

Article

Effects of Aqueous Vermicompost Extracts Produced from Winery Residues on Seedling Growth and Photosynthetic Pigments of *Lepidium sativum* L. and *Cucumis sativus* L.: A Preliminary In Vitro Study

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Abstract

Winery residues generated by the wine industry represent an important sustainability challenge but also a valuable resource for producing vermicompost-derived products with potential agricultural applications through vermicomposting. This study evaluated the effects of aqueous vermicompost extracts produced from winery residues on the early in vitro development and physiological performance of garden cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.) seedlings. Seedlings were cultivated on peat-based pellets (Jiffy-7[®]) under controlled conditions and treated with two extract concentrations (10% and 20%, *w/v*) or distilled water (control). After 30 days, morphological traits (shoot height, leaf number and biomass) and biochemical parameters (photosynthetic pigments and total phenolics) were assessed. Both species showed high survival rates (>85%) and no phytotoxic symptoms. Compared with the control, VE20% increased chlorophyll a, chlorophyll b, and total chlorophyll contents in garden cress by 40.3%, 35.9%, and 38.9%, respectively, whereas VE10% increased chlorophyll a, total chlorophyll, and carotenoid contents in cucumber by 15.7%, 11.5%, and 25.0%, respectively. Total phenolic content did not differ significantly among treatments in either species (*p* > 0.05). These findings indicate that vermicompost extracts produced from winery residues exhibited low phytotoxic potential under the experimental conditions tested and influenced photosynthetic pigment accumulation without adversely affecting seedling growth. These responses warrant further investigation under ex vitro and field conditions to determine whether the observed physiological changes translate into relevant agronomic effects.

Keywords: peat-based pellets; vermicomposting; cucumber; garden cress; phytotoxicity



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1. Introduction

Wine production constitutes a major component of the global agricultural economy; however, throughout the production chain, large volumes of solid by-products, including pruning wood, grape stems, pomace, and liquid effluents, are generated, which may represent up to 30% of processed grape biomass and contribute to the estimated 1.5 million metric tons of solid materials generated annually by the wine industry worldwide [1]. In Portugal, approximately 94,347 tons of waste from agricultural, forestry, and fishing activities were reported in 2020 [2], further emphasizing the need for sustainable strategies for the management and valorization of agro-industrial by-products in order to reduce resource loss and improper disposal [3]. Despite their biochemical richness, these materials are frequently neither treated nor reused, and are often incorrectly disposed of or incinerated, causing significant threats to the environment, such as soil and water contamination [4–6]. Recycling and valorizing these by-products are therefore essential to mitigate environmental impacts and promote the sector's sustainability. From a circular economy perspective, vermicomposting emerges as a promising approach for reusing these by-products [3].

Vermicomposting involves the joint activity of earthworms and microbial communities that convert organic residues into a stabilized material characterized by high organic matter content and improved agronomic properties [7]. During vermicomposting, earthworms enhance the breakdown of organic substrates, stimulate microbial populations, and modify the physicochemical characteristics of the decomposing material [8]. Furthermore, earthworms may contribute to the reduction of the bioavailability of certain toxic compounds, including metals, thereby decreasing their potential environmental impact [9]. By diverting organic residues from landfill disposal, vermicomposting can also contribute to waste reduction, lower the environmental burden associated with residue accumulation, and help limit emissions linked to anaerobic decomposition [10]. The resulting product, vermicompost, provides both nutrients and microbial populations capable of supporting soil biological activity, thereby promoting improved soil structure and water-holding capacity [11]. The incorporation of vermicompost into soil can enhance plant development while increasing tolerance to pests and diseases. Additionally, vermicompost acts as a restorer of degraded areas, promoting biological balance and stimulating beneficial soil microbial life and reducing agriculture dependence on synthetic fertilizers [12–14].

In vitro culture systems offer a controlled approach for assessing how vermicompost-derived products affect plant physiology, as they allow for strict control over environmental conditions and eliminate external variables that could interfere with the results [15]. This system facilitates the observation of early plant development in response to different treatments and is particularly suitable for bioassays with aqueous extracts due to its speed, reproducibility, and capacity to accurately monitor both morphological and biochemical parameters [16,17]. In the context of phytotoxicity assessment, in vitro culture offers a sensitive tool to quantify morphological and physiological effects more rigorously than field systems, where multiple variables may impact results [18]. The use of inert and sterile substrates, such as Jiffy-7[®] peat pellets, has been increasingly explored [19,20]. These substrates provide a stable physical support and uniform moisture distribution, allowing for more accurate monitoring of root and shoot development than the liquid or filter paper systems frequently used in germination and phytotoxicity tests [21]. However, their application in fertilizer phytotoxicity tests is still scarce in the literature, as they are more common in vegetative propagation or rooting protocols [19,20,22]. Therefore, the use of Jiffy-7[®] peat pellets in studies involving vermicompost extracts represents a promising methodological approach for evaluating seedling growth and physiological responses under controlled cultivation conditions.

The use of model species such as garden cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.) is well established in phytotoxicity and plant bioassays due to their rapid germination, short life cycle, and high sensitivity to bioactive compounds [23–26]. These characteristics make both species suitable models for evaluating the biological effects of vermicompost-derived aqueous extracts under controlled in vitro conditions.

