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Physical, microbial and biochemical composition of bio-pesticides based on cow urine and dung with medicinal plants

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Abstract
The use of homemade bio-pesticides in the farming practices is an old age practice which is very much friendly to the environment and can be obtained from nature directly. Bio-pesticides tend to be less toxic, more quickly biodegradable and more targeted to the specific pest. Cow urine and dung has many beneficial properties particularly in the area of agriculture and therapeutics. Cow dung and cow urine enhances the insecticidal activity of panchagavya which can reduce the number of application hazardous chemicals on crops. It has been shown that cow urine extract of certain plants as well as cow urine in combination with certain plant extracts are found to possess marked inhibitory effect on human pathogens as well as plant pathogens because N_2, CP, Urea, Uric acid, hippuric acid, creatinine and mineral contents were significantly higher which work as insecticidal. Presence of total bacterial and total yeast and mould were important factor that determines the quality of bio-pesticides. The cow urine and dung along with medicinal plants extract may be alternative of chemical pesticides.

Keywords: Cow, dung, urine, biochemical parameters, bacteria, fungi, mineral profile

Introduction
Bio-pesticide or Biological Pesticides are pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. It is less toxic and also reduces the pollution problems caused by conventional pesticides. Broadly, it can be categorized into three groups such as (a) Biochemical pesticides, (b) Microbial pesticides and (c) Plant Incorporated Protectants (PIPs) (Kandpal, 2014).

The use of homemade bio-pesticides in the farming practices is an old age practice which is very much friendly to the environment and can be obtained from nature directly. It is almost free of cost and there is no negative impact on human health, soil, animals, plants and environment. Bio-pesticides tend to be less toxic, more quickly biodegradable and more targeted to the specific pest. Now-a-days, it is widely used due to increased environmental awareness and the pollution potential and health hazards from many conventional pesticides, as well as increasing global demand for organically grown food (Islam and Morshed, 2013).

Cow urine has many beneficial properties particularly in the area of agriculture and therapeutics. It has also been observed in scientific research that the urine of Indian cows is highly effective as compared to the urine of other species. Cow urine is a good bio-pesticide and also effective against many diseases including cancer and is a very potent immune enhancer. In *Sushruta Samhita* and *Ashtanga Sangraha*, cow urine has been described as the most effective substance/secretion of animal origin with innumerable therapeutic values (Dhama *et al*., 2005) [7]. Cow urine contains 95% water, 2.5% urea, and 2.5% minerals, salts, hormones and enzymes. It contains iron, calcium, phosphorus, salts, carbonic acid, potash, nitrogen, ammonia, manganese, sulphor, phosphate, potassium, urea, uric acid, amino acids, enzymes, cytokines, lactose etc. Cytokines and amino acids present in urine may play a role in immune-enhancement (Bhadauria, 2002).

Cow urine is one of the components of popular preparations such as Panchagavya, Amrutpani and Jiwamrut used as biofertilizers and for composting farm waste (Dhama *et al*., 2005; Salkinkop *et al*., 2005) [7]. Experts also suggest it tobe used for spraying as an organic fungicide or pesticide. After regular use of cow urine in the crops it is found that soil microorganisms has increased along with the crop production. Cow urine works as plant growth promoter. It is used by the farmers as an effective indigenous method to control crop pests (Banjo *et al*., 2003) and spraying of the cow urine has been recommended to minimize the harmful effects of synthetic pesticides (Chauhan and Singhal, 2006).

For this purpose, stored/fermented stock is preferred over fresh urine because it can cause leaf scorching or burning and plant wilting due to hippuric acid and urea present in cow urine (Peterson *et al*., 2012).

