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Effect of three different vermicomposts on germination of barley (*Hordeum vulgare*), ryegrass (*Lolium perenne*) and chives (*Allium schoenoprasum*).

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Abstract

Vermicompost is a potential soil fertilizer used in sustainable agriculture and its use can increase food resources. In this study, germination and growth of *Allium schoenoprasum* (chives), *Hordeum vulgare* (barley) and *Lolium perenne* var. Squire (English ryegrass) were evaluated in four different vermicomposts: grass clippings (vegetable), seaweed, chicken droppings droppings (chicken droppings) and undisturbed soil (soil) to see which of them gave the best results. The vermicomposts were obtained after three months of vermicomposting using the earthworm *Eisenia sp* .

Keywords vermicompost, germination, growth, chives, barley, reygrass

INTRODUCTION

The application of organic fertilizers is receiving a great deal of attention from the agricultural sector due to its impact on the improvement of soil properties, plant growth and even yield (Masmoudi et al. 2020); in this sense, vermicomposting processes favor the decomposition of the organic fraction of waste until obtaining a product rich in nutrients and with a potential use in agriculture without any adverse effect (Mupanbwa and Mnkeni, 2018); an important part of these vermicomposting processes are earthworms, which ingest the organic matter and defecate it increasingly fragmented, approximately 95% of what is ingested (Hossein et al. 2017), which increases the specific surface area for interaction with microorganisms (Orgiazzi and Bardegett, 2016). The food ingested by earthworms is mineralized in their digestive tract, depending on humidity, temperature and pH, and organic carbon is reduced by 8-24% (Hossein et al., 2017), but in addition, earthworms, which together with ants or larvae of some insects constitute the group of "soil engineers", perform important physical modifications due to the presence of galleries or feces, the so-called biogenetic structures (Lavelle, 1997; Brown et al. 2000). Earthworm activity accelerates the decomposition of plant debris, increasing the rate of nutrient transformation, favors the formation of aggregates, improves porosity, infiltration and aeration. For all these reasons, they constitute a potential resource of great interest in the sustainability of agriculture, as they actively participate in the regulation of soil physical properties (Doan et al. 2013), the dynamics of nutrients and growth regulating substances (Padhhiyar et al. 2017, Arancon et al. 2012) and consequently in the growth and development of plants (Hernández-Rodríguez et al. 2017)

One of the end products of these processes is vermicompost, a product that increases the bioavailability of nutrients favoring plant growth and seedling germination, thus acting as an organic fertilizer (Abernethy, 2017). It is a stable organic compound, homogeneous and rich in nutrients and microorganisms (David-Santoya et al., 2018; Coulibaly, 2021) thanks to a series of processes that occur in the digestive tract of earthworms (Hernández et al. 2010, Castillo et al. 2013). The excellent physical, chemical and biological properties of vermicompost make it a very good organic amendment with beneficial effects on germination (Nava-Pérez et al. 2018), which increases crop yields. On the other hand, it favors aeration, drainage, water retention capacity as well as the formation of a mixture of minerals with ion exchange capacity and growth accelerating substances, which allows to decrease production times (Coulibaly et al. 2016). Research also shows that vermicompost improves electrical conductivity compared to the starting material (Doan et al. 2015), relating this increase to the production of ions and minerals in the digestive tract of earthworms (Garg et al. 2006).

The importance of vermicompost in increasing agricultural productivity of many crops is well documented (Olle, 2016; Zaremanesh et al. 2017); in a study conducted on cotton and groundnut yield it was found that the application of vermicompost favored early seedling growth, increased root length and stem length due to the contribution of nitrate, phosphorus , soluble potassium or calcium, which caused an increase of 14.98% compared to control samples (Chavda and Rajawat, 2015), similar results were found for wheat (Kizilkaya, et al. 2012). There are also studies that evaluate the influence of vermicompost on both the germination and growth of legumes and vegetables and demonstrate beneficial effects in terms of improved germination and seedling development, as well as on the biomass of fruits produced (Lopes-Olivares et al. 2015; Hernández-Rodríguez et al. 2017, Nava-Pérez 2018).

