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GIS-Facilitated Seed Germination, Fertilization Effects on Growth, Nutrient and Phenol Contents and Antioxidant Potential in Three Local Endemic Plants of Crete (Greece) with Economic Interest: Implications for Conservation and Sustainable Exploitation

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Abstract: This multidisciplinary study is focused on the conservation and sustainable utilization of *Lomelosia minoana* (Dipsacaceae; subsp. *minoana* and subsp. *asterusica*) and *Eryngium ternatum* (Apiaceae), three local endemic plants of Crete (Greece) with economic interest. Using Geographical Information Systems and open-source geodatabases, detailed ecological profiles were compiled to illustrate the abiotic environmental conditions prevailing in their wild habitats. We examined for the first time temperature effects (10, 15, 20 and 25 °C) on seed germination and fertilization effects (INM, integrated nutrient management, and chemical fertilization compared to control) on growth parameters and nutrient content of leaves as well as their phenol content and antioxidant potential. *L. minoana* subsp. *asterusica* germinated better at 15 °C (61.25%), subsp. *minoana* at 10 and 15 °C (30% and 27.50%, respectively) while *E. ternatum* did not show significant differences. The seedling fertilization with INM resulted in 10–15-fold higher absorption of copper without toxicity compared with chemical fertilization and the control; INM was also superior to chemical fertilization in most of the macronutrients in leaves. The total phenol content and the antioxidant capacity of leaf extracts were positively affected by chemical fertilization in *L. minoana* subsp. *minoana* and *E. ternatum*. Both fertilization treatments almost equally affected the morphological and physiological characteristics of the examined taxa. In light of the above-mentioned and the research gaps bridged for the studied taxa, we re-evaluated and updated both the feasibility and the readiness timescale for their sustainable exploitation in economic sectors.

Keywords: biodiversity; chlorophyll content; ecological profiles; integrated nutrient management; neglected and underutilized species; nutrient content

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1. Introduction

Aside from the overall appeal and appreciation of the Mediterranean region as an important biodiversity hotspot [1], most of this extraordinarily high plant diversity includes neglected and underutilized plant species (NUPs) [2]. Due to weak documentation of the extant potential and lack of coordinated actions about these important phytogenetic resources, most of the NUPs are not sustainably exploited by the agricultural sector [3,4] or the medicinal-cosmetic [5] and ornamental-horticultural industries [1,6]. During the last decade, increasing attention has been given to integrated conservation actions concerning rare local endemic plant species, such as NUPs, many of which are often threatened with extinction [1,4,5]. After multifaceted evaluation in different economic sectors [1,4,5] and targeted studies to bridge extant research gaps [7–21], the possible utilization of several NUPs has been outlined as important, interesting, or promising in different economic sectors. Currently, at least some of the Mediterranean NUPs, such as *Sideritis syriaca* L. subsp. *syriaca*, are considered as new industrial crops [22]. In this framework, several wild-growing members of the genus *Lomelosia* (including the closely related *Scabiosa*) of the Dipsacaceae family are appreciated in the ornamental/horticultural industry as new flower crops [23] as xerophytes in landscaping [24,25] and for green roof plantations [26]. At the same time, members of the genus *Eryngium* (Apiaceae) are wild-sourced or cultivated as vegetables or edible greens, and they are often used as ornamental species or medicinal plants in folk medicine to treat various inflammatory disorders, fever, diarrhea, hypertension, edema, sinusitis and snake or scorpion bites [27,28].

In general, species-specific information about the most suitable and effective propagation technique for every target species prioritized by biological conservation needs or triggered due to possible economic interest is equally important, and it is needed in every attempt to optimize conservation, to introduce individuals for population restoration efforts or to enable sustainable exploitation strategies [1,4,5]. For many plant species, propagation from seeds is the most common and cheapest method used in nurseries [29]. Information about the prevailing environmental conditions is required to determine the factors controlling the seed germination of target-plant species in wild habitats, as well as an understanding of how these conditions interact [30]. This knowledge may contribute to a better understanding of plant reproduction from seeds, involving life history traits, adaptation to habitats and physiological processes [30]. Cultivation-wise, and especially in harsh Mediterranean environments, the quality of planting stock produced regardless of the propagation method employed is very important to ensure success in conservation or restoration programs and ex situ conservation actions focused on threatened species [1,31]. Various cultivation practices can positively affect the morphological and physiological characteristics of the plants produced in nurseries or ex situ facilities, and therefore, high-quality planting stock can be produced with increased outplanting success [32]. One of these practices is the use of inorganic and organic fertilizers during ex situ plant propagation to increase the nutrient reserves of propagated plants at the nursery stage before any transplantation to wild habitats is attempted [33]. For example, it is known that applications of nitrogen fertilizers in the nursery usually increase the survival and growth of seedlings when transplanted in the field [34,35].

Species-wise, the present investigation focused on three local endemic perennial plants of Crete (Greece) that are threatened with extinction [36], namely *Lomelosia minoana* (P.H. Davis) Greuter and Burdet subsp. *asterusica* (Greuter) Greuter & Burdet, *Lomelosia minoana* P.H. Davis) Greuter & Burdet subsp. *minoana* (both Dipsacaceae) and *Eryngium ternatum* Poir. (Apiaceae). Unfortunately, the seed ecology of the herein-targeted plants is limited [37]. Only an in vivo germination trial for *L. minoana* subsp. *minoana* reporting 38% germination in 60 days has been published [11]. The detailed knowledge of the germination requirements of the Cretan endemic plants studied herein can lead to conclusions about the adaption of the germination process to habitat conditions and about the effect of environmental factors affecting their seed germination. Furthermore, information referring to the production of seedlings in containers of the herein-studied Cretan endemic plants is not

currently available. With the scope to facilitate the introduction of new local endemic plants of Crete in cultivation programs for conservation purposes and sustainable exploitation, the aims of this study were: (i) Unveil the ecological preferences regarding the abiotic conditions prevailing in their wild habitats; (ii) Investigate the effect of temperature on seed germination under controlled conditions; (iii) Evaluate the effect of inorganic and organic fertilizers on seedling growth and compare the effect of fertilizers on the nutrient content of seedlings produced; (iv) Evaluate their total phenolic content and possible antioxidant potential; Finally, (v) re-evaluate the feasibility and readiness timescale for their sustainable exploitation in economic sectors in the light of all the above-mentioned examined herein.

2. Materials and Methods

2.1. Characteristics of the Local Cretan Endemic Plants

Lomelosia minoana subsp. *asterusica* (Dipsacaceae, Figure 1A–D and Table 1) is protected by the Greek Presidential Decree 67/1981 as a range-restricted and Critically Endangered [36] chamaephyte with very localized populations, which are exclusively restricted in the south-facing summit area (Kofinas) of the Asterousia mountain range (perhaps also extant on inaccessible screes at lower altitudes) in limestone habitats [38]. This taxon resembles subsp. *minoana* [38] and is mainly distinguished from the latter by its broader, suborbicular leaves, which are densely covered with long, silky and strigose hairs.

Table 1. Seed collection details and IPEN (International Plant Exchange Network) accession numbers regarding the protected and Critically Endangered endemic plants of Crete (Greece) studied herein.

Scientific Name /Family	IPEN Accession	Collection Date	Collection Site and Area	Latitude (North)	Longitude (East)
* *Eryngium ternatum* Poir./Apiaceae	GR-BBGK-1-19,1140	21 August 2019	Samaria Gorge, Chania	35.2724	23.9616
* *Lomelosia minoana* (P.H. Davis) Greuter and Burdet s subsp. *asterusica* (Greuter) Greuter & Burdet/Dipsacaceae	GR-BBGK-1-20,364	17 August 2020	Kofinas, Asterousia, Heraklion	35.1833	24.2427
Lomelosia minoana (P.H. Davis) Greuter & Burdet subsp. *minoana*/Dipsacaceae	GR-BBGK-1- 19,16	10 July 2018	Kalami, Ano Viannos, Heraklion	35.0301	25.5073

* Protected status according to the Greek Presidential Decree 67/1981.

Lomelosia minoana subsp. *minoana* (Dispacaceae, Figure 1E,F) is a range-restricted chamaephyte with widely scattered and small populations (Table 1), mostly naturally thriving on crevices of limestone cliffs and in coarse screes from 450 m to 1200 (−1800) m above sea level [38], which is assessed as Critically Endangered [36].

Eryngium ternatum (Apiaceae, Figure 1G,H) is a rare range-restricted chamaephyte with scattered populations, growing on crevices of limestone cliffs in narrow gorges from 100 m to 600 m above sea level, which is assessed as Critically Endangered [36] and is protected by the Greek Presidential Decree 67/1981 (Table 1).