Composition of bio-pesticide formulations

Bio-pesticide formulations of cow urine and dung with different plants extracts

Natrajan (2003) Took cow dung mixed with water – 5 kg, cow urine – 3 litres, Cow milk – 2 litres, Curd – 2 litres, Ghee – 1 kg, Ripe yellow Banana – 12 pieces, Tender Coconut water – 3 litres, Sugarcane juice – 3 liters. Wide mouthed mud pots were used for preparation of Panchakavya. The measured amount of cow dung and ghee were added first into the container and kept for about three days for fermentation. The fourth day the remaining products were added to the container and kept for seven more days. The contents were stirred for 20 minutes each, both in the morning as well as evening to facilitate aerobic microbial activity. After ten days of incubation, different concentrations were prepared and used as foliar spray for plants.

Subedi and Vaidya (2003) [26] prepared aqueous extract by soaking 1 kg leaves each of six plants in 5-20 litres of water, *viz*. Neem, *Acoruscalamus L*., *Ageratum conyzoides L*., *Durantarepens L*., *U. dioica* and *Spilanthesacmella L*. The extract was then mixed with 10-20% of cow urine and compared with neem-based product, Neemazal® at 0.01-0.1%. Maximum pest mortality (>60%) was attributed to neem, *A. calamus* or *U. diocaat* 1 kg/20 L of water with 20% urine.

Gupta (2005) [12] prepared different combinations of neem with cow urine, T1-NLE-1% (5 liter cow urine + 1.250 kg neem leaves extract/ha), T2-NLE-2% (10 liter cow urine + 2.500 kg neem leaves extract/ha), T3-NLE-1% (15 liter cow urine + 3.750 kg neem leaves extract/ha), T4-NKE-1% (5 liter cow urine + 1.250 kg neem kernel extract /ha), T5-NKE-2% (10 liter cow urine + 2.500 kg neem kernel extract /ha), T6-NKE-3% (15 liter cow urine + 3.750 kg neem kernel extract /ha), T7-Nil-1% (5 liter neem oil/ha), T8- Phospamidon 04% (Phosphamidon 85 EC- 240 ml /ha) and T9 – Untreated. Treatments were applied three times at 50, 60 and 70 days after sowing and four times at 50, 60, 70 days and 80 days after sowing.

Cow urine as such and/or after addition of neem leaves is a wonderful biopesticide. Such bio-pesticide is safe to use, do not accumulate in the food chain and as such do not have the harmful effects like chemical pesticides. In 10 liters of cattle urine, about 2 kg of neem leaves were soaked with some other vegetable matter and could be used in proportion of 1:50 for spraying. Cow dung mixed with cow urine formed excellent manure and a natural pesticide. Pest repellent prepared from cow urine and neem leaves exhibited excellent insecticidal, fungicidal and pesticidal properties and also exerted excellent plant growth promotion property (Dhama *et al*., 2005) [7].

According to Boomathi *et al*. (2006) [5] the different Treatments Conc. (%) was prepared based on cow urine and dung with Neem seed kernel extract such as NSKE (5%), Cow urine (5%), Cow dung extract (5%), NSKE + cow urine (5+5%), NSKE + cow dung extract (5+5%), NSKE + cow urine + cow dung extract (5+5+5%) and Cow urine + cow dung extract (5+5%) and observe their efficacy on Helicoverpa armigera and compared with Endosulfan 35 EC and control, the data on the toxic effect of organic sources revealed that the mortality (83.3%) was significantly higher in NSKE 5%+cow urine 5%+cow dung extract 5% at different time intervals in all the instars of H. armigera. However, it was significantly lower than endosulfan (100%) followed by Cow urine + cow dung extract (57.0%).

Azadirachta indica extract (biopesticide) is an effective insecticide against the insect pests (Schmutter, 1985; Baidoo *et al*., 2006) [23, 4]. The *Azadirachta indica*, *Vitexnegundo* and *Partheneumhisterophorus* are the medicinal plants and plays insecticidal role. *Azadirachta indica* and *Partheneumhisterophorus* plant extracts are also efficient against the scarab beetles. *Azadirachta indica* and *Partheneumhisterophorus* plays an important role as insecticides (Phal *et al.*, 2012) [20].

