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Changes in Growth and Production of Non-Psychotropic Cannabinoids Induced by Pre-Sowing Treatment of Hemp Seeds with Cold Plasma, Vacuum and Electromagnetic Field

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Received: 26 October 2020; Accepted: 25 November 2020; Published: 28 November 2020

Featured Application: The results of this study have the potential for application in agriculture for increasing the production of industrial hemp biomass and the yield of cannabinoids.

Abstract: In this study, the effects of seed treatments with different stressors, such as cold plasma (CP), a vacuum and an electromagnetic field (EMF), on the in vitro germination of industrial hemp cv. Futura 75 were compared with the effects on germination in the field, plant growth, and the amount of major cannabinoids in the leaves and inflorescences of female plants. CP and EMF (but not vacuum) treatments improved in vitro seed germination, but had no impact on germination in the field. EMF treatment increased the weight of the above-ground part of male and female plants grown for 4 months by 65–70% and the number of female inflorescences by 70%. CP stimulated the growth of male plants (weight increased 1.4 times) but reduced the growth of female plants. Vacuum treatment did not induce changes in the growth of female and male plants. Vacuum and EMF treatments did not change the amount of cannabidiolic acid (CBDA), but CP decreased the CBDA content in hemp leaves by 41%. Vacuum treatment increased the amount of CBDA in female plant inflorescences by 26%. Thus, hemp seed treatment with EMF has a potential application for increasing the biomass of female plants. CP treatment can be used to increase male plant production while vacuum treatment can stimulate CBD production.

Keywords: cold plasma; cannabinoids; electromagnetic field; industrial hemp; seed germination; growth; biomass production

1. Introduction

The improvement of crop agricultural performance and production yields without using chemicals is one of the most important challenges in sustainable and organic agriculture [1,2]. Various pre-sowing

seed treatments are being used to increase seed quality and to stimulate germination [3,4]. In this respect, the effectiveness of physical stressors, such as low-temperature plasma (cold plasma (CP)) and electromagnetic fields (EMFs), has been intensively explored in the last several decades, and numerous studies concluded that such treatments can improve seed germination and enhance the production yields of various crops (reviewed in [5–9]). The majority of studies in this field are focused on germination. However, the importance of long-term observations has become clear by the much larger effects obtained in plant growth and biomass production. For example, it was recently reported that red clover seed treatment with CP and EMFs can increase the biomass by up to 40% [10].

Industrial hemp (*Cannabis sativa*) is a multi-use crop (e.g., fiber, food, pharmaceuticals, and bioenergy production) of high economic value [11–13]. However, only a few short-term studies [14–16] have been published on the effects of seed treatment with physical stressors on industrial hemp until now. Significant environmental benefits of hemp that are relevant for sustainable farming have been demonstrated, including the potential for soil phytoremediation, converting high amounts of atmospheric CO_2 into biomass, use in crop rotation, and the ability to suppress soil pathogens and weeds (reviewed in [17]). In addition, hemp is valued for the synthesis of a wide array of biologically active secondary metabolites (e.g., phytocannabinoids, terpenes, and phenolic compounds) with pharmacological properties [18–20]. Cannabidiolic acid (CBDA) and cannabidiol (CBD) are the most abundant phytocannabinoids in the majority of industrial hemp cultivars, but some of them biosynthesize cannabigerol (CBG) as the main constituent [19]. Among other cannabinoids, CBD, its precursor CBDA, and CBG are particularly valued as non-psychotropic substances with numerous biological effects and potential therapeutic uses, due to their anticonvulsant, anti-spasmodic, anxiolytic, anti-nausea, anti-rheumatoid arthritis, and neuroprotective properties [18,19].

Several studies reported earlier that pre-sowing seed treatment with CP and EMFs can stimulate the synthesis of secondary metabolites in the leaves or roots of different plants [10,21–23]. Taking into account the rapidly rising demand for hemp biomass and pharmacologically important hemp products, we aimed to determine the effects of seed treatment with CP and EMFs on germination, biomass production, and plant growth in the field, as well as on the content of CBD and CBDA in the leaves and inflorescences of female hemp plants. We intended to verify the hypothesis that hemp seed treatments can stimulate biomass production and CBDA (CBD) synthesis. Vacuum treatment was used as an additional control for low pressure CP treatment. The obtained results showed that, although CP and EMFs (but not the vacuum) stimulated hemp seed germination, EMFs strongly stimulated the growth of female plants and CP stimulated the growth of male plants, whereas only the vacuum treatment increased the amount of CBDA in female hemp inflorescences.

2. Materials and Methods

2.1. Plant Material

Seeds of the industrial hemp (*Cannabis sativa*) cultivar (cv.) Futura 75 were received from the Endobiotech company. Seed quality was checked visually, and damaged seeds were removed; thus, only undamaged seeds were used for experiments.

2.2. Seed Treatment with CP, Vacuum, and EMF

In order to determine the optimal duration for seed treatments, pilot experiments were performed to test the effect on germination by using irradiation with cold plasma (CP) for 2, 5, and 7 min, or treatment with radio frequency (RF) EMF for 5, 10, and 15 min. The optimal duration of treatments for seed germination in vitro were 5 min for CP treatment and 15 min for EMF. These durations of treatments were used in the study.

