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Research Article

Impact of planting density and organic fertilization on the cultivation of the Greek endemic *Helichrysum amorginum* Boiss. and Orph.

Georgios STAVROPOULOS[1,2]*, Panayiota PAPASTYLIANOU[1]
Eleftherios KARAPATZAK[3], Evangelos VASSALOS[2],
Eleni MALOUPA[3], Ioanna KAKABOUKI[1]

[1]*Agricultural University of Athens, School of Plant Sciences, Department of Crop Science, Laboratory of Agronomy, 75 Iera Odos St., 118 55 Athens, Greece; giorgos.stavropoulos@korres.com (G.S.); ppapastyl@aua.gr (P.P.); i.kakabouki@aua.gr (I.K.)*
[2]*Korres Natural Products S.A., 57th km NR Athens-Lamia, 32011 Oinofyta, Greece; vvassalos@gmail.com (E.V.)*
[3]*Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Dimitra, 57001 Thessaloniki, Greece; ekarapatzak@gmail.com (E.K.); emaloupa@elgo.gr (E.M.)*

Abstract

Native phytogenetic resources can offer valuable germplasm diversity with potential for sustainable utilization. *Helichrysum amorginum* Boiss. and Orph. (Asteraceae) a Greek endemic, range-restricted neglected and underutilized plant species (NUP) holds significant utilization potential stemming from its known biochemical properties. The current study presents the results of a three-year experimental cultivation scheme of documented Greek *H. amorginum* germplasm aiming at its sustainable exploitation. Different planting densities in combination with ascending levels of organic fertilization in two distinct experimental fields were applied and plant growth coupled with inflorescence emergence and biomass yield were evaluated across a period of three years. The results highlighted the growth enhancing effect of organic fertilization under different planting densities with lower densities presenting a trend to sustain larger plants in terms of increased plant base diameter. Inflorescence emergence increased significantly from the second cultivation year onwards in both experimental fields with increasing levels of organic fertilization delivering higher rates of inflorescence number under decreasing planting densities. The current study provides a basis for a systematized research scheme that can facilitate further domestication and upscaling efforts leading to the sustainable utilization of *H. amorginum* in the pharmaceutical and cosmetics sectors, offering at the same time, local economic development.

Keywords: agronomic utilization; domestication; *Helichrysum amorginum*; neglected and underutilized germplasm; pharmaceutical plants; range-restricted species

Introduction

The native phytogenetic resources of the Mediterranean basin and beyond include an extensive range of plant species with ecological, ornamental or nutraceutical value which stems from the naturally high levels of

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biodiversity, genetic diversity and endemism that characterize the above region (Strid and Tan 1997; Grigoriadou *et al*., 2020; Myers *et al*., 2000). The flora of Greece entails significant diversity of valuable plant germplasm with potential for selection and sustainable agronomic utilization in various sectors (Bourgou *et al*., 2021; Krigas *et al*., 2021; Libiad *et al*., 2021). In addition, the conservation of such valuable plant germplasm can be regarded as a priority considering the current climate change challenges native plants face across temperate regions worldwide in terms of adaptation pressures due to habitat shifts (Parmesan and Hanley, 2015).

During the past decade an array of systematized research efforts has been brought forward on cultivation for conservation and domestication of selected native plant species across Europe and the mediterranean basin, including Greece (Ensslin and Godefroid, 2020; Maloupa *et al*., 2021; Karapatzak *et al*., 2022a; 2022b). The aims of this type of research revolve around the sustainable utilization of phytogenetic resources enhancing at the same time, the conservation status of native plants (Krigas *et al*., 2022); especially following the current climate change and economical challenges of the primary production sector worldwide (Agovino *et al*., 2019; Czyżewski *et al*., 2019; Rokicki *et al*., 2021). The utilization of native phytogenetic resources entails successive stages starting from the evaluation of the target taxa in terms of conservation status and utilization potential (Krigas *et al*., 2022), to ex situ adaptation and conservation coupled with documentation and molecular authentication of the selected material, and development of efficient propagation protocols (sexual or asexual) (Gkika *et al*., 2013; Maloupa *et al*., 2021; Varsamis *et al*., 2023). Based on these primary stages, a further step is the purpose-oriented experimental cultivation of the documented germplasm which will ultimately set the basis for further exploitation and breeding efforts (Chen *et al*., 2016; Kostas *et al*., 2022; Hatzilazarou *et al*., 2023).

The experimental cultivation of native germplasm is an intricate process requiring tailor-made treatments and evaluation protocols to control the ex-situ adaptation challenges such plants usually face (Ensslin and Godefroid, 2020; Karapatzak *et al*., 2023). Therefore, evaluation of parameters like planting density or fertilization have been shown to affect plant growth and yield during trial cultivation of medicinal plants via optimisation of water and nutrient inputs ameliorating possible ex situ adaptation constraints (Chen *et al*., 2016). Particularly for native plants of the Greek flora, the application of external fertilization inputs during ex situ cultivation, has shown promising results in terms of sustainable utilization of target taxa (Kostas *et al*., 2022; Paschalidis *et al*., 2024).

The current study focuses on *Helichrysum amorginum* Boiss. and Orph. (Asteraceae) a range-restricted neglected and underutilized plant species (NUP) which is endemic to Greece and has been characterized as endangered (EN) according to a recent extinction risk assessment of the Greek endemic flora (Kougioumoutzis *et al*., 2021). The natural populations of *H. amorginum* occur in the Cyclades, mainly on the island of Amorgos, Greece as chasmophytes growing in rock crevices close to the seashore (Phitos *et al*., 1995). It is a perennial herb that bears several flowering stems (inflorescences) from a base crown (or several crowns) granting the plant an upward rossete-type appearance. The base leaves are thick, oblanceolate to spathulate, light grey to greenish colored (Figure 1). Flowering heads are round at anthesis, with white or pink bracts and yellow in the middle. The produced achenes are rounded, with sparse vesicular hairs (Phitos *et al*., 1995).

