

Article

Vermicomposting of Lavender Waste: A Biological Laboratory Investigation

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Abstract: In the present work, lavender waste, a residue of the essential oil extract industry, was used to feed *Eisenia andrei* with mature horse manure at ratios of 0:100, 25:75, 50:50, 75:25 and 100:0 on dry weight basis. Vermicomposting was carried out for 70 days in laboratory conditions. Biological parameters such as population build-up, total biomass, mortality and cocoon production were observed and measured. Increasing concentrations of waste affected positively the growth and reproduction of worms in a significant way. The 100% lavender waste combination showed the best cocoon production and even tripled their biomass in the first week. A seed germination test was also made, where no evidence of toxicity was found. The germination index range was, in general terms, above 100. The results indicated that the earthworm *E. andrei* was able to transform lavender waste into compost and thus play a major role in industrial waste management and apply circular economy.

Keywords: essential oil extraction waste; agro-industrial residues; waste valorization; *Eisenia andrei*; sustainable agriculture



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1. Introduction

Aromatic plants have historically been of great importance for the production of essential oils. So much so, that up to almost a hundred families of angiosperms have been used to produce volatile oils [1]. The industrial development of these essential oils has been accompanied by large quantities of agro-waste generation, of which stems, leaves and flowers are the main residues after extraction [2].

Today, huge quantities of agro-waste, generated from the volatile oil extraction industry or other agro-industries, end up illegally dumped or burned, are abandoned in the countryside or are buried in landfill because of the lack of effective ways for their valorization, provoking diverse environmental and socio-economic issues and governance challenges [3,4]. Thus, the development is required of any applications that can benefit from the use of the agro-waste generated from the volatile oil extraction industry [5]. In this way, those materials should be considered as new resources and not as useless materials [6]. This would contribute to reducing the economic and the environmental concerns of this industry and to the valorization of these organic valuable resources, in breach of the waste hierarchy in force in the EU Waste Framework legislation and in accordance with the basic principles of the circular economy, as well as contributing towards sustainable and climate-smart agriculture and reducing the global warming due to greenhouse gas emissions [6–10].

Other kinds of organic waste have almost always had a high potential for recyclability through low-cost aerobic techniques such as composting and other kindred methodologies (e.g., vermicomposting, larvaecomposting and so on), which transform them into a highly added-value natural fertilizer, called customarily compost. In terms of vermicomposting, certain species of earthworms have widely been used since the 1970s to break this organic

matter down and turn it into vermicompost [11]. To date, much livestock, agro-industrial, urban and industrial organic waste has been successfully converted into vermicomposting by using this well-documented technique [12–16]; nonetheless, as far as the essential oil industry is concerned, only a few studies have been carried out. Deka et al. [17] reported a vermicomposting study of java citronella from an aromatic oil yielding plant using *Perionyx excavates* showing an increasing nutrient content in the final product. Distillation waste of the citronella plant (*Cymbopogon winterianus*) was vermicomposted by Deka et al. [18], employing *Eudrilus eugeniae* with better results when cow dung was added. In both studies, vermicompost production was better in summer than in winter. More recently, Boruah et al. (2019) vermicomposted citronella bagasse and paper mill sludge mixture employing *Eisenia fetida*. They suggested the interesting aspect of blending citronella bagasse with other substrate as a way of putting an end to the biomanagement lacuna of said citronella bagasse [19].

An evident lack of knowledge available in the scientific literature on the use of the vermicomposting technique applied to essential oil extraction industry waste has been highlighted. The aim of the study was to evaluate the potential of lavender (*Lavandula h. 'Grosso'*) waste from an essential oil extraction industry to be decomposed through vermicomposting. For this reason, a laboratory investigation was carried out employing *Eisenia andrei* [20] worms blended with mature horse manure (HM). This investigation focused on earthworm bioindicators like earthworm weight, cocoon production, hatchling formation and sexual maturation of earthworms. Seed germination parameters were also considered to state the existence or not of toxic substances in the compost for plants.

2. Materials and Methods

2.1. Materials

HM, which was directly used, was collected in an Equine Center in the town of Labiano (Spain). Lavender (*Lavandula h. 'Grosso'*) was supplied by Montes de Cristal y Acero S.L. in the town of Olite (Spain), whose integrated business is based on agricultural production and subsequent extraction of essential oils from diverse plants. *Eisenia andrei* earthworms were purchased from Vermican Soluciones de Compostaje S.L., a local company specialized in composting in the town of Cordovilla (Spain).

