

Vermicompost and vermiwash add beneficial micro flora that enhance soil quality and sustain crop growth

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ABSTRACT

Recycling agricultural and horticultural crop residues to vermicompost and vermiwash provide a good option for small and marginal farmers to produce solid and liquid organic manure locally for use in their farms. Vermicompost and vermiwash are well known to improve soil health and fertility as they add major and micro nutrients, organic matter, and plant growth promoting substances besides improving the soil structure. However, not much known but equally critical is the ability of vermicompost and vermiwash to add substantial load of plant-beneficial microbial communities to the soil that play an important role in augmenting the soil microbial diversity, nutrient mineralization, pathogen/nematode suppression and organic matter degradation resulting in better soil resistance and resilience. More importantly, addition of the vermicompost and vermiwash provides much needed food for the microorganisms in soil thereby stimulating their activities that is necessary for a soil to be fit for supporting good crop growth. In this article, we highlight the evidence gathered through conventional and molecular protocols on the important perspective of coconut leaf vermicompost and vermiwash being the foci of dissemination of plant-beneficial microorganisms and their ability to sustain the crop production capacities of the soils.

Keywords: coconut, vermicompost, vermiwash, microflora, recycling wastes

INTRODUCTION

It has been reported that the wastes generated annually in India from field and horticultural crop residues, agro-processing residues, animal/poultry excreta and livestock/fisheries wastes amount to about 1566 million tonnes. Producing quality manure through composting and vermicomposting technologies with stringent standards as well as generating bio-energy through anaerobic microbial fermentation and pyrolysis has been suggested for effective utilization of these voluminous agro-wastes (NAAS, 2010).

Recycling wastes to vermicompost and vermiwash

Vermiculture biotechnology for producing vermicompost using earthworms from the agro-wastes is a simple and useful technology and has been proposed as one of the front runner technologies for realizing the second green revolution and sustainable agriculture (Chaudhary *et al.*, 2004; Sinha *et al.*, 2010). Vermicomposting is the bio-oxidation and stabilization of organic matter involving the joint action of earthworms and microorganisms, thereby turning wastes into a valuable soil amendment called vermicompost (Dominguez *et al.*, 2010). The earthworm

species (or composting worms) most often used are Tiger Worms or Red worms (*Eisenia foetida*) or Red Wigglers (*Lumbricus rubellus*) or African night crawlers (*Eudrilus eugeniae*) and Indian blue worm (*Perionyx excavatus*). These species are rarely found in soil and are adapted to the special conditions in rotting vegetation and compost and manure piles (Kale, 1998). A wide range of agri-horti wastes have been used as substrates for production of vermicompost using one or the other above mentioned earthworms (Edwards, 1998; Jeyabal & Kuppaswamy, 2001; Garg *et al.*, 2006; Barik *et al.*, 2010).

Vermiwash is a liquid organic fertilizer generated as a spin off technology from vermicompost production (Ismail, 1997). It can be done basically by two methods. One involves soaking soil + cow dung + earthworms substrate in excess water in plastic tub and siphoning the wash periodically from the bottom of the tub (Ismail, 1997) while another one 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 (Kale, 1998).

Plant-beneficial microorganisms in vermicompost and vermivash: less researched area

It is well known and widely reported that earthworm casts or vermicompost possess high contents of nitrogen (Bansal and Kapoor, 2000), phosphorus (Parmanik *et al.*, 2010), plant growth hormones such as auxins, cytokinins and vitamin B12 (Tomati *et al.*, 1983) and humic acids (Atiyeh *et al.*, 2002; Arancon *et al.*, 2006). Similarly, vermivash too has been reported to be rich in nutrients and plant growth hormones (Ismail, 1997, Suthar, 2010; Gopal *et al.*, 2010). Their application in improving the yield of horticultural (Gajalakshmi and Abbasi, 2002; Gutiérrez-Miceli *et al.*, 2007; Singh *et al.*, 2008, Gopal *et al.*, 2010, Chatterjee *et al.*, 2012) and agronomic crops (Rajkhowa *et al.*, 2000; Jeyabal and Kuppaswamy, 2001; Tejada and Gonzalez, 2009; Roy *et al.*, 2010, Hatti *et al.*, 2012) are well documented.

