

Biotic and abiotic factors influence secondary metabolite accumulation and allelopathic potential of grapevine (*Vitis vinifera*) against cosmopolitan weeds

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Abstract

Viticulture generates significant pruning wastes, which can be valorized as bioherbicides for sustainable weed management, and as a means to advance the circular economy. Allelopathy, an environmentally friendly approach in which plants release secondary metabolites that suppress the growth of other species, presents a means to managing weed growth in agricultural systems. Our study aimed to assess whether biotic (grapevine variety and mycorrhization) and abiotic (atmospheric CO₂ level, air temperature or water availability) factors influence the accumulation of secondary metabolites in grapevine (*Vitis vinifera* L.) pruning wastes and their allelopathic effects on three cosmopolitan weeds (*Sisymbrium irio*, *Solanum nigrum* and *Sonchus oleraceus*). Two grapevine varieties, Tempranillo and Cabernet Sauvignon, inoculated (M) or not (NM) with mycorrhizal fungi, were grown under two environmental conditions: CATA (current CO₂ and temperature conditions i.e., 400 ppm CO₂ and ambient air temperature) and CETE (700 ppm CO₂ and ambient air temperature + 4°C). Within each grapevine variety and environmental condition, half of the M and NM plants were subjected to either full irrigation (WW) (90–100% substrate field capacity, FC) or limited irrigation (D) (cycles from 90–100% to 20–30% FC). Characterization of the methanolic extracts of the grapevine wastes revealed significant variations in phenolics, flavonoids, flavonols, and anthocyanins across treatments, with higher accumulation and free radical scavenging activity under elevated CO₂ and temperature conditions. Within each variety, the accumulation of secondary compounds was also influenced by the level of irrigation and the presence or absence of root-associated mycorrhizal fungi. Aqueous extracts of grapevine leaves used in germination bioassays strongly inhibited seed germination and seedling growth of the weeds, with the pronounced effects observed in *S. irio*. The presence of these secondary metabolites contributes to their allelopathic effect, highlighting the potential of grapevine pruning waste as bioherbicides and an alternative to synthetic herbicides. However, further studies are needed to determine optimal extract concentrations and assess their effects on crops under field conditions.

1. Introduction

Agricultural wastes, otherwise known as agro-wastes, are by-products of farming activities, including crop cultivation, harvesting, livestock rearing, and the industrial processing of farm produce. These waste products emerge from the processing and manufacturing of agricultural commodities and often contain components with potential utility. However, their economic value is typically lower than the costs associated with collection, transportation, and processing for reuse in beneficial applications (Obi et al. 2016). Agricultural wastes can be broadly categorized into organic and inorganic wastes. Organic wastes include crop residues, manures, and by-products of food processing, while inorganic wastes encompass synthetic chemicals such as herbicides, pesticides, and fertilizers (Selvakumar et al. 2021).

Globally, it is estimated that more than 140 billion metric tons of agricultural waste biomass are generated annually (Ufitikirezi et al. 2024). With the increasing global population, agricultural production is expected to rise to meet the growing food demand (Chaichi et al. 2022), inevitably leading to the generation of more agricultural wastes. The indiscriminate disposal of agricultural wastes poses significant environmental risks. The landfilling and burning of crop residues and animal wastes can pollute groundwater (Babu et al. 2022) and release greenhouse gases such as methane, carbon oxides, sulphur oxides, and nitrogen oxides, thereby contributing to global warming and climate change (Rao et al. 2024). Additionally, run-off of chemical wastes into water bodies can lead to water pollution, while excessive application of synthetic chemicals contributes to heavy metal contamination of soils. A sustainable approach to waste management is therefore crucial to mitigate pollution, protect soil health, and promote environmental sustainability.

The concept of circular economy advocates for the optimal utilization of available resources, reducing waste generation, and reintegrating by-products into the production cycle. This approach provides environmental, economic, and social benefits (da Freiria Ferreira et al. 2024). Hence, in line with the circular economy principle, the management of agricultural waste by applying the four “R” – reduce, reuse, recycle and recover, is integral to achieving sustainability. One key strategy is valorization, which involves converting waste materials into raw materials or reusable products to enhance resource use efficiency.

One example of agricultural waste with valorization potential is grapevine (*Vitis vinifera* L.), which produces significant vegetative waste during pruning. Grapevine is a key perennial crop that is cultivated for its fruits which are used to produce vinegar, wine, juice, jelly, jam and other by products (Torregrosa et al. 2015). Global grape production has been estimated at approximately 77.8 million tonnes annually (OIV, 2019), and grapevine viticulture generates up to 29.6 million tonnes of vegetative wastes each year, particularly during pruning (Dwyer et al. 2016; Troilo et al. 2021; Santos et al. 2022). Given these large volume of wastes, efficient waste

management strategies are essential. These wastes can be valorized to produce bioherbicides as an environmentally friendly alternative for weed control in agricultural fields.

Grapevine leaves are rich in biologically active compounds, that is, secondary metabolites, such as tocopherols, carotenoids, polyphenols (phenolics, flavonoids and hydroxycinnamic acids), terpenoids and alkaloids which possess antioxidant and antimicrobial properties (Amarowicz et al. 2016; Torres et al. 2018; Baroi et al. 2022; Nzekoue et al. 2022; Sousa et al. 2024; Kamah et al. 2025), although the levels of these bioactive compounds may vary depending on the environmental or biotic factors affecting the grapevine (Torres et al. 2015). Therefore, the extraction of bioactive compounds is one of the most viable valorization options in grapevine waste management (Singh et al. 2023; de Freitas et al. 2024).

In agricultural systems, weeds pose significant challenges by competing with crops for nutrients, water, light, and space, thereby reducing yields and causing economic losses of up to 100 billion USD worldwide (Le Tourneau et al. 1956; Bates et al. 2012). Among the common and problematic weeds are *Solanum nigrum* L. (black nightshade), *Sonchus oleraceus* (common sow thistle), and *Sisymbrium irio* L. (London rocket). These weeds have developed resistance to synthetic herbicides, making them increasingly difficult to manage in farming systems (Chauhan et al. 2006; Dellow et al. 2006; Hyatt, 2006; Gandia et al. 2021). As such, there is a critical need for sustainable weed control strategies that do not rely on synthetic herbicides. In fact, the European Green Deal aims to reduce the use and risks of chemical pesticides by 50% by 2030 (Schneider et al. 2023). Herbicides, like other pesticides, can damage ecosystems and bee colonies and cause contamination of soil, groundwater, drinking water and food, posing health risks to consumers. Inhalation or ingestion of pesticide residues increases the risk of chronic diseases and cancer (Gensch et al. 2024). There is therefore an urgent need to find alternatives to the use of herbicides (and other pesticides) to ensure food safety.

The application of bioherbicides is an emerging alternative solution for weed management (Islam et al. 2024). Given their rich secondary metabolite content, grapevine leaves hold potential as a natural source of bioherbicides. Allelopathy, a biological phenomenon in which plants release secondary metabolites that inhibit germination and growth of other species by altering their physiology and metabolism offer an environmentally friendly approach for controlling weed. Allelochemicals are released in to the environment from plants via root exudation, litter decomposition and leachates from stems and leaves (Inderjit et al. 2008). This form of natural weed control presents a promising alternative to synthetic chemicals, which can bioaccumulate in the food chain and harm soil health (Hassan et al. 2021). Furthermore, given the growing resistance of certain weed species to synthetic herbicides (Heap, 2025), several authors have highlighted the effectiveness of bioherbicides of plant origin in weed control (Mao et al. 2006; Jabran et al. 2015; Gulzar and Siddiqui, 2017; Dhungana et al. 2019; Đorđević et al. 2022a; Sidhu et al. 2023; Islam et al. 2024). Thus, bioherbicides derived from plants like grapevine may prove to be an effective and sustainable solution.

