



Microbiological and enzymatic properties of diverse *Jaivik Krishi* inputs used in organic farming

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Jaivik Krishi is a system of production and natural agriculture free from all fertilizers, pesticides, herbicides and synthetic harmful substances. Organic Farming is a method which forbids the application of synthetic inputs (such as chemical fertilizers, pesticides, feed additives, hormones, etc.) *Jaivik krishi* products (organic inputs) are organic formulations that boost the biological productivity of crops and the nutritional quality of vegetables and fruits. The use of *Jaivik Krishi* inputs helped in sustaining crop yields in organic nutrient management system. In the present study, various organic formulations were prepared from the various indigenous cow-products and plant based waste materials. Microbial count viz., total bacteria, fungus and actinomycetes count, and enzymatic activities viz., acid phosphatase, alkaline phosphatase and dehydrogenase were also evaluated in different organic liquid formulations. The average microbial count of Panchgavya (14.9×10^8 , 5.8×10^5 , 8×10^5 cfu/mL for total bacteria, fungus and actinomycetes count respectively) was highest among various *Jaivik Krishi* inputs studied followed by Dasparni. In present study, enzymatic activities of *Jaivik Krishi* inputs was directly related and corresponded to the microbial count. The enzyme activities of Panchgavya was highest (29.97, 52.10 and 66.64 $\mu\text{g/mL}$ for acid phosphatase, alkaline phosphatase and dehydrogenase respectively) followed by Dasparni. These *Jaivik Krishi* inputs will benefit in enhancing the soil carbon content of soil and improving the soil fertility and micro-fauna.

Keywords: Acid Phosphatase, Alkaline phosphatase, Dehydrogenase, *Jaivik Krishi*, Microbial count, Organic farming

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Organic farming is a method of farming that emphasizes primarily on cultivating the land and crops in such a way that the soil is kept alive and in good health. It encourages the utilization of organic waste and other natural products, most of which are generated *in situ* along with beneficial microorganisms to release nutrients to crops, representing the 'organic' essence of organic farming. In the Indian context it is also termed as *Jaivik Krishi*. In India, traditional methods are being used for the preparations of different organic inputs formulations utilizes plant based materials, milk based products, animal and farm wastes, aquatic wastes and dead animal carcass etc. In the modern intensive agriculture (1990s), excessive use of chemical

fertilizers and pesticides by replacing traditional Indian methods lead to high crop yield with adverse effects on soil productivity. The soil became less sustainable and healthy productive due to decline in organic matter & microbial community, increase in the soil salinity, soil pH disturbance etc.^{1,2}. The traditional organic (*Jaivik*) formulations are being adopted and utilized to restore the soil fertility and productivity in recent times. These formulations serve crucial role in maintaining and strengthening microflora population, salinity of soil, providing nutrients to soil and pH³⁻⁴.

Soil enzymes play vital role in maintenance of soil ecology, physical and chemical properties of soil, fertility of soil and organic matter decomposition. Soil enzymatic activity catalyses the biochemical activity of microorganisms and thus shows the ability of the soil to permit fundamental biochemical processes

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required for soil fertility. Organic management increases overall enzyme activity⁵. However the activities of some of the specific enzymes depends on the several factors such as quantity and quality of nutrient present in soil, soil type and the characteristics of soils⁶.

Many organic formulations for nutrient, pest and disease management and growth stimulation are used in traditional farming and present modern organic production system. But their protocols of preparation, microbial and enzymatic characteristics are not well defined. Therefore under Rashtriya Krishi Vikas Yojana (RKVY), a study to find out the quality characteristics of different organic inputs was undertaken during 2017-18 to 2019-20. In the present paper, 14 organic inputs formulations viz., Jeevamrut, Panchagavya, Silica enriched Panchagavya, Matka khad, Bhabhut Amrit Pani, Beejamrit, Dasparni, Fafundnashi, Vermiwash, Silica enriched Vermiwash, Compost tea, Silica enriched Compost tea, Teekha Sat and Gomutra were assessed for microbial counts and enzymatic activities.