However, what is not well studied and remains largely unrecognized is the fact that the vermicompost and vermivash act as foci for dissemination of microorganisms, particularly the plant-beneficial communities. Few articles report upon the microbial diversity in the finished compost and vermicompost (Fracchia *et al.*, 2006) or the microbial community size, structure and function (Vivas *et al.*, 2009) or isolation of plant-beneficial microbes from compost and vermicompost (Hameeda *et al.*, 2006) to be used as bioinoculant, but none of them characterize the vermicomposts exhaustively for the plant-beneficial microbial community profile.

Plant-beneficial microbes in coconut leaf vermicompost and vermivash

Coconut (*Cocos nucifera* L.) is an important plantation crop grown in an area of 1.8 million ha in India. The leaf biomass residue produced by this palm is to the tune of 6-8 tonnes annually from one hectare area. High lignin content in the coconut leaves and petioles makes it one of the most recalcitrant organic matters resisting natural decomposition. However, using a local strain of earthworm, *Eudrilus* sp., degradation of the coconut leaves could be hastened to 3 months time and recycled to valuable vermicompost (Prabhu *et al.*, 1998).

An elaborate study was conducted to understand the dynamics of general and plant-beneficial microbial communities during the conversion of coconut leaves mixed with cow-dung to vermicompost using the indigenous earthworm, *Eudrilus* sp. This was compared with the vermicompost produced from cow-dung alone by the same earthworm. Fifteen microbial communities were analyzed in the two sets of substrates (coconut leaf + cow-dung in 10:1 w/w ratio and cow-dung alone), in the gut contents of

the *Eudrilus* sp. feeding on the substrates and in the vermicasts excreted by the earthworm. Similarly the vermivash produced from coconut leaf vermicompost was analyzed for the microbial parameters.

The results of the studies showed that during the vermicomposting process 9 out of 15 microbial communities, particularly the plant beneficial ones were enriched in the vermicompost produced from coconut leaves + cow dung mixture compared to that of 5 communities in vermicompost produced from cow-dung alone. The coconut leaf vermicompost contained significantly high populations of fungi, free-living nitrogen fixers, phosphate solubilizers, fluorescent pseudomonads and silicate solubilizers whereas the cow-dung vermicompost was preponderant with aerobic heterotrophic bacteria; actinomycetes and *Trichoderma* spp. Spore forming and plant growth promoting *Bacillus* spp. were present in similar numbers in both the vermicomposts (Fig. 1). The thickness of the bars in Fig. 1., represents log colony forming units g⁻¹ dry weight samples analyzed; more the thickness, more the population of that particular microbial community. On the other hand, the bacterial content of coconut leaf vermivash was low compared to vermicomposts which had 10⁶⁻⁸ cfu g⁻¹ of sample (Gopal *et al.*, 2009). This may be because of high phenol concentration in the vermivash, which possesses microbicidal attributes. There is a low probability of the vermivash getting spoiled since the microbial load is very low. However, within the low counts of plant-beneficial microorganisms in coconut leaf vermivash, a high population of fluorescent pseudomonads was observed contributing nearly 50% of the plant-beneficial microbial communities consisting N-fixers, P-solubilizers and *Bacillus* spp. (Fig. 2).

