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# Characterising the traditional organic liquid formulations used by the farmers of western agro climatic zone of Tamil Nadu

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The study was conducted to document the preparation method of organic liquid formulations used by the farmers belongs to the western agro climatic zone of Tamil Nadu and to characterise the same. Most of the formulations have been used as manure, pest and disease control agents since ancient times. In this study, for the first time, we attempted to characterise all the organic liquid formulations by measuring its physiochemical properties (pH, EC, OC, TDS), macronutrients (N, P, K) and micronutrients (Zn, Cu, Mn, Fe). The microbial status of the fermented liquid manure was also enumerated (bacteria, fungi, actinobacteria). Physiochemical properties divulged that most of the formulations had an acidic pH except a few. The analysis revealed the presence of micronutrients in liquid manures that could promote plant growth. Microbial analysis proved the preparation has more naturally occurring colonies. Mould formation was studied to check the formulation free from spoiling microbes. The results obtained proved the mineral potential of the liquid formulations, which may replenish the soil's fertility and promote plant growth.

Keywords: Macronutrients, Micronutrients, Organic liquid formulations, Physiochemical properties

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Current farming situation urges to develop an ecofriendly farming technique as the conventional crop production throughout the globe affected the soil fertility environment quality and leading to un-sustainability in production. Consequently, these concerns imparted a way to organic farming<sup>1</sup>. In Tamil Nadu, organic farming occupies a little area of 30,910 acres of land (certified) of all the utilised agricultural area<sup>2</sup>. However, during the past few decades, this organic farming technique has undergone rapid development. Especially in the western agro climatic zone of Tamil Nadu, organic farming is gaining momentum and the farmers are practicing very systematically. In this region, farmers are using different types of organic manures, liquid formulations that improves the plant health, soil quality, pest resistance by inducing systemic resistance through the production of secondary metabolites.

Use of organic liquid formulations has been in practice since the Vedic age, for instance Vrikshayurveda talks about such type of formulations (Panchagavya)<sup>3</sup> for the benefit of plant growth and soil health. Presently, usage of organic liquid formulations prepared from on farm resources is gaining momentum and demand for the same are increased in the study zone. Many of these formulations add organic and mineral matter to soil, rich in beneficial microflora and preferred by the farmers<sup>4</sup>. These liquid formulations have ingredients like cow dung, cow urine, legume flour and jaggery which contain both macronutrients and essential micronutrients, numerous vitamins, essential amino acids, and growth-promoting compounds like indole acetic acid (IAA) and gibberlic acid (GA). This may be primarily responsible for the presence of these beneficial microorganisms in these formulations<sup>5-7</sup>.

Foliar application of these liquid formulations is very important for curing micronutrient deficiencies and enhancing plant performances. Panchagavya, fish amino acid, egg amino acid, vermiwash, jeevamirtham, 5 leaf extract, amirtham solution, EM solution are some of the commonly used organic liquid formulations by the farmers of this region and has grown to be a significant focus of research in the

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realm of organic farming. Very little research has been attempted to reveal their scientific rationale, *i.e.*, Panchagavya<sup>8</sup>, Jeevamirtham<sup>9</sup>, beejamirtham<sup>6</sup>. There is a dearth of scientific rationale to support the usage of other formulations and no prior research has looked into this issue from this area. Therefore, we tried to collect the standard preparation methods of the liquid formulations and basic characterisation of these inputs.

### Methodology

# Study area

The focus areas of study were selected from Sathyamangalam and Gobichettipalayam of Erode District, Tamil Nadu, India. The area for this study, which is part of Tamil Nadu's western agro-climatic zone, was purposefully chosen since more farmers are engaging in organic farming, and the amount of certified organic land is growing daily. Climate of the experimental area was classified as tropical with an altitude of 257 m above MSL. The annual rainfall of the area varies from 524 to 1428 mm with an average of 711mm. Mixed methodology was adopted for the study.

#### Semi-structured interviews

A semi-structured questionnaire was used to collect data. A few selection criteria were used to select the farmer's field. The criteria were: (1) history of field (2) number of years of doing organic farming, and (3) Distinctive practices in the organic field. A questionnaire was prepared in Tamil language and interviews to gather information about organic crop management aspects of the farm and about the fixed about criteria. Respondents were asked the preparation of organic liquid formulations (Fig. 1). We have obtained a consent from the farmers head for conducting the interview. Fifty farmers of 30 different villages of Erode District of Tamil Nadu were interviewed with detailed questionnaire contains the details of the cultivation practices.