The seeds chosen for this study were Barley, *Hordeum vulgare*, the fourth most cultivated cereal in the world behind wheat, corn and rice (FAO, 2018), which is used in both human and animal feed (US Grain Council, 2019) and even in the production of malt beverages (Ponce-Molina et al. 2019); Ray Grass, *Lolium perenne* var. Squire, which is one of the most widely used grasses in the world and has the ability to produce large amounts of biomass of good nutritional quality, making it an important source of animal feed (Alcántara, 2017); and chives, *Allium schoenoprasum*, very important in human food because of its content of vitamins A and C and folates(https://www.mapa.gob.es/es/ministerio/servicios/informacion/cebollino_tcm30-102511.pdf) as well as minerals such as Fe, Ca and Mg, have a high water content and very low in lipids (Alcántara, 2017)

The objective of the study was to evaluate the effect of different types of vermicompost (made from undisturbed soil, seaweed topsoil compost, chicken droppings droppings compost and grass clippings compost) on the germination and growth of barley, English ryegrass and chives.

MATERIAL AND METHODS

The vermicompost was obtained from the 4 substrates mentioned above, elaborated from undisturbed soil, seaweed compost, chicken droppings manure compost and grass cuttings compost (hereinafter soil, seaweed, chicken droppings and vegetable respectively), under different conditions: original substrates -without passing through the digestive tract of the worms- and the final substrates, after three months of vermicomposting during which all the containers were checked weekly -with manipulation- or no checking at all -without manipulation-..

Table 1 shows the main physicochemical parameters of the initial and final substrates, with and without earthworms, after three months.

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Substrate	% Ca	% Mg	% Na	% K	% Mn	% P	% MS	% N	% C	C/N	pH	CE (µS/m)
S_I	0,05	0,19	0,35	0,46	0,03	0,17	97,45	0,21	2,72	12,71	4,80	100,50
S_WFF	0,04	0,14	0,29	0,50	0,04	0,17	97,98	0,25	3,03	12,23	4,63	413
S_WF	0,04	0,14	0,22	0,48	0,04	0,16	95,48	0,26	3,15	12,18	4,44	420,5
A_I	1,31	0,14	0,33	0,15	0,01	0,59	84,61	1,87	37,34	19,97	6,52	166,00
A_WFF	1,11	0,14	0,23	0,14	0,01	0,55	97,98	1,83	38,92	21,3	5,45	757,33
A_WF	1,31	0,16	0,26	0,16	0,01	0,65	95,48	1,72	35,36	20,57	5,13	1121
C _I	0,53	0,25	0,29	0,54	0,06	0,27	94,76	0,77	9,59	12,4	6,10	1736
C _WFF	0,62	0,29	0,53	0,7	0,06	0,36	91,00	0,75	10,13	13,56	6,22	1696
C_WF	0,89	0,3	0,39	0,68	0,07	0,37	89,86	0,74	9,11	12,3	6,19	2062,33
V_I	0,61	0,23	0,29	0,55	0,06	0,29	91,75	0,75	14,37	19,16	5,17	---
V_WFF	0,3	0,12	0,43	0,67	0,06	0,36	93,31	0,84	16,49	19,56	4,98	225
V_WF	0,26	0,13	0,29	0,53	0,05	0,37	86,14	0,93	17,98	19,37	4,73	511

Table 1. Physicochemical parameters of the initial substrates (I) and worm-free finish (WFF) and worm finale (WF) (S: undisturbed soil; A: seaweed compost; C: chicken droppings manure compost; V: grass cuttings compost).

To determine the quality of the vermicompost, germination experiments were carried out with three different species (three replicates per treatment): *Allium schoenoprasum* (chives), *Hordeum vulgare* (barley) and *Lolium perenne* var. Squire (ray grass). The experiment was carried out in the Zooloxía laboratory (Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela).

The test was carried out in 150 ml cells placed inside trays; only one type of substrate was used in each tray (substrates without worm action, final substrates with worm action without manipulation and with weekly manipulation) and three cells for each plant species, which represents a total of 9 cells per tray; each plant species was planted in 36 cells distributed in 9 trays (Figure 1).

