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Katedra agroenvironmentální chemie a výživy rostlin

Vliv výživy na tvorbu biomasy a obsah sekundárních metabolitů u rostlin konopí (*Cannabis sativa* L.)

doktorská disertační práce

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1. ÚVOD

Rostliny konopí jsou pro svou variabilitu využití spojeny s lidskou historií od nepaměti. První zmínky o pěstování konopí se datují do období 10 000 let před naším letopočtem (př.n.l.) do Číny, kde bylo pěstováno zejména pro vlákno. Rozšíření léčebného využívání konopí se datuje do let 2800 (př.n.l.) v Egyptě a 2000 let (př.n.l.) v Číně (Russo 2007). Tyto dva rozdílné způsoby využití jedné rostliny s sebou nesou i rozdílné pěstební metody a přístupy, které mají zásadní vliv na kvalitu i kvantitu sklízeného květu. V evropských zemích se většina konopí pěstuje na poli pro průmyslové účely (Żuk-Gołaszewska & Gołaszewski 2018) a je používáno při výrobě přírodních vláken, lan a oděvů (Pickering et al. 2007). Semena slouží jako vynikající zdroj oleje díky svému obsahu a zastoupení nenasycených mastných kyselin (Mölleken et al. 1997), jsou významným zdrojem proteinu pro člověka a zvířata (Patel et al. 1994). Z tohoto důvodu je pěstební technologie technického konopí relativně dobře prozkoumána a její optimalizace směřuje k maximalizaci výnosu vlákna, semen, nebo celkové biomasy, která má značný potenciál k využití v energetickém průmyslu (Michal et al. 2023). Produkce rostlin konopí určená pro léčebné použití klade na takový materiál velmi vysoké legislativní nároky. Tyto se týkají zejména pěstebních a zpracovatelských procesů, obsahu a poměru aktivních látek. Vyprodukovaný květ léčebného konopí je nehomogenní materiál a jeho složení je ovlivněno řadou faktorů. Mezi faktory ovlivňující výnos, obsah kanabinoidů a jejich poměr patří zejména genetické založení rostliny, pěstební podmínky, fáze vegetace – zralost v době sklizně a v neposlední řadě podmínky skladování a následné manipulace s materiálem (Potter 2014). Faktory pěstebních podmínek je nemožné při polním pěstování řídit a nelze tak zaručit konzistenci produkce a její kvalitativní parametry. Z těchto důvodů se v případě produkce konopí určeného k léčebnému využití, jako nejvhodnější způsob jeví tzv. indoor pěstování, kdy kultivace rostlin probíhá v řízených podmínkách v uzavřené pěstební místnosti.

Oba způsoby využití, jak technické, tak léčebné, se prolínají i v rámci studia a výzkumu konopí prováděného v této práci. První pokusy při řešení této práce byly zaměřeny na alternativní výživu rostlin konopí se snahou prokázat vhodnost využití alternativního hnojení při zachování výnosových parametrů technického konopí. Zároveň byl brán zřetel na environmentální hledisko pěstování této plodiny a jejího následného využití. V dalších letech, díky legislativnímu vývoji v oblasti léčebného konopí, došlo k přesměrování výzkumu na řízenou produkci léčebného konopí pěstovaného pod státní licencí. Pro účely tohoto výzkumu byla vybudována unikátní pěstební laboratoř s řízenými podmínkami prostředí, kde probíhaly

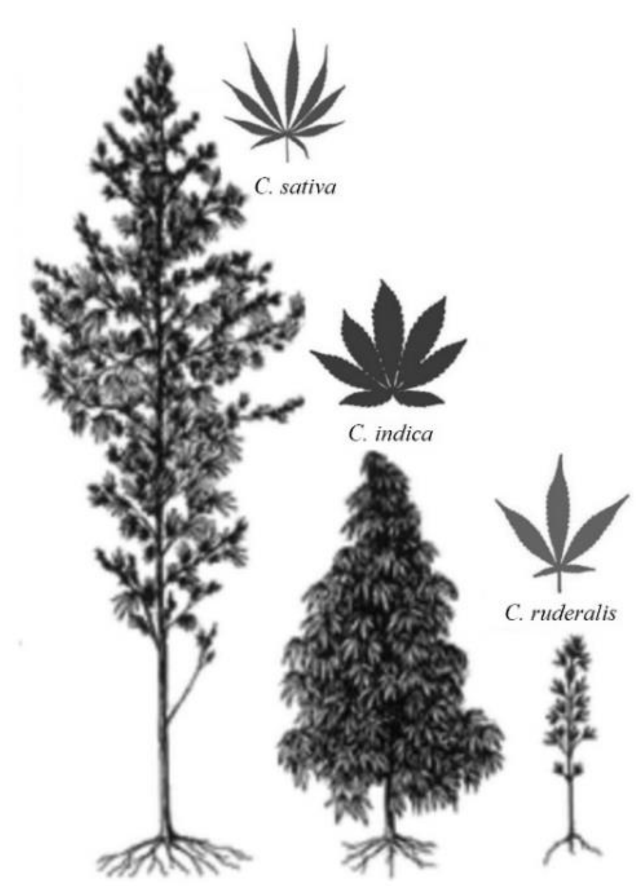
další experimenty zaměřené na výživu a vliv pěstebních podmínek na výnos květenství a biosyntézu sekundárních metabolitů.

2. LITERÁRNÍ PŘEHLED

2.1 Systematika

Genetická rozmanitost a plasticita rostlin konopí způsobuje poměrně obtížnou klasifikaci a ujednocení botanické nomenklatury této rostliny. Historicky byl popsán rod *Cannabis sativa* (Konopí seté) složený z jediného druhu (Linné 1753). Nedlouho poté Lamarck et al. (1783) rozdělil konopí na základě srovnávání psychoaktivních účinků, velikosti a tvaru listů na dva samostatné druhy. V rámci čeledi *Cannabaceae* (konopovité) se tak konopí dělilo na stávající druh *Cannabis sativa* a nově *Cannabis indica (Konopí Indické).* Roku 1924 bylo publikováno, že rostliny konopí rostoucí v oblastech Ruska nesplňují botanické charakteristiky ani jednoho z dosud popsaných druhů. Na základě toho byl popsán třetí samostatný druh *Cannabis ruderalis* (Konopí rumištní), charakteristický malým vzrůstem (Janischevsky 1924). Rozdíly jednotlivých druhů je možné vidět na obrázku č. 1.

