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Effect of Fertilization with Urea and Inhibitors on Growth, Yield and CBD Concentration of Hemp (*Cannabis sativa* L.)

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Abstract: Field experiments were conducted during 2019 in two different locations in Greece (Athens and Farsala) to evaluate the effect of urea and urea fertilization with inhibitors on the agronomic yield and quality characteristics of two cannabis varieties (*Cannabis sativa* L.), "Uso31"and "Fedora 17". The experimental design was split-plot with four different fertilization treatments—control, Urea (U), urea with Urease Inhibitor (UI), and urea with Nitrification Inhibitor (NI) and urease inhibitor (UI). The significance of differences between treatments was estimated by using Tukey's test with a significance level of $p = 0.05$. The plant height was significantly affected by the different fertilizations and different varieties as well as by the two locations. The maximum plant height was 197 cm for "Fedora 17"in Farsala. The seed yield was higher forthe urea with inhibitors treatment in both varieties. The Cannabidiol (CBD) content was significantly affected by the fertilization—it was higher in urea with inhibitors in "Uso31"and "Fedora 17" treatments. The lowest CBD content value was 1.29% (control) and the highest was 1.69% (urea NI + UI). In conclusion, in both varieties, it seems that urea with inhibitors has a positive effect on their growth, as well as on the increase in cannabidiol (CBD) content.

Keywords: agronomic characteristics; *Cannabis*; CBD content; fertilizers; nitrogen inhibitors

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1. Introduction

Industrial hemp (*Cannabis sativa* L.) isone of the earliest domesticated crops [1] and has been cultivated for centuries all over the world. It is a multipurpose crop which is cultivated for textile fibers, biocomposites, food, cosmetics, and medicines [2,3]. Although hemp is widely used in over 25.00 products in the global market [4], its cultivation decreased during the 20th century. Major causes for this decline were the increased use of synthetic fibers and various feedstocks [5].

Nowadays, interest in hemp has increased due to the dissociation of psychotropic varieties. Furthermore, many European countries have reintroduced legal cultivation of varieties with tetrahydrocannabinol (THC) contents lower than 0.2% [6,7]. Hemp has gradually regained ground to meet the demand for nonfood crops and more derivatives [8]. In Greece, parallel advances of medical cannabis and hemp have been observed [9,10].

Customarily, hemp has been cultivated in Europe for its fiber [11]; however, there has been a growing interest for its innovative applications. Hemp is used in the textile sector as a green substitute of synthetic fibers and its products are also an important element of food and pharmaceutical industries. In the food sector, hemp is a component of various products such as esculent seeds, crude oil, and protein cake.

Hemp seeds are considered a superior source of protein, suitable for human consumption, and are endowed with 30–32% contents of fatty acids (ω–3, ω–6) [12,13]. From a pharmaceutical point of view, industrial cultivars produce hundreds of secondary metabolites such as flavonoids, terpenes, and cannabinoids. Most of them are mainly contained in

flowers and can be extracted [14,15]. The most well-known cannabinoids are cannabidiol (CBD) which is the nonpsychoactive part with medicinal properties, and the primary psychoactive chemical, tetrahydrocannabinol (THC). Certain hemp varieties are characterized by high CBD concentration. The higher the ratio of CBD to THC, the higher the medical exploitation [4].

The wide use of hemp products, either as food or medicine, has been recently recognized. However, there is a lack of agronomic information concerning hemp cultivation, such as the genotype selection and several cultivation practices [16,17]. According to a literature review, monoecious dual-purpose varieties are mentioned as the best option for selection and are quite common in many European countries due to the joint fiber and seed productions [2]. Despite the fact that *Cannabis sativa* L. is naturally a dioecious plant with separated male and female members, modern breeding has developed particular monoecious cultivars. The development of cultivars with low THC contents has contributed to the extraction of secondary metabolites, in addition to their use as dual-purposed crops [18,19]. Breeders utilize selective cross-breeding to maximize CBD production although THC augmentation is not desired [20]. Moreover, monoecious cultivars provide higher inflorescence and seed yield in comparison with dioecious cultivars [21]. Thus, satisfactory multiple exploitations of the crop have been achieved and the sustainability of the crop has improved [22].

From an agronomic point of view, hemp has been promoted as a high-yielding crop with low input cultivation techniques [11]. However, experimental results confirm that special attention needs to be paid to nutritional demands of hemp, principally nitrogen fertilization. Nitrogen is undoubtedly a crucial nutrient affecting major functions, such as photosynthesis, plant development, flowering, and senescence [23,24]. Several studies reported that high doses of N increased chlorophyll content and photosynthetic rate [25]. Additional nitrogen supply has a positive impact on plant height and hemp biomass, while negatively affects the fiber content [26]. Moreover, increasing the rate of N fertilizer positively affects the inflorescence indices [27] and leads to length and weight increases [28]. Concerning seed production, nitrogen fertilization has little effect on hemp seed mass. Furthermore, seed oil content is positively affected, while seed protein content is conversely reduced [29]. It is worth mentioning that N uptake also affects the secondary metabolite profile since N is one of their necessary components, as can be observed in aromatic plants [30]. However, there are conflicting statements about the effects of N supply on various secondary metabolites including flavonoids [30].

While the influence of nitrogen in major morphological and quality features of hemp is clear, it is necessary to apply the most appropriate type of fertilizer to achieve the maximum and efficient absorption by the plant. In hemp crop, N fertilizer is applied as a basal dressing; therefore, nitrate leaching and N loss from soil might occur, resulting in absorption rate reduction. Slow-release fertilizers might be the best tool to increase N uptake in stressful semiarid environments where drought frequently occurs along with N deficiency [16,31]. Urea efficiency could be further enhanced with inhibitors, as has been seen for many crops [31]. Urease inhibitors have the capability to protect urea from disintegration, hence releasing NH_3 in the air and avoiding N losses. Moreover, nitrification inhibitors block the conversion of ammonium by soil bacteria, so ammonium remains available in soil for uptake by plants for longer time [32]. As an additional consequence, hemp can be characterized as an environmentally friendly and highly sustainable crop [17].

