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Use of *Conyza canadensis* L. Extracts as Biostimulant in *Cyclamen persicum* Mill.

Eunice R. Batista [1], Andre May [1], Sergio O. Procópio [1], Marcia R. Assalin [1], Helio D. Quevedo [2], Nicole Binhardi [3] and Sonia C. N. Queiroz [1,*]

[1] Embrapa Environment, C.P. 69, Jaguariúna 13918-110, SP, Brazil; eunice.reis@embrapa.br (E.R.B.); andre.may@embrapa.br (A.M.); sergio.procopio@embrapa.br (S.O.P.); marcia.assalin@embrapa.br (M.R.A.)
[2] Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba 13418-900, SP, Brazil; hd.qvdo@gmail.com
[3] Graduate Program in Agroecology and Rural Development, Federal University of São Carlos, Araras 13565-905, SP, Brazil; bnicole@estudante.ufscar.br
* Correspondence: sonia.queiroz@embrapa.br

Abstract: Cyclamen (*Cyclamen persicum* Mill.) is an ornamental plant that is highly susceptible to pathogens, requiring high amounts of phytosanitary products. Therefore, the development of more sustainable alternatives has been required. The present study aimed to analyze the effect of *C. canadensis* root extract (aqueous and with dichloromethane) applied via foliar or soil, in *C. persicum*, on gas exchange and the SPAD index and on the biomass of cyclamen. The aqueous extract treatment increased net CO_2 assimilation, the transpiration rates, and instantaneous carboxylation efficiency. The water use efficiency values were reduced in the treatments with both extracts. The greatest increases in the SPAD index were provided by the aqueous extract. The cyclamens that received the aqueous extract applied in soil or the dichloromethane extract applied in leaves showed an increase in total biomass and number of leaves. To identify the compounds present in the extracts, CG-MS and LC-MS/MS analyses were performed. The positive effects obtained indicated a high biostimulant effect of *C. Canadensis*. Thus, the root extracts of *C. Canadensis*, particularly the aqueous extracts, have the potential to be used to reduce the use of mineral fertilizers and pesticides, promoting agroecological practices and contributing to sustainable agriculture.

Keywords: plant extract; *C. canadensis*; *C. persicum*; gas exchanges; SPAD index; sustainable agriculture; allelopathy; biostimulant

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1. Introduction

The application of plant extracts, which have biostimulant properties, can significantly contribute to sustainable production, promoting greater resistance of cultivated plants to biotic and abiotic stresses, increasing productivity, and improving the quality of harvested products, as well as reducing the use of mineral fertilizers and pesticides [1–3].

The organic and inorganic compounds present in several plant species can influence the metabolism and physiological response of recipient plants, as they are able to stimulate the growth/development rate of these species under normal conditions or under stress conditions. These compounds act on the modulation of phytohormone, water, and soil nutrient absorption, in the control of enzymatic functions, in the photosynthetic rate, in gene expressions, in the defense system against biotic threats, and in the control of water relations [4–6].

The use of biostimulants derived from plants or microorganisms represents an ecological and efficient technology and can be used as an important tool integrated into sustainable agricultural practices [7–9]. An annual growth of 11.24% is estimated in the global biostimulants market [10].

Several plants, including their waste and by-products, can be used in the production of extracts with the aim of allowing the biostimulation of other plant species, and the part of the plant to be used in the production of extracts can also be segmented (leaves, flowers, fruits, roots, stems, and seeds), which can change the result of biostimulation [11]. Furthermore, various extraction methods can be used in the production of plant-derived biostimulants. Selection of the most appropriate method is of fundamental importance to ensure that the final extract has a high content of biologically active compounds. In this sense, simple and traditional methods, or even advanced extraction techniques, which include enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and ultrasound-assisted extraction (UAE) can be used [3]. Water is the most commonly used solvent in the extraction of active compounds from plant biomass, due to the fact that it is more environmentally friendly [12,13].

Plant extracts are normally applied in agriculture by foliar spraying; so, they normally need to go through a filtration process to avoid clogging the spray tips, with concentrations of up to 10% being the most used [3]. The effectiveness of plant extract applications depends on a series of factors, such as the target plant species (culture), cultivar, plant development stage at the time of application, plant extract concentration, number of applications, application method, and environmental conditions before, during, and after applications [14–16].

In addition to the response in the growth of recipient plants, the identification of the bioactive substance (s) present in the plant extract is of fundamental importance for understanding the mechanism (or mode) of action [17,18]. It is common for the same plant extract to contain more than one bioactive compound; these compounds can interact with each other, making it difficult to understand how they act on the receiving plant [19]. Favorable effects on the growth of recipient plants are often attributed to organic substances such as polyphenols, amino acids, phytohormones, vitamins, antioxidants, osmoprotectants, and micro- and macronutrients [3,11]. The function of these molecules is to provide better development and growth and, in turn, to be a defense mechanism; so, they can promote the development and maturation of other plants through biostimulation. [20].

Among the various mechanisms of action of plant biostimulants already studied, the improvement of the performance of the photosynthetic apparatus stands out as it ultimately results in higher growth rates and productivity. For example, the foliar application of *Moringa oleifera* extract in different crops promoted an increase in the synthesis of chlorophyll, carotenoids, and stomatal conductance, resulting in an increase in photosynthesis rates in arugula crops and also in beans grown under saline stress [9]. When applied to geranium (*Pelargonium graveolens*), moringa extract increased the content of chlorophyll and carotenoids, which function as antenna pigments in capturing light energy for the reaction centers of the photosystems, thus favoring the photochemical stage of photosynthesis [21].

Likewise, the foliar application of aqueous extract of garlic (*Allium sativum*) to eggplant (*Solanum melongena*) seedlings promoted an increase in the synthesis of photosynthetic pigments, increasing the rates of carbon assimilation, stomatal conductance, and transpiration [17]. Psyllium (*Plantago ovata*) leaf extract ensured the protection of the photosynthetic apparatus and stomata, reducing oxidative stress in corn seedlings subjected to hydric stress [22]. The treatment of pea (*Pisum sativum*) with *Lolium perennial* leaf extract, which is naturally rich in proline, reduced the effects of NaCl and $NiCl_2$ toxicity, increasing gas exchange rates, water use efficiency, and relative water content in the leaves, and was more effective than the application of exogenous proline [23]. The increase in the electrical conductivity of irrigation water negatively affected the growth rates and gas exchange of *Physalis peruviana*; however, the application of seaweed extract increased the photosynthetic capacity by 43.3% and reduced transpiration (26.5%), attenuating the effects of salinity on specific leaf area and stomatal conductance [24]. In another study with three cultivated species, tomato, melon, and cucumber, the application of seaweed extract (*Ecklonia maxima*) promoted an increase in photosynthetic rates by inducing greater production of photosyn-

thetic pigments, thus promoting better conversion of light energy into chemical energy for the assimilation of carbon and carbohydrate synthesis [25].

The relationship between biostimulants and the SPAD index in plants is a point of great relevance. In many cultivated vegetables, the SPAD index is widely used as a non-destructive estimate of chlorophyll contents and, generally, lower SPAD values are correlated with leaf senescence in response to severe abiotic stress, including salinity [26]. Studies carried out with zucchini (*Cucurbita pepo*) indicated that the application of seaweed extract to the leaves resulted in higher values of leaf area, carbon assimilation, and the SPAD index. The application of two different commercial biostimulants based on plant extracts, in combination or not with nitrogen, promoted leaf expansion and increased the SPAD index in arugula (*Eruca sativa*) [27].

The plant *Conyza canadensis* L. is a species considered invasive in agricultural areas; it is widespread worldwide and can cause significant impacts on biodiversity and the structure and functioning of many ecosystems [28–30]. This plant has a height ranging from 0.8 to 2.3 m and quickly forms high-density populations due to the high production of seeds per plant and the ease of seed dispersal by the wind [31–33]. At high densities, this species can cause productivity losses of up to 90%, with high adaptation in areas cultivated under the no-tillage system (without soil disturbance) [34]. Furthermore, species of the genus *Conyza* spp. release allelopathic compounds into the environment to inhibit the growth of other spontaneous plant species, thus promoting the rapid occupation of agricultural spaces [35–37].

