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Diniconazole Promotes the Yield of Female Hemp (*Cannabis sativa*) Inflorescence and Cannabinoids in a Vertical Farming System

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Abstract: Hemp (*Cannabis sativa*) has gained global attention since being legalized in the USA in 2018. The legalization of hemp is also underway in Korea; however, it requires facility cultivation and an agricultural technological system that can produce high-quality plants. This study investigated the changes in the growth and secondary metabolite content of female hemp following treatment with the plant growth retardant diniconazole (DIN). Treatment with DIN decreased the plant growth rate with increasing concentrations; however, at a concentration of 25 mg·L^{-1}, it increased inflorescence biomass and apical inflorescence size. The high-performance liquid chromatograph analysis of major cannabinoids showed no statistically significant differences in total cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC) contents among the treatment groups (25, 50, 100, 200, and 400 mg·L^{-1}). However, the calculated production of CBD and THC per plant was significantly highest at DIN 25 mg·L^{-1}. Overall, at a concentration of 25 mg·L^{-1}, DIN treatment not only decreased the height of female hemp but also enhanced the production of female hemp inflorescences as well as major cannabinoids (CBD and Δ9-THC). Our results indicate that at a concentration of 25 mg·L^{-1}, DIN is suitable for use in a vertical farming system. However, the Δ9-THC content needs to meet the 0.3% standard for industrial use. These findings can be applied in various fields that use hemp.

Keywords: dwarfism; gibberellin acid; industrial hemp; plant factory; triazole

Citation: Hahm, S.; Lee, B.; Bok, G.; Kim, S.; Park, J. Diniconazole Promotes the Yield of Female Hemp (*Cannabis sativa*) Inflorescence and Cannabinoids in a Vertical Farming System. *Agronomy* **2023**, *13*, 1497. https://doi.org/10.3390/agronomy13061497

Academic Editor: Alessandra Carrubba

Received: 10 May 2023
Revised: 28 May 2023
Accepted: 29 May 2023
Published: 30 May 2023

1. Introduction

Cannabis sativa, a dioecious annual plant from the Cannabaceae family, has been cultivated mainly in central Asia since ancient times [1]. It is a multifaceted plant with diverse applications in both agriculture and industry. For centuries, it has been utilized as a source of fiber, food, oil, and medicine [2,3]. Currently, *Cannabis sativa* has various applications, including industrial, ornamental, nutritional, medicinal, and recreational uses [4–7]. *Cannabis sativa* consists of roughly 540 distinct compounds, including primary metabolites like amino and fatty acids, proteins, sugars, and vitamins, as well as secondary metabolites like cannabinoids, terpenes, flavonoids, and alkanes [8–11]. Cannabinoids were first isolated from *Cannabis sativa* in 1964 [12].

Cannabis sativa produces acidic forms of cannabinoids (C22) via the biosynthetic pathway of cannabinoids in plants. These cannabinoids are later transformed into alkyl-type cannabinoids with a monoterpene isoprenyl moiety (C10) and a pentyl side chain (C5),

mainly through decarboxylation and cyclization [13–15]. The most abundant acidic cannabinoids in Cannabis sativa are cannabigerolic acid (CBGA), cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), and cannabichromenic acid (CBCA), whereas the alkyl-type cannabinoids include Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), and cannabigerol (CBG) [16,17].

Cannabis sativa can be classified into two forms: medicinal hemp and narcotic marijuana. Marijuana contains more than 20% of the psychoactive compound Δ9-THC, whereas hemp contains less than 0.3%. Hemp was excluded from the Controlled Substances Act regulation of controlled plants after the 2018 US *Farm Bill* was signed. The major secondary metabolite of hemp, the non-psychoactive compound CBD, has gained attention. According to clinical studies, CBD has potential therapeutic effects in psychiatric disorders, anxiety, seizures, sleep disorders, diabetes, pain, cancer, and cardiovascular diseases [18–25].

Hemp is a high-value plant primarily grown in controlled environmental production facilities, such as greenhouses or vertical farms, under artificial light conditions [26]. Controlled environmental production facilities offer the advantage of consistent year-round production [27,28]. However, the operating costs of vertical farms are higher than those of greenhouses or open fields; therefore, it is more advantageous to select high-demand or high-value crops [29]. In Korea, leafy vegetables are mainly cultivated on vertical farms; however, efforts are being made to produce high-value crops owing to profitability issues [30]. Generally, marijuana plants reach an average height of 2–3 m and can even reach up to 5 m, depending on the climatic conditions [31–33]. Given the large size of the hemp plant, maximizing profits in agricultural facilities with limited space, such as vertical farms, is difficult. Therefore, dwarf induction of the plant is required to achieve profitability in vertical farms.

