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Vermiliquer as a biostimulant and antioxidant in hydroponic lettuce (*Lactuca sativa*) production

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Abstract: The use of vermiliquers obtained from earthworms in hydroponic crops is well received as an alternative for the fertilisation of leafy vegetables. Vermiliquer boosts growth and defence compounds and increases the uptake of nutrients and minerals by plants. The growth and enzymatic activities related to stress and phenolic compounds were explored in hydroponic lettuce crops treated with different concentrations of vermiliquer. The treatments consisted of three different vermiliquer products, a mesh vermiwash, a direct vermiwash, and a vermileachate, and their combinations as a complement for the complete fertilisation. The addition of vermiliquer to hydroponic lettuce affected the leaf and root fresh weight, and the reactive oxygen species-like superoxide dismutase (SOD) or phenylalanine ammonia-lyase (PAL). Vermileachate (Vl) plus direct vermiwash treatment caused higher growth in lettuce leaf and roots from the first week indicating a biostimulant effect, indicating that it was an effect biostimulant. Vl caused the highest enzymatic SOD and PAL activity, indicating that it was an effect elicitor. In summary, vermiwash proved to improve the hydroponic lettuce crop and enzymatic activities related to stress.

Keywords: earthworm; organic wastes; greenhouse; agriculture; vermicompost

Earthworms in vermicomposting produce vitamins, hormones, and humic substances; as well as causing the elimination of pathogens and improving the natural microbiota (Jindo et al. 2012; Sun et al. 2020). Earthworms hasten the process of organic waste decomposition. Furthermore, earthworms modify the structure and physicochemical properties, such as the porosity, pH, organic matter content, electric conductivity, moisture content and texture, and also increase the nutrient availability (Singh et al. 2020). The presence of earthworms increases the ground biomass and the crop yield by 25%. Vermiliquer and vermicompost are the most common products obtained from earthworms (Churilova, Midmore 2019).

Studies have supported the use of vermicompost, which is an organic fertiliser that improves the structure of the soil, reduces the reliance on external nutrients, thereby reducing the use of agrochemicals, the environmental impact and economic production costs. On the other hand, there are few reports on vermiliquer, including vermiwash (VW) and vermicomposting leachates (Vls). Vermiliquer has been reported to assertively affect

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the plant growth, improve the defence compounds and stimulate the plant, due to the uptake of nutrients and minerals (Kumari et al. 2019). Vl is a nutrient liquid that drains through vermicomposted waste, which includes the worms' food, their microbiota, organic and inorganic material dissolved in water (Churilova, Midmore 2019). As a result, Vl is rich in macronutrients and micronutrients. Plants can absorb Vl directly through their roots, shoots, and fruits. VW is also known as worm tea; it is made from a liquid infusion of a mature vermicompost extract. In addition, VW has different organisms and nutrients, which are derived from the applied biomass in comparison with Vl (Singh et al. 2020). Vermicompost products that enhance plant growth require balances, such as adjusting the pH levels to acidic, diluted nutrient concentrations in the water or nutrient supplementation. VW and Vl applied in the hydroponic cultivation of lettuce (*Lactuca sativa* L.) require a nutrient balance to enhance the plant growth. Lettuce belongs to the Asteraceae family, and it is the most commercially grown leaf in the world and is mainly grown in a hydroponic systems because it grows faster, has a high yield, consumes less water and reduces soil-borne diseases that affect it (Ahmed et al. 2021). Hydroponics is a specialised technique for growing plants without soil. A key for culture hydroponics is the delivery of the nutrient solution, minimising the consumption of water and reducing the labour to ensure the highest yields (Eigenbrod, Gruda 2015; Majid et al. 2020).

Root plants require the availability of oxygen for the correct development, therefore, hydroponic culturing forces the aeration of nutrient solutions and diminishes bacterial colonies; this technique enhances the root biomass in plants grown in this system (Churilova, Midmore 2019), for example, vermileachate used on radishes (*Raphanus sativus* L.) promoted a 15% greater dry root weight (Kumari et al. 2019). In order to incorporate lettuce in hydroponics, the farmer needs to maintain the optimal conditions for lettuce plant growth – water quality, nutrients, temperature, and oxygen, all of which improve the production methods (Spehia et al. 2018; Churilova, Midmore 2019).