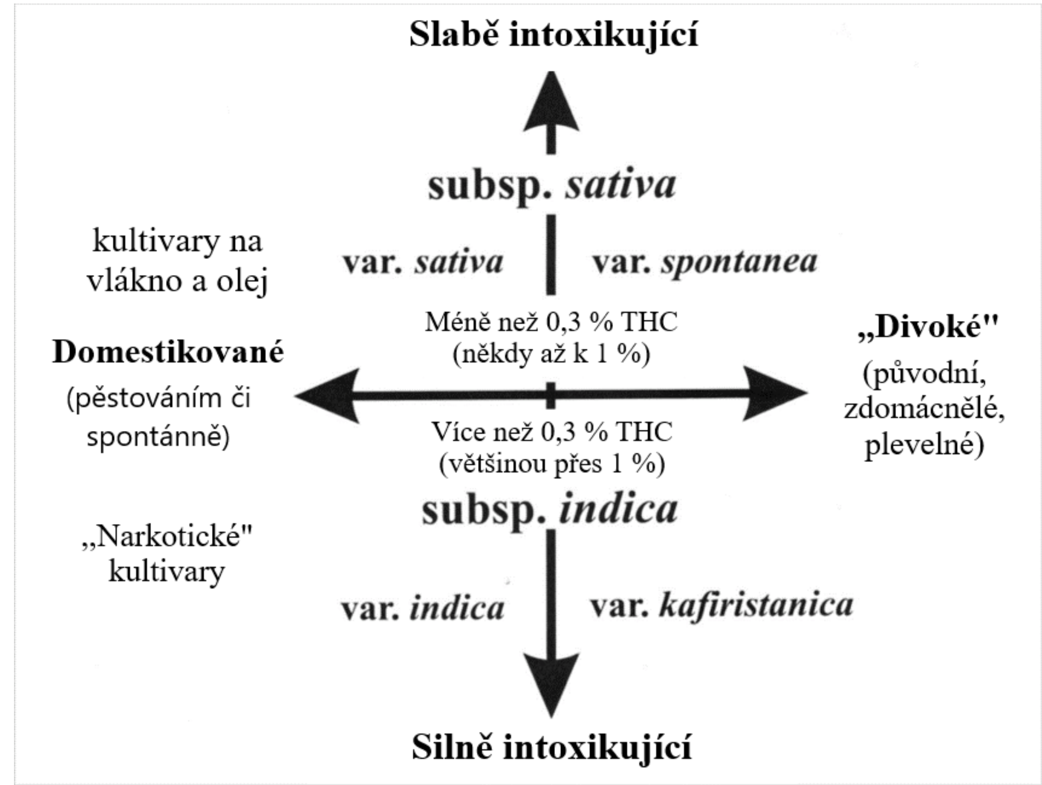
Obrázek č. 1. Druhy konopí (Hartsel et al. 2016)



Dále bylo členění rozšířeno Small et al. (1976) na základě chemických a morfologických rozdílů do čtyř taxonů, které popisuje obrázek č 2. Následující čtyři skupiny se odlišují zejména množstvím obsahových látek (THC a CBD) a zároveň je zohledněn původ, výskyt a rovněž účel, za kterým je daný taxon konopí pěstován.

1. *Cannabis sativa* L. subsp. *sativa* var. *sativa*,
2. *Cannabis sativa* L. subsp. *sativa* var. *spontanea* Vavilov,
3. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *indica* (Lam) Wehmer,
4. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *kafiristanica* (Vavilov) Small & Cronquist.

Obrázek č. 2 Chemotypy konopí (Small et al. 1976)



Small (2015) navrhl další možnost klasifikace pro domestikované konopí, které je ve shodě s Mezinárodním kódem nomenklatury kulturních rostlin. Rozdělení je následující:

1. Non-narkotické rostliny, domestikované pro vlákno a/nebo olejnatá semena v západní Asii a Evropě. Nízký obsah Δ^9-tetrahydrokanabinolu (THC) a vysoký obsah kanabidiolu (CBD)

2. Non-narkotické rostliny, domestikované ve východní Asii, hlavně Číně. Nízký až střední obsah THC a vysoký obsah CBD
3. Psychoaktivní rostliny, domestikované v jižní a střední Asii. Vysoký obsah kanabinoidů, především THC
4. Psychoaktivní rostliny, domestikované v jižní Asii (Afghánistán a sousední země), Vysoký obsah THC i CBD

Mimo výše uvedené třídy, byly navrženy další dvě hybridní:

5. Nenarkotické rostliny, hybridní kultivary mezi skupinami 1 a 2.
6. Psychoaktivní rostliny, hybridní kultivary mezi skupinami 3 a 4.

Dle doporučení (Zhang et al. (2018) by měl být monotypický rod *Cannabis* rozdělen na tři poddruhy *subsp. sativa, subsp. indica, and subsp. ruderalis.* Tento návrh je podložen studií, která se zaměřovala na analýzu sekvencí DNA rostlin konopí. Ke stejnému závěru na základě analýzy genetického kódu dospěl i McPartland (2018), který doporučuje rovněž dělení na poddruhy *subsp. sativa a subsp. indica.*

Výše zmíněné druhy konopí se mezi sebou mohou úspěšně křížit a produkují plodné hybridy (Beutler & Marderosian 1978). V současné době dochází k neustálé produkci nových hybridů ať už pro rekreační užití v zemích, kde to legislativa umožňuje, či pro využití rostlin konopí v medicíně. Rozdíly mezi jednotlivými kříženci je možné detekovat pomocí analýzy profilu obsahových látek, zejména kanabinoidů a terpenů. Tyto rozdíly zvané též chemotaxonomické markery umožňují screening jednotlivých hybridů a mohou tak odhalit podíl zastoupení jednotlivých poddruhů v daném hybridu (Hillig & Mahlberg 2004; Fischedick et al. 2010; Fischedick J 2015).

2.2 Botanický popis konopí

Konopí je jednoletá dvouděložná nahosemenná rostlina patřící do čeledi konopovité (*Cannabaceae*). Přirozeně jsou rostliny konopí dvoudomé, mohou se ale vyskytnout i jednodomí jedinci, zejména v oblastech krátkého dne. Jednodomé odrůdy byly následně

selektovány, aby se omezily problémy spojené s agronomickými zásahy a kvalitou produkce spojené s pohlavním dimorfismem rostlin (Berenji et al. 2013; Faux et al. 2013; Amaducci et al. 2015).

Pohlaví rostlin je možné rozeznat na základě jejich odlišností. Samčí rostliny jsou štíhlejšího, nižšího vzrůstu v porovnání s rostlinami samičími také označovanými jako hlavaté. Dalším rozdílem je dřívější dozrávání samčích rostlin oproti samičím, a to zhruba o 4 – 6 týdnů. Tyto rostliny produkují velké množství pylu, jehož přenos není závislý na opylování hmyzem. Samičí rostliny jsou významnější díky svému hospodářskému využití, většímu obsahu metabolitů a psychoaktivních látek. Jsou také více olistěné a statnější (Valíček 2003). Rostliny konopí se řadí mezi krátkodenní, kdy ke kvetení dochází při zkrácení fotoperiody pod 12 hodin (Knight et al. 2010). Celý životní cyklus konopí trvá v přirozených polních podmínkách 5 – 6 měsíců, přičemž kvetení nastupuje po 3 – 4 měsících růstu (Moliterni et al. 2004).

Květenství konopí setého se rozlišuje podle pohlaví rostlin. Květy nacházející se na samčích rostlinách jsou seskupeny do lat vyrůstajících na stopkách z úžlabí listů. V období květu produkují prašníky samčích květů pyl, který je pomocí větru roznášen až do vzdálenosti 12 kilometrů a je schopný oplodnění po dobu 14 dní. Po době květu, trvající 20 – 25 dní, tyto květy odumírají a samčí rostliny usychají (Miovský 2008; Ruman & Kalvaňová 2008).

Samičí květenství se nachází zejména v horních a koncových částech rostlin v několika vrstvách. Skládá se do hustě olistěných hroznů. Počátek květu nastává u samičích rostlin zpravidla o 3 – 10 dní později než je tomu u rostlin samčích. Doba kvetení se uvádí v rozmezí 15 – 25 dní. Květ samičí rostliny je složen ze dvou pouzdrových semeníků s jedním vajíčkem. Dále se květ skládá ze dvou blizen s pestíky. Listen obalující květenství je silně pokryt žláznatými trichomy (Ruman & Kalvaňová 2008; Ruman 2014).

Sirikantaramas et al. (2005) svým výzkumem potvrdily, že trichomy na samičím květenství jsou zodpovědné za vylučování pryskyřice bohaté na metabolity.

2.3 Sekundární metabolity konopné pryskyřice – terapeutický potenciál

Rostliny konopí obsahují rozličné množství fytochemických sloučenin, které lze zařadit do různých chemických skupin. Konkrétně identifikované sloučeniny v konopí spadají do skupiny kanabinoidů, terpenoidů, flavonoidů, steroidů, lignanů, alkaloidů a dalších. Mezi těmito látkami byla prokázána vzájemná synergie působení (ElSohly & Slade 2005; Mechoulam 2012; Russo 2019).