There are various methods for inducing plant dwarfism. Synthetic substances known as plant growth retardants can modify the growth and development of plants, including leaf expansion, stem elongation, and internode elongation, and they can be effective at low concentrations [34]. These retardants have been applied to plants to reduce accidental stem elongation without affecting productivity [35]. Various types of recognized plant growth retardants, particularly those of the triazole group, such as hexaconazole, tebuconazole, and diniconazole (DIN), suppress the biosynthesis of gibberellic acid (GA) via the inhibition of a precursor in the GA biosynthesis pathway thereby reducing cell elongation and division to produce dwarfed plants [36,37]. The triazole group offers several advantages, including high effectiveness, a wide range of applications, minimal harm to non-target organisms, and low levels of resistance [38]. In particular, DIN is widely used in domestic farms because it is distributed at a lower price than other growth retardants [39]. Moreover, these retardants are used as fungicides for disease control. Hexaconazole is used for controlling powdery and downy mildew in cucurbitaceous vegetables, and DIN and tebuconazole are used for disease control in fruit crops such as apple scabs [40]. However, the moderate lipid solubility, extended durability, and photolytic half-life of triazole group compounds pose a challenge as they have the potential to accumulate in aquatic environments, such as wastewater, lakes, and rivers [38]. As a result, it is essential to ensure the judicious use of DIN in agricultural practices by employing appropriate concentrations to prevent excessive application.

Numerous studies have investigated the application of DIN at various concentrations to different crops for determining the appropriate concentration for optimal effect; however, there have been no reported studies on the application of DIN to female hemp to determine a suitable concentration for treatment. This study aimed to identify the appropriate concentration of DIN for cultivating and producing female hemp, a high-value plant, in a limited-space agricultural facility, by treating it with DIN at various concentrations. This study has considerable implications for the commercial production of female hemp, particularly in areas with limited space and resources.

2. Materials and Methods

2.1. Plant Materials and Cultivation Conditions

Feminized hemp (*Cannabis sativa* L. 'Hot blonde') seeds were purchased from Blue Forest Farms (Blue Forest Farms Co. Ltd., New York, NY, USA) and germinated for four weeks under the growth conditions of 200 ± 10 μmol·m^{-2}·s^{-1} light intensity, ~70–75% humidity, and a photoperiod of 18 h/6 h (light/dark). Thereafter, the seedlings were transplanted into rockwool cubes (10 × 10 × 10 cm, Grodan, Roermond, The Netherlands) and grown in a controlled vertical farming system (Daejeon, South Korea (36°22′11″ N 127°21′10″ E, elevation = 60 m) using Hoagland nutrient solution (irrigated through a drip irrigation system and adjusted to electrical conductivity (EC) of 2.0 dS·m^{-1} and a pH of 6.5). Once the female hemp had grown to a height of 1.7 ± 0.2 m, cuttings were obtained by removing all leaves except for three to four leaves and rooted for three weeks under the growth conditions of 100 ± 6 μmol·m^{-2}·s^{-1} blue light, a photoperiod of 20 h/4 h (light/dark), a temperature of 25 °C, and humidity of 90% (Table 1). Female cuttings with fully developed adventitious roots and a uniform height of 12.6 cm were chosen for further cultivation (Figure 1). These were transplanted into cultivation beds in a fully controlled vertical farming system for vegetative growth for four weeks at a temperature of 25 ± 1.2 °C, a humidity of 70 ± 6%, a photoperiod of 18 h/6 h (light/dark), and a light intensity of 400 ± 31.7 μmol·m^{-2}·s^{-1}. Subsequently, the plants were transitioned to the reproductive growth phase, which lasted for five weeks. During this phase, the temperature and humidity conditions were maintained similar to those of the vegetative phase. However, the photoperiod was adjusted to 12 h/12 h (light/dark) and the light intensity was increased to 500 ± 35.1 μmol·m^{-2}·s^{-1}. The plants were regularly thinned and relocated every three weeks based on their shoot development. The nutrient solution was consistently maintained at an EC of 2.0 ± 0.2 dS·m^{-1} and a pH of 6.5 ± 0.3, with daily measurements taken at 09:00 h to account for any fluctuations in EC and pH levels. The plants were grown for a total of nine weeks.

Table 1. Environmental conditions of adventitious root induction in female hemp (*Cannabis sativa* L. 'Hot blonde') cuttings.

Environmental Conditions of Cutting Growth Chamber	
Light Quality	Blue
Light intensity (μmol·m^{-2}·s^{-1})	100
Temperature (°C)	25
Humidity (%)	90
EC (ds m^{-1})	2.0
Nutrient solution	Hoagland

2.2. DIN Treatment

DIN (Binari®, Youngil Co., Incheon, Korea) was diluted in water as a solvent to concentrations of control (0 mg·L^{-1}), 25 mg·L^{-1}, 50 mg·L^{-1}, 100 mg·L^{-1}, 200 mg·L^{-1}, or 400 mg·L^{-1} and applied as a root-soaking treatment. As far as we know, this is the first paper to apply DIN treatment to female hemp. Therefore, the treatment concentration and method were set based on the DIN conditions provided for indoor farm production of Agastache rugosa by Lam et al. [41]. The treatment was performed once during the cultivation period for 10 min by root-soaking hemp cuttings after 3 weeks of adventitious root induction before transitioning to vegetative growth. Each treatment group comprised three hemp plants (n = 3).