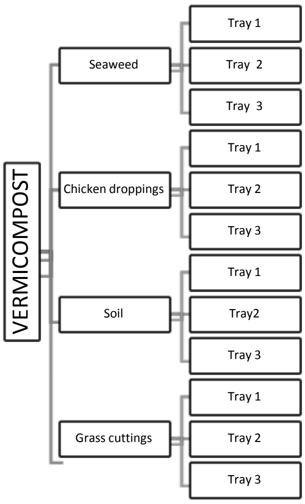

Figure 1. Distribution of trays for each substrate (1: final substrate without worms; 2: vermicompost without manipulation throughout the 3 months; 3: vermicompost with weekly

manipulation; 4: vermicompost with weekly manipulation; 5: vermicompost with weekly manipulation; 6: vermicompost with weekly manipulation).

Each alveolus was filled with 150 ml of substrate and 5 cm of water was placed at the bottom of the trays to moisten the substrate and avoid possible desiccation. Once all the containers were wet, the seeds (100) were placed in each alveolus, making a total, per tray, of 300 seeds of chives, 300 of barley and 300 of ray grass; on top of all, a thin layer of perlite was deposited to prevent the light from spoiling the seeds, as shown in photo 1.

Photo 1. Seed cells (without and with perlite)   Photo 2. Seed germination

Each day the water level of the trays was checked, and the trays were moved in order to achieve homogeneity in the growth and to try to make the plants grow as straight as possible (Photo 2).

After 30 days, the alveoli were emptied to count the germinated seeds and thus obtain the germination rate per species; the length of each shoot was also measured to check if there were differences between the four types of substrates. The length of each sprout was also measured to check if there are differences between the four types of substrates. Likewise, it was visually checked if the plants presented any type of deficiency (nutritional or growth deficiencies) depending on the substrate in which they were grown.

Prior to the germination tests, seed viability was tested by placing 100 seeds of each species in a Petri dish with distilled water for one week, in the dark and at 20-25 ºC (68-86ºF)..

The variables evaluated were seed germination after 30 days and plant length.

Chemical analyses of the initial substrates and vermicompost, with and without earthworm manipulation, were carried out in the USC Plant Production laboratory.

The data obtained were statistically analyzed with SPSS v.20 using analysis of variance (ANOVA) and to determine differences between means, a Tukey mean comparison was performed ($p \leq 0.05$).

RESULTS AND DISCUSSION

Regarding the physicochemical parameters, see Table 1, in general the highest values of each factor were in the vermicompost and not in the initial substrates; in view of the results, it was checked whether there were significant differences in the values of the different physicochemical factors in the different substrates (Table 2).

It can be seen that there are significant differences for Ca, N, C and pH values in all substrates, being the seaweed substrate the one with the highest values, except for pH, which reaches the best records in chicken droppings droppings; these results are very close to those obtained by other authors (Coulibaly et al. 2020). In the undisturbed soil and in the grass cuttings, the pH presented low values, as it corresponds to Galician soils mostly granitic (Barral Silva and Díaz-Fierros, 1996), and also may be related to the use of inorganic fertilizers and pesticides (Sradnick et al. 2013) over the years which would cause an accumulation of acid compounds in the soils.

N= 40	Seaweed	Grass cutting	Chicken droppings droppings	Undisturbed soil
% Ca	1.3±0.2**a**	0.3±0.1**d**	0.8±0.4**b**	0.04±0.01**c**
% Mg	0.2±0.03**b**	0.1±0.01**b**	0.3±0.1**a**	0.1±0.02**b**
% Na	0.3±0.1**b**	0.3±0.1	0.4±0.2**a**	0.3±0.1**b**
% K	0.2±0.03**c**	0.6±0.1**ab**	0.7±0.1**a**	0.5±0.1**b**
% Mn	0.01±0.0**c**	0.1±0.0**a**	0.7±0.01**a**	0.04±0.0**b**
% P	0.6±0.1**a**	0.4±0.1**b**	0.4±0.1**b**	0.2±0.02**c**
% MS	96.3±2.2**a**	88.5±6.9**b**	90.2±4.8**b**	96.3±2.2**a**
% N	1.8±0.2**a**	0.9±0.1**b**	0.7±0.1**c**	0.3±0.02**d**
% C	36.6±2.3**a**	17.5±2.6**b**	9.5±1.1**c**	3.1±0.2**d**
pH	5.2±0.2**b**	4.8±0.2**c**	6.2±0.1**a**	4.5±0.3**d**
CE (µS/m)	999.8±254.4**b**	415.7±172.8**c**	1940.2±254.1**a**	418.0±121.6**c**