Due to their allelopathic effects, extracts from *C. canadensis* plants have been investigated in several research centers, mainly for the use as pesticides, such as possible herbicidal activity. It was found that aqueous extracts of *C. canadensis* impaired the germination and initial growth of the species *Plantago asiatica*, *Digitaria sanguinalis* and *Youngia japonica* [32]. Methyl 4-hydroxybenzoate and hispidulin, isolated from *Conyza bonariensis* plants, were shown to be active in inducing the germination of *Phelipanche ramosa* and *Orobanche cumana* seeds. On the other hand, the authors also isolated two allelochemicals from *C. bonariensis*, (4Z)-lachnophyllum lactone e (4Z,8Z)-matricaria lactone, which inhibited the root growth of *Orobanche crenata*, *Orobanche cumana*, and *Orobanche minor* e *Phelipanche ramosa*. Other authors observed that aqueous extracts of *C. canadensis* (whole plant), in different concentrations, weakened the root activity of Arabidopsis thaliana and caused the accumulation of reactive oxygen species [38]. The lactones, (4Z,8Z)-matricaria lactone and (4Z)-lachnophyllum lactone, found in the aerial part of *C. canadensis*, inhibited the growth of the monocot *Agrostis stolonifera* and the dicot *Lactuca sativa* [39].

Cyclamen (*Cyclamen persicum* Mill.) is an ornamental plant grown in pots that has flowers of the most diverse shades, including white, pink, and red. It is a plant with a relatively long cycle (up to 7 months), compared to other ornamental plants, and is highly susceptible to pathogens, requiring high investments in phytosanitary treatments [40]. The national production of species with flowers and potted plants represents 39% of the revenue of the flower and ornamental plant chains in Brazil [41]. The municipality of Holambra/SP is a major producer of this species in Brazil, representing 0.8% of all sales movement in the largest wholesale market located in the region (Veiling, Holambra/SP).

It is known that allelochemical compounds have an influence on photosynthetic activity, but to date, no studies have been reported on the effects of *C. canadensis* extracts on gas exchange in plants. The main aim of the present study is to analyze the effect of *C. canadensis* root extract (aqueous and with dichloromethane) on gas exchange and the SPAD index of *C. persicum* grown in a greenhouse.

2. Materials and Methods

2.1. Study Location

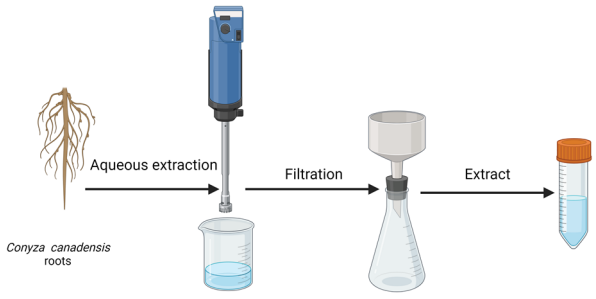
The present study was carried out from August to December 2023, at the experimental facilities of Embrapa Meio Ambiente located in the municipality of Jaguariúna, SP (22°43′ S, 47°01′ W, 570 m altitude).

2.2. Obtaining and Growing Cyclamen

Healthy seedlings of var. Halios® Red Rebelle® cyclamen with approximately 60 days of development, obtained from the BALL seedling production company, based in the city of Holambra (SP), were transplanted into plastic pots 10 cm high by 12 cm in diameter, containing commercial Sphagnum peat-based substrate fertilized with 10 g of NPK 10:10:10. The pots, containing two plants each, were kept in a greenhouse with daily drip irrigation, average relative humidity of 65%, average minimum temperatures of 23.5 °C, and average maximum temperatures of 30.5 °C. The experimental design in completely randomized blocks contained 5 replications per treatment, with each pot considered as 1 replication. The treatments consisted of leaves (foliar) or a soil application of an aqueous extract and a dichloromethane extract obtained from *C. canadensis*. For comparison purposes, a control treatment, without the application of extracts, was also carried out.

2.3. Cultivation and Obtaining C. canadensis Extract

C. canadensis seeds, obtained from a supplier of agricultural products in the city of Engenheiro Coelho (SP), AgroCosmos, were germinated in the greenhouse located at Embrapa Meio Ambiente, in Jaguariúna (SP). With the resulting plants, developed over a period of 110 days, their roots were harvested, aiming to produce the studied extracts. The plants were harvested at the phenological stage of white bell inflorescence, washed in distilled water, and dried on absorbent paper, and the roots were separated to prepare the extracts. The aqueous extract of the roots was prepared from 307.7 g of fresh roots and 1 liter of water, crushed in a high-speed industrial blender for 10 min. The mixture was then filtered through clean, fine-mesh fabric, consisting of viscose and polyester fibers, resin, and antibacterial agents; 750 mL of aqueous extract was recovered at the end of filtration. Figure 1 shows this process. Immediately after preparation, the extracts were applied to cyclamen seedlings. Each of the 5 pots received 5 mL of extract applied to the soil (treatment with aqueous extract applied to the soil, AES), and the other 5 pots received 5 mL of extract sprayed on the leaves (treatment with aqueous extract sprayed on leaves, AEL). The extract from the roots of *C. canadensis*, dried and ground, was prepared using the organic solvent dichloromethane. A total amount of 293 g was extracted with 720 mL of dichloromethane for 3 days at 25 °C and stirring at 150 rpm. The extract was filtered and partially evaporated in a rotary evaporator. The extract obtained was then dried under nitrogen flow, resulting in a final mass of 2.4523 g. To prepare the solution, 1303.3 mg of the extract was dissolved in 254.8 mL of deionized water with the addition of 5.2 mL of Tween-80® surfactant, resulting in a final concentration of 5 mg/mL of extract. The solution was homogenized using an ultrasound bath and magnetic stirrer to ensure complete dissolution. Figure 1 illustrates this procedure. Immediately after preparation, the extracts were applied to the plants, with 5 pots receiving 5 mL of extract in the soil (treatment with organic extract to the soil, OES) and 5 pots receiving 5 mL of extract sprayed on the leaves (treatment with organic extract sprayed on leaves, OEL). The first application of the both extracts was performed at 6 days after transplantation (DAT) and the second at 60 DAT, and both extracts were prepared again for the second application, immediately before the referenced moment.



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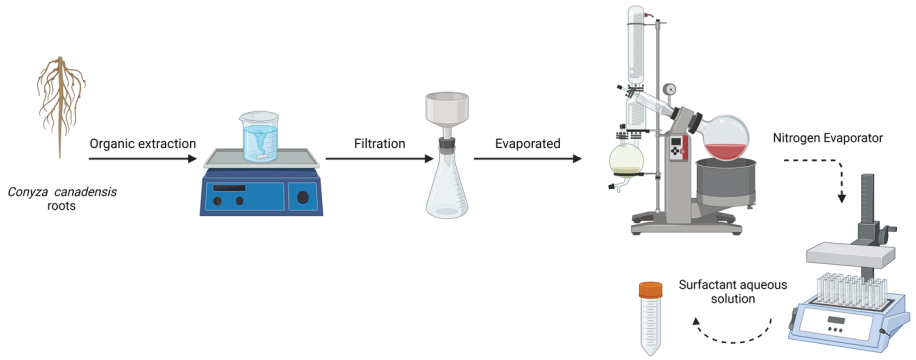

Figure 1. Process of obtaining the studied extracts.

2.4. SPAD Index Measures

The SPAD index readings were taken in the central region of the leaf blade of the 1st leaf completely expanded from the apex, using the SPAD-502 portable chlorophyll meter (Konica Minolta, INC., Japan). All five pots were evaluated and the two plants in each pot were examined, thus obtaining 10 SPAD values per treatment. The SPAD index readings were taken at 30, 50, and 70 days after the first application of the extracts to the plants (carried out in 2 doses, at 6 and 60 days after transplanting the seedlings); the application was carried out in the morning, between 9 am and 11 am.

2.5. Biomass Measurements and Number of Leaves

The same plants evaluated by the SPAD index were also measured, on the same dates, for the total number of leaves. After the last leaf count, at the end of the experiment, three plants were selected and separated into roots and aerial parts (bulbs, branches, leaves, and flowers). The plant organs were dehydrated in an oven with constant circulation of hot air ($\pm$60°C) for 72 h. After cooling to room temperature, the plant tissues were weighed and the results expressed in grams of dry mass (biomass).

