Journal of Crop and Weed 8(1): 47-51 (2012)

Effect of cellulolytic bio-inoculants and their co-inoculation with earthworm on the conversion of plant biomass

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Received: 30.09.2011, Revised: 24.05.2012, Accepted : 25.06.2012

ABSTRACT

Efficiency of cellulose decomposing microorganisms and earthworms either alone or in mixture was evaluated for decomposition of plant biomass viz., paddy *straw, Eichhornia crassipes (water hyacinth) and Ipomoea carnea (Kolmou). The kinetics of decomposition of different plant biomass measured in terms of changes in C:N ratio, per cent dry matter loss and time required for composting revealed that decomposition was affected by type, quality, nutrient content in the plant biomass as well as the decomposing agents. Dual inoculation of earthworms and cellulose decomposing microorganisms (CDMs) resulted in maximum reduction in composting period irrespective of plant biomass used. The compost prepared with dual inoculation of CDMs and earthworms recorded the lowest C:N ratios (9.8 – 16.2) and had maximum N, P, K content and microbial population followed by the compost prepared by using earthworms alone. Among the different plant biomass evaluated, compost produced from I. carnea was superior in terms of nutrient content followed by E. crassipes.*

Key words: Cellulose decomposing microorganisms, earthworms, rice straw, weed biomass

In recent times, the need for application of organic manures is increasingly felt in view of rapid degradation of soil health. As such, the demand for quality organic manures is progressively increasing. Owing to acute shortage of conventional sources of organic manures, conversion of different plant biomass into compost of high quality over a short time is essential. Huge quantity of plant biomass such as crop residue, weed biomass and other bio waste generated in and around farm land every year are not yet fully exploited for their manurial potential and their faulty disposal often become a source of environmental pollution. Rice straw is the most important crop residue in India with annual production reaching more than 105 t. Similarly, biomass of weeds such as *Eichhornia crassipes* and *Ipomoea carnea* are abundantly available in and around farm land which offer unique potentiality for use as a source of organic matter and plant nutrients. Aruna *et al.*, (1996) reported that recycling of organic sources for supplementing chemical fertilizer is a necessary practice in maintaining soil fertility and sustaining crop productivity. Traditional methods of composting generally take more time (6-8 months) and the resultant manure is also poor in nutrient status. Therefore development of technology for faster decomposition of plant biomass is very much important. The role of earthworms in decomposition of plant biomass is well recognized. Activity of earthworms stimulates rate of organic matter decomposition by increasing the surface area and aeration (Edwards, 1983). Vermicomposting, as one of the fastest and effective ways of recycling organic matter, has been reported by many workers (Rajkhowa *et al.*, 2005). Several workers have also reported enhanced decomposition of biowastes with the inoculation of Cellulose Decomposing Microorganisms (Gaur and Mathur, 1990). Gaur (1992) reported that the use of efficient cellulolytic microbes not only helped in preparing compost within 8-10 weeks but also reduced the bulkiness of the compost by 5-10 per cent. As such, combine use of earthworms and cellulolytic microorganisms may hasten the decomposition process due to their cumulative action. Moreover, the efficiency of decomposition may also vary with the nature of plant biomass. In cognizance of the above, the present study was, therefore, undertaken to find out the efficacy of earthworms and cellulose decomposing microorganisms either alone or in combination on decomposition of plant biomass such as rice straw, *E. crassipes* and *I. carnea* biomass.

MATERIALS AND METHODS

The plant biomasses *viz.*, paddy straw, *Eichhornia crassipes* and *Ipomoea carnea were selected* based on their availability and abundance in and around farm land. The respective biomass was collected in sufficient quantity and heaped under Sun for partial wilting and was chopped into 2-4cm sizes manually. The biochemical properties of the selected plant biomass are given in table-1.

