



Discovering Novel Bioherbicides: The Impact of Hemp-derived Phytocannabinoid Applications on *Zea mays* L. and Relevant Weeds

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Abstract

In addition to competition, phytotoxic plant metabolites contribute to the weed-suppressing properties of cover crops, which could be the basis for the development of novel bioherbicides. We investigated the impact of five *Cannabis sativa* L.-derived neutral phytocannabinoids and an aqueous *C. sativa* tissue extract (HE) at six concentrations on the germination rate (GR) and seedling root length (RL) of *Zea mays* L., two monocotyledonous and two dicotyledonous weed species in laboratory Petri dish bioassays. Additionally, the effect of pre-emergence applications of HE, cannabidiol (CBD), and cannabidivarin (CBDV) formulations on GR and shoot dry matter (SDM) were examined in greenhouse pot studies. The effects of phytocannabinoids and HE were analyzed in dose-response curves. For the highest rates, the effects on GR, RL and SDM were calculated by ANOVA and HSD test ($p < 0.05$). HE exhibited the greatest suppression on GR and RL for all plant species in the Petri dish bioassay, with $R_{GR, RL}$ exceeding -90% . Phytocannabinoids reduced mainly RL of all plants and decreased the GR of most weed species. Effects varied among plants and phytocannabinoids, with CBDV and CBD showing similar high inhibitory effects on RL as HE in the Petri dish bioassay. All pre-emergence applications resulted in a positive R_{GR} across all studied plants and in a positive R_{SDM} in *Z. mays* and *Echinochloa crus-galli* (L.) P. Beauv., whereas in the other weed species the R_{SDM} was negative. In conclusion, phytocannabinoids play a major role in weed suppression of HEs. CBDV and CBD are the most promising candidates for bioherbicide development especially against annual dicotyledonous weed species.

Keywords Neutral phytocannabinoids · Aqueous *Cannabis sativa* tissue extracts · Bioherbicide · Petri dish bioassay · Pre-emergence application

Introduction

Hemp (*Cannabis sativa* L.) has a strong ability to suppress weeds by competition and allelopathy (Boulloc et al. 2013; Osipitan et al. 2019). Cover cropping with *C. sativa* can be a successful tactic in integrated weed management and offering numerous benefits for sustainable cropping systems (Sturm et al. 2018). These benefits include the reduction of soil erosion and nitrate leaching (Weinert et al. 2002; Hartwig and Ammon 2002), enhancement of soil fertility, improvement of soil structure, stimulation of microbiological activity, promotion of biodiversity (Wilson et al. 1982; Mendes et al. 1999), and suppression of weeds (Kunz et al.

2016) and pathogens (Wen et al. 2017). The weed-suppressive effect of cover crops (CCs) during their vegetation period is attributed to their rapid emergence (Brust et al. 2011), quick canopy closure (Hiltbrunner et al. 2007), high biomass production (Osipitan et al. 2019), strong competitiveness for water, nutrients, light, and space, and additionally through the production, accumulation, and release of allelochemicals (Belz 2007). Allelochemicals are secondary metabolites that can be released actively through root excretion and volatilization from plant foliage (Weston 1996) or passively enter the soil via leaching from shoot tissues and the decomposition of plant residues (Caamal-Maldonado et al. 2001; Kunz et al. 2016). CCs from several plant families have been found to produce phytotoxic allelochemicals (Table 1).

Additionally, allelochemicals can be the basis for novel herbicidal active substances with potential new modes of action for the development of bioherbicides, all while exhibiting lower toxicity and a shorter environmental

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Table 1 Several identified allelopathic chemical compounds in cover crop (CC) species with their associated plant family

CC family	CC species	Identified allelochemicals	Reference
Asteraceae	<i>Helianthus annuus</i> L.	Phenolic acids, flavonoids, terpenoids	Alsaadawi et al. (2012)
Brassicaceae	<i>Brassica napus</i> L. <i>Brassica oleracea</i> L.	Glucosinolates, flavonoids	Velasco et al. (2011)
Boraginaceae	<i>Phacelia tanacetifolia</i> Benth	Phenolic acids, flavonoids	Puig et al. (2021)
Cannabaceae	<i>Cannabis sativa</i> L.	Phytocannabinoids, terpenes	Shikanai and Gage (2022)
Poaceae	<i>Avena strigosa</i> Schreb. <i>Avena sativa</i> L. <i>Secale cereale</i> L. <i>Sorghum bicolor</i> L.	Phenolic acids, saponins, benzoxazinoids, benzoquinones	Reginatto et al. (2020); Trojanowska et al. (2000); Belz (2007); Baerson et al. 2008
Polygonaceae	<i>Fagopyrum tataricum</i> (L.) Gaertn	Phenolic acids, flavonoids	Uddin et al. (2012)

persistence (Bailey 2014). As society and EU policies advocate for reduced pesticide usage, stricter approval criteria for new herbicidal agents, and lower maximum pesticide residue thresholds, cover crops and their allelochemicals are gaining increasing importance as integral components of weed management (Kristoffersen et al. 2008; Hillocks 2012). Furthermore, approximately 267 weed species worldwide have developed resistance to herbicides, making the discovery of new allelochemicals with strong phytotoxic effects a necessity (Heap 2023). One group of substances that has not yet been studied for their phytotoxic effect, and represents potential candidates for the development of bioherbicides, are phytocannabinoids, which are secondary plant metabolites found naturally in the plant genus *Cannabis* (Cannabaceae), *Rhododendron* (Ericaceae), *Helichrysum* (Asteraceae), *Glycyrrhiza* (Fabaceae), *Amorpha* (Fabaceae) and *Radula* (Radulaceae) (Gülck and Möller 2020). Many phytocannabinoids are found in the cash and cover crop *C. sativa* (>200 identified phytocannabinoids) (Hesami et al. 2022) and are worldwide currently in high demand for medicinal, cosmetic, and lifestyle products (UN 2022), resulting in a global increase in *C. sativa* cultivation (approximately 33,000 ha with a yield of 179,000 t solely in Europe in 2022) (European Commission 2023). The global increase is also driven by the growing demand of the food, textile, and construction industries for *C. sativa* seeds and fibers, for which *C. sativa* has a long-standing tradition of cultivation (Johnson 2014). The phytocannabinoids in *C. sativa*, which are terpeno-phenolic compounds, are biosynthesized and primarily accumulated in glandular trichomes, predominantly distributed in the flowers, leaves, and bracts (Turner et al. 1978; Livingston et al. 2019). However, low levels of phytocannabinoids can also be found in other plant parts of *C. sativa* including roots, stem seeds and pollen (Ross et al. 2000, 2005; Cappelletto et al. 2001; Stout et al. 2012). In *C. sativa*, the two phytocannabinoid precursors cannabigerolic acid (CBGA) and cannabigerovarinic acid (CBGVA) are synthesised through the reaction of geranyl

pyrophosphate with either olivetolic acid (for CBGA) or with divarinolic acid (for CBGVA) (Thomas and ElSohly 2015). CBGA undergoes further biosynthesis resulting in various acidic forms of C₂₁ phytocannabinoids such as cannabichromenic acid (CBCA), cannabidiolic acid (CBDA), and tetrahydrocannabinolic acid (THCA), which are primarily found in *C. sativa* beside CBGA (Thomas and ElSohly 2015). Neutral phytocannabinoids are then formed by acidic cannabinoid decarboxylation, which mainly involves drying, storage or thermal processing where the carboxyl group is removed from the acidic phytocannabinoids (Filer 2022). Decarboxylation mainly takes place after harvest of the plants, nevertheless neutral phytocannabinoids can also be found in small concentrations in several plant parts during the flowering phase (Sánchez-Carnerero Callado et al. 2018). Phytocannabinoids fulfill several functions within the plant, as they protect against UV radiation, desiccation, and act as plant defense compounds (Gülck and Möller 2020). Neutral phytocannabinoids have antimicrobial effects against fungi and bacteria (Russo 2019), have harmful effects on various arthropods (Mantzoukas et al. 2020a, b; Park et al. 2019), and show phytotoxicity in plant tissue cultures in vitro (Sirikantaramas et al. 2005). Nevertheless, the impact of neutral phytocannabinoids on weeds and their potential as bioherbicides have not yet been investigated. Aqueous *C. sativa* tissue extracts (HEs), on the other hand, have already successfully inhibited germination and growth of cash crops and weeds in several studies (Patanè et al. 2023; Sturm et al. 2016). The present study focuses on the phytotoxic potential of different applied hemp-derived neutral phytocannabinoid isolates and HEs on the cash crop *Zea mays* L. and two monocotyledonous and two dicotyledonous weed species in laboratory Petri dish bioassays and additionally in pre-emergent application trials in the greenhouse. It was also tested if the impact depends on the concentration of the compounds. The following four hypotheses were tested: (i) Weeds are more sensitive to the application of HE and phytocannabinoid formulations than *Z. mays*, (ii) phyto-

cannabinoids vary in phytotoxicity, (iii) the application of HEs shows higher phytotoxicity than the phytocannabinoid formulations, (iv) phytotoxicity increases with the rate of HEs and phytocannabinoid formulations.

