International Journal of Recycling of Organic Waste in Agriculture (2023)12: 325-339
Doi: 10.30486/IJROWA.2022.1944906.1371

ORIGINAL RESEARCH

Vermicompost quality and earthworm reproduction in different organic waste substrates

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Received: 13 November 2021 / Accepted: 31 July 2022 / Published online: 08 November 2022

Abstract

Purpose The present study aims to evaluate the changes in parameters affecting the quality of vermicompost produced by the earthworm '*Eisenia fetida*' on different organic waste substrates using multivariate analysis, variance analysis, factor analysis and principal component analysis (PCA).

Method A completely randomized design experiment was conducted with a 2 × 8 factorial arrangement of experimental and control treatments in triplicate per treatment. We investigated the growth and reproduction of earthworms and the characteristics of vermicompost produced on different organic wastes and residues represented by carrot pulp (C), potato peel (P), vegetables (V) and sawdust (S) blended with cattle manure (as the main substrate) at two levels of 100 and 150 grams of each treatment in two kilograms of manure. Chemical parameters include pH, electrical conductivity (EC), carbon/nitrogen ratio (C/N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and copper (Cu) were measured in the vermicompost produced.

Results The results of cluster analysis and PCA grouped nine substrate combinations into three categories with similar qualitative characteristics. The first two principal components in PCA revealed that the major parameters responsible for the qualitative changes in the produced vermicompost were iron, copper, calcium, magnesium, potassium, phosphorus and nitrogen.

Conclusion Current results suggested that the treatment CPVS and the treatment S (sawdust) provided the optimal conditions for the growth and reproduction of earthworms and the production of high-quality vermicompost.

Keywords *Eisenia fetida* reproduction, Multivariate analysis, Organic waste, Physicochemical parameters, Vermicompost quality

Introduction

Organic waste is generated in large quantities annually throughout the world, which causes serious environmental problems and leads to space occupation (Arjaqy and Fataei 2015; Usmani et al. 2019). Waste management, or waste disposal, is one of the challenges in today's life (Fataei and Seiied Safavian 2017; Amirfazli et al. 2019; Samadi Khadem et al. 2020; Ojaghi et al. 2021). One of the solutions inquired by humans for a long time is to transform organic waste to fertilizer and return it to the production cycle (Seiiedsafavian et al. 2014; Kazemi Noredinvand et al. 2016; Babaei et al. 2017; Zangeneh et al. 2021; Mirzade Ahari et al. 2019). Therefore, organic waste

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recycling through composting is a convenient strategy for waste management (Lim and Wu 2016; Goel and Kalamdhad 2017 ; Nikpour et al. 2020).

Vermicomposting technology has two main advantages, reducing organic waste generation on one hand and converting it into a valuable product on the other hand (Daghestani and Niknam 2019). There are several methods for vermicomposting, among which the use of earthworms, especially the Lumbricidae family, including *Eisenia fetida*, is one of the most important methods in the production of fertilizers from organic matter (Ahmadi Mousavi and Ataei 2017).

Cattle manure has been reported to act as an optimal main substrate for vermicomposting (Doan et al. 2013). In a study by Das and Deka (2021) on the vermicomposting of harvested waste biomass of potato crop using *E. fetida* in two treatments, the first treatment containing only potato plant biomass was determined to be the main raw materials. The second treatment consisted of a mixture of potato plant biomass with cattle manure at a ratio of 5:1. Their results revealed that the combination of cattle manure significantly increased the quality and quantity of the final product of vermicompost and had a positive effect on the earthworm population.

Numerous factors affect how the vermicomposting process is performed and organic matter is one of the factors participating in such a process (Gupta et al. 2007). Many studies have been carried out on the stages of vermicomposting and the analysis of quality parameters of harvested compost. Such studies documented a significant relationship between vermicomposting and changes in parameters such as carbon (C) content, nitrogen (N) content, C/N ratio and pH value (Ramnarain et al. 2019). In addition, these parameters were reported to affect vermicomposting process and earthworm activity (Fataei et al. 2011). It should be noted that toxic compounds (such as the presence of heavy metals, residues of antibiotics and other chemical compounds) can reduce the function of microorganisms such as bacteria and other organisms such as earthworms (Jalilzadeh et al. 2017; Shokri et al. 2019; Derakhshan-Nejad et al. 2020; Mehrdoost et al. 2021). Ramnarain et al. (2019) investigated vermicomposting of various organic materials (rice straw, dry grass clippings and cow manure) using *E. fetida* and found that the harvested vermicompost had a very high nutrient content, and contained all the essential macronutrients and micronutrients. Heavy metals in vermicompost can enter the food cycle through the soil, water and plants and adversely affect human health (Senesil et al. 1999; Azizi et al. 2013). According to Ahmadi Mousavi and Ataei (2017), the concentration of heavy metals increased in the exchangeable, carbonate-bound, iron-manganese oxides-bound fractions (bioavailability of heavy metals) due to the activity of earthworms and the decomposition of organic matters into finer compounds. Zn and cadmium (Cd) displayed the highest and Cu the lowest bioavailability in the harvested vermicompost. The change in variation of different fractions showed that the earthworm activity could be an influential factor in the distribution of metals in various fractions and their bioavailability in vermicompost. Therefore, it is necessary to measure and regulate the concentration of these elements for plants before applying vermicompost to the soil (Gupta and Garg 2008). Several methods have been employed to assess stable organic matters, including C/N ratio, physicochemical parameters and chemical structure determination (Dominguez et al. 1997; Bhat et al. 2017)). Limited research has been conducted to compare the structural changes of organic materials in both composting and vermicomposting processes, and the majority of studies have been performed on their chemical properties and nutrient content (Fataei and Hashemimajd 2012).

