Response of drug-type *Cannabis sativa* L. to P and K concentrations in a deep-water system



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Introduction

Drug-type cannabis (*Cannabis sativa* L.) is a booming industry following its legalization in Canada. However, few scientific knowledge on fertilization management constitutes a main challenge for producers who aim to harvest high yields of a high-quality product. The most studied nutrient in *C. sativa* is N because of the nitrophilous characteristic of the plant, which means that a relatively high amount of N is needed to sustain its growth. On the other hand, few studies were conducted with P and K, which may impact the flower yield and its content in secondary metabolites such as the cannabinoids and terpenes (Cockson *et al*., 2020; Yep and Zheng, 2021). Consequently, we have conducted a study on the effects of P and K concentrations on the plant development, flower yield and secondary metabolites of drug-type *Cannabis* grown hydroponically.

Objectives

- To determine the plant response curve of one cultivar of drug-type *Cannabis* to five P concentrations and two K concentrations when plants are grown in a soilless hydroponic system.
- To provide cannabis producers with the optimal concentration of P and K for hydroponic production systems.

Hypothesis

- High P and K concentrations can induce a higher potency and terpene production, and a better inflorescence yield.

Materials and methods

Propagation Cuttings were obtained from the mother plants of one genotype of drug-type *C. sativa* "White Shark" (WS), having high THC and low CBD content. For root development, cuttings were placed in an aeroponic tower under a 16 h photoperiod for about 3 weeks. The plants were propagated by IsoCanMed Inc., a licensed cannabis producer.

Experimental design Five P concentrations (25, 50, 100, 150 and 200 ppm) and two K concentrations (175 and 250 ppm) were studied within a factorial experimental design located in a greenhouse at Université Laval (Quebec City); randomized complete block design with 4 replicates (P X K = 10 treatments; total of 40 experimental units, e.u.). Plants were grown in 40 deep-water systems of 40 L having 3 plants per system (total of 12 plants per treatment and 120 plants). Upon their transplantation, the plantlets were submitted into the flowering phase with a 12 h photoperiod supplied with 1000 W HPS providing a PPFD of 321 µmol m^{-2} s^{-1} at the plant level.

Physiological parameters measured

- Plant development: Height, number of nodes, chlorophyll content index (CCI) (SPAD 502 Plus Chlorophyll Meter), shoot diameter and growth index.
- Destructive growth measurements after 25 and 80 days: fresh and dry biomass of roots, shoot, leaves, and flowers; leaf area; mineral content of the leaves and flowers.

Results and discussion

Table 1. Growth parameters of C. sativa cv White Shark submitted to ten combined treatments of P (25, 50, 100, 150, and 200 ppm) and K (175 and 250 ppm) after 80 days of growth in deep-water systems; data are means of n=8, except for water use efficiency where n=4.

Treatment		Total biomass	Flower yields	Leaf yields	Root yields	Shoot yields	Growth index	Shoot diameter	CCI	Fv/Fm	Foliar area		Number of leaves	Water use efficiency
		(g DM plant^{-1})						(mm)			(cm^2 plant^{-1})		(Nb plant^{-1})	(g L^{-1} plant^{-1})
P	25	163.7	27.90	106.88	12.62	16.97	1683.6	13.7	61.2	0.819	7734		295	1.32
	50	157.9	25.64	106.73	11.01	16.01	1644.2	13.5	60.9	0.822	7353		296	1.29
	100	164.3	26.35	108.59	10.92	17.29	1570.9	12.4	62.9	0.819	12796		305	1.22
	150	158.2	28.67	101.20	11.28	17.30	1699.8	13.9	61.5	0.818	6841		272	1.29
	200	158.2	27.88	101.82	11.62	17.64	1769.1	12.8	62.5	0.821	10036		294	1.30
K	175	157.3	26.89	103.27	11.14	16.72	1696.2	12.8	61.8	0.820	6878	b[z]	277	1.25
	250	163.6	27.69	106.82	11.86	17.36	1650.8	13.7	61.8	0.819	11015	a	308	1.31
ANOVA														
P		0.947	0.853	0.890	0.653	0.900	0.878	0.354	0.385	0.799	0.281		0.986	0.869
K		0.421	0.672	0.527	0.282	0.556	0.707	0.077	0.974	0.661	0.013		0.398	0.306
P × K		0.058	0.742	0.239	0.378	0.071	0.412	0.586	0.255	0.599	0.218		0.944	0.267

[z] Different small letters above the means represent significant differences between treatments by LSD protected by Fisher (P<0.05).

No significant effect of P x K fertilization treatments were observed on growth and yield parameters, except for the leaf area where the higher K concentrations (250 ppm), regardless of the P concentration, increased its value by 1.6 times compared with the 175 ppm K treatment.