There is limited research about the effect of new types of nitrogen fertilizers on cannabis crop. Furthermore, there is a lack of literature concerning the effects of nutritional supplements on the secondary metabolites of hemp. Therefore, the main goal of this study is to shed light on the effect of nitrogen fertilization on the agronomic and quality features of hemp. More specifically, the present study aims to investigate the effect of urea and urea with inhibitor fertilizers on two commonly cultivated monoecious hemp varieties under semiarid Mediterranean conditions.

2. Materials and Methods

2.1. Location and Experimental Design

Field experiments were conducted in two different places in Greece, Athens (latitude: 37°59′1.70″ N; longitude: 23°42′7.04″ E; altitude: 30 m above sea level) and Farsala (Central Greece, latitude: 39°18′ N; longitude: 22°22′ E; altitude: 160 m above sea level) during 2019 to evaluate the effect of fertilizers with inhibitors on growth and yield components of cannabis (*Cannabis sativa* L.). The meteorological data, mean temperature, and monthly precipitation, during the cultivation period are presented in Figure 1. The total precipitation was 120.8 mm in Athens and 142.8 mm in Farsala. The main soil characteristics (at 0–25 cm sampling depth) of the experimental plot are demonstrated as follows: in Athens, the soil was clay loam (29.8% clay, 34.3% silt and 35.9% sand) with a pH of 7.29 (1:1 H_2O), available phosphorus (P)—13.2 mg kg^{-1} soil, available potassium (K)—201 mg kg^{-1} soilmand organic matter percentage—1.47%. In Farsala, the soil was clay (59.77% clay, 30.92% silt, and 9.32% sand) with a pH of 7.8 (1:1 H_2O), available phosphorus (P)—23.39 mg kg^{-1} soil, available potassium (K)—240 mg kg^{-1} soil and organic matter—1.45%. The former cultivation, before the first experimental year, was durum wheat.

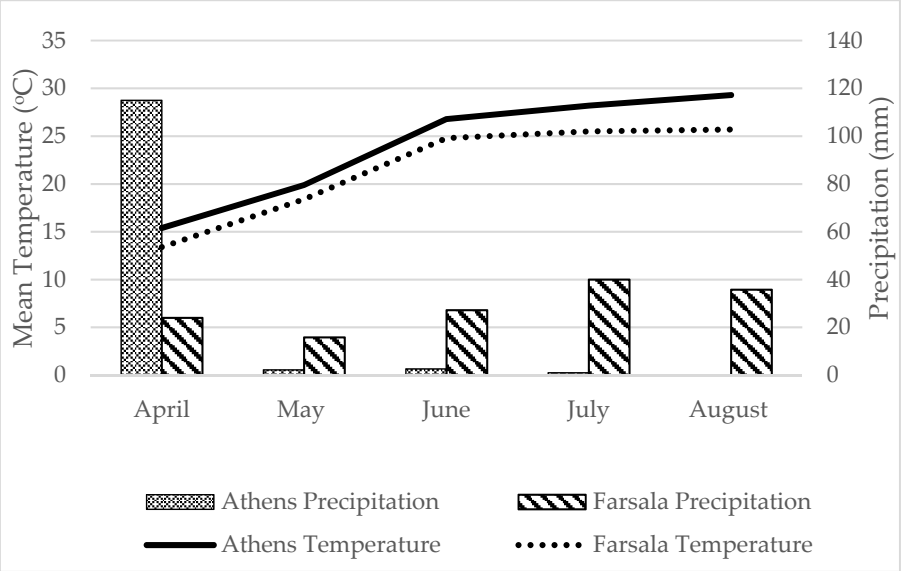

Figure 1. Meteorological data of the experimental areas from April to August (2019).

The experimental design was split-plot, where the whole-plot factor was four different fertilization types (control, Urea (U), urea with Urease Inhibitor (UI), and urea with Nitrification and Urease Inhibitors (U + NI + UI)) and the split-plot factor was two different cannabis varieties ("Fedora 17", "Uso31"). The design had 12 whole plots (20 m^2 each), each of which was split into subplots with two varieties (10 m^2) with three replications. The total applied fertilizer dose was 160 kg N ha^{-1}; 100 kg N ha^{-1} as a basal dressing and 60 kg N ha^{-1} as a top dressing. The type of urea fertilizer was 42-0-0. The nitrification inhibitor was MPA (0.07%) and urease inhibitor was 2-NPT (0.035%) for the fertilizer with urea with double inhibitors (46-0-0). As for the fertilizer of urea with urease inhibitor (40-0-0 + (5.75 S)), the inhibitor used was NBPT, 0.046%. Sowing took place on 29 April and 28 April in Athens and Farsala, respectively, using a HALDRUP SP-25 plot seeder. Basal fertilization was applied the same day of sowing owing to application of fertilizers with inhibitors. Seed rate was adjusted to 130 viable seeds m^{-2}. The row spacing was 40 cm.

Soil tillage encompasses agronomic chisel plow at a depth of 40 cm, followed by secondary tillage with a disc harrow. Additional irrigation was applied through a drip system. Total irrigation applied was 500 mm, divided into five equal doses. Weed control was not required. Harvesting was held at 125 Days After Sowing (DAS).

2.2. Plant Materials

Two cannabis cultivars were selected for this experiment, "Uso31" and "Fedora 17". These varieties are a breeding results from Ukraine and France, respectively. Both varieties are monoecious and are characterized by early flowering periods. The main reason for choosing "Fedora 17" and "Uso31" varieties is their wide use in European environments. Each variety has the main following characteristics: dry matter ranges of 6–8 tonnes ha^{-1} and 10–12 tonnes ha^{-1}; seed yield ranges of 0.8–1 tonnes ha^{-1} and 1–2 tonnes ha^{-1}; seed oil content ranges of 28–30% and 30–32%, for "Uso31", and "Fedora 17", respectively.

2.3. Sampling and Methods

Root samples were collected to 25 cm using a cylindrical auger (25 cm length, 10 cm diameter) and root analyses were conducted. Root density and Arbuscular Mycorrhizal Fungi (AMF) were measured at the start of flower period, 60 DAS. Then, roots were washed over a 5 mm mesh sieve. For their separation from soil, there was used a 0.1% trypan blue FAA staining solution (a mixture of 10% formalin, 50% ethanol, and 5% acetic acid solutions). They were determined in millimeters using a high-resolution scanner using DT-software (Delta-T Scan version 2.04; Delta Devices Ltd., Burwell, Cambridge, UK) [33,34].