Doan et al. (2013) found that the vermicomposting caused more changes in buffalo manure than composting, and that the vermicompost was rich in N-containing compounds, and polysaccharides were reduced to a greater extent; in addition, changes in lignin compounds were higher in the vermicompost. In a study by Vinceslas-Akpa and Loquet (1997), the amount of compounds derived from lignin decomposition was higher in vermicompost, which led to a decrease in the C/N ratio and a high percentage of nitrogen in the vermicompost. The level of dissolved organic carbon was significantly increased during vermicomposting due to the decomposition of organic matter into finer particles with higher water-solubility as well as increased microbial activity. In a study by Mago et al. (2021) on the qualitative characteristics of vermicompost made from banana crop waste biomass blended with the cattle manure as a bulking agent, it was found that the earthworm activity significantly reduced the pH, total organic carbon (TOC), C/N ratio and carbon/phosphorus ratio in the waste biomass, whereas macronutrients and micronutrients increased after vermicomposting.

Sharma and Garg (2017) investigated the management of food and vegetable processing waste spiked with buffalo manure using *E. fetida* and found that nitrogen content, total phosphate content and total potassium content in the harvested vermicompost increased significantly compared to the raw materials. There was also a significant decrease in pH, TOC and organic matter. Moreover, the concentration of metals, including Fe, Cu, Zn and nickel (Ni), was higher in all vermicompost treatments produced from different raw materials. Data on earthworm growth and reproduction indicated that the highest increase in earthworm biomass and fertility was observed in the mixture of food and buffalo manure and the lowest took place in the mixture of vegetable and buffalo manure.

Due to the annual production of large amounts of vegetable waste, carrot pulp and sawdust, improper disposal of these residues results in the generation of high amounts of leachate at the landfill and their penetration into the environment. Accordingly, the present study aims to investigate the feasibility of converting carrot pulp, potato crop waste, vegetable waste and sawdust into valuable compost fertilizer using the vermicomposting method, and subsequently compare the quality of the harvested composts as well as determine the optimal compost fertilizer and the most suitable substrate for earthworm reproduction.

Materials and methods

Experiment design and setup

The study was carried out in one of the breeding halls of *E. fetida* and production of vermicompost fertilizer via basket method in Tonekabon County with coordinates 36°44'03.2"N 50°58'31.9"E, Mazandaran Province, northern Iran in 2021. A full factorial experiment design with two factors was used where the first factor was a combination of four types of wastes together with a control at nine levels and the second factor was their concentration at two levels (100 and 150 g) based on completely randomized design (CRD) in triplicate. The main substrate of earthworm growth and reproduction was cattle manure that after being dried was poured into boxes measuring 30 cm (long), 40 cm (wide) and 60 cm (wide). The number of earthworms was used at a ratio of about 10 worms per 100 grams of waste (Rostami et al. 2008). To prevent the possibility of earthworm extinction in different substrate compositions, the worms were added to the studied treatments in three periods with an interval of three weeks, so that 35 adult *E. fetida* earthworms were inoculated into each box. To prepare the main substrate for the worms, the manure was first dried at 65°C for 48 hours, then ground and subsequently passed through a 0.5-mm sieve. After that, 300 g of dried manure with a ratio of 3 to 1 and 3 to 2 was used as the main substrate for each treatment.

The treatments used with the main substrate (300 g of cattle manure) for earthworm breeding at two levels of 100 and 150 g were as follows:

1-	Control	0	0
2-	Potato (P)	100 g	150 g
3-	Carrot pulp (C)	100 g	150 g
4-	Vegetables (V)	100 g	150 g
5-	Sawdust (S)	100 g	150 g
6-	Potato, carrot pulp, vegetables, sawdust (PCVS)	25 g of each	37.5 g of any of the compounds (PCVS)
7-	Potato, carrot pulp, vegetables (PCV)	33.33 g of each	50 g of any of the compounds (PCVS)
8-	Potato, sawdust, carrot pulp (PSC)	33.33 g of each	50 g of any of the compounds (PCVS)
9-	Carrot pulp, sawdust, vegetables (CSV)	33.33 g of each	50 g of any of the compounds (PCVS)