Although, it has been established that grapevine wastes are rich in secondary metabolites with various bioactive properties, the allelopathic effects of grapevine leaf wastes on the cosmopolitan weeds - *Sisymbrium irio*, *Solanum nigrum* L. and *Sonchus oleraceus* have not been explored, nor have the effects of environmental and other biotic factors modulating the allelopathic potential of grapevine by-products. The present study was therefore designed to (i) determine whether biotic (grapevine variety and mycorrhization) and abiotic (atmospheric CO₂ level, air temperature or water availability) factors influence the accumulation of secondary metabolites in two grapevine varieties (*Vitis vinifera* L.) – Tempranillo and Cabernet Sauvignon; (ii) investigate the potential allelopathic effect of the pruning by-products of these grapevine varieties against three cosmopolitan weeds, by evaluating their effect on seed germination and seedling growth.

2. Material and Methods

2.1. Biological material and experimental design

Sisymbrium irio L. (Brassicaceae), called London rocket, is an annual weed that is widely distributed in Europe, Asia and North Africa and has been naturalized across several hemispheres (Ray et al. 2005; Kim et al. 2021). *Solanum nigrum* L. (Solanaceae), also called black nightshade, is a noxious, annual to short-lived perennial weed found in several tropical and temperate regions of the world (Defelice, 2003). *Sonchus oleraceus* (Asteraceae), called common sow thistle, is a rapidly maturing, highly dispersive and difficult to control annual weed with wide distribution in diverse agroecosystems (Peerzada et al. 2019). These species are widely distributed across tropical and temperate regions, where they cause significant yield losses, particularly in crops such as maize, cotton, vegetables and grapes (Tursun et al. 2006; Shrestha and Fidelibus, 2005; Wang et al. 2019). Seeds of the weeds - *Sisymbrium irio*, *Solanum nigrum* and *Sonchus oleraceus* were procured, respectively, by the Banco de Germoplasma 'César Gómez Campo'

(Universidad Politécnica de Madrid, Spain), the Banco de Germoplasma Hortícola (Centro de Investigación y Tecnología Agroalimentaria de Aragón, CITA, Zaragoza, Spain) and the Centro de Recursos Fitogenéticos (INIA-CSIC, Madrid).

Three-years old grapevine (*V. vinifera* L.) var. Cabernet Sauvignon and Tempranillo grafted in 2020 onto R110 rootstocks (*V. berlandieri* x *V. rupestris*) were used for the experiment. Plants were grown in 13-L pots filled with a mixture of peat, vermiculite, and sand (1: 2.5: 2.5). The peat used (Floragard, Vilassar de Mar, Barcelona) contained nitrogen ($70\text{--}150\text{ mg L}^{-1}$), P_2O_5 ($80\text{--}180\text{ mg L}^{-1}$) and K_2O ($140\text{--}220\text{ mg L}^{-1}$), had a pH of 5.2–6.0 and was previously sterilized at 100°C for 1 h on three consecutive days. In 2021 (one-year old plants), the plants were divided into two groups: (1) half of the plants received 10 g (per plant) of a commercial inoculum (Bioradis Plant, Bioera SLU, Tarragona, Spain) containing approximately 100 spores per gram of a mixture of five arbuscular mycorrhizal fungi (AMF) (*Rhizophagus irregularis* 30 spores g^{-1} , *Funneliformis mosseae* 25 spores g^{-1} , *Septoglomus deserticola* 30 spores g^{-1} , *Claroideoglomus claroideum* 5 spores g^{-1} and *Claroideoglomus etunicatum* 10 spores g^{-1}) accompanied by 4×10^7 CFU (Bacteria Forming Unit) per gram of a mixture of four PGPRs (*Bacillus subtilis*, *B. megaterium*, *B. altitudinis* and *B. licheniformis*) (M plants); (2) the other half of the plants of each grapevine variety only received a filtrate containing the rhizobacteria (NM plants). The filtrate was obtained by washing an equivalent amount of the inoculum with distilled water and vacuum filtering the resulting liquid through 15–20 mm diameter filters with a particle retention capacity of $2.5\text{ }\mu\text{m}$ (Whatman 42; GE Healthcare, Little Chalfont, UK). With this, the differences between M and NM plants could be attributed exclusively to the absence or presence of AMF. The plants were inoculated following the same protocol in the second and third year.

When three-year-old (in 2023), plants from each grapevine variety were grown in temperature gradient greenhouses (TGGs) at the Universidad de Navarra campus (42.80 N, 1.66 W; Pamplona, Spain) under two environmental conditions applied from fruit set (E-L 27) to maturity (E-L 38) (Coombe, 1995): (1) current CO_2 and temperature conditions (CATA, ca. 400 ppm CO_2 and ambient air temperature corresponding to the summer of 2023 in Pamplona) or (2) climate change conditions predicted by the year 2100 (CETE, 700 ppm CO_2 and ambient air temperature + 4°C). The average of the maximum temperatures was 26.6, 28.8, 30.7 and 26.7°C in June, July, August and September 2023, respectively. The average of the minimum temperatures was 15.7, 16.3, 15.5 and 14.9°C in June, July, August and September 2023, respectively. The increase in temperature of 4°C with respect to the current ambient values implemented in the CETE treatment, was chosen in order to simulate the changes projected for the end of the 21st century, as per the SSP5-8.5 greenhouse emission scenario derived from the concentration-driven CMIP6 model simulations (IPCC, 2021). At fruit veraison (E-L 35), within each environmental condition (CATA or CETE), half of the plants of every variety (T or CS) and inoculation treatment (M or NM) were divided into two homogeneous groups and subjected to two levels of water availability: maintained full irrigation (WW) (90–100% substrate field capacity, FC) or restricted irrigation (D) (cycles from 90–100% till 20–30% FC). Soil water content was monitored using EC 5 water sensors (Decagon Devices, Inc., Pullman, WA, USA). Therefore, the total number of treatments applied to each grapevine variety was eight (M/NM plants, CATA/CETE conditions and WW/D water regime: $2 \times 2 \times 2$). The number of biological replicates (plants) per variety and treatment was four. Plants were irrigated with alternating water and nutrient solution (Ollat et al., 1998) and regularly pruned. Leaf material from pruning after water deficit treatment was used to test their allelopathic potential.

The treatments are as follows Tempranillo with Mycorrhiza (TM), Tempranillo with No Mycorrhiza (TNM), Cabernet Sauvignon with Mycorrhiza (CSM) and Cabernet Sauvignon with No Mycorrhiza (CSNM).

2.1. Preparation of methanolic leaf extracts

0.25 g of frozen leaves from each grapevine treatment were pulverized in liquid nitrogen and macerated in a mortar with 2 mL of 2% Methanol in 12N HCl, placed in a tube and kept at room temperature in the dark. This was followed by centrifugation at 3500 rpm 15 min 15°C (three times for 24 h) until a total of 6 mL of 2% Methanol in 12N HCl has been added. The supernatants were then combined and stored at 4°C until determinations.

2.3. Characterization of leaf extracts

The leaf extract was diluted with 2% Methanol in 12N HCl in the ratio 1:5 to facilitate sample collection and measurements, followed by determination with appropriate standard chemicals and spectrophotometric methods.

2.4. Determination of secondary metabolites

Total phenolic content in grapevine leaves was determined by adding 100 μL of the diluted extract (1:20) to 2 mL of distilled water and 500 μL of 0.17 M FeCl_3 . The mixture was shaken, followed by the addition of 400 μL of 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$. After shaking again, the

absorbance was measured at 760 nm after 15 minutes and gallic acid was used as the standard solution (Singleton and Rossi, 1965).

Total flavonoid content was determined following the method of described by Kim et al. (2003). 1 mL of each sample was mixed with 4 mL of deionized water, followed by the addition of 300 µL of NaNO₂ and shaking. The mixture was incubated for 5 min before adding 300 µL of AlCl₃, 2 mL of 1 M NaOH was added after 6 min, and the reaction mixture was diluted with 2.4 mL of deionized water. Absorbance was measured at 510 nm using catechin as the standard.

The quantification of flavonols and hydroxycinnamic acids was performed spectrophotometrically as described by Boulanouar et al. (2013). A 0.5 mL aliquot of each sample was diluted (1:2) in 95% ethanol acidified with 0.1% HCl, and an additional 4 mL of 2% HCl was added to make up to a final volume of 5 mL. The absorbance was measured at 360 nm, 320 nm and 520 nm, using quercetin, caffeic acid and malvidin as standards for derivatives of flavonols, hydroxycinnamic acid and anthocyanins, respectively.