#### Visual observation

Observation of input preparation, compost unit, and preparation of liquid formulations was made during walk to different farms and visited their fields.

# Organic input sample collection

In order to obtain samples from the whole region of Sathyamangalam and Gobichettipalayam, sampling sites were randomly delineated. The organic liquid formulations samples collected from the farmer's field were kept in clean plastic bottles, labelled and brought to the laboratory for analyses (Fig. 1).

# **Physiochemical tests**

Quantitative analysis for total solids, pH, EC of all the organic liquid formulations was done in triplicates. Available N was extracted with 2 M KCl for 1 h and determined by Kjeldahl method<sup>10</sup>. Micronutrients were analysed using triple acid extract method. Three mL of liquid formulation was taken and 15 mL of triple acid is added and kept for cold digestion overnight. Then wet digestion was carried out using block digested at a temperature of 220°C till attaining a clear solution. Then it was filtered using Whatman no 1 filter paper and the volume is made up to 100 mL using Milli pore water. Then this extract was fed into ICP-OES Model: 6500 series (Inductively Coupled Plasma Optical Emission Spectrometry) for analysing micro nutrients (Zn, Fe, Cu, Mn). The extract was measured by flame photometry for total K and total P was quantified by colourimetry.

#### Microbial assay

#### Bacterial assay

The viable/living cells in the liquid formulations were counted using the dilution plate technique<sup>11</sup>. One millilitre of the liquid sample was added to a 9 mL aliquot of sterile distilled water to count the number of bacterial populations present. The liquid sample was then serially diluted, and a 1 mL aliquot of the appropriate dilution was transferred to sterile nutrient agar (HiMedia Labs) using the pour plate technique.



Fig. 1 — Collected 21 nos. of organic liquid formulation

After that, the sample was incubated for 24 h at  $37^{\circ}C^{12}$ .

# Fungal assay

Dilution plate technique was used to enumerate fungi in liquid formulation<sup>13</sup>. Using a plate spreading technique, a 0.1 mL aliquot of the appropriate serially diluted sample was transferred to sterile molten martin rose Bengal Agar (HiMedia Labs) plates. The samples were then incubated at 25°C for 4-5 days.

#### Actinobacteria assay

The technique described by Jeffrey<sup>14</sup> was used to assay actinobacteria. After serially diluting the liquid samples of the organic formulation, 0.1 mL aliquots were placed onto Kenknights agar (HiMedia Labs) plates. The number of colony-forming units (CFU) per ml of each sample was calculated after incubation at 28°C for 7-10 days<sup>12</sup>. Three replicates of each microbiological analysis were made.

#### Solubility nature of liquid formulations

Different solvents namely methanol, hexane, dichloromethane and acetonitrile (HPLC grade) were tried to extract the liquids sample for instrumental analysis. 5 mL sample was taken in 50 mL centrifuge tube containing solvent (20 mL - individually) that was mixed well and vortexed for about 1 min to check the solubility.

#### Mould growth

The surface growth of mould in liquid organic manures was visually observed and noted.

#### Maggot population

The presence of maggots was visually observed.

#### Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics 25 for Windows (IBM, Inc., Armonk, NY, USA) and results were expressed as mean values with standard error (SE) of three technical replicated analysis. The intrinsic relationships between various parameters were determined by the Pearson correlation coefficient.

# **Results and Discussion**

The outcome of our survey, preparation methods of different organic formulations and characterisation of the same is presented below. For most of the liquid formulations, this is the first complete analysis regarding their content on desirable properties.

#### **Organic liquid formulations**

About 21 nos. of organic inputs with different nature (Fig. 1) were analysed. Liquid formulations are mainly applied by farmers through seed treatment, foliar spray and through irrigation, which is meant to enhance soil health by improving biological activity, crop productivity and sustainability of organic crop production. In addition to nutrients, farmers believe that these fermented liquid organic formulations contain plant growth hormones and microbial load which helps to boost the plant growth, improve metabolic activities and impart resistance to pest and diseases. The role of each formulation and the dosage is summarised in Table 1.