A schematic diagram of the experimental setup for seed treatments with RF EMFs and CP with a device for optical emission spectroscopy (OES) analysis of the plasma species and measurement of the discharge characteristics is presented in Figure 1.

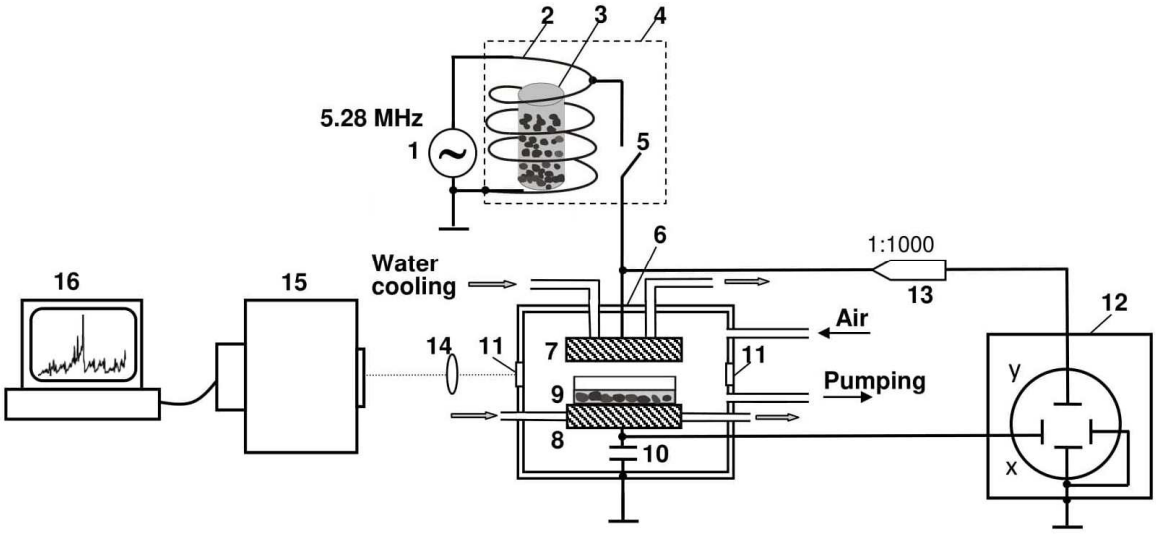

Figure 1. Schematic diagram of the experimental setup. 1 = RF generator, 2 = inductor, 3 = dielectric container with seeds, 4 = screen, 5 = commutator, 6 = vacuum chamber, 7 = powered electrode, 8 = lower electrode, 9 = Petri dish with seeds, 10 = measuring capacitor, 11 = window, 12 = oscilloscope, 13 = voltage probe, 14 = lens, 15 = spectrometer, and 16 = computer.

Seed treatment with radiofrequency (RF) EMF was carried out by placing the dielectric container with seeds in the three-turn, water-cooled coil of the RF generator operating at 5.28 MHz. The characteristics of the EMF strength components in the axial zone of the coil were as described previously [24]. The amplitude values of the magnetic and electric components were 835 A/m ($B \approx 1$ mT) and 17.96 kV/m, respectively. The treatment was performed in ambient air at atmospheric pressure and room temperature for 15 min (this treatment is abbreviated as EMF15).

Low-pressure, capacitively coupled plasma was produced at a frequency of 5.28 MHz in a planar geometry reactor, consisting of two water-cooled copper electrodes (120 mm diameter) placed at a distance of 20 mm from each other in a stainless steel hermetic chamber. The RF voltage was applied to the upper electrode by the commutator (Figure 1). An open, sterile Petri dish with evenly dispersed seeds was placed on a grounded electrode. Seed treatment with CP was performed in open air at a pressure of 200 Pa, and the average value of the input power was 8.4 W (measured as described in [25]). The plasma parameters were as follows. The effective electron temperature $T_e \approx 2.3$ eV, and the electron density $n_e \approx 5 \times 10^8$ cm^{-3}. Before igniting the discharge, the air was pumped from the chamber for about 7 min to reach the working pressure (such seed treatment with a vacuum was used as an additional control for CP treatment). The CP treatment lasted 5 min (this treatment is further abbreviated as CP5).

Optical emission spectroscopy (OES) analysis was used to identify the active species produced in the plasma. Spectra were recorded in a range from 220 to 950 nm by a spectrometer equipped with a CCD (charge coupled device) area image sensor S10141 (Hamamatsu Photonics Norden AB, Sweden). During plasma treatment, molecular bands of nitrogen (second positive system) dominated in the OES spectra (Figure 2), and weak bands of N_2^+ (first negative system) were observed. In the wavelength range of 220–320 nm, a number of oxygen-containing reactive species were recorded, such as nitric oxide and hydroxyl bands. However, the intensity of these molecular bands was relatively low.

All experiments were performed in three replicates. The control, vacuum-, CP-, and EMF-treated seeds were stored at room temperature (19–22 °C) until germination tests.

