down and filtered through Whatman paper no. 42 to remove the suspended solids. A working buffer was prepared by dissolving 1.78 g of $Na_2HPO_4.2H_2O$ (0.1 M), 10.8 g of 50% NaOH in 100 ml of distilled water and 5 g of sodium-potassium tartrate (5%). Also, sodium salicylate (15%) and sodium nitroprusside solution (0.03%) were mixed with 100 ml of distilled water and stored in a dark brown bottle for further use. Additionally, the diluent solution was prepared by adding 2.2 g of catalyst (1.1 M) to 6.1 ml H_2SO_4 in 100 ml of distilled water. Sodium hypochlorite solution was freshly prepared for each experimental run. Hence, the maximum absorbance for AN content was observed at a wavelength range of 650 nm using a UV-Vis spectrophotometer and a calibration curve was prepared for concentration versus absorbance in the range of 0-6 ppm (Walter 1961, Baethgen and Alley 1989). Thereby, AN is represented as mg/L of the entity in the bio-fertilizer digested solution (1 g of Jeevamrutha bio-fertilizer digested in the 3 mL of concentrated H_2SO_4 solution).

Similarly, for P nutrient content, 2 g of the fresh cow dung sample was added to an acid mixture that contains HCl and HNO_3 in a ratio of 1:3. The mixture was shaken vigorously for 30 minutes with a magnetic stirrer. The digestion was carried out for 6 h 30 minutes at 150°C. The digested sample was then cooled and filtered using Whatman filter no. 42. The filtrate was finally mixed with ammonium molybdate and hydrazine sulphate. Thereby, the mixture was kept in a water bath for 30 minutes to turn the sample solutions from yellow to blue. Hence, maximum absorbance was measured at a wavelength range of 880 nm with a UV-Vis spectrophotometer. Thereafter, a calibration curve was plotted for concentration versus absorbance in the 0-5 ppm concentration range (Adelowo, Oladeji et al. 2016). The calibration curves for AN and P nutrient yield are illustrated in the Appendix A section of the Ph.D. thesis.

2.1.5.3 PBD-based Experimental Design for Jeevamrutha Bio-fertilizer System

A set of 12 experimental runs with the Plackett-Burman design were carried out to screen out factors associated with alternate modified Jeevamrutha formulations in a specified parametric range. Minitab 16.0 software (USA) was used to generate the PBD runs and for 5 independent variables (jaggery, gram flour, water, soil and pH). Thereby, following experimental protocol, two critical responses namely AN and P were evaluated and were provided to the database to achieve useful insights based on the PBD. Considering two alternate parametric ranges for the independent variables (S_L and S_H), the PBD involved the evaluation of the primary effects associated with low (-1) and high levels (+1) (Table 2.1). The parametric range was considered for a fixed choice of ambient conditions (temperature, 29-32°C and relative humidity, 70-84%). A primary reason associated with the consideration of lower and higher parametric ranges was to evaluate the influence of the screening criteria and associated sensitivity and fitness variations.

Table 2.1 A summary of parametric range of independent variables for the Plackett-Burman design case (a) S_L (lower range) and (b) S_H (higher range).

(a)

S.No	Independent variables	Parameter Code	Unit	Minimum value (-1)	Maximum value (+1)
1	Jaggery	A	kg	0.02	0.04
2	Gram Flour	B	kg	0.02	0.04
3	Moisture	C	L	0.02	0.04
4	Soil	D	kg	0.2	0.4
5	pH	E	-	3	6

(b)

S.No	Independent variables	Parameter Code	Unit	Minimum value (-1)	Maximum value (+1)
1	Jaggery	A	kg	0.005	0.04
2	Gram Flour	B	kg	0.005	0.04
3	Moisture	C	L	0.005	0.04
4	Soil	D	kg	0.05	0.4
5	pH	E	-	3	8

2.1.5.4 Precursor Parametric Range for CCD-based Bio-fertilizer Formulation Design Studies

After the screening study, the solid Jeevamrutha bio-fertilizer formulation was prepared with a fixed choice of 1 kg cow dung and cow urine constitution (0.02 L). Jaggery, gram flour and soil being identified from the PBD-based screening study were varied as 0.012-0.04 kg, 0.012-0.04 and 0.12-0.5 kg for CCD-based solid Jeevamrutha bio-fertilizer during summer and winter seasons (Table 2.2). For these process parametric variations, the following section delineates the aspects associated with the CCD-based design of experiments. The moisture (water) content and pH were considered as 0.04 L and 8.0 respectively. After preparing the Jeevamrutha bio-fertilizer, the %TKN, AN and %P of the alternate formulations were determined as per the procedure outlined in section 2.1.5.2 of the Ph.D. thesis.

2.1.5.5 CCD Statistical Design-based Compositional Optimality of Jeevamutha Bio-fertilizer System

A preliminary screening study for the components associated with modified Jeevamrutha formulations revealed that jaggery, gram flour and soil have been the influential or critical factors. Further, a 2^3 rotatable CCD of RSM (Design Expert 7.0) was applied to predict the influence of these three independent variables and their interactions to improve the nutrient yield with varied combinations of the identified factors. A three-dimensional response surface plot was thereafter generated to gain useful insights into the associated sensitivities.

For either summer or winter season, twenty experimental sets were investigated to evaluate the coefficients of the second-order polynomial regression model for the three independent variables (jaggery, gram flour and soil) and at five levels -1, 0 +1, -α, +α. The following equation from Design Expert 7.0 was used to optimize the response variables:

$$Y\% = X_0 + X_1A + X_2B + X_3C + X_{12}AB + X_{13}AC + X_{23}BC + X_{11}A^2 + X_{22}B^2 + X_{33}C^2 \quad (2.1)$$

where, Y is the predicted response associated with the independent variables (A, B and C viz., jaggery, gram flour and soil respectively); X_0 is a constant coefficient; X_1, X_2 and X_3 are coefficients of linear terms; X_{12}, X_{13} and X_{23} are coefficients of interaction terms; X_{11}, X_{22} and X_{33} are quadratic terms (Asadu, Egbuna et al. 2019).

During analysis of variance (ANOVA) studies conducted for various cases, $p<0.05$ were considered significant in the CCD-developed model. The best model and the quadratic model were chosen based on the F- and p-values ($p<0.05$). The independent factors lie at a factorial point (±1), center point (0) and axial points (±α) (Table 2.2). The developed model was well-studied for its lack-of-fit and significance and thereby omits non-significant terms. One-way ANOVA was also performed to gain confidence in all experimental data.

Table 2.2: Factor levels deployed for the optimization of independent variables of Jeevamrutha bio-fertilizer formulations

Independent variables	Range and Level				
	-1	0	+1	-α	+α
Jaggery (A)	0.012	0.03	0.04	0.002	0.049
Gram Flour (B)	0.012	0.03	0.04	0.002	0.049
Soil (C)	0.12	0.5	0.4	0.02	0.49

2.1.6 Evaluation of Microbial Count of the Optimally Constituted Jeevamrutha Bio-fertilizer System

The optimized Jeevmarutha bio-fertilizer during the summer and winter seasons were analyzed for their microbial colony that includes bacteria, fungi, actinomycetes, N-fixers and P-solubilizers. The samples were subjected to serial dilution for their colony-forming units (CFU). The plate count method was adopted for this purpose (Devakumar, Shubha et al. 2014). The solidified media plates were streaked using an L-spreader in a laminar hood set up and thereafter the plates were incubated at 28-30°C for 1-3 days respectively. Thereby, the CFU count (CFU/mL) was evaluated as:

$$CFU\ count = \frac{Number\ of\ colonies \times Dilution\ factor}{Amount\ spreaded\ in\ the\ plate(mL)} \tag{2.2}$$

2.1.7 Evaluation of Macro- and Micro-nutrients, Organic Carbon and Electrical Conductivity of the Optimally Constituted Jeevamrutha Bio-fertilizer System

Macro-nutrients such as K, Na, Ca and Mg for the optimal compositional set of Jeevamrutha bio-fertilizer were estimated using a flame photometer and atomic absorption spectrometer (AAS) (Worth 1985, García and Báez 2012). Micro-nutrients (Zn, Cu, Fe and

Mn) were estimated using the AAS method. These elements were determined in flame AAS mode using air/acetylene flame at a furnace temperature of 2200-2000ºC. For Mg, Zn, Cu, Fe and Mn, the corresponding wavelength were 285.2, 213.9, 217.9, 248.3 and 279.5 nm respectively. Before the sample analysis on the same day, we run the standard solution of the micro-nutrients each time and constructed the calibration curve. This is because analytical background including lamp, detectors and atomization source (gases) often change from time to time. So, for every new run, a new calibration curve was obtained and due to this variability, they are not illustrated in the Ph.D. thesis.

The organic carbon content and electrical conductivity of the samples were achieved with a TOC analyzer and conductivity meter respectively.

2.1.8 Phytotoxicity Assay Characteristics of the Optimized Jeevamrutha Bio-fertilizer Formulations

The phytotoxic effect of the best bio-fertilizer constitution was carried out by adopting the literature-reported method for the mung bean plant. Mung bean could be used as a model plant because it can adapt against high metal stress and exhibit short germination time (5 days) (Haq, Kumari et al. 2016). Thereby, the optimized Jeevamrutha bio-fertilizer sample and distilled water were mixed in a ratio of 1:3 and kept in a shaker for 24 h. The sample was centrifuged at 8000 rpm for 10 minutes and the supernatant was collected. The collected liquid extract (supernatant) was varied as 25%, 50%, 75% and 100% (v/v). Thereby, four treatment cases (25%, 50%, 75% and 100%) were selected and for each treatment, ten seeds (in triplicates) were carefully chosen and washed with de-ionized water. 5 mL of each test solution along with the control group (0%) were added to the individual dishes. The seeds were grown in a petri dish soaked with Whatman filter paper no.1. Thereafter, the plates were stored in a dark environment for 5 days at 28°C. For each treatment case, the shoot and root length of the mung bean plant was measured after 5 days using a centimeter scale for each treatment cases (0%, 25%, 50%, 75% and 100% respectively). Thereby, germination index (GI) and seed germination (SE) were determined using the following expression (Haq, Kumari et al. 2016, Patel and Patel 2020):

$$Seed\ germination\ (\%) = \left(\frac{Number\ of\ germinated\ seeds\ in\ test\ solution}{Total\ of\ germinated\ seeds\ in\ control} \right) \times 100 \tag{2.3}$$

$$Root\ elongation = \frac{Sample\ mean\ root\ length}{Control\ mean\ root\ length} \times 100 \tag{2.4}$$

$$GI = \frac{SE\ (\%) \times\ root\ elongation}{100} \tag{2.5}$$

2.2 Optimality of Jeevamrutha Bio-fertilizer Modified with Vermicompost, Neem Cake, Tea Waste and Water Hyacinth

2.2.1 Precursor System Preparation and Targeted Proportions

Other inexpensive precursors such as water hyacinth, tea waste, neem cake and vermicompost were adopted to modify the optimized Jeevamrutha bio-fertilizer and thereby enhance its nutrient yield in terms of nitrogen and phosphate content. Water hyacinth was collected from a lake nearby IIT Guwahati. Tea waste was obtained from Mazbat Tea Estate and was finely ground. Neem cake and vermicompost were purchased from TrustBasket (9.8-13.4% OC, 0.5-1.6% N, 0.19-1% P, 0.15-0.73% K, 1.1-7.6% Ca, 0.09-0.56% Mg, 0.058-0.15% Na, 0.0042-0.1% Zn, 0.002-0.004% Cu, 0.205-1.33% Fe and 0.01-0.2% Mn). These raw materials were mixed with the optimized Jeevamrutha bio-fertilizer in varying proportions and as follows:

a) Jeevamrutha: Vermicompost: 1:1, 1:2, 1:3, 1:4

b) Jeevamrutha: Neem cake: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6

c) Jeevamrutha: Tea waste: 1:1, 1:2, 1:3, 1:4, 1:5

d) Jeevamrutha: Water hyacinth: 1:1, 1:2, 1:3, 1:4, 1:5

The mixed fertilizer system was prepared at an ambient temperature (29-38°C during summer and 12-21°C during winter seasons). Thereby, the seasonal influence can be assessed in terms of the targeted responses.

For the mixed fertilizer system, AN, %TKN, %P, macro- and micro-nutrients, microbial population and phytotoxicity characteristics were determined to evaluate the optimal constitution.

2.2.2 Nutrient Yield of the Precursors Modified Jeevamrutha Bio-fertilizer Systems

The nutrient content such as %TKN, AN and %P content in terms of the best-integrated fertilizer system for vermicompost, neem cake, tea waste and water hyacinth were analyzed with a Kjeldahl nitrogen analyzer and UV-Vis spectrophotometer. The adopted procedures have been outlined in section 2.1.5.2 of the Ph.D. thesis.

2.2.3 Physicochemical, Macro-Nutrient and Micro-Nutrient Characteristics of the Precursors Modified Jeevamrutha Bio-fertilizer Systems

The best optimized compositional set for the integrated-vermicompost, neem cake, tea waste and water hyacinth-based modified Jeevamrutha formulations were characterized by its physicochemical and macro-nutrient content. The adopted procedures have been outlined in section 2.1.7 of the Ph.D. thesis. The moisture content for each treatment case was determined with the AOAC method. For this, a known quantity of sample was weighed and placed for drying in a glass petri plate at 105°C for 14 h. Thereafter, the dried sample was further cooled and the final sample weight was measured. The moisture content (MC) of the sample was determined based on the initial and final weights of the sample using the following equation:

$$MC(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 is the weight of the fresh sample and W_2 is the weight of the dried sample (Horwitz 2010).

2.2.4 Microbial Characteristics of the Precursors Modified Jeevamrutha Bio-fertilizer Systems

The presence of microorganisms such as bacteria, fungi, actinomycetes, N-fixers and P-solubilizers in the vermicompost, neem cake, tea waste and water hyacinth modified Jeevamrutha bio-fertilizer system were determined with plate count method. The adopted procedures have been outlined in section 2.1.6 of the Ph.D. thesis.

2.2.5 Phytotoxicity Characteristics of the Precursors Modified Jeevamrutha Bio-fertilizer Systems

The phytotoxicity assay in the vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer system was subjected for their germination, root and shoot length indices for the mung bean plant. The adopted procedures have been outlined in section 2.1.8 of the Ph.D. thesis.

2.3 Performance of Jeevamrutha Bio-fertilizer with Sugarcane Juice and Starch Water Carbon Source

2.3.1 Constitution of Sugarcane Juice and Starch Rice Water-based Jeevamrutha Bio-fertilizer System

To replace 0.03 kg jaggery content being identified as the best with the CCD-based model, 0.03 L sugarcane juice and starch water respectively were used for the preparation of

Jeevamrutha bio-fertilizer to obtain a maximum nutrient yield during the summer and winter seasons. This is due to the reason that jaggery-based modified Jeevamrutha bio-fertilizer shows nutrient depletion in Assam during the winter season. Therefore, a low-cost organic fertilizer system with a readily available precursor such as starch rice water is necessary that could serve as an efficient factor to address the dynamics of the critical issue. Thus, process optimization targeting the further optimality of either sugarcane juice or starch water constitution in the further modified Jeevamrutha bio-fertilizer formulation was not targeted due to the reason that minimal alteration in the solid formulation was desired and the case study was conducted as a preliminary investigation to gain useful insights into the competence of the alternate carbon source. Thereby, the obtained compositional set for the experimental runs is as follows:

For summer and winter seasons, cow dung, cow urine, pH and moisture content were in a fixed proportion of 1 kg, 0.02 L, 8.0 and 0.04 L respectively. Gram flour content was considered to be 0.03 kg for the summer and winter seasons. Soil concentration was 0.35 kg for summer and 0.36 kg for winter seasons respectively. In other words, the jaggery content in the modified Jeevamrutha bio-fertilizer (0.03 kg) was replaced by 0.03 L sugarcane juice and starch rice water respectively.

2.3.2 Sugarcane Juice and Starch Rice Water Characterization

The raw substrates (sugarcane juice and starch rice water) were analyzed for their sugar content with the HPLC-RID (High-Performance Liquid Chromatography-Refractive Index Detector) for its sucrose, glucose and fructose content and a comparative analysis was conducted with respect to the jaggery-based modified Jeevamrutha bio-fertilizer. The adopted procedures have been outlined in section 2.1.4 of the Ph.D. thesis (Rajakylä and Paloposki 1983).

For both alternate cases, AN, %TKN, %P, macro- and micro-nutrients, microbial population and phytotoxicity assay were determined to evaluate the performance characteristics of the further modified Jeevamrutha bio-fertilizer.

2.3.3 Nutrient Yield, Physicochemical, Macro- and Micro-nutrients Characteristics of Sugarcane Juice and Starch Rice Water-based Modified Jeevamrutha Bio-fertilizer System

The modified sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer was characterized for its %TKN, AN and %P nutrient yield, physicochemical and macro- and micro-nutrient content. The adopted procedures have been outlined respectively in sections 2.1.5.2 and 2.1.7 of the Ph.D. thesis. Similarly, moisture content was determined as per the procedure outlined in section 2.2.3 of the Ph.D. thesis.

2.3.4 Microbial Analysis of Sugarcane Juice and Starch Rice Water-based Modified Jeevamrutha Bio-fertilizer System

The microbial load of sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer were subjected to microbial studies to assess the constitution of the bacteria, fungi, actinomycetes, N-fixers and P-solubilizers. The adopted procedures have been outlined in section 2.1.6 of the Ph.D. thesis.

2.3.5 Evaluation of Phytotoxicity Characteristics of Sugarcane Juice and Starch Rice Water-based Modified Jeevamrutha Bio-fertilizer System

The phytotoxicity assessment of sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer were evaluated for their germination index, and root and shoot length for the mung bean plant. The adopted procedures have been outlined in section 2.1.8 of the Ph.D. thesis.

2.4 Integrated Organic Farming Studies of *Phaseolus vulgaris* with Jeevamrutha Bio-fertilizer-Vermicompost System

2.4.1 Collection and Samples Preparation

In this work, *Phaseolus vulgaris* was chosen as a model plant. The raw seeds were procured from Jaikissan Seeds Corporation near Machkhuwa, Guwahati, Assam. The soil sample used for the experimental pot set-up was collected from a shady place inside the IIT Guwahati campus. Vermicompost was purchased from TrustBasket (Amazon). Solid Jeevamrutha bio-fertilizer formulation was prepared from the screening and optimization study reported by the authors (0.62% OC, 0.67% TKN, 0.14% P, 0.54 % K, 0.07% Ca, 0.0046 % Mg, 0.04 % Na, 0.0058% Zn, 0.0034% Cu, 0.036% Fe and 0.001% Mn) (Bhattacharjee and Uppaluri 2022).

2.4.2 *Phaseolus vulgaris* Pot Culture Set-up

Physical factors such as temperature (27°-31°C), humidity (50-87%) and adequate sunlight were continuously monitored during the entire growth period of *Phaseolus vulgaris* under natural conditions. Prior to seed sowing activity, the soil in the respective pots was adequately moistened with tap water. The seeds were sown in 4 pots (1 kg each in duplicates) and were planted at 1 cm uniform depth to thereby receive optimal sunlight. Three treatments, namely T1 (167 g Jeevamrutha, 666 g soil and 167 g vermicompost), T2 (200 g Jeevamrutha and 800 g soil), T3 (200 g vermicompost and 800 g soil) and T4 (only soil) were considered to evaluate upon the efficacy of the integrated organic farming options and for a time frame of 45 days (April-May).

2.4.3 Evaluation of *Phaseolus vulgaris* Morphological Characterization

Using a centimeter scale, the plant growth and yield parameters such as plant height, number of leaves, number of branches, number of flowers, length of leaves, number of fruits, fruit length, fruit weight and root length for each treatment case were measured at time intervals of 10, 20, 30 and 40 days after germination of the seeds.

2.4.4 Evaluation of Physical and Nutritional Characteristics of Alternate Treatment Cases

For all treatment cases (T1-T4), the pH, OC, EC, %TKN, %P and %K were evaluated to determine their physiological, nutritional and moisture content. The respective procedures have been outlined in sections 2.1.5.2, 2.1.5.7 and 2.2.3 of the Ph.D. thesis (Horwitz 2010, Adelowo, Oladeji et al. 2016).

2.4.5 Evaluation of Microbial Count of Alternate Treatment Cases

The treatments (T1-T4) were analyzed for their microbial colony that includes bacteria, fungi, actinomycetes, N_2-fixers and P-solubilizers. The adopted procedures have been outlined in section 2.1.5.6 of the Ph.D. thesis.

2.4.6 Nutrient Assessment of *Phaseolus vulgaris* System

For nutritional assessment in terms of carbohydrate, soluble protein and vitamin C content in *Phaseolus vulgaris*. The fruit sample was collected from each alternate treatment option and was crushed with a mortar and pestle for its nutritional parameters.

2.4.6.1 Carbohydrate Content Evaluation

The carbohydrate content for the bean samples (T1-T4) was determined using Anthrone's method. To do so, a 1000 ppm sucrose stock solution was used for calibration. 2 g of crushed fresh common beans was dissolved in 100 mL of water as a sample. Thereby, 100 mg of Anthrone reagent was dissolved in 50 mL of ice-cold H_2SO_4 (95%). 2 mL of this prepared solution was added to each of the samples and was mixed at 100 rpm for 15 minutes. The mixture was boiled for 30 minutes, then cooled and finally, the absorbance was measured at 630 nm using a UV-Vis spectrophotometer (Loewus 1952).

2.4.6.2 Determination of Soluble Protein Content

The soluble protein content for the bean sample (T1-T4) was determined using Bradford's method. The adopted procedure has been outlined in section 2.1.4 of the Ph.D. thesis.

2.4.6.3 Determination of Vitamin C Content

Vitamin C content for the bean sample (T1-T4) was evaluated with the titrimetric method (Sadasivam 1996). Using the DCPIP method, the vitamin C content of the sample was estimated which involves titrating the standard and experimental samples with a dye solution (Pal, Kumar et al. 2012). The dye solution was achieved by mixing 52 mg of DCPIP with 42 mg of sodium bicarbonate and further diluting the mixture with 200 mL of pure water. 100 mg of ascorbic acid was mixed with 100 mL of 4 wt.% oxalic acids to prepare a working standard. This was further diluted with 10 mL of the prepared solution with 90 mL of 4 wt.% oxalic acids. Lastly, 10 mL of 4 wt.% oxalic acids were mixed with 5 mL of the standard solution and titrated against the dye solution till it turns pink (A_1). Thereby, 100 mL of 4 wt.% oxalic acid and 1 g of sample were mixed. 5 mL of sample-oxalic acid was taken and diluted with 10 mL of 4 wt.% oxalic acids to titrate with the dye solution. The consumed dye solution was noted as A_2. Hence, the vitamin C content could be expressed as follows:

$$Vitamin\ C = \frac{0.5 \times 100 mL \times A_2 \times 100\ (mg)}{5mL \times A_1 \times weight\ of\ the\ sample\ (100\ g)} \tag{2.4}$$

2.4.7 Evaluation of Photosynthetic Constituents in *Phaseolus Vulgaris* System

After fine-chopping the fresh leaves, 0.5 g of the leaves was measured for each treatment case with an analytical balance. Thereafter, the samples were homogenized using 10 mL of 80% acetone. This extract was further centrifuged for 5 minutes at 2500 rpm. The final extract was diluted using 9 mL of 80% acetone per mL of the extract and was subjected to absorbance measurement using a UV-Vis spectrophotometer to obtain chlorophyll a and b values for all samples (Bojović and Stojanović 2005). The photosynthetic pigment assay and carotenoid content were evaluated at a mean absorbance of 663.6 and 646.6 nm respectively. The total carotenoid content was determined with the same extract by measuring absorbance at 470 nm using a UV-Vis spectrophotometer (Bojović and Stojanović 2005).

2.4.8 Colour and Odour of Stored Samples

On a seasonal basis, the sample's colour was altered from dark green to greenish black. The darkening of the samples during their storage time indicates their fermentation. This is henceforth attributed to microbial activity. Storage time also influences the odour that ranged from pungent to foul smell for the samples.

2.4.9 One-way ANOVA-based Statistical Analysis

Adopting the one-way Analysis of Variance (ANOVA) method, the statistical evaluation of the variations between the associated significance of the independent parameters and their mean values was determined using Origin Pro 8 software (Pytlakowska, Kita et al. 2012). Thereafter, error quantification and confidence levels within the acceptable range have been confirmed in the measured and evaluated data. The statistical evaluations have been outlined in the Appendix C section of the Ph.D. thesis.

In due course of the Jeevamrutha bio-fertilizer preparation, the weights of all ingredients namely, cow dung, cow urine, jaggery, gram flour, soil and rural precursors (vermicompost, neem cake, tea waste and water hyacinth) were measured with a weighing balance (Make: Sartorius) at the room temperature. For this purpose, all measurements were taken thrice to ensure upon the weighed sample accuracy. On the other hand, liquid ingredients (water, sugarcane juice and starch rice water) being used in the Jeevamrutha bio-fertilizer preparation were measured with a measuring cylinder. Since weight alterations may be possible due to seasonal influence, such variations have been considered to be a part of the adopted research strategy. Accordingly, the following methodology has been rendered to be as practical as possible for the corroboration of the obtained data for the benefit of the rural community of Assam, India. In other words, room temperature alterations can alter the weights of the samples and can affect the results to a certain extent. Future research towards this perspective is needed to analyze the pertinent sensitivities.

Chapter 3:

Screening and Optimization of Jeevamrutha Bio-fertilizer Formulations in Assam

Screening and Optimization of Jeevamrutha Bio-fertilizer Formulations in Assam

Targeting the optimality of Jeevamrutha bio-fertilizer formulations in Assam, India, the chapter aims to improvise agricultural systems through enhanced soil health characteristics targeted through the reduced cost and preparation time and wider utility of land in comparison to other organic fertilizer preparation techniques and with statistical design-based screening and optimization tools. After a brief introduction in section 3.1, section 3.2 outlines a comparative investigation of Palekar's unmodified Jeevamrutha bio-fertilizer with the developed modified bio-formulation to infer upon the influence of the incubation period on the TKN and AN content during summer and winter seasons in Assam. Section 3.3 elaborates upon the utility of a screening tool such as Plackett-Burman Design (PBD) for the identification of the critical precursors associated with the bio-formulation through modest experimental suites. Finally, section 3.4 conveys the optimality of the solid Jeevamrutha bio-formulations for the first time using a CCD-RSM-based statistical tool and thereby assesses its efficacy in terms of its phytotoxic attributes on seed growth and germination.

Overview

Large-scale cow dung-based bio-fertilizer development and utilization necessitate the deployment of cheaper protein and sugar sources. In the rural scenarios, these correspond to jaggery and gram flour respectively. Thereby, the chapter addresses the screening, identification and optimization of critical precursors associated with the development of low-cost and macro-nutrient-rich cow dung-based solid Jeevamrutha bio-fertilizer formulations. Plackett-Burman Design (PBD) and Central Composite Design (CCD) statistical tools were deployed to screen, identify and optimize critical precursors in the modified Jeevamrutha bio-fertilizer formulation. Best-screened parameters were identified with the PBD for its compositional fitness towards the improvement of nutrient content in the Jeevamrutha formulation. Further, the optimization of the screened critical precursors was evaluated for their corresponding TKN, AN and P during the summer and the winter seasons in Assam using the CCD-based statistical tool. Thereby, the conducted studies aim to contribute towards enhanced sustainability of the rural economy and target the minimization of the utility of hazardous chemical fertilizers for the sustained restoration of soil health and quality.

3.1 Background

Hazardous impacts due to the vast usage of pesticides and chemical fertilizers detriment long-term agricultural soil quality. To overcome this issue, emerging trends associated with organic farming indicate effective integration of livestock with agricultural practices. Popular cow-dung-based bio-fertilizers in rural scenarios refer to both fertilizers produced in solid and liquid forms. Among the commonly used cow dung-based bio-fertilizers, Jeevamrutha liquid bio-fertilizer received significant attention due to enhanced microbial load, nitrogen fixation through indigenous microbes in the soil and modest requirements in terms of inexpensive precursors such as jaggery and pulse flour (Devakumar et al., 2014; Palekar, 2006). Other ingredients of the fertilizer include cow urine, garden soil and water. Several investigations affirmed the efficacy of Jeevamrutha bio-fertilizers in terms of nutrient characteristics during bio-fertilizer production. Further characterization in terms of field application involving the growth of chosen plant species using mentioned bio-fertilizer and shelf life studies have been as well carried out. Investigations targeting nutrient characteristics during bio-fertilizer production received considerable attention. This indicates the efficacy of specific liquid (0.2 kg: 0.2 kg: 1 kg: 1 L: 20 L of jaggery, pulse flour, fresh cow dung, cow urine and water respectively along with a handful of soil) and solid Jeevamrutha formulations (1 kg: 0.05 L: 0.01 kg: 0.01 kg and 0.2 kg soil without water). The bio-fertilizer preparation referred to the hosting of the sample mixture in iron or a plastic vessel. Thereby, the vessel was covered with a wet gunny cloth and the system was kept in a shady environment for seven days with intermittent stirring of the mixture (thrice a day) using a wooden stick. All investigations in due course of time targeted the same composition but with varied incubation periods.

The study was conducted in the northeastern region of India, Assam, a well-known home and niche hotspot for a variety of flora and fauna along with a rich microbial consortium. Thus, the organic farming scope is apparent in terms of the promotion of organic bio-fertilizers through the systematic exploration of regional conditions (temperature, relative humidity and organic resources) for enhanced crop productivity (Munda et al., 2014). In summary, these physical factors need further validation for their seasonal influence on microbial formulation. Considering this fact, emphasis can be laid by adopting a holistic approach to develop indigenous and low-cost green manure using natural resources available in the region of interest. The formulation's nutrient content can differ from region to region based on the source of the raw precursors procured and pertinent variations in temperature and humidity. Nonetheless, the methodology and outcomes have been anticipated to serve as a generalized framework to confirm relevant variable trends in any region of the world.

Further insights into the available state-of-the-art associated with Jeevamrutha formulations are as follows. Firstly, nutrient characteristics during bio-fertilizer production did not follow a rigorous and scientific or rational methodology. Secondly, while there is a consensus towards the incubation period of 5-7 days, solid formulations have not been investigated with the fact that liquid formulations have detrimental aging effects in comparison with solid formulations. Thirdly, the literature does not specify a common incubation period based on the maximum macro-nutrient content of N or P. Thereby, perplexing inferences could be drawn with respect to the incubation period which could vary between 7–10 days (Devakumar et al., 2014; Palekar, 2006). Therefore, a single incubation period based on overall macro-nutrient content needs to be identified. Fourthly, Assam is an acclimatized zone for organic farming and henceforth, investigations need to address the sensitivity of the macro-nutrient range in the region vis-à-vis the data available in other agricultural regions. Fifthly, liquid Jeevamrutha formulations have been targeted to date and these do have a primary limitation in terms of the pungent and intense odour due to significant microbial activity. Further, they are also vulnerable to lower shelf life. Hence, solid Jeevamrutha formulations need to be targeted.