Figure 1. (**A**) Wild habitat of a difficult-to-access *Lomelosia minoana* subsp. *asterusica* individual at the Kofinas summit area, Asterousia mountain range, (**B**) with flowering inflorescences (**C**) and ripe seeds collected (**D**) (photos: (**B**–**E**). Edwards, NHMC-UOC, reproduced with permission); a *Lomelosia minoana* subsp. *minoana* individual in Kalami, Ano Viannos, Crete, in late flowering (**E**) and fruiting (**F**); and an *Eryngium ternatum* individual as a rock-dweller in Samaria gorge, Crete, (**G**) from which fruiting heads were collected (**H**).

2.2. Seed Collections

Mature seeds from about 10–15 wild-growing individuals of each studied taxon were collected by hand in 2019 and 2020 just before dispersion (Table 1). The seeds were collected using special permission (182336/879 of 16 May 2019 and 64886/2959 of 6 July 2020) issued by the national competent authority, namely the Greek Ministry of Environment and Energy. After collection, the seeds of each taxon were manually cleaned, and then they were stored dry in glass containers at 3–5 °C before using them in germination experiments.

2.3. Ecological Profiles

The ecological profiles of the studied local endemic plants of Crete were generated in Geographical Information Systems (GIS), and the methodology followed was described in previously published studies [10,12,14,16,20,39]. In brief, historical climatic data of 30 s pixels were taken from WorldClim (https://www.worldclim.org/data/worldclim21.html, accessed on 13 December 2022), and ecological profiles were created for each taxon based on its distribution points of natural occurrence in the wild of Crete [38]. In this way, a variety of characteristic temperatures (minimum, maximum and average) for these distribution sites were data-mined, as well as the means of 19 standard bioclimatic variables for the taxon's distribution sites.

2.4. Germination Tests and Seed Appearance

The germination experiments were initiated in December 2020 and were conducted in the Laboratory of Floriculture, School of Agriculture, Aristotle University of Thessaloniki (Thermi, Greece). Seed germination of each taxon was investigated using temperature-controlled growth chambers. Specifically, their germination responses at four constant temperatures of 10, 15, 20 and 25 °C were evaluated. For the two subspecies of *L. minoana*, four replications of 20 seeds were used per temperature treatment. In *E. ternatum*, four replications of 15 seeds per temperature treatment were used due to the limited seed amount initially sourced from the wild (Figure 2A). The seeds were placed in 9 cm sterile plastic Petri dishes, and they were allowed to germinate on two filter papers moistened with distilled water. The Petri dishes were randomly arranged on the shelves of the growth chambers, with a 12 h light/12 h dark photoperiod, and the filter paper was kept moist as required throughout the whole experimental period. Any germinated seeds were counted and removed every five days for a period of 45 days, except for the germination test of *E. ternatum*, which lasted 65 days. An individual seed was considered germinated upon evident radicle protrusion emergence from its seed coat (Figure 2B).

E. ternatum sets flattened obovate fruits (schizocarpia with mericarps) in July and August [38], which are 2.8–4.3 mm long and 1.7–2.5 mm wide (Figure 2A,B), crowned with the lobes of the remaining hyaline calyx at the margins (calyx lobes up to 2.7 mm long and approximately 1 mm wide) with outer ridges covered lengthwise with membranous scales (comparatively longer on lateral ridges) and almost flat inner sides (suture side of mericarps).

L. minoana forms a spherical bundle (head) of densely aggregated achenes from June to September [38], which are surrounded by calyx and epicalyx; the latter has a coating tube without grooves but with elongated pits near the apex and is densely hairy with divergent hairs, and the calyx is asymmetrical with 28–30 setae (Figure 2C,D). The two subspecies (subsp. *asterusica* and subsp. *minoana*) have similar seed morphology.

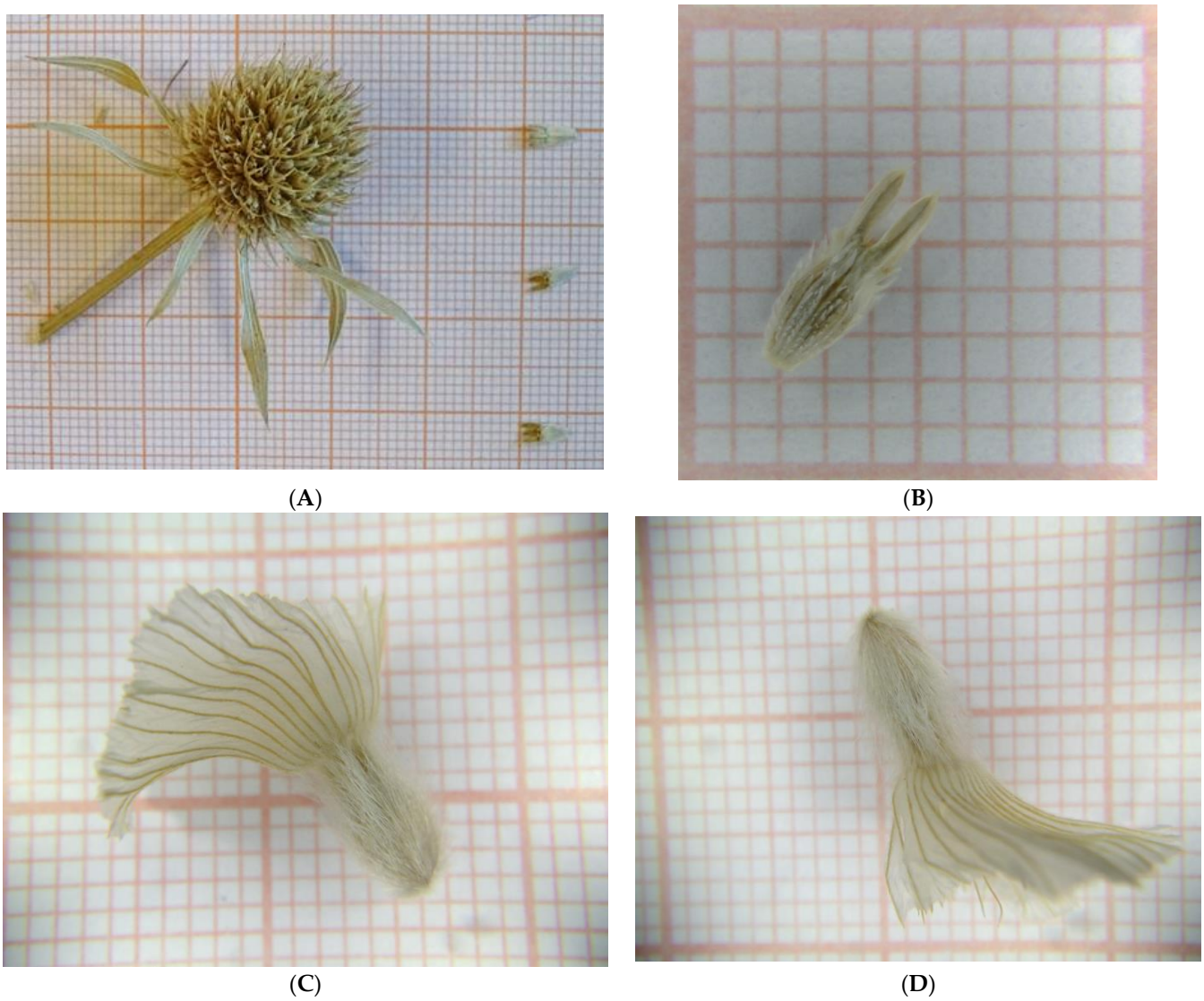


Figure 2. (**A**) Fruiting inflorescence of *Eryngium ternatum* with a limited number of extracted seeds and morphology of individual seed (**B**). (**C**,**D**) Different sides of *Lomelosia minoana* seeds.

2.5. Plant Production

The germinated seeds were used for plant production. The seeds were sown in plastic pots (6 × 6 × 6.5 cm) filled with a 3:1 ratio (*v*/*v*) mixture of enriched peat (TS1, Klassmann) and perlite. The sown seeds were carefully covered with sand, and the containers were placed on a bench in the greenhouse. Adequate amounts of water were supplied to maintain the proper moisture conditions for seedling growth.

The seedlings of the three species and subspecies (taxa) were grown in the above-mentioned pots allowing the development of a good root system. In mid-March, the seedlings of each taxon were carefully transplanted into larger pots (8.5 × 8.5 × 9.5 cm). The new pots were filled with a mixture of soil, enriched peat (TS2, Klassmann), and perlite that was prepared in a ratio of 4:5:1 (*v*/*v*). A soil sample of approximately 1.5 kg was taken and transferred to the laboratory for chemical analysis to estimate the soil fertility before the substrate preparation. The results of the chemical analysis, as well as the mechanical analysis of the soil used, are presented in Table 2.

