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INTERNSHIP REPORT

The Impacts of "Effective Microorganisms" and Sauerkraut on Earthworm Growth

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Summary:

Commercially available "Effective Microorganisms" (EM) are believed to enhance soil microbial diversity in agricultural systems. Since microorganisms are among the components of the diet of *Eisenia fetida*, we were interested here in the potential of EM and sauerkraut (a more readily available product containing beneficial bacteria) in increasing earthworm growth. Our results showed that EM had no effect on earthworm growth, whereas sauerkraut had a comparative negative impact on *E. fetida*, which may be explained by the presence of allyl isothiocyanate in *Brassicaceae* species.

Introduction:

"Effective Microorganisms" (EM) are an assemblage of microorganisms containing photosynthetic bacteria, lactobacilli, actinomycetes and yeasts, developed in the 1990s (Higa and Parr, 1994). They are believed to improve plant germination, as well as having positive effects on plant growth and quality and enhancing soil microbial diversity (Olle and Williams, 2013). The earthworm species *Eisenia fetida* has an important role in soil quality and plays an integral part in nutrient recycling (Gupta and Garg, 2012). Moreover, considering that "a diversified base of microorganisms will be fed on by earthworms" (Joshi et al., 2019), we wanted to test here whether EM applied to soil surface could serve as a food source to increase earthworm growth. Indeed, there have been previous studies focusing on the effect of EM on plant growth (Olle and Williams, 2013 ; Bzdyk et al., 2018 ; Cui et al., 2021) and studies focusing on the effects of different substances such as pesticides on earthworm growth (Espinoza-Navarro and Bustos-Obregón, 2005 ; Zhang et al., 2019) but there have never been any studies on the effect of EM on earthworm growth. Therefore, the protocol used here is an assemblage of different methods chosen in previous studies.

This experiment was conducted in conditions similar to those that may be found in earthworm composting systems and earthworm farms such as Biotica visited by the Giacche Verdi Bronte organisation on October 3rd 2022, in Maletto, with rich soil and high humidity which is different to open crop fields in the region of Bronte. Therefore, the use of this experiment is to establish whether or not "Effective Microorganisms" may be used as a way of enhancing earthworm growth in composting systems.

One component of EM, lactobacilli, are largely available in lactofermented commercial products. Therefore, as a cheaper, more easily implementable option, we also tested the effect of lactofermented cabbage in the form of commercialised sauerkraut containing lactobacilli bacteria to see if it could also induce a difference in growth.

Materials and methods:

The earthworms used are of the species *Eisenia fetida,* they were purchased from the company *Biotica* based in Maletto, province of Catania. The "Effective Microorganisms" used are sold by EMIKO under the name EM1 and were "activated" during a period of four weeks after adding water and 600 ml of sugar cane treacle to 600 ml of the EM1 product for a final volume of 20 l, kept at room temperature. The sauerkraut is of the brand Herbert Lechner. The method used here is largely based on OECD recommendations (OECD, 2016).

The soil used for the experiment was collected at La Difesa, Bronte (37°805396′ N, 14°868860′ E), on October 12th 2022 in the afternoon, the last rain-shower having occurred the day before. The soil was extracted from a several year-old pile of horse manure having undergone compostation.

In order to establish the percentage of humidity of the soil used for the experiment, 4 x 50 g of it were placed in a kitchen oven at around 120°C for 3 hours and moisture was taken as the average of all 4. The quantity of soil necessary for each replicate (600 g of equivalent dry soil) was thus determined. Moisture was also measured after experimentation for each bag with 1 x 10 g of soil used and placed at the same temperature, for the same time.

The soil pH was measured by using an electronic pH-meter, adding 1 part of soil for 5 parts of water. This was done with the soil used in the experiment before, and with every bag afterwards.

4 replicates were made for each of the control (no microorganisms or sauerkraut), treatment 1 (microorganisms applied to soil surface) and treatment 2 (sauerkraut): 12 bags of just under 200 cm² in surface were therefore prepared containing the 600 g of dry soil. Triplets of the open bags were set to rest in 4 plastic boxes with a perforated lid and gauze beneath the lid to limit loss of humidity (Additional Photo 1).

