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Posted Date: 13 December 2023

doi: 10.20944/preprints202312.0913.v1

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Dynamic of Agronomic Characteristics and Plant Diversity in Lemon Verbena (*Aloysia citrodora* Paláu.) Cultivation in Greece

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Abstract: The purpose of this investigation was to study the evaluation of a biological fertilizer use on growth and on agronomic characteristics of *Aloysia citrodora*. For this aim a two – year Completely Randomized Design (CRD) field experiment was carried out in central Greece. The experiments included four biological fertilizer levels, BF0: 0 N kg ha^{-1}, BF50: 50 N kg ha^{-1}, BF100: 100 N kg ha^{-1} and BF150: 150 N kg ha^{-1} with 3 replication. Plant height, dry stem, dry leaves, dry total yield and Leaf Area Index (LAI) were measured during the two growing years. The results showed that in the 1st studied the maximum plant height was observed in BF150 treatment (51.8 cm) while the 2nd year the BF150 level provoked a raise of 16.08% and 1.09% compared to BF50 and BF100, respectively. The 1st year the values of total dry yield were better in BF150 application (1945 kg ha^{-1}). The 2nd year the total yield increased by 50.27% in BF100 and 50.09% BF150 treatments. Moreover, LAI was ameliorated by applied the BF100 and BF150 treatments both in two studied years. A key finding in this study is that *A. citrodora* ecosystem favors herbaceous plant species richness. Also, soil factors (soil organic matter, P and K) promote positive herbaceous plant diversity within the *A. citrodora* ecosystem. Finally, *A. citrodora* can be a promising medicinal plant, cultivated under the Mediterranean climatic conditions.

Keywords: medicinal plant; LAI; yield; plant diversity; environment; sustainability; Greece

1. Introduction

Greece is considered one of the most biologically diverse countries of the European continent with high plant diversity and endemism. Greece accounts for 6% of the Mediterranean area and 26% of its flora. Medicinal and aromatic plants play a crucial role in sustainable development, environmental protection, and human health [1,2]. There's a plant called *A. citrodora* Paláu. with important health benefits and ecosystem services. *A. citrodora*, more specifically, originated in South America [3] and was introduced to Europe by the Spaniards. Today, it is cultivated in many Latin and Central American countries, southern European countries, such as Greece and France, northern Africa (Algeria and Morocco), China, and Iran. It is used as both a culinary and a medicinal herb. Alloysia is a genus in the *Verbenaceae* family, which contains many species and genera. Botanical synonyms include *Aloysia triphylla* (L'Hér.) Britton, *Lippia citriodora* Kunth, *Lippia triphylla* (L'Hér.) Kuntze, *Verbena triphylla* L'Hér. and *Zappania citrodora* Lam [4]. There are many medicinal and

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aromatic properties associated with this shrub. Alloysia is an evergreen perennial shrub, a deciduous sub-shrub, and there are about 2300 species in the genus Alloysia. A warm, moist environment with plenty of sunlight is ideal for it. It becomes deciduous when exposed to frost. *A. citrodora* prefers light, sandy, medium loamy, well-drained acid, neutral, and basic alkaline (pH of 4.5–7.8.) soils [5,6] and has an annual water requirement of 500 to 1.300 mm. The lemon-scented essential oil from the *A. citrodora* have been widely studied for its calming, digestive, abdominal-discomfort, lemony flavour properties [5–7]. *A. citrodora* traditionally has been utilized as a remedy for gastrointestinal and respiratory disorders. Besides antimalarial properties, some species also possess antiviral, antispasmodic, antibacterial, antioxidant, and cytostatic properties [8–11].

A. citrodora leaves can be used as an ingredient in stews and soups as well as fresh leaves [12]. Several studies have demonstrated that lemon verbena's related curative properties are due to essential oils and flavonoids [13]. Approximately 0.22% to 1.00% of *A. citrodora* leaves are hydrodistilled to yield the essential oil. Volatiles are influenced by harvesting season, time of day, and particle size [14]. The literature indicates that in Greece more attention is paid to the essential oil of *A. citrodora*, while their effects on parameters influencing ecosystem structure (such as biodiversity, soil properties, etc.) are neglected.

Nitrogen is an essential element which applied to the agricultural fields to increase the total crop biomass [15]. Synthenic fertilizers can provoke pollution and contamination of soil and waters [16,17]. At present there is the trend of using biological fertilizer instead of chemical ones. The organic fertilizers contains bacteria and fungus that can help to nutrients' uptake by plants and can also, provoke a significant quantitative and qualitative crop production increase [18].

Consumers' concern about food security has led to be prompted chemical free products by the farmers. Biofertilizers are able to motivate the useful elements for the plants, improve the nutrients' availability supply and enhance the nutrient process in soil through biological actions. The mechanisms of bioferitizers' fuction are direct or indirect. The fisrt ones affect the growth of plants directly by nitrogen fixation, phosphate solibilazation etc. The second ones protect the plants from the harmful effects of pathogens [16,19–21].