Therefore, the present study evaluated the effects of aqueous extracts obtained from vermicompost produced from grape pomace, grape stems, and grapevine pruning residues on the in vitro growth and physiological performance of *Lepidium sativum* L. and *Cucumis sativus* L. cultivated in Jiffy-7® peat pellets.

2. Materials and Methods

2.1. Vermicomposting Process

The winery residues used for vermicomposting included grape marc, grape stems, and grapevine pruning wood obtained from vineyards in Vila Real, Portugal. Before use, pruning wood was manually reduced to fragments of approximately 1–2 cm. Equal amounts of each residue (100 g fresh weight) were mixed and distributed into 1 L plastic containers, with three replicate containers prepared for the experiment. No pre-composting phase was performed prior to vermicomposting. *Eisenia fetida* earthworms, obtained from a laboratory colony maintained at UTAD (Vila Real, Portugal), were added at a biomass of 15 g per 300 g of fresh substrate. The experiment was conducted under room temperature, in darkness, for 91 days. Moisture content was maintained at approximately 70–80% by periodic addition of distilled water when required. The plastic containers were perforated to allow passive aeration, and the substrate was gently mixed when necessary to avoid compaction. Earthworm biomass was monitored at five timepoints during the vermicomposting process.

2.2. Vermicompost Characterization

The resulting vermicompost was characterized by physicochemical analysis at the Soil and Plant Analysis Laboratory of UTAD [27]. Analyzed parameters included pH, electrical conductivity, organic matter, macro- and micronutrient content, and trace metals (Table 1).

The phytotoxic potential of the vermicompost was determined through a seed germination bioassay using *Lepidium sativum* L. seeds. Dried VC samples (80 °C, 24 h) were extracted with distilled water at a ratio of 10% (*w/v*). The suspensions were shaken for 3 h at 180 rpm in an orbital shaker (SH-200, Cole-Parmer Ltd., Stone, UK) and filtered through Whatman no. 2 filter paper. For each treatment, twenty seeds were distributed in 90 mm Petri dishes with Whatman no. 42 filter paper. Then, a volume of 3 mL of distilled water (control) or VC aqueous extract was added to each dish. All treatments were performed in triplicate. Petri dishes were incubated under dark conditions at 28 °C for 4 days (E07086, MEMMERT, Schwabach, Germany). After incubation, seed germination was assessed by counting the number of seeds that had germinated. Radicle length was evaluated from digital images using Digimizer version 5.7.2 software (Digimizer, MedCalc Software, Ostend, Belgium). Phytotoxicity was expressed as the germination index (GI) [28], which integrates relative radicle elongation according to the equation $GI (\%) = (NSGT/NSGC) \times (CRT/CRC) \times 100$, where NSGT and NSGC denote the number of germinated seeds in the treatment and control groups, respectively, whereas CRT and CRC correspond to the mean radicle length measured in the treatment and control.

Microbial activity was assessed through basal respiration measurements based on CO₂ evolution, according to Anderson [29]. Briefly, fresh vermicompost (5 g) was incubated in sealed 1 L glass vessels with NaOH (1 M; 30 mL) traps at 25 °C for 4 days. After incubation, the amount of CO₂ released was quantified by titration with HCl (1 M), using phenolphthalein as the indicator following the addition of BaCl₂.

Table 1. Physicochemical properties; content in macronutrients, micronutrients and metals; basal respiration; germination index (GI); and total phenolic content of the final vermicompost. Data are presented as mean $\pm$ SD ($n = 3$).

Parameter	Value
C/N ratio	35.9 $\pm$ 2.5
Electrical conductivity (dS m ⁻¹)	0.73 $\pm$ 0.09
Organic matter (g kg ⁻¹ DW)	681 $\pm$ 17
pH (H ₂ O; 1:5)	6.3 $\pm$ 0.1
N-NH ₄ ⁺ /N ⁻ NO ₃ ⁻ ratio	4.09 $\pm$ 3.46
P (g element kg ⁻¹ DW)	2.31 $\pm$ 0.35
K (g element kg ⁻¹ DW)	28.1 $\pm$ 2.1
Ca (g element kg ⁻¹ DW)	9.02 $\pm$ 2.20
Mg (g element kg ⁻¹ DW)	2.44 $\pm$ 0.33
S (g element kg ⁻¹ DW)	1.54 $\pm$ 0.34
B (mg kg ⁻¹ DW)	20.1 $\pm$ 3.6
Fe (mg kg ⁻¹ DW)	2364 $\pm$ 1437
Cu (mg kg ⁻¹ DW)	43.8 $\pm$ 5.1
Zn (mg kg ⁻¹ DW)	89.54 $\pm$ 11.36
Mn (mg kg ⁻¹ DW)	148.3 $\pm$ 20.6
Ni (mg kg ⁻¹ DW)	2.99 $\pm$ 1.16
Cd (mg kg ⁻¹ DW)	0.05 $\pm$ 0.01
Pb (mg kg ⁻¹ DW)	1.16 $\pm$ 0.77
Cr (mg kg ⁻¹ DW)	4.96 $\pm$ 2.06
Hg (μ g kg ⁻¹ DW)	11.16 $\pm$ 2.65
Basal respiration (mg CO ₂ g ⁻¹ OM d ⁻¹)	5.05 $\pm$ 0.88
Germination index (GI, %)	85.0 $\pm$ 5.5
Total phenolic content (mg GAE g ⁻¹ DW)	5.79 $\pm$ 0.24

DW: dry weight; GAE: gallic acid equivalent.