Preparation methods for the liquid formulations were received from the farmer. All these collected inputs were categorised into 3 groups based on the ingredients used to prepare. 1. Animal-based formulations (consist of animal parts along with cow urine). 2. Liquid-based formulations (comprised of plant leaves and animal wastes), and 3. Microbialbased formulations (microbes and their food sources). After a month of preparation, for ensuring the highest microbial load in the liquid formulation, farmers used to add wood apple in the solution as a food source for the microbes by which it magnifies. These formulations are practiced by farmers throughout the Sathyamangalam and Gobichettipalayam region of Erode District of Tamil Nadu. These were easy and simple to make using locally available material and application was eco friendly in nature. It was compatible with other farm resources, technically viable and economically feasible. The preparation of individual formulations are given the in supplementary material as Annexure I.

#### Physiochemical properties

The pH of different samples shows the indication of fermentation. In our study pH of organic formulations are ranges from 3.9 to 8.7 (Table 2).

The higher pH was noticed in bandihoot solution (8.7), lantana+calotropis (8.5) and 5 leaf extract (8.4), whereas other formulations were slightly acidic to acidic. This was corroborated with the findings of Saelee<sup>15</sup>, who found that the majority of liquid organic fertilisers had a pH between 3-5 that were suitable for crop growth and development. The high sugar content of the substrate was used by the microbes to produce lactic or acetic acid caused the pH readings to be so acidic. Acetic acid is produced by bacteria through the fermentation of lactate and

S. NoInputsDosageUsesManure formulations1.Jeevamirtham200 litres per acreImproves soil health2.Ganajeevamruth200 kg/acreImproves soil healthPlant growth promoters3.Placenta solution5-10% foliar sprayPlant growth improves4.Fish amino acid6% foliar sprayNutrient for the plants5.Bandihoot solution5-10% foliar sprayPlant growth promotion
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5. Bandihoot solution 5-10% foliar spray Plant growth promotion
6. Egg amino acid 2% foliar spray Growth promotion
7. Archaebacterial solution 10% foliar spray Growth promotion
Irrigation - 200-300 Improves soil health
litres per acre
8. Panchagavya 3% foliar spray It provides all kinds of micronutrients, enhances plant growth, repels
3% seed treatment insects, and helps increase disease resistance in plants.
9. Goat aavottam 2% foliar spray Improves branching, leafing, flowering and fruiting.
10. Vermiwash Vermiwash:cow's urine: Controls deflowering and increases the yield
water in ratio of 1:1:6 Effective management of aphids and caterpillars in vegetables
Pest control
11. Treated cow urine 5% foliar spray Controls pests and diseases
12. Fermented plant extract 5-10% solution (a) rectifies micronutrient deficiency, (b) acts as pest repellent, (c)
(FPE) 10-20 litres per acre prevents pests from feeding and (d) induces disease resistance.
13. Agniasthra 2-3% foliar spray Useful against leaf roller and stem/pod/fruit borers
14. Amirtham solution 10% foliar spray Pest repellent, anti feedant supplies nitrogen quick growth promoter)
15. 5 leaf extract 10% foliar spray Pest repellent
16. Neem + Cow urine 3-6% foliar spray Controls leaf eating caterpillars, grubs, locusts and grasshoppers
Disease control
17. Pseudomonas + Buttermilk 10% foliar spray Controls fungal disease Solution
18. EM2 solution 2-5% foliar spray Controls fungal and bacterial diseases.
1% seed treatment Crop residue decomposition
19. Butter milk solution 10% foliar spray Against mosaic virus and fungal diseases
20. Arappu + Buttermilk 10% foliar spray This helps plant growth, repels insect, and adds resistance to fungal diseases
21. Lantana + Calotropis 5-10% solution Fungi diseases and pest control

Table 1 — Organic liquid formulations used by the western agro climatic zone of Tamil Nadu and their utility

citrate or the metabolism of amino acids. Our finding was in line with accordance of Hepsibha and Geetha<sup>16</sup>.

The electrical conductivity of liquid organic formulations is furnished in Table 2. The highest EC of 9.85 dS m<sup>-1</sup> was recorded in egg amino acid and the lowest was found in pseudomonas + buttermilk solution (2.38 dS m<sup>-1</sup>). The highest EC was due to the presence of soluble salts in the egg amino acid formulation. This corroborated with the findings of Natarajan<sup>17</sup>.

TDS or dissolved salt concentrations decide the electrical conductivity of the formulation. Dissolved concentration for the formulations ranged from 1.7 to 156.3 ppm. Highest TDS was found in egg amino acid (156.3 ppm) and the lowest was found in pseudomonas + buttermilk solution (1.7 ppm).

