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EFFECT OF VERMICOMPOST LEACHATE ON LETTUCE GERMINATION *IN VITRO*

EFECTO DEL LIXIVIADO DE VERMICOMPOST EN LA GERMINACIÓN DE LECHUGA *IN VITRO*

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ABSTRACT

Given the pressing global population growth and the escalating demand for food, the adoption of sustainable agricultural practices has become a necessity. In this context, the use of vermicompost leachate (VCL), a nutrient and microorganism-rich organic alternative to chemical fertilizers, has gained significant attention. However, its application necessitates careful consideration due to the potential phytotoxicity it may pose. The experiment was designed to evaluate the effects of various VCL concentrations on lettuce seed germination, vegetative development, photosynthetic pigments, and phytotoxicity in vitro. The concentrations tested ranged from 2.0% to 4.0%. The results revealed a concentration-dependent effect, with lower concentrations (2.0%-2.5%) showing neutral or positive effects on germination and growth, and higher concentrations (3.5%-4.0%) demonstrating phytotoxic effects, leading to reduced germination rates and impaired seedling growth. The analysis of photosynthetic pigments further supported these findings, showing a decrease with increasing VCL concentrations. Principal component analysis further highlighted the concentration-dependent effects of VCL on seed germination and growth. While lower concentrations correlated positively with germination and growth parameters, higher concentrations exhibited negative correlations, indicating greater phytotoxicity. These findings highlighted the complex interplay between VCL concentrations and their effects on seedling growth and development.

Keywords: Lettuce; Germination; Phytotoxicity; Sustainable agriculture, Worm leachate.

RESUMEN

Dado el apremiante crecimiento de la población mundial y la creciente demanda de alimentos, la adopción de prácticas agrícolas sostenibles se ha convertido en una necesidad. En este contexto, el uso de lixiviados

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de vermicompost (VCL), una alternativa orgánica rica en nutrientes y microorganismos a los fertilizantes químicos, ha ganado significativa atención. Sin embargo, su aplicación requiere una cuidadosa consideración debido a la potencial fitotoxicidad que puede plantear. El experimento fue diseñado para evaluar los efectos de varias concentraciones de VCL sobre la germinación de semillas de lechuga, el desarrollo vegetativo, los pigmentos fotosintéticos y la fitotoxicidad in vitro. Las concentraciones probadas oscilaron entre 2.0% y 4.0%. Los resultados revelaron un efecto dependiente de la concentración, con concentraciones más bajas (2.0%-2.5%) que muestran efectos neutros o positivos sobre la germinación y el crecimiento, y concentraciones más altas (3.5%-4.0%) que demuestran efectos fitotóxicos, lo que conduce a tasas de germinación reducidas y deterioro. crecimiento de plántulas. El análisis de los pigmentos fotosintéticos apoyó aún más estos hallazgos, mostrando una disminución al aumentar las concentraciones de VCL. El análisis de componentes principales destacó aún más los efectos dependientes de la concentración del VCL sobre la germinación y el crecimiento de las semillas. Mientras que las concentraciones más bajas se correlacionaron positivamente con los parámetros de germinación y crecimiento, las concentraciones más altas mostraron correlaciones negativas, lo que indica una mayor fitotoxicidad. Estos hallazgos resaltaron la compleja interacción entre las concentraciones de VCL y sus efectos sobre el crecimiento y desarrollo de las plántulas.

Palabras claves: Lechuga; Germinación; Fitotoxicidad; Agricultura sustentable; Vermicomposta.

1. INTRODUCTION

In recent decades, there has been an accelerated increase in the global population, estimated to reach 9.7 billion by 2050, with a 70% increase in the demand for food and water (Chen *et al*., 2016). Intensive farming and the excessive use of agrochemicals such as fertilizers, pesticides, and water to meet food demand have led to water pollution and soil degradation, which are fundamental for production (Riedo *et al*., 2023; Campos *et al*., 2024). In this context, the shift towards more sustainable and environmentally friendly agriculture has led to the research and use of practices and materials that preserve soil health and biodiversity (Crist *et al*., 2017; Lehmann *et al*., 2020).