This study gives first insights about the impact of phytocannabinoid applications on *Z. mays* and relevant weeds and their potential use in integrated weed management.

Material and Methods

Laboratory (experiment 1) trials were conducted at the University of Hohenheim (Stuttgart, Germany) in 2021 and 2022 to evaluate the biochemical effects of the five different hemp-derived neutral phytocannabinoid isolates (Fig. 1, see description below) cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabivarin (CBDV) (Pharmabiond, Wageningen, Netherlands) with a purity of >98% and aqueous *C. sativa* tissue extract (HE) in six different concentrations (including controls with 0%) on the germination and root growth of *Z. mays*, the monocotyledonous weed species *Alopecurus myosuroides* Huds. and *Echinochloa crus-galli* (L.) P. Beauv. and the dicotyledonous weed species *Chenopodium album* L. and *Stellaria media* (L.) Vill.. Additionally, pre-emergence trials were conducted in the greenhouse (experiment 2) with HE and the neutral phytocannabinoids CBD and CBDV with the same six concentrations to investigate the germination and biomass production of *Z. mays* and the relevant weeds.

A completely randomized design with one factor and four repetitions was chosen for experiment 1 and a completely randomized block design (RCBD) with four blocks (repetitions) was selected for experiment 2. All experiments were additionally repeated for two years. The weed species investigated were selected due to their global economic relevance and dominant occurrence in *Z. mays* cultivation. Due to the complex and expensive extraction of phytocannabinoid isolates from the *C. sativa* biomass by using specialized chemical equipment for the fractionation of the *C. sativa* extract, the phytocannabinoid isolates used in the study were purchased. Nevertheless, the phytocannabinoid isolates offered by the company derived from *C. sativa* biomass.

Experimental Preparations

For all experiments, the shoot biomass, required for HEs, was taken from greenhouse-grown *C. sativa* plants (Monoecious fiber-type (industrial) hemp genotype: Futura 75). The *C. sativa* plants were cultivated, according to Sturm et al. (2016), with a seed density of 45 kg ha⁻¹ for 10 weeks in 5 L plastic pots with a soil mixture consisting of 50%

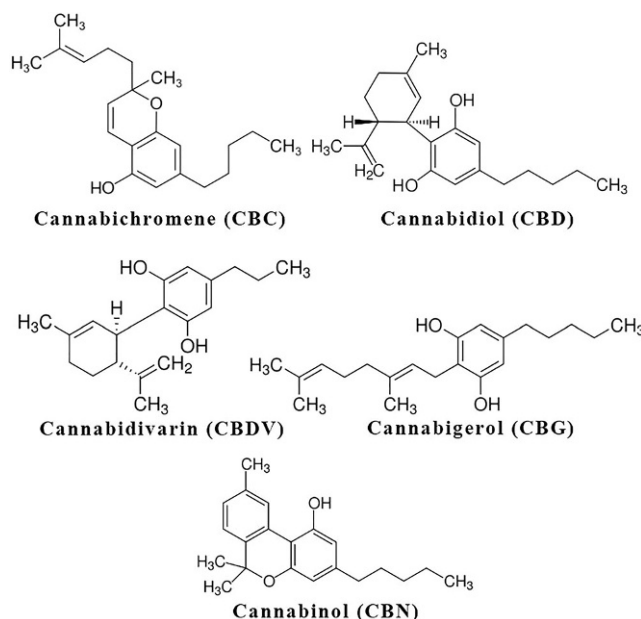


Fig. 1 Chemical structure of the investigated neutral phytocannabinoids cannabichromene, cannabidiol, cannabivarin, cannabigerol, and cannabinol

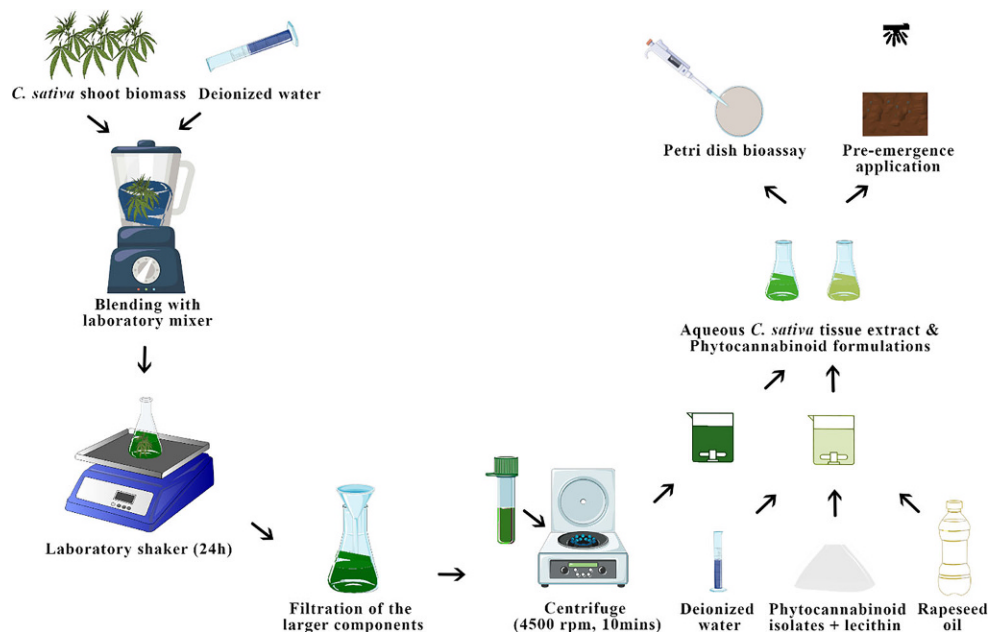
steam-sterilized compost, 25% clay and 25% sand. From the time of sowing, the plants were fertilized with a NPK fertilizer (14-7-17) with 2 g per pot in a two-week cycle and irrigated on a daily basis. Furthermore, the cover crops were additionally supplied with light from sodium vapor lamps (40,000 lm) for a 12 h photoperiod at a temperature of 22/15 °C day/night. The harvested aboveground shoot biomass was stored in the freezer (−20 °C) until further aqueous tissues extract processing two weeks after harvesting.

For the preparation of the HEs, the frozen hemp shoot biomass including leaves, flowers, and stem was crushed with a blender under the addition of deionized water (dH₂O) to generate a stock solution of 500 mg biomass per ml dH₂O. After the agitation with an orbital laboratory shaker for 24 h, the coarse particles were sieved off, then the stock solution was vacuum filtered through a nylon filter using a Büchner funnel (Ø 1.2 mm) and finally centrifuged (4500 rpm, 10 min) (Fig. 2).

Experiment 1: Petri Dish Bioassays with Phytocannabinoid Solutions and Aqueous *Cannabis sativa* Tissue Extracts

From the obtained stock solution, a dilution series with six concentrations of 0 (0%) 31.25 (6.25%), 62.5 (12.5%), 125 (25%), 250 (50%) and 500 (100%) mg ml⁻¹ were prepared by adding dH₂O. Due to the hydrophobic properties of the neutral phytocannabinoids, pure ethanol was used to prepare the stock solution (100%: 2 mg ml⁻¹). To create

Fig. 2 Preparation steps of aqueous *Cannabis sativa* tissue extracts for all trials and preparation of the phytocannabinoid formulations for the greenhouse trial



the neutral phytocannabinoid dilution series, six concentrations of 0 (0%, control), 0.125 (6.25%), 0.25 (12.5%), 0.5 (25%), 1 (50%) and 2 (100%) mg ml⁻¹ were prepared by adding pure ethanol. After mixing with the magnetic stirrer, 1 ml of each of the prepared concentrations was applied per petri dish (Ø 60 mm, Sarstedt AG & Co, Nümbrecht, Germany), which were equipped with filter paper (Macherey-Nagel, Düren, Germany) and placed under the fume hood for 30 min to volatilize the ethanol. In the process, only the phytocannabinoids remained on the filter. For the preparation of the control (0%) 1 ml of pure ethanol was applied, which vaporized subsequently. Either four *Z. mays* seeds or 20 weed seeds per each selected weed species were placed separately on the filter paper in the Petri dishes. For the neutral phytocannabinoid treatments (+ control), 3 ml of dH₂O and in the case of the HE treatments, 3 ml of the HEs were applied to each Petri dish, sealed (Parafilm® M, Sigma-Aldrich, Munich, Germany) and positioned in the climatic chamber (KBF 720, Binder GmbH, Tuttlingen, Germany). The temperatures during the trial were 20 °C during the day and 15 °C at night with a daily 12 h of artificial lighting by LEDs (15,000 lx). The experiment was conducted with four replicates. Ten days after the application, the root length and germination rate were measured. Seeds with a root length of ≥ 2 mm were measured and classified as germinated.