Lettuce cultivation on a hydroponic bed has been studied over the world and to improve the yields and quality. We set out an experiment combining vermiliquers to supplement nutrients for lettuce production. The hypothesis for this work is that the combinations of vermiliquers will increase the antioxidant enzymes and the yield of the lettuce.

MATERIAL AND METHODS

Physicochemical composition and vermicompost innocuity. The vermicomposts were analysed to determine their electrical conductivity (EC) and pH in aqueous extracts using a portable meter (Hanna Instruments, USA) (Czekała et al. 2016).

The moisture and organic matter content were determined through the weight loss at 105 °C for 24 hours (Silva et al. 2014).

The total nitrogen (TN) and the total phosphates (TPs) were determined according to Kjeldhal (Czekała et al. 2016) and the molybdovanadate phosphate method (AOAC 1990). The mineral content was determined by atomic absorption.

Escherichia coli concentration was evaluated by aseptic membrane of minerals and *Salmonella spp.* concentration was analysed in a local laborator.

Preparation of the vermiliquers. The vermiliquers were obtained from composted cattle manure and then vermicomposted with *E. foetida*

The vermiwash was obtained from two productions processes:

– The direct vermiwash (VwD) was derived from a vermicompost:water ratio (1 : 5) that remained in continuous stirring for 48 hours.
– The mesh vermiwash (VwM) was created from a vermicompost on meshwater (1 : 5) while maintaining agitation for 48 hours.
– The vermicompost leachate (Vl) was obtained from the excess moisture in the vermicomposting process.
– Sulphurous (S) concentration was determined by turbidimetry method and potassium (K) concentration was evaluated by atomic absorption. The same production batch were used for all the analyses.

Hydroponic system. *L. sativa* plants were established in eight wood tanks colocated on a metal base. The dimensions of the eight wood tanks were 80 × 80 × 20 cm, which was lined with plastic with a capacity for 25 heads of lettuce for each tank. The hydroponic roots that were submerged all the time needed an auxiliary oxygen supply, which was provided through individual aerators. Each aerator supplied oxygen to the crop's roots. A Steiner fertilizer solution at 100% was used.

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Experimental design. The hydroponic treatments were set up in a completely random factorial design. Each treatment was carried out in a separate hydroponic tank. The details of the treatments are presented in Figure 1. The treatments consisted of adding the complement of each vermiliquer to the complete fertilisation (CF).

Growth analysis. The crop lettuce growth was determined by destructive sampling, which was carried out every week, starting after transplant. The leaves and roots were separated from the plant to obtain the fresh weight (FW).

Protein content and antioxidant enzyme activity. Frozen lettuce leaf samples were homogenised with liquid nitrogen. The enzyme extract was prepared with 0.3 g of lettuce leaves that were homogenised with 1 mL of an extraction buffer according to the technique: potassium phosphate 0.05 mM, ph 7.8 for the superoxide dismutase (SOD) activity; Tris-HCL 100 mM, pH 8 for the catalase (CAT) activity; 0.1 mM borate to 0.1% v/v with 2mercaptoethanol, pH 8.8 for the phenylalanine ammonia-lyase (PAL) activity, which were then vortexed for two minutes and centrifuged at 12 000 rpm for 15 minutes at 4 °C. The supernatant was used as a crude enzyme extract (CEE). The protein concentrations were determined by the Bradford assay.

To measure the SOD activity, 0.3 mL of EDTA-Na_2, 0.1 mM, 0.3 mL of Methionine at 0.13M, 0.3 mL of NBT at 0.75 mM, 0.3 mL of Riboflavin, 0.05 mL of CEE and 0.25 mL of distilled water were mixed in a tube by inversion and exposed to fluorescent light for 30 min. The absorbance was measured at 560 nm. The control reaction contained all the solutions except CEE (Hayat et al. 2018).

To measure the CAT activity, 0.2 mL of a potassium phosphate reaction buffer (50 mM, pH 8), 0.2 mL of H_2O_2 (100 mM), and 0.1 mL of CEE. The absorbance λ = 240 nm was read for 6 min at 25 °C.

The PAL activity was determined for an absorbance of λ = 290 nm according to Toscano et al. (2018) with some modifications. The quantities of the solutions 0.23 uL of potassium phosphate reaction buffer (50 mM, pH 8) and 0.2 uL of CEE were mixed and incubated at 40°C for 40 min. Then, 50 uL of 1N HCL were added to the mix and ten minutes later the absorbance was measured and only the CEE varied.

