Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 2277-2284
Received: 16-03-2020
Accepted: 18-04-2020

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# Importance of soil microbes on panchagavya based fertilizer for sustainable agriculture: A review

## Sumit Pal and Neelam Patel

### Abstract
Panchagavya is an organic formulation prepared from milk, curd, cow ghee, cow urine, cow dung, and other optional ingredients. Panchagavya also contains plant growth regulatory substances such as indole acetic acid and gibberellic acid, as well as other essential plant nutrients. Due to chemical fertilizers pollution and detrimental effect on the soil health, panchgavya plays a major role in the organic farming as a replacement of chemical one. Moreover, studies revealed the presence of several beneficial microorganisms such as *Actinomycetes*, *Pseudomonas*, photosynthetic bacteria and fungi in panchagavya which plays a major role as growth enhancer for crops and make soil more productivity.

**Keywords:** Cow dung, cow milk, cow urine, panchagavya

### Introduction
Due to the negative impact of chemical inputs, organic farming gained more attention from last few decades. The Food and Agricultural Organization of the United Nations (FAO) estimated that the area under organic agriculture was 37.2 million ha in 2011, which was three times higher than that of 1999. In organic farming, synthetic pesticides, mineral fertilizers, genetically modified organisms and sewage sludge applications are strictly excluded. Nonetheless, biofertilizers or traditional organic formulations prepared by using organic materials/wastes are encouraged to use as a plant nutrition (Badgley *et al*. 2007) [3]. Panchagavya plays a major role in organic farming. Panchagavya is the blend of five products obtained from cow dung, curd, ghee, milk and urine. These products are mixed together and called panchagavya. In Hindu vedas like Vrikshayurveda, Panchagavya has been mentioned as the source of nutrients for the soil. (Natarajan, 2002) [42]. In India, use of panchagavya in organic farming is gaining popularity in recent years in most of states. It is a special formulation made from cows by products with certain ingredients has the potential to play the role of promoting growth and providing immunity in plant system. In Panchagavya, Effective Micro Organisms (EMO) were the mixed culture of naturally occurring, beneficial microbes mostly lactic acid bacteria (Lactobacillus), yeast (Saccharomyces), actinomyces (Streptomyces), photosynthetic bacteria (Rhodopsuedomonas) and certain fungi (Aspergillus) and that improved the soil quality, growth and yield of sweet corn, which was equal to or higher than what was obtained from chemical fertilizers. In the present review we focused on the panchagavya based fertilizer and the importance of soil microorganism in organic farming.
In this section various organic based fertilizers prepared from the cow products like Panchgavya, Jeevamurth and Beejamurth have been discussed and reviewed under different heads.

### Panchagavya
For preparation of panchagavya, Fresh cow dung(7kg)plus Cow Gheeincubate for 2 days then add cow urine (3:1) and 10 litre of water and stir it properly twice a day for 1 week. After 1 week, add sugarcane juice (3 litre) or jiggery mixed in water at 1:6 ratio with Cow milk (2 litre), Cow curd (2 litre), Coconut water (3 litre), Yeast (100 g) and 12-ripe banana all things stir properly twice a day for atleast 3 weeks. Sugarcane juice and coconut water are added to accelerate fermentation. Toddy also accelerates fermentation and helps in minimizing the bad odor. To prepare toddy two liters of tender coconut water has to be kept in a sealed airtight plastic bottle for a week. However, 100 g of yeast powder can be made use of in case of non-availability of toddy. After 3 weeks of procedure, panchagavya has been ready to use. (Gopakkali, 2013) [15].

