

Do vermicompost applications improve pharmaceutically important alkaloids, growth performance, phenolic content, and defense enzyme activities in summer snowflake (*Leucojum aestivum* L.)?

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Abstract

Leucojum aestivum L. includes galanthamine and lycorine, which are two pharmaceutically valuable alkaloids. Vermicompost (VC), an organic waste product created by earthworms enhances soil quality and can improve the medicinal quality of the plant that is crucial to the pharmaceutical industry.

Purpose

The aim of this study was to determine the effects of four different VC concentrations (5%, 10%, 25%, and 50%) on *L. aestivum* growth parameters, alkaloid levels (galanthamine and lycorine), total phenol-flavonoid content, free radical scavenging potential, and defense enzyme activities (SOD and CAT) compared to control (no VC).

Methods

A 2,2-diphenyl-1-picrylhydrazil (DPPH) radical assay was performed for antioxidant activity. Folin-Ciocalteu and aluminum chloride colorimetric methods were applied for the determination of total phenol and flavonoid content, respectively. Alkaloid amounts (galanthamine and lycorine) were analyzed by the HPLC-DAD system.

Results

The width, length, and fresh weight of the leaves were improved by 10% VC treatment. The highest total phenolic content was found in bulbs and leaves treated with 50% VC. HPLC-DAD analysis of alkaloids showed that 10% and 50% VC treatments contained the most galanthamine in the bulb and leaf extracts, respectively. The application of 25% VC was the most efficient in terms of lycorine content in both extracts. CAT activity was elevated at 10%, 25%, and 50% VC.

Conclusions

Based on the growth performance and galanthamine content of the bulbs and leaves, it can be concluded that a 10% VC application was the most effective in the cultivation of *L. aestivum*.

1 Introduction

Leucojum aestivum subsp. *aestivum*, a bulbous perennial plant, is part of the Amaryllidaceae family. Its common names are Loddon Lily, snowflake lily, and summer snowflake. *L. aestivum* grows naturally in alluvial soils near rivers, lakes, and humid and semi-shaded fields, such as swamps, wetlands, and floodplains, from sea level to 1000 m elevation (Parolo et al., 2011; Arslan et al., 2020; Ateş et al., 2021). The flower stalk has two to five umbrella-shaped bell-like flowers placed at equal intervals and blooms between March and June (Seyidoğlu, 2009). *L. aestivum* is a threatened plant because it has been collected from its natural habitat for the pharmaceutical industry. It is a medicinal and ornamental plant native to southern Europe, Turkey, the Balkans, Caucasias, and northern Iran (Çiçek et al. 2007; Arslan et al.

2020). Galanthamine, an isoquinoline alkaloid, is a long-acting, selective, reversible, and competitive acetylcholinesterase (AChE) inhibitor used to treat neurological disorders, primarily senile dementia of Alzheimer's type, poliomyelitis, and other neurological diseases (Diop et al., 2006;

Berkov et al., 2009; Ivanov et al., 2011). Lycorine is a pyrrollophenanthridine alkaloid that exhibits antiretroviral, antimitotic, anticancer, antiparasitic, anti-inflammatory, analgesic, emetic, and cytotoxic activity. In addition, several studies have shown that lycorine has strong antiviral effects against poliovirus, measles, severe acute respiratory syndrome-associated coronavirus, and Middle East respiratory syndrome-associated coronavirus (Ivanov et al., 2011; Wang et al., 2014). Recent studies have indicated that lycorine is a strong COVID-19 inhibitor (Jin et al., 2021).

Overharvesting destroys and endangers the natural populations of *L. aestivum*, which contain the pharmaceutically valuable alkaloid galanthamine. This plant has been cultivated in licensed fields in Turkey to export bulbs to pharmaceutical manufacturers. Although galanthamine in *L. aestivum* has commercial significance in the pharmaceutical industry, and the impact of vermicompost on secondary metabolite augmentation is well known, to our knowledge, no studies have investigated the effectiveness of VC on this pharmaceutically famous alkaloid.