Plant height (cm) at 120 DAS, leaf area index (LAI) at 90 DAS using SunScan (Delta-T Devices Ltd., Cambridge, UK), total dry matter (kg ha^{-1}) at 100 DAS, and one thousand seed weights (g) and seed yields (kg ha^{-1}) at 140 DAS were measured in terms of agronomic characteristics.

Yield components were also reported. Number of buds per plant, bud weight (g), and length (cm) were measured at 90 DAS. The seed oil content was determined using a laboratory cold pressure machine and seed oil yield at 90 DAS. CBD content as well as the yield per plant (g $plant^{-1}$) at 90 DAS was determined using the GemmaCert device machine (GemmaCert Ltd., Ra'anana, Israel).

Seed protein (g $plant^{-1}$) was distilled using a Buchi 316 device (Buchi, Flawil, Switzerland) and was determined by the Kjeldahl method [35]. Protein yield (kg ha^{-1}) was subsequently calculated at 140 DAS.

2.4. Calculations and Statistics

Bud compact index (g cm^{-1}) was determined using the equation:

$$\text{BudcompactIndex} = \frac{\text{Budweight (g)}}{\text{Budlength (cm)}} \quad (1)$$

Analysis of variance was carried outon data using the STATISTICA v10 (StatSoft, Inc., Tulsa, OK, USA, 2011) logistic package as a split-plot design36. The estimation of significance of differences among treatments was carried out by using Tukey's test on significant level (p = 0.05). The tests of correlation coefficients and linear regression by Statistica software were set at two levels of significance (p = 0.05) and remarkable significance (p = 0.01).

3. Results

Agronomic Characteristics

Concerning root density, control had no statistically significant differences with urea in both varieties (Table 1). Furthermore, urea UI and urea UI + NI had no statistically significant differences in both varieties and places. The highest value was 9.56 mm cm^{-3} in the urea with double inhibitors treatment (Athens area), and the lowest was 5.65 mm cm^{-3} in control in Farsala for "Uso 31" (Table 1). Only fertilization influenced AMF. Treatment of urea NI + UI had no significant difference with urea UI and control in both locations. AMF varied between 19.67 (urea, "Fedora 17", in Athens) and 31% (control, "Uso31", in Farsala).

Table 1. Agronomic characteristics as affected by different treatments for "Uso31"and "Fedora 17" cannabis varieties.

Fertilization	Root Density (mm cm^{-3})		AMF (%)		Plant Height (cm)		LAI		DM (kg ha^{-1})	
	Athens									
	"Uso31"	**"Fedora 17"**	**"Uso31"**	**"Fedora 17"**	**"Uso31"**	**"Fedora 17"**	**"Uso31"**	**"Fedora 17"**	**"Uso31"**	**"Fedora 17"**
Control	5.71 ^{a}	6.12 ^{a}	26.33 ^{a}	27.67 ^{a}	172.6 ^{a}	175.0 ^{a}	3.76 ^{a}	3.93 ^{a}	3.689 ^{a}	3.729 ^{a}
Urea	6.11 ^{a}	5.95 ^{a}	20 ^{b}	19.67 ^{b}	176.0 ^{a}	182.0 ^{a}	4.05 ^{b}	4.18 ^{b}	4.002 ^{b}	3.916 ^{b}
Urea UI	8.26 ^{b}	8.14 ^{b}	24.33 ^{c}	25.67 ^{c}	182.6 ^{b}	187.6 ^{b}	4.39 ^{b}	4.36 ^{b}	4.212 ^{c}	4.148 ^{c}
Urea NI + UI	9.56 ^{c}	9.37 ^{c}	26.33 ^{a}	25.67 ^{c}	190.7 ^{c}	193.0 ^{c}	4.71 ^{c}	4.76 ^{c}	4.400 ^{d}	4.369 ^{d}
	Farsala									
Control	5.65 ^{a}	6.06 ^{a}	31 ^{a}	29.7 ^{a}	176.6 ^{a}	177.67 ^{a}	4.16 ^{a}	4.33 ^{a}	3.30 ^{a}	3.940 ^{a}
Urea	5.91 ^{a}	6.44 ^{a}	22 ^{b}	22 ^{b}	179.6 ^{a}	183.33 ^{a}	4.55 ^{b}	4.92 ^{b}	4.186 ^{b}	4.117 ^{b}
Urea UI	7.64 ^{b}	7.71 ^{b}	24 ^{c}	25 ^{c}	186.3 ^{b}	188.33 ^{b}	4.82 ^{b}	4.80 ^{b}	4.413 ^{c}	4.342 ^{c}
Urea NI + UI	8.51 ^{c}	8.70 ^{c}	26.7 ^{ac}	27 ^{ac}	195.3 ^{c}	197.00 ^{c}	5.13 ^{c}	5.12 ^{c}	4.605 ^{d}	4.694 ^{d}
F^{Fert}	92.95 ***		12.43 ***		34.44 ***		39.36 ***		81.42 ***	
$F^{Variety}$	ns		ns		4.86 *		ns		ns	
$F^{Location}$	4.30 *		ns		5.13 *		61.70 ***		44.49 ***	
$F^{Fert \times Variety}$	ns		ns		ns		ns		ns	
$F^{Fert \times Location}$	ns		ns		ns		ns		ns	
$F^{Variety \times Location}$	ns		ns		ns		ns		ns	
$F^{Fert \times Variety \times Location}$	ns		ns		ns		ns		ns	

F-test ratios are from ANOVA. Different letters (a, b, c and d) within a column indicate significant differences according to Tukey's test. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Moreover, plant height was significantly affected by fertilization, variety, and location (Table 1). In both locations and varieties, the lowest values were observed for control (172.67 cm). Higher plants were reported for the "Fedora 17" variety; however, the highest value was 197 cm in Farsala. Moreover, Leaf Area Index (LAI) was affected by fertilization and location. Control had no statistically significant difference with urea in both varieties and locations. In Farsala, the highest LAI values were observed for "Uso 31" (15.13), and "Fedora 17 (1 15.12 under urea NI + UI treatment. Additionally, dry matter was significantly affected by fertilization and location. All treatments had statistically significant differences among them. The lowest value recorded was 3689 kg ha^{-1}(control—Athens area, "Uso31" variety), and the highest was 4694 kg ha^{-1} (urea NI + UI treatment—Farsala area, "Uso31"; Table 1).