The use of organic fertilizers, such as biochar, manures, compost, or vermicompost, among others, represents an ecological alternative for food production, as they help enrich the soil with nutrients, improve its structure water retention capacity, and promote biological activity (Singh *et al*., 2020; Shaji *et al*., 2021; Fan *et al*., 2023). Within this category, vermicompost leachates (VCL) emerges as a highly effective and sustainable option. VCL is a brown liquid resulting from the passage of water through vermicomposting devices that contains beneficial microorganisms, nutrients, hormones, and enzymes that can stimulate plant growth (Aremu *et al*., 2015; Rehman *et al*., 2023; Wongkiew *et al*., 2023). Unlike other organic fertilizers that require time to decompose and release nutrients, VCL makes them immediately available to plants, promoting their growth and even helping control phytopathogens (Sivasabari *et al*., 2023; Vambe *et al*., 2023).

Several studies have documented the benefits of using VCL during the development and production of horticultural crops, with positive results (Benazzouk *et al*., 2020; Alcívar *et al*., 2021; Alemán *et al*., 2021; Torres *et al*., 2024). However, despite the multiple benefits of VCL, its application is challenging, especially those related to appropriate dosing. Undiluted VCL can harm germination and seedling growth (Gutiérrez *et al*., 2011; Kandari *et al*., 2011). Inappropriate concentrations can cause phytotoxic effects, such as inhibition of root growth and reduced germination, due to the presence of salts and other compounds in high concentrations (Sanadia *et al*., 2019). The concentrations of VCL in various studies vary widely, from 0.2% to greater than 40%, and they are used in different ways and in different phenological states of the crops (Aremu *et al*., 2015; Gutiérrez *et al*., 2011; Ameen, 2020). Therefore, it is essential to determine the appropriate concentration of VCL to maximize its benefits without harming the plants, especially during germination.

Since germination is a critical phase for crop establishment, it depends on intrinsic seed factors, environmental factors, and stimulants and inhibitors (Kaur *et al*., 2018). In this context, choosing lettuce (*Lactuca sativa*) as a model crop to study the effect of VCL during germination and its phytotoxic effect is strategic, given that it is a crop consumed fresh of significant economic importance worldwide and recommended for standard toxicity tests (ISO, 2012; De Corato, 2019). Lettuce is a crop with rapid growth and sensitive to environmental conditions or management changes, making it an excellent indicator for studying the impacts of different agricultural treatments (Galieni *et al*., 2016).

This study aimed to evaluate the efficacy of different concentrations of VCL on lettuce germination and phytotoxicity in vitro to understand how this biostimulants can be optimally used during seedling production, contributing to sustainable agricultural practices.

2. MATERIALS AND METHODS

2.1 Experiment

The assay was conducted in the Applied Microbiology, Plant Pathology, and Postharvest Physiology Laboratory of the Autonomous University of Chihuahua (UACH). The methodology of Sobrero and Ronco (2014) was modified to establish the assay. Firstly, lettuce seeds (*Lactuca sativa* cv. 'Black Seeded Simpson'; KristenSeed®) were sterilized using a 5% (v/v) NaClO solution for 5 min. Subsequently, three rinses with distilled water were conducted, and the seeds were left to dry.

The VCL was obtained from vermicompost boxes (1.0 m x 1.2 m x 0.80 m) with *Eisenia* sp. and elaborated from grass and garden pruning residues. The analysis of the physicochemical composition of the VCL was carried out in the UACH soil laboratory. The analysis indicated that the VCL contained 0.19% nitrogen, 1.60% phosphorus, 0.22% potassium, 0.73% calcium, 0.12% magnesium, 125.5 mg•L^{-1} copper, 606.50 mg•L^{-1} iron, 99.50 mg•L^{-1} manganese, and 300.50 mg•L^{-1} zinc, with a pH of 6.78 and an EC of 7.49 mS•cm. VCL concentrations were prepared as treatments at 2.0%, 2.25%, 2.50%, 3.0%, 3.25%, 3.5%, 3.75%, and 4.0%, while distilled water was used as a control. A volume of 5 mL from each VCL concentration was taken and placed in sterile Petri dishes (9 mm x 11 mm) containing sterile paper. Sterile lettuce seeds were placed in the Petri dishes treated with different concentrations of CVL, using 25 seeds per dish. These dishes were sealed with Parafilm® to prevent moisture loss and were placed in a germination chamber at 25 °C ± 2 for 6 days.