To better achieve a scientific or rational methodology associated with the optimality of Jeevamrutha formulations, statistical design tools need to be deployed. Among alternate statistical design tools, the PBD tool is well known to screen out and identify significant factors that contribute towards the critical responses through modest experimental suites (Plackett & Burman, 1946). Similarly, the CCD-based model shall corroborate with the sensitivity of the significant factors of the solid Jeevamrutha formulations during the summer and winter seasons on the nutrient yield. Sensitivity analysis based on a three-dimensional response surface plot can be also conducted for the chosen research theme. This is very important to identify the associated criticality of the chosen degrees of freedom. However, the developed product could be only established in commercial aspects by evaluating its phytotoxic attributes on seed growth and germination (Haq et al., 2016). In the present work, the phytotoxic effects of various constituents in the optimized formulation have been targeted for in situ characteristics on the seed germination rate of a mung bean plant and hence its suitable soil applicability.

It is well known that both nitrate and ammonium nitrogen (AN) are essential for plant growth and metabolism, signaling protein accumulation and root elongation. However, in this work, only AN was considered to corroborate with the TKN trends. The appropriate justification for the same is that in flooded or acidic soil, ammonium becomes the major nitrogen form (Wenzhu et al. 2023). Considering the fact that the developed bio-fertilizer has been in the acidic pH range (5.0-5.5), such non-consideration of nitrate evaluation is justified. Also, the pH range has been formally adjusted to neutral pH and alkaline range (pH of 7-8) by considering resilience to higher temperatures, rainfall and drought. Nonetheless, future research investigations can devote towards the role of nitrate constitution in the bio-fertilizer and its influence on the evaluated characteristics.

Hence, the carried-out investigations considered AN and phosphate (P) as responses to the fact that the evaluation of Kjedahl nitrogen on a timely basis is tedious. Also, it is justified by the fact that the AN has a greater role in plant metabolism-related factors (Liu & von Wirén, 2017). However, explicit findings related to the significance and correlation of both TKN and AN are also not available in the prior art to judge the influence of microbial activity on macro-nutrient enhancement.

3.2 Nutrient Characteristics of Palekar's Jeevamrutha Formulation

3.2.1 Precursor NPK Characteristics

The overall nitrogen-phosphate-potassium (NPK) content along with the pH range has been summarized for various precursors during the summer and winter seasons in Table 3.1. In this table, gram flour and cow urine have maximum nitrogen content (0.52 and 0.50%)

followed by cow dung (0.43%). Similarly, a higher P nutrient yield was observed for cow urine and gram flour (0.11 and 0.10%) followed by cow dung (0.08%) and soil (0.08%) respectively. Gram flour and cow urine exhibited better K content (0.46 and 0.36% respectively) followed by soil (0.34%). Such data is required to assess the nutrient yield alterations through the appropriate incorporation of other substrates. Similarly, an acidic pH for jaggery (6.15), gram flour (6.62) and nearby neutral pH for soil (6.98) was obtained. On the other hand, the pH for cow-excreta i.e., cow dung and cow urine varied in the alkaline range (8.17 and 8.26).

Table 3.1: Nutrient characteristics of Jeevamrutha bio-fertilizer precursors.

Samples	pH	TKN (%)	P (%)	K (%)
Cow dung	8±1	0.4±0.1	0.08±0.05	0.2±0.1
Cow urine	8±0.5	0.5±0.07	0.1±0.06	0.3±0.2
Jaggery	6±1	0.1±0.06	0.06±0.02	0.1±0.07
Gram Flour	6±0.2	0.5±0.2	0.1±0.04	0.4±0.1
Soil	6±0.6	0.3±0.1	0.08±0.04	0.3±0.06

3.2.2 Sugar and Protein Content of Carbon and Nitrogen Sources

The raw materials such as jaggery and gram flour were characterized for sugar and protein content using HPLC-RID and Bradford’s method. The sucrose, glucose and fructose content in jaggery were 30.6, 5.42 and 1.35 mg/L respectively. The soluble protein content in gram flour was 48.3 mg/L.

3.2.3 Nutrient Characteristics of Palekar’s Jeevamrutha Bio-formulation

An assessment of Palekar's unmodified Jeevamrutha formulation during the winter and summer seasons was targeted to visualize the influence of the incubation period on the TKN, AN and P in Assam. It was observed that for an incubation period of 7 days, the AN and TKN of Palekar's solid-based composition varied proportionally and as 124.8 and 289.6

mg/L and 0.13 and 0.21% for winter and summer seasons respectively. Also, P nutrient content with an alkaline pH (8.1) varied as 0.04 and 0.05% for the seasons respectively. Incidentally, the reported values of the liquid Jeevamrutha bio-fertilizer varied as 0.03-1.96% for TKN and 0.06-0.17% for P nutrient yield (Anandan et al., 2016; Devakumar et al., 2014; Gore & Sreenivasa, 2011). A reduced winter temperature range (12-21°C) could be the reason for the slower decomposition process of soil organic matter that decreased the accumulation of available nutrients required by the microbial diversity. Thereby, a significant nutrient decline in terms of %TKN and %P yield in the bio-fertilizer is evident during the winter season. Sample calculations for AN, %TKN and P nutrient yield have been shown in Appendix D section of the Ph.D. thesis.

For Palekar's solid Jeevamrutha bio-fertilizer formulation, the observed microbial count for bacteria, N-fixers and P-solubilizers (CFU/mL) were 42×10^6 and 27×10^6 mL^{-1}, 14×10^4 and 8×10^4 mL^{-1} and 21×10^4 and 15×10^4 mL^{-1} respectively during the summer and winter seasons. Hence, soil nutrient characteristics are highly influenced by the presence of beneficial microflora that holds variable effects on the overall nitrogen and phosphate availability.

3.3 Screening Study of Palekar's Bio-fertilizer System with Plackett- Burman Design (PBD)-based Statistical Model

A first-order model was deployed to screen the design variables using the expression:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i \tag{3.1}$$

where Y corresponds to the response (AN and P); β_0 is the model intercept; βi is the linear co-efficient and X_i corresponds to the level of independent variables (Plackett & Burman, 1946; Warda et al., 2016). Tables 2.1(a) and 2.1(b) respectively (Chapter 2) summarize the lower

(S_L) and higher (S_H) parametric range data of all independent variables being set to generate alternate data sets using the PBD approach. For the S_L case, they have been specified as 0.02-0.04 kg/L for jaggery (A), gram flour (B) and moisture content (C). Corresponding variations in soil (D) and pH (E) have been specified as 0.2-0.4 kg and 3-6 respectively. For the S_H case, the range for A-C, D and E have been specified as 0.005-0.04 kg/L, 0.05-0.4 kg and 3-8 respectively. Accordingly, 12 experimental runs (in duplicate) have been conducted to evaluate the influence of A-E independent variables on AN and P responses for each case. A primary reason associated with the consideration of lower and higher parametric ranges was to evaluate the pertinent influence upon the screening criteria and associated sensitivity and fitness alterations.

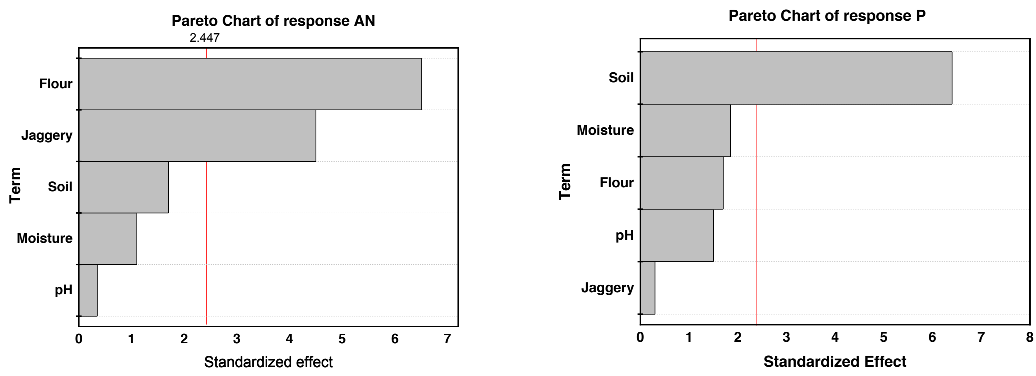
For the S_L case (data in Table 3.2), the statistical design-based variation refers to 151.8-315.7 mg/L and 867.2-1221.2 mg/L for AN and P for a variation in A-C, D and E from 0.02-0.04 kg/L, 0.2-0.4 kg and 3-6 respectively. Similarly, for the S_H case (Table 3.4), the PBD design variation inferred that the AN varied from 49.8-388.6 mg/L and P varied from 606.5-1248.1 mg/L for a variation in A-C, D and E from 0.005-0.04 kg/L, 0.05-0.4 kg and 3-8 respectively.

Parameters associated with the ANOVA of the PBD-based data sets have been summarized for the S_L and S_H cases in Tables 3.3 and 3.5 respectively. Thereby, pertinent variations in the measured average AN and P values were recorded. Table 3.3 elucidates the fitness parameters of the statistical design best-fit model. It assures upon the high significance level of the model and the associated rank of each process variable. For the S_L case, the overall model fitness parameters R^2 and Adj. R^2 were 0.91 and 0.84 respectively for the AN. For the AN case and independent variables A and B, high F-values (20.7 and 41 respectively) and low p-values (0.004 and 0.001) for A and B respectively. These are significantly lower than the standard p-value of 0.05 and thereby confirm the significance of the data sets. On the other hand, for the P sub-case, only for variable D, a high F-value (40.6) and low p-value (less than 0.001) were obtained and thereby indicated its significance.

Further, for the S_H case in Table 3.5, the overall model fitness parameters R^2 and Adj. R^2 were 0.91 and 0.85 respectively for AN (The pertinent formulae are shown in Appendix section E of the Ph.D. thesis). Corresponding significance indices for the A, B and C independent variables can be analyzed from the high F-value (40, 15.1 and 9.7 respectively) and low p-values (0.001, 0.008 and 0.02 respectively). On the other hand, for the P sub-case, the overall model fitness parameters R^2 and Adj. R^2 were 0.92 and 0.85 respectively. Corresponding significance indices referred to B independent variable due to its high F-value (14.1) and lower p-value (less than 0.009).

Pareto charts (Fig. 3.1 and 3.2) affirm the standardized effects from largest to smallest effect for the absolute values that are statistically significant. A reference line indicates its statistical significance that depends on α (significance level).

Thus, the mentioned variables for the chosen cases have maximum influence and can be screened as the critical variables influencing the chosen responses. For both S_L and S_H cases, AN have shown a high R^2 of 0.91 and thereby affirms a very good fitness of the PBD. Given the fact that the Jeevamrutha formulation experiments were not conducted in a fully controlled laboratory environment, marginally lower correlation coefficient values are justified. Also, the variations in temperature and relative humidity have been significant in due course of the entire duration of the summer and monsoon seasons in Guwahati, Assam, India. Thus, all these factors have been accounted to be responsible to achieve a lower yet acceptable range of correlation coefficient values.



(a) **(b)**

Fig. 3.1: Pareto charts depicting standardized effect on **(a)** AN and **(b)** P nutrient yield of S_L case.

Table 3.2: PBD-based independent and dependent variable data summary for S_L case.

S.No.	A (kg)	B (kg)	C (L)	D (kg)	E	AN (mg/L)	P (mg/L)
1	0.04	0.02	0.04	0.2	3	151.8±1.25	878.45±1.17
2	0.04	0.04	0.02	0.4	3	183.46±3.37	962.16±2.31
3	0.02	0.02	0.02	0.4	6	159.23±0.12	883.2±1.53
4	0.04	0.04	0.02	0.4	6	183.26±1.37	958.23±4.32
5	0.04	0.02	0.04	0.4	3	203.41±2.14	893.57±2.76
6	0.02	0.02	0.02	0.2	3	162.23±1.75	871.26±4.28
7	0.02	0.04	0.04	0.4	3	164.87±0.56	1173.29±3.48
8	0.04	0.02	0.02	0.2	6	171.5±0.18	869.37±2.18
9	0.02	0.02	0.04	0.4	6	157.28±1.29	1221.2±1.63
10	0.02	0.04	0.02	0.2	3	153.37±4.31	870.58±1.52
11	0.04	0.04	0.04	0.2	6	315.7±2.36	867.2±2.25
12	0.02	0.04	0.04	0.2	6	156.23±0.84	874.17±1.38

Table 3.3: ANOVA data summary for the PBD and S_L case.

	AN					P				
Source	**DF**	**Adj SS**	**Adj MS**	**F-value**	**p-value**	**DF**	**Adj SS**	**Adj MS**	**F-value**	**p-value**
Main effects	5	37729.3	7545.9	13.2	0.003	5	132221	26444	9.8	0.007
A	1	11800.8	11800.8	20.7	0.004	1	348	348	0.1	0.7
B	1	23359.8	23359.8	41	0.001	1	8150	8150	3	0.1
C	1	689.3	689.3	1.2	0.313	1	8401	8401	3.1	0.1
D	1	1798.5	1798.5	3.1	0.126	1	109330	109330	40.6	0.001
E	1	80.9	80.9	0.1	0.719	1	5992	5992	2.2	0.1
Residual Error	6					6				
Total	11					11				

Table 3.4: PBD-based independent and dependent variable data summary for S_H case.

S.No.	**A (kg)**	**B (kg)**	**C (L)**	**D (kg)**	**E**	**AN (mg/L)**	**P (mg/L)**
1	0.005	0.04	0.04	0.05	8	193.4±0.17	721±6.28
2	0.04	0.04	0.005	0.4	8	292.1±3.27	1109.6±2.56
3	0.04	0.005	0.04	0.05	3	178.1±1.13	715.7±1.07
4	0.04	0.04	0.005	0.4	3	209.1±1.07	1139±2.98
5	0.04	0.005	0.04	0.4	3	262.1±2.78	947±1.34
6	0.005	0.04	0.04	0.4	3	198.5±2.11	1248.1±1.89
7	0.04	0.04	0.04	0.05	8	388.6±0.73	688.3±2.45
8	0.005	0.005	0.005	0.05	3	49.8±0.18	608.8±2.93
9	0.005	0.005	0.005	0.4	8	59.4±1.23	734.6±1.85
10	0.04	0.005	0.005	0.05	8	211±3.47	606.5±3.04
11	0.005	0.04	0.005	0.05	3	99.3±2.79	684.5±3.18
12	0.005	0.005	0.04	0.4	8	110.2±5.28	885.6±1.54

Table 3.5: ANOVA data summary for the PBD and S_H case.

	AN					P				
Source	**DF**	**Adj SS**	**Adj MS**	**F-value**	**P-value**	**DF**	**Adj SS**	**Adj MS**	**F-value**	**p-value**
Main effects	5	98769.7	19753.9	13.7	0.003	5	476787	95357	14.2	0.003
A	1	57462.3	57462.3	40	0.001	1	10221	10221	1.5	0.2
B	1	21725.2	21725.2	15.1	0.008	1	94629	94629	14.1	0.009
C	1	14030.9	14030.9	9.7	0.020	1	7304	7304	1	0.3
D	1	10.8	10.8	0.01	0.934	1	337466	337466	50.3	0.000
E	1	5540.6	5540.6	3.8	0.097	1	27166	27166	4.05	0.09
Residual Error	6					6				
Total	11					11				

3.3.1 Effect of Independent Variables on the Nutrient Yield for S_L Case

Fig. 3.1 (a) and (b) depict the Pareto charts for the S_L case and AN and P respectively. Since the plots correspond to those being depicted for a 95% confidence level and an alpha of 0.05, they do confirm that the independent variables (gram flour (B) and jaggery (A)) have a significant effect but not the pH (E) on the AN characteristics. This could be explained by the fact that gram flour consists of protein, enzymes and nucleic acid for microbial cell development and growth. Similarly, jaggery is a well-established carbon substrate and is known to serve as an energy source for the microbial population. Thus, jaggery and gram flour critically enhance the metabolic activity of nitrogen-fixing bacteria and enhance the AN content of the formulation (Stres et al., 2008). Also, the phosphate content was critically influenced with the soil (D) and jaggery (A) had a minimal influence on the case. Higher P content in the bio-formulation by D and B is justified with that shown in Table 3.1. However, D may have an inhibitory response towards AN nutrient yield in comparison to jaggery. This is due to the fact that jaggery is a rich carbon source and could establish a high microbial association that influences nitrogen fixers in soil.

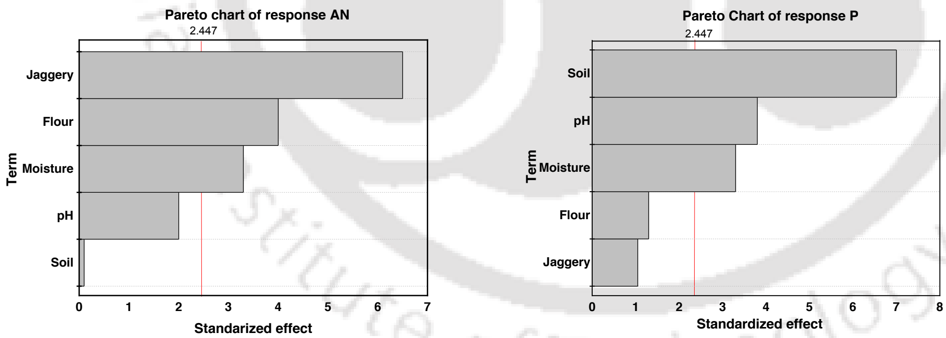
Thus, the study affirms that soil microflora profusely contributes to P productivity. Further, the figures affirm a lower contribution of all other components toward the P yield. These findings could also be interpreted using normal plots and thereby affirm the standardized influence of the screened variables with the PBD. The findings are also in agreement with the higher R^2 value reported for AN in the S_L case thereby denoting that over 90% of the variation in the response could be considered by the model. However, despite varying pH in a fairly wider range of 3–6, the S_L case refers to the narrow range of all other independent variables A–D. Therefore, it would be interesting to consider the analysis for the wider range i.e., the S_H case. A detailed account of the statistical terms and their formulae (F-test, sum of squares, df, errors, Adj SS, F- and p-value) has been presented in the Appendix F section of the Ph.D. thesis.

3.3.2 Effect of Independent Variables on the Nutrient Yield for S_H Case

Fig. 3.2 (a) and (b) illustrate the Pareto charts and the influence of formulation precursors at a 95% confidence level (α=0.05) for the S_H case. The illustrations depict that for the AN content, A, B and C independent variables influenced AN significantly and variables D and E had minimal influence. As discussed in the earlier section, both A (jaggery) and B (gram flour) have a significant influence on the AN for the S_L case. However, for the wider parametric range (S_H), apart from these, higher water content indicated better AN nutrient yield. This is due to the influence of higher water content on the microbial community population and their activities and hence better AN characteristics for the wider S_H range (Stres et al., 2008).

On the other hand, for the P case, both D and B exhibited significant influence on the P nutrient characteristics and all other factors namely A, C and E can be concluded to be insignificant. Also, for the S_L case, D was a vital factor. This conveys that the wider parametric range is necessary to affirm upon the criticality of all screening factors during the PBD. The normal plots corroborate these findings associated with the standardized effect of each screened variable. In summary, for the S_L case, B and A have critically influenced AN and D critically influenced P. However, for the S_H case, A, B and C can be screened to be effective variables to influence AN and D and B critically influenced P characteristics. Among both AN and P, it shall be noted that P cannot be considered to be the most critical dependent variable as it is reasonably abundant in nature. Further, AN trends also indicate its sensitive dependence upon the identified screened independent variables.

Since A and B are excellent precursors for carbon and nitrogen content respectively, these parameters to a certain extent can be considered to enhance P productivity in addition to soil constitution. The parametric variation of the factors provides a very useful analysis associated with sensitivity. Thereby, the exact influence of the chosen independent variables on the AN and P nutrients can be visualized. It also provided a greater understanding of the critical independent variables and this was not the case for the lower parametric range (S_L).



(a) **(b)**

Fig. 3.2: Pareto charts depicting standardized effect on **(a)** AN and **(b)** P nutrient yield of S_H case.

Moisture content (water) and pH were considered at a higher concentration in all conducted investigations. This is due to their significant outcome in the AN and P nutrient yield. Thus, the water content may not exclusively contribute towards the intended purpose of solid Jeevamrutha bio-fertilizer formulation. Similarly, the pH adjustment in real-field application scenarios is not possible as the natural pH of the system is difficult to deviate significantly by considering naturally occurring pH additives. Hence, only jaggery, gram flour and soil have been considered as the significant factors in the PBD optimization study. Also, the initial pH of the modified solid-based Jeevamrutha bio-fertilizer formulation was in the acidic pH range (5.0-5.5). Considering the possible scope for feasible pH alteration, the pH range of the system has been adjusted to neutral pH and alkaline range (7-8) in all experimental runs. This was also considered to soil stress and create a favorable environment for the optimal growth of the microbes.

3.3.3 TKN and AN Characteristics

Experimental investigations were conducted to gain insights into the influence of the incubation period on the TKN and AN for Palekar's unmodified Jeevamrutha formulation. It was observed that for an incubation period of 7 days, the AN and TKN of Palekar's solid-based composition varied proportionally as 124.8 and 289.6 mg/L and 0.13 and 0.21% for winter and summer seasons respectively. Similar data reported in the literature refers to 0.19% TKN and 508 mg/L AN for buffalo manure (Faugno et al., 2012). Other prior art emphasized TKN during cow dung-based bio-fertilizer formulations and affirmed 0.05% TKN for a neutral pH system after 7 days of incubation (Rameeza et al. (2016)) and 1.96% TKN for an acidic pH system after 9 days of the incubation period (Devakumar et al. (2014)). Thus, it is apparent that the TKN in the literature for the cow dung-based Jeevamrutha compatible formulations varied significantly from 0.05–1.96% and the obtained best value in this work of 0.26% is within the range. This is possibly due to the significant influence of cattle fodder nutrition and other regional factors in influencing the sourced cow dung TKN and subsequently the TKN of the Jeevamrutha. It is opined that the cow dung TKN will have a significant influence on the TKN of the Jeevamrutha formulation and hence the quality of the source is an issue. Along with this, temperature and relative humidity also play an important and key role to influence both AN and TKN.

3.3.4 Incubation Period

To date, the time-dependent variation of AN and P and its optimality did not receive detailed attention for the Jeevamrutha formulation in the literature. The highest AN and P nutrient content reported in the PBD-based experimental data (Tables 3.1 and 3.2) affirms that the AN and P nutrient content varied significantly for a variation in the incubation period from 1–9 days respectively. For the S_L-based experimental investigations, the incubation period varied from 7–9 days with the AN varying from about 245.6–315.7 mg/L. On the other hand, for P yield, the incubation period of 9 days indicated the best nutrient yield (1221.2 mg/L).

Similarly, for the S_H parametric range, the incubation period was 7 days for AN (388.6 mg/L) and 9 days for P (1248.1 mg/L) productivity. Thus, the highest AN and P values can be obtained for the 7 and 9 days incubation period respectively for the chosen constitution of the Jeevamrutha formulation. However, since AN is more sensitive than P, the incubation period for the mentioned Jeevamrutha formulation has been inferred to be 7 days for all further experiments.

3.3.5 Qualitative Analysis of Temperature and Relative Humidity Factors

For both S_L and S_H cases, the experimental runs generated by the PBD model were conducted within a temperature range of 29-32°C and relative humidity of 70-84%. For the S_L case, a maximum AN yield concentration (315.7 mg/L) was obtained at a temperature and relative humidity of 31°C and 82% respectively.

On the other hand, for the S_H case, the AN value was higher (388.6 mg/L) for a similar temperature (32°C) and relative humidity (84%). Further, it was observed that the lowest AN yield (49.8 mg/L) was obtained for a case with a relative humidity of 70% and a temperature of 31°C.

Nonetheless, P productivity remained unaffected for both highest (1248.1 mg/L) and lowest values (606.5 mg/L) and similar temperature and relative humidity ranges. Hence, it can be interpreted that among both physical parameters namely temperature and relative humidity, higher temperature and higher humidity are preferred to obtain enhanced AN trends. Further investigations need to be addressed to gain useful insights to corroborate such qualitative analysis and inference.

3.3.6 Literature Comparison

Table 3.6 elucidates the compositional influence on Jeevamrutha and similar formulations on the AN, TKN and P yield. In this work, A, B and cow dung composition was similar to that mentioned in the literature (Anandan et al., 2016; Devakumar et al., 2014; Gore & Sreenivasa, 2011). However, soil constitution was not provided appropriately in the mentioned literature and was chosen as 0.2 kg in the formulation. Similarly, the cow urine content was reduced from 0.05 to 0.02 L to minimize its utilization and achieve a solid manure formulation. This is due to the reason that cow urine has medicinal and bio-enhancer applications and is not an abundantly produced waste product in the farm community. Hence, the thesis focused on the nutritional augmentation of the developed formulation using other influential precursors such as jaggery, gram flour and soil for its effectiveness. Further, the moisture content was added to enhance the long-term storage capacity of the developed product. Further investigations could provide more analysis in this regard. Photographic images for a study that investigates the role of cow urine in the growth profile characteristics of *Phaseolus vulgaris* and its root health have been illustrated in Appendix B of the Ph.D. thesis.

Nonetheless, with a higher concentration of the precursors in the formulation, the obtained results may not vary linearly. For the mentioned formulation, a reasonably higher TKN (0.26%) was obtained in comparison with that reported by Anandan et al. (2016) (0.03%) with liquid Jeevamrutha formulation. Similarly, better P productivity (1034 mg/L) was achieved in comparison with that reported by Gore et al. (2011) (166 mg/L). Considering these aspects, it can be inferred that the findings are in accord with those stated in the relevant literature, with the fact that regional factors, fodder quality and the health profile of the cows do influence these parameters significantly.

Table 3.6: A comparative summary of best reported Jeevamrutha bio-fertilizer formulations and their nutrient yield.

S. No	**A (kg)**	**B (kg)**	**C (L)**	**D (kg)**	**E**	**Cow dung (kg)**	**Cow urine (L)**	**TKN (%)**	**P (mg/L)**	**Location**	**Sources**
1.	0.2	0.2	20	0.1	7	1	1	0.03	177.1	Tamil Nadu	Anandan et al. 2016
2.	0.2	0.2	20	Handful	4.9	10	1	1.96	1730	Karnataka	Devakumar et al. 2014
3.	0.2	0.2	20	Handful	7	10	1	0.07	166	Karnataka	Gore and Sreenivasa 2011
4.	0.02	0.02	0.04	0.2	7.8	1	0.02	0.26	1248.1	Assam	**This work**

The conducted research studies with respect to compositional choice and optimality were based on the control experiment with Palekar's Jeevamrutha composition for the summer and winter seasons (1 kg cow dung, 0.05 L cow urine, 0.01 kg jaggery, 0.01 kg gram flour and a handful of soil and without pH and water alteration). In rural scenarios that do not favor the abundant availability of cow dung and cow urine, their alteration is restricted. Hence, a fixed quantity of cow dung and a lower amount of cow urine (0.02 L) were considered. Soil constitution alteration is relatively inexpensive and hence, soil can be altered. The other two ingredients namely jaggery and gram flour are expensive and hence they need to be considered for possible alteration. Thus, jaggery, gram flour and soil were the choices for parametric variation and subsequent analysis. The thesis considered a lower (S_L) and higher (S_H) parametric range of these variables. For the cases, jaggery and gram flour constitution were 0.02-0.04 kg and 0.005-0.04 kg respectively for both cases. Similarly, soil constitution was 0.2-0.4 and 0.05-0.4 kg respectively. pH and water content were 3-5 and 3-8 and 0.02-0.04 L and 0.005-0.04 L for S_L and S_H cases. Such a consideration of low and higher parametric ranges has been based on the following reasons:

a) As additives, it is often desired to have a minimal constitution of additives such as gram flour and jaggery. Hence S_L is justified. The constitution of soil for the range has been based on a few trials and error-based experimental investigations.

b) The highest constitution of additives such as gram flour and jaggery also needs to be explored from the perspective of sensitivity. Hence, S_H is justified. The constitution of soil for the range has been based on a few trials and error-based experimental investigations.

c) Parametric range other than the mentioned cases did not yield appropriate alteration in AN and P and possible scope for the model-based analysis was not possible.

Thus, considering the pertinent limitations of statistical modelling and economic feasibility for the rural community, S_L and S_H were considered.

3.4 Optimality of Jeevamrutha Bio-fertilizer Constitution and its Phytotoxicity Assay Characteristics

3.4.1 CCD-based Experimental Design

The results from the CCD model generated alternate datasets for the summer and winter Jeevamrutha formulations and with Design Expert (7.0). Thereby, the primary influences were determined. For all formulations, the independent variables such as jaggery (A) and gram flour (B) varied from 0.012-0.04 kg along with soil (C) from 0.12-0.4 kg. The parameters chosen for the summer season did confirm greater deviation and significant influence on the AN and P responses in comparison with those evaluated for the winter season. The primary target involving the differential influence of the seasonal aspects in the CCD model was devoted to the identification of the pertinent critical interactions of the response variable on the nutrient yield of the modified Jeevamrutha formulations.

Twenty experimental sets were investigated to evaluate the coefficients of the second-order polynomial regression model for the three independent variables (jaggery, gram flour and soil) at five levels -1, 0 +1, -α, +α. The following equation from Design Expert 7.0 was used to optimize the response variables.

$$Y\% = X_0 + X_1A + X_2B + X_3C + X_{12}AB + X_{13}AC + X_{23}BC + X_{11}A^2 + X_{22}B^2 + X_{33}C^2 \quad (2.2)$$

A brief discussion of the methodology has been outlined in Chapter 2 of the thesis. The response data recorded and presented in Tables 3.7 and 3.9 were used to develop appropriate model equations.

The evaluated response variables (AN and P) were interrelated to the independent variables and through a second-order polynomial (equation (3.2)). The coefficient with a single factor (A, B and C) solely represents the effects of that factor. Further, the coefficients of two factors, i.e., AB, AC, BC and second-order terms (A^2, B^2 and C^2) depict the interaction between any two factors and the individual squared effects of each factor. A negative value of the regression terms affirms an antagonistic effect while a positive sign specifies a synergistic effect in the best-fit model expressions. The coefficients in the regression equation can be used to evaluate the sensitive influence of each element with respect to others and with the coded values (Danbaba et al., 2015).

Substituting the values obtained in the coded factors of equation 1, the following equations were obtained for summer (eq. 3.3 and 3.4) and winter seasons (eq. 3.5 and 3.6) to represent AN and P nutrient content as functions of A, B and C.

$$AN = +50A + 60.88B + 8.46C - 1.77AB - 2.72AC + 13.42BC - 2.28A^2 + 8.15B^2 + 13.14C^2 \tag{3.3}$$

$$P = -3.84A + 28.11B + 155.33C - 5.53AB - 4.7AC + 2.31BC - 13.18A^2 - 15.13B^2 + 29.56C^2 \tag{3.4}$$

$$AN = +11.73A + 20.55B + 14.76C + 2.61AB - 1.14AC + 3.90BC + 2.99A^2 + 4.96B^2 + 4.84C^2 \tag{3.5}$$

$$P = -1.06A + 22.51B + 137.49C - 0.36AB + 0.90AC + 2.09BC - 10.37A^2 - 5.44B^2 + 0.025C^2 \tag{3.6}$$

In the above expressions, AN and P are the response variables, and A, B and C refer to the coded terms representing jaggery (A), gram flour (B) and soil (C).

3.4.2 Seasonal Influence on Nutritional Characteristics

The CCD-based design for twenty alternate datasets was investigated for various combinations of the process variables (Tables 3.7 and 3.9).

Table 3.7: CCD-RSM-based data summary of Jeevamrutha bio-fertilizer system in the summer season.