Total phenolic compounds were quantified in lyophilized and milled vermicompost samples ($n = 3$). For each sample, 0.1 g was extracted three consecutive times with 1.4–1.5 mL of 50% acidified ethanol under continuous shaking (1 h). After each extraction, samples were centrifuged at 1800 $\times$ g (10 min), and the supernatants were pooled for subsequent analysis.

Total phenolics were measured using the Folin–Ciocalteu colorimetric assay [30]. Briefly, a portion of the combined extract (100 μ L) was mixed with distilled water (1.5 mL) and, after a 3 min incubation period, 100 μ L of Folin–Ciocalteu reagent and 300 μ L of 20% Na₂CO₃ were added. The reaction mixtures were kept at room temperature for 2 h, after which absorbance was read at 765 nm (Evolution 201, Thermo Scientific, Waltham, MA, USA). Results were expressed as milligrams of gallic acid equivalents (GAE) g⁻¹ of vermicompost dry weight (DW), using a gallic acid calibration curve [30].

2.3. In Vitro Assays

Aqueous vermicompost extracts (VEs) were prepared at two concentrations (10% or 20% *w/v*) following the same extraction procedure described for the phytotoxicity bioassay (Section 2.2). The 10% and 20% concentrations were selected based on concentrations commonly used in previous phytotoxicity and plant biostimulant bioassays, allowing for the evaluation of both moderate and relatively high extract strengths while avoiding excessive osmotic effects. The phenolic content of each VE was quantified using the Folin–Ciocalteu colorimetric method and reported as mg gallic acid equivalents g⁻¹ of DW [30]. The physicochemical characterization of the VE included pH and electrical conductivity, both measured in triplicate at room temperature. A portable pH meter (WTW

ProfiLine pH 3110, Xylem Analytics, WTW, Weilheim, Germany) coupled to a SenTix® 41 electrode and a conductivity meter (WTW ProfiLine Cond 3110, Xylem Analytics, WTW, Weilheim, Germany) fitted with a TetraCon® 325 electrode was used for these analyses (Xylem Analytics, WTW, Weilheim, Germany). To ensure sterility before application, the extracts were filtered through 0.2 µm membrane filters (Whatman® Puradisc 30/0.2 CA S MLL, Maidstone, UK).

Seeds of *Lepidium sativum* L. and *Cucumis sativus* L. cv. Marketer (Rocalba, Girona, Spain) were surface sterilized using standard aseptic laboratory protocols before germination on sterile 0.8% agar. Following radicle emergence, individual seedlings were aseptically transferred to sterilized Jiffy-7® [31], following the procedure described previously [32]. According to the manufacturer's specifications, the Jiffy-7® peat pellets used in this study had a pH of approximately 6.0 and an electrical conductivity of 0.2 mS cm⁻¹. The culture jars remained sealed throughout the 30-day cultivation period, minimizing moisture loss and maintaining adequate substrate hydration without the need for additional irrigation or extract application. Three treatments were established for each species: distilled water control (CNT), VE10% and VE20%. Each treatment included 15 biological replicates ($n = 15$), with each replicate consisting of one seedling grown in an individual Jiffy-7® pellet placed in a separate culture jar (Figure 1). Under aseptic conditions, 1 mL of the corresponding extract or distilled water for control was added to each Jiffy-7®, followed by seedling transfer. The jars were kept for 30 days at 25 ± 1 °C with a daily light/dark cycle (16 h light and 8 h darkness) with a photosynthetic photon flux density of approximately 55 µmol m⁻² s⁻¹ at explant level in a controlled-environment growth chamber (Fitoclima 'Walk-in' 4600, Aralab, Lisbon, Portugal). No additional applications were performed during the 30-day experimental period.

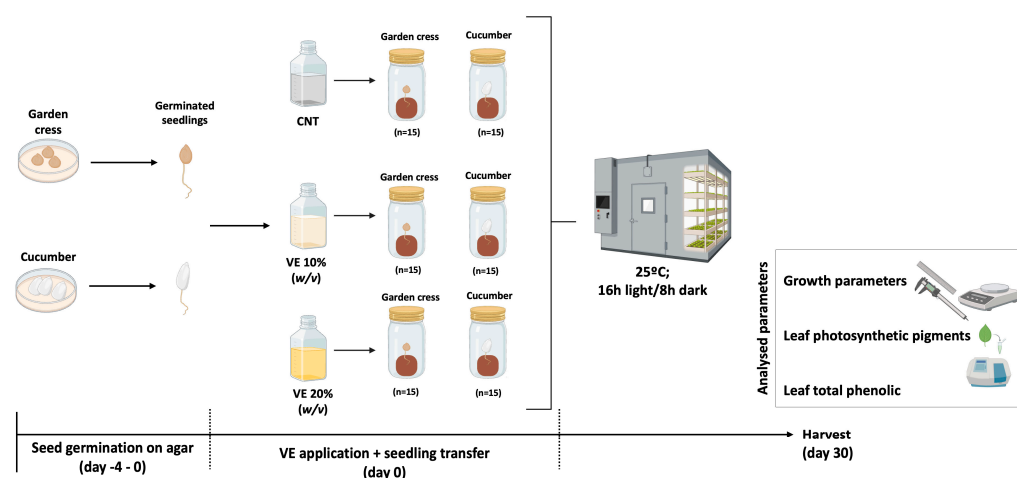


Figure 1. Schematic representation of the experimental protocol (CNT: control; VE: aqueous vermicompost extract).