The treatments are as follow:

- box 1:
  - bag 1: “Effective Microorganisms”
  - bag 2: control
  - bag 3: sauerkraut
- box 2:
  - bag 4: control
  - bag 5: “Effective Microorganisms”
  - bag 6: sauerkraut
- box 3:
  - bag 7: “Effective Microorganisms”
  - bag 8: sauerkraut
  - bag 9: control
- box 4:
  - bag 10: “Effective Microorganisms”
  - bag 11: control
  - bag 12: sauerkraut

10 adult worms were added to each of the 12 replicates, making for a total of 120 worms with 40 per treatment (those that did not burrow after 15 minutes were removed and replaced). These worms had previously been washed in water and weighed using a scale of precision 0.01 g (Additional Photo 2). 4 mL of the solution containing activated

EM1 were then applied to each of the 4 “treatment 1” bags on October 18th 2022, approximately 4 hours after worm burrowing. 24 mL of a solution containing 5 parts of water for 1 part of sauerkraut juice (equivalent to 4mL of sauerkraut) were applied at the same time to each of the 4 “treatment 2” bags.

The amount of watering for each bag was kept the same during experimentation and the worms were fed every week with old horse manure (5 g per bag) that was made slightly wet.

After 28 days, the earthworms were weighed again using the same scale and same method (washing and slight drying before weighing).

Statistical analysis

The statistical analysis was conducted in R version 4.0.1.

In order to determine whether there was any difference in the weight means between treatments, both before experimentation and after experimentation, we conducted an ANOVA using *aov* function. Normality of residues was verified with *shapiro.test* and equality of variance with *bartlett.test.* Post-hoc analysis was conducted with the *TukeyHSD* function. The weights of the worms were also compared for each treatment before and after with an unpaired t-test using the function *t.test*. Equality of variance was tested with *var.test*.

We tested if any differences were present in the median moisture and pH of the bags, between treatments, after experimentation, by conducting a Kruskal-Wallis test (*kruskal.test*). We also verified if the moisture or pH of the soil could explain the differences in weights by testing the correlation between weights and moisture or pH using a non-parametric Spearman correlation (*cor.test* function).

Graphics were produced with *ggplot2*. All tests were conducted with two-sided alternatives.

Results:

There were 120 worms at the beginning of experimentation and 114 worms at the end of experimentation, which is a mortality inferior to 10%, in accordance with OECD testing standards (OECD, 2016).

At the beginning of experimentation, the soil moisture was 20%: 750 g of soil were therefore added to each bag in the preparation phase. The soil pH was around 7.6. The pH of the microorganism solution is 3.6. The sauerkraut pH is 3.5. After experimentation, no differences were observed in the median moisture and pH of bags compared between treatments ($p = 0.37$ for moisture and $p = 0.31$ for pH).

The comparison of weights before experimentation between the three conditions (control, sauerkraut and EM) revealed no significant difference in means: the ANOVA test produced a p-value of 0.253 ($n = 120$). The conditions of normal distribution were verified with Shapiro test ($p = 0.07$) and homoscedasticity was verified with a Bartlett test ($p = 0.88$).

The comparison of weights after experimentation revealed a significant difference in means (verified ANOVA produced p = 0.006 with n = 114). The post-hoc Tukey test revealed a significant difference in means between the EM treatment and sauerkraut (weight(EM) > weight(sauerkraut) with p = 0.005) and a nearly significant difference between control and sauerkraut (weigh(control) > weight(sauerkraut) with p = 0.07). The means of the weights of the EM and control earthworms are considered the same (p = 0.61) (Fig. 1).

The comparison of weights for each treatment, before and after, using unpaired t-tests, reveals that there is a significant increase in the average weight among the worms in the control (p = 0.009) and those subject to "Effective Microorganisms" (p = 0.009). However, there was no change in the average weight of the sauerkraut worms before and after (p = 0.28) (Fig. 2).