Metagenomic analysis of coconut leaf vermicomposting process using pyrosequencing technology, a next-generation sequencing platform, also revealed the bacterial diversity at higher resolution. Genomic DNA was extracted using Power Soil DNA kit (MoBio, Carlsbad, USA) from the coconut leaf + cow dung substrate at different stages of decomposition and the final mature vermicompost. The bacterial 16S rRNA gene in the genomic DNA was amplified using barcoded primers for pyrosequencing (454 Gs-FLX). The output files from the platform were analyzed using QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso *et al.*, 2010) and MOTHUR (Schloss *et al.*, 2009), two well documented open-source, platform-independent bioinformatics programmes used for assessing the bacterial diversity in any given area, community or ecosystem. The computational analysis generated 68,438 unique sequences and 18,637 OTUs (Operational Taxonomic Units). The 16S rRNA amplicons were

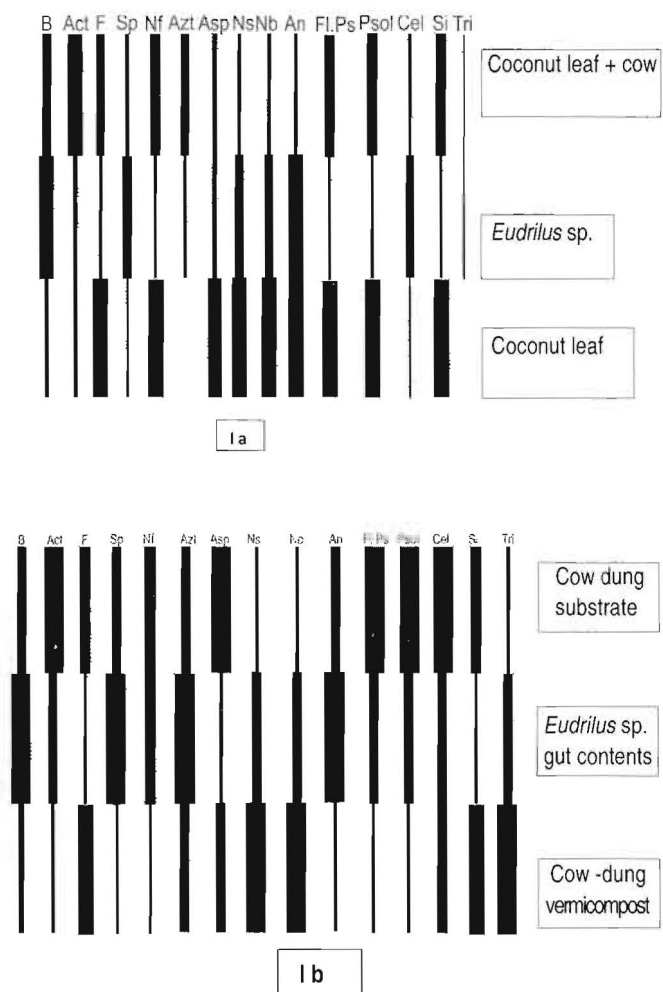


Fig. 1. Qualitative bar coding microbial dynamics during the production of Coconut Leaf Vermicompost (Fig. 1a) and Cow dung Vermicompost (Fig. 1b) from substrate to earthworm gut contents and in vermicompost . (The acronym details- B-bacteria, Act-actinomycetes, F-fungi, Sp-spore formers, Nf-free living N-fixers, Azi-Azotobacter, Asp-Azospirillum, Ns-Nitrosomonas, Nb-Nitrobacter, An-Ammonifiers, Fl Ps-fluorescent pseudomonads, Cel-celulose degraders, Si-silicate solubilizers, Tri-Trichoderma)

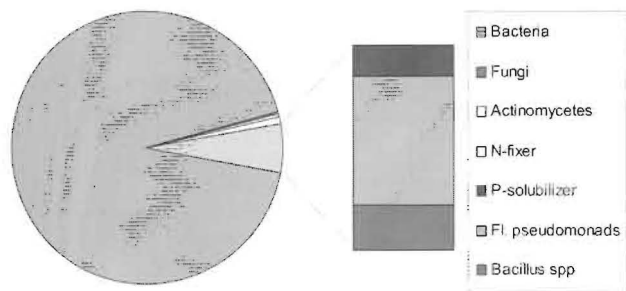


Fig. 2. Pie-chart depicting the microbial composition of coconut leaf vermivash showing preponderance of plant growth promoting Fluorescent pseudomonads within the plant-beneficial microbial communities

