

**SCIENTIFIC VALIDATION OF JEEVAMRUTHA  
FOR SUSTAINABLE AGRICULTURE**

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**CENTRE FOR RURAL DEVELOPMENT & TECHNOLOGY  
INDIAN INSTITUTE OF TECHNOLOGY DELHI  
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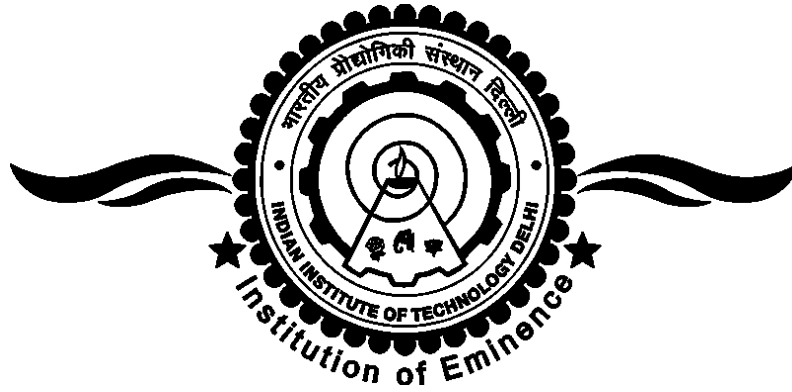
**SCIENTIFIC VALIDATION OF  
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by

**DURAIVADIVEL P  
CENTRE FOR RURAL DEVELOPMENT & TECHNOLOGY**

Submitted

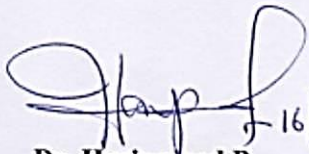
**in fulfillment of the requirements of degree of Doctor of Philosophy  
to the**



**INDIAN INSTITUTE OF TECHNOLOGY DELHI  
DECEMBER 2022**

## CERTIFICATE

This is to certify that the thesis entitled “**Scientific Validation of Jeevamrutha for Sustainable Agriculture**”, being submitted by **Mr. Duraivadivel P.** to the **Indian Institute of Technology Delhi** for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge, the results contained in this thesis has not been submitted in part or full to any other university or institute for the award of any degree or diploma.



16/08/2022

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by

Duraivadivel P

## ABSTRACT

Jeevamrutha is a traditional organic bioformulation used for several decades by farmers. It is prepared using locally available resources such as cow dung, cow's urine, jaggery, pulse flour, soil, and water. Though jeevamrutha is said to possess various beneficial properties related to soil and plant health, till date detailed studies highlighting its microbial dynamics, functional properties and long-term field studies are lacking. To address several issues associated with jeevamrutha, the present study was carried out under five objectives to understand jeevamrutha preparation, structural and functional diversity of microbes and their role in promoting plant growth and soil fertility. Further, long-term field studies were conducted to understand the impact of jeevamrutha application on soil and plant health.

A field survey was conducted at several places in India, during which a questionnaire comprising 19 questions about jeevamrutha was used to document farmers' opinions/experiences related to its use. The survey's significant finding was that most farmers use jeevamrutha as it is low cost, prepared using local resources, and organic. However, due to the lack of scientific validation of jeevamrutha, young farmers showed apprehension regarding its future use. The survey was followed by physicochemical and microbial characterization of jeevamrutha in the laboratory. The pH, EC, TDS, DO, C/N ratio, protein, microbial load, and activity was assessed for 12 days of jeevamrutha incubation. It was found that, as the incubation progressed, a pH decline was observed along with an increase in EC value.

Further, the microbial characterization revealed that jeevamrutha is a fermentative product harboring various microbes dominated by *Lactobacillus spp.* During incubation, bacterial succession happens, facilitating the survival of only those microbes that can withstand the unique physico-chemical and biological conditions of jeevamrutha. Moreover, metagenomics, metabolomics, and proteomics analysis of eight-day old jeevamrutha revealed the presence of phylum Firmicutes, Bacteroidetes, and Proteobacteria as the dominant microbial communities. The presence of bacteriophages, fungi, and unclassified microbes in jeevamrutha indicated its diversity and potential for further research. Further, various metabolites such as lactic acid, ascorbic acid, caproic acid, succinic acid, etc. and proteins such as chaperone proteins, heat shock proteins, elongation factor-Tu, etc. were found in jeevamrutha. The diverse microbial communities and the metabolites and proteins present in jeevamrutha were reported to promote plant and soil health directly or indirectly.

Moreover, field experiments were conducted to understand the impact of long-term jeevamrutha application on soil parameters such as soil organic carbon (SOC), pH, microbial load, and water holding capacity (WHC). It was found that jeevamrutha application along with basal application of farmyard manure (FYM) for four consecutive years increased the SOC and microbial load, thereby improved the soil quality and fertility. Further, jeevamrutha application along with FYM was found to increase growth of medicinal plant *Centella asiatica* and enhanced the yield from the 102 to 120 kg/acre. Overall, the present study is an attempt to scientifically validate jeevamrutha and evaluate its impact on soil health upon long-term application.