Vermicompost is a biological fertilizer created by the continuous and slow movement of decomposing organic matter in the gastrointestinal systems of certain earthworm species. Before excretion, substances pass through the earthworm's body and are filled with gastrointestinal mucosa, vitamins, and enzymes (Jahanbakhshi and Kheiralipour, 2019). Vermicomposting is a useful, environmentally friendly, and economically viable waste recycling method, in which organic waste material is transformed into usable compost through the combined activities of microorganisms and earthworms (Zhang et al., 2015; Gopal et al., 2017). VC is often generated by epigeic earthworms that feed on fresh organic materials in the litter layer and the first centimeter of the soil. The most well-known earthworm used in vermicomposting is *Eisenia fetida*, which has a remarkable ability to change the physical, biological, and chemical characteristics of organic substances, as well as its resilience and wide temperature tolerance (Lazcano and Domínguez, 2011; Domínguez et al., 2019; Ghorbani and Sabour, 2021; Przemieniecki et al., 2021). It is a nutrient-rich peat-like substance with high porosity, water-holding capacity, low C: N ratio (Domínguez et al., 2019), ventilation, drainage (Hosseinzadeh et al., 2016), cation exchange capacity, and hormone-like activity (Amooaghaie and Golmohammadi, 2017). Furthermore, VC improves the microbial diversity of the soil and stores nutrients for an extended period without negatively affecting the environment (Lim et al., 2015). Plant roots are generally unable to adapt to mineral N unless the carbon: nitrogen (C: N) ratio is $\leq 20:1$. Earthworms contribute to the decline in the C: N ratio of fresh organic material during respiration (Thakur et al., 2021).

Nutrients are secreted and transformed into soluble and accessible forms throughout vermicomposting, supplying nutrients such as accessible N, soluble K, exchangeable Ca, Mg, and P, as well as microelements such as Fe, Mo, Zn, and Cu that plants can easily absorb (Mistry et al., 2015). An organism that enhances the ecological and biological state of the soil is known as an eco-biological

engineer. Thus, earthworms alter the characteristics of soil that has been contaminated by pesticides or heavy metals and prevent the contamination of these substances caused by humans (Singh et al., 2020; Thakur et al., 2021).

VC inhibits soil-borne diseases and provides microorganisms with antimicrobial activity. Earthworms release plant growth regulators, such as gibberellin, auxin, and cytokinin, during vermicomposting, which work synergistically with microorganisms. It also synthesizes plant growth hormone-like molecules including fulvic acid, humic acid, and humates. These conditions can enhance plant growth and yield (Adhikary, 2012; Amooaghaie and Golmohammadi, 2017; Arancon et al., 2019, Domínguez et al. 2019; Liu et al., 2019; Makkar et al., 2023). The mechanism by which VC alters secondary metabolites remains unclear. Investigations on the use of humic compounds in medicinal plants have shown that they can boost the production of secondary metabolites and the activity of bioactive substances, such as flavonoids, coumarins, alkaloids, phenylpropanoids, total phenols, and anthocyanins (Pereira et al., 2019). This is explained by the chemical composition of VC, which usually includes several types of non-hormonal plant growth stimulants, phytohormones, and soluble phenolic compounds, together with a variety of microorganisms and macronutrients that can affect plant physiology (Souffront et al., 2022).

In this context, it was hypothesized that (i) various VC applications can enhance growth parameters and alkaloid levels, (ii) the phenolic content and free radical scavenging power can be improved with different VC treatments, and (iii) stress endurance capacity may be supported by VC implementation in *L. aestivum*.

2 Materials and Methods

2.1 Cultivation of *L. aestivum* with different VC concentrations

L. aestivum was collected from Bolu-Gölcük when it reached approximately 5 cm in diameter in March 2021. Nearly the same size of the *L. aestivum* bulbs was chosen randomly and placed one bulb into each pot (18.5 cm x 15.5 cm). Combinations of VC: soil mixture with ratios of 5:95, 10:90, 25:75, and 50:50 (w/w), as well as a control (only soil mixture with no VC) were prepared. The soil mixture contained 4:1:1 (v:v) ratios of peat (Terradena®, 65% peat, and 35% soil), sand, and vermiculite (Agrekal®), respectively. All preparations were very well homogenized.

The supply of VC (made from cow manure and *Eisenia fetida*) was Labfarm Inovatif Organik Tarım® and VC analysis was performed by AgrioLabEN®. Characteristics of VC were as follows: pH- 7.1; EC- 5.1 dS/m; organic matter content- 65.04%; moisture- 34.4%; organic carbon (C)- 50.4%; organic nitrogen (N)- 2.1%; C/N ratio- 17.3; total nitrogen- 2.9%; total diphosphorus trioxide (P₂O₃)- 1.7%; total humic + fulvic acid- 31.5%; water-soluble potassium oxide (K₂O)- 0.86%; cadmium (Cd)- 0.4 mg/kg; copper (Cu)- 50.9 mg/kg; nickel (Ni)- 12.4 mg/kg; lead (Pb)- 3.57 mg/kg; zinc (Zn)- 238.5 mg/kg; mercury (Hg)- <0.01 mg/kg; chromium (Cr)- 8.89 mg/kg; tin (Sn)- 4.4 mg/kg. Analysis of VC and soil mixture were also