Furthermore, number of buds per plant differed due to fertilization treatments (Table 2). Control statistically differed in all treatments, while the highest number of buds was observed under urea NI + UI in both locations and varieties. The highest value was 6.03 buds per plant^{-1} in Farsala within the variety "Uso31") (Table 2). In addition to bud weight, there were no significant differences between urea and urea UI, nor between urea UI and urea NI + UI, for both locations and varieties. Specifically, the lowest values in both places and varieties were observed in control 161.67 g and the highest 181.67 g in urea + UI (Table 2).

Bud length was significantly affected by location and fertilization, whereas there were no differences between the two varieties (Table 2). The highest values were 32.33 cm under urea UI treatment for "Uso31" (Athens) and 36.33 cm under urea NI + UI, in the "Fedora 17" variety (Farsala). Importantly, bud compact index was affected by location. The highest value was observed in Athens for "Fedora 17" (6.02 g cm^{-1}). On the other hand, the lowest value was observed in Farsala in urea NI + UI (4.79 g cm^{-1}) (Table 2).

Regarding one thousand seed weight, there was no statistically significant difference neither after the urea with UI treatment nor the urea with NI + UI for the varieties, "Uso 31" and "Fedora 17", in both locations. The lowest value was observed in control (13.13 g) and the highest value was observed in urea NI+UI (15.65 g) for "Uso 31" variety in both experimental areas. Concerning seed yield, the control showed a statistically significant difference with all treatments in both varieties and locations. The highest value was 2895 kg ha^{-1} in urea NI+UI and the lowest value was observed in control (2149 kg ha^{-l}) for "Uso 31" in Athens and in Farsala, respectively (Table 3). Moreover, regarding seed protein, the control showed no statistically significant difference with urea and the urea UI showed no statistically significant difference with the urea NI + UI. The lowest value was 16.83% in control in Athens and the highest was 19.32% in urea NI + UI treatment for

"Uso 31" in Farsala Furthermore, referring to protein yield, all treatments had statistically significant differences among them for "Uso 31" and "Fedora 17". The lowest value was 363.63 kg ha^{-1} in control ("Uso 31" variety, in Farsala). On the other hand, the highest value was 550.83 kg ha^{-1} in urea UI ("Fedora 17", in Athens; Table 3).

Table 2. Bud Characteristics Affected by Different Treatments for "Uso 31" and "Fedora 17" Cannabis Varieties.

Fertilization	No Buds/Plant		Weight/ Bud (g)		Bud Length (cm)		Bud Compact Index (g cm^{-1})	
Athens								
	"Uso 31"	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**
Control	4.27 ^{a}	4.47 ^{a}	168 ^{a}	161.67 ^{a}	30.33 ^{a}	28.33 ^{a}	5.57 ^{a}	5.70 ^{a}
Urea	5.03 ^{b}	4.77 ^{b}	176.67 ^{b}	171.33 ^{b}	31.33 ^{a}	30 ^{a}	5.72 ^{a}	5.78 ^{a}
Urea UI	5.43 ^{c}	5.27 ^{c}	181.67 ^{c}	175.33 ^{bc}	32.33 ^{b}	32 ^{b}	5.68 ^{a}	5.58 ^{a}
Urea NI+UI	5.90 ^{d}	5.80 ^{d}	181.33 ^{c}	181.67 ^{c}	32 ^{b}	30.67 ^{a}	5.75 ^{a}	6.02 ^{b}
Farsala								
Control	4.53 ^{a}	4.77 ^{a}	165 ^{a}	161.67 ^{a}	30.33 ^{a}	29.67 ^{a}	5.46 ^{a}	5.46 ^{a}
Urea	5.27 ^{b}	4.97 ^{b}	172.33 ^{b}	169 ^{b}	32.33 ^{a}	32.67 ^{a}	5.36 ^{a}	5.18 ^{a}
Urea UI	5.60 ^{c}	5.33 ^{c}	177.33 ^{c}	170.33 ^{bc}	33.33 ^{b}	35.67 ^{b}	5.35 ^{a}	4.79 ^{a}
Urea NI+UI	6.03 ^{d}	5.83 ^{d}	177 ^{c}	179.33 ^{c}	34.67 ^{b}	36.33 ^{b}	5.16 ^{a}	4.96 ^{a}
F_{Fert}	64.85 ***		11.91 ***		3.26 *		ns	
$F_{Variety}$	ns		ns		ns		ns	
F_{Place}	5.75 **		ns		5.27 *		7.51 **	
$F_{Fert \times Variety}$	ns		ns		ns		ns	
$F_{Fert \times Location}$	ns		ns		ns		ns	
$F_{Variety \times Location}$	ns		ns		ns		ns	
$F_{Fert \times Variety \times Location}$	ns		ns		ns		ns	

F-test ratios are from ANOVA. Different letters (a, b, c and d) within a column indicate significant differences according to Tukey's test. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Table 3. Seed characteristics affected by different treatments of "Uso 31" and "Fedora 17" cannabis varieties.