2.6. Determination of Gas Exchange Rates

The gas exchange rates were determined between 9 am and 11 am in the fully expanded leaves of three plants, from different pots, per treatment. The following parameters were quantified: (a) net CO_2 assimilation rates (A, µmol CO_2 m^{-2} s^{-1}); (b) stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}); (c) transpiration rates (E, mmol H_2O m^{-2} s^{-1}); (d) intracellular CO_2 concentration (Ci, µmol CO_2 mol^{-1}); water use efficiency (WUE, µmol CO_2/mmol H_2O), calculated as the A/E ratio; and instantaneous carboxylation efficiency (ICE, µmol CO_2 mol air^{-1}), calculated as the A/Ci ratio. All the measurements were carried out using an infrared gas analyzer (IRGA, mod. Li-Cor® 6400 XT). Inside the leaf chamber, the following conditions were maintained: (PAR) of 1000 µmol de fótons m^{-2} s^{-1}, previously determined after light response curve analysis; relative humidity of 60%; internal CO_2 concentration of 420 ppm; and air flow of 400 mL s^{-1}.

2.7. LC-MS/MS and GC-MS Analysis

The organic and aqueous extracts of *C.canadensis* roots were injected into a liquid chromatography system (Waters Acquity Ultra Performance LC; Milford, MA, USA) connected to a Synapt Quadrupole Time-of-Flight mass detector (Waters Synapt HDMS; Milford, MA, USA) equipped with an electrospray ion source. Separation of compounds was performed using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm; 1.7 µm, waters). The mobile phase was composed of solvent A: 0.1% formic acid aqueous solution and solvent B: methanol.

The following gradient program was used: 0––2 min 5% B, 2.1––6 min 5––40% B, 6.1–10 min 40–45% B, 10–11 min 45% B, 11.1–18 min 45––75% B, 18.1–20 min 75% B, 20.1–21 min 75–98% B, 21.1–22 min 98% B, 22.1 min 5% B, min 5% B. The mobile phase

flow rate was 0.3 mL min^{-1}, the column temperature was 30 °C, and the injection volume of the samples and blanks was 10 µL. Positive and negative ion modes were recorded (separately), and the instrument was operated in both MS and MS/MS (low energy: 10 V, high energy: 35 V) modes in the m/z range of 50–1000, under the following conditions: capillary voltage, 3.0 kV (positive mode of ionization) or 2.0 kV (negative ionization mode); cone voltage, 30 V; source temperature, 110 °C; desolvation gas temperature (nitrogen), 400 °C; desolvation gas flow (nitrogen), 500 L/h.

A sodium formate solution (10% formic acid/0.1% sodium hydroxide/ACN, 1:1:8, $v/v/v$) was used for calibration, and sodium formate ions m/z 566.8891 and 588.8970 in positive and negative ion modes, respectively, were used as a lock mass for accurate mass measurements. MassLynx V4.1 software (Waters, Milford, MA, USA, 2005) was used for data acquisition and processing.

The organic extract was also analyzed using gas chromatography (Agilent 7890A) coupled with a mass spectrometry system (triple quadrupole-Quattro Micro, Waters) (GC-MS) equipped with an automatic injector (CombPal). The software for system control and acquisition was MasslynxV4.1. The DB-5MS capillary column (length: 30 m × 0.250 mm × 0.25 µm, J&W Scientific, Folson, CA, USA) was used to separate the compounds. The oven temperature started at 50 °C (kept for 2 min) and was increased at a rate of 8 °C/min to 250 °C (kept for 5 min); then, it was increased at a rate of 30 °C/min to 300 °C (kept for 2 min). The source used was electron ionization (EI) and electron energy of 70 eV, with a temperature source of 200 °C and a transfer line of 280 °C. Helium was used as a carrier gas at 1 mL/min, and argon was used as a collision gas (2.5×10^{-3} mBar). The injection volume was 1 µL, and the injector temperature was 220 °C.

2.8. Statistical Analysis

Analysis of variance was applied to all the data using R software version 4.2.3 [42]. The normality of the distribution of residuals was checked with the Shapiro–Wilk test, while the homogeneity of variance was checked with the Levene test; means were compared using the Tukey test, and differences were considered significant at 5% probability.

3. Results and Discussion

3.1. Gas Exchange Parameters

The treatment of cyclamen seedlings with extracts from *C. canadensis* roots resulted in specific changes in gas exchange parameters. The treatment with aqueous extract applied to the leaves (AEL) showed a 27% increase in CO_2 assimilation rates (A), whose average values were 18.4 µmol CO_2 m^{-2} s^{-1}, while in the untreated plants the average values were 14.5 µmol CO_2 m^{-2} s^{-1} (Figure 2A). The same treatment (AEL) increased the transpiration rates (E) by 81%, and the average values were 2.5 mmol H_2O m^{-2} s^{-1}, whereas in the untreated plants the averages were 1.4 mmol H_2O m^{-2} s^{-1} (Figure 2C). The average water use efficiency values (*WUE*) in the AEL treatment (7.2 µmol CO_2/mmol H_2O) were reduced by 23% since this variable is the result of the A/E ratio (Figure 2E). As a result of the increase in A, the instantaneous carboxylation efficiency (*ICE*) values increased by 142% in the AEL treatment (Figure 2F). The treatment with organic extract applied to the soil (OES) reduced the *WUE* values by 22% without significant changes in the A and E values (Figure 2E). No significant changes were observed in the stomatal conductance (g_s) (Figure 2B) and internal net CO_2 concentration (Ci) (Figure 2D).

Among the different ways of calculating water use efficiency (WUE), in this study this variable was measured at the leaf scale, considering the ratio between net CO_2 assimilation and transpiration [43]. Although the WUE values were negative in the AEL and OES treatments, there was no negative effect on the biomass gain of the cyclamen; conversely, there was a positive effect of the AES and OEL treatments on the number of leaves and total biomass. Despite the occurrence of negative WUE values, there was no negative effect on the biomass gain variables in the cyclamen grown under controlled conditions with unrestricted water availability.