Following the method of Arnous et al. (2001), flavan-3-ols in leaf samples were analyzed using the p-dimethylaminocinnamaldehyde (DMACA). Briefly, 0.2 mL aliquot of a 1:20 diluted sample (in 80% aqueous acidified methanol containing 2% HCl 12N) was combined with 1 mL of DMACA solution (0.1% in 1 N HCl in methanol). The mixture was vortexed and incubated at room temperature for 10 min, after which absorbance was measured at 640 nm, with catechin as the standard. All absorbance measurements were performed using a UV-VIS spectrophotometer (UV-1800, Shimadzu, Tokyo). The results were expressed as milligrams of the corresponding standard for each type of phenolics per gram of leaf fresh weight (FW).

2.5. DPPH radical scavenging activity

Free radical scavenging activity of grapevine leaves was evaluated by using DPPH (α, α-diphenil-β-picrylhydrazyl) reagent. 50 µL of the sample was added to a cuvette containing 950 µL of 80 µM in methanol. The tubes wrapped in aluminium, shaken, covered with parafilm to prevent evaporation. The mixture was kept in the dark for 30 min after which absorbance measured at 517 nm. Scavenging activity was evaluated following the method of Barros et al. (2007) using the formula below:

$$\text{Scavenging activity (\%)} = 100 \times \frac{A_0 - A_1}{A_0} \dots\dots\dots \text{Eq. 1}$$

where A₀ = Absorbance of the control, A₁ = Absorbance of the sample.

2.6. Germination assays

2.6.1. Preparation of aqueous leaf extracts

Grapevine leaves (TM, TNM, CSM and CSNM) under CETE conditions were collected from the growth chamber greenhouses of the Department of Environmental Biology, University of Navarra, Spain. They were stored in the refrigerator (4°C) until when ready for use. Leaves were grounded to fine powder with a mixer mill (Retsch MM 400 GmbH, Haan, Germany). 12 g of powdered grapevine leaves from each treatment were added into 600 mL of distilled water in a beaker with and placed in a water bath at 100°C for 45 min for extraction. The extracts were then filtered with a vacuum filter lined Whatman filter paper No. 1. This served as the germination assay for the experiment. The treatments are as follows Tempranillo with Mycorrhiza (TM), Tempranillo with No Mycorrhiza (TNM), Cabernet Sauvignon with Mycorrhiza (CSM) and Cabernet Sauvignon with No Mycorrhiza (CSNM). The osmotic potentials (MPa) of extracts were measured at 25.2°C with a dew point potentiometer (Nobel, 1983).

2.6.2. Determination of germination percentage, root length, shoot length and seedling vigour index (SVI)

Seeds of each weed were surface sterilized with 95% ethanol and 10% sodium hypochlorite for 5 min and then washed thoroughly with distilled water. Petri dishes (9 cm) were lined with two layers of Whatman No. 1 filter paper and autoclaved overnight for sterilization. Six surface sterilized seeds of each weed species were placed in each Petri dish. Thereafter, 7 mL of each germination assay (TM, TNM, CSM and CSNM) was added to the Petri dish. Petri dishes that received only distilled water were taken as control. The experiment was replicated three times and kept in a climate-controlled room at 25°C for 14 days with 12 h light per day. The number of germinated seeds was recorded daily up to 14 days, and each seed was considered germinated when the radicle emerged (Wang et al. 2016).

Germination percentage was calculated by using the equation by Rashidi et al. (2021):

$$GP = 100 \times \frac{NG}{NT} \dots\dots\dots \text{Eq. 2}$$

where NG is germinated seeds and,
 NT is total number of seeds sown.

A metric ruler was used to take daily measurements of both the root and shoot length of all germinated seeds

Seedling vigor index was estimated with equation below:

$$SVI = (S + R) G \dots\dots\dots \text{Equation 3}$$

where S and R are the shoot and root length (in cm) and G is the germination percentage (Kulkarni et al. 2007).

2.7. Statistical analysis

The germination experiment was conducted using a completely randomized design (CRD) with all experiments in triplicates. Results are expressed as the mean ± SE (standard error). Data were subjected to analysis of variance (ANOVA) by using R statistical package version 4.3.2 (R Development Core Team, 2024). A one-way ANOVA was used to compare secondary metabolite accumulation between CATA and CETE conditions and, a three-way ANOVA to evaluate the main effects of the water availability, grapevine variety and mycorrhization. For the data on allelopathic potential, one-way ANOVA was used to compare the effects of different grapevine extracts on germination and seedling growth of different weed species and, a two-way ANOVA to evaluate the main effects of treatment with grapevine extracts and weed species. Significant differences were assessed with Duncan’s multiple range tests post-hoc test at $p \leq 0.05$. A correlation analysis was conducted to evaluate the interrelationships among secondary metabolites under CATA and CETE conditions. Bar graphs were constructed in GraphPad Prism 8.0.1.

3. Results

3.1 Secondary metabolites content and DPPH radical scavenging activity under varying environmental conditions

The results obtained from the phytochemical analysis methanolic extracts of the two varieties of grapevine Cabernet Sauvignon (CS) and Tempranillo (T) under mycorrhizal (M) or non-mycorrhizal inoculation (NM) and subjected to different water availability (well-watered, WW, and drought, D) are presented in Table 1 and Table 2. Characterization of grapevine leaf extracts and under different environmental conditions (CATA and CETE) showed the presence of significant amounts of total phenols and total flavonoids with free radical scavenging activity.

Under CATA, the levels of secondary metabolites varied significantly in both grapevine varieties across all treatments except for hydroxycinnamic acids. Total phenolics was highest in WW TM grapevines (4.89 mg/g FW) while the lowest value was recorded in D CSNM (1.75 mg/g FW). Mycorrhization did not significantly affect total phenolics content in T both in D and WW conditions. The highest level of total flavonoids was recorded in WW CSM (1.74 mg/g FW) while the lowest was recorded in D CSNM (1.04 mg/g FW). Across the drought treatments, mycorrhization did not significantly affect total flavonoid content in CS while there was a significant difference in total flavonoid levels in CS under WW condition. In the same vein mycorrhization, significantly affected total flavonoids content in CS in both D and WW conditions.

The three factors investigated viz; grapevine variety, mycorrhization and water availability had a significant effect on the levels of flavan-3-ols except for the drought stress treatment where there was no significant difference in the levels of flavan-3-ols in M and NM CS.

Leaves of drought stressed, TNM grapevine recorded the highest amount of hydroxycinnamic acids (0.40 mg/g FW) similar to that of WW TM (0.35 mg/g FW) and D CSM (0.35 mg/g FW). In a similar trend, flavonols were highest in D TNM; WW TM and; D CSNM grapevines with 0.67 mg/g FW, 0.58 mg/g FW, 0.51 mg/g FW, respectively. In CS and T, there were significant differences in flavonoid contents irrespective on mycorrhization and water availability. Anthocyanins level was highest in D TNM (1.46 mg/g FW). The study recorded significant differences in the levels of anthocyanins both CS and T across all treatments. Free radical scavenging activity (% DPPH) was highest in WW CSM (83.33%) and lowest in D CSM grapevines (81.04%). In both CS and T grapevine varieties,

mycorrhization had no significant impact on free radical scavenging activity in WW condition but had a significant impact in D conditions.

Concentration of secondary metabolites and antioxidant activity in grapevine leaf extracts as influenced by mycorrhization and water availability under elevated temperature and CO₂ conditions (CETE) are presented in Table 2. T grapevine variety generally showed lower levels of phenolic compounds and antioxidant activity compared to the CS under WW and mycorrhizal conditions. Total phenolic content showed significant variation among treatments, with the highest levels recorded in WW CSM grapevines (6.80 mg/g FW) and the lowest in D CSNM (2.20 mg/g FW). Similarly, total flavonoid content varied across treatments in CS, with no statistically significant differences observed in WW and D TNM leaves. The highest concentration of total flavonoids was obtained WW CSM (1.77 mg/g FW) and the lowest in D TM (0.83 mg/g FW). There was significant difference in the levels of flavan-3-ols across all treatments. The lowest concentration was observed in D CSM (0.19 mg/g FW) and the highest concentration in WW CSM (0.81 mg/g FW).