Methodology

Preparation of Organic Inputs

The different types of organic products and formulations are prepared from the products of indigenous cow and plant based materials. These ingredients are properly mixed in various proportions and may be used as fresh concoctions or boil or fermented to use them as organic formulations for enhancing soil health and plant performance.

1. **Jeevamrut**- The cow dung and urine, jaggery, pulse powder and live soil were added in 200 L of water. The solution was kept for 2-7 days in shade for fermentation and should be stirred daily twice.

2. **Panchagavya**- Panchagavya consists of five different products viz. cow dung, cow urine, milk, curd and ghee and was mixed in the ratio of 5:3:2:2:1. In the pot the cow dung and cow ghee is thoroughly mixed in morning and evening hours and kept it for 3 days. After 3 days cow urine, cow milk, curd and jaggery was added. The mixture was kept for 15 days with regular mixing both in morning and evening. After 15 days the Panchagavya was ready.

3. **Silica enriched Panchagavya**-It was prepared with additional 1% silica through diatomaceous earth with the similar methodology and ingredients used for Panchagavya.

4. **Matka khad**- It was prepared by mixing cow dung, cow urine, jaggery in 1:1:1 ratio and water. Then fermentation was carried for 7-10 days.

5. **Bhabhut Amrit Pani**: Bhabhut Amrit Pani consists of four different products viz. cow dung, cow ghee, honey and water. Ten kg cow dung was mixed properly with 500 g of honey and then 250 g of cow ghee was thoroughly mixed and used fresh for seed treatment or as spray formulation @25 kg/ha.

6. **Beejamrit**- It was prepared by mixing desi cow dung (0.5 kg), urine (0.5 L), limestone (5 g), live soil (10 g) and 2 L water in a bowl. All ingredients were mixed thoroughly in a particular sequence and was kept for one day.

7. **Dasparni**- About 2 kg of each plant leaves viz. neem, karanja, seetafal, dhatura, *Lantana*, tulsi, mango, papaya, marigold and guava were soaked in 200 L of water and mixed well with 10 L cow urine and 2 Kg cow dung. Dried tobacco leaves, green chilli paste, garlic paste, dried ginger powder, and dried turmeric powder (about 200 to 500 g each) were added and mix thoroughly^{7,8}. The container was covered and placed in shade for incubation for 40 days with routine stirring after every 12 h. The plants used for the preparation of Dasparni might vary for different regions and mainly depend on the availability of plants in region.

8. **Fafundnashi**- It was prepared by taking 200 g black pepper powder in 5 L of cow milk and mixed thoroughly. This product was used fresh and did not require fermentation.

9. **Vermiwash**- Vermiwash, a yellow-brownish leachate which is produced during the vermicomposting process. A 20 L capacity earthen pot having plastic tap at the lower side is used for production of vermiwash. Barrel consists of tri-layered structure from inside i.e., bottom to top of which first layer is made up of brick pieces (7"), second layer consists of coarse sand (6") and last layer is made up of fine sand (5"). A mosquito mesh made up of nylon is placed over it just after the third layer. The entire setup is fitted into an iron mount stand. Cow dung amended with earthworms were used for the retrieval of vermiwash. After 15 days of incubation, water drops are poured on material inside the pot and the watery extract collected is vermiwash. The watery yellowish to black extract of vermicompost (vermiwash) drainage out off drum.

10. **Silica enriched Vermiwash**- It was prepared with additional 1% silica with the similar methodology and ingredients used for vermiwash.

11. **Compost tea**- For the production of compost tea, compost and water is brewed in the ratio of 1:5 w/v using a 20 L brewing tank having an air pump for continuous aeration. In order to allow volatilization of chlorine, tap water was supplied to the brewing tank approximately 24 h prior to use.

12. **Silica enriched Compost tea**- It was prepared with additional 1% silica with the similar methodology and ingredients used for compost tea.