Most members of the genus *Helichrysum* hold significant ornamental value, due to the prolonged vase life of their flowers (Argyriou *et al*., 2020). However, *H. amorginum* like other members of the genus *Helichrysum*, holds additional exploitation potential stemming from the secondary metabolites found in its inflorescences which entail terpenoids and other phenolic compounds with antioxidant, anti-inflammatory, neuroprotective, and cognitive function potency (Chinou *et al*., 1997, 2004; Akaberi *et al*., 2019). As such, domestication efforts of *H. amorginum* have been initiated in terms of the development of seed propagation protocols for conservation and sustainable exploitation of the germplasm studied herein (Argyriou *et al*., 2020).

Figure 1. *H. amorginum* flowering plant from the experimental cultivation

Based on the above framework, the current work studied the effects of planting density and organic fertilization on *H. amorginum* plants during three years of experimental cultivation. The overall aim of the work was to set the basis for the cultivation of Greek *H. amorginum* germplasm in agronomical terms. This has been attempted via specific objectives set as follows: (a) to evaluate the vegetative growth of *H. amorginum* plants under different planting densities in combination with ascending levels of organically certified fertilization regimes in two distinct experimental fields across a period of three years, (b) to evaluate the inflorescence emergence and growth under the same treatment scheme which represent the final commercial product of interest. The study aspires to initiate a novel research scheme for setting the framework of the sustainable exploitation of Greek *H. amorginum* that can facilitate further breeding and upscaling efforts leading to the creation of a novel value chain for the pharmaceutical and cosmetics sectors.

Materials and Methods

Plant material

The initial, wild-occurring material of *Helichrysum amorginum* was located and collected during botanical expeditions by the Institute of Plant Breeding and Genetic Resources (IPBGR, ELGO-Dimitra, Thessaloniki, Greece) under a special permit (Permit 82336/879 updated in 18 May 2019, and 26895/1527 of 21 April 2021) issued by the Greek Ministry of Environment and Energy, in its natural habitats on the island of Amorgos in the Aegean Sea following taxonomic identification using established diagnostic keys (Strid, 2016). The collected material was then transferred to the laboratory of IPBGR for asexual reproduction using in vitro methods. The propagation work resulted in the production of 350 ex-situ adapted young plants that were transferred in 1 L pots with peat: perlite 3:1 (v/v) substrate back to the island of Amorgos early in 2016 for the experimental cultivation. The plants were then randomly divided into two groups of 175 plants each for field establishment into the two trial cultivation locations.

Experimental cultivation locations

The study took place across a period of three years (2016, 2017 and 2018) on the island of Amorgos, Aegean Sea, Greece. Two separate field locations were used for the experimental cultivation of *H. amorginum* on the island of Amorgos, in Aegiali and in Katapola, that were of different altitude, distance from the sea, soil organic matter, chemical and mechanical properties (Table 1), both however, had access to irrigation water.

Table 1. Topographic and soil characteristics of the two experimental cultivation locations of *Helichrysum amorginum* in the island of Amorgos, Greece

Topographic / soil property	Aegiali	Katapola
Coordinates (HGRS87) (Lat, Lon)	36° 54′ 55,6 N 26° 00′ 22,4 E	36° 49′ 36,8 N 25° 52′ 39,9 E
Altitude (m)	247	20
Distance from sea (m)	1500	400
Soil type	SCL	SCL
Organic matter (%)	4.83	3.27
Soil pH	7.64	7.5
Electrical conductivity (μS/cm)	160.6	188.6
Water saturation point (%)	50	45
Salinity (mg/Kg)	251	120.7
$CaCO_3$ (g/100 g)	0.58	0.58
NO_3-N (mg/Kg)	77	74
P (mg/Kg)	100	36
K (mg/Kg)	179	426
Ca (mg/Kg)	28488.1	3152.38
Mg (mg/Kg)	1539.9	1820.6
Fe (mg/Kg)	24488.9	18257

Experimental cultivation set-up and treatment application

The climatic conditions between the two experimental cultivation locations were recorded for the entirety of the three years of the study in terms of average, minimum and maximum temperatures, and rainfall (Table 2). During the three years of the study, the annual mean temperatures fluctuated between 11.6 °C in December and January (which are typically the area's coldest months) to 27.3 °C in July and August (typically the hottest months of the area). In both experimental cultivation locations minor monthly temperature differences were recorded between the three years of the study apart from May 2018 that showed slightly higher temperatures (1.6-2.1 °C average temperature difference) than the previous two years and the 10-year average, and December 2016 that showed lower ambient temperatures (1.9-3.7 °C on average) than the next two years and the 10-year average. In terms of precipitation, June – September were the driest months whereas December – March were the wettest months throughout the study, which is typical for the area. However, in contrast to the temperature conditions, significant variation in the monthly precipitation volumes was observed between the three years of the study for both experimental cultivation areas. For the months of February, November and December 2018 more than double rainfall volumes were recorded than the region's 10-year average compared to 2016 which was the driest year of the last decade.

The topsoil of both experimental fields was cultivated before planting at a depth of 20 cm, following which a drip irrigation system was installed according to the experimental plan. Consecutively the plants were established in both experimental fields in April 2016 and were irrigated. The irrigation water in both experimental fields was made available via nearby artesian wells. The available water on the island of Amorgos, like the rest of the Cyclades, is of moderate to high salinity, which traditionally poses a limitation on commercial horticultural practice on the available land. As such, the electrical conductivity (EC) of the irrigation water was monitored throughout the experimental period and showed values that ranged between 0.688-0.993 dS/m in Katapola and between 0.543-0.781 dS/m in Aegiali, which is considered within acceptable levels for plant growth (Munns and Tester, 2008; Sheldon *et al*., 2017). Additional pervious research on *Helichrysum petiolatum,* has shown tolerance to irrigation water salinity for cultivated germplasm (4.0 to 5.1 dS/m) (Niu *et al*., 2010). The plants in both experimental fields were irrigated continually throughout the experimental period every 2-3 days during the spring and summer months when higher temperatures and low precipitation rates prevailed, and every 5-7 days during the autumn and winter months. The experimental cultivation was set-up based on the principles of organic horticulture (Ferguson, 2004), and as such biological methods for pests and disease management were applied when needed (biological fungal-based fungicides and *Bacillus thuringiensis* (Bt) based pesticides were used) coupled with manual weeding between the planting rows.