V rostlinách konopí bylo identifikováno více než sto jedinečných kanabinoidů, které se nejhojněji nachází v květenství neoplozených samičích rostlin. Zejména této skupině sekundárních metabolitů charakteristických pro konopí je přisuzován léčebný potenciál s tím, že mezi nejčetněji zastoupené kanabinoidy patří Δ9-tetrahydrocannabinol (THC) a cannabidiol (CBD). (Mechoulam et al. 1970; Fischedick J 2015).

V rostlině se kanabinoidy syntetizují a kumulují ve formě karboxylových kyselin. Dekarboxylace nastává v procesu skladování neenzymatickou cestou díky teplotě a oxidaci. Se zvyšující se teplotou roste zároveň rychlost dekarboxylace a dochází k uvolňování oxidu uhličitého (Shoyama et al. 1970; Kimura & Okamoto 1970). Kyselina tetrahydrocannabinolová (Δ^9-THCA), CBDA (kyselina canabidiolová) a CBCA (kyselina canabichromenová) jsou označovány za tzv. primární kanabinoidy právě z důvodu, že ostatní kanabinoidy jsou z těchto tří prekurzorů generovány převážně neenzymatickými degradačními procesy (ElSohly & Slade 2005).

Léčebný účinek kanabinoidů z konopí (fytokanabinoidy) byl na fyziologické úrovni lépe pochopen až díky objevu endokanabinoidního systému v lidském těle (Devane et al. 1992). Endokanabinoidní systém sestává z receptorů CB1 a CB2 a také z endogenně produkovaných kanabinoidů, které s nimi reagují. Mezi dva hlavní endokanabinoidy patří N-arachidonoyolethanolamin (AEA), též zvaný anandamind, a 2-arachidonoylglycerol (2-AG). Terapeutické účinky konopí vyplývají právě z interakce fytokanabinoidů obsažených v konopí s receptory endokanabinoidního systému. (Hanuš 2009; Šulcová 2015). Indikace, na které probíhal, či stále probíhá, výzkum v oblasti humánní medicíny, jsou nevolnost a zvracení (Duran et al. 2010), stimulace k jídlu při infekci HIV/AIDS (Whiting et al. 2015), chronická bolest (Lynch & Campbell 2011; Portenoy et al. 2012), spasticita v důsledku roztroušené sklerózy nebo paraplegie (Pooyania et al. 2010; Corey-Bloom et al. 2012), deprese (Selvarajah et al. 2010), úzkostná porucha a poruchy spánku (Russo et al. 2007; Bonn-Miller et al. 2014; Babson et al. 2017), glaukom (Järvinen et al. 2002), psychóza, dále poruchy způsobené v důsledku Tourettova syndromu či Parkinsonovy choroby (Lotan et al. 2014; Whiting et al. 2015) a v neposlední řadě zánětlivé onemocnění střev (Allegretti et al. 2013), či selektivní cytotoxický účinek a potenciální léčba různých typů rakoviny (Abrams & Guzman 2015; Heider et al. 2022).

2.4 Pěstování technického konopí – outdoor

Konopná biomasa se k energetickým účelům používá již po staletí. Dosud bylo v mnoha zemích navrženo komerční využití biomasy technického konopí pro energetické účely. Konopná biomasa lze využívat k výrobě tepla a energie přímým spalováním biomasy z celých rostlin, nebo lze energii vázanou na biomasu přeměnit na kapalná nebo plynná biopaliva, jako je bioetanol a bioplyn (Burczyk et al. 2008; Finnan & Styles 2013).

2.4.1 Nároky na prostředí

V případě technického konopí se bavíme o polním pěstování, též označovaném jako outdoor produkce. Stejně jako ostatní polní plodiny je produkce konopí zcela závislá na venkovních podmínkách a průběhu vegetace během pěstební sezóny.

Konopí je považováno za rostlinu, která je schopna přizpůsobit se mnoha stanovištím a podmínkám. Jedná se o plodinu mírného pásma, s relativně vysokými nároky na teplo a zásobenost půdy vodou. Za optimální pro růst konopí se považují teploty mezi 16 a 27 °C. Půda by měla být úrodná, dobře zásobená organickými látkami, středně hluboká až hluboká, hlinitá až hlinitopísčitá s neutrální až zásaditou reakcí v rozmezí pH 6 – 7,6. Je třeba se vyhnout půdám s vysokou salinitou, kyselostí a zhutněním (Amaducci et al. 2015; Kostuik & Williams 2019; Rehman et al. 2021; Visković et al. 2023).

Délka dne je v úzkém spojení s nástupem rostlin do fáze kvetení. Pokud jsou rostliny konopí osvětleny během dne méně než 14 hodin, dochází k urychlení nástupu fáze kvetení (Lisson et al. 2000). Z výzkumu provedeného (Hall et al. 2014) vyplývá, že dochází k nárůstu podzemní i nadzemní biomasy při prodloužené fotoperiodě. Výsledky ukazují na to, že konopí pěstované za účelem produkce biomasy by mělo být vystaveno osvětlení více než 13 hodin denně, aby bylo dosaženo maximálního výnosu. V případě, že rostliny nemají dostatek světla, nebo je perioda osvětlení příliš krátká, dochází u rostlin konopí ke snížení kořenové aktivity, což má za následek snížený příjem živin v důsledku redukce fotosyntézy. Takové rostliny nedosahují dostatečných výnosů. Citlivost na fotoperiodu je tedy zásadním rysem adaptace konopí na určitou oblast a určuje dobu kvetení, která je dominantním faktorem pro výnos konopí jak z hlediska kvantity, tak z hlediska kvality (Struik et al. 2000; Amaducci et al. 2008, 2012).

Vzhledem k proměnlivým a těžko předvídatelným podmínkám venkovního prostředí při outdoor pěstování není možno docílit požadované homogenity sklizně ve smyslu kvality a

množství obsahových látek. Sklizeň jde ve venkovním prostředí realizovat pouze jednou ročně. Navíc konopí rostoucí venku je též vystaveno vyššímu riziku v podobě škůdců a chorob (Potter 2014).

2.4.2 Agrotechnika pěstování technického konopí

2.4.2.1 Příprava půdy a setí

Honzík et al. (2012) uvádějí, že pro konopí je vhodné, aby předplodina zanechala půdu čistou, dobře zásobenou živinami, zejména dusíkem. Dále aby půda byla po předplodině kyprá a v bezplevelném stavu. Tyto podmínky splňují luskoviny, okopaniny, jeteloviny, vojtěška a kukuřice. Konopí není náročné na zařazení v osevním postupu a lze jej pěstovat bez chemické ochrany v případě, že nedochází k pěstování monokultury více let po sobě (Kostuik & Williams 2019).

Po předplodině se doporučují běžné agrotechnické zásahy, jako je podzimní hluboká orba (25 – 30 cm) se zapravením organických hnojiv. Ke konopí je vhodné kromě statkových hnojiv zapravit i digestát ze zemědělských bioplynových stanic(Sladký 2004; Visković et al. 2023). Doba výsevu konopí a příprava semenné vrstvy jsou významné z hlediska založení porostu. Důležité je také myslet na očekávané počasí předpovídané krátce po výsadbě pro případ nízké vitality sazenic. To je důležité zejména při použití tradičních metod zpracování půdy s přímým výsevem (Kostuik & Williams 2019).