Table 2. Chemical and physical properties of the soil used in the experimentation.

pH	Organic Matter (%)	Soluble Salts (mS/cm)	CaCO3 (%)	Mechanical Analysis		
				Sand (%)	Silt (%)	Clay (%)
8.12	0.36	0.35	5.50	56.00	28.00	16.00
Macronutrient Concentrations (ppm)						
	$N\text{-}NO_3$	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Calcium (Ca)	
	8.00	8.00	104.00	842.00	>2000	
Micronutrient Concentrations (ppm)						
	Fe	Zinc (Zn)	Manganese (Mn)	Copper (Cu)		
	4.7	2.00	7.06	0.77		

After transplanting, the seedlings of each species or subspecies were randomly divided into three groups. In *L. minoana* subsp. *minoana* and *E. ternatum*, there were five seedlings' replicates per group, whereas in *L. minoana* subsp. *asterusica* there were 15 seedlings' replicates per group. In the seedlings of the first group, integrated nutrient management (INM) was applied; in the seedlings of the second group, chemical fertilization (ChF) was applied; and in the seedlings of the last group, no fertilization was applied (control). Both types of fertilizers were applied through a foliar spray. The INM fertilization consisted of a nutrient solution of THEOCOPPER at 7 mL/L, THEOCAL at 1.5 g/L, THEOFAST at 5 mL/L, 10-47-10 (AGRI.FE.M. LTD Fertilizers, Aspropyrgos, Greece) at 3.2 g/L, K_2SO_4 (0-0-52, AGRI.FE.M. LTD Fertilizers, Aspropyrgos, Greece) at 2.07 g/L, micronutrients (Plex Mix, AGRI.FE.M. LTD Fertilizers, Aspropyrgos, Greece) at 1.5 mL/L and $MgSO_4$ (Mg 25.6%, AGRI.FE.M. LTD Fertilizers, Aspropyrgos, Greece) at 0.6 g/L [13,19]. For the conventional fertilization (ChF), the nutrient solution that was used consisted of NH_4NO_3 (34,4-0-0, Neofert®, Neochim PLC, Dimitrovgrad, Bulgaria) at 2.7 g/L, $Ca(NO_3)_2$ (NITROCAL, Agrohimiki, Greece) at 1.7 g/L, 10-47-10 at 3.2 g/L, K_2SO_4 (0-0-52) at 2.27 g/L, micronutrients at 1.5 mL/L and $MgSO_4$ (Mg 25.6%) at 0.6 g/L [13,19]. The fertilizations were initiated in late March and were applied each week until mid-June. The plants were grown inside the glasshouse of the Laboratory of Floriculture, School of Agriculture, farm campus of the Aristotle University of Thessaloniki (Thermi, Greece). During the experimental period, the plants were irrigated every three days.

2.6. Morphological and Physiological Measurements of Seedlings

At the end of June, the effect of fertilization treatment on morphological and physiological variables was evaluated. The main shoot height (SH), root collar diameter (RCD) and the leaf number of all plants per treatment were measured using a ruler and a digital caliper, respectively. In addition, for the measurement of the root dry biomass (RDB) and the above-ground dry biomass (AGDB), three plants per treatment were randomly sampled in *L. minoana* subsp. *minoana* and *E. ternatum*, whereas four plants were sampled per treatment in *L. minoana* subsp. *asterusica*. Dry weights were determined after drying in the oven at 74 °C for 48 h.

At the same time, gas exchange parameters, chlorophyll and chlorophyll fluorescence were determined. A chlorophyll meter CCM 200 (Opti-sciences, Tyngsboro, MA, USA), which calculated chlorophyll content index (CCI) based on the ratio of transmittance measurement at 660 and 940 nm, was used [40]. Chlorophyll fluorescence parameter F_v/F_m was measured by OS30p+ Rapid Plant Stress Screening Device (Opti-sciences, Tyngsboro, MA, USA). In addition, F_v/F_m was calculated as the ratio of variable (v) to maximum (m) fluorescence after dark adaptation, representing the maximum photochemical efficiency of photosystem II which is widely used for detecting various stress conditions in plants [41].

In total, 13 measurements with CCM 200 and ten measurements with OS30p+ were taken on fully expanded young leaves of the plants in each treatment.

2.7. Plant Tissue Analyses of Seedlings

The plant tissues of the young experimental plants were finely grounded to pass through a 40-mesh sieve after being dried in the oven at 74 °C for 48 h. More precisely, all the leaves of the dried plants for each treatment were ground to determine their leaf nutrient concentration. For each taxon, three samples of fine powder were formed, corresponding to the three fertilization treatments applied to the experimental plants (ChF, INM or control). Subsequently, from each finely powdered and homogenized sample, three subsamples of ca. 0.25 g each were randomly formed. Each subsample was digested by the method of wet oxidation until a transparent solution was obtained, using a triple-acid mixture of H_2SO_4, HNO_3 and $HClO_4$ in a ratio of 5:1:1 at 80 °C [42]. The solutions resulting from the digested subsamples were colorimetrically analyzed for total phosphorus (P) determination according to the Molybdenum Blue Method by using a Shimadzu spectrophotometer, model UV-1201V [43]. The concentrations of magnesium (Mg), potassium (K), calcium (Ca), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) were determined by an atomic absorption spectroscopy (Perkin-Elmer Analyst 300, Norwalk, USA). Furthermore, the total nitrogen (N) was determined by the Kjeldahl method [44].

2.8. Determination of Total Phenol Content and Antioxidant Capacity

Fresh young leaves from the three studied taxa were collected per fertilization treatment and were lyophilized with liquid nitrogen. One (1) mL methanol was added to a quantity of 0.1 g lyophilized leaves, followed by a 20 min vortexing and centrifugation at 10,000× *g* at room temperature for 5 min. The liquid portion was transferred to a fresh glass vial and placed in a freezer (−20 °C) until measured.

The Folin–Ciocalteu method was used in samples of the studied taxa for the determination of total phenols content (TPC) as described in previous studies [45]. An amount of 0.5 mL extracted sample was dissolved in MeOH (1:50 *v*/*v*) with the addition of 2.5 mL of the Folin–Ciocalteu solution (1:10 *v*/*v*). To this mixture, 2 mL Na_2CO_3 (7.5% *w*/*v*) solution was also added after 6 min, and the mixture was vortexed. Finally, the sample mixture remained for 5 min at 50 °C in a water bath (LabTech Digital Water Bath, Gurgaon, India). The absorbance of each sample was measured at 760 nm by a spectrophotometer (Thermo Spectronic Helios Alpha, Cambridge, UK). TPC was based on a gallic acid calibration curve (100, 50, 25, 12.5 and 0 μg/mL) and was expressed in mg of gallic acid equivalent per g of fresh weight (mg GAE/g FW).

The antioxidant capacity (AC) in the samples of the studied taxa was determined by the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) method [46]. In 0.2 mL of samples dissolved in (1:20 *v*/*v*) in 80% *v*/*v* MeOH, 2.8 mL 4% *w*/*v* DPPH was added. The final solution was vortexed and kept in darkness for 30 min at room temperature. AC was determined at 517 nm in a spectrophotometer, compared to an ascorbic acid calibration curve (0.1, 0.05, 0.025, 0.01 and 0 μg/mL), and was expressed in mg of ascorbic acid equivalents per g of fresh weight (mg AAE/g FW).

2.9. Statistical Analysis

A completely randomized experimental design was used for each taxon. The data were subjected to a one-way analysis of variance (ANOVA), and the comparisons of the means were made using Duncan's test at a significance level of $p \leq 0.05$ [47]. Prior to the ANOVA, only the germination percentage data were transformed to arc-sine square root values [48]. All statistical analyses were carried out using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA).

The results of TPC and AC determination represent the mean of three separate measurements for each sample, and they are presented as mean ± standard deviation. Analysis

of variation (ANOVA) was used for comparisons of means using SPSS 27 and Duncan's multiple range test at $p \leq 0.05$.

3. Results

3.1. Seed Germination Success

The germination percentages of *L. minoana* subsp. *asterusica* and *L. minoana* subsp. *minoana* seeds were significantly affected by temperature ($p = 0.022$ and $p = 0.008$, respectively). More precisely, the seeds of *L. minoana* subsp. *asterusica* presented the highest germination percentage (61.25%) at 15 °C (Figure 3). No significant differences were observed in the germination percentages of seeds incubated at 10, 20 and 25 °C (35.00%, 47.50% and 45.00%, respectively). Regardless of temperature incubation, the seeds germinated after the 10th day, and germination was complete after a period of a month.