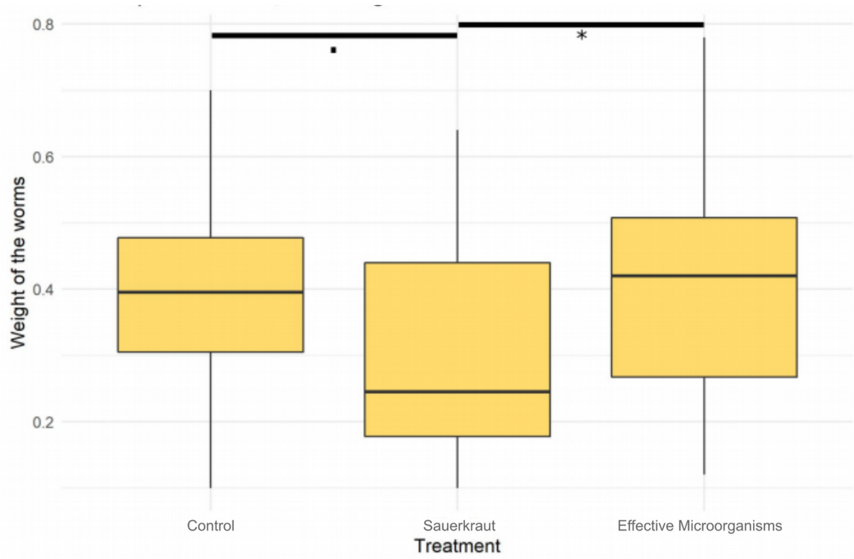

Figure 1: Comparison of the weights of the worms (in grams) between treatments after experimentation. The star shows a significant difference (p < 0.05) and the dot shows a nearly significant difference (0.05 < p < 0.1).

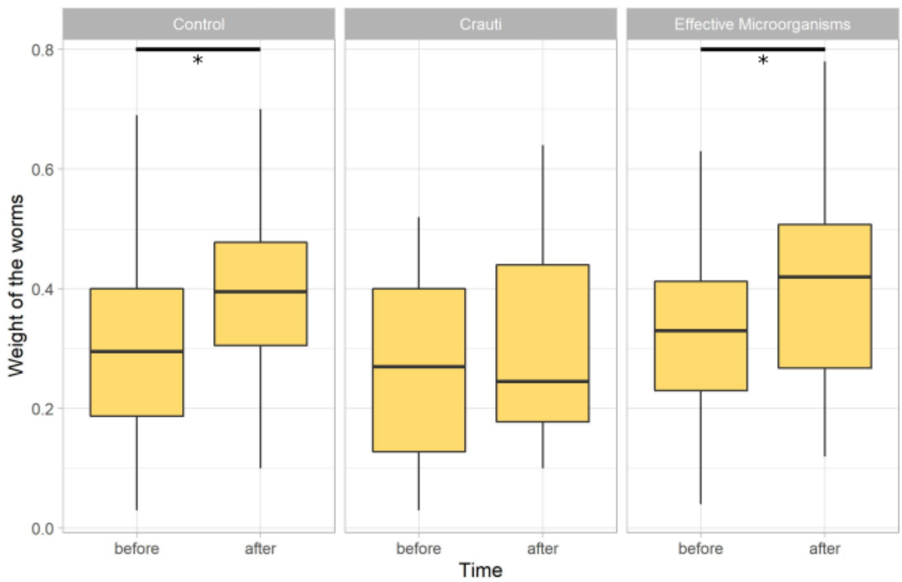

Figure 2: Comparison of the weights of the earthworms (in grams) according to treatment, before and after experimentation. The stars show a significant difference (p < 0.05) between before and after.

The non-parametric Spearman correlation test between the worm weights and moisture or pH of the bag revealed no significant correlation ($p = 0.08$ for moisture and $p = 0.06$ for pH, Fig. 3).

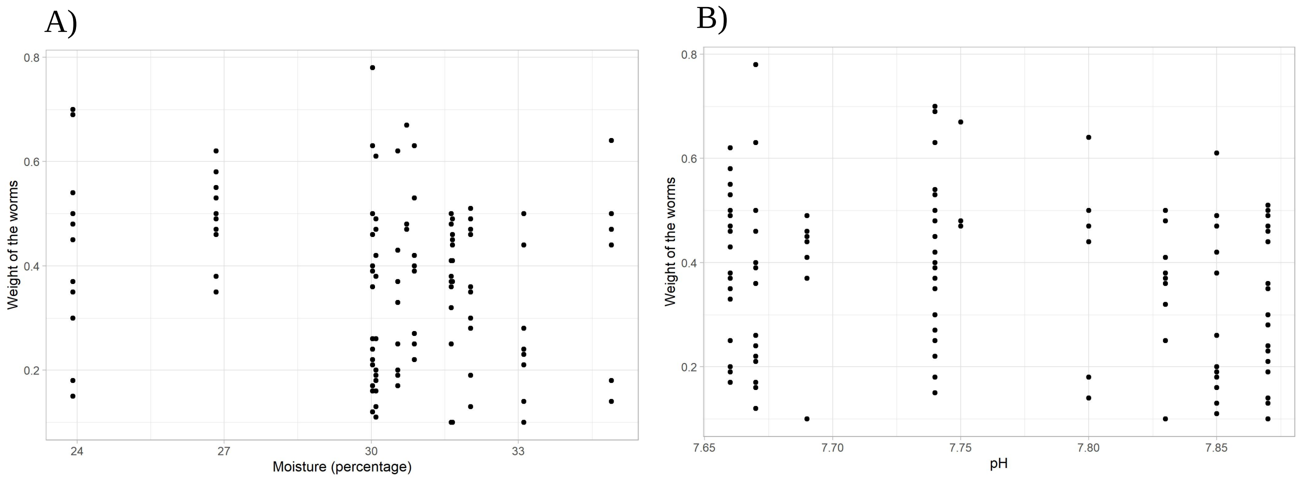

Figure 3: Weights of the earthworms according to A) moisture and B) pH.