2.2. Experimental Design and Vermicomposting

To design the experimental trial, an adaptation of a previous study by Elvira et al. [21] was carried out. The trial used 0.75 liter plastic cups filled with 200 g of waste without any kind of bed, besides eight young non-clitellated worms per cup. HM and lavender were mixed and subjected to vermicomposting in the concentrations of 0:100 (T1), 25:75 (T2), 50:50 (T3), 75:25 (T4) and 100:0 (T5) on a dry weight basis, as is shown in Table 1. Those five treatments were carried out in triplicate during 70 days in total. The combinations' moisture content was maintained in the range $70 \pm 10\%$ by spraying the surface with water once per week, whereas environmental temperature was constantly maintained at 22 ± 2 °C.

Table 1. Description of different treatments of horse manure (HM) and lavender waste used for the vermicomposting trial.

No. of Treatment	HM (%)	Lavender (%)
T1	0	100
T2	25	75
T3	50	50
T4	75	25
T5	100	0

2.3. Biological Analysis

Changes in each individual biomass of earthworm and newly produced cocoons were measured at weekly intervals in the plastic cups, following the method used by [22–24].

Earthworms and cocoons were separated from the parental waste mixture by a hand sorting method. Worms were washed with tap water, to remove adhered material from their bodies, and then weighed. After that, both were returned to their original test container. At the end of the experiment, worms, cocoons and hatchlings were taken out and put back in separate stock culture. A similar procedure was used by Bhat et al. [25], although in our experiment they only lasted 70 days. Biological parameters, such as number of alive and clitellated earthworms, total biomass, and number of cocoons and hatchlings, were measured every week.

2.4. Seed Germination

This test was considered for the assessment of the maturity of the vermicompost. Employing an identical method of seed germination assay published in earlier studies [26,27], 5 g samples from each combination at the age of 70 days were mixed with 50 g of deionized water. Fresh extract of each sample and deionized water in 1:1 proportion were poured into a Petri dish lined with filter paper. Deionized water was considered as control. Ten bread wheat (*Triticum aestivum* hybrid cultivar Hybiza) and Pardina lentil seeds were placed on the filter papers and were kept for 5 days under dark conditions at 25 °C. Each combination was performed in triplicate.

Germination percentage (GP), percentage of relative seed germination (RSG), relative root growth (RRG), relative shoot growth (RShG) and germination index (GI) were calculated by Equations (1)–(5), based on previous studies [26,28–37]:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds tested}} \times 100 \quad (1)$$

$$RSG = \frac{\text{Number of germinated seeds in vermicompost extract}}{\text{Number of germinated seeds in control}} \times 100 \quad (2)$$

$$RRG = \frac{\text{Mean root length in vermicompost extract}}{\text{Mean root length in control}} \times 100 \quad (3)$$

$$RShG = \frac{\text{Mean shoot length in vermicompost extract}}{\text{Mean shoot length in control}} \times 100 \quad (4)$$

$$GI = \frac{RSG \times RRG}{100} \quad (5)$$

To consider a positive seed germination, following US Environmental Protection Agency guidelines [38], a primary root ≥ 5 mm was used. This condition has already been used in prior studies [26,32,39].

2.5. Statistical Analysis

Statistical analysis was carried out using the R computer software package. One-way analysis of variance (ANOVA) was performed to analyze the significant differences between combinations during vermicomposting at 5% level of significance. Tukey's test was used to determine any significant differences between the combinations in the variables of interest involved. An identical germination assay analysis was carried out as well. The normality and homoscedasticity of variance for all the variables involved were corroborated in all the cases.

3. Results and Discussion

Parameters such as growth and reproduction are two bioindicators of interest in vermicomposting to attest to the efficiency of the process [40,41]. In the current study, the number of earthworms, cocoons, hatchlings and total biomass were measured.

3.1. Earthworm Development

According to Negi and Suthar [42], feed mixtures have influence in bioindicators of growth and activity of the earthworms. In this study, population growth, based on the

number and biomass of earthworms in the different combinations of lavender and HM, was significantly different (p -value < 0.05).

The number of earthworms started rising on week 7 for the combinations T1 and T4, and this went on until the last week of the experiment, as shown in Figure 1. In the T2 combination, the population of earthworms decreased a little on week 2, and then increased again on week 6, maintaining this trend until the end of the vermicomposting period. The T3 mixture showed that the number of earthworms increased on weeks 8 and 9, maintaining this value until the final week. The T5 combination followed a different trend though, since the population decreased until the 8th week, rising again on week 9 and keeping this value on the final week. Thus, the maximum value of the number of earthworms was observed on week 10 for the T1 combination (38.67 ± 19.14), followed by T2 (15.67 ± 14.36) and T4 (15.00 ± 6.25). The lowest values were observed for the T3 (8.67 ± 1.15) and T1 (5.67 ± 2.08) mixtures. The lowest value was reached by T1 (5.33 ± 1.15) on week 8. In the present study, it is not clear if the number of earthworms reached its maximum value at the end of the experiment. Nevertheless, a significant declining trend was observed in the T5 combination for almost all the vermicomposting period.