In year 2007, Maheshwari *et al*. analyzed Panchagavya and they reported that pH (5.45 - 6.46), EC (6.15 - 10.80 dS/m), total N (385 - 410 ppm), total P (230 - 255 ppm,), total K (345 - 370 ppm), QC (0.80 -1.20%), Na (75.0- 82.0 ppm), Ca (37-32 ppm), Mn (15- 24 ppm), Fe (12 -18 ppm), Zn (0.26 ppm), Mn (0.23 ppm), Cu (0.20 ppm), total sugars (165 - 255 ppm), reducing sugars (85 - 115 ppm), IAA (7.55 - 9.15 ppm), GA (3.50 - 4.50 ppm), phenols (0.75 µg/ml). The biochemical analysis of Panchagavya using GC-MS resulted in compounds of fatty acids, alkanes, alkanol and alcohols.

In microbiological study, Lactobacillus (22 x $10^6$ cfu/ml), Methylotrophs (5 x $10^3$ cfu/ml), Pseudomonas (45 x $10^3$ cfu/ml), total anaerobes (9 -11.5 x $10^4$ cfu/ml), bacteria (35-42 x$10^9$ cfu/ml), fungi (13 -16.5 x 10 cfu/ml), actinomycetes (6- 9 x $10^2$ cfu/ml), yeast (2- 22.5x $10^5$ cfu/ml) found in Panchagavya.

In year 1996, Singh, found that cow dung had 82% of water, 18% of solid matter and minerals (0.1%), silica (1.5%), ash (2.4%), organic matter (14 6%), Calcium sulphate (0.05%), and Magnesium (0.4% each), Nitrogen (0.5%), Phosphorus (0.2%) and Potassium (0.5%). Reddy (1998) [58] observed that cow urine was rich in urea and acted as nutrient as well as growth hormone. Nene (1999) [44] stated that Cow's milk reported to have an excellent sticker and spreader protein (casein); a good medium for saprophytic bacteria and also inhibit viruses. The ghee contains vitamin A, vitamin B, calcium, fat and also rich in glycosides, which protects cut wounds from infection.

As it's already fact that the curd is rich in microbes that are responsible for fermentation (Chandha, 1996) [8]. Somasundaram and singaram (2006) [66] found total N (302 mg/kg), total P (218 mg/kg), total K (355 mg/kg), total sugars (205 µg/ml), glucose (6 mUdl), sodium (96 mg/kg), calcium (27 mg/kg), total organic carbon (0.80%), IAA (9.15 mg/kg), GA (4. mg/kg), phenols (0.75µg/ml), Zn (0.26 mg/kg), Fe (0.83 mg/kg), Mn (0.23 m_plkg) and Cu (0.2 mg/kg), pH (5.62), EC (1 0.3 dS/m), bacteria (34 x $10^6$ cfu /ml), fungi (22 x $10^4$ cfu /ml), actinomycetes (3 x $10^2$ cfu/ml), Pseudomonas (45 x $10^3$ cfu/ml), yeast (35 x $10^4$ cfu/ml), lactic acid bacteria (22 x $10^6$ cfu/ml), Methylotrophs (5 x $10^3$ cfu/ml), Azospirillum (2 x $10^2$ cfu/ml), Acetobacter (43 x $10^3$ cfu/ml), ammonium oxidizers (24 x $10^5$ cfu/ml) and nitrite oxidizers (2 x $10^2$ cfu/ml) in Panchagavya.

Pathak and Ram (2007) [54] reported that Panchagavya krishi - a system of agriculture by using Panchagavya from five products obtained from cow i.e., cow dung (7 kg), cow ghee (1 kg), cow urine (10 litres), water (10 litres), cow milk (3 litres), cow curd (2 hires), tender coconut (3 litres), jaggary (3 kg) and well ripened banana (12 Nos.). The preparation was rich in IAA, GA and microflora i.e., bacteria (19x10 /ml), anaerobes (1x10/ml), acid formers (360/ml) and methanogens (250/ml).

In year 2007, Swaminathan et.al. reported that Panchagavya is a fermented liquid of 5 main ingredients which are urine, dung, milk, ghee and curd of cow (5.7 kg, 3.46 litres, 2.3 litres, 2.3 litres and 1.15 kg, respectively). Further, they reported that the ultimate product had total N (302 g/kg), total P (219 mg/kg), total K (355 mg/kg), total organic carbon (0.80%), Zn (0.26 mg/kg), Fe (0.83 mg/kg), Mn (0.23 mg/kg), Cu (0.20 mg/kg), pH (6.02) and electrical conductivity (3.02 dS/m).