Table 2. Monthly means of precipitation (mm), maximum, minimum and average temperatures (°C) for 2016, 2017 and 2018* and the 10-year average at experimentation sites

Average temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2016	13.0	15.5	15.2	18.5	20.3	24.4	25.3	25.6	24.0	21.0	17.6	11.9
2017	11.6	13.8	14.9	16.8	20.7	24.5	25.9	25.4	24.5	20.1	17.1	15.6
2018	13.8	15.2	16.8	18.7	22.4	25.0	27.3	26.2	24.5	20.6	17.9	13.8
AVG 10 years	13.3	14.2	14.8	17.3	20.8	24.1	25.5	25.9	24.2	20.7	18.1	15.0
Maximum temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2016	14.9	17.7	17.1	21.6	23.3	27.9	27.8	27.7	26.3	22.9	19.3	13.5
2017	13.5	15.4	17.2	19.6	23.8	27.4	28.8	27.9	27.6	22.3	19.5	17.4
2018	15.1	16.0	16.7	19.9	23.8	26.9	28.2	28.4	26.6	22.7	19.8	16.6
AVG 10 years	15.6	16.9	18.8	21.9	25.4	28.1	30.0	28.3	22.2	19.3	15.6	15.2
Minimum temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2016	10.8	13,2	13.2	15.5	17.6	21.9	23.3	23.8	22.1	18.9	15.2	9.9
2017	8.9	12,2	12.4	14.0	18.0	22.0	23.6	23.6	22.0	18.1	14.5	13.7
2018	11.6	12.9	14.7	15.8	20.2	22.5	25.2	24.7	22.6	18.9	16.5	11.7
AVG 10 years	11.0	11.9	12.4	14.6	18.0	21.7	23.3	23.9	22.2	18.8	16.1	12.9
Precipitation (mm)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2016	59.2	19.0	28.4	0	7.4	0.8	0	0	1.2	3.4	24.2	25.8
2017	98.6	46.4	101.0	4.8	26.8	1.2	0	0	0	17.4	25.8	31.8
2018	46.6	80.8	6.0	0.0	0.0	2.8	0.0	0.0	6.2	7.4	58.8	113.2
AVG 10 years	110.5	69.4	54.7	16.1	6.6	5.5	0.0	0.0	4.4	12.1	35.3	71.6

*Source data: *www.meteo.gr*, accessed on 12 April 2023

The experimental design entailed three planting densities and three organic fertilization regimes and was set as a split-plot in randomized complete block design where the planting density was the main plot assigned in three replicate blocks with each planting density within each block containing the three fertilization regimes as the sub-plot. The design resulted in nine combinations of planting density and fertilization (3 × 3) with 6 plants each which was replicated three times in the three blocks for every experimental field utilizing the block as the experimental replicate (n = 3). Thus, 162 plants were planted in each experimental field resulting in 324 plants in total with 12 extra plants in each field used as guard plants.

The planting densities were as follows: 0.3 m (D30, high density), 0.5 m (D50, medium density) and 0.7 m (D70, low density) plant distance within rows, with 0.7 m distance between rows in all cases. The applied fertilization consisted of an organically certified, ready-made commercial nutrient mix, Biogen 7-4-7 (organic N-P_2O_5-K_2O) with Mg 3%, B 0.2%, organic matter 33% and micronutrients (Phytothreptiki S.A., Athens, Greece) which was applied in three ascending levels. The fertilization regimes were as follows: 20-5-16.5 (organic N-P-K-OF/1a, basic level), 30-7.5-25 (organic N-P-K- OF/1.5a, medium level), and 40-10-33 (organic N-P-K-OF/2a, high level) in each experimental field. The fertilization regimes were applied during planting in 2016 and the same application was repeated at the start of the growth season in 2017 and 2018.

Plant measurements

The effects of planting density and organic fertilization were assessed continually throughout the three years of the experimental cultivation via morphological measurements of plant growth, coupled with inflorescence emergence and growth, and inflorescence dry weight which is the final yield element of commercial interest for *H. amorginum*, since it is the plant part that contains various secondary metabolites of interest (Chinou *et al*., 2004). Plant base diameter and total plant height were measured at regular intervals from spring through winter throughout the three years of experimental cultivation. The main flowering period of *H. amorginum* during the experimental cultivation herein was determined during mid-late spring until early summer. The number and length of emerged inflorescences were measured, at full bloom before seed set in all cases, following which, the inflorescences were harvested (cut from their base) for dry weight assessment and the plants continued their growth until the next flowering period the following spring. At the first year of experimental cultivation (2016), since the newly established plants were young, inflorescence measurement and harvest was conducted following their emergence and development in May until July 2016, whereas for the following years of 2017 and 2018, inflorescence emergence, measurement and harvest took place from late March / mid-April until late June. The harvested inflorescences were collected and labelled separately for each experimental field, treatment and replicate and were transferred to a warehouse where they were left to dry under natural ventilation in purpose-built shelves. The drying inflorescences were ruffled daily, and their weight was measured every two days. The material was considered fully dry, when no difference in weight was recorded for three consecutive measurements.

Statistical analysis

The plant growth data of plant height and base diameter were measured continually across the three years of the study and were subjected to analysis of variance (GLM-ANOVA) to establish overall treatment effects including the effect of time (date of measurement). Following the initial ANOVA results, mean separation was conducted for each measurement date using Tukey's HSD post hoc test ($p<0.05$). Similarly, the treatment effects on inflorescence number, length and weight data that were measured on a yearly basis, were assessed by analysis of variance (GLM-ANOVA) and means were compared via Tukey's HSD post hoc test ($p<0.05$). All analyses were conducted using the SigmaPlot 12 software (Systat Software Inc., San Jose, CA, USA), and graphs were drawn using Microsoft Excel 365 (Microsoft corp., Washington, USA).

Results

Planting density and organic fertilization effects on plant growth during cultivation of Helichrysum amorginum

In general, *H. amorginum* plants showed higher rates of plant growth in the experimental field of Aegiali compared to that of Katapola in terms of plant base diameter and plant height (Figures 2-5). Across the three years of the study, during the winter and early spring months, plants demonstrated higher values of base diameter, whereas during the summer months limited plant base growth was observed in all applied treatments in both experimental fields (Figures 2 and 3).