Table 1: Biochemical composition plant biomass used

Particulars	*I. carnea*	*E. crassipes*	Rice straw
Nitrogen (%)	1.90	1.50	0.65
Phosphorus (%)	0.75	0.72	0.12
Potassium (%)	2.50	2.20	0.96
Organic carbon (%)	56.40	48.20	40.40
C:N	29.6:1	32.1:1	60.2:1

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Four sets of experiments were conducted with different plant biomasses viz., paddy straw, *Eichhornia*, *Ipomoea* and their mixer (paddy straw + *Eichhornia* + *Ipomoea*) in the ratio of 2:1:1 to evaluate the effect of CDMs and earthworm on decomposition. Treatments consisted of biomass inoculated with earthworms, earthworms + CDM and CDM alone. Each set was arranged in CRD design with four replications. For this study five kg capacity earthen pot with bottom hole was used. The pots were filled with five hundred gram of respective plant biomass along with two hundred gram of cowdung in alternate layers on dry weight basis. In order to minimize error, equal amount of the bedding material was also kept in all the pots. Ten adult earthworms of the species *Eudrillus eugineae* were added to the respective pots as per the treatment. Consortium of CDMs (*Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., *Penicillum* sp. and *Trichoderma harzaianum*) @ 2 ml of cell suspension containing $10^8 - 10^9$ cfu ml^{-1} for every kg of plant biomass was applied as per treatment. The pots were then covered with Hessian cloth. Sprinkling of water was done as and when necessary to keep the substrate moist.

Organic carbon was estimated following the procedure described by Waggoner (1972) and the total N, P and K content was determined by the procedures described by Jackson (1973). The fungal and bacterial population was determined as per the procedure given by Johnson and Curl (1972) and Waksman's (1922) respectively.

The per cent loss in dry matter was calculated by the following equation.

$$\text{Dry matter loss (\%)} = \frac{\text{(Initial Dry Matter)} - \text{(Final Dry Matter)}}{\text{Initial Dry matter}} \times 100$$

Data obtained from the experiments were analyzed through Completely Randomized Design using standard statistical procedure (Gomez and Gomez, 1981).

RESULTS AND DISCUSSION

Kinetics of decomposition

The kinetics of decomposition of different plant biomass measured in terms of changes in C:N ratio, per cent dry matter loss and time required for composting have revealed that decomposition was affected by type, quality, nutrient content in the plant biomass as well as the decomposing agents. The age of the plant, its lignin content and the degree of disintegration of the substrate presented to microflora also govern the role of decomposition. In general, organic matter with high C:N ratio decompose more slowly than those with a low C:N ratio (Parr and Papendick, 1978). Irrespective of plant biomass used, the maximum decomposition was recorded in pots that received dual inoculation of earthworms and cellulose decomposing microorganisms. This may be due to the role of earthworms in hastening the rate of decomposition of organic wastes in comparison to decomposition in conventional manner as they feed on the decomposing plant biomass there by increased the surface area available for colonization by CDMs (Edwards, 1983). Presence of earthworms in the composting system accelerates the activity of cellulose decomposing microorganisms (Atiyeh *et al.*, 2011). The intestinal mucus which consists of easily metbolizable compounds was considered to result in a priming effect of earthworms to microbial decomposition (Vinceslas – Akpa and Loquat, 1997). Decomposition of plant biomass resulted in substantial reduction in C:N ratio of the decomposed plant biomass from their respective original values. The C:N ratio in the decomposed material of rice straw, *Eichhornia*, *Ipomoea* and their mixer varied respectively from 16.2 -19.5, 10.6-12.8, 9.6-10.2 and 9.8-12.2 (Table 2). The compost prepared due to the activity of dual inoculation of CDMs and earthworms recorded the lowest C:N ratio followed by the treatment receiving earthworms alone, irrespective of the type of plant biomass used. The highest value of C:N ratio was recorded when compost was prepared by using CDMs only. Variation in C: N ratio was also recoded with the type of plant biomass used. Gaur and Sadasivam (1993) reported that the C:N ratio narrowed down as nitrogen remained in the system and some of the carbon was released as CO_2.