In microbiological study, Swaminathan et.al. was reported that, bacteria (34 x $10^6$ cfu/ml), fungi (22 x $10^4$ cfu/ml) and actinomycetes(3 x $10^2$ cfu/ml). At CCRI, Bangalore, Salkinkop *et al*. (2008) [60] prepared the Panchagavya for bio-compositing. Panchagavya was used in the rate of 2.51 diluted in fifty litres of water for composting 1 tone of farm yard wastes, cherry husk and pulp. while preparing compost heaps, it was sprinkled on each layer of moist FYM

**Jeevamruth**

Jeevamruth is a fermented liquid product prepared by mixing cow dung (10 kg) with cow urine (10 hires), Jaggary (2 kg), legume flour (2 kg) and a handful of soil brought from the bunds of the cultivated lands Jeevamruth also contains enormous amount of beneficial microbial load. The application of Jeevamruth as reported to enhance microbial activity in soil and ultimately ensuring the availability and uptake of nutrients by the crops.(Palekar, 2006) [51]. In year 2007, Pathak and Ram observed that natural farming by using Jeevamruth prepared by using cow dung (10 kg), cow urine (10 litres), jaggary (2 kg/4 litres sugar cane juice), virgin soil (1 kg), pulse flour (2 kg) and water (20 litres) in a container (plastic/earthen/cement). The Jeevamruth was analyzed on 5th day after preparation and had sample Azospirillum (2 x $10^6$), PSM (2 x $10^6$), Pseudomonas (2 x $10^2$), Trichoderma (2 x $10^6$), yeast and moulds (2 x $10^7$) per ml of sample.

**Beejamruth**

According to Palekar (2006) [51], Beejamruth is not a source of nutrients, but it is a product which contains ingredients *viz*., cow dung (5 kg), cow urine (5 litre), lime (50 g) and 20 litre water. It is being used by the organic farmers for seed or seedling treatment which was found to increase seed germmation and seedling growth as it contains growth hormones and beneficial microflora.

In this section we discuss and review the role of microorganism and its functional enzymes associated with them to enhance the quality of panchgavya based biofertilizers. In this context, following study has been reviewed to prove the role of microbial organism and its enzymes.

**Rhizosphere and Phyllosphere microorganisms**

Schroth and Hancock (1992) [62] defined rhizobacteria as the microorganism which is effectively colonized with the roots. Kloepper *et al*. (1989) [22] stated that the root colonization is the process where bacteria survive on seeds, multiply in spermosphere in response to seed exudates rich in carbohydrates and amino acids attach on to the root surface (Suslow, 1982) [69] and colonize the developing root system. Thus, colonization of roots is an active process and not a transitory relation between bacteria and roots in the soil. The rhizobacteria are of interest because they can be potentially beneficial (nitrogen fixation, phosphate solubilization, production of plant growth promoting substances, biocontrol) or detrimental (causing diseases, immobilization of plant nutrients). Many microbes are colonizing plant parts without eliciting defense responses from host plants or causing disease symptoms (Bacon and White, 2000) [2]. Microorganisms form a characterstic component of the phylloplane of the majority of plants (Leben, 1964) [26]. Phyllosphere forms a characteristic habitat for microorganisms (Beattie and Lindow, 1995) [4]. The surface of plants is always covered by epiphyllic microorganisms such as bacteria, yeast, filamentous fungi belonging to different systematic categories (Morris et. al., 1996) [38]. Mostly phyllosphere bacterial species belonging to the genera Corynebacterium, Erwinia, Pseudomonas, Xanthomonas and bacillus (Ercolani 1991; Morris *et al*., 1998) [12, 37].