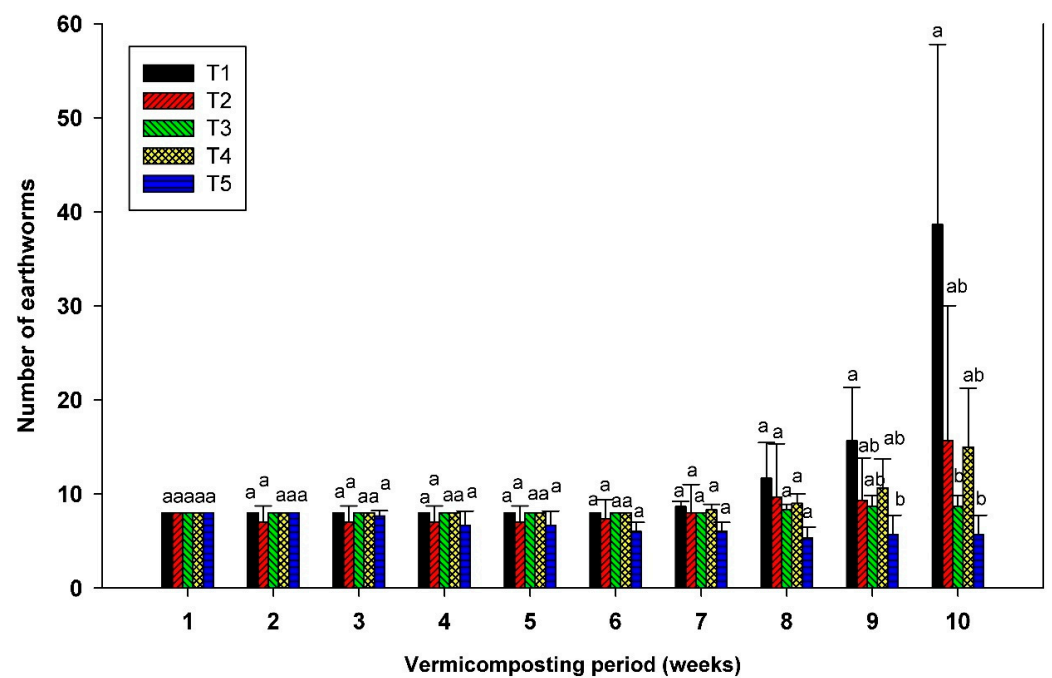


Figure 1. Mean \pm SD values of number of earthworms followed by different letters in the same week are different in terms of statistical significance (p -value < 0.05) between the combinations involved.

In addition, the highest values for the number of earthworms were reached in the combination containing the highest proportion of lavender. Regarding the earthworm biomass, there was a huge increase on week 2 with respect to the first one in all the cases, but the trend that the different combinations followed from that point forward varied considerably between each one. In the T1 combination, the earthworm biomass followed an oscillating and declining trend until week 8, to finally increase again until week 10. T2, T3 and T4 maintained an increasing trend overall for the earthworm biomass until the final week of the experiment, in which they yielded the maximum value for this parameter. Finally, the T5 mixture presented high oscillations in the earthworm biomass, its value being very similar in the final week compared to the first one. The values of the earthworm biomass for the T1, T2, T3, T4 and T5 combinations on week 10 were 3.37 ± 0.23 g, 2.43 ± 0.84 g, 2.67 ± 0.11 g, 2.51 ± 0.16 g and 1.21 ± 0.18 g, respectively. All the measured values are gathered in Figure 2. The highest values for the earthworm biomass were reached in T1,

followed by T2, T3 and T4, which yielded relatively similar values between them. Finally, the lowest values were once again yielded by the T5 combination which served as control.

