МИКРОБИОЛОГИЯ ПОЧВ

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GROWTH AND REPRODUCTIVE PERFORMANCE OF EISENIA FOETIDA IN COW MANURE, SUGARCAN BAGASSE AND SAWDUST WASTE AND ITS EFFECTS ON CO_2 EVOLUTION

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Abstract. Sawdust and Sugarcane bagasse waste are causing severe environmental problems. Growth and reproductive performance of Eisenia foetida in cow manure (CM), sugarcane bagasse (SB) and sawdust (S) waste and its effects on CO_2 evolution was studied under determined laboratory conditions. The results showed that the biomass gain for Eisenia foetida in CM+SB and CM waste were greater than the CM+S waste. Cocoons produced were in the order: CM+SB > CM+S >CM. After adding worms to CM, CM+SB, CM+S, the highest CO_2 emissions related to the fifteenth day. Statistical analysis showed that vermicompost produced after 90 days in terms of nitrogen, phosphorus, potassium and pH had no significant difference with compost. Our trials showed that vermicomposting as an alternate technology for the recycling of S and SB with CM wastes using by Eisenia foetida.

Keywords: Earthworms, Cocoon, physicochemical characteristics vermicompost.

INTRODUCTION

Over the last few years, that regulation of held application and disposal of animal manure has become more rigorous, interest in using earthworms as an ecologically conservative system for manure management has increased tremendously. Various researchers have examined the potential utilization of earthworm processed wastes, commonly referred to as vermicompost, in the horticultural and agricultural industries [1-3]. Vermicompost as an excellent product is considered; because was homogeneity, reduces pollution, and over a long period tend to keep more nutrients, without influence on the environment. The potential of Perionyx excavates to vermicomposting of different wastes such as sheep dung, cow dung, biogas sludge and poultry manure was reported with Kale et al., [4]. Loh et al., were reported more cocoon production and biomass gain by Eisenia foetida in cattle waste than the goat waste [5]. Gunadi and Edwards were studied growth, fecundity and mortality of Eisenia foetida for more than one year on cattle manure solids, pig manure solids and supermarket waste [6]. Worms could not survive in fresh cattle solids, pig solids, fruit wastes and vegetable wastes. The growth of Eisenia foetida in pig wastes was faster than in cattle solids. The several additions of substrates prolonged the fecundity of worms, but there was a tendency of decreasing of the weight by worms after 60 weeks of the experiment. Organic matter, microorganisms and plants are some components of the environment that continually influence agricultural systems [7]. During vermicomposting earthworm eat, grind and some anaerobic micro flora, convening it increasing the surface area for microbial colonization of the substrate and enzymatic action through commuting the organic residues [8]. Eisenia foetida is an epigamic earthworm spice which lives in organic wastes, and it requires high moisture content, adequate amounts of suitable organic material and dark conditions, for proper growth and development [6, 9]. Earthworms play a major part in determining the greenhouse gas balance of soils throughout the world and their influence is expected in the coming decades has to grow [10]. The respiratory CO^2 involved (a measure of metabolic activities) and the enzymes involved in various chemical transformations are commonly used as indices for the some laboratory studies, in which manure was amended with earthworm and incubated for several weeks. It has revealed that earthworm severely reduces soil respiration and enzyme activities [11].