Soil microorganisms play an important role in increasing phosphorus availability of plants by dephosphorylating. Phosphate bearing organic compounds and also by bringing about favourable changes in the soil reaction and soil micro-environment leading to solubilization of inorganic phosphates. Besides, the metabolic by products of soil microorganisms such as organic acids and humic acid substances primarily produced during organic matter decomposition, form complexes with calcium, iron and aluminium compounds, resulting in reduction of phosphorus fixation and increases its availability indirectly.

The Systematic study on phosphate solubilization was made by Pikovskaya (1948) [56] for the first time, which showed dissolution of tricalcium phosphate by pure culture of 'Bacterium p'. Microorganisms could dissolve tricalcium phosphate (TCP) to a high degree in acidic, neutral and alkaline culture media but rock phosphate was dissolved to a lesser extent than TCP by isolates (Muromtsev, 1958) [39]. But, maximum solubilization of TCP in liquid medium was observed within a week by different phosphate solubilizing microorganisms as reported by Goswami and Sen (1962) [16]. The Phosphate Solubilizmg microorganisms have been isolated in the recent years from the soils and rhizosphere of crop plants by various workers (Halder et. al., 1990; Krishnaraj and Gowda, 1990; lllmer and Schinner, 1992; Maheshkumar, 1997) [18, 25, 27, 31]. One of the mechanisms in these organisms to solubilize the insoluble mineral phosphate is the production of organic acids (Gaur, 1990) [13]. lllmer *et al*. (1995) [28] reported that four species *viz*., Aspergillus niger, Penicillium simplicissimum, Penicillium awantiogriseum and Pseudomonas sp. were very effective in solubilizing rarely soluble aluminium phosphate. lllmer and Schinner (1992) [27] reported that solubilization of tricalcium phosphate occurs even in the absence of release of organic acids. Some bacteria like Fluorescent pseudomonads also present and are Gram negative, aerobic rods, motile bacteria with polar flagella and have the ability to produce water soluble yellow green pigments (Palleron1 *et al*., 1973) [52] The species of fluorescent pseudomonads include P. fluorescens, P. putida, P, aeruginosa. P. chlororaphus, P. aureofaciens and P. syringe (Schipper el al, 1987) [61]. They possess characteristics that make them ideal for biological control (Weller, 1985) [76]. The worldwide interest in this group of rhizobactina was sparked off by the studies initiated at the University of Calfornia, Berkeley, USA during the 1970s. Burr et. al. (1978) [7] reported that strains of P. fluorescens and P. putida applied to seed tubers improved the growth of potato. These findings were confirmed and later exemplified in radish (Kloepper and Schroth, 1978) [23], cotton (Sakuuvel *et al*., 1986) [58], vegetables (Elad *et al*., 1987) [11], tomato and eggplant (Dileep and Dubey, 1991) [10] and groundnut (Vikram, 1997) [75]. On other study with nitrogen fixers microorganism, as its universally clear that nitrogen play a critical role in the growth of plants as well as microorganisms as it is required for the synthesis of many compounds, including amino acids, purines, pyrimidines and lipids, enzyme cofactors and proteins all of which are essential for growth processes.(Tejswini *et al*. 2020) [71] The microorganisms have the ability to fix atmospheric nitrogen which is restricted to a limited number of bacteria. The asymbiotic nitrogen fixing bacteria were first discovered by Beijerinck (1901) [5] which represent the main group of heterotrophic free living nitrogen fixers that contribute to global nitrogen fixation considerably. Azotobacters represent the main group of aerobic, heterotrophic, free-living, nitrogen fixing soil bacteria. (M Sharath *et al*. 2019, Prajwal *et al*., 2018) [30, 57] The first observation made by Hiltner (1904) [19] was that the bacteria were more abundant in rhizosphere than in non rhizosphere. Hiltner (1904) [19] discovered that free living nitrogen fixing bacteria were stimulated by plant root growth.