2.2 Germination parameters

Seed germination was monitored daily and expressed as a germination percentage using the equation (1) described by Al-Ansari and Kiski (2016). Seeds that had completed germination were considered when hypocotyls emerged.

$$Germination\ percentage\ (G\%) = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} x\ 100 \qquad (1)$$

The mean germination time (MGT) was calculated using the equation (2) proposed by Ranal and Garcia (2016) as indicated below:

$$MGT\ (\text{day}) = \sum_{i=1}^{k} \left(\frac{Ni\ x\ Ti}{Ni} \right) \qquad (2)$$

Where, MGT=mean germination time (day); Ni=number of germinated seeds on day Ti. Ti= time from the start of experiment to the *i* observation and k= the last time of germination.
Finally, the mean germination speed (MGS) was determined following the equation (3) proposed by Wardle *et al*., (1991):

$$MSG\left(seed\ day^{-1}\right) = \frac{N1}{T1} + \frac{N2}{T2} + \frac{N3}{T3} + \ldots \frac{Nn}{Tn} \qquad (3)$$

Where, N1, N2, N3,...,Nn = numbers of geminated seeds observed at time (days); T1,T2,T3,…,Tn= time after sowing (numbers of seeds that germinated at the specific time).

2.3 Morphological parameters

The morphological characteristics of the lettuce seedlings evaluated were hypocotyl length, collar diameter and radical length. For this, 1,200 dpi digital images of the seedlings were created (Epson EcoTank® L5590 C11CK57301, Seiko Epson Corp., Philippines), and ImageJ 1.46r software was used to perform the measurements.

2.4 Photosynthetic pigments

The determination of photosynthetic pigments was according to the methodology described by Lichtenthaler and Wellburn (1983). A sample of 0.1 g of fresh cotyledon leaves were ground in 4 ml of 80% acetone (v/v) (JT Baker, Estado de México, México). The mixture was centrifuged at 3,000 rpm for 5 min (Eppendorf centrifuge 5430R, Eppendorf AG, 22331 Hamburg, Germany). The absorbance of the supernatant was measured at 663, 645, and 470 nm using a UV spectrometer (Evolution 60 S; Thermo Fisher Scientific Inc., Waltham, MA, USA) to determine chlorophyll and carotenoids. As a black, 80% acetone (v/v) was used. The calculation of the total amount of chlorophyll a and b and carotenoids was using the following equations (4, 5, 6):

$$Chl\ a\ (mg{\bullet}g^{-1}FW) = (12.21\ x\ A_{663}\text{-}2.81\ x\ A_{645})\ x\ V/(1000\ x\ W) \qquad (4)$$

$$Chl\ b\ \left(mg{\bullet}g^{-1}FW\right) = (20.13\ x\ A_{645}\text{-}5.03\ x\ A_{663})\ x\ V/(1000\ x\ W) \qquad (5)$$

$$Carotenoids\ \left(mg{\bullet}g^{-1}FW\right) = \left[\frac{(1000\ x\ A_{470}\text{-}3.27\ x\ Chl_a\text{-}104\ x\ Chl_b)}{229}\right] x\ V/(1000\ x\ W) \qquad (6)$$

Where V= volume in mL of 80 % acetone (v/v), FW= fresh weight of hypocotyl in g of the sample.

2.5 Phytotoxicity

To assess the low toxicity of CVL on root growth and high toxicity on germination, the relative germination percentage (RGP), relative root growth (RRG), and germination index (GI) were calculated according to the following equations (7, 8, 9) (Tiquia, 2000).

$$RGP(\%) = \frac{Gs}{Gc}\ x\ 100 \qquad (7)$$

Where: Gs = number of germinated seeds in the samples (VCL concentrations) and Gc = number of germinated seeds in the control (distilled water).

(8)

$$RRG(\%) = \frac{Ls}{Lc} \; x \; 100$$

Where: Ls = length of the radicle in the germinated seeds in the samples and Lc = length of radicle in the germinated seeds in the control.

