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Research Article

Comparison and potential utilization of sugarcane bagasse, date palm wastes and grape waste mixed with cow manure for the production of vermicompost and as feed material for earthworms

S. H. Hosseini Jafari[1], M. J. Zarea[*]

Department of Agronomy and Crop Breeding, Faculty of Agriculture, Ilam University, Ilam, I. R. Iran

* Corresponding Author: mj.zarea@ilam.ac.ir
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ABSTRACT- In this study, various agricultural and industrial organic wastes were mixed with cow manure (CM) as the main raw material to compare their properties for the production of vermicompost and as feed material for earthworms. Cow manure, alone and in mixed with sugarcane bagasse (BG), date palm wastes (DW) and grape waste (GW) at the ratios of 3:1, 1:1, and 1:3 were used for culturing of the earthworm, *Eisenia foetida*. Total number, final biomass and the final length of earthworms and resulting vermicomposts were analyzed for their properties and chemical compositions after 70 days. Results showed that the growth of the earthworms in terms of the total number, final biomass and final length in CM mixed with GW at the ratio of 3:1 were significantly greater than those in other mixture treatments probably due to the neutral pH condition (pH = 7.1). The mixture of cow manure and bagasse resulted in the highest rate of vermicompost production that was 24.6 Kg m^{-3}. The mixture of CM and GW provided the best conditions for *E. foetida* growing. CM mixed with GW produced the best results in terms of the chemical composition of the resulting vermicompost. Results of this study showed that vermicomposting was an adequate process for the degradation of sugarcane bagasse, grape waste and date fruit waste. The aforementioned organic wastes could be mixed with cow dung to produce quality vermicompost.

INTRODUCTION

Intensive modern agriculture based on intensive cropping and applying higher chemical fertilizer has gradually reduced the fertility of the soil in croplands worldwide. Fertilizers, derived from organic substances such as agricultural organic waste, would be important to restore soil fertility and improve soil physical and chemical conditions.

Animal waste can act as a suitable nutrient source for plants (Bhardwaj, 1995). Organic waste in the soil is naturally recycled by a variety of decomposer organisms and detritivore invertebrates especially earthworms (Oyedele et al., 2005). Involving earthworms in converting organic waste to valuable resources (vermicomposting) can accelerate the provision of organic sources of nutrients in a relatively short period (Pramanik et al., 2007). The health and nutrient status of the soil could be improved through the amendment of vermicomposts (Orozco et al., 1996; Jack and Thies, 2006). The positive effect of vermicomposts on various crops has been demonstrated (Mba, 1996; Atiyeh et al., 2000; Singh and Varshney, 2013). Applying vermicomposts can improve the nitrogen (N) content in soil (Singh and Varshney, 2013; Wang et al., 2017). Application of vermicomposts has been shown to enhance N-fixing microorganisms (Mackay et al., 1982), soil microbial biomass (Arancon et al., 2003) and enzymatic activities in soils (Lavelle and Martin, 1992). *Eisenia foetida* is the most eurythermal species of epigeic earthworm (Reinecke et al., 1992) preferring neutral to slightly alkaline pH (Pramanik et al., 2007).

Vermicompostingis a simple and cost-effective technique. Composting different substrates by earthworms has been frequently reported (Garg et al., 2006; Singh et al., 2006; Ramnarain et al., 2019). Animal wastes and industrial by-products of agriculture can act as suitable substrates for vermicomposting. Buffalo manure can influence the quality and quantity of organic matter content in degraded soil (Ngo et al., 2011). Involvement of earthworms in the composting process has been reported to lead to a stronger transformation of cow manure to compost than conventional composting (Ngo et al., 2011). Cow manure has been shown to affect soil bacterial diversity and activity (Ngo et al., 2013).