## सारांश

जीवामृत एक पारंपरिक जैविक बायोफॉर्म्यूलेशन है जिसे किसानों द्वारा कई दशकों से उपयोग किया जाता रहा है। इसे स्थानीय संसाधनों गोबर, गोमूत्र, गुड़, पिसी हुई दाल, मिट्टी और पानी के द्वारा तैयार किया जाता है। यद्यपि जीवामृत के बारे में कहा जाता है कि इसमें मिट्टी और पौधों के स्वास्थ्य से संबंधित विभिन्न लाभकारी गुण होते हैं, लेकिन इसके सूक्ष्मजीवी गतिकी, कार्यात्मक गुणों और मिट्टी और पौधों के स्वास्थ्य पर जीवामृत के उपयोग का दीर्घकालिक प्रभाव सम्बंधित गहन अध्ययनों और वैज्ञानिक प्रमाणिकता की कमी है। तथैव वर्तमान अध्ययन को पांच उद्देश्यों के द्वारा जीवामृत उत्पादन, जीवामृत में सूक्ष्मजीवों की संरचनात्मक और कार्यात्मक विविधता और मिट्टी और पौधों के स्वास्थ्य में सुधार में उनकी भूमिका को समझने के लिए किया गया है। इसके अलावा, मिट्टी और पौधों के स्वास्थ्य पर जीवामृत के उपयोग का दीर्घकालिक प्रभाव को व्यापक रूप से समझने के लिए खेती आधारित अध्ययन भी किए गए।

सर्वप्रथम जीवामृत के उपयोग से सम्बंधित किसानों की राय/अनुभव जानने के लिए भारत में कई स्थानों पर 19 प्रश्नों की प्रश्नावली के द्वारा एक सर्वेक्षण किया गया। सर्वेक्षण में अधिकांश किसानों ने स्वीकार किया कि पारंपरिक, जैविक और कम लागत में स्थानीय संसाधनों से बनने की वजह से वो जीवामृत का उपयोग करते हैं। हालांकि, जीवामृत की वैज्ञानिक मान्यता की कमी के कारण, युवा किसानों ने इसके भविष्य के उपयोग के प्रति आशंका व्यक्त किया। सर्वेक्षण के बाद प्रयोगशाला में जीवामृत के भौतिक-रासायनिक और सूक्ष्मजीवों की संरचनात्मक और कार्यात्मक विवरण प्राप्त किया गया। इसके लिए जीवामृत सैंपल के 12 दिनों के इन्क्यूबेशन के दौरान pH, EC, TDS, DO, C/N अनुपात, प्रोटीन, और सूक्ष्मजीवों की उपस्थिति और उनकी गतिविधि का मूल्यांकन किया गया। इन्क्यूबेशन अवधि के दौरान pH में कमी और EC में वृद्धि दर्ज की गई।

इसके अलावा, सूक्ष्मजीवों की उपस्थिति और उनकी गतिविधि के मूल्यांकन से पता चला कि जीवामृत एक किण्वक उत्पाद है जिसमें लैक्टोबैसिलस बहुतायत मात्रा में उपलब्ध होता है। इन्क्यूबेशन के दौरान केवल जीवामृत की अनूठी भौतिक-रासायनिक और जैविक स्थितियों में जीवित रह सकने वाले बैक्टीरिया ही पनपते हैं। इसके अतिरिक्त, 8 दिनों के इन्क्यूबेशन के बाद जीवामृत के मेटागेनोमिक्स, मेटाबोलामिक्स और प्रोटीओमिक्स अध्ययनों से जीवामृत संयोजन में फाइलम फर्मिक्यूट्स, बैक्टीरियोडेट्स, और प्रोटोबैक्टीरिया प्रचुर मात्रा में मौजूदगी

का पता चला। साथ ही बैक्टीरियोफेज, कवक और अवर्गीकृत सूक्ष्मजीवों की उपस्थिति से जीवामृत में जटिल सुक्ष्मजैव विविधता और आगे के शोध की जरूरत का पता चलता है। इसके अलावा, जीवामृत में विभिन्न मेटाबोलाइट्स जैसे कि लैक्टिक एसिड, एस्कॉर्बिक एसिड, कैप्रोइक एसिड, स्यूसिनिक एसिड, आदि और प्रोटीन जैसे कि चैपरोन प्रोटीन, हीट शॉक प्रोटीन, आदि भी पाए गये। जीवामृत में मौजूद विविध सूक्ष्मजीव, मेटाबोलाइट्स और प्रोटीन एक्सोपॉलीसेकेराइड प्रत्यक्ष और अप्रत्यक्ष रूप से मिट्टी के स्वास्थ्य और पौधों के पोषण के लिए लाभकारी होते हैं।