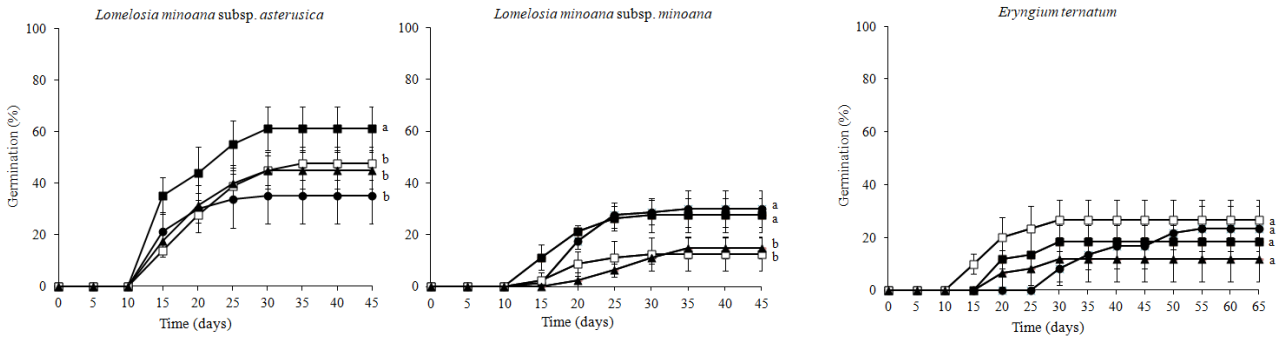


Figure 3. Cumulative germination percentage diagrams of *Lomelosia minoana* subsp. *asterusica*, *Lomelosia minoana* subsp. *minoana* and *Eryngium ternatum* seeds incubated at 10, 15, 20 and 25 °C (● 10, ■ 15, □ 20 and ▲ 25 °C). In each taxon, means are statistically different at $p < 0.05$ when they do not share a common letter. The comparisons were made using Duncan's test.

The seeds of *L. minoana* subsp. *minoana* incubated at 10 and 15 °C germinated at a comparatively higher percentage (30.00% and 27.50%, respectively) than seeds incubated at 20 or 25 °C (12.50% and 15.00%, respectively). No significant differences were observed between the germination percentages of seeds incubated at 10 and 15 °C as well as in seeds incubated at 20 and 25 °C. For seeds incubated at 15 and 20 °C, germination started after the 10th day, and it was completed on the 30th day from the beginning of the test. At 10 °C, the germination started after the 10th day, whereas at 25 °C, it started after the 15th day. In both incubation temperatures (10 and 25 °C), seed germination was completed on the 35th day from the beginning of the test (Figure 3).

The germination of *E. ternatum* seeds was not affected by the temperature ($p = 0.098$) because no significant differences were observed in the germination percentages of seeds incubated at 10, 15, 20 and 25 °C (see Figure 3). For seeds incubated at 20 °C, the germination percentage was 26.67% starting on the 10th day and was completed on the 30th day from the beginning of the test. In seeds incubated at 15 and 25 °C, the germination percentages were 18.33% and 11.67%, respectively. At both temperatures, germination started after the 15th day, and it was completed on the 30th day from the beginning of the test. Seeds incubated at 10 °C germinated with a percentage of 23.33%. Their germination started after the 25th day, and it was completed on the 55th day from the beginning of the test.

3.2. Seedlings Growth

The applications of fertilizers did not affect the main shoot height ($p = 0.277$) and the number of leaves ($p = 0.216$), whereas they affected the root collar diameter ($p = 0.001$), the root and above dry biomass ($p = 0.017$ and $p = 0.049$, respectively) and physiological characteristics ($p = 0.000$ for chlorophyll content index, and $p = 0.001$ for chlorophyll fluorescence) of *L. minoana* subsp. *asterusica* plants. However, the root collar diameter of fertilized plants (regardless of the type of fertilizer) was significantly higher than that of

the unfertilized plants (control) (Table 3). As far as dry biomass (root and above ground) is concerned, the plants fertilized with INM exhibited the significantly lowest values. Between plants fertilized with ChF and control plants, no significant difference was found in the root and above-ground dry biomass. According to Table 3, the lowest values of chlorophyll content and chlorophyll fluorescence were recorded in control plants.

Table 3. Effect of fertilization on morphological and physiological characteristics of *Lomelosia minoana* subsp. *asterusica* plants. Means ± standard deviation values are provided.

Attribute (Unit)	Control	Integrated Nutrient Management (INM)	Chemical Fertilization (ChF)
Shoot height (cm)	10.83 ± 1.29 ^{a*}	11.43 ± 1.21 ^{a}	11.57 ± 1.44 ^{a}
Root collar diameter (mm)	3.30 ± 0.45 ^{b}	3.94 ± 0.48 ^{a}	3.93 ± 0.50 ^{a}
Number of leaves	15.93 ± 1.62 ^{a}	17.60 ± 2.80 ^{a}	16.53 ± 3.11 ^{a}
Root dry biomass (g)	0.52 ± 0.12 ^{a}	0.31 ± 0.06 ^{b}	0.53 ± 0.09 ^{a}
Above-ground dry biomass (g)	1.93 ± 0.39 ^{ab}	1.52 ± 0.29 ^{b}	2.10 ± 0.14 ^{a}
Chlorophyll content index (CCI)	46.16 ± 7.99 ^{c}	85.58 ± 9.56 ^{b}	103.34 ± 13.97 ^{a}
Chlorophyll fluorescence (F_v/F_m)	0.741 ± 0.011 ^{b}	0.762 ± 0.015 ^{a}	0.760 ± 0.010 ^{a}

* Values in the same row followed by the same letter are not significantly different ($p > 0.05$) according to Duncan's test.

In *L. minoana* subsp. *minoana* plants, the fertilization affected all the morphological characteristics except for the number of leaves ($p = 0.001$ for shoot height, $p = 0.002$ for root collar diameter, $p = 0.000$ for root dry biomass, $p = 0.000$ for above-ground dry biomass, and $p = 0.056$ for the number of leaves) and physiological characteristics ($p = 0.000$ for chlorophyll content index, and $p = 0.001$ for chlorophyll fluorescence). The plants fertilized with INM showed the highest shoot height (Table 4). No significant difference was found in shoot height between control plants and plants fertilized with ChF. The lowest values of root collar diameter and the number of leaves were observed in plants fertilized with ChF. As far as dry biomass (root and above ground) is concerned, the unfertilized plants (control) exhibited significantly higher values. Between plants fertilized with ChF and INM, no significant difference was found in the root and above-ground dry biomass. The highest values of chlorophyll content and chlorophyll fluorescence were recorded in plants fertilized with INM.

Table 4. Effect of fertilization on morphological and physiological characteristics of *Lomelosia minoana* subsp. *minoana* plants. Means ± standard deviation values are given.

Attribute (Unit)	Control	Integrated Nutrient Management (INM)	Chemical Fertilization (ChF)
Shoot height (cm)	13.80 ± 1.48 ^{b*}	16.90 ± 1.19 ^{a}	13.30 ± 1.09 ^{b}
Root collar diameter (mm)	3.36 ± 0.25 ^{a}	3.24 ± 0.17 ^{a}	2.63 ± 0.33 ^{b}
Number of leaves	17.00 ± 2.45 ^{a}	16.20 ± 2.77 ^{ab}	13.40 ± 0.89 ^{b}
Root dry biomass (g)	0.55 ± 0.04 ^{a}	0.22 ± 0.04 ^{b}	0.19 ± 0.03 ^{b}
Above-ground dry biomass (g)	2.66 ± 0.05 ^{a}	1.32 ± 0.07 ^{b}	1.51 ± 0.20 ^{b}
Chlorophyll content index (CCI)	50.81 ± 9.16 ^{c}	74.50 ± 11.22 ^{a}	65.67 ± 9.09 ^{b}
Chlorophyll fluorescence (F_v/F_m)	0.750 ± 0.010 ^{b}	0.765 ± 0.011 ^{a}	0.747 ± 0.011 ^{b}

* Values in the same row followed by the same letter are not significantly different ($p > 0.05$) according to Duncan's test.

In *E. ternatum*, fertilizer applications did not affect the shoot height ($p = 0.081$), root collar diameter ($p = 0.085$) or the number of leaves ($p = 0.253$) of plants. As far as the dry biomass is concerned, the plants were affected by fertilizer applications ($p = 0.000$) for root dry biomass and $p = 0.000$ for above-ground dry biomass). Specifically, the plants fertilized with ChF exhibited the significantly highest values of dry biomass (Table 5), whereas the lowest values of dry biomass were observed in non-fertilized plants (control). Furthermore,

fertilization affected the physiological characteristics (p = 0.000 for chlorophyll content index and p = 0.000 for chlorophyll fluorescence). According to Table 5, the highest values of chlorophyll content index (CCI) and chlorophyll fluorescence were recorded in plants fertilized with INM or ChF.

Table 5. Effect of fertilization on morphological and physiological characteristics of *Eryngium ternatum* plants. Means ± standard deviation values are given.

