

Effects of Different Doses of Liquid Vermicompost on The Seed Germination of *Nigella sativa* L.

Sıvı Solucan Gübresinin Farklı Dozlarının *Nigella sativa* L. Türevinin Tohum Çimlenmesine Etkileri

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ABSTRACT: The gradual decrease in agricultural lands and the increase in the need for food have made the use of fertilizers widespread in the agricultural sector in order to obtain high quantity and quality products. However, since chemical fertilizers cause environmental pollution, the use of organic fertilizers has become undeniable. In this study, the effects of different liquid vermicompost (VCL) doses (0.1%, 0.25%, 0.5%, 1%, and 2%) on the seed germination and seedling growth on *Nigella sativa* L. (black cumin) were investigated. Seeds were left to germinate in the dark at 20 ± 2 °C in the climate chamber. After the 14-day development process, germination rate, plumule length, radicle length, seedling length, and radicle fresh weight, plumule and radicle dry weight were measured. Especially 0.25% VCL treatment had the most positive effect on parameters such as germination rate, plant height, plumule and radicle length, fresh and dry weight. On the other hand, 2% VCL treatment suppressed germination likely due to osmotic stress, increased microorganism activity, pH changes or impaired air circulation.

Keywords: Black cumin, liquid vermicompost, germination, seedling growth

ÖZ: Tarım alanlarının giderek azalması ve gıdaya olan ihtiyacın artması, tarım sektöründe yüksek miktarda ve kalitede ürün elde etmek amacıyla gübre kullanımı yaygınlaştırmıştır. Ancak kimyasal gübrelerin çevre kirliliğine yol açması nedeniyle organik gübre kullanımı yadsınamaz hale gelmiştir. Bu çalışmada, farklı Sıvı Solucan Gübresi (SSG) dozlarının (%0,1, %0,25, %0,5, %1 ve %2) *Nigella sativa* L. (Çörek otu) bitkisinin tohum çimlenmesi ve fide büyümesi üzerine etkileri araştırılmıştır. Tohumlar 20 ± 2 °C'lık iklim odasında karanlıkta çimlenmeye bırakılmıştır. 14 günlük gelişme süreci sonunda çimlenme oranı, plumula uzunluğu, radikula uzunluğu, fide uzunluğu, radikula taze ağırlığı, plumula ve radikula kuru ağırlığı ölçülmüştür. Özellikle %0,25 SSG uygulaması çimlenme oranı, bitki boyu, plumula ve radikül uzunluğu, taze ve kuru ağırlık gibi parametreler üzerinde olumlu etki yaptığı belirlenmiştir. Öte yandan, %2 SSG uygulaması muhtemelen ozmotik stres, artan mikroorganizma aktivitesi, pH değişiklikleri veya bozulmuş hava sirkülasyonu nedeniyle çimlenmeyi bastırmıştır.

Anahtar Kelimeler: Çörek otu, sıvı solucan gübresi, çimlenme, fide gelişimi

1. INTRODUCTION

Medicinal and aromatic plants have historically been used for medicine, food, spices and cosmetics since ancient times. In recent years, the growing demand from the pharmaceutical, food, and cosmetic industries has made it increasingly important to adopt proper farming techniques to ensure the production of high-quality medicinal and aromatic plants. To ensure this, it is important to create a suitable growing environment for plants, determine the correct harvest time, and use appropriate fertilizers (Pakdemirli, 2020).

