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MICROBIAL EFFECTIVENESS THROUGH VERMICOMPOSTING TECHNIQUE FOR THE BIOLOGICAL STABILIZATION OF SOLID WASTE

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ABSRACT

Bacterial and fungal isolates were isolated and their effectiveness in composting on different substrates with the aid of earthworms *Eisenia foetida* (Red Wigglers) was performed at Nepal Academy of Science and Technology (NAST). Different parameters such as isolation of microorganisms, earthworm multiplication, composting effectiveness, analysis of micro-flora, their antimicrobial activity and nutrient content were performed. Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Azotobacter* sp., *Beijerinckia* sp. were isolated, where as the fungal isolates include *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp., *Geotrichum* sp. etc and the isolated actinomycetes were *Streptomyce*s sp., *Micromonospora* sp etc. The treatment prepared by using the saw dust as a substrate and inoculation with *Rhizobium* sp. showed better for the earthworm multiplication, followed by biogas slurry with *Rhizobium*, *Lantana camara* with both *Trichoderma* and *Rhizobium, Ageratina adenophora* with both *Trichoderma* and *Rhizobium* and finally control. The treatment prepared from biogas slurry with inoculum *Trichoderma* + *Rhizobium* showed the fast composting and decomposition than other combinations. The harvested vermicomposts were higher in microbial diversity, nutrient content and showed effective antimicrobial activity towards different plant and human pathogens. Among treatments, the treatment prepared from *Ageratina* inoculated with the combination of *Trichoderma* and *Rhizobium* showed the higher increment of Potassium (K) content in comparison to the control. Similarly, Phosphorous (P) increment was found to be higher in the treatment with *Lantana* inoculated with both *Trichoderma* and *Rhizobium* sp. Likewise, Nitrogen (N) increment was found to be higher in *Lantana* inoculated with *Rhizobium* sp. In comparison to the control, organic matter was found to be in higher increment on *Ageratina* inoculated with both *Trichoderma* and *Rhizobium* sp. Composts formed from all these combination were found to be basic in nature.

Key-words: antimicrobial properties, earthworms, microcosm, microorganisms, vermicompost

INTRODUCTION

Available organic wastes from different sources in real sense are not the waste but wealth. With the aid of macro and micro organisms, it can be converted into rich vermicompost and compost which is highly valuable natural resource for the plant nutrition. The technique of using specific kinds of earthworms and microorganisms to convert the biodegradable solid organic waste into black earthy-smelling nutrient enriched humus (Cook 2000) is vermicomposting and it stabilizes the variety of wastes. It is an efficient technique of recycling the animal waste, crops residues and agro-industrial wastes using low energy (Talashilkar *et al.* 1999). Simply, it is a biooxidation and stabilization of organic material involving the joint action of earthworms and microorganisms. The earthworms provide ideal temperature, pH and oxygen concentration

for the speedy growth of useful bacteria and actinomycetes and thus have a higher microbial density of about 100 times greater than in surrounding soil (Subler 1998, Yami *et al.* 2003). It is very useful in the management of the organic wastes of the industrial areas that have been deteriorating our environment through the noxious smell. This methodology of biosolid waste management has shifted from conventional disposal strategies to conversion into value added products (Liang *et al.* 2003) forming different types of useful byproducts.

The vermicomposting process includes two different phases regarding the activity of earthworms: (i) an active phase during which earthworms process the organic waste, thereby modifying its physical state and microbial composition (Lores *et al.* 2006), and (ii) a maturation- like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the waste processed by the earthworms (Aira *et al.* 2007). The efficiency towards vermicomposting depends upon the age, species of earthworms and also the foods that are placed during the feeding. *Eisenia foetida* is the best converter of bio wastes into vermicompost. During vermicomposting, the earthworms maintain aerobic conditions in the organic wastes through proper mixing. Moreover, the biochemical process is enhanced by microbial decomposition of the substrate in the intestines. The earthworms convert a portion of the organic present in the wastes into worm biomass and excrete undigested/partially-digested matter as worm cast, which are rich in nutrient source (Benitez *et al.* 1999). The earthworms also enhance the biological activity in the soil by improving the environment suitable for microbes (Mulongoy *et al.* 1989).