According to the study, the materials used in the preparation of liquid manures exerted a significant effect on the nutritional status of organic liquid manures (Table 3). Results showed that vermiwash had the highest N content (0.89%) followed by bandihoot solution (0.42%) and ganajeevamruth had the lowest N content of 0.02%. among the studied organic manures, few had an N content higher than most conventional organic fertilizers and few are equivalent. The source of the nitrogen in the liquid manures might be due to the inherent N-containing ingredients. This suggests that even a minimum application of liquid manures is more sufficient as nutritive substitute than bulky organic manures.

From the finding of the study, that egg amino acid possessed the highest P content of (8.53 ppm) which was comparable with fish amino acid (8.48 ppm)

Table 2 — Physio chemical properties of organic liquid formulations								
S.No.	Input	рН	EC	TDS				
1	5 leaf extract	8.4±0.52	$8.81 \pm 0.61$	$18.6 \pm 1.60$				
2	Agniasthra	$6.8 {\pm} 0.61$	$8.76 \pm 0.81$	$18.6 \pm 1.71$				
3	Amirtham solution	$6.6 {\pm} 0.39$	$4.22 \pm 0.25$	$6.70{\pm}0.40$				
4	Arappu + Buttermilk	$6.6 \pm 0.33$	$4.54 \pm 0.32$	20.7±1.47				
5	Archae bacterial solution	4.7±0.15	$3.51 \pm 0.13$	3.40±0.17				
6	Bandihoot solution	$3.9 \pm 0.39$	$8.75 \pm 0.40$	12.5±0.38				
7	Butter milk solution	4.8±0.33	$7.22 \pm 0.49$	8.70±0.46				
8	Egg amino acid	$4.8 \pm 0.38$	9.85±1.61	121±17.9				
9	EM2 solution	$4.0{\pm}0.20$	$2.58 \pm 0.12$	21.3±1.02				
10	Fermented plant extract (FPE)	$4.2 \pm 0.59$	$4.77 \pm 0.38$	5.50±0.28				
11	Fish amino acid	$7.4{\pm}0.19$	4.91±0.29	7.20±0.62				
12	Ganajeevamruth	$3.2{\pm}0.57$	$4.00{\pm}0.44$	13.5±0.99				
13	Goat aavottam	$4.2{\pm}0.16$	$8.38 \pm 0.36$	$10.9 \pm 0.86$				
14	Jeevamirtham	$5.3 \pm 0.37$	$3.50 \pm 0.24$	17.6±17.6				
15	Lantana + Calotropis	8.5±0.53	$7.20{\pm}0.97$	20.2±2.20				
16	Neem + Cow urine	$9.0{\pm}0.92$	$6.50 \pm 2.01$	$18.1{\pm}0.70$				
17	Panchagavya	$4.4{\pm}0.46$	$8.46{\pm}0.88$	$9.80{\pm}0.68$				
18	Placenta solution	8.7±0.54	$4.83 \pm 0.30$	$7.90{\pm}0.50$				
19	Pseudomonas + Buttermilk Solution	$7.6 \pm 0.57$	$2.8{\pm}0.18$	$1.70\pm0.17$				
20	Treated cow urine	3.7±0.17	$3.4{\pm}0.55$	13.3±1.38				
21	Vermiwash	$7.2{\pm}0.45$	$3.47 \pm 0.22$	8.50±0.53				
Data are t	he mean values of three replicates with±standar							

Data are the mean values of three replicates with±standard error (n=3).