conducted by the Bolu Directorate of Provincial Agriculture and Forestry, Turkey. Properties of VC and soil mixture were as follows: soil texture-clay and clay loam; pH- 6.71 and 6.99, EC- 1.9 dS/m and 1.62 dS/m; total salt- 0.21% and 0.66%; organic matter content- 25.22% and 4.93%; total nitrogen- 5.04% and 0.98%; phosphorus (P)- 326 mg/L and 0.02 mg/L; potassium (K)- 746.10 mg/L and 53.28 mg/L, respectively. The experimental design was completely randomized in triplicate in pots (a total of 15). The experiments were performed twice. The maximum pot water holding capacity for each treatment was calculated as described by Gutiérrez-Miceli et al. (2007). Irrigation of the treatments was adjusted according to the maximum soil moisture using a soil moisture meter (Extech Instruments®, M0750), and the pots were controlled every other day. The experiment was performed under plant room conditions at $22 \pm 1^\circ\text{C}$ using a 16/8 h (light/dark) photoperiod (cool-white, fluorescent lights, $22\text{--}28 \mu\text{mol}/\text{m}^2/\text{s}$) with 60% relative humidity. After collecting *L. aestivum* bulbs from natural habitats in March at the vegetative stage, they were planted in pots one day after collection for a 1-week acclimatization process. At the end of 1 week, the bulbs were planted in pots for 2.5 months and plant samples were harvested separately at the vegetative stage by the end of 2.5 months. After harvesting, all plant samples were lyophilized at -65°C (Christ®) and stored at -20°C until extraction and biological activity investigations. The length, width, and weight of the bulbs and leaves were individually specified.

2.2 Preparation of methanolic extracts

L. aestivum leaves and bulbs were freeze-dried (Christ®) and powdered prior to extraction. The methanolic extracts were prepared in a water bath for 24 h at 40°C . Subsequently, the extract was concentrated using a rotary evaporator and the extraction yields for each treatment were determined.

2.3 Determination of alkaloid content

The bulb and leaf extracts of *L. aestivum* were analyzed using a VWR-Hitachi LaChrom Elite® HPLC-DAD system. Galanthamine hydrobromide and lycorine (Sigma® and TCI America®, respectively) were used as the alkaloid standards. All the plant extracts and alkaloid standards were prepared using trifluoroacetic acid (TFA) (0.1%). The HPLC operating parameters were utilized as in a previous report by Arslan et al. (2020), and analyses were carried out using isocratic elution.

2.4 Free radical scavenging potency

The free radical scavenging capacity of *L. aestivum* extracts was measured spectrophotometrically (UV-VIS spectrophotometer Hitachi U-1900®) using a modified version of the Blois (1958) method, as described by Basay et al. (2021). The 2,2-diphenyl-1-picrylhydrazil (DPPH, Sigma-Aldrich Chemie®, Steinheim, Germany) radical assay was performed.

2.5 Total phenol and flavonoid content

Folin-Ciocalteu and aluminum chloride colorimetric methods described by Turker et al. (2021) were used to assess the total phenolic and flavonoid contents of *L. aestivum* extracts, respectively. Calibration curves were generated using gallic acid and quercetin as the reference phenols and flavonoids, respectively.

2.6. Enzymatic antioxidant activities

2.6.1 Enzyme Extraction and Protein Determination

Enzymes and proteins were obtained from the bulbs and leaves of *L. aestivum* to evaluate SOD and CAT enzyme activities. The enzyme extraction was performed as described by Ulgen et al. (2021). After enzyme extraction, The Lowry technique (Lowry et al., 1951) was used to determine the protein content in the bulbs and leaves. Bovine serum albumin (BSA) was used as the reference protein.

2.6.2 Superoxide dismutase (SOD) activity

A modified method for measuring SOD activity was developed based on van Rossum et al. (1997). The absorbance of the plant samples was measured against distilled water at 560 nm using a UV-Vis Spectrophotometer (Hitachi U-1900®). One unit of SOD was determined as the quantity of protein that caused a 50% decrease in the NBT in the reaction, and activity was expressed as units/mg protein.

2.6.3 Catalase (CAT) activity

CAT activity was measured according to the procedure described by Lartillot et al. (1988) by observing the decline in absorbance at 240 nm caused by the decomposition of hydrogen peroxide (H_2O_2) by CAT. An H_2O_2 extinction coefficient of 0.0392 mM/cm was used to compute the activity, which was reported as mmol H_2O_2 /mg protein.