Figure 1. Pictures of female hemp cuttings (*Cannabis sativa* L. 'Hot blonde') after the induction of adventitious root was completed. The adventitious root induction was carried out for 21 days. The average length is 12.6 cm.

2.3. Measurement of Plant Growth Parameters

The growth analysis was conducted based on Anderson et al. [42] as the fundamental basis. Growth analyses included measuring the length and width of the top (apical), middle (11th node), and bottom (3rd node) leaves of the female hemp plants harvested after treatment using a ruler. Additionally, the non-destructive chlorophyll content of leaves was measured using a SPAD-502 (Minolta Camera Co. Ltd., Osaka, Japan) to calculate the chlorophyll content per unit area for the leaves of each treatment group. Stem diameter and internode length were measured at the same position using a caliper (SD500-300PRO; Shin Con Co. Ltd., Bucheon, Korea), and plant height was measured from the soil surface to the apical meristem using a ruler. Leaf area was measured using a leaf area meter (Li-3100, LICOR, Lincoln, NE, USA), and plant biomass (fresh weight) was measured using an electronic balance (MW-2N, CAS Co. Ltd., Seoul, Korea). To measure dry weight, the harvested plants were dried in a freeze dryer (TFD550, Ilshin BioBase Co. Ltd., Dongducheon, Korea) for seven days and then weighed using an electronic balance (MW-2N).

2.4. Measurement and Analyses of Cannabinoid Content

The cannabinoids in female hemp were analyzed by modifying the method described by Hädener et al. [43]. Briefly, the aboveground parts of the nine-week-old female hemp plants were separated into leaves and inflorescences and dried in a freeze dryer (TFD550, Ilshin BioBase Co. Ltd., Dongducheon, Korea) for seven days. The dried samples were ground into powder using a mortar and pestle. The powder (100 mg) was placed into a 2.0 mL tube and mixed with a solution of MeOH: hexane (9:1; 2 mL) by vortexing for 1 min, followed by sonication (powersonic420, Hwashin Tech Co. Ltd., Daegu, Korea) at 25 °C for 20 min to extract the cannabinoids. After sonication, the mixture was vortexed for 1 min and centrifuged at 13,000 rpm for 5 min to extract the supernatant. The extracted supernatant was filtered through a 0.45 μm syringe filter (25HP020AN, Advantech Co. Ltd., Asan, Korea) (1 mL per sample) and transferred to high-performance liquid chromatography (HPLC) vials for analysis. The HPLC system consisted of an Agilent 1260 Infinity II binary pump (G7112B, Agilent Technologies Inc., Santa Clara, CA, USA), Agilent 1260 Infinity II Vialsampler (G7129C Agilent Technologies Inc., Santa Clara, CA, USA), Agilent 1260 Infinity II multicolumn thermostat (G7116A, Agilent Technologies Inc., Santa Clara, CA, USA) and Agilent 1260 Infinity II diode array detector HS (G7117C, Agilent Technologies Inc., Santa Clara, CA, USA). Chromatographic separation was achieved using a Poroshell 120 EC-C18 column (4.6 × 50 mm, 2.7 μm, Agilent Technologies Inc., Santa Clara, CA, USA) and gradient elution with 0.1% formic acid (reagent grade, >96%, Sigma-Aldrich, Saint Louis, MO, USA) in water (HPLC grade, Ducsan Pure Chemical Co. Ltd., Incheon, Korea) as mobile phase A, and 0.1% formic acid in acetonitrile (HPLC grade, Ducsan pure chemical Co. Ltd., Incheon, Korea) as mobile phase B. The flow rate and oven temperature were 1 mL·min^{-1} and 25 °C, respectively and the gradient conditions were as follows: 0–5 min, held at 55% B; 5–25 min, increased to 85% B; 25–30 min, held at 85% B; 30–30.1 min, decreased to 55% B; 30.1–35 min, held at 55% B. The injection volume was 10 μL, and full spectra were recorded from 200 to 800 nm. For quantification, the detection wavelength was set at 210 nm.

The cannabinoid standards for CBDA (CAS No. 1244-58-2) and CBD (CAS No. 13956-29-1) and THCA (CAS No. 23978-85-0) were acquired from Lipomed (Arleisheim, Switzerland). Δ9-THC (CAS No. 1972-08-3) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). These were used to determine the retention times of each cannabinoid and prepare a six-point calibration curve. A calibration verification standard was injected at the start of each analysis day to verify retention times and quantitation. Quantification was performed using calibration curves ranging from 50 to 1,000 μg·mL^{-1}. The linear equations were y = 32.251x + 19.131 for CBDA, y = 83.907x − 24.125 for CBD, y = 31.180x + 35.423 for THCA and y = 86.082x − 0.954. The experiment was performed three times.