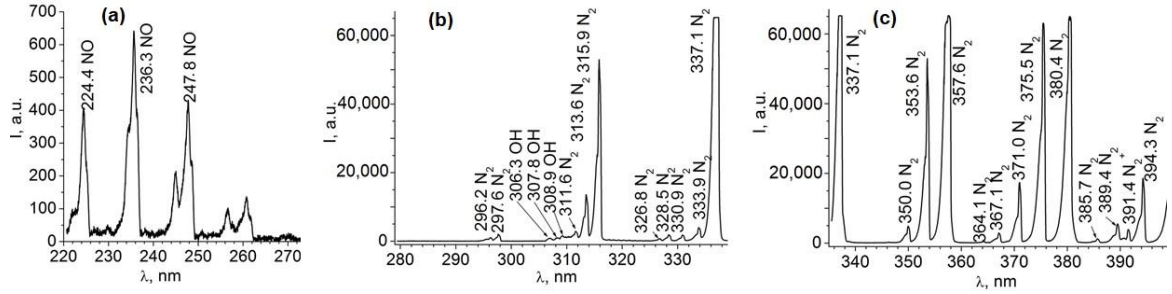

Figure 2. Emission spectra of radio frequency (RF) air plasma in the wavelength range of (**a**) 220–270 nm, (**b**) 280–340 nm, and (**c**) 340–400 nm.

2.3. Seed Germination Tests

In vitro germination tests were started four days after seed treatment with CP, a vacuum, and EMFs. Seeds were distributed on two layers of filter paper in plastic Petri dishes 12 mm in diameter and filled with 5 mL of distilled water. For each experimental group, three replicates of 30 seeds (90 seeds in total) were used for germination testing. Petri dishes with germinating seeds were placed in a climatic chamber with automated control over the light, temperature, and moisture (60%). The following regime of alternating light and temperature was used: in darkness, 14 °C for 10 h, and in light, 21 °C for 14 h. To prevent drying, seeds in the Petri dishes were given additional water, if necessary. Germinated seeds were counted every day until their number stopped increasing.

For the analysis of the germination results of each replicate, Richards' function [26] was used for the analysis of the germinating seed population [27]. For the control and treated seed groups, the following indices of germination kinetics were calculated: the final germination percentage or seed viability (Vi (%)), the median germination time or the germination halftime of a seed lot or germination rate (Me (hours)), and the quartile deviation, or the dispersion of germination time in a seed lot as described earlier in more detail (Qu (hours)) [22,24].

2.4. Plant Cultivation in the Field and Morphometric Analysis

Field experiments were carried out in the central lowland of Lithuania in the Sakiai district (54°94′49″ N, 22°88′50″ E). The soil in the experimental site was Endocalcari-Epihypogleyic Cambisol with a pH of 7.4. For each experimental group, 200 seeds were sown at a distance of 20 cm in rows 20 cm apart, with 25 seeds per row and 8 rows per plot. The size of the basic plot of each experimental group was ~10 m^2 with 1 m of distance between the plots. Herbicides or fertilizers were not applied in the field tests. Four months after sowing, the above-ground part of the plants was cut and used for morphometric analysis. Although industrial hemp variety Futura 75 is considered as monoecious, only a few hermaphroditic plants developed, and it was possible to separate the plants into two groups with different sexual phenotypes: male plants, tiny staminate plants with male flowers (Figure 3a), and female plants, large pistillate plants with female inflorescences (Figure 3b). For the morphometric analysis, at the end of the vegetation season, 30–37 female plants and 20–23 male plants were used. The leaves and inflorescences of the female plants were collected and used to prepare extracts for the estimation of radical scavenging activity and for CBDA and CBD analysis.

The dry matter yield was determined by drying separate plant samples at 70 °C to a constant moisture (at least 72 h). The number of inflorescences per plant was measured by counting these morphological structures for each plant by hand.

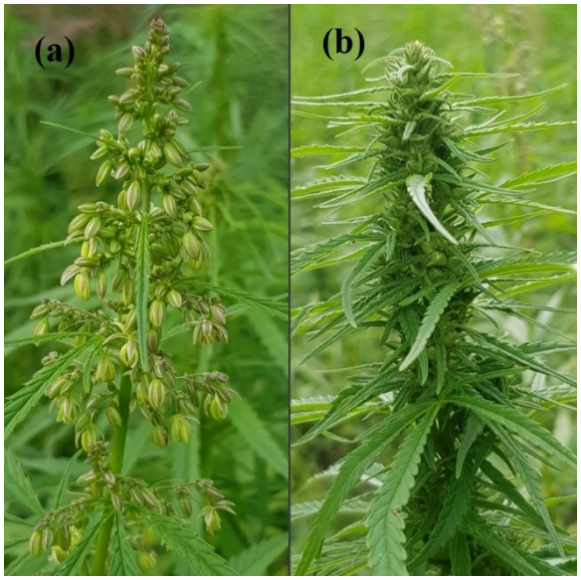

Figure 3. Sexual dimorphism of industrial hemp cv. Futura 75. (**a**) Inflorescences of a staminate male plant. (**b**) Inflorescences of a pistillate female plant.