OBJECTS AND METHODS

Three treatment groups with three reproduces were investigated that consisted of Cow manure (CM) alone, Cow manure (CM) + Sugarcane bagasse (SB) and Cow manure (CM) + Sawdust (S) apiece in a ratio of 4: 1 (V/V) mixtures. Growth, sexual development and cocoon production. Nine 1 liter plastic containers (diameter 12 cm, depth 10 cm) were filled with 150 g (Air-dried) of CM + EW (earthworms), CM+ SB + EW and CM + S + EW. Waste turned over by hand every day for 15 days to eliminate the volatile toxic gases. After fifteen days, seven non-clitlellated hatchling of Eisenia foetida, weighing 200-250 mg (live weight) were entered into a container. During the study period the wastes moisture by spraying adequate quantities of distilled water was adjusted in 70-80 %. All containers were kept in a dark growth chamber at 25±1° C temperature. Biomass gain, clitellum development and cocoon production were recorded weekly for twelve weeks. The feed was removed from container and earthworms and cocoons were separated from feed by hand sorting. After that they were counted, examined for clitellum development and weight after washing with water and drying them by tissue. The worms were weighed without removing their gut contents. For any obtained data in this study correction for gut contents were not used. Then, all earthworms feed (but no cocoons) was returned to the respective container. No more feed was added at any stage during the study period. Cocoon viability was determined weekly for 12 weeks by cocoon harvesting from each plastic container and placing them in a Petri dish filled with distilled water. All petri dishes were kept in dark with temperature 25±1° C. To prevent bacterial growth and avoid negative impact on the results, water of these dishes was replaced daily. Cocoons and hatchings for each cocoon were recorded over a period of 12 weeks.

Co_2 evolution, eighteen 1 litr plastic containers (diameter 12 cm, depth 10 cm) were filled with 150 g (Air- dried) of CM, CM+ SB, CM + S, CM + EW (earthworms), CM+ SB + EW and CM+ S + EW. The moisture content of Wastes was adjusted to 70-80 % during the study period by spraying adequate quantities of distilled water. All containers were kept in dark at temperature 25° C. Wastes were turned over manually every day for 15 days in order to eliminate volatile toxic gases. After 15 days, in treatment with earthworms, 7 non- clitlellated hatchling of E, foetida, weighing 200-250 mg (live weight) were introduced into a container. Samples were drawn at 0, 15, 30, 45, 75, 90 days. The 0 days refer to the time of initial mixing of the waste before preliminary decomposition. The earthworms were removed by hand sorting and treatments were analyzed for CO2 evolution. The alkali trap method was used to quantify the released CO_2, A 500 ml conical respiration flasks contained treatments and assimilation vials containing 10 ml of 0.3 M NaOH [12]. Flasks containing the alkali traps alone served as controls. The alkali traps were replaced at each sampling data and titrated with 0.1 M HCl [12]. The evolved CO_2 was derived from titration data, corrected for the control. All experiments were performed in triplicate and the results were averaged.

Physicochemical analysis, the pH was determined in a double distilled water suspension of each mixture with ratio of 1:5 (W/V) that had been shake mechanically for 30 min and filtered through whatman №1. The same solution was used for measuring the electrical conductivity by a conductivity meter [13]. Total nitrogen was determined by Bremner and Mulvaney procedure after digesting the sample with concentrated H_2SO_4 and $HClO_4$ (9:1, V/V) [14]. For determination of other nutrient each sample (2 g) was ashed in a muffle furnace at 550° C, then white ash dissolved in 2 N HCl and reached to 100 ml volume with distilled water [15]. Total P was determined by Murphy and Riley procedure by using the colorimetric method, with molybdenum in sulfuric acid by spectrophotometer [16]. Total K was measured by flame photometer with using Houba, et al., method, after digesting the sample in the diacid mixture (concentrated HNO_3: $HClO_4$, 4:1, V/V) [17]. Nelson and Sommer method was used to measure total organic carbon [18].

RESULTS AND DISCUSSION

Growth of Eisenia foetida in the wastes, no mortality was observed in any waste during the study period. In our experiments, all wastes every day for 15 days was stirred by hand, which all toxic gases produced probably stopped. Eisenia foetida died after two weeks in fresh manure was reported by Gunadi and Edwards (2003), while other factors such as pH, electrical conductivity, ratio of C:N, content of NH4+ and NO_3 was suitable for the growth of earthworms [6]. They believed that the deaths of earthworms due to the anaerobic conditions which were created after two weeks in fresh cow dung. It is established that pre-composting is essential to avoid the mortality of worms. The growth curves of Eisenia foetida in studying wastes during the observation period are given in Figure 1.