Table 2. Significant differences ($p \leq 0.05$) between the different parameters (different letters indicate significant differences between substrates; lack of letters indicates no significance; DM: dry matter; EC: electrical conductivity).

On the other hand, regarding the C/N ratio, it can be observed that only in seaweed vermicompost the value of 20 is exceeded (although in vegetables it is quite close); lower values indicate an advanced degree of stabilization of organic matter, which would provide a good degree of fertility (Masmoudi et al. 2020).

As already mentioned in material and methods, viability tests were carried out beforehand on the seeds to be used; in these tests, 98% germination was achieved for barley, 95% for Ray Grass and 90% for chives, which shows that the seeds used were viable.

In Table 3 we can observe the germination percentages in the different substrates (final without worms; final with worms without manipulation; final with worms and with weekly revision) and it can be seen that, in the case of chives, they were very low, or null, which may be indicative that these seeds are sensitive to the presence of phytotoxic metabolites that can inhibit their germination capacity (Warman and AngLopez 2010) and cause nitrogen deficiencies, which causes competition between edaphic microorganisms and plant roots for nitrogenous compounds (Majlessi, 2012).

For the ray gras we can observe that, in the seaweed substrate with earthworms, the germination percentage drops considerably, which may indicate that there is some kind of reaction that prevents the seeds from thriving.

Substrate	Barley	Ray Grass	Chives
Seaweed_WFF	90.33	82.66	1,00
Seaweed_FNM	90,00	41.66	0.66
Seaweed_FYM	82.66	13.66	1,00
Chicken droppings_WFF	73.33	66.33	0.33
Chicken droppings_FNM	84.33	64.33	0,00
Chicken droppings_FYM	86.66	66.66	1.33
Soil_WFF	86.66	84.66	1,00
Soil_FNM	87.66	78.33	0,00
Soil_FYM	89.66	68,00	0.33
Grass cuttings_WFF	85,00	85,00	5,00
Grass cuttings_FNM	89.66	86.33	3.66
Grass cuttings_FYM	86.66	91,00	0.66

Table 3. Percentage of germination in the different composts (WFF: worm-free finish) and vermicompost (FNM: final with worms without manipulation; FYM: final with worms and with handling)

In order to test whether there were significant differences between treatments, tests were done to see what influenced growth more, the treatment or the manipulation. worm-free substrates, with worms, but without manipulation and with worms with manipulation). A two-way ANOVA was performed

In Table 4 we can see that both the treatment and the manipulation influence the final result, but if we observe the F values, the variable with the greatest influence in the three cases was the treatment, which indicates that the most important in germination are the substrates used (there are authors who point out that a higher germination rate obtained in vermicompost may be related to the capacity of these substrates to retain water, since water plays a very important role in the viability and maintenance of seeds which triggers their germination (Obroucheva, 1999)) and not the fact that they have been, or not, in contact with earthworms, although there are studies that show that earthworms decrease phytotoxicity by 50% which can cause an increase in germination (Masciandaro et al. 2010).