S.No.	Jaggery (kg)	Gram flour (kg)	Soil (kg)	AN (mg/L)	P (mg/L)
1.	0.012	0.012	0.12	161.67±0.17	830.67±2.98
2.	0.040	0.012	0.12	289.56±1.16	829.36±3.66
3.	0.012	0.040	0.12	275.34±2.67	887.21±1.48
4.	0.040	0.040	0.12	356.98±0.86	883.56±0.74
5.	0.012	0.012	0.40	189.45±3.06	1116.23±1.35
6.	0.040	0.012	0.40	267.31±1.05	1115.89±0.87
7.	0.012	0.040	0.40	317.64±4.39	1161.77±0.39
8.	0.040	0.040	0.40	427.56±0.73	1159.55±1.73
9.	0.002	0.03	0.26	194.26±2.23	1001.34±2.04
10.	0.05	0.03	0.26	364.32±1.14	998.43±3.83
11.	0.03	0.002	0.26	201.34±0.54	951.58±2.68
12.	0.03	0.05	0.26	416.56±0.33	1037.17±1.69
13.	0.03	0.03	0.02	323.89±2.29	835.78±0.84
14.	0.03	0.03	0.50	322.19±0.45	1405.78±1.26
15.	0.03	0.03	0.26	263.24±3.57	1002.34±0.35
16.	0.03	0.03	0.26	261.47±0.23	1005.89±0.67
17.	0.03	0.03	0.26	268.57±0.68	1045.45±0.21
18.	0.03	0.03	0.26	269.34±0.39	1015.18±1.04
19.	0.03	0.03	0.26	291.45±1.27	1019.45±0.17
20.	0.03	0.03	0.26	289.57±2.66	1003.21±1.26

In Table 3.7, it could be analyzed that the AN is varied from 427.56-161.67 mg/L. The measured AN refers to the value evaluated after 9 days of the incubation period for the summer season case. In the earlier PBD section (Section 3.3), a similar trend for AN nutrient yield was recorded for variations in jaggery, gram flour and soil content. Comparable results were achieved for AN during the winter season. During this period, the variable altered proportionally from 135.86-59.82 mg/L after 11 days' incubation period for 0.012-0.04 kg alteration of A and B and 0.12-0.4 kg alteration of C (Table 3.9).

Table 3.8: ANOVA and best fit model parameter data of best fit model representing Jeevamrutha bio-fertilizer system in the summer season.

Components	DF	Sum of squares	Ammonium Nitrogen		DF	Sum of Squares	Phosphate	
			F value	p value			F value	p value
Model	9	69206.25	26.73 (Quadratic)	< 0.0001 (Quadratic)	9	2.790E+005	50.73 (Quadratic)	< 0.0001 (Quadratic)
A	1	14916.46	90.56	< 0.0001	1	11.28	0.25	0.62
B	1	36579.38	134.29	< 0.0001	1	8659.14	13.63	0.0042
C	1	17344.96	2.59	0.13	1	2.700E+005	416.33	< 0.0001
AB	1	13.55	0.067	0.80	1	2.23	0.31	0.59
AC	1	3.45	0.16	0.70	1	0.72	0.22	0.64
BC	1	1.61	3.82	0.07	1	58.00	0.05	0.82
A^2	1	346.45	0.20	0.66	1	20.54	3.16	0.10
B^2	1	2.31	2.54	0.14	1	142.28	4.17	0.06
C^2	1	1.65	6.60	0.02	1	47.74	15.91	0.002
Residual	6	188.11			10	4.93		
Lack of fit	5	139.69	3.37	0.10	5	5.54	4.96	0.051
R squared				0.96				0.97
Pure Error	5	48.42			5	4.32		
Adequate Precision				17.7				26.7
Total	34	69394.36			38	3.070E+005		

Thereby, the study confirmed the critical dependence of microbial growth on the enrichment of the developed formulations. Also, the metabolism rate of microorganisms got adversely affected during the winter season. Thereby, it requires additional supplements for the nutritional enhancement of the Jeevamrutha bio-fertilizer (Vigneron et al., 2019).

The phosphate content was found to be highest after 12 days of the incubation period for both the summer and winter seasons. The P varied from 829.36-1405.78 mg/L and 573.78-1046.75 mg/L during the summer and winter season cases respectively. The significance of soil for maximum P nutrient yield is justified by findings of the prior screening study summarized for the PBD case in section 3.3 of the thesis. The nutritional characteristics of the raw precursor viz., jaggery, gram flour, cow urine, cow dung and soil have been evaluated using literature-reported procedures (Devakumar et al., 2014; Gore & Sreenivasa, 2011).

Table 3.9: CCD-RSM-based data summary of Jeevamrutha bio-fertilizer system in the winter season.

S.No.	Jaggery (kg)	Gram flour (kg)	Soil (kg)	AN (mg/L)	P (mg/L)
1.	0.012	0.012	0.12	59.82±1.47	631.28±1.24
2.	0.040	0.012	0.12	76.28±2.34	627.29±1.37
3.	0.012	0.040	0.12	89.24±1.06	667.54±0.52
4.	0.040	0.040	0.12	119.48±1.78	663.41±0.48
5.	0.012	0.012	0.40	78.92±4.68	895.21±0.07
6.	0.040	0.012	0.40	94.19±2.58	896.13±1.02
7.	0.012	0.040	0.40	127.29±0.44	941.14±2.64
8.	0.040	0.040	0.40	135.86±1.34	939.32±1.53
9.	0.002	0.03	0.26	72.17±1.65	782.47±1.29
10.	0.05	0.03	0.26	117.27±2.94	779.24±0.86
11.	0.03	0.002	0.26	69.28±1.26	751.45±1.58
12.	0.03	0.05	0.26	131.28±0.43	838.19±1.07
13.	0.03	0.03	0.02	71.29±3.19	573.78±0.37
14.	0.03	0.03	0.50	128.62±0.65	1046.75±1.93
15.	0.03	0.03	0.26	89.86±2.07	809.21±0.87
16.	0.03	0.03	0.26	81.29±1.45	807.32±0.32
17.	0.03	0.03	0.26	85.26±1.38	791.03±3.28
18.	0.03	0.03	0.26	88.92±0.97	801.01±2.31
19.	0.03	0.03	0.26	86.18±1.55	805.29±1.55
20.	0.03	0.03	0.26	87.18±2.27	803.67±1.93

Table 3.10: ANOVA and best fit model parameter data of best fit model representing Jeevamrutha bio-fertilizer system in the winter season.

Components	DF	Sum of squares	Ammonium Nitrogen		DF	Sum of squares	Phosphate	
			F value	p value			F value	p value
Model	9	106234.21	73.75 (Quadratic)	< 0.0001 (Quadratic)	9	2.986E+05	335.82 (Quadratic)	< 0.0001 (Quadratic)
A	1	35673.86	108.35	< 0.0001	1	10.92	0.17	0.68
B	1	78943.72	332.85	< 0.0001	1	8649.07	78.31	< 0.0001
C	1	54123.67	171.73	< 0.0001	1	2.188E+05	2922.30	< 0.0001
AB	1	38.43	3.13	0.10	1	2.44	0.01	0.91
AC	1	6.4	0.45	0.67	1	0.60	0.07	0.79
BC	1	1.45	0.02	0.77	1	56.92	0.40	0.54
A^2	1	3.47	7.44	0.001	1	5502.81	17.55	0.001
B^2	1	1.8	0.001	0.73	1	6654.68	4.82	0.05
C^2	1	1.74	0.001	0.77	1	52343.21	9.83E-005	0.99
Residual	6	176.46			10	23972.08		
Lack of fit	5	147.38	2.74	0.13	5	23683.97	3.20	0.11
R squared				0.985				0.996
Pure Error	5	61.49			5	288.10		
Adequate Precision				31.96				69.58
Total	34	275414.08			19	3.226E+05		

3.4.3 Statistical Evaluation of Regression Models

The F-value analysis was considered to ensure the significance of a regression model in due course of the prediction of the effects of independent factors on the response variables. The variance distributed by the observed model is statistically larger than the random error with a higher F-value at a specific significance level (α) (Danbaba et al., 2015). Tables 3.8 and 3.10 summarize ANOVA results for the best fit quadratic regression model for the summer and winter seasons respectively. For the AN, the F-value range for each response variable altered from 0.16-134.29 during summer and 0.001-332.85 during the winter seasons respectively. For the AN, a higher F-value was observed for B during the summer (134.29) and winter (332.85) seasons. Similarly, the F-value for P varied from 0.05-416.33 and 0.01-2922.30 during the summer and winter seasons respectively. In contrast, for P, the F-value was highest for C (416.33 and 2922.30) followed by B (13.63 and 78.31) during the summer and winter seasons. Hence, it is evident that B and C are the crucial variables for AN and P nutrient yield in Jeevamrutha bio-fertilizer formulations.

Consequently, the p-value confirms the model significance of the corresponding coefficients. For the AN, the linear terms A and B possessed appropriate p-value ($p<0.0001$) and are significant for both seasons. Similarly, for the P, the term C ($p<0.0001$) assured good model fitness and significance. For both cases, the lack-of-fit was statistically insignificant and was not described at the level of probability. Additionally, the coefficient of determination (R^2) was evaluated to confirm the reliability of the model to predict the AN and P nutrient content.

The R^2 value of 0.96 and 0.97 has been obtained for both AN and P during the summer season. On the other hand, the R^2 value of 0.98 and 0.99 has been obtained for AN and P during the winter season. Hence, 96-99% of the response variables could be explained by the design model and thereby confirmed upon its very good fitness characteristics.

3.4.4 Three-dimensional Response Surface Plots

The three-dimensional response plot assures upon the implication of the coefficients of the equation and thereby analyzes the maxima and minima of the evaluated responses (Danbaba et al., 2014). Further, the associated sensitivities can be examined from the graphical representation, to thereby infer the criticality and non-criticality of the independent variables.

As shown in Fig. 3.3 (a-f), the response surface plots were obtained through the variation of any two independent variables and for a fixed choice of the other independent variable. For the summer season, the AN varied as 161.67-427.56 mg/L to reach an optimal point (maximum) for the binary variation of A (0.02-0.04 kg), B (0.02-0.04 kg) and C (0.2-

0.4 kg) independent variables (Fig. 3.3 (a-c)). Further, the AN altered considerably for B followed by A in the B and A binary interaction. For the P case, both C and B could be analyzed to potentially alter the nutrient yield that varied as 829.36-1405.78 mg/L (Fig. 3.3 (d-f)). Thus, A had the lowest influence on the P due to the low intake of energy substrate by the P-solubilizing microorganism (Fig. 3.3 (d-e)) (Vijayendra et al., 2001). Similarly, for the winter case, both B and C affirmed their strong influence on the P and this is in agreement with the trend evaluated for the summer season (Fig. 3.4 (a-f)).

Gram flour (B) is a good source of nitrogen and has a critical role in the rapid metabolism pathway of microbes (Salar-García et al., 2017). Similarly, soil content (C) with a variation of 0.2-0.4 kg also has a critical role with the fact that the soil bacteria (N_2-fixers) may have contributed to the nitrogen breakdown of substrates along with the utilization of carbon sources such as jaggery (A) (Vijayendra et al., 2001). However, the lower AN trend (59.82-135.86 mg/L) for A and B for a variation of 0.02-0.04 kg during the winter season have been due to the reason that the microbial consortium is largely affected due to the significant alterations in the climatic conditions favorable for their growth and breakdown of the available substrates. The conducted study legitimates that with a variation in the concentration of C from 0.12-0.04 kg and B from 0.012-0.04 kg, a significant improvement in P can be achieved. Hence, it can be concluded that gram flour (B) and soil (C) are the most crucial factors to influence the overall nutritional composition of the solid Jeevamrutha formulations. Also, jaggery (A), being a rich carbon source, has a profound role in the precursor breakdown and subsequent enrichment pathway in the addressed formulations (Vijayendra et al., 2001).

In this work, the scope of response variables to possibly explore available nutrients in the Jeevamrutha bio-fertilizer formulations has been targeted for the best combinations of nutritional characteristics. It could also be analyzed from this study that the P concentration has been substantially higher in comparison with the AN concentration. This suggests that the bio-fertilizer developed in this study is an effective P-solubilizing bio-fertilizer during both seasons. Thus, it will be an interesting proposition to appropriately blend the optimized formulation with nitrogen-rich bio-enhancers to further enhance its utility in field application.

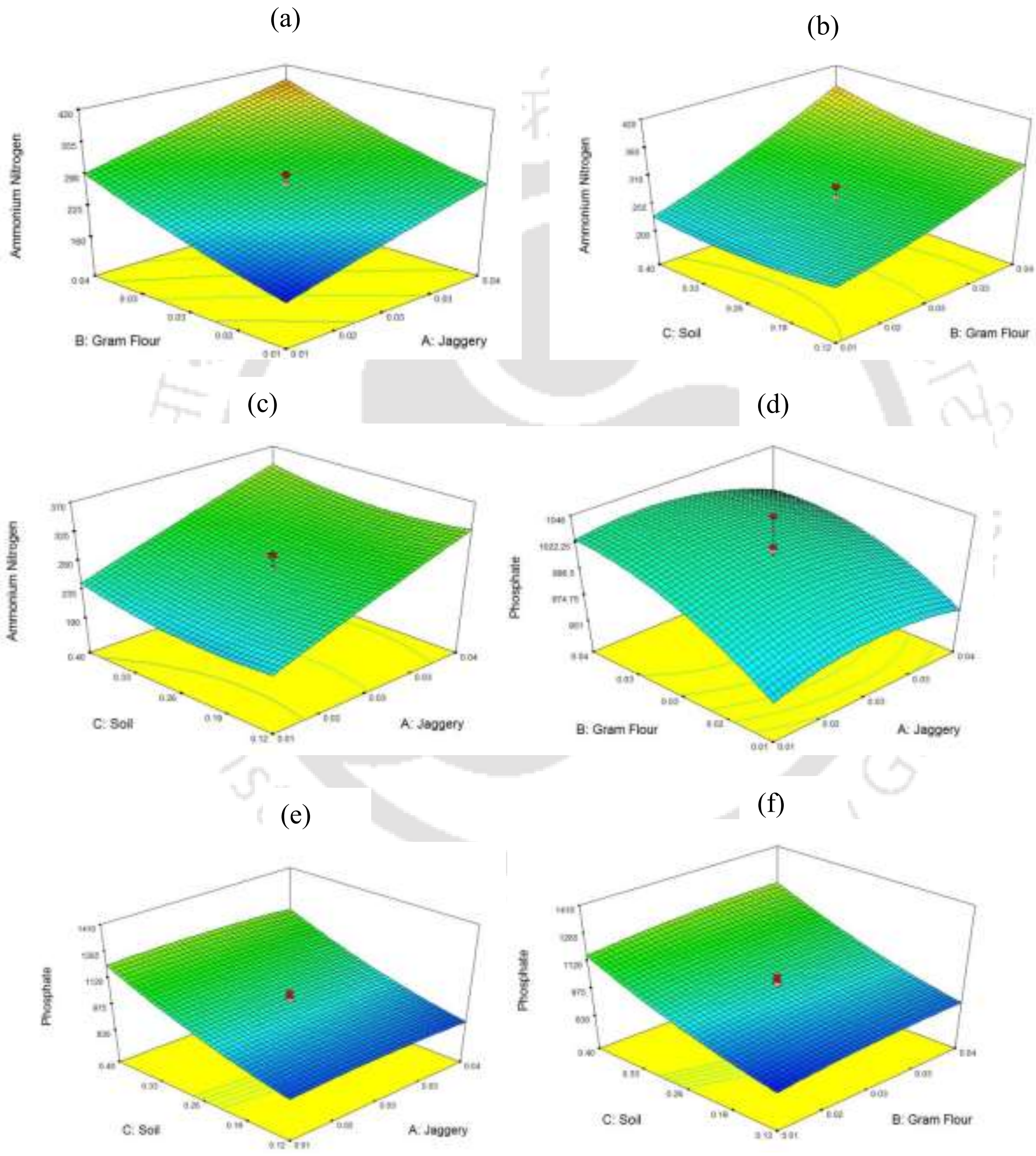


Fig. 3.3 (a-f): 3D surface plots depicting the influence of independent (jaggery, gram flour and soil) variables on the nutrient characteristics (AN and P) in the summer season.

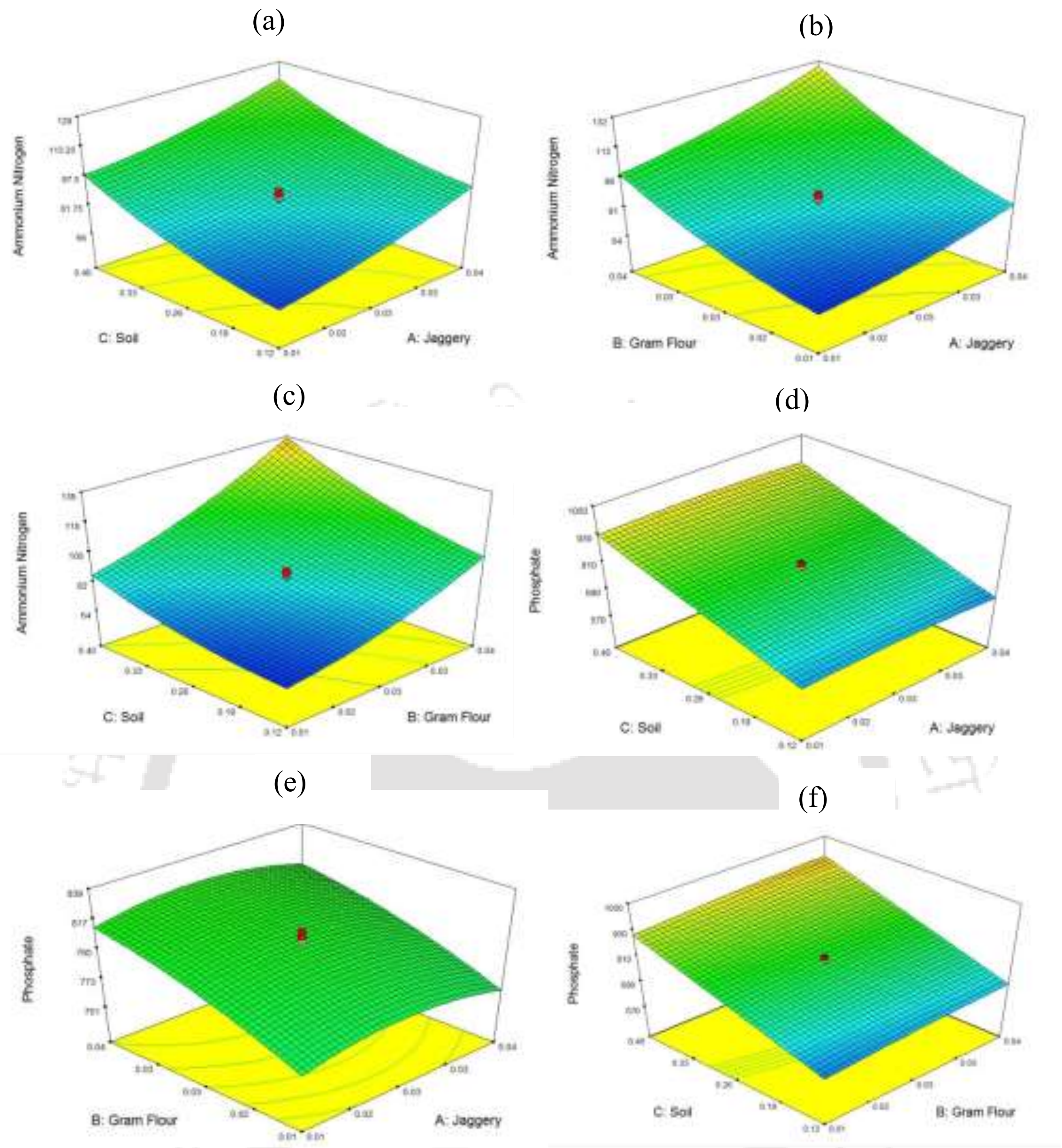


Fig. 3.4 (a-f): 3D surface plots depicting the influence of independent (jaggery, gram flour and soil) variables on the nutrient characteristics (AN and P) in the winter season.

As shown in the depicted 3D plots (Fig. 3.3 and 3.4 (a-f)), highly non-linear variation of the responses existed with respect to the variation in A, B and C. For the summer case, with an increase in jaggery (A) and gram flour (B), the AN concentration increased from 0.02-0.04 kg and 0.02-0.04 kg respectively (Fig. 3.3 (a-c)). Thus, both A and B had a positive influence on the AN nutrient yield. However, this has not been the case for the C from 0.2-0.4 kg. On the other hand, the P content increased with an increase in soil amount (Fig. 3.3 (d-f)).

For the winter case, all the factors i.e., B, C and A critically influenced the linear variation of AN nutrient yield (Fig. 3.4 (a-c)). However, for the P case, C and B but not A exhibited significant influence (Fig. 3.4 (d-f)). A photographic image of the modified Jeevamrutha bio-fertilizer formulation is shown in the Appendix B section of the Ph.D. thesis.

3.4.5 Microbial Growth Count of Optimally Constituted Jeevamrutha Bio-fertilizer Systems

The observed microbial counts (CFU/mL) for bacteria were 85×10^6 mL^{-1} and 47×10^6 mL^{-1} during summer and winter cases respectively. For fungi, actinomycetes, N-fixers and P-solubilizers during both seasonal periods, the microbial population were 9×10^4 mL^{-1} and 3×10^4 mL^{-1}; 7×10^4 mL^{-1} and 4×10^4 mL^{-1}; 18×10^4 mL^{-1} and 11×10^4 mL^{-1} and 36×10^4 mL^{-1} and 23×10^4 mL^{-1} respectively for the optimized Jeevamrutha formulations (Fig. 3.5). The findings are comparable with the prior art data with a marginal alteration in the microbial growth characteristics.

Also, the physicochemical characterization of the optimum composition of Jeevamrutha bio-fertilizer formulations has been summarized in Table 3.11. Macro- and micro-nutrients such as K, Ca, Mg, Na, Zn, Cu, Fe and Mn were characterized for their constitution in the bio-formulations and for their appropriate significance in plant growth and metabolism. On a similar note, electrical conductivity (EC) and Total Organic Carbon (TOC) along with pH, odour and colour were evaluated. Among all metals in the bio-fertilizer formulation, Zn concentration (0.0002%) was minimum whereas K and Ca concentrations (0.54 and 0.07%) were maximum.

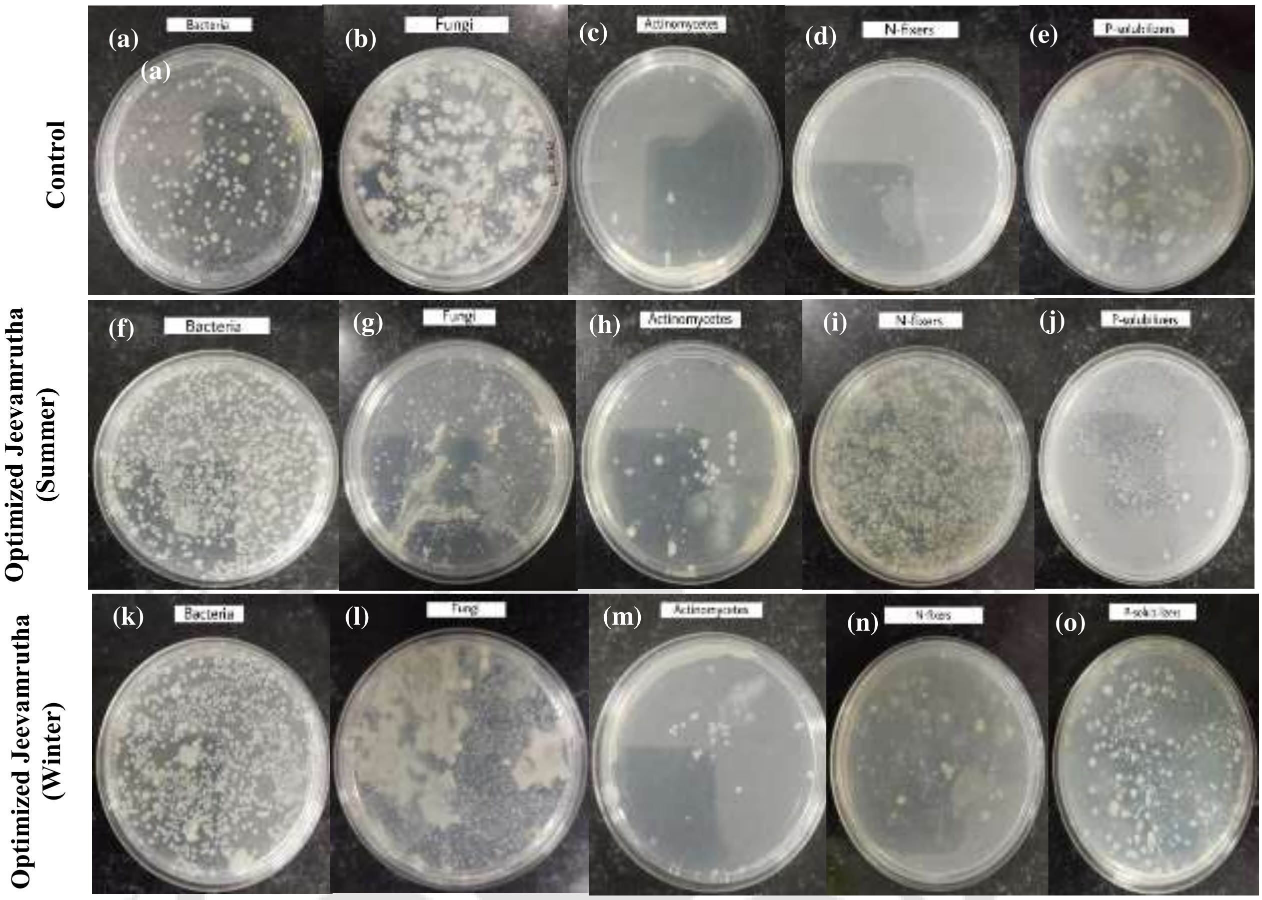


Fig. 3.5 Petri plate images depicting microbial count for various cases - control case (**a-e**); optimized Jeevamrutha in the summer season (**f-j**) and optimized in the winter season (**k-o**).

Table 3.11: Physicochemical characteristics of optimized Jeevamrutha bio-fertilizer formulation in the summer and winter seasons.

S.No.	Parameters	Methodology	Results
1.	pH	pH meter	S:8.07±0.5 W: 7.52±1.2
2.	EC	Conductivity meter	2.01±0.21 dS/m W:1.53±0.45dS/m
3.	OC	TOC Analyzer	S:0.62±0.04% W:0.31±0.08%
4.	K	Flame photometer	S:0.54±0.17% W:0.43±0.28%
5.	Ca	Flame photometer	S:0.07±0.04% W:0.04±0.02%
6.	Mg	AAS	S:0.0046±0.002% W:0.0021±0.0003%
7.	Na	Flame photometer	S:0.04±0.01% W:0.002±0.001%
8.	Zn	AAS	S:0.0058±0.001% W:0.0002±0.0001%
9.	Cu	AAS	S:0.0034±0.002% W:0.009±0.001%
10.	Fe	AAS	S:0.036±0.01% W:0.018±0.010%
11.	Mn	AAS	S:0.003±0.001% W0.003±0.001%
12.	Odour	Sensory analysis	Foul
13.	Colour	Visual analysis	Greenish black

3.4.6 Phytotoxicity Characteristics of Optimized Jeevamrutha Bio-fertilizer System

The overall influence of phytotoxic effects of the optimized Jeevamrutha bio-fertilizer on the mung bean plant for 5 days has been observed. The findings confirm 100% seed germination for all treated samples (25%, 50%, 75% and 100%) and the control group (0%). These findings henceforth confirmed that the treated solutions exhibited maturity and were non-toxic. After 5 days of the incubation period, the highest shoot length of the seedlings was observed for 75% (v/v) (3.6 cm) followed by 100% (v/v) (3.4 cm) test solutions and the lowest was achieved for 50% (v/v) (2.8 cm) and 25% (v/v) (3.2 cm) cases. Similarly, root length achieved the best results with 50% (v/v) (5 cm) and 25% (v/v) (4.1 cm) in comparison with 100% (v/v) (3.3 cm) and 75% (v/v) (3.5 cm) respectively. Therefore, maximum shoot and root length were achieved for 75% (v/v) and 50% (v/v) concentrations of the formulation (Fig. 3.6). The germination index (GI) of the mung bean plant has been highest for 50% (v/v) (87.87%) followed by 75% (v/v) (82.85%) treated sample. It was also observed that the GI was lowest for 25% (v/v) (58.04%) cases (Table 3.12). A major reason for not achieving a higher GI (90-95%) could be due to the presence of other heavy metals such as arsenic, cadmium or chromium in the optimized Jeevamrutha bio-fertilizer. Hence, 50% (v/v) was considered optimal for the modified Jeevamrutha bio-fertilizer compositional set with a higher GI. Thereby, the maturity of the optimized bio-fertilizer formulation in terms of the disappearance of phytotoxins can be inferred (Zucconi, 1981). A similar trend was achieved for the winter case. Sample calculation has been shown in Appendix D section of the Ph.D. thesis.

Table 3.12: A summary of the influence of optimized Jeevamrutha concentrations on the germination index, shoot and root length of mung bean plants in the summer season.

Sample Concentration (%)	**Germination Index (%)**	**Shoot length (cm)**	**Root length (cm)**
0	35	2.3±1.3	2.9±0.7
25	58	3.2±0.5	4.1±0.3
50	87.87	2.8±0.7	5.0±0.6
75	82.85	3.6±0.2	3.5±1.2
100	70.83	3.4±0.7	3.3±0.8

Fig. 3.6: Photograph depicting phytotoxicity characteristics of mung bean plant for various Jeevamrutha bio-fertilizer concentration cases (0%, 25%, 50%, 75% and 100%) in the summer season.

3.4.7 Literature Comparison

Devakumar et.al (2014) reported 1.96% N and 1730 mg/L P content with higher cow urine (0.1 L) content in the liquid Jeevamrutha bio-fertilizer formulation. Owing to its chemotherapeutic and other pharmaceutical uses, cow urine-based bio-fertilizers have a greater challenge for their generous inclusion as a key ingredient (Randhawa & Sharma, 2015). In this regard, the greater constitution of accessible resources such as soil in the formulation as a potent substrate would be helpful to achieve good quality formulations. In this study, with a reduced cow urine (0.02 L) content and enhanced soil quantity (0.36 kg), the optimized compositional set generated from the CCD design software were 0.03 kg jaggery, 0.03 kg gram flour and 0.35 kg soil during summer and 0.03 kg jaggery, 0.03 kg and 0.36 kg soil during winter cases (Table 3.13).

An incubation time of 9 days is required for the maximum constitution of TKN and AN. For P nutrient yield, 12 days of incubation was required. These modified optimized compositions for Assam have been compared with Palekar's Jeevamrutha formulation reported for Maharashtra, India. For the research conducted in this work, the AN, P and TKN were 289.6 mg/L, 976 mg/Land 0.21% respectively during the summer and 102.8 mg/L, 793 mg/L and 0.13% respectively during the winter seasons. The obtained results signified nutritional improvement with a small amendment in the developed formulations. Hence, they provide an insight into wider aspects of its commercial utility in organic farming and soil amendment. However, physical factors such as temperature, humidity and the quality of cattle feed on farms are highly responsible for the wider variations in the nutrient characterization of the bio-fertilizer.