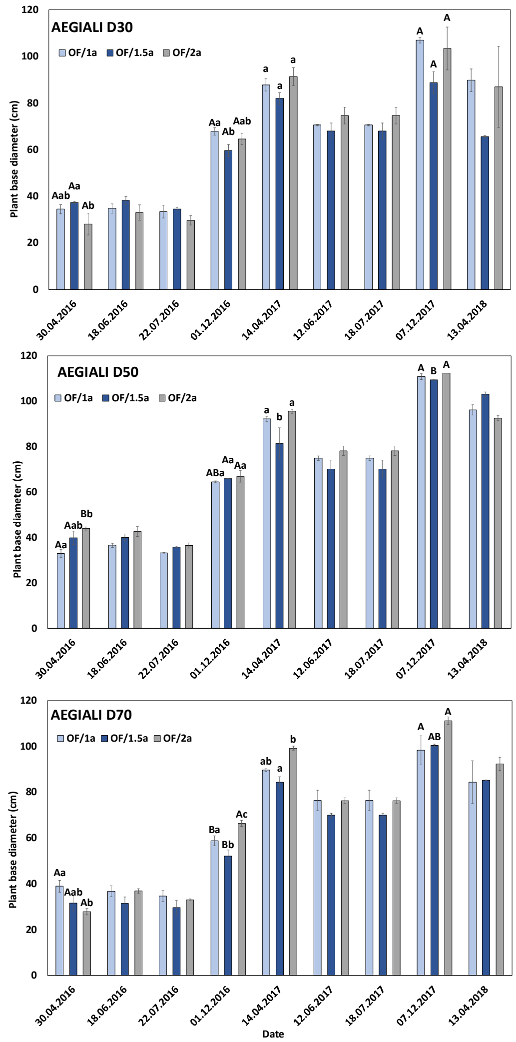

Figure 2. Effect of the application of organic fertilization (OF/1a, OF/1.5a, and OF/2a) in interaction with planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance) on the base diameter (cm) of *Helichrysum amorginum* plants under cultivation conditions across three years of experimentation in the field of Aegiali

Standard error bars are shown on the graphs. Capital letters denote differences among planting densities within each fertilization treatment. Lowercase letters denote differences between fertilization treatments within each planting density and date (Tukey HSD, $p<0.05$). In cases where no letters can be seen, no significant effects of the applied factors or their interaction were observed.

Concerning the applied treatments, generally the planting density affected plant base diameter in interaction with the organic fertilization (Figures 2 and 3). In the field of Aegiali, the organic fertilization levels showed limited effects during the summer months, however, towards the end of the first year (December 2016) and the beginning of the second year (March 2017), both the lower and the higher fertilization levels (OF/1a and OF/2a) showed higher values of plant base diameter in the medium and lower planting densities (D50 and D70) with no effect on plant height during the same period (Figures 2 and 4). Comparatively in the field of Katapola the medium fertilization level (OF/1.5a) showed higher planet base diameter in the lower planting density (D70) in July 2016 whereas in July and December of the following year (2017) the higher fertilization level (OF/2a) showed higher plant base diameter with similar plant height in the lower planting density (D70) (Figures 3 and 5). At the same time, limited fertilization effects were observed in the two higher planting densities (D30 and D50) (Figures 3 and 5). Generally, the lower planting density in the field of Katapola demonstrated higher plant base diameter in conjunction with the higher fertilization level from the second year (2017) of the experimental cultivation onwards (Figure 3).

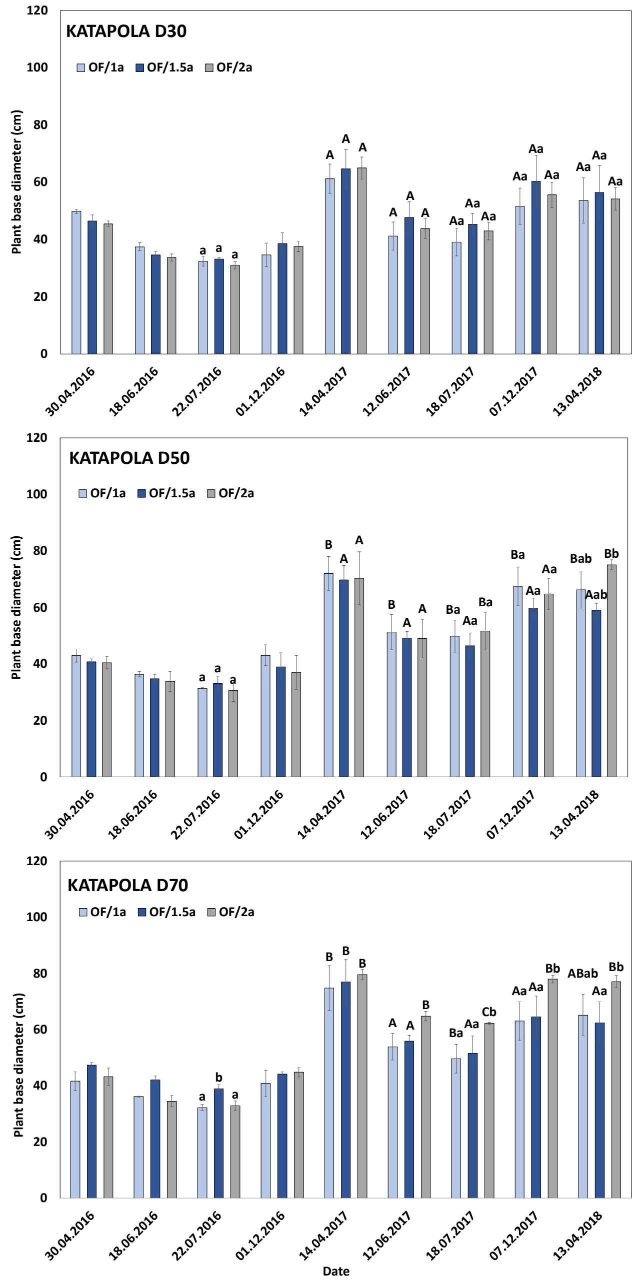

Figure 3. Effect of the application of organic fertilization (OF/1a, OF/1.5a, and OF/2a) in interaction with planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance) on the base diameter (cm) of *Helichrysum amorginum* plants under cultivation conditions across three years of experimentation in the field of Katapola

Standard error bars are shown on the graphs. Capital letters denote differences among planting densities within each fertilization treatment. Lowercase letters denote differences between fertilization treatments within each planting density and date (Tukey HSD, $p<0.05$). In cases where no letters can be seen, no significant effects of the applied factors or their interaction were observed.