Hydroxycinnamic acid content was highest in D CSM (0.46 mg/g FW) and lowest in D TM (0.12 mg/g FW). Water availability and mycorrhization significantly impacted the levels of hydroxycinnamic acid in CS and T varieties. Flavonol concentrations significantly increased in D CSNM (0.74 mg/g FW), while the lowest levels were recorded in D TM (0.24 mg/g FW). Anthocyanin content varied significantly across all treatments, with the highest concentration in D TNM (2.19 mg/g FW) and the lowest in D TM (0.01 mg/g FW). Anthocyanin content varied significantly across all treatments, with the highest concentration in D TNM (2.40 ± 0.72 mg/g FW) and the lowest in WW CSNM (0.08 ± 0.27 mg/g FW). Antioxidant activity (% DPPH) was similar across treatments, although a notable decline was observed in D TNM (71.68%) when compared to other treatments.

In Table 3, a three-way analysis of variance (ANOVA) for secondary metabolites in grapevine leaves under CATA revealed that only grapevine variety had a significant effect on the total phenolics content while water availability had a significant effect on total flavonoids content. Mycorrhization, the interaction of grapevine variety and mycorrhization had significant effects on flavan-3-ols level. Anthocyanin levels were greatly impacted by the three factors under consideration, that is, grapevine variety, water availability and mycorrhization and their interactions. Mycorrhization significantly impacted DPPH radical scavenging activity while however, neither of the three factors had a significant effect on hydroxycinnamic acids and flavonol levels. Under CETE, water availability, the interactions of grapevine variety and water availability, grapevine variety and mycorrhization, mycorrhization and water availability had a significant effect on total phenolics content. Flavan-3-ols levels and DPPH scavenging activity were significantly impacted by mycorrhization; the interaction of grapevine and water availability had a significant effect on the levels of hydroxycinnamic acids, flavonols, anthocyanins and DPPH scavenging activity (Table 4). In addition, DPPH scavenging activity, anthocyanin and flavonoids content were significantly impacted by the interaction of grapevine variety and mycorrhization. Lastly, the interaction of the three factors studied viz grapevine variety, water availability and mycorrhization significantly influenced anthocyanin, and hydroxycinnamic acid levels but not on DPPH scavenging activity total phenolics and total flavonoids, flavan-3-ols and flavonol contents.

Table 1

Concentration of secondary metabolites and antioxidant activity in grapevine leaf extracts as influenced by mycorrhization (myc) and water availability under ambient conditions (CATA)

Grapevine variety	Water	Myc	Total Phenolics	Total Flavonoids	Flavan-3-ols	Hydroxycinnamic acids	Flavonols	Anthocyanins	%DPPH
CS	WW	M	4.36 ± 0.04 ^b	1.75 ± 0.17 ^b	0.83 ± 0.00 ^c	0.31 ± 0.08 ^a	0.47 ± 0.12 ^{ab}	0.14 ± 0.06 ^a	83.83 ± 0.28 ^b
		NM	4.29 ± 0.37 ^a	1.39 ± 0.07 ^{ab}	0.71 ± 0.19 ^{bc}	0.26 ± 0.07 ^a	0.40 ± 0.09 ^a	0.05 ± 0.04 ^a	83.09 ± 1.20 ^b
	D	M	3.57 ± 0.84 ^{ab}	1.26 ± 0.10 ^a	0.40 ± 0.04 ^a	0.35 ± 0.10 ^a	0.51 ± 0.11 ^{ab}	1.10 ± 0.05 ^c	81.35 ± 0.38 ^b
		NM	1.87 ± 0.29 ^a	1.04 ± 0.26 ^a	0.22 ± 0.01 ^a	0.31 ± 0.03 ^a	0.47 ± 0.05 ^{ab}	0.84 ± 0.02 ^b	81.04 ± 1.71 ^{ab}
T	WW	M	5.47 ± 0.64 ^b	1.43 ± 0.04 ^{ab}	0.52 ± 0.08 ^{ab}	0.35 ± 0.04 ^a	0.58 ± 0.90 ^{ab}	1.04 ± 0.02 ^c	82.40 ± 1.14 ^b
		NM	4.05 ± 0.24 ^b	1.21 ± 0.19 ^a	0.50 ± 0.02 ^{ab}	0.27 ± 0.05 ^a	0.37 ± 0.23 ^a	0.12 ± 0.01 ^a	83.21 ± 0.69 ^b
	D	M	4.39 ± 0.92 ^b	1.31 ± 0.07 ^{ab}	0.43 ± 0.11 ^{ab}	0.23 ± 0.03 ^a	0.47 ± 0.06 ^{ab}	1.28 ± 0.10 ^d	77.63 ± 1.80 ^a
		NM	4.89 ± 0.90 ^{2b}	1.25 ± 0.06 ^a	0.37 ± 0.10 ^a	0.40 ± 0.03 ^a	0.67 ± 0.12 ^b	1.46 ± 0.01 ^e	81.10 ± 0.59 ^{ab}

Values represent means (n = 3) in mg/g FW separated by Duncan test ($P \leq 0.05$). Different letters across columns, indicate significant differences as affected by the main factors - Grapevine variety, water availability and mycorrhization. CS = Cabernet Sauvignon; T = Tempranillo; WW = well-watered; D = drought; M = with mycorrhiza; NM = no mycorrhiza; FW = fresh weight.

Table 2

Concentration of secondary metabolites and antioxidant activity in grapevine leaf extracts as influenced by mycorrhization (myc) and water availability under elevated temperature and CO₂ conditions (CETE)

Treatment	Water	Myc	Total Phenolics	Total Flavonoids	Flavan-3-ols	Hydroxycinnamic acids	Flavonols	Anthocyanins	%DPPH
CS	WW	M	6.80 ± 0.06 ^c	1.77 ± 0.06 ^c	0.81 ± 0.24 ^c	0.34 ± 0.05 ^{ab}	0.55 ± 0.10 ^{abc}	0.99 ± 0.07 ^b	82.40 ± 1.46 ^b
		NM	4.64 ± 0.20 ^b	1.02 ± 0.17 ^{ab}	0.42 ± 0.06 ^{abc}	0.20 ± 0.03 ^a	0.31 ± 0.03 ^{ab}	0.12 ± 0.04 ^a	84.08 ± 0.33 ^b
	D	M	2.47 ± 0.04 ^a	1.44 ± 0.24 ^{bc}	0.19 ± 0.04 ^a	0.46 ± 0.12 ^b	0.74 ± 0.17 ^c	2.15 ± 0.21 ^c	82.71 ± 0.39 ^b
		NM	2.20 ± 0.04 ^a	0.96 ± 0.12 ^a	0.22 ± 0.03 ^{ab}	0.16 ± 0.02 ^a	0.33 ± 0.01 ^{ab}	0.28 ± 0.02 ^a	83.52 ± 0.68 ^b
T	WW	M	4.28 ± 0.14 ^b	0.96 ± 0.08 ^a	0.63 ± 0.15 ^{abc}	0.19 ± 0.07 ^a	0.30 ± 0.09 ^{ab}	0.19 ± 0.05 ^a	84.94 ± 0.11 ^b
		NM	4.64 ± 0.25 ^b	1.67 ± 0.08 ^c	0.76 ± 0.16 ^{bc}	0.31 ± 0.05 ^{ab}	0.52 ± 0.06 ^{abc}	1.14 ± 0.01 ^b	82.28 ± 1.33 ^b
	D	M	2.56 ± 0.64 ^a	0.83 ± 0.00 ^c	0.33 ± 0.14 ^{abc}	0.12 ± 0.06 ^a	0.24 ± 0.09 ^a	0.01 ± 0.00 ^a	81.91 ± 1.18 ^b
		NM	3.64 ± 0.55 ^b	1.61 ± 0.20 ^c	0.52 ± 0.29 ^{abc}	0.35 ± 0.12 ^{ab}	0.58 ± 0.14 ^{bc}	2.19 ± 0.21 ^c	71.68 ± 5.44 ^a

Values represent means (n = 3) in mg/g FW separated by Duncan test (P ≤ 0.05). Different letters across columns, indicate significant differences as affected by the main factors - Grapevine variety, water availability and mycorrhization. CS = Cabernet Sauvignon; T = Tempranillo; WW = well-watered; D = drought; M = with mycorrhiza; NM = no mycorrhiza; FW = fresh weight.