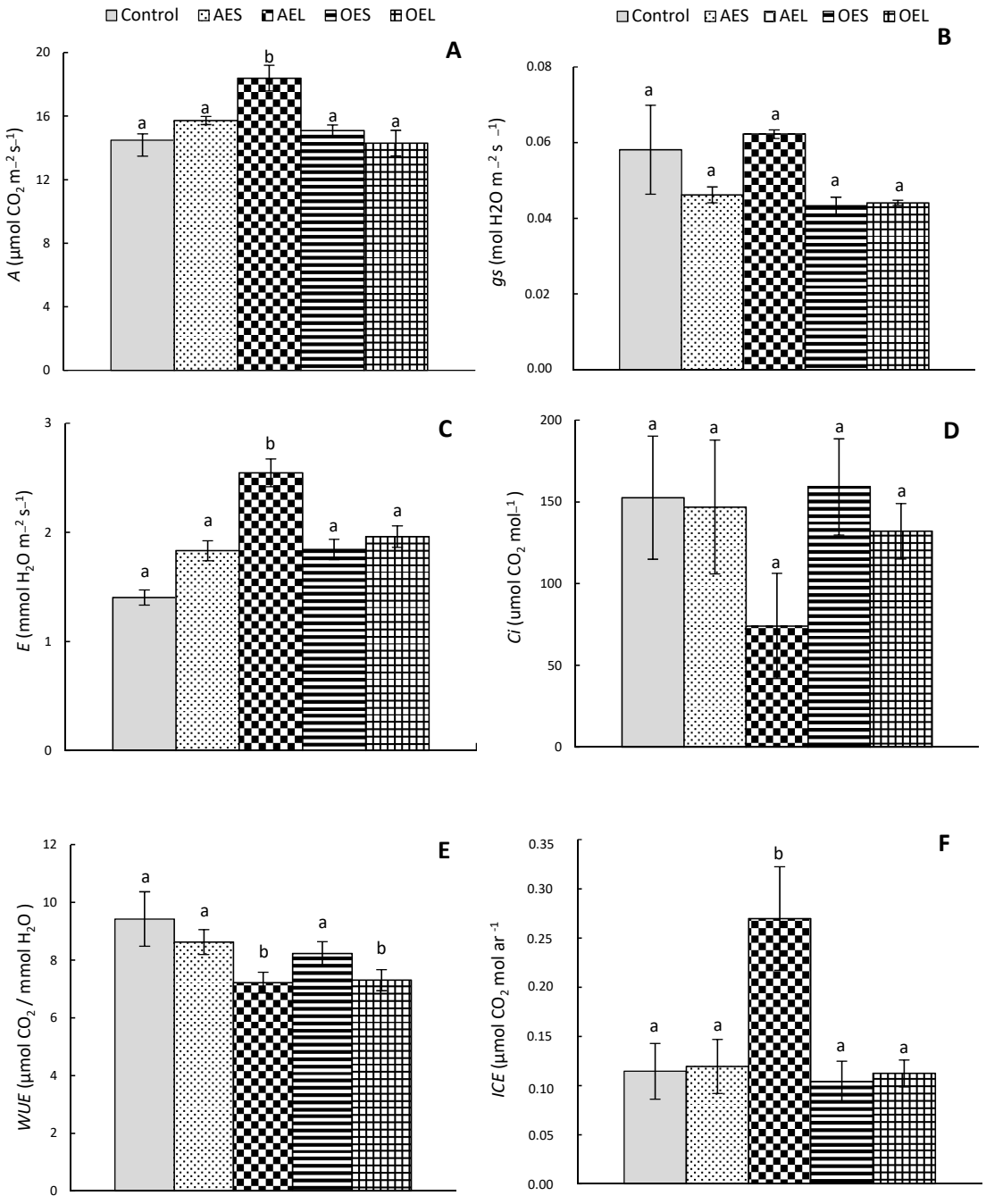

Figure 2. Effect of *C. canadensis* root extract application on net CO_2 assimilation (**A**), stomatal conductance (**B**), transpiration (**C**), intercellular CO_2 concentration (**D**), water use efficiency (**E**), and instantaneous carboxylation efficiency (**F**) of *C. persicum* at 30 days after the second application of aqueous extract in the soil (AES), aqueous extract on the leaves (AEL), organic extract in the soil (OES), or organic extract on the leaves (OEL). The values are the mean of (n = 3) ± SD. Different lowercase letters above the error bars indicate significant differences between treatments ($p < 0.05$).

Several studies indicate that the application of biostimulants formulated from plant extracts can promote physiological processes, including water absorption, photosynthesis, nutrient assimilation, hormone synthesis, water use efficiency, germination, and delay of senescence [44]. As observed in our study, alterations in the *A* and *WUE* values were related to the application of *Lolium perenne* foliar extract to pea leaves, which reversed the negative effects from water and chemical stress [23]. In spinach leaves treated with biostimulants, increases in *A* and *E* were reported, without changes in *gs* [45]. The high *E* values observed

in cyclamen treated with AEL may have reflected the cultivation conditions in a greenhouse, since, in situations of optimal water availability, plants generally have high transpiration rates [46]. The increase in *ICE*, also observed in the AEL treatment, indicated higher rates of carbon fixation by the Rubisco enzyme, which was reflected in higher *A* values, an effect that was also observed in physalis (*Physalis peruviana* L.) treated with biostimulant by foliar application [24]. The positive effects on several corn gas exchange parameters were amplified when the application of biostimulants to the grains, before planting, was complemented with foliar application at a certain vegetative stage, resulting in greater efficiency in water absorption and nutrient assimilation by the plants [46]. As observed in other studies, the application of biostimulants via soil is subject to interference from soil composition and texture, which may interfere with the community of microorganisms and root–soil interaction [45]. Thus, the insignificant effect of the other treatments, on some gas exchange parameters, may be related to the type of application, foliar or soil, which influences the mode of action of the plant extract, or even to the type of solvent used in the treatment extract preparation (aqueous or organic) [45,47]. Furthermore, the effect of plant extracts can be species-specific, and it also depends on the age of the treated plant [47]. This study showed that foliar application, unlike soil application, had positive effects on gas exchange, corroborating studies carried out with extracts from other plant species applied to corn [45], stevia [48], eggplant [17], squash [49], arugula [50], and countless other grown vegetables [11]. Some studies indicate that allelochemical compounds, as well as environmental factors, are also capable of significantly influencing photosynthesis [17]. It is known that *C. canadensis*, as well as other species of the genus *Conyza*, contains several allelochemical compounds that contribute to the phytotoxicity of its exudates and extracts, inhibiting the germination of plants around it [28,32,36,37,51] and promoting resistance to both herbicides [51–53] and pathogenic microorganisms [39,54,55]. Furthermore, it has been demonstrated that *C. canadensis* root exudates are capable of modifying nutrient cycling, enzymatic activity, and soil microbiota under different occupancy densities [28]. Therefore, the results of this study indicate that components of *C. canadensis* root extracts applied to the soil may have affected the cyclamen roots by interfering with gas exchange rates.

3.2. SPAD Index Assessment

The first evaluation, carried out at 30 days after transplanting (DAT) of the seedlings, indicated that cyclamens treated with extracts of *C. canadensis*, aqueous or organic, via foliar or soil application, increased the average value of the SPAD index when compared to the cyclamens that did not receive *C. canadensis* extracts (Figure 3). The SPAD values increased by 17.5%, 13.8%, 12.5%, and 11.6% in the AEL, AES, OEL, and OES treatments, respectively, compared to the control. In the second evaluation, at 50 DAT, the SPAD values of the treatments were also higher than those of the control. The increases were 28.8%, 13.6%, 9.9%, and 7.3% in the AES, OEL, AEL, and OES treatments, respectively. In the third and final evaluation, at 70 DAT, all the treatments with *C. canadensis* extract again resulted in increases in SPAD values in relation to the control. The values were 28.8%, 15.3%, 9.8%, and 5.8% higher in the AES, AEL, OES, and OEL treatments, respectively, in relation to the average value observed in cyclamens that did not receive *C. canadensis* extracts. Greater increases in the SPAD index were provided by the aqueous extracts, via foliar or soil application, to the detriment of organic extracts, which also increased the index, though in smaller proportions.

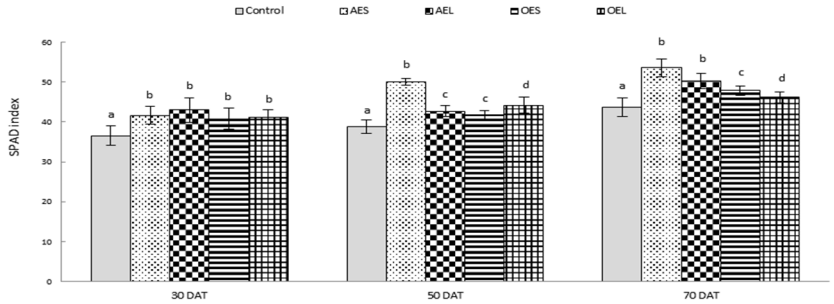

Figure 3. Effect of *Conyza canadensis* root extract application on the SPAD index of *Cyclamen persicum* subject to aqueous extract in the soil (AES), aqueous extract on the leaves (AEL), organic extract in the soil (OES), or organic extract on the leaves (OEL). Seedlings received extracts at 6 and 60 days after transplanting (DAT). The SPAD index was determined at 30, 50, and 70 DAT. The values are the mean of (n = 10) ± SD. Different lowercase letters above the error bars indicate significant differences between treatments ($p < 0.05$).

3.3. Biomass and Leaf Quantity Measurements

The treatments with *C. canadensis* root extract showed different effects on cyclamen growth variables. According to the data, the seedlings that received aqueous extract via soil (AES) and organic extract via foliar (OEL) had 49% and 29% increases, respectively, in the number of leaves, compared to the control (15 leaves): 22 leaves in the AES treatment and 19 leaves in the OEL treatment (Figure 4A). Additionally, in the same treatments, AES and OEL, increases of 30% and 26%, respectively, in the amount of total biomass were observed, compared to the cyclamens that did not receive *C. canadensis* extracts (Figure 4B).

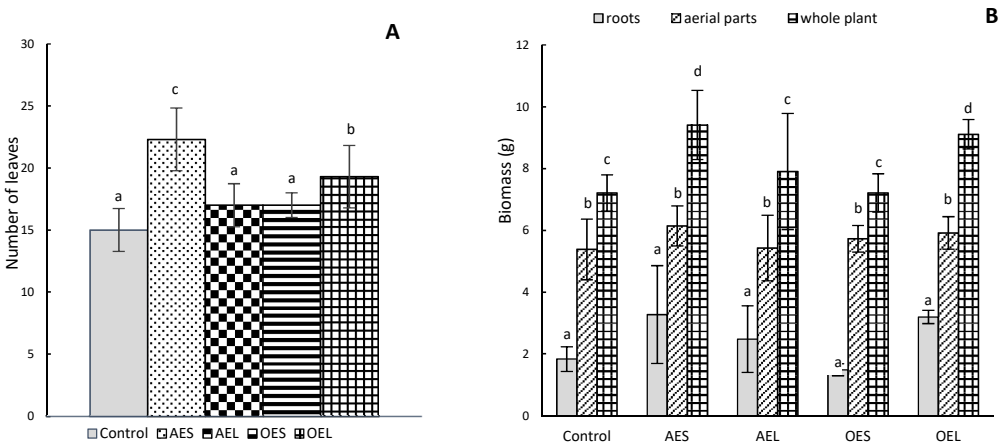

Figure 4. Effect of *C. canadensis* root extract application on the number of leaves (**A**) and the biomass (**B**) of the root, aerial part, and total of *C. persicum*. The treatments were aqueous extract in the soil (AES), aqueous extract on the leaves (AEL), organic extract in the soil (OES), or organic extract on the leaves (OEL). Seedlings received extracts at 6 and 60 days after transplanting. The values are the mean of (n = 3) ± SD. Different lower case letters above the error bars indicate significant differences between treatments ($p < 0.05$).