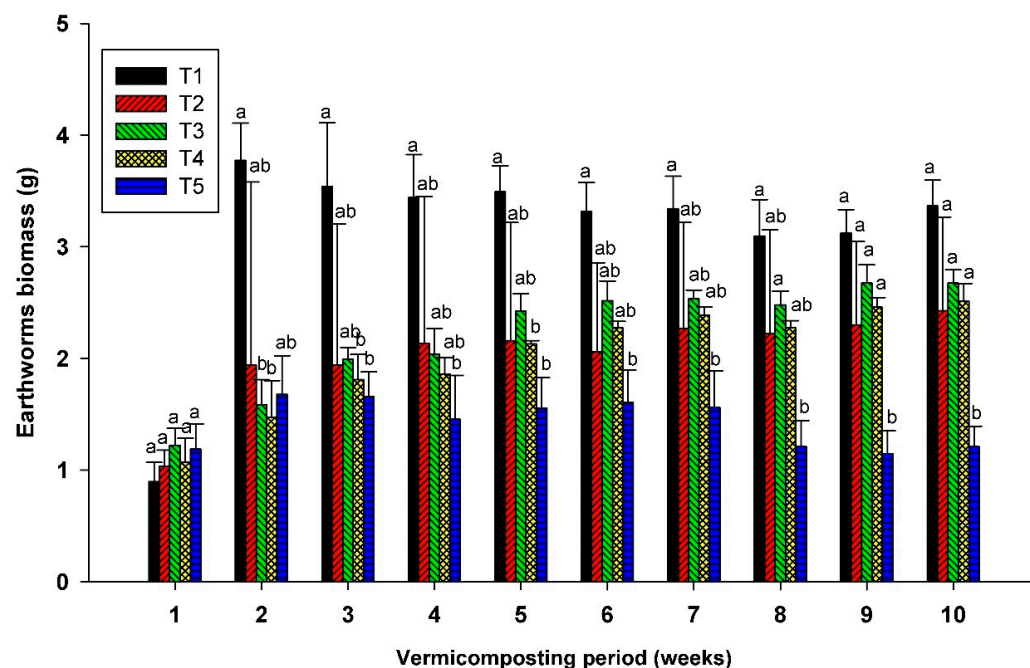


Figure 2. Mean \pm SD values of the earthworm biomass (g) followed by different letters in the same week are different in terms of statistical significance (p -value $<$ 0.05) between the combinations involved.

Historically, there have been poor results in mixtures composed of 100% by-product. For instance, Varma et al. [43], vermicomposting water hyacinth with three different kinds of earthworms, or Gong et al. [44], who obtained better results when garden waste was mixed with cattle manure and/or spent mushroom substrate than using garden waste alone. González-Moreno et al. [26] obtained appalling results using coffee silverskin and spent coffee grounds too. Suthar et al. [45], vermicomposting water lettuce spiked with manure, showed worse results with a higher quantity of waste. This fact indicates that lavender waste is initially an appetizing and non-toxic food for earthworms.

3.2. Cocoon Production

Edwards et al. [46] stated that cocoon production rates are directly related to the quality of the feed, which obviously depends on the type of waste. Figure 3 shows the cocoon production results. The number of cocoons produced (NC) by the earthworms in the present study was statistically different among the various combinations (p -value $<$ 0.05). The cocoon production started at week 2 in the T5 mixture, on week 3 in combinations T1 and T4, and on week 4 in the T2 and T3 combinations. In the T1, T2 and T3 mixtures, NC increased through the different weeks, reaching its highest value on week 10. In combination T4, NC followed an upward trend until week 8, in which the maximum value was reached, descending in the last two weeks. NC declined from week 4 to week 8 in the T5 mixture, being null at that moment and increasing again on week 9. The maximum value of NC was 33.00 ± 5.29 for the T1 combination on week 10, followed by combinations T2 (20.67 ± 19.14), T3 (20.00 ± 10.39) and T4 (9.00 ± 5.29). These combinations showed relatively higher values than the one yielded by the T5 combination (1.00 ± 1.73). The mixtures composed with low or null proportion of lavender yielded lower values for NC, especially the control composed entirely of HM.