Table 3 — Macronutrients present in organic liquid formulations

S. No.	Input	N (%)	P (mg/kg)	K (mg/kg)
1	5 leaf extract	$0.04{\pm}0.003$	2.27±0.19	29±2.47
2	Agniasthra	$0.09{\pm}0.008$	$0.23 \pm 0.02$	25±2.30
3	Amirtham solution	$0.08 \pm 0.005$	$1.53 \pm 0.09$	26±1.55
4	Arappu + Buttermilk	$0.04{\pm}0.003$	$0.45 \pm 0.03$	$118 \pm 8.37$
5	Archae bacterial solution	$0.28 \pm 0.011$	$1.62 \pm 0.06$	$10\pm0.38$
6	Bandihoot solution	$0.42{\pm}~0.022$	$0.42 \pm 0.02$	68.3±3.54
7	Butter milk solution	$0.03 \pm 0.002$	$0.88 \pm 0.05$	$96 \pm 5.07$
8	Egg amino acid	$0.18 \pm 0.023$	8.53±1.11	$109 \pm 14.2$
9	EM2 solution	$0.03 \pm 0.001$	$0.33 \pm 0.02$	$6.5 \pm 0.31$
10	Fermented plant extract	$0.03 \pm 0.002$	$0.73 \pm 0.06$	28±2.31
11	Fish amino acid	$0.02 \pm 0.002$	$8.48 \pm 0.73$	97±8.34
12	Ganajeevamruth	$0.02{\pm}0.001$	$0.46 \pm 0.46$	23.4±1.73
13	Goat aavottam	$0.18 \pm 0.033$	$0.95 \pm 0.17$	92±16.6
14	Jeevamirtham	$0.13 \pm 0.008$	$0.49{\pm}0.03$	$107\pm6.38$
15	Lantana + Calotropis	$0.13 \pm 0.011$	$0.45 \pm 0.04$	$118 \pm 10.2$
16	Neem + Cow urine	$0.07{\pm}~0.003$	$0.51 \pm 0.02$	112±4.35
17	Panchagavya	$0.38 \pm 0.026$	$5.04 \pm 0.35$	56±3.86
18	Placenta solution	$0.06 \pm 0.004$	$0.20{\pm}0.01$	$18.6 \pm 1.16$
19	Pseudomonas + Buttermilk Solution	$0.06 \pm 0.003$	$0.32 \pm 0.02$	$2.5 \pm 0.14$
20	Treated cow urine	$0.20 \pm 0.023$	$0.73 {\pm} 0.08$	91±10.6
21	Vermiwash	$0.89 \pm 0.055$	$7.80{\pm}0.48$	$90{\pm}5.58$
Data are th	ne mean values of three replicates with $\pm$ standard error (n=3).			

followed by vermiwash (7.8 ppm). All the analysed organic liquid fertilizers had higher P content than most common bulky organic manures used by the farmers in general.

Lantana + calotropis liquid manure had the highest potash content of 118 ppm which was on par with arappu + buttermilk solution (118 ppm) followed by neem + cow urine (112 ppm) and pseudomonas + buttermilk solution had the lowest (2.5 ppm). The potassium content in all liquid manures is greater than that of cattle manure.

Micronutrients (Fe, Mn, Cu and Zn) concentrations showed significant difference among the different liquid organic formulations. The micronutrient content of the organic formulations is furnished in Table 4.

Iron concentrations were higher for Panchagavva (378 ppm), fish amino acid (118 ppm) and 5 leaf extract (105 ppm) than the other formulations whereas, the lower concentration was found in neem + cow urine (4. 83 ppm). Remaining formulations had iron concentrations in the range of 13.4 to 117.8 ppm. Ingredients of organic formulations and its fermentation process significantly influenced the zinc concentration. Total zinc content was significantly higher for Panchagavya (232 ppm) and lower value was found in neem + cow urine. Total copper concentration ranged from 0.30 to 5.63 ppm with an average of 1.69 ppm in the studied liquid formulations. Incase of microbial based formulations. EM2 has a higher zinc concentration (54.6 ppm) which might be due to the presence of Zn solubilising bacteria. According to the results obtained on Mn content, it was inferred that, regardless of different formulations, Panchagavya recorded higher Mn content (13.3 ppm) and the lowest value was found in egg amino acid (0.17 ppm).

In general, micronutrient concentration might have varied depending on the ingredients used in preparation *via* cow dung, cow urine, milk, jaggery, gram flour, etc. The changes in the micronutrients could be the result of fermentation and subsequent reactions. When using organic source of manures, the binding nature of organic materials, prevents micronutrients from precipitation, fixation, oxidation, and leaching and also increases the availability of micronutrients in soils. Hence, these formulations may act as a strong source for providing micronutrients through foliar supplementation.

### Microbial population

Data regarding total microbial population in organic liquid formulations stated that there was a significant variation among the formulations (Table 5 and Fig. 2).

