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Microbial characterization of on-farm produced bio-enhancers used in organic farming

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ABSTRACT

Different bio-enhancers commonly used in organic production of various crops were prepared for microbial characterization. *Panchagavya* had been utilized since long time by the farmers to provide nutrients to the plants and soil micro-organisms. Microbial analysis of bio-enhancers revealed that *Panchagavya* contained highest number of total bacteria (6.25×10^9 cfu/ml) as compared to *Jeevamrita* (3.24×10^9 cfu/ml), biodynamic liquid pesticide (2.27×10^9 cfu/ml) and *Amritpani* (5.49×10^8 cfu/ml). *Panchagavya* had also higher number of *Pseudomonas* (4.7×10^7 cfu/ml), *Rhizobium* (2.43×10^6 cfu/ml), *Azotobacter* (1.4×10^5 cfu/ml) and *Azospirillum* (1.03×10^5 cfu/ml). Fungi population was highest in *Jeevamrita* (1.20×10^7 cfu/ml) as compared to biodynamic liquid pesticide (2.64×10^6 cfu/ml), *Amritpani* (0.46×10^5 cfu/ml) and *Panchagavya* (0.20×10^5 cfu/ml). Actinomycetes, *Pseudomonas*, p-solubilizing microbes, *Azotobacter* and *Azospirillum* population were highest in biodynamic liquid pesticide (1.37×10^8, 3.28×10^8, 8.50×10^6, 2.00×10^6 and 1.40×10^5 cfu/ml). Similarly, gram positive bacteria, gram negative bacteria and *Rhizobium* were highest in *Jeevamrita* (1.60×10^8, 2.20×10^8, 7.51×10^7 cfu/ml). *Amritpani* had higher number of actinomycetes (1.31×10^7 cfu/ml), gram negative bacteria (1.35×10^8 cfu/ml) and p-solubilizing microbes (4.80×10^6 cfu/ml). Among all bio-enhancers, *Panchagavya* was rated most effective bio-enhancer followed by biodynamic liquid pesticide, *Jeevamrita* and *Amritpani.* Results reveal that these bio-enhancers could play a potent source of beneficial microbes which could improve soil fertility, crop productivity and produce quality.

Key words: *Amritpani*, Biodynamic liquid pesticide, *Jeevamrita*, Microbial population, *Panchagavya*

Use of organic liquid preparations is age old practice in India. Preparation of *Kunapajala* which involves boiling of flesh, fat and marrow of animals such as deer, pig, fish, sheep, goat in water, placing it in earthen pot, and adding milk, powders of sesame oil cake, blackgram boiled in honey, decoction of pulses, ghee and hot water used to be the common booster of plant vigour (Nene 2007). Bio-enhancers are organic preparations, obtained by active fermentation of animal and plant residues over specific duration, e.g. *Panchagavya.* These are rich source of microbial consortia, macro and micronutrients and plant growth promoting substances including immunity enhancers. They are used for seeds/seedlings treatment and fast decomposition of organic wastes in composting. *Panchagavya* is the most effective bio-enhancer demonstrated by many researchers (Sangeetha and Thevanatham 2010).

Nutrient management in organic farming is one of the most critical aspects. Organic inputs are less dense in plant nutrients and bulky in nature and needs in larger quantity to meet nutritional requirements of the crops. The organic inputs must have excellent quality regarding their nutrients composition or to supply plant nutrient by the activity of viable microbes. In India, availability of quality organic inputs, viz. organic manures, bio-enhancers, bio-pesticides etc. for organic farming is challenging. That's why organic inputs must be produced at the farm itself. Analysis of organic inputs for their quality is very essential aspect for organic production. In this study we have analyzed bio-enhancers viz. *Panchagavya, Amritpani, Jeevamrita* and *biodynamic liquid pesticides* microbiologically which were prepared at the farm.

MATERIALS AND METHODS

Inputs required for organic production, viz. *Panchagavya, Amritpani, Jeevamrita* and *biodynamic liquid pesticides* were produced from locally available materials in the month of May, 2016. This study was done under returore project on organic horticulture.

Panchagavya was prepared by mixing five products of cow, i.e. dung (5 kg), urine (3 l), milk (2 l), curd (2 l) and ghee (1 l). To this, sugar cane juice (3 l), jaggery (0.5 kg), tender coconut water (3 l), toddy (2 l) and ripe bananas (12) were mixed in a wide mouthed mud container. It was ready for use in 18 days.