Maximum worm biomass was gained in CM+SB waste (621±119 mg/earthworm) and minimum in CM+S (513±166 mg/earthworm). The maximum weight of earthworms was gained of the 7th week in CM wastes, whereas it took 9 and 12 weeks in CM+SB and CM+S waste respectively. At first worms gained biomass, but later after a few weeks, weight loss by earthworms was observed in all the tested animal wastes. The loss in worm biomass can be attributed to exhausting food. The biomass gain for Eisenia foetida for each g dry weight of feed (DW) was highest in CM+SB waste (39±0.66 mg/g) and smallest in CM+S waste (34±1.05mg/g). Edwards et al., (1998) have reported a biomass gain of 292 mg/g cattle waste by P. excavatus at 25 °C [17]. But in our experiments, the biomass gain was only 37±0.66 mg/g by Eisenia foetida species in CM at 25 °C. This difference could be due to difference in species morphology and first characteristics of the feed waste. Neuhauser, et al., (1980) reported that rate of biomass gain by Eisenia foetida was dependent on people density and food type [18]. Net biomass gain/earthworm per unit feed material in different feeds followed the order: CM+SB > CM > CM+S. Net biomass gain by earthworms in CM+SB waste was 1.1 times higher than in CM+S waste (Figure 1).

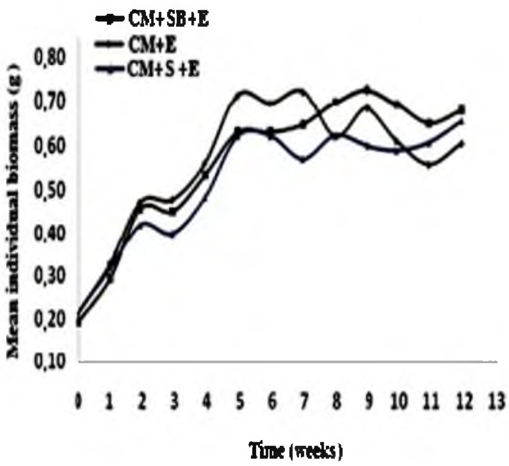

Figure 1 - The Eisenia foetida growth on different wastes.
Abbreviations:CM, cow manure; SB, sugarcane bagasse; S, sawdust; E, earthworms

According to Edwards, et al. (1998), growth rate (mg weight gained/ day/ earthworm) is a good indicator for comparing the growth of earthworms in different wastes [19]. The supported the least growth of Eisenia foetida; CM+S and CM waste was marginally better than CM+SB (61±1.96 mg/ day/ earthworm).

Table 1 summarizes the sexual development and cocoon production by Eisenia foetida in different feeds. After the start of the experiment, all individuals in all feeds developed clitellum before day 28. Cocoon production of earthworms was started with 35 days in all wastes. Table 1 shows the cumulative cocoon production by earthworm in different feeds. After 12 weeks maximum cocoons (215±30) were counted in CM+SB waste and minimum (191±20) in CM waste.

Table 1 - Cocoon production by Eisenia fetida in CM, SB and S waste

Treatments	Clitellum development started in	Cocoon production started in	Total no. of cocoons produced after 12 weeks	No. of cocoons produced/ worm	No. of cocoons produced/ worm /day	Cocoon production stopped after
CM	3th week	5th week	191±20	30.2±1.70	0.36±0.02	12th week
CM+S	4th week	5th week	209±46	30.8±4.26	0.37±0.05	12th week
CM+SB	4th week	5th week	215±30	31.5±6.61	0.38±0.08	12th week

CM, cow manure; SB, sugarcane bagasse; S, sawdust; All values was introduced as the mean ± SD (standard deviation)

Cocoons produced for each earthworm per day in different wastes was in the order: CM+SB > CM+S > CM. The difference between rates of cocoon production could be about the biochemical quality of the feeds, which is an important factor in determining the time taken to reach sexual maturity and onset of reproduction [8, 19]. Feeds which provide earthworms with enough and easily metabolizable organic matter and non-assimilated carbohydrates, favor growth and reproduction of earthworms [8]. We suggest that CM+S and CM+SB wastes are a good biomass supporting medium and good for reproduction.