Regardless of different formulations, fungi population was higher in Amirtham solution and bandihoot solution (19 CFU  $\times 10^4$ ) which was followed by egg amino acid (18 CFU  $\times 10^4$ ) and the lowest was found in fish amino acid (4 CFU  $\times 10^4$ ). The data on bacterial population depicted that, irrespective of different organic formulations. Placenta solution was found to have a higher bacteria (281) colony forming units/mL of solution which was followed by arappu + buttermilk solution (265 CFU  $\times 10^6$ ) that was on par with pseudomonas +

	Table 4 — Micronutrients present in organic liquid formulations								
S. No.	Input	Cu	Zn	Mn	Fe				
1	5 leaf extract	$2.90{\pm}0.18$	30.4±1.90	2.03±0.13	106±6.59				
2	Agniasthra	$1.03 \pm 0.10$	$3.30 \pm 0.30$	$0.73 {\pm} 0.07$	$15.9 \pm 1.46$				
3	Amirtham solution	$1.73 \pm 0.10$	$2.57 \pm 0.15$	$1.40{\pm}0.08$	29.7±1.77				
4	Arappu + Buttermilk	$0.87 {\pm} 0.06$	11.7±0.83	$2.87 \pm 0.20$	$73.3 \pm 5.20$				
5	Archae bacterial solution	$0.93 {\pm} 0.04$	$3.43 \pm 0.13$	$2.87 \pm 0.11$	47.3±1.79				
6	Bandihoot solution	$1.17 \pm 0.05$	$2.17 \pm 0.10$	$0.53{\pm}0.02$	$38.0{\pm}1.72$				
7	Butter milk solution	$2.40{\pm}0.16$	$3.40{\pm}0.23$	$1.70\pm0.12$	$42.4 \pm 2.90$				
8	Egg amino acid	$0.30{\pm}0.03$	$7.90{\pm}0.75$	$0.17{\pm}0.02$	$8.40{\pm}0.80$				
9	EM2 solution	$1.57{\pm}0.08$	54.6±2.62	$1.90{\pm}0.09$	85.8±4.10				
10	Fermented plant extract	$3.33 \pm 0.27$	4.27±0.34	$2.43 \pm 0.19$	36.2±2.88				
11	Fish amino acid	$3.07{\pm}0.18$	38.2±2.28	$4.83 \pm 0.61$	118±0.29				
12	Ganajeevamruth	$9.30 \pm 1.01$	13.1±1.43	$7.50{\pm}0.61$	$23.1 \pm 0.82$				
13	Goat aavottam	$0.57{\pm}0.02$	5.17±0.20	$2.00 \pm 0.61$	$58.9 \pm 0.08$				
14	Jeevamirtham	$5.63 \pm 0.39$	30.5±2.10	$1.93 \pm 0.61$	$53.2 \pm 0.13$				
15	Lantana + Calotropis	$0.77 {\pm} 0.05$	2.67±0.17	$1.00{\pm}0.61$	$21.4 \pm 0.06$				
16	Neem + Cow urine	$0.33 \pm 0.03$	$1.20\pm0.12$	$0.30 \pm 0.61$	$4.83 \pm 0.03$				
17	Panchagavya	4.93±0.51	232±24.2	$13.3 \pm 0.61$	$378 \pm 1.38$				
18	Placenta solution	$1.53 \pm 0.10$	$12.3 \pm 0.76$	$0.93 \pm 0.61$	$37.5 \pm 0.06$				
19	Pseudomonas + Buttermilk Solution	$0.67 \pm 0.05$	$1.23 \pm 0.09$	$0.50 \pm 0.61$	$13.4 \pm 0.04$				
20	Treated cow urine	$0.63{\pm}0.03$	$2.17 \pm 0.10$	$0.50 \pm 0.61$	$17.8 \pm 0.02$				
21	Vermiwash	$0.47 \pm 0.03$	$0.14{\pm}0.01$	$0.07{\pm}0.005$	$3.14 \pm 0.20$				
Data are th	ne mean values of three replicates with±star	ndard error (n=3).							