13. **Teekha Sat**- It was prepared by mixing the 500 g green chilli, 500 g garlic, 1000 g dhatura leaves and 500 g neem leaves were crushed in 10 L cow urine. This mixture was boiled till the volume of the mixture remains half. The mixture was squeezed through muslin cloth and the filtrate was ready to use and then it was filled in the bottle.

14. **Gomutra**- Fresh cow urine was stored in 200 L container for use within a week time.

Microbial analysis

The 10 mL of organic liquid formulation was added in 90 mL sterilized distilled water to prepare 10^1 dilution and subsequent dilutions up to 10^{10} were made by transferring serially 1 mL of each dilution to the test tube containing 9 mL of sterilized water blanks. The total bacterial count (TBC), total fungal count (TFC) and total actinomycetes count (TAC) were estimated by standard spread-plate dilution method in triplicate. Nutrient agar, Potato dextrose and actinomycetes isolation agar media were used for estimation of TBC, TFC and TAC, respectively. The plates were kept for 24-48 h for incubation at $30^\circ\text{C}\pm 1^\circ\text{C}$ for bacteria and actinomycetes and at $25^\circ\text{C}\pm 1^\circ\text{C}$ for fungus. After incubation the colony forming unit (CFU) was calculated. The plates that had colony counts in the range of 30-300 CFU present were used for calculating the CFU of the samples.

$$\frac{\text{CFU}}{\text{ml}} = \frac{\text{no. of colonies} \times \text{dilution factor}}{\text{volume of culture plate}}$$

Enzyme analysis

a. **Phosphatase activity**: Phosphatase activity was estimated by Tabatabai and Bremner procedure⁹. The acid and alkaline test procedure was same except in the step of MUB buffer used. The MUB buffer of pH 6.5 and 11 was used in acid and alkaline test respectively. About 1 mL of sample in test tube or conical flask was taken. Then 4 mL of MUB buffer (pH 6.5 and 11; depending on acid and alkaline test respectively), 250 μL toluene and 1 mL of p-nitrophenyl phosphate (0.115 M PNP) solution were

added to the tubes and shaken for 30 seconds. Test tubes were incubated at 37°C for one hour. Following incubation period, 1 mL of calcium chloride (0.5 M) and 4 mL of sodium hydroxide (0.5 M) was added to the test tubes and filtered through Whatman filter paper no. I. The optical density (O.D.) of the filtrate was measured at 440 nm. Blank was also maintained separately without adding liquid formulation. The phosphatase activity (microgram of p-nitrophenol released per mL per hour) was calculated from the standard curve of p-nitrophenol.

b. **Dehydrogenase activity**: For estimating dehydrogenase activity in liquid formulations the TTC (2-3-5-Triphenyl tetrazolium chloride) reduction method was used¹⁰. 1 mL of fresh sample was taken in test tube and then 1 mL of tris-buffer (2.5 mL) and 1 mL of 1% TTC solution was added. The pH was adjusted to 7.0 using 1.0 N HCl. The mixture was vortexed and incubated it at 30°C for 24 h. The tubes were centrifuged for 10 min and then extraction with carried out three times using 2.5 mL ethanol each times. The OD of the filtrate was measured at 485 nm using nano-spectrophotomete (Implen, Germany), using methanol as a blank and the enzyme activity was in microgram formazan per mL.

Results

Microbial properties

The microbial populations of different *Jaivik Krishi* inputs or organic formulations are presented in Table 1 (Fig. 1). Average counts of microorganisms are expressed as CFU per 1 mL liquid formulations.