2.5. Measurement of Radical Scavenging Activity

Radical scavenging activity was determined in methanol extracts prepared from fresh leaves. Ten leaves from the tops of 12 plants from each group were weighed and grinded with ice-cold 85% methanol (using the proportional 10 mL methanol solution for 1 g of leaves). The homogenate was sonicated for 15 min (4 °C) and centrifuged at 13,000× *g* for 10 min. Supernatants were immediately tested for radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [28] as described earlier [22]. Six milligrams of the DPPH radical were dissolved in 200 mL of an acetonitrile-methanol (1:1, *v/v*) solution and mixed with 200 mL of a 0.1 M sodium acetate buffer (pH 5.5). Absorbance was measured at 515 nm using a spectrophotometer. From the sample, 50 µL was added to 1.95 mL of the DPPH buffered solution and left in the dark at an ambient temperature for 15 min. A control sample was prepared using the same procedure, replacing the leaf extract with the same amount of 85% methanol. A calibration curve was obtained using 0.05–0.25 mg/mL rutin solutions. Radical scavenging activity was expressed in milligrams of rutin equivalents per 1 g of dry leaf weight.

2.6. Detection of Cannabinoid Amount

The extracts for the cannabinoid analysis were prepared from dried hemp leaves grinded in a Retsch ZM200 centrifugal high-speed mill (Haan, Germany) using a 0.5 mm sieve. From that, 500 mg of powder were extracted with a mixture of methanol:chloroform (9:1 *v/v*) (UNODC, 2009), using the following procedure: mixing for 10 s in a vortex, then performing extraction for 15 min in an ultrasound bath and vortexing for 10 s after 5, 10, and 15 min. The mixture was centrifuged at 14,000 rpm for 20 min, 100 µL of centrifugate was diluted with 900 µL of methanol, and 100 µL of the solution obtained was diluted with 900 µL of methanol. The latter solution was used for HPLC (high performance liquid chromatography) analysis.

Quantitative determination of the CBD and CBDA contents was performed on an HPLC system, consisting of a Waters 2795 separation module and a 2487 UV detector (Milford, MA, USA). Separation was performed in a 150 µm length column Pro C18, S-3, 12 nm YMC (Kyoto, Japan), 4.0 mm I.D using a CH_3CN/ultra-pure H_2O mobile phase (4:1) with 0.1% formic acid (*v/v*) as a mobile phase. The flow rate was set to 1.0 mL/min and the oven temperature to 30 °C. The mobile phase was continuously degassed with an on-line degasser. The injection volume was 10 µL. Isocratic elution was completed in 20 min.

2.7. Statistical Analysis

Statistica 10 software was used for statistical analysis of the obtained results. Differences between the control and treatment groups were compared using Student's t-tests for independent samples, and results were considered statistically significant at $p \leq 0.05$. The number of repetitions in the experiments is indicated in the legends of the figures and tables.

3. Results

3.1. Effects on In Vitro Germination

The results of in vitro germination tests for the control and treated seeds of industrial hemp cv. Futura 75 are presented in Figure 4.

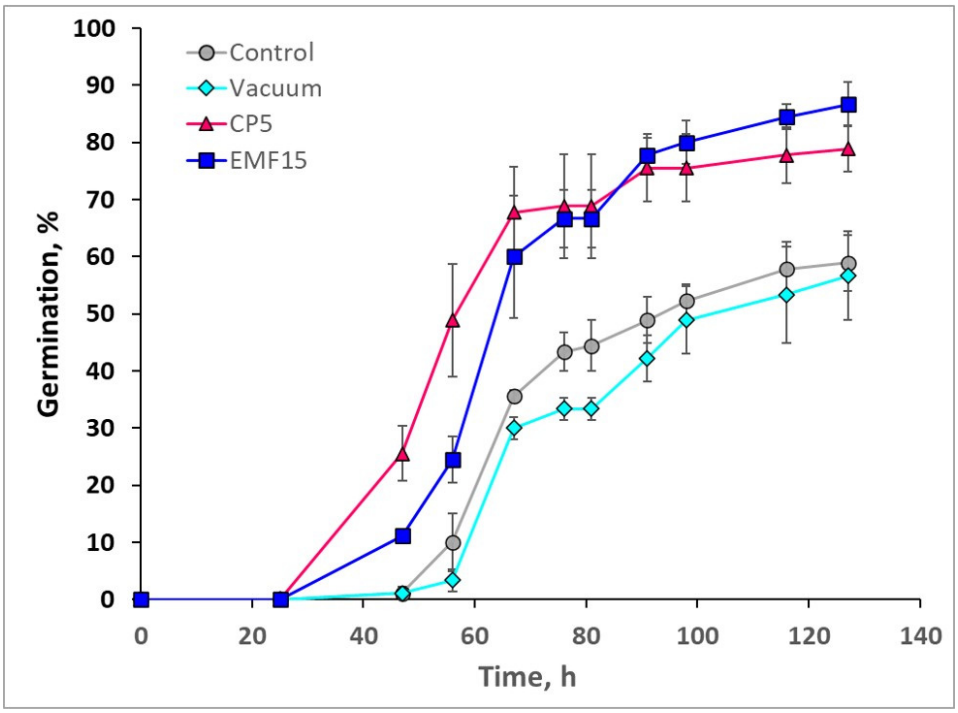

Figure 4. Germination dynamics of the control and treated seeds of industrial hemp. Mean values of the three replicates ± standard error are presented. The number of seeds in each replicate was 30 (n = 3).