2.5. Total Yield of Major Cannabinoids

To calculate the total production of major cannabinoids (mg·g^{-1} DW) (mg/plant DW), HPLC analysis results were combined with plant growth results. The total CBD and total Δ9-THC content (mg·g^{-1} DW) present in the inflorescences and leaves of the control and treatment groups were multiplied by the dry weight (mg/plant DW) of the inflorescences and leaves.

$$\begin{aligned}&\text{Total major cannabinoid yield } [(\text{mg g}^{-1}\text{ DW}) \times \left(\tfrac{\text{mg}}{\text{plant}}\text{DW}\right)\\&= \text{cannabinoids content } (\text{mg g}^{-1}\text{ DW}) \times \text{dry weight } \left(\tfrac{\text{mg}}{\text{plant}}\text{DW}\right)\end{aligned}$$

2.6. Statistical Analyses

The growth and cannabinoid content of the female hemp plants were analyzed using the Analysis of Variance test with Tukey's multiple comparison test for significance ($p \leq 0.05$) using the SPSS program (Version 22.0.0.1, SPSS Inc., Chicago, IL, USA). Addition-

ally, to determine the trends in growth and cannabinoid content, first- and second-order regression analyses were performed using SPSS.

3. Results

3.1. Analyses of Female Hemp Growth Parameters According to DIN Concentration

The overall growth of the female hemp, except for the stem diameter, tended to decrease with increasing concentration of DIN (Figure 2). Growth analyses of DIN-treated plants revealed that stem-related factors, such as plant height, stem diameter, and fresh and dry weights of stem, were the highest in the control group, with mean values of 103.9 cm, 9.7 mm, 183.4 g/plant, and 29.2 g/plant, respectively. There were no significant differences in internode length, and fresh and dry weight of stem among the DIN 100 mg·L^{-1}, 200 mg·L^{-1}, and 400 mg·L^{-1} treatment groups, and no significant differences in stem diameter among the treatment groups. Values of leaf-related factors, such as leaf number, length, width, area, and fresh and dry weight, were the highest in the control group, similar to the stem-related factors. The apical inflorescence size increased at the DIN concentration of 25 mg·L^{-1} and then decreased as the concentration increased thereafter (Figure 3). Nevertheless, the fresh and dry weights of inflorescences showed the highest values in the DIN 25 mg·L^{-1} treatment group, with mean values of 123.1 g/plant and 22.7 g/plant, respectively, and increased by 24.46% and 24.04%, respectively, compared to the control group. Inflorescence weight showed a tendency to decrease at DIN concentrations of 50 mg·L^{-1}, 100 mg·L^{-1}, 200 mg·L^{-1}, and 400 mg·L^{-1} compared to the control group (Table 2).

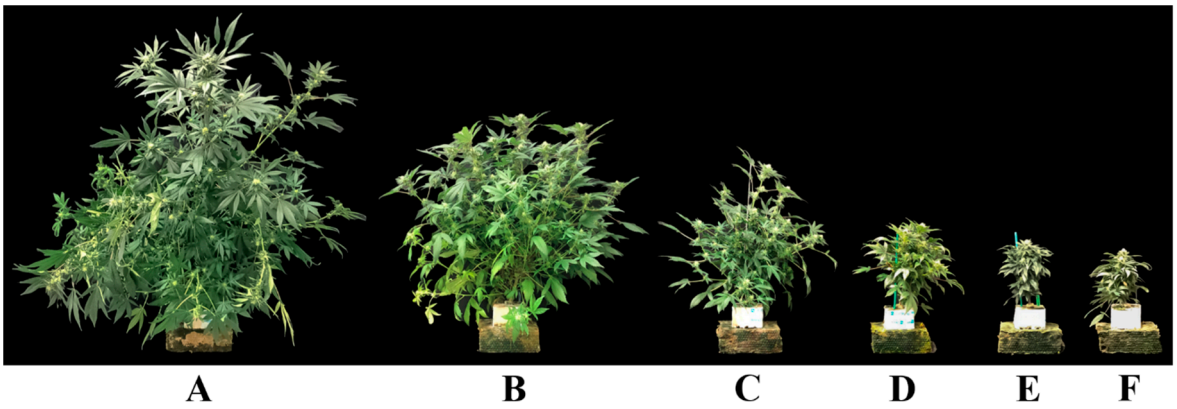

Figure 2. Pictures of female hemp (*Cannabis sativa* L. 'Hot blonde') on harvest day of diniconazole applications. The control group (**A**) did not receive the additional diniconazole treatment. Harvest day is 35 days after reproductive growth transition. (**A**) Control; (**B**) 25 mg·L^{-1}; (**C**) 50 mg·L^{-1}; (**D**) 100 mg·L^{-1}; (**E**) 200 mg·L^{-1}; and (**F**) 400 mg·L^{-1}.