Table 5 — Microbial load in the organic liquid formulations										
S. No.	Input	Bacteria (10 <sup>6</sup> CFU mL <sup>-1</sup> )	Fungi (10 <sup>4</sup> CFU mL <sup>-1</sup> )	Actinomycetes (10 <sup>2</sup> CFU mL <sup>-1</sup> )						
1	5 leaf extract	230±14.4	$13 \pm 0.81$	$176 \pm 110.0$						
2	Agniasthra	$174 \pm 16.0$	$7 \pm 0.64$	59±5.43						
3	Amirtham solution	42±2.50	19±1.13	56±3.34						
4	Arappu + Buttermilk	$265 \pm 18.8$	$17 \pm 1.21$	$158 \pm 11.2$						
5	Archae bacterial solution	176±6.66	$8 \pm 0.30$	223±8.44						
6	Bandihoot solution	$110 \pm 4.97$	$19 \pm 0.86$	$48 \pm 2.17$						
7	Butter milk solution	138±9.46	$4{\pm}0.27$	97±6.65						
8	Egg amino acid	$197{\pm}18.8$	$15\pm1.43$	$323 \pm 30.8$						
9	EM2 solution	$80{\pm}3.83$	9±0.43	$167 \pm 8.00$						
10	Fermented plant extract (FPE)	72±5.73	$11\pm0.88$	61±4.85						
11	Fish amino acid	226±13.5	$10\pm0.60$	$198 \pm 11.8$						
12	Ganajeevamruth	$130{\pm}14.1$	$5\pm 0.54$	$55 \pm 5.98$						
13	Goat aavottam	92±3.57	$13\pm0.50$	111±4.31						
14	Jeevamirtham	$178 \pm 12.3$	9±0.62	$130 \pm 8.97$						
15	Lantana + Calotropis	69±4.32	$4 \pm 0.25$	20±1.25						
16	Neem + Cow urine	3±0.31	5±0.51	9±0.92						
17	Panchagavya	196±20.4	11±1.15	203±21.1						
18	Placenta solution	281±17.4	$6\pm 0.37$	$58 \pm 3.60$						
19	Pseudomonas + Buttermilk Solution	256±19.2	$9{\pm}0.67$	150±11.2						
20	Treated cow urine	$194 \pm 9.09$	$10\pm0.47$	$113\pm 5.30$						
21	Vermiwash	$81{\pm}5.08$	$7{\pm}0.46$	25±6.15						
Data are th	Data are the mean values of three replicates with+standard error $(n=3)$									

Data are the mean values of three replicates with±standard error (n=3).



Fig. 2(a) — Fungi colony investigated on different liquid formulations, (b) Actinobacteria colony investigated on different liquid formulations

buttermilk solution and meagre population on was found in neem+cow urine (3 CFU ×10<sup>6</sup>). Irrespective of different liquid formulations, actinobacteria count was higher in egg amino acid (323 CFU ×10<sup>3</sup>) and the lowest was found in neem + cow urine (9 CFU ×10<sup>3</sup>).

Placenta acts as a potential source for many microbes. The addition of wood apple and cow urine to the mixture might have increased the amount of bacteria present overall in the fermented solution. From the results, it is concluded that any material can be fermented without the inclusion of commercial inoculums. The higher microbial population present in these liquid organic formulations aids in rapid decomposition of bulky organic manures. This leads to the maintenance of soil fertility and to enhance nutrient availability.

Table 6 — Solubility nature of liquid organic formulations								
S. No.	Inputs	Methanol	Dichloromethane	Acetonitrile	Hexane			
1	5 leaf extract	$\checkmark$	×	×	×			
2	3G extract	$\checkmark$	×	×	×			
3	Agniasthra	$\checkmark$	×	×	×			
4	Amirtham solution	$\checkmark$	×	$\checkmark$	×			
5	Lantana + Calotropis	$\checkmark$	×	×	×			
6	Arappu Buttermilk solution	$\checkmark$	×	×	×			
7	Archae bacterial solution	$\checkmark$	×	×	×			
8	Bandihoot solution	$\checkmark$	×	×	×			
9	Butter milk solution	$\checkmark$	×	×	×			
10	Egg amino acid	$\checkmark$	×	×	×			
11	EM2 solution	$\checkmark$	×	×	×			
12	Fermented plant extract (FPE)	$\checkmark$	×	×	×			
13	Fish amino acid	$\checkmark$	×	×	×			
14	Goat aavottam	$\checkmark$	×	$\checkmark$	×			
15	Jeevamirtham	$\checkmark$	×	$\checkmark$	×			
16	Neem + Cow Urine	$\checkmark$	×	$\checkmark$	×			
17	Panchagavya	$\checkmark$	×	$\checkmark$	×			
18	Placenta solution	$\checkmark$	×	×	×			
19	Pseudomonas + Buttermilk Solution	$\checkmark$	×	$\checkmark$	×			
20	Treated cow urine	$\checkmark$	×	×	×			
21	Vermiwash	✓	×	$\checkmark$	×			

ble 6 — Solubility nature of liquid organic formulation

Most of the formulations are having acidic pH. According to Somasundaram *et al.*,<sup>3</sup> more beneficial microorganisms were detected in panchagavya due to its acidic nature. They act as catalysts with a synergistic effect to promote all the beneficial bacteria in the environment, besides enhancing the existing microbes in the environment. In the presence of organic matter, these microbes secrete proteins, organic acids, and antioxidants that are then converted into energy by the soil microflora and fauna, transforming the soil from one that promotes disease to one that suppresses it.