Results and discussion

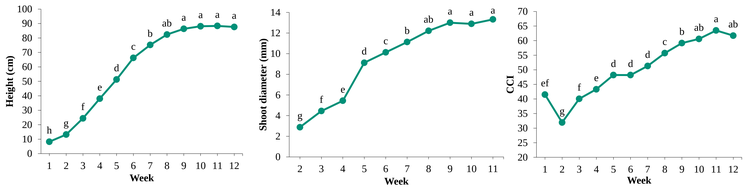

Figure 1. Weekly evolution of the plant height, the shoot diameter and the chlorophyll content index (CCI) of *C. sativa cv* White shark grown in deep-water systems. Data are means of n=40 (10 treatments x 4 replicates). Different small letters above the means represent significant differences between treatments by LSD protected by Fisher (P<0.05).

The plants had grown rapidly until week 8 where they reached a plateau (Figure 1). For example, between weeks 4 and 8, the height of the plants has more than doubled (from 38.0 cm to 82.4 cm). This fast growing can be associated to the "flowering stretch" often observed shortly after the first flowers appeared, at week 3 in this study. The other growth parameters (growth index and stem diameter) reached a plateau at around the same time as the final height; at weeks 8 and 9. The number of nodes and CCI increased until weeks 9 and 11, respectively.

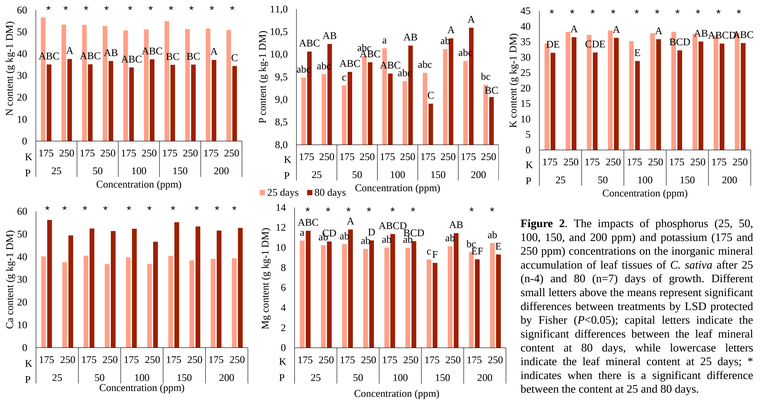

Figure 2. The impacts of phosphorus (25, 50, 100, 150, and 200 ppm) and potassium (175 and 250 ppm) concentrations on the inorganic mineral accumulation of leaf tissues of *C. sativa* after 25 (n-4) and 80 (n=7) days of growth. Different small letters above the means represent significant differences between treatments by LSD protected by Fisher (P<0.05); capital letters indicate the significant differences between the leaf mineral content at 80 days, while lowercase letters indicate the leaf mineral content at 25 days; * indicates when there is a significant difference between the content at 25 and 80 days.

Leaf content for N, K, Ca and Mg were significantly different at days 25 and 80, excepted for Mg at 150 ppm P. After 80 days, N and K leaf content decreased compared with their content at 25 days, while Ca and Mg increased (Figure 2).

- **N**: The N content in the leaves decreased after 80 days due to the N increase observed in the flowers (data not shown), which suggest that flowers may constitute a sink for N due to the high energy demand for the biosynthesis of cannabinoids and terpenes as Gershenzon (1994) stated that terpene biosynthesis is one of the most energy-demanding metabolite to synthesis.
- **P**: There was 37% more P in the inflorescences (data not show), which can be attributed to the high demand in the flowers for the biosynthesis of the secondary component such as terpenes and cannabinoids (Gershenzon, 1994).
- **K**: The high K concentration (250 ppm) increased the leaf K content after 25 and 80 days of growth compared with 175 ppm K. Paired with the absence of a significant impact of K on the plant development, it appears that *C. sativa* was subjected to "luxury consumption" of K.
- **Ca**: The leaf Ca content at 80 days was 36% higher than its content at 25 days of growth, which might be due to a higher transpiration rate as Ca transportation from the roots to the leaves is highly related to transpiration rates (White, 2012).
- **Mg**: Increase of 5% of the Mg content in the leaves between 25 and 80 days, which was related to the increase of CCI (Mg being a component of the chlorophyll).