Figure 3. Pictures of female hemp (*Cannabis sativa* L. 'Hot blonde') apical inflorescences on harvest day of diniconazole applications. The control group (**A**) did not receive the additional diniconazole treatment. Harvest day is 35 days after reproductive growth transition. Scale bars 1 cm; (**A**) Control; (**B**) 25 mg·L^{-1}; (**C**) 50 mg·L^{-1}; (**D**) 100 mg·L^{-1}; (**E**) 200 mg·L^{-1}; and (**F**) 400 mg·L^{-1}.

Table 2. The growth parameters of female hemp (*Cannabis sativa* L. 'Hot blonde') grown in the different diniconazole concentrations. The control group did not receive the additional diniconazole treatment.

Diniconazole Concentration ($mg \cdot L^{-1}$)	Plant Height (cm)	No. of Nodes	Stem Diameter (mm)	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm^2)	[w]SFW (g/plant)	LFW (g/plant)	FFW (g/plant)	SDW (g/plant)	LDW (g/plant)	FDW (g/plant)
Control	103.9 ± 0.6 a	25.0 ± 0.1 a	9.7 ± 0.6 a	791.0 ± 10.0 a	14.3 ± 0.1 a	15.0 ± 1.2 a	11580.5 ± 1269.1 a	183.4 ± 13.2 a	244.9 ± 12.0 a	98.9 ± 4.3 a	29.2 ± 2.8 a	45.9 ± 4.2 a	18.3 ± 0.2 b
25	68.0 ± 1.6 b	22.6 ± 0.3 b	6.6 ± 0.4 ab	651.6 ± 32.9 b	11.9 ± 0.2 ab	11.6 ± 0.6 ab	7544.0 ± 405.6 b	108.1 ± 4.4 b	162.7 ± 6.5 b	123.1 ± 7.9 a	18.6 ± 1.1 b	35.0 ± 1.6 ab	22.7 ± 1.6 a
50	54.4 ± 2.8 c	23.3 ± 0.3 b	5.7 ± 0.3 b	529.3 ± 53.4 b	10.4 ± 0.7 b	10.4 ± 0.7 bc	5455.5 ± 913.2 b	57.7 ± 11.4 c	110.5 ± 20.3 c	58.5 ± 12.8 b	9.9 ± 2.3 c	23.0 ± 4.0 bc	13.2 ± 0.3 c
100	28.2 ± 2.5 d	22.0 ± 0.5 bc	3.9 ± 0.2 b	300.0 ± 18.5 c	7.6 ± 0.5 c	7.6 ± 0.2 cd	2007.2 ± 43.37 c	12.4 ± 3.0 d	48.6 ± 3.0 d	34.2 ± 7.4 bc	1.9 ± 0.5 d	10.2 ± 2.2 cd	7.2 ± 0.6 d
200	22.1 ± 0.8 de	21.0 ± 0.1 cd	4.3 ± 0.0 b	164.0 ± 16.0 cd	5.9 ± 0.8 c	5.9 ± 1.0 d	656.2 ± 133.16 c	4.5 ± 0.6 d	19.2 ± 3.7 d	23.4 ± 2.9 c	0.8 ± 0.0 d	5.4 ± 0.7 d	4.6 ± 0.4 de
400	17.8 ± 1.4 e	20.3 ± 0.3 d	6.9 ± 1.6 ab	74.0 ± 19.0 d	6.9 ± 0.1 c	6.9 ± 0.1 d	298.7 ± 72.1 c	2.1 ± 0.4 d	8.4 ± 2.7 d	13.2 ± 1.2 c	0.5 ± 0.1 d	3.8 ± 1.2 d	3.1 ± 0.3 e
Significance[x]	***	***	**	***	***	***	***	***	***	***	***	***	***
L[y]	***	***	*	***	***	***	***	***	***	***	***	***	***
Q[z]	***	***	NS	***	***	***	***	***	***	***	***	***	***

[w]SFW—Stem fresh weight; LFW—Leaf fresh weight; FFW—Inflorescence fresh weight; SDW—Stem dry weight; LDW—Leaf dry weight; FDW—Inflorescence dry weight—NS: not significant ($p > 0.05$); Significance[x] at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$; L[y], linear; Q[z], quadratic in regression analysis. The data represent the means and standard errors ($n = 3$). Different letters indicate significant differences among treatments at the level of 5%, according to Tukey's test.