Table 3

Main effects of grapevine variety (V), water availability (W) and mycorrhization (M) on secondary metabolites and antioxidant activity under ambient conditions (CATA)

	Total phenolics	Total Flavonoids	Flavan-3-ols	Hydroxycinnamic acids	Flavonols	Anthocyanins	%DPPH
V	*	ns	ns	ns	ns	Ns	ns
W	ns	*	ns	ns	ns	Ns	ns
M	ns	ns	**	ns	ns	**	**
V × W	ns	ns	ns	ns	ns	Ns	ns
V × M	ns	ns	*	ns	ns	Ns	ns
W × M	ns	ns	ns	ns	ns	Ns	ns
V × W × M	ns	ns	ns	ns	ns	Ns	ns

(*), (**), (***) indicates p values are significant at p < 0.05, 0.01 and 0.001 respectively; ns – not significant according to Duncan multiple range test.

Table 4

Main effects of grapevine variety (V), water availability (W) and mycorrhization (M) on secondary metabolites and antioxidant activity under elevated temperature and CO₂ (CETE)

	Total phenolics	Total Flavonoids	Flavan-3-ols	Hydroxycinnamic acids	Flavonols	Anthocyanins	%DPPH
V	ns	ns	ns	ns	ns	Ns	ns
W	***	ns	ns	ns	ns	Ns	ns
M	ns	ns	**	ns	ns	Ns	*
V × W	***	ns	ns	**	***	***	*
V × M	***	***	ns	ns	ns	***	*
W × M	*	ns	ns	ns	ns	Ns	ns
V × W × M	ns	ns	ns	*	ns	***	ns

(*), (**), (***) indicates p values are significant at $p < 0.05$, 0.01 and 0.001 respectively; ns – not significant according to Duncan multiple range test.

3.2. Correlation analysis of bioactive compounds under varying environmental conditions

The correlation plot suggests that different classes of phenolic compounds are interrelated, with total phenolics, flavonoids, and flavan-3-ols forming a closely linked group (Figs. 5 and 6). Under CATA, Total phenolics and total flavonoids showed high positive correlation ($r = 0.8-10$), as indicated by the large dark blue circle. Flavan-3-ols also showed a strong positive correlation with total phenolics and total flavonoids ($r = 0.8-10$). Hydroxycinnamic acids had a strong positive correlation with flavan-3-ols and Flavonols ($r = 0.8-10$). Similarly, flavonols showed moderate positive correlations with total phenolics, total flavonoids, and flavan-3-ols ($r = 0.3-0.7$), while anthocyanins showed a moderate positive correlation with hydroxycinnamic acids ($r = 0.3-0.7$). However, anthocyanins exhibited a weak to negative correlation with total phenolics and flavan-3-ols, as indicated by the light reddish circle ($r < 0$).

Under CETE, a strong positive correlation was observed between total phenolics, total flavonoids, and flavan-3-ols ($r = 0.8-10$), similarly, hydroxycinnamic acids and flavonols showed a strong positive correlation ($r = 0.8-10$). However, moderate correlations were observed between hydroxycinnamic acids, flavan-3-ols, and flavanols ($r = 0.3-0.7$). On the other hand, anthocyanins showed weak negative correlations with flavan-3-ols and total phenolics as shown by the light reddish circle ($r < 0$).

3.3. Effect of grapevine leaf extracts on germination and seedling growth in the weeds – *S. irio*, *S. nigrum* and *S. oleraceus*

The effects of bioassays of M and NM T and CS grapevine varieties on germination of the weeds - *S. irio*, *S. nigrum* and *S. oleraceus*, are presented in Fig. 1. The bioassays were carried out using aqueous extracts of cut leaves from WW plants grown under CETE conditions. This plant material was selected on the basis of the highest levels of total phenolics found in these leaves.

The results clearly show that more than 80% and 79% of *S. nigrum* and *S. oleraceus* control seeds germinated, while germination was much lower in *S. irio* seeds (37%) (Fig. 1). The response of the weed species to all grapevine leaf extract was significant irrespective of grapevine variety and mycorrhization. Seed germination was however highly inhibited by treatments with TNM extracts across the weed species. In *S. nigrum*, germination in seeds treated with TM extracts was significantly higher than the those of the control.

Regarding seedling growth, extracts from both CS and T leaves had a significant effect on root length of *S. irio* and *S. oleraceus* but not on *S. nigrum*. In the control treatment, was highest in *S. oleraceus* (2.8 cm) followed by *S. nigrum* (2.02 cm) and lowest in *S. irio* (1.62 cm) (Fig. 2). Root growth was more affected in *S. irio* across all treatments. Extracts from CSNM, CSM and TM totally inhibited shoot growth in *S. irio* with very reduced growth in seedlings treated with extracts from TNM (Fig. 3). In *S. nigrum*, no significant difference in root length was noted among the treatments with the four grapevine leaf extracts, however there was a significant difference between the control and the treatments with grapevine leaf extracts. Treatments with control recorded highest shoot length across the three weed species. Grapevine leaf extracts had a slighter effect on shoot growth compared to root growth across the three weed species studied.

The effects of the different grapevine leaf extracts on seedling vigour index in the three weeds studied are presented in Fig. 4. In the control treatments across the three weed species, similar seedling vigour index was observed in *S. oleraceus* and *S. nigrum*, 358 and 318 respectively but much lower in *S. irio* (51.8). As observed in the study, seedling vigour across the weed species was significantly influenced by treatment with different combinations of grapevine leaf extracts. Treatments with CSNM, CSM and TM extracts had a severe impact on seedling vigour of *S. irio*. Treatments with CSM extracts recorded the highest seedling vigour index in *S. nigrum* (147) followed by *S. oleraceus* (75). Although *S. nigrum* treated with TM extracts had a similar seedling vigour index (126) compared to that treated with CSM.

In a Table 5, a two-way analysis of variance (ANOVA) showed that the weed species, treatment with grapevine extracts and the interaction of the two factors significantly influenced germination percentage and root length. On the other hand, the effect of weed species and treatment with grapevine extracts significantly impacted shoot length and seedling vigour index.

Table 5

Main effects of weed species (W) and grapevine leaf extracts (E) on seed germination, root length and shoot length and seedling vigour index

	% Germination	Root length	Shoot length	Seedling vigour index
W	***	***	***	***
E	**	***	*	***
W × E	*	*	ns	ns

(*), (**), (***) indicates p values are significant at $p < 0.05$, 0.01 and 0.001 respectively; ns – not significant according to Duncan multiple range test.

3.4. Osmotic potentials of leaf extracts

The osmotic potentials (MPa) of leaf extracts determined at 25.2°C were – 0.19 for CS, independently of mycorrhizal colonization, -0.23 for TNM and – 0.34 for TM.

4. Discussion

The present study demonstrates that valorization of grapevine leaves revealed appreciable level of secondary metabolites in form of allelochemicals such as total phenolics and total flavonoids, existing as catechin, quercetin, gallic acid, hydroxycinnamic acid, caffeic acid, malvidin with a higher concentration under CETE (elevated temperature and CO₂) conditions. The study also revealed secondary metabolite accumulation in grapevine leaves is influenced by the water availability, mycorrhization and grapevine variety. The interaction of these factors has been shown to affect phenolic composition and antioxidant activity, both of which are crucial for moderating plant response to stress and achieving metabolic balance (Rani et al.,2016; Zhang et al. 2017). Environmental conditions can affect the allelopathic potential of plants (Talmot et al. 2024).

4.1. Influence of environmental factors on secondary metabolite accumulation

Air temperature, CO₂ concentration in the atmosphere and water availability played a critical role in determining the levels of secondary metabolites in grapevine leaves, but the effects were dependent on grapevine variety and mycorrhizal association. For example, CSM accumulated higher levels of total phenolics in leaves when grown under CETE conditions with optimal irrigation. Leaves of WW grapevine showed higher concentrations of total phenolics compared to D plants under both CATA and CETE conditions, especially in CS variety, regardless of mycorrhizal colonization. This is consistent with previous findings that mycorrhizal symbiosis can increase, decrease or not influence the phenolic accumulation in grapevine leaves depending on environmental conditions and clones within a given grapevine variety (Torres et al. 2015, 2018).