In the reported optimized formulations, gram flour and soil are the most critical independent variables for AN and P respectively. From a precursor cost perspective, a marginal increase is relevant due to the comparatively higher constitution of jaggery and gram flour (0.03 kg) concerning Palekar's solid Jeevamrutha formulation (0.02 kg). The present study considers 0.167 kg A and B per kg soil, 0.056 L cow urine per kg soil and 2.78 kg cow dung per kg soil in comparison to the best-reported data by Devakumar et al. (2014) (0.04 kg A and B, 0.1 L cow urine and 1 kg cow dung with a handful of soil respectively) (Devakumar et al., 2014).

In this regard, it shall be noted that the findings report optimal solid Jeevamrutha formulations for the first time using a CCD-RSM-based statistical tool. The conducted investigations will not only sensitize upon the environmental aspects but also explore the economic benefits of the rurally available less expensive precursors in the developed formulations for improved soil health.

Table 3.13: Comparative assessment of optimized Jeevamrutha bio-fertilizer formulations with the best reported literature data.

S. No	A (kg)	B (kg)	C (L)	D (kg)	pH	CD (kg)	CU (L)	TKN (%)	P (mg/L)	Location	Sources
1.	0.2	0.2	20	0.1	7	1	1	0.03	177.1	Tamil Nadu	Anandan et al. 2016
2.	0.02	0.02	2	Handful	4.9	1	0.1	1.96	1730	Karnataka	Devakumar et al. 2014
3.	0.02	0.02	2	Handful	7	1	0.1	0.07	166	Karnataka	Gore and Sreenivasa 2011
4.	0.02	0.02	0.02	0.2	7.8	1	0.02	0.26	1248.1	Assam	**This work (PBD)**
5(i)S	0.03	0.03	0.02	0.35	8	1	0.02	0.67	1405.78	Assam	**This work**
(ii)W	0.03	0.03	0.02	0.36	8	1	0.02	0.28	1046.7		**(CCD)**

*A:Jaggery; B:Gram Flour; C:Water; D:Soil; CD:Cow dung; CU:Cow urine; S:Summer and W:Winter

3.5 Summary

A critical aspect of the thesis was to suggest robust analytical procedures and subsequent replacement of AN to serve as a marker representing TKN in the mentioned formulation. In the near future, key precursors such as cow urine and cow dung need detailed validation for their compositional influence on AN, TKN and P. To do so, firstly, the chosen reference composition was modified with lower cow urine and moisture content. This facilitates research toward greater utilization of farm waste stocks and indigenous protein and sugar sources. Secondly, the PBD proved to be an effective tool (91 and 92% R^2 for AN and P respectively and p less than 0.05) for the screening and identification of independent variables such as A (jaggery), B (gram flour) and C (water) for AN and B (gram flour) and D (soil) for P respectively. Thus, screened (critical) factors for Jeevamrutha were jaggery, gram flour and moisture content. Thirdly, among both S_H and S_L, the former provided a useful range to assist the screening process which was otherwise dubious for the lower parametric range. Fourthly, for Assam, the best TKN, AN and P referred to 0.26%, 388.1 mg/L and 1248.1 mg/L respectively. Fifthly, for another case, AN and TKN with Palekar's composition agreed proportionally to about 289.6 mg/L and 0.21% in the region. Finally, a temperature of 32–34°C and a humidity of 82–84% have been effective to achieve good quality Jeevamrutha formulations.

The optimized compositions of solid and modified Jeevamrutha formulations referred to 0.03 kg for jaggery and gram flour and 0.35-0.36 kg soil during the summer and winter seasons in Assam respectively. The sensitivity of individual components may be explained with the combination of A and B for AN and B and C for P. Further, phytotoxicity assay analysis inferred maturity and non-toxic status for the formulation. These findings provide needful support and justification for the wider application of the formulations as low-cost scalable bio-fertilizer due to their reduced preparation time to dynamically serve rural agricultural and sustainable soil fertility needs.

Chapter 4:

Optimality of Jeevamrutha Bio-fertilizer Modified with Vermicompost, Neem Cake, Tea Waste and Water Hyacinth

Optimality of Jeevamrutha Bio-fertilizer Modified with Vermicompost, Neem Cake, Tea Waste and Water Hyacinth

To further improvise upon the nutrient yield associated with the optimized modified Jeevamrutha bio-fertilizer formulations, investigations were addressed through the incorporation of commercial and rurally available substrates such as vermicompost, neem cake, tea waste and water hyacinth into the optimally modified Jeevamrutha bio-formulation. The findings are presented in this Chapter. After presenting a brief overview in section 4.1, section 4.2 delineates the results associated with the optimization of the dominant substrates deployed in the optimized modified Jeevamrutha bio-fertilizer formulations. Section 4.3 details respect to essential and trace elements during the bio-fertilizer preparation process and their probable benefit towards plant-soil interactions. Following this, section 4.4 devotes to the microbial diversity of the mentioned precursors with optimized modified Jeevamrutha bio-fertilizer and their specific role in nitrogen fixation and phosphate solubilization. Section 4.5 summarizes the phytotoxic effect of optimally constituted precursors-based modified Jeevamrutha fertilizer in the conducted research work. A comparison of the findings with the best available prior art has been addressed in section 4.6. Section 4.7 details respect to the conceptual cost indices of the mentioned precursors-modified optimal Jeevamrutha bio-fertilizer. Finally, a summary of the findings has been briefly presented in section 4.8 of the chapter.

Overview

In the previous chapter, screening and optimization efforts of the solid Jeevamrutha bio-fertilizer formulations with PBD and CCD-based statistical tools have proven to be promising in terms of physicochemical constituents, macro and micro-nutrients and organic carbon content. Nonetheless, further improvement of the optimized bio-fertilizer formulations for both summer and winter seasons in terms of enhanced soil fertility, N-fixing and P-solubilizing bacteria constitution and associated phytotoxic effects can be addressed. Such sub-objectives have been targeted in this chapter. Thereby, varied constitution ratio of vermicompost, neem cake, tea waste and water hyacinth has been considered for the summer and winter seasons respectively. In such efforts, commercial and raw substrates were ground and uniformly mixed with the Jeevamrutha bio-fertilizer. Thereby, the system was assessed in terms of %TKN, AN and P. An interesting feature of this work was to identify the optimized ratio of these substrates in terms of mentioned characteristics such as macro- and micro-nutrients, pH, EC and %OC, microbial plate count and phytotoxic effects. The seasonal variation is anticipated to have a critical role in the microbial population and its associated nutritional benefits for the optimally modified Jeevamrutha bio-fertilizer formulations.

4.1 Background

Organic bio-fertilizers such as Jeevamrutha, Beejamrutha and Panchagavya are judicious combinations of cow manure and can be easily prepared from rurally available substrates. Jeevamrutha bio-fertilizer is a good plant growth enhancer and catalyzes and promotes nutrient sustainability and agro-ecosystems (Sakthivel, Dhanapal, et al. 2022). Further, it shall be noted that green manures such as vegetable waste, fruit peels and dried leaves have been predominantly comprehended for their utility to alter the dynamics of soil properties through the composting mechanism (Mercy, Mubsira, et al. 2014, Panwar 2015). Similarly, rural resources such as tea waste and water hyacinth along with commercial precursors such as vermicompost and neem cake can replenish the soil fertility and thereby overcome the limiting factor and issues associated with macro- and micro-nutrient deficiencies. Low yields and poor quality of the crops could be further addressed through the incorporation of these precursors in the modified Jeevamrutha bio-fertilizer formulations. Thereby, pertinent agricultural practices that support organic and natural farming can be widely explored.

In a relevant prior art, the incorporation of vermicompost and G5-soil enricher granules into liquid Jeevamrutha bio-fertilizer did convey a better growth profile for *Amaranthus caudataus* (Rosi and Thatheyus, 2018). Also, neem cake amended with biofertilizer (Phosphate solubilizing bacteria, Azotobacter and Azospirillum) and weed manure has enhanced the soil fertility and crop yield of *Phaseolus aureus* (Bhalshakar 2020).

Further, tea powder which is considered to be a biodegradable waste was proven to be a good source of compost. Thereby, the prepared compost enhanced the phosphorus, calcium, magnesium, chloride and sulphate concentration in the soil (Gurav and Sinalkar 2013). Similarly, plant growth and germination period exhibited better increment during the composting process. Also, milk tea waste blended with chemical fertilizer has been proven to improve the biochemical properties of desert soil and wheat growth (Sial, Liu, et al. 2019). On the other hand, the applications of the natural substrate such as water hyacinth in terms of deduced organic liquid fertilizer blend with chemical fertilizer affirmed higher productivity of *Crotalaria ochroleuca* vegetable (Andika, Ogada, et al. 2016). Thereby, it can be hypothesized that the water hyacinth can also serve as a good organic source for the enhancement of critical characteristics of modified Jeevamrutha bio-fertilizer formulations.

In summary, the integration of commercial and rural precursors such as vermicompost, neem cake, tea waste and water hyacinth into the modified Jeevamrutha bio-fertilizer formulations could further improve the nutrient characteristics in terms of their physicochemical characteristics, macro-nutrients, organic carbon, microbial load, crop productivity and soil health, especially during the nutrient decline observed during the winter seasons. Thereby, useful benchmarks can be set for the production of superior, cost-effective and nutrient-rich optimal bio-fertilizer formulations for deployment in demanding circumstances such as the winter seasons of Assam, India. With such efforts, sustainable plant nutrition needs and demands can be effectively met by reducing or mitigating chemical fertilizers.

4.2 Commercial and Rural Resources Constitution in the Modified Jeevamrutha Bio-fertilizer Systems

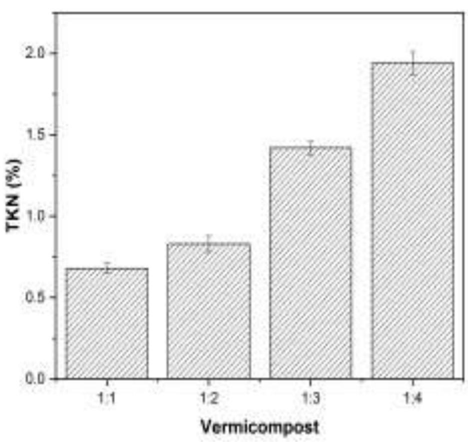
Unconventional commercial and rurally available resources such as vermicompost, neem cake, tea waste and water hyacinth have been subjected to optimization studies with the previously optimized modified Jeevamrutha bio-fertilizer formulations. Thereby, the ratio of the formulations to the precursors was altered as 1:1-1:4, 1:1-1:6, 1:1-1:5 and 1:1-1:5 respectively and for a fixed constitution of the Jeevamrutha. Accordingly, the study was targeted to invoke further nutrient improvement and enhancement in the microbial count in terms of N-fixing and P-solubilizing bacteria in the targeted formulations.

4.3 Optimal Constitution of Commercial and Rural Precursors in the Alternate Jeevamrutha Bio-fertilizer Formulations

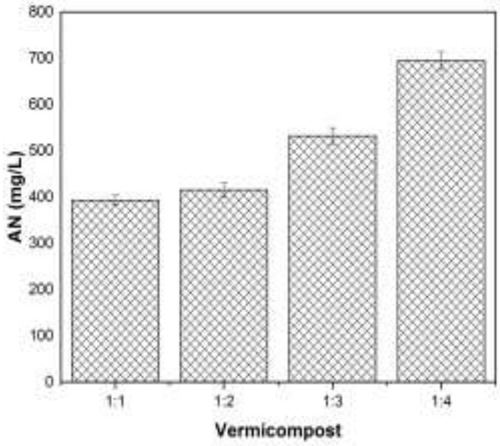
For a variation in the ratio of optimal Jeevamrutha bio-fertilizer (cow dung, cow urine, jaggery, gram flour, soil, water and pH as 1 kg, 0.02 L, 0.03 kg, 0.03 kg, 0.35 kg, 0.04

L and 8 respectively during summer and 1 kg, 0.02 L, 0.03 kg, 0.03 kg, 0.35 kg, 0.04 L and 8 respectively during winter seasons), with respect to vermicompost (1:1-1:4), neem cake (1:1-1:6), tea waste (1:1-1:5) and water hyacinth (1:1-1:5), relevant studies were conducted.

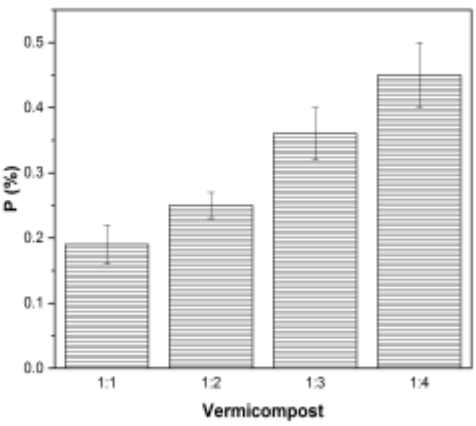
(a)



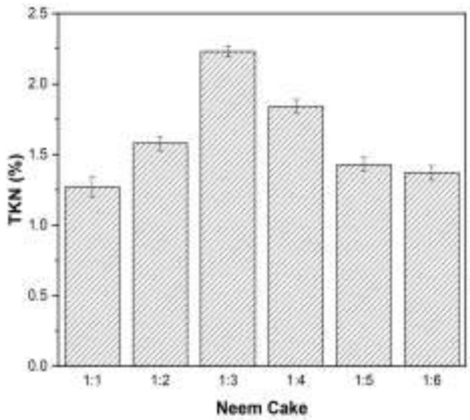
(b)



(c)



(d)



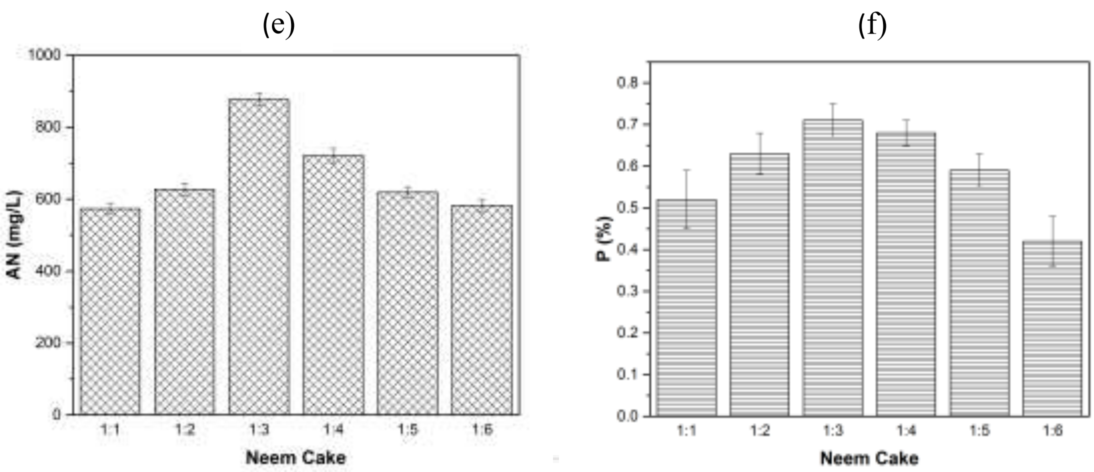
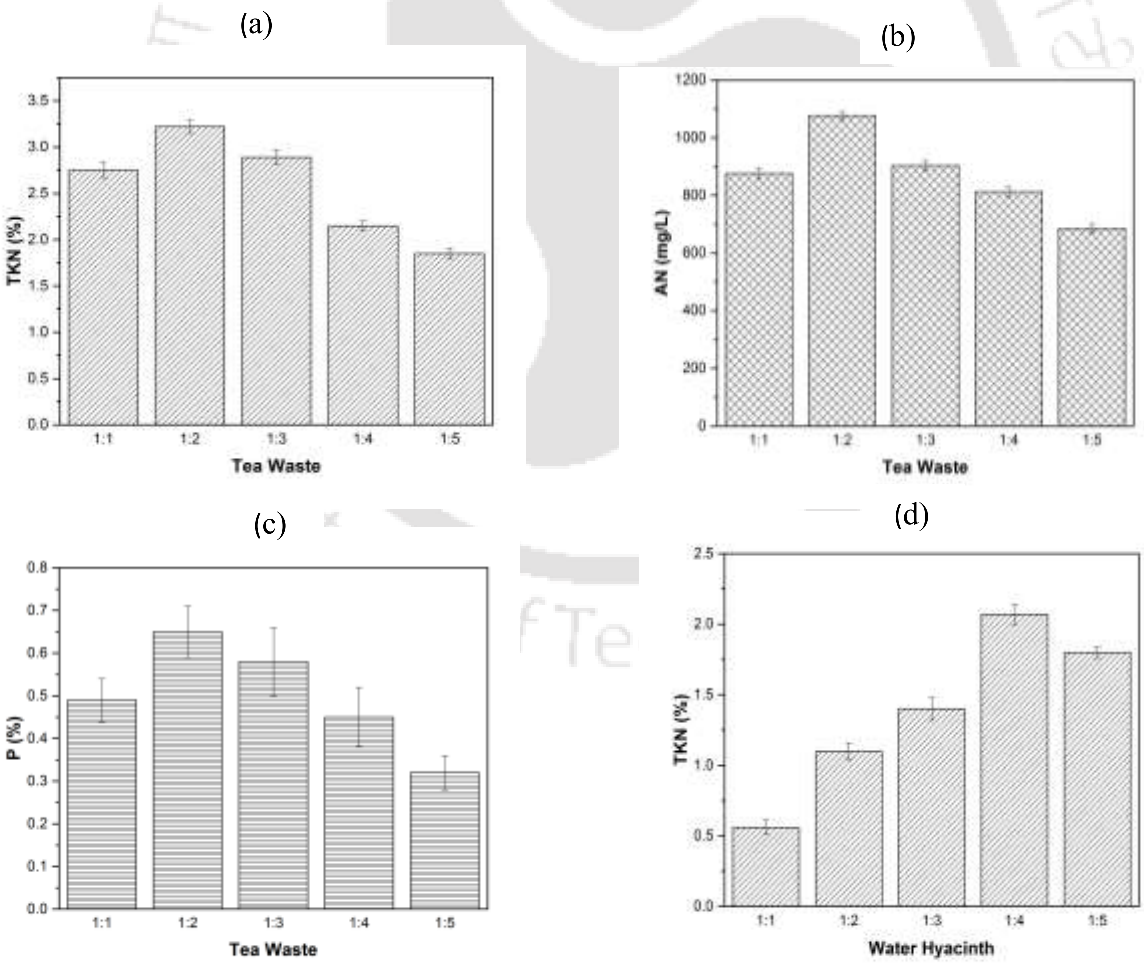


Fig. 4.1: Bar charts depicting the influence of commercial precursors proportion on the nutrient yield (% TKN (a & d); AN (b & e) and %P (c & f)) with modified Jeevamrutha bio-fertilizer system - Vermicompost (a-c); Neem cake (d-f).



(e) (f)

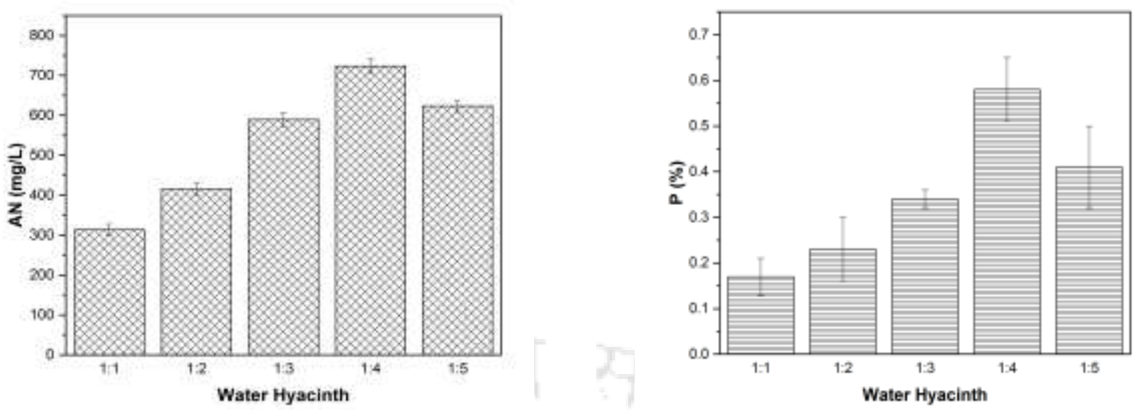


Fig. 4.2: Bar charts depicting the influence of rural precursors proportion on the nutrient yield (% TKN (a & d); AN (b & e) and %P (c & f)) with modified Jeevamrutha bio-fertilizer system – Tea waste (a-c); Water Hyacinth (d-f).

Fig. 4.1 and Fig. 4.2 (a-c) and (d-f) illustrate the graphical representation of %TKN, AN and %P nutrient yield of precursors with modified Jeevamrutha bio-fertilizer. It is evident that %TKN is highly comparable with the trends associated with AN content. Better %TKN and AN nutrient yield was achieved for tea waste (1:2) followed by neem cake (1:3). On the other hand, for P content, neem cake (1:3) followed by tea waste (1:2) was considered best among all treatments. A similar trend was obtained for all the precursors with modified Jeevamrutha bio-fertilizer during the winter season. Further, tea waste could be a potential resource for garden and landscape farming because of the presence of tannic acid residues that could aid in the decomposition process in the Jeevamrutha bio-fertilizer (Gurav and Sinalkar 2013).

Thereby, based on the nutrient characteristics such as %TKN, AN and P content, the optimal Jeevamrutha bio-fertilizer formulations with optimal ratio of vermicompost, neem cake, tea waste and water hyacinth were 1:4, 1:3, 1:2 and 1:4 respectively during the summer season and 1:4, 1:3, 1:2 and 1:3 respectively during the winter season.

For both seasons, the optimum dataset for each precursor was evaluated in terms of the physicochemical characteristics and macro-nutrient composition (Table 4.1). The macro-nutrients such as %TKN, AN and P have shown higher nutrient uptake for vermicompost, neem cake, tea waste and water hyacinth during the summer season (1.94%, 695.1 mg/L and 0.45%; 2.24%, 873.56 mg/L and 0.71%; 3.22%, 1076.4 mg/L and 0.65%; 2.07%, 723.81 mg/L and 0.58% respectively) in comparison with the winter season (0.85%, 416.6 mg/L and 0.32%; 1.78%, 623.4 mg/L and 0.64%; 1.94%, 726.8 mg/L and 0.57%; 1.42%, 525.7 mg/L and 0.44% respectively). The seasonal variation in the nutritional properties might be associated with the rapid nutrient release owing to N-fixation, denitrification and solubilizing and mineralizing inorganic and organic P content by a large soil microflora for the summer season case due to its higher temperature and relative humidity (Bhattacharyya, Dey et al. 1984, Meng, Ding et al. 2005). Similarly, %moisture content for vermicompost, neem cake, tea waste and water hyacinth varied proportionally as 22.6-25.8, 11.2-19.4, 15.3-21.3 and 21.6-27.2 respectively. A higher moisture level along with organic carbon content was observed for water hyacinth (27.2% and 3.3% respectively) for a temperature and relative humidity range of 32-37°C and 88-92% respectively.

Soil nutrition and health are highly influenced by its essential micro-nutrient constitution. The constitution of micro-nutrients such as Cu, Zn, Ca, Fe, Mn and Mg in optimally formulated vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer formulations for summer and winter seasons have been summarized in Table 4.2. It is well-known that the presence of these elements in the form of salts is beneficial for the soil. Soil deficient in Cu, Fe and Mn could lead to a decline in photosynthesis, colouration abnormalities and depletion in plant electron transport mechanism.

Table 4.1: Physicochemical characteristics of commercial and rural precursors with optimally modified Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Precursors	**AN (mg/L)**		**TKN (%)**		**P (%)**		**Moisture Content (%)**		**pH**		**EC (dS/m)**		**OC (%)**	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Vermicompost -modified Jeevamrutha	695.1 ±3.25	416.6 ±2.23	1.94 ±0.05	0.85 ±0.03	0.45 ±0.07	0.32 ±0.02	25.8 ±1.24	22.6 ±1.37	7.78 ±1.5	7.65 ±1.1	2.9 ±0.4	2.8 ±0.08	2.6 ±0.03	1.6 ±0.07
Neem Cake - modified Jeevamrutha	873.56 ±4.72	623.4 ±5.89	2.24 ±0.07	1.78 ±0.04	0.71 ±0.02	0.64 ±0.08	19.4 ±1.3	11.2 ±0.78	8.0 ±0.8	7.8 ±0.5	0.48 ±0.15	0.52 ±0.06	2.1 ±0.51	1.9 ±0.14
Tea Waste - modified Jeevamrutha	1076.4 ±5.87	726.8 ±7.26	3.22 ±0.31	1.94 ±0.07	0.65 ±0.03	0.57 ±0.06	21.3 ±1.56	15.3 ±2.96	6.4 ±0.5	6.5 ±0.74	1.54 ±0.26	1.13 ±0.09	1.8 ±0.07	1.5 ±0.17
Water Hyacinth - modified Jeevamrutha	723.81 ±4.62	525.7 ±5.87	2.07 ±0.15	1.42 ±0.04	0.58 ±0.11	0.44 ±0.08	27.2 ±1.45	21.6 ±2.25	6.8 ±0.3	7.1 ±0.5	0.57 ±0.04	0.53 ±0.13	3.3 ±0.23	2.7 ±0.03
Jaggery-based Jeevamrutha	427.56 ±3.55	135.8 ±2.75	0.67 ±0.04	0.28 ±0.03	0.14 ±0.02	0.10 0.01	17.8 ±0.67	15.3 ±1.78	8.07 ±0.5	7.52 ±1.2	2.01 ±0.21	1.53 ±0.45	0.62 ±0.04	0.31 ±0.08

*S:Summer;W:Winter

Also, soil lacking Zn content causes depletion of plant growth hormones such as auxin which leads to a decline in plant leaf size. Thereby, it also influences several molecular and physiological mechanisms (Umair Hassan, Aamer, et al. 2020). Ca deficiency results in poor nitrogen absorption in soil and a defective plant root system (Russell 2002). Similarly, Mg is an outstanding activator for multiple enzymatic reactions associated with phosphorus-rich components in the soil (Ishfaq, Wang, et al. 2022). Hence, unfolding the interactions between the precursors-modified Jeevamrutha bio-fertilizer formulations and the soil nutrient uptake of the plant can settle many relevant questions along with justification. This includes solubility and soil pH level, and a mycorrhizal association between fungal colonies and plant rhizosphere (Rillig 2004).

Table 4.2: Macro- and micro-nutrient characteristics of commercial and rural precursors with optimally modified Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Metals (%)	Vermicompost		Neem cake		Tea waste		Water Hyacinth	
	S	W	S	W	S	W	S	W
Cu	0.056 ±0.003	0.042 ±0.01	0.042 ±0.02	0.031 ±0.004	0.048 ±0.03	0.032 ±0.02	0.022 ±0.01	0.007 ±0.004
Zn	0.003 ±0.001	0.02 ±0.004	0.004 ±0.001	0.002 ±0.0001	0.0002 ±0.001	0.004 ±0.001	0.01 ±0.005	0.07 ±0.03
Ca	0.084 ±0.003	0.032 ±0.002	0.18 ±0.006	0.07 ±0.03	0.035 ±0.02	0.038 ±0.002	0.074 ±0.02	0.06 ±0.01
Fe	0.06 ±0.004	0.04 ±0.01	0.12 ±0.004	0.08 ±0.05	0.18 ±0.008	0.07 ±0.03	0.24 ±0.008	0.17 ±0.008
Mn	0.008 ±0.001	0.003 ±0.001	0.006 ±0.001	0.002 ±0.001	0.032 ±0.01	0.026 ±0.01	0.065 ±0.01	0.061 ±0.002
Mg	0.04 ±0.005	0.01 ±0.007	0.06 ±0.02	0.02 ±0.004	0.02 ±0.005	0.009 ±0.001	0.008 ±0.002	0.002 ±0.001

*S:Summer;W:Winter

Thus, as summarized in Table 4.2, %Cu, %Zn and %Ca altered as 0.042-0.056%, 0.031-0.042%, 0.032-0.048% and 0.007-0.022%; 0.003-0.02%, 0.002-0.004%, 0.004-

0.0002%, 0.01-0.07% and 0.032-0.084%, 0.07-0.18%, 0.035-0.038% and 0.06-0.07% respectively for vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha for both seasons. Similarly, %Fe, %Mn and %Mg for Cu residues vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha varied as 0.04-0.06%, 0.08-0.12%, 0.07-0.18% and 0.17-0.24%; 0.003-0.008%, 0.002-0.006%, 0.026-0.032% and 0.061-0.065% and 0.01-0.04%, 0.02-0.06%, 0.009-0.02% and 0.002-0.008% respectively during the summer and winter seasons.

Cu residues were highest in vermicompost-modified Jeevamrutha bio-fertilizer formulation (0.056%), Zn and Mn in water hyacinth (0.07% and 0.065%), Ca and Mg in neem cake (0.18% and 0.06%) and Fe in water hyacinth (0.24%) followed by tea waste (0.18%). Hence, it could be observed that the utilization of loamy soil in the conducted study for the development of precursors-modified Jeevamrutha bio-fertilizer formulations was a good choice for its rich constitution of macro- and micro-nutrients. Thereby, further investigation of the bio-fertilizer system for plant-soil interaction can provide an inherent advantage to minimizing the consumption of chemical fertilizers.

4.4 Microbial Characteristics of Optimally Constituted Jeevamrutha Bio-fertilizer System with Commercial and Rural Precursors

Table 4.3 presents an account of various microbial populations in the optimally prepared vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer formulations. Thus, the precursors-modified Jeevamrutha bio-fertilizer formulations conveyed a higher constitution of naturally occurring microbes such as bacteria, fungi, actinomycetes, N-fixers and P-solubilizers. The microbial density significantly varied from the day after preparation (DAP) (CFU/mL) ((Vermicompost: Bacteria, 21-74×10^5; Fungi, 2-6×10^4; Actinomycetes, 3-7×10^2; N-fixers, 6-18×10^4 and P-solubilizers, 11-21×10^5), (Neem cake: Bacteria, 107-219×10^5; Fungi, 2-1×10^4; Actinomycetes, 5-11×10^2; N-fixers, 74-126×10^4 and P-solubilizers, 37-95×10^5), (Tea waste: Bacteria, 97-321×10^5; Fungi, 2-4×10^4; Actinomycetes, 9-22×10^2; N-fixers, 105-178×10^4 and P-solubilizers, 57-104×10^5) and (Water Hyacinth: Bacteria, 113-192×10^5; Fungi, 3-5×10^4; Actinomycetes, 3-9×10^2; N-fixers, 32-103×10^4 and P-solubilizers, 25-96×10^5) mL^{-1} respectively). A similar trend was achieved for the precursors-modified Jeevamrutha bio-fertilizer systems during the winter season. All the precursors except water hyacinth exhibited higher microbial count for day 7 during the summer and day 12 during the winter seasons respectively. For the water hyacinth case, the maximum CFU for the microbes was obtained between days 10-14 and 11-13 respectively. This is due to the fact that water hyacinth slowly releases nutrients and thereby significantly alters time-dependent microbial activity (Andika, Ogada, et al. 2016).