3.2. Analysis of Female Hemp Cannabinoids Content According to DIN Concentration

The CBDA content of the leaves and inflorescences of the female hemp plants were the lowest in the control group, at 20.54 $mg \cdot g^{-1}$ and 56.80 $mg \cdot g^{-1}$, respectively, but there was no statistically significant difference among the groups (including the control group (0 $mg \cdot L^{-1}$)). The THCA content of female hemp leaves was the lowest in the control group at an average of 0.96 $mg \cdot g^{-1}$, but there was no significant difference among the treatment groups. The THCA content of the inflorescences too was the lowest in the control group; the THCA contents of the DIN 25 $mg \cdot L^{-1}$, 50 $mg \cdot L^{-1}$, and 100 $mg \cdot L^{-1}$ treatment group showed increases of 23.96%, 23.18%, and 22.00%, respectively, compared to the control group. However, there were no significant differences in THCA content among the treatment groups (25 $mg \cdot L^{-1}$, 50 $mg \cdot L^{-1}$, 100 $mg \cdot L^{-1}$, 200 $mg \cdot L^{-1}$, and 400 $mg \cdot L^{-1}$), except for the control group. The total CBD and CBDA content of the leaves and inflorescences did not show statistically significant differences among all treatment groups. Similarly, there was no significant difference observed in the total Δ9-THC and THCA content of the leaf and inflorescence. The total Δ9-THC content was similar to the THCA content. The control group had the lowest total Δ9-THC content of 0.85 $mg \cdot g^{-1}$ for leaves on average, and the highest value of 1.24 $mg \cdot g^{-1}$ was observed in the DIN 100 $mg \cdot L^{-1}$ treatment group. However, no statistically significant differences were observed between the control and treatment groups. The total Δ9-THC content of the inflorescences was the lowest in the control group at 4.95 $mg \cdot g^{-1}$, whereas the highest was observed in the DIN 25 $mg \cdot L^{-1}$ treatment group at 5.15 $mg \cdot g^{-1}$ (Table 3).

Table 3. The cannabinoid contents of female hemp (*Cannabis sativa* L. 'Hot blonde') leaves and inflorescences grown in the different diniconazole concentrations. The control group did not receive the additional diniconazole treatment.

Diniconazole Concentration ($mg \cdot L^{-1}$)	[w]CBDA ($mg \cdot g^{-1}$)		CBD		Δ9-THC		THCA		Total CBD		Total Δ9-THC	
	Leaf	Inflorescence	Leaf	Inflorescence	Leaf	Inflorescence	Leaf	Inflorescence	Leaf	Inflorescence	Leaf	Inflorescence
Control	20.54 ± 1.08	56.80 ± 2.24	0.12 ± 0.022	0.76 ± 0.08 c	0.0124 ± 0.0009 ab	0.09 ± 0.0007	0.96 ± 0.045	5.56 ± 0.027	18.14 ± 0.9781	50.57 ± 2.057	0.85 ± 0.041	4.97 ± 0.024
25	23.20 ± 1.61	62.73 ± 1.16	0.13 ± 0.019	1.32 ± 0.07 a	0.0107 ± 0.0003b	0.11 ± 0.0056	1.10 ± 0.107	5.75 ± 0.216	20.48 ± 1.4305	56.34 ± 1.484	0.97 ± 0.094	5.15 ± 0.193
50	24.40 ± 1.12	62.45 ± 1.90	0.14 ± 0.025	1.34 ± 0.07 a	0.0106 ± 0.0013b	0.10 ± 0.0087	1.17 ± 0.065	5.71 ± 0.278	21.54 ± 1.0001	56.12 ± 1.694	1.04 ± 0.057	5.11 ± 0.252
100	25.33 ± 0.62	58.61 ± 2.68	0.14 ± 0.027	1.33 ± 0.02 a	0.0132 ± 0.0013 ab	0.09 ± 0.0008	1.39 ± 0.153	5.77 ± 0.081	22.36 ± 0.5554	52.73 ± 2.335	1.24 ± 0.134	5.15 ± 0.072
200	25.35 ± 0.32	61.47 ± 0.92	0.07 ± 0.001	0.85 ± 0.04 bc	0.0171 ± 0.0001 a	0.09 ± 0.0001	1.16 ± 0.010	5.74 ± 0.118	22.31 ± 0.2901	54.76 ± 0.767	1.03 ± 0.009	5.14 ± 0.104
400	24.43 ± 1.37	60.98 ± 0.09	0.07 ± 0.024	1.08 ± 0.01 ab	0.0169 ± 0.0013 a	0.10 ± 0.0030	1.21 ± 0.110	5.64 ± 0.102	21.49 ± 1.2308	54.56 ± 0.065	1.08 ± 0.098	5.05 ± 0.087
Significance[x]	NS	NS	NS	***	**	NS	NS	NS	NS	NS	NS	NS
L[y]	*	NS	NS	NS	***	NS	NS	NS	*	NS	NS	NS
Q[z]	**	NS	*	*	***	NS	*	NS	**	NS	*	NS

[w]CBDA—Cannabidiolic acid; CBD—Cannabidiol; Δ9-THC—Δ9-Tetrahydrocannabinol; THCA—Tetrahydrocannabinolic acid; NS: not significant ($p > 0.05$); Significance[x] at * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$; L[y]—linear; Q[z]—quadratic in regression analysis. The data represent the means and standard errors ($n = 3$). Different letters indicate significant differences among treatments at the level of 5%, according to Tukey's test.