After 30 days of cultivation, seedling survival (%) was determined by calculating the proportion of viable plants in relation to the initial number of germinated seedlings. Morphological development was assessed through the measurement of shoot height, stem diameter, number of leaves, and shoot fresh biomass. For biochemical determinations, leaf material was harvested on the same day during the morning period, under the same experimental conditions. Samples were immediately immersed in liquid nitrogen and preserved at −80 °C until further processing. Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoids were extracted from leaf material with 80% (*v/v*) acetone and quantified according to Sesták et al. [33] and Lichtenthaler [34]. Leaf total phenolic content was determined following the methodology of Singleton and Rossi [30]. A portion (20 µL)

of the acetone extract was mixed with 100 μL of Folin–Ciocalteu reagent and 80 μL of 7.5% (w/v) sodium carbonate and incubated for 30 min at 25 $^{\circ}\text{C}$. Afterwards, absorbance was recorded at 765 nm using a PowerWave X2S microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Phenolic content was determined from a gallic acid calibration curve and expressed as mg GAE g^{-1} leaf DW.

2.4. Statistical Analysis

Statistical analyses were performed with GraphPad Prism 10.4.1 (GraphPad Software, Boston, MA, USA). Prior to hypothesis testing, normality and homogeneity of variance were checked using the Shapiro–Wilk and Levene tests. When these assumptions were satisfied, treatment groups were compared using one-way ANOVA followed by Tukey’s multiple comparisons test, and values are presented as mean $\pm$ standard deviation (SD). When the assumptions for parametric testing were not fulfilled, the Kruskal–Wallis test was used, followed by Dunn’s multiple comparisons test, and results are shown as median with the 25th and 75th percentiles. Seedling survival was followed throughout the assay and, at the final timepoint, calculated as the proportion of viable seedlings relative to those initially germinated. Differences in seedling survival rates among treatments were evaluated using Fisher’s exact test. Significant differences were considered when $p < 0.05$.

3. Results and Discussion

3.1. Evolution of Earthworm Biomass and Assessment of Vermicompost Quality

Earthworm biomass was monitored throughout the vermicomposting process as an indicator of earthworm adaptation and substrate suitability (Figure 2). Biomass increased from 15.00 g to 17.10 ± 1.72 g by day 35, indicating good initial adaptation to the substrate. Thereafter, biomass gradually declined during the later stages of vermicomposting, reaching 10.98 ± 0.90 g by day 91. This pattern suggests that earthworm growth was favored during the early phase of active substrate transformation, whereas the subsequent decrease may be associated with reduced availability of easily degradable organic fractions and changes in substrate quality as the process progressed. Similar trends have been described in vermicomposting systems, where earthworm biomass commonly increases during active substrate degradation and subsequently declines as available resources become limited [35–37].

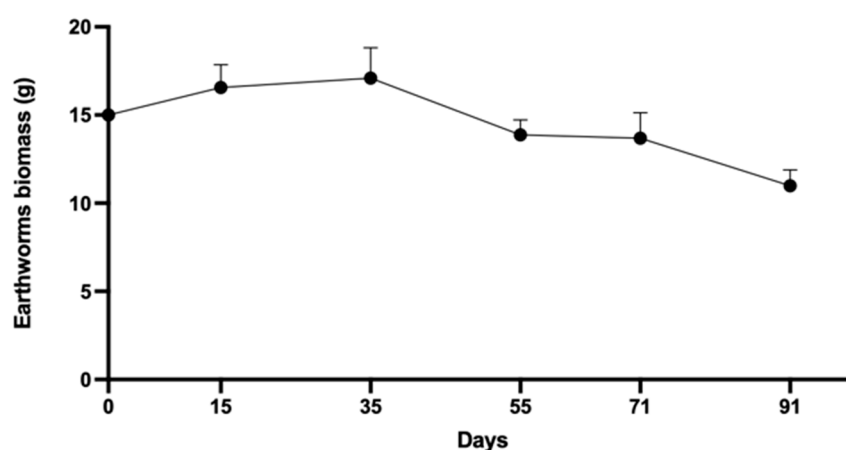


Figure 2. Earthworm biomass throughout the 91-day vermicomposting process.

The physicochemical characteristics of the vermicompost are presented in Table 1. The pH remained within the range considered suitable for plant growth, and the electrical conductivity (0.73 ± 0.09 dS m^{-1}) indicated a low risk of salinity stress during seed germination and early seedling development [38–40]. The C/N ratio was higher than the

values commonly reported for highly stabilized vermicomposts, which are generally below 20 [41,42]. This can be explained by the presence of lignocellulosic materials, particularly grapevine pruning wood, which are characterized by high carbon content and slow decomposition rates [43]. Similar C/N values have been reported in vermicomposts produced from woody or pruning residues, where the degradation of recalcitrant organic matter may delay stabilization [28,44,45]. Likewise, the relatively high basal respiration indicates active microbial metabolism and ongoing organic matter decomposition [46,47]. This apparent discrepancy may be explained by the simultaneous occurrence of microbial degradation of more labile organic fractions and the persistence of recalcitrant lignocellulosic compounds, which maintain a higher C/N ratio despite active microbial activity. Although these characteristics may indicate that the vermicompost had not yet reached complete stabilization, the germination index exceeded the 80% threshold commonly considered indicative of low phytotoxicity and acceptable compost maturity for plant bioassays [48]. The vermicompost total phenolic content corresponded to 5.79 ± 0.24 mg GAE g⁻¹ DW.

