# **Coconut leaf vermiwash: a bio-liquid from coconut leaf vermicompost for improving the crop production capacities of soil**

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**Coconut leaf vermiwash (CLV) was produced from actively vermicomposting coconut leaf litter + cow dung substrate (10 : 1 w/w basis) by** *Eudrilus* **sp. It significantly increased the seedling vigour index of cowpea and paddy at 1 : 10 and 1 : 15 dilutions in laboratory trials. Field trials carried out in red sandy loam soil (Arenic Paleustults) resulted an increase of 36% fresh biomass weight of cowpea with application of CLV at 1 : 10 dilution. In maize, increase in cob yield by 5–10% and in bhendi (okra) 22–33% increase in fruit yield were recorded at 1 : 5 dilutions of CLV. A concomitant increase in populations of general and plant beneficial microorganisms and soil enzyme activities in the rhizosphere of CLV-applied plants were also recorded. Soil organic carbon content increased in the CLV-applied plots in all the crops studied, but the total N, available P and K content in soil varied in different crops. The study indicated that CLV must be used in graded doses. Its application increased the crop production capacities of soil by (i) enhancing the organic carbon contents in the soil and (ii) increasing the populations of the soil microorganisms, particularly plant beneficial ones, and their activities which would have facilitated increased uptake of the nutrients by the plants resulting in higher growth and yield.** 

**Keywords:** Bio-liquid, coconut leaf vermiwash, soil enzymes, soil microflora, soil nutrients, vermicompost.

CONVERSION of agricultural, urban and industrial refuse into vermicompost by employing specific earthworms is quickly becoming a favoured method of recycling wastes in many countries $1,2$ . Wastes from plantation crops like coconut<sup>3</sup>, arecanut and cocoa<sup>4</sup>, coffee<sup>5</sup> and acacia<sup>6</sup>, which contain high percentage of lignin and phenols, have also been successfully converted to vermicompost using different species of epigeic earthworms. Application of vermicompost rejuvenates the depleted soil fertility, enriches the available pool of nutrients, maintains soil quality and conserves more water and biological resour- $\cos^{7,8}$ . The vermicomposting technology can also be utilized for generating a bio-liquid termed as vermin wash or vermiwash<sup>9</sup>. Vermiwash is a liquid leachate collected by allowing excess water to saturate the actively vermicomposting substrate in such a way that the water washes the nutrients from the vermicast excreted by the earthworms feeding on the substrate as well as the earthworm's body surface. This bio-liquid is rich in nutrients and plant growth hormones<sup>9</sup> and its application has been reported to stimulate anthurium<sup>10</sup>, increase soil nutrient status and  $\mu$  is sumatate and and  $\mu$ <sup>11</sup> and biological productivity of mari- $\text{gold}^{12}$ . Similarly, vermicompost leachate has also been reported to be suitable as formulation for liquid fertilizer<sup>13</sup> and other agricultural uses<sup>14</sup>. Zaller<sup>15</sup> had reported that foliar applications of vermicompost leachate improved certain quality parameters of tomatoes besides suppressing *Phytophthora* disease. However, most of the studies mentioned here report the beneficial effects of vermiwash or vermicompost leachate produced from animal manure + earthworms or from the earthworms alone.

 In this article, we present the production of vermiwash from actively composting coconut leaf litter  $+$  cow dung substrate (10 : 1 w/w basis) by *Eudrilus* sp., its chemical, biochemical and microbiological properties, and its potential as a bio-liquid to increase crop growth and yield accompanied with soil, microbial, enzyme and nutrient properties.

# **Materials and methods**

# *Coconut leaf vermiwash production*

Vermiwash production can be done basically by two methods. One method involves soaking  $\text{soil} + \text{cow}$ dung + earthworms substrate in excess water in plastic tub and siphoning the wash periodically from the bottom of the tub<sup>9</sup> whereas the other involves releasing the earthworms in lukewarm water and agitating them gently so as to shock them to secrete higher amount of body fluids and mucus<sup>16</sup>, which actually is the true vermin wash.