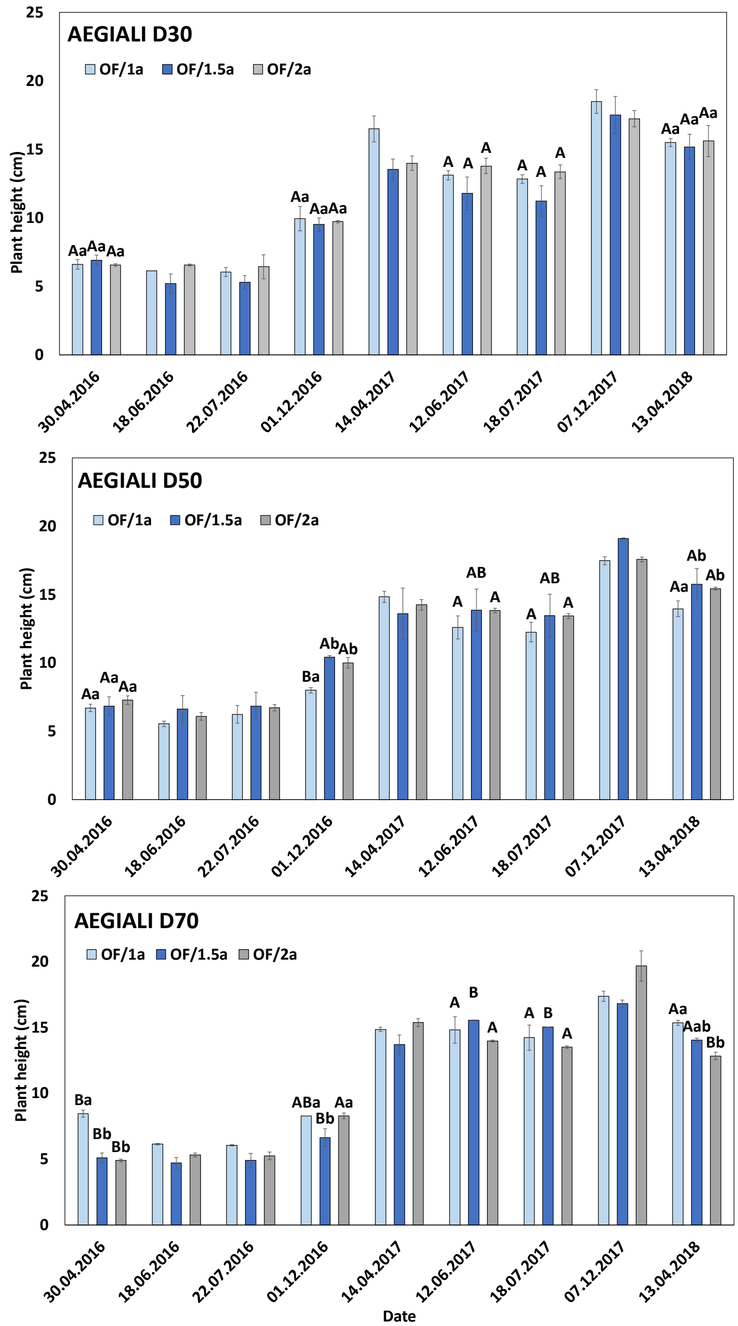

Figure 4. Effect of the application of organic fertilization (OF/1a, OF/1.5a, and OF/2a) in interaction with planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance) on plant height (cm) of *Helichrysum amorginum* under cultivation conditions across three years of experimentation in the field of Aegiali

Standard error bars are shown on the graphs. Capital letters denote differences among planting densities within each fertilization treatment. Lowercase letters denote differences between fertilization treatments within each planting density and date (Tukey HSD, $p<0.05$). In cases where no letters can be seen, no significant effects of the applied factors or their interaction were observed.