According to literature the knowledge of the application of a biological fertilizer recommendation program in *A. citrodora* cultivation is lacking [22]. Most of the published papers are mentioned to the determination of its chemical oil composition [23–27].

For the above purpose the aim of this study was to evaluate the use of a biofertilizer in different doses on agronomic characteristics and herbaceous plant diversity of *A. citrodora* in central Greece.

2. Materials and Methods

2.1. Study area

The field experiments were established in 2014 using *A. citrodora* cuttings which were obtained from a local nursery. Two growing periods (2014 and 2015) of *A. citrodora* plants were studied. The experiments conducted in the Velestino city (Volos, Magnesia). The studied area is located at an altitude of 120 m above sea level (Figure 1). The area has a Mediterranean climate with hot, dry summers and cool, humid winters. There is a high amount of calcium in this clay loam soil as well as good drainage [28].

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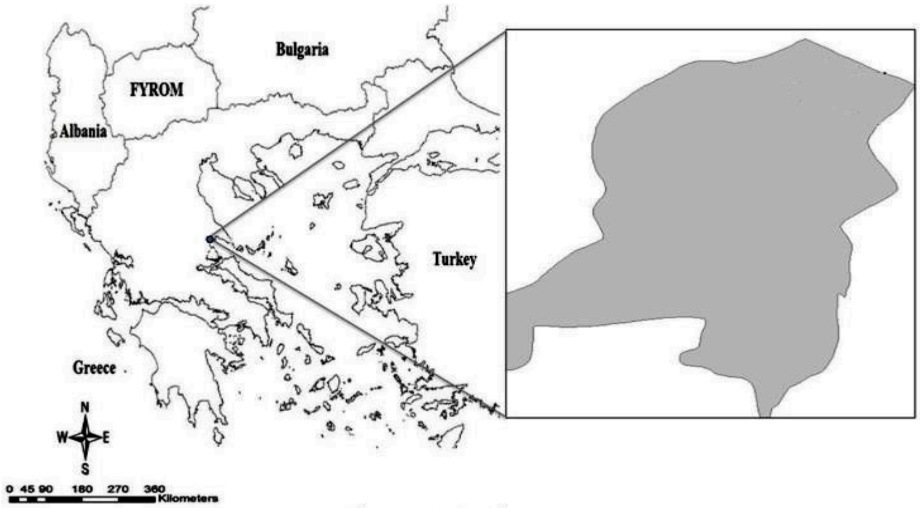

Figure 1. Study area.

2.2. Soil analysis

The 1st year two soil samples, from two depths (0-30 and 30-60 cm) were collected using the proper soil sampler. The 2nd year one soil sample was collected from one depth (0 – 40 cm). Each soil sample was consisted from five soil subsamples. The soil samples were transported to the Soil laboratory of the Institute of Industrial and Forage Crops (Larissa), air-dried and sieved using a 2-mm sieve. Soil samples were analyzed for pH (1:2.5 d. H_2O), electrical conductivity (1:5 d. H_2O), calcium carbonate ($CaCO_3$) using a calcimeter, the percentage (%) of sand, clay and silt using the Bouyoukos method and organic matter with Walkley – Black method , Available P (Olsen method, analyzed with ammonium vanadomolybdate / ascorbic blue and measured in a UV spectrophotometer at 882 nm) and Exchangeable K (1:10 at 1M CH_3COONH_4 pH 7, analyzed in a flame photometer) according to Rowell (1994) [29].

The soil was loam with pH 7.6, 1.8% organic matter and 11.5 – 12% $CaCO_3$. The physicochemical properties of the soil are presented in Table 1.

Table 1. Physicochemical properties of the used soil the 1st and 2nd cultivation year.

Year	Depth	pH	Organic matter (%)	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)	P (mg kg^{-1})	K (mg kg^{-1})
2014	0 – 30 cm	7.6	1.8	11.5	38	15	47	40	172
2014	30 – 60 cm	7.6	1.8	12	40	13	47	35	170
2015	0 - 40 cm	7.5	1.83	13	39	14	47	43	175

2.3. Meteological data

The meteorological data are presented in Figure 2. Total precipitation levels were 162.3 mm and 264.5 mm from May until September the 1st and 2nd year, respectively. The higher rainfall events were on May 2015 (62.5 mm) and on September 2015 (100 mm). The average temperature was at least 3^oC higher the 1nd year compared to 2st year.