Experiment 2: Pre-emergence Application of Phytocannabinoid Formulations and Aqueous *Cannabis sativa* Tissue Extracts

In the pre-emergence trial, only HE and the neutral phytocannabinoids CBD and CBDV were investigated in comparison to the Petri dish bioassay, as they showed the high-

est suppression potential when tested in the laboratory. In contrast to experiment 1, for the preparation of the neutral phytocannabinoid stock solutions, the lipophilic CBD and CBDV isolates (2 mg ml⁻¹) were first dissolved in rapeseed oil (60 µl per ml stock solution) with a magnetic stirrer and then dH₂O and lecithin (10 mg per ml stock solution) were added under constant stirring (Fig. 2). With the help of the emulsifier, the water-fat-phytocannabinoid mixtures were homogenized. Subsequently, a phytocannabinoid solution series with six concentrations 0 (0%, control), 0.125 (6.25%), 0.25 (12.5%), 0.5 (25%), 1 (50%) and 2 (100%) mg ml⁻¹ was prepared by adding dH₂O. HEs and the respective dilutions series with six concentrations were prepared as in experiment 1 (Fig. 2).

The controls (0%) were formulations consisting of rapeseed oil (60 µl ml⁻¹), lecithin (10 mg ml⁻¹) (Sigma Aldrich, Steinheim, Germany) and dH₂O. For the pre-emergence trial, plastic pots (12 × 12 cm) were first filled with the Hohenheim soil, four *Z. mays* seeds or 20 weed seeds were sown per pot, covered with soil and irrigated with tap water. Immediately after sowing and irrigation, the formulations were applied with an automatic spray chamber (Schachtner, Ludwigsburg, Germany) (2001 ha⁻¹; speed: 788 mm s⁻¹; pressure: 3.6 bar). The spray rate of the nozzle during application was 0.8 L/min⁻¹ with a pressure of 3 bar and an application height of 900 mm. Starting from the second day after application, the irrigation of the pots with tap water was done daily, and no fertilizers were applied. Pots were investigated 21 days after application to determine the germination rate, then the shoot biomass was harvested, dried in paper boxes in a drying oven (80 °C, 48 h) and then weighed with a precision scale. To determine the dry matter

of the individual plants, the total dry matter was weighed and calculated for a single plant.

Total Phenolic Content (TPC) Determination of Aqueous *Cannabis sativa* Tissue Extracts

For the determination of the total phenolic content (TPC) of the investigated HEs (0.5 g fresh shoot biomass/ml deionized water) the Folin-Ciocalteu-Micro method described by Slinkard and Singleton (1977) was used. For the analysis, the required gallic acid stock solution (0.5 g of dry gallic acid per 10 ml ethanol) and the sodium carbonate solution (200 g anhydrous carbonate solved in 800 ml of autoclaved water) were first prepared. Then the aliquots (20 µL) of the HEs were mixed with 1.58 ml of autoclaved water and 100 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany). Subsequently, 300 µL of sodium carbonate solution was added after 8 min and homogenized. The final mixture was then incubated at 37 °C for 35 min. A spectrophotometer (PM6, Analysen Technik Graete, Germany) at 760 nm was used to measure the absorbance. A gallic acid calibration curve (0, 50, 100, 150, 200, 300, 400 µg mL⁻¹) ($R^2=0.9612$) was prepared and used for the comparison with the measured values. Findings were presented in mg gallic acid equivalent (GAE) g⁻¹ fresh weight of *C. sativa* shoot biomass and performed in triplicate.

Statistical Analysis

R Studio (version 2022.07.2+576, RStudio Team, Boston, MA, USA) with ‘lme4’, ‘nlme’, ‘lmerTest’, and ‘emmeans’ packages was used for the statistical data analysis and Origin PRO (Origin Lab Corporation, Northampton, MA, USA) for the preparation of the graphs (+ for the non-linear regression). To create the image for the test procedure, the open-source editing program GIMP (GIMP 2.10, The GIMP Team, USA) was used. For the statistical analysis of the data collected in the laboratory, a one-factorial analysis of variance (ANOVA) followed by a post-hoc TukeyHSD test at a significance level of $p < 0.05$ was performed. For the greenhouse experiment a two-factorial ANOVA containing the Kenward-Roger method (Kenward and Roger 1997) was chosen to determine p -values in the two factors (Factor 1: Phytocannabinoid and HE treatment; Factor 2: Investigated plants) and in the interaction between these two factors. Afterwards, post-hoc tests with Tukey adjustments for pairwise mean comparison were performed using the ‘emmeans’ R-package to identify significant differences ($p < 0.05$) between treatments. Data from the two experiments were tested visually for variance homogeneity with q-q plots, followed by the Levene’s test and for normal distribution the Shapiro-Wilk test was done. In the case of variance heterogeneity and non-normally distributed data,

the outliers were removed and, if necessary, the data were transformed by log transformation or the square root to create the conditions for ANOVA. Treatment values with a difference of more than 5% ($p < 0.05$) were considered statistically significant.

For nonlinear regression, Streibig’s (1980) four parameter log-logistic model was used:

$$y = \frac{k}{1 + ax^b} + d$$

y = average response to the dose x ; x = phytocannabinoid and aqueous *C. sativa* tissue extract dose; a and b = determine the way the yield decreases with dose parameters; d = lower asymptote; k = upper asymptote.

When hormesis did occur, Brain and Cousens’ (1989) modified model was used:

$$y = \frac{k + fx}{1 + e^{bgx^b}} + d$$

For the analysis of the greenhouse data, the following statistical linear mixed model was used:

$$y_{iju} = \mu + \alpha_i + \beta_j + (a\beta)_{ij} + \rho_u + \varepsilon_{iju}$$

y_{iju} = the result (germination rate or shoot dry matter) of phytocannabinoid or aqueous *C. sativa* tissue extract treatment i in plant j at block u

μ = baseline mean; ρ_u = block effect of block u ; α_i = fixed effects of aqueous *C. sativa* tissue extract and phytocannabinoid treatments i ; β_j = fixed effect of *Z. mays* and the weed species; $(a\beta)_{ij}$ = effect of the interaction between treatment i and plant j ; ε_{iju} = residual error of the observation

Since the highest concentration of applied HEs and phytocannabinoids achieved either the strongest stimulating effect or the greatest suppressing effect, the highest applied concentration (100%) was selected for the calculation of the response (R) of the plants and seeds to obtain information about the effect of the treatments in relation to the measured values in the untreated controls. The formula described by Rasmussen (1991) for the calculation of the weed control efficacy was used as the basic and modified for the calculation of the responses regarding the germination rates, root lengths or the shoot dry matters. Positive R values show stimulatory effects, while negative R values represent suppressive effects of the treatments.

$$R_{GR,RL,SDM}(\%) = -1 \left(100 - \omega_t(0.01x\omega_c)^{-1} \right)$$

ω_t corresponds to the germination rate, root length and shoot dry matter of the respective phytocannabinoid and aque-

ous *C. sativa* tissue extract treatments and ω_c is the germination rate, root length or shoot dry matter of the untreated control.

Results

Laboratory Petri Dish Bioassays by the Application of Aqueous *Cannabis sativa* Tissue Extracts and Phytocannabinoids On Seeds of *Zea mays* and Relevant Weeds

Influence On the Germination Rate

The application of aqueous *C. sativa* tissue extract (HE) at the highest concentration resulted in greater suppression of the germination rate (higher negative R_{GR}) in all investigated plants (crop and weed species) compared to the respective phytocannabinoids (Table 2).

Zea Mays A significantly reduced germination rate of *Z. mays* was measured solely through the application of HE in the highest concentration (500 mg ml⁻¹) compared to the control (Fig. 3).

Alopecurus Myosuroides All applied phytocannabinoids resulted in a positive R_{GR} showing stimulating effects on the germination rate of *A. myosuroides*. However, the application of CBN and CBG resulted in 1.3–5.7 times significantly higher positive R_{GR} compared to the other phytocannabinoids (Table 2). Compared to the control, the germination rate of *A. myosuroides* was significantly increased by the application of HE at a low concentration of 32.5 mg ml⁻¹, while significant decreases compared to the aqueous control occurred above a concentration of 250 mg ml⁻¹ (Fig. 3).

Echinochloa Crus-galli Application of all phytocannabinoids resulted in a negative R_{GR} , with the exception of CBD, where a significant stimulating effect on germination rate

of *E. crus-galli* was measured with a positive R_{GR} (Table 2). For HE concentrations of 250 mg ml⁻¹ and higher, *E. crus-galli* showed significantly reductions in the germination rate (Fig. 3).

Chenopodium Album Although, in *C. album*, application of all phytocannabinoids in the highest concentration resulted in negative R_{GR} , the application of CBD showed a 1.4 and 1.5 times significantly stronger suppression compared to CBC and CBN (Table 2). All concentrations of the HE significantly decreased the germination rate of *C. album* (Fig. 3).