$$GI = \frac{RGP \; x \; RRG}{100} \qquad (9)$$

2.6 Statistic Analysis

The trial was established under a completely randomized experimental design, where concentrations of VCL at 2.0%, 2.25%, 2.50%, 3.0%, 3.25%, 3.5%, 3.75%, and 4.0% were tested as treatments on lettuce seeds, with distilled water employed as control. Each treatment was replicated five times, using a Petri dish per replication. The data obtained from the germination, morphological, and phytotoxicity parameters were subjected to the Shapiro-Wilk tests to determine their normal distribution and the Levene test to determine the homogeneity of variance. The MSG data was transformed to meet normality (Box-Cox transformation). Subsequently, an analysis of variance (ANOVA) and Tukey's means separation tests were performed ($p < 0.05$). GMT was analyzed with the Kruskal-Wallis/Conover-Iman test. Simple regressions were also performed between VCL concentrations, photosynthetic pigment content, and VCL and germination rate. To determine the influence of leachate concentrations on germination, the development of lettuce seeds, and their phytotoxicity, a principal component analysis (PCA) was carried out using the most relevant variables. Prior to this, Bartlett's efficiency test was performed to test the validity of the data set ($p < 0.01$). The suitability of the data set to perform the PCA was tested using the Kaiser-Meyer-Olkin (KMO), which considered KMOs greater than 0.60 acceptable. The data were processed using InfoStat software (InfoStat 2021v. Grupo InfoStat, Argentina) and JAMOVI 2.5.2.0 software.

3. RESULTS

The leachate concentrations evaluated in this trial significantly influenced ($p \leq 0.05$) the gemination parameters of lettuce seeds (Table 1). The germination percentage was not affected by CVL concentrations at 2.25%, 2.75%, and 3.0%. However, increasing CVL concentrations to 4.0% reduced germination by 27.05%. In the case of MGT, it was observed that CVL concentrations of 2.0% to 2.5% showed an average germination time similar to the control. However, with increasing CVL concentration, the average germination time increases to 56.6% compared to the control. On the other hand, MSG decreased with the use of CVL from 7.5 to 2.7 germinated seeds/day with the maximum concentration of CVL.

Table 1. Germination parameters of lettuce seeds treated with different concentrations of vermicompost leachate *in vitro.*

Leachate concentration	G%	MGT [1] (days^{-1})	MSG (Number of seeds germinated day^{-1})
0%	98.7 ± 1.81 a	4.3 ± 0.05 a	12.4 ± 0.58 a
2.0%	96.0 ± 2.81 ab	5.1 ± 0.29 abc	7.5± 1.24 bc
2.25%	98.0 ± 2.99 a	5.1 ± 0.18 abc	7.6 ± 0.92 bc
2.5%	96.0 ± 1.52 ab	4.9 ± 0.09 ab	8.5 ± 0.46 b
2.75%	96.0 ± 2.37 a	5.3 ± 0.13 bc	6.8 ± 0.55 cd
3.0%	96.7 ± 2.37 a	5.3 ± 0.12 bcd	6.6 ± 0.50 cd
3.25%	94.0 ± 2.81 ab	5.5 ± 0.07 cde	5.7 ± 0.20 d
3.5%	91.3 ± 1.81 b	6.5 ± 0.49 de	3.8 ± 0.83 e

Leachate concentration	G%	MGT [1] (days^{-1})	MSG (Number of seeds germinated day^{-1})
3.75%	84.0 ± 2.81 c	6.8 ± 0.45 e	3.1 ± 0.53 ef
4.0%	72.0 ± 1.81 d	6.8 ± 0.22 e	2.7 ± 0.29 f

Means ± SD values are shown (n=5). Means followed by the same letter in the column do not differ significantly at ($p \leq 0.05$), based on Tukey test and [1]Conover-Iman test. G%= germination percentage, MGT=mean germination time, MSG= mean speed of germination.

The vegetative development of lettuce seedlings was significantly affected ($p \leq 0.05$) by vermicompost leachate concentrations (Figure 1). The hypocotyl length was not affected by VCL of 2 to 2.5%, which was equal to the control. However, increasing the concentration to 4.0% reduced the length by up to 35% compared to the control. Similarly, the collar diameter was similar when using concentrations of up to 2.5%; however, the maximum concentration of VCL reduced the collar diameter by up to 25.84%. The length of the radicle decreased significantly, with a reduction of 45.94% with concentrations of 2 to 2.75% of VCL and up to 72.30% compared to the control.