Bagasse is obtained from fibrous residue of the sugarcane stalk after extraction of the juice. Bagasse as the by-product of the Sugarcane industry is used in animal feeding and as a fuel source. Bagasseis characterized by low crude protein and nutritive value, poor palatability and high content of ligno-cellulose (Berndtet al., 2002). This fibrous residue of the sugarcane stalk can be considered as earthworm food.

Industries related to date processing are continuously increasing worldwide (Chandrasekaran and Bahkali, 2013). The increasing industrialization of dates worldwide causes huge amounts of date palm fruit wastes. Daily discarded palm fruit wastes by the industries cause serious problems to the environment. Date fruit has enormous sugar content (50-60%), K, Ca, Mg and Fe as well as Niacin and vitamins. Seeds of the date palm also contain P, K, Ca, S, Mg, Fe, Zn, Cu, Mn, Al, Br, Co, and fluorine (Chandrasekaran and Bahkali, 2013). Grape pomace (solid remains of grapes) as a by-product of grape fruit after extraction of juice can be considered as an organic waste for composting and vermicomposting (Domínguez et al., 2014). Grape pomace constitutes 20% of the total weight of the grape fruit (Domínguez et al., 2017).

The quality of composts would be determined by several factors such as type of substrate (Pramanik et al., 2007). This study aimed to investigate the different sources of agricultural and industrial organic waste, sugarcane bagasse, date palm wastes and grape waste, as earthworm food on the composition and chemical properties of the resulting vermicompost.

MATERIALS AND METHODS

Experiment Design and Procedure

The current study was conducted in late 2020. Recently-deposited cow manure used in the current study was collected from alocal dairy farm located near Ahvaz, central city of Khuzestan Province, sugarcane bagasse (BG) from a sugar factory in Ahvaz, grape waste (grape pomace + dried leaves + dried clusters) (GP) from grape gardens located along Karun river in Ahvaz, Iran and date palm fruit waste + date fruit seeds (DW) were collected from date palm groves in Ahvaz, Khuzestan Province Iran.

Various organic waste mixes were used for vermicomposting including cow manure alone and cow manure mixed with BG, GP, DW at ratios of 3:1, 1:1 and 1:3. By mixing different rations of cow manure (CM) with BG, GP and DW 9 different feed mixtures (different treatments) were prepared (Table 1). Cow manure was used as the main raw material that was air dried before use. Date palm waste consisted of date fruits and date seeds. The date seeds were left to dry completely before grinding (Fig. 1). Grape waste consisted of dried leaves, cluster and grape pomace. Grape clusters were allowed to be completely dried and then were grinded (Fig. 1). The experiment was designed in a completely randomized factorial design with 3 replications. To produce vermicompost in the experiments, earthworms (*E. foetida*) were reared on the above mentioned culture media.

Ten Kg of the manure (ona dry weight basis, 77 % moisture content) was placed into each plastic box with dimensions of 0.5 ×0.30 × 0.22 m. One hundred juveniles and adult earthworms, *E. foetida*, (0.4±0.5, mg live weight per earthworm) were added into the perforated plastic boxes. All boxes were kept at room temperature (appx. 25 °C) for 70 days. The moisture of the substrate material was maintained at about 75% (w.b.) with the daily temperatures that fluctuated between 25 and 28 °C. After four months, *E. foetida* casting from each box was analyzed for its chemical and properties. Productivity of vermicompost was calculated using the formula described by Ramnarain et al. (2019):

$$\text{Productivity of vermicompost (\%)} = \frac{\text{Harvested vermicompost (kg)}}{\text{Total mass of feed (kg)}} \times 100$$

Chemical properties of the resulted vermicomposts including EC, pH, moisture content (%), organic matter (%), organic carbon (%), total nitrogen (%), phosphorus (P_2O_5) and potassium (K_2O) contents and C:N ratios were determined. Total organic carbon (TOC in %) was measured by Titrimetry using the Walkley-Black method. Total nitrogen was measured using the Kjeldahl method. Total phosphorus (P in %) was determined by the colorimetric method using a spectrophotometer. Total potassium and C:N ratio were measured according to the absorption method using an Atomic Absorption Spectrophotometer.pH-H_2O was measured using a pH meter. Electrical conductivity (EC in dS/m) was measured using a conductivity meter. The chemical analysis of feeding materials and vermicompost was done to determine the rates of C, N, P, K, C/N ratio, organic matter, pH and EC using the aforementioned methods.