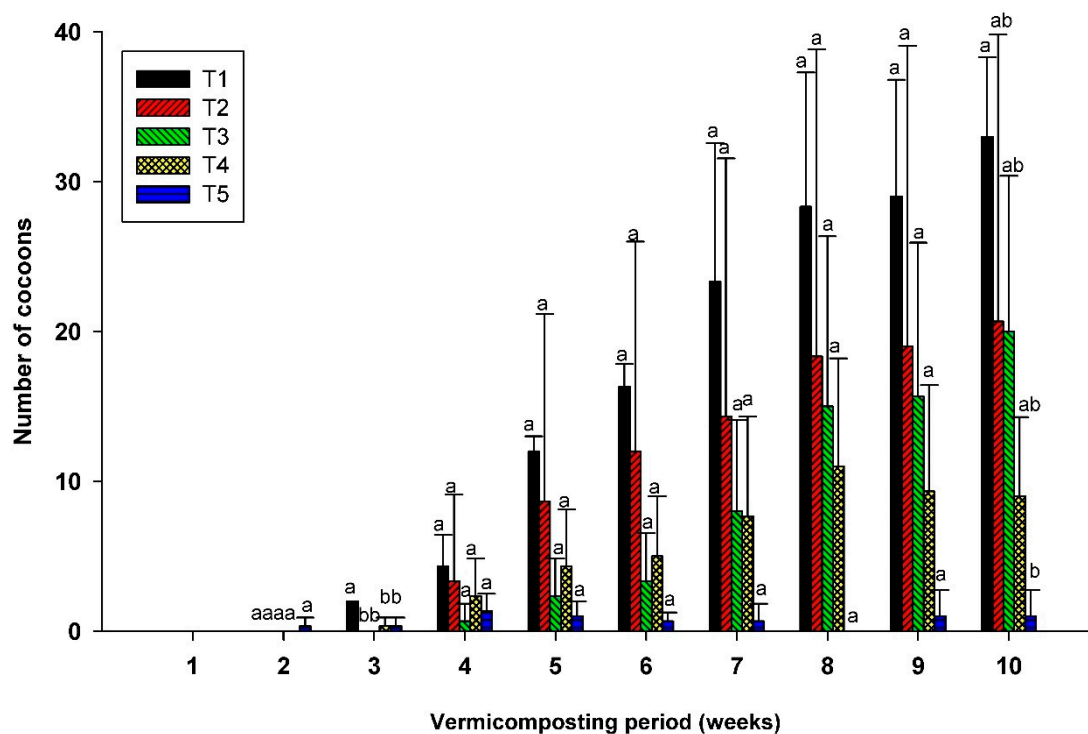


Figure 3. Mean values of the number of the cocoons produced by the earthworms followed by different letters in the same week are different in terms of statistical significance (p -value < 0.05) between the combinations involved.

Huge differences were showed by Domínguez et al. [47] employing *E. andrei* cocoon production in the sewage sludge and in the mixtures with the different bulking agents, with ratios between 0.05 and 3.16 cocoons/earthworm/week. In relative terms of cocoon production, all the results in this study are within the range aforementioned. T1 showed a maximum ratio of 2.75 ± 1.30 cocoons/worm/week, followed by T3 (2.23 ± 0.84), T2 (1.85 ± 1.23), T4 (1.17 ± 0.70) and T5 (0.25 ± 0.43). Frederickson et al. [48] obtained ranges between 0.46 and 1.56 cocoons/worm/week vermicomposting green waste.

Other values reported in earlier studies were 1.82 [49] or 2.14 [50] cocoons/worm/week. The results clearly illustrate that high lavender waste combinations showed better ranges that those aforementioned. However, the value of 3.08 cocoons/worm/week reported by Haimi et al. [51] was not reached.

3.3. Hatchling Formation and Number of Clitellated Earthworms

The numbers of hatchlings and clitellated earthworms were statistically different between the various mixtures (p -value < 0.05). Hatchlings appeared for the first time on week 6 in the T2 combination, on week 7 in the T1 and T4 combinations, and on week 8 in the T3 and T4 mixtures. All the results are shown in Figure 4. In all the cases the number of hatchlings increased until the final week, in which the maximum value was observed in the T1 combination (30.33 ± 19.22), followed by T2 (8.67 ± 13.32) and T4 (7.00 ± 6.25). The lowest values were reached in combinations T5 (1.00 ± 1.00) and T3 (0.67 ± 1.15). A higher number of hatchlings was found in mixtures with 100% lavender, whereas lower values were observed in the combinations without it. Similar results were observed by Bhat et al. [25] employing *E. fetida* in cattle dung and bagasse.