 For production of coconut leaf vermiwash (CLV), we developed a batch method that was minor modification of

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the one reported by Ismail<sup>9</sup>. A food grade plastic barrel (200 l) with a tap fitted just above the base of the barrel was used for CLV production. Inside the barrel, smooth pebbles were filled at the bottom up to 10 cm height followed by 10 cm each of clean, coarse gravel and then clean beach sand forming the vermiwash filtration and accumulation compartment of the system. Water was then allowed to flow through these layers and drain without any blockage from the tap. This permitted the filtration layers to settle properly. The tap was then closed. Above the filtration strata, layers of matured coconut leaf vermicompost, partially decomposed coconut leaf  $+$  cow dung substrate  $(10:1 \text{ w/w } \text{ basis})$  and fresh cow dung, totally amounting to 100 kg, were added such that the substrates filled just up to the brim of the barrel. Water was added in quantities enough to keep the substrates at level of 40% moisture content. Then 500 earthworms (*Eudrilus* sp.) having the capacity to degrade the coconut leaves were introduced into the barrel. The unit was allowed to remain as such for 10 days without further addition of water, allowing the earthworms to migrate to centre of the barrel where the partially decomposed feed was available, and start active decomposition of the substrates. After 10 days, a 25 l capacity earthen pot was hung over the barrel. The bottom of the pot had a perforation in which a cotton wick was inserted, permitting water to trickle out in drops. Clean water was filled in the pot and the trickle was regulated in such a way that the pot needed refilling once in two days. After 30 days, the CLV that had collected at the bottom of the barrel was drawn from the tap and stored in 10 l plastic cans for further studies. Subsequent collections were made at weekly intervals for a month. The CLV production can also be carried out in mud pots in similar way.

#### *Analysis of CLV*

Freshly collected CLV was analysed for chemical, biochemical and microbiological properties. The pH of the vermiwash was measured by taking 50 ml of fresh solution in 100 ml beaker and read with a pH meter (Accumet Research AR 25, Fischer Scientific). The major and minor soluble nutrients N, P, K, Ca, Mg, Zn, Mn, Fe and Cu were estimated by standard methods.

 The CLV was also analysed for total sugar by anthrone method $17$  and the resulting dark green colour was read at 630 nm (Shimadzu UV 1601 spectrophotometer), reducing sugars using DNSA (dinitrosalicylic acid) method<sup>18</sup> and the dark red colour produced was read at 510 nm. The total phenol was estimated using Folin–Ciocalteau<sup>19</sup> reagent and the blue colour developed was read at 650 nm. The protein content in the vermiwash was estimated by reduction of phosphomolybdic–phosphotungstic acid components in the Folin–Ciocalteau reagent by the amino acids tyrosine and tryptophan as well as the protein with alkaline cupric tartarate resulting in blue colour development measured at  $660 \text{ nm}^{20}$ , whereas the total free amino acids was estimated using ninhydrin solution and the bluish purple product was measured at  $570 \text{ nm}^2$ .

 The vermiwash was also evaluated for the plant growth hormones like indoleacetic acid (IAA) by modified Salkowski's method<sup>22</sup> and gibberellic acid (GA) was estimated following the method of Holbrook and coworkers<sup>23</sup>. The humic acid content in the vermiwash was estimated by Centre for Applied Research and Development (CARD), Neyveli Lignite Corporation (NLC) by ISO 2000 method described by International Humic Substances Society (IHSS). Briefly, the humic acids in the vermiwash were precipitated with concentrated hydrochloric acid until the pH dropped to 1.5–2.0. The precipitation was allowed to stand overnight for complete settling of the humic acids which is then separated from solution by filtration using ash-free filter paper. The precipitate was further washed three to four times with hydrochloric acid, transferred to crucible and heated at  $815 \pm 10^{\circ}$ C for 1 h. The humic acid content in the vermiwash was determined by:

Humic acid content (%) = 
$$
\frac{100(m_1 - m_2)}{m}
$$

where  $m_1$  is the mass of dry humic acid in grams;  $m_2$  the mass of ash residue of humic acid in grams; *m* the mass of dry solid substances taken for estimation of humic acid in grams.