Among these plants, *N. sativa* stands out due to its historical and medicinal significance. This annual herbaceous plant, belonging to the Ranunculaceae family which has 20 species in total, has 14 species in the flora of Turkey (Dönmez et al., 2010). *N. sativa* is primarily cultivated mainly in the dry and semi-arid regions of the Middle East, Western Asia, the Mediterranean and Central Europe (Ozer et al., 2020). *N. sativa* seeds were discovered preserved in a small container unearthed during archaeological excavations at the site of a Hittite temple in present-day Türkiye (Salih et al., 2009). This finding dates back to approximately 1650 BC, providing evidence of the historical use and cultural significance of *N. sativa* in ancient Anatolian civilizations. The germination of *N. sativa*, whose growing period is from November to April, varies between 25 - 30 days under ideal conditions (Chaouche et al., 2014). The most commercially cultivated species is *N. sativa* (Can, 2021). It is a valuable medicinal plant due to the fixed oil, volatile oil and nutrients contained in its seeds. The thymoquinone exists in its volatile compound contributes to its various pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antihistamine, and anticancer properties (Güzelsoy et al., 2018).

In light of its value, *N. sativa* has become an economically significant crop in Turkey. Recent statistics report an annual production of 6,435 tonnes. In 2022, 10,802 decares yielded 10,089 tonnes of *N. sativa* seed (TÜİK, 2022). Although the production of black *N. sativa* has increased over the years, it still cannot meet the need sufficiently. One of the major constraints in *N. sativa* cultivation is its slow and uneven germination, primarily due to its small seed size. Additionally, external factors such as weeds, diseases, and pests exacerbate this issue, causing irregular germination and consequently reducing plant development, yield, and quality (Muhyaddin and Wiebe, 1989).

These early stages—germination and soil emergence—are particularly critical for successful agricultural production (Özkara et al., 2014; Durmaz and Kara, 2023). However, in the seed production of medicinal and aromatic plants, development is often slow, seed germination problems might arise, and large variations in most morphological and chemical properties occur (Baydar, 2019). For this reason,

various applications are made to encourage seed germination in many plant species. Fertilization is one of the most widely used strategies, as it supplies essential macro- and micronutrients that may be deficient in the soil. Fertilizers support the macro- and micronutrients that plants need at the point where they are insufficient in the soil. The correct fertilizer form and dose contribute positively to the growth and development of plants and increase the amount of product per unit area. Fertilizers can be applied in organic form as well as in chemical form. However, the chemical or organic origin of the fertilizer and the application doses are important (Kaya and Erdönmez, 2020).

Liquid vermicompost (VCL), which has emerged as a promising fertilizer, obtained by composting solid vermicompost. This fertilizer, which is rich in bacteria, can be used for drip irrigation or as foliar fertilizer (Hussain and Abbasi 2018). VCL has no harmful effects on humans, animals or soil. It has an accelerating effect on the growth of seeds, stimulates the rooting of plants, and allows them to form strong roots (Thuraisingham and Seran, 2019). Some researchers have been undertaken to explore the impact of different organic fertilizers on germination and yield parameters in *N. sativa*. Studies have revealed that organic fertilizers have a positive effect on plant development and yield. (Salehi et al., 2017; Chelariu et al., 2020; Sohrabi Rinani et al., 2021; Karer and Beyzi, 2022; Ekren and Koç, 2024; Sayed et al., 2024).

Given these information above-mentioned and the economic importance of *N. sativa* in Turkey, exploring effective pre-germination treatments has the potential to significantly improve its cultivation. Therefore, this study aims to investigate the effect of VCL on the germination of *N. sativa* seeds.

2. MATERIALS and METHODS

2.1. Germination Experiments

N. sativa seeds used in the study were obtained commercially. The VCL to be used was obtained from a private company that was registered and licensed. The study was conducted in the Laboratory of Forestry Faculty, Cankiri Karatekin University.