**Microbial activity in organic fertilizers**

Jenkinson and Ludd, (1981) [20] stated that, Soil micro-organisms play a very important role in soil fertility not only because of their ability to carry out bio-chemical transformations but also due to their importance as a source of sink for mineral nutrients. Several groups of microorganisms have the potential to enhance growth and improve the health of crops.(N Murugalatha *et al*., 2018) [40] Microbial biomass is the total sum of all microorganisms present in the soil. The number and activity of these microbes (Tilak *et al*., 1995) [72] exhibit variable responses to different agricultural management practices. The presence of beneficial effective microbes (EMOs) in Panchagavya of lactic acid bacteria, yeast, actinomycetes, photosynthetic bacteria, Azotobacter, Azospirillum and Phosphobacterium and some fungi were detected which are known to improve the soil health, growth and yield of crops (Xu and Xu, 2000) [77]. The use of fermented liquid organic fertilizers (Panchagavya, Jeevamruth, Beejarnruth, Sasyamruth, vermiwash, Amrutpani etc.) prepared from cow dung, urine, leguminous leaves or vermiwash are effective in rapid buildup of soil fertility through enhanced activity of soil micro-flora and fauna (Yadav and Mcwade, 2004) [78].

Use of Beejamrutha, a mix of cow dung, cow urine, water, lime and a handful of soil has been given importance in sustainable agriculture since age old days. Bacteria were isolated from beejamrutha and tested for their beneficial traits. Sreemvasa *et al*. (2010) [68] conducted an experiment on Beejamruth, a source of beneficial bacteria at the Institute of Organic Farming, UAS, Dharwad. The beneficial microorganisms present in Beejamruth were tested for their beneficial traits. The isolates were capable of nitrogen fixation and phosphate solubilization. The isolate Az82 showed highest amount of nitrogen fixation (13.71 mg/g carbon source utilized) whereas BPS3 showed highest amount of phosphate solubilization (8.15%). Inoculation of these beneficial isolates resulted in the improvement of seed germination, seedling length and seed vigour index in soybean.

In one of the experiment, Shwetha (2008) [64] found significantly higher microbial activity in treatments given organic manures such as compost, verrmicompost and green leaf manure with fermented organics *viz*., Beejamruth, Jeevamruth and Panchagavya over RDF + FYM and fermented organics alone.

Meena et. al. (2000) [35] in two years of experimentation did not found any pest and diseases in the crops sprayed with Panchagavya. The Panchagavya contained Pseudomonas (45 x $10^3$ cfu/ml) and saprophytic yeasts (35 x $10^4$ cfu/ml), which might have contributed to plant protection because the presence of Pseudomonas on plant surfaces have been found to induce the production of pathogenesis related protein, sidercphores, antibiotics and HCN in groundnut and rice. Thus, can be used as a biocontrol agent.

In the organic farming, to replace the chemical fertilizers by organic sources, the quantity required per hectare shall be 37 to 50 tonnes per hectare calculated on the basis of the NPK content. But, on the basis of microbial content one need only 50 kg cow dung per ha (in split dose of 25 kg twice) as the millions of microorganisms present are multiplied to billions during fermentation. The numerous microorganisms present and growing in the soil are capable of providing all nutrients required by the crop. The Soil is living only when it will have a microbial population of $10^8$ per cubic centimeter of soil. These microfloras need adequate quantity of organic manures as feed for their survival and multiplication (Babalad *et al*., 2008) [1]. Nagaraj and Sreenivasa (2009) [41] conducted an experiment to study the influence of bacteria isolated from Panchagavya on seed germination and seed vigour in wheat. On 8th day after sowing, significantly highest percentage germination (99%) was noticed in the seeds treated with bacterial culture PB9 and PB15 while lowest germination was recorded in uninoculated seeds (85%) which indicated positive role of bacterial isolates in promoting seed germination. The bacterial culture PB9 has registered significantly higher seedling length (28.5 cm) and vigor index (2822) which were markedly lowest in the control (16.5cm and 1403 respectively).

