International Journal of Tropical Agriculture
Volume 42, Numbers (3-4) • 2024 : pp. 223-228
ISSN: 0254-8755

Effect of Magnesium on Cannabis Biomass Yield and Cannabinoids Content: Evaluating Different Application Rates and Methods

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Abstract: Magnesium deficiency (MGD) is a severe problem in plants. Magnesium (Mg) is one of the important nutrients involved in many enzymatic activities and rarely studied in cannabis. In this study, the effect of application of different concentrations (0.75 ml L^{-1} of 'Advanced Nutrients Bud Factor X' equivalent to 3.75 mg L^{-1} of Mg, 2ml L^{-1} of 'Advanced Nutrients Bud Factor X' equivalent to 10 mg L^{-1} of Mg, and 4ml L^{-1} of 'Advanced Nutrients Bud Factor X' equivalent to 20 mg $L^{-1}$of Mg), and methods of application (Soil Drench vs. Foliage Application) of magnesium was studied on useable biomass yield and cannabinoids content on two chemovars (high CBD and high CBG) of *Cannabis sativa* L. Plants grown through feminized seeds were divided into two groups, control (no treatment) and treated with different concentrations of Mg (10 mg L^{-1} soil drench, 20 mg L^{-1} soil drench and 3.75 mg L^{-1} foliage spray). Plants of both groups were grown side by side in an identical environmental condition in a polytunnel and, were watered and fertilized normally. The application of different concentrations of magnesium began in the first week of flowering and continued until maturity. Both groups of plants were harvested at maturity, processed for usable dry biomass, and compared for biomass production per plant and cannabinoid content. Our results show that among all treatments (Soil Drench and Foliage Application), the maximum increase in cannabis biomass per plant in CBD-rich chemovars was achieved with the application of 10 mg L^{-1} Mg, whereas the highest increase in biomass per plant in CBG-rich chemovars was achieved with the application of 20 mg L^{-1} Mg. On the other hand, the maximum increase in CBD content per plant (in the CBD-rich chemovar) and CBG content per plant (in the CBG-rich chemovar) was observed with the application of 10 mg L^{-1} Mg to the soil, compared to the 'control' plants.

Keywords: Biomass yield, *Cannabis sativa* L., Cannabidiol, GC-FID, Δ^9- Tetrahydrocannabinol, Magnesium

INTRODUCTION

Taxonomically, cannabis is a single but highly variable species, *Cannabis sativa* L. It is an annual and dioecious but occasionally monoecious plant. Also, cannabis is a wind pollinated plant which is highly allogamous in nature. It is widely distributed in nature and can be found in all kinds of habitats from tropics to foot hills of alpines. Cannabis is cultivated for millennia for the use of grain, fiber as well as for recreational, medical, and ritual purposes. Traditionally, the plant has been used for the treatment of a variety of ailments such as headache, asthma, diarrhea, constipation, pain and anxiety, just to name a few, since ancient times in different forms (Russo 2017, Zuardi 2006).

Cannabis has been reported to contain more than 550 different compounds (ElSohly et al. 2017, ElSohly 2023) belonging to a diverse group

Article History: Received: 12 August 2024; Revised: 15 September 2024; Accepted: 20 September 2024; Published: 30 December 2024

of chemical classes, the most important of which is the cannabinoids. There are 120 cannabinoids reported so far (ElSohly et al. 2017, ElSohly 2023), among which Δ^9-Tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD) are the two major natural cannabinoids having very different pharmacological profiles with a tremendous therapeutic potential. It accumulates mainly in the glandular trichomes of the plant (Hammond and Mahlberg 1977). Cannabidiol (CBD) was initially isolated by Adams et al. in the 1940, but it was Mechoulam and Shvo who fully elucidated its chemical structure in 1963. The structure of THC was determined by Gaoni and Mechoulam in 1964.

Magnesium (Mg) is a vital nutrient involved in numerous essential physiological and biochemical processes in plants, including chlorophyll synthesis, the production, transportation, and utilization of photoassimilates, enzyme activation, and protein synthesis. Due to the widespread adoption of high-yielding, fertilizer-responsive cultivars, intensive cropping without adequate Mg replenishment, soil acidification, and the leaching of exchangeable Mg (Ex-Mg), magnesium has become a limiting nutrient for optimal crop production. Despite this, research has primarily focused on more commonly limiting macro-elements, such as nitrogen (N), phosphorus (P), and potassium (K), while the importance of other mineral nutrients like magnesium has often been overlooked in soil and plant testing, as well as fertilization programs. As a result, Mg deficiency is not typically considered a significant concern in agricultural productivity. Nonetheless, Mg is an essential component in a wide range of critical physiological and biochemical processes throughout plant growth and development.