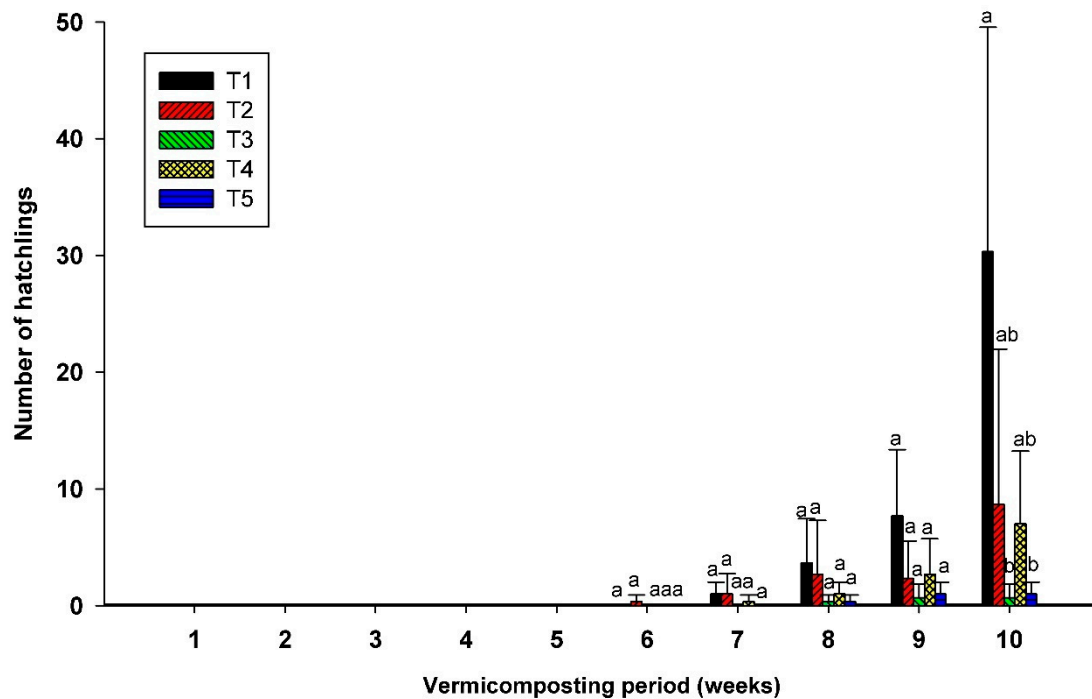


Figure 4. Mean \pm SD values of the number of hatchlings followed by different letters in the same week are different in terms of statistical significance (p -value $<$ 0.05) between the combinations involved.

Clitellum development is a less used but another interesting bioindicator which shows the degree of maturity of the worms. The first clitellated earthworms appeared on week 2 in all the cases (Figure 5). The trend was very oscillating in the different mixtures, although a general increase in the overall number of clitellated earthworms was observed, except for the T5 mixture. Higher values were yielded by combination T1, which reached its maximum value on week 10 (8.33 ± 0.58), followed by T3 (7.33 ± 1.15), T4 (5.67 ± 1.53) and T2 (5.33 ± 0.58). The lowest value was reached in T5 (0.67 ± 1.15). Once again, T1 yielded better results, followed by the other mixtures. Lower values were reached in the combinations composed of 100% of HM.

Except for the T5 combination, these data agree with previous studies like Elvira et al. [50] who reported that *E. andrei* acquired the clitellum first on day 24 in their study; on day 40 all individuals had the clitellum and seventy days were necessary for 50% of individuals of *E. andrei* to have a well-developed clitellum. Nogales et al. [52] reported 100% of clitellated worms after the 35-day incubation period in all except one of their combinations, employing *E. andrei* in cattle manure and different wastes. In this study, the combination of only lavender waste was also shown to have all clitellated worms on the 5th week (35 days), whereas T3 needed seven weeks. Frederickson et al. [48] observed that *E. andrei* fed on fresh waste were all clitellated after 6 weeks, whereas those fed material pre-composted for 2 weeks needed 7 weeks to reach maturity. Nevertheless, in the results of this research, it can be seen how not all the combinations were optimal enough to achieve sexual maturation in all the worms.