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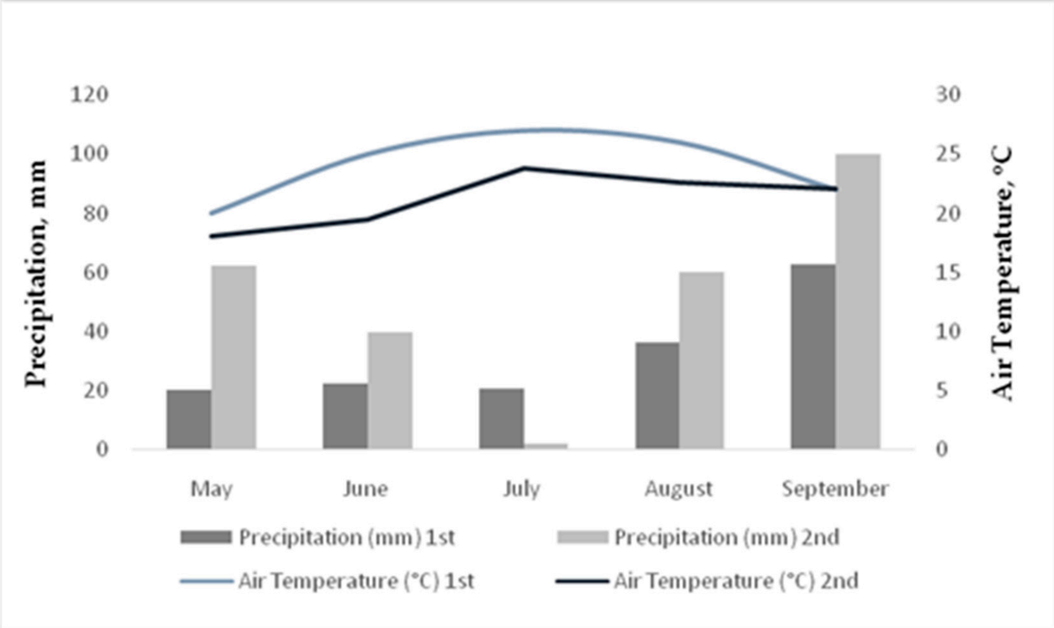

Figure 2. Air temperature and total precipitation in studied area during the growing periods (1st and 2nd growing years).

2.4. Field experiment

A two – year field experiment was established and the experimental design was a split-plot based on randomized complete blocks. Before the transplant the plants was raised for three months in polyethylene bags. The transplant was carried out on 3rd May 2014 in rows with spacing of 60 cm and 60 cm and each plot had 18 plants. The experiments included four nitrogen treatments of a biological fertilizer (6-0.5-0.3), BF0: 0 kg ha^{-1}, BF50: 50 kg ha^{-1}, BF100: 100 kg ha^{-1} and BF150: 150 kg ha^{-1}. The fertilizer was manually applied at the base of plants. The composition of the biological fertilizer was: 85% organic matter, 6% total N, 0.5% P_2O_5 and 0.3% K_2O (named Biosol). Also, during the experiments the plants were watered according to their needs so that the plots maintain their moisture level constant, using the drip irrigation system.

In total, the treatments were 12 with three replicates each and a total of 216 plants were transplanted in field.

Plant height, dry stem weight, dry leaves weight, dry total yield and Leaf Area Index were measured during the two growing years. The plant height of each plot was calculated from the average of five randomly plants. Then, these five plants were harvested by hand at 10 cm above the soil surface and immediately were weighted to record the fresh total weight, using a mobile balance. Furthermore, the plants were separated into leaves, flowers and stems and each edible part was weighted. Then, the plants were transported to the Lab for further measurements. Plant tissue of each plot was dried at 40°C until constant weight. Each plant part and the total biomass were weighted so that the dry weight to be calculated.

LAI measurements were performed during three different cutting periods from two plants of every different plot. Leaf area index was determined using an automatic LI-COR (model LI-3000A). The measured agronomic data are summarized in Table 2.

Table 2. Agronomic data of *Aloysia citrodora* cultivation in two growing years.

	Velestino	
	2014	**2015**
Date of transplant	**03/05/2014**	**-**

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Height	**29/09/2014**	**29/08/2015**
Fresh and dry weight	**30/06/2014, 89/07/2014, 29/09/2014**	**20/05/2015, 2/07/2015, 29/08/2015**
LAI measurement	**30/06/2014, 89/07/2014, 29/09/2014**	**20/05/2015, 2/07/2015, 29/08/2015**
Date of harvest	**30/06/2014, 89/07/2014, 29/09/2014**	**20/05/2015, 2/07/2015, 29/08/2015**

2.5. Sampling of herbaceous plants

A sampling of herbaceous plants was conducted during May – June in 12 plots of 0.25 m^2, every year. In each plot, herbaceous plant species richness and density in an organic *Aloysia citrodora* were recorded [30]. Also, 12 soil samples, were collected using the proper soil sampler. The physicochemical properties of the studied soil samples for the two years are presented in Table 3 (2014) and Table 4 (2015).

Table 3. Physiochemical properties of the 12 soil samples for the 1st studied year (2014).