For surface sterilization, seeds were treated with 5% sodium hypochlorite for 5 min and then washed with distilled water. Each trial was carried out with 10 replicates, and each replicate contained 50 seeds. The seeds were placed between two layers of blotting paper in sterile petri dishes. The treatments were composed of control (VCL0), 0.1% (v/v) (VCL0.1), 0.25% (v/v) (VCL0.25), 0.50% (v/v) (VCL0.5), 1% (v/v) (VCL1) and 2% (v/v) (VCL2). Distilled water was used as the control and in the preparation of VCL doses. VCL doses were applied directly to the seeds. The seeds were incubated in darkness at 20 +/- 2°C. There was no plant nutrients applied during the germination stage in the experiment. The treatments were made regularly at

the same time every day to ensure that the seeds did not remain dry out, and the germinated seeds were counted and recorded every day. Throughout the experiment, blotting papers were replaced every two days, and 10 ml of solution was added to the petri dishes during each change. As a result of the germination of all seeds, the determination of the number of germinated seeds was finalized. After making sure that all seeds germinated, phenological observations and quality parameters were measured. Seeds with a root length of 1 mm were accepted as germinated.

2.2. Measurements

The number of seeds that germinated each day was used to calculate the germination rate (GR). The number of seeds that germinated in petri dishes ten days after germination was divided by the total number of seeds tested to determine GR (Akıncı and Çalışkan 2010). Seedling, plumule and radicle length of 10 plants randomly selected from the petri dishes were measured with the help of a millimeter ruler. Plumule and radicle wet and dry weights were determined by weighing 10 plants randomly selected from the petri dishes on a precision balance (Bilgili et al., 2018).

2.3. Statistical Analysis

The Kolmogorov-Smirnov test was run to see if the data distributions were normally distributed. The homogeneity of the variance was controlled using the Levene's test. As a result, the germination % followed a normal distribution, even though all other seedling traits did not. In the event of non-normally distributed characteristics, the Kruskal-Wallis test with independent samples was employed to compare the means of treatments, including different VCL concentrations. In order to identify similar groups where there was a significant difference among the means, Dunn's test was utilized as a post-hoc test. Bonferroni correction was used for p-value adjustment. The germination percentage was compared between the groups using one-way analysis of variance, and if a difference was found, the Duncan test was used to identify similar groups. SPSS version 25 was used to conduct these analyses (IBM Corp., 2017).

3. RESULTS

Commercially obtained *N. sativa* seeds were evaluated in two groups as control and treatment. While only 10 mL of pure water was applied to the control group, 10 mL of VCL prepared at 50% to 0.5% levels was added to five different treatment groups.

3.1. Germination Percentage (%)

As a result of germination of all seeds, the determination of the number of germinated seeds was finalized. No germination was observed for the first three days after the beginning of the experiment. The first germination was observed on the fourth day. All seed completed germination within 14 days. Not all seeds used in the

experiment germinated.

The mean germination rates of *N. sativa* seeds at different VCL doses are given in Figure 1. The highest germination rate was found in VCL-0.1 and the lowest germination rate was determined in VCL-2. There is a significant difference between the groups. According to the Pairwise comparisons showed, the treatments were divided into four homogenous groups. The first group consisted of VCL-0 and VCL-0.1, VCL-0.5 and VCL-2 were in the second and third groups, respectively. The fourth group included VCL-0.25 and VCL-1 as transition treatments. A large portion of the seeds germinated at 2% VCL concentration died without forming stems and roots. Due to the very low germination rate in VCL-2, this group was excluded from subsequent statistical analyses.

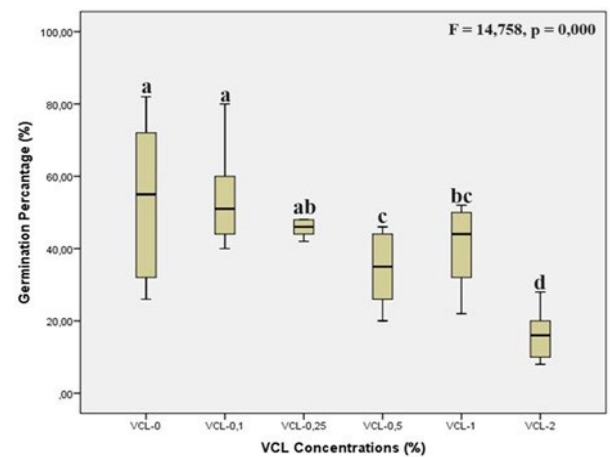


Figure 1. Boxplot diagram of germination percentage of *N. sativa* (n=100; Different letters indicate significant differences (p < 0.05) among the germination percentage means of treatments)