The beneficial microorganisms from Panchagavya and their establishment in the rhizosphere with the use of compost will improve the plant growth, crop yield and suppresses plant pathogens. The total population (cfu's) of bacteria, fungi, yeast and aclinomycetes in compost treated with Panchagavya ranged from 269 - 294 x $10^7$ cfu/g, 44 - 47 x $10^6$ cfu/g, 14 - 23 x $10^5$ clu/g and 9 - 13 x $10^3$ cfu/g, respective and in control their respective population was significantly lower (68 x $10^1$ cfu/g, 14 x $10^0$ cfu/g, 7 x $10^5$ cfu/g and 3 x $10^5$ cfu/g) (Salkinkop *et al*., 2008) [60].

Boomiraj and Christopher (2007) [6] used the Panchagavya and botanical spray on the soil microbial population. Higher bacterial and fungal population were recorded in treatment given poultry manure and Panchagavya (145 x $10^6$ cfu and $10^3$ x $10^4$ cfu), followed by the application of neem cake and Panchagavya (140.0 x $10^6$ cfu and $10^2$ x $10^4$ cfu) and poultry manure and herbal leaf extract (138.5 x $10^6$ cfu and 101.5 x $10^4$).

**Soil microbial enzymatic activity**

The soil enzymes are remarkable molecules that show a high degree of specificity in catalyzing biological reactions the various activities associated with biotic and abiotic components contribute to overall soil enzyme activities. (Pal, *et al*. 2012a; Pal,. *et al*. 2011; Pal, *et al*. 2012b) [45, 46, 47]

The enzymes produced by proliferating microorganism mediate many processes occurring in soil. The variation in the microbial population might result m an alteration of the enzyme activity. The enzymes have biological significance as they participate in the biological cycling of elements. (Pal, S. *et al*. 2016b; Pal, S. *et al*. 2015) [48, 49] They play an important role in the decomposition of organic residues and transformation of some of the mineral compounds (Kiss *et al*., 1975; Neelam *et al*. 2019) [21, 43]. Some of the important soil enzymes are dehydrogenase, urease and phosphatase.(Upma *et al*. 2019; Tripathi VK,*et al*. 2019) [74, 73]

Kondapanaidu (2008) [24] observed significant differences between different treatments with respect to dehydrogenase activity in soil after the harvest of chilli. The treatment given with 50 per cent RON through chemical fertilizer + 50 per cent of recommended N through vermicompost + biofertilizers + Panchagavya showed highest enzyme activity.

Pascual *et al*. (1999) [53] conducted 8 years of experimentation on compost and recorded higher dehydrogenase, urease and phosphatase activity as compared to control. The organic amendments had a positive effect on the activities of these enzymes, particularly when the amendment was at the highest dose. The response was probably due to the higher microbial biomass produced in the system.

Shwetha (2008) [64] reported that the combined application of fermented organics *viz*., Beejamruth, Jeevamruth, Panchagavya along with organic manures such as compost, vermicompost, green leaf manure recorded higher soil biological activity.

Similarly, dehydrogenase activity was higher with the combined application of organic manures and fermented liquid organics than their individual application and RDF + FYM. The highest dehydrogenase activity 34.84 µg TPF per g soil per day was observed with the application of compost + vermicompost + green leaf manure + Jeevamruth + Beejamruth and 11 was on par with the treatment given vermicompost + green leaf manure + Jeevamruth + Beejamruth + Panchagavya. The lowest dehydrogenase activity of 24.27 µg TPF per g soil per day was noticed with the application of RDF + FYM at 60 DAS of soybean in soybean-wheat cropping system. Manjunath (2006) [33] observed a marked increase in dehydrogenase activity in the soil of organic farms than that of conventional farms in the selected major cropping system i.e., cotton, sugarcane, jowar and vine yard.