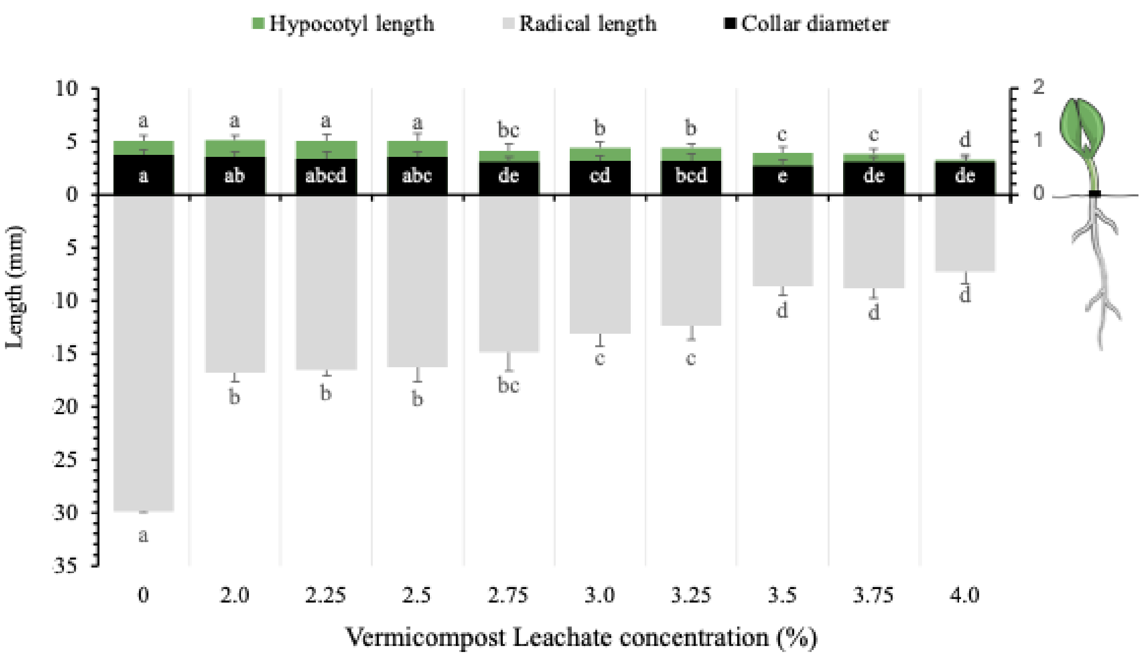

Figure 1. Morphological parameters of lettuce seedling treated with different concentrations of vermicompost leachate *in vitro*. Means ± SD values are shown (n=5 replications with 8 seedlings each). Means followed by the same letter in the bar do not differ significantly at ($p \leq 0.05$), based on Tukey test.

The content of photosynthetic pigments was significantly affected ($p \leq 0.05$) by leachate concentrations (Table 2). The 2.0% concentration did not affect the content of photosynthetic pigments; However, from 2.25% to the maximum concentration, the pigments will decrease between 5.37% and 84.0%. When analyzing the relationship between the content of each pigment and its reduction due to VCL concentrations, the regression models found an inversely proportional relationship between the decrease in pigment contents and the concentration of leachates evaluated. All regression models were adjusted to an r^2 above 85%, with a strong negative correlation of -0.923 and $p < 0.001$ (Table 3). Regression equations could be useful in predicting the behavior of photosynthetic pigment content at VCL concentrations lower or higher than those evaluated in this study.

Table 2. Photosynthetic pigments of lettuce seedlings treated with vermicompost leachate *in vitro.*

Leachate concentration	Chl a ($mg•g^{-1}$WF)	Chl b ($mg•g^{-1}$WF)	Carotenoids ($mg•g^{-1}$WF)
0%	0.918 ± 0.07a	1.432 ± 0.10a	0.319 ± 0.02a
2.0%	0.794 ± 0.14ab	1.355 ± 0.14a	0.296 ± 0.03a
2.25%	0.624 ± 0.06bc	1.068 ± 0.06b	0.237 ± 0.01b
2.5%	0.621 ± 0.07bc	0.999 ± 0.10b	0.222 ± 0.02b
2.75%	0.513 ± 0.05c	0.956 ± 0.05b	0.208 ± 0.01b
3.0%	0.320 ± 0.08d	0.497 ± 0.15c	0.114 ± 0.03c
3.25%	0.220 ± 0.06d	0.332 ± 0.08cd	0.074 ± 0.02cd
3.5%	0.191 ± 0.09d	0.291 ± 0.11cd	0.065 ± 0.03cd
3.75%	0.202 ± 0.04d	0.321 ± 0.07cd	0.071 ± 0.02cd
4.0%	0.146 ± 0.02d	0.234 ± 0.02d	0.052 ± 0.01d

Means ± SD values are shown (n=4). Means followed by the same letter in the column do not differ significantly at ($p \leq 0.05$), based on Tukey test. Chl a= chlorophyl a, Chl b= chlorophyl b.

Table 3. Regression models for leached concentration (Y) and pigments photosynthetic (X) according to sample concentration.