2.7 Data Analysis

The investigations were conducted using a completely randomized design. Analysis of variance (ANOVA) and Duncan's Multiple Range Tests using SPSS version 26 (IBM Corp, NY, USA) were performed. All results in the tables were presented as mean $\pm$ standard error (SE). Means with the same letter within columns are not significantly different at $P > 0.05$. Pearson's correlation analysis was used for the indication of the relationship.

3 Results

3.1 Growth parameters and water accumulation with VC treatments

After 2.5 months of culture in VC treatments, the width, length, and weight of *L. aestivum* bulbs and leaves were determined (Table 1; Fig. 1; Fig. 2). Compared with the control, 5%, 10%, and 25% VC applications increased bulb width by 21.6%, 13.5%, and 13.5%, and individual fresh weight by 7.7%, 22.4%, and 30%, respectively. Interestingly, the bulb length was not influenced by VC application. Application of 10% VC contributed the most to the development of the width (22% increase), length (6.3% increase), and individual fresh weight (5.3% increase) of the leaves (Table 1) compared to the control. It was evident that VC treatments of 10%, 25%, and 50% caused a slight increase in water accumulation

capacity in the bulbs compared to the control, but the water content of all VC-treated leaves was lower than that of the control (Fig. 3).

3.2 Alkaloid accumulation with VC treatments

Quantification of alkaloid content in 10 different methanolic extracts (bulbs and leaves) was carried out using HPLC-DAD analysis, and the extraction yield of the methanol extract is presented in Table 2. The chromatograms of the standards used are shown in Fig. 4a. All tested VC concentrations enhanced the galanthamine content in the bulb extracts in comparison with the control. Additionally, it was found that 5%, 25%, and 50% VC treatments increased lycorine content compared to the control in the bulb extracts. The maximum galanthamine content was detected in the 10% VC treatment (Fig. 4b), while the highest amount of lycorine was identified in the bulb extracts treated with 25% VC compared to the control (44% increase in both) (Table 2).

All VC treatments increased the amount of galanthamine in the leaves, except for the 5% VC application. Compared to the control, leaf extracts treated with 50% VC had the highest galanthamine content (37% increase). (Table 2, Fig. 4c). The application of 10% VC was the same as that of the control. Treatment with 25% and 50% VC increased lycorine content in the leaf extracts, with the 25% VC treatment providing the greatest increase (84%).

3.3 Phenolic content and free radical scavenging potency with VC treatments

Phenolics and flavonoids are strong scavengers of free radicals because of their hydroxyl groups. The total phenolic and flavonoid contents of *L. aestivum* bulb and leaf extracts were assessed using gallic acid and quercetin calibration curves, respectively ($R^2 = 0.998$). The total phenolic and flavonoid contents of all extracts are shown in Table 3. All concentrations of VC enhanced the total phenolic content in the bulbs compared to the control (no VC). The maximum total phenolic and flavonoid contents were identified in the bulbs treated with 50% VC, with increases of 30.46% and 55.8%, respectively, compared with the control. Although the total phenolic content was the highest with 50% VC (44.13% increase), the total flavonoid level was the highest with 5% VC in the leaves (17.22% increase) in comparison with the control (Table 3).

Free radical-scavenging activity has been shown to have a half-maximal inhibitory concentration (IC_{50}). Quercetin was utilized as the antioxidant standard. The best antioxidant activity was observed with the 50% VC treatment in both bulbs and leaves (Table 3). The highest antioxidant activity with 50% VC treatment was related to the highest total phenolic contents in both (Table 3).

3.4 Defense enzyme activities with VC treatments

Superoxide dismutase is an enzyme that catalyzes the dismutation of the superoxide anion ($\bullet O_2^-$) into H_2O_2 and O_2 . When both oxidation and reduction reactions occur in the same reactant ($\bullet O_2^-$) in a biological system, dismutation reactions occur and produce two compounds: one with a higher oxidation

state (O_2) and one with a lower oxidation state (H_2O_2). This enzyme is one of the most important enzymatic systems in plants that scavenges stress-generated free radicals ($\bullet O_2^-$) (Rajput et al., 2021).

Alterations in SOD and CAT activities in the bulbs and leaves are shown in Table 4. All VC treatments decreased SOD activity in bulbs and leaves. Catalase is a tetrameric heme-containing enzyme that directly dismutates H_2O_2 into H_2O and O_2 . They are essential for reactive oxygen species (ROS) detoxification under stress conditions (Gill and Tuteja, 2010). CAT activity in the bulbs increased 2.17-, 2.02, and 2.35 folds, respectively, with 10%, 25%, and 50% VC treatments. The administration of 5% VC had no effect on CAT activity (Table 4).