Nepal is potential for composting and vermicomposting (Pradhan and Tamrakar 1999). Large quantities of organic wastes are produced from different sectors viz., agriculture and horticulture, agro-industries and as municipal waste. Vermicomposting technology is a great potential on the solid waste management which is environmental friendly and productive by reducing biodegradable solid waste, while in another hand it will benefit the marginal farmers in rural and suburban areas by producing organic manure and is likely to substitute the chemical fertilizer's demand. Biocomposting of solid waste brings about stabilization of the organic matter and effectively reduces pathogen concentrations in sludges to very low levels (Burge *et al.* 1987). However, absolute removal of pathogens becomes difficult to achieve and many survives the composting process (Sidhu *et al.* 2001). This paper basically aims to study the effectiveness of different substrate degradation enhancing fungi, *Trichoderma* and *Rhizobium* at the time of vermicomposting with the aid of earthworms, *Eisenia foetida.*

MATERIALS AND METHODS

Assemblage of Materials: The required materials for bedding were assembled from several places adjoining to Satdobato, Khumaltar, Lalitpur, Nepal. Different plant materials such as *Lantana camara* L. and *Ageratina adenophora* (Spreng.) King and H. Rob. L. were collected from Chobhar, Lalitpur, Nepal. Bio-gas slurry (75% moisture content) and rice straw (dried) were collected from Chapagaun, Lalitpur, Nepal.

Structuring of Beds: Buckets were uniformly pored at the bottom and on the sides for good aeration. 100 gm of dried chopped rice straw (2 inches long) was placed in each bucket. *Lantana* and *Ageratina* powders, biogas slurry and saw dust each of 100 gm were placed

in different buckets. A control set of experiment was run in parallel for every treatment. The buckets were kept moist and were placed in the laboratory at 24 ± 2 °C for a period of 120 d away from the direct sunlight. There were four replicate containers for each treatment, giving a total of 64 dishes.

Treatment Combinations: 4 treatments inoculums x 4 substrates x 4 replications = 64 combinations

Earthworm and Microorganism Inoculation: Twenty five numbers of morphologically similar earthworms were added for the initiation of the experiment in every bucket except the control set. Similarly, 5 ml of microorganism's suspension (10^7 cfu/ml bacteria and 1×10^6 – 5×10^6 spores/ml fungi) were added in each bucket. Biodegradable solid wastages especially the fruits, except the limes were continuously feed at every fifteen days till the last of the experimentation. The addition of the kitchen and fruit wastes were done and regularly stirred for the uniform degradation of the material. Care was taken regularly to ensure that the buckets are not devoid of food to feed for the developing earthworms.

Earthworm's Number: Counting of the earthworms was done at each month interval starting from the first month of feeding till the last. Four months later, after the whole wastages (feeding) turned blackish, the harvesting of the vermicompost was done.

Microfloral Diversity: Microbial diversity of the vermicompost and compost were done by serial dilution method followed by agar plating method in Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Starch Casein Agar (SCA) for bacteria, fungi and actinomycetes respectively. Bacteria and actinomycetes were identified according to latest edition of Bergey's Manual of Determinative Bacteriology (Holt *et al.*1994). Actinomycetes were identified by their delayed appearance of dry, firm and tenacious colonies (Cross 1976). Morphologically, they were identified by cover slip culture method (Kawato and Shinobu 1959). Further identification was done by biochemical tests (Collins *et al*. 1989). Fungal colonies were identified on the basis of colony morphology, hyphae, asexual spores and sexual spores by cellophane tape method and cotton blue staining method (Aneja 1993, Alexopolus and Mims 1962).

The antimicrobial and antifungal properties of the obtained vermicompost solution and isolated microorganisms were performed against different human and plant pathogenic bacteria and fungi.