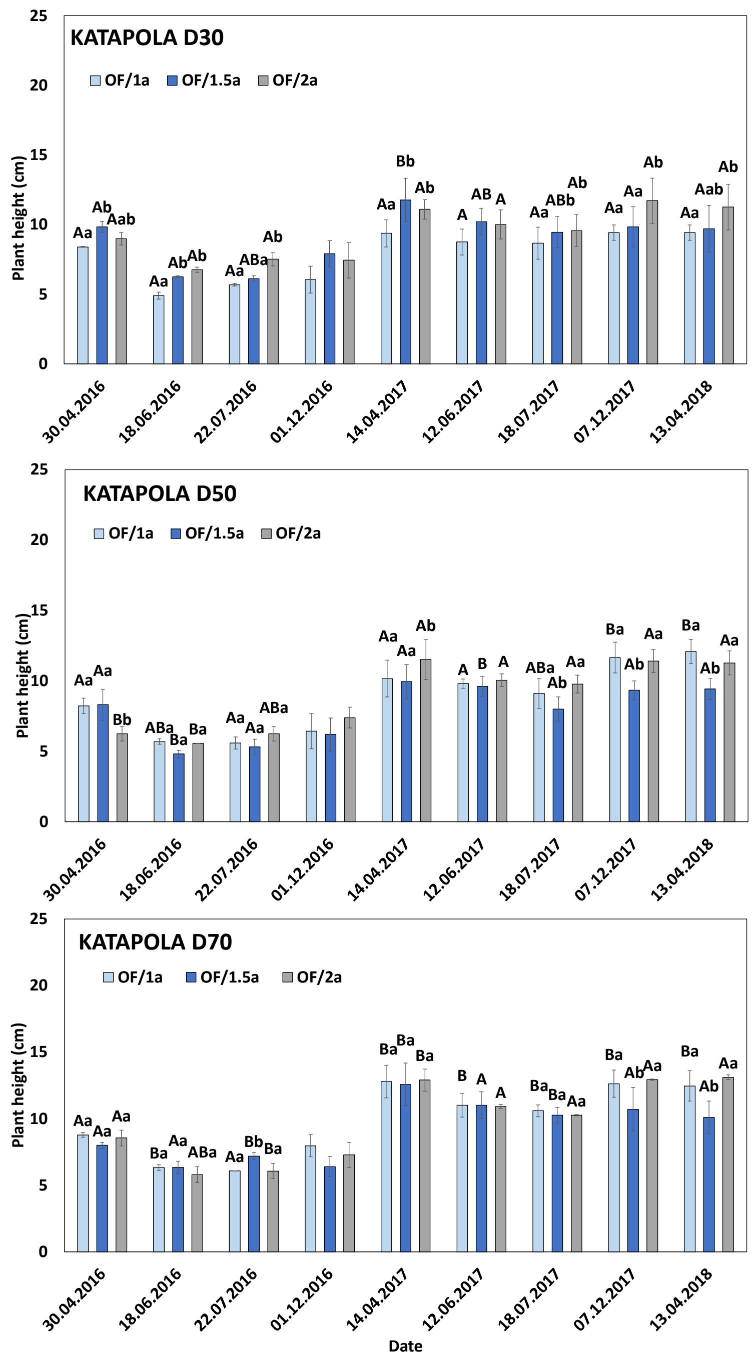

Figure 5. Effect of the application of organic fertilization (OF/1a, OF/1.5a, and OF/2a) in interaction with planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance) on plant height (cm) of *Helichrysum amorginum* under cultivation conditions across three years of experimentation in the field of Katapola

Standard error bars are shown on the graphs. Capital letters denote differences among planting densities within each fertilization treatment. Lowercase letters denote differences between fertilization treatments within each planting density and date (Tukey HSD, $p<0.05$). In cases where no letters can be seen, no significant effects of the applied factors or their interaction were observed

Inflorescence production during cultivation of Helichrysum amorginum

During the first year of experimental cultivation, inflorescence production was limited in both experimental fields with mean inflorescence number per plant ranging from 1.3 in D30, OF/1a treatment to 9.1 in D30, OF/1.5a treatment in Aegiali, whereas in Katapola, mean inflorescence number per plant ranged from 0 in D70, OF/1.5a treatment to 4.76 in D30, OF/1.5a treatment (Table 3). Inflorescence emergence increased significantly in the second and third year of experimental cultivation with the higher fertilization level (OF/2a) under the medium planting density (D50) showing the highest number of emerged inflorescences in both experimental fields in 2017 and 2018 (37.1 mean inflorescence number per plant in 2017 to 56.4 in 2018 in Aegiali and 33.4 in 2017 to 51 in 2018 in Katapola, Table 3).

The growth rate of inflorescences in terms of inflorescence length showed similar results following their emergence with higher lengths in the second and third years of experimental cultivation (Table 3). The higher level of organic fertilization (OF/2a) under the medium planting density (D50) seemed to deliver longer inflorescences in Katapola across the first two years of the study with 14.5 cm mean inflorescence length per plant in the first year, and 29.6 cm in the second year (Table 3), whereas in the third year the respective average inflorescence length of the above treatment combination was 40.2 without being affected by fertilization (Table 2). Similarly, in Aegiali the higher level of applied fertilization showed higher inflorescence length in the medium and high planting densities in 2017 (31.8 cm and 30.7 cm respectively) and in the lower planting density (D70) in 2018 (50.2 cm) (Table 3). During the third year of experimentation, fertilization was the factor affecting inflorescence length in Aegiali, whereas in Katapola the planting density affected this parameter in the third year (Table 3).

Considering the harvested biomass of the inflorescences in terms of dry weight, more and longer inflorescences showed higher dry biomass yield as expected (Figure 6). The higher level of organic fertilization (OF/2a) applied under the medium planting density (D50), delivered higher dry biomass yield across the three years of experimentation in both fields with values ranging between 10.9 kg/1000 m^2 in 2016, 158.8 in 2017 and 265 in 2018 in Katapola and 9.7 in 2016, 251.7 in 2017 and 338.6 in 2018 in Aegiali (Figure 6).

Table 3. Inflorescence emergence expressed as mean inflorescence number per plant (± SEM) and inflorescence growth expressed as mean inflorescence length per plant (cm ± SEM) of the Greek endemic *Helichrysum amorginum* as affected by planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance) and organic fertilization (OF/1a, OF/1.5a, and OF/2a) measured on a yearly basis across the three years of experimental cultivation in the fields of Aegiali and Katapola

Treatments		Aegiali					
		Year					
Planting density	Fertilization	2016		2017		2018	
		Inflorescence number	Inflorescence length (cm)	Inflorescence number	Inflorescence length (cm)	Inflorescence number	Inflorescence length (cm)
D30	**OF/1$_a$**	1.30 (±0.40) Aa	10.59 (±2.01)	20.83 (±1.45) a	27.35 (±0.14) Aa	29.50 (±6.64) Aa	47.85 (±0.40) a
	OF/1.5$_a$	9.09 (±3.36) Ab	12.98 (±0.40)	18.54 (±6.71) a	29.00 (±0.12) Ab	27.74 (±2.86) Aa	48.05 (±0.70) a
	OF/2$_a$	2.09 (±0.31) Aa	13.00 (±0.00)	25.56 (±0.80) a	30.75 (±0.38) Ac	28.00 (±6.64) Aa	48.50 (±1.20) a
D50	**OF/1$_a$**	2.30 (±0.40) Aa	13.95 (±0.89)	26.40 (±3.46) a	27.55 (±0.38) Aa	28.48 (±0.29) Aa	47.70 (±0.10) a
	OF/1.5$_a$	2.46 (±1.13) Ba	14.80 (±3.00)	16.35 (±7.59) b	28.90 (±0.46) Ab	34.47 (±2.87) Aa	48.00 (±0.00) a
	OF/2$_a$	4.97 (±1.11) Aa	12.55 (±0.14)	37.14 (±3.06) c	31.80 (±0.23) Ac	56.44 (±3.71) Bb	48.90 (±1.20) a
D70	**OF/1$_a$**	5.38 (±2.53) Aa	13.40 (±0.12)	18.47 (±5.46) a	32.15 (±0.26) Ba	20.17 (±5.68) Aa	47.25 (±0.50) a
	OF/1.5$_a$	2.38 (±1.08) Ba	12.35 (±0.78)	20.50 (±1.73) a	31.15 (±0.78) Ba	27.27 (±7.89) Aa	47.75 (±0.50) a
	OF/2$_a$	3.17 (±1.25) Aa	13.00 (±0.46)	30.00 (±2.69) b	30.90 (±0.06) Aa	43.33 (±8.85) ABb	50.25 (±0.30) b