The time required for composting varied with the biomass used. The plant biomass irrespective of their type, required least time for composting when they were inoculated with earthworms and CDMs together (Table 2.). Maximum time for composting was taken when CDMs were used alone followed by the treatment receiving only earthworms. The loss in dry weight of different plant biomass revealed (Table 2) that irrespective of plant biomass used; the maximum dry weight loss was recorded in the treatment that received dual inoculation of earthworms and CDMs. The minimum dry weight loss however, was obtained in case of pots treated with CDMs only, which might be due to sole inoculation. Variations in loss in dry weight due to different treatments were statistically significant.

Table 2: Composting period, C:N ratio and dry weight loss (%) as influenced by different treatments

Treatment	Composting period (days)	C:N ratio	Loss of dry weight (%)
Rice straw			
Earthworm	54	18.1	53.8
Earthworm + CDM	48	16.2	54.3
CDM	78	19.5	42.5
LSD (0.05)	**3.2**	**0.7**	**0.8**
Eichhornia biomass			
Earthworm	54	11.2	54.0
Earthworm + CDM	45	10.6	57.8
CDM	68	12.8	46.9
LSD (0.05)	**5.5**	**0.2**	**0.5**
Ipomoea biomass			
Earthworm	60	9.8	47.2
Earthworm + CDM	49	9.6	49.4
CDM	70	10.2	37.9
LSD (0.05)	**3.4**	**0.3**	**1.4**
Mixed (Rice straw + *Eichhornia* + *Ipomoea*) biomass			
Earthworm	58	10.5	50.5
Earthworm + CDM	50	9.8	52.4
CDM	70	12.2	40.5
LSD (0.05)	**6.5**	**0.3**	**0.9**

Table 3: Influence of earthworm and cellulose decomposing microorganisms (CDMs) on nutrient content in the compost from different plant biomass

Treatment	Nutrient content (%)		
	N	P	K
Rice straw			
Earthworm	1.32	1.04	1.20
Earthworm + CDM	1.38	1.05	1.20
CDM	1.14	0.98	1.04
LSD (0.05)	**0.07**	**0.02**	**0.02**
Eichhornia biomass			
Earthworm	2.02	1.30	2.18
Earthworm + CDM	2.04	1.34	2.19
CDM	1.98	1.20	1.98
LSD (0.05)	**0.02**	**0.03**	**0.03**
Ipomoea biomass			
Earthworm	2.46	1.81	2.60
Earthworm + CDM	2.45	1.82	2.62
CDM	2.36	1.75	2.46
LSD (0.05)	**0.02**	**0.03**	**0.02**
Mixed (Rice straw + *Eichhornia* + *Ipomoea*) biomass			
Earthworm	1.96	1.34	2.08
Earthworm + CDM	2.01	1.36	2.10
CDM	1.82	1.25	1.90
LSD (0.05)	**0.08**	**0.02**	**0.09**

Nutrient content in the compost

Decomposition of plant biomass resulted in substantial increase in nutrient content over their original levels. The nutrient content in compost varied significantly with the type of plant biomass and decomposing agents used. The inoculation of earthworms either alone or with CDMs resulted in significantly higher level of nitrogen, phosphorus and potash content in the compost over sole inoculation of CDMs. Faster decomposition and rapid mineralization of nutrients in the compost due to the synergistic activity of earthworms and CDMs might have resulted