Statistical Analysis

F-test was carried out to test the significance of treatment differences at 5% level of probability, using the PROC ANOVA procedure of SAS, by SAS software version 9.1. The least significant differences were calculated to identify the differences among treatment means using LSD 0.05 analysis.

Table 1. Description of the treatments (feed mixtures)

Treatment	Description
CM	Cow manure
$CM_{75}BG_{25}$	75% CM mixed with 50% sugarcane bagasse
$CM_{50}BG_{50}$	50% CM mixed with 50% sugarcane bagasse
$CM_{25}BA_{75}$	25% CM mixed with 75% sugarcane bagasse
$CM_{75}DW_{25}$	75% CM mixed with 50% date palm wastes
$CM_{50}DW_{50}$	50% CM mixed with 50% date palm wastes
$CM_{25}DW_{75}$	25% CM mixed with 75% date palm wastes
$CM_{75}GW_{25}$	75% CM mixed with 50% grape waste
$CM_{50}GW_{50}$	50% CM mixed with 50% grape waste
$CM_{25}GW_{75}$	25% CM mixed with 75% grape waste

Grape waste consisted of grape pomace + dried leaves + dried clusters

RESULTS AND DISCUSSION

The resulting composts of different organic wastes produced by *E. foetida* within 70 d are shown in Fig. 2. The chemical analysis shows the percentage of nutrient contents, C/N, OM, WC, pH and EC of the feeding materials including cow manure alone and cow manure in combination with sugarcane bagasse, date palm waste and grape waste is represented in Table 2. By analysis of variance (ANOVA), significant differences between various vermicomposts were observed (Table 3). Least Significant Difference (LSD) test was further employed to separate significantly different means between vermicomposts prepared from different vermin beds (Table 4). The effect of the combination of cow manure and organic wastes (sugarcane bagasse, grape waste and date palm wastes) on the rate of vermicompost production is shown in Fig. 3. In the present study, cow manure mixed with grape waste at the ratio of 3:1 and 1:1 caused higher amounts of vermicompost productivity (85%) as compared to other vermi beds (Fig. 3).

Properties and chemical compositions of provided vermicompost, i.e. contents of C, N, P, K, C/N, organic matter, water content, pH and EC are presented in Table 4. The composition chemical of vermicasts was significantly different for the vermicompost. Total C in cow manure (CM) vermicast combined with sugarcane bagasse (BG) mixed at a ratio of 3:1 on a dry matter basis was higher as compared to the other vermicasts. Total C content in cow manure vermicast combined with grape waste (GW) was significantly ($P < 0{:}05$) lower than those in other vermicasts, regardless of their mixture ratio level (Table 4).