2014	**pH**	**Organic matter**	**$CaCO_3$**	**Sand**	**Silt**	**Clay**	**P**	**K**	**Mg**
Units				%				(mg kg^{-1})	
Treatment									
1	7.6	1.80	11.5	38	15	47	40	172	326
2	7.6	1.80	12	40	13	47	38	170	330
3	7.5	1.71	13	39	14	47	43	175	335
4	7.7	1.79	11.1	41	15	44	41	176	320
5	7.6	1.70	10	40	15	45	40	172	322
6	7.4	1.61	12	42	16	42	36	168	328
7	7.5	1.80	12.5	42	15	43	44	173	321
8	7.5	1.70	13	40	16	44	45	174	325
9	7.7	1.80	13.5	43	15	42	42	170	329
10	7.6	1.69	11.4	41	14	45	43	174	327
11	7.5	1.70	11.5	41	15	44	40	175	326
12	7.6	1.80	13	38	16	46	39	172	330

Table 4. Physiochemical properties of the 12 soil samples for the 2nd studied year (2015).

2015	**pH**	**Organic matter**	**$CaCO_3$**	**Sand**	**Silt**	**Clay**	**P**	**K**	**Mg**
Units				%				(mg kg^{-1})	
Treatment									
1	7.5	1.82	12	40	16	44	40	171	328
2	7.7	1.83	12.5	41	15	44	38	171	331
3	7.6	1.73	13.2	40	15	45	43	174	334

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4	7.7	1.81	11.5	42	15	43	41	175	321
5	7.5	1.72	10.5	39	14	47	39	172	325
6	7.5	1.64	12	40	13	47	36	169	329
7	7.5	1.81	12	41	15	44	44	174	322
8	7.6	1.72	13.5	42	16	42	45	175	326
9	7.6	1.82	13	42	15	43	42	171	330
10	7.5	1.72	11.5	39	17	44	44	173	326
11	7.5	1.71	11.5	41	16	43	40	174	327
12	7.5	1.81	13.5	38	15	47	39	173	328

2.6. Statistical analysis

Species Diversity and Richness IV software was used to calculate the Shannon plant diversity index [30]. The Shannon index (SH) index takes into account both species abundance and species richness and it is the most commonly used index [31,32]. The SH is calculated for any sample population as follows:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i \tag{1}$$

where, s equals the number of species and pi is the relative cover of its species [33,34] (for a detailed description for this index, see Seaby and Henderson [30]). Correlations between the measured variables (soil organic matter (%), pH, $CaCO_3$, texture-clay, silt, sand, P, K and Mg) and SH in an organic *L. citriodora* were analyzed using Pearson correlation coefficients. Before the calculation of the Pearson correlation matrix, the data had to be transformed to logarithms to conform to the normal distribution criterion.

All statistical analyses for the data were performed using the software package Statgraphics plus 18 to the LSD test about the level of significance 95% ($p<0.05$).

3. Results

3.1. Plant height

Figure 3 shows the results regarding the height of the plants as they were measured during the harvest on 29/09/2014 and the second final harvest on 29/08/2015. As shown in Figure 3, the maximum height of the plants during the 1st year and 2nd year reached a value of 51.8 and 61.2 cm in BF150 plots. Fertilization seems to have had a statistically significant effect in the 1st and 2nd year of establishment. Specifically, both in two cultivation years between the BF0 and the other treatments there was statistically significant difference. Furthermore, statistically significant difference was observed among BF50 and BF100 and BF150 fertilization. Furthermore, the 2nd year was noticed an increase in plant height of BF150 compared to BF100 (1.09%).