phylogenetically assigned according to their best matches to sequences in SILVA database (Pruesse *et al.*, 2007). The results indicated that the diversity of the bacteria increased as the substrates were converted to vermicompost by the earthworms and then decreased in the finished product to stabilize on par with the initial stage. The α -proteobacteria and Bacteroidetes were the dominant phyla present in the samples followed by γ -proteobacteria and Actinobacteria. Unlike Verrucomicrobia, a significant increase in the Firmicutes numbers was recorded from the first to fourth stage of the vermicompost production (Gopal *et al.*, 2011). These results indicated that the diversity of the bacteria increased as the substrates were converted to vermicompost by the earthworms and then decreased in the finished product to stabilize on par with the initial stage. It has been reported that vermicomposting modifies the original microbial community structure of the substrates in different way and vermicomposting process produces greater bacterial diversity, greater bacterial numbers and greater functional diversity (Vivas *et al.*, 2009).

These microbial communities increase the fitness of the soils for sustainable production through (i) effective nutrient mineralization, (ii) inducing systemic and acquired resistance in plants towards pest and diseases, (iii) enhancing the cation exchange capacity of soil and (iv) improving water holding capacity of soil. The results clearly indicated that important groups of microorganisms beneficial for soil health, fertility and plant growth were present abundantly in the coconut leaf vermicompost. Schmidt *et al.* (1997) reported when food was tested as carrier and inoculum source for *Pseudomonas corrugata*, a biocontrol agent, high numbers (10^5 - 10^7 cfu g^{-1} fresh weight) of the bacteria were recovered from fresh casts of three earthworm species, and similar results were obtained in our earlier work when we inoculated *Metarhizium anisopliae* along with coconut leaves for the control of *Oryctes rhinoceros*, which multiplied in coconut leaf substrate during vermicompost production (Gopal *et al.*, 2006). Both these reports support our observation of increased densities of fluorescent pseudomonads and other beneficial microorganisms during the production of vermicompost from the coconut leaf substrate. Evidences are now coming in confirming our findings that the plant-beneficial microbial communities present in the vermicompost play a key role in imparting systemic resistance to plants against insect pests and diseases (Gopalakrishnan *et al.*, 2011).

Our studies thus highlights the fact that vermicompost not only adds nutrients to the soil, but also large and diverse pool of plant-beneficial microorganisms.

Effect of coconut leaf vermicompost/vermiwash on soil microflora

A soil-incubation study was conducted to evaluate the effect of addition of coconut leaf vermicompost on the microflora of sandy loam soil taken from coconut garden. The following treatments were set up in triplicates (i) soil alone, (ii) coconut leaf vermicompost alone and (iii) soil + coconut leaf vermicompost at recommended dosage of 20 kg/palm. The duration of the study was for 60 days. Sub sampling was done on 0, 15, 30, 45 and 60th day and the observations recorded were:

- Enumeration of general and plant-beneficial microbial communities in different treatments
- Estimation of moisture content of the samples and evaluate its effect on the microbial populations

Decimal dilutions of three sample aliquots were inoculated into King's B medium for fluorescent pseudomonads enumeration, Rose Bengal for fungus enumeration, Nutrient agar media for bacteria and bacillus enumeration, Ken Knight and Munaier's medium for actinomycetes enumeration, Pikovskaya's media for phosphate solubilising bacteria enumeration and Jenson's media for nitrogen fixing bacteria enumeration. The inoculated plates were incubated at 36 °C for 48 hours for bacillus, fluorescent pseudomonads and bacteria, two days for fungi, three days for phosphate solubilising bacteria and six days for actinomycetes and nitrogen fixing bacteria. After incubation, the colonies were counted.