The obtained germination curves showed that the germination of the vacuum-treated seeds did not differ from the control, but both the CP5 and EMF15 treatments stimulated germination. Two days after sowing, when the first seeds started to germinate in the control and vacuum-treated groups, the percentage of germinated seeds was 26% in the CP5 group and 11% in the EMF15 group. To quantify the induced changes in germination, indices of germination kinetics Vi, Me, and Qu were calculated for each seed group (Table 1). There were no differences between the vacuum and the control groups in the indices of germination kinetics. However, compared with the control, the maximal germination percentage Vi was 25% higher in the CP5 group and 32% higher in the EMF15 group. CP5 treatment increased the germination rate (reduced Me) by 21%.

Table 1. Indices of the germination kinetics of industrial hemp seeds in the experimental groups.

Treatment	V_i (%)	M_e (Hours)	Q_u (Hours)
Control	58.9 ± 4.8	64.1 ± 1.1	8.6 ± 2.7
Vacuum	56.7 ± 7.7	68.7 ± 3.8	8.6 ± 2.1
CP5	78.9 ± 4.0 *	50.8 ± 2.2 *	9.6 ± 3.1
EMF15	86.7 ± 3.8 *	61.7 ± 3.4	9.0 ± 1.4

V_i = the final germination percentage, M_e = the median germination time, and Q_u = the quartile deviation. Results are presented as mean values ± standard errors. * Significantly different from the control group ($p \leq 0.05$).

The percentage of germinated seeds sown in the field was counted two weeks after sowing, and the obtained results were different from those obtained in vitro. Maximal germination in the control, vacuum, CP5, and EMF15 groups was 72%, 63%, 67% and 66%, respectively. Thus, pre-sowing seed treatments did not stimulate germination of the industrial hemp cultivar Futura 75 in the field.

3.2. Changes in Growth of Female and Male Plants

Four months after sowing in the field, female and male plants were cut, and morphometric analysis of their above-ground parts was performed by measuring the length and weight and counting the number of female inflorescences per plant. The results are presented in Figure 5.

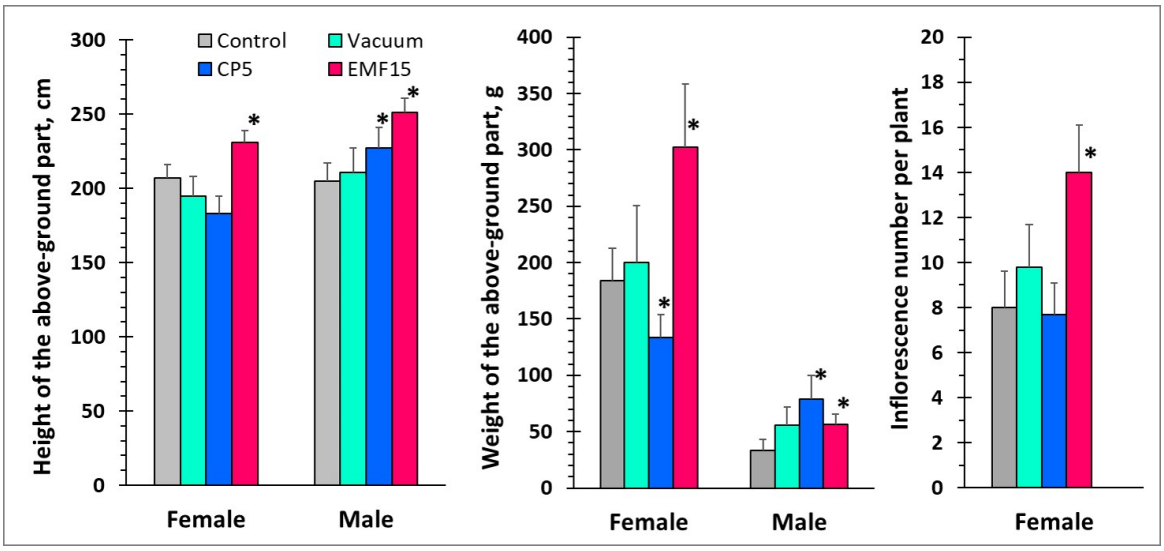

Figure 5. Morphometric parameters of female and male hemp plants four months after sowing and growth in field conditions. The means ± standard errors are presented (n = 20–37). * Significantly different from the control group ($p \leq 0.05$).

In the control, the heights of the above-ground parts of the male and female plants did not differ, but female plants were 5.6 times heavier (Figure 5). Seed treatment with a vacuum did not induce changes in the morphometric parameters of female and male plants. CP5 treatment stimulated the growth of male plants (height increased by 11% and weight 1.4 times greater) and reduced female plant growth (27% lower weight compared with the control). In contrast, EMF15 treatment had a strong positive effect on both male and female plants. In comparison to the controls, the heights of the above-ground parts of the female plants in the EMF15 group increased by 9%, the weight increased by 65%, and the number of inflorescences increased by 70%. EMF15 treatment increased male plant height by 22% and weight by 70% compared with the control.