Amritpani was prepared by incubating 10 kg cow dung

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along with 250 g cow ghee and 100 g honey in 200 liters plastic container. Preparation was ready for use in 7-10 days.

Jeevamrita was prepared by fermenting 10 kg cow dung, 5 liters urine, 2 kg jaggery, 2 kg pulse flour and 250 g virgin soil and 150 liters water in a plastic/mud/cemented container by simple facilities created in the village with minimum expenditure. This formulation is used within 7-10 days of preparation.

Biodynamic liquid pesticide was prepared with locally available materials, i.e. cow dung, urine and neem leaves. Besides cow dung, cow urine and one set of biodynamic preparations (502-507) were also incorporated. Biodynamic preparation helps in harnessing cosmic energy from different planets to improve nutritive value of preparation and enhance fermentation process. The liquid pesticide is used for the management of insect and pest.

Enumeration of different beneficial microbial populations, viz. bacteria, fungi, actinomycetes, *Pseudomonas*, gram positive bacteria, gram negative bacteria, p-solubilizing bacteria, *Rhizobium*, *Azotobacter* and *Azospirillum* were carried out by using dilution plate count method using selective media *viz*. Nutrient agar, Rose Bengal Chloramphenicol Agar (RBCA), actinomycetes isolation agar, King's B (King *et al.* 1954), methyl red agar (Hagedorn and Holt 1975), crystal violate agar (Goud *et al.* 1985), Pikovskaya's agar (Pikovskaya 1948), Yeast extract mannitol agar with congo red (CRYEMA, Fred *et al.* 1932), modified Jenson's agar (Jensen 1954, Norris and Chapman 1968) and N-free malate medium (Okon *et al.* 1977), respectively.

Petri dishes were made by pouring each specific solid medium. Then 10 ml of each bio-enhancers sample were diluted to 90 ml sterile water and that was considered being 10^{-1} dilution factor. Transferring of 1 ml of 10^{-1} dilution to 9 ml sterilized water with the help of a sterilized pipettes yielded 10^{-2} dilution. In this way, a series of up to 10^{-8} dilutions were prepared under aseptic condition. Point one ml (0.1 ml) of the suspension from required dilution (e.g. 10^{-8}) was taken and poured into the respective agar media on Petri dish and spread with L-spreader with the help of Plate Master (Hi-Media). Then plates were incubated at 28 ± 2°C for 3-5 days. The numbers of visible colonies were counted. The total count was obtained by multiplying number of visible colonies on the plate by the dilution factor.

RESULTS AND DISCUSSION

Microbial population dynamics in Panchagavya

Bacterial population gradually increased during the fermentation period and highest number was recorded at 18th day (62.50 × 10^7cfu/ml) (Table 1). Population of fungi initially increased up to 6th day (4.0 × 10^5 cfu/ml), but then gradually decreased at maturity stage. Interestingly, actinomycetes population increased from 0 day (0.19 × 10^6 cfu/ml) to 20th day (8.0 × 10^6 cfu/ml). Gram positive bacterial population decreased towards maturity stage of *Panchagavya*. Gram negative bacteria increased over time and reached at a very high level (35.8 × 10^6 cfu/ml) on 20th day. Similar phenomenon was also observed with *Pseudomonas*. Population of *Rhizobium* was almost stable at the initial period but, slightly increased and highest was recorded at 20th day (4.14 × 10^6 cfu/ml). The numbers