The results of the analysis are given in **Tables 1 & 2**. It is clearly seen that among the three treatments, the coconut leaf vermicompost had the highest microbial load as well as moisture content. Mixing the vermicompost into sandy loam soils significantly increased the population of the microorganisms, particularly, the plant-beneficial ones compared to the soil alone. Moreover, it was seen that, the moisture content of the soil was also maintained at higher level when the vermicompost was mixed into it. The enhanced moisture content helped the microorganisms to mine its nutrient requirement from the substrate and multiply vigorously.

Table 2. Percentage moisture content of samples

Treatments	% Moisture content of samples at different incubation period				
	0 day	15 day	30 day	45 day	60 day
Soil	9	8.3	8.3	8.3	8.0
VC*	57.6	57.6	56.3	56.0	54.6
Soil + VC*	9	9	9	9	8.6

It is clear from the above results that addition of the coconut leaf vermicompost rich in plant beneficial microorganisms increases the same in the soils too. However, to know if really vermicompost added its native plant-beneficial microbial load to that of the soil or if the soil's native plant-beneficial microbial load increased because of addition of vermicompost would require a detailed experiment.

But a molecular analysis with PCR based T-RFLP studies, done usually to understand microbial community structure, conducted in vermicomposts produced from different palm wastes and upon their incubation for 100 days period mixed with sandy loam soil showed that there was a significantly different microbial community structures in the soil and vermicomposts (**Fig. 3**) (Gopal, 2008). The incubated samples showed that microbial community structure moved closer to that of the vermicompost upon mixing with the soil indicating that the addition of vermicompost altered the microbial structure of the soil and pulled it closer to its own structure. However, by the 100th day of the incubation, it was observed that the microbial community structure in soil + coconut leaf vermicompost mixture slowly returned to that present in soil alone (**Fig. 3**). What emerged from this molecular study was that vermicompost addition significantly altered the microbial community structure of the soil in the initial period. It points to the fact that there was addition of the microbial communities from the vermicompost to soil which precisely proved that vermicomposts are foci for disseminating microorganisms, more so of the plant-beneficial nature that leads to spurt of activities in soil which could improve the mineralization and nutrient availability to the plants, prevent build up of soil pest/pathogens, allow stronger plant establishments besides several other functions that improve the soil fitness for sustainable crop productivity.

Table 1. Effect of addition of coconut leaf vermicompost on the micro flora of sandy loam soil

Treatment	Bacteria ($n \times 10^6$)	Fungi ($n \times 10^6$)	Actino- mycetes ($n \times 10^4$)	Phosphate solubilizer ($n \times 10^4$)	Nitrogen fixers ($n \times 10$)	Fluorescent pseudomonades ($n \times 10$)	Bacillus spp. ($n \times 10^4$)
Soil	6.34 ^c	3.98 ^c	6.06 ^c	3.48 ^c	3.20 ^c	0.54 ^c	6.17 ^b
VC	7.73 ^a	4.67 ^a	7.10 ^a	4.48 ^a	3.66 ^a	1.44 ^a	6.66 ^a
Soil + VC	7.13 ^b	4.25 ^b	6.33 ^b	4.22 ^b	3.33 ^b	0.93 ^b	6.59 ^a

Means followed by the same letter in a column, do not differ significantly according to Duncan's multiple range test at P=0.05. (log cfu g⁻¹ dry sample)