The experiment was conducted in a greenhouse with dimensions of 4 × 6 m. During the experiments, the moisture content of the substrate was maintained at about 70% and the temperature at 15-20°C. To raise this aim, a mechanical aeration method was applied during preparation through a pipe with a diameter of 10 cm in which several holes with a diameter of 5 cm were made. The pipe, which was connected to the air blower, was embedded under the platforms of the samples. The moisture content was maintained and controlled by irrigating the treatments using a drip irrigation system daily and in the early morning (between 8 and 10 am). The moisture content of the substrate was kept at about 70% as further increase in humidity increased the mortality rate of earthworms. Decreased humidity also reduced respiration and made it difficult for worms to move and perform other activities. In fact, the worms move and function easily in humid environments.

Sampling and measurements

Sampling was performed by combining different points of each treatment at the end of the 90-day compost processing period. The amount of sample was selected to be sufficient for all tests and was considered as an average of 5 g sample. Hence, after ripening the vermicompost, the samples were taken and transferred to the laboratory to measure the levels of chemical parameters of vermicompost produced in different treatments including pH, EC, C, N, C/N ratio, P, K, Ca, Mg, Fe and Cu. The elements in the produced vermicompost were measured in each of the treatments using the devices as shown in Table 1.

A property of the resulting vermicompost included pH was measured by a digital pH meter (Model 335, Systronics, India) and electrical conductivity was measured by an EC meter (Model 303, Systronics, India) using distilled water at a ratio of 10: 1 (w/w) after being shaken for 30 minutes and passed through Whatman Gr. 42 filter paper. Total organic carbon (TOC in%) was determined by weighing 1 g of each sample and stirring with 5 mL of distilled water for 24 h, followed by centrifugation at 4000 rpm for 30 minutes. The supernatant was passed through a 0.45-micron filter, and the measurement was performed by a TOC meter (Shimadzu 5000).

The Ca and Mg concentrations were measured by complexometric titration using EDTA. Potassium concentration was determined by flame photometer. To obtain the concentration of potassium present in the vermicompost extract, the extract was read by flame photometer without dilution and then the potassium concentration (in ppm) was calculated by placing the number read in the standard potassium equation. The concentrations of micronutrients including Fe and Cu were determined with the DTPA extractant and element concentrations were read via the Atomic Absorption Spectrophotometer (AAS) (Shimadzu 6200). Also, in the end of vermicomposting period, the number of cocoons was counted.

Table 1 Measured chemical parameters of the harvested vermicompost and analytical methods and instruments that were used

Parameters	Analytical methods	Device Number, Company, Country
pH-H_2O	pH meter	Model 335, Systronics, India
EC (mS/cm)	Conductivity meter	Model 303, Systronics, India
Total-N (%)	Kjeldahl method-(Alkaline Permanganate Method)	Kajaldal HANON model K1100, China
Total-P (mg/kg)	The colorimetric method using a spectro-photometer	HACH DR5000, USA
Total organic carbon (%)	Titrimetric using the Walkley–Black method	Dry combustion method
$CaCo_3$ (ppm)	The colorimetric method using a spectro-photometer	HACH DR5000, USA
Total-Mg (ppm)	Atomic absorption spectrophotometer (AAS).	Thermo Fisher. Model iCE 3000 Series AA System
Total-K (ppm)	Flame photometrical	XP model, BWB UK
Total-Fe (ppm)	Atomic absorption spectrophotometer (AAS).	Thermo Fisher. Model iCE 3000 Series AA System
Total-Cu (ppm)	Atomic absorption spectrophotometer (AAS).	Thermo Fisher. Model iCE 3000 Series AA System
C/N ratio	Carbon/Nitrogen	

Reference: Authors

Data treatment and statistical analysis

Data were analyzed by SPSS version 22 and Minitab software version 16. Differences in the quality of produced compost were investigated by analysis of variance (ANOVA) at the level of 1% probability ($P<0.01$), 5% probability ($P<0.05$) and 10% probability ($P<0.1$). Normality of data distribution and equality of variances were assessed by Kolmogorov-Smirnov test and F-test, respectively. Cluster analysis and principal component analysis (PCA) were used to define the relationship among the group of substrate combinations in vermicomposting based on the measured chemical parameters. Data comparison was performed by Duncan's test and deemed statistically significant at $p < 0.05$. Cluster analysis is one of the multivariate analysis methods whose main purpose is to group vermicomposting treatments based on their qualitative characteristics so that vermicomposts with similar quality characteristics are placed in the same group. According to predetermined criteria, the grouping will be appropriate when in-group treatments have more homogeneity and inter-group treatments have more heterogeneity. Investigation of the relationship between nine substrate compounds (eight treatments + one control) for 13 measured parameters requires a correlation matrix of 13 × 9. On the other hand, it is necessary to check the quality of compost by including all parameters. Therefore, due to the large volume of data, cluster analysis was performed based on the dissimilarity matrix, grouping parameters and treatments, and using Minitab v.22 software. Cluster analysis was performed using Ward's minimum-variance method based on the minimum squared Euclidean distance after data standardization. The treatments were classified using the mean quantitative (number of worms and cocoon) and qualitative (physicochemical indices) parameters measured in each treatment in triplicate.