3.3. DIN Treatment Contributes to the Contents of Major Cannabinoids in Female Hemp

The total contents of major cannabinoids in the female hemp grown in a fully controlled plant factory for nine weeks varied depending on the concentration of DIN during treatment (Figure 4). The total CBD and total Δ9-THC contents of the inflorescences were 1276.67 ($mg \cdot g^{-1}$ DW)·(mg/plant DW) and 116.76 ($mg \cdot g^{-1}$ DW)· (mg/plant DW), respectively, at a DIN concentration of 25 $mg \cdot L^{-1}$, representing an increase of 37.3% and 27.6% compared to the control group. Except for the DIN 25 $mg \cdot L^{-1}$ treatment group, the total contents of CBD and total Δ9-THC of the inflorescences decreased for all treatment groups compared to the control group, and the decrease became more pronounced as the DIN concentration increased. Although the total CBD and total Δ9-THC contents of the leaves were highest in the control group at 839.96 ($mg \cdot g^{-1}$ DW)·(mg/plant DW) and 39.65 ($mg \cdot g^{-1}$ DW)·(mg/plant DW), respectively, there were no significant differences compared to the DIN 25 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$ treatment groups. None of the DIN treatment groups showed an increase in the total contents of CBD and total Δ9-THC in the leaves, unlike that in the case of the inflorescences, and there was a tendency for the contents to decrease as the DIN concentration increased.

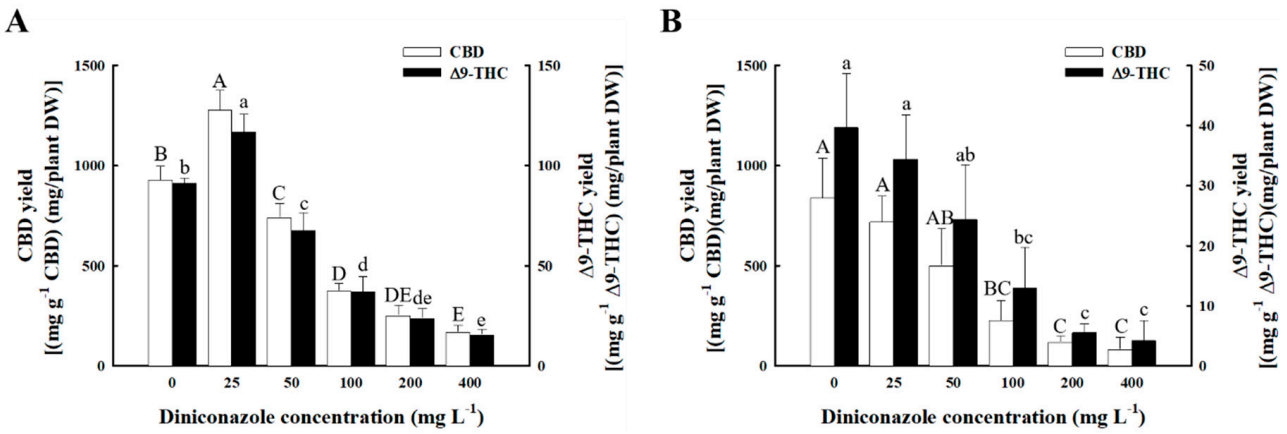

Figure 4. Total yield of major cannabinoids of female hemp (*Cannabis sativa* L. 'Hot blonde') inflorescence (**A**) and leaf (**B**) following diniconazole applications. The control group did not receive the additional diniconazole treatment. Harvest day is 35 days after reproductive growth transition. Data are means ± SD (n = 3). Different letters indicate significant differences among treatments at the level of 5%, according to Tukey's test.

4. Discussion

DIN is a plant growth regulator belonging to the triazole class of fungicides that inhibits GA biosynthesis, resulting in reduced cell elongation and division, ultimately leading to the production of dwarf plants [37]. By inhibiting the oxidation and methylation of *ent*-kaurene, a precursor of GA, via phytochrome P450, the application of triazole suppresses the biosynthesis of GA [36]. Treating peanut plants (*Arachis hypogaea* L.) with DIN led to decreases of 33%, 16%, and 19% in plant height, leaf area, and leaf dry weight, respectively, when compared to the control group [44]. Similarly, treatment of tomato seedlings with DIN at a concentration of 30 mg·L^{-1} resulted in reductions of 27.40%, 4.80%, 23.20%, 20.70%, and 27.05% in plant height, leaf number, leaf area, shoot fresh weight, and dry weight, respectively, compared to the control group [45]. These results show a trend similar to those of this study and are commonly attributed to the general effects of DIN. Interestingly, in this study, inflorescence growth showed the highest increase in the DIN 25 mg·L^{-1} treatment group compared to the control group. Paclobutrazol, a triazole similar to DIN, at a concentration of 500 mg·L^{-1}, when exogenously applied, increased flower bud size and flowering rate in apples (*Malus domestica*), whereas treatment with GA_3 (750 mg·L^{-1}) had the opposite effect [46].

