

Article

Integrative Seed and Leaf Treatment with Ascorbic Acid Extends the Planting Period by Improving Tolerance to Late Sowing Influences in Parsley

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Abstract: Abnormal production of reactive oxygen species (ROS) is an undesirable event which occurs in plants due to stress. To meet this event, plants synthesize ROS-neutralizing compounds, including the non-enzymatic oxidant scavenger known as vitamin C: ascorbic acid (AsA). In addition to scavenging ROS, AsA modulates many vital functions in stressed or non-stressed plants. Thus, two-season (2018/2019 and 2019/2020) trials were conducted to study the effect of integrative treatment (seed soaking + foliar spray) using 1.0 or 2.0 mM AsA vs. distilled water (control) on the growth, seed yield, and oil yield of parsley plants under three sowing dates (SDs; November, December, and January, which represent adverse conditions of late sowing) vs. October as the optimal SD (control). The ion balance, osmotic-modifying compounds, and different antioxidants were also studied. The experimental layout was a split plot in a completely randomized block design. Late sowing (December and January) noticeably reduced growth traits, seed and oil yield components, and chlorophyll and nutrient contents. However, soluble sugar, proline, and AsA contents were significantly increased along with the activities of catalase (CAT) and superoxide dismutase (SOD). Under late sowing conditions, the use of AsA significantly increased growth, different yields, essential oil fractions, CAT and SOD activities, and contents of chlorophylls, nutrients, soluble sugars, free proline, and AsA. The interaction treatments of SDs and AsA concentrations indicated that AsA at a concentration of 2 mM was more efficient in conferring greater tolerance to adverse conditions of late sowing in parsley plants. Therefore, this study recommends 2.0 mM AsA for integrative (seed soaking + foliar spraying) treatment to prolong the sowing period of parsley seeds (from October up to December) and avoid damage caused by adverse conditions of late sowing.

Keywords: ascorbic acid; sowing dates; *Petroselinum crispum*; low temperature stress

1. Introduction

Parsley, *Petroselinum crispum* Mill, belonging to Umbelliferae/Apiaceae, is a biennial plant native to the Mediterranean region (Spain, Italy, Greece, Malta, Algeria, Tunisia, and Morocco) and has been introduced into cultivation worldwide in limestone and rocky areas [1]. It has three common varieties (*P. crispum* var. *crispum*, var. *neapolitanum*, and var. *tuberosum*) used for many purposes, including a garnish, in tabbouleh, and as a root vegetable, respectively [2]. Additionally, it is considered an aromatic, culinary, and medicinal plant. It is used in the pharmaceutical, perfume, and cosmetic industries [3,4]. It is an important dietary source of vitamins and essential nutrients, and its volatile compounds (e.g., myristicin, apiole) are responsible for medicinal uses [4]. In traditional medicine, parsley is considered a diuretic, uterine stimulant, sedative, emollient, and antiparasitic agent. It functions to treat many human diseases [5].

One of the factors that determine the biometric features of the plant is the date of sowing [6]. The sowing date plays a fundamental role in the performance, the production, and thus the yield of medicinal and aromatic plants [7], which in turn affects the income of farms, as the planting date may affect the productivity of the crop and its inner components, especially when exposed to low temperature stress [8] as one of the adverse climatic conditions under late sowing. The steady increase in the world's population with the need to secure food from economic food crops may lead to the occupation of agricultural land for long periods of the year; thus, other crops such as parsley may be planted at inappropriate dates (late planting). Therefore, the seeds when they are planted and the seedlings as they grow are exposed to unsuitable growth conditions, including a low light intensity and temperature, especially with the current event called climatic change in dry (arid and semi-arid) regions. In this regard, temperature, light, air relative humidity, etc., are essential climatic factors that affect the growth of any plant. Therefore, the planting time controls the crop phenological development, total biomass production, and the efficient conversion of biomass into economic yield [9]. Additionally, the biosynthesis of secondary metabolites is genetically controlled and strongly influenced by eco-factors [10].

Plants grown in open field conditions often go through periods of abiotic stress throughout all their life stages [11]. Under stress conditions, in addition to ensuring food security, it is necessary to provide the right amount of medicinal crops. Therefore, it is possible to cultivate some medicinal crops outside the official dates using some exogenous bio-stimulants to prolong the time for planting economic food crops.

When planting crops, including parsley, are delayed (from October up to December and January), their exposure to optimal growing conditions is reduced. This is detrimental to the plant's growth rate due to the reduction in average air and soil temperatures as well as the continuous decrease in light quality and intensity. Additionally, soil moisture may increase and can adversely affect soil trafficability, which is critical for machines to work and sow seeds of crops [12], including parsley. As one of the adverse climatic conditions caused by late parsley sowing (December and January), low temperature (LT) causes a decrease in plant growth and productivity along with the contents of photosynthetic pigments, mineral nutrients, and unsaturated and total essential oils. LT also generates an unfavorable increase in signs of oxidative stress (reactive oxygen species; ROS) and cell membrane permeability. All these consequences of LT cause a decrease in the efficiency of plant performance and eventually lead to cell death [13,14]. Subsequently, the plant stress tolerance potential is harnessed through undergoing various osmotic resistance (soluble sugars, proline, etc.) and antioxidant (proline, ascorbate, superoxide dismutase, peroxidase, catalase, etc.) mechanisms for adaptation and protection. These mechanisms cannot effectively attenuate the adverse influences of climatic factors, including LT and a low quality and intensity of light, but with exogenous application of antioxidants, these mechanisms are improved and can maximize plant tolerance to adverse climatic conditions under late sowing [15–19].

Among the metabolites worthy of being involved in the ascorbate-glutathione cycle and in some vital processes, including cell division and osmotic modification, ascorbic

acid (AsA), called “vitamin C”, has potent antioxidant potential and is involved in the scavenging and controlling the formation of ROS [14,20–23]. It acts as an enzyme co-factor, as an antioxidant that inhibits the activity of superoxide, singlet oxygen, ozone, and hydrogen peroxide, and as a donor/acceptor in electron transport at the plasma membrane or in chloroplasts. It also replenishes the lipophilic antioxidant α -tocopherol (vitamin E) from α -chromanoxyl radicals [24]. Under different eco-stress conditions, the exogenous application of AsA can improve the endogenous content of AsA [14], which contributes to an increase in plant tolerance to many stresses, including LTS by optimizing the mechanisms of the plant antioxidant system [14,21–23].

The improving role of integrative seed plus leaf treatments with AsA to attenuate adverse climatic condition-induced damage in parsley plants due to late sowing is rarely available. Therefore, the aim of this investigation was to investigate the potential enhancing influences of integrative treatment (seed soaking + foliar spray) using 1.0 or 2.0 mM AsA on the growth, seed and essential oil yields, osmotic-modifying compounds, and different antioxidants (soluble sugars, proline, AsA, superoxide dismutase, peroxidase, and catalase) of parsley plants grown under different sowing dates (SD) such as November, December, and January vs. October as an optimal SD (control). We hypothesized that AsA might lead to a systemic tolerance in parsley under adverse conditions of late sowing. Therefore, growth, yields, chlorophyll, osmoprotectant contents, and antioxidative activities would be enhanced under the integrative (seed priming + foliar spray) impact of AsA, particularly 2.0 mM under late parsley sowing. How AsA can maintain parsley plants' ability to withstand damage caused by adverse conditions of late sowing and survive is explained.

2. Materials and Methods

2.1. Experimental Dates, Site, Soil Analysis, and Meteorological Data

October–January (sowing dates) until May (harvest date for all sowing dates) during 2018/2019 and 2019/2020 were the dates for conducting two consecutive main trials. During the 2016/2017 and 2017/2018 seasons, the main trials were preceded by two preliminary experiments in the same open field (September–May). All experiments were carried out in the experimental farm of the Faculty of Agriculture (Demo village; 29°17' N; 30°53' E), Fayoum University, Fayoum Governorate, Egypt.