 The population of general (aerobic heterotrophic bacteria, filamentous actinomycetes and fungi) and function specific (free-living  $N_2$  fixers, phosphate solubilizers and fluorescent pseudomonads) microorganisms were determined in the CLV. The samples were serially diluted in sterile water blanks to produce several dilutions and 1 ml of aliquot was pour plated in different media. Three sets of samples were drawn from each treatment. Five replications for each group of microorganisms were maintained. Total number of culturable aerobic heterotrophic bacteria was counted on nutrient agar<sup>24</sup> after incubating for 48 h at 28°C, filamentous actinomycetes on Ken Knights and Munaier's agar<sup>24</sup> counted after  $5-7$  days of incubation at 28 $\degree$ C, fungi on Martin's rose Bengal agar<sup>25</sup> counted after 4 days of incubation at 28°C. The free-living nitrogen fixing bacteria were counted after 4 days on N-free medium<sup>26</sup>, phosphate solubilizers were enumerated by locating the clear halo formed around the colonies on Pikovskaya agar<sup>27</sup>, for fluorescent pseudomonads producing green or greenish blue fluorescence, King's B agar<sup>28</sup> was used and the colonies were counted under UV light after incubation for 24–48 h at 28°C. The results of the microbial analysis were given as CFU  $ml^{-1}$  of vermiwash. Each CFU value was the average of three sample replicates and five plate replications per sample replicates.

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#### *Laboratory bioassay*

Laboratory bioassay was performed in petri dishes to compare the seedling vigour index of cowpea (test crop for dicotyledonous plants) and paddy (test crop for monocotyledonous plant) at full strength, half strength, 1 : 5, 1 : 10, 1 : 15 and 1 : 20 dilutions of CLV. Bottom plates of petri dish were layered with Whatman no. 1 filter paper and sterilized in an autoclave. Cowpea and paddy seeds (uniform size and weight) were washed once with de-ionized water and then with sterilized water and placed on the filter paper of sterilized dishes. Ten replications per treatment, with 10 seeds in each replication were maintained. Vermiwash (5 ml) was applied twice a day to each plate. Once the seeds germinated and started growing, the lids of the dishes were removed. This was followed by vermiwash application three times a day. After 10 days, the length of the individual seedlings was measured and their fresh and dry weights recorded. The vigour index of the seedlings was calculated by multiplying germination percentage with seedling length $^{29}$ .

#### *Field studies*

Based on the results of the laboratory bioassays, field studies were carried out with  $1:5$ ,  $1:10$  and  $1:20$  dilutions of the CLV to evaluate the growth and yield of some field and horticultural crops. The first study was on cowpea, grown as green-manure crop, conducted during May to July 2004 in  $100 \text{ m}^2$  plot. Forty eight rows of cowpea plants with 12 plants in each row were grown. Four treatments with 12 rows/treatment were taken. The treatments included irrigation with water (control) and vermiwash at 1 : 5, 1 : 10 and 1 : 20 concentrations. After the experiment, fresh plant biomass and the number of nodules in the roots of cowpea in each treatment were recorded. The second study was on maize, conducted from July to September 2004. Four rows of maize plants with 60 plants in each row were grown for experiment. Similar vermiwash treatments and modes of application were adopted. After the experiment, the cob yield per plant, cob fresh weight and fresh biomass weight of plants were recorded. The third study was conducted with bhendi during October to December 2004. Four rows of bhendi plants with 450 plants per row were grown for the experiment. The same treatments were imposed. The bhendi fruit yield was recorded at the end of the experiment. In all the studies, CLV was applied as irrigation once in 10 days and approximately 25–30 l of vermiwash applied to drench the total number of plants in one treatment. No other source of nutrients was applied to any of the crops.

 Pre- and post-treatment rhizosphere soil samples were collected from all the treatments from the three field experiments. They were analysed for populations of heterotrophic bacteria, filamentous actinomycetes, fungi, free-living nitrogen fixers and phosphate solubilizers as mentioned earlier; and the associative nitrogen fixer *Azospirilla* was estimated by serial dilution and most probable number (MPN) method $30$ . Statistical analysis of the microbial populations was carried out through analysis of variance (ANOVA) following Gomez and Gomez $3<sup>1</sup>$ .