Table 2 showed that changes in CO_2 evaluation of SB and S wastes mixed with CM in absent and present of earthworms during 90 days. Respiration rate rapidly decreased after 15 days in introducing waste, Satchell (1967) showed the effect of earthworms in the readily degradable organic matter, which already contains a high population of microorganisms, which is likely to be less significant than in soil [20]. Earthworm activity in soil usually enhances microbial numbers and biomass Edwards and Bohlen, (1996) [21]. Bautista, et al., (2011) suggested that more than 70 % of the total CO_2 evolution occurred during the first week in the composting process [22]. Zhang, et al, (2000) reported that earthworms are used micro-organisms as a secondary food source; transit time of the earthworm gut reduced total soil microbial biomass and increases the active components of microbial biomass [23].

Table 2 - Change in CO_2 evolution (μg CO_2 g-1 h-1) during composting of organic wastes

Moisture Content (%)	PH (1:5)	EC (dS/m)	OC (%)	N (%)	C: N ratio	K (%)	P (%)	Waste
14.40	8.15	2.06	47.30	1.24	38.12	0.62	0.29	CM
14.85	7.37	0.46	57.18	0.31	181.81	0.61	0.34	S
15.05	7.79	3.19	50.23	0.45	111.75	0.40	0.04	SB

Abbreviations: CM, cow manure; SB, sugarcane bagasse; S, sawdust.

The results of these experiments are conforming to Grappelli et al. (1983), who suggested that earthworms selectively support the microorganisms which are responsible for the transformation of organic substances in soils [24]. Physicochemical properties results in Table 3 indicated that the pH of waste containing earthworms was decreased, which may be due to the effect of earthworms on the accumulation of organic acids derived from microbial metabolism or produced during the decomposition of humic acids and folic [25, 26]. Similar results on vermicomposting of cow manure, fruit and vegetable wastes have been reported by Azizi et al., 2008; Gunadi and Edwards (2003) and Mitchell (1997) [3, 6, 27]. There wasn't more N, P and K in worm-inoculated compost than in the compost without earthworms (Table 3).

Table 3 - Physicochemical properties of treatments after 90 days of composting

PH (1:5)	Total K (%)	Total P (%)	Total N (%)	Treatments
8.11 ab	1.01 ac	0.48 ab	1.75 a	CM
8.00 b	1.13 ab	0.50 a	1.81 a	CM+ E
7.30 c	0.88 bd	0.38 bc	1.44 ab	CM+S
7.20 c	1.15 a	0.39 b	1.47 ab	CM+S+ E
8.46 a	0.74 df	0.46 ab	1.53 a	M+SB
8.18 ab	0.82 ce	0.46 ab	1.67 a	CM+SB+ E
Abbreviations:CM, cow manure; SB, sugarcane bagasse; S, sawdust; E, earthworms. Means followed by the same letters do not signi?cantly differ (p = 0.05)				

CONCLUSIONS

The biomass gain for Eisenia foetida (live weight) per g dry weight of the feed source (DW) was in CM+SB (39±0.66mg/g) and CM waste (37±0.36 mg/g) greater than the CM+S waste (34±1.05mg/g). Cocoons produced per earthworm in each day in treatments were in the order: CM+SB > CM+S > CM wastes supported the growth and reproducing E. foetida, so could be used as feed materials in large scale vermicomposting facilities. Further studies are required to explore potential use S and SB wastes in mixture with other dung materials. Another parameter that demonstrates rapid stabilization of manure with earthworms is the respiration rate (i.e. CO_2 production) [28]. In this experiment, earthworms and the microbial activity within the first four weeks of processing promoted, it seems rapidly destroy most of the easily biodegradable substances, as indicated by the rapid reduction in the amounts of CO_2 evolving from the CM, CM +S and CM+SB. CO_2 evolution decreased rapidly two weeks after earthworm introduction and continued in lower rate throughout the 12 weeks indicating increasing stability of the organic matter. Our trials showed vermicomposting as an alternate technology for the recycling of S and SB with CM wastes by using an earthworm Eisenia foetida.