Before sowing for each season of the main experiments, soil samples were collected at a depth of 30 cm from the experimental site to analyze the initial physical and chemical characteristics. The procedures described in [25,26] were applied, and the results are presented in Table S1.

Table S2 and Figure S1 show the environmental conditions during the two main experimental seasons in Fayoum Governorate according to the Central Laboratory of Agricultural Climatology, Agricultural Research Center (ARC), Egypt.

2.2. Experimental Design, Treatments, and Experimental Setup

The experimental layout was a completely randomized block design, arranged as a split plot with three replications. In each season, a total area of 216 m² was divided into 36 plots (4 sowing dates (October, November, December, and January) \times 3 ascorbic acid levels (0, 1.0, and 2.0 mM) \times 3 replications = 36). The area of each plot was 2.50 \times 2.40 = 6 m², including four ridges (rows) 60 cm apart and 2.50 m long, and each row had ten hills (25 cm apart).

Viable seeds of parsley (*Petroselinum crispum* Mill, var. *neapolitanum* cv. Balady) were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Before seeding, the experimental area was disked and harrowed. Before sowing for each sowing date in each season of the main experiments, seeds were soaked in 1.0 and 2.0 mM ascorbic acid (AsA) vs. soaking in distilled water as a control for 8 h (based on a preliminary study; Table S3).

Seeding was carried out in rows at the rate of 5 seeds per hill with a depth of 1–2 cm. Seedlings were thinned to two per hill at 45 days after sowing (DAS) on October 1, 40 DAS

on November 1, 35 DAS on December 1, and 30 DAS on January 1. The seeds were sown directly in the open field at four different sowing dates (SDs), including the optimum sowing date (1 October based on a preliminary study; Table S3). Three non-ideal SDs of 1 November, December, and January were chosen to represent adverse conditions of late sowing during both the 2018/2019 and 2019/2020 seasons. These three SD treatments vs. the optimal SD (1 October) randomly occupied the main plots, while the sub-main plots received the treatments of the selected AsA levels (0, 1.0, or 2.0 mM). The AsA levels were foliar sprayed three times, to run off. For the first sowing date (1 October), spraying was conducted at 60, 80, and 100 DAS. Foliar spraying was performed at 55, 75, and 95 DAS for the second sowing date (1 November). Foliar spraying was conducted at 50, 70, and 90 DAS for the third sowing date (1 December), and 45, 65, and 85 DAS were the dates for foliar spraying for the fourth sowing date (1 January). A 20 L dorsal sprayer was utilized with a few drops from Tween-20 as a surfactant.

For fertilization of parsley trials, complete (100%) recommended doses of nitrogen (N), phosphorus (P), and potassium (K) were applied at a rate of 480 kg ammonium sulfate (20.5% N), 480 kg calcium super-phosphate (15.5% P₂O₅), and 240 kg potassium sulfate (48% K₂O) ha⁻¹. During soil preparation for sowing, calcium super-phosphate (31 units of P₂O₅ ha⁻¹) and organic manure (20 ton ha⁻¹) were added as a base dose in both seasons. All plots were also fertilized by 23.5 N units ha⁻¹ and 5.0 K₂O units ha⁻¹, which were side banded at three equal doses: 20, 40, and 60 DAS for all the sowing dates. Irrigation for the experiments was carried out regularly according to the field capacity, taking into account the amounts of water added as a result of rainfall. Other basic agricultural management practices were applied for the cultivation of parsley following the recommendations of the Egyptian Ministry of Agriculture and Soil Reclamation.

2.3. Sampling

At the flowering of 25% of plants per sowing date treatment (130, 110, 108, and 95 DAS for the 4 sowing dates: 1 October, November, December, and January, respectively), 9 plants were chosen from each treatment (sub-plot) for growth and nutrient determinations, as well as physiological and antioxidant capacity. In May of each season, all plants were harvested for seed and oil yield determinations.

2.4. Morphological Characters, and Seed and Oil Yield Attributes

Plant height (cm) was measured using a graduated ruler (100 cm). Plant fresh weight (g) was measured using an electronic balance. After air drying and oven drying at 60 °C for 72 h, plant dry weight (g) was weighed using an electronic balance.

At the full maturity fruit stage (May in both seasons), all remaining plants in all plots were harvested to evaluate average seed weight (g plant⁻¹) and total seed yield (ton ha⁻¹). The seed yield was used to estimate oil yield by using the following formula:

$$\text{Oil yield plant}^{-1} \text{ (mL)} = (\text{seed yield plant}^{-1} \times \text{oil content, \%})/100$$

2.5. Essential Oil Distillation (Extraction)

The oil was quantified gravimetrically by hydro-distillation according to the European Pharmacopoeia (EP) [27]. Seed samples of each plot were hydro-distilled for 2 h using a Clevenger-type apparatus. Each sample was analyzed in three replications, and the average was used for statistical analyses. The oil concentration was calculated as the amount (g) of oil per weight (g) of dry parsley seed, while the oil yield per area was computed from the seed yields per area and oil content. To assess the oil content, 100 g of powdered samples in 0.5 L of water was extracted from each plant population for determining the oil content (*v/w*, %). The oil was dried with anhydrous sodium sulfate, Na₂SO₄. The oil was kept at 4 °C in dark glass containers for further laboratory analyses. Each 1.0 mL equals a density of 1.04 g mL⁻¹ [28].

2.6. GC/MS Analysis of Essential Oil

GC/MS analysis was performed on a Varian CP 3800 GC equipped with a Varian Saturn 2200 MS detector and a capillary column (30 m length and 0.25 mm I.D., 0.25 μm film thickness) (Walnut Creek, CA, USA). The stationary phase was 5% phenyl-methyl polysiloxane. The instrument was operated in EI mode at 70 eV. The transfer line and injector temperatures were set to 250 °C and 200 °C, respectively. High-purity helium functioned as a carrier gas (2 mL min^{-1}). The GC oven temperature was set at 100 °C for 7 min, programmed to 250 °C at a rate of 5 °C min^{-1} , and kept constant at 250 °C for 1 min. The split ratio was 1:10.

2.7. Determinations of Chlorophyll and Nutrient Contents

A chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Osaka, Japan) was used to measure leaf greenness (SPAD). The total contents of macronutrients (N, P, and K) were determined in parsley leaves after being ground and washed with water. Total N content was determined using the semi-micro Kjeldahl method [29]. Total P content was quantitatively assessed using the spectrophotometry method of molybdenum-reduced molybdophosphoric blue color [30]. The K content was photometrically estimated using a flame photometer, as described in [30]. The dried powdered plant leaves were used to measure the contents of micronutrients (Fe, Mn, and Zn), using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer [31].

2.8. Determinations of Total Soluble Sugars, Free Proline, and Ascorbic Acid

The procedures in [32] were applied to evaluate the contents of total soluble sugars (mg g^{-1} DW). After extraction and centrifugation for extracts, the supernatant was collected and combined with anthrone (freshly prepared), and incubation for 10 min at 100 °C followed. After recording the readings at 625 nm, the contents of total soluble sugars were calculated following a standard curve made using pure glucose. The procedures in [33] were applied to assess leaf contents of free proline (mmol g^{-1} DW). Using 3% (*v/v*) sulphosalicylic acid, leafy samples (0.5 g each) were extracted, and the extracts were then centrifuged (at 10,000 $\times g$ for 10 min). Then, the supernatant (2 mL) was mixed with 2 mL acid ninhydrin solution (freshly prepared). After incubation of the mixture at 90 °C for 30 min, an ice bath was used to terminate the reaction. After an additional extraction using toluene, the mixture was separated in the dark for 20 min at 25 °C, and the toluene phase was used to read the absorbance at 520 nm. The procedures described in [34] were followed to evaluate the leafy contents of ascorbic acid (AsA). After the homogenization and extraction of leafy samples (1.0 g each) using liquid N_2 and 5% (*w/v*) trichloroacetic acid (TCA), centrifugation (15,600 $\times g$ at 4 °C for 5 min) was carried out. The assay of AsA was carried out using 1.0 mL of reaction mixture containing the supernatant, 10 mM DTT, 0.5% (*v/v*) Nethylmaleimide, 10% (*w/v*) TCA, 42% (*v/v*) H_3PO_4 , 4% (*v/v*) 2,2'-dipyridyl, 3% (*w/v*) FeCl_3 , and 0.2 M phosphate buffer, pH 7.4.