VARIABLE	Barley	Ray-Grass	Chives
Treatment	$F=575.55$ $\mathbf{p \leq 0.05}$	$F= 60.49$ $\mathbf{p \leq 0.05}$	$F= 3.22$ $\mathbf{p \leq 0.05}$
Manipulation	$F= 130.5$ $\mathbf{p \leq 0.05}$	$F= 2$ $p= 0.14$	$F= 2.26$ $p= 0.11$
Treatment *Manipulation	$F= 67.67$ $\mathbf{p \leq 0.05}$	$F= 9.80$ $\mathbf{p \leq 0.05}$	$F= 1.53$ $P= 0.19$

Table 4. Variables with the greatest influence on the three types of seeds

It is known that vermicompost can favor rapid seedling growth through increased root formation and stem elongation (Chavda and Rajawat, 2015), so stem lengths were measured in each of the substrates (Figure 2) and the results of growth in length in the different treatments were statistically analyzed to establish the existence, or not, of significant differences. The results of these tests can be seen in Table 5, where we observe that for Barley all substrates have significant differences, reaching the highest growth (36.14 cm) in the Chicken droppings substrate; in the case of Ray Grass, significance is only seen in the soil substrate (19.76 cm) and in the case of chives it can be seen that the chicken droppings and soil substrates show no differences between them and that the seaweed and vegetable substrates (7.21) do not show significant differences either, but the vegetable does show significant differences with the chicken droppings and soil substrates.

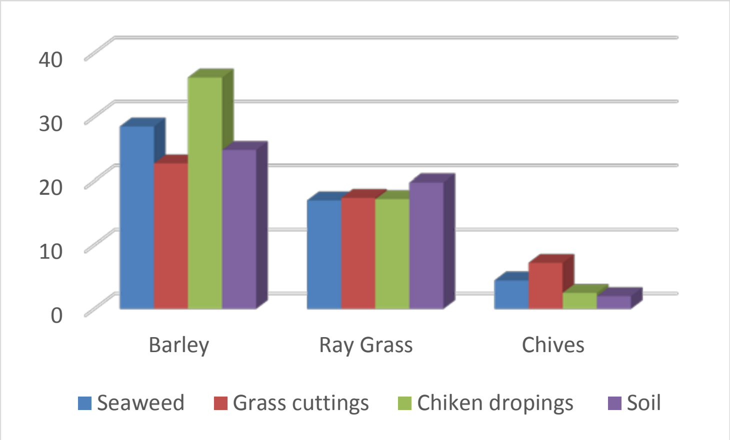

Figure 2. Effect of different vermicompost on stem length growth.

Treatment	Seaweed	Grass cuttings	Chicken droppings	Soil
Barley	28.47±8.81**b**	22.69±6.28**d**	36.14±8.05**a**	24.79±6.66**c**
Ray Grass	16.99±4.05**b**	17.40±4.03**b**	17.18±4.22**b**	19.76±4.69**a**
Chives	4.46±4.92**ab**	7.21±4.28**a**	2.50±3.56**b**	2.00±4.03**b**

Table 5. Statistically significant differences to growth by treatment and for each specie. (Tukey b test, p≤ 0,05). Different letters indicate statistical differences.

The results of growth in the substrates without earthworms, with earthworms without manipulation and with earthworms with manipulation were analyzed (Table 6). In the case of barley, it can be said that the manipulation favors the growth of the seeds reaching a value of 29, 4 cm in the substrates in which there was previously manipulation of the earthworms. In the case of Ray Gras, it is the non-manipulation that favors growth (18.32 cm), and in chives it is in the substrates that did not have earthworms where better growth is observed.

Manipulation	Final substrate (non-worms)	Final substrates (worm and non-manipulation)	Final substrates (earthworms and manipulation)
Barley	25.09±8.16**b**	29.32±9.43**a**	29.40±8.90**a**
Ray Grass	17.80±4.22**b**	18.32±4.58**a**	17.17±4.47**b**
Chives	6.50±5.02**a**	3.48±4.10**b**	4.56±4.44**ab**

Table 6. Statistically significant differences to growth by manipulation and for each specie. (Tukey b test, p≤ 0,05). Different letters indicate statistical differences.

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Note that these experimental results were obtained without any mixing of vermicompost with crop soil; the effectiveness of vermicompost depends on the type of soil, as well as the rate and timing of application (Coulibaly et al. 2020). Excessive applications of organic vermicompost have an adverse effect like that of over-application of inorganic fertilizers.

A principal component analysis (PCA) was performed to see the relationships between the different substrates and the parameters of germination and stem length (Figure 4). Results from PCA are show in Table 7 and Table 8.