Attribute (Unit)	Control	Integrated Nutrient Management (INM)	Chemical Fertilization (ChF)
Shoot height (cm)	20.80 ± 3.11 ^{ab*}	20.00 ± 2.74 ^{b}	24.10 ± 2.35 ^{a}
Root collar diameter (mm)	4.09 ± 0.89 ^{a}	3.97 ± 0.66 ^{a}	4.92 ± 0.31 ^{a}
Number of leaves	6.80 ± 1.30 ^{a}	7.40 ± 1.14 ^{a}	8.00 ± 0.71 ^{a}
Root dry biomass (g)	0.69 ± 0.22 ^{c}	2.16 ± 0.03 ^{b}	2.79 ± 0.43 ^{a}
Above-ground dry biomass (g)	0.35 ± 0.07 ^{c}	0.85 ± 0.06 ^{b}	1.08 ± 0.06 ^{a}
Chlorophyll content index (CCI)	12.01 ± 1.17 ^{b}	18.03 ± 2.02 ^{a}	18.94 ± 3.90 ^{a}
Chlorophyll fluorescence (F_v/F_m)	0.696 ± 0.018 ^{b}	0.750 ± 0.009 ^{a}	0.748 ± 0.012 ^{a}

* Values in the same row, followed by the same letter, are not significantly different (p > 0.05) according to Duncan's test.

3.3. Leaf Nutrient Concentration

Macronutrient concentrations in leaves of *L. minoana* subsp. *asterusica* were affected by fertilization (p = 0.002 for nitrogen, p = 0.000 for P, p = 0.000 for Ca, p = 0.000 for Mg, p = 0.000 for K and p = 0.000 for Na). The macronutrient content of *L. minoana* subsp. *asterusica* leaves showed an increased concentration of P in plants treated with INM as compared with unfertilized plants (control) or ChF, while K, Ca, Mg and Na were detected in higher concentrations in the leaves of untreated plants (Table 6). However, the N concentration was almost the same in plants either treated with INM or control. The fertilization affected the concentration of micronutrients (p = 0.000 for iron, p = 0.003 for zinc and p = 0.000 for copper) except for manganese (Mn) (p = 0.263). A remarkable increase—15-fold—was detected in the concentration of Cu in plants treated with INM compared with the control (Table 7). The same trend was also noticed in the concentration of Fe, while with Zn, no statistical differences were found between the INM and the untreated (control) plants. Except for manganese, the concentration of the micronutrients in the leaves of plants treated with ChF was lower compared with the other two treatments.

The fertilization affected the concentrations of micronutrients (p = 0.000 for N, p = 0.000 for P, p = 0.000 for Ca, p = 0.000 for Mg, p = 0.000 for K and p = 0.000 for Na) in the leaves of *L. minoana* subsp. *minoana* plants. The concentration of N, P, Ca and Mg in the leaves was higher in those treated with INM than in those treated with ChF or the untreated ones (Table 6). Both fertilization treatments increased the concentration of K in the leaves of the plants as compared with the control, while ChF-treated plants showed an increased concentration of Na in their leaves in comparison with the control or INM treatment. Furthermore, fertilization affected the concentration of micronutrients (p = 0.003 for Fe, p = 0.019 for Zn and p = 0.000 for Cu) except for Mn (p = 0.540). The Cu concentration of the *L. minoana* subsp. *minoana* leaves in the plants treated with INM showed a 10-fold higher absorption than ChF fertilization treatment or the control (Table 7). On the other hand, the application of ChF fertilizers increased the Fe content of the leaves as compared with the other two treatments. In addition, a higher concentration of Zn was measured in the leaves of both the control and ChF-treated plants as compared with the plants treated with INM fertilizers.

The analysis of the macronutrient content of *E. ternatum* leaves showed that the fertilization affected the concentrations of micronutrients (p = 0.000 for N, p = 0.002 for P, p = 0.000 for Ca, p = 0.000 for K and p = 0.000 for Na) except for Mg (p = 0.129). An increased concentration of N and K in plants treated with INM fertilizers, while in the leaves of untreated plants (control), a higher concentration of Ca and P was detected (Table 6). It is

worth mentioning that the Mg absorption was almost the same for all treatments. A higher Na concentration was noticed in the leaves of the plants treated with ChF fertilizers, while both fertilizers over-doubled the content of Na in leaves as compared with the control. Regarding micronutrient contents, fertilization affected their concentrations (p = 0.003 for Fe, p = 0.015 for Mn and p = 0.000 for Cu). The INM-treated plants increased more than 12-fold the concentration of Cu in plant leaves as compared with ChF-treated plants (Table 7). Additionally, Fe was measured at almost two times the levels in the leaves of untreated plants compared with the other two treatments, while Mn was noticed in higher concentration in both the control and INM treatments. The concentration of Zn was not affected by fertilizer applications (p = 0.404).

Table 6. Effect of fertilizers (INM: integrated nutrient management; ChF: chemical fertilizers; control: no fertilization) on macronutrient concentration in leaves of *Lomelosia minoana* subsp. *asterusica*, *Lomelosia minoana* subsp. *minoana* and *Eryngium ternatum* plants derived from seedlings. Means ± standard deviation values are given.

Treatment	Nitrogen (N) (%)	Phosphorus (P) (mg/g)	Potassium (K) (mg/g)	Calcium (Ca) (mg/g)	Magnesium (Mg) (mg/g)	Sodium (Na) (mg/g)
			Lomelosia minoana subsp. *asterusica*			
Control	1.91 ^{a*}	2.77 ^{b}	21.79 ^{a}	10.84 ^{a}	3.53 ^{a}	4.80 ^{a}
INM	1.96 ^{a}	2.92 ^{a}	21.11 ^{b}	10.41 ^{b}	3.35 ^{b}	3.63 ^{c}
ChF	1.55 ^{b}	2.58 ^{c}	16.55 ^{c}	9.19 ^{c}	3.16 ^{c}	3.95 ^{b}
			Lomelosia minoana subsp. *minoana*			
Control	1.54 ^{c}	1.88 ^{c}	13.98 ^{b}	10.11 ^{c}	3.39 ^{c}	5.07 ^{b}
INM	2.19 ^{a}	2.90 ^{a}	17.70 ^{a}	12.57 ^{a}	3.77 ^{a}	4.12 ^{c}
ChF	1.98 ^{b}	2.51 ^{b}	17.18 ^{a}	10.96 ^{b}	3.64 ^{b}	5.70 ^{a}
			Eryngium ternatum			
Control	1.69 ^{b}	3.77 ^{a}	15.42 ^{c}	14.55 ^{a}	3.59 ^{a}	7.90 ^{c}
INM	2.30 ^{a}	2.88 ^{b}	22.87 ^{a}	11.92 ^{b}	3.80 ^{a}	14.93 ^{b}
ChF	1.70 ^{b}	3.07 ^{b}	20.95 ^{b}	11.34 ^{c}	3.61 ^{a}	15.86 ^{a}

* In the same column, the means are statistically different at $p < 0.05$ when they do not share a common letter. The comparisons were made using Duncan's test.

Table 7. Effect of fertilizers (INM: integrated nutrient management; ChF: chemical fertilizers; control: no fertilization) on micronutrient concentration in leaves of the *Lomelosia minoana* subsp. *asterusica*, *Lomelosia minoana* subsp. *minoana* and *Eryngium ternatum* plants derived from seedlings.

Treatment	Iron (Fe) (ppm)	Manganese (Mn) (ppm)	Zind (Zn) (ppm)	Copper (Cu) (ppm)
		Lomelosia minoana subsp. *asterusica*		
Control	213.41 ^{b*}	16.06 ^{a}	15.48 ^{a}	7.32 ^{b}
INM	385.61 ^{a}	16.53 ^{a}	15.53 ^{a}	102.74 ^{a}
ChF	227.15 ^{b}	11.76 ^{a}	10.00 ^{b}	1.54 ^{b}
		Lomelosia minoana subsp. *minoana*		
Control	181.83 ^{b}	15.54 ^{a}	26.50 ^{a}	6.73 ^{b}
INM	205.53 ^{b}	15.43 ^{a}	14.18 ^{b}	76.02 ^{a}
ChF	263.84 ^{a}	13.00 ^{a}	20.32 ^{ab}	7.54 ^{b}
		Eryngium ternatum		
Control	377.08 ^{a}	48.58 ^{a}	13.17 ^{a}	10.83 ^{b}
INM	161.23 ^{b}	44.82 ^{a}	14.07 ^{a}	131.89 ^{a}
ChF	170.15 ^{b}	35.36 ^{b}	12.20 ^{a}	2.18 ^{c}

* In the same column, the means are statistically different at $p < 0.05$ when they do not share a common letter. The comparisons were made using Duncan's test.

Figures 4 and 5 illustrate the studied plants in the fertilization experiments that were used for nutrient analysis.