Stellaria Media As in *E. crus-galli*, the application of all phytocannabinoids reduced the germination rate in *S. media*, which was reflected by a negative R_{GR} except for the application of CBD, which resulted in a positive R_{GR} , indicating an increased germination rate (Table 2). Furthermore, CBG and CBN showed a 3.8–5.6 times significantly higher suppression of the germination compared to CBC and CBDV. With an HE concentration of 125 mg ml⁻¹ and higher, the germination rate was significantly decreased (Fig. 3).

Impact On the Root Length

The application of HE in the highest concentration resulted in the strongest suppression of root growth by showing the highest negative R_{RL} in *Z. mays* and all weed species examined, while the application of CBDV resulted in the second highest negative R_{RL} and the application of CBD in the third highest negative R_{RL} (Table 3).

Zea Mays All applied phytocannabinoid formulations and HE at the highest concentration had a suppressive effect on root growth, while the application of the CBG formulation resulted in a significantly different positive R_{RL} , which was shown by an increased root length (Table 3). While the lower concentration of 62.5 mg ml⁻¹ significantly increased

Table 2 Responses based on germination rate (R_{GR}) in % of *Zea mays*, *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Chenopodium album* and *Stellaria media* with standard deviation after the application of the phytocannabinoids cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabigerol (CBG), and cannabitol (CBN) concentrated with 2 mg ml⁻¹ or aqueous *Cannabis sativa* tissue extract (HE) using a concentration of 500 mg ml⁻¹. Positive values show stimulating effects on germination, while negative values represent suppressive effects. Means with the same small letter within the *Zea mays* or the weed species are not significantly different regarding the post hoc test ($p < 0.05$)

Formulation	<i>Z. mays</i>	<i>A. myosuroides</i>	<i>E. crus-galli</i>	<i>C. album</i>	<i>S. media</i>
HE	-34.4 ± 6.2b	-76.2 ± 8.6a	-95.6 ± 8.2c	-91.0 ± 3.3d	-89.7 ± 2.6d
CBC	0 ± 0a	24.9 ± 7.0c	-36.7 ± 6.4b	-38.3 ± 5.9ab	-6.7 ± 3.1b
CBD	0 ± 0a	9.7 ± 5.6b	79.5 ± 7.3a	-53.4 ± 4.3c	15.7 ± 5.5a
CBDV	0 ± 0a	34.3 ± 8.7c	-39.9 ± 7.3b	-45.9 ± 7.4bc	-6.4 ± 3.7b
CBG	0 ± 0a	46.0 ± 4.8d	-44.6 ± 2.2b	-45.1 ± 6.0bc	-25.4 ± 6.0c
CBN	0 ± 0a	55.2 ± 6.4d	-35.7 ± 7.3b	-36.1 ± 5.8a	-36.1 ± 5.8c
<i>p</i> value	<0.001	<0.001	<0.001	<0.001	<0.001

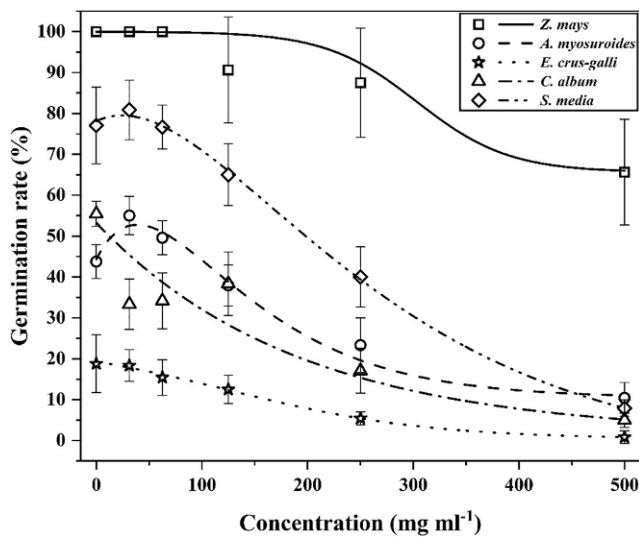


Fig. 3 Dose-response showing the mean germination rate (%) of *Zea mays*, *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Chenopodium album* and *Stellaria media* with standard deviation in relation to the six aqueous *Cannabis sativa* tissue extract concentrations ranging from 0–500 mg/ml⁻¹. For the non-linear regressions, the log-logistic growth model of Streibig (1980) and for hormesis the modified one of Brain and Cousens (1989) were used. Means of the germination rate were proofed for significant differences using the TukeyHSD post hoc test ($p < 0.05$)

root length of *Z. mays* by 8% compared to the control, concentrations of 250 mg ml⁻¹ (–48%) or higher led to, in comparison to the control, significantly reduced root lengths (Fig. 4a). In contrast, the application of CBD (Fig. 4b) and CBDV (Fig. 4c) at concentrations of 0.25 mg ml⁻¹ and 0.5 mg ml⁻¹ (–30% and –25%) and higher significantly reduced root length of *Z. mays* compared to the control.

Alopecurus Myosuroides The application of all neutral phytocannabinoids resulted in negative R_{RL} , while the application of CBN showing a 4.4–12.2 times significantly lower suppression of root growth compared to the other phytocannabinoids (Table 3). Furthermore, the application of HE at a concentration of 62.5 mg ml⁻¹ and higher led to a sig-

nificant reduction in root length (–17%) compared to the control (Fig. 4a). Whereas the root lengths of *A. myosuroides* were significantly reduced compared to the control by the application of CBD (Fig. 4b) and CBDV (Fig. 4c) at concentrations of 0.125 mg ml⁻¹ (–20% and –86%) and higher.

Echinochloa Crus-galli Similar to *A. myosuroides*, all applied neutral phytocannabinoids led to negative R_{RL} , while the application of CBN exhibited a 1.9–5.9 times significantly lower suppression of root growth compared to the other phytocannabinoids (Table 3). Applying HE resulted in significantly reduced root length for concentrations of 125 mg ml⁻¹ (–72%) and higher (Fig. 4a) compared to the control. Furthermore, even the lowest applied concentration of CBD (Fig. 4b) and CBDV (Fig. 4c) significantly reduced the root length of *E. crus-galli* by –47% and –89%, respectively, compared to the control.

Chenopodium Album The application of CBN resulted in a positive R_{RL} , indicating increased root length in *C. album*, which significantly differs from the other applied neutral phytocannabinoids that suppressed root growth (Table 3). All concentrations of the applied HEs significantly decreased root length of *C. album* compared to the control, while the lowest concentration of 31.25 mg ml⁻¹ already showed a reduction in root length of –33% (Fig. 4a). In contrast, the application of even the lowest concentrations (0.125 mg ml⁻¹) of CBD (Fig. 4b) and CBDV (Fig. 4c) significantly reduced root length of *C. album* by –49% and –84%, respectively, compared to the control.

Stellaria Media Similar to *C. album*, the application of CBN at the highest concentration resulted in a positive R_{RL} , while all other applied neutral phytocannabinoids resulted in significantly different negative R_{RL} , representing a suppression of root growth (Table 3). From 62.5 mg ml⁻¹ (–45%) onwards, all concentrations of the applied HEs showed a significant reduction in root length in *S. media* (Fig. 4a). In

Table 3 Plant response (R_{RL}) in % based on root length of *Zea mays*, *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Chenopodium album* and *Stellaria media* with standard deviation after the application of the phytocannabinoids cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabigerol (CBG), and cannabiol (CBN) concentrated with 2 mg ml⁻¹ or aqueous *Cannabis sativa* tissue extract (HE) application using a concentration of 500 mg ml⁻¹. Positive values show stimulating effects on root growth, while negative values represent suppressive effects. With regard to TukeyHSD ($p < 0.05$) means with the same letter within the *Zea mays* or the weed species showing no significant differences

Solution	<i>Z. mays</i>	<i>A. myosuroides</i>	<i>E. crus-galli</i>	<i>C. album</i>	<i>S. media</i>
HE	–61.4 ± 5.1d	–93.6 ± 2.0e	–95.9 ± 4.4d	–91.9 ± 2.1e	–92.9 ± 2.2d
CBC	–35.0 ± 6.7c	–33.4 ± 5.2b	–29.4 ± 7.2b	–45.3 ± 5.0c	–20.4 ± 9.2b
CBD	–39.1 ± 8.0c	–56.7 ± 5.8d	–79.7 ± 7.8c	–75.0 ± 2.5d	–68.0 ± 4.4c
CBDV	–52.7 ± 4.8d	–91.7 ± 0.9e	–90.0 ± 2.4d	–90.0 ± 1.7e	–76.1 ± 3.1c
CBG	28.7 ± 7.4a	–47.9 ± 8.0c	–34.4 ± 7.6b	–32.9 ± 4.3b	–23.8 ± 9.4b
CBN	–9.4 ± 6.1b	–7.5 ± 5.1a	–15.3 ± 4.2a	10.9 ± 2.4a	23.8 ± 5.5a
<i>p</i> value	<0.001	<0.001	<0.001	<0.001	<0.001