In the leaves, CAT activity was 1.02-, 1.25, and 1.04 folds higher in the 10, 25, and 50% VC treatments, respectively. As with the bulbs, VC treatment reduced CAT levels in the leaves (Table 4).

4 Discussion

An increase in water accumulation capacity in the bulbs should be related to an increase in bulb width with VC application (Table 1). Due to the hydrophilic groups included in VC, the soil temperature rises, water retention increases, and plant development accelerates, improving yield (Singh et al., 2020). By using VC, it is possible to improve soil structure, porosity, air intake, and water holding capacity. Moreover, VC aids in the greater completion of root formation in plants, resulting in plants that take nutrients from the soil more readily and perform better in terms of growth (Kayabaşı and Yılmaz 2021). Due to the presence of humic acid, which is a chemical similar to plant growth hormone, VC treatments have been proven in several studies to be able to improve plant growth parameters such as height, fruit yield, stem width, water content, flower width, leaf length, leaf number, and leaf area (Gutierrez-Miceli et al., 2007; Azarmi et al., 2008; Lazcano et al., 2009; Wang et al., 2010; Garcia et al., 2012; Ali and Çiğ, 2018; Rashtbari et al., 2020; Ose et al., 2021; Esringü et al., 2022; Makkar et al., 2023). Balmori et al. (2019) explained that the foliar application of humic extract from VC at a 1:40 (v:v) on *Allium sativum* L. was the best treatment for growth parameters. They found that the humic substance increased the fruit quality and improved the bulb diameter. Akram et al. (2020) found that the bulbs of *Narcissus pseudonarcissus* that were treated with humic acid grew faster and were heavier than the control bulbs. According to Babarabie et al. (2018), the addition of VC to the soil enhanced flower life, germination rate, and stem diameter of *Narcissus* flowers.

Kahraman and Akcal (2018) investigated the effects of different nutrient solution recipes containing NPK (nitrogen, phosphorus, and potassium) on *L. aestivum* growth in soilless agriculture. They found the highest bulb diameter in nutrient solutions with 200% NPK and 125% NPK when compared with the control groups. Bakian et al. (2020) investigated how *L. aestivum* bulbs from different zones responded to organic and biological fertilizers. They used both biochar fertilizers and biofertilizers. They discovered that a biochar treatment of 12 tons/h resulted in the maximum root weight and total plant weight. Other findings corroborate the results of this study.

El-Attar et al. (2022) observed a significant increase in the width of *Narcissus tazetta* bulbs and their fresh weights with the application of K sources (K-nano or K-humate) and soil treated with VC. Srivastava et al. (2012) reported that 50% VC + 50% NPK treatment significantly increased the size and fresh weight of *Allium cepa* bulbs.

Garcia et al. (2012) indicated that 34 and 46 mg humic acid/L increased the water accumulation in rice seedlings under water stress when compared with the control groups.

Several studies have reported that VC application positively affects the production of various secondary metabolites, including scopolamine, caffeic, gallic, ellagic acid, phytol, lupeol, β -amyrin, lycopersene, β -phellandrene, and rosmarinic acid (Gholami et al., 2018; Alinejad et al., 2020; Celikcan et al., 2021; Souffront et al., 2022). Souffront et al. (2022) analyzed secondary metabolite production in tomato crops using GC/MS analysis. They found that 20% VC tea treatment increased the production of lycopersene and β -phellandrene. In addition, Alinejad et al. (2020), VC treatment may mitigate the negative effects of water shortage stress in *Datura stramonium*. They reported that the leaves with the highest scopolamine content were found in the 15% water deficit treatment with 15% VC. Celikcan et al. (2021) reported that 2.5%, 5%, and 10% VC treatments increased the caffeic acid content, whereas 2.5%, 5%, 10%, and 20% VC treatments decreased the estragole and eucalyptol contents in *Ocimum basilicum*. Rasa et al. (2017) indicated that 30% VC treatment enhanced alkaloids in *Fumaria vialantii*, such as fumaryn and synaktyn. Gholami et al. (2018) determined that the highest caffeic acid content was found in a mixture of humic acid (0.3 kg per hectare) and VC (5 t per hectare) treatments in *Cichorium intybus*. They reported that the highest ellagic acid content was observed in shoots when only VC (5 t per hectare) was applied.