Fertilization	1000 Seed Weight (g)		Seed Yield (kg ha^{-1})		Seed Protein (%)		Protein Yield (kg ha^{-1})	
Athens								
	"Uso 31"	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**
Control	13.13 ^{a}	13.60 ^{a}	2335 ^{a}	2423 ^{a}	16.83 ^{a}	17.07 ^{a}	393.27 ^{a}	412.96 ^{a}
Urea	13.55 ^{b}	13.98 ^{b}	2514 ^{b}	2580 ^{b}	17.68 ^{ab}	17.77 ^{ab}	444.11 ^{b}	457.66 ^{b}
Urea UI	14 ^{c}	14.23 ^{c}	2721 ^{c}	2703 ^{c}	18.36 ^{bc}	18.36 ^{bc}	499.42 ^{c}	495.56 ^{c}
Urea NI+UI	14.32 ^{c}	14.56 ^{c}	2895 ^{c}	2860 ^{c}	18.77 ^{c}	19.26 ^{c}	544.15 ^{d}	550.83 ^{d}
Farsala								
Control	13.67 ^{a}	14.04 ^{a}	2149 ^{a}	2220 ^{a}	17 ^{a}	17.06 ^{a}	363.63 ^{a}	379.12 ^{a}
Urea	14.19 ^{b}	14.59 ^{b}	2325 ^{b}	2385 ^{b}	17.74 ^{ab}	17.52 ^{ab}	411.94 ^{b}	417.93 ^{b}
Urea UI	15.17 ^{c}	14.94 ^{c}	2530 ^{c}	2512 ^{c}	18.63 ^{bc}	18.61 ^{bc}	470.62 ^{c}	467.33 ^{c}
Urea NI+UI	15.65 ^{c}	15.23 ^{c}	2730 ^{c}	2721 ^{c}	19.32 ^{c}	19.25 ^{c}	526.14 ^{d}	524.23 ^{d}
F_{Fert}	27.42 **		14.48 **		9.47 ***		32.32 ***	
$F_{Variety}$	ns		ns		ns		ns	
F_{Place}	47.12 ***		9.69 **		ns		6.87 *	
$F_{Fert \times Variety}$	ns		ns		ns		ns	
$F_{Fert \times Location}$	ns		ns		ns		ns	
$F_{Variety \times Location}$	ns		ns		ns		ns	
$F_{Fert \times Variety \times Location}$	ns		ns		ns		ns	

F-test ratios are from ANOVA. Different letters (a, b, c and d) within a column indicate significant differences according to Tukey's test. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

In connection with CBD content, the control showed no statistically significant difference with the urea and urea UI. In addition, urea and urea NI + UI for both varieties and locations did not differ. The lowest value was 1.29% (control) and the highest was 1.69% (urea NI + UI, "Uso 31" variety, Farsala; Table 4). With reference to CBD yield per plant, a statistically significant difference among all treatments for both varieties and locations was observed. The lowest value was 9.59 g in control, for the "Fedora 17" variety, in Athens, and the highest value was 18 g in urea NI + UI for "Uso 31" in Farsala (Table 4).

Table 4. Cannabidiol (CBD) as affected by different treatments for "Uso 31" and "Fedora 17" cannabis varieties.

Fertilization	CBD Content (%)		CBD Yield/Plant (g)	
		Athens		
	"Uso 31"	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**
Control	1.37 ^{a}	1.33 ^{a}	9.83 ^{a}	9.59 ^{a}
Urea	1.41 ^{ab}	1.41 ^{ab}	12.49 ^{b}	11.55 ^{b}
Urea UI	1.54 ^{bc}	1.51 ^{bc}	15.15 ^{c}	13.98 ^{c}
Urea NI+UI	1.66 ^{c}	1.53 ^{c}	17.65 ^{d}	16.07 ^{d}
		Farsala		
Control	1.29 ^{a}	1.38 ^{a}	9.65 ^{a}	10.61 ^{a}
Urea	1.35 ^{ab}	1.45 ^{ab}	12.24 ^{b}	12.15 ^{b}
Urea UI	1.54 ^{bc}	1.53 ^{bc}	15.18 ^{c}	13.93 ^{c}
Urea NI+UI	1.69 ^{c}	1.64 ^{c}	18 ^{d}	17.15 ^{d}
F_{Fert}	10.23 ***		78.037 ***	
$F_{Variety}$	ns		ns	
F_{Place}	ns		ns	
$F_{Fert \times Variety}$	ns		ns	
$F_{Fert \times Location}$	ns		ns	
$F_{Variety \times Location}$	ns		ns	
$F_{Fert \times Variety \times Location}$	ns		ns	

F-test ratios are from ANOVA. Different letters (a, b, c and d) within a column indicate significant differences according to Tukey's test. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Concerning seed oil content, urea UI showed no statistically significant difference with urea NI + UI in both varieties and experimental locations. The lowest value was 24.83% for "Fedora 17" and the highest value was 29.90% in urea NI+UI for "Uso 31" (Table 5). Regarding oil yield, all fertilizer treatments had statistically significant differences among them for "Uso 31" and "Fedora 17" in both locations. The lowest value was 557.81 kg ha^{-1} in control for "Uso 31" (Farsala) and the highest value was 865.27 kg ha^{-1} in urea NI + UI, in "Uso 31" (Athens) (Table 5).

Table 5. Oil content and oil yield as affected by different treatments for "Uso 31" and "Fedora 17" cannabis varieties.

Fertilization	Oil Content (%)		Oil Yield (kg ha^{-1})	
		Athens		
	"Uso 31"	**"Fedora 17"**	**"Uso 31"**	**"Fedora 17"**
Control	26.47 ^{a}	24.83 ^{a}	618.89 ^{a}	599.38 ^{a}
Urea	27.68 ^{b}	26.83 ^{b}	695.52 ^{b}	691.95 ^{b}
Urea UI	29.84 ^{c}	27.66 ^{c}	811.54 ^{c}	748.47 ^{c}
Urea NI + UI	29.90 ^{c}	28.34 ^{c}	865.27 ^{d}	811.08 ^{d}
		Farsala		
Control	25.96 ^{a}	25.69 ^{a}	557.81 ^{a}	570.31 ^{a}
Urea	27.97 ^{b}	27.23 ^{b}	650.24 ^{b}	648.25 ^{b}
Urea UI	29.26 ^{c}	28.29 ^{c}	739.98 ^{c}	710.50 ^{c}
Urea NI + UI	29.48 ^{c}	29.38 ^{c}	804.45 ^{d}	799.78 ^{d}
F_{Fert}	32.53 ***		38.73 ***	
$F_{Variety}$	14.02 ***		ns	
F_{Place}	ns		7.71 **	
$F_{Fert \times Variety}$	ns		ns	
$F_{Fert \times Location}$	ns		ns	
$F_{Variety \times Location}$	ns		ns	
$F_{Fert \times Variety \times Location}$	ns		ns	

F-test ratios are from ANOVA. Different letters (a, b, c and d) within a column indicate significant differences according to Tukey's test. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

4. Discussion

The differences in root density values between the two locations were attributed to soil-climatic conditions. Precipitation in Farsala area was greater than in Athens area. Lower rainfall causes root to spread and extent greater. As a result, its density increases so as absorb water and nutrients. Root system is adapted to corresponding conditions for the purpose of increasing nutrients absorption [26]. Moreover, differences in root density may be due to plasticity of hemp and not necessarily to soil-climatic conditions [36]. Uniformity of root could be explained by the plasticity of hemp root development despite variance of soil characteristics. In hemp cultivation, increased root density was positively correlated with plant height ($r = 0.74$, $p < 0.001$; Table 6) and LAI ($r = 0.61$, $p < 0.001$; Table 6). It is remarkable that AMF and root density did not have a correlation with cannabis crop (Table 6).