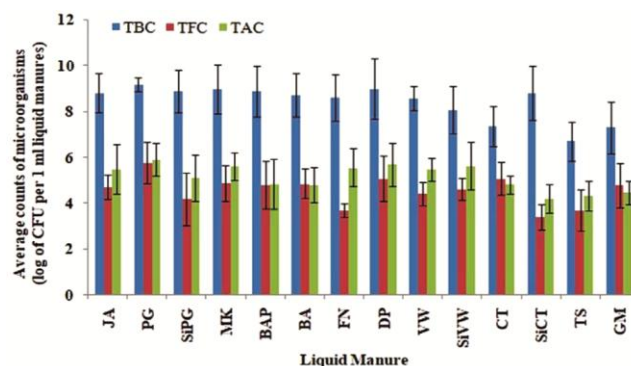


Fig. 1 — Microbiological analysis of 14 formulations of liquid organic formulations. TBC – total bacteria count; TFC- total fungal counts; TAC- total actinomycetes counts; JA-Jeevamrut; PG-Panchgavya; SiPG- Silica in Panchgavya; MK-Matka khad; BAP- Bhabhut Amrit pani; BA-Beejamrut; FN-Fafundnashi; DP- Dasparni; VW-Vermiwash; SiVW-Silica enriched Vermiwash; CT-Compost tea; SiCT-Silica enriched compost tea; TS-Teekha sat; GM-Gomutra

Average counts of total bacteria were 8.41 log of CFU per 1 mL liquid formulations. Counts of fungal and actinomycetes were 4.56 and 5.13 log of CFU per 1 mL organic liquid formulations respectively. There were significant differences in microbial count observed among tested liquid organic inputs in number of these bacteria. Qualitative estimation of microorganism in sample is represented by Total viable count (TVC) on different agar media.

Total bacterial counts

The mean total bacterial counts (TBC) of liquid formulations sample ranged from 0.05-14.9 x 10⁸ colony forming units (cfu) per milliliter of liquid formulations. There were significant differences in the averages total bacterial counts of the different liquid formulations sample. The highest counts were observed in panchgavya (14.9 x 10⁸) and lowest count was observed in teekha sat (0.05 x 10⁸).

Total fungal counts

The mean total fungal counts (TFC) of liquid formulations sample ranged from 0.025-5.8 X 10⁵ cfu per milliliter of organic liquid. Highest counts were observed in panchgavya (5.8 X 10⁵), lowest counts were observed in silica enriched compost tea (0.025 X 10⁵) as shown in Table 1. Differences in the average total fungal counts in various liquid organic inputs were significant.

Total actinomycetes counts

The mean total actinomycetes counts (TAC) of liquid formulations sample ranged from 0.15-8.0 X 10⁵ cfu per milliliter of liquid formulations. Highest counts were observed in panchgavya (8 X 10⁵) and lowest counts were observed in silica enriched compost tea (0.15X 10⁵) as shown in Table 1. Differences in the average total actinomycetes counts in various liquid organic inputs were significant.

Enzyme activity

Concentration of acid phosphatase, alkaline phosphatase and dehydrogenase enzymes in different organic formulations are presented in Table 2. In the present investigation, the acid phosphatase activity of organic liquid formulations sample ranged from 0.31 to 29.97 µg/ mL and these differences were statistically significant. The highest activity of acid phosphatase enzyme was observed in panchgavya and lowest activity was observed in teekha sat. The alkaline phosphatase activity of formulations ranged from 0.72 to 52.10 µg/ mL. There was significant difference in the alkaline phosphatase activity of the different liquid formulations. The highest activity of

alkaline phosphatase was observed in panchgavya and lowest activity was observed in teekha sat. The dehydrogenase activity of formulations ranged from 0.91 to 66.64 µg/ mL and differences in the dehydrogenase activity of the different organic liquid organic formulation were statistically significant. The highest activity was observed in panchgavya and lowest activity was observed in teekha sat.

Pearson correlation matrix among microbial counts and enzyme activities

Results of the Pearson correlation matrix among microbial counts and activities of enzymes of various organic liquid formulations are shown in Table 3. Bacterial count in the different liquid formulations showed significant correlation with fungi and actinomycete (r=0.35 and r=0.48, respectively). Correlation coefficient between total fungal counts and total actinomycetes counts (r=0.72) was highly significant. The activities of different enzymes were significantly correlated to the microbial counts. The acid, alkaline phosphatase and dehydrogenase enzyme activities were strongly correlated with bacterial counts (r=0.74, r=0.81 and r=0.71 respectively) as compared to fungal (r=0.33, r=0.57 and r=0.62, respectively) and actinomycete counts (r=0.50, r=0.60 and r=0.63 respectively). Among various enzymes, alkaline phosphatase showed strong significant correlation with dehydrogenase (r=0.92).