 Along with microbial enumeration, dehydrogenase, phosphatase and urease enzyme activities were also estimated in the soils. The activity of dehydrogenase enzyme was estimated spectrophotometrically at 485 nm (Shimadzu UV 1601 spectrophotometer) through the production of triphenyl formazan from triphenyl tetrazolium chloride<sup>32</sup>. Dehydrogenase activity was expressed in terms of milligrams of triphenyl formazon (TPF) produced  $h^{-1}g^{-1}$ of air dried soil, calculated from a standard curve using TPF (Sigma) in methanol. Phosphatase activity was assayed by spectrophotometric estimation of p-nitrophenol (at 400 nm) released by phosphatase activity when soil was incubated with buffered (pH 6.5) sodium p-nitrophenyl phosphate (PNP) solution and toluene at 37°C for 1 h (ref. 33). Phosphatase activity was expressed in terms of μg of p-nitrophenol released  $h^{-1}g^{-1}$  of air dried soil. The urease enzyme activity was measured in 0.5 M tris(hydroxymethyl)aminomethane (THAM) buffer  $(pH 9)^{34}$ .

Pooled topsoil (0–15 cm) samples taken from each treatment of the field studies were air-dried, sieved through 2 mm sieve and stored at room temperature for further analysis. Organic C was quantified according to Walkley–Black procedure<sup>35</sup>, total  $N^{36}$ , available P by the Bray-P 1 extractant<sup>37</sup> was determined by using  $1:10$ (w/v) soi1: solution ratio and an extraction time of 5 min and extracted P was analysed colourimetrically by ascorbic acid technique of Murphy and  $Riley^{38}$ . Available potassium was determined using a flame photometer in the 1 N  $NH<sub>4</sub>OAC<sup>39</sup>$ .

#### **Results and discussion**

#### *CLV production*

About 8–10 l of the vermiwash was collected from the unit during the first draw after 30 days period. The vermiwash was clear, dark brown, odourless and free of any debris. Subsequent collections at weekly intervals yielded 4–5 l of vermiwash and had a progressive decrease in the intensity of brown colour. When the colour of the vermiwash had turned pale brown and also the substrate level had gone down to almost half the height of the barrel, the collection was terminated. The brown colour was due to the phenol content in the vermiwash. About 35–40 l of vermiwash was collected from one complete run of the unit. The number of earthworms had multiplied 3–4 folds with an average weight of 1.0 g per worm during the total run.

#### *Analysis of CLV*

The chemical, biochemical and microbiological properties of freshly collected CLV are given in Table 1. The CLV had an alkaline pH range of 7.6–8.9. This was a substantial shift from the pH of the coconut leaf  $+$ cowdung substrate used, which had a pH range of 4.5– 5.2. Application of vermiwash will be able to produce a positive effect on the availability of plant nutrients because of its ability to neutralize the predominantly acidic soils of Kerala that has a pH ranging from 5.4 to 6.1 depending upon the type of crops being grown and simultaneously enhance the nutrient availability to the plants.

 Weathered coconut leaves contain 8900, 600, 4500 ppm of N, P and K respectively<sup>40</sup>. The CLV produced from the substrate however has only 2.8, 10.28 and 205 ppm of N, P and K. This reduction in the nutrient contents, we presume, was primarily due to the assimilation of the N, P and K by the earthworm biomass whose number increased from three to four folds from the initial 500 added. In addition to loss due to assimilation, the action of nitrification and de-nitrification bacteria present in the substrate and earthworms gut could also be responsible for the transformation of organic N to gaseous N, which is a well-established  $fact<sup>41</sup>$ . Zaller<sup>15</sup> too reported similar reduction in total N content from 640 to 48.06 mg  $l^{-1}$  when aqueous extract was obtained by steeping tap water in vermicompost produced by *Eisenia fetida* from fruit, vegetable and cotton waste using windrow composting. In comparison to P  $(3.08 \pm 0.10 \text{ ppm})$ , K  $(60.49 \pm 1.94 \text{ ppm})$ , Ca  $(58.91 \pm 2.62 \text{ ppm})$  and Mg  $(18.19 \pm 0.58 \text{ ppm})$  in aqueous vermicompost extract produced from fruit and vegetable wastes<sup>15</sup>, we found P, K, Ca and Mg concentrations in CLV to be very high. However, N, P, Ca, Mg and IAA contents in CLV were less than vermiwash produced from pure cowdung, which was reported to contain 61, 18, 179, 198 and 15 ppm of the components respectively<sup>12</sup>. Compared to its pH  $(7.52)$ and K content (55 ppm), CLV had higher pH (7.6 to 8.9) and four times more potash (205 ppm). Thus, it was evident that the chemical compositions of the substrates were responsible for the differences in the nutrient contents of the vermiwash produced.