3.3. Changes in the Content of CBDA (CBD) and in Radical Scavenging Activity

The amount of CBDA and CBD was determined only in the leaves and inflorescences of the female plants, since females accumulate significantly greater cannabinoid content than male plants (predominantly in the inflorescences) and are therefore commonly used for cannabinoid production [27]. This study was focused on CBDA and CBD, because the detected amounts of THC (tetrahydrocannabinol) and other cannabinoids were much lower. The results of the HPLC analysis of CBDA and CBD content in the leaves and inflorescences of female plants are presented in Figure 6a.

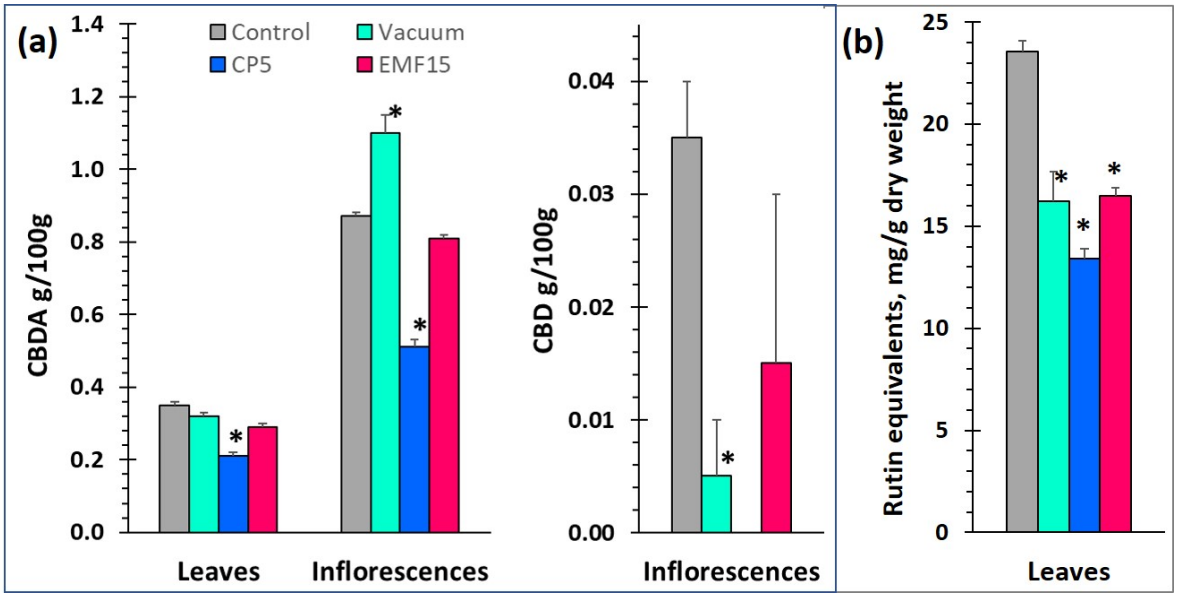

Figure 6. (**a**) The content of cannabidiolic acid (CBDA) and cannabidiol (CBD) in leaves and inflorescences of female plants, and (**b**) the radical scavenging activity of female leaves four months after sowing in the field. The means ± standard errors are presented (n = 10–12). * Significantly different from the control group ($p \leq 0.05$).

The amount of CBDA in the leaves of the hemp growing from vacuum- and EMF15-treated seeds did not differ from the control, but the CBDA in the leaves of the CP5 group decreased by 41%. No CBD was detected in the leaves. The inflorescences of the control plants contained 2.5 times more CBDA compared with the leaves, but the amount of CBD was 10 times lower compared with the amount of CBDA. Seed treatment with a vacuum increased the amount of CBDA by 26% in the inflorescences, but it reduced the amount of CBD by 7 times. The average amount of CBD in the inflorescences of the EMF15 group was more than two times lower compared with that of the control; however, this difference was not statistically significant. There was no CBD found in the inflorescences of the CP5 group. It is assumed that the acidic forms of cannabinoids (e.g., CBDA) are synthesized in hemp tissues, and their neutral forms (such as CBD) originate from the non-enzymatic decarboxylation of corresponding carboxylated forms [18,19], particularly during the extraction process. Since the CBD detected in the extracts was formed from CBDA, the total amount of CBDA + CDB could be regarded as the important marker of changes in cannabinoid production. The amount of CBDA + CBD was 0.91 ± 0.02, 1.11 ± 0.05, 0.51 ± 0.02, and 0.83 ± 0.03 in the extracts of the inflorescences of female plants in the control, vacuum, CP5 and EMF15 groups, respectively. Thus, despite the decrease in the CBD amount, the CBDA + CBD content in the extract of the vacuum group was 22% larger compared with the control ($p < 0.05$). The reason why the extent of CBDA decarboxylation to CBD was decreased in the vacuum group remains to be established.