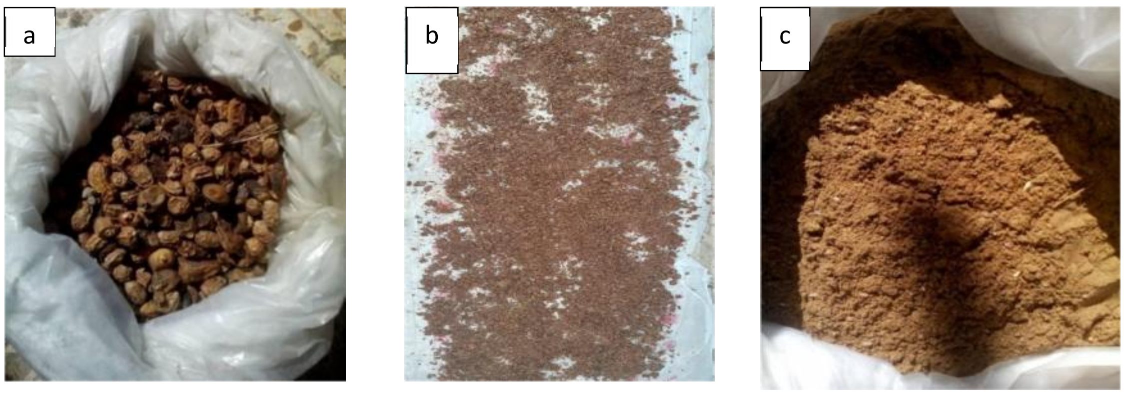

Fig. 1. Date palm wastes consisting of date fruit (**a**) and grinded date seeds (**b**) and grape waste consisting of grinded grape cluster (**c**) which were used as feed stocks *Eisenia foetida* in this study.

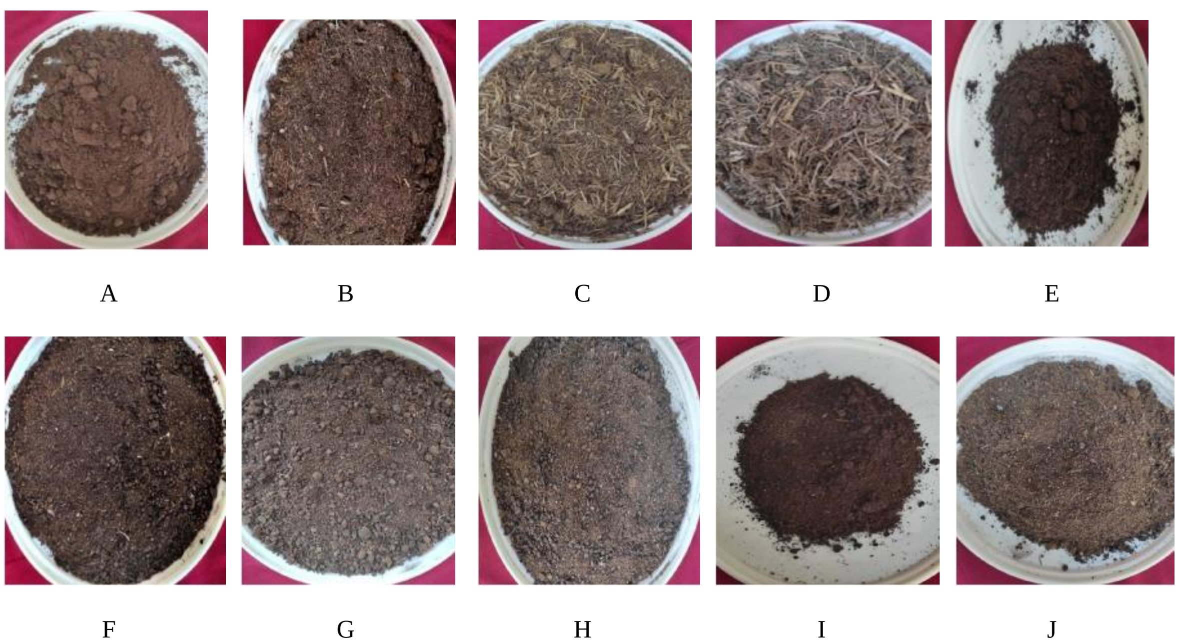

Fig. 2. Composting of organic wastes through vermicompost processing by earthworms *Eisenia foetida* within 70 days. (**A**), Cow manure (CM); (**B**), $CM_{75}BG_{25}$, 75% CM mixed with 25% BG; (**C**), $CM_{50}BG_{50}$,50% CM mixed with 50% BG; (**D**), $CM_{25}BA_{75}$, 25% CM mixed with 75% BG; (**E**), $CM_{25}GW_{75}$,25% CM mixed with 75% GW; (**F**), $_{50}GW_{50}$,50% CM mixed with 50% GW; (**G**), $CM_{25}GW_{75}$, 25% CM mixed with 75% CG; (**H**), $CM_{75}DW_{25}$, 75% CM mixed with 25% DW, (**I**) $CM_{50}DW_{50,}$ 50% CM mixed with 50% DW and (**J**) $CM_{50}DW_{50}$, 25% CM mixed with 75% DW.