2.9. Extraction and Assaying Antioxidant Enzyme Activities

The method applied to extract the enzymes from parsley leaf samples was described in [35]. Catalase activity (CAT; EC 1.11.1.6) was assayed according to the method in [36] using appropriate volumes of the enzyme extract and phosphate buffer, and H_2O_2 was used as a reaction substrate. Superoxide dismutase activity (SOD; EC 1.15.1.1) was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described in [37]. Peroxidase activity (POD; EC 1.11.1.7) was assayed following the method in [36], with a slight modification, using appropriate volumes of the enzyme extract and phosphate buffer, and guaiacol and H_2O_2 were used as reaction substrates.

2.10. Statistical Analysis

All values as means of three replications were subjected to two-way ANOVA after testing for homogeneity of error variances according to the procedure outlined in [38] using

InfoStat software estadístico [39]. Differences between means were tested using the least significant difference (LSD) test [40] at the 1% and 5% probability levels ($p \leq 0.01$ and $p \leq 0.05$).

3. Results

3.1. The Preliminary Study

Two-season preliminary trials were conducted during the 2016/2017 and 2017/2018 seasons to identify the optimum sowing date and soaking period for parsley seeds, the optimum concentrations of ascorbic acid (AsA), and the optimum foliar spray times for application in the main experiments (Table S3). The selected identifications were dependent on some tested variables such as the percentage of seedling emergence, plant dry weight, and plant seed weight. The optimal sowing date was 1 October, the optimal soaking period was 8 hrs, the optimal concentrations of AsA were 1.0 and 2.0 mM, and three spray applications were optimal.

3.2. The Main Study

Since all tested data obtained from the 2018/2019 season matched the corresponding data from the 2019/2020 season, the average of the two seasons was processed. Four sowing dates were used in this study from October to January to represent the negative effect of low temperature stress, and to study the potential enhancing effect of ascorbic acid on low-temperature-stressed parsley plants.

3.2.1. Effect of Integrative Seed and Leaf Treatment with Ascorbic Acid (AsA) on Growth Traits of Parsley Plants Grown under Different Sowing Dates (SDs)

For SDs, there was a gradual decrease in plant height (pHt), fresh weight (pFWt), and dry weight (pDWt) by gradually delaying the sowing of parsley seeds from October to January. The highest values of pHt, pFWt, and pDWt were obtained in October (the optimal planting date), while the lowest values were obtained in January (Table 1).

Table 1. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on growth traits of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	Plant Height (cm)	% of Cont.	Fresh Weight (g Plant ⁻¹)	% of Cont.	Dry Weight (g Plant ⁻¹)	% of Cont.
Sowing date (SD)	*		*		**	
Oct.	110.5 ^a ± 9.7	108.8 ^a ± 9.4	28.0 ^a ± 1.7
Nov.	107.3 ^a ± 9.3	−2.90	103.6 ^a ± 8.7	−4.78	27.0 ^a ± 1.7	−3.57
Dec.	96.9 ^b ± 8.4	−12.3	82.8 ^b ± 6.6	−23.9	18.7 ^b ± 0.8	−33.2
Jan.	64.3 ^c ± 5.0	−41.8	52.9 ^c ± 4.1	−51.4	10.4 ^c ± 0.6	−62.9
AsA levels	*		*		**	
0 mM (AsA ₀)	82.5 ^c ± 7.0	71.8 ^c ± 5.6	15.5 ^c ± 0.9
1.0 mM (AsA ₁)	94.3 ^b ± 8.0	+14.3	85.9 ^b ± 7.3	+19.6	20.4 ^b ± 1.3	+31.6
2.0 mM (AsA ₂)	107.4 ^a ± 9.2	+30.2	103.4 ^a ± 8.7	+44.0	27.6 ^a ± 1.8	+78.1
SD × AsA levels	*		*		**	
Oct. × AsA ₀	97.9 ^c ± 8.4	97.0 ^b ± 7.9	24.8 ^c ± 1.2
Oct. × AsA ₁	111.1 ^b ± 9.8	+13.5	110.2 ^a ± 9.9	+13.6	28.5 ^b ± 1.8	+14.9
Oct. × AsA ₂	122.4 ^a ± 10.9	+25.0	119.1 ^a ± 10.3	+22.8	32.3 ^a ± 2.2	+30.2
Nov. × AsA ₀	90.4 ^{cd} ± 7.8	−7.66	80.9 ^c ± 6.0	−16.6	19.4 ^d ± 1.1	−21.8

Table 1. Cont.

Source of Variation	Plant Height (cm)	% of Cont.	Fresh Weight (g Plant ⁻¹)	% of Cont.	Dry Weight (g Plant ⁻¹)	% of Cont.
Nov. × AsA ₁	109.0 ^b ± 9.3	+11.3	110.5 ^a ± 9.8	+13.9	28.7 ^b ± 1.8	+15.7
Nov. × AsA ₂	122.4 ^a ± 10.7	+25.0	119.4 ^a ± 10.3	+23.1	32.8 ^a ± 2.1	+32.3
Dec. × AsA ₀	81.8 ^d ± 7.2	−16.4	60.0 ^{de} ± 4.6	−38.1	10.5 ^f ± 0.7	−57.7
Dec. × AsA ₁	88.4 ^{cd} ± 7.5	−9.70	70.8 ^d ± 5.5	−27.0	14.1 ^e ± 0.8	−43.1
Dec. × AsA ₂	120.5 ^a ± 10.4	+23.1	117.6 ^a ± 9.8	+21.2	31.6 ^a ± 2.0	+27.4
Jan. × AsA ₀	59.9 ^e ± 4.7	−38.8	49.4 ^{ef} ± 3.9	−49.1	7.4 ^g ± 0.5	−70.2
Jan. × AsA ₁	68.5 ^e ± 5.5	−30.0	52.0 ^{ef} ± 3.8	−46.4	10.1 ^f ± 0.6	−59.3
Jan. × AsA ₂	64.4 ^e ± 4.7	−34.2	57.4 ^{ef} ± 4.5	−40.8	13.8 ^e ± 0.8	−44.4

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “*” and “***” indicate significant differences at the $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

For AsA levels, the 2.0 mM level significantly outperformed the 1.0 mM level, and both levels significantly increased pHt, pFWt, and pDWt compared to the control (0 mM AsA). The level of 2.0 mM AsA was superior to the level of 1.0 mM (Table 1).

Concerning the combination of SDs and AsA, the combination treatments of Oct. × AsA₂, Nov. × AsA₂, and Dec. × AsA₂ had higher values for pHt (122.4, 122.4, and 120.5 cm, respectively), pFWt (119.1, 119.4, and 117.6 g plant⁻¹, respectively), and pDWt (32.3, 32.8, and 31.6 g plant⁻¹, respectively) than the other combination treatments and significantly exceeded the control values (optimal sowing date without any treatments with AsA). However, AsA at any concentration (1 mM or 2 mM) failed to enable plants grown in January to give results close to the control values (Table 1). This means that the planting of parsley can be delayed from its optimum date (October) until December and obtain higher growth traits with the application of AsA as an integrative treatment (seed soaking + foliar spray).

3.2.2. Effect of Integrative Seed and Leaf Treatment with Ascorbic Acid (AsA) on Seed and Oil Yield components of Parsley Plants Grown under Different Sowing Dates (SDs)

With respect to SDs, seed weight per plant (SW-P), seed yield per hectare (SY-H), oil content (OC, %), and oil yield per hectare (OY-H) were gradually decreased by gradually delaying the sowing of parsley seeds from October to January. The highest values of SW-P, SY-H, OC, and OY-H were obtained in October (the optimal sowing date), while the lowest values were obtained in January (Table 2).