Mohapatra *et al*. (2009) [17] used vitex leaf extract (5%), *Lantana camara* leaf +Tulsi leaf extract (5%), Nerium + Ipomoea leaf extract (5%), Agave leaf flesh extract + kerosene (2.5%), Jatropha leaf extract + cow urine (5%), Adathoda leaf extract + cow dung slurry (2%), ricebran + kerosene, and Brammathandu leaf extract + toddy (3%) to control rice pests. They used vitex leaf extract (5%), lemongrass +tulsi leaf extract (5%), garlic + kerosene + chilli fruit extract (2%), Agave leaf flesh extract (2.5%), Eucalyptus leaf extract (5%), fenugreek + betel vine + onion + butter milk + castor oil (3%), and tobacco leaf extract (5%) against groundnut pests. For pests of pulses they used vitex leaf extract (5%), Datura leaf extract (3%), sweetflag leaf and rhizome extract (2.5%), chilli + garlic + kerosene (2%), Neem oil + Vetiver extract (4%), fenugreek + betel vine + onion + butter milk +castor oil (3%), and Brammathandu leaf extract (5%). Likewise, For vegetable pests they used vitex leaf extract (5%), Agave leaf flesh extract (5%), Jatropha leaf extract (5%), Anna leaf + Aduthinapalai leaf extract (3%), Aloe vera flesh + turmeric + chilli powder (2.5%), Calotropis leaf extract + garlic + onion + chilli powder (3%), and Nerium leaf extract (5%).

Hegde and Nandihalli (2009) [14] used NSKE (5%), GCKE, sweet flag extract (5%), garlic extract (5%), cow dung (10%) and cow urine (10%) and tried individually and in combinations along with endosulfan (0.07%) and untreated control for fruit borers (*H. armigera and E. vittella)* and found the repeated sprays of GCKE and NSKE (5%) alternated with cow urine (10%) were significantly superior in recording least fruit damage (14.50% and 15.80%, respectively) with higher reduction of damage (64.83 and 63.26%, respectively) and were at par with each other and significantly lower than the Endosulfan treated.

Sangeetha and Thevanathan (2010) [22] prepared Seaweed based panchagavya a modified preparation containing the aqueous extract of the algae and Sargassum wightii. The preparation contained cow dung - 5.0 Kg; cow urine - 3.0 L; cow milk - 2.0 L; cow curd - 2.0 L; cow ghee - 1.0 Kg; sugarcane juice - 3.0 L; tender coconut water - 3.0 L; banana - 12 nos; yeast powder -100 g; jaggery - 100 g; water - 2.0 litre. The above composition gives approximately 20.0 L of panchagavya. The extract was allowed to cool, filtered through a layer of muslin cloth and dried in vacuum and the dry residue was used.

Farmers have invented different dilution levels for different crops and application techniques. The dilution levels vary between approximately 2:1 (2 parts urine to 1 part water) to 1:4 (one part urine to 4 parts water). A dilution level of 1:1 (one part of urine to one part of water) was found to be the most common practice among the farmers met by members of Grameen Foundation Community Knowledge Worker network in Uganda to mitigate pests and diseases (FAO, 2012) [8].

Ashwini *et al*. (2014) [3] studied efficiency of biopesticides on *Aedesaegypti* adults. Four different solutions of biopesticide were prepared as follows Solution 1: *Azadirachtaindica*, *Vitexnegundo* and *Partheneumhisterophorus* fresh leaves taken in equal proportion that was 1: 1: 1 ground and mixed in ethanol. Solution 2: *Azadirachtaindica*, *Vitexnegundo* and *Partheneumhisterophorus* fresh leaves were collected, ground in mortal- pestle and mixed in ethanol. All these plant materials were taken in 2:2:1 proportion but dissolved in ethanol and fermented for overnight. Solution 3: *Azadirachtaindica*, *Vitexnegundo* and *Partheneumhisterophorus* fresh leaves were collected and ground in mortal- pestle. After grinding this biopesticide, it dissolved in ethanol with 1: 2: 2 proportions, fermented overnight and filtered solution used for test. Solution 4: *Azadirachtaindica*, *Vitexnegundo* and *Partheneumhisterophorus* fresh leaves were collected, ground and dissolved in ethanol in 2: 1: 2 proportions, fermented overnight, and filtered for the test. These four solutions were diluted in ethanol because the prepared solutions were tested by electrical vaporizer. Each biopesticide solution was taken in an electrical vaporizer and tested for 24 hours. The repellency of four different solutions of *Azadirachtaindica*, *Vitexnegundo* and *Partheneumhisterophorus* on mosquito, *Aedesaegypti* was showed about 55%, 95%, 30% and 20%, respectively.