Such results are in line with several studies in which the application of plant extracts and other plant-based biostimulants promoted an increase in several growth and production parameters in different cultivated species [2,48,50,56]. As verified in our study, increases in chlorophyll content and gas exchange rates were reported, resulting in greater growth and production of arugula *(Eruca sativa*) treated with *Moringa oleifera* leaf extract [50]. Similarly, photosynthetic activity, leaf chlorophyll levels, and number of leaves per plant were increased after application of moringa leaf extract to *Freesia x hybrida* leaves; in this case, the authors attributed the effects to the increase in growth-promoting substances and minerals, which were naturally contained in the extract [56]. Finally, the application of

moringa leaf extract increased the growth, photosynthetic pigment content, and SPAD index of *Stevia rebaudianna* [48].

3.4. Identification of Bioactive Compounds

Biostimulants based on plant extracts may contain various bioactive compounds such as phenolics, organic acids, phytohormones, minerals, photosynthetic pigments, amino acids, nucleotides/nucleosides, and lipids, which can act as signaling molecules and regulators of metabolic processes, activating gene transcription and promoting the growth, development, yield, and resistance to water and biotic stress of cultivated plants, making it difficult to identify the exact mechanism of action [57]. The literature reports numerous studies on plant extracts used in various crops; however, most of the time, the extracts are produced from leaves or aerial parts of plants. Few studies have been reported on extracts produced from roots, especially *C. canadensis*, as reported in this study. More comprehensive chemical analyses of the components of the aqueous/organic root extracts of *C. canadensis* would allow a more in-depth analysis of the results obtained in this study. Therefore, analyses of the extracts were carried out using GC-MS and LC-MS/MS chromatographic techniques.

Important factors that influence the phytochemical compounds and bioactivity of plant extracts include the different parts of the plant and the type and experimental conditions of the extraction methods, including the polarities of the solvent. In this work, the biostimulant effect of extracts from the roots of *C. canadensis* obtained using different types of extraction solvents (organic and aqueous) was investigated. Between the comparative profiles obtained for the two extractions, significant differences in the chemical composition could be observed. Table 1 shows the LC-MS data for compounds detected in aqueous and organic extracts of *C. canadensis* L. roots. The m/z 193.0501 was found in both extracts, and it was attributed to ferulic acid ($C_{10}H_{10}O_4$). It is a phenolic compound, widely found in species of the genus *Conyza* spp., also known as horseweed [58]. This compound is related to the protection of plants against pathogens, UV radiation, and environmental stress [58–60]. Other phenolic compounds were found in the aqueous extract (Table 1). Phenolic compounds are organic compounds that are usually soluble in water. They include a wide range of plant substances and are considered to be one of the more abundant secondary metabolites synthesized in plants during normal development and in response to stress conditions. Resveratrol ($C_{14}H_{12}O_3$), like other compounds identified in *Conyza* spp. extracts, is strongly related to the antioxidant bioactivity that protects the plant against infections and oxidative stress [59]. Other classes of compounds, such as carboxylic acids and disaccharides, were also detected and may contribute to the biostimulating effect of the extracts.

The major volatile compounds identified in the organic extract by GC-MS were the well-known (2Z,8Z)-matricaria acid methyl ester ($C_{11}H_{10}O_2$ (M^+, m/z 174)); (4Z,8Z)-matricaria lactone ($C_{10}H_8O_2$ (M^+, m/z 160)); (4Z)-lachnophyllum lactone ($C_{10}H_{10}O_2$ (M^+, m/z 162.)); and *cis*-lachnophyllum ester ($C_{11}H_{12}O_2$; (M^+, m/z 176)). The mass spectra are in accordance with those previously described by [39,61]. These compounds have antifungal and herbicidal activities.

Table 1. The identified compounds present in the aqueous and organic extracts by LC-MS/MS.

Entry	*m/z* Experimental	*m/z* Calculated	Error (ppm)	iFit	Retention Time	Proposed Formula	Compound Class	Compound
1	193.0494	193.0501	−3.6	0	6.26	$C_{10}H_{10}O_4$	Phenolic	Ferulic acid [a,b]
2	199.0613	199.0606	3.5	0	1.14	$C_9H_{10}O_5$	Phenolic	Syringic acid [a]
3	209.0789	209.0814	−12	0	9.00	$C_{11}H_{14}O_4$	Phenolic	Ethyl 2,4-dihydroxy-5,6-dimethylbenzoate [a]
4	215.1287	215.1283	1.9	0	14.46	$C_{11}H_{20}O_4$	Carboxilic acid	Undecanedioic acid [a]
5	229.0861	229.0865	−1.7	0	9.04	$C_{14}H_{12}O_3$	Phenolic	Resveratrol [a]
6	229.1436	229.1440	−1.7	0.2	16.10	$C_{12}H_{22}O_4$	Carboxilic acid	Dodecanedioic acid [a]
7	245.0798	245.0814	−6.5	0.9	14.10	$C_{14}H_{12}O_4$	Phenolic	trans-piacetannol [a]
8	291.0864	291.0869	−1.7	0.6	6.20	$C_{15}H_{14}O_6$	Phenolic (flavonoid)	Epicathechin [a]
9	341.1108	341.1084	7	0	13.99	$C_{12}H_{22}O_{11}$	Disaccharide	Sucrose [a]
10	577.1339	577.1346	−1.2	0.7	10.20	$C_{30}H_{24}O_{12}$	Phenolic (flavonoid)	Procianidin [a]
11	607.1677	607.1663	2.3	0.1	6.30	$C_{28}H_{32}O_{15}$	Phenolic (glycoside flavonoid)	Sorbifolin 6-O-α-rhamnopyranosyl(1‴→6″)-β-glucopyranoside [a]

[a] = Aqueous extract; [b] = Organic extract.

Figure 5 shows the main compounds identified in the aqueous extract. To confirm the biological activity, as well as the mode of action of each compound, further investigation would be necessary. However, compounds that exhibit structural similarities tend to also share similar physicochemical attributes and biological functionalities [62]. Thus, the possible modes of action of the classes of molecules detected in the extracts may have the following biostimulant functions, among others: (i) Phenolic acids serve as structural units in lignin biosynthesis and cell wall formation [63], as a growth regulator involved in root development, plant growth, and crop yield [64,65], and as a plant growth-promoting natural compound that stimulates cell division and expansion [66]; (ii) Flavonoids function as indirect growth regulators through interaction with phytohormone signaling [67]; they regulate nodulation and symbiotic nitrogen fixation [68] and stimulate fungal growth in arbuscular mycorrhizal symbiosis and phosphorus acquisition [69]; (iii) Soluble sugars eliminate reactive oxygen species and are osmoprotectors that stabilize membranes [70].

Although *C. canadensis* L. has previously been evaluated due to its allelopathic effects, using more sensitive indicator plants, such as lettuce, and applied to more delicate structures, such as seeds [39], this research describes for the first time studies involving the application of root extracts of *C. canadensis* L. to cyclamen plants, with a significant positive effect on their growth and development. Thus, it was possible to demonstrate that there may be a differentiated positive response in other plants when using different parts of the plant, such as the root, different solvents for extraction, application at different stages of plant development, and different methods of application to the plant, such as foliar or soil. All these issues highlight the importance of new research to generate extracts with beneficial compounds for economically important cultivated plants.