Treatment	Equation	r^2 (%)	*R*-Pearson	Significance
Chlorophyl a	Y= -0.334x + 1.405	85.3	-0.923	<0.001
Chlorophyl b	Y= -0.585x + 2.426	86.6	-0.931	<0.001
Carotenoids	Y= -1.28x + 0.533	87.5	-0.936	<0.001

The concentrations of VCL evaluated in this study showed a variable phytotoxic effect on lettuce seed germination (Table 4). Regarding RRG, concentrations of 2.0, 2.25, and 2.5% had the same effect on radicle development. But, from 2.75% to 4.0%, a decrease of up to 50.62% in radicle development was recorded. On the other hand, RGP was similar, with concentrations from 2.0 to 3.25% having values greater than 95%. However, as the percentage of VCL increased, a significant decrease in RGP was observed. Regarding IG, concentrations of 2.0, 2.25, and 2.5% had the highest germination indices. However, with increasing concentrations, a decrease in germination index of up to 57.55% was recorded.

Table 4. Phytotoxicity parameters of lettuce seeds treated with different concentrations of vermicompost leachate *in vitro.*

Leachate concentration	RRG (%)	RGP (%)	GI (%)
2.0%	56.1 ± 3.05 a	98.0 ± 2.39 a	54.9 ± 2.90 a
2.25%	55.2 ± 1.94 a	99.3 ± 3.02 a	54.9 ± 3.35 a
2.5%	54.3 ± 4.74 a	97.3 ± 1.51 ab	52.9 ± 4.99 a
2.75%	49.6 ± 5.92 ab	98.0 ± 2.39 a	48.6 ± 6.37 ab
3.0%	43.9 ± 4.11 b	98.0 ± 2.38 a	43.0 ± 4.28 b
3.25%	41.5 ± 6.67 b	95.3 ± 2.83 ab	39.6 ± 6.48. b
3.5%	29.0 ± 5.27 c	92.6 ± 1.85 b	26.9 ± 5.07 c

Leachate concentration	RRG (%)	RGP (%)	GI (%)
3.75%	29.7 ± 2.67 c	85.1 ± 2.83 c	25.2 ± 2.47 c
4.0%	24.4 ± 3.02 c	73.0 ± 1.85 d	17.8 ± 2.17 c

Means ± SD values are shown (n=5). Means followed by the same letter in the column do not differ significantly at ($p \leq 0.05$), based on Tukey test. RRG=relative root growth, RGP=relative germination percentage, GI= germination index.

The analysis of the relationship between the germination index values and the concentrations of VCL using a regression model indicated an inversely proportional relationship between the decrease in the GI and the increase in the concentration of leachates evaluated. The regression model fitted an r^2 of 86.3%, with a strong negative correlation of -0.929 and a p-value < 0.001 (Figure 2).

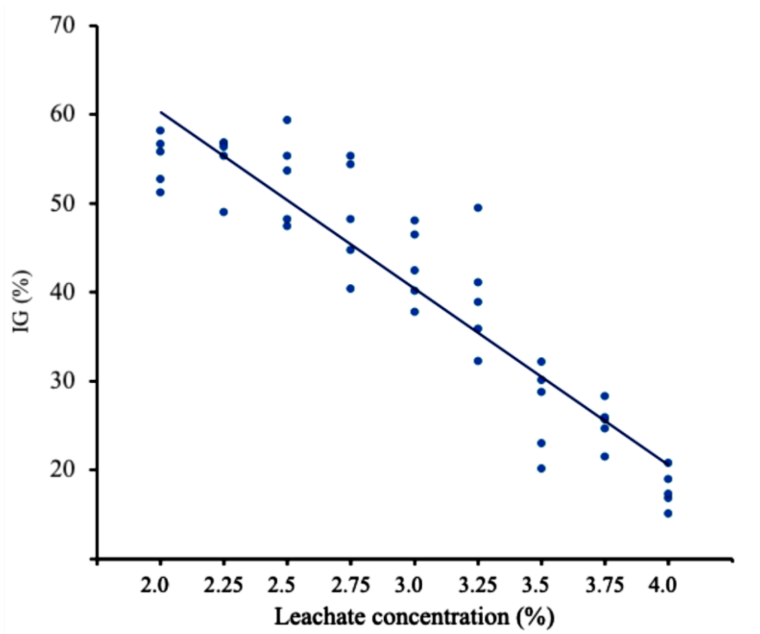

Figure 2. Germination index (GI) of lettuce seeds at different concentrations of vermicompost leachate *in vitro*. Independent variable: leachate concentration (%). Dependent variable: GI (%), y= -19.9x + 100.1; r^2= 86.3%.