Termíny výsevu jsou většinou určovány klimatickými faktory. Konopí klíčí již při teplotách 1 až 2 °C, nemělo by se však vysévat na začátku sezóny. Výsev by měl být odložen, dokud teplota půdy nedosáhne 10-12 °C, aby byl zajištěn rychlý vývoj, který zvyšuje jeho schopnost překonat plevele. Většina druhů konopí vyklíčí za 3 až 5 dní, pokud je zaseto do teplé půdy (>10 °C) s dostatečnou půdní vlhkostí (Visković et al. 2023).

Rozteč rostlin u konopí se řídí typem pěstovaného konopí a účelem jeho využití. Obecně platí, že hustě osázené konopí podporuje větší výšku rostlin a omezuje kvetení. Konopí pěstované primárně na vlákno se vysazuje těsně vedle sebe, aby se podpořilo prodlužování stonků a zároveň omezilo větvení, čímž se získají delší a silnější vlákna. Zejména při pěstování konopí na vlákno nebo na semena se konopí často vysazuje pomocí secích strojů s roztečí řádků od 7,6 do 17,8 cm. Doporučené výsevky se však značně liší, přičemž ideální hloubka výsevu se pohybuje od 1,9 do 3,2 cm v závislosti na typu půdy, přípravě půdy, dostupné vodě a termínu výsevu (Visković et al. 2023). Při pěstování na biomasu se doporučuje set do řádků běžných pro obiloviny o šířce 12,5 – 25 cm. Výsevek se v tomto případě pohybuje v rozmezí 50 – 80

kg/ha. Pro kombinovaný směr pěstování konopí na semeno i vlákno je doporučen výsevek 35 – 70 kg/ha (Honzík et al. 2012). Podle jiné studie se rozestupy mezi rostlinami konopí pěstovaného na vlákno pohybují od 20 do 40 cm (Bosca & Karus 1997; Liu et al. 2017). Maximálního výnosu stonků technického konopí při polním pěstování lze dosáhnout při hustotě rostlin 90 rostlin/m2 (van der Werf et al. 1995)

2.4.2.2 Hnojení rostlin konopí setého

Pokud jde o výživu konopím dusíkem, aplikační dávky se liší v závislosti na způsobu využití biomasy. Doporučené dávky se pohybují v rozmezí od 60 – 160 kg N/ha, v závislosti na půdních vlastnostech a klimatických podmínkách (Thouminot et al. 2017; Visković et al. 2023). Hnojení rostlin konopí dusíkem v dávce 150 kg N/ha zajišťuje optimální výšku rostlin, vyšší výnos semen, vyšší pevnost stonků (Johnson 2011) a celkově vysokou produkci biomasy (Barron et al. 2003; Vera et al. 2004, 2010; Finnan & Burke 2013). Struik et al. (2000) dosáhli v tříletém polním pokusu s konopím průměrného výnosu 14 t/ha. Příjem dusíku je největší v raných fázích růstu (Landi 1997). Dostatečné zásobení dusíkem je zajištěno, pokud se obsah dusíku v sušině rostliny pohybuje v rozmezí 5-6 % v mladých listech (Iványi 2005). Potřeba dusíku je závislá na odrůdě (Finnan & Burke 2013). Výsledky pokusů s využitím dusíku rostlinami ve variantách, kde byl aplikován dusičnan amonný, komunální čistírenský kal a hovězí hnůj ve shodné dávce 100 kg N/ha ukázaly vhodnost použití kalů jako organického hnojiva při pěstování konopí pro energetické účely. Naopak aplikace hovězího hnoje nedokázala zajistit maximální výnos biomasy (Alaru et al. 2013).

Fosfor z půdy je rostlinou přijímán rovnoměrně a jeho spotřeba se zvyšuje v období kvetení a dozrávání semen. Příjem fosforu rostlinami konopí se pohybuje v rozmezí 25-38 kg/ha v závislosti na výnosu (Vera et al. 2010). Dostatečná zásoba fosforu v konopí je při obsahu 0,5-0,6 % P v mladých plně vyvinutých listech. Potřebné množství fosforu pro vytvoření jedné tuny sušiny rostlinné hmoty je 1,7 kg (Iványi 2005; Iványi & Izsáki 2009). Vliv fosforu na produkci konopí byl dosud zkoumán jen v omezené míře a je uváděno, že příjem fosforu přepočteno pro oxid fosforečný (P_2O_5) se pohybuje v rozmezí 52 až 67 kg/ha (Amaducci et al. 2015). Jako doporučená dávka fosforu v závislosti na využití produkce je 56 – 67 kg/ha (Visković et al. 2023). Pokud je půda bohatá na fosfor, lze hnojení touto živinou vynechat (Iványi & Izsáki 1996).

Draslík je důležitou živinou pro tvorbu stonků a vláken konopí. Vzájemné působení dusíku a fosforu zvyšuje kvalitu vláken a výnos konopných stonků. Draslík rostliny konopí

přijímají především v období intenzivního růstu (Landi 1997; Aubin et al. 2015). Požadavky rostlin konopí na draslík jsou vysoké. Běžně se pohybují v rozmezí 75 až 100 K kg/ha, někdy dosahují ale hodnot až 336 kg/ha (Visković et al. 2023). Naopak jiný zdroj uvádí, že nebyla zjištěna korelace mezi výnosem konopí a dávkou draslíku ani jeho hladinou v půdě. Autoři dospěli k závěru, že konopí má nižší nároky na K než jiné plodiny, a navrhují roční potřebu 65 kg ha-1 (Finnan & Burke 2013; Amaducci et al. 2015). Luxusní odběr byl pozorován (podobně jako u trav) při vysoké hladině K v půdě. Konopí je však schopno využívat draslík z hlubších vrstev půdního profilu. Konopí koncentruje většinu draslíku ve stonku, a to až 70-75 % (Finnan & Burke 2013). Jako optimální obsah draslíku v rostlině je uvedeno 2,7-3,0 % (Iványi & Izsáki 2009).

Pro půdy s neutrálním pH je nezbytný také vápník, a to kvůli jeho vysoké spotřebě při růstu kořenového systému, stonků a semen. Uvádí se, že potřeba vápníku je spolu s dusíkem a draslíkem pro konopí z hlediska makronutrientů dominantní. V závislosti na výnosu se spotřeba vápníku pohybuje v rozmezí 151-227 kg/ha při výnosech 8-10 t/ha. V půdách s jeho nedostatkem je nutné kompenzační hnojení. Rostliny konopí přijímají vápník především na konci vegetačního období. (Landi 1997; Johnson 2011)

Hořčík se podílí na zajištění dobrého zdravotního stavu rostlin a je přijímán rostlinami konopí v rozmezí 36-54 kg K/ha v závislosti na výnosu (Landi 1997).

Z mikroživin konopí zpočátku akumuluje zinek a měď do vegetativních orgánů rostliny; později je transportuje do generativních orgánů, zatímco železo, bór a mangan se akumulují především ve vegetativních orgánech (Velechovský et al. 2021).

Z literatury vyplývá, že vedlejší suroviny ze zemědělských bioplynových stanic je možné aplikovat na zemědělskou půdu za účelem výživy a hnojení rostlin. Na základě dostupných znalostí o výživě rostlin technického konopí se tyto suroviny nabízejí jako vhodná alternativa k běžně používaným minerálním hnojivům (Kolář et al. 2010; Makdi et al. 2012; Coelho et al. 2018).