In this study, we investigate the effect of different concentrations and application methods of magnesium on cannabis biomass yield and cannabinoids content of two different (high CBD and high CBG) chemovars of *Cannabis sativa*.

MATERIAL AND METHODS

Two different chemovars (high in CBD and high in CBG) of cannabis was grown from seeds. On flowering, male plants were removed and female plants were kept for further study. Plants of each variety were divided in four groups: (1) control (with no Mg application), (2) treated with 2ml L^{-1} Advanced Nutrients Bud Factor X, soil drench application, equivalent to 10 mg L^{-1} of Mg, (3) treated with 4ml L^{-1} of Advanced Nutrients Bud Factor X, soil drench application, equivalent to 20 mg $L^{-1}$of Mg, and (4) treated with 0.75ml L^{-1} Advanced Nutrients Bud Factor X, foliage application, equivalent to 3.75 mg L^{-1} of magnesium. Treated plants were supplemented with Mg every week starting from first week of flowering till harvest. Plants were harvested on maturity and data were collected.

Analysis of Cannabinoids Content

Biomass samples of cannabis plants (control and treated with Advanced Nutrients Bud Factor X) were used for cannabinoids content. Seven cannabinoids (Δ^8- tetrahydrocannabinol, Δ^8-THC; Δ^9- tetrahydrocannabinol, Δ^9 –THC; cannabidiol, CBD; tetrahydrocannabivarin, THCV; cannabinol, CBN; cannabigerol, CBG and cannabichromene, CBC (Figure 1**)** were analyzed using our previously published GC-FID methods (ElSohly et al. 2000, 2016, Geweda et al. 2024).

GC-FID Instrumentation and Conditions for Cannabinoids Analysis

A gas chromatography (GC) analyses were performed using Varian CP-3380 gas chromatographs, equipped with Varian CP-8400 autosamplers, capillary injectors, dual flame ionization detectors, and DB-1MS columns (15 m × 0.25 mm × 0.25 µm) (J&W Scientific, Folsom, CA). Data was recorded using a Dell Optiplex GX1 computer and Varian Star workstation software (version 6.1). Helium was used as carrier and detector makeup gas with an upstream indicating moisture trap and a downstream indicating oxygen trap. Hydrogen and compressed air were used as the combustion gases. The following instrument parameters were employed: air, 30 psi (300 mL / min); hydrogen, 30 psi (30 mL/ min); column head pressure, 15 psi (1.0 mL / min); split flow rate, 100 mL /min; split ratio, 50:1; septum purge flow rate: 5 mL / min; makeup gas pressure, 20 psi (30 mL / min); injector temperature, 240°C; detector temperature,

270°C; oven program, 170°C (hold 1 min) to 250°C at 10°C / min (hold 3 min); run time, 12 min; injection volume, 1 µL. The instruments are daily maintained and calibrated to ensure a Δ^9- THC / internal standard response factor ratio of one.

Calculation of Concentrations

The concentration of a specific cannabinoid is calculated as follows:

Cannabinoids (%) =
{GC area (cannabinoid) / GC area (ISTD)} ×
{Amount (ISTD) / Amount (sample)} × 100

Standards and Reagents

All reference cannabinoids standards were purchased from Cayman Chemicals as 1 mg/mL solutions in MeOH with purity ≥95%. The purity of cannabinoids was confirmed by GC/MS.

RESULTS AND DISCUSSION

Mineral nutrition plays a crucial role in both vegetative and reproductive growth of plants (Kirkby 2023, Marschner 2011, Aibara and Miwa 2014, Fageria et al. 2008). Magnesium is a vital macronutrient for plant growth and metabolism (Chen et al. 2018, Li et al. 2001).