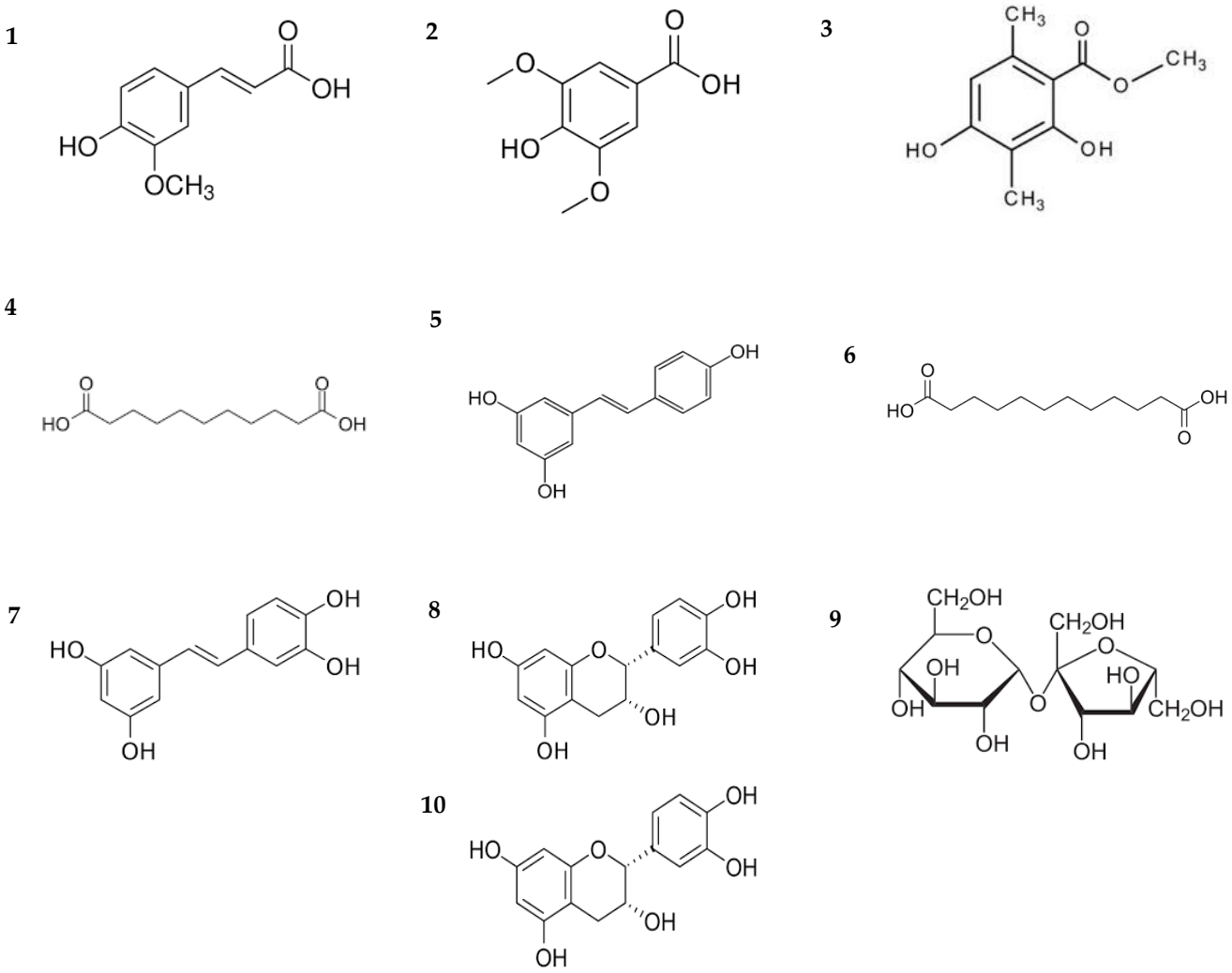

Figure 5. Structural formulas of the compounds found in the aqueous extract ((**1**) Ferulic acid; (**2**) Syringic acid; (**3**) Ethyl 2,4-dihydroxy-5,6-dimethylbenzoate; (**4**) Undecanedioic acid; (**5**) Resveratrol; (**6**) Dodecanedioic acid; (**7**) Trans-piacetannol; (**8**) Epicathechin; (**9**) Sucrose; (**10**) Procianidin).

4. Conclusions

Both extracts from the roots of *C. canadensis* (aqueous and dichloromethane) showed biostimulant effects when applied to *C. persicum*; however, the effects were dependent on the composition and application method of the extracts. The aqueous extract showed a greater positive effect when compared to dichloromethane. Furthermore, it has the advantage of using water as the extraction solvent, which can be considered more environmentally friendly and less expensive. Therefore, the natural compounds extracted from the roots of *C. canadensis* have biostimulant properties and have the potential to be explored in agriculture to reduce the application of agrochemicals, promoting agroecological practices, towards sustainable agriculture.

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References

1. Cozzolino, E.; Giordano, M.; Fiorentino, N.; El-Nakhel, C.; Pannico, A.; Di Mola, I.; Mori, M.; Kyriacou, M.; Colla, G.; Rouphael, Y. Appraisal of biodegradable mulching films and vegetal-derived biostimulant application as eco-sustainable practices for enhancing lettuce crop performance and nutritive value. *Agronomy* **2020**, *10*, 427. [CrossRef]
2. Rouphael, Y.; Colla, G. Biostimulants in agriculture. *Front. Plant Sci.* **2020**, *11*, 511937. [CrossRef]
3. Godlewska, K.; Ronga, D.; Michalak, I. Plant extracts-importance in sustainable agriculture. *Ital. J. Agron.* **2021**, *16*, 1851. [CrossRef]
4. Bogatek, R.; Gniazdowska, A. ROS and phytohormones in plant-plant allelopathic interaction. *Plant Signal. Behav.* **2007**, *2*, 317–318. [CrossRef] [PubMed]
5. Cheng, F.; Cheng, Z. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Front. Plant Sci.* **2015**, *6*, 1020. [CrossRef]
6. Farooq, M.; Rizwan, M.; Nawaz, A.; Rehman, A.; Ahmad, R. Application of natural plant extracts improves the tolerance against combined terminal heat and drought stresses in bread wheat. *J. Agron. Crop Sci.* **2017**, *203*, 528–538. [CrossRef]
7. Dipak Kumar, H.; Aloke, P. Role of biostimulant formulations in crop production: An overview. *Int. J. Agric. Sci. Vet. Med.* **2020**, *8*, 38–46.
8. Rouphael, Y.; Colla, G. Toward a sustainable agriculture Through plant biostimulants: From experimental data to practical applications. *Agronomy* **2020**, *10*, 1461. [CrossRef]
9. Castiglione, A.M.; Mannino, G.; Contartese, V.; Bertea, C.M.; Ertani, A. Microbial biostimulants as response to modern agriculture needs: Composition, role and application of these innovative products. *Plants* **2021**, *10*, 1533. [CrossRef] [PubMed]
10. Shahrajabian, M.H.; Chaski, C.; Polyzos, N.; Petropoulos, S.A. Biostimulants application: A low input cropping management tool for sustainable farming of vegetables. *Biomolecules* **2021**, *11*, 698. [CrossRef]
11. Zulfiqar, F.; Casadesús, A.; Brockman, H.; Munné-Bosch, S. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Sci.* **2020**, *295*, 110194. [CrossRef] [PubMed]
12. Li, S.; Zhihui, C. *Allium Sativum* extract as a biopesticide affecting pepper blight. *Int. J. Veg. Sci.* **2009**, *15*, 13–23. [CrossRef]
13. Roy, S.; Mukhopadhyay, A.; Gurusubramanian, G. Field efficacy of a biopesticide prepared from *clerodendrum viscosum* vent. (Verbenaceae) against two major tea pests in the sub himalayan tea plantation of North Bengal, India. *J. Pest Sci.* **2010**, *83*, 371–377. [CrossRef]
14. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
15. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguier, R.; Rouphael, Y. Protein hydrolysates as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 28–38. [CrossRef]
16. Ertani, A.; Pizzeghello, D.; Francioso, O.; Tinti, A.; Nardi, S. Biological activity of vegetal extracts containing phenols on plant metabolism. *Molecules* **2016**, *21*, 205. [CrossRef]
17. Ali, M.; Cheng, Z.H.; Hayat, S.; Ahmad, H.; Ghani, M.I.; Liu, T. Foliar spraying of aqueous garlic bulb extract stimulates growth and antioxidant enzyme activity in eggplant (*Solanum melongena* L.). *J. Integr. Agric.* **2019**, *18*, 1001–1013. [CrossRef]
18. Rinschen, M.M.; Ivanisevic, J.; Giera, M.; Siuzdak, G. Identification of bioactive metabolites using activity metabolomics. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 353–367. [CrossRef]
19. Saa, S.; Del Rio, A.O.; Castro, S.; Brown, P.H. Foliar application of microbial and plant based biostimulants increases growth and potassium uptake in almond (*Prunus dulcis* [Mill.] D. A. Webb). *Front. Plant Sci.* **2015**, *6*, 87. [CrossRef] [PubMed]
20. Del-Toro-Sánchez, C.L.; Rodríguez-Félix, F.; Cinco-Moroyoqui, F.J.; Juárez, J.; Ruiz-Cruz, S.; Wong-Corral, F.J.; Borboa-Flores, J.; Castro-Enríquez, D.D.; Barreras-Urbina, C.G.; Tapia-Hernández, J.A. Recovery of phytochemical from three safflower (Carthamus tinctorius L.) by-products: 20, protective effect of human erythrocytes and profile by UPLC-DAD-MS. *J. Food Process. Preserv.* **2021**, *45*, 3. [CrossRef]
21. Ali, E.F.; Hassan, F.A.S.; Elgimabi, M. Improving the growth, yield and volatile oil content of *Pelargonium graveolens* L. herit by foliar application with moringa leaf extract through motivating physiological and biochemical parameters. *S. Afr. J. Bot.* **2018**, *119*, 383–389. [CrossRef]
22. Nawaz, M.; Wang, X.; Saleem, M.H.; Khan, M.H.U.; Afzal, J.; Fiaz, S.; Ali, S.; Ishaq, H.; Khan, A.H.; Rehman, N.; et al. Deciphering *Plantago Ovata* Forsk leaf extract mediated distinct germination, growth and physio-biochemical improvements under water stress in maize (*Zea mays* L.) at Early Growth Stage. *Agronomy* **2021**, *11*, 1404. [CrossRef]
23. Balal, R.M.; Shahid, M.A.; Javaid, M.M.; Anjum, M.A.; Ali, H.H.; Mattson, N.S.; Garcia-Sanchez, F. Foliar Treatment with *Lolium perenne* (Poaceae) leaf extract alleviates salinity and nickel-induced growth inhibition in pea. *Rev. Bras. Bot.* **2016**, *39*, 453–463. [CrossRef]
24. Figueiredo, F.R.A.; Nóbrega, J.S.; de Fátima, R.T.; Ferreira, J.T.A.; da Silva Leal, M.P.; Melo, M.F.; Dias, T.J.; de Albuquerque, M.B. Impact of biostimulant and saline water on cape gooseberry (*Physalis peruviana* L.) in Brazil. *Physiol. Mol. Biol. Plants* **2021**, *27*, 2141–2150. [CrossRef]
25. Lefi, E.; Badri, M.; Hamed, S.B.; Talbi, S.; Mnafgui, W.; Ludidi, N.; Chaieb, M. Influence of brown seaweed (*Ecklonia maxima*) extract on the morpho-physiological parameters of melon, cucumber, and tomato plants. *Agronomy* **2023**, *13*, 2745. [CrossRef]