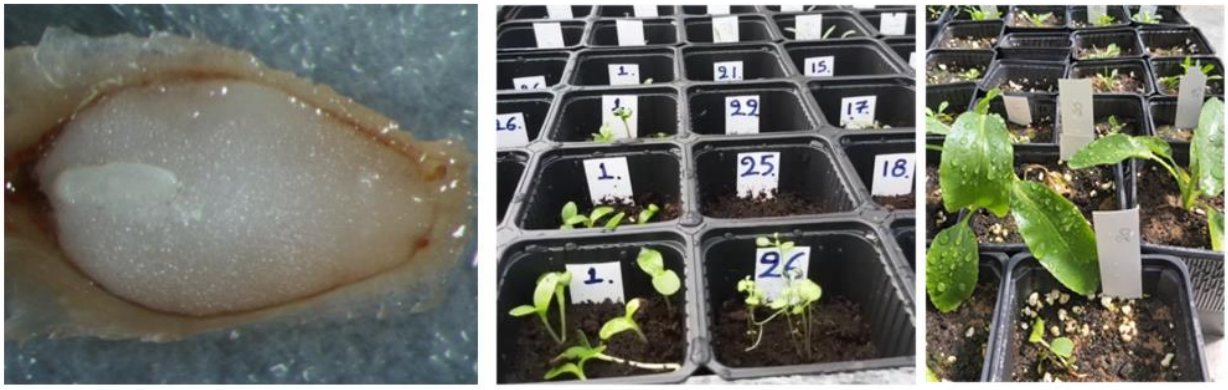


Figure 4. Mature embryos (**A**), seedlings (**B**) and young plants (**C**) of *Eryngium ternatum* used in fertilization experiments and nutrient analysis.

Figure 5. Plants raised from seedlings of *Lomelosia minoana* subsp. *minoana* (**A**) and *L. minoana* subsp. *asterusica* (**B**) after the end of fertilization experiments, including individuals treated with inorganic fertilizer (**left**), the control individuals (**middle**) and the individuals treated with integrated nutrient management fertilizer (**right**).

3.4. Total Phenol Content and Antioxidant Capacity

The effect of fertilization on the TPC and AC of the studied taxa is shown in Table 8. For *L. minoana* subsp. *asterusica*, both TPC and AC did not require the application of fertilizers to reach the highest levels (3.084 mg GAE/g FW and 2.279 mg AAE/g FW, respectively). Concerning the other two taxa, *L. minoana* subsp. *minoana* and *E. ternatum* were positively influenced by fertilization for TPC; however, AC was higher for ChF-fertilized plants compared with the control and INM treatments.

Table 8. Effect of fertilizers (INM: integrated nutrient management; ChF: chemical fertilizers; and control: no fertilization) on the total phenol content expressed as gallic acid equivalents (GAE) and the antioxidant capacity expressed as ascorbic acid equivalents (AAE) in young leaves of the Cretan endemic plants *Lomelosia minoana* subsp. *asterusica*, *Lomelosia minoana* subsp. *minoana* and *Eryngium ternatum*.

Treatment	Total Phenol Content (mg GAE/g FW)	Antioxidant Capacity (mg AAE/g FW)
	Lomelosia minoana subsp. *asterusica*	
Control	3.084 ± 0.065 ^{a*}	2.279 ± 0.047 ^{a}
INM	1.572 ± 0.090 ^{c}	2.037 ± 0.035 ^{b}
ChF	2.694 ± 0.044 ^{b}	2.197 ± 0.044 ^{a}
	Lomelosia minoana subsp. *minoana*	
Control	2.096 ± 0.152 ^{b}	2.312 ± 0.025 ^{b}
INM	2.167 ± 0.080 ^{b}	2.085 ± 0.041 ^{c}
ChF	2.658 ± 0.071 ^{a}	2.419 ± 0.024 ^{a}
	Eryngium ternatum	
Control	0.748 ± 0.004 ^{c}	0.101 ± 0.0005 ^{c}
INM	0.897 ± 0.017 ^{b}	0.109 ± 0.0013 ^{b}
ChF	1.210 ± 0.007 ^{a}	0.122 ± 0.0008 ^{a}

* In the same column, the means are statistically different at $p < 0.05$ when they do not share a common letter. The comparisons were made using Duncan's test.

4. Discussion

4.1. Seed Germination Protocols

The Mediterranean climate is characterized by seasonality in temperature and precipitation, resulting in hot, dry summers and wet, cool winters [49]. Although soil moisture is critical for seedling emergence and survival in nature, temperature is the main environmental factor controlling the timing of seed germination [50]. According to their climate origin, plant species have developed different requirements for their seed germination [30]. In Mediterranean-type ecosystems, the optimal temperature for seed germination is relatively low (generally ranging between 10 °C and 20 °C), and the period with this temperature in the Mediterranean's natural environment coincides with early winter when rainfall is adequate to allow seed germination and seedling survival [51–53].

The results of the present study clearly showed that temperature affects seed germination in both *Lomelosia minoana* subspecies. In both subspecies, the seed germination success was higher at low temperatures (10 and 15 °C) compared with higher temperatures (20 and 25 °C). In particular, the highest germination percentage of *L. minoana* subsp. *asterusica* seeds was observed at 15 °C, whereas *L. minoana* subsp. *minoana* seeds exhibited the highest germination percentages at 10 and 15 °C. Due to relatively low germination percentages, stored seeds of both subspecies were subjected to estimation of their viability by cutting test. A random sample of 60 seeds from each taxon (three replications of 20 seeds) was taken, and then the seeds were longitudinally cut to assess their viability by visual inspection. According to the results of the cutting test, the percentage of viable seeds of *L. minoana* subsp. *asterusica* and *L. minoana* subsp. *minoana* were 68.33% and 36.66%, respectively. Therefore, the germination percentages of *L. minoana* subsp. *asterusica* and *L. minoana*

subsp. *minoana* seeds observed in the present study were close to the potential germination capacity of the seed lots used in the germination experiments. According to previous studies [11], seeds of *L. minoana* subsp. *minoana* sowed in plastic trays at 20 °C may exhibit a 38% germination percentage (however, with no cutting test performed). Furthermore, a similar low-germination percentage was also reported for *L. graminifolia* (L.) Greuter & Burdet seeds that were incubated at alternate temperatures (day/night) of 25/15 °C [54]. It has been reported that the temperature of 15 °C is optimal for seed germination of *Lomelosia minoana* subsp. *asterusica*, however, seeds may also germinate well at temperatures of 10 and 20 °C [37].

As the germination requirements of these two *Lomelosia* taxa have been adapted to the climatic conditions of their Cretan natural habitats, this adaption ensures that in the harsh Mediterranean conditions of Crete Island, their seed germination takes place at the right time when the established seedlings will have more possibilities to survive. The GIS-derived ecological profiles can provide significant information regarding the climate conditions under which a plant species thrive in its natural habitat [12,14,16,20]. According to the ecological profiles generated herein for *L. minoana* subsp. *asterusica* and *L. minoana* subsp. *minoana* (see Supplementary Materials Figures S1 and S2), the favorable temperatures for their seeds' germination prevail in autumn months (October and November). Furthermore, the increased amount of precipitation during this period creates the optimum soil moisture conditions for seed germination, and the relatively mild temperatures of winter may allow the growth of emerged seedlings; these claims are in line with the observed natural fruiting period of these taxa from June to September, depending on altitude and aspect [38].

To the best of the authors' knowledge, no propagation protocol describing the germination requirements of *E. ternatum* seeds has ever been reported in the literature. In this investigation, the seeds of *E. ternatum* germinated at low percentages and the incubation temperature did not affect the seed germination. This finding is probably related to a common problem often reported in several members of the Apiaceae family with poor seed germination [30]. Consequently, poor seed quality sourced from the wild and/or the existence of innate seed dormancy may have contributed to the poor germination percentages found in this study. Possibly, the seed lot used in the germination experiments consisted of a high percentage of non-viable seeds, and unfortunately, due to the limited seed amount sourced in the wild, their viability by the cutting test was not estimated. A similarly high non-viable seed percentage (62.5%) has also been observed in *E. viviparum* J. Gay [55]. Vis-à-vis the dormancy issue, previous studies have reported that seeds of many species of Apiaceae are often morphologically or morphophysiologically dormant [56]. In studies that investigated the germination of other members of the genus *Eryngium*, the existence of morphophysiological dormancy has already been reported [55,57–59]. In general, seeds with morphophysiological dormancy have to remain for a period under specific conditions (moisture, suitable temperatures) to germinate. However, the assumed morphophysiological dormancy of the seeds of *E. creticum* Lam. is reportedly overcome by a two- and half-month period after ripening, and germination may occur at low temperatures [30,60]. In regions with a Mediterranean climate, the morphophysiological dormancy in seeds of herbaceous species is usually broken during dry storage (after ripening) at 20 °C or higher [30]. In the case that the seeds of *E. ternatum* are characterized by morphophysiological dormancy, similarly to other species of this genus, their exposure to high temperature and dry conditions after seed dispersal during the summer may result in the breaking of the physiological portion of the assumed complex of morphophysiological dormancy. According to the ecological profile of *E. ternatum* (see Supplementary Materials Figure S3), it was shown that the dry and hot conditions during August and September are optimum for the after-ripening of seeds. The increased amount of precipitation and the relatively high temperature prevailing in October across its natural habitats may create the optimum conditions for further embryo growth. Probably, under these conditions, the embryos of *E. ternatum* grow and germinate in four weeks or less. However, the investigation of the germination requirements of *E. ternatum* seeds still remains a challenge.