For AsA levels, the 2.0 mM level significantly outperformed the 1.0 mM level, and both levels significantly increased SW-P, SY-H, OC, and OY-H compared to the control (0 mM AsA). The level of 2.0 mM AsA was superior to the level of 1.0 mM (Table 2).

Regarding the combination of SDs and AsA, the combination treatments of Oct. × AsA₂, Nov. × AsA₂, and Dec. × AsA₂ had higher values for SW-P (13.17, 13.25, and 13.11 g plant⁻¹, respectively), SY-H (1.49, 1.49, and 1.47 t h⁻¹, respectively), OC (1.32, 1.32, and 1.31%, respectively), and OY-H (17.24, 17.32, and 17.22 L ha⁻¹, respectively) than the other combination treatments and significantly exceeded the control values. However, AsA at any concentration (1 mM or 2 mM) failed to enable plants grown in January to give results close to the control values (Table 2). This means that the planting of parsley can be delayed from its optimum date (October) until December and obtain higher growth traits with the application of AsA as an integrative treatment (seed soaking + foliar spray).

Table 2. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on seed and oil yield components of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	Seed Weight (g Plant ⁻¹)	% of Cont.	Seed Yield (t h ⁻¹)	% of Cont.	Oil Content (%)	% of Cont.	Oil Yield (L ha ⁻¹)	% of Cont.
Sowing date (SD)	*		*		*		**	
Oct.	12.05 ^a ± 0.84	1.35 ^a ± 0.10	1.21 ^a ± 0.09	15.48 ^a ± 1.4
Nov.	11.77 ^a ± 0.82	−2.32	1.32 ^a ± 0.10	−2.22	1.17 ^a ± 0.08	−3.31	15.34 ^a ± 1.4	−0.90
Dec.	10.30 ^b ± 0.67	−14.5	1.15 ^b ± 0.08	−14.8	0.97 ^b ± 0.07	−19.8	10.97 ^b ± 0.93	−29.1
Jan.	7.05 ^c ± 0.47	−41.5	0.78 ^c ± 0.05	−42.2	0.73 ^c ± 0.05	−39.7	5.54 ^c ± 0.40	−64.2
AsA levels	*		*		*		**	
0 mM (AsA ₀)	8.92 ^c ± 0.57	0.99 ^c ± 0.07	0.89 ^c ± 0.06	9.40 ^c ± 0.77
1.0 mM (AsA ₁)	10.17 ^b ± 0.71	+14.0	1.13 ^b ± 0.08	+14.1	1.01 ^b ± 0.07	+13.5	11.64 ^b ± 0.98	+23.8
2.0 mM (AsA ₂)	11.79 ^a ± 0.82	+32.2	1.32 ^a ± 0.11	+33.3	1.17 ^a ± 0.09	+31.5	14.46 ^a ± 1.31	+53.8
SD × AsA levels	*		*		*		**	
Oct. × AsA ₀	10.67 ^b ± 0.74	1.19 ^{bc} ± 0.09	1.09 ^{bc} ± 0.07	12.85 ^b ± 1.10
Oct. × AsA ₁	12.30 ^a ± 0.86	+15.3	1.37 ^{ab} ± 0.09	+15.1	1.23 ^{ab} ± 0.09	+12.8	16.34 ^a ± 1.48	+27.2
Oct. × AsA ₂	13.17 ^a ± 0.91	+23.4	1.49 ^a ± 0.13	+25.2	1.32 ^a ± 0.10	+21.1	17.24 ^a ± 1.68	+34.2
Nov. × AsA ₀	9.67 ^b ± 0.64	−9.37	1.07 ^{cd} ± 0.07	−10.1	0.97 ^{cd} ± 0.06	−11.0	12.43 ^b ± 1.00	−3.27
Nov. × AsA ₁	12.40 ^a ± 0.88	+16.2	1.39 ^{ab} ± 0.11	+16.8	1.23 ^{ab} ± 0.08	+12.8	16.28 ^a ± 1.56	+26.7
Nov. × AsA ₂	13.25 ^a ± 0.94	+24.2	1.49 ^a ± 0.13	+25.2	1.32 ^a ± 0.10	+21.1	17.32 ^a ± 1.67	+34.8
Dec. × AsA ₀	8.68 ^c ± 0.50	−18.7	0.96 ^{de} ± 0.06	−19.3	0.78 ^e ± 0.05	−28.4	7.49 ^c ± 0.58	−41.7
Dec. × AsA ₁	9.11 ^c ± 0.62	−14.6	1.01 ^{de} ± 0.07	−15.1	0.81 ^{de} ± 0.06	−25.7	8.19 ^c ± 0.61	−36.3
Dec. × AsA ₂	13.11 ^a ± 0.90	+22.9	1.47 ^a ± 0.12	+23.5	1.31 ^a ± 0.10	+20.2	17.22 ^a ± 1.67	+34.0
Jan. × AsA ₀	6.67 ^d ± 0.41	−37.5	0.74 ^f ± 0.05	−37.8	0.72 ^e ± 0.05	−33.9	4.81 ^d ± 0.38	−62.6
Jan. × AsA ₁	6.87 ^d ± 0.49	−35.6	0.76 ^f ± 0.04	−36.1	0.75 ^e ± 0.06	−31.2	5.76 ^d ± 0.39	−55.2
Jan. × AsA ₂	7.62 ^c ± 0.52	−28.6	0.83 ^{ef} ± 0.06	−30.3	0.73 ^e ± 0.05	−33.0	6.05 ^{cd} ± 0.43	−52.9

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “*” and “**” indicate significant differences at the $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

3.2.3. Effect of Integrative Seed and Leaf Treatment with Ascorbic Acid (AsA) on Macro- and Micronutrient Contents of Parsley Plants Grown under Different Sowing Dates (SDs)

Regarding SDs, there was a gradual decrease in chlorophyll (Chls), macronutrient (N, P, and K), and micronutrient (Fe, Mn, and Zn) contents by gradually delaying the sowing of parsley seeds from October to January. The highest values of Chls, N, P, K, Fe, Mn, and Zn were obtained in October (the optimal planting date), while the lowest values were obtained in January (Tables 3–5).

Concerning AsA levels, the 2.0 mM level significantly outperformed the 1.0 mM level, and both levels significantly increased the contents of Chls, N, P, K, Fe, Mn, and Zn compared to the control (0 mM AsA). The level of 2.0 mM AsA was superior to the level of 1.0 mM (Tables 3–5).

For the combination of SDs and AsA, the combination treatments of Oct. × AsA₂, Nov. × AsA₂, and Dec. × AsA₂ had higher values for Chls (49.9, 49.7, and 49.5, respectively), N (3.29, 3.31, and 3.28%, respectively), P (0.74, 0.75, and 0.75%, respectively), K (3.07, 3.07, and 3.03%, respectively), Fe (33.3, 33.0, and 33.3 mg 100 g⁻¹ DW, respectively), Mn (20.5, 20.9, and 20.4 mg 100 g⁻¹ DW, respectively), and Zn (9.92, 9.98, and 9.93 mg 100 g⁻¹ DW, respectively) than the other combination treatments and significantly exceeded the control values. However, AsA at any concentration (1 mM or 2 mM) failed to enable plants grown in January to give results close to the control values (Tables 3–5). This means that the planting of parsley can be delayed from its optimum date (October) until December

and obtain higher growth traits with the application of AsA as an integrative treatment (seed soaking + foliar spray).