Antioxidant activity of the female hemp leaf extracts was determined using a DPPH radical scavenging assay, and the results (Figure 6b) showed that it was significantly lower in the leaves of

plants growing from treated seeds compared with the control. DPPH radical binding was reduced by 31%, 43%, and 27% in the vacuum, CP5, and EMF15 groups, respectively.

4. Discussion

Pre-sowing seed treatments with various dormancy-breaking agents are traditionally applied to stimulate crop germination and to increase the uniformity of germination [3,4]. In addition to changes in germination (tested in vitro under laboratory conditions), CP and EMF treatments can decrease seed microbial contamination and exert positive effects on early seedling growth. However, rather moderate effects (5–20%) on early growth have been achieved for the majority of annual crops [5–9]. The application of such innovations in agriculture requires knowledge on how plants respond to such treatments over a longer time scale and under more natural conditions. It is important to estimate if the effects observed on seed germination and growth in the laboratory are followed by changes in plant behavior throughout the entire vegetation cycle while cultivated in the field. A small number of long-term studies on the effects of CP [10,29–32] and EMFs [10,32–36] have been performed, and the results show that the effects of seed treatments on crop growth are retained for the whole vegetation period in most cases. However, for industrial hemp, studies have been limited on the effects of CP on germination and early seedling growth. It has been shown that gliding arc plasma treatment can increase the growth of seedlings (estimated up to 5 days of cultivation) in both cv. Finola and cv. Bialobrzeskie hemp, while seed treatment with CP, generated by a downstream microwave device, inhibited seedling growth [14,15]. It has been reported recently [16] that hemp seed treatment with DBD (dielectric barrier discharge) plasma stimulated 8-day-old seedling growth and enhanced the transcription rates of the transcription factor WRKY1 and four key genes involved in the biosynthesis of cannabinoids in the leaves of 30-day-old seedlings. The expression of olivetolic acid cyclase was increased 42 times, olivetol synthase 19 times, cannabidiolic acid synthase 12.4 times, and Δ9-tetrahydrocannabinolic acid synthase 25.6 times. The latter finding implied that hemp seed treatment with CP may have a strong effect on the biosynthesis of hemp's secondary metabolites, in line with findings reported on other plants [10,21–23]. However, the amount of cannabinoids was not measured in this study [13].

In this study, the effects of seed treatments with three different stressors (CP, a vacuum, and EMFs) on the in vitro germination of industrial hemp cv. Futura 75 were compared, both with the effects on germination in field conditions and on plant growth for the entire vegetation season (4 months). In addition, the amounts of CBDA and CBD, the major cannabinoids biosynthesized by industrial hemp cv. Futura 75 [37,38], in the leaves and inflorescences of female plants were estimated in the control and treated groups.

The obtained results showed that both the CP and EMF treatments increased the maximal germination percentage of hemp seeds, but only the CP treatment increased the rate of germination in vitro under laboratory conditions (Table 1). However, maximal germination in the field was not affected by seed treatments. Thus, the results obtained in the laboratory had low prognostic value concerning the effects on germination in the field. This finding is in line with the observations reported earlier [32,39], suggesting that the effects of seed treatments on germination in vitro and under other conditions (e.g., in the substrate) are different.

Treatment with CP5 significantly improved seed germination kinetics, but had an adverse effect on female plant height, antioxidant leaf activity, and cannabinoid content in the leaves and inflorescences. A decrease in CBD and CBDA content seems to be in apparent contradiction with the reported CP-induced increase in the expression of genes involved in the biosynthesis of cannabinoids [13]. This could be explained by the different types of equipment used (the capacitively coupled low-pressure CP device in this study and the atmospheric DBD plasma device in [13]), as well as the parameters used for seed treatment (e.g., treatment dose) or the dependence of the effects on plant cultivars. All mentioned factors could cause different treatment outcomes (as shown in [14,15]).

Sexual dimorphism was observed in the response of hemp to CP5 treatment, as the same treatment increased the height and weight of the male plants (Figure 5).

A strongly positive effect from the EMF15 treatment on hemp germination and growth was observed. An increase in the germination yield in vitro (by >30%) was followed a substantial increase in the growth of the above-ground part in both female and male plants, as well as the increased number of female inflorescences (by 70%) (Figure 5). Thus, the obtained results indicate that seed treatment via EMFs could be used to increase the bioproduction of industrial hemp. The antioxidant activity of the leaves was negatively affected by EMFs (but to a lesser extent when compared with CP5), and the observed decrease in the amount of CBDA and CBD was not statistically significant. It remains to be established if such changes can affect the defense mechanisms of plants. Taking into account a strong positive effect on the leaf biomass and the number of inflorescences, it might be concluded that the yield of CBD and CBDA produced per plant in the EMF15 group could be accordingly higher when compared with the control.

Seed treatment with a vacuum did not induce changes in seed germination and plant morphometric parameters (Table 1 and Figure 5). However, biochemical analysis revealed a follow-up response of plants to vacuum treatment. Radical scavenging activity in the leaves was reduced, whereas the amount of CBDA was increased and the amount of CBD decreased in the female inflorescences. Such a finding provides an interesting example of a hidden plant response to stress experienced by the seeds, and this has to be studied in more detail in the context of possible applications of seed treatment with a vacuum to increase the amount of biosynthesized CBD in industrial hemp.