4.2. Fertilization Protocols

There is a large amount of literature regarding the effect of fertilization (especially N) on the phenolic compound content and antioxidant potential of plants [61–63]. Even though there is no general trend reported to date, fertilization seems to function either positively or negatively. Thus, the findings of the present study indicate a variety of responses rather than species-specific trends. Such patterns are also shown in other studies examining the fertilization of three *Helianthus tuberosus* L. cultivars in terms of both total phenolics and antioxidants [63]. Yet, some studies have reported a negative effect of increased N fertilization levels on total phenolic content in the leaves of two mustard genotypes [61], while others report that fertilizers may increase both antioxidants and total phenols in the berries of *Berberis microphylla* G. Forst. [62]

4.3. Leaf Nutrient Content

The effect of ChF fertilization treatments was not positively correlated with either macronutrient or micronutrient content of the leaves of *L. minoana* subsp. *asterusica*. On the other hand, the application of INM fertilization increased the P, Fe and Cu concentrations in the leaves as compared with the control plants. In general, it is known that P fertilization treatments favor nutrient synthesis as well as transportation in plants, and thus the latter may advance their growth, dry matter accumulation and light use efficiency [64]. On the other hand, plants often show a negative correlation between the level of P supply and the concentration of N and K macronutrients [65]. This may explain the lower values of N and K in our results as compared with the control. Similarly, Fe is one of the most important transition metals in plants, which is required for essential cell functions. In plants, the photosynthetic apparatus has a particularly high need for Fe cofactors containing redox enzymes [66]. Thus, foliar Fe homeostasis plays a crucial role in the physiological status of the plants since autotrophy relies on the availability of Fe [67]. In addition, Fe deficiency-induced chlorosis depends on P availability [68]. This was indeed confirmed by the findings of the present study as the photosynthetic rate of *L. minoana* subsp. *asterusica* was positively affected when INM fertilization was used.

The comparison of INM and ChF fertilization treatments as compared with the control (no fertilization) in *L. minoana* subsp. *minoana* plants showed many positive aspects, except for the micronutrient absorption of Mn and Zn. In most of the macronutrients and in Cu, INM fertilization was superior to chemical fertilization in terms of the total content of these nutrients in leaves. Only in the cases of Na and Fe nutrients did the ChF fertilization achieve increased contents in leaves compared to INM-treated or control plants. Since modern agriculture strives to manage fertilizers sustainably for both economic and environmental reasons [65], monitoring any nutritional deficiency in growing plants is a challenge for precision farming technology [69]. Thus, it seems that for *L. minoana* subsp. *minoana* plants, the use of the INM scheme by foliar application was the most appropriate and ecologically friendly method for the cultivation of these plants.

The INM and ChF fertilization treatments were negatively correlated with P, Ca and Fe concentrations in the leaves of *E. ternatum* plants, while the application of INM fertilizers increased the concentration of N, K and Cu. Generally, nutrient limitation to plant growth is widespread in terrestrial ecosystems, and N, P and K are the most common limiting nutrients, both individually and in combination [70]. The determination of nutrient concentration in plants is of great reference value for understanding plant health, precise fertilization, and ecological adaptation [71]. In a commercial cultivation protocol, leaf nutrient concentration and resorption often play an important role in determining plant nutrient use strategies [72]. Thus, it seems that the INM fertilization treatments may be useful in improving the cultivation techniques of *E. ternatum* plants.

In the last 20 years, research in agriculture has developed distinct groups of materials with innovative properties to improve farming and increase crop production. The INM fertilization used in this study consisted of commercial chemicals, polysaccharides, systemic copper, biostimulants, organic Ca and urea as an N source. It is known that

polysaccharide-based resources can enhance fertilizer efficiency by being non-toxic and water-soluble and may increase the availability of nutrients, thus playing an important role in plant growth and development in agricultural applications [73,74]. Plant biostimulants include any material applied to plants with the ability to increase nutrient absorbance, abiotic stress tolerance and/or improve crop quality characteristics, regardless of its content of nutrients [75]. Among nutrients, Cu is an essential micronutrient but is also toxic to plants when in excess [76]. Specifically, Cu is an important micronutrient in photosynthesis by binding to the plastocyanin protein, participates in carbon metabolism, and acts as a protector against oxidative stress [77,78]; moreover, Cu plays an important role in controlling fungal diseases in plants [79]. The application of Cu-based foliar fertilizers with the regulated release of urea [79] and polysaccharides with Cu has been found to be advantageous for tomato cultivation [80]. The results of this study herein indicated that the INM fertilization treatments increased in all three taxa the concentration of the Cu at much higher levels compared with the control or the ChF fertilization treatment (Table 7). In our results, the copper concentration was 10- to 15-fold higher than other treatments, without any toxicity problems. It seems that the three taxa studied herein tolerated high levels of Cu without any adverse effect on their growth; however, further investigation is certainly needed into the different developmental stages of these three taxa prior to conclusions. Similar results after using INM fertilization with foliar and soil application compared with the control and chemical treatment have also been reported for some Tunisian local endemic plants, namely *Teucrium luteum* (Mill.) Degen subsp. *gabesianum* (S. Puech) Greuter with 19-fold higher Cu concentration [14] and *Marrubium aschersonii* Magnus with 34-fold higher Cu concentration [20]. Thus, the results of this study using a foliar INM fertilization system may suggest the systemic absorbance of copper from the plant cells of the studied taxa with no toxicity observed.

4.4. Re-Evaluation of Feasibility and Readiness Timescale for Sustainable Exploitation

The knowledge and data generated herein for *L. minoana* subsp. *minoana*, *L. minoana* subsp. *asterusica* and *E. ternatum* can be taken into consideration to re-evaluate the feasibility of value chain creation for these taxa with economic interest (Level II evaluation after [1]). When such re-evaluation of the attributes reported in our previous investigation is performed, the feasibility for value chain creation for *L. minoana* subsp. *minoana* and subsp. *asterusica* are both considerably improved from 40.28% and 50.00% to 70.83% and 63.89%, respectively, thus upgrading both in the respective ranking (both previously ranked in the very low class, see [1]). In light of the findings reported herein, the first is upgraded to the highest class (>70%), and the second is to the above average to the high class (55–70%) (see Table 9). The differences in the Level II re-evaluations were generated due to the increased threat status assigned to date (both Critically Endangered according to [36], which is equivalent to a score of six compared with zero): the increased ex situ conservation (a score of four, at least two stored accessions in two different countries for the first taxon), the development of the seed propagation protocols (for both taxa, a score of six compared with five for the first taxon), the effective vegetative propagation published for the first taxon [11] (resulting in a score of six compared with zero), the documented knowledge on species-specific cultivation needs (for both taxa, a score of six compared with four), and the development of species-specific cultivation protocol for both taxa (a score of five compared with three for the first and zero for the second taxon). These Level II re-evaluations indicate noteworthy upgrades in terms of the readiness timescale for their sustainable exploitation (Level III evaluation according to [1]). The latter was previously estimated as "achievable in the long-term" regarding *L. minoana* subsp. *minoana* and "achievable in the medium-term" for *L. minoana* subsp. *asterusica* [1]; however, these estimations may be upgraded herein to "already achieved" for the first taxon (class >70%) and to "achievable in the short-term" for the second one (Table 9).

Table 9. Overview of the multifaceted evaluation (general and special interest in different economic sectors, feasibility and readiness timescale for sustainable exploitation) regarding the three studied local endemic plants of Crete with upgrades of individual scores and previous assessments (Level II and III evaluations) after the data herein presented.