The specific activity of the enzymes was expressed as U/mg of protein.

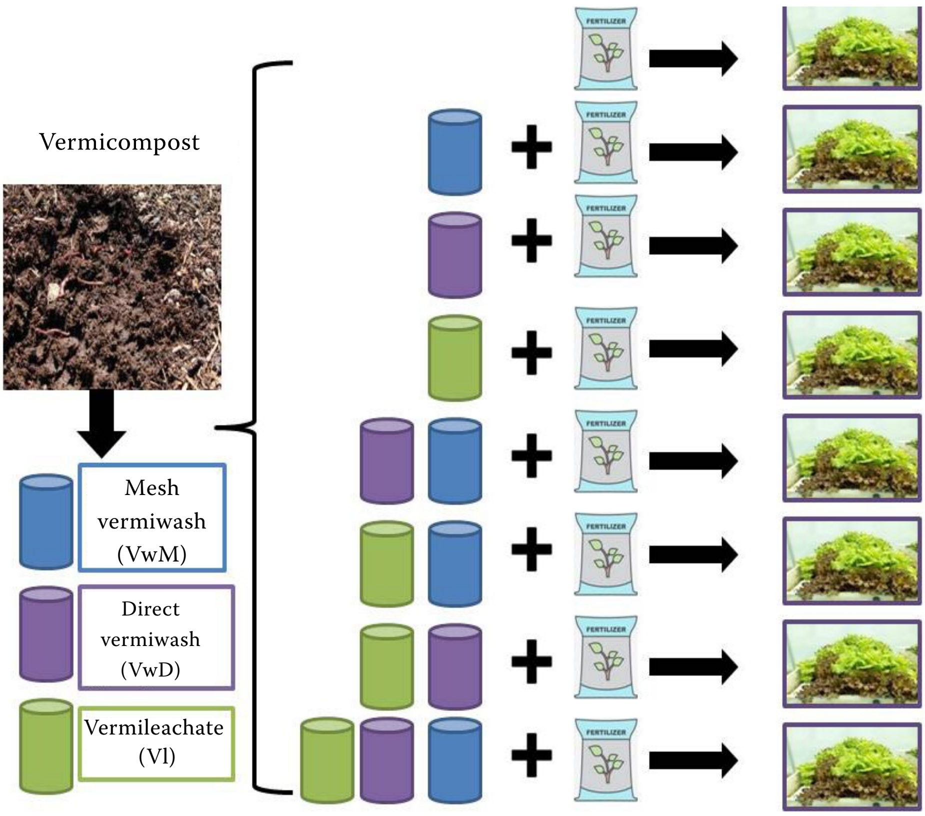

Figure 1. Schematic of the vermiliquers as a complement fertiliser for the hydroponic lettuce production

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Table 1. Physicochemical composition and innocuity of the studied vermicompost

Parameter	Unit	Vermicompost
pH	–	7.4
Electrical conductivity	ds m^{-1}	2.3
Moisture	%	25.24
Organic matter	%	28.892
Nitrogen	%	1.08
Phosphorus (P_2O_5)	%	0.42
Potassium (K_2O)	%	2.16
Calcium (CaO)	%	2.79
Magnesium (MgO)	%	0.87
Sodium (Na_2O)	%	0.11
Sulfur	%	0.22
Iron	mg/L	3 032
Copper	mg/L	21.74
Manganese	mg/L	173.73
Zinc	mg/L	132.89
Estrechia coli	NMP/g	< 3
Salmonella spp.	CFU/25 g	abscence

Statistic analysis. The experiment of adding vermiliquers to the hydroponic lettuce was randomly divided and each sample had three replications. The data were analysed as a randomised complete block design. The data were subjected to an analysis of variance (ANOVA) and Tukey's post-hoc test was used for the statistical evaluation of the differences among the means ($\alpha < 0.05$) using the software Statgraphics.

RESULTS AND DISCUSSION

Physicochemical composition and vermicompost innocuity. The results are shown in Table 1, the results obtained for the organic matter content and the physicochemical composition reflect the transformation of organic nutrients into inorganic nutrients by the earthworms and microorganisms (Saba et al. 2019). In the same way, the cattle manure utilised for the vermicompost is related to the nutrient quality obtained, which is an important factor for earthworm production and for supplementing the hydroponic production (Das et al. 2021).