3.2. Plant Height of Germinated Seed

The plant height varied depending on the treatment of different doses of VCL (Figure 2). The research results showed that VCL-0.25 had the highest shoot length with 7.31 cm and VCL-0.5 had the lowest shoot length with 5.66 cm. There was a significant difference between the groups. Statistical analysis indicated that VCL-0.5 and VCL-0, VCL-0.1 and VCL-0.25 treatments were similar, VCL-1 and VCL-0.1 and VCL-0.25 treatments were similar, and VCL-0.1 and VCL-0.25 treatments were similar.

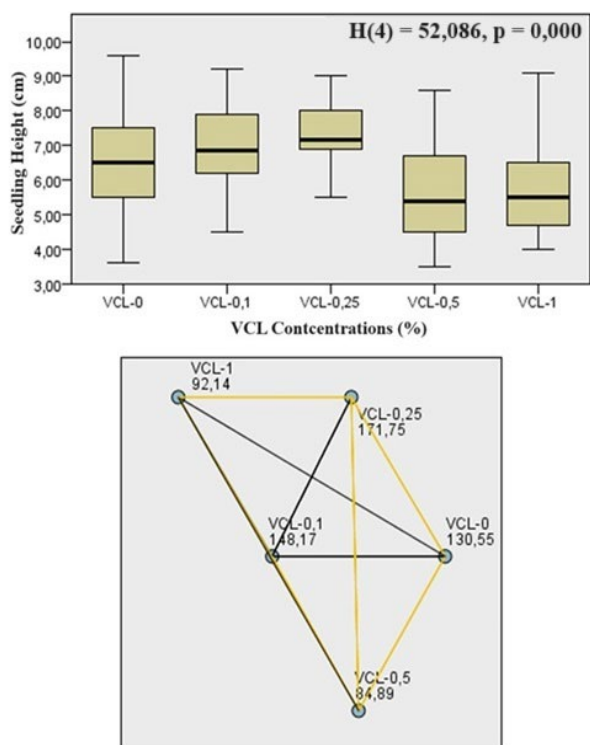


Figure 2. Boxplot diagram of plant height germinated seed of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

3.3. Plumule Length of Germinated Seed

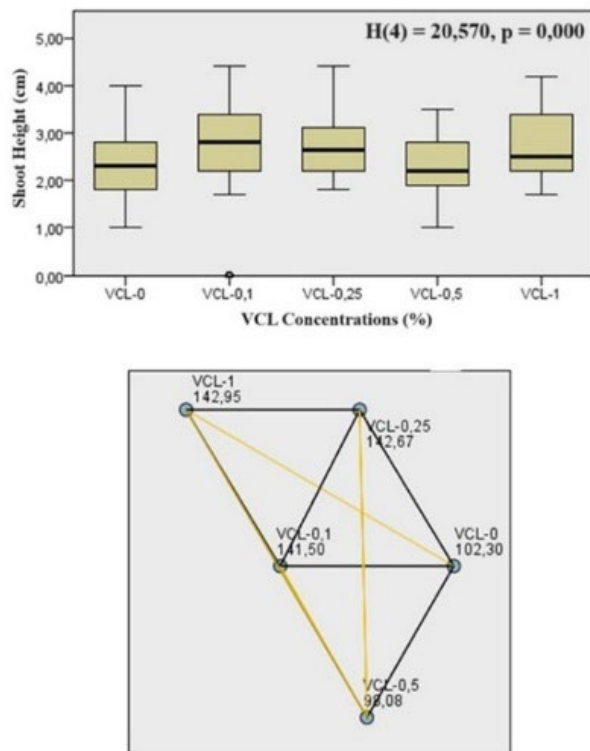


Figure 3. Boxplot diagram of plant length germinated seed of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