 The existence of higher content of total sugars, reducing sugars, proteins and free amino acids in the CLV adds to its wholesome nutrient supplying capacity. Inclusion of plant hormones, viz. IAA (0.52–1.15 ppm) and GA (0.23– 1.61 ppm) too, commonly found in vermicomposts $42$ , makes it a good bio-liquid for plant growth promotion, and the occurrence of total phenol (10.2–14.8 ppm) can enhance the resistance capacity of plants to pests and diseases $43$ . Total humic acid content in CLV was in the range of 100–142 ppm. This constituent is reported to be commonly present in vermicomposts and is known for its positive influence on the growth of plants $44$ .

 The microbial content of CLV was low compared to vermicomposts which contain  $10^6 - 10^8$  CFU  $g^{-1}$  of sample45. This may be because of high phenol concentration in the solution which possesses microbicidal attributes. There is a low probability of the vermiwash getting spoiled since the microbial load is low. However, predominance of fluorescent pseudomonads, amounting to 40% of the total microbial load in the vermiwash, is of great importance, as this bacterial community is well known for plant growth promotion properties.

#### *Laboratory bioassay*

The results of laboratory bioassay of CLV on the percentage of seed germination and seedling vigour index of cowpea and paddy seeds are given in Figure 1 *a* and *b* respectively. It was clearly seen that higher concentrations of vermiwash (1 : 0 and 1 : 1 dilutions) were phytotoxic to both cowpea and paddy as it significantly reduced the percentage of germination and seedling vigour index of both the test crops. We observed that, at higher concentrations of vermiwash, the roots had turned completely brown and had a charred appearance. Even the filter paper had turned dark brown in colour. The probable reason was the presence of high concentration of phenols, which was toxic to the tender roots and shoots emerging

**Table 1.** Chemical, biochemical and microbiological properties of coconut leaf vermiwash (CLV)

Chemical constituent (ppm)		N pH	P	K	Ca	Mg	Zn	Mn	Fe	Cu
<b>CLV</b>		$7.6 - 8.9$ 2.8	10.28	205	37.9	6.4	0.07	0.17	<b>Traces</b>	Traces
Biochemical constituents $(\mu g/ml)$	Total sugars	Reducing sugars	Proteins		Total free amino acids	Indole acetic acid		Gibberellic acid	Total phenols	Total humic acid
<b>CLV</b>	$61.6 - 111.2$	$41.9 - 88.4$	615-890		$20.8 - 32.3$	$0.52 - 1.15$		$0.23 - 1.61$	$10.2 - 14.8$	$100 - 142$
Microbial populations (Cfu/ml)	Bacteria	Fungi	Actinomycetes			Phosphate solubilizers		Free living nitrogen fixers		Fluorescent pseudomonads
<b>CLV</b>	$2 \times 10^4$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$			$2 \times 10^2$		15		$8 \times 10^2$

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from the germinated seeds. The diluted vermiwash  $(1:5)$ to 1 : 20) however was able to increase the percentage of seed germination and the seedling vigour index when compared to water. Cowpea showed a maximum vigour index of 11.55 at 1 : 10 dilution, whereas paddy gave 11.9 at 1 : 20 dilution. This indicated that appropriately diluted vermiwash had a positive effect on the percentage of seed germination and growth of the cowpea and paddy seedlings, which was one of the most important findings from this study. In response to vermiwash application, both crop seedlings produced a profuse growth of fine root hairs, which was absent in the seedlings that received plain water. The profuse root hair growth was direct indication of involvement of IAA present in CLV. Our findings corroborate the report of  $Zambare<sup>46</sup>$  who found that application of vermiwash to cowpea plant grown in soil extract agar medium increased the length of the plant.





**Figure 1.** Effect of coconut leaf vermiwash on (*a*) the percentage of seed germination and (*b*) seedling vigour index of cowpea and paddy. The error bars represent standard deviation.