Table 1 Different microbial populations in *Panchagavya*

Type of microbe	Multiplication factor	Microbial population (cfu/ml) after days of preparation (Mean ± sd)								CD (P = 0.0.5)
		0	3	6	9	14	18	20	25	
Bacteria	10^8	3.40± 0.51	4.90± 0.67	3.50± 0.85	7.15± 1.48	5.30± 0.70	62.50± 5.52	25.90± 4.76	29.50± 2.52	4.60
Fungi	10^5	0.01± 0.01	0.50± 0.23	4.00± 0.58	1.20± 0.31	0.56± 0.12	0.20± 0.07	0.15± 0.03	0.1± 0.08	0.42
Actinomycetes	10^6	0.19± 0.03	0.30± 0.07	1.70± 0.29	1.80± 0.22	1.40± 0.43	2.20± 0.13	8.00± 1.38	7.00± 1.00	1.14
Gram positive bacteria	10^7	1.38± 0.24	2.04± 0.22	1.10± 0.22	0.23± 0.31	0.13± 0.71	0.11± 3.53	0.19± 6.73	12.00± 0.51	4.50
Gram negative bacteria	10^6	0.55± 0.12	1.20± 1.86	2.50± 0.26	3.20± 0.70	6.10± 0.30	17.40± 1.03	35.80± 0.25	0.90± 0.61	1.51
Pseudomonas	10^6	1.89± 0.03	1.20± 0.04	1.42± 0.03	2.40± 0.02	6.00± 0.06	47.00± 0.61	57.00± 0.10	3.10± 0.63	0.59
Rhizobium	10^6	1.48± 0.85	6.74± 0.40	1.55± 0.01	2.05± 0.01	1.92± 0.02	2.43± 0.01	4.14± 0.01	2.42± 0.01	0.56
p-solubilizing microbes	10^6	0.29± 0.26	0.15± 0.04	0.15± 0.03	0.16± 0.11	1.40± 0.36	3.20± 0.42	2.42± 0.07	2.13± 1.16	0.83
Azotobacter	10^6	4.50± 0.07	3.93± 0.59	0.01± 0.15	0.09± 0.12	0.07± 0.01	0.14± 0.01	0.15± 0.03	0.15± 3.36	2.10
Azospirillum	10^5	1.12± 0.10	0.29± 0.52	0.06± 0.14	0.49± 0.32	0.72± 0.46	1.03± 2.57	1.60± 3.81	4.00± 0.20	2.50

of p-solubilizing microbes were also increased gradually from initial number of 0.29×10^6 (0 day) to 2.42×10^6 (20^{th} day). *Azotobacter* population decreased (4.50×10^6 at 0 day to 0.14×10^6 at 18^{th} day. *Azospirillum* population also decreased from 0 day to 14^{th} day (1.12 to 0.72×10^5 cfu/ml) but then increased gradually (Table 1). Cow dung is an active ingredient of *Panchagavya* and it is a rich source of beneficial microbes as reported earlier by many workers (Girija *et al.* 2013). Cow milk also contains beneficial microbes (Crielly *et al.* 1994). Cow curd is rich source of *Lactobacillus* sp. The highest number of bacteria in *Panchagavya* might be due to nutrient richness of the mixture obtained from ingredients of cow origin, viz. cow milk, curd and milk etc. Amalraj *et al.* (2013) had reported highest population of total bacteria (22×10^9 cfu/ml), actinomycetes (60×10^4 cfu/ml), p-solubilizers ($10^3 \times 10^6$ cfu/ml), fluorescent pseudomonas (151×10^5 cfu/ml). Chadha *et al.* (2012) also reported highest load of viable bacterial populations, *Azotobacter* sp., actinomycetes as well as p-solubilizers in *Panchagavya.* Microbial and biochemical analysis of *Panchagavya* were also worked out by Radha and Rao (2014).

Microbial population dynamics in Amritpani

Bacterial population increased in the preparation from an initial value of 2.40×10^8 cfu/ml at 0 day to 5.49×10^8 cfu/ml at 9^{th} day but then gradually decreased (Table 2). Fungi population decreased gradually from 0.12×10^6 cfu/ml at 0 day to 0.046×10^6 cfu/ml at 9^{th} day. Similarly, actinomycetes population decreased up to 6^{th} day but increased at 9^{th} day then again decreased. Population of gram positive bacteria decreased with fermentation. Gram negative bacteria had increased at 9^{th} day. Interestingly, the *Pseudomonas* population gradually increased from an initial value of 0.71×10^7 cfu/ml at 0 day to 4.80×10^7 cfu/ml at 14^{th} day. *Rhizobium* and p-solubilizing microbes gradually increased during preparation process (0.50, 0.72×10^6 cfu/ml at 0 day to 3.03, 4.80×10^6 cfu/ml at 9^{th} day). The population of *Azotobacter* and *Azospirillum* showed a gradual reduction with fermentation process up to 9^{th} day even became nil in case of *Azospirillum*.