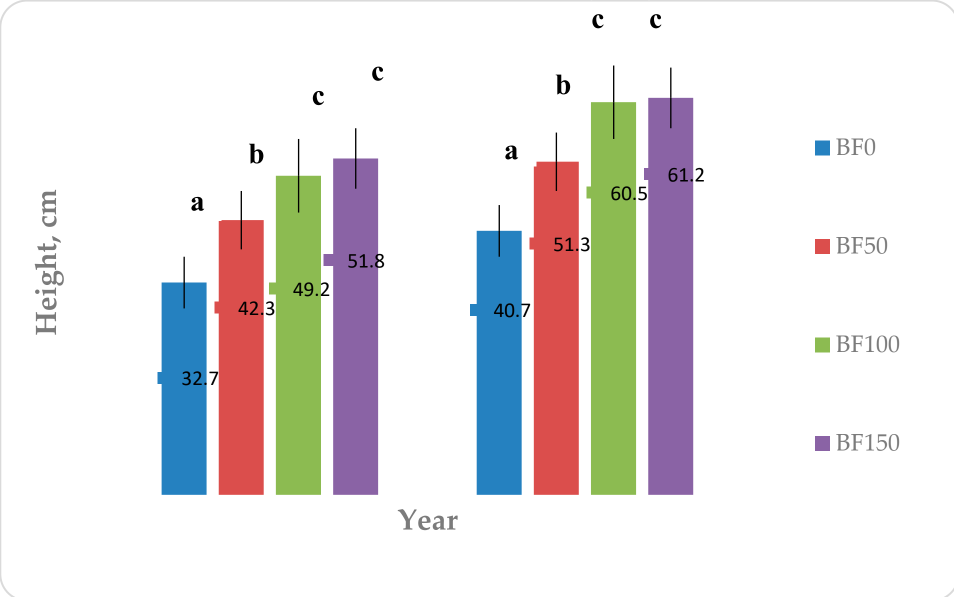

Figure 3. Plant height (cm) of the *Aloysia citrodora* cultivation in two growing years. *Different letters at each column denote statistically significant difference of means according to the LSD test for 95% significance level ($p < 0.05$).

3.2. Dry plant biomass

Table 5 shows the dry weight results of the steams, leaf and the total dry biomass, as measured in 2014 and 2015. In 2014 three cuttings of plants took place on 30/06/2014, 28/07/2014 and 29/09/2014, specifically 57, 85 and 146 days after planting. In 2015 plants harvested three times on 20/05/2015, 2/07/2015 and 29/08/2015, 382, 424 and 511 days after planting. In 2014 the maximum amount of dry stem (955 kg ha^{-1}), dry leaf (990 kg ha^{-1}) and total dry weight (1945 kg ha^{-1}) was observed in the BF150 fertilization. In 2015, the fertilization that gave the largest amount of dry total weight was the BF150 treatment, with yield of 3935.83 kg ha^{-1}, followed by BF100 and BF50 with 3233.83 and 2797.50 kg ha^{-1}, respectively. Furthermore, in two studied years statistically difference was noticed between the BF150 level and other treatments.

Table 5. Effect of the different fertilization on dry stem, dry leaf and total dry weight (kg ha^{-1}) of the *Aloysia citrodora* cultivation in two growing years.

	2014			2015		
Treatment	Dry stem weight, kg ha^{-1}	Dry leaf weight, kg ha^{-1}	Total dry weight, kg ha^{-1}	Dry stem weight, kg ha^{-1}	Dry leaf weight, kg ha^{-1}	Total dry weight, kg ha^{-1}
N0	583,33 a*	576.67 a	1160.00 a	1245.83 a	1174.50 a	2420.33 a
BF50	723,33 b	713.33 b	1436.67 b	1391.33 b	1406.17 b	2797.50 a
BF100	830.00 c	855.00 c	1685.00 b	1624.50 c	1609.33 c	3233.83 b
BF150	955.00 d	990.00 d	1945.00 c	1952.83 d	1983.00 d	3935.83 c
$LSD_{0.05}$	24.768	21.683	34.185	28.949	32.228	48.619

*Different letters at each column denote statistically significant difference of means according to the LSD test for 95% significance level ($p < 0.05$).

3.3. Leaf area index

The results of LAI are illustrated in Figure 4. Three measurements of LAI were conducted, in the 1st year on 30/06/2014, 28/07/2014 and 29/09/2014 and the in 2nd year on 20/05/2015, 2/07/2015 and

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29/08/2015. The 1st growing year the higher LAI values observed in the BF150 application and a significant increase in LAI values of BF150 plots was noticed which were 62.55%, 47% and 39.49% in 1st, 2nd and 3rd measurements, respectively, compared to BF0. In 2nd growing year the BF100 and BF150 treatments provoked the highest values of LAI and between these fertilizations there was no statistically difference. Moreover, in 2nd year the LAI value raised by 27.18% in BF100 treatment.