जीवामृत उपयोग का मिट्टी के pH, जल धारण क्षमता, मृदा जैविक कार्बन, सूक्ष्मजीवों की उपस्थिति और उनकी गतिविधि जैसे मापदंडों पर दीर्घकालिक प्रभाव का आकलन के लिए खेती आधारित अध्ययन किए गए। प्रायोगिक अध्ययन में पाया गया कि लगातार चार वर्षों तक खेत में जैविक खाद के साथ जीवामृत के प्रयोग से मृदा जैविक कार्बन, सूक्ष्मजीवों की उपस्थिति में वृद्धि हुई, जिससे मिट्टी की गुणवत्ता और उर्वरता में सुधार हुआ। इसके अलावा, जैविक खाद के साथ जीवामृत के उपयोग औषधीय पौधे *संटेला एशियाटिका* के विकास के लिए अनुकूल पाया गया और उपज 102 किलोग्राम प्रति एकड़ से बढ़कर 120 किलोग्राम प्रति एकड़ हो गई। वर्तमान अध्ययन जीवामृत को वैज्ञानिक रूप से मान्य करने और मिट्टी और पौधों के स्वास्थ्य पर जीवामृत के उपयोग के दीर्घकालिक प्रभाव का मूल्यांकन करने का यह पहला प्रयास है।

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## **Jeevamrutha**

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## LIST OF SYMBOLS /ABBREVIATIONS

Symbol/Syntax	Description
%	Percentage
°C	Celsius
μ	Micron
μg	Microgram
μL	Microliter
μm	Micrometer
μS/cm	Micro siemens Per Centimeter
2D-LC MS/MS	Two-dimensional liquid chromatography/tandem mass spectrometry
AP	Andhra Pradesh
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
BOD	Biological Oxygen Demand
bp	base pair
BSA	Bovine Serum Albumin
C/N	Carbon and nitrogen
CaCl <sub>2</sub>	Calcium chloride
CAGRs	Cumulative Annual Growth Rates
CFU	Colony Forming Units
CHN	Carbon hydrogen and nitrogen
cm	Centimeter
COG	Clusters of Orthologous Group
CRDT	Centre for Rural Development and Technology
CRF	Central Research Facility
DAP	Diammonium phosphate
DAS	Days after sowing
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
DTT	Dithiothreitol
EC	Electrical Conductivity
EM	Effective Microorganisms
EPS	Extracellular polymeric substances
FAI	Fertiliser Association of India
FAO	Food and Agriculture Organization
FDA	Fluorescein diacetate
FDR	False discovery rate
FiBL	Forschungsinstitut für biologischen Landbau
FYM	Farmyard Manure
g	Gram
Gb	Gigabyte

GC-MS	Gas chromatography–mass spectrometry
GHG	Greenhouse gas
GO	Gene Ontology
GPS	Global Positioning System
GRAS	Generally Recognized as Safe
Gt	Gross Tonnage
ha	Hectare
HCl	Hydrochloric acid
HP-TLC	High Performance-Thin Layer Chromatography
HQ	High Quality
HR-LCMS	High Resolution Liquid Chromatograph Mass Spectrometer
HS	High Sensitivity
HYV	High Yield Variety
IAA	Indole acetic acid
ICAR	Indian Council of Agricultural Research
ICH	Isochorismate synthase
IITD	Indian Institute of Technology Delhi.
INR	Indian rupee
IPL	Isochorismate pyruvate-lyase
JA	Jeevamrutha
K <sub>2</sub> O	Potassium oxide
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilogram
kg/ha	Kilogram Per Hectare
kg/ha/yr	Kilogram Per Hectare per Year
L	Litre
LA	Leaf area
LAB	Lactic Acid Bacteria
LCA	Life Cycle Assessment
M	Molar
mg	Milligram
Mg	Magnesium
MGGP	Mahatma Gandhi Grammodaya Parisar
Mha	Million hectares
min	Minute
mL	Milliliter
mM	Millimolar
MMT	Million metric tons
mol	Moles
mS/cm	Milli Siemens per centimeter
MT	Metric tons
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
N	Normality
N	Nitrogen

NA	Nutrient Agar
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NF	Natural Farming
ng/μl	Nanogram Per Microlitre
NGOs	Non-governmental organizations
NITI	National Institution for Transforming India
nm	Nanometer
NRF	Nano Research Facility
NSSO	National Sample Survey Office
OAs	Organic Acids
OD	Optical Density
OTUs	Operational Taxonomic Units
P	Phosphorus
P <sub>2</sub> O <sub>5</sub>	Phosphorus pentoxide
PBS	Phosphate buffer saline
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
Pfam	Protein families
PKVY	Paramparagat Krishi Vikas Yojana
QS	Quorum sensing
RNA	Ribonucleic acid
rpm	Revolutions per minute
S	Sulphur
SAIF	Sophisticated Analytical Instrument Facility
SOC	Soil Organic Carbon
SRL	Sisco Research Laboratories
TCA	Tricarboxylic acid cycle
TDS	Total Dissolved Solids
TE	Triennium Endings
TOC	Total Organic Carbon
UN	United Nation
UNEP	United Nations Environment Programme
UP	Uttar Pradesh
USA	United States of America
VC	Vermicompost
w/v	Weight by volume
w/w	Weight by weight
WHC	Water holding capacity
WHO	World Health Organization
ZBNF	Zero Budget Natural Farming