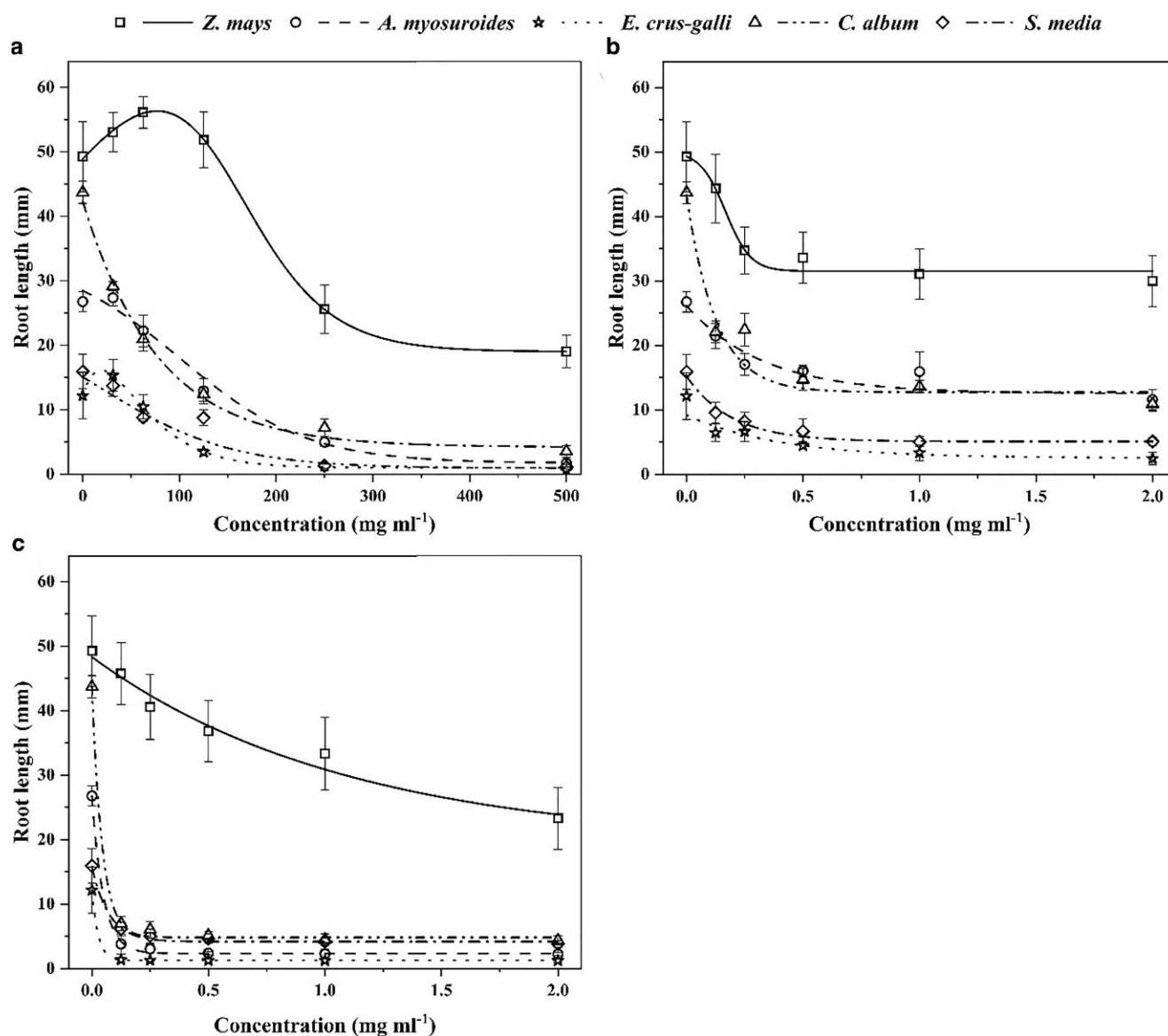


Fig. 4 Dose-response diagram showing the mean root length (mm) of *Zea mays*, *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Chenopodium album* and *Stellaria media* with standard deviation in relation to the applied six aqueous *Cannabis sativa* tissue extract (HE) (a) concentrations ranging from 0 mg ml⁻¹–500 mg ml⁻¹ and six cannabidiol (CBD) (b) and cannabidivarin (CBDV) (c) concentrations ranging from 0 mg ml⁻¹–2 mg ml⁻¹ 10 days after the application. For the non-linear regressions, the log-logistic growth model of Streibig (1980) and for hormesis the modified one of Brain and Cousens (1989) were used. Means of the germination rate were proofed for significant differences using the TukeyHSD post hoc test ($p < 0.05$)

contrast, applications of CBD (Fig. 4b) and CBDV (Fig. 4c) starting from the lowest concentration (0.125 mg ml⁻¹) exhibited a significant suppressive effect on root length compared to the control of –40% and –62%, respectively.

Pre-emergence Trials by the Application of the Phytocannabinoids Cannabidiol and Cannabidivarin, and Aqueous *Cannabis sativa* Tissue Extracts On *Zea mays* and Relevant Weeds

In the pre-emergence trials, the ‘treatment’ factor showed significant differences in R_{SDM} , while the ‘plant’ factor and the interaction of the two factors showed significant differences in R_{GR} and R_{SDM} (Fig. 5).

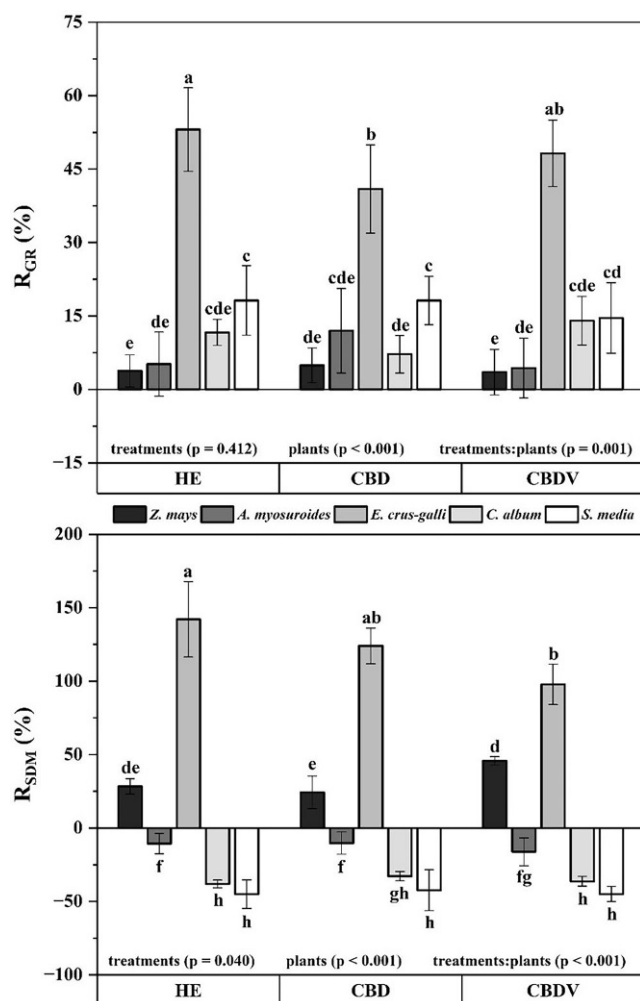


Fig. 5 Response (R_{GR}) based on germination rate (top) and response (R_{SDM}) based on shoot dry matter (bottom) of *Zea mays*, *Alopecurus myosuroides*, *Echinochloa crus-galli*, *Chenopodium album* and *Setaria media* with standard deviation 21 days after the application of the phytocannabinoids cannabidiol (CBD) and cannabidiol (CBDV) concentrated with 2 mg mL^{-1} or the aqueous *Cannabis sativa* tissue extract (HE) application using a concentration of 500 mg mL^{-1} in comparison to a control with dH_2O . Mean values with the same lowercase letter within *Zea mays* or the weed species show no significant differences regarding the Tukey-adjusted ‘emmeans’ post-hoc test ($p < 0.05$)

Zea Mays

Pre-emergence application of CBD, CBDV, and HE at the highest concentration resulted in increased germination and shoot dry matter with a positive R_{GR} and R_{SDM} (Fig. 5). The application of CBDV resulted in a 1.9 times significantly higher R_{SDM} in *Z. mays* than the application of CBD and HE (Fig. 5).