Gorinova et al. (1993 and 1995) noticed that galanthamine biosynthesis was likely controlled by soil fertility levels. They found that *L. aestivum* grew best in soils with a pH close to neutral and rich in organic matter and that galanthamine levels were highest in soils with ample supplies of K, N, B, Mg, Zn, Mo, Cu, and Fe. Similarly, Demir et al. (2022) reported that soils that were neutral to slightly alkaline with a high organic matter content were effective in increasing the alkaloid content in *L. aestivum* bulbs and leaves. Moreover, they demonstrated that the amount of galanthamine present in *L. aestivum* might be correlated with soil K levels.

The galanthamine and lycorine contents of *L. aestivum* were enhanced with different VC concentrations in this study (Table 2), as in previous studies, owing to the organic nature of the VC applied to the soil, which increases soil nutrients (Kayabaşı and Yilmaz, 2021). Akram et al. (2020) reported that higher levels of humic acid resulted in a higher level of alkaloids (galanthamine and haemanthamine) in *Narcissus pseudonarcissus*. Humic acid in VC (Gholami et al., 2018; Akram et al., 2020) may also have increased the alkaloid content in *L. aestivum* in this study.

A strong negative correlation was observed between free radical scavenging potential and total phenolic content of bulbs and leaves ($r = -0.77$ and -0.92 , respectively, $P < 0.05$), indicating that an increase in the overall phenolic content generated by the impact of VC caused an increase in antioxidant capability.

Similar to the results of this study, Demir et al. (2022) reported that high levels of organic matter in the soil were beneficial for enhancing the phenolic constituents and antioxidant capacity of *L. aestivum*.

Several studies have investigated the effects of VC on various plants. Mardani-Talaei et al. (2016) reported the highest phenol activity in *Capsicum annuum* with 30% VC. Souffront et al. (2022) determined that a 10% VC tea treatment resulted in a higher concentration of total phenolic compounds than the control and 20% VC tea treatments in tomato crops. Omar et al. (2012) indicated that VC treatment on cassava tubers had the greatest total phenolic content (10.88 mg GAE/g fw) when compared with the inorganic fertilizer (8.35 mg GAE/g fw). Lujan-Hidalgo et al. (2015) determined the total phenol content in *Annona purpurea* leaf samples. The flavanone content in the leaf samples increased from 2.45 mg/g leaf dry weight in the unamended soil to 5.06 mg/g leaf dry weight after adding 100 g of VC.

Several studies have revealed that VC-applied application generally increases the antioxidant activity of plants due to humic acid substances (Cordeiro et al., 2011; Elmongy et al., 2018; Zuo et al., 2018). Wang et al. (2010) indicated that mixing VC and soil at a ratio of 4:7 (w/w) showed the best DPPH radical-scavenging activity on Chinese cabbage leaves, which was enhanced by 92% compared with the activity of the full soil treatment (0:7 w/w). Omar et al. (2012) found that VC treatment resulted in the highest DPPH scavenging activity (67.30%) in cassava tubers. Choirunnisa et al. (2022) observed that all VC treatments (40, 60, and 80 g/plant) showed positive antioxidant activity in *Echinacea purpurea*.

Antioxidant enzymes have been frequently shown to be elevated in VC-applied trials with various plants. Xu et al. (2016) reported that 15% VC treatment increased SOD and CAT enzyme activities in the aerial parts of *Silybum marianum*. In addition, they determined that 15% VC and 1% NaCl + 5% VC treatments enhanced SOD activity in the roots. In *Mentha haplocalyx*, all VC treatments enhanced SOD activity in the aerial parts. However, all VC treatments decreased CAT activity in the roots. Ahmad et al. (2022) determined that under drought conditions, both SOD and CAT activities were enhanced by the application of 8 tonnes per hectare (t/ha) VC on wheat straw. Lahbouki et al. (2022) indicated that *Opuntia ficus-indica* infected with arbuscular mycorrhizal fungi (AMF), treated with VC, and their combination demonstrated higher activities of SOD and CAT than control plants.

They revealed that SOD and CAT activities increased when AMF and VC were added to soils. García et al. (2012) determined that the association of humic acid with the radicular system in plants stimulates antioxidative enzymatic functions in rice plants. Zuo et al. (2018) indicated that the SOD activity of strawberries was significantly enhanced by the application of 20% and 30% VC. Lower SOD activity with all VC applications in comparison with the control (no VC application) showed that these treatments did not impose too much stress on *L. aestivum*. On the other hand, CAT activity was the lowest in the 5% VC treatment, and activity of this enzyme was elevated in the 10%, 25%, and 50% VC treatments compared with the control. Application of 5%, 10%, and 25% VC increased the bulb width and fresh weight. The lowest level of CAT activity with 5% VC application (low stress) may be associated with greater bulb enlargement (Table 1). The accumulation of secondary plant products is strongly influenced by growing conditions such as temperature, light regime, and nutrient availability. Various stress conditions have an