Potassium was the predominant macronutrient in the vermicompost, reaching 28.13 ± 2.14 g kg⁻¹ DW, whereas calcium was present at 9.02 ± 2.20 g kg⁻¹ DW and phosphorus and magnesium at 2.31 ± 0.35 and 2.44 ± 0.33 g kg⁻¹ DW, respectively. The high potassium content may be attributed to the presence of grape pomace in the vermicomposting mixture, since this agro-industrial residue is typically characterized by elevated K concentrations [49]. Among micronutrients, Fe showed the highest concentration (2364 ± 1437 mg kg⁻¹ DW), although with considerable variability among replicates. This variability likely reflects the inherent heterogeneity of the vermicompost matrix derived from mixed winery residues. Manganese, zinc and copper were present at 148 ± 21 , 90 ± 11 and 44 ± 5 mg kg⁻¹ DW, respectively, while boron reached 20.1 ± 3.6 mg kg⁻¹ DW. Metals including Ni, Cd, Pb, Cr and Hg were detected only at trace concentrations of 2.99 ± 1.16 mg kg⁻¹ DW, 0.05 ± 0.01 mg kg⁻¹ DW, 1.16 ± 0.77 mg kg⁻¹ DW, 4.96 ± 2.06 mg kg⁻¹ DW and 11.16 ± 2.65 µg kg⁻¹ DW, respectively, remaining well below the maximum limits established for organic fertilizers under Portuguese legislation (Portaria n° 185/2022 de 21 July 2022), and did not pose environmental or agronomic risks. These results indicate that the vermicompost presents an adequate nutritional and environmental profile for potential agricultural use.

3.2. Aqueous Vermicompost Extract Characterization

The physicochemical profile of the aqueous vermicompost extracts (VE10% and VE20%) is presented in Table 2.

Table 2. pH, electrical conductivity (EC) and total phenolic content of the aqueous vermicompost extract (VE). Data are presented as mean $\pm$ SD ($n = 3$). For each parameter, different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Parameter	VE10%	VE20%
pH	6.380 ± 0.004 ^a	6.356 ± 0.008 ^b
EC (mS cm ⁻¹)	1.486 ± 0.001 ^a	2.830 ± 0.010 ^b
Total phenolic content (mg GAE g ⁻¹ DW)	0.868 ± 0.015 ^a	0.730 ± 0.01 ^a

The pH values were close to neutrality and differed slightly between treatments ($p < 0.05$), ranging from 6.356 ± 0.008 in VE20% to 6.380 ± 0.004 in VE10. Electrical conductivity significantly increased from 1.486 ± 0.001 mS cm⁻¹ in VE10% to 2.830 ± 0.010 mS cm⁻¹ in VE20% ($p < 0.05$), reflecting higher concentration of dissolved ions in the more concentrated extract.

No significant differences were observed in total phenolic content between VE10% and VE20%, with values of 0.868 ± 0.015 and 0.730 ± 0.010 mg GAE g⁻¹ DW for VE10% and VE20%, respectively. Unexpectedly, the more concentrated extract did not exhibit a higher total phenolic content. The reasons for this observation remain unclear but may be related to limitations associated with the extraction procedure or interactions among extract constituents at higher concentrations. However, the present study was not designed to investigate the mechanisms underlying this response.

3.3. Effect of Aqueous Vermicompost Extract on Garden Cress In Vitro Growth

Garden cress survival was high in all treatments, reaching 93.3% in the CNT, 100% in VE10%, and 86.7% in VE20%. According to Fisher's exact test, no significant differences were observed among treatments ($p > 0.05$). Likewise, none of the morphological parameters evaluated differed significantly following VE application ($p > 0.05$; Table 3). These findings indicate that the tested vermicompost extracts did not exert phytotoxic effects on garden cress seedlings during the experimental period [50–52].

Table 3. Garden cress morphological parameters at the end of the experimental period (30 days). Data are presented as mean $\pm$ SD for parametric data or median (25th percentile–75th percentile) for non-parametric data. For each parameter, different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Parameter	CNT ($n = 14$)	VE10% ($n = 15$)	VE20% ($n = 13$)
Shoot length (cm)	4.10 ± 1.52^a	3.43 ± 1.44^a	3.58 ± 1.33^a
Stem diameter (mm)	0.57 ± 0.07^a	0.54 ± 0.20^a	0.55 ± 0.09^a
Shoot fresh weight (g)	$0.05 (0.03–0.07)^a$	$0.03(0.02–0.05)^a$	$0.04 (0.03–0.06)^a$
Number of leaves	$5.50 (4.88–6.50)^a$	$5.50 (4.50–6.00)^a$	$5.50 (4.50–6.00)^a$