Table 2. The chemical analysis of nutrient contents, C/N, OM, WC, pH and EC of the prepared feeding materials.

Treatment	N (%)	P (%)	K (%)	C (%)	C:N	OM (%)	WC (%)	pH	EC (dS/m)
CM_{100}	0.95	0.77	1.062	57.8	81.1	91	12.4	6.71	5.63
$CM_{75}BG_{25}$	0.6	0.25	0.98	58.8	131.9	101	8.4	8.19	2.99
$CM_{50}BG_{50}$	0.23	0.16	0.79	69.7	168.9	94	9.8	6.89	5.56
$CM_{25}BA_{75}$	0.11	0.12	0.65	74.4	162.7	103	8.7	6.27	5.48
$CM_{75}DW_{25}$	0.59	0.25	1.82	64.7	97.9	93	12.7	5.29	6.57
$CM_{50}DW_{50}$	0.41	0.22	0.99	62.9	97.2	97	11.3	5.43	8.0
$CM_{25}DW_{75}$	0.46	0.18	1.18	80.6	99.6	106	9.1	5.31	7.09
$CM_{75}GW_{25}$	1.3	0.36	4.11	55.5	61.1	90	15.5	8.15	5.76
$CM_{50}GW_{50}$	1.09	0.33	2.43	70.1	68.4	96	12.6	7.44	6.19
$CM_{25}GW_{75}$	0.94	0.29	3.57	70.8	71.4	92	8.7	7.79	6.07

CM, cow manure; BA, sugarcane bagasse; DW, date palm waste; GW, grape waste.
Grape waste consisted of grape pomace + dry leaves + dried cluster
Date palm wastes consisted of fruits waste and grinded fruit seed

Table 3. ANOVA of the provided vermicompost production and physiochemical parameters

SOV	Vermicompost production	C	N	P	K	C:N	OM	WC	pH	EC
Replication	104.54*	96.42**	0.28**	0.0003	0.47	30.00	4.93	0.10	0.0005	0.003
Vermicompost	455.88**	82.58**	1.76**	0.04**	20.88**	3775.87**	30.20**	6.06**	0.78**	5.71**
Error	29.42	0.18	0.011	0.003	0.23	13.17	6.89	0.069	0.0016	0.0087
CV	7.92	1.12	6.04	10.19	14.18	10.46	3.60	1.86	0.54	2.05

Table 4. The percentage of nutrient contents, C/N, OM, WC, pH and EC of resulting vermicompost of cow manure (CM) mixed differently with sugarcane bagasse (BG), data palm waste (DW) and grape waste (GW)

Treatments	CM	CM:BG			CM:DW			CM:GW		
		3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3
C (%)	31.4i	48.6a	45b	38.8e	40.5d	36.8f	41.7c	35.3g	34.8g	35.5g
N (%)	1.8d	0.49f	0.44f	1.4a	2.3b	2.5a	2.1c	1.8d	2.1c	2.58a
P (%)	0.56cd	0.36f	0.43ef	0.50de	0.50ed	0.56cd	0.6bc	0.7a	0.73a	0.66ab
K (%)	2.4b	1.0d	0.9b	1.5d	2.5d	1.7bcd	2.3bc	6.8a	6.9a	7.6a
C/N	16.7cd	99.3a	103.9a	25.8b	17.2cd	14.4cd	20.0bc	18.8cd	16.5cd	13.7d
OM (%)	67.6c	75.3a	76.3a	69.3bc	75.3a	75.6a	73.0ab	70.6bc	70.3bc	75.6a
Water content (%)	16.3a	12.5e	12.7e	13.2d	13.6d	14.1c	14.2c	12.6e	15.6b	16.0ab
pH	7.5d	7.4d	7.6c	7.7b	6.3g	6.8f	7.1e	7.9a	7.9a	7.4d
EC (ds m^{-1})	4.3e	2.4g	3.4f	2.2h	5.7b	5.4c	5.3c	5.3c	6.0a	4.9d