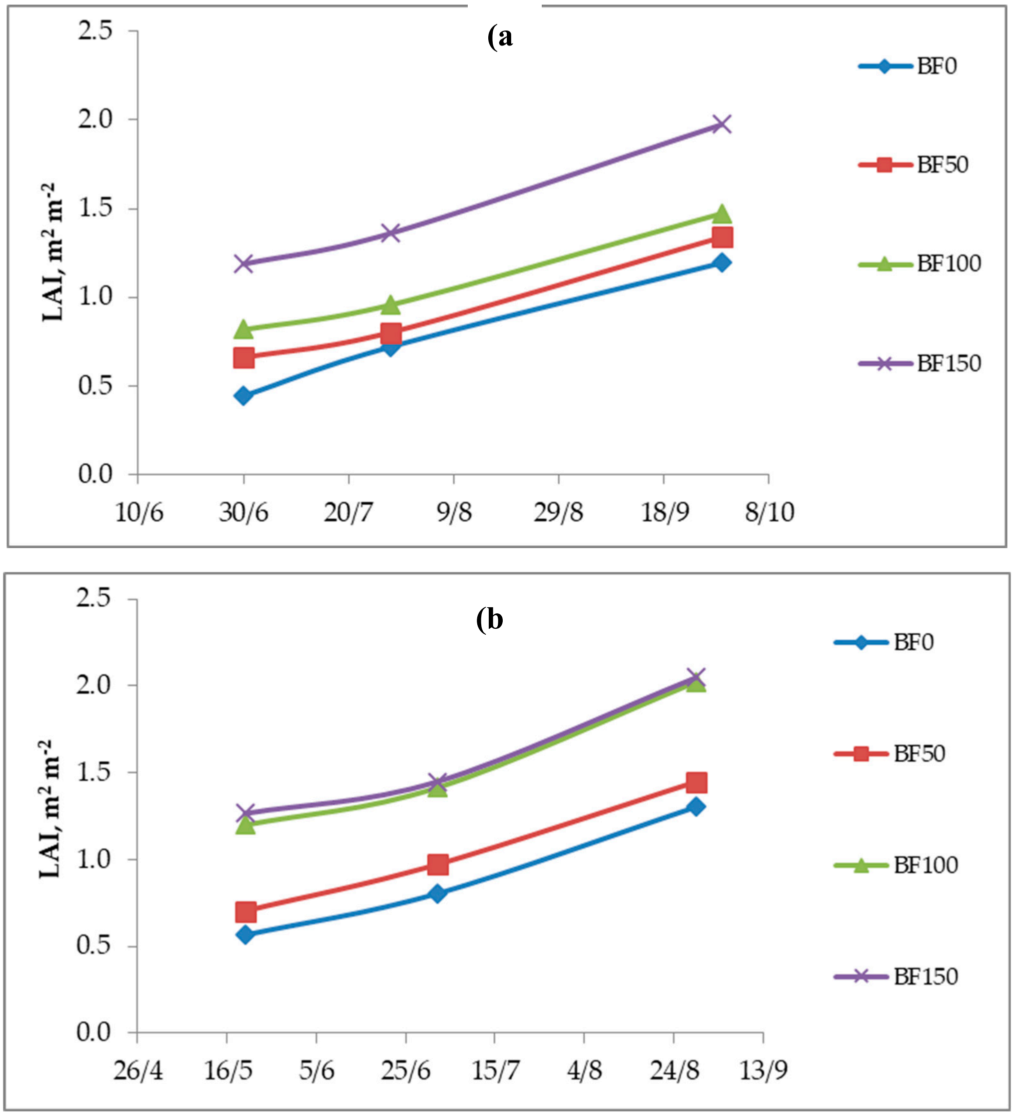

Figure 4. Leaf area index (LAI) of the *Aloysia citrodora* cultivation in two growing years (a) 2014 and (b) 2015.

3.4. Herbaceous plant composition

In total, 20 plant species in the *A. citrodora* ecosystem were recorded in the study area (Table 4). The most frequently occurring plant was *Avena sterilis* L. (Family: *Poaceae*) (Status: Native, Chorology: Mediterranean-SW Asian, Life-form: Therophyte, Habitat: Agricultural and Ruderal habitats) and *Chenopodium album* (Family: *Chenopodiaceae*) (Status: Native, Chorology: Cosmopolitan, Life-form: Therophyte, Habitat: Agricultural and Ruderal habitats) in *Aloysia triphylla* L. ecosystem (Figure 5).

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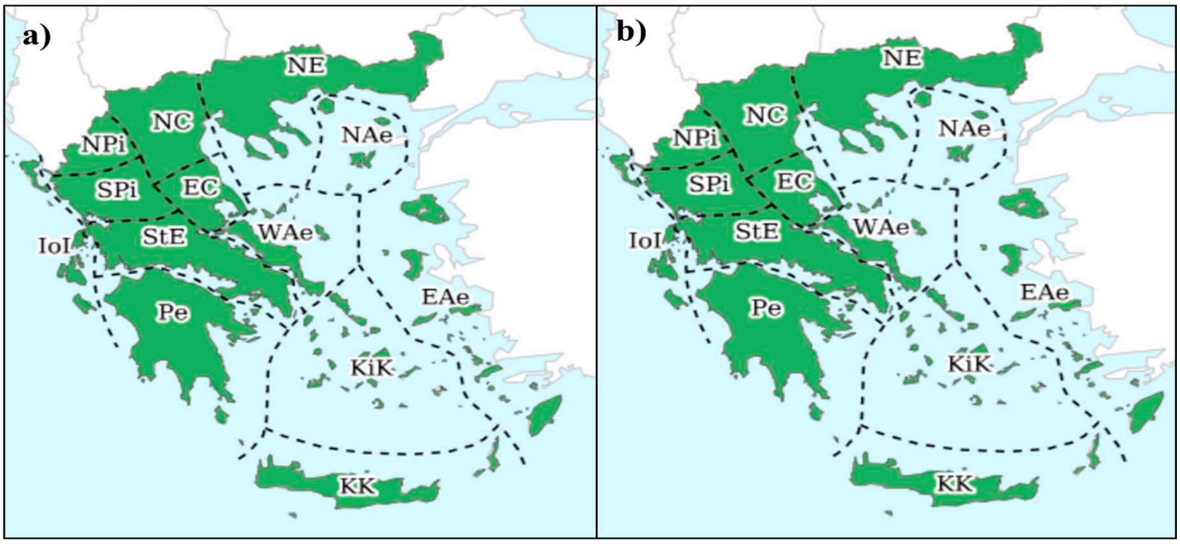