2.4.2.2.1 Digestát

Jedná se o materiál zbývající po anaerobní digesci (kvašení), které probíhá bez přístupu vzduchu a jehož hlavním cílem je výroba bioplynu. Digestát obsahuje širokou škálu živin a lze jej využít jako organické hnojivo s pozitivním vlivem na půdní organickou hmotu (Nkoa 2014; Tambone et al. 2015; García-Sánchez et al. 2015). Jeho recyklace v zemědělském systému hraje důležitou roli tím, že snižuje používání minerálních hnojiv (Alburquerque et al. 2012). Má

podobný obsah dusíku v čerstvé hmotě jako kejda (0,2-1 %), ale vyšší hodnotu pH v rozmezí 7-8 (Faisal-Cury & Menezes 2006; Kratzeisen et al. 2010; Makdi et al. 2012). Obsah živin v sušině digestátu je uváděn následovně: celkový N: 3,1-14 %, P: 0,6-1,7 % a K: 1,9-4,3 % (Möller & Müller 2012). Aplikace digestátu jako organického hnojiva na zemědělskou půdu je již považována za standardní způsob jeho využití (Teglia et al. 2011; Lijó et al. 2015). Studie ukazují, že použití digestátu ze zemědělských bioplynových stanic (BPS) snižuje environmentální rizika, která jsou obecně spojena s používáním minerálních hnojiv, a zároveň je dosahováno srovnatelných výnosových parametrů zemědělských plodin, jako jsou *Medicago sativa* L. a *Triticum aestivum* L a *Cannabis sativa* L. Na druhé straně je obsah živin závislý na vstupních surovinách do procesu anaerobní digesce a nelze obecně říci, že použitím vedlejších produktů BPS dosahujeme lepších výnosů polních plodin (Möller & Müller 2012; Koszel & Lorencowicz 2015; Sogn et al. 2018; Tsachidou et al. 2019; Velechovský et al. 2021; Michal et al. 2023). Mechanickou separací digestátu vznikají dvě homogenní složky, tzv. pevná fáze digestátu (separát) a kapalná fáze digestátu tzv. fugát.

2.4.2.2.2 Separát

Obsah makroživin a mikroživin v separátu je rovněž ovlivněn složením vstupních surovin do fermentačního procesu a dobou zdržení surovin ve fermentoru (Kolář et al. 2010; Abubaker et al. 2012). Obsah dusíku v separátu v rozmezí 2,2 - 3 %, obsah fosforu 1,9 % a draslíku 3,6 % v sušině. Obsah sušiny se pohybuje od 20 do 30 % s vysokým obsahem fosforu a uhlíku (Rehl & Müller 2011; Makdi et al. 2012; Möller & Müller 2012; Wellinger et al. 2013; Tambone et al. 2015). Separát je možné kompostovat, sušit a spalovat, nebo přímo aplikovat do půdy (Kolář et al. 2010; Tambone et al. 2015). Vzhledem k chemickému složení a fyzikálním vlastnostem může aplikovaný separát do půdy pozitivně ovlivnit její strukturu a výnos biomasy (Makdi et al. 2012; Dubský et al. 2019).

2.4.2.2.3 Fugát

Fugát je označován jako zředěný roztok obsahující širokou škálu živin ve formě přijatelné pro rostliny (Kolář et al. 2010). Fugát se jeví jako vhodná surovina pro aplikaci na ornou půdu v průběhu vegetace a lze využít jeho hnojivých a závlahových účinků (Kolář et al. 2010; Makdi et al. 2012). Schievano et al. (2009) charakterizují fugát jako organické hnojivo, které obsahuje minerální živiny spolu s organickou hmotou. Fugát představuje 80 – 90 % hmotnosti digestátu. Jeho sušina se pohybuje od 2 do 6 % a má vysoký obsah amoniakálního

dusíku a draslíku (Wellinger et al. 2013; Tambone et al. 2015; Vondra et al. 2018). Literatura uvádí následující koncentrace základních živin v suché hmotě: N (6,6 - 24,1 %, průměr 11,7 %), P (0,81 - 3,28 %, průměr 1,74 %) a K (0,81 - 17,35 %, průměr 6,15 %) (Coelho et al. 2018). Další studie uvádí hodnoty v sušině následovně: 7,7 - 9,2 % N, 0,4 - 0,7 % P a 3,9 % K (Möller & Müller 2012). Protože se podíly N-P-K v jednotlivých digestátech liší, je nutné před vlastní aplikací na pole zajistit analýzu konkrétních digestátů (Coelho et al. 2018). Ostatní živiny jsou přítomny ve výrazně nižších koncentracích (Holm-Nielsen et al. 2009).

2.4.2.3 Sklizeň a výnos

Sklizeň se dá označit za problematickou fázi pěstování konopí vzhledem ke stále obtížné dostupnosti specializované mechanizace, která by byla schopna efektivně podříznout pevné stonky s vysokým obsahem vlákna, a sklidit obecně hustý porost konopí (Sladký 2004; Huang et al. 2017)

Při sklizni konopí určeného na výnos biomasy je vhodné porost sklízet v období, kdy obsahuje přibližně 25 % sušiny, což v podmínkách ČR odpovídá období plné zralosti květu (Honzík et al. 2012)

Prade et al. (2011, 2012) ve svých studiích uvádějí, že při pěstování konopí určeného k energetickým účelům pro výrobu bioplynu s cílem maximálního výnosu rostlinné biomasy, je ideální termín sklizně na podzim. V podmínkách střední až severní Evropy doba sklizně odpovídá termínu od počátku září až konci října.

Vzhledem k náročnosti sklizně konopí a různým způsobům využití porostů, se volí odlišné způsoby sklizně prováděné buďto ručně, či mechanizovaně, případně děleně (Sladký 2004).

Z literárních zdrojů vyplývá, že konopí je schopné dobře růst a dosahovat vysokých výnosů biomasy na hektar ve velice rozmanitých agroekologických podmínkách spolu s velice nízkým množstvím vstupů a zásahů, jako je hnojení, agrotechnika a chemická ochrana rostlin (Struik et al. 2000; Amaducci et al. 2012; Zatta et al. 2012).

Při sklizni konopí v říjnu, což odpovídá termínu uváděnému autory v přechozí kapitole, byl na základě dvouletého experimentu zjištěn průměrný výnos suché hmoty 15 tun na hektar (Kreuger et al. 2011).

Prade et al. (2011) uvádějí průměrný výnos v oblastech severní Evropy 14 t/ha při pěstování konopí pro využití v bioplynových stanicích.

V podmínkách České republiky se výnosy konopí setého pohybují v rozmezí 8 – 12 tun suché hmoty nadzemních částí rostlin na hektar. Tyto hodnoty jsou průměrem výnosů od několika autorů při pěstování na odlišných stanovištích, ale vždy s hnojením dusíkem v dávce odpovídající 80 – 120 kg/ha (Honzík et al. 2012).

2.5 Pěstování léčebného konopí - indoor

Indoor kultivace se stává stále sofistikovanější, kdy se běžně využívá automatizovaných systémů osvětlení, ventilace, zavlažování a komplexní výživy. Aktuální znalosti o indoor produkci konopí se dají stále získat hlavně z tzv. šedých zdrojů (Vanhove et al. 2011; Caplan et al. 2017a). Jako nespornou výhodu indoor pěstování lze uvést, že pěstitel je schopen díky řízeným podmínkám ovlivnit délku vegetace, potažmo vegetativní (růstovou) fázi a fázi generativní (květovou). Tyto fáze pěstitel dokáže řídit zejména pomocí délky fotoperiody, a tím i dosáhnout většího počtu pěstebních cyklů za jeden kalendářní rok (Farag & Kayser 2015). Při uplatnění moderních pěstebních postupů lze dosáhnout průměrně tří až šesti sklizní za rok (Leggett 2006).

V indoor pěstebním zařízení můžou být rostliny konopí pěstovány pomocí mnoha systémů. V této práci se ovšem detailním rozdělením a popisem rozdílů jednotlivých systémů nebudu zabývat, a proto uvedu pouze základní dělení.