Microbial population dynamics in Jeevamrita

In this bio-enhancer, bacterial population increased rapidly from an initial value of 1.80×10^7 cfu/ml at 0 day to 324.2×10^7 cfu/ml at 9^{th} day after that it was rapidly decreased (Table 3). Population of fungi increased gradually up to 14^{th} (4.42×10^7 cfu/ml). Actinomycetes population increased at 3^{rd} day but again decreased on 6^{th}, 9^{th} day and further reduced to 0.50×10^6 cfu/ml on 14^{th} day. Gram positive and gram negative bacteria increased up to 9^{th} day but then decreased. Similar trend was observed in case of *Pseudomonas* population. *Rhizobium* population increased during preparation period and maintained at a very high level at 9^{th} (75.1×10^6 cfu/ml) day but then gradually decreased. Number of p-solubilizing microbes rapidly increased from 0 day (1.20×10^6 cfu/ml to 9^{th} (5.04×10^6 cfu/ml). *Azotobacter* and *Azospirillum* population reduced during the process. Probably for this reason *Jeevamrita* and *Amritpani* were being used for seed treatment in different crops (Phate *et al.* 2014).

Microbial population dynamics in biodynamic liquid pesticide

Bacterial population slowly increased throughout the fermentation period. Fungi population had also a similar trend of increase up to 9^{th} day but after that it decreased. Actinomycetes population maintained exceptionally very high level from initial time (1.90×10^8 cfu/ml at 0 day) to 1.65×10^8 cfu/ml at 14^{th} day (Table 4). Population of gram negative bacteria increased rapidly from 1.68×10^7 cfu/ml at 0 day to 6.86×10^7 cfu/ml at 9^{th} day and decreased thereafter. *Rhizobium* and P- solubilizing microbial population increased and reached maximum at 14^{th} day for *Rhizobium* and 9^{th} day for p-solubilizers. Interestingly, the initial *Azotobacter* population was

Table 2 Different microbial populations in *Amritpani*

Type of microbe	Multiplication factor	Microbial population (cfu/ml) after days of preparation (Mean ± sd)						CD (P = 0.05)
		0	3	6	9	14	20	
Bacteria	10^8	2.40±0.18	2.74±0.59	3.29±0.29	5.49±0.21	1.60± 0.93	1.20± 0.45	0.86
Fungi	10^6	0.12±0.01	0.11± 0.06	0.001±0.01	0.046±0.001	0.05± 0.07	0.18± 0.04	0.08
Actinomycetes	10^7	0.66±0.16	0.73±0.37	0.10±0.10	1.31± 0.19	0.37± 0.14	2.00± 0.15	0.40
Gram positive bacteria	10^8	0.50±0.14	1.70±0.34	0.30±0.14	0.30± 0.12	0.07± 0.57	0.29± 0.51	0.53
Gram negative bacteria	10^8	0.10±0.24	0.29±0.14	0.58± 0.22	1.35± 0.50	0.06± 0.03	0.12± 0.33	0.55
Pseudomonas	10^7	0.71±0.18	1.47±0.07	1.29± 0.05	1.53± 1.45	4.80± 0.35	3.10± 0.43	1.07
Rhizobium	10^6	0.50±0.46	0.40±0.03	1.20± 0.01	3.03± 0.01	1.12± 0.15	1.64± 0.03	0.36
p-solubilizing microbes	10^6	0.72±0.24	2.20±0.19	3.20± 0.02	4.80± 0.001	2.93± 0.04	2.00± 0.01	0.20
Azotobacter	10^7	2.97±0.05	0.26±0.35	0.01± 0.02	0.001± 0.18	0.28± 0.01	0.27± 0.04	0.30
Azospirillum	10^6	2.01±0.06	0.80±0.05	0.02± 0.17	Nil	0.20 ± 0.04	0.006±0.03	0.35

Table 3 Different microbial populations in *Jeevamrita*

Type of microbe	Multiplication factor	Microbial population (cfu/ml) after days of preparation (Mean ± sd)						CD (P = 0.05)
		0	3	6	9	14	20	
Bacteria	10^7	1.80± 0.38	43.00± 7.44	148.1± 15.50	324.20± 28.76	52.60± 7.21	7.20± 1.65	20.04
Fungi	10^7	0.01± 0.01	0.02± 0.01	0.50± 0.21	1.20± 0.33	4.42± 0.85	3.50± 0.21	0.67
Actinomycetes	10^6	2.70± 0.43	8.00± 1.51	3.10± 0.43	3.10± 0.51	0.50± 0.11	0.30± 0.07	1.11
Gram positive bacteria	10^8	0.12± 0.12	0.45± 0.38	0.18± 0.06	1.60± 0.56	0.14± 0.01	1.20± 0.06	0.47
Gram negative bacteria	10^7	0.50± 0.27	3.13± 1.28	11.90±2.57	20.19±5.09	0.13± 5.18	1.00± 0.12	8.09
Pseudomonas	10^7	0.19± 0.25	2.60± 0.89	2.89± 0.44	5.09± 0.40	0.005±0.14	0.30± 0.02	0.70
Rhizobium	10^6	1.66± 0.43	10.80±0.07	12.41±0.09	75.10±0.45	35.0± 0.08	0.71± 0.01	0.50
p- Solubilizing microbes	10^6	1.20± 0.83	3.80± 0.18	3.94± 0.19	5.04± 0.01	1.40±0.001	0.03±0.001	0.65
Azotobacter	10^5	5.00± 0.01	0.10± 0.09	0.30± 0.04	1.12± 0.20	0.30± 0.03	0.10± 0.54	0.43
Azospirillum	10^5	9.00± 0.07	0.50± 0.47	0.60± 1.90	0.01± 4.45	Nil	Nil	3.61