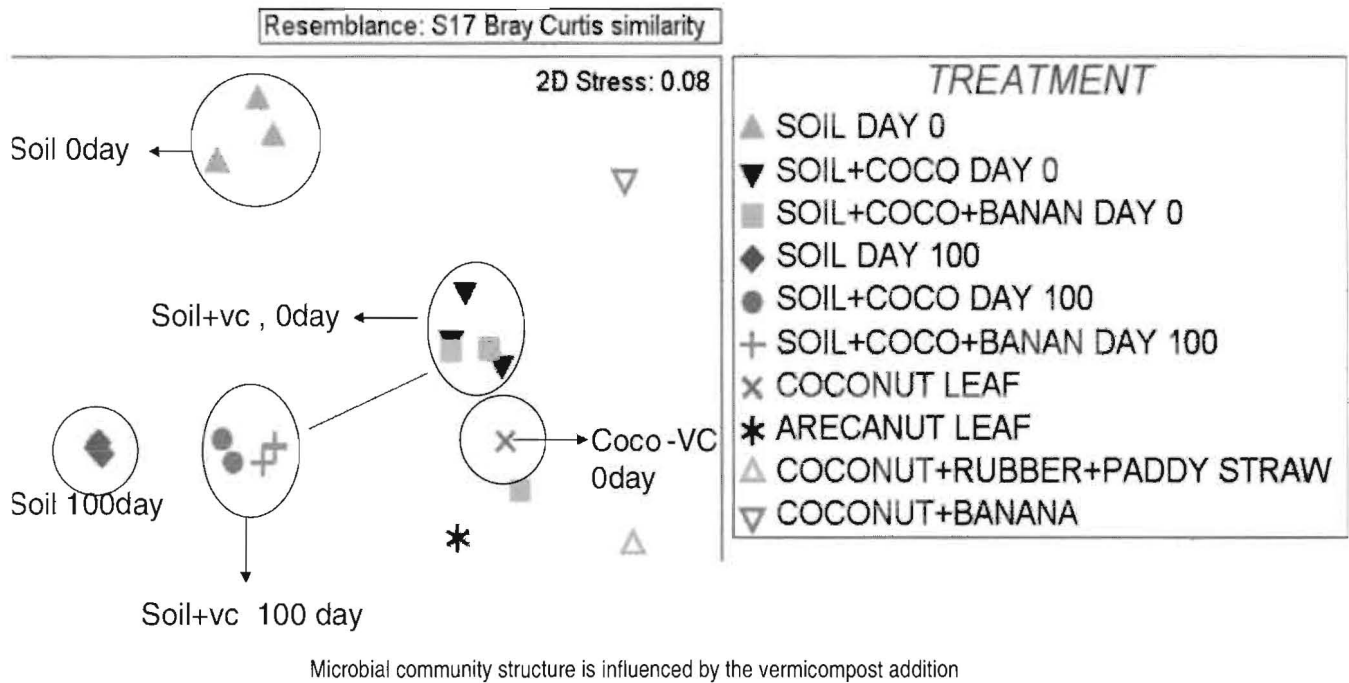


Fig. 3. Changes in the microbial community structure of soil on addition of coconut leaf vermicompost revealed by T-RFLP studies. The encircled symbols show the interaction effect of addition of coconut leaf vermicompost to sandy loam soils in the incubation studies.

Coconut leaf vermiwash acts in multitude ways to improve soil health and fertility. An important one among them is that it adds the vital fluorescent pseudomonad community to soil besides priming them and other microbial groups to increase in population that carry out critical activities in soil. A field study with application of coconut leaf vermiwash at 1:5, 1:10 and 1:20 dilution in water applied to root region of okra resulted in enhanced microbial populations in the all the treatments compared to the control plot. (Table 3). Several modes of action could be attributed to the enhanced microbial populations such as (a) vermiwash is wash of the composting substrates and of earthworm body rich in the plant growth promoting nutrient content which must be acting as a liquid fertilizer getting

immediately and quickly absorbed by the plant roots (b) though the vermiwash by itself was having very low microbial population, on its application in soil, it was observed to potentiate the soil microbial population, particularly the beneficial ones like the nitrogen fixers and the phosphate solubilizers and (c) increase in organic C contents of soil after vermiwash application could boost the rhizodeposition of C from the plants that add to the rhizosphere effect (Gopal *et al.*, 2010).