Thus, our results show that short (5–15 min) seed exposure to three different physical stressors (CP, a vacuum, and EMFs) resulted in stressor-specific effects on seed germination, plant growth, and secondary metabolism. In comparison with the vacuum or EMFs, low-pressure CP is a more complex stressor consisting of multiple components, such as low pressure, UV radiation, electrical discharge, electromagnetic field, and reactive plasma particles [40]. The performed OES analysis showed that nitric oxide NO· and hydroxyl radical OH· (Figure 2) were the dominating forms of reactive particles produced by the equipment used for hemp seed treatment with CP in this study. These two highly reactive species are also known as universal regulators of signaling processes in plants [41,42] and, together with numerous other ROS (reactive oxygen species) species, may contribute to the stimulation or inhibition of seed germination [43]. However, most studies have attempted to explain the interaction between plasma particles and seeds by the processes that occur on the seed surface or the treated seed coat. The simplest and most popular explanation is that seed germination is stimulated by etching or chemical modifications of the seed coat, followed by an increase in seed hydrophilicity (wettability) and the facilitated penetration of water in a dry seed after imbibition [44–46]. However, other reports do not support this explanation, since germination can be stimulated by CP in the absence of surface etching [24,47]. Moreover, supra-optimal CP doses inhibit seed germination, despite the increased wettability [48].

Numerous reports have been published recently showing that the effects of physical stressors on seed germination are far more complex and involve multiple responses to internal seed processes. Both the CP and EMF treatments increase a seed's EPR (electron paramagnetic resonance) signal [32,49], induce changes in the balance of seed phytohormones involved in the control of germination [23,39,50,51], and modulate ROS production in germinating seeds [32]. These changes have an impact not only on germination and early seedling growth, but also on plant growth and development processes on a longer time scale, including protein expression in seedling leaves [51], photosynthetic activity [39,51], the secondary metabolism [10,16,21–23], and biomass production [10,24]. The intriguing finding is that EMF treatment can induce similar or sometimes even stronger enhancement of agronomic plant properties (e.g., biomass production, as shown for hemp in this study and for red clover in [10]), compared with CP. The similarity of the response to different stressors leads to a hypothesis that plants have developed universal molecular mechanisms in seeds for sensing environmental changes that could be dangerous for the survival of seedlings, as well as for responding to such signals by mobilizing

internal resources and the defensive potential, leading to improved plant fitness and competitiveness (stimulated growth, defense, and reproduction). The complexity of such a response is only beginning to be understood, and detailed knowledge of these mechanisms needs to be gained to apply them in the development of innovative technologies in sustainable agriculture.

5. Conclusions

The performed long-term observations revealed that the pre-sowing treatment of industrial hemp seeds with CP, a vacuum, and EMFs induced stressor-specific changes in important agronomic plant properties, such as germination, biomass production, and secondary metabolite synthesis. Although CP5 and EMF15 treatments positively affected germination in vitro, the germination of treated and control seeds was not different in the field. This shows the limited value of laboratory germination tests for estimating the effects on germination in the field. Furthermore, the effects of treatments on the growth of the above-ground parts of the plants and the secondary metabolism also did not correlate with the effects on germination in vitro. The growth of industrial hemp was affected differently by the stressors used for seed treatment. EMF15 strongly increased (up to 70%) the weight of both the female and male plants and increased the number of female inflorescences, the main part used for the extraction of CBD and other cannabinoids. The molecular mechanisms underlying such strong stimulations of hemp growth by seed treatment with EMFs remain to be elucidated, and we hypothesize that such changes are related to a shift in the balance of growth regulating phytohormones. The observed gender-dependent effects of CP5 treatment on hemp growth (growth stimulation in male plants and inhibition in female plants) also deserve further investigation and point to the potential use of CP for increasing the production of high-quality fiber, since the stems of male plants are valued as a source of fine fiber, whereas crude fiber is made from female stems [52]. Finally, only the vacuum treatment increased the amount of CBDA in female inflorescences, and this result is highly relevant in the context of applied science, since the vacuum has obvious advantages compared with CP and is not only cheaper, but a technically simpler mode of seed treatment.

Author Contributions: Conceptualization, V.M., I.F., and P.R.V., methodology, Z.N., R.Z., and V.L.; investigation, A.I., L.D.-F., A.M., R.Z., Z.N., and P.K.; data curation, A.I. and P.K.; writing—original draft preparation, V.M.; writing—review and editing, V.M., P.R.V., and I.F.; visualization, Z.N.; supervision, V.M., P.R.V., and I.F.; project administration, V.M. and I.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Lithuanian Research Council grant No. S-LB-19-3 and grant No. F19LITG-008 from the Belarusian Republican Foundation for Fundamental Research.

Acknowledgments: We are grateful to agronomist Elvyra Gruzdeviene and the Agrobiotech company for help with the field experiments.

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

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