Goldberg-Moeller et al. [47] demonstrated that treatment with GA resulted in a decrease in the number of flower buds in *Citrus reticulata* Blanco × *Citrus temple* Hort. ex Y. Tanaka. Additionally, the application of GA_3 at a concentration of 40 mg·L^{-1} as a flower induction agent to sweet orange (*Citrus sinensis* L. Osbeck) decreased the number of flowers per 100 nodes by 72% compared to the control group. In contrast, treatment with paclobutrazol at 2000 mg·L^{-1} increased the number of flowers per 100 nodes by 123% [48]. According to Yamaguchi et al. [49], elevated levels of gibberellin lead to the cessation of vegetative growth and upregulation of transcription factor-encoding genes, such as SPLs and LFY. An elevation in gene expression results in a decline in the GA concentration and an amplification in the activity of the DELLA protein. Consequently, the DELLA protein stimulates SPL9 and LFY, which trigger the formation of flowers by activating AP1 [49]. Inhibiting gibberellin with DIN is anticipated to have a positive impact on the floral biomass of hemp by increasing the growth rate, bud size, and number of buds. Nonetheless, it is important to note that the effects of chemical growth regulators like DIN on flowering may differ depending on the plant species and application timing [50].

Female *Cannabis sativa* is predominantly grown for the production of cannabinoids as they can accumulate the highest concentration of cannabinoids in their unaltered flowers [51]. Two pathways, the fatty acid oxidation pathway and the methylerythritol

4-phosphate (MEP) pathway, are involved in the biosynthesis of cannabinoids in Cannabis sativa. These pathways result in the production of olivetolic acid (OA) and geranyl diphosphate (GPP), respectively [52]. CBGA is produced through the prenylation of OA and GPP in the plastids, with the involvement of several enzymes, including DXP synthase (DXS), an enzyme in the MEP pathway [53]. The activity of DXS was found to decrease when exogenous GA_3 was applied to flowering Cannabis sativa, particularly in male plants, compared to female plants [54]. Moreover, applying GA_3 to the leaves of *Cannabis sativa* during vegetative growth led to a reduction in DXS activity [55]. The study did not find a significant difference in the total CBD and Δ9-THC contents after DIN treatment, which may be due to the increase in DXS caused by GA inhibition. However, the lack of statistical significance is thought to be due to the use of female *Cannabis sativa* in the study.

In a vertical farming system, plants with smaller sizes, like leafy vegetables and herbs, can be cultivated in multiple layers, resulting in higher yields per unit of height [56,57]. To accommodate larger plants such as tomatoes or peppers, alternative cultivation techniques, specific plant varieties, and gene editing technology can be employed to regulate their size [56,58]. This allows for the creation of dwarf plants, which maximizes cultivation space and enhances overall efficiency [59]. Environmental factors, including light, temperature, humidity, carbon dioxide (CO_2) levels, and nutrients, are precisely controlled in a vertical farming system to ensure optimal plant growth and productivity in a multi-tiered indoor setup [60]. By effectively managing these factors, morphological changes can be induced in tall plants, contributing to their successful integration into the vertical farming system [61–64]. However, it is crucial to select varieties that are well-suited for each specific vertical farming setup when integrating tall plants like hemp. This careful selection significantly influences the plant's growth, yield, and overall profitability of the vertical farming system. Additionally, choosing the most appropriate cultivation methods tailored to the target crop is essential for maximizing efficiency and profitability. In summary, in a vertical farming system, the choice of suitable plant varieties, utilization of cultivation techniques, and precise control of environmental factors are key considerations for integrating tall plants and ensuring successful and profitable cultivation.

5. Conclusions

The decrease in plant stature holds significance in vertical farming setups that employ artificial illumination, as it can result in enhanced yield per unit area. Triazole, encompassing diniconazole (DIN), stands out as one of the most effective agents in the realm of agriculture. The results of this study exhibited the capability of DIN to curtail the vertical growth of hemp within vertical farming systems, thereby economizing cultivation space. We identified the optimal concentration of DIN (25 $mg \cdot L^{-1}$) that promotes increased inflorescence production, which is a vital organ in hemp. Our findings put forth potential avenues for exploring treatment methodologies applicable to hemp cultivation in vertical farming systems. Although we assessed growth parameters and cannabinoid content in response to the DIN application, ascertaining the potential existence of pesticide residues within hemp remains beyond our scope. Moreover, the agricultural implementation of triazoles occasionally carries the risk of environmental toxicity. Consequently, the utilization of DIN should be determined in compliance with pesticide regulations, which may exhibit variability across different countries.

Author Contributions: S.H. and B.L.: constructing the idea, setting up the experiments, data collection and analysis, writing—original manuscript, and writing—review and editing. G.B.: preparation for the manuscript and writing—review and editing. S.K.: Setting up the experiments, data analysis. J.P.: project administration, supervision, constructing the idea, experimental design, data analysis, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by [Cooperative Research Program for Agriculture Science and Technology Development] grant number [PJ017063022023] and the APC was funded by [PJ017063022023].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ017063022023)" Rural Development Administration, Republic of Korea.

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

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