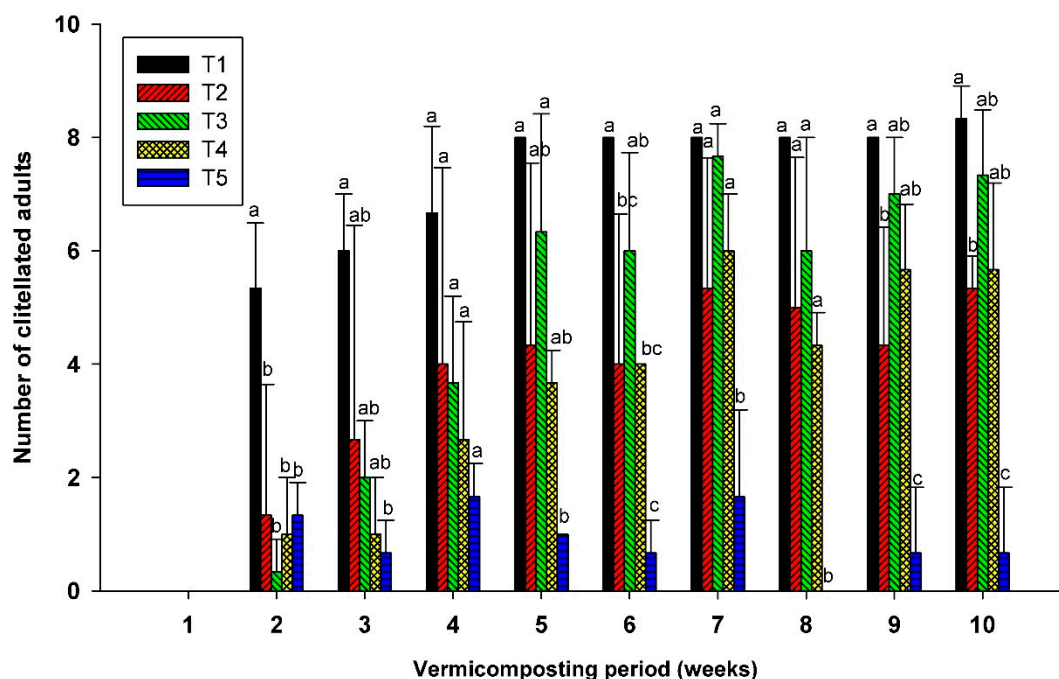


Figure 5. Mean \pm SD values of the number of clitellated earthworms followed by different letters in the same week are different in terms of statistical significance (p -value $<$ 0.05) between the combinations involved.

3.4. Mortality

In this study, no evidence of mortality of earthworms in lavender combinations was found. T1, T3 and T4 showed 100% survival, whereas T2 mortality rose to $12.5 \pm 21.7\%$ because of an anomalous mortality of 37.5% in one of the three samples. In T5 more deaths occurred ($29.17 \pm 26.02\%$), as the authors expected, as a consequence of using a manure that was already quite mature and from which the residue was drained with the passage of time.

Palatability and suitability of waste as earthworm feed may be displayed through another biological indicator: the mortality rate [25,53,54]. Hence, lavender waste can be said to have an excellent palatability to *E. andrei*.

3.5. Germination Results

Traditionally, the evaluation of the degree of maturity and the verification of the absence of phytotoxicity of composts for agricultural use has been carried out by means of a seed germination test [40]. Table 2 depicts the effect of the different combinations on germination parameters of bread wheat and lentil seeds. Specifically, the mean values and standard deviations (SDs) of the GP, RSG, RRG, RShG and GI are shown. According to Zucconi et al. [29] a GI of $\geq 80\%$ indicates the disappearance of phytotoxins in composts.

In the bread wheat seed assay, the ranks of the parameters GP, RSG, RRG, RShG and GI were (26.7 ± 5.8 – 46.7 ± 20.8 %), (100.0 ± 100.0 – 186.1 ± 144.9 %), (74.7 ± 31.0 – 266.2 ± 149.2 %), (52.8 ± 37.5 – 775.3 ± 644.6 %) and (70.4 ± 2.3 – 245.5 ± 91.4 %), respectively. Likewise, the trends associated with those parameters were: T4 > T1 > T5 > T3 > T2, T4 > T1 > T3 > T2 = T5, T2 > T1 > T5 > T4 > T3, T2 > T1 > T5 > T4 > T3 and T1 > T2 > T4 > T5 > T3, respectively. T1 yielded high mean values for all the germination parameters, as opposed to T3, which yielded low mean values in general.

Table 2. The effect of the different combinations of the lavender with HM on germination parameters of bread wheat seeds (A) and Pardina lentil seeds (B) is evaluated. Specifically, the mean values and SDs of the germination percentage (GP), relative seed germination (RSG), relative root growth (RRG), relative shoot growth (RShG) and germination index (GI) are shown. Different letters indicate significant differences between treatments ($p < 0.05$).