4.2. Correlation analysis of bioactive compounds

The correlation analysis showed the interrelationships among the classes of bioactive compounds under varying conditions. The strong positive correlations observed among total phenolics, flavonoids and flavan-3-ols suggests that they share common

biosynthetic pathways and regulatory mechanisms, while also contributing to the overall anti-oxidant capacity of the grapevine leaves (Singleton and Rossi, 2016). This also aligns with the study of Teixeira et al. (2013), who reported a biochemical link between total phenolic content and flavonoid accumulation, particularly under drought stress conditions. The positive correlation of hydroxycinnamic acids with flavan-3-ols and flavonols also supports their synergistic roles in plant defense against environmental stresses such as drought (Ju et al. 2023). The weak and negative correlation of anthocyanins with some other phenolics may suggest that its biosynthesis is influenced by environmental triggers such as temperature fluctuations and CO₂ rather than the phenolic biosynthetic pathway (Castellarin et al. 2007). Torres et al. (2015) found that air temperature, mycorrhizal colonization and their interaction can affect anthocyanin accumulation in Tempranillo grapevine leaves, but the effects were dependent on the Tempranillo clone.

4.3. Antioxidant activity and ROS free radical scavenging mechanism

In this study, the ability of the bioactive compounds to scavenge free radicals was evaluated using the DPPH assay. The high DPPH percentage due to the presence of the allelochemicals such as phenols and flavonoids contributes to scavenging reactive oxygen species (ROS), thus preventing oxidative damage (Rashidi et al. 2022). The antioxidant activity conferred by these allelochemical inhibits of molecular oxidation by preventing the initiation of oxidative chain reactions, thus facilitating the formation of stable, non-reactive radicals (Mittal et al. 2014). The interaction effects of grapevine variety, water availability and mycorrhization, as shown by three-way ANOVA further emphasize the complexity of the regulation of secondary metabolites in grapevine varieties. This aligns with earlier studies that have opined that plant-microbe interactions and water availability during growth collectively shape metabolic responses in grapevines (Shreiner et al. 2007; Cesco et al. 2010).

4.4. Allelopathic potential of grapevine leaf extracts

Plant aerial parts, especially leaves, have been recognized to be a high source of allelochemicals (Tanveer et al. 2010; Scavo et al. 2018; Đorđević et al. 2022a). The current study establishes the efficacy of bioassays of grapevine leaf waste as bioherbicides to control the three noxious weeds – *S. irio*, *S. nigrum*, and *S. oleraceus*. Growth of *S. irio* was largely inhibited by both T and CS in the bioassays. However, the percentage of germination of the control seeds that received water was also low (about 50%). This suggests that other factors besides the presence of allelochemicals in the grapevine extracts were involved in the inhibition of germination. Seeds of *S. irio*, as well as those of *S. nigrum* and *S. oleraceus*, are classified as orthodox (Seed Information Database), which means that their rate of loss of vigour and viability depends mainly on temperature and seed moisture content. The lower the moisture content and storage temperature, the longer the longevity (Corbineau, 2024). As the seeds for this study were provided by seed banks, it can be assumed that the storage conditions were adequate. However, as a member of the Brassicaceae family, *S. irio* seeds accumulate a relevant amount of oil, with an average oil content of 22.78% (Seed Information Databases). Orthodox oilseeds have a short longevity (Corbineau, 2024). In contrast to the low germination percentages achieved by the control seeds of *S. irio*, the germination percentages of seeds of *S. nigrum* and *S. oleraceus* that received water (control) reached almost 100%, demonstrating their high viability.

The application of leaf extracts had very different effects on germination depending on the weed, the grapevine variety and the mycorrhizal condition of the plants. The strongest inhibition was observed in *S. nigrum* treated with TNM leaf extracts and in *S. oleraceus* seeds treated with T (NM and M) grapevine leaf extracts. Leaf extracts from T (and especially from TM) showed the most negative osmotic potentials, which could theoretically reduce seed germination. However, according to Ali et al. (2020), the germination of *S. oleraceus* at day/night temperatures of 25/15°C and exposed to osmotic potentials of -0.38 MPa reached about 96%. The strongest osmotic potential in our study was that of the TM extract, which reached - 0.34 MPa and seeds were exposed to 25°C, minimising the negative effect of osmotic potential on seed germination in our study. Despite the apparent negative results of CS (NM and M) and TM extracts on inhibiting the germination of *S. nigrum*, the seedling vigour index showed that seedlings could not complete their development process correctly when they were irrigated with any grapevine extract. Similar results were obtained when the seedling vigour index of *S. oleraceus* was studied. When compared the growth of roots and shoots, roots appeared as a more sensitive organ to the application of grapevine leaf extracts, in agreement with findings of Wang et al. (2022). The presence of bioactive compounds emphasizes the allelopathic potential of both grapevine varieties, inhibiting seed germination and/or growth of the weeds under study by disrupting seed metabolism, water uptake, and root elongation due to alteration in auxin synthesis (Tawaha and Turk, 2003, Ullah et al. 2017). This highlights their potential in replacing synthetic herbicides in agricultural lands. Furthermore, flavonoids disrupt the structure and function of the mitochondrial, thus making stored materials unavailable for cell growth and plant development (Sharma et al., 2013). This is corroborated by the findings of Gulzar and Siddiqui (2017), who revealed that the aqueous extracts of the leaves of *Calotropis procera* significantly reduced germination percentage, radicle length, plumule length of *Brassica*

oleracea. Previous studies have suggested that allelochemicals such as phenolic compounds may alter gibberellic activity, which is well known for regulating amylase production during seed germination (Knox et al. 2014).

In allelopathy studies, several authors have linked the degree of inhibition of seed germination and seedling growth suppression with extract concentrations (Sarkar et al. 2012; Ayeni and Kayode, 2013; Dadhkar, 2013; Patanè et al. 2023). Extracts with higher concentrations exert stronger allelopathic effects, suppressing plant growth (Đorđević et al. 2022b) while lower concentrations tend to stimulate the plant's antioxidant defense system by enhancing enzymatic activity, maintaining redox balance and thus exhibiting lesser allelopathic effect (Talukder et al., 2020). This underscores the dose-dependent nature of the allelopathy mechanism in weed management. In the present study, only one concentration of grapevine leaf extract was tested: 2% (w:v). The promising data obtained in our study invite further research and testing of different doses of the extracts to establish the best dose-response effect on each weed. In addition, the application of aqueous extracts of grapevine leaves should also be evaluated on the crop species that usually share habitats with these weeds, to test the feasibility of applying these extracts in the field without damaging the crops (Đorđević et al. 2022a).

For all the weed species studied, inoculation of grapevine with AMF influenced the effects of leaf extracts on seed germination and seedling vigour, but the effect was in some cases enhancing and in others inhibiting. For example, mycorrhization counteracted the inhibitory effect of TNM extract on germination and seedling vigour of *S. nigrum*, but increased the reduction in seedling vigour of *S. oleraceus* watered with T extract. The levels of flavonoids, hydroxycinnamic acids and flavonols in TNM plants were slightly higher than in TM plants, but these differences do not explain the weed-dependent result. Furthermore, in other studies, mycorrhizal symbiosis induced the accumulation of secondary compounds in host plants, especially when the host plants were then subjected to abiotic stress (Baslam and Goicoechea, 2012). However, this effect was not as evident when plants were grown under elevated CO₂ (Baslam et al. 2012), as in the present study.