Discussion

Results of 14 organic inputs and formulations indicate a different range of microbial population of bacteria, actinomycetes and fungi and varying concentrations of acid phosphatase, alkaline phosphatase and dehydrogenase enzymes (Table 1 and Table 2). Among the 14 organic inputs, population of bacteria varied from 0.05 x 10⁸ – 14.9 x 10⁸, fungi from 0.025 x 10⁵ – 5.8 x 10⁵ and actinomycetes from 0.15 x 10⁵ – 8 x 10⁵ (Table 1). Similarly in case of enzymes in organic inputs/formulations, the acid phosphatase concentration varied from 0.31 to 29.97 µg/ mL, alkaline phosphatase concentration varied from 0.72 to 52.10 µg/ mL and dehydrogenase concentration varied from 0.91 to 66.64 µg/ mL (Table 2). In the present study, total microbial count was found to be higher in panchgavya, matka khad, jeevamrit and beejamrit as compared to other organic inputs or formulations. The reason for the above observation is that incorporation of high carbon (dung and jaggery) and

Table 1 — Microbial populations in *Jaivik krishi* inputs used in organic agriculture

S. No.	Organic formulations	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Total Actinomycetes Count (cfu/mL)
1.	Jeevamrut (JA)	6.33×10^8	0.51×10^5	3×10^5
2.	Panchagavya (PG)	14.9×10^8	5.8×10^5	8×10^5
3.	Silica in Panchgavya (SiPG)	7.6×10^8	0.15×10^5	1.25×10^5
4.	Matka khad (MK)	9.32×10^8	0.76×10^5	4.1×10^5
5.	Bhabhut Amrit pani (BAP)	7.01×10^8	0.6×10^5	0.7×10^5
6.	Beejamrit (BA)	5.16×10^8	0.7×10^5	0.6×10^5
7.	Fafundnashi (FN)	3.98×10^8	0.05×10^5	3.5×10^5
8.	Dasparni (DP)	9.65×10^8	1.15×10^5	4.8×10^5
9.	Vermiwash (VW)	3.7×10^8	0.26×10^5	2.9×10^5
10.	Silica enriched Vermiwash (SiVW)	1.15×10^8	0.415×10^5	4.15×10^5
11.	Compost tea (CT)	0.23×10^8	1.2×10^5	0.65×10^5
12.	Silica enriched compost tea (SiCT)	6.1×10^8	0.025×10^5	0.15×10^5
13.	Teekha sat (TS)	0.05×10^8	0.05×10^5	0.2×10^5
14.	Gomutra (GM)	0.2×10^8	0.6×10^5	0.29×10^5

Table 2 — Enzyme activities of various organic liquid formulations

S. No.	Organic formulations	Acid Phosphatase ($\mu\text{g/mL}$)	Alkaline Phosphatase ($\mu\text{g/mL}$)	Dehydrogenase ($\mu\text{g/mL}$)
1.	Jeevamrut	6.72	13.34	2.77
2.	Panchagavya	29.97	52.10	66.64
3.	Silica in Panchgavya	1.14	1.21	5.58
4.	Matka khad	24.66	14.10	8.74
5.	Bhabhut Amrit pani	7.62	10.24	4
6.	Beejamrit	24.86	21.69	11.90
7.	Fafundnashi	9.62	1.69	7.33
8.	Dasparni	16.86	34.66	46.46
9.	Vermiwash	5.55	1.97	2.49
10.	Silica enriched Vermiwash	1.21	1.28	5.58
11.	Compost tea	1.14	2.52	14.53
12.	Silica enriched compost tea	0.79	0.92	4.70
13.	Teekha sat	0.31	0.72	0.91
14.	Gomutra	0.93	1.07	1.90

Table 3 — Pearson correlation matrix among microbial counts and enzymatic activities in organic formulations