A higher microbial population was observed for tea waste (CFU/mL) (Bacteria, 243-321×10^5; Fungi, 2-4×10^4; Actinomycetes, 17-22×10^2; N-fixers, 124-178×10^4 and P-solubilizers, 82-104×10^5) followed by neem cake (Bacteria, 164-219×10^5; Fungi, 2-1×10^4; Actinomycetes, 9-11×10^2; N-fixers, 108-126×10^4 and P-solubilizers, 67-95×10^5) and water hyacinth (Bacteria, 157-192×10^5; Fungi, 2-5×10^4; Actinomycetes, 5-9×10^2; N-fixers, 84-103×10^4 and P-solubilizers, 58-96×10^5 mL^{-1}) precursors-modified Jeevamrutha bio-fertilizer cases. Maximum N-fixers and P-solubilizers were obtained for tea waste-modified Jeevamrutha bio-fertilizer during summer (178×10^4 and 104×10^5 mL^{-1}) and winter (124×10^4 and 82×10^5 mL^{-1}) seasons respectively. In the prior art, several bacterial isolates such as *Actinobacteria, Bacillus cereus* and *Cyanobacteria* have been extracted from tea leaves biochar and were opined to enhance soil properties, organic carbon, enzymatic activity and crop productivity (Azeem, Hassan, et al. 2021).

Table 4.3: Microbial plate count data of commercial and rural precursors-modified optimal Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Precursors	Bacteria (10^{-5})		Nitrogen fixers (10^{-4})		Phosphate Solubilizers (10^{-5})		Actinomycetes (10^{-2})		Fungi (10^{-4})	
	S	W	S	W	S	W	S	W	S	W
Vermicompost	74	56	18	11	21	16	7	5	6	8
Neem Cake	219	164	126	108	95	67	11	9	1	2
Tea Waste	321	243	178	124	104	82	22	17	4	2
Water Hyacinth	192	157	103	84	96	58	9	5	5	2
Control	56	48	7	5	14	11	2	1	8	7

All standard deviations for the microbes (bacteria, N-fixers, P-solubilizers, actinomycetes and fungi) were in the range 2-4 and 4-7 respectively.

Following this, neem cake-modified Jeevamrutha bio-fertilizer exhibited a higher bacterial N-fixation and P-solubilization during summer (219×10^4 and 164×10^5 mL^{-1}) and winter (95×10^4 and 67×10^5 mL^{-1}) seasons respectively. Also, the least fungal growth was observed for the neem cake modified case and henceforth justified its utility as a fungicide. In the previous study, plant growth-promoting rhizobacteria have shown a better multiplication of growth with the application of neem cake to improve tea cultivation and yield (Bagyalakshmi, Balamurugan, et al. 2012). Therefore, the conducted studies support the need for the precursors-modified Jeevamrutha bio-fertilizer systems to improve soil health characteristics and thereby facilitate their application as reliable components in sustainable agriculture.

4.5 Phytotoxicity Characteristics of Optimally Constituted Jeevamrutha Bio-fertilizer System with Commercial and Rural Precursors

Table 4.4 (a) details the phytotoxic effects of vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer systems and for the summer season case. GI was highest for vermicompost-modified Jeevamrutha bio-fertilizer case (29.11-87.87%) with a peak at 50% sample concentration and tea waste-modified Jeevamrutha bio-fertilizer case (29.21-87.87%) with a peak at 50% sample concentration. This was followed by the neem cake case (25.12-81.23%) with a peak at 25% sample concentration. Maximum shoot and root length were achieved for 50% vermicompost case (7.3 and 5.3 cm) followed by 25% neem cake case (6.2 and 10.1 cm). Similarly, additional substrates such as tea waste (50%) exhibited higher root length (10.9 cm) whereas, water hyacinth (25%) have shown maximum shoot length (5.5 cm). Hence, it could be interpreted that 50% of vermicompost and tea waste constitution were promising and 25% of neem cake and water hyacinth-modified Jeevamrutha cases were promising due to better root and shoot length in the summer season.

The data presented in Table 4.4 (b) represents the phytotoxicity assay data of the winter season bio-fertilizer systems and for alternate commercial and rurally available precursors. It was observed that the GI was highest for vermicompost (37.23-82.85%) and tea waste (41.75-81.45%)-modified Jeevamrutha bio-fertilizer cases with a peak at 50% sample concentration. This was followed by the neem cake-modified Jeevamrutha bio-fertilizer case (38.26-77.84%) with a peak at 50% sample concentration. Also, maximum shoot and root length were achieved for 50% neem cake and tea waste-modified Jeevamrutha bio-fertilizer cases (6.6 and 9.2 cm) followed by 50% water hyacinth and neem cake-modified Jeevamrutha bio-fertilizer cases (5.1 and 10.1 cm).

Table 4.4 (a): Phytotoxicity characteristics (germination index, shoot length and root length) of commercial and rural precursors with modified Jeevamrutha bio-fertilizers and mung bean plant system in the summer season.

Precursors	Sample Concentration (%)	Germination Index (%)	Shoot Length (cm)	Root Length (cm)
Vermicompost	0	29.11	3.4±1.2	3±0.1
	25	81.27	6.1±0.3	3.2±0.3
	50	87.87	7.3±0.4	5.3±1.1
	75	72.34	5.6±0.7	3.6±0.2
	100	68.17	4.4±0.4	3.5±0.1
Neem cake	0	25.12	5.4±0.1	4.8±0.4
	25	81.23	6.2±0.1	10.1±0.2
	50	69.31	4.1±0.5	6.6±1.2
	75	53.17	3.8±1.0	5.4±1.2
	100	32.62	3.5±0.5	1.9±0.3
Tea waste	0	29.21	3.4±0.2	3.2±0.4
	25	86.38	4.7±0.3	10.2±0.4
	50	87.87	4.4±0.1	10.9±0.3
	75	85.26	5.3±0.3	10.1±0.4
	100	78.64	4.3±0.4	6.4±0.1
Water hyacinth	0	24.04	2.4±0.2	5.1±0.4
	25	74.58	5.5±0.1	8±1.3
	50	62.65	4.5±0.4	7.8±1.4
	75	56.71	4±0.2	6±0.4
	100	48.37	3.6±0.3	4±0.3

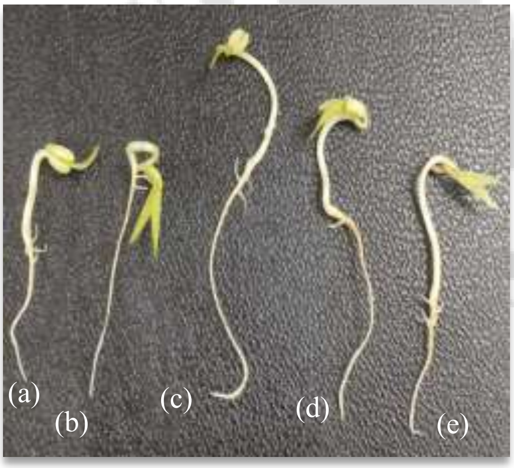
*All standard deviations for the germination index were in the range of 0.5-0.7 and 5-8 respectively.

Hence, 50% vermicompost and neem cake concentration in the precursors-modified Jeevamrutha bio-fertilizers affirmed promising characteristics in terms of higher shoot and root length (2.7 and 3.7 cm; 6.6 and 7.6 cm respectively). On the other hand, at the 50% concentration of tea waste and water hyacinth-modified Jeevamrutha bio-fertilizers, higher shoot and root lengths were achieved (4.8 and 9.2 cm; 5.1 and 9.8 cm respectively). Therefore, it could be concluded that the 50% concentration affirming commercial and rurally available precursor-modified Jeevamrutha bio-fertilizers possessed maturation on the mung bean plant. Thereby, to ensure the farmer's appropriate application of precursors-modified Jeevamrutha bio-fertilizers into the agricultural scenario, the concentration of the matured bio-fertilizer system should be as per the dosage identified in the conducted case studies.

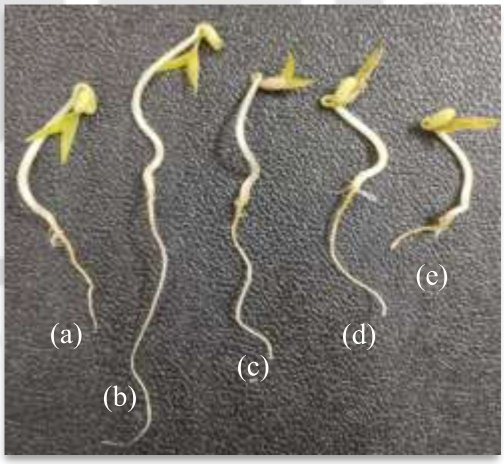
Table 4.4 (b): Phytotoxicity characteristics (germination index, shoot length and root length) of commercial and rural precursors with modified Jeevamrutha bio-fertilizers and mung bean plant system in the winter season.

Precursors	Sample Concentration (%)	Germination Index (%)	Shoot Length (cm)	Root Length (cm)
Vermicompost	0	37.23	1.3±0.5	2.3±0.2
	25	67.23	1.6±1.2	2.6±1.1
	50	82.85	2.7±0.7	3.7±0.3
	75	41.68	1.4±0.1	2.5±0.7
	100	39.41	1.3±0.6	2.4±0.4
Neem cake	0	38.26	3.5±0.1	4.5±0.4
	25	56.31	4.7±0.1	6.3±0.2
	50	77.84	6.6±0.5	7.6±1.2
	75	42.46	3.4±1.0	5.6±1.2
	100	36.87	1.9±0.5	2.6±0.4
Tea waste	0	41.75	1±0.4	7±1.2
	25	52.44	1.5±0.6	8±0.1
	50	81.45	4.8±0.2	9.2±1.4
	75	72.04	3.5±1.3	5.5±0
	100	70.53	3.4±0.3	4.5±0.8
Water hyacinth	0	23.43	2.2±1.5	2.4±1.6
	25	32.21	3.1±0.3	4.7±1.1
	50	65.27	5.1±0.5	9.8±0.7
	75	41.75	4.2±0.6	3.8±1.3
	100	36.82	3.6±0.8	3.5±1.1

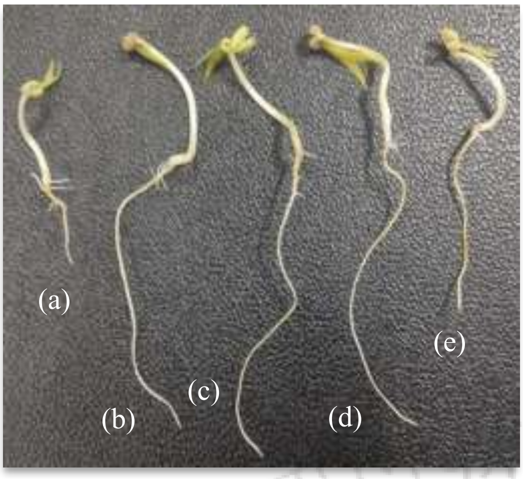
*All standard deviations for the germination index were in the range of 0.5-0.7 and 5-8 respectively



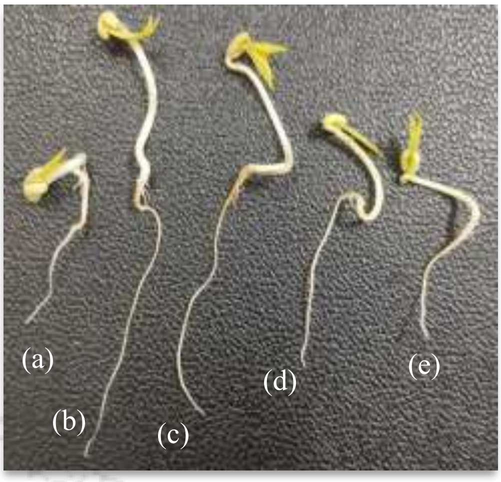
(i) Vermicompost



(ii) Neem Cake

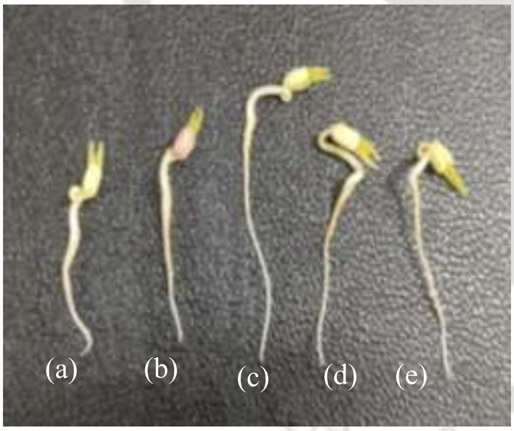


(iii) Tea waste

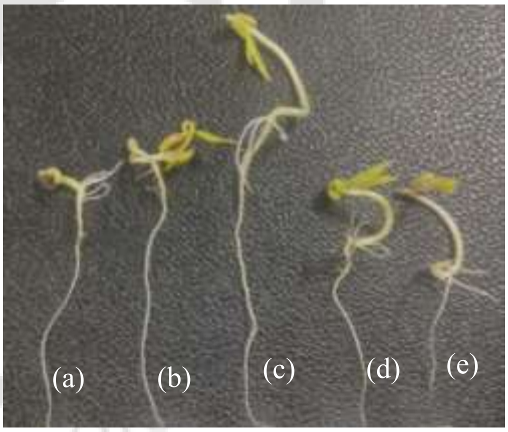


(iv) Water Hyacinth

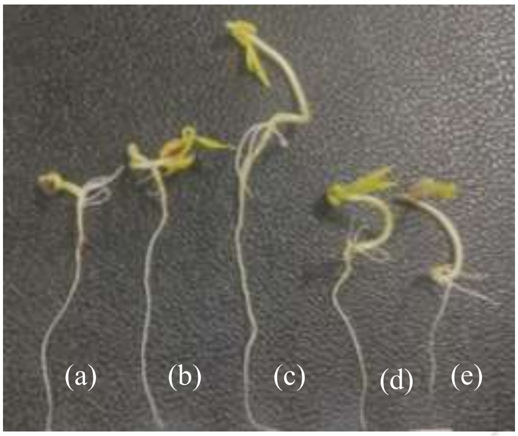
Fig. 4.3: Photographs depicting the phytotoxicity assessment characteristics of **(i)** Vermicompost, **(ii)** Neem cake, **(iii)** Tea waste and **(iv)** Water Hyacinth with optimal modified Jeevamrutha bio-fertilizers and mung bean plant system in the summer season. Labels a, b, c, d and e refer to 0, 25, 50, 75 and 100% concentration cases.



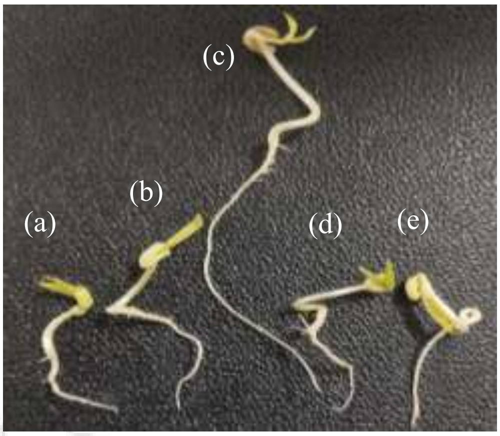
(i) Vermicompost



(ii) Neem Cake



(iii) Tea waste



(iv) Water Hyacinth

Fig. 4.4 Photographs depicting the phytotoxicity assessment characteristics of **(i)** Vermicompost, **(ii)** Neem cake, **(iii)** Tea waste and **(iv)** Water Hyacinth with optimal modified Jeevamrutha bio-fertilizers and mung bean plant system in the winter season. Labels a, b, c, d and e refer to 0, 25, 50, 75 and 100% concentration cases.

Fig. 4.3 and 4.4 illustrates the phytotoxic influence on the shoot and root length for various concentrations of vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizers. The conducted study affirmed the maturity of the developed bio-fertilizer systems for their application on the mung bean plant. Thereby, for all cases, no inhibition or adverse impact has been confirmed towards the plant growth pattern by the constituents of the precursors-modified Jeevamrutha bio-fertilizer systems.

4.6 Literature Comparison

Rosi and Thatheyus (2018) conducted a study targeting the integrated farming system of Jeevamrutha, vermicompost along with G5-soil enricher granules. Thereby, the authors assessed the growth profile of *Amaranthus aritis* (Rosi and Thatheyus, 2018). For the case, %TKN and %P were 1.5 and 0.3% respectively. Thereby, the current work signifies a better nutrient yield without any additional soil enrichers. In this work, for all cases, the %TKN and

%P yield varied as 0.85-1.94% and 0.32-0.45% during the summer and winter seasons respectively. On the other hand, another investigation involved the integration of 15% urea with 6 mg of neem seed cake for soil properties and for the evaluation of microflora along with %N and %P nutrient content (5.5 and 0.55% respectively) (Elnasikh, Osman, et al. 2011). In this work, the %TKN and %P yield for neem cake-modified Jeevamrutha bio-fertilizer varied proportionally as 1.78-2.23% and 0.64-0.71% respectively for both summer and winter seasons.

Similarly, in another report, a higher %N (3.7%) and %P (0.42%) yield were achieved for milk tea waste that was amended with chemical fertilizer (Sial, Liu, et al. 2019). The present work targeted the incorporation of tea waste with Jeevamrutha bio-fertilizer formulations and enabled %TKN and %P content alteration as 1.94-3.22 and 0.57-0.65% respectively during the summer and winter seasons. This is significantly higher as no inorganic resources were utilized during the preparation process. Also, Andika et al. (2016) used water hyacinth as an organic liquid fertilizer for the growth study of *Chrotalaria ochroleuca* vegetables. For the case, the %P and nitrate concentration were 0.003 and 0.00016% respectively (Andika, Ogada, et al. 2016). On the contrary, this work targeted the modified Jeevamrutha with water hyacinth, a rurally available resource and an annual water plant. Thereby, the %TKN and %P content of the modified bio-fertilizer altered as 1.42-2.07% and 0.44-0.58% respectively.

In summary, all precursors-modified Jeevamrutha bio-fertilizers were better than the vermicompost-modified Jeevamrutha bio-fertilizer case (1:4) during the summer season. Also, the conducted research work not only targets cost-effective precursors readily available in rural scenarios but emphases on the nutrient yield variation for two consecutive seasons i.e., summer and winter seasons respectively. Thus, the reported fertilizer systems are novel, low-cost and eco-friendly fertilizer systems.

Table 4.5: Comparative assessment of best nutrient yield data achieved for commercial and rural precursors-modified Jeevamrutha bio-fertilizer systems with best reported organic bio-fertilizer systems and precursors in the literature.

S.No.	Precursors	TKN (%)	AN (mg/L)	P (%)	Sources
1.	Tea waste - modified Jeevamrutha (1:2)	S:3.22 W:1.94	S:1076.4 W:726.8	S:0.65 W:0.57	**This work**
2.	Neem cake - modified Jeevamrutha (1:3)	S:2.23 W:1.78	S:876.56 W:623.4	S:0.71 W:0.64	**This work**
3.	Water hyacinth - modified Jeevamrutha (S:1:4; W:1:3)	S:2.07 W:1.42	S:723.81 W:525.7	S:0.58 W:0.44	**This work**
4.	Vermicompost - modified Jeevamrutha (1:4)	S:1.94 W:0.85	S:695 W:416.6	S:0.45 W:0.32	**This work**
5.	Vermicompost (VC) (1:4)	1.5	-	0.3	Rosi and Thatheyus, 2018
6.	Water hyacinth (WH) (200 kg/ha)	0.00016 (Nitrate)	-	0.003	Andika at al. 2016
7.	Neem seed cake (NC) (60 g soil+15%urea and 6mg neem cake)	5.5 *Enhanced N-fixers*	-	0.55	Elnasikh et al. 2011
8.	Milk Tea waste (TW) (5%)	3.7	-	0.42	Sial et al. 2019

Table 4.6: Conceptual cost indices and nutrient yield summary of commercial and rural precursors-modified Jeevamrutha bio-fertilizer formulations.

S. No.	Precursors	Precursors Used/kg of modified system (g)	Jeevamrutha used/kg of modified system (g)	Cost of Precursors without labour charge per kg (Rs.)	Cost of Jeevamrutha without labour charge per kg (Rs.)	Cost of precursors modified Jeevamrutha bio-fertilizer system with labour charge (Rs.)	TKN (%)	AN (mg/L)	P (%)
1	Vermicompost-modified Jeevamrutha	200	800	2.94	9.76	16.51	1.94	695	0.45
2	Neem cake-modified Jeevamrutha	250	750	92.2	9.15	131.75	2.23	876.56	0.71
3	Tea waste-modified Jeevamrutha	334	667	-	8.13	10.56	3.22	1076.4	0.65
4	Water hyacinth-modified Jeevamrutha			-					
	(Summer)	200	800		9.76	12.68	2.07	723.81	0.58
	(Winter)	250	750		9.15	11.89	1.42	525.7	0.44
5.	Jaggery-based modified Jeevamrutha	-	1000		17.3	22.49	0.67	427.56	0.14

* While procuring raw samples of tea waste and water hyacinth, no cost was associated. Hence, only labour cost was considered here.
(Labour charge, 30% of total cost)

4.7 Conceptual Cost Analysis

Table 4.6 illustrates the cost associated with the incorporation of the alternate rural substrates. Readily available precursors such as tea waste and water hyacinth affirmed the least investment in terms of their cost as Rs. 10.56/kg and Rs. 12.68/kg respectively and with good performance in the modified bio-fertilizer systems (%N of 3.22% and 2.07% and %P of 0.65% and 0.58% respectively). Similarly, the vermicompost exhibited a total cost of Rs. 16.31/kg as the expenditure of the developed modified Jeevamrutha system and with good %N and %P content (1.94% and 0.45%). A higher %P content (0.71%) in the neem cake-modified Jeevamrutha system was achieved which requires an even higher preparation cost. In summary, both tea waste and water hyacinth can be explored for their good performance and as alternate solutions to expensive vermicompost and highly expensive neem cake in the organic fertilizer or resources-based Jeevamrutha bio-fertilizer systems.

4.8 Summary

Organic bio-fertilizers on their own do not have the capacity to provide essential nutrients for plant growth and development. Henceforth, integration with other bio-fertilizers is required. Thereby, the present study categorically demonstrated the need for an integrated farming technique using readily available rural substrates that could improvise the stimulation mechanism for nutrient response in the developed modified Jeevamrutha bio-fertilizer formulations. The optimal precursors' constitution in precursors-modified Jeevamrutha bio-formulations were vermicompost, neem cake, tea waste and water hyacinth cases respectively (1:4, 1:3, 1:2 and 1:4 ratios and 1:4, 1:3, 1:2 and 1:3 ratios respectively during summer and winter seasons).

The percentage alteration of TKN for vermicompost, neem cake, tea waste and water hyacinth cases with respect to the modified Jeevamrutha bio-fertilizer (control formulation) was 1.27%, 1.57%, 2.55% and 1.4% during the summer and 0.57%, 1.5%, 1.66% and 1.14% during the winter season. Similarly, the percentage alteration of P for vermicompost, neem cake, tea waste and water hyacinth cases with respect to the modified Jeevamrutha bio-fertilizer (control formulation) was 0.31%, 0.57%, 0.51% and 0.44% during the summer and 0.22%, 0.54%, 0.47% and 0.34% during the winter season.

The primary macro-nutrients such as N, P, K, Ca and Mg in the bio-formulations do create a favorable soil environment for higher nutrient uptake by plants, stimulate plant growth regulators and restore fertility for a longer duration of time. Similarly, micro-nutrients such as Fe, Zn, Cu and Mn may aid in the electron transport system, and physiological and photosynthetic mechanism in plants. Besides biochemical characteristics, the microbial properties can contribute to growth-yielding factors due to the presence of nitrogen fixers and phosphate solubilizers in the bio-formulations. Also, enhanced soil microflora is due to the abundant carbon and nitrogen sources provided by the natural precursors that act as an energy powerhouse for their heterotrophic buildup.

Additionally, the phytotoxic effects of the mature precursors modified Jeevamrutha bio-fertilizer systems comprehend their utility due to benefits in agricultural economics. Hence, it could be summarized that the optimal desired concentration for vermicompost and tea waste-modified Jeevamrutha bio-fertilizer is 50% whereas the desired concentration for neem cake and water hyacinth-modified Jeevamrutha bio-fertilizers were 25% during the summer season. On the other hand, a 50% concentration of vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizers were optimal during the winter season to achieve higher shoot and root length of the mung bean plant.

The findings from the economic analysis reinforce the generalized inference that tea waste and water hyacinth could be targeted for the production of an integrated organic farming system. This is due to their excellent decomposition characteristics and cost-effectiveness that improve the efficacy of the modified Jeevamrutha bio-fertilizer in terms of nutritive values.

Therefore, the conducted study established future scope for the modest application of organic substances readily available in the rural scenario and thereby realized desired synergistic effects. These refer to soil health, better absorption of nutrients and water, stimulation of plant growth regulators, improve crop yield and as well minimize inappropriate usage of conventional chemical fertilizers in recent times. Also, by conducting pot experiments, it would be interesting to evaluate the integration of these precursors into the modified Jeevamrutha bio-fertilizer formulations and investigate plant growth characteristics and their associated nutritional benefits.

Chapter 5:

Performance of Jeevamrutha Bio-fertilizer with Sugarcane Juice and Starch Water Carbon Source

Performance of Jeevamrutha Bio-fertilizer with Sugarcane Juice and Starch Water Carbon Source

The chapter summarizes the experimental findings of jaggery-based optimal modified Jeevamrutha bio-fertilizer formulations being targeted with the replacement of carbon source (jaggery) with either sugarcane juice and starch rice water (obtained after cooking rice through the traditional method). Section 5.1 presents a summary background of the raw precursors followed by their elementary characterization in section 5.2. Section 5.3 details the preparation-related information for the mentioned carbon source-based Jeevamrutha formulation. Following this, sections 5.4 and 5.5 respectively devotes to the physicochemical properties and microbial load of sugarcane juice and starch rice water of the optimized bio-formulation. Section 5.6 briefly elucidates the phytotoxicity characteristics for the altered carbon substrate-based Jeevamrutha for the evaluation of its optimal constitution based on the maximum shoot and root length of the mung bean plant. Thereafter, section 5.7 presents a literature comparison of the conducted study. Subsequently, the conceptual cost analysis data of the alternate formulations has been presented in section 5.8. Finally, section 5.9 presents a summary of the findings.

Overview

To further analyze the nutrient efficacy of the carbon source altered optimized modified Jeevamrutha bio-fertilizer, sugarcane juice and starch rice water (obtained after cooking rice with the traditional method) were deployed to replace jaggery in the Jeevamrutha bio-formulation. The objective of the conducted work is to evaluate the influence of seasonal variation in the depletion or improvement of nutrients through the targeted replacement of jaggery with other raw carbon sources readily available in rural India. The significance of N-fixers and P-solubilizers was briefly explored for both seasons in the context of their contribution to the nutritional values of the developed bio-fertilizer formulations. Thereby, the alternate mentioned carbon precursor-based Jeevamrutha were evaluated for their physicochemical factors, microbial diversity and phytotoxicity assay during the summer and winter seasons respectively. The possibility to replace naturally occurring low-cost carbon sources to address the nutrient decline during the winter season has been targeted in the conducted research.

5.1 Background

Sugarcane is mainly grown in Sonitpur, Nagaon, Karbi Anglong and Golaghat districts of Assam, India. In these districts, the soil distribution varies from clay to sandy loam under upland and rainfed conditions (Begum, Singha, et al. 2016). The prevalence of higher crop yield achievement in the region catalyzes its rationality to serve as an alternative carbon source (Buah, Tachie Menson, et al. 2011). This is due to the obvious fact that sugarcane juice contains a large number of sugars such as glucose and fructose (de Souza, de Menezes et al. 2015). In the context of dwindling soil improvement through organic means, replacing jaggery with a carbon-rich substrate such as sugarcane juice could enhance the sustainability and nutrient enhancement in the modified Jeevamrutha bio-fertilizer.

Another investigation was conducted to develop bio-fertilizer using rice by-products such as rice hulls and grits. The study revealed higher growth performance of the microbial community for the case and affirmed its possible effectiveness as a carbon source (Leite, Melo, et al. 2020). Thereby, the replacement of jaggery with starch rice water in the Jeevamrutha could result in desired availability of nutrients and carbon for the achievement of Jeevamrutha with good characteristics.

The rationale behind the application of sugarcane juice and starch rice water (obtained usually as a waste product after cooking rice with the traditional method) in the modified Jeevamrutha bio-fertilizer is to achieve higher crop yield through the release of plant nutrition demanding organic matter. Further, the developed bio-fertilizer could address the quicker transfer of the constituent essential elements such as N, P, K, Mg, Ca, Mn, Fe, Zn and Cu in the bio-formulation and thereby influence the soil microflora and its chemical attributes.

Deploying alternate carbon sources, the pertinent nutrient characteristics such as AN, TKN and P were evaluated to achieve needful analyses and introspection into the phytotoxicity assessment in the developed product. Finally, this work could establish promising and desirable traits in the development of Jeevamrutha bio-fertilizer appropriately with higher nutrient yield, microbial population and soil health. Also, seasonal influence on nutrient characteristics was targeted for sugarcane juice or starch rice water-based Jeevamrutha bio-fertilizer. Thereby, betterments in the agronomic systems in rural India can be targeted.

5.2 Carbon Source Characterization with HPLC-RID

Both sugarcane juice and starch rice water (obtained after cooking rice with the traditional method) were analyzed for their sugar content. The findings have been presented in Table 5.1. It can be observed that higher sucrose content exists in jaggery (30.6 mg/L) followed by sugarcane juice (25.6 mg/L). On the other hand, the maximum glucose content was analyzed for starch rice water (8.92 mg/L) followed by jaggery (5.42 mg/L). Further, better fructose content was obtained in the sugarcane juice sample (2.78 mg/L).

Table 5.1: Characterization parameters of sugarcane juice and starch rice water systems.

Precursors	Sucrose (mg/L)	Glucose (mg/L)	Fructose (mg/L)
Jaggery	30.6±1.26	5.42±0.15	1.35±0.21
Sugarcane juice	25.6±0.57	0.42±0.04	2.78±0.17
Starch rice water	0.78±0.07	8.92±1.12	-

5.3 Composition of Sugarcane Juice and Starch Water-based Jeevamrutha Bio-fertilizer

The optimal jaggery-based modified Jeevamrutha bio-fertilizer formulation constituted 1 kg cow dung, 0.02 L cow urine, 0.04 L water, 0.35 kg soil, 0.03 kg jaggery and 0.03 kg gram flour for the summer season case and 1 kg cow dung, 0.02 L cow urine, 0.04 L water, 0.36 kg soil, 0.03 kg jaggery and 0.03 kg gram flour for the winter season case. Thereby, 0.03 kg jaggery was replaced by 0.03 L of sugarcane juice and starch rice water (obtained after cooking rice with the traditional method) in the modified Jeevamrutha bio-fertilizer formulation along with a fixed choice of all other constituents in the summer and winter seasons cases. The basis for such a carbon source constitution is to ensure that minimal alteration occurs in the solid Jeevamrutha formulation which would otherwise tend to become a liquid Jeevamrutha formulation. Since only preliminary studies were carried out, the conducted studies have been targeted to provide useful insights for further research in the alternate carbon source based modified Jeevamrutha formulations in the near future.

5.4 Physicochemical, Macro- and Micro-Nutrients Characteristics of Alternate Bio-fertilizer Systems

The physicochemical and macro- and micro-nutrient characteristics of alternate formulations were evaluated for each season (Table 5.2 and 5.3). As shown in Table 5.2, the AN, %TKN, %P, %OC and moisture content varied as 517.3-623.67 mg/L, 0.67-1.35%, 0.31-0.38%, 1.6-2.6% and 19.6-20.7% respectively for the sugarcane juice-based formulation during winter and summer seasons. Corresponding pH and EC altered as 6.4-6.7 and 4.8-5.7 dS/m respectively.