Monocotyledonous Weeds

Pre-emergence application of CBD, CBDV, and HE resulted in a slightly increased germination rate in *A. myosuroides* with a positive R_{GR} while decreased shoot dry matter was measured in all treatments with a negative R_{SDM} (Fig. 5). In *E. crus-galli*, however, pre-emergence application of CBD, CBDV, and HE resulted in an increased germination rate and shoot dry matter (Fig. 5). The application of HE in *E. crus-galli* increased the R_{GR} significantly by a factor of 1.3 compared to CBD and the R_{SDM} by a factor of 1.5 compared to CBDV (Fig. 5).

roides with a positive R_{GR} while decreased shoot dry matter was measured in all treatments with a negative R_{SDM} (Fig. 5). In *E. crus-galli*, however, pre-emergence application of CBD, CBDV, and HE resulted in an increased germination rate and shoot dry matter (Fig. 5). The application of HE in *E. crus-galli* increased the R_{GR} significantly by a factor of 1.3 compared to CBD and the R_{SDM} by a factor of 1.5 compared to CBDV (Fig. 5).

Dicotyledonous Weeds

On the one hand, the germination rate was enhanced by the application of CBD, CBDV, and HE at the highest concentration, as evidenced by a positive R_{GR} in *C. album* and in *S. media*. On the other hand, the application of CBD, CBDV, and HE at the highest concentration resulted in reduced shoot dry matter, which was shown by a negative R_{SDM} in *C. album* and in *S. media*.

Total Phenolic Content (TPC) of the Aqueous *Cannabis sativa* Tissue Extract

The total phenolic content of the HE with a stock solution of 0.5 shoot biomass per ml deionized water was $12.1 \pm 2.7\text{ mg gallic acid equivalent g}^{-1}$ fresh weight.

Discussion

Influence of Phytocannabinoid and Aqueous *Cannabis sativa* Tissue Extract Applications On the Germination and Root Growth of *Zea mays* and Relevant Weeds

The application of aqueous *C. sativa* tissue extract (HE) resulted in the highest suppression of germination and root growth in all the investigated plants in the Petri dish bioassay, and the suppressive effects increased with rising concentrations of the aqueous extract, but the effect was stronger in the weed species than in the cash crop. Additionally, the application of lower concentrations of HE stimulated the germination of *A. myosuroides* and the root growth of *Z. mays* (inducing hormesis). The same effects were observed by Rueda-Ayala et al. (2015) in their study, where the application of low concentrations of HE stimulated the root growth of *Z. mays*, *Amaranthus retroflexus* L., and *Setaria viridis* (L.) P. Beauv., while with increased concentrations, the root growth became increasingly suppressed. Similarly, *Z. mays* was less suppressed in germination in their study compared to the weeds. The stronger observed suppressive effects on the weeds in our Petri dish bioassays can be attributed to the higher surface-to-volume ratio of the small seeds. Weed

seeds were more exposed to HEs than the larger *Z. mays* seeds. Additionally, it is assumed that the larger crop seeds may have better abilities to detoxify biochemicals, as suggested by several authors in studies involving aqueous *Secale cereale* L. (Burgos and Talbert 2000) and *Trifolium pretense* L. (Liebmann and Sundberg 2006) extracts. Laboratory Petri dish bioassays from other researchers also demonstrated that the application of HE derived from shoot biomass exhibited germination-inhibiting effects on the weeds *Cyperus rotundus* L. (Srivastava and Das 1974), *Matricaria chamomilla* L. (Sturm et al. 2016), *Lepidium sativum* L. (Stupnicka-Rodzynkiewicz 1970), *S. media* (Sturm et al. 2016), *C. album* (Sturm et al. 2016), and *Matricaria chamomilla* L. (Sturm et al. 2016). Additionally, Sturm et al. (2016) investigated the root growth of the weeds and observed suppression effects up to 46%. In contrast, the application of neutral phytocannabinoids suppressed the germination rate but had a stronger effect on the root growth of the weeds, with the exception of *A. myosuroides*, where the germination rate was increased. Furthermore, the phytocannabinoid applications inhibited the root growth of *Z. mays* less compared to the weeds, as also observed in HE, without having an influence on the crop's germination rate. Therefore, root growth appears to be a more sensitive indicator of the phytotoxicity of HE and phytocannabinoids than the germination rate. This is further supported by the study of Patanè et al. (2023), who observed that the application of aqueous *C. sativa* leaf extracts resulted in a stronger inhibition of root growth in *Triticum durum* Desf. and *Hordeum vulgare* L. compared to shoot growth and germination rate. According to Nishida et al. (2005) who investigated the allelopathic effects of volatile monoterpenoids from *Salvia leucophylla* Greene on *Brassica campestris* L. seedlings, one reason for the stronger suppression of root growth by allelopathic substances may be the higher permeability of root apex compared to shoot apex. Moreover, the allelopathic substances in HE, such as the phytocannabinoids, may have affected root growth by inhibiting the mitotic process in the root tips and causing changes in the ultrastructure (three major layers: the middle lamella, the primary wall, and the secondary wall) of the cells, as observed by the application of aqueous leachates of *Sicyos deppei* G. Don. to seedling roots of *Phaseolus vulgaris* L. in the study by Cruz-Ortega et al. (1998). This, however, was not investigated further in our study. Additionally, among the phytocannabinoid applications, CBD and CBDV in the highest concentration (2 mg ml⁻¹) exhibited the most pronounced suppressive effect on root growth, which was comparable to the effect of HE in the highest concentration (500 mg ml⁻¹), and in the case of CBDV and CBD, even the lowest concentration (0.125 mg ml⁻¹) decreased the root length of all weed species by 62–89% and 20–51%, respectively, compared to the control. De

Vita et al. (2022) demonstrated in their study, through phytochemical analyses, that methanol-extracted essential oils from the leaves of the hemp genotype 'Futura 75', the same genotype as used in our study, contain besides the phytocannabinoids CBD, cannabicyclol (CBL) and CBDV mainly sesquiterpenes and its oxygenated derivatives such as β -caryophyllenes, caryophyllene oxide, α -humulenes, α -bisabolol oxide B, and allo-aromatidene epoxides. Patanè et al. (2023), on the other hand, detected phenols in aqueous leaf extracts derived from the hemp variety 'Futura 75' as in the present study and assumed that these phenols are primarily responsible for the observed seedling growth and germination suppressive effects on *H. vulgare* and *T. durum* in their bioassays. The total phenolic content of the aqueous *C. sativa* extract in the present study was 12 mg gallic acid equivalent g⁻¹ fresh weight, which was similar high as in the study of Patanè et al. (2023) with 13 mg gallic acid equivalent g⁻¹ dry weight, who used 20 mg dried *C. sativa* leaves per ml water for the aqueous extracts instead of 500 mg *C. sativa* fresh shoot biomass per ml water. Furthermore, Shikanai and Gage (2022) conducted bioassays to investigate the suppression of *Brassica napus* L. germination through the application of an extract from leaves and inflorescences of the *C. sativa* genotype '21 Mother PH4'. They attributed the phytocannabinoids CBDA, THCA, CBD, and CBN, as well as the monoterpenes (-)- β -pinene and the sesquiterpenes β -caryophyllene, α -humulene, and guaiol as primarily responsible for the phytotoxic effects because these secondary metabolites constituted the major proportion in the performed chemical analysis of the extract. Therefore, we assume that besides the phytocannabinoids CBD and CBDV, terpenes and other phenolic compounds seem to be mainly responsible for the suppression of germination and root growth of the investigated plants by HE in our Petri dish bioassays, which however cannot be completely confirmed due to the lack of a chemical analysis of HE regarding the identification of these secondary metabolites.

Impact of Applied Pre-emergence Formulations Based On the Phytocannabinoids Cannabidiol and Cannabidivarin, and Aqueous *Cannabis sativa* Extracts On *Zea mays* and Relevant Weeds

In contrast to the germination test in the laboratory, the application of HE, CBD, and CBDV formulations in the pre-emergence greenhouse trial stimulated the germination rate of all plants examined, but especially of *E. crus-galli*, which additionally was the only plant where significant differences between the formulations ($R_{GR}(\text{CBD}) < R_{GR}(\text{HE})$) were observed. Likewise, the biomass production of *Z. mays* and *E. crus-galli* was stimulated in the pre-emergence trial through all formulations, while the shoot dry matter was

decreased in the other weed species. Particularly remarkable was that the plants reacted differently to HE and phytocannabinoid formulations by the pre-emergence application, but there were only minimal differences, or none, in the effect between the formulations in the individual plants. All these are indicators that the phytocannabinoids CBD and CBDV are partly responsible for the visible stimulatory as well as suppressive effects observed in HE. Furthermore, one reason for the stimulation was the concentration of the formulations, which had a suppressive effect on germination under laboratory conditions but was probably too low for greenhouse application. We assume that this could be attributed to the physicochemical properties of the soil, such as pH, temperature, soil moisture (Patni et al. 2023), and organic matter content (Bayja et al. 2023), as well as biological conversion and degradation processes, mainly driven by microorganisms (Fisher 1978), which may have led to the detoxification or conversion of the chemical substances. In addition, we assume according to Tanase et al. (2019), phenolic compounds, which are present in *C. sativa* extracts and to which phytocannabinoids also belong chemically, act in low concentrations as bioregulators that influence the hormonal balance of plants by showing auxin effects potentially resulting in increased biomass production. Therefore, HE, CBD, and CBDV formulations could be used, for example, in the form of a pre-emergence bioherbicide to break seed dormancy by stimulating weed seed germination prior to sowing the main crops, like the mechanical integrated weed management methods of the false seedbed or stale seed (Messelhäuser et al. 2022). These germinated weeds can then be controlled by integrated weed management methods before sowing the cash crop. For the final evaluation of the use of phytocannabinoids as a pre-emergent bioherbicide for breaking the seed dormancy of weeds under practical farming conditions, future field trials with different concentrated phytocannabinoid formulations at different locations with different soil types and under different climatic conditions are required.