Treatments		Katapola					
		Year					
Planting density	Fertilization	2016		2017		2018	
		Inflorescence number	Inflorescence length (cm)	Inflorescence number	Inflorescence length (cm)	Inflorescence number	Inflorescence length (cm)
D30	**OF/1$_a$**	1.33 (±0.58) Aa	13.50 (±1.73) Aa	9.917 (±2.93) Aa	26.30 (±0.20) Aa	11.90 (±0.46) A	37.60 (±0.90) A
	OF/1.5$_a$	4.76 (±0.68) Ab	15.05 (±0.14) Aa	15.27 (±4.21) Aa	27.00 (±0.80) Aa	31.54 (±14.9) A	37.75 (±1.20) A
	OF/2$_a$	1.91 (±0.84) ABa	12.60 (±1.21) Aa	15.04 (±1.21) Aa	26.95 (±0.20) Aa	24.05 (±2.52) A	36.15 (±0.30) A
D50	**OF/1$_a$**	0.75 (±0.24) Aa	11.40 (±1.39) Aa	21.17 (±4.52) Ba	27.70 (±1.00) Ba	34.40 (±5.20) B	38.65 (±0.20) A
	OF/1.5$_a$	1.17 (±0.00) Ba	13.05 (±0.61) Aab	17.58 (±6.30) Aa	27.20 (±0.60) Aa	26.54 (±0.16) A	37.15 (±0.10) A
	OF/2$_a$	3.58 (±1.30) Ab	14.50 (±0.06) Ab	33.42 (±6.11) Bb	29.65 (±0.10) Bb	51.03 (±6.71) B	40.25 (±0.40) B
D70	**OF/1$_a$**	1.42 (±0.05) Aa	13.40 (±0.06) Aa	22.67 (±7.22) Ba	29.55 (±0.10) Cab	35.49 (±13.30) B	38.30 (±1.20) A
	OF/1.5$_a$	0.0 (±0.0) Ba	0.00 (±0.00) Bb	16.63 (±4.26) Aa	28.20 (±0.50) Aa	28.00 (±1.15) A	37.35 (±0.50) A
	OF/2$_a$	1.08 (±0.53) Ba	13.10 (±0.81) Aa	32.58 (±0.63) Bb	30.30 (±0.20) Bb	45.84 (±3.60) B	38.60 (±0.30) B

Capital letters denote differences among planting densities within each fertilization treatment. Within each measurement year, lowercase letters denote differences between fertilization treatments within each planting density (Tukey HSD, $p<0.05$). In cases where no letters can be seen, no significant effects of the relevant applied factor were observed.

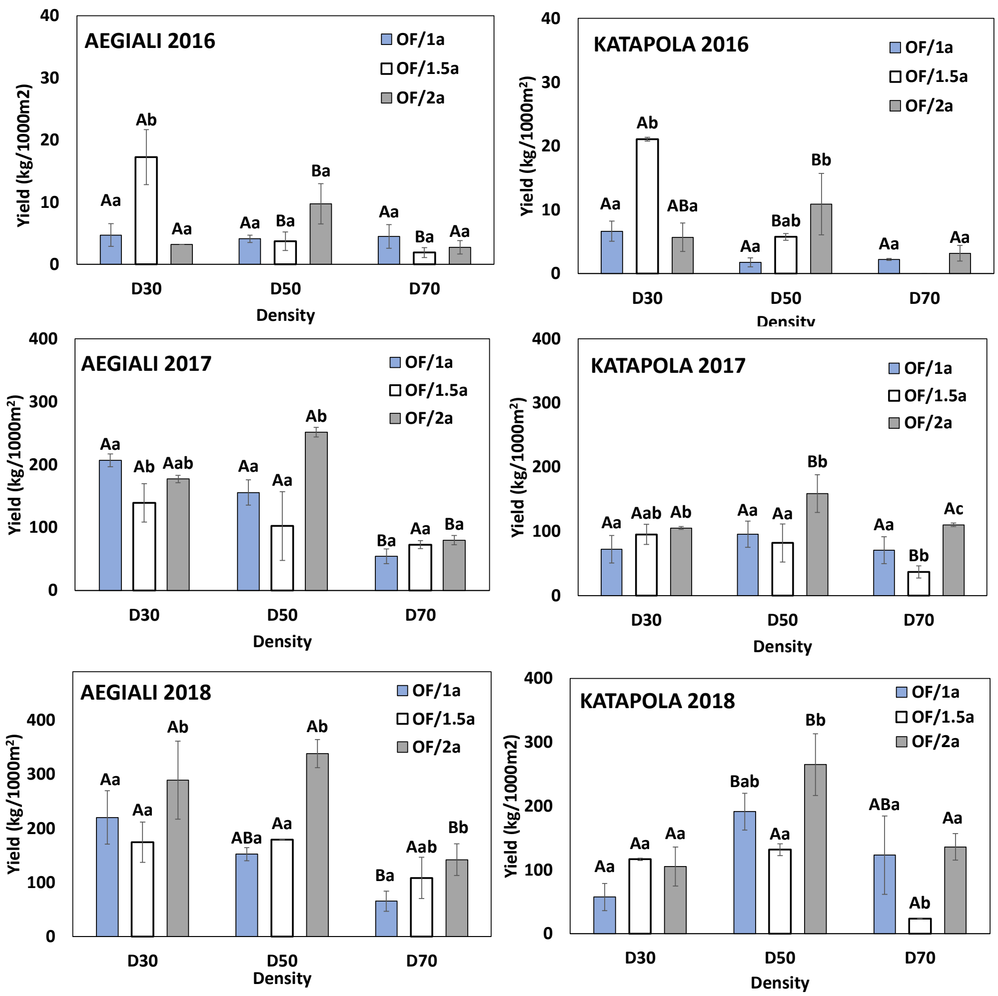

Figure 6. Dry inflorescence biomass accumulation (yield – kg/1000m^2) of *Helichrysum amorginum* across three years of experimentation in the fields of Aegiali and Katapola as affected by the application of organic fertilization (OF/1a, OF/1.5a, and OF/2a) in interaction with planting density (D30 – 0.3 m, D50 – 0.5 m and D70 – 0.7 m within row distance). Standard error bars are shown on the graphs

Capital letters denote differences among planting densities within each fertilization treatment. Lowercase letters denote differences between fertilization treatments within each planting density (Tukey HSD, $p<0.05$).