Chandrakala (2008) [9] reported that dehydrogenase activity was significantly influenced by the application of organic manures and liquid organic manures at both 120 DAT and 160 DAT. Among liquid organic manures, the treatment given Beejamruth + Jeevamruth + Panchagavya realized significantly higher dehydrogenase activity of 26.69 and 22.24 µg TPF per g soil per day at 120 DAT and 160 DAT respectively as compared to Panchagavya and control, but it was on par with Beejamruth + Jeevamruth at 120 DAT. The interaction effects of manures and liquid manures did not show any significant difference.

Boomiraj and Christopher (2007) [6] opined that the organic and inorganic sources of nutrition increased soil dehydrogenase, phosphatase and urease activities. The initial level of soil dehydrogenase activity was 8.07 µg TPF/g soil/day while the highest activity was observed in plots applied with poultry manure + Panchagavya (24.26 µg TPF/g soil/day) and the lowest activity was observed in plots given neem cake alone (20.92 µg TPF/g soil/day). The highest phosphatase and urease activities were observed in plots given poultry manure+ Panchagavya (25.90 µg P- nitrophenol/g soil/day and 45.64 µg of NH/g soil/day respectively). The lowest soil phosphatase and urease activities were observed in plots given neem cake alone (21.81 µg P-nitrophenol/g soil/day and 40.53 µg NH:/g soil/day respectively).

**Diversity, Richness, Similarity and Evenness of bacterial population present in panchagavya samples** (Gunwantrao & Patil, 2015) [17].

Shannon diversity index has been one of the most widely used parameters to access biodiversity; it measures the average degree of distribution of species of a given individual within a randomly chosen population. Shannon diversity (H) index is a mathematical measure of species diversity in a given community based on the species richness (the number of species present) and species abundance (the number of individuals per species).(Shannon,1948) [63] This index provides important information about rarity and commonness of species in a community. When both diversities and richness increase Shannon diversity index value also increases. (Pal, S. *et al*. 2016a) [50] In the study, the highest Shannon diversity index (H) was observed in the panchagavya sample drawn on 15th day (2.96) followed by the sample drawn on 21st day (2.82) indicating that panchagavya samples drawn on 15th and 21st days were having more species richness and species abundance. This is likely to provide an insight into diverse microbial groups present in similar proportion and their relative distribution at given space and time. The lowest value of Shannon index (2.35) was observed with metagenome of panchagavya sample drawn on 30th day. This possibly occurred as the bacterial species and their number might have decline from 21st day until 30th day. In the preparation of panchagavya in this study ingredient at the starting included, 600 g of cow dung and 100 g of ghee along with 400 ml of cow urine and 1000 ml of water, Shannon diversity was lower during the early stages of panchagavya till 7th day possibly because of microflora represented mainly from cow dung. Other ingredients like sugarcane juice 300 ml, 200 ml of cow's milk, 200 ml of curd, 200 ml of coconut water, 25 g jaggery and one ripened banana were added and mixed thoroughly on 15th day. The total microbial diversity from all ingredients was appeared to have contributed for higher Shannon value (2.96) on 15th day. The species richness in the metagenomic DNA was calculated based on the number of bands (OTUs) present per sample between 30-70 per cent denaturing gradient. Critically analyzed bands were used to calculate species richness and expressed as range weighted richness (Rr). The Rr values were observed to be more than 30, which implied that panchagavya is a typical and very habitable environment with broad carrying capacity, having high microbial diversity. Hence, it could be called as high range weighted richness sample.

Besides this Pielou's evenness values of panchagavya at all the stages were observed to be more than 0.75 per cent. Results showed that species were more evenly distributed at all the stages of panchagavya. Pielou's evenness index value of one and closer to it indicated highly even distribution of species in a sample and index value of zero and closer values indicated highly uneven distribution of species in a sample (Pielou, 1966) [55]. It was very clear that highly even bacterial species distribution occurred at all stages of panchagavya preparation. (Gunwantrao & Patil, 2015) [17].