Although growth traits remained unchanged, VE treatment altered the photosynthetic pigment profile of garden cress seedlings (Figure 3). VE20% significantly increased chlorophyll *a*, chlorophyll *b*, and total chlorophyll in comparison with CNT ($p < 0.05$). VE10% resulted in intermediate pigment levels, with no significant difference from either CNT or VE20%. Earlier reports have also described increased pigment accumulation and changes in bioactive compound profiles following vermicompost application, even when the phenolic concentration of the extract is low [53–55]. The increase in chlorophyll content in VE20% may reflect an effect of nutrient availability on photosynthetic pigment synthesis; however, this response was not accompanied by changes in growth-related parameters [56,57]. The absence of concomitant changes in biomass-related traits despite increased pigment accumulation may indicate that the enhanced photosynthetic machinery was not sufficient to promote measurable growth within the 30-day experimental period. During early seedling development, plants may preferentially allocate available resources towards metabolic adjustments and the synthesis of photosynthetic pigments rather than towards structural biomass accumulation. Additionally, a single initial application of 1 mL of VE may have provided a transient stimulus that influenced physiological processes but was insufficient to sustain long-term growth responses. Therefore, the observed increase in pigment content may represent an early physiological adaptation preceding detectable morphological changes.

The chlorophyll *a/b* ratio differed significantly between CNT and VE10%, while no other differences were observed among treatments. Total phenolic content and carotenoid levels tended to increase following VE application, particularly in VE20%, although these differences were not statistically significant. Carotenoid concentration remained stable or tended to increase following VE application, without adverse effects on seedling growth. Carotenoids, in addition to assisting in photosynthesis, are essential in the protection

against oxidative stress [58]. Similar results have been described by Fesendouz et al. [59] and Pant et al. [60], who observed stable carotenoid contents in horticultural crops supplemented with vermicompost, particularly during the early stages of development.

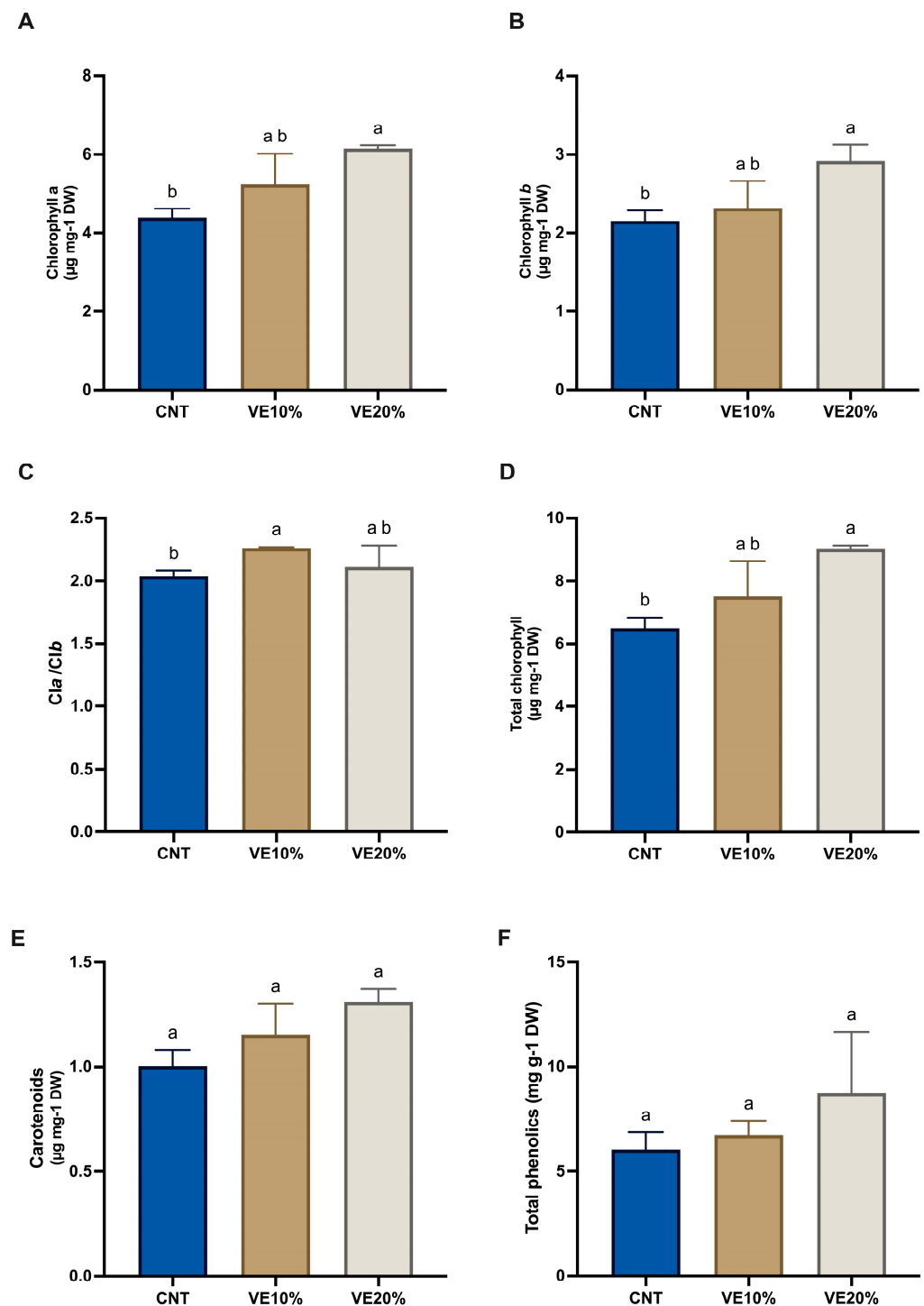


Figure 3. Biochemical parameters of garden cress leaves determined at the end of the experimental period: (A) chlorophyll a; (B) chlorophyll b; (C) chlorophyll a/b ratio (Cla/Clb); (D) total chlorophyll; (E) carotenoids; (F) total phenolics. Data are presented as mean $\pm$ SD ($n = 3$). For each parameter, different lowercase letters within the respective line indicate significant differences among the treatments ($p < 0.05$).