Table 3. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on macronutrient contents of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	N (%)	% of Cont.	P (%)	% of Cont.	K (%)	% of Cont.
Sowing date (SD)	*		*		*	
Oct.	2.98 ^a ± 0.09	0.71 ^a ± 0.02	2.92 ^a ± 0.11
Nov.	2.78 ^a ± 0.09	−6.71	0.70 ^a ± 0.02	−1.41	2.89 ^a ± 0.11	−1.03
Dec.	2.44 ^b ± 0.07	−18.1	0.66 ^b ± 0.02	−7.04	2.66 ^b ± 0.09	−8.90
Jan.	1.72 ^c ± 0.05	−42.3	0.54 ^c ± 0.01	−23.9	2.37 ^c ± 0.06	−18.8
AsA levels	*		*		*	
0 mM (AsA ₀)	2.10 ^c ± 0.06	0.60 ^c ± 0.02	2.53 ^c ± 0.08
1.0 mM (AsA ₁)	2.43 ^b ± 0.07	+15.7	0.64 ^b ± 0.02	+6.67	2.69 ^b ± 0.09	+6.32
2.0 mM (AsA ₂)	2.91 ^a ± 0.10	+38.6	0.72 ^a ± 0.03	+20.0	2.91 ^a ± 0.11	+15.0
SD × AsA levels	*		*		*	
Oct. × AsA ₀	2.71 ^b ± 0.07	0.68 ^{bc} ± 0.02	2.80 ^{abc} ± 0.10
Oct. × AsA ₁	2.95 ^{ab} ± 0.09	+8.86	0.71 ^{ab} ± 0.02	+4.41	2.90 ^{ab} ± 0.11	+3.57
Oct. × AsA ₂	3.29 ^a ± 0.11	+21.4	0.74 ^{ab} ± 0.03	+8.82	3.07 ^a ± 0.13	+9.64
Nov. × AsA ₀	2.10 ^c ± 0.06	−22.5	0.64 ^{cd} ± 0.02	−5.88	2.67 ^{bcd} ± 0.08	−4.64
Nov. × AsA ₁	2.92 ^{ab} ± 0.09	+7.75	0.71 ^{ab} ± 0.02	+4.41	2.93 ^{ab} ± 0.11	+4.64
Nov. × AsA ₂	3.31 ^a ± 0.11	+22.1	0.75 ^a ± 0.03	+10.3	3.07 ^a ± 0.13	+9.64
Dec. × AsA ₀	1.90 ^c ± 0.05	−29.9	0.58 ^d ± 0.02	−14.7	2.37 ^{ef} ± 0.07	−15.4
Dec. × AsA ₁	2.15 ^c ± 0.06	−20.7	0.64 ^{cd} ± 0.02	−5.88	2.57 ^{cde} ± 0.08	−8.21
Dec. × AsA ₂	3.28 ^a ± 0.11	+21.0	0.75 ^a ± 0.03	+10.3	3.03 ^a ± 0.12	+8.21
Jan. × AsA ₀	1.69 ^c ± 0.04	−37.6	0.50 ^e ± 0.01	−26.5	2.27 ^f ± 0.06	−18.9
Jan. × AsA ₁	1.71 ^c ± 0.05	−36.9	0.51 ^e ± 0.01	−25.0	2.37 ^{ef} ± 0.05	−15.4
Jan. × AsA ₂	1.75 ^c ± 0.05	−35.4	0.62 ^{cd} ± 0.02	−8.82	2.47 ^{def} ± 0.07	−11.8

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “***” indicates significant differences at the $p \leq 0.05$ probability level. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

Table 4. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on micronutrient contents of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	Fe (mg 100 g ^{−1} DW)	% of Cont.	Mn (mg 100 g ^{−1} DW)	% of Cont.	Zn (mg 100 g ^{−1} DW)	% of Cont.
Sowing date (SD)	*		*		*	
Oct.	30.6 ^a ± 1.3	18.4 ^a ± 0.9	9.10 ^a ± 0.31
Nov.	30.6 ^a ± 1.3	−0.00	18.2 ^a ± 0.9	−1.09	9.01 ^a ± 0.31	−0.99
Dec.	27.7 ^b ± 1.2	−9.45	16.7 ^b ± 0.9	−9.24	8.20 ^b ± 0.25	−9.89
Jan.	24.2 ^c ± 1.0	−20.9	14.0 ^c ± 0.7	−23.9	7.28 ^c ± 0.19	−20.0
AsA levels	*		*		*	

Table 4. Cont.

Source of Variation	Fe (mg 100 g ⁻¹ DW)	% of Cont.	Mn (mg 100 g ⁻¹ DW)	% of Cont.	Zn (mg 100 g ⁻¹ DW)	% of Cont.
0 mM (AsA ₀)	24.8 ^c ± 1.0	14.7 ^c ± 0.7	7.42 ^c ± 0.19
1.0 mM (AsA ₁)	28.9 ^b ± 1.2	+16.5	16.7 ^b ± 0.8	+13.6	8.23 ^b ± 0.25	+10.9
2.0 mM (AsA ₂)	31.3 ^a ± 1.4	+26.2	19.1 ^a ± 1.0	+29.9	9.55 ^a ± 0.35	+28.7
SD × AsA levels	*		*		*	
Oct. × AsA ₀	27.0 ^b ± 1.1	16.2 ^d ± 0.8	8.14 ^{cd} ± 0.25
Oct. × AsA ₁	31.6 ^a ± 1.3	+17.0	18.5 ^c ± 0.9	+14.2	9.25 ^{ab} ± 0.31	+13.6
Oct. × AsA ₂	33.3 ^a ± 1.5	+23.3	20.5 ^{ab} ± 1.0	+26.5	9.92 ^a ± 0.36	+21.9
Nov. × AsA ₀	26.6 ^b ± 1.1	-1.48	15.0 ^{def} ± 0.8	-7.41	7.76 ^{cde} ± 0.21	-4.67
Nov. × AsA ₁	32.6 ^a ± 1.3	+20.7	18.8 ^{bc} ± 0.9	+16.0	9.29 ^{ab} ± 0.33	+14.1
Nov. × AsA ₂	33.0 ^a ± 1.5	+22.2	20.9 ^a ± 1.1	+29.0	9.98 ^a ± 0.40	+22.6
Dec. × AsA ₀	23.6 ^{bc} ± 0.9	-12.6	14.4 ^{def} ± 0.7	-11.1	7.19 ^{def} ± 0.18	-11.7
Dec. × AsA ₁	26.3 ^b ± 1.1	-2.59	15.2 ^{de} ± 0.8	-6.17	7.48 ^{cdef} ± 0.20	-8.11
Dec. × AsA ₂	33.3 ^a ± 1.6	+23.3	20.4 ^{abc} ± 1.1	+25.9	9.93 ^a ± 0.37	+22.0
Jan. × AsA ₀	22.0 ^c ± 0.9	-18.5	13.1 ^f ± 0.6	-19.1	6.58 ^f ± 0.13	-19.2
Jan. × AsA ₁	25.0 ^{bc} ± 1.0	-7.41	14.2 ^{ef} ± 0.7	-12.3	6.90 ^{ef} ± 0.15	-15.2
Jan. × AsA ₂	25.6 ^{bc} ± 1.0	-5.19	14.6 ^{def} ± 0.7	-9.88	8.35 ^{bc} ± 0.28	-2.58

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “*” indicates significant differences at the $p \leq 0.05$ probability level. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