Coconut leaf vermiwash application also had significant impact on the suppression of the nematode *Meloidogyne incognita* infestation in okra. In the above mentioned field studies it was observed that vermiwash application significantly reduced the gall index, egg mass g⁻¹ of root,

Table 3. Effect of coconut leaf vermiwash (CLV) at different dilutions on the rhizosphere microflora (cfu/g dry soil) of okra (*Abelmoschus esculentus*)

Okra						
Treatment	Bacteria (n x 10 ⁴)	Fungi (n x 10 ³)	Actino. (n x 10 ²)	Free N-fixers (n x 10 ²)	Azospirilla (n x 10 ⁴)	Phosphate solubili- zers (n x 10 ³)
Pre-treatment	30	14	21	14	5.2	03
Post- treatment						
Control	54	17	30	18	13	02
CLV 1:5	50	29	41	36	160	14
CLV 1:10	91	22	20	23	54	09
CLV 1:20	44	30	18	13	35	03
CD (p=0.05)	15.4	7.7	8.8	6.3	—	4.1

and nematode population g^{-1} of root and per 250 cc soil (**Table 4**). Upon uprooting the okra for dry weight analysis, the absence or reduction in the number of root galls was clearly seen in the vermiwash applied plants compared to the large number of galls in the control plants (**Fig. 4**). The coconut leaf vermiwash contains very high counts of fluorescent pseudomonads known for plant growth promotion activities. Though not proved directly, it is possible that these microorganisms present in the vermiwash could have played an important role in the suppression of the nematode population and activities in the rhizosphere of okra. There are several reports that support our presumption that plant growth promoting bacteria, particularly the pseudomonad community, is involved in the suppression of pathogens, pests and nematodes in field conditions (Ramamoorthy *et al.*, 2001; Dong and Zhang, 2006, Kumar *et al.*, 2009). It was also reported that vermiwash contained thiocarbamic acid which was known to possess pesticidal properties (Parmanik,

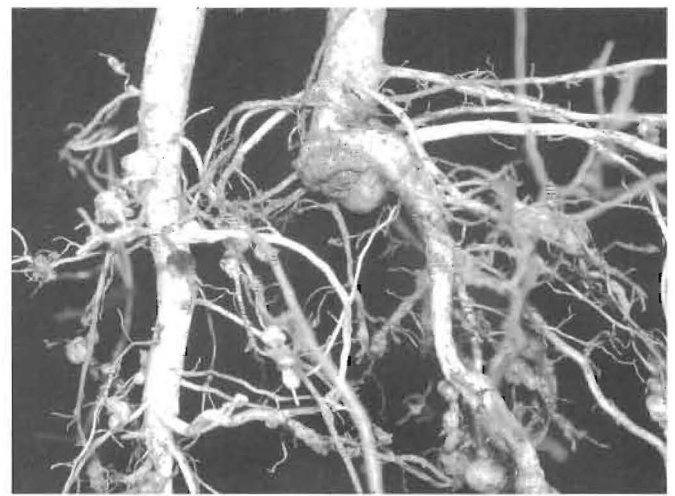
2010). Possibly, the coconut leaf vermiwash may also contain this pesticide component enabling it to have pest suppression property along with the action of the plant-beneficial communities.

Conclusion

Our studies with coconut leaf vermicompost and vermiwash on their microbiological contents and profiles give ample and convincing proof that these solid and liquid organic fertilizers carry significantly high load of plant-beneficial microorganisms and act as focal point for their dissemination into the soils. Their addition is able to alter the microbial community structure of the soils, increase the microbial populations in the soil, specifically those belonging to plant-beneficial communities, and suppress nematode populace and infestation. The overall effects of their addition result in improving the fitness of the soils and make it sustainable for crop growth.

Table 4. Effect of application of coconut leaf vermiwash on nematode infestation in okra

Treatments	Gall index	Eggs mass /g root	Nematode population /g root	Nematode population /250 cc soil
Control	3.0	23	72	230
CLV 1:5	1.0	2	12	120
CLV 1:10	1.0	11	37	148
CLV 1:20	2.0	17	55	157



4 a. Absence of any nematode caused root galls in vermiwash applied okra plants. **4 b.** Profuse root galls caused by the nematode infestation in control treatment.

Fig. 4. Vermiwash application reduces nematode infestation in okra indicated by absence of root galls (4a) compared to control (4b).

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