Properties of the vermiliquer. The macronutrient values for the vermiliquer preparation are shown in Table 2. The difference between the vermiliquers' macronutrient concentration was derivated for the preparation formula, of note, the difference in the vermiliquers can be seen with the P, K and S content as reported by Churilova and Midmore (2019).

Growth analysis. The growth analysis revealed significant differences in the roots and leaves among the vermiliquer treatments on the hydroponic bed (Figure 2).

There is a difference between the CF with 224.2 g/plant and the VwM + VwD with 196.5 g/plant in the fresh weight of the leaves; this difference may be attributed to the VwM + VwD activating the lettuce immune system. On other hand, the highest root fresh weight were obtained for VwM + VwD, VwM + Vl and CF with a global average of 69 g/plant; which could be due to presence of the phytohormones and humic susbtances represented by the combination of VwM plus the other vermiliquer. Arancon et al. (2019) reported a greater yield in hydroponic grown lettuce under vermicompost treatment.

The obtained results showing a significant difference in the lettuce could be due to the management of the hydroponic systems, as the nutrient solution or the aeration on the bed impacts the lower yield per plant values (Majid et al. 2020; Tomov et al. 2021)

Antioxidant capacity. The antioxidant enzyme system maintains low and harmless levels of . oxygen free radicals in the cells. Furthermore, the defensive properties of the antioxidants and specialised metabolites in the plant cells provide broad tolerance to the abiotic stress. The antioxidative stress response of *L. sativa* L. cultivated in a hydroponic system and treated with vermiwash and ver-

Table 2. Macronutrient content of the different vermiliquer preparations

Parameter	Unit	VwM	VwD	Vl
Nitrogen	%	0.076	0.053	0.038
Phosphorus (P_2O_5)	%	0.370	0.110	0.190
Potassium (K_2O)	%	0.213	0.188	0.285
Sulphur	%	0.037	0.026	0.150

VwM – the mesh vermiwash; VwD – the direct vermiwash; Vl – the vermicompost leachate

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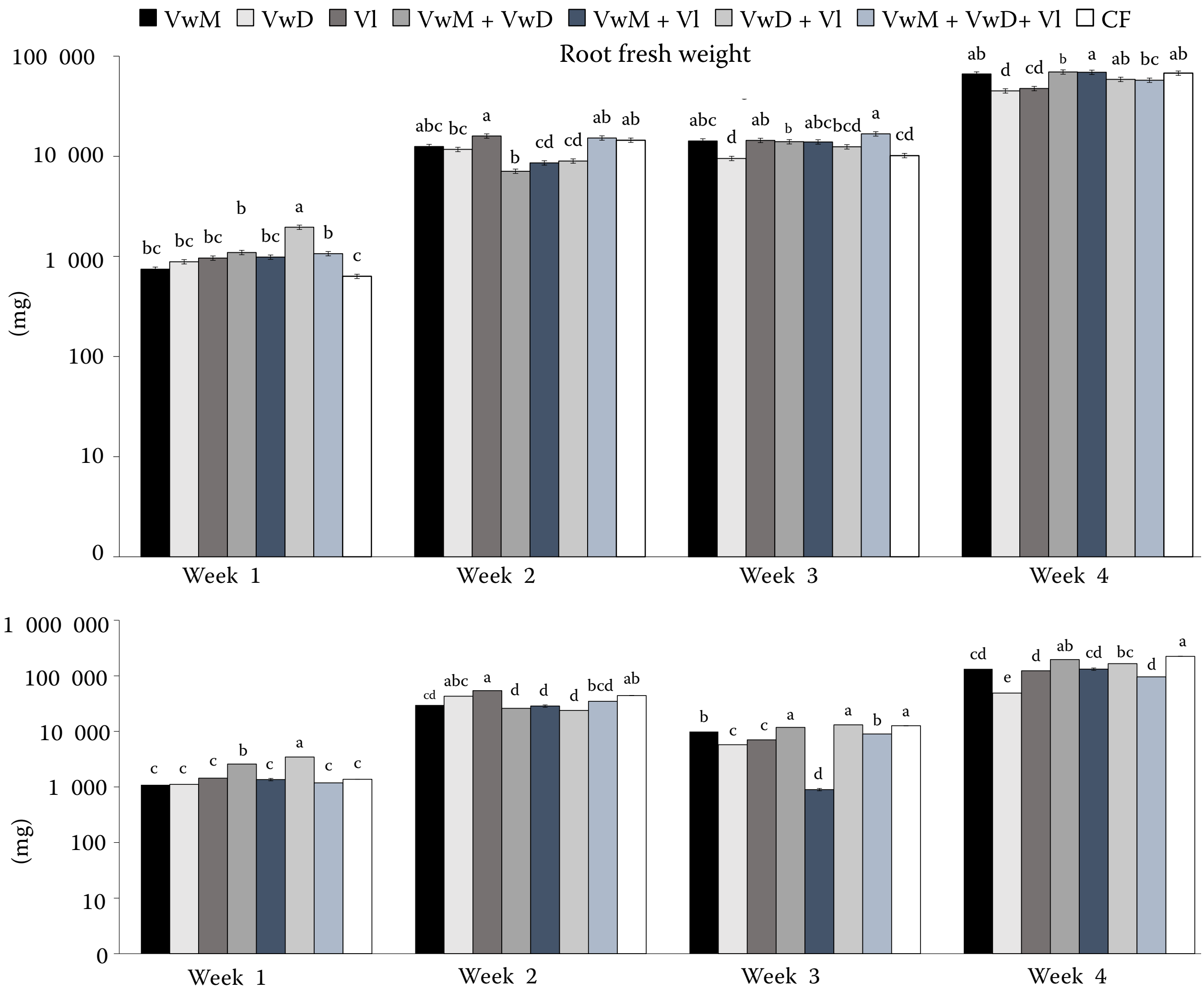