Results and discussion

Analysis of variance test and comparison of mean data

The effects of different combinations of organic wastes were studied on the changes in number and reproduction rate of cocoon and earthworm. Table 2 present the results of ANOVA test for the number of cocoon and worms and physicochemical properties of compost resulted from the activity of *E. fetida* in different treatments. The ANOVA table of data (Table 2) showed a significant difference at the level of 1% probability ($P<0.01$) for C content and C/N ratio, at the level of 5% probability ($P<0.05$) for calcium (Ca) and magnesium (Mg) levels for the treatment × level interaction for Fe and C parameters (Table 2). To compare the mean data, the maximum probability level of judgment regarding their significance is considered to be 5%, but the probability level of significance can be considered exceptionally up to 10% in some cases due to the high importance of the studied parameter.

The ANOVA test results indicated that the diversity of substrate combinations had no significant effect on the rate of earthworm reproduction during the vermicomposting process due to the diversity of nutrient compositions. However, Garg et al. (2005) also reported a difference in the number of cocoons when fed with different substrates in their experimental results. It was also observed that changes in the type of substrate for vermicomposting partly had an effect on the quality of harvested vermicompost, so that the concentrations of C, Ca, Mg and C/N ratio varied in the vermicompost produced from the different substrates. Aynehband et al. (2019) found that the type of wastes used for vermicomposting had a significant effect on the chemical parameters of N, P, K, sodium (Na), pH, EC, C and C/N ratio at the level of 1% probability.

In the present study, the mean comparison test was performed using Duncan's method to encode the mean value of the treatments by the letters so that the treatments with identical letters do not differ significantly. Due to insignificant differences in some traits between the studied treatments, further analysis was performed via the mean comparison using Duncan's method at the level of 5% probability. Table 3 presents the results of comparing the mean data from the number of worms and cocoons as well as qualitative parameters (pH, EC, C, N, P, K, Ca, Mg, Fe and Cu) measured in the final compost produced.

The results of mean data comparison revealed the individual effects of experimental treatments on the chemical parameters of the final vermicompost (Table 3). The maximum number of cocoons was observed in the treatments 5 (sawdust) and 6 (potato, carrot pulp, vegetables, sawdust) with the means of 605 and 552.5, respectively, and the minimum number of cocoons in the treatment 4 (vegetable) with the mean of 185.

Based on the mean data comparison, the highest C content in vermicompost was obtained from PCVS (31.66 mg/kg) and the lowest values in PCV (22.77 mg/kg) treatments. The results of mean comparison revealed that the highest C/N ratio in the harvested vermicompost was related to PVSC (19.66) and S (19.60) treatments, respectively. The C/N ratio below 20 has been reported to be an appropriate marker for vermicompost maturation (Srivastava et al. 2020). On the other hand, the C/N ratio is of particular importance for plant growth because the plants are unable to absorb mineral nitrogen unless the ratio is 20:1 or less (Biabani et al. 2018). As mentioned, the maximum number of both cocoons (n=605) and worms (n=426) were found in sawdust treatment. This can be explained by the fact that earthworms require carbon to provide energy for new cells (Mehrjo et al. 2014), and that sawdust treatment was suitable for providing the required C for the growth and reproduction of cocoons and worms.A comparison of the mean Ca content in the final product of vermicompost displayed that the highest Ca content, behind the control treatment, was related to PSC treatment (11835.04 mg/kg). The highest Mg content (5117.00 mg/kg) was found in PCV treatment. Ramnarain et al. (2019) found no significant difference in Mg content between treatments. According to Edwards et al. (2011), the presence of Ca and Mg in the final vermicompost indicates its high nutritional value.

Table 2 The resuls of ANOVA test for the number of cocoons and worms counted and qualitative parameters measured in different treatments

Source	**Cocoon**			**Worm**			**Fe**		
of Variation	Ms	F	Sig	Ms	F	Sig	Ms	F	Sig
Treatment	44012.478	1.650	0.155	39231.142	1.658	0.153	4770.232	1.629	0.161
Level	2796.785	0.105	0.748	468.796	0.020	0.889	2178.234	0.744	0.394
Treatment × level	13432.985	0.503	0.825	14056.770	0.594	0.756	5566.334	1.901	0.100
Error	26680.780			23656.051			2927.428		

Source	**Cu**			**Ca**			**Mg**		
of Variation	Ms	F	Sig	Ms	F	Sig	Ms	F	Sig
Treatment	132.564	1.239	0.309	270046.916	1.548	0.185	400401.442	1.169	0.346
Level	30.448	0.285	0.597	652546.248	3.741	0.051*	1377500.251	4.023	0.053*
Treatment × level	96.628	0.903	0.515	229745.268	1.317	0.272	388275.953	1.134	0.366
Error	106.980			174451.826			342412.827		