Magnesium takes part in key physiological functions and metabolic processes in plants, and its deficiency or toxicity can disrupt these functions. It plays a critical role in photosynthesis by activating ribulose-1,5-bisphosphate carboxylase-oxygenase, a key enzyme in the photosynthesis process and the most abundant enzyme on Earth (Lorimer et al. 1976). Additionally, magnesium is a central component of the chlorophyll molecule (Verbruggen and Hermans 2013). It is involved in photosynthesis by activation of ribulose-1,5-bisphosphate carboxylase-oxygenase, which is a key enzyme of the photosynthesis proocess (Lorimer et al. 1976), and as a central component of the chlorophyll molecule (Verbruggen and Hermans (2013). In general, Mg deficiency results in shorter roots, smaller shoots, necrotic spots on leaves, and interveinal chlorosis (Verbruggen and Hermans 2013, Hermans et al. 2010, Hermans and Verbruggen 2005). The lack of information pertaining to the response of cannabis plants to Mg nutrition limits the development of optimal fertilization practices.

For cannabis, optimal nutrient supply rates during the vegetative phase of cannabis were found to be 175 mg L^{-1} of K (Saloner et al. 2019), 30 mg L^{-1} of P (Shiponi & Bernstein 2021), and 160 mg L^{-1} of N (Saloner & Bernstein 2020). On the other hand, optimal nutrient supply rates for the reproductive phase were reported to be 60 mg L^{-1} of K (Saloner and Bernstein 2022), 30–90 mg L^{-1} of P depending on the genotype (Shiponi and Bernstein 2021), and 160 mg L^{-1} of N (Saloner and Bernstein 2021). However, with the exception of a few studies, such as Veazie et al. (2021) on two

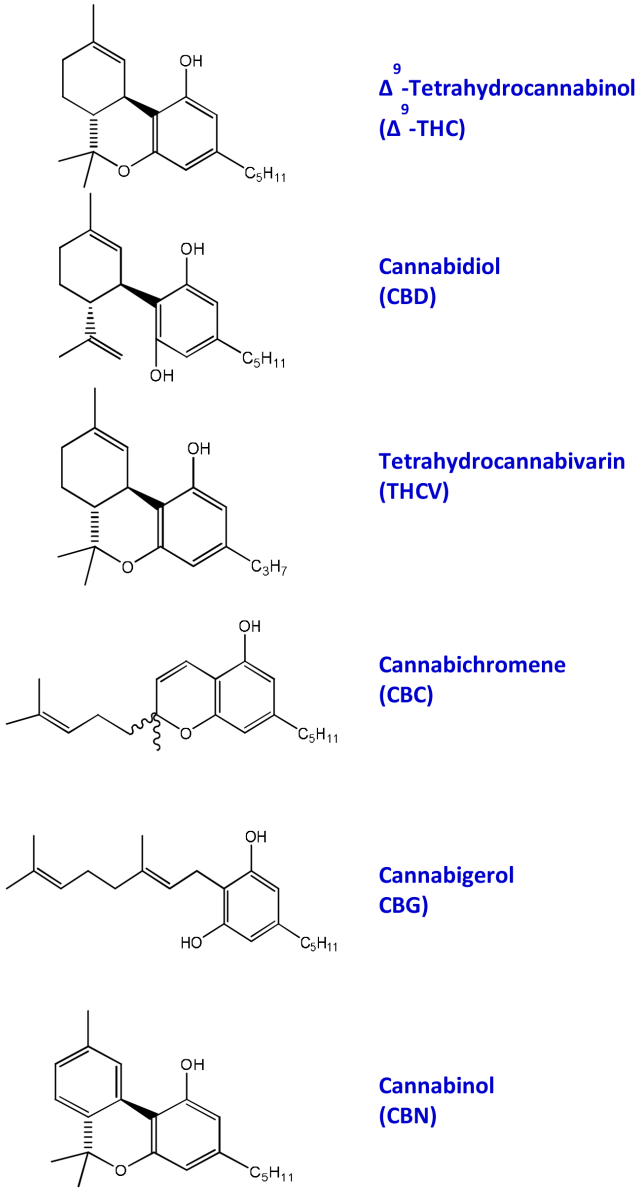

Figure 1: Chemical structures of major cannabinoids found in *Cannabis sativa* L.

Table 1: Effect of different methods of application and application rates of 'Advance Hemp Factor x' on 'number of buds (flowers) per plant' and 'weight of buds per plant' of a high CBD chemovar of *Cannabis sativa* L.