RESULTS AND DISCUSSION

Vermicompost samples were blackish moist and highly porous with the smell of earth. Different range of microbial diversity (bacteria, fungi and actinomycetes) was found to be present within small microcosm (vermicompost). The dominant microbial organisms were bacterial strains followed by fungi and actinomycetes which are similar to the work of Yami *et al.* (2003). Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Azotobacter* sp., *Beijerinckia* sp. were isolated, where as the fungal isolates include *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Fusarium* sp., *Geotrichum* sp. etc. The isolated actinomycetes were *Streptomyce*s sp., *Micromonospora* sp. The presence of *Fusarium* sp. is found to be an active organism in the organic matter decomposition in vermicompost (Mori *et al.* 2000). They also

produce growth promoting substances called as 'gibberellins' that enhance other beneficial microorganisms in soil (ISSAC 1999). *Curvularia lunata* and *Geotrichum candidum* have the ability to metabolize organic compounds (Daigel *et al.*1999)

The beneficial microorganisms found in vermicompost made the compost nutrients rich which is valuable for better plant growth. *Aspergillus* sp. are capable of degrading an enormous variety of substance to use them as food because of the large number of enzymes they produce (Alexopolus and Mims 1962). *Mucor* and *Rhizopus* produced different kinds of acids such as citric acid, succinic acid, oxalic acid and other chemicals that help in the degradation of the wastes. Cellulolytic fungi such as *Alternaria hemicola, Fusarium* sp., *Aspergillus niger* and cellulolytic bacteria such as *Bacillus circulans*, *Cellulomonas uda* and *Micrococcus roseus* present in vermicompost were found to decompose the organic substances by secreting the different types of secondary metabolites.

Table 1. Multiplication of earthworms at different months with different substrates and treatments.

Different treatments	**Month**	**Substrates ***			
		Lantana	***Ageratina***	**Biogas slurry**	**Sawdust**
Control	1 , 2 , 3 and 4	0	0	0	0
Trichoderma sp.	1	17.00±6.21	10.50±7.00	26.50±4.50	19.75±3.59
	2	20.50±4.02	21.50±5.25	31.50±4.50	40.50±3.87
	3	26.75±3.30	28.25±6.70	36.75±4.34	42.50±2.88
	4	29.25±3.86	29.50±6.80	39.25±4.19	43.50±2.64
Rhizobium sp.	1	19.00±6.21	10.00±5.47	22.25±3.304	20.00±2.16
	2	23.75±5.12	15.50±2.08	24.75±3.50	44.50±2.88
	3	26.75±2.87	20.75±2.36	37.5±4.434	45.75±2.75
	4	29.00±2.94	24.25±2.62	41.5±4.20	47.50±3.10
Trichoderma sp.+ *Rhizobium* sp.	1	19.00±5.59	12.25±6.02	22.25±4.57	17.00±4.96
	2	24.25±10.27	18.00±4.96	20.75±4.27	19.50±4.65
	3	29.50±8.22	25.75±4.99	33.75±6.50	23.75±4.57
	4	32.50±7.937	29.50±4.20	40.00±6.16	30.25±5.37

** x ± SD (n = 4)*

The earthworm numbers were found to be significantly increasing from the initial of the experiment till the last. Several red cocoons were present in the vermicompost. Earthworm numbers were significantly high in the treatment using saw dust provided with *Rhizobium* (*x*= 47.5) which was followed by biogas slurry with *Rhizobium* (*x* = 41.5), *Lantana* with both *Trichoderma* and *Rhizobium* (*x*= 32.5)*, Ageratina* with *Trichoderma* + *Rhizobium* (*x* = 29.5) and control (Table 1). This work is dissimilar to the work of Pant and Yami (2008); they found saw dust to be worst for the earthworm's multiplication. For rapid earthworm multiplication, saw dust did not play vital role, other factors such as good aeration, addition of other wastes made the saw dust to be favorable for the better reproducing environment for the earthworms. Using of substrate such as *Lantana* and *Ageratina* were found to have the least number of earthworms. The extracts *Lantana* and *Ageratina* showed the high antagonistic properties against bacterial and fungal isolates (Shrestha *et al.* 2009, Bhattarai and Shrestha 2009) which is found to be toxic for the earthworm multiplication. So it may be highly toxic for the earthworm multiplication.

Table 2. Change in the weight of buckets with substrates at different months.