Source	**K**			**P**			**N**		
of Variation	Ms	F	Sig	Ms	F	Sig	Ms	F	Sig
Treatment	3091233.875	0.874	0.537	238778.074	1.633	0.160	0.038	1.286	0.287
Level	4379658.241	1.239	.274	17101.130	0.117	0.734	0.002	0.052	0.821
Treatment × level	5080977.585	1.437	0.223	263793.745	1.804	0.119	0.001	0.044	1.000
Error	3535853.757			146244.747			0.029		

Source	**C**			**C/N**			**EC**			**pH**		
of Variation	Ms	F	Sig	Ms	F	Sig	Ms	F	Sig	Ms	F	Sig
Treatment	18.421	2.194	0.00**	6.239	1.563	0.000**	1.406	2.079	0.737	0.142	1.399	0.238
Level	14.330	1.707	0.200	3.728	0.934	0.460	0.000	0.000	0.984	0.089	0.884	0.354
Treatment × level	2.662	0.317	0.006**	0.759	0.190	.5470	0.098	0.145	0.994	0.036	0.360	0.919
Error	8.394			3.991			0.678			0.101		

Reference: Authors

Table 3 Comparison of the mean number of cocoons and worms as well as chemical parameters measured in vermicomposting among different treatments

Treatments		**Cocoon**		**Worm**		**Fe**		**Cu**
1(Control)	c	177.33	ab	176.67	a	7400	b	31
2 (P)	a-c	396	ab	230.89	b	648.98	a	94.30
3 (C)	a-c	371.89	ab	195.11	b	644.68	a	90.93
4 (V)	c	185	b	58.5	b	649.14	a	87.82
5 (S)	a	605	a	426	b	640.95	a	85.05
6 (PCVS)	ab	552.5	ab	373.5	b	654.44	a	86.68
7 (PCV)	bc	240.5	b	61.5	b	660.92	a	92.89
8 (PSC)	a-c	345	ab	166	b	681.50	a	87.83
9 (CSV)	a-c	401	ab	222	b	656.85	a	86.68

Treatments		**Ca**		**Mg**		**K**		**P**
1(Control)	a	21854.00	c	1520.00	b	1437.00	a	7980.00
2 (P)	bc	11219.06	ab	4018.33	a	11652.67	b	3004.89
3 (C)	bc	11513.26	ab	4454.78	a	11338.11	b	3153.39
4 (V)	C	10869.85	b	3780.00	a	11028.00	b	2988.50
5 (S)	bc	10868.85	b	3919.00	a	11028.00	b	3387.00
6 (PCVS)	bc	11396.25	ab	4219.00	a	11984.00	b	3133.50
7 (PCV)	bc	11391.65	a	5117.00	a	13432.00	b	3450.00
8 (PSC)	b	11835.04	ab	4122.00	a	10726.00	b	3150.00
9 (CSV)	bc	11255.30	b	3697.00	a	10082.50	b	3114.00

Treatments		**N**		**EC**		**pH**		**C**		**C/N**
1(Control)	a	1.81	ab	13.40	a	7.6	a-c	26.83	b	14.79
2 (P)	a-c	1.54	ab	13.46	a	7.67	a-c	26.04	ab	16.98
3 (C)	a-c	1.59	a	13.62	ab	7.61	a-c	28.36	ab	17.84
4 (V)	ab	1.78	a	13.85	ab	7.61	ab	30.73	ab	17.19
5 (S)	c	1.31	b	11.99	a	7.65	bc	25.75	a	19.60
6 (PCVS)	a-c	1.61	a	14.15	ab	7.25	a	31.66	a	19.66
7 (PCV)	a-c	1.61	a	14.85	b	7.00	c	22.77	b	14.20
8 (PSC)	a-c	1.52	a	13.91	ab	7.35	a-c	27.17	ab	17.86
9 (CSV)	bc	1.45	a	13.60	ab	7.55	bc	25.56	b	17.56

Treatments with identical letters within the same column indicate no significant differences (P = 0.05).
Refrence: Authors

Based on the mean data comparison (Table 3), the highest Fe content (7400 µg/kg) was seen in the control treatment. The ANOVA test results in a study by Alikhani et al. (2011) on the levels of Fe, Zn and Cu micronutrients in vermicomposts obtained from treatments with cattle manure substrate and different ratios of plant residues showed that the mentioned micronutrients were not significantly different between various treatments. They explained that earthworm activity can remove micronutrients from the reach of leaching and can convert these elements into various usable plant forms. The results illustrated that the composition of vermicompost in different treatments was very different compared to the control. Fe content was higher in the control treatment (Table 3), while the highest amount of nutrients needed by the plant (such as N, Mg, K and Cu) was found in other treatments. This indicates the effect of vermicomposting on the quality of harvested vermicomposts by increasing its quality for use in agricultural lands as an organic modifier and soil enrichment. High levels of N, Mg and K in organic fertilizers are one of the methods of soil enrichment with this type of fertilizer (Sharifi et al. 2019).