It is crucial that AMF under fertilization with double inhibitors did not differ with control. No interrelation between mycorrhiza and cannabis growth and yields was observed. According to our results, AMF did not affect crop development or yields (seed yield and oil yield). Conversely, Kakabouki et al. observed that AMF significantly affected yield-contributing characteristics. This difference is owed to application of commercial AMF products [32]. A result of this is that the symbiont fungus AMF did not possibly adequately colonize the hemp root.

Plant height was affected by variety, location, and last fertilization and values varied. Similar results were reported by Papastylianou et al. and Folina et al. [17,27]. These enhancements increase DM ($r = 0.76$, $p < 0.001$) and LAI ($r = 0.69$, $p < 0.001$). Height trait has been previously reported to be genetically controlled [17,37,38]. According to our study, hemp height is affected by multiple factors. LAI was affected by location and fertilization. Rich foliage of hemp was observed by many researchers [10], albeit crop ability to produce their biomass depends on nutrient disposal [24]. Extended nitrogen availability due to slow release led to a lush foliage of hemp. In cannabis crop, while LAI increased, DM also increased ($r = 0.83$, $p < 0.001$; Table 6). As the leaf area increases, so does the CBD yield ($r = 0.70$, $p < 0.001$; Table 6). Increase in LAI with the use of N inhibitors was probably in virtue of increases in the number of leaves per plant and an increase in inflorescences per plant; bract, sugar leaves, and fan leaves are included in buds.

As shown in Table 6, slow-release fertilizer obviously enhanced DM compared to the control and conventional urea treatment. DM was enhanced with increased nitrogen rates [10]. This could be explained by the physical reality that plants use the available nutrients and adapt their metabolic processes [26]. DM positively correlates with oil content ($r = 0.75$, $p < 0.001$; Table 6). It is crucial that while hemp grew, its oil content did not decrease. Not only oil content but also CBD yield per plant had a positive correlation with DM ($r = 0.83$, $p < 0.001$; Table 6). In hemp, use of inhibitors offer an increase in biomass development and CBD yield per plant.

Number of buds per plants had a considerable turn-up with slow-release fertilizers in contrastto urea. Moreover, location significantly increased the number of buds per plant. The effectiveness of nitrogen doses in terms of bud growth was significantly affected by weather conditions after fertilizer application. It is remarkable that the number of buds per plant had a positive correlation with oil content ($r = 0.81$, $p < 0.001$; Table 6) and oil yield ($r = 0.75$, $p < 0.001$; Table 6). Additionally, the number of buds has a positive correlation with DM ($r = 0.89$, $p < 0.001$, Table 6). On the contrary, Papastylianou et al. reported that as the dry matter was increased, inflorescences were declined. This variance of results can be ascribed to hemp plasticity [27].

With respect to bud weight, significant differences were only recorded in relation to the applied fertilizer. Utilization of inhibitors prolonged nitrogen availability. As a result, bud weight was significantly increased. There is much debate among researchers; the first case studies demonstrate that bud weight is a genetic trait [16], whereas other research indicates that it is influenced by cultivation practices [38], and others show that it is influenced by interaction of cultivar and fertilization [27].

Table 6. Correlation matrix between agronomic and quality characteristics for "Fedora 17" and "Uso 31" cannabis varieties.