Values followed by different letters in the same row are significantly different (*P*< 0:05). CM, cow manure; BA, sugarcane bagasse; DW, date palm wastes; GW, grape waste. Grape waste consisted of grape pomace + dry leaves + dried cluster
Date palm wastes consisted of fruits waste and grinded fruit seed

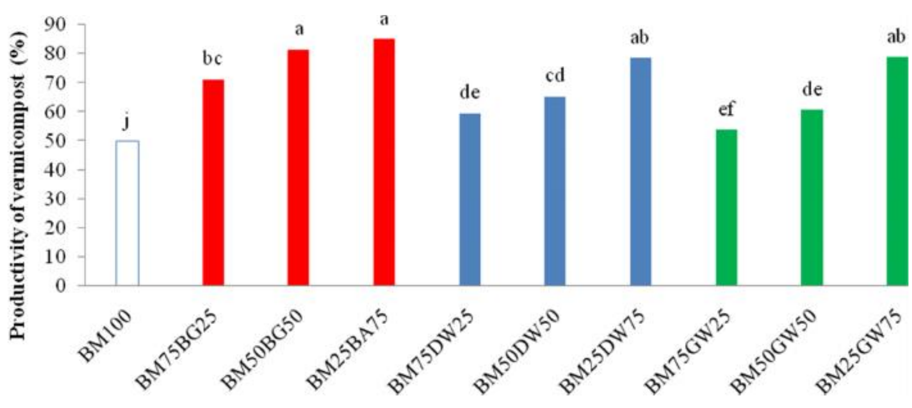

Fig. 3. Vermicompost production resulted from composition of various waste organic materials. Cow manure (CM), 100% (control treatment); (2) $CM_{75}BG_{25}$, 75% CM mixed with 25% BG, (3) $M_{50}BG_{50}$,50% CM mixed with 50% BG; (4) $CM_{25}BA_{75}$, 25% CM mixed with 75% BG; (5) $CM_{25}GW_{75}$,25% CM mixed with 75% GW; (6) $M_{50}GW_{50}$,50% CM mixed with 50% GW; (7) $CM_{25}GW_{75}$, 25% CM mixed with 75% BG; (8) $CM_{75}DW_{25}$, 75% CM mixed with 25% DW, (9) $CM_{50}DW_{50,}$ 50% CM mixed with 50% DW and (10) $CM_{50}DW_{50}$, 25% CM mixed with 75% DW. Grape waste consisted of grape pomace + dry leaves + dried cluster, Datepalm wastes consisted of fruits waste and grinded fruit seed. Different small characters (letters) on the top of each bar show significant differences between treatments at 5% level of probability (*P < 0:05*).

In the present study, N contents in $CM_{25}BA_{75}$, $CM_{50}DW_{50}$ and $CM_{25}GW_{75}$ were significantly higher (*P*< 0:05) than those in the other vermicasts. The lower N content was obtained in cow manure as the main raw material. Combined CM with GW, regardless of mixed ration level, contained significantly higher (*P*< 0:05) P and K contents than those in other vermicasts. The C:N ratios in CM of the worm vermicast mixed with BG at a ratio of 3:1 and 1:1 were higher than those in other vermicasts. The result for vermicast water content indicated that the vermin bed containing cow manure had the highest water content followed by the vermin bed containing combination of cow manure and grape wastes at the ratio of 1:3. pH in various vermicasts was significantly different. Cow manure combined with sugarcane bagasse at the ratio of 3:1 had the lowest pH (6.3), followed by mixed ratio of 1:1 of CM:BG (6.8). pH in other vermicasts was above neutral condition (pH 7.00). $CM_{50}GW_{50}$ had higher pH and EC than the other vermicasts.