A					
Mean Values (and Standard Deviations) of Bread Wheat Seed Assay Germination Parameters					
Treatment	GP (%)	RSG (%)	RRG (%)	RShG (%)	GI (%)
T1	36.7 ± 11.5 ^a	130.6 ± 48.8 ^a	218.4 ± 129.7 ^a	584.0 ± 500.2 ^a	245.5 ± 91.4 ^a
T2	26.7 ± 5.8 ^a	100.0 ± 50.0 ^a	266.2 ± 149.2 ^a	775.3 ± 644.6 ^a	223.9 ± 116.1 ^a
T3	30.0 ± 10.0 ^a	105.6 ± 41.9 ^a	74.7 ± 31.0 ^a	52.8 ± 37.5 ^a	70.4 ± 2.3 ^a
T4	46.7 ± 20.8 ^a	186.1 ± 144.9 ^a	101.1 ± 51.0 ^a	156.8 ± 118.7 ^a	205.7 ± 225.1 ^a
T5	33.3 ± 30.6 ^a	100.0 ± 100.0 ^a	112.6 ± 139.4 ^a	326.4 ± 423.9 ^a	202.2 ± 292.2 ^a
B					
Mean Values (and Standard Deviations) of Pardina Lentil Seed Assay Germination Parameters					
Treatment	GP (%)	RSG (%)	RRG (%)	RShG (%)	GI (%)
T1	83.3 ± 5.8 ^a	89.6 ± 10.0 ^a	114.0 ± 29.8 ^a	145.2 ± 45.7 ^a	101.8 ± 26.6 ^{ab}
T2	96.7 ± 5.8 ^a	103.7 ± 6.4 ^a	208.1 ± 101.3 ^a	325.6 ± 193.3 ^a	215.3 ± 100.4 ^a
T3	90.0 ± 0.0 ^a	96.7 ± 5.8 ^a	108.7 ± 44.5 ^a	156.8 ± 112.0 ^a	106.7 ± 47.8 ^{ab}
T4	83.3 ± 11.5 ^a	90.0 ± 17.3 ^a	134.3 ± 22.1 ^a	164.8 ± 79.4 ^a	123.4 ± 40.8 ^{ab}
T5	63.3 ± 25.2 ^a	67.0 ± 22.8 ^a	91.6 ± 85.7 ^a	98.4 ± 115.7 ^a	56.7 ± 50.1 ^b

In the Pardina lentil seed assay, the ranks of the parameters GP, RSG, RRG, RShG and GI were (63.3 ± 25.2–96.7 ± 5.8 %), (67.0 ± 22.8–103.7 ± 6.4 %), (91.6 ± 85.7–208.1 ± 101.3 %), (98.4 ± 115.7–325.6 ± 193.3 %) and (56.7 ± 50.1–215.3 ± 100.4 %), respectively. The trends associated with those parameters were: T2 > T3 > T1 = T4 > T5, T2 > T3 > T1 > T4 > T5, T2 > T4 > T1 > T3 > T5, T2 > T4 > T3 > T1 > T5 and T2 > T4 > T3 > T1 > T5, respectively. T2 was the combination where the highest mean values were found for all the germination parameters, whereas T5 yielded the lowest values in all the cases.

The results showed that there are statistically significant differences between the combinations T2 and T5 for the germination parameter GI in the assay with Pardina lentil seeds. Nevertheless, there was no statistical evidence in the rest of the cases to consider that the differences between the mean values of the treatments were different (p -value > 0.05). This can be explained by the very high SD associated with the data.

In general, these results are above the values obtained in previous studies. Karak et al. [35], who employed wheat seeds (*T. aestivum* cv. PBW 3) besides Indian mustard (*Brassica campestris* cv. *Pusa jaikisan*), in a traditional municipal solid waste compost, reported 89–96 and 86.9–98.25% of GI respectively. In vermicomposting, Karmegam et al. [40] presented a study where GP and GI ranged from 86 to 98% and 279–360 for maize (*Zea mays* var. Co 6), and 84–98% and 285–372 for cowpea (*Vigna unguiculate* var. VBN), respectively. The best results in our study are similar to those published by González-Moreno et al. [26] employing hybrid wheat seeds, with ranges from 124.3% to 384.3% with coffee industry waste vermicomposts. Other studies, such as Zhang and Sun [55], who employed pakchoi (*Brassica rapa* L.) and had values of 72–162%, Che et al. [56], who observed 86% on day 20, or Unuofin and Mnkeni [57], who showed values of 98–162%, 48–81% and 51–128% for tomato, carrot and radish, respectively, showed lower values than those seen in this research.

4. Conclusions

The study shows that vermicomposting lavender waste employing the epigeic earthworm *E. andrei* was successful, obtaining a new value-added material in the form of compost. The results suggest that it can be used either on its own without any pre-treatment or mixed with manure independently, since no negative evidence has been observed in this regard. Moreover, a high palatability towards this residue can be stated.

Author Contributions: M.Á.G.-M.: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing—Original Draft, Writing—Review and Editing. B.G.G.: Methodology, Formal analysis, Investigation, Writing—Review and Editing. S.M.S.: Methodology, Investigation, Funding acquisition, Project administration. E.P.C.: Methodology, Data curation, Writing—Review and Editing, Supervision. A.S.M.: Conceptualization, Methodology, Investigation, Formal analysis, Supervision. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

GI	germination index
GP	germination percentage
HM	horse manure
NC	number of cocoons
PR	cocoon production ratio
RRG	relative root growth
RSG	relative seed germination
RShG	relative shoot growth
SD	standard deviation

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