As shown in Figure 3, plumule length values varied between 2.29 cm and 2.77 cm as a result of the application of different VCL doses. Among the VCL doses, the highest plumule length was found in plants treated with VCL-1, while the lowest plumule length was found in VCL-0.5. There is a significant difference between the groups. Based on the results of the comparison test, VCL-0.1, VCL-0.25, and VCL-1 treatments did not differ significantly from each other, nor did VCL-0 and VCL-0.5 treatments.

3.4. Radicle Length of Germinated Seed

According to the results of the analysis of variance (Figure 4), the difference between the averages of VCL treatments was found to be statistically significant. As a result of VCL treatments at different doses, radicle lengths varied between 3.08 cm and 4.67 cm. Among the VCL doses, the highest radicle length was observed at VCL-0.25, and the lowest radicle length was determined at VCL-1. From the results of the statistical comparison, VCL-1 and VCL-0, VCL-0.1, and VCL-0.25 were similar, VCL-0.5 and VCL-0 and VCL-0.25 were similar, and VCL-0.1 and VCL-0.25 were similar.

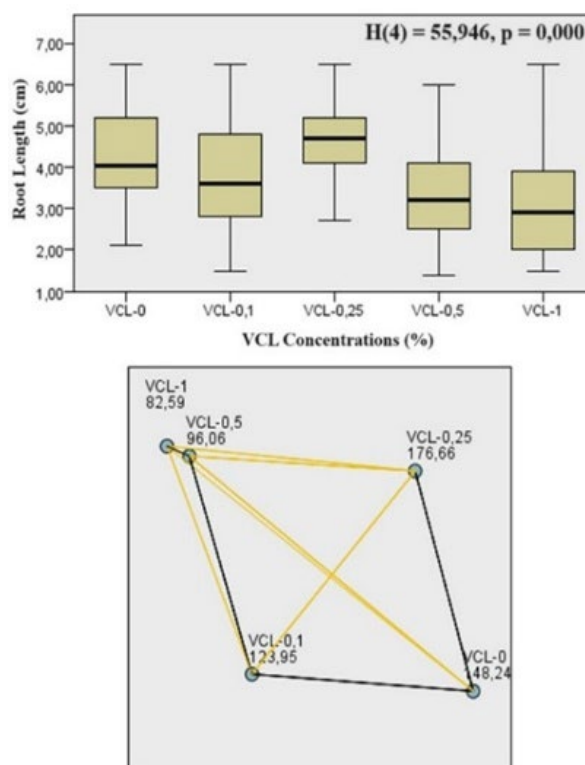


Figure 4. Boxplot diagram of radicle length of germinated seed of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

3.5. Plumule Fresh Weight

The shoot fresh weight values of *N. sativa* seeds treated with different doses of VCL vary between 0.0115 and 0.0150 g. The highest shoot fresh weight was obtained from the VCL-0.25 treatment, while the lowest shoot fresh weight was obtained from the control group. There is a significant difference between the groups. As demonstrated by the comparison analysis, VCL-0 and VCL-0.1, VCL-0.25 and VCL-1 treatments were similar (Figure 5).