On the other hand, the starch rice water-based formulation affirmed AN, %TKN, %P, %OC and moisture content alteration as 358.9-489.4 mg/L, 0.54-0.82%, 0.27-0.35%, 1.5-1.8% and 20.5-21.3% respectively during winter and summer seasons. Corresponding pH and EC altered as 6.8-7.2 and 3.2-3.3 dS/m respectively. In contrast, jaggery-based modified Jeevamrutha bio-fertilizer had the least nutritional influence in terms of AN, %TKN and %P, especially during the winter seasons. For the case, these three integral terms varied as 135.8-427.56 mg/L, 0.28-0.67% and 0.10-0.14% respectively. Hence, the replacement of jaggery with rich carbon sources such as sugarcane juice and starch rice water could ensure good on-field performance and a significant improvement in soil health and plant nutrition.

Also, tea-integrated Jeevamrutha bio-fertilizer exhibited excellent nutrient characteristics during the winter season and thereby affirmed higher AN, %TKN and %P nutritional attributes as 726.8 mg/L, 1.94% and 0.57%. Hence, in the near future, it would be interesting to evaluate starch rice water-integrated tea waste-modified Jeevamrutha for its outstanding performance, integrity and cost-effectiveness for various field applications and crop productivity.

Table 5.2: Physicochemical characteristics of sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Precursors	**AN (mg/L)**		**TKN (%)**		**P (%)**		**Moisture Content (%)**		**pH**		**EC (dS/m)**		**OC (%)**	
	S	W	S	W	S	W	S	W	S	W	S	W	S	W
Sugarcane Juice-based Jeevamrutha	623.67 ±3.48	517.3 ±2.32	1.35 ±0.42	0.67 ±0.05	0.38 ±0.02	0.31 ±0.06	20.7 ±2.25	19.6 ±1.57	6.4 ±0.3	6.7 ±0.5	5.7 ±0.21	4.8 ±0.18	2.6 ±0.07	1.6 ±0.14
Starch Rice Water-based Jeevamrutha	489.41 ±2.91	358.9 ±4.12	0.82 ±0.18	0.54 ±0.08	0.35 ±0.07	0.27 ±0.17	21.3 ±2.26	20.5 ±3.85	7.2 ±1.59	6.8 ±1.66	3.3 ±0.78	3.2 ±0.36	1.8 ±0.19	1.5 ±0.52
Jaggery-based Jeevamrutha	427.56 ±3.55	135.8 ±2.75	0.67 ±0.04	0.28 ±0.03	0.14 ±0.02	0.10 0.01	17.8 ±0.67	15.3 ±1.78	8.07 ±0.5	7.52 ±1.2	2.01 ±0.21	1.53 ±0.45	0.62 ±0.04	0.31 ±0.08
Vermicompost-based Jeeavamrutha	695.1 ±3.25	416.6 ±2.23	1.94 ±0.05	0.85 ±0.03	0.45 ±0.07	0.32 ±0.02	25.8 ±1.24	22.6 ±1.37	7.78 ±1.5	7.65 ±1.1	2.9 ±0.4	2.8 ±0.08	2.6 ±0.03	1.6 ±0.07
Neem Cake-based Jeeavamrutha	873.56 ±4.72	623.4 ±5.89	2.24 ±0.07	1.78 ±0.04	0.71 ±0.02	0.64 ±0.08	19.4 ±1.3	11.2 ±0.78	8.0 ±0.8	7.8 ±0.5	0.48 ±0.15	0.52 ±0.06	2.1 ±0.51	1.9 ±0.14
Tea Waste-based Jeeavamrutha	1076.4 ±5.87	726.8 ±7.26	3.22 ±0.31	1.94 ±0.07	0.65 ±0.03	0.57 ±0.06	21.3 ±1.56	15.3 ±2.96	6.4 ±0.5	6.5 ±0.74	1.54 ±0.26	1.13 ±0.09	1.8 ±0.07	1.5 ±0.17
Water Hyacinth-based Jeeavamrutha	723.81 ±4.62	525.7 ±5.87	2.07 ±0.15	1.42 ±0.04	0.58 ±0.11	0.44 ±0.08	27.2 ±1.45	21.6 ±2.25	6.8 ±0.3	7.1 ±0.5	0.57 ±0.04	0.53 ±0.13	3.3 ±0.23	2.7 ±0.03

*S:Summer; *W: Winter

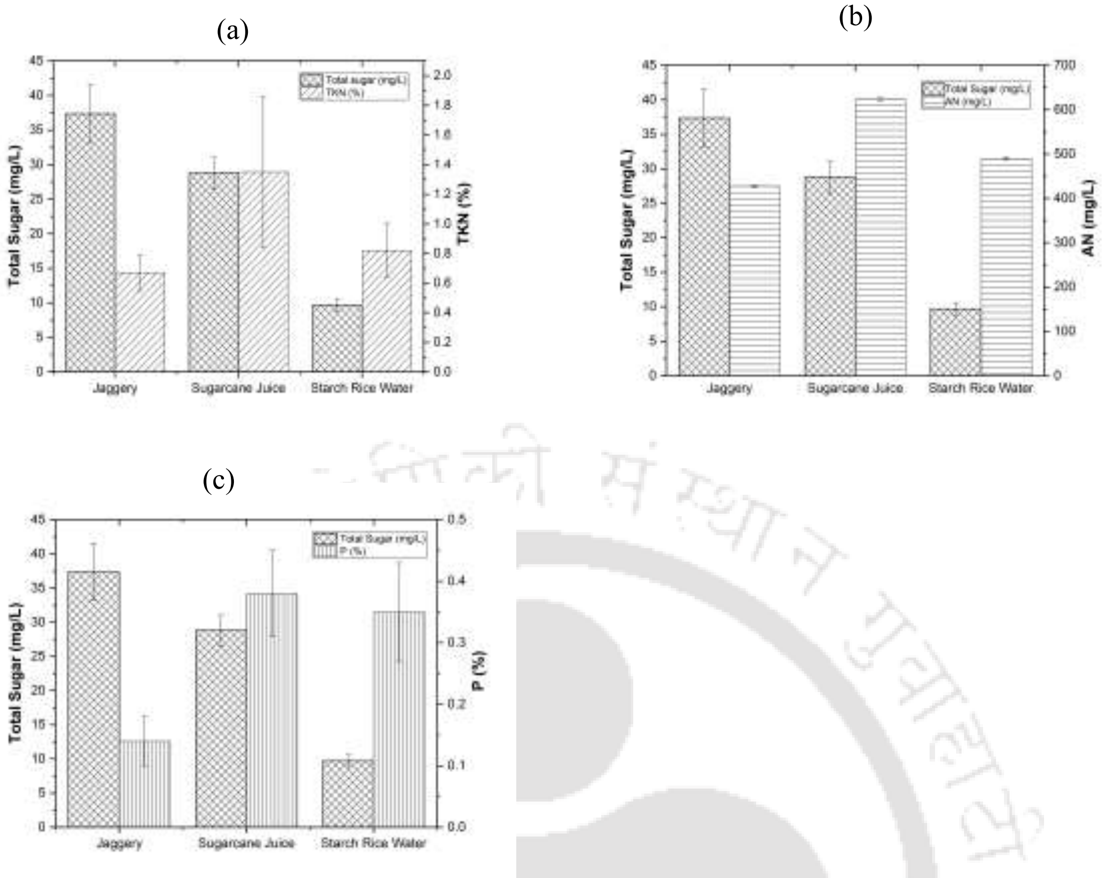


Fig. 5.1: Bar charts depicting the total sugar content and nutrient yield (%TKN (a); AN (b) and %P (c)) of sugarcane juice, starch rice water and jaggery carbon sources in the modified Jeevamrutha bio-fertilizer system during the summer season.

A graphical representation of the influence of total sugar content on AN, %TKN and %P for sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer has been shown in Fig. 5.1. The total sugar content of jaggery, sugarcane juice water and starch-rich water were 37.37, 28.8 and 9.7 mg/L respectively and thereby affirmed that sugarcane juice has highest total sugar content followed by starch rice water. Hence, it is evident that sugarcane juice and starch rice water exhibited higher nutrient yield in terms of AN, %TKN and %P in comparison with jaggery-based modified Jeevamrutha bio-fertilizer. In this regard, the moisture level in the formulations has a significant role that could establish a higher microbial count to thereby improve nutritional characteristics. A similar trend was obtained for the nutrient parameters during the winter season.

Further, micro- and macro-elemental constitution of Cu, Zn, Ca, Fe, Mn and Mg in the sugarcane juice-based formulation conveyed an alteration as 0.069-0.071%, 0.003-0.004%, 0.003-0.074%, 0.009-0.013%, 0.002-0.004% and 0.051-0.066% respectively for winter and summer seasons (Table 5.3). Similarly, a variation of Cu, Zn, Ca, Fe, Mn and Mg micro- and macro-elements in starch rice water-based formulation altered as 0.002-0.0021%, 0.003-0.0004%, 0.008-0.021%, 0.013-0.024%, 0.0009-0.005% and 0.002-0.003% respectively during winter and summer seasons. In contrast, a variation of Cu, Zn, Ca, Fe, Mn and Mg micro- and macro-elements in jaggery-based modified Jeevamrutha bio-fertilizer formulation altered as 0.0009-0.0034%, 0.0002-0.00058%, 0.04-0.07%, 0.018-0.036%, 0.003-0.0031% and 0.0021-0.0046% respectively during winter and summer seasons. Hence, replacing jaggery with other carbon sources such as sugarcane juice and starch rice water could serve the nutritional requirements in terms of plant health and soil fertility.

Table 5.3: Macro- and micro-nutrient characteristics of sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Metals (%)	Sugarcane juice-based modified Jeevamrutha(%)		Starch rice water-based modified Jeevamrutha(%)		Jaggery-based modified Jeevamrutha	
	S	W	S	W	S	W
Cu	0.069±0.04	0.071±0.01	0.0021±0.001	0.002±0.001	0.0034±0.002	0.009±0.001
Zn	0.003±0.001	0.004±0.002	0.0004±0.0001	0.003±0.001	0.0058±0.001	0.0002±0.0001
Ca	0.074±0.01	0.003±0.001	0.021±0.01	0.008±0.001	0.07±0.04	0.04±0.02
Fe	0.013±0.006	0.009±0.001	0.024±0.003	0.013±0.009	0.036±0.01	0.018±0.002
Mn	0.002±0.001	0.004±0.001	0.005±0.001	0.0009±0.0002	0.0031±0.001	0.003±0.001
Mg	0.066±0.01	0.051±0.007	0.003±0.001	0.002±0.001	0.0046±0.002	0.0021±0.0003

*S:Summer; *W: Winter

5.5 Microbial Plate Count of Sugarcane Juice and Starch Water-based Jeevamrutha Bio-fertilizer Systems

Table 5.4 summarized the obtained findings. During the summer season, significant multiplication of the microbial population has been observed for both sugarcane juice and starch rice water cases in conjunction with the jaggery case. This is due to the fact that moisture content has an influential role in the rapid growth of microbes. The bacterial, fungal, actinomycetes, N-fixers and P-solubilizers varied from 76-93×10^6 mL^{-1}, 6-9×10^4 mL^{-1}, 3-5×10^2 mL^{-1}, 22-37×10^4 $mL^{-1}$and 28-41×10^5 mL^{-1} respectively for sugarcane juice, and 54-87×10^6 mL^{-1}, 5-7×10^4 mL^{-1}, 2×10^2 $mL^{-1}$and 2×10^2 mL^{-1}, 19-24×10^4 mL^{-1} and 25-38×10^5 mL^{-1}respectively for starch rice water.

Table 5.4: A summary of microbial density of sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer systems in the summer and winter seasons.

Precursors	Bacteria (10^{-6})		Nitrogen fixers (10^{-4})		Phosphate Solubilizers (10^{-5})		Actinomycetes (10^{-2})		Fungi (10^{-4})	
	S	W	S	W	S	W	S	W	S	W
Sugarcane juice	93	76	37	22	41	28	5	3	9	6
Starch Rice Water	87	54	24	19	38	25	2	2	7	5
Jaggery	85	47	18	11	36	23	7	4	9	3

All standard deviations for the microbes (bacteria, N-fixers, P-solubilizers, actinomycetes and fungi) were in the range 2-4 and 4-7 respectively.

Comparatively, for jaggery-based Jeevamrutha, these have been 47-85 $mL^{-1}\times10^{6}$ mL^{-1}, 3-9$\times10^{6}$ mL^{-1}, 4-7$\times10^{2}$ mL^{-1}, 11-18$\times10^{4}$ mL^{-1} and 23-36 $\times10^{3}$ mL^{-1} respectively) for both summer and winter seasons. Thereby, the efficacy of the bio-formulations altered significantly with carbon substrates due to the alteration and enhancement of the microbial load. Hence, both N-fixers and P-solubilizers have a critical role in the nutritional characteristics of carbon-source-altered Jeevamrutha bio-fertilizers.

5.6 Phytotoxicity Characteristics of Sugarcane Juice and Starch Rice Water-based Jeevamrutha Bio-fertilizer Systems

For the summer case, the phytotoxicity assay of the sugarcane juice case conveyed a GI alteration of 15.62-71.37% with a peak at 75% (v/v). The maximum shoot and root length were obtained for 75% (v/v) cases (4.1 and 7.7 cm respectively). Similarly, GI altered as 17.43-79.13% with a peak at 75% (v/v) achieved for the starch rice water case. Higher shoot and root length were obtained at 50% (v/v) and 75% (v/v) respectively (2.3 and 7.5 cm respectively) (Table 5.5 (a), Fig 5.2 (i) and Fig. 5.3 (i)). Thereby, 75% (v/v) could be considered as the optimal concentration for sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer during the summer season owing to its higher germination rate and root length of the sample plant.

On the other hand, for the winter season and sugarcane juice case, GI varied as 17.47-70.18% with a peak at 75% (v/v). For the case, maximum shoot and root length were achieved at 25% (v/v) and 75% (v/v) respectively (5.8 and 5.1 cm respectively). Also, the starch rice water case conveyed the highest GI peak at 50% (v/v) which varied from 16.32-61.65%. The shoot and root length were highest for 25% (v/v) and 50% (v/v) sub-cases respectively (5.6 and 4.8 cm respectively) (Table 5.5 (b), Fig. 5.2 (ii) and Fig. 5.3 (ii)).

Hence, for the winter case, 75% (v/v) and 50% (v/v) for sugarcane juice and starch rice water-based Jeevamrutha biofertilizer was considered optimal due to better root length and GI at the same concentration.

Therefore, for the summer case, the optimal concentration of sugarcane juice and starch rice water-based Jeevamrutha biofertilizer has been 75% (v/v) whereas for the winter case, the best dataset for sugarcane juice-based Jeevamrutha bio-fertilizer has been 75% (v/v) and starch rice water-based Jeevamrutha bio-fertilizer has been 50% (v/v). The least plant growth for the higher (100% v/v) and lower cases (25% v/v) for the winter season could be the accumulation of higher concentrations of metals during the sprouting phase of the mung bean plant which leads to a lower maturity level.

Table 5.5 (a): Phytotoxicity assay data (germination index, shoot length and root length) of sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer and mung bean plant systems in the summer season.

Precursors	Sample Concentration (%)	Germination Index (%)	Shoot Length (cm)	Root Length (cm)
Sugarcane juice	0	15.62	2.1±0.3	3.5±0.5
	25	28.26	2.4±0.2	5.1±0.1
	50	49.79	3.2±0.1	5.5±0.3
	75	71.37	4.1±1.2	7.7±0.4
	100	67.81	2.5±0.8	7.2±0.3
Starch Rice Water	0	17.43	1.2±1.1	3.1±0.2
	25	57.34	1.9±0.5	4.7±0.4
	50	75.27	2.3±0.7	4.9±0.1
	75	79.13	2.1±0.4	7.5±0.3
	100	71.37	1.8±0.3	6.4±0.4

*All standard deviations for the germination index were in the range of 0.5-0.7 and 5-8 respectively

Table 5.5 (b): Phytotoxicity assay data (germination index, shoot length and root length) of sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer and mung bean plant systems in the winter season.

Precursors	Sample Concentration (%)	Germination Index (%)	Shoot Length (cm)	Root Length (cm)
Sugarcane juice	0	17.47	2.1±0.4	3.5±0.2
	25	67.83	5.8±2.23	4±1.1
	50	65.24	5±1.1	4.8±0.5
	75	70.18	5.4±0.9	5.1±0.6
	100	56.31	2.8±0.5	4.2±0.3
Starch Rice Water	0	16.32	1.8±0.3	3.1±0.5
	25	54.68	5.6±1.3	4.2±0.3
	50	61.65	5±0.3	4.8±0.7
	75	32.54	2.4±0.1	3.5±0.1
	100	42.33	3.5±0.6	2.8±1.1

*All standard deviations for the germination index were in the range of 0.5-0.7 and 5-8 respectively

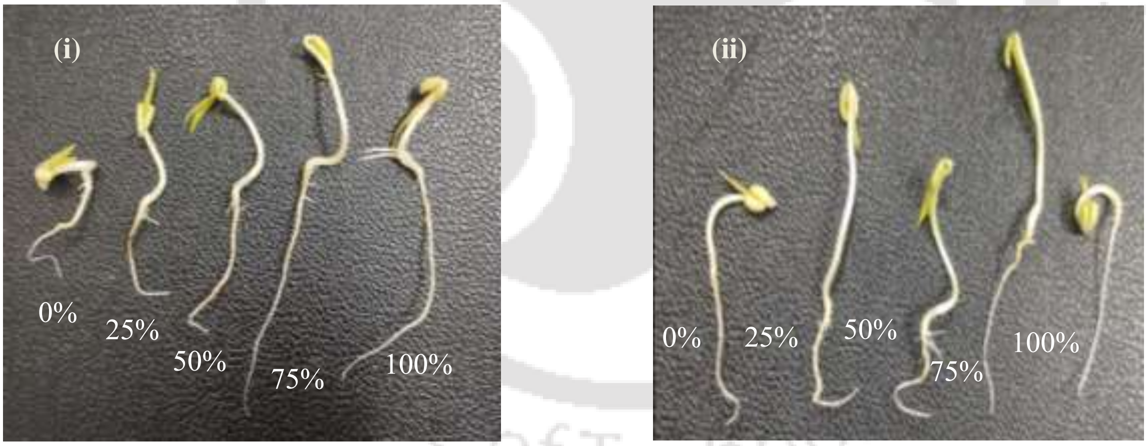


Fig. 5.2: Photograph depicting phytotoxicity characteristics of sugarcane juice modified Jeevamrutha bio-fertilizer system during (i) summer and (ii) winter seasons.

Fig. 5.3: Photograph depicting phytotoxicity characteristics of starch rice water modified Jeevamrutha bio-fertilizer system during (i) summer and (ii) winter seasons.

Fig. 5.2 and 5.3 depict photographs of the sugarcane juice and starch rice water-based Jeevamrutha biofertilizer. It could be observed that for the summer case, shoot and root length was obtained for 75% (v/v) for both cases. On the other hand, sugarcane juice and starch rice water-based Jeevamrutha bio-fertilizer affirmed better shoot and root length at 75% (v/v) and 50% (v/v) respectively during the winter season. In contrast, GI for jaggery-based modified Jeevamrutha bio-fertilizer was 87.87% and at 50% (v/v) with a shoot and root length of 2.8 and 5 cm respectively for both seasons.

5.7 Literature Comparison

Table 5.6 summarizes the literature comparison of sugarcane juice and starch water cases with the best-reported data. Radha and Rao (2014) considered sugarcane juice and jaggery in the Panchagavya bio-formulations that facilitated the growth of several bacterial isolates such as *Bacillus cereus*, *B. safensis*, *B. subtilis*, etc. Thereby, the organic bio-formulation promoted maize productivity (Radha and Rao 2014). Comparatively, the present study conveyed an improvement in microbial plate count (CFU/mL) (bacteria, 93×10^6; N-fixers, 37×10^4 and 41×10^5 mL^{-1}) for the sugarcane juice and starch rice water-based modified

Jeevamrutha bio-fertilizer in comparison with jaggery-based modified Jeevamrutha bio-fertilizer (bacteria, 85×10^6; N-fixers, 18×10^4 and 36×10^5 mL^{-1}). Thereby, these findings corroborated the optimal and higher combinations of AN, TKN and P yield for the case in comparison to Radha and Rao (2014). Incidentally, the authors did not delineate these findings and hence further insights could not be gained.

In another prior art, rice waste compost and sawdust were utilized as raw substrates and integrated into a cow dung-based bio-fertilizer that enabled substantial nutritive values and microbiological properties (Oladejo and Fasan 2015). However, the present research targets to reduce the compost preparation time through the direct addition of starch rice water and thereby improve nutrient content through an amendment of the physical and chemical properties of soil. In summary, no literature exists for a near comparison with the findings of this work. Also, the nutrient yield detriments during the winter season. Thereby, addressing such issues with readily available carbon sources in the rural sector serves the purpose of organic farming. Thereby, the novelty of the Ph.D. thesis is the affirming of the greater utility of starch rice water during the winter season for the betterment of plant growth characteristics with the modified Jeevamrutha bio-fertilizer.

Table 5.6: Comparative assessment of sugarcane juice and starch rice water modified Jeevamrutha bio-fertilizer system with the best reported literature data for organic bio-fertilizer precursors.

Precursors	TKN (%)	AN (mg/L)	P (%)	Sources
Sugarcane juice and Panchagavya	-	-	- (Enhanced microbial count)	Radha and Rao, 2014
Sugarcane juice	S: 1.35±0.42 W: 0.67±0.05	S: 623.67±3.48 W: 517.3±2.32	S: 0.38±0.02 W: 0.31±0.06	**This work**
Rice waste, cow dung and timber sawdust	1.9	-	0.6	Oladejo and Fasan, 2015
Starch rice water	S: 0.82±0.18 W: 0.54±0.08	S: 489.41±2.91 W: 358.9±4.12	S: 0.35±0.07 W: 0.27±0.17	**This work**

5.8 Conceptual Cost Analysis

Both yield and economic benefit have a crucial role in the Indian agricultural system. The adoption of cheaper raw substrates indicates cost efficacy along with improvement in the agroecological factors and soil microbiology. In this study, the conceptual cost of sugarcane juice and starch rice water-based bio-fertilizer were evaluated and summarized in Table 5.7. The labour charge for the Jeevamrutha bio-fertilizer preparation (per kg. of each sample) was considered to be 30% of total cost expenses. Hence, cost per kg. Jeevamrutha bio-fertilizer with labour charge and sugarcane juice and starch rice water was Rs. 26 and Rs. 20.15/kg respectively. Comparatively, the jaggery-based optimal Jeevamrutha bio-fertilizer cost was about Rs. 22.49/kg. Thus, starch rice water can be opined to be the most cost-effective precursor among all experimented carbon sources in terms of AN, %TKN and %P nutrient yield.

Additionally, the cost associated with the integration of the alternate rural substrates such as tea waste and water hyacinth into the modified Jeevarutha bio-fertilizer affirmed the least investment in terms of their cost as Rs. 10.56/kg and Rs. 12.68/kg respectively and with good performance (%TKN of 3.22% and 2.07% and %P of 0.65% and 0.58% respectively). Similarly, vermicompost exhibited a total cost of Rs. 16.31/kg for the expenditure of the developed modified system and a good %TKN and %P content (1.94% and 0.45%). A higher %P content (0.71%) in neem cake modified Jeevamrutha bio-fertilizer system was achieved which requires a higher preparation cost. In summary, both tea waste and water hyacinth along with starch rice water can be explored to compete with the nutrient decline during harsh winter climatic conditions in Assam. Sample calculation of the jaggery-based modified Jeevamrutha and other precursors' associated conceptual cost indices are shown in Appendix E of the Ph.D. thesis.

Table 5.7: Conceptual cost indices and nutrient yield data summary of sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer formulations.

S. No.	Precursors modified Jeevamrutha bio-fertilizer	Quantity Used	Cost of bio-formulation without labour charge per kg (Rs.)	Cost of bio-formulation with labour charge per kg (Rs.)	Cost of precursors without labour charge (Rs.)	Cost per kg. of the product (Rs.)	AN (mg/L)	TKN (%)	P (%)
1.	Sugarcane Juice	0.03 L	20	26	4.5	26	S:623.6±3.48 W:517.3±2.32	S:1.35±0.42 W:0.67±0.05	S:0.38±0.02 W:0.31±0.06
2.	Starch rice water	0.03 L	15.5	20.15	-	20.15	S:489.4±2.91 W:358.9±4.12	S:0.82±0.18 W:0.54±0.08	S:0.35±0.07 W:0.27±0.17
3.	Tea waste	0.3 kg	8.13	10.56	-	10.56	S:1076.4±5.87 W:726.8±7.26	S:3.22±0.31 W:1.94±0.07	S:0.65±0.03 W:0.57±0.06
4.	Water Hyacinth	0.2 kg 0.25 kg	S: 9.76 W:9.15	12.68 11.89	-	12.68 11.89	S:723.81±4.61 W:525.7±5.87	S:2.07±0.15 W:1.42±0.04	S:0.58±0.11 W:0.44±0.08
5.	Vermicompost	0.2 kg	9.76	12.68	2.94	16.51	S:695.1±3.25 W:416.6±2.23	S:1.94±0.05 W:0.85±0.03	S:0.45±0.07 W:0.32±0.02
6.	Neem cake	0.25	9.15	11.8	92.2	131.75	S:873.56±4.72 W:623.4±5.89	S:2.24±0.07 W:1.78±0.04	S:0.71±0.02 W:0.64±0.08
7.	Jaggery	0.03 kg	17.3	22.49	3.6	22.49	S:427.56±3.55 W:135.8±2.75	S:0.67±0.04 W:0.28±0.03	S:0.14±0.02 W:0.10±0.01

*Labour charge, 30% of total cost

5.9 Summary

This work targeted and affirmed the possible scope to utilize nutrition-rich carbon sources that are readily available in rural communities of Assam for bio-fertilizer formulations. The following can be stated as the summary of this chapter.

Among the two carbon sources, sugarcane juice conveyed a higher %TKN, %P and %OC concentration (1.35%, 0.67% and 1.6% respectively) along with a higher microbial density (CFU/mL) (bacteria, 93×10^6; N-fixers, 37×10^4 and P-solubilizers, 41×10^5 mL^{-1}) in comparison with jaggery-based modified Jeevamrutha bio-formulation (%TKN, 0.67%; %P, 0.14%; %OC, 0.62%; bacteria, 85×10^6; N-fixers, 18×10^4 and P-solubilizers, 36×10^5 mL^{-1}).

The starch rice water system effectively enhanced the nutritive values of the developed bio-formulation. The %TKN, %P and %OC content (0.82%, 0.35% and 1.5%) along with higher microbial load (CFU/mL) (bacteria, 87×10^6; N-fixers, 24×10^4 and P-solubilizers, 38×10^5) have been reasonably good in comparison with jaggery-based modified Jeevamrutha bio-fertilizer formulations (%TKN, 0.67%; %P, 0.14%; %OC, 0.62%; bacteria, 85×10^6; N-fixers, 18×10^4 and P-solubilizers, 36×10^5 mL^{-1}).

Phytotoxicity assessment for the sugarcane juice case reveals that for the summer case, 75% and 50% (v/v) were optimum for root and shoot growth of mung bean. On the other hand, 25% and 75% (v/v) were the best among all treatments (0-100% (v/v)) for both sugarcane juice and starch rice water cases during the winter season.

In terms of nutrient status, the AN, %TKN and %P in starch rice water-based Jeevamrutha bio-fertilizer have shown good performance (489 mg/L, 0.82% and 0.35% respectively) in comparison with jaggery-based modified Jeevamrutha bio- fertilizer (427.56 mg/L, 0.67% and 0.14% respectively) during the summer season. Similarly, the AN, %TKN and %P in starch rice water-based Jeevamrutha exhibited better nutrient yield (358.9 mg/L, 0.54% and 0.27%) in contrast with jaggery-based modified Jeevamrutha bio- fertilizer (135.8

mg/L, 0.28% and 0.10% respectively) during the winter season. Therefore, to address the detrimental winter nutrient depletion in soil and microbial growth profile, replacing jaggery with starch rice water in the Jeevamrutha bio-fertilizer could be beneficial in terms of its cost-effectiveness and field applications. Major nutrient characteristics such as AN, %TKN and %P are critical determinants for evaluating plant root health and its morphology and are extrinsic constituents for soil fertility factors. Hence, the further integration of tea waste into the starch-based Jeevamrutha bio-fertilizer could establish an organic platform to reduce the hazardous impact of chemical fertilizers during the winter season scenarios.

In summary, this work conveyed that the replacement of jaggery with sugarcane juice and starch rice water in the modified Jeevamrutha bio-fertilizer affirmed outstanding nutritive values and provides a basis to further understand the beneficial effects of utilizing rich carbon sources readily available in rural regions of India and other developing countries. In this regard, starch rice water was proven to exhibit an enriching independent effect and can be opined to be the best in conjunction with sugarcane juice and jaggery by considering AN, %TKN, %P and %OC content for the modified Jeevamrutha bio-formulation. This is evident from the even conceptual cost perspective that conceptualizes its role to replace jaggery in the Jeevamrutha bio-fertilizer. Henceforth, starch rice water could serve as a relevant precursor with due insights from the bio-formulation's nutritional values. It further justifies its ability to evolve an even better optimal constitution of the modified Jeevamrutha bio-fertilizer formulations to address the decline in nutritional characteristics during winter climatic conditions in Assam. However, it may be possible that carbon substrates such as sugarcane juice would be a source of attraction for insects and pests. Hence, the perspective necessitates further investigations in the near future.

Chapter 6:

Integrated Organic Farming Studies of *Phaseolus vulgaris* with Jeevamrutha Bio-fertilizer-Vermicompost System

Integrated Organic Farming Studies of *Phaseolus vulgaris* with Jeevamrutha Bio-fertilizer-Vermicompost System

This chapter addresses a multidisciplinary organic farming approach for the effective resolution of sustainable soil health-related difficulties being faced by small and marginal farmers. Integrated organic farming practices being addressed in the chapter corroborate with the associated benefits of soil health, fertility, and sustainable productivity. After a brief introduction in section 6.1, section 6.2 delineates the preparation of Jeevamrutha and vermicompost combined fertilizer system in different ratios for the subsequent Phaseolus vulgaris plant. Thereafter, section 6.3 discusses the growth, yield and deployment after seed sowing of the common beans that were monitored for 45 days and were analyzed. Section 6.4 provides a summary of the nutrient composition of the Phaseolus vulgaris plant after 45 days. Section 6.5 delineates the nutrient characteristics of the soil health in terms of plant growth and development, physicochemical constituents and microbial analysis to infer the broader scope for better crop cultivation and combined fertilizer system for germination. Further, section 6.6 summarizes ANOVA-based findings for various organic treatment (T1-T4) cases. Thereby, a literature comparison with relevant prior art has presented in section 6.7. Finally, section 6.8 presents a chapter summary of the importance of natural farming practices in terms of soil health and crop yield in Assam.