Figure 5. The distribution of plant species with the highest relative abundance in Greece: a) *Avena sterilis* L and b) *Chenopodium album* (IoI:Ionian Islands, NPi:North Pindos, SPi:South Pindos, Pe:Peloponnisos, StE:Sterea Ellas, EC:East Central Greece, NC:North Central Greece, NE:North-East Greece, NAe:North Aegean islands, WAe:West Aegean islands, Kik:Kiklades, KK:Kriti and Karpathos, EAe:East Aegean islands) [35].

Table 6. Herbaceous plant species in Aloysia citrodora.

Herbaceous plant species	**Family**	***Aloysia citrodora* Ecosystem**
Aegilops geniculata Roth.	Poaceae	+
Amaranthus deflexus L.	Amaranthaceae	+
Anthemis arvensis L.	Asteraceae	+
Arctium lappa L.	Asteraceae	+
Avena sterilis L.	Poaceae	+
Bellis perennis L.	Asteraceae	+
Calystegia sepium (L.) R. Br.	Convolvulaceae	+
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	+
Chenopodium album L.	Chenopodiaceae	+
Fumaria officinalis L.	Fumariaceae	+
Glaucium flavum Crantz	Papaveraceae	+
Heliotropium europaeum L.	Boraginaceae	+
Lamium amplexicaule L.	Lamiaceae	+
Lolium perenne L.	Poaceae	+
Polygonum aviculare L.	Polygonaceae	+
Sinapis arvensis L.	Brassicaceae	+
Sonchus arvensis L.	Asteraceae	+
Sorghum halepense (L.) Pers.	Poaceae	+
Stellaria media (L.) Vill.	Caryophyllaceae	+
Veronica persica Poir.	Veronicaceae	+
Total		20

3.5. Environmental Factors affecting the Shannon plant diversity index

In our study, the Pearson correlation coefficients (Table 7) showed that there were soil parameters such as the soil organic matter (SOM), the Phosphorus (P) and the Potassium (K), on which the SH plant diversity index depended significantly for organic A. citrodora.

Table 7. Pearson correlation coefficients of soil parameters with the Shannon diversity index.

Soil parameters	Shannon diversity index
Soil organic matter	0.83*
pH	0.15
$CaCO_3$	0.13
texture-clay	0.23
texture-silt	0.20
texture-sand	0.19
P	0.92*
K	0.81*
Mg	0.17

*denote significance level at 0.05 probability level.

4. Discussion

4.1. Plant height

The 1st year the plant height was ranged from 32.7 cm (control) to 51.8 cm (BF150), while the 2nd year the height was between 40.7 (control) – 61.2 cm (BF150). The BF100 level had a significant increase on plant height (18.73%) the 2nd year while BF150 fertilization increased the plant height 15.26% compared to the 1st year. Kassahun et al. [36] found that the *A. citrodora* plants' height varied from 61.67-87.14 cm, results that are in disagreement with our study. According to the literature biological fertilizers can improve the plant growth by improving the soil fertility [37]. The beneficial effects of bio-fertilizers have been investigated in many crops (38-39).

4.2. A. citrodora total dry yield

The total dry production (stem, leaf) was increased after the use of nitrogen via the biological fertilizer. The 2nd year an average increase of 49.8% in total biomass was observed in all the treatments. That increase was expected because A. citrodora is a perennial scrub which come to full production after the 2nd year of cultivation. It is remarkable that the BF150 treatment provoked the most effectively raise in 2nd year (50.58%) compared to 1st. Until now, only few investigations have studied the use of organic fertilization in A. citrodora and its positive effects in dry biomass [18,22]. Afonso et al. [22] mention that the application of a biological fertilizer in A. citrodora cultivation from 0 to 100 kg N ha-1 increased the leaf and total yield, while between 100 to 150 kg N ha-1 there was a stabilization in yield. Studies showed that the use of bio-fertilizer (Azospirilum and Azotobacter) increased the Salvia plants' dry weight [40].

4.3. LAI

The BF100 and BF150 biological fertilizer levels have a significantly positive effect in Leaf Area Index, in second studied year. Specifically, in 2nd year the increase of LAI using the BF100 fertilizer reached an average value of 27.18%. No publications are found to have studied the impact of bio-fertilization (chemical or organic) in leaf Area Index of *A. citrodora*. The bio-fertilizer application plays an important role in the photosynthesis and in the process of the green surface production [41,42].