26. Rouphael, Y.; De Micco, V.; Arena, C.; Raimondi, G.; Colla, G.; De Pascale, S. Effect of *Ecklonia maxima* seaweed extract on yield, mineral composition, gas exchange, and leaf anatomy of zucchini squash grown under saline conditions. *J. Appl. Phycol.* **2017**, *29*, 459–470. [CrossRef]
27. Di Mola, I.; Ottaiano, L.; Cozzolino, E.; Senatore, M.; Giordano, M.; El-Nakhel, C.; Sacco, A.; Rouphael, Y.; Colla, G.; Mori, M. Plant-based biostimulants influence the agronomical, physiological, and qualitative responses of baby rocket leaves under diverse nitrogen conditions. *Plants* **2019**, *8*, 522. [CrossRef]
28. Zhang, H.Y.; Goncalves, P.; Copeland, E.; Qi, S.S.; Dai, Z.C.; Li, G.L.; Wang, C.Y.; Du, D.L.; Thomas, T. Invasion by the weed *Conyza canadensis* alters soil nutrient supply and shifts microbiota structure. *Soil Biol. Biochem.* **2020**, *143*, 107739. [CrossRef]
29. Ruiz, M.R.; Mangolin, C.A.; Oliveira Júnior, R.S.; Mendes, R.R.; Takano, H.K.; Eisele, T.G.; Machado, M.F.P.S. Mechanisms that may lead to high genetic divergence and to the invasive success of tall fleabane (*Conyza sumatrensis*; Asteraceae). *Weed Sci.* **2022**, *70*, 64–78. [CrossRef]
30. Sahil, L.D.; Florentine, S.; Chauhan, B.S. Germination ecology of hairy fleabane (Conyza bonariensis) and its implications for weed management. *Weed Sci.* **2020**, *68*, 411–417. [CrossRef]
31. Huang, H.; Ye, R.; Qi, M.; Li, X.; Miller, D.R.; Stewart, C.N.; Dubois, D.W.; Wang, J. Wind-mediated horseweed (*Conyza canadensis*) gene flow: Pollen emission, dispersion, and deposition. *Ecol. Evol.* **2015**, *5*, 2646–2658. [CrossRef] [PubMed]
32. Hu, G.; Zhang, Z.H. Aqueous tissue extracts of inhibit the germination and shoot growth of three native herbs with no autotoxic effects. *Planta Daninha* **2013**, *31*, 805–811. [CrossRef]
33. Bhowmik, P.C. Horseweed (*Conyza canadensis*) seed production, emergence, and distribution in no-tillage and conventional-tillage corn (*Zea mays*). *Agron. Trends Agric. Sci* **1993**, *1*, 67–71.
34. Shields, E.J.; Dauer, J.T.; VanGessel, M.J.; Neumann, G. Horseweed (*Conyza canadensis*) seed collected in the planetary boundary layer. *Weed Sci.* **2006**, *54*, 1063–1067. [CrossRef]
35. Peralta, A.C.; Soriano, G.; Zorrilla, J.G.; Masi, M.; Cimmino, A.; Fernández-Aparicio, M. Characterization of *Conyza bonariensis* allelochemicals against broomrape weeds. *Molecules* **2022**, *27*, 7421. [CrossRef] [PubMed]
36. Zhou, J.; Xu, Z.; Zhong, S.; Yu, Y.; Xu, Z.; Du, D.; Wang, C. Nitrogen influence to the independent invasion and the co-invasion of *Solidago canadensis* and *Conyza canadensis* via intensified allelopathy. *Sustainability* **2022**, *14*, 11970. [CrossRef]
37. Fernández-Aparicio, M.; Soriano, G.; Masi, M.; Carretero, P.; Vilariño-Rodríguez, S.; Cimmino, A. (4Z)-lachnophyllum lactone, an acetylenic furanone from *Conyza bonariensis*, identified for the first time with allelopathic activity against *Cuscuta campestris*. *Agriculture* **2022**, *12*, 790. [CrossRef]
38. Zhang, H.; Rutherford, S.; Qi, S.; Huang, P.; Dai, Z.; Du, D. Transcriptome profiling of *Arabidopsis thaliana* roots in response to allelopathic effects of *Conyza canadensis*. *Ecotoxicology* **2022**, *31*, 53–63. [CrossRef]
39. Queiroz, S.C.N.; Cantrell, C.L.; Duke, S.O.; Wedge, D.E.; Nandula, V.K.; Moraes, R.M.; Cerdeira, A.L. Bioassay-directed isolation and identification of phytotoxic and fungitoxic acetylenes from *Conyza canadensis*. *J. Agric. Food Chem.* **2012**, *60*, 5893–5898. [CrossRef]
40. Marque, J.M.; Kobori, R.F.; Kato, S.M. Ocorrência de *Colletotrichum gloeosporioides* em ciclamem cultivado em vaso sob estufa no Estado de São Paulo. *Fitopatol. Bras.* **2006**, *31*, 323. [CrossRef]
41. Neves, M.F.; Pinto, M.J.A. *Mapeamento e Quantificação da Cadeia de Flores e Plantas Ornamentais Do Brasil*; OCESP: São Paulo, Brazil, 2015; 132p, Available online: https://www.ibraflor.com.br (accessed on 1 January 2024).
42. R CORE TEAM. R Foundation for Statistical Computing: Vienna, Austria, 2020. Available online: https://www.r-project.org (accessed on 3 February 2024).
43. Jones, H. Whats is water use efficiency. In *Water Use Efficiency in Plant Biology*; Bacon, M., Ed.; Blackwell Publishing: Oxford, UK, 2004; pp. 27–41.
44. Ahmad, A.; Blasco, B.; Martos, V. Combating salinity through natural plant extracts based biostimulants: A review. *Front. Plant Sci.* **2022**, *13*, 862034. [CrossRef] [PubMed]
45. Castronuovo, D.; Comegna, A.; Belviso, C.; Satriani, A.; Lovelli, S. Zeolite and *Ascophyllum nodosum*-based biostimulant effects on spinach gas exchange and growth. *Agriculture* **2023**, *13*, 754. [CrossRef]
46. Luiz Piati, G.; Ferreira de Lima, S.; Lustosa Sobrinho, R.; dos Santos, O.F.; Vendruscolo, E.P.; Jacinto de Oliveira, J.; do Nascimento de Araújo, T.A.; Mubarak Alwutayd, K.; Finatto, T.; AbdElgawad, H. Biostimulants in corn cultivation as a means to alleviate the impacts of irregular water regimes induced by climate change. *Plants* **2023**, *12*, 2569. [CrossRef] [PubMed]
47. Mutlu-Durak, H.; Arikan, Y.; Kutman, B.Y. Willow (*Salix babylonica*) extracts can act as biostimulants for enhancing salinity tolerance of maize grown in soilless culture. *Plants* **2023**, *12*, 856. [CrossRef] [PubMed]
48. Sardar, H.; Nisar, A.; Anjum, M.A.; Naz, S.; Ejaz, S.; Ali, S.; Ahmad, R. Foliar spray of moringa leaf extract improves growth and concentration of pigment, minerals and stevioside in stevia (*Stevia rebaudiana* Bertoni). *Ind. Crops Prod.* **2021**, *166*, 113485. [CrossRef]
49. Abd El-Mageed, T.A.; Semida, W.M.; Rady, M.M. Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation. *Agric. Water Manag.* **2017**, *193*, 46–54. [CrossRef]
50. Mona, M.A. The potential of moringa oleifera extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) Plants. *Int. J. Plant Physiol. Biochem.* **2013**, *5*, 42–49. [CrossRef]
51. Lazaroto, C.A.; Fleck, N.G.; Vidal, R.A. Biologia e ecofisiologia de buva (*Conyza bonariensis e Conyza canadensis*). *Ciência Rural* **2008**, *38*, 852–860. [CrossRef]