5. Conclusions and future perspectives

Pruning residues are rich in secondary metabolites with allelopathic activity against the weeds *S. oleraceus*, *S. nigrum* and *S. irio* when applied as aqueous extracts. This opens up the possibility of reusing these plant by-products as bioherbicides, thus revealing new perspectives for integrated vineyard management, which is not only focused on grape yield but also on the recycling of pruning residues. In addition, our results suggest that the accumulation of bioactive products in grapevine leaves may increase under future CO₂ and temperature conditions, thus enhancing the potential of pruning residues as allelopathic agents against weeds. In contrast, the severe and prolonged droughts predicted in the climate change scenarios may reduce the accumulation of secondary metabolites in the leaves of grapevines grown under elevated CO₂ and temperature conditions. The levels of allelopathic compounds also depend on the grape variety and the mycorrhizal conditions of the vines, making it difficult to obtain a homogeneous raw material for an efficient recycling process focused on the production of bioherbicides. The different responses observed between weed species when treated with aqueous extracts of grapevine leaves suggest that these extracts may provide selective control of specific weed species, which could be advantageous in integrated weed management systems. Experimental trials are needed to determine the best dose-response effect of vine leaf extracts on each weed and to test the effect of these extracts on crop germination and growth.

Declarations

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Figures

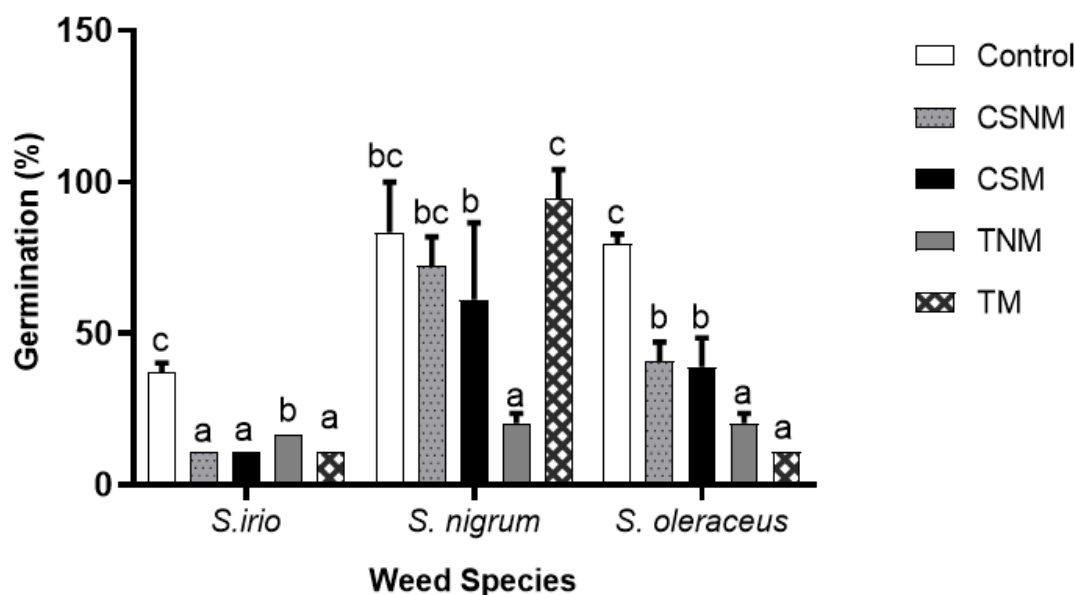


Figure 1

Germination percentage of weed species – *S. irio*, *S. nigrum* and *S. oleraceus* as a response to treatment with grapevine leaf extracts. Bars represent the means of 3 replicates $\pm$ SE. Bars topped by the different letters indicate significant difference between treatments at the 5% level using Duncan’s multiple-range test. CSM – Cabernet Sauvignon with Mycorrhiza, CSNM - Cabernet Sauvignon with No Mycorrhiza, TM – Tempranillo with Mycorrhiza, TM – Tempranillo with No Mycorrhiza.

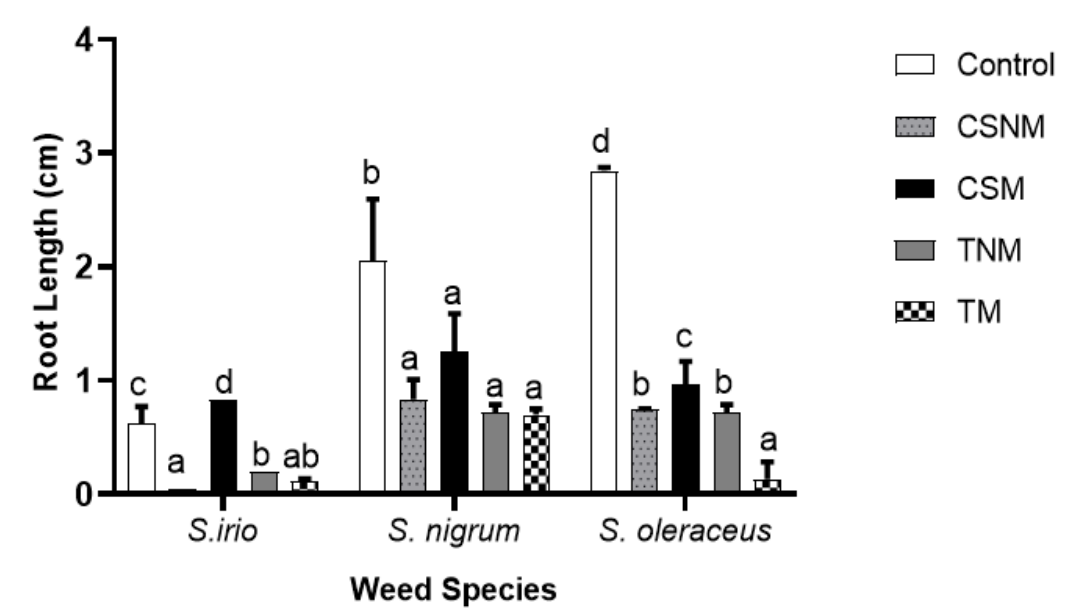


Figure 2

Root length of weed species – *S. irio*, *S. nigrum* and *S. oleraceus* as a response to treatment with grapevine leaf extracts. Bars represent the means of 3 replicates $\pm$ SE. Bars topped by the different letters indicate significant difference between treatments at the 5% level using Duncan’s multiple-range test. CSM – Cabernet Sauvignon with Mycorrhiza, CSNM - Cabernet Sauvignon with No Mycorrhiza, TM – Tempranillo with Mycorrhiza, TM – Tempranillo with No Mycorrhiza.

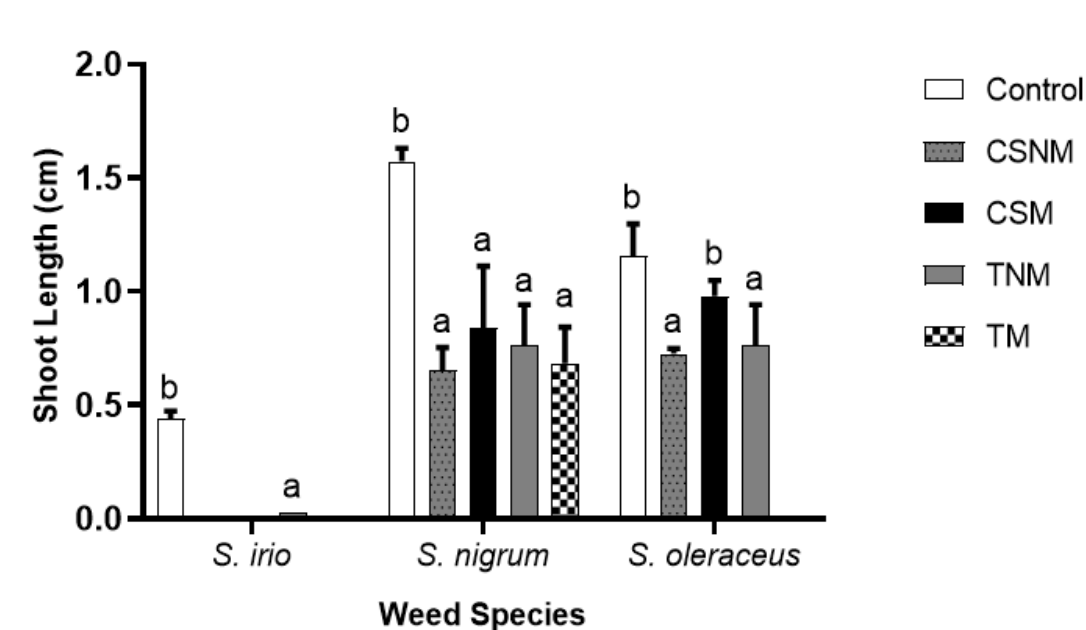


Figure 3

Shoot length of weed species – *S. irio*, *S. nigrum* and *S. oleraceus* as a response to treatment with grapevine leaf extracts. Bars represent the means of 3 replicates ± SE. Bars topped by the different letters indicate significant difference between treatments at the 5% level using Duncan’s multiple-range test. CSM – Cabernet Sauvignon with Mycorrhiza, CSNM - Cabernet Sauvignon with No Mycorrhiza, TM – Tempranillo with Mycorrhiza, TM – Tempranillo with No Mycorrhiza.