Different treatments	**Months**	**Substrates***			
		Lantana	***Ageratina***	**Biogas slurry**	**Sawdust**
Control	1	0.855±0.196	1.093±0.130	0.945±0.017	0.784±0.075
	2	0.830±0.082	0.785±0.102	0.769±0.071	0.963±0.161
Trichoderma sp.	1	0.961±0.034	0.995±0.058	0.981±0.096	1.045±0.085
	2	0.851±0.113	0.762±0.107	0.830±0.141	1.129±0.083
Rhizobium sp.	1	0.964±0.046	0.990±0.133	1.018±0.092	1.094±0.060
	2	0.755±0.141	0.751±0.141	0.810±0.102	1.071±0.195
Trichoderma sp.+ *Rhizobium* sp.	1	1.025±0.170	0.926±0.068	1.163±0.484	1.121±0.152
	2	0.810±0.077	0.861±0.036	0.787±0.190	1.076±0.113

** x ± SD (n = 4)*

Weight of the substrates and wastes were found to get reduced except on control at the end of the experiment (40 d). This might be due to the proper utilization of various nutrients by the earthworms and decomposition of wastes by cellulolytic fungi and bacteria. The treatment prepared from biogas slurry inoculating with both *Trichoderma* + *Rhizobium* sp. showed higher reduced weight (0.376 kg) being the better composting, followed by *Ageratina* inoculated with *Trichoderma, Lantana* inoculated with *Rhizobium* + *Trichoderma*, saw dust inoculated with *Trichoderma* and control. That indicates the presence of cellulolytic microorganisms in

the slurry and spores of *Trichoderma* contributing effectively for the fast decomposition of the vermicompost (Table 2).

Isolated organisms from vermicompost and their solution showed the antagonistic properties towards the bacterial and fungal isolates. Among the tested 14 bacterial isolates *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, S*almonella typhi, Shigella* sp. and *Shigella dysenteriae* F44DH were susceptible towards the vermicompost solution. Similarly, among four fungal isolates *Fusarium moniliforme* was also susceptible for this solution which is similar to the work of Manandhar and Yami (2008). *Pseudomonas* sp. produce different kinds of hydrolytic enzymes that was responsible for the decomposition of organic compounds. Except degradation, *Pseudomonas* sp. showed antibiotic properties that plays a defensive role for the bio-controlling mechanisms of diseases.

Table 3. Nutrient analysis of the vermicompost.

Different treatments	**Parameters**	**Green manures used as substrates***			
		Lantana	***Ageratina***	**Biogas slurry**	**Sawdust**
Control	OG	4.06	3.96	4.14	3.61
	pH	9.1	7.3	9.0	7.8
	N	0.16	0.18	0.14	0.16
	P (kg/hectare)	554.12	1042.12	1002.12	636.41
	K(kg/hectare)	38756.23	1083.86	17732.68	10284.20
Trichoderma sp.	OG	6.25	4.07	4.59	4.29
	pH	8.5	8.9	8.3	8.3
	N	0.31	0.20	0.23	0.21
	P (kg/hectare)	663.53	1359.84	1114.08	1196.00
	K(kg/hectare)	52426.00	32427.84	23705.28	22890.24
Rhizobium sp.	OG	5.95	4.29	4.56	5.10
	pH	9.6	8.0	9.1	8.0
	N	0.30	0.21	0.23	0.26
	P (kg/hectare)	1032.17	1359.84	1359.84	745.45
	K(kg/hectare)	50549.28	14306.40	19075.20	16213.92

Trichoderma sp. +*Rhizobium* sp.	OG	4.44	5.19	4.86	4.59
	pH	8.5	8.4	7.9	8.0
	N	0.22	0.26	0.24	0.23
	P (kg/hectare)	1196	1196.00	1359.84	950.25
	K(kg/hectare)	41965.44	32427.84	55318.08	15260.16

**OG: Organic matter, pH: Negative logarithm of Hydrogen ion concentration, N: Nitrogen, P: Phosphorous, K: Potassium*

**Values represent the mean of four replications of each*

The harvested vermicompost were higher in nutrients content. In comparison to the control, the treatment prepared from biogas slurry inoculated with both *Trichoderma* + *Rhizobium* showed better increment (55318.08), followed by *Ageratina* inoculated with both *Trichoderma* + *Rhizobium, Lantana* camara with *Trichoderma* and finally saw dust with *Trichoderma* (Table 3). The hydrolysis of cellulose by cellulolytic microorganisms occurs mainly through the concentrated action of several hydrolytic enzymes (Coughlan 1985). Cellulose enzyme complex decomposes cellulose into disaccharides, cellobiase to glucose that can be easily be assimilated as a carbon source. Fungi such as *Aspergillus* sp. *Fusarium* sp. and *Geotrichum* sp. and bacteria such as *Micrococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. are phosphate solubilizing microorganisms. They play a key role in the conversion of insoluble phosphate to the soluble form which is highly indispensible for the plant growth.