	TBC	TFC	TAC	AcP	AIP	Dehyd
TBC	0					
TFC	0.35	0				
TAC	0.48	0.72	0			
AcP	0.74	0.33	0.50	0		
AIP	0.81	0.57	0.60	0.84	0	
Dehyd	0.71	0.62	0.63	0.67	0.92	0

TBC – total bacteria count; TFC- total fungal counts; TAC- total actinomycetes counts, AcP- acid phosphatase, AIP- alkaline phosphatase, Dehyd- Dehydrogenase

nitrogen (urine, gram flour and milk) as ingredients in the organic inputs facilitates the growth of bacteria present in the microflora. Palekar¹¹ reported that Jeevamruth contains a large amount of microbial load that multiplies in the soil and serves as a tonic to increase the soil's microbial activity. Sreenivasa *et al.*¹² revealed that Panchagavya, Jeevamruth and Beejamruth developed using cow products, are

known to possess abundant numbers of beneficial microorganisms such as *Azotobacter*, *Azospirillum*, phosphobacteria, lactic acid bacteria, *Pseudomonas* and Methylophs, as well as some useful actinomycetes and fungi. An indication of the occurrence of easily degradable organic compounds such as simple sugars is given by the enzymes activity in various organic inputs^{13,14}.

Various organic inputs (*Jaivik krishi* products) had a substantial impact on activity of phosphatase. Use of organic nutrient sources at varying levels in panchgavya, dasparni, matka khad, jeevamrut, beejamrut and bhabhut amrit paani supported the activity of microbes which in turn increased the acid, alkaline and dehydrogenase activity. In the present study, panchgavya recorded highest amount of acid phosphatase (29.97 µg/mL) and alkaline phosphatase (52.10 µg/mL). Similar findings have been reported showing increased phosphatase activity in the presence of organic nutrient sources¹⁵. The above findings are in close accordance with the fact that after the incorporation of energy sources, enzyme activities can be increased¹⁶. Waldrop *et al.*¹⁷ suggested that enzyme activities are correlated with the amount of organic matter and with the composition of microbial communities. Among all the liquid formulations under study, panchgavya had highest total microbial, fungal and actinomycetes growth. Dehydrogenase activity, a marker of microbial activity engaged in oxidative phosphorylation was reported to be significantly higher in Panchgavya (66.64 µg/mL) followed by Dasparni (46.46 µg/mL). Further enhancement of the activity of dehydrogenase due to panchgavya and other liquid formulations could be due to the higher microbial population and their stimulatory impact on indigenous microorganisms in these formulations.

Significant correlation between microbial activity (bacteria, fungi and actinomycetes) and enzymatic activity (acid phosphatase, alkaline phosphatase and dehydrogenase) was noted under the study which indicate that population of microbes in liquid formulations decide the concentration of enzymes in the liquid formulations. The population of microbes in liquid formulations varied with concentration of source of energy, nitrogen and types of microbes and days of incubation, boiling and days of fermentation which varies in different organic formulations.

Conclusion

The principal bio-quality parameters used for monitoring liquid organic input quality are microbial counts and enzyme activities. The type and dose of input material used, period of incubation and process of preparation varied in different formulations which resulted in different microbial population and hence enzymatic activities (acid phosphatase, alkaline phosphatase and dehydrogenase) varied significantly.

The study provides useful findings with the use of *Jaivik krishi* inputs with insight of their microbial count which might be one of the reasons for their activity. There are millions of resource-poor farmers who routinely encounter restricted access to readily accessible and affordable chemical fertilizers. Such farmers also face the pressure of over-reliance on costly and environmentally unfriendly and detrimental synthetic fertilizers to tackle the challenges of land degradation on their fields. These *Jaivik krishi* inputs serve as both relief and economically beneficial to farmers and will be the great aids to the organic farming.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

Author Contributions

DJ and SKS conceived and designed the experiments; PJ and AAB performed experiments; RJ and SKY prepared organic manures; DJ, SKS, RC and GJ wrote the manuscript. All authors read and approved the final manuscript.

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