Table 5 shows the ANOVA of growth performance of *E. foetida* among different feed mixture treatments, demonstrating the significant effect of feed mixture on earthworm growth. Growth of the earthworms in terms of the total number, final biomass and final length in cow manure mixed with grape waste at the ratio of 3:1 were greater than those in other mixture treatments (Table 6).*Eisenia fetida* is the most eurythermal species of epigeic earthworm (Reinecke et al., 1992) preferring neutral to slightly alkaline pH (Pramanik et al., 2007). In the present study, pH in cow manure amended with grape waste at the ratio of 3:1 was higher than those in other treatments.

The increase in mineral nutrients (P, Na and K) of the castings in cow manure amendment with grape waste indicated that earthworms accelerated the mineralization of organic matter. It seems that the addition of grape waste had a positive effect on the acceleration of nutrient mineralization. The C:N ratio in cow manure combined with date palm wastes and grape waste was lower than that in cow manure mixed with sugarcane bagasse. Organic substances during the vermicomposting are transformed to the stable form i.e., humic compounds. Thus, higher humification of castings resulted in lower level in C:N ratio (Riffaldi and Levi-Minzi, 1983). Increased organic matter decomposition due to the feeding actions of earthworms has been reported to cause lower the C:N ratio (Talashilkar et al., 1999; Loh et al., 2005). Although the pH value of the vermicast has been reported to be decreased due to production of organic acids and CO_2 during microbial metabolism (Hartenstein and Hartenstein 1981) but it is substrate-dependent and dynamic (Ndegwa et al., 2000). The different pH in the resulting vermicomposts might be due to the initial substrate. On the other hand, the pH change during vermicomposting is not only dynamic but is substrate-dependent. Ndegwa et al., (2000) indicated that a different substrate would result in the formation of a different intermediate, accordingly causing a difference in the pH value of the vermicompost formed.

Cation exchange capacity (CEC) of the vermicasts in cow manure (CM) combined with grape waste (GW) and date palm wastes (DW) was higher than that in cow manure combined with sugarcane bagasse (BG). Higher CEC may be related to the higher rate of mineralization and increased humic acid. The EC could be an indicator of the mineralization rate of organic matter (Hartenstein and Hartenstein, 1981). Humic acid which increases during the vermicomposting process (Albanell et al. 1988) has been reported to have high ECE (Holtzclaw and Sposito, 1979).

Table 5. ANOVA table of the growth performance of *Eisenia foetida* among different feed mixture treatments

Mean square				
SOV	df	Final number	Final biomass	Final length
Replication	2	237.73ns	0.082ns	0.078
vermicompost	9	3644.20**	0.31**	0.28**
Error	18	152.91	0.048	0.055
CV	-	6.004	2.79	15.34

ns: non-significant. * and ** significant at P ≤ 0.05 and P ≤ 0.01, respectively

Table 6. Growth performance of *Eisenia foetida* among different feed mixture treatments

Treatments	CM	CM:BA			CM:DW			CM:GW		
		3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3
Final number (per box)	232.0b	219.0bc	169.3gf	154.3g	253.6a	208.0cd	177.6ef	257.6a	197.6de	190.0def
Final biomass (g/worm)	1.80ab	1.77ab	1.40bcd	1.20cd	1.89a	1.54abc	1.09d	1.87a	1.59abc	1.13d
Final length (cm)	7.83bc	7.83bc	7.13d	7.60c	8.0ab	7.80bc	8.0ab	8.23a	8.1ab	8.16ab

Values followed by different letters in the same row are significantly different (*P*< 0:05).