### *Field studies*

For the field experiments, three dilutions of the CLV, viz. 1 : 5, 1 : 10 and 1 : 20 were tried based on the outcome of the laboratory seedling bioassays. The results of the field experiments conducted on cowpea, maize and bhendi are given in Tables 2–4. It was observed that application of CLV enhanced the different parameters recorded in cowpea at 1 : 10 and 1 : 20 dilutions, with the latter dilution giving the highest percentage of increase in fresh biomass weight (36%), higher nodule numbers (30%) and nodule fresh weight (43%) when compared to control and other vermiwash treatments. In maize, the total cob yield increased with increasing dilution of the vermiwash, however the weight of cobs and fresh biomass weight of the plants were recorded highest (64% and 30% above control) at  $1:5$  dilution of the CLV. Similarly, an increase in bhendi yield by 33% over control treatment was also recorded when CLV was applied at 1:5 dilution. The field studies, thus, amply supported the fact that application of CLV increased the biomass and yield of a range of crops. Field experiments with vermiwash and vermicast extract have also been reported to increase the growth and yield of paddy<sup>11</sup>. Few others have reported the ability of vermiwash to induce vegetative growth of anthurium $10$ .

 The population of soil microflora is given in Table 5 and enzyme activities in Table 6. It is observed that at concentrations where there was increase in yield and biomass, concurrent increase in the numbers of beneficial microorganisms particularly nitrogen fixers and phosphate solubilizers was also recorded. The capacity of CLV to promote growth and yield of these three crops could be because of two possible modes of actions: (a) CLV constituted washing of the composting substrates and the earthworm body rich in plant growth promoting nutrient content<sup>47</sup>, which might have acted as a liquid fertilizer getting immediately and quickly absorbed by the plant roots, (b) though the vermiwash by itself had low microbial population, on its application in soil, it was observed to potentiate the soil microbial population, particularly the plant-beneficial ones like the nitrogen fixers and the phosphate solubilizers. It was also observed to enhance the soil dehydrogenase, phosphatase and urease enzyme activities, which are direct indicators of enhanced microbial activity in the root region. It is well documented that higher microbial and enzyme activity is a positive sign of soil fertility and health<sup>48</sup>.

 The soil type in which the field experiments were conducted was red sandy loam (Arenic Paleustults) possessing a pH of 5.3 and EC of  $0.123 \text{ d} \text{sm}^{-1}$ . Application of CLV increased the soil organic carbon content in the soil (Table 7), along with a corresponding increase in the biomass production in the test crops. Increased growth and yield of these crops compared to control plants must have resulted in higher amounts of rhizodeposition,

**Table 2.** Effect of CLV on fresh biomass yield and nodulation of cowpea (fresh biomass values are for total 144 plants per treatment, while nodule number is for plant per average in each treatment)

Treatment	Fresh biomass (kg)	No. of nodules (% change)	Fresh nodule weight in g (% change)
Control (water)	25	20	24
CLV1:5	$23(-8%)$	$18(-10\%)$	$23.4(-2.5\%)$
CLV1:10	$28 (+12\%)$	$21 (+5%)$	$28.6 (+16%)$
CLV1:20	$34 (+36%)$	$26 (+30\%)$	$34.3 (+43%)$

**Table 3.** Effect of coconut leaf vermiwash on cob yield and fresh biomass of maize (results are values of 50 plants per treatment)

Treatment	Cob yield (% change)	Cob weight $(kg)$ (% change)	Fresh biomass (kg) $(% \mathbf{A})$ (% change)
Control	42	3.1	9.07
CLV1:5	$44 (+5%)$	$5.1 (+64%)$	$11.33 (+30\%)$
CLV1:10	$45 (+7%)$	$4.4(+42%)$	$9.87(+9\%)$
CLV1:20	$46 (+10\%)$	$4.8 (+29%)$	$9.24 (+2%)$

**Table 4.** Effect of CLV on fruit yield of bhendi (results are values of 30 plants per treatment)

Treatment	Bhendi yield in kg (% change)	
Control	2.82	
CLV1:5	$3.76 (+33%)$	
CLV1:10	$3.46 (+23%)$	
CLV1:20	$3.43 (+22%)$	

**Table 5.** Effect of CLV on soil microbial population in cowpea, maize and bhendi rhizospheres (results expressed in  $n \times 10^n$  cfu/g dry soil are average of 3 sample replicates each replicated 3 times on petri plate)