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РЕЗЮМЕ

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РОСТ И РЕПРОДУКТИВНОЕ РАЗВИТИЕ EISENIA FOETIDA В КОРОВЬЕМ НАВОЗЕ, ОТХОДАХ САХАРНОГО ТРОСТНИКА И ДРЕВЕСНЫХ ОПИЛКАХ И ИХ ЭФФЕКТИВНОСТЬ НА ЭМИССИЮ CO_2

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Древесные опилки и отходы сахарного тростника вызывают серьезные проблемы для охраны окружающей среды. В лабораторных условиях было изучено влияние коровьего навоза, отходов сахарного тростника, древесных опилок на рост и репродуктивное развитие Eisenia foetida, а также их эффективность на выход Co_2.

Результаты показали, что выход биомассы для Eisenia foetida в коровьем навозе +отходы сахарного тростника и отдельно коровьем навозе был больше, чем там, где применялся коровий навоз + древесные опилки.

Количество коконов червей было в следующем порядке: коровий навоз + отходы сахарного тростника > коровий навоз + древесные опилки > коровий навоз.

После добавления червей к коровьему навозу, коровий навоз + отходы сахарного тростника, коровий навоз + древесные опилки наибольшая эмиссия углекислого газа пришлась на 15 день. Статистический анализ показал, что вермикомпост через 90 дней по содержанию в нем азота, фосфора, калия и pH не имел сильных отличий в зависимости от используемых отходов.

Наши исследования показали, что вермикомпостирование является альтернативной технологией для переработки отходов сахарного тростника и древесных опилок с помощью Eisenia foetida.

ТҮЙІН

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СИЫР ҚИЫНДА, ҚАНТ ҚҰРАҒЫНЫҢ ҚАЛДЫҚТАРЫ ЖӘНЕ АҒАШ ҮГІНДЕЛЕРІНДЕГІ EISENIA FOETIDA ӨСУІ ЖӘНЕ РЕПРОДУКТИВТІК ДАМУЫ ЖӘНЕ ОЛАРДЫҢ CO_2 ЭМИССИЯСЫНА ТИІМДІЛІГІ

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Ағаш ұнтақтары және қант құрағының қалдықтары қоршаған ортаны қорғау үшін елеулі проблемалар тударады. Зертханалық жағдайларда сиыр көңінің, қант құрағының қалдығының, ағаш үгінділерінің Eisenia foetida өсуіне және репродуктивтік дамуына, сондай-ақ олардың CO_2.шығу тиімділігі зерттелді.

Зерттеулер Eisenia foetida үшін биомассаның шығымы сиыр көңі+ағаш үгінділері пайдаланылғандарға қарағанда, сиыр көңі+қант құрағының қалдықтары және жеке сиыр қиында көп болды.

Құрттардың коконының мөлшері мынадай ретпен болды: сиыр көңі+қант құрағының қалдықтары > сиыр көңі+ағаш үгінділері > сиыр көңі.

Құрттарды сиыр көңіне, сиыр көңі+қант құрағының қалдықтарына, сиыр көңі+ағаш үгінділеріне қосқаннан кейін көмір қышқыл газының ең үлкен эмиссиясы 15-ші күні болды. Статистикалық талдау вермикомпост 90 күннен кейін оның құрамындағы азот, фосфор, калий және pH мөлшері бойынша пайдаланылатын қалдықтардан елеулі өзгешелігі болмайтынын көрсетті.

Біздің зерттеулеріміз Eisenia foetida көмегімен қант құрағы мен ағаш үгінділерін қайта өңдеу үшін вермикомпосттау балама технология болып табылатындығын көрсетті.