Obecně pěstování indoor probíhá v hydroponických systémech, buďto bez použití pěstebního substrátu (bez obsahu zeminy), nebo za využití půdního substrátu. V prvním případě jsou rostliny fixovány v inertním médiu (minerální vlna tzv. rockwool, kokosová vlákna, perlit či keramzit) a výživa je spolu se závlahou zajištěna formou živných roztoků aplikovaných ke kořenům rostlin (Vanhove et al. 2011). Druhým způsobem je pěstování v substrátech na bázi zeminy a rašeliny. Tyto mohou být již vyhnojené na celou vegetační dobu či její počáteční fáze, a v takovém případě se provádí přihnojování rovněž formou živného roztoku během růstu rostlin. Uvádí se, že využití hydroponických systémů nevykazuje zvýšení produktivity rostlin léčebného konopí ani jejich potenci (množství aktivních metabolitů) a navíc jsou takovéto systémy komplikovanější a pracovně náročnější (Potter 2014). Stejně tak Vanhove (2014) zmiňuje, že v indoor pěstování stále ještě převládá využívání půdních pěstebních substrátů na rozdíl od hydroponických systémů využívajících inertních substrátů z důvodu nižší náročnosti na odbornost pěstitele. V pokusu, kdy byl sledován vliv složení pěstebního substrátu na růstové parametry, výnos biomasy, obsah CBD a CBDA bylo zjištěno, že složení substrátu má

signifikantní vliv na tyto sledované parametry (Burgel et al. 2020). Autoři konstatují, že použití rozdílných substrátů nemá signifikantní vliv na obsah sledovaných kanabinoidů (CBD, CBDA) a má pouze částečný vliv na morfologii rostlin s tím, že tento efekt je odrůdově specifický. Pro studium byly použity byly následující substráty: standardní rašelinový mix, rašelinový mix s 30 % náhradou dřevní štěpky jehličnatých stromů (borovice, smrk) a třetí sledovaný substrát byl čistě z kokosového vlákna.

2.5.1 Pěstování a mikroklima

Pěstební proces léčeného konopí lze rozdělit do dvou základních fází, které se řídí periodou světla během dne a noci. U konopí jakožto krátkodenní rostliny přirozeně kvetoucí na podzim, je kvetení indukováno specializovanými fotoreceptorovými proteiny tzv. fytochromy. Vegetativní (růstová) fáze, by měla být udržována v rozmezí 18 – 24 hodin světla a 0 – 6 hodin tmy a trvá po dobu 1 – 4 týdnů. V tomto období rostlina vyžaduje relativní vzdušnou vlhkost v rozmezí 70 – 80% při teplotě 21 - 28 °C (Vanhove et al. 2011). Oproti tomu fáze generativní (květová) je vyvolána zkrácením délky dne na 12 hodin světla spolu s 12 hodinami tmy. První květy začínají rašit zhruba týden po změně fotoperiody. Rovněž dochází ke zpomalení růstu stonků a listů až do chvíle úplné stagnace růstu přibližně po 3 týdnech indukce kvetení. V publikaci, kde bylo sledováno 200 odrůd konopí s vysokým obsahem THC se ukázalo, že medián i průměrná doba kvetení je 57 dní. U 88 % odrůd byla doba kvetení v rozmezí 7 - 9 týdnů (Toxicodependência 2012). Nároky na teplotu jsou obdobné jako u fáze vegetativní, ale je doporučováno snížení relativní vzdušné vlhkosti na maximální hodnotu 40 %. Při vyšší vlhkosti je rostlina, zejména květenství, náchylná k houbovým chorobám (Vanhove et al. 2011, 2012). Chandra et al. (2008) uvádějí, že při koncentraci CO_2 350 ± 5 μmol/mol (678 mg/m^3) by měla být relativní vlhkost vzduchu udržována v rozmezí 55 ± 5 %.

Farag & Kayser (2015) ve své publikací uvádějí, že pěstitel je schopný díky řízení fotoperiody ovlivnit délku vegetativní a generativní fáze rostlin konopí a dosáhnout tak většího počtu pěstebních cyklů za jeden kalendářní rok. Oproti tomu při pěstování na poli je přirozeně možná pouze jedna sklizeň ročně. Tuto informaci potvrzuje i Leggett (2006), který tvrdí, že při uplatnění moderních pěstebních postupů lze v kontrolovaných podmínkách dosáhnout 3 – 6 sklizní za kalendářní rok.

2.5.2 Osvětlení

Kromě závislosti vegetační fáze na délce fotoperiody je při pěstování rostlin konopí v kontrolovaných podmínkách nutné brát v úvahu rovněž vlnovou délku světelného zdroje. V růstové fázi je pro rostliny optimální rozpětí vlnové délky 420 - 460 nm, což spadá do spektra modrého světla. Ve fázi generativní je jako optimální uváděno spektrum červené, dobře absorbované chlorofylem, v rozsahu vlnových délek 600 – 680 nm (Adams 2012).

Jako zdroje světelného záření se v indoor pěstebních prostorech využívají výbojky metal - halogenidové (MH) pro fázi vegetativní a pro fázi generativní nejčastěji vysokotlaké sodíkové výbojky tzv. HPS (high pressure sodium). HPS výbojky nejsou pro vegetativní fázi vhodné právě díky nedostatku vyzařování modrého světla, což má za následek nevyrovnaný růst stonků a listů, a vede k morfologickým rozdílům u jednotlivých rostlin (Tibbitts et al. 1983, Wheeler et al. 1991). Deficit modrého světla zvyšuje aktivitu transkripčních faktorů, které se podílejí na aktivaci genů podílejících se na biosyntéze auxinu, což má za následek zrychlený dloužívý růst. Modré světlo tak podporuje růst nových výhonků, elongaci internodií a tím i zvyšuje expanzi listů ke světelnému zdroji (Huché-Thélier et al. 2016). Rozdíl mezi těmito dvěma druhy výbojek je, krom vlnové délky vyzařovaného světla, také v plynu v nich obsaženém. HPS i MH výbojky je možné používat současně. Vzhledem k vysoké produkci tepelné energie těchto typů svítidel je nutné zajistit dostatečné odvětrávání pěstebních prostor na optimální teplotu pro danou fázi vegetace. Při nedostatečné chladící kapacitě vzduchotechnického zařízení může dojít k negativnímu ovlivnění růstu zejména v důsledku zpomalení fotosyntézy pro níž je u konopí uváděna optimální teplota v rozmezí 25 °C – 30 °C (Bazzaz et al. 1975). Další možností osvětlení jsou technologie LED (Light-Emitting Diode), které jsou, dle některých autorů, účinnější (Bessho & Shimizu 2012). Tyto diody vyzařují méně tepla, nevyžadují předřadníky jako výše zmíněné výbojky, a poskytují lineární fotonový výstup s dobrou vlnovou specifitou vyzařovaného světla (Akutsu & Yoshiro 1981; Szántó 1987; Massa et al. 2008). Výsledky různých experimentů ovšem neukazují jednoznačné závěry ohledně použití ideálního zdroje světla pro pěstování konopí. Podle Magagnini et al. (2018) rostliny pěstované pod HPS světly dosahovaly vyššího vzrůstu i výnosu. Oproti tomu rostliny pěstované pod LED svítidly měly vyšší obsah THC a CBD. Toto tvrzení potvrzuje i výzkum Namdar et al. (2019), kde bylo publikováno zvýšení koncentrace CBGA v květenství konopí v porovnání s HPS výbojkami.