Table 5. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on chlorophyll, total soluble sugar, and antioxidant (AsA: vitamin C and proline) contents of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	Chls (SPAD)	% of Cont.	S. Sugars (mg g ⁻¹ DW)	% of Cont.	Proline (mmol g ⁻¹ DW)	% of Cont.	AsA (mg 100 g ⁻¹ FW)	% of Cont.
Sowing date (SD)	*		**		*		*	
Oct.	46.3 ^a ± 2.3	15.8 ^c ± 0.5	0.31 ^c ± 0.02	84.4 ^c ± 2.8
Nov.	44.1 ^a ± 2.2	-4.75	22.4 ^b ± 0.7	+41.8	0.37 ^b ± 0.02	+19.4	94.4 ^b ± 3.3	+11.8
Dec.	37.9 ^b ± 1.8	-18.1	28.7 ^a ± 0.9	+81.6	0.41 ^a ± 0.03	+32.3	102.4 ^a ± 3.6	+21.3
Jan.	29.9 ^c ± 1.3	-35.4	22.7 ^b ± 0.7	+43.7	0.32 ^c ± 0.02	+3.23	94.0 ^b ± 3.4	+11.4
AsA levels	*		**		**		*	
0 mM (AsA ₀)	33.9 ^c ± 1.5	16.1 ^c ± 0.5	0.28 ^c ± 0.01	83.5 ^c ± 2.8
1.0 mM (AsA ₁)	38.5 ^b ± 1.9	+13.6	22.3 ^b ± 0.7	+38.5	0.35 ^b ± 0.02	+25.0	92.6 ^b ± 3.2	+10.9
2.0 mM (AsA ₂)	46.4 ^a ± 2.3	+36.9	28.8 ^a ± 1.0	+78.9	0.43 ^a ± 0.03	+53.6	105.3 ^a ± 3.8	+26.1
SD × AsA levels	*		**		**		*	
Oct. × AsA ₀	43.2 ^c ± 2.1	11.2 ^h ± 0.3	0.24 ^g ± 0.01	74.5 ^e ± 2.3
Oct. × AsA ₁	45.9 ^{bc} ± 2.3	+6.25	16.7 ^{fg} ± 0.5	+49.1	0.31 ^f ± 0.02	+29.2	84.9 ^d ± 2.9	+14.0
Oct. × AsA ₂	49.9 ^a ± 2.5	+15.5	19.6 ^{def} ± 0.6	+75.0	0.39 ^{cd} ± 0.02	+62.5	93.7 ^c ± 3.3	+25.8
Nov. × AsA ₀	36.6 ^d ± 1.7	-15.3	16.4 ^g ± 0.5	+46.4	0.30 ^f ± 0.01	+25.0	82.7 ^d ± 2.8	+11.0
Nov. × AsA ₁	46.0 ^{bc} ± 2.4	+6.48	23.5 ^c ± 0.7	+110	0.37 ^d ± 0.02	+54.2	93.8 ^c ± 3.2	+25.9
Nov. × AsA ₂	49.7 ^{ab} ± 2.5	+15.0	27.2 ^b ± 0.8	+143	0.45 ^b ± 0.03	+87.5	106.7 ^{ab} ± 3.9	+43.2
Dec. × AsA ₀	31.5 ^e ± 1.3	-27.1	19.8 ^{de} ± 0.6	+76.8	0.34 ^e ± 0.02	+41.7	93.9 ^c ± 3.1	+26.0

Table 5. Cont.

Source of Variation	Chls (SPAD)	% of Cont.	S. Sugars (mg g ⁻¹ DW)	% of Cont.	Proline (mmol g ⁻¹ DW)	% of Cont.	AsA (mg 100 g ⁻¹ FW)	% of Cont.
Dec. × AsA ₁	32.7 ^{de} ± 1.5	−24.3	28.1 ^b ± 0.8	+151	0.40 ^c ± 0.03	+66.7	99.6 ^{bc} ± 3.5	+33.7
Dec. × AsA ₂	49.5 ^{ab} ± 2.5	+14.6	38.1 ^a ± 1.4	+240	0.48 ^a ± 0.03	+100	113.8 ^a ± 4.1	+52.8
Jan. × AsA ₀	24.1 ^f ± 1.0	−44.2	17.1 ^{efg} ± 0.5	+52.7	0.25 ^g ± 0.01	+4.17	82.9 ^d ± 2.9	+11.3
Jan. × AsA ₁	29.3 ^e ± 1.2	−32.2	20.8 ^{cd} ± 0.6	+85.7	0.32 ^{ef} ± 0.02	+33.3	92.1 ^c ± 3.2	+23.6
Jan. × AsA ₂	36.3 ^d ± 1.6	−16.0	30.1 ^b ± 1.1	+169	0.39 ^{cd} ± 0.02	+62.5	107.1 ^{ab} ± 4.0	+43.8

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “**” and “***” indicate significant differences at the $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

3.2.4. Effect of Integrative Seed and Leaf Treatment with Ascorbic Acid (AsA) on Osmoprotectant and Antioxidant Contents of Parsley Plants Grown under Different Sowing Dates (SDs)

For SDs, there was a gradual increase in osmoprotectant and antioxidant (e.g., total soluble sugars, free proline, and AsA) contents by gradually delaying the sowing of parsley seeds from October to December, while the contents tended to decrease in January, but they still exceeded the control (Table 5).

Regarding AsA levels, the 2.0 mM level significantly outperformed the 1.0 mM level, and both levels significantly increased the contents of total soluble sugars, free proline, and AsA compared to the control (0 mM AsA). The level of 2.0 mM AsA was superior to the level of 1.0 mM (Table 5).

For the combination of SDs and AsA, the combination treatment of Dec. × AsA₂ had the highest values for total soluble sugars (38.1 mg g⁻¹ DW), free proline (0.48 mmol g⁻¹ DW), and AsA (113.8 mg 100 g⁻¹ FW) compared to the other combination treatments and significantly exceeded the control values. With the higher concentration outperforming the lower concentration, either 1.0 mM or 2.0 mM AsA succeeded in increasing the contents of soluble sugars, proline, and AsA in plants grown in January more than the control (Table 5). This means that the planting of parsley can be delayed from its optimum date (October) until December and obtain higher growth traits with the application of AsA as an integrative treatment (seed soaking + foliar spray).

3.2.5. Effect of Integrative Seed and Leaf Treatment with Ascorbic Acid (AsA) on Enzymatic Antioxidant Activities of Parsley Plants Grown under Different Sowing Dates (SDs)

For SDs, there was a gradual increase in catalase (CAT) and superoxide dismutase (SOD) activities by gradually delaying the sowing of parsley seeds from October to December, while the activities tended to decrease in January, but they still exceeded the control. However, peroxidase (POD) activity was not affected by any SD (Table 6).

Regarding AsA levels, the 2.0 mM level significantly outperformed the 1.0 mM level, and both levels significantly increased the activities of CAT and SOD, while POD activity was not affected compared to the control (0 mM AsA). The level of 2.0 mM AsA was superior to the level of 1.0 mM (Table 6).

For the combination of SDs and AsA, the combination treatment of Dec. × AsA₂ had the highest activities for CAT (36.0 Unit g⁻¹ FW min⁻¹) and SOD (2.67 Unit g⁻¹ FW min⁻¹) compared to the other combination treatments and significantly exceeded the control. With the higher concentration outperforming the lower concentration, either 1.0 mM or 2.0 mM AsA succeeded in increasing the activities of CAT and SOD in plants grown in January more than the control (Table 6). This means that the planting of parsley can be delayed from its optimum date (October) until December and obtain higher growth traits with the application of AsA as an integrative treatment (seed soaking + foliar spray).

Table 6. Effect of integrative treatment of seeds and leaves with ascorbic acid (AsA) on enzymatic antioxidant activities of parsley plants under different sowing dates vs. October. All values are the average of the 2018/2019 and 2019/2020 seasons.