Discussion

The conservation of native phytogenetic resources, especially for recognized biodiversity hotspots like the Mediterranean and Greece, is pivotal for safeguarding valuable genotypic diversity under the current climate change in a global context, but also for securing economically potent germplasm sources in a local context (Grigoriadou *et al*., 2020; Sharrock, 2020; Maloupa *et al*., 2021). The current study provides for the first-time valuable knowledge towards the sustainable exploitation of a range-restricted neglected and underutilized plant species (NUP), *Helichrysum amorginum* enhancing at the same time, its conservation status. The 3-year experimental cultivation trial conducted herein demonstrated the effects of organic fertilization and planting density on enhancing vegetative plant growth and inflorescence emergence. External fertilization inputs have been shown to enhance plant growth in both cultivated and wild originated plant germplasm (Ensslin and Godefroid, 2020). In case of newly introduced species in controlled cultivation schemes, the applied cultivation practices which remain unknown must be extensively studied as they affect plant growth and yield (Chrysargyris *et al*., 2022). In the current study, the application of organic fertilization enhanced plant growth in terms of plant base diameter and plant height. Similar responses of integrated nutrient application on plant growth have been observed during experimental cultivation of other range-restricted Mediterranean NUPs, like *Lomelosia minoana* (Hatzilazarou *et al*., 2023), or *Teucrium luteum* (Kostas *et al*., 2022). However, in *H. amorginum* plants herein, the response to fertilization was affected by season following the annual growth patterns of the plants which were restricted during the summer months. In addition, the magnitude of the fertilization effect was influenced by planting density.

In the lower planting densities applied herein, the application of higher levels of organic fertilization, delivered larger plants with more inflorescences which was more expressed in the field of Katapola where the soil contained less organic matter than Aegiali, implying a minor difference in optimal planting density between the two experimental fields. In addition, the organic fertilization applied affected plant growth and development differently under different planting densities. According to Badawy (2015) the interaction of fertilization with the parameter of plant density affected the number of inflorescences/plant, inflorescence diameter, fresh and dry weight of spray, in *Helichrysum bracteatum*, with the maximum values attributed to the lower density (40 cm spacing) combined with the higher levels of Nitrogen (200 kg/fed) and Potassium (75 kg/fed) application.

The level of fertilization herein was mainly linked to increasing inputs of the main macronutrients N, P and K, with N being in organic form. Plants, in general, possess the capacity to utilize organic N and related amino acids via the root system but it can encounter competition by the soil microbiota and the presence of symbiotic mycorrhizal fungi which can facilitate amino acid transport to the root cells enhancing uptake (Näsholm *et al*., 2009; Moran-Zuloaga *et al*., 2015). The application of organic N via an integrated fertilization scheme, has been shown to enhance photosynthetic activity during experimental cultivation of local range restricted herbaceous perennials leading to enhanced tissue growth and flowering rates (Kostas *et al*., 2022; Hatzilazarou *et al*., 2023). Although the photosynthetic activity was not evaluated herein, the current results suggest an effect of organic fertilization and planting density in flower initiation of *H. amorginum* coupled with biomass production of the flowering stems.

The timing of fertilization may also contribute to the fertilizer use efficiency (Hooper *et al*., 2015). In the current experiment, although the applied fertilization was rich in K, that generally enhances flowering, its initial application was conducted at spring of 2016, when flower initiation within the crown buds of the young plants had most probably already taken place. Consecutively, the application was repeated early in 2017 which was long before flower initiation for the following year. However, from the current results it cannot be concluded whether the timing of application was strongly involved in flower initiation and flowering of *H. amorginum* since the periodic application in a yearly basis can be considered as an investment to the soil's fertility profile rather than a direct uptake from the plants like, for instance a foliar application, especially for slow-release elements like P and K (Marschner and Rengel, 2023).

The research on cultivated *Origanum vulgare subsp. hirtum* in pots suggested that both application levels of nitrogen fertilization 0.6 N g/pot and 1,2 N g/pot increased the dry matter compared to the control (Ninou *et al*., 2017). Moreover, a field study of the species *Cnicus benedictus* L. of Asteraceae family concluded that that addition of 100 kg N/ha with 15-20 plants/m^2 led to a significant increase in vegetative and growth variables compared to higher and lower levels of fertilization and plant density respectively (Ghiasy-Oskoee *et al*., 2020). From the current results the precise mechanism under which the applied fertilization acted on *H. amorginum* plants remains unclear. Nevertheless, the positive effect of the applied fertilization on plant growth, inflorescence production and ultimately dry mass yield in combination with an appropriate planting density can set an informed basis for an efficient cultivation protocol, providing, at the same times, grounds for further research on the agronomic utilization of *H. amorginum*.

Conclusions

The conservation of wild germplasm resources for sustainable exploitation has been rendered more relevant than ever over the latest years as the climate change and economic pressures have been intensified both in agroecology and commercial horticulture The current study evaluates the effects of planting density and organic fertilization and provides a basis for the sustainable cultivation of *H. amorginum*, a range restricted taxon with known potential for the pharmaceutical and cosmetic sectors.

Authors' Contributions

Conceptualization GS, PP; Data curation GS, EV; Formal analysis GS; Funding acquisition GS; Investigation IK, EK; Methodology GS, PP; Supervision PP, EM; Validation GS, EK; Visualization PP; Writing - original draft GS, EK; Writing - review and editing GS, PP, EM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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