Treatment	Dehydrogenase (mg TPF/h/g dry soil)	Phosphatase (µg PNP/g dry soil)	Urease $(\mu g NH_4^{\dagger}/g$ dry soil)
Cowpea			
Pre-treatment	12.6	30.1	2.72
Post-treatment			
Control	13.2	47.4	2.36
CLV1:5	12.6	51.5	2.00
CLV1:10	14.5	54.9	3.17
CLV1:20	15.5	56.3	2.41
Maize			
Pre-treatment	15.4	22.9	3.6
Post-treatment			
Control	22.7	23.4	3.2
CLV1:5	12.5	38.3	4.9
CLV1:10	12.7	31.0	4.5
CLV1:20	11.4	42.6	4.1
Bhendi			
Pre-treatment	13.0	44.7	6.9
Post-treatment			
Control	27.9	37.6	6.6
CLV1:5	33.5	44.9	11.0
CLV1:10	26.6	46.8	11.4
CLV1:20	24.8	45.1	3.9

**Table 6.** Effect of CLV on soil enzyme activities in cowpea, maize and bhendi rhizospheres (results are average value of pooled soil samples of each treatment)

Table 7. Effect of application of CLV at different dilutions on soil nutrient properties in the rhizosphere of cowpea, maize and bhendi (values are average of pooled soil samples from each treatment)

Total $N$ $(\% )$			Available P (ppm)			Available K (ppm)			Organic-C $(%)$			
Treatments	Cowpea	Maize	Bhendi	Cowpea	Maize	Bhendi	Cowpea	Maize	Bhendi	Cowpea	Maize	Bhendi
Pre-treatment	0.056	0.043	0.063	54.5	54	62.1	45.9	41.3	68.6	0.38	0.54	0.68
Post-treatment												
Control	0.066	0.053	0.067	89.8	57.2	70.3	50.5	45.9	78	0.46	0.57	0.68
CLV1:5	0.075	0.074	0.064	47	51.8	71.4	41.3	45.9	91.7	0.71	1.1	1.12
CLV1:10	0.053	0.056	0.082	48.1	45.7	87.2	41.3	45.9	73.4	0.71	0.9	0.78
CLV1:20	0.051	0.062	0.096	52	54.1	78.1	59.6	55	96.3	0.86	0.83	1.01

which is a key source of soil organic  $C^{49}$ . Further, the vermiwash could have changed the soil environment in such a way that the rhizosphere microflora could have synthesized enzymes or metabolites that could alter the integrity of root cells or the permeability of their membrane leading to significant increase in the root exudates<sup>50</sup>. Higher organic C of the soil could be due to increased microbial population or biomass in the CLV-treated plots. Studies in maize by Zandonadi and co-workers<sup>51</sup> have indicated that humic substances isolated from vermicomposts can induce plant growth and productivity by functioning as an environmental source of auxinic activity. They showed that humic acids isolated from different sources improved root growth through a marked proliferation of lateral roots along with a differential activation not only of the plasmalemma but also of vacuolar  $H^+$ -ATPases and  $H^+$ -pyrophosphatase. Such an effect could also have been the reason for increased crop growth and yield, in our studies, with CLV which contained humic acid to the tune of 100–142 μg/ml.

 CLV-treated plots had a differential effect on the different plant–soil system. It could be noted from Table 7 that the total N%, available P and K values decreased in rhizosphere soil of cowpea and maize in those CLV treatments which resulted in production of higher biomass or yield compared to control and other treatments. In contrast, in rhizosphere soil of bhendi an increase in content of the N–P–K in response to application of the CLV was noticed. We attribute these changes possibly due to the (i) differences in nutrient needs and varied nutrient uptake capacities of the plants studied coupled with (ii) different concentrations and quality of rhizodeposition from these plants. For getting an accurate explanation, elaborate radio-labelled studies are needed which can give information on how the nutrients are distributed in the soil–plant system.

#### **Conclusion**

Application of CLV was observed to boost the biomass yield and nodule numbers of cowpea grown as greenmanure, cob and fruit yields of maize and bhendi respectively, when cultivated in red sandy loam soils (Arenic Paleustults) having a pH of 5.3. The mode of actions was primarily due to improvement of the crop production capacities of the soil through (i) enhanced organic C content of the soils (ii) increase in the microbial numbers, particularly the beneficial ones, as well as key soil enzyme activities in the root region of the crops and (iii) direct effect of plant growth-promoting substances and nutrients present in CLV. Proper dilution of CLV was, however, important for getting desirable results. For vegetative growth, CLV at 1 : 10 dilution and for economic yield output like fruits and cobs, 1 : 5 dilution was observed to be appropriate in these experiments.

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