Source of Variation	Catalase (Unit g ⁻¹ FW min ⁻¹)	% of Cont.	Peroxidase (Unit g ⁻¹ FW min ⁻¹)	% of Cont.	Superoxide Dismutase (Unit g ⁻¹ FW min ⁻¹)	% of Cont.
Sowing date (SD)	*		ns		*	
Oct.	24.7 ^c ± 0.5	2.02 ^a ± 0.04	1.68 ^c ± 0.03
Nov.	28.5 ^b ± 0.6	+15.4	2.02 ^a ± 0.05	1.97 ^b ± 0.04	+17.3
Dec.	33.2 ^a ± 0.7	+34.4	2.04 ^a ± 0.05	2.26 ^a ± 0.05	+34.5
Jan.	28.8 ^b ± 0.6	+16.6	2.05 ^a ± 0.05	1.93 ^b ± 0.04	+14.9
AsA levels	*		ns		*	
0 mM (AsA ₀)	26.0 ^c ± 0.5	2.03 ^a ± 0.05	1.64 ^c ± 0.03
1.0 mM (AsA ₁)	29.4 ^b ± 0.6	+13.1	2.03 ^a ± 0.05	1.99 ^b ± 0.04	+21.3
2.0 mM (AsA ₂)	31.0 ^a ± 0.6	+19.2	2.05 ^a ± 0.05	2.26 ^a ± 0.05	+37.8
SD × AsA levels	*		ns		*	
Oct. × AsA ₀	23.3 ^e ± 0.5	2.02 ^a ± 0.05	1.40 ^e ± 0.02
Oct. × AsA ₁	24.5 ^{de} ± 0.5	+5.15	2.01 ^a ± 0.04	1.67 ^{de} ± 0.03	+19.3
Oct. × AsA ₂	26.3 ^{cd} ± 0.5	+12.9	2.03 ^a ± 0.04	1.98 ^{bc} ± 0.04	+41.4
Nov. × AsA ₀	25.9 ^{de} ± 0.5	+11.2	1.99 ^a ± 0.04	1.67 ^{de} ± 0.03	+19.3
Nov. × AsA ₁	28.9 ^{bc} ± 0.6	+24.0	2.03 ^a ± 0.05	1.98 ^{bc} ± 0.04	+41.4
Nov. × AsA ₂	30.8 ^b ± 0.6	+32.2	2.04 ^a ± 0.05	2.27 ^b ± 0.05	+62.1
Dec. × AsA ₀	29.3 ^b ± 0.6	+25.8	2.03 ^a ± 0.05	1.88 ^{cd} ± 0.04	+34.3
Dec. × AsA ₁	34.3 ^a ± 0.7	+47.2	1.99 ^a ± 0.04	2.23 ^b ± 0.05	+59.3
Dec. × AsA ₂	36.0 ^a ± 0.7	+54.5	2.10 ^a ± 0.06	2.67 ^a ± 0.06	+90.7
Jan. × AsA ₀	25.3 ^e ± 0.5	+8.58	2.07 ^a ± 0.05	1.60 ^{de} ± 0.03	+14.3
Jan. × AsA ₁	30.0 ^b ± 0.6	+28.8	2.07 ^a ± 0.05	2.07 ^{bc} ± 0.04	+47.9
Jan. × AsA ₂	31.0 ^b ± 0.6	+33.0	2.01 ^a ± 0.04	2.13 ^{bc} ± 0.04	+52.1

Values (means ± SE) with similar letters are not significantly different at $p \leq 0.05$. “*” indicates significant differences at the $p \leq 0.05$ probability level, and “ns” indicates no significant difference. As an integrative treatment, AsA was applied to seeds as a seed soaking treatment and plant leaves as a foliar spray.

3.2.6. Essential Oil Fractions of the Obtained Seeds of Parsley Plants as Affected by Integrative Seed + leaf Treatment with Ascorbic Acid (AsA) under Different Sowing Dates (SDs)

The analysis of the essential oils by GC-MS identified about 42 components (oil fractions) in different percentages (Table S4). There were many fluctuations in the oil fractions under different SDs. Under the SDs, especially in December (14.4–15.7 °C) and January (12.6–13.6 °C), the integrative treatment of seeds + leaves with 2.0 mM AsA altered the ratios of oil fractions present in the essential oil of the yielded seeds. Moreover, at the beginning of the RT time, there was an increase in some fractions using the control (optimal sowing date; 24.0 °C) such as α -Pinene, Limonene, β -Pinene, Farnesole, o-Pyrocatechuic acid, D-Limonene, γ -Terpinene, α -Fenchene, 3-Carene, α -Asarone, Cis-Verbenone, Apiol, and p-Thymol, while this increase shifted to other fractions at the end of the RT time such as Phytanic acid, Epimedin A, B, and C, Quercetin, Schaftoside, Geranyl isovalerate, Glyceryl Monooleate, and 9-Octadecenoic acid (Z)-. The highest fraction of the parsley oil composition was Myristicine, which was greatly affected by the integrative seed + leaf treatment with 2.0 mM AsA with sowing in December (15.7 °C) by giving a

high value (48.05 of the peak area; Figure S2) compared to the control (24.0 °C). However, this oil fraction tended to decrease in January (13.6 °C) compared to the control (24.0 °C). Additionally, Apiol had the second highest value (29.11% of the peak area; Figure S2) under the control conditions with the integrative treatment with 2.0 mM AsA. β -Pinene and α -Terpinolene contents considerably increased in November (19.4 °C) with the integrative treatment with 2.0 mM AsA. The maximum values of Limonene and Farnesole were recorded for the plants grown under the control conditions with the integrative treatment with 1.0 mM AsA. For other oil fractions, in general, the coldest sowing dates with AsA treatment collected higher oil fraction contents compared to the control (24.0 °C).

4. Discussion

The steady increase in the world's population with the need to secure food from economic food crops may lead to the occupation of agricultural land for long periods of the year, resulting in the cultivation of other crops such as parsley at inappropriate sowing dates (late sowing). The sowing date (SD) is one of the essential agronomic factors that can affect crop growth and productivity due to fluctuations in environmental conditions. Therefore, the right SD is a prerequisite [41]. An optimal SD paves the way for better exploitation of time, light, temperature, precipitation, and other factors. As obtained from a preliminary study (Table S3), the optimal SD for parsley is during October. This finding is probably due to the temperature prevailing during October, which maintains the optimum relative humidity and light intensity, which may have given the plants a sufficient opportunity for photosynthesis and been more favorable for better germination, thus improving seed germination and the emergence of seedlings [42]. As a result, there was an improvement in growth traits, seed and oil yields, nutrient and antioxidant homeostasis, and osmotic compounds (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6 and Table S4). Additionally, the improvement in the chlorophyll content (Table 5) leads to an increase in net photosynthesis towards the reproduction structure, which may increase the attributes of all yields significantly [43]. Additionally, the increase in nutrient (e.g., N, P, K, Fe, Mn, and Zn) contents and soluble sugars due to cultivation in the optimum SD is probably because early cultivated plants had a relatively long time for their vegetative growth. Therefore, the uptake of these nutrients is improved, obtaining higher plant growth qualities because the environmental conditions are more favorable, thus giving higher yields of seeds and essential oils. This finding is consistent with the results obtained on some other medicinal plants [44–46].

Late sowing of parsley caused a significant decrease in the growth traits (Table 1), which was attributed to fluctuations in climatic conditions, especially the lower temperature and light intensity, as well as the reduced growth period [47]. Early cultivation of crops may have enjoyed better environmental conditions, especially temperature and solar radiation, which resulted in the best plant growth [48]. Additionally, late sowing of parsley caused a significant decrease in seed and oil yield components (Table 2), which was attributed to the lower production of photosynthates due to the short growing period and low light intensity [47,48]. As one of the adverse conditions caused by late parsley sowing (December and January), low temperature (LT) delays the plant physiological response due to an inhibition caused by stress of plant metabolism and photosystem II activity. It also damages cell membranes while destabilizing the phospholipid layers [44]. Late planting reduces the yield as it harms plant growth, the flowering duration, and seed formation and size. Therefore, January sowing gave the lowest seed yield due to the short lifespan and lack of plant canopy. Additionally, some reports have shown that the negative influences of LT reflect reduced elongation of seedling roots and shoots, and accumulated biomass, leading to reduced seed and oil yields and essential oil composition [14,49,50]. These negative findings may be due to the negative impacts on seedling water relations and nutrient uptake [51,52]. Under LT, disturbance in seedling morphology is a secondary expression of LT-stimulated damage to cellular organelles and its interference with major physiological processes [13]. LT might have reduced seedling growth by inhibiting cell

elongation, cell division, and metabolism in plant tissues [53]. The degree of the negative impact of LT on the vegetative growth of the plant depends on the growth stage [54] and planting date, as shown in the current study (Table 1).