The results of cluster analysis

Cutting a dendrogram based on the farthest Euclidean distance divided the different combinations of production substrates into three groups according to the different combinations of production substrates Fig. 1 shows the dendrogram obtained from cluster analysis of different substrates in terms of the measured parameters.

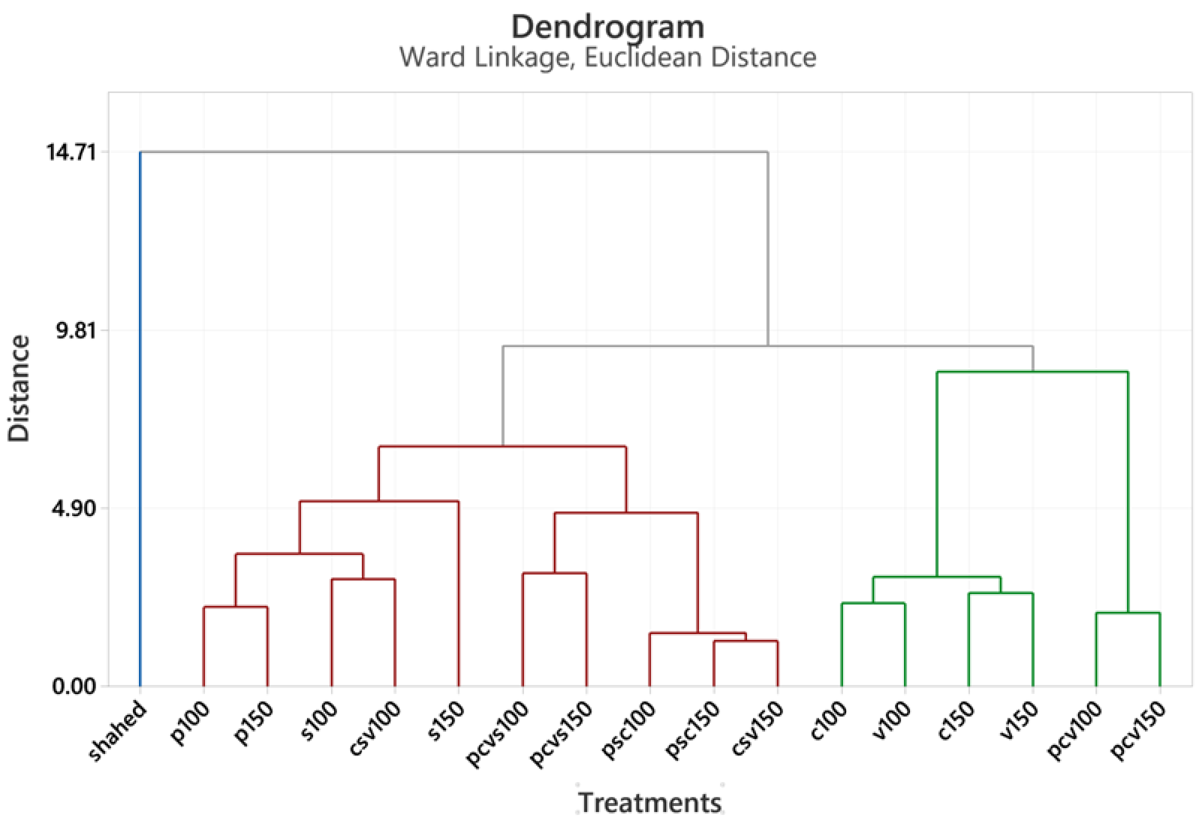

Fig. 1 Dendrogram obtained from cluster analysis of vermicompost produced on different organic waste substrates based on PC1 and PC2 values for quantitative and qualitative parameters

Reference: Authors

The first group (first cluster) included control treatment. This substrate had higher levels of pH, EC, P, Ca, N and Fe than the total average, and had lower levels of C, K, Mg, Cu, and the number of worms and cocoons than the total average (Table 4).