Cretan Endemic Plant	General Ornamental Interest [1]	* Special Ornamental Interest [1]	Agro-Alimentary Interest [4]	Medicinal Interest [5]	Feasibility and Readiness Timescale for Sustainable Exploitation (Level II/III Assessments **)	Increase in the Sum of Individual Scores (+) and Upgraded Percentage (%) Compared with Previous Level II/III Assessments
Eryngium ternatum	Low	Low-to-below average	Very low	Below average	54.17%/ Achievable in medium-term	+13.89% (68.06%)/ Achievable in short-term
	33.33%	34.37%/38.70%/ 47.31%/49.41%	23.81	44.44%		
Lomelosia minoana subsp. *asterusica*	Average	Very high	No	Very low	50%/ Achievable in medium-term	+13.89% (63.89%)/ Achievable in short-term
	56.67%	65.62%/63.64%/ 69.89%/71.54%	0	20.37%		
Lomelosia minoana subsp. *minoana*	Average	Very high	No	Very low	40.28%/ Achievable in long-term	+30.55% (70.83%)/ Already achieved
	51.67%	62.50%/62.08%/ 68.28%/70.75%	0	20.37%		

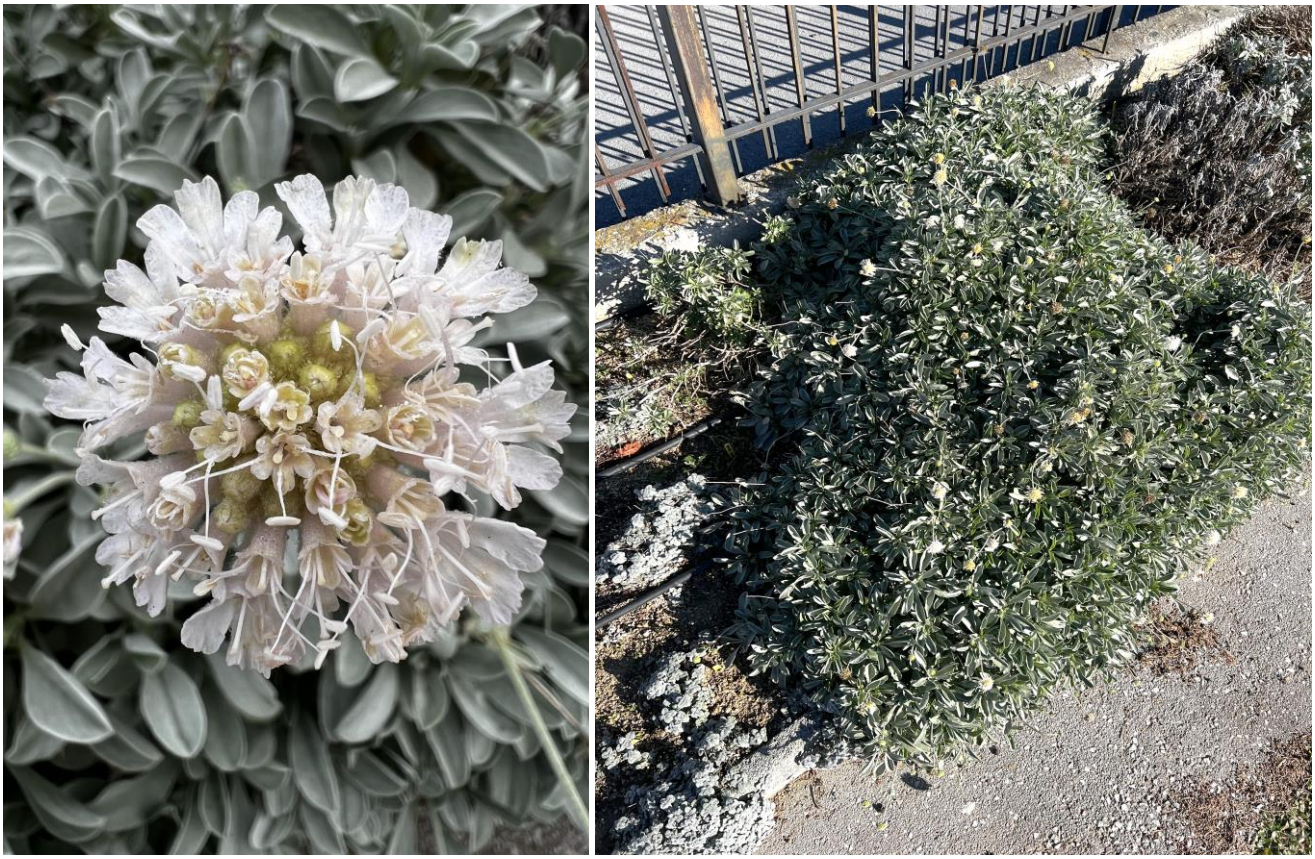
* Special ornamental interest refers to pot/patio plant (1st percentage), home gardening (2nd percentage), landscaping (3rd percentage), and xeriscaping (4th percentage), as estimated in [1], and these are separated with strokes. ** Assessments regarding the feasibility (Level II evaluation) and readiness timescale (Level III evaluation) for the sustainable exploitation of the studied taxa, as estimated in [1].

In the same fashion, the feasibility of value chain creation for *E. ternatum* can be reported as considerably improved from 50.17% to 68.06%, thus upgrading it from the average class to the above average to the high class (>55–70%). The differences in the Level II re-evaluations were generated due to the increased threat status assigned to date to this taxon, namely Critically Endangered (according to [36]), which is equivalent to a score of six compared with zero; the development of the seed propagation protocol and the documented knowledge on species-specific cultivation needs (a score of six compared with four); and the development of the species-specific cultivation protocol (a score of five compared with tow). These Level II re-evaluations indicate a noteworthy upgrade in terms of the readiness timescale for its sustainable exploitation (Level III evaluation according to [1]). This was previously assessed as "achievable in the medium-term" for *E. ternatum* [1], which may be herein upgraded to "achievable in the short-term" (Table 9).

Previous studies examining a plethora of local endemic plants of three Mediterranean regions have shown that the two subspecies of *L. minoana* studied herein (subsp. *asterusica*, subsp. *minoana*) are associated with above-average general interest (56.67% and 51.67%, respectively) and high special interest (65.62–71.54% and 62.08–70.75%, respectively) in different subsectors of the ornamental-horticultural industry [1]. Among other attributes assessed in a comparative and coherent way, all these assessments consider its main natural flowering period occurring in summer (from late May till early September, [38]). Herein, we may further document that the flowering of subsp. *minoana* may also be considerably extended throughout autumn until mid-winter as partial flowering when cultivated ex situ, especially during warm autumns and mild winters (Figure 6), thus adding another important feature of high ornamental interest.

(**A**) (**B**) (**C**)

Figure 6. *Cont.*



(**D**) (**E**)

Figure 6. Inflorescence of *Lomelosia minoana* subsp. *minoana* during the main flowering period in early June ((**A**) photo: P. Tsiklakis, reproduced with permission) and at the beginning (**B**) and during fruiting in late June (**C**) (photos: I. Stoila, reproduced with permission). (**D**): Four plant individuals in partial flowering extended until winter (January), showing increased vegetative growth and comparatively high ground covering potential (**D**) as contrasted with the limited growth and covering potential of other local Cretan endemic plants (**E**), such as *Origanum dictamnus* L. (left in photo (**E**)) and *Carlina diae* (Rech. f.) Meusel & A. Kástner (right in photo (**E**)). All plants of the three taxa in the ornamental flowering bed were of the same age (four years old), were ex situ raised from seeds, and were planted for the same purpose at the same time, soil and ex situ location (the sea-level campus of the Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter in Thermi, metropolitan Thessaloniki, Northern Greece).

5. Conclusions

This study reported for the first time detailed ecological profiling using Geographical Information Systems to provide insight into the abiotic environmental conditions prevailing in the wild habitats of *L. minoana* subsp. *asterusica*, *L. minoana* subsp. *asterusica* and *E. ternatum.* This kind of information about their natural conditions can be imitated during their cultivation and acclimatization in a man-made environment, thus facilitating the development of detailed species-specific cultivation guidelines for these Critically Endangered plants. Furthermore, the effective seed propagation of the studied taxa, as first reported herein, can be perceived as a basic step enabling both in situ conservation efforts and ex situ conservation or sustainable exploitation strategies for these local endemic plants of Crete. Altogether, this novel data concerning the detected effects of chemical and integrated nutrient management fertilizers on the growth and pilot cultivation of the derived seedlings and their first phytochemical evaluation in terms of total phenolics and antioxidant potential may also inform and update the feasibility and readiness timescale of

the sustainable exploitation process for these unique plants with promising medicinal/agro-alimentary potential and ornamental-horticultural value. Undoubtedly, more coordinated efforts and applied research are needed prior to attempts to attract stakeholder attention and before creating an effective value chain for these neglected and underutilized phytogenetic resources of Crete, Greece.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030335/s1, Figure S1: Ecological profile across the natural distribution range of *Lomelosia minoana* subsp. *asterusica* wild-growing populations in Crete; Figure S2: Ecological profile across the natural distribution range of *Lomelosia minoana* subsp. *minoana* wild-growing populations in Crete; and Figure S3: Ecological profile across the natural distribution range of *Eryngium ternatum* wild-growing populations in Crete.

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