To cope with late sowing influences (LSI), plants develop/adopt many rigorous regulating mechanisms, using several biostimulators known to minimize harmful LSI [47,48,55] such as ascorbic acid (AsA). Among the strategies used to induce tolerance to abiotic stress in plants are seed soaking or foliar spray treatments [14,50]. As shown in the current study, 1.0 or 2.0 mM AsA was used as an integrative treatment (seed soaking + foliar spraying) for parsley planted under LSI (November–January) vs. the ideal SD (October). Plants use multiple pathways to synthesize AsA, reflecting the importance of this molecule in plant health [56]. However, plants require additional exogenous AsA to support their endogenous mechanisms under the stress of LSI [14,21–23]. In this study, all results show that both levels (1.0 and 2.0 mM) of AsA significantly improved growth traits, seed and oil yield components, the contents of nutrients, chlorophyll, osmotic compounds, and antioxidants, including endogenous AsA, and enzymatic activities. However, 2.0 mM AsA outperformed the 1.0 mM AsA level in all the obtained results (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6 and Table S4). AsA applied at 2.0 mM succeeded in conferring similar results under LSI of November and December, which significantly exceeded the results obtained by the control (October). However, it failed to confer satisfactory results (similar to the control) under LSI of January.

The importance of AsA for plants grown under stress is that it is an essential component of the ascorbate-glutathione pathway [57]. It detoxifies developmental reactive oxygen species (ROS) generated during photosynthesis or metabolism under stress [56,57]. The participation of AsA in the regulation of cell elongation and development during the cell cycle [58] and also in gibberellic acid (GA₃) biosynthesis [59] can enable the plant to have the best growth parameters (Table 1). AsA also regulates the flowering time, cell division (during embryonic development), the cell cycle, and different stress responses. Azoz et al. [60] reported similar results on *Ocimum basilicum*.

Stressed plants create and accumulate various osmoprotectant substances and compatible solutes as an effective mechanism to protect them from stress damage [61]. Osmoprotectant substances as components of the antioxidant system are among the master controllers of ROS levels in plant cells [62]. Under LSI, in this study, the contents of osmoprotectant substances (soluble sugars and proline) and the levels of antioxidants (soluble sugars, proline, and AsA) were significantly increased and further increased by the integrative AsA treatment (Table 5). As an essential amino acid, proline participates in protecting plants from the effects of stress (LSI) due to its biologically active participation in the regulation of osmosis and antioxidant activity [63]. It mediates stress signaling, eliminates ROS, and maintains cellular osmolarity and photosystem II functioning under stress conditions [13,14]. In this study (Table 5), AsA-mediated enhancement in AsA-metabolizing pathways and proline biosynthesis and accumulation strengthened the antioxidant system [64–66]. The elevation in proline associated with stress and AsA treatment could be because it is a compatible solute to maintain osmotic regulation to enable plants to adapt to undesirable conditions [67]. One of the reasons that lead to the maintenance of osmotic regulation is the ionic balance caused by the increase in parsley plant nutrients by AsA under normal or stress conditions (Tables 3 and 4). However, the mechanism of AsA-mediated ion homeostasis remains obscure, but it is likely due to the enhanced stability of cellular membranes and the plasma membrane Na⁺-H⁺ antiporter SOS1 [68]. Thus, AsA could be involved in promoting the uptake of nutrients, which are the main growth-limiting components to attenuate LSI. As another conceivable explanation, the higher uptake of nutrients (e.g., N, P, K, Fe, Mn, and Zn) in plants treated with AsA may have directly affected the metabolism by regulating essential functions such as the activity of enzymes, biosynthesis of hormones, proteins, and chlorophyll, and regulation of osmosis [69,70].

Under LSI, the contents of osmoprotectants and antioxidants and the activities of antioxidant enzymes (e.g., superoxide dismutase (SOD) and catalase (CAT)) in parsley

plants were noticeably increased (Tables 5 and 6). Merwad et al. [71] found that, under stress conditions, different enzymatic and non-enzymatic antioxidants increase and evolve in the plant to scavenge different types of ROS (e.g., superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)). As is known, SOD dismantles $O_2^{\bullet-}$ to H_2O_2 [72], which in turn is decomposed to O and H_2O by CAT [73] via the ascorbate-glutathione cycle in association with antioxidant enzymes [74]. AsA enhances the scavenging activities of metal ion chelators and ROS, which is a fascinating part of abiotic stress responses in plant cells. As a result, the damage caused by ROS overproduction will mitigate and repair [71]. This finding may allow light to permeate smoothly, delay leaf senescence, increase enzymatic activity and chlorophyll contents by blocking the destruction of chlorophyll, and increase photosynthesis to supply developing tissues, which are associated with the mitigation of stress damage [75]. Additionally, AsA is used as an electron donor by ascorbate peroxidase to remove H_2O_2 . These findings indicate that the role of the ascorbate-glutathione cycle and the improved contents and activities of different antioxidants in LSI-stressed parsley plants treated with AsA (Tables 5 and 6) is to remove excessive ROS. These mechanisms (removal of excess ROS) protect plants from the accumulation of ROS, as well as from reducing growth traits and yield (seed and oil) components (Tables 1 and 2) [76]. AsA enhances the protective mechanisms in parsley plants to counteract LSI. It enhanced the uptake of different nutrients under LSI and thus chlorophyll biosynthesis and contents (Tables 3–5). As a result, there were improvements in plant growth and yield performances under LSI (Tables 1 and 2). Desoky et al. [22] reported the same findings on *Vicia faba* plants under drought stress.

In this study, the integrative AsA treatment significantly increased different essential oil components of parsley seeds, particularly Myristicine or Apiol (Table S4). A similarity was found between the chemical composition of Myristicine ($C_{11}H_{12}O_3$) or Apiol ($C_{12}H_{14}O_4$) and AsA ($C_6H_8O_6$). This result could be due to the effect of AsA, which is known as a plant growth regulator, on the growth rate and the quality and quantity of essential oils of medicinal and aromatic plants [77]. Additionally, AsA participates in regulating several biological processes such as enzymatic reactions. It is a co-factor for enzymes necessary for the regulation of photosynthesis, biosynthesis of hormones, cell division and elongation, and participation in signal transduction [56,78]. A study was conducted in [79] to emphasize that the optimization of the role of AsA in promoting essential oil production is reached by regulating the enzyme activity cascade and facilitating enzymatic reactions. Additionally, Reda et al. [80] added that the beneficial effects of AsA could be attributed to the enhanced capacity of meristematic cells to synthesize the bioactive substrates required for essential oil biosynthesis. Ranjbar et al. [81] also reported that the positive effects of AsA on both the oil content and *Matricaria chamomilla* (L.) yields are enhanced by AsA. Similar results have been reported by other researchers on some medicinal plants [82–85].

5. Conclusions

It is preferable to cultivate parsley early (1 October) to achieve abundant growth and yields (seeds and oil). However, higher growth and yields can be obtained under late sowing influences (November and December) by integrative seed and leaf treatment with ascorbic acid, especially at a level of 2.0 mM. Therefore, parsley can be grown at late dates with integrative ascorbic acid treatment, in the interest of cultivating economic food crops to meet the steady increase in the world's population and climatic changes. However, the planting of parsley cannot be delayed until January, as severe late sowing influences lead to an unsatisfactory loss of parsley yield, even with integrative treatment with ascorbic acid. This loss in parsley yields is due to lower growth parameters, chlorophyll contents, and nutrient uptake. This study recommends using 2.0 mM AsA as an integrative (seed soaking + foliar spraying) treatment with the possibility of delaying the planting of parsley until December in favor of economic food crops.

Supplementary Materials: The following are available online at: <https://www.mdpi.com/article/10.3390/horticulturae8040334/s1>. Figure S1: Monthly average of meteorological data of the experimental farm, Figure S2: GC-MS chromatograms of parsley seed oil as affected by AsA treatments and low-temperature stress, Table S1: Initial physico-chemical characteristics of the studied soils at a depth of 0–25 cm in two growing seasons, Table S2: Monthly average of meteorological data of the experimental farm during 2018/2019 and 2019/2020 seasons, Table S3: A preliminary study to identify the optimal sowing date, seed soaking period, foliar spray times, and ascorbic acid (AsA) concentrations for parsley, and Table S4: Fractionation of essential volatile oil of the produced parsley seeds (identified by Gas Chromatography-Mass Spectroscopy).

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