Principal Component Analysis (PCA) was performed to determine the effect of VCL concentrations on the germination of lettuce seeds *in vitro*, followed by a k-means cluster analysis, with the variables representative of vegetative development (CD, HL, RL), germination (G%, MSG, MGT) and the phytotoxic effect of VCLs on seeds (GI). The PCA explained 91.1% of the variability in the data; Dim1 explained 79.3%, and Dim2 11.8% (Figure 3). The graphical representation of the two dimensions of the PCA, shown in Figure 3, suggests the presence of distinct substructures within each data set.

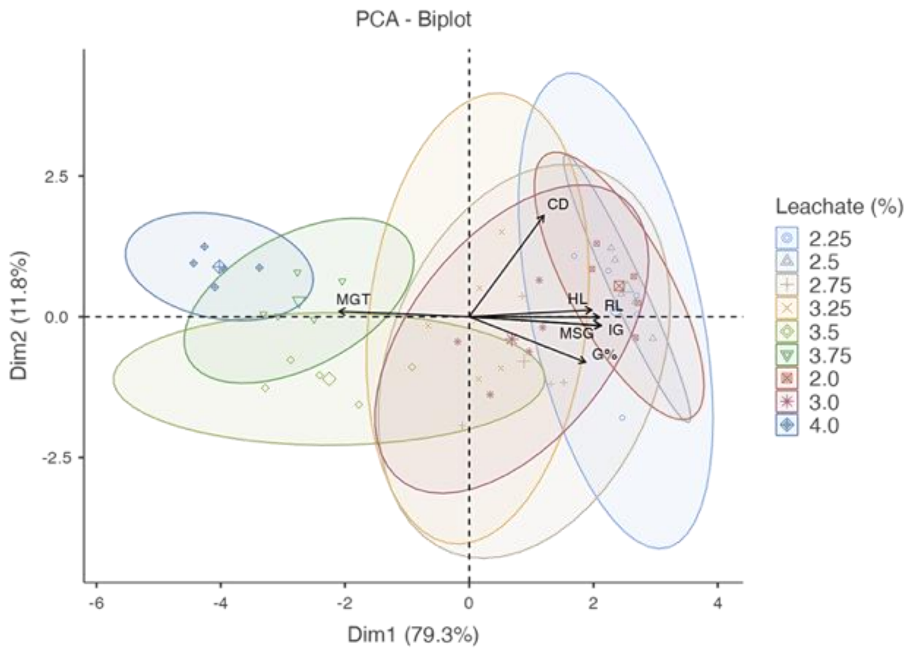

Figure 3. Results of the Principal Component Analysis (PCA) of the effect of vermicompost leachate concentrations (VCL) on the germination development of lettuce seeds *in vitro*. Concentrations of CVLs and parameters combination are color coded. Parameters abbreviations: G%= germination percentage, MGT=mean germination time, MSG= mean speed of germination, GI=germination index, HL=hypocotyl length, RL=radical length, CD= collar diameter.

The analysis segmented the data and identified the behavior of VCL concentrations in differentiated colored groups concerning the loadings of the individual parameters, which are represented graphically with arrows. In the PCA-biplot, VCL concentrations of 2.0, 2.25, and 2.5% positively related to the vegetative and germination parameters, except for MGT, which indicates that these concentrations do not delay the germination process and seedling development. Likewise, a strong relationship is observed with the GI load, indicating a mild phytotoxicity. In contrast, concentrations of 3.5 to 4.0% of VCL show a negative relationship with the mentioned parameters, except MGT, suggesting a germination delay, a negative influence on germination parameters, little development, and high phytotoxicity.

4. DISCUSSION

The findings of this study are important, as they reveal a profound effect of vermicompost leachate concentrations on various aspects of lettuce seedling growth and development. The observed decrease in germination percentage with increasing VCL concentrations, particularly from 3.5% onwards, suggests a phytotoxic effect on the seeds. This finding aligns with previous studies, underscoring the potential inhibitory effects of high leachate concentrations on seed germination in various plant species. For instance, García *et al*., (2008) reported a decrease in maize germination percentage when using VCL at 5.0% and 10%, while Gutiérrez *et al*., (2011) observed a decrease in radish (*Raphanus sativus* L.) germination with VCL concentrations of 10%, 20%, 30%, or 40%. Similarly, Ameen (2020) reported a negative impact on wheat germination with concentrations higher than 10.5% of VCL. Conversely, Ameen (2020) also found that very low concentrations of VCL at 0.2% and 0.5% could negatively affect wheat seed germination.