very high (7.66×10^6 cfu/ml at 0 day) which gradually decreased but maintained subsequently up to 20th day (1.54×10^6 cfu/ml). *Azospirillum* population was very low initially (0.01×10^6 cfu/ml at 0 day, but slightly increased on 9th day (0.14×10^6 cfu/ml), negligible at 14th day but surprisingly, increased at 20th day (1.43×10^6 cfu/ml). Results revealed that the microbial consortium changed with fermentation process advances and after a specific period of time it become almost stable for a period of time and then decreased based on nutrient availability in the medium. Kishun *et al.* (2004) have isolated *Pseudomonas fluorescence* from biodynamic liquid pesticides for the management of mango canker disease. Gupta *et al.* (2013) have isolated antagonists from biodynamic liquid pesticides and observed anti fungal property against *Fusarium* sp.

Microbial population dynamics in all bio-enhancers: a comparative analysis

Every bio-enhancer had higher microbial populations. *Panchagavya* recorded highest number of total bacteria (62.5×10^8 cfu/ml) at 18th day as compared to *Jeevamrita* (324.2×10^7 cfu/ml) at 9th day, biodynamic liquid pesticide (22.73×10^8 cfu/ml) at 9th day and *Amritpani* (5.49×10^8 cfu/ml) at 9th day. *Panchagavya* had also higher number of *Pseudomonas* (47×10^6 cfu/ml), *Rhizobium* (2.43×10^6 cfu/ml), *Azotobacter* (0.14×10^6 cfu/ml) and *Azospirillum* (1.03×10^5 cfu/ml) (Figs 1, 2 & 3). Actinomycetes, *Pseudomonas*, p-solubilizing microbes, *Azotobacter* and *Azospirillum* population were highest in biodynamic liquid pesticide (1.65×10^8, 3.28×10^8, 8.50×10^6, 3.75×10^6 and 0.140×10^6 cfu/ml). Similarly, Gram positive bacteria, gram negative bacteria, and *Rhizobium* were highest in *Jeevamrita* (1.6×10^8, 20.19×10^7, 75.1×10^6 cfu/ml). *Amritpani* had higher number of actinomycetes (2.00×10^7 cfu/ml), gram negative bacteria (1.35×10^8 cfu/ml), and p-solubilizing microbes (4.80×10^6 cfu/ml) (Table 1, 2, 3 and 4)

The days required to obtain a beneficial microbial consortium through natural fermentation process (mixed microbes which includes bacteria, fungi and actinomycetes and possibly others) for highest possible beneficial effect (HPBE) is depends on the type of bio-enhancers and the ingredients used for preparation. Generally, 18-20 days

Table 4 Different microbial populations in biodynamic liquid pesticide

Type of microbe	Multiplication factor	Microbial population (cfu/ml) after days of preparation (Mean ± sd)						CD (P = 0.05)
		0	3	6	9	14	20	
Bacteria	10^8	10.50± 2.50	8.40± 1.00	16.13± 1.48	22.73± 6.86	14.52± 5.03	24.30± 3.91	7.86
Fungi	10^6	0.01± 0.01	0.07± 0.04	0.75± 0.10	2.64± 0.88	0.03± 0.01	0.05± 0.01	0.65
Actionomycetes	10^8	1.90± 0.39	0.91± 0.34	0.55± 0.26	1.37± 0.45	1.65± 0.17	0.47± 0.27	0.55
Gram positive bacteria	10^8	2.60± 0.02	2.50± 0.06	0.04± 0.24	0.02± 0.23	0.01± 0.20	0.01± 0.35	0.29
Gram negative bacteria	10^7	1.68± 0.03	4.30± 0.13	5.26± 0.29	6.86± 0.13	5.42± 0.57	4.93± 0.48	0.57
Pseudomonas	10^8	0.30± 0.73	0.59± 0.30	1.25± 0.29	3.28± 1.01	1.10± 0.09	1.50± 0.29	0.76
Rhizobium	10^6	0.159± 0.29	1.10± 0.38	1.92± 0.20	1.94± 0.37	3.65± 0.26	3.09± 0.32	0.57
p-solubilizing microbes	10^6	4.43± 0.01	4.55± 0.01	5.50± 0.06	8.50± 0.01	0.26± 0.001	2.00± 0.15	NS
Azotobacter	10^6	7.66± 0.81	3.75± 0.48	1.36± 0.01	2.00± 0.01	1.80± 0.01	1.54± 0.01	0.75
Azospirillum	10^6	0.01± 0.16	0.01± 1.04	0.10± 0.47	0.14± 0.74	Nil	1.43± 0.45	1.00