Conclusion

In summary, the study shows that HE and especially the phytocannabinoids CBD and CBDV had a strong suppressive effect on the plants in the laboratory, especially on root growth but also on germination. However, partly stimulating effects on germination and biomass formation of the plants could be observed in the greenhouse experiment. The individual plants reacted differently to the various phytocannabinoid formulations, and significant differences in the responses to the applied phytocannabinoids among the plants became evident. In both trials, the weeds exhibited a much more sensitive response to the application of HE

and the different phytocannabinoids compared to *Z. mays*. HE in the highest concentration demonstrated the strongest suppressive effect in the laboratory concerning the germination rate and root length of the investigated plants. Whereas, applied CBD and especially CBDV, as opposed to HE, already displayed a very strong suppressive effect on root growth already in lowest concentration. In addition, further research is needed in the future regarding the weed suppression properties of higher concentrated phytocannabinoid formulations and the acidic forms of phytocannabinoids such as CBDA and CBDVA, which are mainly found in the living *C. sativa* plants.

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Conflict of interest M. Merkle and R. Gerhards declare that they have no competing interests.

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References

- Alsaadawi IS, Sarbout AK, Al-Shamma LM (2012) Differential allelopathic potential of sunflower (*Helianthus annuus* L.) genotypes on weeds and wheat (*Triticum aestivum* L.) crop. Arch Agron Soil Sci 58(10):1139–1148. <https://doi.org/10.1080/03650340.2011.570335>
- Baerson SR, Dayan FE, Rimando AM, Nanayakkara NPD, Liu C-J, Schröder J, Fishbein M, Pan Z, Kagan IA, Pratt LH, Cordonnier-Pratt M-M, Duke SO (2008) A functional genomics investigation of 57 allelochemical biosynthesis in *Sorghum bicolor* root hairs. J Biol Chem 283(6):3231–3247. <https://doi.org/10.1074/jbc.M706587200>
- Bailey KL (2014) The bioherbicide approach to weed control using plant pathogens. In: Abrol DP (ed) Integrated pest management. Academic Press, Cambridge, pp 245–266 <https://doi.org/10.1016/B978-0-12-398529-3.00014-2>
- Bayja S, Jakhar RS, Bhateshwar V, Dudwal M (2023) Role of soil organic matter in soil health and crop productivity improvement. In: Choudhary M, Choudhary RM, Jat ML, Meena VK, Tak JK (eds) Advancement and innovations in agriculture. Iterative International, Karnataka, pp 135–147
- Belz RG (2007) Allelopathy in crop/weed interactions—an update. Pest Manag Sci 63(4):308–326. <https://doi.org/10.1002/ps.1320>

- Boulou P, Serge A, Arnaud L (2013) Hemp. Industrial production and uses. CABI, Wallingford, p 313
- Brain P, Cousens R (1989) An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Res* 29(2):93–96. <https://doi.org/10.1111/j.1365-3180.1989.tb00845.x>
- Brust J, Gerhards R, Karanisa T, Ruff L, Kipp A (2011) Why undersown and cover crops become important again for weed suppression in European cropping systems. *Gesunde Pflanzen* 63:191–198. <https://doi.org/10.1007/s10343-011-0263-9>
- Burgos NR, Talbert RE (2000) Differential activity of allelochemicals from *Secale cereale* in seedling bioassays. *Weed Sci* 48(3):302–310. [https://doi.org/10.1614/0043-1745\(2000\)048%5B0302:DAOAFS%5D2.0.CO;2](https://doi.org/10.1614/0043-1745(2000)048%5B0302:DAOAFS%5D2.0.CO;2)
- Caamal-Maldonado JA, Jiménez-Osornio JJ, Torres-Barragán A, Anaya AL (2001) The use of allelopathic legume cover and mulch species for weed control in cropping systems. *Agron J* 93:27–36. <https://doi.org/10.2134/agronj2001.93127x>
- Cappelletto P, Brizzi M, Mongardini F, Barberi B, Sannibale M, Nenci G (2001) Italy-grown hemp: yield, composition and cannabinoid content. *Indust Crop Prod* 13:101–113. [https://doi.org/10.1016/S0926-6690\(00\)00057-1](https://doi.org/10.1016/S0926-6690(00)00057-1)
- Cruz-Ortega R, Anaya AL, Hernández-Bautista BE, Laguna-Hernández G (1998) Effects of allelochemical stress produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. *J Chem Ecol* 24:2039–2057. <https://doi.org/10.1023/A:1020733625727>
- De Vita S, Finamore C, Chini MG et al (2022) Phytochemical analysis of the methanolic extract and essential oil from leaves of industrial hemp Futura 75 cultivar: Isolation of a new cannabinoid derivative and biological profile using computational approaches. *Plants* 11(13):1671. <https://doi.org/10.3390/plants11131671>
- European Commission (2023) Hemp production in the EU. https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/hemp_en. Accessed 20 Sept 2023
- Filer CN (2022) Acidic cannabinoid decarboxylation. *Cannabis Cannabinoid Res* 7(3):262–273. <https://doi.org/10.1089/can.2021.0072>
- Fisher RF (1978) Juglone inhibits pine growth under certain moisture regimes. *Soil Soc Am J* 42:801–803. <https://doi.org/10.2136/sssaj1978.03615995004200050030x>
- Gülck T, Möller BL (2020) Phytocannabinoids: origins and biosynthesis. *Trends Plant Sci* 25(10):985–1004. <https://doi.org/10.1016/j.tplants.2020.05.005>
- Hartwig NL, Ammon HU (2002) Cover crops and living mulches. *Weed Sci* 50:688–699. [https://doi.org/10.1614/0043-1745\(2002\)050%5B0688:AIACCA%5D2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050%5B0688:AIACCA%5D2.0.CO;2)
- Heap I (2023) The international herbicide-resistant weed database. <https://www.weedscience.org>. Accessed 31 Aug 2023
- Hesami M, Pepe M, Baiton A, Jones AMP (2022) Current status and future prospects in cannabinoid production through in vitro culture and synthetic biology. *Biotechnol Adv* 62:108074. <https://doi.org/10.1016/j.biotechadv.2022.108074>
- Hillocks RJ (2012) Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Prot* 31(1):85–93. <https://doi.org/10.1016/j.cropro.2011.08.008>
- Hiltbrunner J, Liedgens M, Bloch L, Stamp P, Streit B (2007) Legume cover crops as living mulches for winter wheat: components of biomass and the control of weeds. *Eur J Agron* 26:21–29. <https://doi.org/10.1016/j.eja.2006.08.002>
- Johnson R (2014) Hemp as an agricultural commodity. Congressional research service. Library of Congress, Washington
- Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983–997. <https://doi.org/10.2307/2533558>
- Kristoffersen P, Rask AM, Grundy AC et al (2008) A review of pesticide policies and regulations for urban amenity areas in seven European countries. *Weed Res* 48(3):201–214. <https://doi.org/10.1111/j.1365-3180.2008.00619.x>
- Kunz C, Sturm DJ, Sökefeld M, Gerhards R (2016) Weed suppression and early sugar beet development under different cover crop mulches. *Plant Prot Sci* 52:183–193. <https://doi.org/10.17221/109/2016-PPS>
- Liebman M, Sundberg DN (2006) Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from red clover shoots. *Weed Sci* 54(2):340–345. <https://doi.org/10.1614/WS-05-54.2.340a>
- Livingston SJ, Quilichini TD, Booth JK et al (2019) Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. *Plant J* 79:37–56. <https://doi.org/10.1111/tpj.14516>
- Mantzoukas S, Ntoukas A, Lagogiannis I, Kalyvas N, Eliopoulos P, Poulas K (2020a) Larvicidal action of cannabidiol oil and neem oil against three stored product insect pests: effect on survival time and in progeny. *Biology* 9(10):321. <https://doi.org/10.3390/biology9100321>
- Mantzoukas S, Kalyvas N, Ntoukas A, Lagogiannis I, Farsalinos K, Eliopoulos PA, Poulas K (2020b) Combined toxicity of cannabidiol oil with three bio-pesticides against adults of *Sitophilus zeamais*, *Rhyzopertha dominica*, *Prostephanus truncatus* and *Trogoderma granarium*. *Int J Environ Res Public Health* 17(18):6664. <https://doi.org/10.3390/ijerph17186664>
- Mendes IC, Bandick AK, Dick RP, Bottomley PJ (1999) Microbial biomass and activities in soil aggregates affected by winter cover crops. *Soil Sci Soc Am J* 63:873–881. <https://doi.org/10.2136/sssaj1999.634873x>
- Messelhäuser MH, Saile M, Sievernich B, Gerhards R (2022) Exploring the effects of different stubble tillage practices and glyphosate application combined with the new soil residual herbicide cinmethylin against *Alopecurus myosuroides* Huds. in winter wheat. *Agronomy* 12(1):167. <https://doi.org/10.3390/agronomy12010167>
- Nishida N, Tamotsu S, Nagata N, Saito C, Sakai A (2005) Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *J Chem Ecol* 31:1187–1203. <https://doi.org/10.1007/s10886-005-4256-y>
- Osipitan OA, Dille JA, Assefa Y, Radicetti E, Ayeni A, Knezevic SZ (2019) Impact of cover crop management on level of weed suppression: a meta-analysis. *Crop Sci* 59(3):833–842. <https://doi.org/10.2135/cropsci2018.09.0589>
- Park SH, Staples SK, Gostin EL et al (2019) Contrasting roles of cannabidiol as an insecticide and rescuing agent for ethanol-induced death in the tobacco hornworm *Manduca sexta*. *Sci Rep* 9(1):10481. <https://doi.org/10.1038/s41598-019-47017-7>
- Patanè C, Pellegrino A, Cosentino SL, Testa G (2023) Allelopathic effects of *Cannabis sativa* L. Aqueous leaf extracts on seed germination and seedling growth in durum wheat and barley. *Agronomy* 13(2):454. <https://doi.org/10.3390/agronomy13020454>
- Patni B, Bhattacharyya M, Pokhriyal A (2023) The role of signaling compounds in enhancing rice allelochemicals for sustainable agriculture: an overview. *Planta* 258(5):90. <https://doi.org/10.1007/s00425-023-04241-w>
- Puig CG, Valencia-Gredilla F, Pardo-Muras M, Souto XC, Recasens i Guinjuan J, Pedrol N (2021) Predictive phytotoxic value of water-soluble allelochemicals in plant extracts for choosing a cover crop or mulch for specific weed control. *Ital J Agron* 16:4. <https://doi.org/10.4081/ija.2021.1872>
- Rasmussen J (1991) A model for prediction of yield response in weed harrowing. *Weed Res* 31(6):401–408. <https://doi.org/10.1111/j.1365-3180.1991.tb01780.x>