	R Density	AMF	Plant Height	LAI	DM	1000 Seed Weight	Seed Yield	Oil Content	Oil Yield	No Buds/Plant	Weight/Bud (g)	Bud Length (cm)	Bud Comp. Index	CBD (%)	CBD Yield/Plant	Seed Protein (%)	Protein Yield	THC Content	THC Yield/Plant (g)	Harvest Index	CBD/THC
R Density	1	0.04 ^{ns}	0.74 ***	0.61 ***	0.75 ***	0.52 ***	0.61 ***	0.67 ***	0.74 ***	0.81 ***	0.59 ***	0.40 **	−0.10 ^{ns}	0.63 ***	0.84 ***	0.62 ***	0.75 ***	−0.72 ***	−0.24 ^{ns}	0.13 ^{ns}	0.73 ***
AMF (%)	0.04 ^{ns}	1	0.11 ^{ns}	−0.04 ^{ns}	−0.06 ^{ns}	0.10 ^{ns}	0.04 ^{ns}	−0.22 ^{ns}	−0.05 ^{ns}	−0.16 ^{ns}	−0.24 ^{ns}	−0.31 *	0.20 ^{ns}	0.18 ^{ns}	−0.01 ^{ns}	−0.21 ^{ns}	−0.06 ^{ns}	−0.04 ^{ns}	−0.41 **	0.06 ^{ns}	0.18 ^{ns}
Plant height (cm)	0.74 ***	0.11 ^{ns}	1	0.69 ***	0.76 ***	0.70 ***	0.52 ***	0.52 ***	0.61 ***	0.65 ***	0.58 ***	0.35 *	−0.05 ^{ns}	0.64 ***	0.77 ***	0.65 ***	0.69 ***	−0.78 ***	−0.49 ***	0.01 ^{ns}	0.81 ***
LAI	0.61 ***	−0.04 ^{ns}	0.69 ***	1	0.83 ***	0.76 ***	0.33 *	0.60 ***	0.49 ***	0.75 ***	0.32 *	0.55***	−0.38 **	0.54 ***	0.70 ***	0.58 ***	0.52 ***	−0.68 ***	−0.30 *	−0.23 ^{ns}	0.66 ***
DM (kg ha^{-1})	0.75 ***	−0.06 ^{ns}	0.76 ***	0.83 ***	1	0.78 ***	0.47 ***	0.75 ***	0.66 ***	0.89 ***	0.50 ***	0.50 ***	−0.24 ^{ns}	0.58 ***	0.83 ***	0.58 ***	0.62 ***	−0.84 ***	−0.36 *	−0.21 ^{ns}	0.80 ***
1000 seed weight	0.52 ***	0.10 ^{ns}	0.70 ***	0.76 ***	0.78 ***	1	0.52 ***	0.54 ***	0.61 ***	0.66 ***	0.36 *	0.21 ^{ns}	−0.02 ^{ns}	0.44 *	0.62 ***	0.39 **	0.57 ***	−0.73 ***	−0.48 ***	0.01 ^{ns}	0.69 ***
Seed Yield	0.61 ***	0.04 ^{ns}	0.52 ***	0.33 *	0.47 ***	0.52 ***	1	0.48 ***	0.93 **	0.56 ***	0.43 **	−0.19 ^{ns}	0.44 **	0.34 *	0.55 ***	0.35 *	0.90 ***	−0.71 ***	−0.49 ***	0.77 ***	0.61 ***
Oil content (%)	0.67 ***	−0.22 ^{ns}	0.52 ***	0.60 ***	0.75 ***	0.54 ***	0.48 ***	1	0.77 ***	0.81 ***	0.68 ***	0.41 **	−0.08 ^{ns}	0.51 ***	0.80 ***	0.44 **	0.56 ***	−0.74 ***	−0.17 ^{ns}	−0.02 ^{ns}	0.68 ***
Oil Yield (kg ha^{-1})	0.74 ***	−0.05 ^{ns}	0.61 ***	0.49 ***	0.66 ***	0.61 ***	0.93 **	0.77 ***	1	0.75 ***	0.61 ***	0.03 ^{ns}	0.30 *	0.47 ***	0.74 ***	0.45 ***	0.89 ***	−0.83 ***	−0.43 **	0.55 ***	0.73 ***
No buds/plant	0.81 ***	−0.16 ^{ns}	0.65 ***	0.75 ***	0.89 ***	0.66 ***	0.56 ***	0.81 ***	0.75 ***	1	0.52 ***	0.45 ***	−0.19 ^{ns}	0.57 ***	0.89 ***	0.55 ***	0.67 ***	−0.81 ***	−0.19 ^{ns}	−0.02 ^{ns}	0.74 ***
Weight/bud (g)	0.59 ***	−0.24 ^{ns}	0.58 ***	0.32 *	0.50 ***	0.36 *	0.43 **	0.68 ***	0.61 ***	0.52 ***	1	0.27 ^{ns}	0.20 ^{ns}	0.32 *	0.62 ***	0.49 ***	0.55 ***	−0.55 ***	−0.04 ^{ns}	0.09 ^{ns}	0.53 ***
Bud length (cm)	0.40 **	−0.31 *	0.35 *	0.55 ***	0.50 ***	0.21 ^{ns}	−0.19 ^{ns}	0.41 **	0.03 ^{ns}	0.45 ***	0.27 ^{ns}	1	−0.88 ***	0.36 *	0.45 ***	0.55 ***	0.12 ^{ns}	−0.26 ^{ns}	0.12 ^{ns}	−0.56 ***	0.32 *
Budcomp. index	−0.10 ^{ns}	0.20 ^{ns}	−0.05 ^{ns}	−0.38 **	−0.24 ^{ns}	−0.02 ^{ns}	0.44 **	−0.08 ^{ns}	0.30 *	−0.19 ^{ns}	0.20 ^{ns}	−0.88 ***	1	−0.22 ^{ns}	−0.15 ^{ns}	−0.28 ^{ns}	0.19 ^{ns}	−0.03 ^{ns}	−0.17 ^{ns}	0.64 ***	−0.05 ^{ns}
CBD (%)	0.63 ***	0.18 ^{ns}	0.64 ***	0.54 ***	0.58 ***	0.44 **	0.34 *	0.51 ***	0.47 ***	0.57 ***	0.32 *	0.36 *	−0.22 ^{ns}	1	0.86 ***	0.37 *	0.43 **	−0.61 ***	−0.37 *	−0.03 ^{ns}	0.80 ***
CBD yield/plant	0.84 ***	−0.01 ^{ns}	0.77 ***	0.70 ***	0.83 ***	0.62 ***	0.55 ***	0.80 ***	0.74 ***	0.89 ***	0.62 ***	0.45 ***	−0.15 ^{ns}	0.86 ***	1	0.56 ***	0.67 ***	−0.82 ***	−0.31 *	0.01 ^{ns}	0.89 ***
Seed protein (%)	0.62 ***	−0.21 ^{ns}	0.65 ***	0.58 ***	0.58 ***	0.39 **	0.35 *	0.44 **	0.45 ***	0.55 ***	0.49 ***	0.55 ***	−0.28 ^{ns}	0.37 *	0.56 ***	1	0.72 ***	−0.44 ***	−0.03 ^{ns}	−0.03 ^{ns}	0.45 ***
Protein yield	0.75 ***	−0.06 ^{ns}	0.69 ***	0.52 ***	0.62 ***	0.57 ***	0.90 ***	0.56 ***	0.89 ***	0.67 ***	0.55 ***	0.12 ^{ns}	0.19 ^{ns}	0.43 **	0.67 ***	0.72 ***	1	−0.73 ***	−0.38 **	0.54 ***	0.66 ***
THC content (%)	−0.72 ***	−0.04 ^{ns}	−0.78 ***	−0.68 ***	−0.84 ***	−0.73 ***	−0.71 ***	−0.74 ***	−0.83 ***	−0.81 ***	−0.55 ***	−0.26 ^{ns}	−0.03 ^{ns}	−0.61 ***	−0.82 ***	−0.44 **	−0.73 ***	1	0.68 ***	−0.17 ^{ns}	−0.92 **
THC yield/plant	−0.24 ^{ns}	−0.41 **	−0.49 ***	−0.30 *	−0.36 *	−0.48 ***	−0.49 ***	−0.17 ^{ns}	−0.43 **	−0.19 ^{ns}	−0.04 ^{ns}	0.12 ^{ns}	−0.17 ^{ns}	−0.37 *	−0.31*	−0.03 ^{ns}	−0.38 **	0.68 ***	1	−0.28 ^{ns}	−0.68 ***
Harvest index	0.13 ^{ns}	0.06 ^{ns}	0.01 ^{ns}	−0.23 ^{ns}	−0.21 ^{ns}	0.01 ^{ns}	0.77 ***	−0.02 ^{ns}	0.55 ***	−0.02 ^{ns}	0.09 ^{ns}	−0.56 ***	0.64 ***	−0.03 ^{ns}	0.01 ^{ns}	−0.03 ^{ns}	0.54 ***	−0.17 ^{ns}	−0.28 ^{ns}	1	0.09 ^{ns}

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant ($p > 0.05$).