3.4. Effect of Aqueous Vermicompost Extract on *Cucumis sativus* L. In Vitro Growth

Cucumber survival was high across all treatments, reaching 93.3% in CNT and VE10%, and 100% in VE20%. Fisher's exact test showed no significant differences among treatments ($p > 0.05$). The evaluated morphological traits of cucumber seedlings did not differ significantly among treatments (Table 4). These findings indicate that the tested vermicompost extracts did not exert phytotoxic effects on cucumber seedlings under the experimental conditions.

Table 4. Cucumber morphological parameters at the end of the experimental period. Data are presented as mean $\pm$ SD for parametric data or median (25th percentile–75th percentile) for non-parametric data. For each parameter, different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Parameter	CNT ($n = 14$)	VE10% ($n = 14$)	VE20% ($n = 15$)
Shoot length (cm)	8.19 $\pm$ 2.02 ^a	8.29 $\pm$ 1.50 ^a	8.45 $\pm$ 1.41 ^a
Stem diameter (mm)	2.16 $\pm$ 0.35 ^a	2.34 $\pm$ 0.38 ^a	2.09 $\pm$ 0.39 ^a
Shoot fresh weight (g)	0.05 $\pm$ 0.03 ^a	0.04 $\pm$ 0.03 ^a	0.04 $\pm$ 0.02 ^a
Number of leaves	7.00 (6.75–8.00) ^a	7.00 (6.75–8.25) ^a	7.00 (7.00–8.00) ^a

These results indicate that the VE did not affect shoot length, stem diameter, leaf number, or shoot fresh weight, supporting the absence of phytotoxic effects under the experimental conditions. Comparable findings were reported by Shafique et al. [61] and Azarmi et al. [62], who found that vermicompost application improved seedling growth or maintained it at control levels without toxic effects. Although growth parameters remained unchanged (Table 4), VE influenced the photosynthetic pigment profile of cucumber seedlings (Figure 4). VE10% significantly increased chlorophyll *a*, total chlorophyll, and total carotenoids compared with CNT ($p < 0.05$). No significant differences were observed in total phenolic content among treatments. Comparable responses of photosynthetic pigment levels to different vermicompost concentrations have been reported in other horticultural species, including cucumber and chickpea [48,62,63]. These findings indicate that the aqueous vermicompost extracts influenced photosynthetic pigment accumulation without significantly affecting seedling growth.

The different physiological responses observed between *Lepidium sativum* and *Cucumis sativus* highlight the importance of species-specific responses to aqueous vermicompost extracts. Although both species exhibited low phytotoxicity, garden cress responded more markedly to the higher extract concentration, whereas cucumber showed greater sensitivity to the lower concentration, suggesting that optimal application rates may vary according to plant species and possibly among botanical families. *L. sativum* is widely used as a sensitive model species in phytotoxicity bioassays, while *C. sativus* represents an economically important horticultural crop. However, the present work evaluated only the early seedling stage under controlled in vitro conditions. Therefore, it remains uncertain whether the observed changes in photosynthetic pigments would translate into improved plant growth, productivity or yield under greenhouse or field conditions. Further studies using mature plants and economically relevant crops are required to evaluate the agronomic significance of these physiological responses.