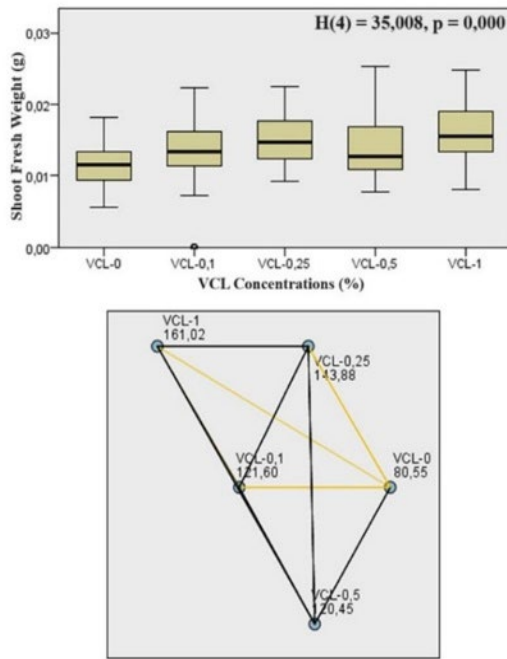


Figure 5. Boxplot diagram of plumule fresh weight of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

3.6. Radicle Fresh Weight (g)

When the radicle fresh weight averages were examined, the highest value was observed in VCL-0.25 with 0.0090 g, while the lowest weight was observed in the control group with 0.0063 g. As demonstrated by the comparison analysis, VCL-0 and VCL-0.5, VCL-0.25 and VCL-1 processes are similar, and VCL-0.1 and VCL-0.25 treatments were similar (Figure 6).

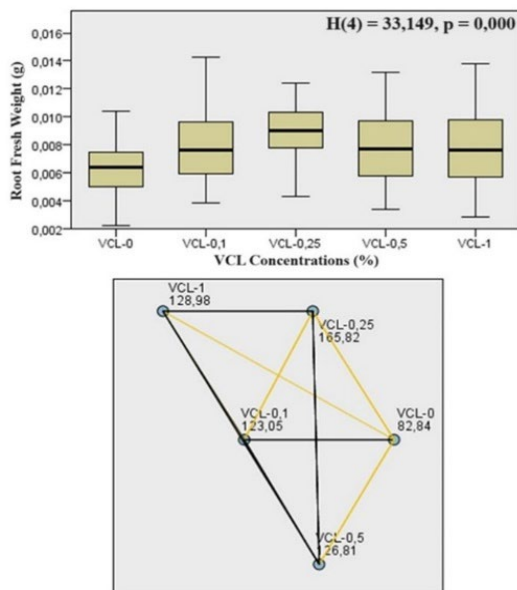


Figure 6. Boxplot diagram of radicle fresh weight of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

3.7. Plumule Dry Weight (g)

The effect of different doses of VCL applied to *N. sativa* plant on plumule dry weight (g) is given in Figure 7. VCL-1 was found to have the most positive effect on plumule length. The outcome of the comparison test indicated that VCL-0.1 and VCL-1 treatments were similar.

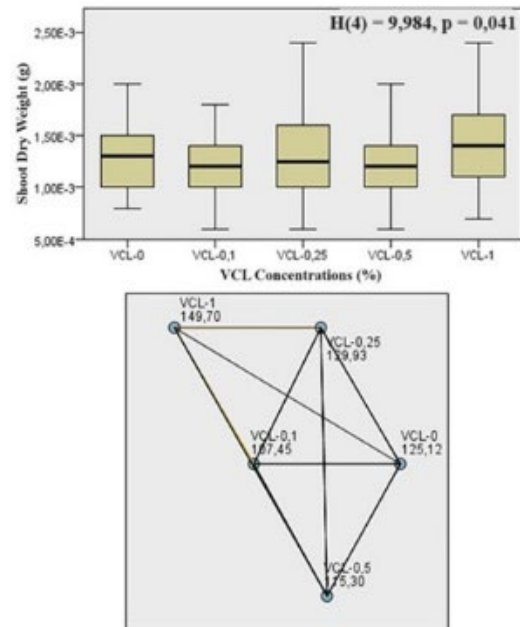


Figure 7. Boxplot diagram of plumule dry weight of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

3.8. Radicle Dry Weight (g)

In terms of radicle dry weight, VCL-0.25 had a higher value than the other groups (Figure 8). According to the comparison test result, VCL-0.25 and VCL-0, VCL-0.5 and VCL-1 treatments were similar.