# Solubility nature

The solubility nature of each organic liquid formulation was carried to study its dissolving nature in various solvents for metabolite characterization. This study found that Panchagavya, Amirtham solution, goat aavoottam, jeevamirtham, neem+cow urine, pseudomonas+buttermilk solution, vermiwash were completely dissolved in methanol and 90% in acetonitrile to isolate active compounds with a minimum number of impurities from metabolites. The remaining organic liquid formulations have dissolved only in methanol. Based on this preliminary study, solvent will be chosen for the extraction of sample analysing in high end instruments (Table 6).

# Mould growth

Freshly prepared organic liquid formulations were not having any mould growth on their surface (Fig. 3) whereas, mould growth was observed after 15 days of preparation on the surface of Panchagavya and goat aavoottam and sloe on the sides of the containers. A slight mouldy growth appeared on amirtham solution, buttermilk solution, arappu buttermilk solution, fish amino acid and fermented plant extract. There was no appearance of mould growth in other liquid formulations.

#### Maggot population

There were no maggots in Panchgavya up to 12 weeks of storage. However the maggot population was started to increase after 4 month onwards. And also observed small and big flies were present in the surface of liquid formation. In general, formulations which had dung as an ingredient in their preparation reported to have maggots population. No maggots were observed in other liquid formulations.

# Relationship among the various properties of organic liquid formulations

The relationship between various properties of different organic liquid formulations was established using Pearson correlation (Table 7). The result revealed soil pH was negatively correlated with phosphorous (r=-0.337), potassium (r=-0.151), iron

Table 7 — Relationship among the various properties of organic liquid formulations											
	pН	Bacteria	Fungi	Actinobacteria	Ν	Р	К	Fe	Zn	Cu	Mn
pН	1	-0.235	-0.096	-0.646**	0.069	-0.337	-0.151	-0.300	-0.272	-0.175	-0.379
Bacteria		1	0.084	0.545*	-0.182	0.135	-0.126	0.246	0.180	0.067	0.171
Fungi			1	0.304	0.082	0.143	0.025	0.139	0.047	-0.187	-0.031
Actinobacteria				1	-0.114	0.487*	-0.014	0.383	0.348	-0.016	0.262
Total N					1	0.456*	0.177	0.090	0.143	-0.211	0.000
Total P						1	0.299	0.263	0.271	-0.048	0.201
Total K							1	-0.057	-0.077	-0.195	-0.132
Total Fe								1	0.967**	0.341	0.854**
Total Zn									1	0.373	0.847**
Total Cu										1	0.675**
Total Mn											1

\*\* correlation is significant at the 0.01 level; \* correlation is significant at the 0.05 level



Fig. 3 — Mould growth on organic liquid formulations

(r=-0.300), zinc (r=-0.272), copper (r=-0.175) and manganese(r=-0.379) while, there was significant and positive correlation with nitrogen (r=0.069). The study apparently confirms the higher micronutrient availability under medium-to-strong acidic conditions

of organic liquid fertilizers. Bacterial population correlated moderately with nitrogen (r=0.082), phosphorous (r=0.143), potassium(r=0.025), iron(r=0.139) and zinc (r=0.047).

# Conclusion

The application of these organic liquid formulations will be a substitute for other organic manures by supplying various nutrients, plant growth promoting substances and microbial cultures. In rural areas, farmers can easily prepare them using resources that are readily available. Scientific rationale is essential in designing the proper use of these solutions in crop which definitely increase the crop yield by supplying all the essential nutrients, growth promoters and bio-control agents.

# **Supplementary Data**

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK\_22(02)(2023) 297-306 SupplData.pdf.

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# **Conflict of Interest**

The authors declare that there is no conflict of interest.

# **Authors' Contributions**

DUN and ES: Conceptualization, DUN: survey, documentation & drafting; ES: review and editing.

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