Physical property of different formulations

Measurement pH of formulation

Revathi *et al. (*2016) [21] was recorded pH of Panchagavya sample by using Digital pH meter and he found 5.10 ± 0.05 in 15th day, 4.43 ± 0.12 in 30th day, 3.97 ± 0.06 in 45th day and 3.73 ± 0.03 in 60th day of fermentation. The pH was found to decline from 15th day to 60th day of fermentation in Panchagavya. Changes of pH value through the maturation process of Sanjeevak are shown. Pandi-Perumal *et al.* (2006) studied the physico chemical properties of Panchakavya and found pH 3.7.

pH values in Sanjeevak decreased steadily after 18 days. However, between day 25 and day 45, there was no significant difference in pH values. This has contributed to the observed decreased of pH values from 8.54 at initiation to 7.1 after 45 days of Sanjeevak anaerobic digestion. These results reflect the fact that the reduction of alkalinity is most likely influenced by VFA, total ammonia (NH_4+ + NH_3) and CO_2 release (Orendo, 2012) [19]. The pH of panchagavya was lowered to 4.52 at 30 days of fermentation and this might be due to *Lactobacillus* bacteria in panchagavya, which produced more organic acids during fermentation (Mathivanan, *et al.*, 2006) [16].

Total bacterial and total yeast and mould count of different formulations

The microbial population changes during aerobic composting, Chang and Hudson (1967) [6] and Golcuke (1954) have described a typical pattern. The fungi and acid producing bacteria appear during the initial mesophilic stage. As the temperature increases above 40 ºC, these are replaced by thermophilic bacteria, actinomycetes and fungi. Any aerobic mesophilic bacteria initially present in the composting material multiply and show increased activity. As the temperature is raised, their numbers decrease due to change in environment. Gaur *et al.*, (1990) [9] when organic materials are broken down in presence of oxygen, the process is called as aerobic decomposition. Under aerobic conditions, living organisms which utilize oxygen, decompose organic matter and assimilate some of the carbon, nitrogen, phosphorus, sulphur and other nutrients for synthesis of their cell protoplasm. Fermentation is one of the oldest and most economical methods of producing, preserving, processing food and improving food safety. In addition to preservation, fermentation can also contribute to the improvement of nutritional value. During fermentation the unique properties of the bacteria and fungi present that increase the levels of proteins, vitamins, essential amino acids and fatty acids in the food. Some microorganisms produce flavouring compounds, complex polysaccharides or organic acids and development of desirable colour (Harlander, 1992) [13].

Ali *et al.*, (2011) [1] Prepared microbial variation in *Sanjibani*, solutions by using raw material which obtained from the two different cow breeds (Native and Jersey), following the same procedure with mixing of cow dung (1 kg), cow urine (1 litre) and water (2 litre) *Sanjeevani* stock solution was prepared. *Panchagavya* was prepared by mixing cow dung (500 gm), cow urine (300 ml), cow milk (200 ml), cow curd (200 ml) and cow ghee (100 ml), both the preparation was kept in a separate plastic container The microbial population in *Sanjibani* prepared from Native cow was comparatively higher than the solution from Jersey cow. Maximum microbial population was recorded on 9th day in both Native (137.33x10^6 ± 13.9) and Jersey (93.33 x10^6 ±4.80). It shows the microbial population of *Sanjibani* attained its higher count on 9-10 days of incubation. After 10th day, the microbial population decline in successive days of decomposition shows, the time of applying *Sanjibani* in the field should be between 9 and 10 days to get better results.