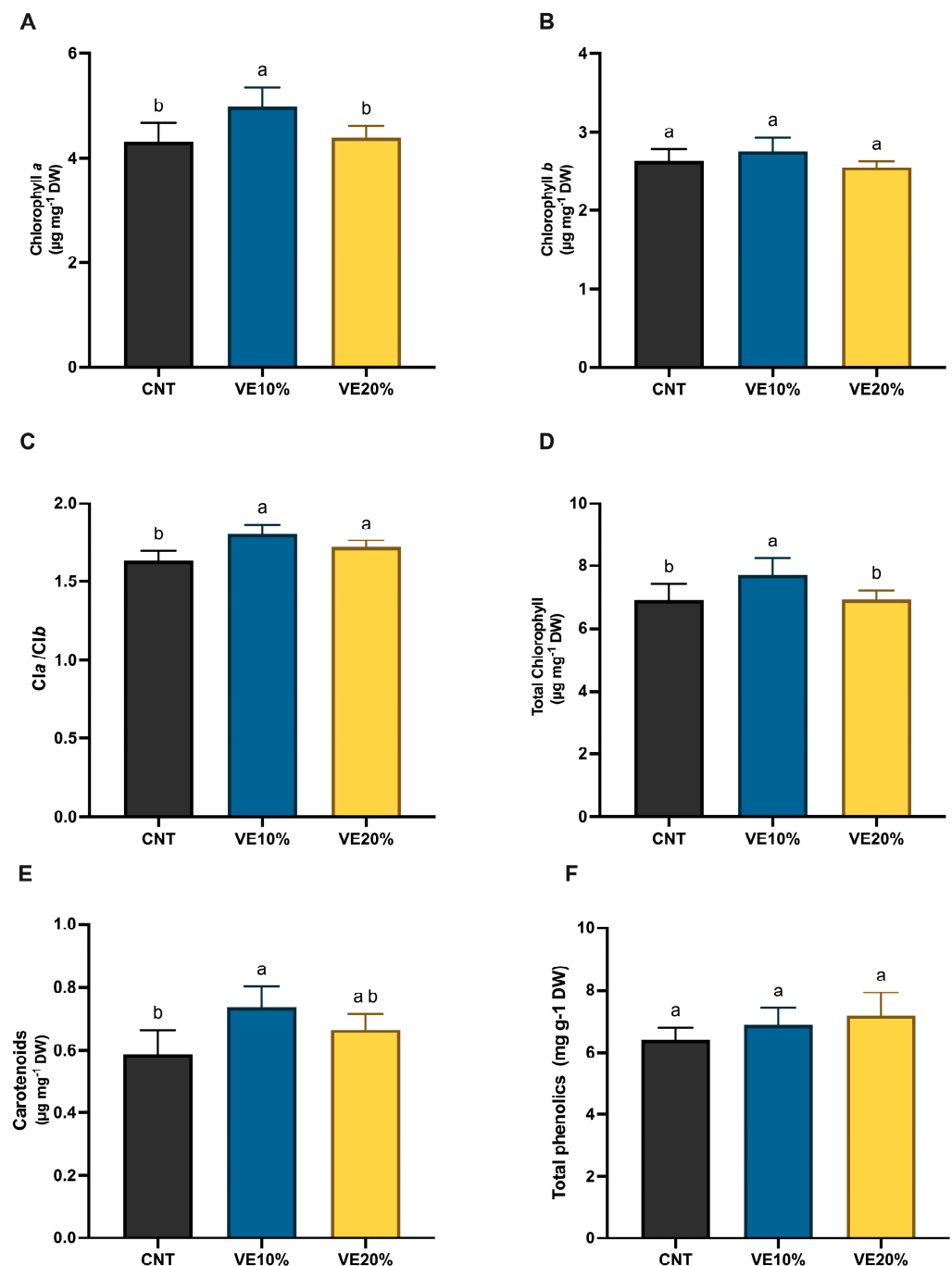


Figure 4. Biochemical parameters of cucumber leaves determined at the end of the experimental period (30 days): (A) chlorophyll a; (B) chlorophyll b; (C) chlorophyll a/b ratio; (D) total chlorophyll; (E) carotenoids; (F) total phenolics. Data are presented as mean $\pm$ SD ($n = 3$). For each parameter, different lowercase letters within the respective line indicate significant differences among the treatments ($p < 0.05$).

3.5. Study Limitations and Future Perspectives

Despite the encouraging physiological responses observed, the present study has several limitations that should be considered when interpreting the results. The aqueous vermicompost extracts were characterized only in terms of pH, electrical conductivity and total phenolic content. Therefore, it was not possible to identify the specific nutrients, dissolved ions or other bioactive compounds potentially responsible for the observed changes in physiological responses. Although the higher electrical conductivity of VE20% is consistent with a greater concentration of dissolved ions, the ion composition was not

determined, and the observed changes in photosynthetic pigment accumulation cannot therefore be attributed to specific nutritional or biochemical mechanisms. In addition, the present work represents a preliminary in vitro assessment based on a single extract application and the evaluation of only two extract concentrations, which do not allow for the establishment of a complete concentration–response relationship or the assessment of repeated applications. Moreover, the experimental design was intended as a controlled in vitro assessment rather than to reproduce agricultural management practices. The absence of a positive control treatment and the relatively limited biological replication used for biochemical analyses should also be considered when interpreting the results. Future studies should include a comprehensive chemical characterization of the aqueous extracts, a wider range of extract concentrations, repeated applications, appropriate positive controls, increased biological replication, and ex vitro and field evaluations to better understand the mechanisms underlying the observed physiological responses and to further assess their potential agricultural applications.

4. Conclusions

This preliminary in vitro study showed that aqueous vermicompost extracts obtained from winery residues exhibited low phytotoxic potential under the experimental conditions tested, as no adverse effects were observed on the early development of *Lepidium sativum* L. and *Cucumis sativus* L. The use of Jiffy-7[®] together with an extended in vitro cultivation period enabled the assessment of seedling growth and physiological responses beyond the germination stage.

Although the extracts did not significantly affect morphological development, they influenced selected physiological traits, particularly chlorophyll and carotenoid contents, depending on extract concentration and plant species. In garden cress, the higher extract concentration induced more evident changes in chlorophyll content, whereas in cucumber, the lower concentration resulted in stronger pigment responses. These findings indicate that aqueous vermicompost extracts may induce specific physiological changes without adversely affecting seedling growth under the tested conditions.

However, further studies including comprehensive extract characterization, broader experimental conditions, and ex vitro and field validation are required to determine the agronomic relevance of these responses.

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Abbreviations

The following abbreviations are used in this manuscript:

VE Aqueous vermicompost extracts
CNT Distilled water control

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