52. Albrecht, A.J.P.; Pereira, V.G.C.; Souza, C.N.Z.D.; Zobiole, L.H.S.; Albrecht, L.P.; Adegas, F.S. Multiple resistance of *Conyza sumatrensis* to three mechanisms of action of herbicides. *Acta Scientiarum. Agron.* **2020**, *42*, e42485. [CrossRef]
53. Da Silva, Í.A.; Campelo, L.H.D.B.P.; de Fátima Padilha, M.D.R.; Shinohara, N.K.S. Mecanismos de resistência das plantas alimentícias não convencionais (PANC) e benefícios para a saúde humana. *An. Da Acad. Pernambucana De Ciência Agronômica* **2018**, *15*, 77–91. Available online: https://www.journals.ufrpe.br/index.php/apca/article/view/1950 (accessed on 1 January 2024).
54. Franzener, G.; da Silva Martinez-Franzener, A.; Stangarlin, J.R.; Czepak, M.P.; Schwan-Estrada, K.R.F.; Cruz, M.E.S. Atividades antibacteriana, antifúngica e indutora de fitoalexinas de hidrolatos de plantas medicinais. *Semin. Ciências Agrárias* **2007**, *28*, 29–38. [CrossRef]
55. Chen, K.; Khan, R.A.A.; Cao, W.; Ling, M. Sustainable and ecofriendly approach of managing soil born bacterium *Ralstonia solanacearum* (Smith) using dried powder of *Conyza canadensis*. *Pathogens* **2020**, *9*, 327. [CrossRef] [PubMed]
56. Ahmad, I.; Tanveer, M.U.; Liaqat, M.; Dole, J.M. Comparison of corm soaks with preharvest foliar application of moringa leaf extract for improving growth and yield of cut *Freesia hybrida*. *Sci. Hortic.* **2019**, *254*, 21–25. [CrossRef]
57. Akram, N.A.; Saleem, M.H.; Shafiq, S.; Naz, H.; Farid-ul-Haq, M.; Ali, B.; Shafiq, F.; Iqbal, M.; Jaremko, M.; Qureshi, K.A. Phytoextracts as Crop Biostimulants and Natural Protective Agents—A Critical Review. *Sustainability* **2022**, *14*, 14498. [CrossRef]
58. Barhoumi, L.M.; Shakya, A.K.; Al-Fawares, O.L.; Al-Jaber, H.I. Conyza canadensis from Jordan: Phytochemical Profiling, Antioxidant, and Antimicrobial Activity Evaluation. *Molecules* **2024**, *29*, 2403. [CrossRef]
59. Shaukat, S.S.; Munir, N.; Siddiqui, I.A. Allelopathic responses of *Conyza canadensis* (L.) Cronquist: A cosmopolitan weed. *Asian J. Plant Sci.* **2003**, *2*, 1034–1039. [CrossRef]
60. Vo, Q.V.; Bay, M.V.; Nam, P.C.; Quang, D.T.; Flavel, M.; Hoa, N.T.; Mechler, A. Theoretical and experimental studies of the antioxidant and antinitrosant activity of syringic acid. *J. Org. Chem.* **2020**, *85*, 15514–15520. [CrossRef] [PubMed]
61. Ayaz, F.; Küçükboyacı, N.; Demirci, B. Chemical composition and antimicrobial activity of the essential oil of Conyza canadensis (L.) Cronquist from Turkey. *J. Essent. Oil Res.* **2017**, *29*, 336–343. [CrossRef]
62. Lo, Y.-C.; Rensi, S.E.; Torng, W.; Altman, R.B. Machine learning in chemoinformatics and drug discovery. *Drug Discov. Today* **2018**, *23*, 1538–1546. [CrossRef]
63. Antonova, G.F.; Varaksina, T.N.; Zheleznichenko, T.V.; Stasova, V.V. Changes in phenolic acids during maturation and lignification of Scots pine xylem. *Russ. J. Dev. Biol.* **2012**, *43*, 199–208. [CrossRef]
64. Hassoon, A.S.; Abduljabbar, I.A. Review on the role of salicylic acid in plants. In *Sustainable Crop Production*; Hasanuzzaman, M., Filho, M.C.M.T., Fujita, M., Nogueira, T.A.R., Eds.; IntechOpen: London, UK, 2020. [CrossRef]
65. Bagautdinova, Z.Z.; Omelyanchuk, N.; Tyapkin, A.V.; Kovrizhnykh, V.V.; Lavrekha, V.V.; Zemlyanskaya, E.V. Salicylic acid in root growth and development. *Int. J. Mol. Sci.* **2022**, *23*, 2228. [CrossRef] [PubMed]
66. Steenackers, W.; El Houari, I.; Baekelandt, A.; Witvrouw, K.; Dhondt, S.; Leroux, O.; Gonzalez, N.; Corneillie, S.; Cesarino, I.; Inzé, D.; et al. cis-Cinnamic acid is a natural plant growth-promoting compound. *J. Exp. Bot.* **2019**, *70*, 6293–6304. [CrossRef] [PubMed]
67. Laoué, J.; Fernandez, C.; Ormeño, E. Plant flavonoids in Mediterranean species: A focus on flavonols as protective metabolites under climate stress. *Plants* **2022**, *11*, 172. [CrossRef] [PubMed]
68. Okazaki, S.; Kaneko, T.; Sato, S.; Saeki, K. Hijacking of leguminous nodulation signaling by the rhizobial type III secretion system. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17131–17136. [CrossRef] [PubMed]
69. Tian, H.; Wang, R.; Li, M.; Dang, H.; Solaiman, Z.M. Molecular signal communication during arbuscular mycorrhizal formation induces significant transcriptional reprogramming of wheat (*Triticum aestivum*) roots. *Ann. Bot.* **2019**, *124*, 1109–1119. [CrossRef]
70. Keunen, E.; Peshev, D.; Vangronsveld, J.; Van Den Ende, W.; Cuypers, A. Plant sugars are crucial players in the oxidative challenge during abiotic stress: Extending the traditional concept. *Plant Cell Environ.* **2013**, *36*, 1242–1255. [CrossRef] [PubMed]