in significant increase in nutrient content of the compost. Nitrogen contents in the compost ranged from 1.14-1.38, 1.98-2.04, 2.36- 2.46 and 1.82- 2.01 per cent in rice straw, *Eichhornia*, *Ipomoea* and their mixture biomass, respectively. Irrespective of the type of plant biomass used, highest level of content was recorded in pots given dual inoculation of cellulose degrading microorganisms and earthworms. Nitrogen content in the compost did not differ significantly between pots treated either with earthworms and CDMs or earthworms alone. Compost obtained due to the activity of cellulose decomposing microorganisms alone recorded the lowest nitrogen content. Atiyeh *et al.*, (2000) reported that earthworms play a significant role in processing organic wastes, since the activity of earthworms accelerate the process of decomposition and stabilization and, promoted biochemical characteristics that were favourable for growth of microbes. Increased mineralization and conservation of nutrients by earthworms is due to the biocatalytic role of earthworms in the decomposition and conservation mechanism (Senapati and Dash, 1984). The intestinal mucus, which consists of easily metabolizable compounds, is considered to cause a priming effect to microbial activity (Vinceslas-akpa and Loquat, 1997). Further nitrogen fixing bacteria indirectly help in decreasing C:N ratio by making more nitrogen available from added organic matter (Shinde *et al.*, 1992). The phosphorus content of the compost obtained from rice straw, *Eichhornia*, *Ipomoea* and their mixer varied from 0.98-1.05, 1.20-1.34, 1.75-1.82 and 1.25-1.36 respectively and the trend was similar to that of nitrogen content. The increase in phosphorus content might be due to higher phosphorus content in the worm casts, which in turn may be due to higher P content (7-10%) in the earthworm tissues (Bhatnagar and Palta, 1996). The potash content in the compost varied with the type of plant materials used. The potash content in rice straw, *Eichhornia*, *Ipomoea* and mixed plant biomass varied from 1.04-1.20, 1.98- 2.19, 2.46- 2.62 and 1.90- 2.10 per cent respectively. The variation in potash content in compost due to different treatments was statistically significant. Compost prepared due to the combined action of CDMs and earthworms recorded the highest potash content, which was statistically at par with pots that received only earthworms. The lowest potash content in the compost was obtained in the treatment receiving only cellulose degrading microorganisms. Bhatnagar and Palta (1996) also reported that the earthworm tissue contained 50-75 percent proteins, 7-10 per cent fats, calcium, phosphorus and other minerals. These are added to vermicompost after the death of the mature earthworms. All these factors helped in increasing the nutrient content in vermicompost. Several trials conducted with different organic resources under varied agro-climatic conditions have shown that inoculation of cellulolytic microbes significantly improved the nutrient status of decomposed material (Gaur, 2005).The nutrient content of the decomposed material also varied due to type of plant biomass used. This could be due to variation in original nutrient levels. Similar results were also reported by Jeyabel and Kuppuswamy (2001).

Table 4: Effect of earthworm and CDMs on microbial population in the compost

Treatment	Bacteria ($\times 10^6$ g^{-1})	Fungi ($\times 10^5$ g^{-1})
Rice straw		
Earthworm	14.0	4.0
Earthworm + CDM	16.0	6.0
CDM	7.3	4.0
LSD (0.05)	**4.7**	**1.6**
Eichhornia biomass		
Earthworm	15.6	9.0
Earthworm + CDM	18.0	12.0
CDM	12.0	8.0
LSD (0.05)	**1.9**	**2.6**
Ipomoea biomass		
Earthworm	14.0	6.0
Earthworm + CDM	18.0	10.0
CDM	7.3	6.0
LSD (0.05)	**0.9**	**2.5**
Mixed (Rice straw + *Eichhornia* + *Ipomoea*) biomass		
Earthworm	14.0	5.0
Earthworm + CDM	20.0	4.6
CDM	12.0	4.0
LSD (0.05)	**1.1**	**3.8**

Microbial population number in the compost

The bacterial and fungal population in the compost varied significantly due to different treatments (Table 4). Irrespective of plant biomass used, bacterial and fungal population was maximum when composting was done with dual inoculation of earthworms and CDMs while, the lowest population was recorded in the treatments receiving inoculation of CDMs alone. Relatively higher bacterial and fungal population was recorded in *Eichhornia* biomass. Higher microbial population observed in dual inoculation treatment can be attributed to enhanced decomposition brought about by cumulative action of earthworm and CDM, which might have resulted in increased availability of easily utilizable substrates for soil microbes

In view of the growing emphasis on use of compost/organic manure in agriculture to achieve the dual goal of sustaining soil health and improving crop productivity, efficient conversion of plant biomasses into quality compost is of immense importance. Results of our study suggest that dual inoculation of earthworms and CDMs could result in faster decomposition of bio-wastes and improved nutritional quality of resultant compost with enriched microbial population.

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