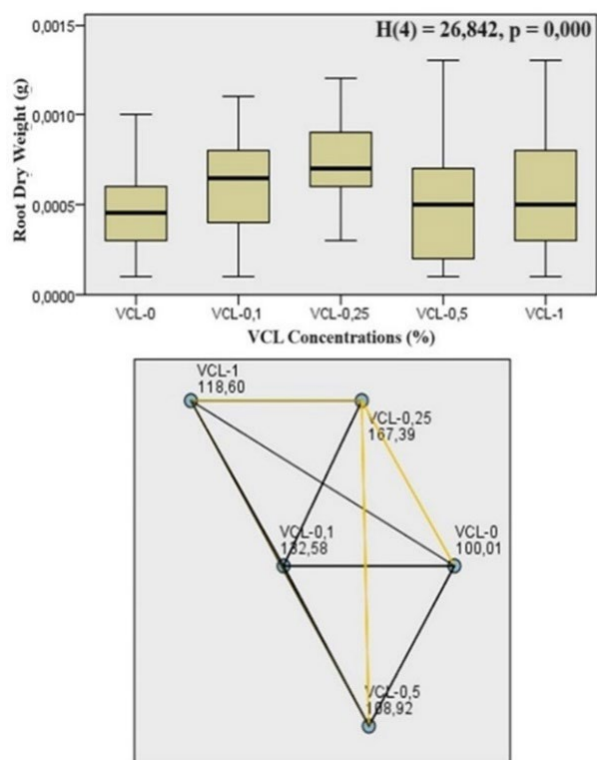


Figure 8. Boxplot diagram of radicle dry weight of *N. sativa* (n=90; Different letters indicate significant differences ($p < 0.05$) among the germination percentage means of treatments)

4. DISCUSSION

In this study, the effects of six different VCL treatments (Control, 0.1%, 0.25%, 0.5%, 1%, and 2% VCL) on germination and growth parameters of *N. sativa* were investigated. Statistical analysis was performed to determine whether there were significant differences in germination rate, radicle and plumule length (cm), seedling radicle and plumule wet and dry weight (g).

The germination of the seeds of the *N. sativa* plant was completed after 14 days. In a study investigating the effects of different fertilizer types on the yield and quality of the *N. sativa* plant, it was determined that the emergence period of the *N. sativa* seeds varied between 15 - 18 days (Ekren and Koç, 2024). Vermicompost fertilizer applied to the seeds of the *N. damascena* L. plant also showed that the germination period of the seeds of this plant was 14 days (Ulus and Şahin, 2020). The number of days determined in our study is compatible with the mentioned studies and supports the accuracy of our results. However, Ekren and Koç (2024) stated that organomineral and inorganic fertilizers did not affect seed emergence periods.

In our study, VCL treatments had a statistically significant effect on germination percentage ($p < 0.05$). The highest germination rates were observed in VCL-0.1 (54%) and the control group (53%), while the lowest rate was seen in the VCL-2 group. Previous studies similarly reported control group germination rates between 40–60% (Gholami et al.,

2015; Endes, 2018), with some noting rates up to 70% (Tan, 2024).

The decreased germination observed in the VCL-2 treatment may be attributed to its high concentration inhibiting water uptake, thereby delaying or preventing seed germination. This is consistent with findings by Amooaghaie (2015), who reported reduced germination metrics at higher vermicompost concentrations.

In our study, plant height, plumule and radicle length were found to be statistically significant ($p < 0.05$) in VCL treatments. The highest plumule length was determined in VCL-1 concentration. Therefore, *N. sativa* seedlings that found sufficient amount of nutrients in the environment after the germination stage may have shown better development in terms of plumule compared to other groups. It was observed that plumule lengths generally increased from the lowest concentration to the highest concentration. However, VCL-0.5 concentration showed a different trend. This difference is thought to be due to reasons not related to concentration.