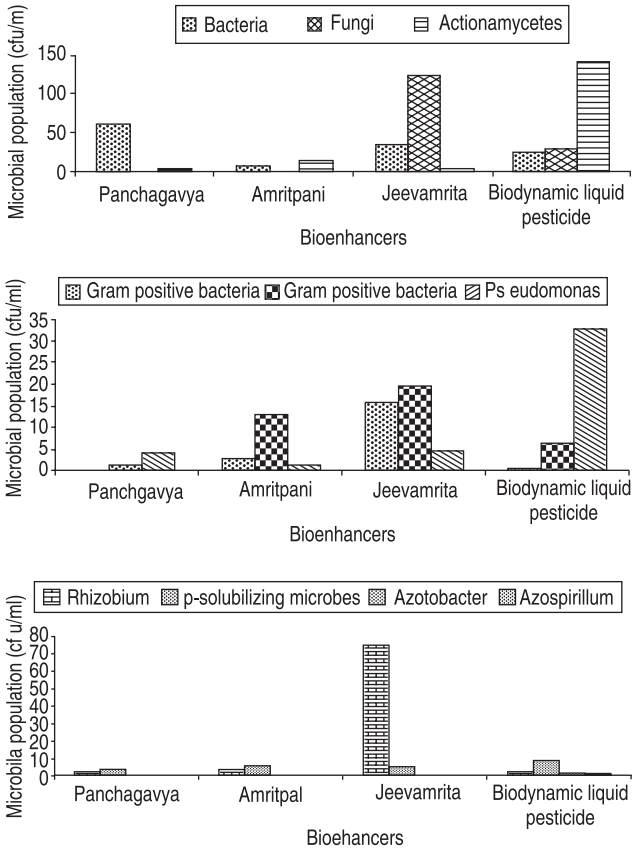

Fig.1. Bacteria ($\times 10^8$), fungi ($\times 10^5$), actinomycetes ($\times 10^6$), gram positive bacteria ($\times 10^7$), gram negative bacteria ($\times 10^7$), *Pseudomonas* ($\times 10^7$), *Rhizobium* ($\times 10^6$), p- solubilizing microbes ($\times 10^6$), *Azotobacter* ($\times 10^6$) and *Azospirillum* ($\times 10^5$) in bio-enhancers.

required for *Panchagavya,* 9-10 days for *Amritpani, Jeevamrita* and *biodynamic liquid pesticide* preparation. Our result showed that the total bacterial populations constantly increased during the fermentation period in all cases and recorded highest (6.25×10^9 cfu/ml in *Panchagavya,* 3.24×10^9 cfu/ml in *Jeevamrita,* 5.49×10^8 cfu/ml in *Amritpani* and 2.27×10^9 cfu/ml in Biodynamic liquid pesticide) at the day when the bio-enhancers suggested to use for crop production (18th day for *Panchagavya* and 9th day for Amritpani, Jeevamrita and biodynamic liquid pesticide). Results also showed that the *Panchagavya* and biodynamic liquid pesticide can be used up to 20th day and 14th day respectively without any quality reductions regarding their microbial populations.

Based on results, it can be concluded that among all bio-enhancers, *Panchagavya* was the best preparation followed by *biodynamic liquid pesticide*, *Jeevamrita and Amritpani.* Results also supported that these bio-enhancers could play a potent source of beneficial microbes which could improve soil fertility, crop productivity and produce quality. Bio-enhancers act as a biofertilizers and bio-pesticides which must be combined with manures so that they can address many challenges of today agriculture and will be helpful to show a way for sustainable production through organic resources.

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