2.5.3 Spon rostlin v indoor pěstebním systému

Téma optimálního sponu rostlin léčebného konopí pěstovaného v indoor podmínkách je v současné době málo diskutovanou oblastí ve vědecké literatuře. Studie publikována Toonen et al. (2006) nabízí přehled 77 ilegálních pěstíren konopí, kde hodnotí způsob indoor pěstování v těchto prostorech. Vzhledem k faktu, že většina informací o indoor pěstování konopí pochází z neoficiálních zdrojů (Vanhove 2014; Caplan et al. 2017a), lze tuto studii považovat za jakýsi náhled do pěstitelské praxe v podmínkách indoor pěstíren. Ze studie (Toonen et al. 2006) vyplývá, že nejběžnější hustota rostlin v ilegálních pěstírnách v Nizozemí je 9 – 16 rostlin/m^2. Tento spon byl zjištěn u 30 sledovaných pěstíren. Ve 20 případech se jednalo o spon 17 – 24 rostlin/m^2. Obecně byl stanoven průměrný počet rostlin na 18,1 rostlin/m^2 s tím, že medián sponu byl 15,3 rostlin/m^2. Na základě výše zmíněné studie provedli (Vanhove et al. 2011) experiment, který měl za cíl zhodnotit spon rostlin konopí (16 a 20 rostlin/m^2), jakožto jeden z faktorů určujících výnos a kvalitu květenství. Autoři došli ke zjištění, že výnos květenství na rostlině je vyšší při sponu 16 rostlin/m^2, nicméně pokud se hodnotí výnos z plochy 1 m^2, nedochází ke statisticky významnému rozdílu při porovnání těchto dvou sponů. Jako možné vysvětlení tohoto jevu uvádí (Van der Werf 1997) to, že si rostliny při menším sponu nekonkurují z hlediska prostoru a tím pádem mohou efektivněji vstřebávat světlo, jehož intenzita je významným faktorem při tvorbě biomasy květů (Potter 2009; Potter & Duncombe 2012; Rodriguez-Morrison et al. 2021). Vanhove et al. (2012) hodnotí vliv sponu obdobně. Zatímco při sledování výnosu květenství v g/rostlinu došlo ke statisticky významnému nárůstu ve variantě 12 rostlin/m^2 oproti 16 rostlinám/m^2, při hodnocení výnosu květenství z 1 m^2 se tento rozdíl stírá. Autor tedy dochází ke stejným závěrům jako předchozí studie. Meta - analýza dat hodnotící faktory určující výnos konopí v indoor podmínkách prezentuje závěr, že pro maximalizaci výnosu je vhodné zvolit spon $\leq$ 12 rostlin/m^2 (Backer et al. 2019). Přístupnost a intenzita světla jsou tedy limitujícím faktorem pro optimalizaci výnosu na určité ploše, stejně tak jako vliv konkrétní odrůdy (Toonen et al. 2006; Vanhove et al. 2011, 2012; Potter & Duncombe 2012). Pro potřeby komerčních pěstitelů je nutné stanovit optimalizované limity sponů rostlin pro jednotlivé odrůdy konopí pěstovaného indoor tak, aby bylo možné efektivně využít pěstební prostor a optimalizovat výnos květenství (Jin et al. 2019).

2.5.4 Množení rostlinného materiálu

Rostliny konopí se přirozeně šíří osivem, ovšem je možné využít vegetativního množení (řízkování) a rovněž se rozvíjejí metody množení in vitro. Vegetativní množení je v současnosti

preferovaný způsob množení rostlin konopí. Jedná se o metodu, kdy se z matečních rostlin, které jsou kultivované právě za účelem poskytování řízků, odebírají terminální výhonky, které se nechají zakořenit a dále se dopěstují do plné zralosti. Tato metoda je vhodná nejen díky snížení nákladů při pořizování nových rostlin, ale rovněž poskytuje geneticky uniformní jedince s vyváženým růstem a relativně vyrovnanou produkcí kanabinoidů na rozdíl od rostlin pěstovaných ze semen (Coffman & Gentner 1979; Lata et al. 2008, 2009, 2011; Potter 2009; Farag & Kayser 2015).

Při pokusech Caplan et al. (2018) byla vyhodnocována úspěšnost zakořenění při různých metodách řízkování z matečních rostlin a ošetření samotných klonů. Dle výsledků studie se jeví jako nejvhodnější řízkování z terminálních výhonů mateční rostliny s tím, že řezům jsou ponechány 3 plně vyvinuté listy a jejich kořenění je podpořeno kořenícím hormonem IBA (Indol – 3 – Butyric Acid) o koncentraci 0,2 % ve formě gelu, do kterého se řezy namáčí. Tento způsob prokázal 2,1x vyšší úspěšnost zakořenění, než při použití přírodního extraktu z vrbových kořenů, který je rovněž určen jako kořenící stimulant. V případě, že se řízkům odstranila 1/3 listové plochy z důvodu snížení transpirace byla úspěšnost kořenění 53 % oproti 71 % u řízků, kterým byla ponechána celá listová plocha. Tento zásah ovšem neměl vliv na kvalitu kořenů. Pokud je nutné z důvodu zvýšeného proudění vzduchu, či klimatických podmínek snižovat plochu listů kvůli nadměrné transpiraci, doporučuje se odstranění celého listu a ponechání pouze dvou plně vyvinutých listů spíše, než odstranění 1/3 plochy listů z celého klonu.

2.5.5 Výživa rostlin léčebného konopí

V oblasti výživy léčebného konopí je pouze malé množství vědeckých publikací založených na experimentálně ověřených postupech. Optimální výživa rostlin a jejich hnojení během vegetačního cyklu jsou významnými parametry v procesu pěstování rostlin pro zajištění jejich optimálního růstu, dosažení maximálního výnosu a požadovaných hodnot obsahu kanabinoidních látek (Caplan et al. 2017a, 2017b).

Z literatury týkající se technického konopí vyplývá, že výživa rostlin, potažmo některá z živin, může ovlivňovat konečný obsah kanabinoidních látek v rostlině stejně jako její celkový výnos (Coffman & Gentner 1975; Bócsa et al. 1997). Tyto informace naznačují, že obdobný význam by výživa mohla hrát i pro léčebné konopí pěstované v kontrolovaných podmínkách. Nicméně z literatury rovněž vyplývá, že technické konopí bylo selektivně šlechtěno na zcela odlišné vlastnosti, zejména na vlákno a semeno. Dalším faktorem je zcela odlišný způsob

pěstování, kdy technické konopí je pěstováno na poli. Tyto skutečnosti činí velkou obtíž při snaze vztahovat znalosti o pěstební technologii a zejména výživě těchto rozdílných, řekněme typů konopí (Amaducci et al. 2015). Obdobné závěry vyplývají i z dalších výzkumů, kde bylo publikováno, že geneticky se oba typy rostlin jen velmi málo podobají (Hillig & Mahlberg 2004; van Bakel et al. 2011). V oblasti výživy technického konopí je známo, že rozsah optimální dávky dusíku se pohybuje v rozmezí 50 – 200 kg/ha (Vera et al. 2004; Aubin et al. 2015). Tyto hodnoty jsou obtížně interpretovatelné pro indoor hydroponické pěstování. Studie probíhající s využitím technologií podobných těm využívaným pro indoor pěstování konopí uvádějí koncentraci dusíku v rozmezí 190 – 400 mg/l. Tato hodnota byla publikována v souvislosti s hnojením rajčat organickým hnojivem, která byla pěstována ve skleníku (Surrage et al. 2010; Zhai et al. 2009).

Informace ohledně výživy rostlin konopí pěstovaných indoor jsou omezené a ve většině případů se čerpají z neoficiálních zdrojů, jako jsou pěstitelská online fóra, stránky tzv. growshopů. Ty rovněž distribuují hnojiva a aditiva často využívaná ilegálními pěstiteli. Jedná se tedy o tzv. šedé zdroje informací, které se v současnosti teprve začínají systematickým výzkumem vědecky ověřovat (Vanhove 2014; Caplan et al. 2017a).