In contrast, the most favourable effect on radicle length was observed at the 0.25% VCL concentration, suggesting this as the optimal dose for root development. It is thought that the difference between the concentration values affecting the plumule and radicle lengths may be due to the differences in the radicle and plumule development of the plant. The differing responses between plumule and radicle development may reflect physiological trade-offs, where nutrient abundance can promote shoot over root growth (Karban, 2008; Dalkılıç, 2020).

Plant height was also significantly enhanced at the 0.25% VCL concentration, consistent with its positive effects on both root and shoot elongation. Additionally, the highest fresh weights for both radicle and plumule were recorded at 0.25% and 1% VCL. These findings indicate that these concentrations improve early seedling vigour by supporting balanced growth and sufficient nutrient uptake.

Usually, small seeds cannot emerge and form a strong seedling due to low nutrient reserves (Hojjat, 2011). Application of organic fertilizer such as vermicompost is considered as the effective approach to increase P solubility (Hejazi Mehrizi et al., 2015). On the other hand, vermicompost has also been reported to play an effective role in lowering soil pH and increasing available P (Azarmi et al., 2008). In our study, it was observed that VCL, which was mostly applied at increasing doses, positively affected the development of *N. sativa* seeds after germination. It was determined that the most appropriate dose for the development of *N. sativa* seeds was 0.25%. The 2% concentration used in the study was found to be a very high dose because it prevented germination. In agreement with our findings, Amooaghaie (2015) reported that high concentrations of vermicompost decreased germination parameters.

As a result, it was determined that VCL treatment positively affected plant germination and development; however, higher concentrations negatively impacted germination rates. This study was carried out only in petri dishes. Therefore, it was observed that it prevented the seed from getting water to some extent. It is assumed that this limitation would not occur under soil conditions. It is predicted that germination percentages will be higher in greenhouse or field trials. Greenhouse and field trials are recommended for future studies.

5. CONCLUSIONS

This study has revealed the effects of different concentrations of VCL on the germination and seedling development of *N. sativa* seeds. In conclusion, VCL treatments positively influenced the germination and seedling development of *N. sativa*, with 0.25% identified as the most effective concentration. Conversely, higher concentrations, particularly 2%, negatively affected germination, likely due to limited water uptake. These results highlight the potential of vermicompost-based treatments in enhancing early plant growth. This clearly shows the critical role of organic fertilizer concentrations in seed germination and plant development. In this context, the study contributes to sustainable agricultural practices by revealing the potential of organic fertilizers in the production of a plant with economic and medical importance, such as *N. sativa*.

The results obtained contribute to the scientific literature in terms of the evaluation of germination and early seedling development processes of *N. sativa* seeds, which are small and had losses during germination. It can be thought that the use of organic fertilizers at the right concentration, especially in species with small seeds, can increase yield and quality. A limitation of this study is the absence of biochemical analyses—such as enzyme activity or nutrient content measurements—which could have provided further insight into the physiological and metabolic responses of *N. sativa* to VCL applications. Incorporating such parameters in future research would help clarify the mechanisms underlying the observed effects and support more comprehensive evaluations

under greenhouse and field conditions.

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Author Contributions

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Tuğba GÜRKÖK TAN: (a) Idea, Concept, (b) Study Design, Methodology, (c) Literature Review, (d) Supervision, (g) Analyses, (h) Writing Text

Nuray ÇİÇEK: (a) Idea, Concept, (b) Study Design, Methodology, (c) Literature Review, (g) Analyses, (h) Writing Text

Ayşenur KAYABAŞ AVŞAR: (c) Literature Review, (h) Writing Text

Elena Liliana CHELARIU: (c) Literature Review, (i) Critical Review

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the 'Higher Education Institutions Scientific Research and Publication Ethics Directive' are complied with, and that none of the actions stated under the heading 'Actions against Scientific Research and Publication Ethics' are not carried out.

Conflict of Interest

No conflict of interest was declared by the authors.

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