Table 4 The biochemical composition of the initial mixture (DM basis %) (n = 3)

Clusters	Statistical parameters	Fe	Cu	Ca	Mg	K	P	N	EC	pH	C	C/N ratio	Worm	Cocoon
1	$\bar{x}$	7400.00	31.00	21854.00	1520.00	1437.00	7980.00	1.81	13.40	7.67	26.72	14.79	177.33	176.70
2	$\bar{x}$	657.01	90.06	11347.38	4198.18	11376.71	3143.46	1.48	13.29	7.52	27.03	18.23	323.23	155.66
3	$\bar{x}$	647.70	85.90	11205.60	4069.00	11771.00	3260.25	1.69	13.09	7.63	27.54	16.23	578.75	399.75
Mean	Total	1404.16	82.57	12483.27	3871.90	10359.92	3706.81	1.66	13.26	7.61	27.10	16.42	363.80	212.24
One-way ANOVA test results		**	**	**	**	**	**	**	**	**	**	**	**	**

* and ** = Significance between clusters at the level of 5% and 1% probability, respectively.
Reference: Authors

The second group (second cluster) consisted of eight treatments, including P (at two levels), S (at two levels), V (150 kg), PCVS (at two levels) and CSV (100 kg). These substrates used for vermicomposting had higher levels of C/N ratio, EC, K, Mg and Cu than the total average and had lower levels of other parameters than the total average.

The third group (third cluster) contained the remaining eight treatments including C (at two levels), V (100 kg), PCV (at two levels), PSC (at two levels) and CSV (150 kg). This cluster had higher levels of C, pH, N, K, Mg, Cu and number of worms and cocoons than the total average.

Overall, it can be stated that the order of the residual substrates included the third, second and first groups, respectively, based on the nutritional requirements of soil and plants and the number of worms and cocoons. One-way ANOVA test results confirmed a significant difference between the groups in terms of most of the studied parameters at the levels of 1 and 5% probability (Table 4). The analysis of differences between the groups suggests that the treatment substrates within each cluster were not significant in terms of the evaluated parameters, while there was a significant difference between the clusters in terms of most of the evaluated parameters at the levels of 1% and 5% probability.

Principal component analysis (PCA)

The PCA is another multivariate analysis technique for analyzing large volumes of data. The purpose of these analyzes is to reduce the amount of data so that the reduced data can cover almost all of the information aspects of the original data. In other words, the purpose of PCA is to describe a set of dependent data as a set of independent variables that are a linear combination of the principal variables. In this method, the components are obtained in such a way that the first component justifies the greatest amount of data changes and the second component in the next order and to the last. In this case, the variability in the source of variation (such as substrates used for vermicomposting) can be justified by a small number of principal components (PCs) with significant variances. An important aspect of using PCA in research, like this project, is that the PCs can be applied as metrics to represent different aspects of data. To this end, if a small number of components justify more than 70% of the total change, the PC can be identified based on the eigenvector coefficients of each PC (for each parameter), to determine which parameters have the greatest impact on the process of change in the source of variation in different combinations of substrates used for vermicomposting. It should be noted that the number 70% is conventional and variable for various designs with different susceptibilities. The PCA of the measured parameters in different substrates used for vermicomposting was performed based on the table of correlation coefficients of 9 × 13 (9 treatments and 13 parameters) obtained from the average of data from triplicate measurements for nine different substrates. Table 5 shows the results of the analysis of principal components including eigenvectors, eigenvalues, relative and cumulative variance of the principal components of the studied parameters. In the PC_1 and PC_2, the coefficients related to the parameters of C, C/N ratio, Fe, Cu, Ca, Mg, K, P and N, as well as the number of worms and cocoons were almost high, indicating the greater influence of these parameters in creating differences between different treatments in vermicomposting.

It can be seen that most of the parameters with almost the same coefficients appeared in PC_1, indicating a greater role of PC_1 in the existence of differences between different treatments.

The eigenvalues of PCA (Table 5) indicated that the high coefficient parameters in the first three components explain more than 90% of the differences between various treatments, so that PC_1 and PC_2 with the eigenvalues of 7.27 and 3.54, respectively, justified a total of 83% of the qualitative changes between the treatments. The share of each of PC_1 and PC_2 was 56 and 27%, respectively. PC_3 had a minor contribution in justifying the qualitative changes of the substrates used for vermicomposting. Ebadi et al. (2007) examined the growth and reproduction of *E. fetida* in substrates of different agricultural and industrial wastes and found that the first two components explained 88.92% of the differences between different treatments. A comparison of the coefficients of each parameter (eigenvector coefficients) for PC_1 and PC_2 revealed that the first component had the highest contribution to model changes, indicating the susceptibility and large differences between substrates due to the parameters of Fe, Cu, Ca, Mg, K, P and N. The negative coefficients mean that if the value of the parameter with a negative coefficient increases, the susceptibility and the difference between the treatments due to that parameter will decrease. The positive coefficients indicate that if the value of a parameter with a positive coefficient increases, the susceptibility and the difference between the treatments of various combinations of different substrates will increase. High susceptibility means a large difference between values in various substrates; this indicates a difference in the quality of vermicompost produced in each substrate. The treatments 5 (S) and 6 (PVSC) had the highest value of PC_1, indicating the highest susceptibility of the quality of this substrate to the essential nutrients for plant nutrition.

Fig. 2 shows the Biplot graph for PC_1 and PC_2 values along with the cluster analysis of substrates used for vermicomposting (treatments) based on PC_1 and PC_2 values for the measured parameters.