Figure 2. Effect on the hydroponic lettuce leaf and root fresh weight treated with the different vermiliquers (error bar according to the standard deviation)

Different letters indicate a significance difference between the means at a $P < 0.05$; VwM – the mesh vermiwash; VwD – the mesh vermiwash; Vl – the vermicompost leachate; VwM + VwD – the mesh vermiwash and the direct vermiwash; VwM + Vl – the mesh vermiwash and the vermicompost leachate; VwD + Vl – the direct vermiwash and vermicompost leachate;VwM + VwD + Vl – the mesh vermiwash, the direct vermiwash and the vermicompost leachate; CF – the complete fertilisation

miliquer gave a higher growth rate and metabolite content (Tomov et al. 2021) pigment content and antioxidative stress response of *Pisum sativum* L., *Zea mays* L. and *Lactuca sativa* L., cultivated in hydroponic and aquaponic systems. It was observed that aquaponic farming of maize and lettuce together with goldfish (*Carassius auratus* L.). The addition of vermiliquer and vermiwash triggered oxidative stress conditions, such as SOD and catalase (CAT) activities (Table 3). Furthermore, the PAL activity was quantified as an indirect indicator of the total phenolic compounds through the phenylpropanoid pathway (Takahashi et al. 2021) (Figure 3).

The enzymes associated with regulating the reactive oxygen species (ROS) levels, preserving the cells from stressful environments, and neutralising the free radicals are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), etc. (Tomov et al. 2021). As shown in Table 1, the activity of SOD in the lettuce leaves was the highest with the Vl treatment in both weeks (as shown in Table 3). The change in the SOD activity in week two was higher than in week 4, indicating that the SOD was time responsive. The CAT activity shifted significantly with the vermiwash treatments (Table 1). The VwM + VwD + Vl treatment enhanced the antioxidant enzyme activity, thus

Table 3. Superoxide dismutase and catalase activities of the leaves in the hydroponic lettuce treated with vermiliquer

Treatments	Week 2		Week 4	
	SOD	CAT	SOD	CAT
	(U/mg protein)			
VwM	2.48E–05***	125*	5.44E–04 *	172*
VwD	1.27E–05**	45*	4.35E–04*	35*
Vl	3.11E–03*	450*	1.06E–07*	25*
VwM + VwD	1.52E–03*	100**	1.51E–03*	645*
VwM + Vl	3.19E–05*	64*	4.14E–05*	350*
VwD + Vl	2.22E–06*	113*	1.95E–06*	73*
VwM + VwD + Vl	6.14E–04*	756*	1.51E–03*	175*
CF	3.20E–09**	183*	1.58E–03*	160*