Overview

The addressed research of the chapter was carried out to evaluate the influence of alternate treatments with Jeevamrutha bio-fertilizer and vermicompost-integrated compositional fertilizer systems. Targeted perspectives referred to the growth, development and nutritional content of *Phaseolus vulgaris* and soil health characteristics improvement. A pot experiment for the model plant, *Phaseolus vulgaris* was conducted for 45 days to examine the effect of each treatment during the early spring season. Thereby, the morphological, physical, nutritional and microbial assessment was targeted for an alternate constitution of the combined organic fertilizer system. It was assessed that the soil fertility improved significantly in terms of the %TKN, %P and %K of the integrated farming system. Likewise, better growth and yield parameters were obtained for the integrated system followed by vermicompost and Jeevamrutha stand-alone treatments respectively.

6.1 Background

The sustained depletion of soil nutrition is a primary drawback to constraining agricultural productivity in India and other developing countries. The prolonged use of hazardous chemical fertilizers detrimentally affected sustainable environmental conditions. Also, a considerable decline in crop yield and associated environmental issues have been witnessed in recent years and these especially refer to atmospheric pollution, bio-magnification in the food web, bio-accumulation and deterioration in soil health. To mitigate and overcome these issues, organic farming techniques received considerable attention due to their ability to offer apparent solutions that mitigate associated challenges in the agricultural sector (Varghese and Prabha 2014). Thereby, organic farming ensures environmental and agricultural integrity without compromising productivity (Ignacimuthu and Vendan 2008). Organic farming primarily involves the adaptation of sustainable precursors that exist in nature. These refer to microbes that enrich the soil, earthworms (red wigglers) for sustainable soil health, decomposed food or vegetable waste, etc. that have great potential to reduce agrochemical dependency.

Among alternate sustainable organic farming techniques, due to their systematic sustaining ability towards the nutritional health of the agricultural soil, bio-fertilizers have been confirmed to be promising and viable alternatives to chemical fertilizers. Being environmentally benign, microbe-rich bio-fertilizers provide numerous benefits such as soil protection against pathogenic attack and drought, stimulation of plant growth hormone, restoration of soil fertility and cost-effectiveness. Microbe-based bio-fertilizers can be nitrogen, phosphate or potassium-rich fertilizers. Henceforth, appropriate customization with other bio-fertilizers will be necessary to achieve desired sustainability of soil health (Anandaraj and Delapierre 2010, Gopinathan and Prakash 2014, Kumar, Singh, et al. 2014). These bio-fertilizers transform vital nutrient components present in the soil such as nitrogen (N) and phosphorus (P) into their accessible form through nitrogen fixation and phosphate solubilization respectively. Thereby, they aid the plant systems in terms of their rapid growth and restricted pest development (Ignacimuthu and Vendan 2008, Kumar, Singh, et al. 2014).

Jeevamrutha, a cow-excreta-based bio-fertilizer, has been well-established in a relevant prior art for its bio-pesticidal property, influence on plant growth and development, faster decomposition and ability to engage readily available rural substrates (Amareswari and Sujathamma 2014). Similarly, vermicomposting is an organic system that utilizes the combined activity of indigenous earthworm species such as *Eisenia fetida* and microbes in the soil to transform and stabilize organic constituents into humus that gradually enhance soil porosity, oxygen availability, water retention, growth, yield and quality of crops (Arora, Singh et al. 2011). Typically, vermicompost comprises 2-3% N, 1.55-2.25% P, humus and other micro-nutrients (Rekha, Kaleena, et al. 2018).

Also, the nutritional requirements of these bio-formulations are not comparable with those of chemical fertilizers in terms of field applications. Hence, bio-fertilizer enrichment targeting a balanced constitution of macro- and micro-nutrients is the need of the hour to ensure its wider commercial utility. Several studies have been addressed to evaluate the effect of bio-fertilizers modified with vermicompost on the growth and development characteristics of the associated crops. Such fertilizer management has been termed an Integrated Farming System (IFS) and has been widely accepted by farmers due to coupled budget optimization and enhanced productivity of the farming system (Upendranaik, Rao, et al. 2018).

IFS provides a better knowledge framework and a greater understanding of the associated ecological and economic benefits. This is achieved through the usage of soil microflora for the rapid growth and development of cultivated crop cultivation (Yifan, Tiaoyan, et al. 2023). Thereby, the organic amendments offer innovative strategies that exhibit substantial betterment in terms of soil organic matter to improve crop yield. Eventually, they contribute to reduced environmental damage due to the extensive usage of chemical fertilizers (Raza, Zhu, et al. 2022).

In the prior art, several studies have been conducted to elucidate the efficacy of the vermicompost-integrated bio-fertilizer system on the yield of tomato plants (Gopinathan and Prakash 2014). The authors observed a 97% improvement in plant mineralization concentration and growth characteristic pattern in comparison with individual vermicompost and bio-fertilizer systems (Gopinathan and Prakash 2014). Similarly, a bio-fertilizer mixed flower waste vermicompost revealed better output (2.29% N) in comparison with the application of vermicompost alone (1.9% N and 14.8% P) in terms of both growth (62%) and biochemical parameters (38%) for groundnut (Kumar, Kumar, et al. 2014).

A combined application of liquid organic formulations Jeevamrutha (at a rate of 500 L/ha soil application), Panchagavya (at a rate of 3% foliar spray), Beejamrutha (seed treatment) and cow urine (10%) and bulky manures such as vermicompost and farm yard manure (through 50 and 100% of the recommended dose of nitrogen) in *Phaseolus vulgaris* revealed better nutrient uptake, growth and yield (pod number, 24.12; pod weight, 193.57 g; pod length, 15.52 cm) and economy (reduced from 44896-152353 to 27775-93275 Rs./ha) (Gowthamchand and Soumya 2020). Amareswari and Sujathamma (2015) inferred the efficacy of Jeevamrutha, Panchagavya and vermicompost on the cost-to-benefit ratio of *Phaseolus vulgaris*.

Similarly, another investigation revealed that the growth parameters of *Phaseolus vulgaris* were higher (plant height, 26.7 cm; leaf area, 1325 cm^2; the number of pods per plant, 15.89) in comparison to the cultivation with farm yard manure (plant height, 26.5 cm; leaf area, 1249 cm^2; the number of pods per plant, 15.33). For this, the authors targeted the foliar spray of Panchagavya and the soil application of Jeevamrutha (Basavaraj, Devakumar, et al. 2016). The combined application of NPK fertilizer and rhizobium culture along with micronutrients were investigated for *Phaseolus vulgaris* and the yield parameters were significantly higher (number of pods per plant, 35.64; pod weight, 6.7 g; the number of nodules per plant, 99.0) in comparison with alternate levels of fertilizers being deployed without rhizobium culture (number of pods per plant, 17.66; pod weight, 5.2 g; the number of nodules per plant, 57.82) (Jena, Pattnaik, et al. 2020).

Furthermore, a field study was targeted to investigate the effect of alternate organic nitrogen-rich sources (vermicompost, neem cake, farm yard and poultry manure) on the growth and yield of *Phaseolus vulgaris*. The results confirmed a higher plant height (33.63 cm), number of branches per plant (5.40) and leaf area/plant (11.69) in comparison with the absolute control treated sample (plant height, 27.11 cm; the number of branches per plant, 5.40; leaf area/plant, 6.22 cm^2) (Sondawale, Kamble, et al. 2023).

However, the agriculture sector requires needful introspection into the influence of climate on the integrated farming system as the microbial population in the deployed organic fertilizer is significantly influenced by the temperature and humidity (Etchells, Bell, et al. 1973, Jansson and Hofmockel 2020). Needless to convey, the diversity and alteration of climatic temperature and humidity are significant in Assam in comparison to other regions such as south India. Additionally, the effect of NPK and other physicochemical properties on the soil is necessary and requires further investigations that hold the relevance of organic fertilizers to ascertain potential economic benefits and feasibility for plant growth. To address the issues associated with a nutrient decline during the extreme winters, the integrated farming system may contribute profitable attributes in terms of crop productivity and soil health.

In summary, IFS involving microbial inoculants, chemical fertilizers and vermicompost were studied in the literature in terms of the nutrient yield, growth of tomato, *Phaseolus vulgaris,* foxtail millet, *Glycine max*, *Vigna mungo,* groundnut, etc. In this regard, the solid Jeevamrutha bio-fertilizer-integrated-vermicompost system was not widely explored despite its proven performance in several south Indian states (Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, etc.). Previously, an integrated system consisting of vermicompost, G5 soil enricher granules (mixture of amino acid, anti-root substance, herbal and seaweed extracts) and modified Jeevamrutha (jaggery replacement with coconut water) were investigated for the growth study of *Amaranthus caudatus* (Rosi and Thatheyus). However, the traditional practices of coconut water and soil enricher are not viable raw and inexpensive resources in terms of their wider availability and utility in rural India. Also, the contribution of soil microflora was not targeted in the article. Thereby, the scope of solid Jeevamrutha bio-fertilizer towards low-cost IFS-based crop cultivation needs to be addressed in conjunction with the available data for other IFS systems.

Considering these lacunae, this chapter aims to evaluate the efficacy of the growth characteristic pattern and nutrient quality of *Phaseolus vulgaris* with the solid Jeevamrutha bio-fertilizer and commercial vermicompost-integrated bio-fertilizer system. In summary, the utilization perspectives of the solid Jeevamrutha bio-fertilizer have been targeted in terms of its cost-effectiveness towards organic farming in Assam. A key issue in this regard is the winter climatic condition that possesses severe restrictions towards the sustained microbial population in the soil. Thereby, the IFS can significantly catalyze such desirable soil health characteristics for better farming. Henceforth, the investigation involving soil health, nutritional characteristics, etc. was targeted during the winter season in Guwahati, Assam, India. Such studies will widen the scope of livestock-based fertilizer systems in terms of the value-added exploration of the available resources.

6.2 Preparation of *Phaseolus vulgaris* Initial Pot Culture

Four alternate treatment cases were considered that involved alternate ratios of the mixed fertilizer system and as Jeevamrutha (J):soil: vermicompost (VC) ratio of 1:4:1 (T1), J: soil ratio of 1:4 (T2), VC: soil ratio of 1:4 (T3) and soil (T4). A screening study was conducted by the authors prior to the consideration of the T2 compositional set (1:4 ratios of Jeevamrutha bio-fertilizer to soil) for the IFS case study. In such a screening study, AN and P nutrient yield were considered as primary responses. Thereby, for J: soil ratio variation of 1:1-1:5, a maximum AN and P were obtained as 135 mg/L and 0.14% for the optimal choice of the ratio as 1:4.

Similarly, considering the case of vermicompost (VC) to soil variation (1:1-1:5), higher AN and P concentration (416 mg/L and 0.32%) were achieved for the optimal choice of ratio as 1:4 (Shozeb and Aruna 2013). The authors reported the mentioned optimal constitution of Jeevamrutha: soil (T2 analog) and vermicompost: soil (T3 analog). Thereby, the growth profile study of *Vigna mungo* and *Glycine max* in conjunction with the chemical fertilizer-based IFS system (Biofertilizer+Vermicompost+Chemical fertilizer) was assessed and chosen as the basis for the optimality (Shozeb and Aruna 2013).

Subsequently, the pot culture for each treatment case was conducted in April and May at an ambient temperature of 29-31°C for 45 days. The growth and yield parameters for T1-T4 in *Phaseolus vulgaris* have been summarized in Table 6.1. Additionally, the %TKN, %P and %K of the treated samples along with the control group were analyzed for their nutrient uptake along with their moisture content. Among all four treated and control samples, T1 yielded the best fruit nutritional content followed by T2.

6.3 Growth and Yield Characteristics of *Phaseolus vulgaris*

After 45 days of pot experimentation for each treated sample, the T1 case recorded better growth characteristics in terms of shoot length (11-42.3 cm), leaf and root length (19.3 and 15.5 cm respectively), number of leaves (32) and branches (14) respectively followed by T2 and T3 treatments (Table 6.1).

Table 6.1: A summary of growth parameters (0 – 40 days) of *Phaseolus vulgaris*.

Treatments	Shoot Length (cm)		Leaf Length (cm)		No. of Leaves		No. of Branches		No. of fruits
	0-20	20-40	0-20	20-40	0-20	20-40	0-20	20-40	20-40
T1	18.1±0.2	42.3±1.4	7.8±3.4	19.3±2.8	8±0	32±0	4±0	14±0	12
T2	17.7±1.1	26±2.6	8.3±1.7	9.6±2.2	8±0	17±1	5±1	12±1	9
T3	15±0.3	24.7±1.7	8.5±0.4	13.6±3.8	8±0	21±2	5±1	12±0	7
T4	8±0.6	16.8±1.8	4.8±1.3	9±1.4	8±0	12±0	3±1	8±2	4

Fig. 6.1: Photograph depicting the initial growth pattern of *Phaseolus vulgaris* for alternate treatments (Jeevamrutha + Soil + Vermicompost (T1); Jeevamrutha + soil (T2); Vermicompost + soil (T3); Soil (T4)).

T2 treated samples exhibited better results in terms of leaf length between days 10 and 20 and with a maximum number of leaves and branches for 30 (11 and 9 respectively) and 40 days (17 and 12 respectively) followed by the T3 case (Fig. 6.1). On the other hand, the control group (T4) didn't exhibit any significant growth and yield in comparison with the treated samples. This is due to the reason that plant growth regulators were highly affected due to the deficient uptake of plant nutrients during various stages of plant growth (Rekha, Kaleena, et al. 2018).

The slower nutrient release by the Jeevamrutha treated sample was evident from the apparent growth characteristics. Also, the yield factors such as the number of flowers and fruits were maximum for the T1 case (26 and 12) followed by T2 cases (24 and 9) for 30 and 40 days respectively. Similarly, the fruit and root length was highest for the T1 case (11.5 and 15.5 cm) and then for the T3 case (9.2 and 9 cm). Thereby, the study affirmed that plant growth hormones have been better regulated to essentially enhance crop production with the utilization of bio-fertilizer treatment systems (T1 and T2).

Fig. 6.2: Photographs depicting the root growth characteristics for alternate treatment cases (Jeevamrutha + Soil + Vermicompost (T1); Jeevamrutha + soil (T2); Vermicompost + soil (T3); Soil (T4)).

However, T3 treated group confirmed a higher number of branches in addition to leaf, fruit and root length. Since yield parameters depend upon the assimilatory regulation of the plant in terms of the utilization and availability of nutrients, significantly higher bean production was achieved for the T1 case followed by T2 and T3 cases respectively (Murthy 2012). After 45 days, the plants were uprooted for the analysis of their root health characteristics (Fig. 6.2).

Also, the T4 treatment case referred to weakened and rotted roots. This was also evident from its wilted and early leaf drop observations in comparison with the treated samples. Such detrimental effects have been inferred due to the presence of soil fungus. Henceforth, it could be justified that T1, T2 and T3 could act as fungicide agents for root rotting in plants (Ovsyankina, Savchenko, et al. 2019).

6.4 Nutrient Characteristics of *Phaseolus vulgaris*

After 45 days of the pot experiment, the nutritional characteristics of the bean samples were analyzed for their respective carbohydrate, soluble protein, vitamin C, macronutrients, chlorophyll, carotenoids and moisture content.

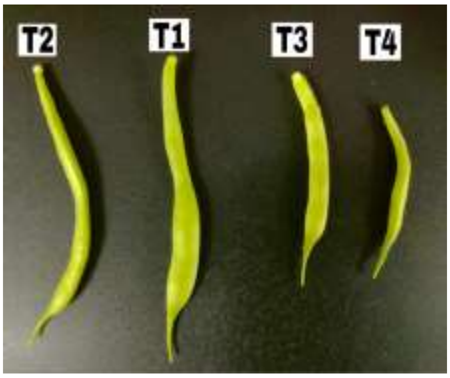


Fig. 6.3: Day 45 fruit yield of *Phaseolus vulgaris* for alternate treatment cases (Jeevamrutha + Soil + Vermicompost (T1); Jeevamrutha + soil (T2); Vermicompost + soil (T3); Soil (T4)).

Fig. 6.3 depicts fruit yield after 45 days of growth study for alternate treatments (T1-T4). A higher fruit length and weight were obtained for the T1 case (11.5 cm and 4.6 g) in comparison with the control (T4 case) (6.4 cm and 5.1 g respectively).

The obtained nutritive values for the bean samples have been summarized in Table 6.2 (a) and (b) respectively. For T1 and T2 treatment cases, maximum carbohydrate (2.7 and 1.7 per unit g of fruit), carotenoids (37 and 35 μg/g), soluble protein (0.037 and 0.026 mg/g) and vitamin C (84.2 and 56.4 mg/100g) along with %TKN (1.8 and 1.7%) and %P (0.18 and 0.15%) content were achieved. Hence, the feasibility of using Jeevamrutha bio-fertilizer solely or in combination with vermicompost for an improvement in the nutritional content is evident. This investigation supports the hypothesis that the dual integration of Jeevamrutha bio-fertilizer and vermicompost was beneficial in terms of enhanced cumulative crop yield and nutritional characteristics (Abdelhafez, Eid, et al. 2021).

Table 6.2: **(a)** Nutritional characteristics of *Phaseolus vulgaris* fruit (day 45); **(b)** chlorophyll (leaf), moisture content (fruit) and macro-nutrient (fruit) characteristics of *Phaseolus vulgaris*.

(a)

Treatment	A (per unit g of fruit)	B (µg/g)	C (mg/g)	D (mg/100g)
T1	2.7	37	0.037	84.2
T2	1.7	35	0.026	56.4
T3	1.5	29	0.024	51.2
T4	1.2	22	0.014	28.2

*A:Carbohydrate; B:Carotenoids; C: Soluble Protein; D: Vitamin C

(b)

Treatment	E (µg/ml)			F (%)	G		
	Chl a	Chl b	Total chl		TKN %	P %	K %
T1	19	7	26	38	1.8	0.18	1.6
T2	19	4	23	32.6	1.7	0.15	1.1
T3	16	8	25	36	1.5	0.16	0.19
T4	12	7	13	26	1.1	0.06	0.58

*E:Chlorophyll; F:Moisture Content; G:Macro-nutrients

*All standard deviations for A, B, C, D, E, F and G were in the range 1-3, 0.1-0.5 and 0.04-0.08 respectively.

On the other hand, T1 and T3 treatments confirmed better chlorophyll (26 and 25 µg/g), moisture (36 and 38%) and P content (0.18 and 0.16%) respectively. Inadequate moisture level in the soil leads to reduced photosynthetic efficiency. The presence of humic acid substances in the vermicompost could exhibit a better photosynthesis rate in the plant physiological systems to thereby enhance chlorophyll content in the plant system (Samantara and Reddy 2009, Baghbani-Arani, Modarres-Sanavy et al. 2017, Rekha, Kaleena et al. 2018).

6.5 Soil Quality Assessment of Alternate Treatment Cases

The alternate organic treatments being deployed for the growth profile study of *Phaseolus vulgaris* were analyzed for the soil nutritional content and microbial population. The presence of beneficial microbes and phytohormones in the treatments does have a role in root initiation and plant morphological features. These have been outlined as follows.

6.5.1 Nutrient Characteristics of the Treated Soil

Among T1, T2, T3 and T4 cases, nutrient traits were evaluated in terms of their physicochemical characteristics. A higher %TKN, % P, %K and %OC were achieved for T1 (1.86%, 0.86%, 0.71% and 2.1%) followed by T3 (1.45%, 0.74, 0.64% and 1.58%) respectively (Table 6.3). Jeevamrutha fertilizer in the integrated formulation catalyzed potential microbial interactions and thereby ensured the ready availability of nutrients in the soil. Similarly, vermicompost is recommended for organic amendment due to its capacity for the transformation, retention and absorption of essential micro- and macro-nutrients in soil (Ravindran, Karmegam, et al. 2021, Raza, Zhu, et al. 2022).

Table 6.3: A summary of physicochemical characteristics of the soil and for alternate treatment cases (Jeevamrutha + Soil + Vermicompost (T1); Jeevamrutha + soil (T2); Vermicompost + soil (T3); Soil (T4)).

Treatment	Moisture content (%)	TKN (%)	P (%)	K (%)	pH	EC (dS/m)	OC (%)
T1	28.6	1.86	0.86	0.71	8.41	3.2	2.1
T2	19.4	0.62	0.71	0.58	7.46	2.1	0.67
T3	24.7	1.45	0.74	0.64	7.68	2.7	1.58
T4	20.3	0.26	0.62	0.46	6.77	3.2	0.32

*All standard deviations for moisture content, %TKN, %P, %K, pH, EC and OC were in the range 2-4, 0.3-0.7 and 0.05-0.07 respectively.

Thereby, this study suggested that the Jeevamrutha bio-fertilizer-vermicompost IFS provided better nutrient supplements for soil fertility and effective plant productivity. Also, the higher moisture content in the integrated farming system confirmed better water retention capacity of the optimal IFS formulation. Sample calculation for the moisture content and nutritive values along with chlorophyll content has been shown in Appendix D of the Ph.D. thesis.

6.5.2 Microbial Characteristics of the Treated Soil

The microbial population of the treated and control samples was evaluated in terms of the CFU count (Table 6.4). Thereby, it was revealed that bacterial growth was highest for T1 and T2. This is due to the fact that bio-fertilizers offer better microbial consortiums. The microbial activity in the Jeevamrutha bio-fertilizer could convert the essential soil-constituted elements such as nitrogen, phosphate and potassium into their accessible forms and thereby promote biodiversity and crop productivity (Mahmud and Chong 2021). However, the fungal colony was highest for T4 and T2 cases. Certain bioactive compounds such as enzymes, proteins and hormones present in the vermicompost have a distinctive and detrimental function toward soil pathogens and could serve as antifungal agents (Gudeta, Bhagat, et al. 2022). Hence, T1 and T3 do have fungicidal properties due to the inclusion of vermicompost.

Also, N-fixers and P-solubilizers were higher for T1 (CFU/mL) (24×10^4 and 28×10^4 mL^{-1} respectively) and T3 (16×10^4 and 17×10^4 mL^{-1} respectively) cases followed by the T2 case (14×10^4 and 13×10^4 mL^{-1} respectively). The addition of Jeevamrutha bio-fertilizer into the system enormously increased the microbial demand for the T1 case in terms of nitrogen fixation and phosphate solubilization (Mahmud and Chong 2021). Similarly, maximum actinomycetes count was achieved for both T1 and T2 cases. This signified an enriched soil rhizosphere that enhances nitrogen availability and its metabolism for crop yield (AbdElgawad, Abuelsoud, et al. 2020).

The IFS utility was evident in terms of the improved growth profile of *Phaseolus vulgaris*. This is due to the reason that the physicochemical properties are qualitatively enhanced due to higher microbial activity and micro- and macro-nutrient availability in the soil. Hence, the combined farming system exhibited a positive influence in terms of chlorophyll, plant growth and nutritional values along with nutrient uptake. Also, vermicompost reduces the release of essential nutrients into the soil and aid in the absorption of organic and inorganic nutrients along with a balanced moisture content (Rekha, Kaleena, et al. 2018). The IFS-treated soil had a loamy texture and possessed an alkaline pH (8.41), 2.1 %OC and good %TKN, %P and %K values (1.86, 0.86 and 0.71 respectively).

Table 6.4: Microbial density data for alternate treatment cases (Jeevamrutha + Soil + Vermicompost (T1); Jeevamrutha + soil (T2); Vermicompost + soil (T3); Soil (T4)).

Treatment	Bacteria (10^{-5})	Fungi (10^{-4})	Actinomycetes (10^{-2})	N-Fixers (10^{-4})	P-Solubilizers (10^{-4})
T1	160	3	15	24	28
T2	104	5	9	14	13
T3	80	3	3	16	16
T4	68	11	4	9	6

*All standard deviations for the microbes (bacteria, N-fixers, P-solubilizers, actinomycetes and fungi) were in the range 2-4 and 4-7 respectively.

6.6 One-way ANOVA Statistical Analysis

For all responses of the sample, the one-way ANOVA demonstrated a 95% confidence interval level and henceforth assured confidence in the obtained results. Each sample measurement (for plant, fruit and soil samples) was subjected to a minimum of three replications. Thereby, it has been analyzed that for all sample responses (shoot length, leaf length, number of leaves, number of branches and number of fruits of plant sample; carbohydrate, carotenoids, chlorophyll, moisture content, macronutrients, soluble protein and Vitamin C content of fruit sample; moisture content, %TKN, %P, %K, pH, EC, %OC, bacteria, fungi, actinomycetes, N-fixers and P-solubilizers of soil sample), high F-values and a low-p value (<0.0001) was achieved and confirmed upon the significance of the findings. The Appendix C section of the Ph.D. thesis summarizes ANOVA-based findings for various cases.

6.7 Literature Comparison

Table 6.5 presents a summary of the findings associated with various microbial bio-fertilizers and their combinations with organic nitrogen sources. The moisture content of the IFS system being reported to be the best in this work (T1 case) has been higher (38%) in comparison to that reported by Meena et al. using microbial bio-fertilizer (12%) (Meena, Ram, et al. 2018). This is due to the incorporation of vermicompost which improves the water retention capacity of the soil. Similarly, a maximum plant height was achieved (54.62 cm) in comparison to that reported by Sondawale et al. (2023) (33.63 cm). Hence, Jeevamrutha bio-fertilizer marks its excellence in promoting the plant growth regulators in the T1 treatment case. Besides this, soluble protein, nutritional characteristics such as carbohydrates, vitamin C and carotenoids and photosynthetic pigment such as chlorophyll content in the plant were not targeted in the prior art. Also, soil microflora was not reported in the literature in conjunction with the plant growth characteristics. This is an important issue to delineate as the sustained soil fertility and health parameters do influence the health and growth characteristics of the cultivated crop. Thereby, a holistic and global methodology has been presented in this article that delineates the need for soil health analysis in conjunction with plant growth characteristics.

Table 6.5: A comparative assessment of nutritional and morphological/yield data of *Phaseolus vulgaris* and Jeevamrutha farming system with best reported data in the literature for alternate farming systems.

Model Plant	Farming System	Nutritional Characteristics	Morphological/Yield factors	Sources
Phaseolus vulgaris	*Rhizobium*+*P*hosphorus Solubilising Bacteria)+vermicompost	Crude protein content (%)-42.31	Plant height (cm)-57.92 Shoot length (g)-43.70 Fruit length (cm)-15.55	Barcchiya and Kushwah 2017
Phaseolus vulgaris	Azotobacter, Phosphate solubilizing bacteria, Rhizobium, Azospirillum and Vesicular-arbuscular mycorrhizae	Moisture (%)-12 Total sugar (%)-6.35 Protein (%)-18.9	Number of pods-23 Pod length (cm)-11.22 Pod weight(g)-4.64	Meena et al., 2018
Phaseolus vulgaris	NPK+ Rhizobium+ Micronutrients	-	Pod length (cm)-3.42 Pod weight (g)-6.7	Jena et al., 2020
Phaseolus vulgaris	Farm Yard Manure, Vermicompost, poultry manure and neem seed cake	Protein content (%)-20.10	Plant height (cm)-33.63 Number of branches/plant-5.40 Dry matter/plant (g)-16.29 Number of pods/plant-15.27	Sondawale et al., 2023
Phaseolus vulgaris	Jeevamrutha,Vermicompost and Soil	Moisture (%)-38 Soluble protein-0.037 mg/g Carbohydrate-2.7 mg/g Vitamin C-84.2 mg/100g	Plant height- 54.62 cm Shoot length-42.3 cm Fruit length-11.5 cm Number of leaves-32 Number of branches-14	**This work**

6.8 Summary

For progressive organic farming, seed germination and high yield of crops are some of the associated primary factors. The adoption of appropriate organic practices is required to boost the level of crop productivity. Jeevamrutha bio-fertilizer is capable to tolerate moisture stress and stimulate plant growth hormone along with the improvement in the soil environment. Similarly, vermicompost ensures the ready availability of nutrients required for the plants. The incorporation of vermicompost into the Jeevamrutha bio-fertilizer offers benefits in terms of the improvement in plant root health and water uptake capacity, lesser fungal growth and improved nitrogen fixation.

In addition to effective waste management strategies, the vermicompost-integrated Jeevamrutha bio-formulation ensured excellent improvement in biological and physicochemical properties. Such an integrated organic farming system also fosters the production of nutritional products that are beneficial for human health. All these reasons confirm a positive and synergic influence of the integrated organic farming system in terms of plant growth yield and enriched nutritional benefits.

A 45-day experimental investigation was conducted to ascertain the possibility of using integrated organic farming techniques on the yield and nutritional quality of *Phaseolus vulgaris* and its alteration in the biological properties of soil. A higher growth characteristic was observed for the case involving vermicompost integration with Jeevamrutha bio-fertilizer in comparison with the control case (soil). This is due to the presence of growth regulators and an adequate supply of essential nutrients at various plant growth stages.

Apart from its bio-pesticidal property, this microbe-rich formulation has better efficacy on plant productivity. Among all cases, T1-T4, the best case corresponds to the T1 case that has an apparent constitution of Jeevamrutha and vermicompost. The biological fertility factors improved through microbial consortiums that exist in the Jeevamrutha bio-fertilizer. Hence, they encompass the utility of discarded waste as an efficient means of sustainability through diversified utilization of raw low-cost rural resources in the agricultural sectors of India and other developing countries with a sub-tropical climate.

The combination of organic bio-fertilizer and vermicompost would have excellent effectiveness for natural agricultural practices in terms of low-cost nutrient enhancement technology exploration and rapid crop growth. Such studies could promote strategic research for the invention of integrated bio-systems in agriculture and thereby investigate the mechanism associated with plant growth promoters and soil fertility. For the affirming of suitable and green agriculture, sufficient awareness in terms of protocol-based field application of Jeevamrutha bio-fertilizer and vermicompost is required to demonstrate the ability of this system to reduce the alarming usage of chemical fertilizers by the farming community of India.

Chapter 7:
Conclusions and Future Work

Conclusions and Future Work

In section 7.1 of the chapter, subjective and objective inferences being deduced in the Ph.D. thesis have been summarized. The subsequent section details the possible scope for future work in the chosen field of research.

7.1 Conclusions

Based on the experimental investigations being carried out to judge the efficacy of modified Jeevamrutha bio-fertilizer formulation in Assam, the resolved objectives of the Ph.D. thesis refer to screening and optimization; incorporation of commercial and rural precursors into the bio-formulation through the replacement of jaggery with other readily available carbon precursors and integrated Jeevamrutha-vermicompost-based organic farming system with model plant, *Phaseolus vulgaris* and its nutritive benefits. Thereby, the following sub-sections outline prominent inferences that can be drawn from the conducted research.

7.1.1 Screening and Optimization of Jeevamrutha Bio-fertilizer Formulations in Assam

- A robust analytical procedure involving the replacement of TKN with the AN has been addressed to serve as an important marker to assess nitrogen nutrient characteristics of the bio-fertilizer formulations. In Assam (North-east India), the best %TKN, AN and P referred to 0.67%, 427.56 mg/L (1 g of Jeevamrutha in 3 mL of concentrated H_2SO_4 digested solution) and 0.14% respectively (1 kg cow dung, 0.02 L cow urine, 0.03 kg of jaggery and gram flour, 0.04 L water 0.36 kg soil). For another case, AN, %TKN and %P with Palekar's composition (1 kg cow dung, 0.05

L cow urine, 0.01 kg of jaggery and gram flour and a handful of soil) agreed proportionally to about 0.13-0.21%, 124.8-289.6 mg/L (1 g of Jeevamrutha in 3 mL of concentrated H_2SO_4 digested solution) and 0.04-0.05% in the region for winter and summer seasons. A temperature of 32–34°C and a humidity of 82–84% have been effective to achieve good quality Jeevamrutha bio-formulations.