Bud length differed significantly due to fertilization and location. Inflorescence beganto form on plant around 90 DAS. If direction of production is toward inflorescences and not stems for fiber, early flowering genotypes are suggested [28]. This suggestion is made for higher temperature conditions, longer day lengths, and higher light intensities. These conditions are experienced during seed development of late-flowering cultivars. Bud length has a negative correlation with bud compact index ($r = -0.88$, $p < 0.001$; Table 6). Yield components declined with higher nitrogen supplies whereas oil content might not have affected [39]. This negative affect could be mitigated by using fertilizers with nitrogen inhibitors, either with coating technique or inhibitors [40]. Indeed, referring to our results, utilization of inhibitors enriched all yield components and at the same time increased the oil content. The increase in yield components under nitrogen application can be attributed to the positive effect of nitrogen on plant development. These findings are in conformity with the findings of other studies [17,27].

An important index for yield assessment of cannabis is the bud compact index which is considered an essential trait related to CBD content [9]. Referring to our results, the bud compact index was affected by location. On the contrary, Folina et al. [9] observed that bud compact index was influenced by variety. Bud compact index values for "Fedora 23"and "Futura 75" varieties were reported as 4.4 g and 5.1 g cm^{-1}, respectively [9]. In our results, the bud compact index was 5.5 for "Uso 31" and 5.56 for "Fedora 17" (Table 2). These are mean values of repeated treatments. Furthermore, a positive correlation was observed between bud compact index and Harvest Index ($r = 0.64$, $p < 0.001$; Table 6). Nitrogen, as the most crucial nutrient in crop production, increased seed yield and quality [41]. The application of fertilizers characterized by a gradual N release enhances the efficiency of fertilizer on the plant and is thus beneficial for seed yield and quality. This fact is well depicted in our experiment after the use of urea fertilizer. Moreover, one thousand seed weight and seed yield were tended to be higher after urea application and even higher after urea with inhibitors fertilizer in comparison with control. One thousand seed weight also had a positive correlation with seed yield ($r = 0.52$, $p < 0.001$) (Table 6). A recent study reported a positive effect of fertilization with urea on hemp yield [42]. On the other hand, urea with urease inhibitors did not present any significant impact on the one thousand seed weight of oilseed rape crop [43].

Even though the two experimental fields are in resemblant semiarid environments, growing conditions importantly affected one thousand seed weight and seed yield. In oil crops, high temperatures at the flowering stage are injurious to seed yield due to the development of the embryo and then seed filling [29,44,45]. Furthermore, in our study, seed yield was higher in Athens, a fact that is contradictory to the former statement. This result could be justified by the fact that both cultivars are early-flowering and the high temperatures probably did not negatively affect the seed mature.

Seed oil content was significantly affected by urea in contrast to control. Previous studies verify the beneficial effect of N on seed oil content [27]. It is also important to mention that in some cases the effect of genotype surpassed the effect of nitrogen and consequently the content in oil decreased [46]. In our study, genotype did not cause any negative effect and an important impact on the content was reported.

All treatments of fertilization significantly affected seed oil yield. The fertilizer of urea with urease and nitrification inhibitors caused the greatest increase of seed oil yield. Xie et al. mentioned that N fertilizer increased the oil content and oil yield in most of the oil crops [40]. Similar results present the positive effect of urea with inhibitors fertilizer on oilseed of flaxseed crop [32]. Seed protein content and seed protein yield were positively affected by fertilization treatments (Table 3). A high impact is mentioned in the case of urea with inhibitors for both cultivars and locations. A previous study mentions that increased rates of N led to a gradual increase in seed protein of hemp seeds [29]. However, there are conflicting statements in the literature about the effect of N on seed protein. A recent survey showed that urea decreased the protein content of hemp seed [42]. Some researchers also observed that seed oil and seed protein have an inversely proportional

relationship [29]. The limited precipitation and the high temperatures seem to increase the yield of seed protein.

Nitrogen is not only a primary element which is absorbed from plants for primary growth but is also a necessary component in many plant secondary metabolites. Nevertheless, conflicting results have been published for the effects of N nutrition on secondary metabolites. While some surveys recognized effect of N supplementation on secondary metabolites of sage crop [31], others reported no significant effects on secondary metabolites of oregano crop [47].

CBD content was only positively affected by fertilization (Table 4). A previous study mentioned that cannabinol (CBN) concentration augmented with organic fertilizer [48]. However, a recent study declares that the NPK fertilization, raises cannabigerol (CBG) content; cannabinoid content was not increased [13]. Similar studies mention that the efficiency of nitrogen at the stage of flowering augmented the alkaloid yield of capsules in poppy crops increasing the levels of morphine [49]. Although alkaloids are also contained in hemp, cannabinoids are produced via another biosynthetic pathway [50]. The influence of nutritional supplements on these pathways has not clarified yet [51]. Furthermore, CBD yield per plant was only affected by fertilization; urea with urease and nitrate inhibitors had the highest values.

5. Conclusions

This study evaluated the impact of new types of nitrogen fertilization with inhibitors, on growth, yield, and quality of cannabis crop. The application of urea with double inhibitors had the most spectacular results in most of the characteristics such as seed yield, seed oil content and seed oil yield and CBD content, as well as seed and agronomic characteristics. In addition, urea with urease inhibitors significantly affects the agronomic and qualitative characteristics of cannabis. However, the lowest values for all the characteristics in the studies were observed in the control treatment. In addition, the two varieties did not significantly differ between both locations. To sum up, both monoecious cultivars, "Uso 31" and "Fedora 17", seem to have been affected positively mainly by urea + NI + UI and urea UI treatments.

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Abbreviations

CBD	Cannabidiol
CBN	Cannabinol
CBG	Cannabigerol
DASs	Days After Sowing
LAI	Leaf Area Index
NI	Urea with nitrification Inhibitor
THC	Tetrahydrocannabinol

U Urea
UI Urea with urease Inhibitor

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