impact on the metabolic pathways responsible for the accumulation of these products in plants (Selmar and Kleinwächter, 2013), such as alkaloid (galanthamine and lycorine) levels and phenolic content (Arslan et al., 2020; Ates et al., 2021; Demir et al., 2022). The highest galanthamine and lycorine levels were observed with 10% and 25% VC in the bulbs, and 50% and 25% VC in the leaves, respectively. It can be deduced that these VC concentrations (10%, 25%, and 50%) caused moderate stress in *L. aestivum* (higher CAT activity) and were associated with higher levels of galanthamine and lycorine.

CAT activity and galanthamine levels in the bulbs showed a strong positive correlation ($r = 0.90$, $P < 0.05$) in response to various VC applications. Plants treated with VC are resistant to adverse environmental conditions (Celikcan et al. 2021). Enhanced CAT activity was noted in response to oxidative stress with 10%, 25%, and 50% VC administration. These concentrations may improve endurance (resistance) to abiotic stress conditions owing to increased antioxidant capacity.

In this study, we demonstrated for the first time that VC application in *L. aestivum* cultivation caused remarkable increases in galanthamine and lycorine levels, bulb and leaf expansion, phenolic content, and free radical scavenging power. It was also verified that increased CAT activity with some VC treatments supports the stress endurance capacity of *L. aestivum*. Overall, it has been shown that increased biomass with much higher galanthamine levels can be obtained from the same field with VC application in the cultivation of *L. aestivum*.

5 Conclusion

Augmentation with 5%, 10%, and 25% VC enhanced the width, length, and fresh weight of the bulbs and leaves. Application of 50% VC increased the total phenol and flavonoid contents and, consequently, the free radical scavenging activity of the bulbs and leaves. Galanthamine levels in the bulbs and leaves were improved by 10% and 50% VC application, respectively. Elevated CAT activity (enzymatic antioxidant defense system) with 10%, 25%, and 50% VC applications may provide a greater resistance to abiotic stress conditions in this species. When all outcomes were considered together, this plant can be cultivated successfully in soil amended with 10% VC to increase bulb galanthamine production with eco-friendly approach. Future research should focus on alleviation of the adverse effects of various abiotic stresses with VC treatments and their influences on alkaloid accumulation in *L. aestivum*.

Declarations

Author contribution

Ayca Cimen: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. Yavuz Baba: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft. Arzu Birinci Yildirim: Investigation, Methodology, Data curation, Formal analysis, Validation. Arzu Ucar Turker: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Visualization, Supervision, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Tables 1-4 is available in the Supplementary Files section.

Figures



Figure 1

Vermicompost treatments.



Figure 2

(A) 0% VC (control); (B) 5% VC treatment; (C) 10% VC treatment; (D) 25% VC treatment; (E) 50% VC treatment.