*Indicates significance differences at a $P < 0.05$; SOD – the superoxide dismutase; CAT – the catalase; VwM – the mesh vermiwash; VwD – the mesh vermiwash; Vl – the vermicompost leachate; VwM + VwD – the mesh vermiwash and the direct vermiwash; VwM + Vl – the mesh vermiwash and the vermicompost leachate; VwD + Vl – the direct vermiwash and vermicompost leachate;VwM + VwD + Vl – the mesh vermiwash, the direct vermiwash and the vermicompost leachate; CF – the complete fertilisation

improving the stress resistance of the lettuce plants in the second week. Like, Tomov et al. (2021), we saw a significant difference in the CAT activity with the hydroponic system in lettuce (7 U/mg of protein). The increasing antioxidant enzyme activity could enhance the plants' stress resistance (Vázquez-hernández et al. 2019). Hence, Zheng et al. (2020) applied an ozonated nutrient solution in hydroponic lettuce; where it was found that the CAT activity at 4 mg/L had a significant difference at 10 days of VL treatments, which may enhance the PAL activity and accumulate the abundance of some phenolic compound activities. Besides, it was determined that the PAL activity in the hydroponic lettuce is upregulated by the VL treatments. This result can be connected with the higher concentration of the SOD activity and also the PAL increment could be influenced by wounding (Mampholo et al. 2019).

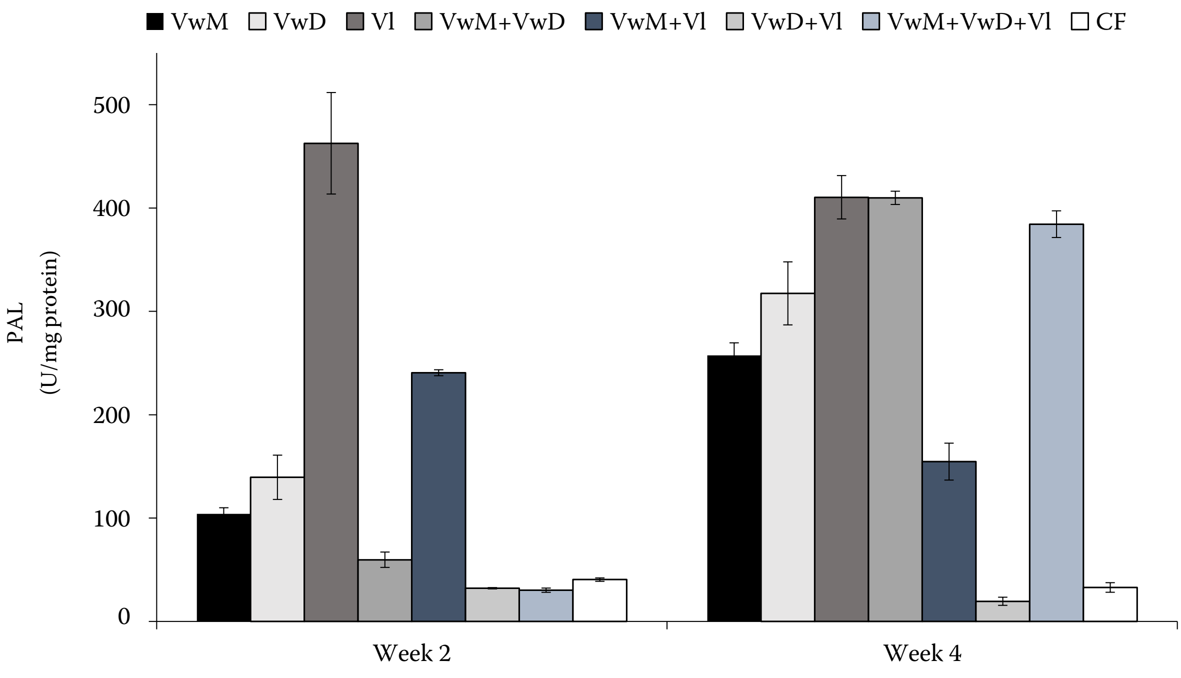

Figure 3. Phenylalanine ammonia-lyase activities (PAL) in the hydroponic lettuce leaves after adding, at the initial time, the vermiliquer treatments (error bar according to the standard deviation)
Different letters indicate significance differences between the means at a $P < 0.05$; for abbreviations see Figure 2

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CONCLUSION

The results demonstrated that the organic nutrients in vermiliquers have the potential to complement the chemical nutritional formula for hydroponics.

Vermiliquers have biostimulant properties to increase the fresh leaf weight and fresh root weight. In addition, vermiliquers have an action on the immune system to trigger the PAL, SOD, and CAT activities that are indicators of stress in plants.