**Functional organization of bacterial species present in panchagavya using Pareto Lorenz (PL) curve**

Pareto Lorenz curves for panchagavya samples drawn at different stages showed that the curve values for all the samples were more than 80 per cent on Y axis at a 20 per cent intercept on Xaxis. This implied that more than 80% bacterial population in panchagavya was belonging to only 20 bacterial species (Lorenz, 1905) [29]. It is likely that a specialized microbial community could exist in each sample.(Miura, 2007) [36] As observed (Marzorati, 2008) [34] in their study such microbial community could be functionally highly organized but fragile to external changes because disruption might require longer recovery time by the existing bacteria and to rebuild their favourable environment. (Gunwantrao & Patil, 2015) [17].

**Bacterial community dynamics present at different stages of panchagavya**

Dynamics of a microbial community in a sample is a measure of the average rate of change in parameter and degree of change between consecutive DGGE profiles of the same community over a fixed time interval. Based on moving window analysis, the rate of change (D*t*) in parameter was calculated at all the consecutive stages of panchagavya at defined time interval. It was observed that the rate of change in parameters was 17.9 per cent. The values for overall per cent change ranged between 13 and 25 per cent. This situation was assumed to represent a medium level of bacterial population dynamics present at all stages of panchagavya. Further, it could imply that in panchagavya sample a new species can enteredinto pre existing bacterial community but cannot interfere with the functionality of the pre-existing population as observed in this study. (Gunwantrao & Patil, 2015) [17].

In metagenomic study of cow dung microbiota by Girija et.al. (2012) [14] observed that bacteria belonging to the phyla *Bacteroidetes* (38.3%), *Firmicutes* (29.8%), *Proteobacteria* (21.3%) and *Verrucomicrobia* (2%) were identified. Bacteroidetes clones included the genera *Bacteroides*, *Alistipes* and *Paludibacter*; while *Clostridium*, *Ruminococcus*, *Anaerovorax* and *Bacillus* were predominant in *Firmicutes* and proteobacterial genera included *Acinetobacter*, *Pseudomonas*, *Rheinheimera*, *Stenotrophomonas* and *Rhodobacter*.

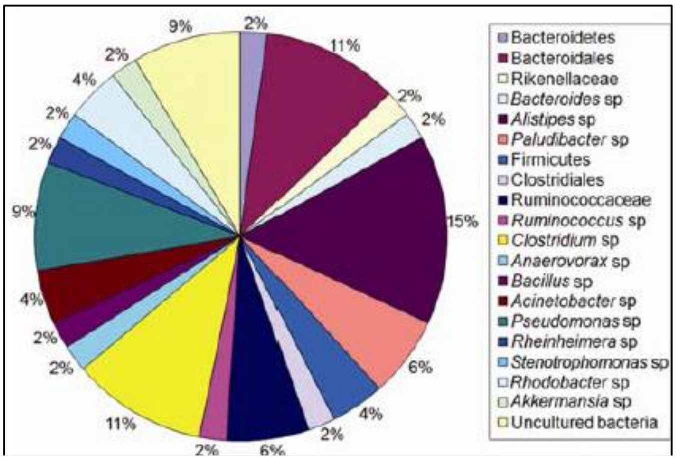


**Fig 1:** Diagrammatic representation of Cow's dung Microbiota.

The Verrucomicrobial clone showed high similarity to *Akkermansia*. Unculturable bacteria constituted 83.3% in the phylum Bacteroidetes and 87.5% in Firmicutes. All clones under phylum Proteobacteria were culturable bacteria.

**Conclusion**

Due to ill effects of chemical compounds like pesticides and fertilizers on soil is a serious issue at present time. The increasing concern for environmental safety and global demand for pesticide residue free food has evoked keen interest in crop production using eco-friendly products. So it is necessary to use ecofriendly and natural products like Panchagavya to produce chemical residue free food crops and hence it can play a major role in organic farming. The review on the enzyme activity and microorganism proved that the quality of panchgavya based fertilizers has been increased and also based on the potential of microorganism present in soil where it implemented. So, the role of microorganism is very crucial for the sustainable organic farming.

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