No significant effect of the P and K studied concentrations on cannabinoids were observed for the cultivar WS (Table 2). The total averaged potency was 14.08-16.24 % for THC and 0.05% for CBD. The concentration of the other cannabinoids did not significantly differ between the treatments with 1.20-1.36 %, 0.10-0.11 % and 0.14-0.15 % for CBGA, CBG and CBC, respectively. The averaged terpene content of the flowers was 19.56 mg/g. Seventy-four percent of the terpenes were sesquiterpenes followed by the monoterpenes (25%), while the remaining 1% were diterpenes. From the 60 terpenes identified by our analysis, we selected only the ones that had more than 0.50 mg/g and known as having pharmacological metabolites of interest. This resulted in 11 terpenes, which represented 72% of the total terpene content. β-caryophyllene was the terpene found at the highest concentrations with an averaged value of 2.64 mg/g. Germacrene B, selina-3,7(11)-diene and α-pinene were the other important terpenes with 2.45, 1.72 and 1.50 mg/g, respectively. Significant effects of the P X K interaction were found in three terpenes: (E)-nerolidol (P=0.027), germacrene B (P=0.042) and γ-elemene (P=0.034). However, the principal effect was due to the 50 ppm P X 175 ppm K treatment (Figure 3).

Table 2. Cannabinoid concentrations of the WS flowers harvested after 80 days from plants submitted to different P (25, 50, 100, 150 and 200 mg L^{-1}) and K (175 and 250 mg L^{-1}) concentrations. Data are means of n = 3.

P	K	THCA	Δ9-THC	CBDA	CBD	CBGA	CBG	CBC	Total THC	Total CBD
mg L^{-1}	mg L^{-1}	%								
25		17.01	0.09	0.06	0.00	1.20	0.10	0.15	15.01	0.05
50		16.03	0.09	0.05	0.00	1.22	0.10	0.14	14.15	0.05
100		17.05	0.09	0.06	0.00	1.24	0.10	0.15	15.04	0.05
150		17.54	0.10	0.06	0.00	1.32	0.11	0.15	15.48	0.05
200		17.27	0.10	0.06	0.00	1.30	0.10	0.15	15.25	0.05
	175	16.83	0.09	0.06	0.00	1.25	0.10	0.15	14.85	0.05
	250	17.13	0.09	0.06	0.00	1.26	0.10	0.15	15.12	0.05
25	175	16.45	0.09	0.06	0.00	1.18	0.10	0.15	14.51	0.05
	250	17.58	0.10	0.05	0.00	1.21	0.11	0.15	15.51	0.05
50	175	16.11	0.09	0.05	0.00	1.21	0.10	0.14	14.22	0.05
	250	15.95	0.09	0.05	0.00	1.23	0.09	0.14	14.08	0.05
100	175	16.95	0.09	0.06	0.00	1.21	0.10	0.15	14.96	0.05
	250	17.15	0.08	0.06	0.00	1.27	0.11	0.15	15.12	0.05
150	175	16.67	0.10	0.06	0.00	1.36	0.10	0.14	14.72	0.05
	250	18.41	0.09	0.06	0.00	1.29	0.11	0.16	16.24	0.05
200	175	17.97	0.10	0.06	0.00	1.30	0.11	0.16	15.85	0.05
	250	16.58	0.10	0.06	0.00	1.30	0.09	0.14	14.64	0.05
ANOVA										
P		0.794	0.625	0.311	-	0.189	0.943	0.817	0.796	0.311
K		0.704	0.959	0.572	-	0.898	0.717	0.671	0.705	0.572
P × K		0.760	0.840	0.983	-	0.840	0.491	0.571	0.767	0.983

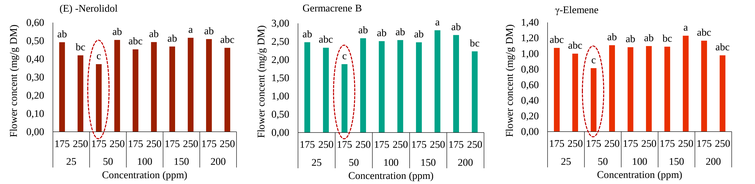

Figure 3. Concentrations of the three terpenes that were impacted by a significant P X K interaction. Data are means of n=3. The inflorescence of *C. sativa* grown cv. White Shark grown in deep-water systems were harvested after 80 days of treatments. Different small letters above the means represent significant differences between treatments by LSD protected by Fisher (P<0.05).

Conclusions

Hypothesis: False. We did not observe any higher cannabinoid and terpene content in the inflorescences of plants submitted to higher P and K concentrations. Only three main terpenes were affected by P X K fertilization; the 50 ppm P x 175 ppm K treatment reduced their content. Furthermore, P and K fertilization did not significantly improve the studied plant growth parameters and flower yield, except for the leaf area under 250 ppm K.

Overall, our results suggest that for a deep-water growing system, relatively low concentrations of P and K were sufficient to support an optimal plant development and yield. However these levels may vary according to the phenotype and the light growing condition, as our experiment was conducted during the fall.

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