Natarajan (2003) [18]. Panchagavya was prepared by mixing fresh cow dung-5 kg, Cow urine -3 lit, Cow milk-2 lit, Cow curd-2 lit, Cow ghee-1 lit, Sugarcane juice-3 lit, tender coconut water-3 lit, ripped banana-12 Nos and toddy-2 lit. and Revathi *et al. (*2016) [21] evaluate the efficiency of Panchagavya as probiotics by enumerating the total bacterial in nutrient agar using spread plate technique and the number of bacteria was expressed as CFU/ml. The bacterial count was found to be 12.90 ± 1.56 x 10^3 in 15th day, 18.80 ± 0.92 x10^3 in 30th day, 10.70 ± 1.42 x10^3 in 45th day and 7.43 ± 1.69 x 10^3 in 60th day of fermentation of Panchagavya. The bacterial count observed to increase from 15th day till 30th day of fermentation of Panchagavya and the count decreased gradually from 45th day. For the composition of panchagavya, Somasundaram and Singaram (2006) [24] analyzed panchagavya solution and author found total N (302.0 mg/kg), total P (218.0 mg/kg), total K (355.0 mg.kg), total sugars (205.0 µg/ml), glucose (6.00 mg.dl), sodium (96.0 mg/kg), calcium (27.0 mg/kg), total organic carbon (0.80 per cent). IAA (9.05 mg/kg), GA (4.0 mg/kg), phenols (0.756 µg/ml), bacteria (34 X 10^6cfu/ml), fungi (22 X10^4 cfu/ml), actinomycetes (3 X 10^{12} cfu/ml), yeast (35 X 10^4cfu/ml pH(5.62), Zn (0.26 mg/kg), Fe (0.83 mg/kg), Mn (0.23 mg/kg) and Cu (0.2 mg/kg). Kate and Pathe (2009) [15] prepared a different formulations *viz*., *Panchagavya and Amritpani.* The microbiological analysis of the different formulations of organic growth promoters highlighted the presence of Total bacteria, fungi and actinomycetes found that in *Panchagavya* (count/ml) were 1.51 x 10^8, 2.50 x 10^7 and 2.13 x 10^6 (count/ml) respectively while in *Amritpani* (count/ml) 1.03 x 10^6, 1.12 x 10^5 and < 30 colonies were reported respectively. Panchagavya is a fermented liquid of 5 main ingredients *viz*., cow dung, cow urine, cow's milk, ghee and curd (5.70 kg, 3.46 litres, 2.30 litres, 2.30 litres and 1.15

kg, respectively). Further, they reported that the ultimate product had total N (302.00 g/kg), total P (219.00 mg/kg), total K (355.00 mg/kg), total organic carbon (0.80%), bacteria (34 X 10^6cfu/ml), fungi (22 X 10^4cfu/ml), actinomycetes (3 X 10^2cfu/ml), Zn (0.26 mg/kg), Fe (0.83 mg/kg), Mn (0.23 mg/kg), Cu (0.20 mg/kg), pH of 6.02 and electrical conductivity 3.02 ds/m. Swaminathan *et al.* (2007b)

The total microbial population from fresh cow dung, cow urine and different formulations (Treatments) was analyzed by serial dilution technique and plated in suitable media and summarized results in given below table.

Table 1: Total bacterial population from fresh cow dung, cow urine and different formulations

Treatments	Different days interval (population No. x10^8cfu / ml)			
	0 Day	10 Day	20 Day	30 Day
T1	21.67c	30.00c	18.67d	11.67e
T2	17.00d	24.00d	13.00e	8.67f
T3	27.00b	32.00bc	21.33d	15.67d
T4	28.67b	34.33ab	24.67c	18.33e
T5	28.33b	32.00bc	28.67ab	22.33b
T6	29.67b	35.67ab	27.33bc	22.67b
T7	28.67b	32.67bc	29.00ab	24.00b
T8	33.00a	37.00a	31.33a	26.67a
S.Em±	± 0.67	0.84	0.67	0.51
CD@ 5%	1.99	2.52	2.02	1.54

(Ananda, 2011) [2].