Treatments (Advanced Nutrients Bud Factor X)	*Number of buds (flowers)/plant*	*Dry weight (g) of buds (flowers) /plant*
Control	198.83±37.27	79.33±27.90
Drench (2ml L^{-1})	176.33±32.45	119.50±42.76
Drench (4ml L^{-1})	154.67±41.21	79.00±21.99
Foliage application (0.75 ml L^{-1})	257.67±69.89	94.17±35.29

Data represent: Mean ± SD, *n*=6

Table 2: Effect of different methods of application and application rates of 'Advance Hemp Factor x' on 'number of buds (flowers) per plant' and 'weight of buds per plant' of a high CBG chemovar of *Cannabis sativa* L.

Treatments (Advanced Nutrients Bud Factor X)	*Number of buds (flowers)/plant*	*Dry weight (g) of buds (flowers) /plant*
Control	38.50 ± 8.92	25.17 ± 15.67
Drench (2ml/L)	68.50 ± 19.93	44.67 ± 16.27
Drench (4ml/L)	68.50 ± 14.57	61.17 ± 16.76
Foliage application (0.75 ml/L)	67.83 ± 8.04	50.33 ± 10.67

Data represent: Mean ± SD, *n*=6

Table 3: Effect of different methods of application and application rates of 'Advance Hemp Factor x' on 'Cannabinoids content' of a high CBD Chemovar of *Cannabis sativa* L.

Treatment (Advanced Nutrients Bud Factor X)	*CBDV*	*THCV*	*CBD*	*CBC*	*Δ□ THC*	*Δ□ THC*	*CBG*	*CBN*
Control	0.06±0.06	0.02±0.01	10.13±0.96	0.94±0.51	0.05±0.02	0.42±0.04	0.20±0.04	0.01±0.01
Drench (2ml L^{-1})	0.07±0.04	0.01±0.01	10.66±1.73	0.87±0.40	0.11±0.02	0.49±0.10	0.21±0.07	0.01±0.00
Drench (4ml L^{-1})	0.10±0.06	0.01±0.01	8.90±1.46	0.99±0.47	0.09±0.02	0.40±0.07	0.15±0.09	0.01±0.00
Foliage application (0.75 ml L^{-1})	0.11±0.12	0.01±0.00	10.34±1.58	0.96±0.37	0.06±0.02	0.43±0.07	0.21±0.08	0.01±0.01

Data represent: Mean ± SD, *n*=6

Table 4: Effect of different methods of application and application rates of 'Advance Hemp Factor x' on cannabinoids content of a high CBG chemovar of *Cannabis sativa* L.

Treatments (Advanced Nutrients Bud Factor X)	*CBDV*	*THCV*	*CBD*	*CBC*	*Δ□ THC*	*Δ□ THC*	*CBG*	*CBN*
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.01±000	0.01±0.00	0.05±0.03	3.31±1.94	0.05±0.02
Drench (2ml L^{-1})	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.01±0.01	0.09±0.01	5.66±0.92	0.05±0.08
Drench (4ml L^{-1})	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.10	3.3±3.40	0.1±0.00
Foliage application (0.75 ml L^{-1})	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00	0.05±0.02	3.08±1.60	0.05±0.02

Data represent: Mean ± SD, *n*=6

different high-CBD-type cultivars and Morad and Bernstein (2023) on medical cannabis at the vegetative stage, there is limited information available on the response of drug-type cannabis to other macro and micronutrients, including magnesium (Mg). Therefore, in the present study we focus to investigate the effect of different concentrations and application methods of magnesium (during the flowering stage) on the biomass yield and cannabinoid content of two distinct chemovars (CBD type and CBG type) of *Cannabis sativa* L.

Our results show that, across all treatments (Drench and Foliage application), the highest increase in cannabis biomass per plant in 'CBD-rich chemovar' was achieved with the application of 10 mg L^{-1} Mg (Table 1). In contrast, in 'CBG-rich chemovar,' the maximum increase in biomass per plant was attained with the application of 20 mg L^{-1} Mg (Table 2).

On the other hand, across all the treatments (Drench and Foliage application), maximum increase in CBD content/plant (in 'CBD rich chemovar) and CBG content/plant (in CBG rich chemovar) was observed by the application of 10 mg L^{-1} Mg drench as compared to those as 'control' plants (Table 3 and Table 4). These findings are very valuable for optimizing nutrient supply, maintaining chemical composition, and biomass production while maintaining a cost-effectiveness in cannabis cultivation. However, a further in-depth research is required for a better understanding.

ACKNOWLEDGMENTS

This study was supported in part by 'Advanced Nutrients, West Hollywood, CA'. The authors appreciate the company providing the 'Advanced Nutrients Bud Factor X' used in this study.

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