REFERENCES

Ahmed Z.F., Alnuaimi A.K., Askri A., Tzortzakis N. (2021): Evaluation of lettuce (*Lactuca sativa* L .) production under hydroponic dystem: Nutrient solution derived from fish waste vs . inorganic nutrient solution. Horticulturae, 7: 292.

Arancon N.Q., Owens J.D., Converse C. (2019): The effects of vermicompost tea on the growth and yield of lettuce and tomato in a non-circulating hydroponics system. Journal of Plant Nutrition, 42: 2447–2458.

Churilova E.V., Midmore D.J. (2019): Vermiliquer (Vermicompost Leachate) as a complete liquid fertilizer for hydroponically-grown Pak Choi (*Brassica chinensis* L.) in the tropics. Horticulturae, 5: 26.

Czekała W., Malińska K., Cáceres R., Cáceres R., Dach J., Lewicki A. (2016): Co-composting of poultry manure mixtures amended with biochar – The effect of biochar on temperature and C-CO_2 emission. Bioresource Technology, 200: 921–927.

Das D., Abbhishek K., Banik P., Bhattacharya P. (2021): A valorisation approach in recycling of organic wastes using low-grade rock minerals and microbial culture through vermicomposting. Environmental Challenges, 5: 100225.

Hayat S., Ahmad H., Ali M., Ren K., Cheng Z. (2018): Aqueous garlic extract stimulates growth and antioxidant enzymes activity of tomato (*Solanum lycopersicum*). Scientia Horticulturae, 240: 139–146

Jindo K., Sánchez-Monedero M.A., Hernández T., García C., Furukawa T., Matsumoto K., Sonoki T., Bastida F. (2012): Biochar influences the microbial community structure during manure composting with agricultural wastes. The Science of the Total Environment, 416: 476–481.

Kumari A., Ahli F. (2019): Vermitechnology leachate: an eco-friendly innovative technology for better crop production. Journal of AgriSearch, 6: 25–27.

Majid M., Khan J.N., Shas Q.M.A., Masoodi K.Z., Afrozo B., Parvaze S. (2020): Evaluation of hydroponic systems for the cultivation of lettuce (*Lactuca sativa* L., var. Longifolia) and comparison with protected soil-based cultivation. Agricultural Water Management, 245: 106572.

Mampholo B.M., Maboko M., Soundy P., Sivakumar D. (2019): Postharvest responses of hydroponically grown lettuce varieties to nitrogen application rate. Journal of Integrative Agriculture, 18: 2272–2283.

Saba S., Zara G., Bianco A., Garau M., Bonono M., Deroma M., Pais A., Budroni M., (2019): Bioresource technology comparative analysis of vermicompost quality produced from brewers' spent grain and cow manure by the red earthworm Eisenia fetida. Bioresource Technology, 293: 122019.

Silva M.E.F., de Lemos L.T., Nunes O.C., Cunha-Queda A.C. (2014): Influence of the composition of the initial mixtures on the chemical composition, physicochemical properties and humic-like substances content of composts. Waste Managenement, 34: 21–27.

Spehia R.S., Devi M., Singh J., Sharma. S., Negi A., Singh S., Chauhan N., Sharma D., Sharma J.C. (2018): Lettuce growth and yield in hoagland solution with an organic concoction. International Journal of Vegetable Science, 24: 557–566.

Singh R.S., Devi M., Singh J., Sharma A., Negi A., Singh S., Chauhan N., Sharma D., Sharma J.C. (2020): Soil properties changes earthworm diversity indices in different agroecosystem. BMC Ecology, 20: 27.

Takahashi S., Namioka Y., Azis H.R., Sano T., Aono M., Koshiyama M., Fujisawa H., Isoda H. (2021): Prohydrojasmon promotes the accumulation of phenolic compounds in red leaf lettuce. Plants, 10: 1920.

Tomov A., Petrova D., Yocheva L., Georgieva Z., Karcheva Z., Chaneva G. (2021): Antioxidant status of hydroponically and aquaponically grown plant species. Oxidation Communications, 44: 397–408.

Vázquez-Hernández M.C., Parola-Contreras I., Montoya-Gómez L.M., Torres-Pacheco I., Schwarz D., Guevara-González R.G. (2019): Eustressors : Chemical and physical stress factors used to enhance vegetables production. Scientia Horticulturae, 250: 223–229.

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