In this study, concentrations from 2.0% to 3.0% did not affect germination compared with control but did not improve it, contrasting with the findings of Ameen (2020), who found the best germination rates in wheat with concentrations of 1.5% and 3.5%. Additionally, García *et al*., (2021) demonstrated a significant improvement in germination rates, up to 70%, in *Salicornia bigelovii* (Torr.) when evaluating VCL at 4.78%. However, it is important to note that lettuce is more sensitive to environmental substances that could affect its germination (Bowers, 1997). The potential toxicity of VCL is attributed to high salt content, pH or toxic substances (Asciutto *et al*., 2006; Pittaway 2011; Frederickson 2022). But the inhibition varies depending on the plant species. For example, cow mature leachate inhibited radish germination but did not affect watercress (Gutiérrez *et al*., 2011). Additionally, low concentrations of VCL decrease the number of toxic substances (Benazzouk *et al.*,2020).

Adverse effects were also manifested in the vegetative development of seedlings, with a significant reduction in hypocotyl length, neck diameter and radicle length. These results are consistent with previous research demonstrating the negative impact of high concentrations of vermicompost leachate on plant growth (Gutiérrez *et al*., 2011; Aremu *et al*., 2015). For instance, Ameen (2020) reported that VCL at 25.5% reduced root and shoot length in wheat. Furthermore, a decrease in the content of photosynthetic pigments was observed as VCL concentration increased, suggesting an impairment in the photosynthetic capacity of lettuce seedlings exposed to higher leachate concentrations, with potential implications for their energy production and growth, as pigment content influences plant growth (Sabeti *et al*., 2019). Principal component analysis supports this relationship, showing a positive correlation between lower VCL concentrations (2.0% - 2.5%) and vegetative and germination parameters and a negative correlation with higher concentrations (3.5% - 4.0%), indicating greater phytotoxicity.

The results of this study are consistent with existing literature that has highlighted the variable effects of vermicompost leachate on different plant species and at different concentrations. While some studies have shown positive effects of leachate on plant growth and development (García *et al*., 2008; Aremu *et al*., 2015; Ameen, 2020), others have pointed out inhibitory effects, especially at high concentrations (Gutiérrez *et al*., 2011; Pittaway 2011). However, it is essential to note that the effect varies not only by concentration and plant type but also by the application method, such as direct watering (Benazzouk *et al*., 2020), plant spraying (Čabilovski *et al*., 2023; Torres *et al*., 2024), or seed immersion (David et al., 2023); extraction methods, such as vermicompost bed washing or vermiwash (Patnaik *et al*., 2022), or aerated and non-aerated compost tea (Hanc *et al*., 2017); substrate for obtaining vermicompost, such as cow manure, sheep manure, vegetable residues, among others (Warman and AngLopez, 2010); and the crop development stage (germination, emergence, seedling, or adult plant (Ievinsh, 2020)). Therefore, further research is needed to fully understand these effects and determine the best practices for vermicompost leachate application, especially in the seed germination process, as this is an essential factor in successful crop establishment and yield (Buriro *et al*., 2011; Ameen, 2020). In this context, there is a need for more research to understand better the underlying mechanisms behind the effects observed in this study.

5. CONCLUSIONS

This study investigated the influence of different concentrations of vermicompost leachate on lettuce seed germination and seedling development *in vitro*. The results demonstrate the VCL significantly impacts these processes. At low concentrations (2.0%-2.5%), VCL does not affect germination percentage or growth; however, higher concentrations (3.5%-4.0%) reduce the germination percentage, decrease the number of seeds germinated daily, and increase the germination time. Moreover, concentrations above 2.5% of VCL cause phytotoxicity, negatively affecting radicle development, reducing length, impacting the hypocotyl collar diameter, and decreasing photosynthetic pigment content. These finding suggest that VCL, in controlled doses, could be a sustainable alternative to chemical fertilizers, although its effectiveness may vary under field conditions. Future studies should investigate the underlying mechanisms that produce these effects and determine the optimal VCL application practices across different crop and growth stages.

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DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work presented in this article.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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