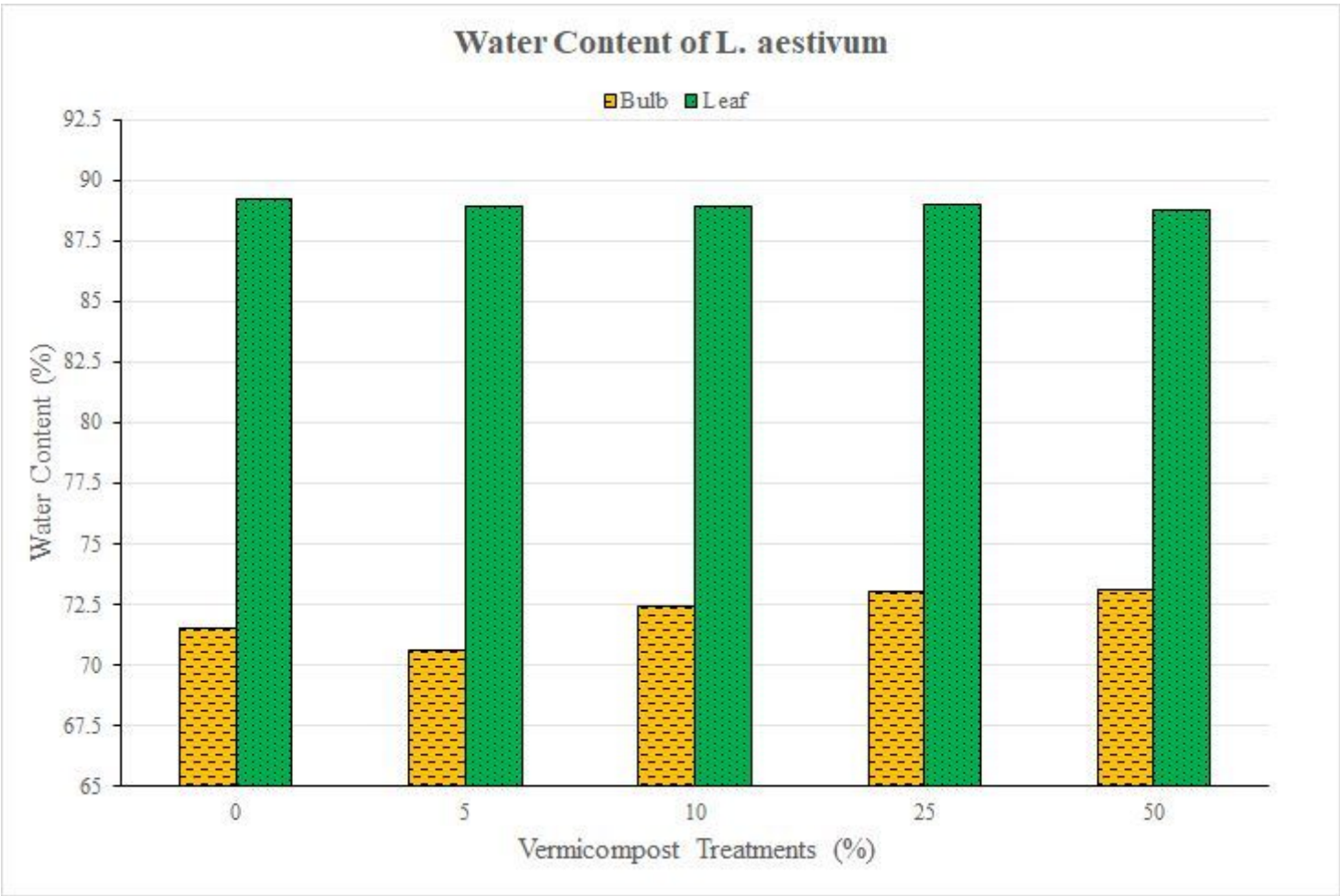


Figure 3

Effect of VC treatments on water content (%) of *L. aestivum* bulbs and leaves.

*Water Content (%) = [Total fresh weight (g) - Total dry weight (g)] / Total fresh weight x 100]

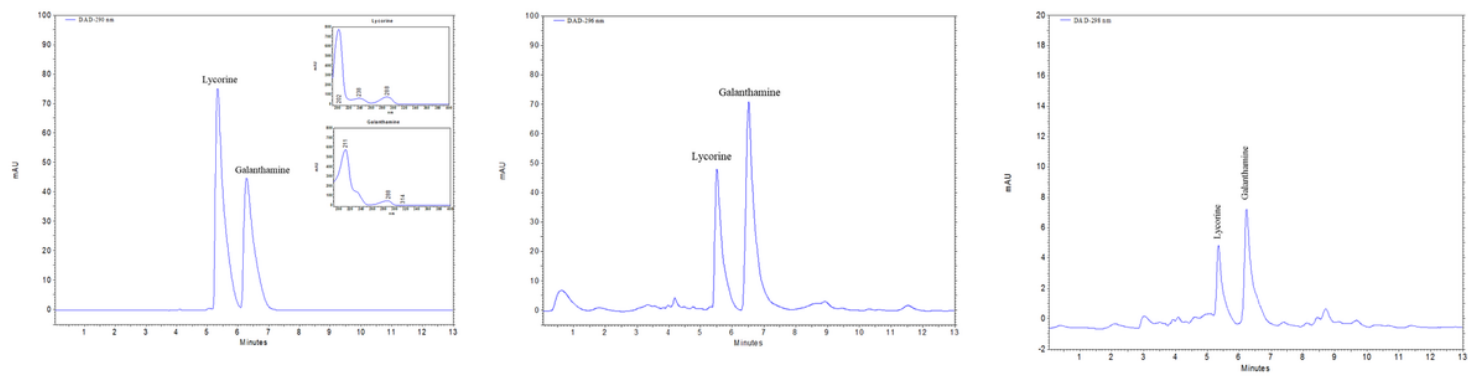


Figure 4

a HPLC chromatogram of alkaloid standards and their spectrums. Retention times: 1. Lycorine-5.31 min, 2. Galanthamine-6.29 min.

b HPLC chromatogram of bulb extracts obtained from 10% VC treatment.

c HPLC chromatogram of leaf extract obtained from 50% VC treatment.

Supplementary Files

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