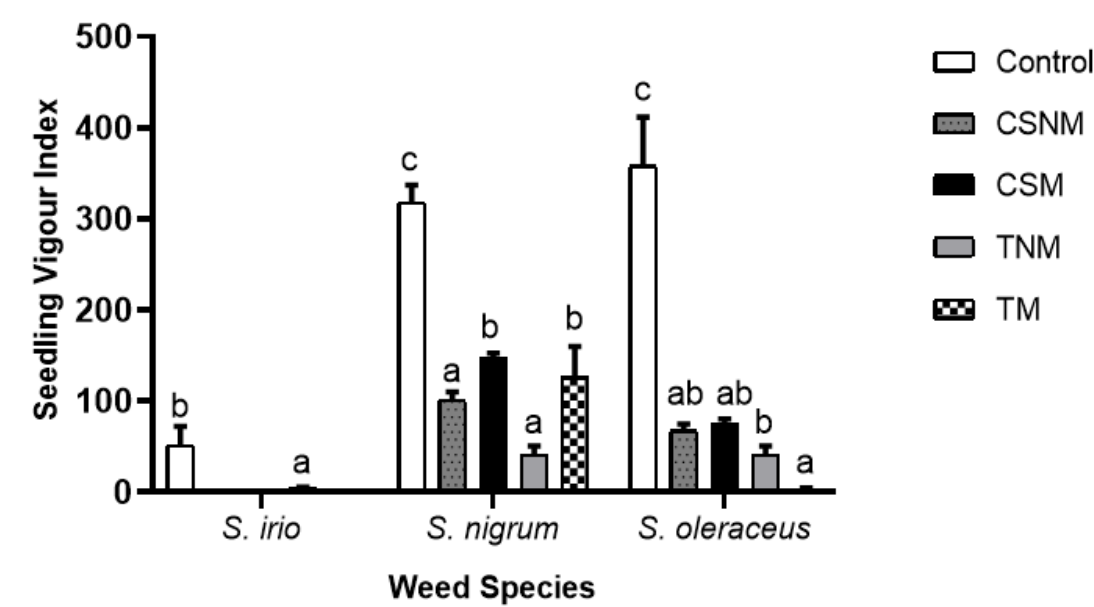


Figure 4

Seedling vigour index of weed species – *S. irio*, *S. nigrum* and *S. oleraceus* as a response to treatment with grapevine leaf extracts. Bars represent the means of 3 replicates ± SE. Bars topped by the different letters indicate significant difference between treatments at the 5% level using Duncan’s multiple-range test. CSM – Cabernet Sauvignon with Mycorrhiza, CSNM - Cabernet Sauvignon with No Mycorrhiza, TM – Tempranillo with Mycorrhiza, TM – Tempranillo with No Mycorrhiza.

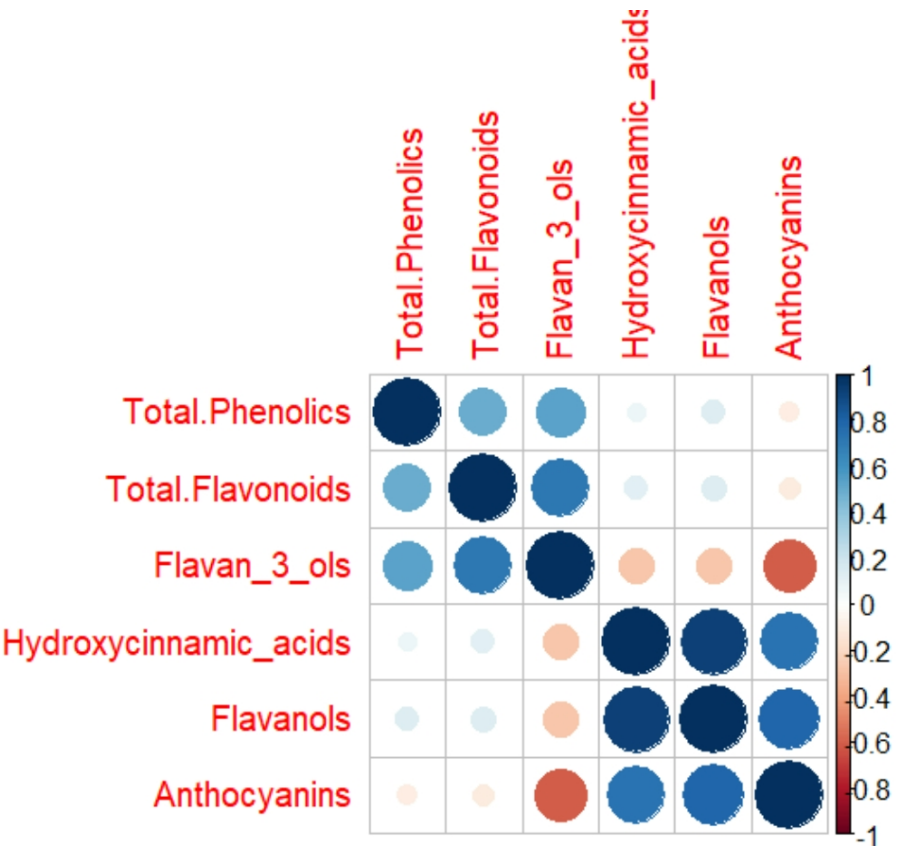


Figure 5

Correlation matrix showing interrelationships among bioactive compounds under CATA

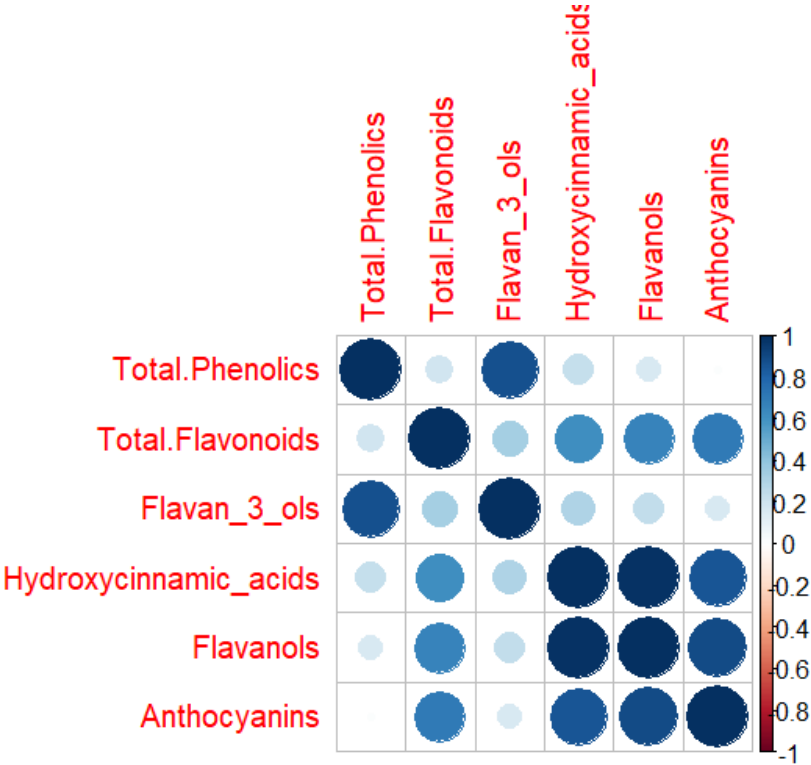


Figure 6

Correlation matrix showing interrelationships among bioactive compounds under CETE