Table 5 Eigenvectors of parameters in principal component analysis and rotation matrices of factor analysis along with eigenvalues and percentage of their variance explained

Variables	**Principal component analysis**		
	PC_1	**PC_2**	**PC_3**
Number of cocoon	0.194	0.419	-0.078
Number of worm	0.062	0.500	-0.083
Fe	-0.363	0.035	0.185
Cu	0.359	-0.089	-0.183
Ca	-0.361	0.024	0.212
Mg	0.350	-0.121	0.172
K	0.366	-0.046	0.008
P	0.280	0.336	0.041
N	0.337	-0.085	-0.008
EC	-0.141	0.269	-0.660
pH	-0.261	-0.018	-0.468
C	0.081	0.421	0.107
C/N ratio	-0.157	0.422	0.420
Eigenvalues	7.2718	3.5395	0.9284
Variance percentage	0.559	0.272	0.071
Cumulative percentage	0.559	0.832	0.903

Reference: Authors

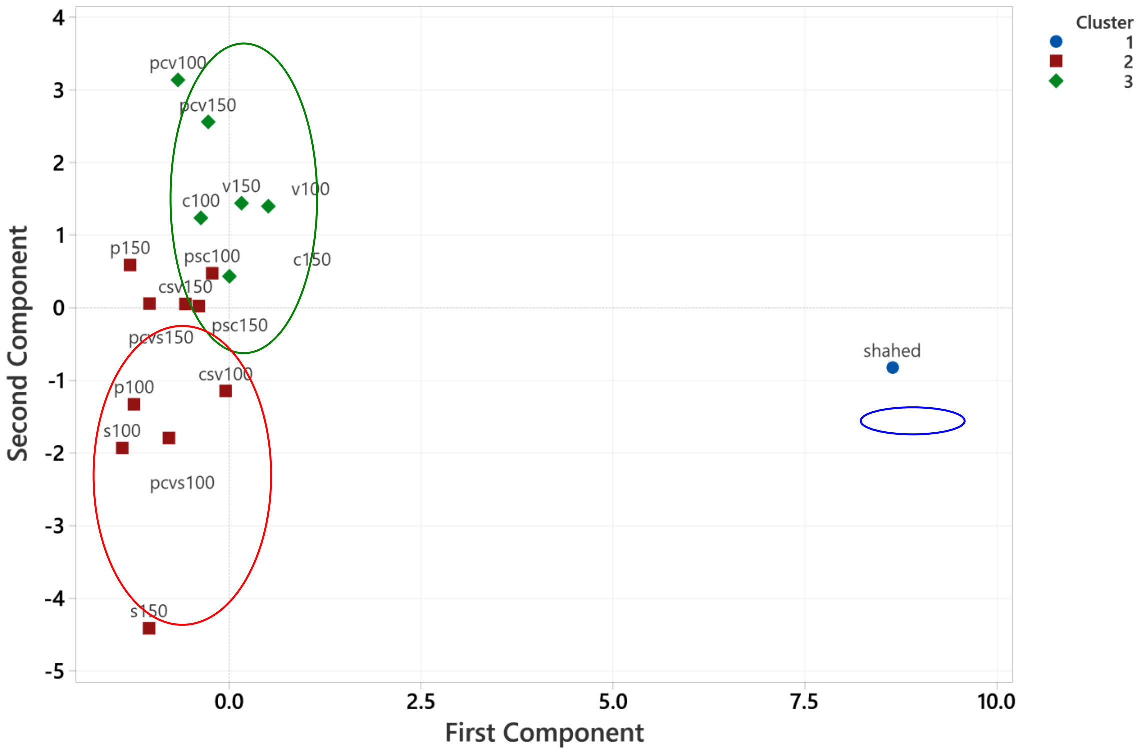

Fig. 2 Dispersion of different substrates under study (Biplot) based on the first and second components of principal component analysis in vermicomposting from various organic wastes

Conclusion

The results of this study demonstrated that the main supplemental substrates blended with animal manure (as the main substrate) were the treatment PCVS (potato, carrot pulp, vegetables and sawdust) and the treatment S (sawdust), which provided the best conditions for the growth and reproduction of earthworms and vermicomposting with suitable qualitative characteristics. In our study, the dispersion of the treatments obtained in the principal components indicated that the cluster analysis was consistent with PCA. So that the two-dimensional representation of different substrates based on the first two principal components confirmed the grouping of the results obtained from cluster analysis and principal component analysis, and thus the studied substrates were separated in the principal component analysis similar to the cluster analysis. By applying cluster analysis and principal component analysis methods, the nine substrate combinations were grouped into three clusters with similar qualitative characteristics. The principal component analysis divided the vermicomposts produced on different substrates into three different groups based on the studied qualitative parameters. The first two components of the principal component analysis revealed that the major parameters responsible for the qualitative changes in the vermicompost produced were iron, copper, calcium, magnesium, potassium, phosphorus and nitrogen.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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