Discussion:

As previously mentioned, no previous study has been conducted on the effects of EM on earthworm growth, therefore the method used here is an assemblage of protocols found in articles relating to earthworm growth and EM separately. In the literature, the maximum dosage of EM often observed in open field systems is of 100 litres of activated “Effective Microorganisms” applied per hectare of crops (Olle and Williams, 2013 ; Bzdyk et al., 2018) but for potted plants there are more extreme cases of volumes used such as 4 mL for each 100 cm^2 surface for potted *Sesbannia cannabina* (Cui et al., 2021). Considering that the surfaces of earthworm composting systems and earthworm farms are relatively small and the use of large quantities of EM is therefore possible, this last concentration was chosen here.

The period over which to observe change in worm weight due to pesticides or biochar is about 1 month (Espinoza-Navarro and Bustos-Obregón, 2005 ; Zhang et al., 2019) as is recommended by the OECD guidelines for testing on *E. fetida* (OECD, 2016). The period over which to observe change in plant-related traits after application of EM is variable: Bzdyk et al. sprayed trees twice (14 days separation) with EM and measured mycorrhizal density 3 months after the first spray ; plants were left to grow over 4 months in Cui et al. but biochar was also systematically added in every treatment. We have chosen here to conduct a first phase of experimentation over 28 days to account for the earthworms life cycle and in order to follow standard guidelines but, in future studies, it might be relevant to stretch the experiment over a longer period of time.

The results therefore obtained after 28 days show that, contrary to the other treatments, the earthworms contained in the sauerkraut bags did not grow (Fig. 2). And, indeed, the sauerkraut worms were smaller than those subject to “Effective Microorganisms” and almost significantly smaller than control earthworms (Fig. 1). This seems to suggest that the sauerkraut affected negatively the growth of the earthworms and, moreover, the “Effective Microorganisms” did not induce any particular growth compared to the control earthworms. On a more subjective note, it was also observed during the weighing of the worms that three bags seemed to contain earthworms that were moving more slowly than in other bags: all three of these had been exposed to sauerkraut.

Moreover, these differences in worm weights were not explained by soil moisture (Fig. 3 A), leaving only the treatments and pH as a possible explanation. The correlation test for pH also revealed no relationship between pH and worm weight (Fig. 3 B). However this result is to be interpreted carefully as the pH-meter used for the experiment did not provide great accuracy and the method used consisting in adding 5 parts of water for 1 part of soil may smooth out any differences in soil pH by dilution in water.

Therefore, one source of explanation for the negative impact induced by the sauerkraut could be linked to the use of mustard as a common extractant of earthworms from the soil. Indeed, mustard is often used as a way of inducing earthworms to rise to soil surface in participatory science programmes (Pélosi et al., 2014) because of the presence of allyl isothiocyanate contained in the product. Since the substance is present in many species of the *Brassicaceae* family after the breakdown of their characteristic glucosinolates (Pélosi et al., 2009), it could also be present in the sauerkraut used in this experiment, as cabbage (*Brassica oleracea*) is part of this plant family. After application of the product, it may have induced skin irritation among the earthworms which negatively impacted their growth.

The “Effective Microorganisms” on the other hand did not induce any difference in growth compared to the control. Therefore, although EM are believed by some sources to have an impact on crop yield and quality (Olle and Williams, 2013), it may be that they cannot enhance soil microbial diversity in such a way that is beneficial for *E. fetida*. It could also be that the chosen concentration of EM applied to the soil was too small. However, the absence of official recommendations in the application of the product prevents any general guidelines for the application of EM from being established. This led us to rely on previous studies using EM where an effect was observed to decide on an appropriate concentration. Thus, the lack of positive impact induced by EM in this study could be explained by an absence of potential in enhancing soil microbial diversity in such a way that increases earthworm growth.

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Additional photos

Photo 1: Experimental setup with the 4 boxes containing the 12 bags and the electronic scale.

Photo 2: Worm weight measurement using an electronic scale of precision 0.01g.

Photo 3: Applying the microorganisms to the 4 treatment 1 replicates.