Similarly, P increment was found to be higher (1196.00) in that treatment which was prepared from *Lantana* inoculated with both *Trichoderma* + *Rhizobium*, followed by saw dust with *Trichoderma*, biogas slurry inoculated with both *Trichoderma* + *Rhizobium* and *Ageratina* inoculated with both *Trichoderma* + *Rhizobium* (Table 3). *Lantana* with *Rhizobium*, biogas slurry treated with both *Trichoderma* + *Rhizobium*, saw dust with *Rhizobium* and *Ageratina* with *Trichoderma* +*Rhizobium*. This finding was similar to the work of the Yami *et al.* (2003), Pant and Yami (2008), Subler *et al.* (1998).

Table 4. Assay of vermicompost against different pathogenic bacterial strains.

SN	Organisms	Zone of Inhibition (ZOI, mm)
1.	*Escherichia coli*	35
2.	*Staphylococcus aureus*	30
3.	*Klebsiella pneumoniae*	25
4.	*Salmonella typhi*	13
5.	*Shigella* sp.	20
6.	*Shigella dysentriae* F44D1	18
7.	*Bacillus subtilis*	--
8.	*Proteus mirabilis*	--

9	*Salmonella paratyphi*	--
10	*Acinetobacter* sp.	--
11	*Enterococcus faecalis*	--
12	*Citrobacter freundi*	--
13	*Pseudomonas aeruginosa*	--
14	*Shigella dysentriae*1002	--

Likewise, Nitrogen (N) content was found to be in higher increment in the inoculum of *Trichoderma* sp. with *Lantana* as substrate (0.31%), followed by slurry inoculated with both *Trichoderma* + *Rhizobium*, saw dust with *Rhizobium* and *Ageratina* inoculated with both *Trichoderma* + *Rhizobium*. Except control, all vermicomposts harvested from different treatments showed the higher NPK contents which was similar to the work of the Yami *et al.* (2003), Pant and Yami (2008), Subler *et al.* (1998). It means bacteria and fungi played pivotal role for the decomposition of organic wastes and conversion in valuable nutrients. The formation of N_2 fixing bacteria such as *Azotobacter*, *Beijerinckia* and *Derxia* produced slime, which helps for the aggregation of the soil. Inoculation of *Azotobacter* sp. in the vermicompost causes decrease in C: N ratio (Sing *et al.* 2000) and increase in N_2 content significantly (Kumar and Singh 2001).

As far as the nutrient compositions are concerned, the pH of the every set of buckets was found to be very alkaline (>7.0). The highest value for pH was recorded on the inoculums provided with *Rhizobium* sp. alone with *Lantana* followed by biogas slurry using as substrate. The minimum value was recorded for control treatment with *Ageratina* substrate followed by sawdust on the same.

The organic matter was found to be highest (6.25) in the treatment of *Trichoderma* sp. alone with *Lantana* used as substrate followed by the inoculums of the *Rhizobium* sp. (5.95) on the same substrate. The least value for organic matter (4.07) was recorded for the inoculums of *Trichoderma* with *Ageratina* substrate followed by the control treatment (4.13). In a sum, using of the different inoculums do harbor good nutrient contents then the control treatments.

Vermicomposting could be widely used for the stabilizing and recycling of agricultural wastes and domestic wastes reducing the bulk and level of pollution at the generation site itself. It can be used as biocontrol agent and best organic fertilizer for producing organic vegetables, organic fruits and ornamental plants. Since vermicompost produces high population of beneficial microflora its application to cultivated land will also increase their population increasing soil fertility.

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