- PBD proved to be an effective tool (91 and 92% R^2 for AN and P respectively and p-value <0.05) for the screening and identification of independent variables such as jaggery, gram flour and water for AN and gram flour and soil for P nutrient yield respectively. Thus, the final screened factors for Jeevamrutha bio-fertilizer were jaggery, gram flour and soil.
- Among the higher (S_H) and lower (S_L) parametric ranges, the former provided a relevant range to assist the screening process which was otherwise dubious for the lower parametric range. The parametric range referred to the case of 0.02 kg jaggery and gram flour, 0.04 L water, 0.2 kg soil with a fixed proportion of 1 kg cow dung and 20 mL cow urine and pH 7.8.
- Also, optimized compositions of solid and modified Jeevamrutha formulations referred to 0.03 kg for jaggery and gram flour and 0.35-0.36 kg soil during the winter and summer seasons in Assam, India respectively. Thereby, the AN, %TKN and %P of the optimal compositional set were 135.86-427.56 mg/L (1 g of Jeevamrutha in 3 mL of concentrated H_2SO_4 digested solution), 0.28-0.67% and 0.10-0.14% respectively for both seasons.
- The phytotoxicity assessment using the mung bean plant revealed a good germination index of 87.87% and root length (5 cm) at a 50% (v/v) sample concentration. The results inferred the maturity and non-toxic status of the jaggery-based optimal bio-

fertilizer formulation during the summer season. A similar trend was achieved during the winter season.

7.1.2 Optimality of Jeevamrutha Bio-fertilizer Modified with Vermicompost, Neem Cake, Tea Waste and Water Hyacinth

- The deployed precursors for further modification of Jeevamrutha bio-fertilizers were vermicompost, neem cake, tea waste and water hyacinth. For the precursors modified Jeevamrutha bio-fertilizer systems, the optimal ratios were:
  - 1:4 for Jeevamrutha: Vermicompost in both summer and winter seasons,
  - 1:3 for Jeevamrutha: Neem cake in both summer and winter seasons,
  - 1:2 for Jeevamrutha: Tea waste in both summer and winter seasons and
  - 1:4 and 1:3 for Jeevamrutha: Water Hyacinth in summer and winter seasons respectively.
- The macro-nutrient constituents such as %TKN, AN and P have shown higher nutrient uptake for vermicompost, neem cake, tea waste and water hyacinth-modified Jeevamrutha bio-fertilizer systems during the summer season (1.94%, 695.1 mg/L and 0.45%; 2.24%, 873.56 mg/L/0.08% and 0.71%; 3.22%, 1076.4 mg/L/0.1% and 0.65%, and 2.07%, 723.81 mg/L/0.07% and 0.58% respectively) in comparison with the winter season (0.85%, 416.6 mg/L/0.04% and 0.32%; 1.78%, 623.4 mg/L/0.06% and 0.64%; 1.94%, 726.8 mg/L/0.07% and 0.57%, and 1.42%, 525.7 mg/L/0.05% and 0.44% respectively). For AN, it is represented as mg/L (1 g of Jeevamrutha in 3 mL of concentrated H_2SO_4 digested solution).
- Similarly, %moisture content for vermicompost, neem cake, tea waste and water hyacinth modified Jeevamrutha bio-fertilizer system cases varied proportionally as 22.6-25.8, 11.2-19.4, 15.3-21.3 and 21.6-27.2 respectively for both the seasons. A

higher moisture level along with organic carbon content was achieved for the water hyacinth case (27.2% and 3.3% respectively). For the case, the temperature and relative humidity variation were about 32-37°C and 88-92% respectively.

- A higher microbial population was achieved for the tea waste case followed by the neem cake case and water hyacinth case. Maximum N-fixers and P-solubilizers (CFU/mL) were obtained for tea waste during summer (178×10^4 mL^{-1} and 104×10^5 mL^{-1}) and winter (124×10^4 mL^{-1} and 82×10^5 mL^{-1}) seasons respectively. Also, the least fungal growth was observed for the neem cake case which justifies its utility as a fungicide.

- Additionally, the phytotoxic effects of the mature precursors modified Jeevamrutha bio-fertilizer systems comprehend its utility to foster benefits in agricultural economics. Thereby, the optimal desired concentration for vermicompost and tea waste is 50% (v/v) and the desired concentration for neem cake and water hyacinth was 25% (v/v) during the summer season. On the other hand, 50% (v/v) concentration of vermicompost, neem cake, tea waste and water hyacinth were optimal choices in terms of higher shoot and root length during the winter season.
- The findings from the conceptual cost analysis affirm that tea waste and water hyacinth could be targeted as alternate rural resources for the production of the integrated organic farming systems during the winter season which witnessed a significant reduction in the AN and %TKN nutrient trends. Also, these nitrogen-rich precursors have excellent decomposition characteristics and greater cost-effectiveness that improves the efficacy of the modified Jeevamrutha bio-fertilizer in terms of its nutritive values. Thereby, they have a promising potential to reduce the detrimental use of chemical fertilizers in field applications.

7.1.3 Performance of Jeevamrutha Bio-fertilizer with Sugarcane Juice and Starch Water Carbon Source

- Among the two alternate carbon precursors, sugarcane juice-based modified Jeevamrutha bio-fertilizer case demonstrated a higher %TKN, %P and %OC concentration (1.35%, 0.67% and 1.6% respectively) along with a higher microbial density (CFU/mL) (bacteria, 93×10^{6}; N-fixers, 37×10^{4} and P-solubilizers, 41×10^{5} mL^{-1}) in comparison with jaggery-based modified Jeevamrutha bio-formulation (%TKN, 0.67%; %P, 0.14%; %OC, 0.62%; bacteria, 85×10^{6} mL^{-1}; N-fixers, 18×10^{4} mL^{-1} and P-solubilizers, 36×10^{5} mL^{-1}) during the summer season. On the other hand, for the winter case, %TKN (0.67%), %P (0.31%), %OC (1.6%) and a better microbial load (CFU/mL) (bacteria, 76×10^{6} mL^{-1}; N-fixers, 22×10^{4} mL^{-1} and P-solubilizers, 28×10^{5} mL^{-1}) were achieved in comparison with jaggery-based optimal Jeevamrutha biofertilizer during the winter (%TKN, 0.28%; %P, 0.10; %OC, 0.31%; bacteria, 47×10^{6} mL^{-1}; N-fixers, 11×10^{4} mL^{-1} and P-solubilizers, 28×10^{5} mL^{-1}).
- The consideration of starch rice water effectively enhanced the nutritive values of the developed bio-formulation. For both seasons, better combinations of %TKN, %P and %OC content (0.54-0.82%, 0.27-0.35% and 1.5-1.8%) along with higher microbial load (CFU/mL) (bacteria, 54-87$\times10^{6}$ mL^{-1}; N-fixers, 19-24$\times10^{4}$ mL^{-1} and P-solubilizers, 25-38$\times10^{5}$ mL^{-1}) were achieved in comparison with jaggery-based modified Jeevamrutha bio-fertilizer formulations (%TKN, 0.28-0.67%; %P, 0.10-0.14%; %OC, 0.31-0.62%; bacteria, 47-85$\times10^{6}$ mL^{-1}; N-fixers, 11-18$\times10^{4}$ mL^{-1} and P-solubilizers, 28-36$\times10^{5}$ mL^{-1}) during winter and summer seasons respectively.
- Micro- and macro-elements content such as Cu, Zn, Ca, Fe, Mn and Mg for the sugarcane juice-based modified Jeevamrutha bio-fertilizer case exhibited a variation of 0.069-0.071%, 0.003-0.004%, 0.003-0.074%, 0.009-0.013%, 0.002-0.004% and

0.051-0.066% respectively for both summer and winter seasons. Similarly, the variation of Cu, Zn, Ca, Fe, Mn and Mg micro- and macro-elements in starch rice water altered as 0.002-0.0021%, 0.0004-0.003%, 0.008-0.021%, 0.013-0.024%, 0.0009-0.0031% and 0.002-0.003% respectively during summer and winter seasons.

- Phytotoxicity assessment for the sugarcane juice based modified Jeevamrutha bio-fertilizer case revealed that for the summer case, 75% and 50% (v/v) were optimum for root and shoot growth of mung bean respectively. On the other hand, 25% and 75% (v/v) were the best among all treatments (0-100% (v/v)) for both sugarcane juice and starch rice water-based modified Jeevamrutha bio-fertilizer cases during the winter season.
- From the perspective of the conceptual cost analysis, starch rice water was the most cost-effective precursor (Rs. 20.15/kg) in comparison with jaggery-based Jeevamrutha bio-fertilizer (Rs. 22.49/kg) and terms of AN, %TKN and %P nutrient yield. Hence, to address the reduction in the nutritional characteristics of modified jaggery-based Jeevamrutha bio-fertilizer during the winter season, the inclusion of starch rice water could favour and enhance desired characteristics such as %TKN, AN and P nutrient yield. Thereby, soil fertility can be improved to provide adequate plant nutrition.

7.1.4 Integrated Organic Farming Studies of *Phaseolus vulgaris* with Jeevamrutha Bio-fertilizer-Vermicompost System

- Four alternate treatments namely integrated Jeevamrutha bio-fertilizer-vermicompost system (T1), Jeevamrutha system (T2), vermicompost system (T3) and soil-only system (T4) were assessed in terms of the growth, development and nutritional content of *Phaseolus vulgaris* along with the improvement in soil fertility. A 45 days pot experiment for *Phaseolus vulgaris* was conducted for the T1-T4 samples in April and May (Jeevamrutha:soil: vermicompost as 1:4:1 (T1), Jeevamrutha: soil as 1:4 (T2), vermicompost: soil 1:4 (T3) and soil (T4) and at an ambient temperature of (29-31°C). Maximum shoot and root length were achieved for the T1 case (42.3 and 19.3 cm respectively) along with the number of leaves, branches and fruits (32, 14 and 12 respectively).

- The soil fertility (%TKN, %P, %K and %OC) improved significantly for T1 (1.86%, 0.86%, 0.71% and 2.1% respectively) followed by T3 evaluated parameters (%TKN, %P and %OC of 1.45%, 0.74, 0.64% and 1.58% respectively). Likewise, T1 and T3 treatments confirmed better chlorophyll (26 and 25 μg/g), moisture (36 and 38%) and P content (0.18 and 0.16%) respectively during the mid-summer season.
- T1 and T2 treatments exhibited maximum carbohydrate (2.7 and 1.7 per unit g), carotenoids (37 and 35 μg/g), soluble protein (0.037 and 0.026 mg/g) and vitamin C (84.2 and 56.4 mg/100g) along with %TKN (1.8 and 1.7%) and %P (0.18 and 0.15%) during mid-summer season.
- N-fixers and P-solubilizers were higher for T1 (24×10^4 and 28×10^4 mL^{-1}) and T3 (16×10^4 and 17×10^4 cfu/mL) cases followed by T2 case (14×10^4 and 13×10^4 mL^{-1}) with maximum actinomycetes count for T1 (15×10^2 cfu/mL) and T2 (9×10^2 mL^{-1}) cases respectively during the mid-summer season.

7.2 Future Work

Based on the quantum of work conducted in this thesis, the following research directions could be targeted for the needful consideration in the near future for the address of subsequent challenges in the development of cost-effective bio-fertilizer formulations with easily accessible rural commodities.

- Efficacy of the solid Jeevamrutha bio-fertilizer with a variation in cow breeds, soil and water sources that could be considered to evaluate their competence in terms of nutrient yield variant source conditions.
- Evaluate the influence of pH range (alkaline/acidic) using banana peel paste or lemon peel extract to further correlate upon soil characteristics, nutrient characteristics and compositional optimality.
- Target the addition of vegetable waste or fruit peel in the Jeevamrutha formulation and scope upon its efficacy in terms of evaluated characteristics.
- Replace optimized nitrogen sources such as gram flour with soybean waste or onion peel and carbon source with starch rice water to quantify its cost-effectiveness on the Jeevamrutha bio-fertilizer formulations.
- Incorporate nitrogen-rich precursors such as tea waste and water hyacinth into starch rice water-based Jeevamrutha bio-fertilizer as an integrated system to evaluate its nutritional characteristics in terms of plant growth and soil fertility.
- Target similar studies with modified Sanjibani formulation (with only cow dung and cow urine and starch water as key precursors) to develop a super-efficient low-cost bio-fertilizer with a combination of minimal cow dung and cow urine and maximum starch water constitution.
- Conduct integrated farming system (IFS) studies with soya bean flour/gram flour and starch rice water-based optimally modified Jeevamrutha and Sanjibani bio-fertilizer system.
- Microbial strain identification to further gain insights into the metabolic capacities of microorganisms supporting different biochemical processes that could contribute to plant tolerance against stress and soil pathogens.

- Scale-up studies to further validate the process and suggest innovative practices in the agro-rural sector of India.
- Target field studies at pilot scale level and for alternate plant crops.

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Publications

1. Publications

a. **Bhattacharjee, U.**, Uppaluri, R., 2023. Growth and nutritional characteristics of *Phaseolus vulgaris* and Jeevamrutha bio-fertilizer-vermicompost system. Bioresource Technology Reports 22, 101416. https://doi.org/10.1016/j.biteb.2023.101416

b. **Bhattacharjee, U.**, Uppaluri, R., 2023. Production and Optimization of Jeevamrutha Bio-fertilizer Formulations for Soil Fertility and its Role in Waste Minimization. Sustainable Chemistry for Climate Action, 2, 100025. https://doi.org/10.1016/j.scca.2023.100025.

c. **Bhattacharjee, U.**, Uppaluri, R., 2023. Utility of cow excreta-based bio-enhancer: Boon substrate for the growth study in *Phaseolus vulgaris* & its variability in the modified Jeevamrutha bio-formulation. Conservation of Biodiversity in the North Eastern States of India. NERC 2022. Springer, Singapore. https://doi.org/10.1007/978-981-99-0945-2_3.

d. **Bhattacharjee, U.**, Uppaluri, R., 2022. Screening and scoping of precursors associated to the production of jeevamrutha bio-fertilizer. Materials Today-Proceedings 68, 679-685. https://doi.org/10.1016/j.matpr.2022.05.410.

2. Articles to be Submitted

a. **Bhattacharjee, U.**, Uppaluri, R. Incorporation and Efficacy of Nutrient-rich Precursors in Jeevamrutha Bio-fertilizer for Organic Farming Application.

b. **Bhattacharjee, U.**, Uppaluri, R. Replacing Jaggery-based Jeevamrutha Bio-fertilizer in Assam with Sugarcane juice and Starch Rice Water to Improve Plant Nutritional Factors and Soil Fertility.

Appendices

Appendix A: Calibration Curves for Determination of Ammonium Nitrogen, Phosphate and Soluble Protein Content

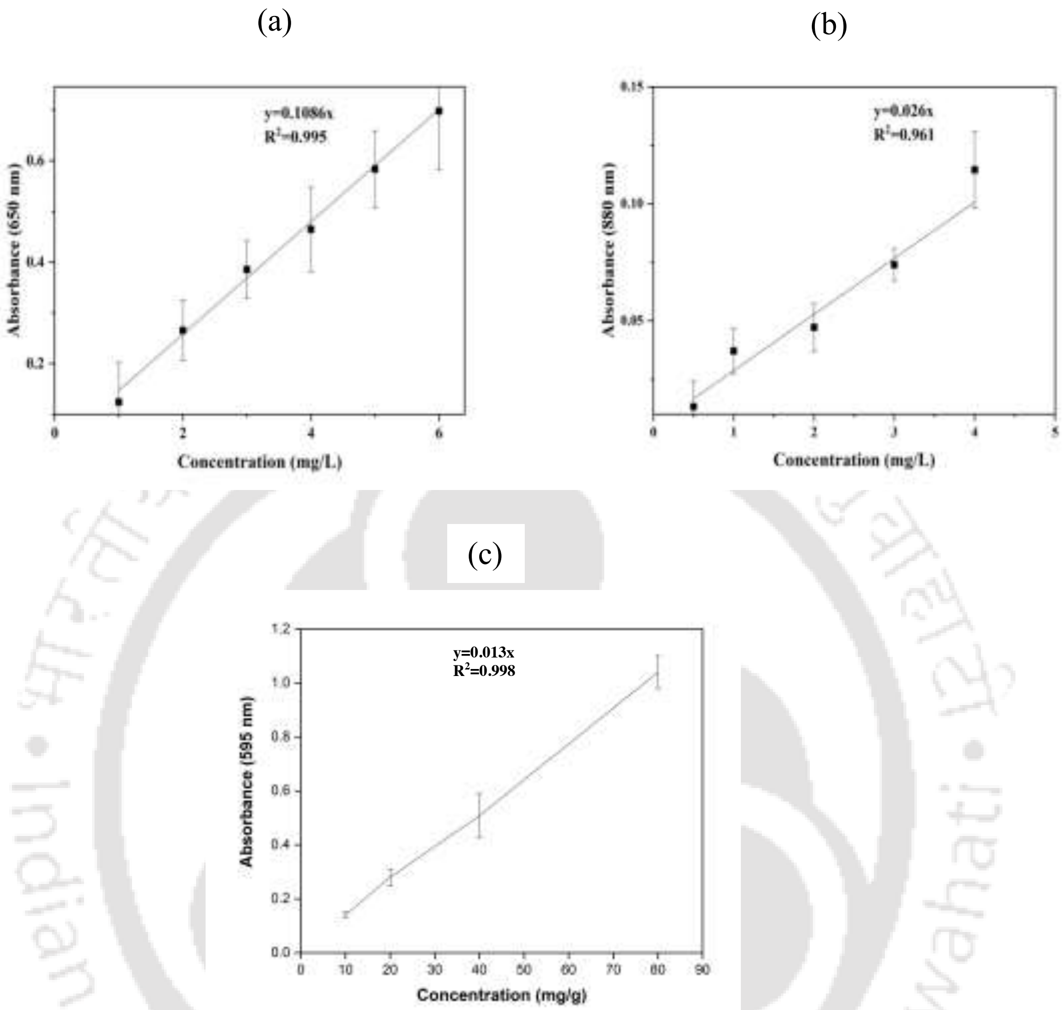


Fig. A1: Calibration curves for determination of **(a)** AN, **(b)** P and **(c)** Soluble protein content using UV-Vis spectrophotometer.

Appendix B: Images of Equipment and Samples used in this Ph.D Studies along with the Bio-product Achieved

UV—Vis Spectrophotometer

Kjeldahl Nitrogen Analyzer

HPLC-RID

TOC Analyzer

AAS

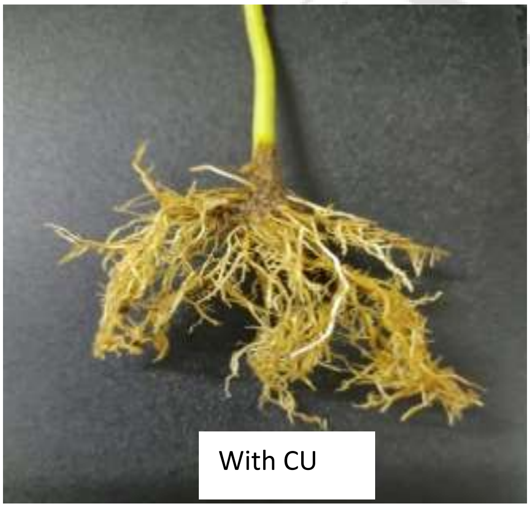
Muffle Furnace

Laminar Flow hood

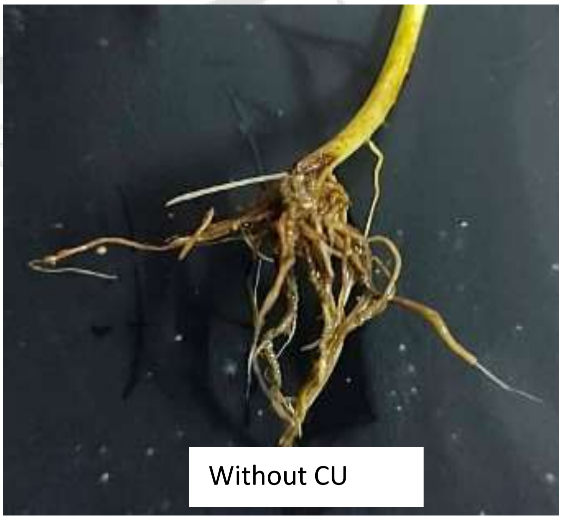
pH meter

Flame photometer

Root health of soil



Root health of sample 1



Root health of sample 2

Day 45 fruit yield

Prepared Jeevamrutha Bio-fertilizer

Appendix C: One-way ANOVA Data Analysis for Pot Experiments with *Phaseolus vulgaris*

Table C1: ANOVA-based statistical data summary for various cases **(a)** Shoot length, **(b)** Leaf length, **(c)** Number of leaves, **(d)** Number of branches and **(e)** Number of fruits of plant sample; **(f)** Carbohydrate, **(g)** Carotenoids, **(h)** Chlorophyll, **(i)** Moisture Content, **(j-l)** Macronutrients: %N, %P and %K, **(m)** Soluble protein and **(n)** Vitamin C of fruit sample and **(o)** Moisture content, **(p)** %N, **(q)** %P, **(r)** %K, **(s)** pH, **(t)** EC, **(u)** %OC, **(v)** Bacteria, **(w)** Fungi, **(x)** Actinomycetes, **(y)** N-fixers and **(z)** P-solubilizers of soil sample.

(a) Shoot length of plant sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	1129.05	282.26	2776.36	1.34182E-12
2	Error	8	0.81	0.1		
3	Total	11	1129.86			

(b) Leaf length of plant sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	199.53	66.51	1308.39	4.20E-11
2	Error	8	0.4	0.05		
3	Total	11	199.93			

(c) Number of leaves of plant sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	544.25	181.4	217.7	5.25E-8
2	Error	8	6.66	0.83		
3	Total	11	550.9			

(d) Number of branches of plant sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	544.25	181.4	217.7	5.25E-8
2	Error	8	6.66	0.83		
3	Total	11	550.9			

(e) Number of fruits of plant sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	83.66	27.88	55.77	1.046E-5
2	Error	8	4	0.5		
3	Total	11	87.66			

(f) Carbohydrate of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	3.58	1.19	143.3	2.72E-7
2	Error	8	0.06	0.008		
3	Total	11	3.64			

(g) Carotenoids of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	376.25	125.4	150.5	2.24-7
2	Error	8	6.66	0.83		
3	Total	11	382.92			

(h) Chlorophyll of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	353.66	117.88	235.77	3.83E-8
2	Error	8	4	0.5		
3	Total	11	357.66			

(i) Moisture Content of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	243.29	81.09	154.22	2.03E-7
2	Error	8	4.2	0.52		
3	Total	11	247.49			

(j) Macronutrients: %N of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	1.35	0.45	36	5.41E-5
2	Error	8	0.1	0.01		
3	Total	11	1.45			

(k) Macronutrients: %P of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	0.03	0.001	120.1	5.43E-7
2	Error	8	6.66E-4	8.33E-5		
3	Total	11	0.03			

(l) Macronutrients: %K of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	3.85	1.28	343.40	8.64E-9
2	Error	8	0.02	0.003		
3	Total	11	3.88			

(m) Soluble protein of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	7.54E-4	2.51E-4	502.83	1.90E-9
2	Error	8	4E-6	5E-7		
3	Total	11	7.58E-4			

(n) Vitamin C of fruit sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	4760.76	1586.92	47607.6	0
2	Error	8	0.26	0.033		
3	Total	11	4761.02			

(o) Moisture content of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	157.82	52.60	751.56	3.84E-10
2	Error	8	0.56	0.07		
3	Total	11	158.38			

(p) %N of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	4.87	1.62	1203.08	5.88E-11
2	Error	8	0.01	0.001		
3	Total	11	4.88			

(q) %P of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	0.08	0.02	884.25	2.0E-10
2	Error	8	2.6E-4	3.33E-5		
3	Total	11	0.08			

(r) %K of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	0.09	0.03	477.66	2.33E-9
2	Error	8	5.33E-4	6.66		
3	Total	11	0.09			

(s) pH of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	4.15	1.38	159.83	1.77E-7
2	Error	8	0.06	0.008		
3	Total	11	4.22			

(t) EC of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	2.19	0.73	219	512E-8
2	Error	8	0.026	0.0033		
3	Total	11	2.21			

(u) %OC of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	5.76	1.92	1302.46	4.28E-11
2	Error	8	0.01	0.001		
3	Total	11	5.77			

(v) Bacteria of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	14874.5	4958.08	3718.56	6.48E-13
2	Error	8	10.66	1.33		
3	Total	11	14884.91			

(w) Fungi of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	99.66	33.22	66.44	5.36E-6
2	Error	8	4	0.5		
3	Total	11	103.66			

(x) Actinomycetes of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	259.58	86.52	129.79	4E-7
2	Error	8	5.33	0.66		
3	Total	11	264.91			

(y) N-fixers of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	344.25	114.75	344.25	8.56E-9
2	Error	8	2.66	0.33		
3	Total	11	346.91			

(z) P-solubilizers of soil sample

S.No.		DF	Sum of Squares	Mean Square	F-value	Prob>F
1	Model	3	775	258.33	516.66	1.70E-9
2	Error	8	4	0.5		
3	Total	11	779			

Appendix D: Sample Calculation for the Evaluation of Various Parameters

In this section, a sample calculation has been presented for the determination of various parameters of Jeevamrutha bio-fertilizer samples.

a. AN of Palekar's Jeevamrutha=> $x=y/0.1063$;

$=0.308/0.1063\times100$ (Dilution factor)

$=289.7$ mg/L

b. %TKN of Optimized Jeevamrutha=14.01 (Blank titration-Sample titration) ×0.1×100/Weight of the sample×1000

%TKN=14.01 (2.7-0.2) ×0.1×100/0.525×1000

=0.67%

c. P nutrient yield of Palekar's Jeevamrutha =>$x=y/0.02$;

$=0.1/0.02\times100$ (Dilution factor)

=500 mg/L

=0.05%

d. Germination Index (GI) of optimized Jeevamrutha (%)

Seed Germination (%) = No. of germinated seeds in test solution/No. of germinated seeds in control solution×100

$=0.51\times100$

=51%

Root elongation=Mean root length in sample/ Mean root length in control×100

$=1.72\times100$

=172.4

GI=SE (%) ×root elongation/100

=87.8%

e. Moisture content of optimized Jeevamrutha (%) = W_1-W_2/W_1×100

=5.8-4.8/5.8×100

=17.8%

f. Carbohydrate content in the integrated system =25×absorbance of sample/ absorbance of standard solution

=25×0.11

=2.75 mg/g

g. Soluble Protein content in the integrated system =>x=y/0.02;

=0.49/0.013 mg/L

=0.037 mg/g

h. Vitamin C content in the integrated system=0.5×V_2×10×100/V_1×5×W_2;

V1=blank titration; V2=sample titration; W2=weight of the sample;

=0.5×15×10×100/3.55×5×5

=84.5 mg/100g

i. Chlorophyll content in the integrated system=

Chlorophyll a =12.25×$A_{663.6}$ – 2.25×$A_{646.6}$

=12.25×2-2.25×5.4

=19

Chlorophyll b =20.31×$A_{646.6}$– 4.91×$A_{663.6}$

=20.31×0.5-4.91×0.6

=7

Total Chlorophyll = Chlorophyll a+ Chlorophyll b

=19+7

=26 μg/g

Appendix E: Precursors Associated Conceptual Cost Analysis

In this section, cost associated during the preparation of precursors'-based modified Jeevamrutha bio-fertilizers are outlined.

a. Jaggery-based Jeevamrutha Bio-fertilizer

For 1 kg Jeevamrutha,
The cost of 0.03 kg jaggery is Rs.1.8; 0.03 kg gram flour is Rs. 2.4; 0.04 L cow urine is Rs.10 and 0.056 kg cow dung is Rs. 3.1 respectively.
Total Amount is Rs.17.3;
Labour Charge is 30% of total cost,
Cost for 1 kg Jeevamrutha including labour cost is Rs.(17.3+5.19)
Therefore, grand total amount is Rs. 22.49 per kg.

b. Vermicompost (VC)-based modified Jeevamrutha (J) Bio-fertilizer

For 1:4 (VC,200:J,800),
For 0.8 kg Jeevamrutha;
The cost of 0.02 kg jaggery is Rs. 1.44; 0.02 kg gram flour is Rs. 1.92; 0.01 L cow urine is Rs. 4 and 0.04 kg cow dung is Rs. 2.4 respectively.
Total amount for J is Rs. 9.76,
Total amount for VC is Rs. 2.94,
Grand total +30% labour charge is Rs. 16.51 per kg

c. Sugarcane juice-based Jeevamrutha Bio-fertilizer,

For 1kg sample,
The cost of 0.03 L sugarcane juice is Rs. 4.5; 0.03 kg gram flour is Rs. 2.4; 0.04 L cow urine is Rs.10 and 0.056 kg cow dung is Rs. 3.1
Total Amount is Rs.20,
Labour Charge is 30% of total cost,
Cost for 1 kg Jeevamrutha including labour cost @30% is Rs. 26.

Appendix F: Statistical Terms and their Formulae

In this section, a detailed account of the relevant statistical terms and their pertinent formulae has been presented as follows.

a. F-test statistics

The F-test parameter is determined using the expression from the book Beginning statistics (v.1.0) (Mosteller, Frederick, Stephen E. Fienberg, and Robert EK Rourke. Beginning statistics with data analysis. Courier Corporation, 2013).

$$\text{F - test} = \frac{\text{MST}}{\text{MSE}}$$

MST= Mean square for treatment
MSE= Mean square for error

$$\text{MST} = \frac{n_1(x_1 - x)^2 + n_2(x_2 - x)^2 + \ldots + n_k(x_k - x)^2}{k - 1}$$

$$\text{MSE} = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \ldots + (n_k - x_0)s_k^2}{n - k}$$

Where, n_1, n_2… and n_k are the sample sizes, x_1, x_2… x_k are the sample mean, s_1^2, s_2^2 and s_k^2 are the sample variance and k is the population.

b. Sum of squares (SS)

The sum of squares (SS) is expressed as:

$$SS = \sum(x - x_1)^2$$

where, x=variable in a set of sample data,
x_1= average/mean of whatever the variable represents.

Accordingly, the highest order polynomial is selected for which the additional terms are significant and the model is not aliased (p-value < 0.05).

c. Degree of freedom (df)

The degree of freedom term (df) is expressed as:

$df = (n - 1)$ where n=sample size.

For the model to be significant, a minimum of three lack-of-fit df and four pure error df terms are required.

d. Errors

With the measurement of pure error from replicated runs, it is possible to examine the F-test findings for the lack of fit. Degrees of freedom for residual error must be available to determine the power factor. Hence, for unreplicated factorial designs, the primary model effects will be specified.

e. (i) Adjusted sum of squares, F and p-value

Adjusted sums of squares in the Minitab does not depend on the order when the factors are entered into the model. It is a part of Sum of Square Regression elucidated by a factor, given all other factors in the model, irrespective of the order they were entered into the model. Also, the sequential and adjusted sums of squares are always the same for the last term in the model.

Additionally, Minitab uses the F-value to calculate the p-value, which can be used to make a decision about the statistical significance of the test.

The p-value is a probability that measures the evidence against the null hypothesis. Lower probabilities provide stronger evidence against the null hypothesis.

(ii) R^2 and Adjusted R^2

$$R^2 = 1 - \frac{\text{SS Error}}{\text{SS Total}}$$

$$\text{Adj.}R^2 = 1 - ((1 - R^2)\frac{N-1}{N-M-1}$$

where, SS=the sum of squares,
N=no. of rows
M=no. of columns.