CM, buffalo manure; BA, sugarcane bagasse; DW, date palm wastes; GW, grape waste

Grape waste consisted of grape pomace + dry leaves + dried cluster

Datepalm wastes consisted of fruits waste and grinded fruit seed

CONCLUSIONS

Interest in applying organic fertilizers in agricultural systems has been growing in recent decades. In this study, cow manure as the main raw material was mixed with various organic wastes including sugarcane bagasse, grape waste and date fruit waste, at the different ratios of 3:1, 1:1 and 1:3. The addition of these organic wastes in the preparation of organic compost by vermicomposting affected the chemical composition of the resulting cow dung vermicomposts. This study revealed when cow dung manure was mixed with grape waste at the ratio of 3:1 caused the higher vermicompost productivity with the better status of chemical composition. Mixing date palm wastes with cow manure provided better conditions for earthworm growth, demonstrating they are better materials among other used vermin beds for culturing of the earthworm, *E. foetida.*

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مقایسه و ارزیابی پتانسیل کاربرد باگاس نیشکر، دورریزهای خرما و انگور در مخلوط با کود گاوی برای تولید ورمیکمپوست و به عنــوان مواد غذایی کرم خاکی (*Eisenia foetida*)

سید هادی حسینی جعفری، محمد جواد زارع*

گروه زراعت و اصلاحات نباتات دانشکده کشاورزی، دانشگاه ایلام، ایلام، ج.ا. ایران

*نویسنده مسئول:

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کرم خاکی(*Eisenia foetida*)

ورمی کمپوست

چکیده- در این مطالعه دورریزهای آلی حاصل از بخش کشاورزی و صنعتی با کود دامی حاصل از گاومیش به عنوان ماده اولیه اصلی مخلوط شدند تا خواص آنها برای تولید و رمیکمپوست و به عنوان ماده خوراک کرم خاکی مقایسه شوند. کود گاومیش بطور جداگانه و مخلوط با باگاس نیشکر، دورریزهای خرما و انگور به نسبتهای ۱:۳، ۱:۱ و ۳:۱ برای آماده سازی بستر پرورش کرم خاکی *Eisenia foetida* استفاده شدند. تعداد، وزن و طول نهایی کرمها و ویژگیها و ترکیبات شیمیایی در پایان آزمایش (۷۰ روز) در ورمی کمپوست های حاصل شده اندازه‌گیری شدند. نتایج نشان داد که رشد کرمها از نظر تعداد، وزن و طول در بستر آماده شده از تلفیق کود گاومیش با دورریزهای انگور در نسبت ۱:۳بیش از سایر تیمارها ثبت گردید که علت آن میزان اسیدیته نزدیک به خنثی بستر کشت تهیه شده بود. تلفیق کود گاومیش و باگاس نیشکر منجر به تولید میزان بیشتری از ورمی کمپوست به میزان 24/6 کیلوگرم در مترمربع ورمی کمپوست گردید. مخلوط دورریزهای خرما با کود گاومیش منجر به فراهم آمدن بهترین شرایط جهت فعالیت کرمها گردید که می‌تواند به علت شرایط اسیدیته نزدیک به خنثی ($pH = 7.1$) باشد. بر اساس تجزیه آنالیز شیمیایی حاصل از ورمی کمپوستهای حاصله، ورمی کمپوست حاصل شده از ترکیب کود گاومیش با دوریزهای انگور بهترین ترکیبات شیمیایی را داشت. همچنین آزمایش انجام گرفته قابلیت تجزیه باگاس نیشکر، دوریزهای انگور و خرما را از طریق ورمیکمپوست و امکان مخلوط دوریزهای آلی نامبرده شده را با کود گاوی به جهت تولید ورمیکمپوست نشان داد.