4.4. Herbaceous plant composition

The agricultural landscape is a cultural landscape. Agroecosystems are the basic components of rural landscapes. There are large numbers of flora in Agroecosystems, and those systems are considered agricultural systems possessed of a high ecological value for biodiversity (high-nature-value farming systems). The most frequently occurring plant was *Avena sterilis* L. and *Chenopodium album* in *A. citrodora* ecosystem. These plant species are characteristic of agroecosystems, according to Dimopoulos et al. [35]. Several suitable regions of Greece have already been invaded by *Chenopodium album* and *Avena sterilis*. The most important factors explaining herbaceous plant species composition, based on literature [43] are management practices and environmental factors. It is noteworthy, plants are an important indicator of environmental health since they connect the ground to the air. Particularly, they draw most of the data they need from the ground, but their peaks are also directly related to the air because their components make up gas collectors. As a result, comparing plant chemical composition with that of plants that grow in a healthy environment is an important sign of contamination in plant growth areas. According to the literature *Chenopodium album* is an indicator plant in the *A. citrodora* ecosystem, as it indicates soil nitrogen and humus levels [44] which is very important for farmers regarding crop management decision making.

4.5. Environmental Factors affecting the Shannon plant diversity index

The physical characteristics of soil are a key factor, which defines its own physical consistency, a physical consistency that is actively influenced by biological processes, while chemical elements are used to specify soil properties [45–47]. In our study, SH plant diversity correlated positively with the soil organic matter (SOM), the Phosphorus (P) and the Potassium (K). This is probably due to that organic matter in soils preserves nutrients, upgrades the nutrient circle, constructs soil composition, improves water permeability, decreases soil density, resists rapid changes in soil pH, serves as a power source for microorganisms and increases the rate of assimilating copper, magnesium, and zinc into the soil, therefore all of the above promotes the composition and plant diversity [48]. It is noteworthy that organic soil provides important nutrients such as phosphorus and potassium used by plants in large quantities for their growth and survival. Soil Phosphorus (P) being an organic and inorganic substance, it is found in soils, water, and most living organisms. Many P chemical mixtures are in harmony and extend from solution P (absorbed by plants) to stable, unstable or even unobtainable compounds (the most common). As the most important nutrient for plants, phosphorus plays a crucial role. Phosphorus plays many roles in plants, but the biggest one is for storing and transferring energy [40–50]. Furthermore, potassium (K) is an important element in determining soil fertility and plant diversity. The accessibility of plants in soil solution is separated into interchangeables and irreplaceables and exists in soil crystal lattices [51,52]. Among its many functions, it maintains plant turgor, stomatal movement, cell expansion, pH, phloem transport and protein synthesis. According to Maestre et al. [53] and Korol et al. [54] stressed the importance of P cycling with regard to plant diversity. Potassium increases the resistance of plants to dry climates such as in the Mediterranean zones and P gives the energy to all biological reactions and therefore both elements contribute to the increase of biodiversity [55].

5. Conclusions

Generally, the BF100 application provoked almost the same results in plant height and in dry weight of the edible parts of the cultivation compared to BF150. This means that the best bio-fertilizer for the *A. citrodora* cultivation is the BF100 nitrogen level instead of BF150, although the results of the BF150 was a little bit better. The use of BF100 fertilizer can help positively the agronomic characteristics of *A. citrodora* with the minimum cost not only for the producers but also for the environment generally.

A. citrodora ecosystems provide a variety of functions and services that are beneficial to humans and animals. There is a significant finding in this study that soil factors (soil organic matter, P and K) promote positive herbaceous plant diversity within the *A. citrodora* ecosystem. These results are crucial for understanding the ecology and dynamics of this ecosystem. As aromatic and medicinal plants have beneficial effects, as well as their contributions to the development of several sectors like medicine, there is a need to continue the study in the future. It is notable that the data from this study is relevant to health-care programme development, aromatherapy, phytotherapy, economic agricultural policy development, alternative food program, and ethnobotany.

The climate and physicochemical properties of the soils in Greece favour the growth of aromatic, medicinal plants that can produce products of excellent quality, even if cultivated in mountainous and semi-mountainous areas. There are many such lands in our country and the cultivation of these plants can provide a serious income to rural residents.

Author Contributions: Conceptualization, A.M, A.S. and E.S; methodology, A.M., A.S. and A.L.; validation, A.M., A.S. and A.L; software, A.L.; investigation, A.M, A.S., M.T, A.L, and E.S..; data curation, A.M, A.S. and M.T. and A.L.; writing—original draft preparation, A.M., A.S., M.T., A.L. and E.S.; writing—review and editing, A.M., A.S., M.T., A.L. and E.S. ; supervision, A.M A.S., A.L. and E.S.; project administration, A.M., A.S. and E.S.. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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