- Reginatto M, da Silva Bonome LT, Giovanetti LK, von Hertwig Bittencourt H, Tormen L, Conceição PC (2020) Allelopathic potential from cover crops aqueous extract on weeds and maize. *Res Soc Dev* 9(10):e5859108579–e5859108579. <https://doi.org/10.33448/rsd-v9i10.8579>
- Ross SA, Mehmedic Z, Murphy TP, ElSohly MA (2000) GC-MS analysis of the total Δ^9 -THC content of both drug- and fiber-type *Cannabis* seeds. *J Anal Toxicol* 4:715–717. <https://doi.org/10.1093/jat/24.8.715>
- Ross SA, ElSohly MA, Sultana GNN, Mehmedic Z, Hossain CF, Chandra S (2005) Flavonoid glycosides and cannabinoids from the pollen of *Cannabis sativa* L. *Phytochem Anal* 16:45–48. <https://doi.org/10.1002/pca.809>
- Rueda-Ayala V, Jaecck O, Gerhards R (2015) Investigation of biochemical and competitive effects of cover crops on crops and weeds. *Crop Prot* 71:79–87. <https://doi.org/10.1016/j.cropro.2015.01.023>
- Russo EB (2019) The case for the entourage effect and conventional breeding of clinical cannabis: No “strain,” no gain. *Front Plant Sci* 9:1969. <https://doi.org/10.3389/fpls.2018.01969>
- Sánchez-Carnerero Callado C, Núñez-Sánchez N, Casano S, Ferreira-Vera C (2018) The potential of near infrared spectroscopy to estimate the content of cannabinoids in *Cannabis sativa* L.: a comparative study. *Talanta* 190:147–157. <https://doi.org/10.1016/j.talanta.2018.07.085>
- Shikanai A, Gage KL (2022) Allelopathic potential of hemp: implications for integrated weed management. *Front Agron* 4:832471. <https://doi.org/10.3389/fagro.2022.832471>
- Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y (2005) Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol* 46:1578–1582. <https://doi.org/10.1093/pcp/pci166>
- Slinkard K, Singleton VL (1977) Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic* 28(1):49–55. <https://doi.org/10.5344/ajev.1977.28.1.49>
- Srivastava PP, Das LL (1974) Effect of certain aqueous plant extracts on the germination of *Cyperus rotundus* L. *Sci Cult* 40:318–319
- Stout JM, Boubakir Z, Ambrose SJ, Purves RW, Page JE (2012) The hexanoyl-CoA precursor for cannabinoid biosynthesis is formed by an acyl-activating enzyme in *Cannabis sativa* trichomes. *Plant J* 71:353–365. <https://doi.org/10.1111/j.1365-3113X.2012.04949.x>
- Streibig JC (1980) Models for curve-fitting herbicide dose response data. *Acta Agric Scand* 30(1):59–64. <https://doi.org/10.1080/00015128009435696>
- Stupnicka-Rodzinkiewicz E (1970) Phenomena of allelopathy between some crop plants and weeds. *Acta Agrar Silv* 10(2):75–105
- Sturm DJ, Kunz C, Gerhards R (2016) Inhibitory effects of cover crop mulch on germination and growth of *Stellaria media* (L.) Vill., *Chenopodium album* L. and *Matricaria chamomilla* L. *Crop Prot* 90:125–131. <https://doi.org/10.1016/j.cropro.2016.08.032>
- Sturm DJ, Peteinatos G, Gerhards R (2018) Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res* 58(5):331–337. <https://doi.org/10.1111/wre.12316>
- Tanase C, Bujor O-C, Popa VI (2019) Phenolic natural compounds and their influence on physiological processes in plants. In: Polyphenols in plants, pp 45–58. <https://doi.org/10.1016/b978-0-12-813768-0.00003-7>
- Thomas BF, ElSohly MA (2015) Biosynthesis and pharmacology of Phytocannabinoids and related chemical constituents. In: Thomas BF, ElSohly MA (eds) The analytical chemistry of cannabis. Quality assessment, assurance, and regulation of medicinal marijuana and cannabinoid preparations. Elsevier, Amsterdam, pp 27–41. <https://doi.org/10.1016/C2014-0-03861-0>
- Trojanowska MR, Osbourn AE, Daniels MJ, Threlfall DR (2000) Biosynthesis of avenacins and phytosterols in roots of *Avena sativa* cv. Image. *Phytochemistry* 54(2):153–164. [https://doi.org/10.1016/S0031-9422\(00\)00062-5](https://doi.org/10.1016/S0031-9422(00)00062-5)
- Turner JC, Hemphill JK, Mahlberg PG (1978) Quantitative determination of cannabinoids in individual glandular trichomes of *Cannabis sativa* L. (Cannabaceae). *Am J Bot* 65:1103–1106
- Uddin MR, Li X, Won OJ, Park SU, Pyon JY (2012) Herbicidal activity of phenolic compounds from hairy root cultures of *Fagopyrum tataricum*. *Weed Res* 52(1):25–33. <https://doi.org/10.1111/j.1365-3180.2011.00894.x>
- UN (2022) Conference on trade and development: commodities at a glance: special issue on hemp. UNCTAD, Geneva
- Velasco P, Francisco M, Moreno DA, Ferreres F, García-Viguera C, Cartea ME (2011) Phytochemical fingerprinting of vegetable Brassica oleracea and Brassica napus by simultaneous identification of glucosinolates and phenolics. *Phytochem Analysis* 22(2):144–152. <https://doi.org/10.1002/pca.1259>
- Weinert TL, Pan WL, Moneymaker MR, Santo GS, Stevens RG (2002) Nitrogen recycling by nonleguminous winter cover crops to reduce leaching in potato rotations. *Agron J* 94:365–372. <https://doi.org/10.2134/agronj2002.3650>
- Wen L, Lee-Marzano S, Ortiz-Ribbing LM, Gruver J, Hartman GL, Eastburn DM (2017) Suppression of soilborne diseases of soybean with cover crops. *Plant Dis* 101(11):1918–1928. <https://doi.org/10.1094/PDIS-07-16-1067-RE>
- Weston LA (1996) Utilization of allelopathy for weed management in agroecosystems. *Agron J* 88:860–866. <https://doi.org/10.2134/agronj1996.00021962003600060004x>
- Wilson GF, Lal R, Okigbo BN (1982) Effects of cover crops on soil structure and on yield of subsequent arable crops grown under strip tillage on an eroded alfisol. *Soil Tillage Res* 2:233. [https://doi.org/10.1016/0167-1987\(82\)90013-7](https://doi.org/10.1016/0167-1987(82)90013-7)

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