Table 2: Total yeast and fungi population of different fermentation of cow products

Treatments	Different days interval (population No. x10^2cfu / ml)			
	0 Day	10 Day	20 Day	30 Day
T1	8.33c	12.67d	10.67d	6.67c
T2	5.33d	9.67e	7.67e	5.33d
T3	9.67c	13.67d	11.33d	8.67b
T4	11.33c	16.33c	14.33bc	9.33b
T5	14.33b	16.00c	13.33c	12.00a
T6	15.67a	18.33ab	15.67b	12.33a
T7	14.33a	17.00bc	15.00b	12.67a
T8	15.67a	19.67a	17.67a	12.33a
S.Em±	0.34	0.40	0.37	0.31
CD@ 5%	0.99	1.23	1.12	0.93

(Ananda, 2011) [2].

Sreenivasa *et al.,* (2009) [25] Study was carried out to enumerating total microorganisms in beejamrutha and found the population (cfu/ml) of total bacteria, fungi, actinomycetes, free living N_2-fixers and phosphate solubilising microorganisms in beejamrutha was 15.4×10^5, 10.5×10^3, 6.8×10^3, 3.1×10^2 and 2.7×10^2 respectively.

Bio-chemical composition of different formulations

Ali *et al.,* (2011) [1] Study was conducted on chemical properties of *Sanjeevani* stock solution which was prepared by mixing of cow dung **(**1 kg), cow urine (1 litre) and water (2 litre) and *Panchagavya* was prepared by mixing cow dung (500 gm), cow urine (300 ml), cow milk (200 ml), cow curd (200 ml) and cow ghee (100 ml) and reported that N, P and pH of *Panchagavya* was 1.4%, 0.8% and 5.6 respectively while N, P and pH of *Sanjeevani* was 1.03%, 0.04% and 7.8 respectively.

Gore and. Sreenivasa (2011) [11] Work was done in liquid organic manures (panchagavya, jeevamrutha and beejamrutha) which were analyzed for the nutrient status of different organic liquid manures; the results are presented in table 3.

Table 3: Nutrient status of different formulations

Parameter	Panchagavya	Beejamruth	Jeevamruth
pH	6.82	8.20	7.07
Total Nitrogen	1000	40.00	770
Total Phosphorus (ppm)	175.40	155.30	166
Total Potassium (ppm)	194.10	252.00	126
Total Zinc (ppm)	1.27	2.96	4.29
Total Copper (ppm)	0.38	0.52	1.58
Total Iron (ppm)	29. 71	15.35	28.2
Total Manganese (ppm)	1.84	3.32	10.7

Orendo, S. R., (2012) [19] Prepared Sanjeevak by using Cattle faeces and urine were mixed with water in the following proportions (1:1:18), with a handful of sugar; then fermented. It kept for a period of ± 45 days after analyzed results found 0.007%, 0.03±0.01ppm, 4.74±0.92 ppm and 0.03 ± 0.01 ppm for P, As, Mn, Zn and Cu respectively using atomic absorption spectroscopy (AAS). However, Sanjeevak content in total nitrogen (TN) (0.11%) was very low.

Conclusion

Biopesticides are an important tool for managing the increasing concern over pesticide residues on food and the always-present problem of pest resistance. Biochemical investigation of cow urine and dung sample and medicinal plant extracts will definitely prove the presence of bioactive compounds like urea, uric acid hippuric acid and creatinine which are the main constituents promoting antimicrobial activity. However cow urine and dung based formulations were promising in the control of pests in fodder crops and it may be substitute of the chemical pesticide.

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