Varimax rotation	Communalities	Component 1	Component 2	
% GERM BARLEY	0.68	-0.12	,819	
LGHT BARLEY	0.91		-0.55	-,780
% GermRAY	0.66		0.29	,761
LGHT RAY	0.87		-,552	-,780
% GermCHIVES	0.93		,962	,058
LGHT CHIVES	0.93		,966	,023

Table 7. Eigenvalues and % of Variance explanation.

Axes	Eigenvalues	Variance (%)
1	2.72	45.39
2	2.27	37.8
Total		82.21

Table 8. Communalities for each treatment and by two components extracted after Varimax rotation from PCA.

Axis 1 and 2 explained 82.21% of the total variance; biplots allowed evaluation of the correlations of variables shown with their position from x, y lines (closer correlations) (Figure 3). The analysis grouped the different treatments according to their effect on the germination and stem length parameters obtained, observing that the length and germination of chives is correlated with the grass cuttings vermicompost substrate with manipulation (VYM), while the length of barley stems (LGHT Barley) is correlated with the substrates of chicken droppings droppings with and without manipulation (CYM, CNM); the rest of the parameters show a much less marked correlation, but with a slight tendency towards the undisturbed soil substrate (with and without manipulation, SYM,SNM).

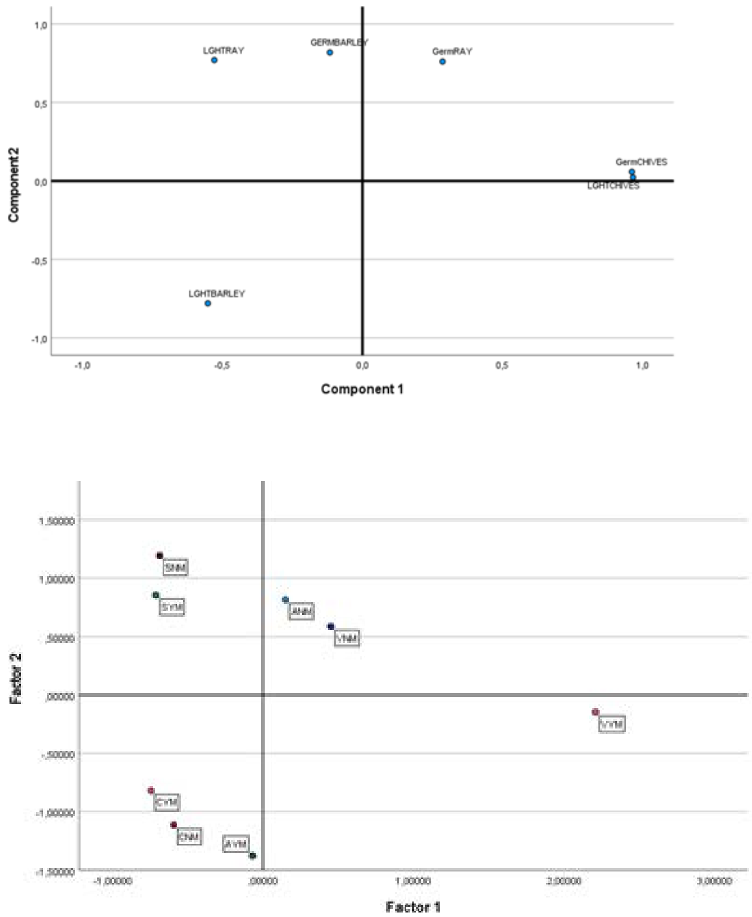

Figure 3. Biplots form PCA show the relationships between the length and germination of ray, chives and barley and different substrates (vermicomposting, raw soil and seaweed) with and without manipulation treatment.

CONCLUSIONS

The most important factor in the germination of seeds is the substrates used and not the fact that they have been in contact, or not, with earthworms.

Regarding the length of the stems after germination, the results varied depending on the seed and the vermicompost.

Principal component analysis showing relationships between plant germination and stem length parameters and different vermicompost and composts, with and without manipulation mainly for vermicomposting between manipulation treatment and germination and length of chives, and between chicken droppings droppings and ray length.

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