Informace z těchto šedých zdrojů jsou často vágního charakteru, kde je uveden pouze obsah makro a mikro prvků. Zpravidla se jedná o N, P, K hnojiva s přídavkem Mg, Ca, S spolu s často nespecifikovanými mikroprvky pro fázi vegetativní. Ve fázi generativní bývá navýšen podíl P a K. Velké množství výživných schémat doporučovaných prodejci hnojiv doporučuje i používání aditiv pro zlepšení růstu kořenů a zejména zvýšení výnosu. Produkty ze skupiny aditiv často neposkytují dostatečné informace o složení, a tudíž je jejich efekt zpochybnitelný (Vanhove, 2014).

Bernstein et al. (2019) ve své práci tvrdí, že minerální výživa je jedním z majoritních faktorů ovlivňujících růst a schopnost rostlin konopí produkovat sekundární metabolity. Tvorba sekundárních metabolitů je v rostlině založena primárně geneticky, což určuje potenciál rostliny tyto látky tvořit. Oproti tomu environmentální faktory ovlivňují kvalitu, množství a distribuci sekundárních metabolitů v rostlině. Úrodnost půdy potažmo minerální výživa rostlin jsou tedy hlavními faktory, které ovlivňují vývoj rostlin a jejich metabolismus. Rostliny v největším množství odebírají dusík (N), fosfor (P) a draslík (K), které tím pádem hrají zásadní roli v jejich metabolismu. Podle výzkumu je patrné, že makroprvky mají vliv na produkci sekundárních metabolitů u aromatických rostlin, zejména na produkci a profil terpenů u Šalvěje lékařské (*Salvia Officinalis)* (Piccaglia et al. 1989; Rioba et al. 2015). Rovněž salinita půdy a

zastoupení mikroživin mohou ovlivňovat profil sekundárních metabolitů (Preety et al. 2000; Bernstein et al. 2010). Z toho lze usuzovat, že obdobný vliv bude mít výživa i na kanabinoidy, přestože se jedná o specifickou skupinu sekundárních metabolitů. V rostlinné říši se vyskytují látky, které mají obdobnou funkci jako kanabinoidy v konopí. Tyto látky se nazývají kannabimimetika a jsou chemicky odlišné, produkují se v jiných (různých) rostlinných částech, ale mají společnou schopnost vázat se na kanabinoidní receptory, či jinak vstupovat do interakce s enzymy zapojenými do metabolizace v rámci endokanabinoidního systému. Nicméně jedná se o širokou skupinu látek (terpeny, deriváty mastných kyselin, polyfenoly a další), které se nachází v mnoha rozličných rostlinách (Smil písečný - *Helichrysum umbraculigerum,* Mechorost *Radula marginita*, Amazonská liána z čeledi bobovité *Machaerium multiflorum,* Netvařec křovitý - *Amorpha fruticosa* a další). Určit souvztažnost mezi výživou těchto rostlin a tvorbou fytokanabinoidů či kanabimimetik je v současné době díky nedostatku vědeckých informací na toto téma téměř nemožné (Gertsch et al. 2010; Kumar et al. 2019; Gonçalves et al. 2020)

Pří sledování vlivu výživy obohacené o 15 % N, P, K oproti kontrolní variantě, kde bylo v zálivce dodáno 65 ppm N, 17 ppm P a 90 ppm K, bylo zjištěno, že vliv jednotlivých živin se projevuje specificky v jednotlivých rostlinných orgánech a rovněž se liší jejich vliv na jednotlivé kanabinoidy. Obohacení živného roztoku o fosfor (20 ppm) neovlivnilo produkci THC, CBD, CBN ani CBG v květenství, na celé rostlině ovšem došlo k poklesu koncentrace THC v okvětních listech o 16 %. Navýšení dávky o všechny tři hlavní makroživiny (75 ppm N, 20 ppm P, 104 ppm K) vedlo k navýšení koncentrace CBG v květenství o 71 % v porovnání s kontrolní variantou. Naopak hodnoty CBN v tomto případě klesly o 38 % v květenství a o 36 % v okvětních listech. V případě akumulace prvků se ukázalo, že vyšší koncentrace P vedla k navýšení obsahu Ca v květenství ze 13,2 na 29, 4 mg/g (nárůst o 55 %). Zvýšená suplementace P rovněž způsobila zvýšený obsah zinku v květech, okvětních listech i samotných listech. Zároveň způsobila pokles hmotnosti biomasy okvětních listů o 10 %. Zajímavé rovněž je, že došlo ke zkrácení délky rostlin o 23 %. Naopak zvýšená dávka NPK způsobila navýšení celkové hmotnosti rostlin o 41 %. Zvýšená dávka fosforu nevedla k navýšení koncentrace žádného ze sledovaných kanabinoidů (THC, CBD, CBN, CBG) v květech léčebného konopí (Bernstein et al. 2019b). Při dávkách fosforu přesahujících 5 mg P/l došlo ke snížení obsahu THCA a CBDA v květenství až o 25 % (Shiponi & Bernstein 2021a). Při navýšení koncentrace na 30 mg P/l byly pozorovány optimální fyziologické procesy rostlin ve vegetativní fázi (rychlost

fotosyntézy, stomatální vodivost a transpirace). Při této hladině bylo rovněž dosaženo 80 % maximálního výnosu květenství (Shiponi & Bernstein 2021b).

Vliv výživy na výnos květenství a obsah kanabinoidů potvrzují i další vědecké práce. Caplan et al. (2017a) ve své studii zabývající se optimalizací úrovně hnojení v průběhu vegetativní fáze pěstebního cyklu léčebného konopí pěstovaného v substrátech na bázi kokosového vlákna uvádějí, že výnos biomasy rostlin a obsahu kanabinoidů narůstal až do maxima při použití koncentrace celkového dusíku 389 mg N/l. Nicméně tato studie byla zaměřena pouze na vegetativní část růstového cyklu rostlin, ale produkce kanabinoidů dosahuje maxima až ve fázi generativní, kdy dochází k tvorbě květů a trichomů, kde je koncentrace kanabinoidů nejvyšší (Vogelmann et al. 1988; Muntendam et al. 2012). Aplikované rozpětí dávek dusíku v experimentu (Caplan et al. 2017a) bylo následující: 117, 234, 351, 468, 585 mg N/l. V pokusu bylo použito komerční tekuté organické hnojivo ve vyšších dávkách, než je doporučeno při použití minerálních hnojiv z důvodu zpomalené přístupnosti dusíku při aplikaci v organickém hnojivu. Při použití organického hnojiva organické látky stimulují biologickou aktivitu a dochází k postupnému uvolnění dusíku s tím, že během vegetačního cyklu (140 dní) dojde k uvolnění 25 – 60 % dodaného dusíku (Prasad et al. 2004). I to je důvod, proč je nutné stanovit přesnější schémata pro použití jednotlivých druhů a forem hnojiv pro léčebné konopí pěstované indoor.

Vlivu dusíku během vegetativní fáze se ve své práci věnují rovněž (Saloner & Bernstein 2020), kteří došli k závěru, že během této fáze lze u sledovaných kultivarů považovat za optimální hladinu výživy dusíkem v rozpětí 80 – 160 mg/l (80% N ve formě nitrátové, 20% ve formě amonné). Při vyšším poměrném zastoupení amonné formy dusíku než 10 – 30 % se zvyšuje riziko vážného poškození rostlin toxickým NH_4^+ (Saloner & Bernstein 2022b). Při hodnotách v rozsahu 30 – 80 mg N/l byla pozorována snížená pigmentace fotosyntetických pletiv, snížená fixace uhlíku a morfologické poruchy pramenící z těchto poruch. Naopak excesivní příjem dusíku při použití živného roztoku s obsahem N >160 mg/l vedl k růstové retardaci spojené s toxicitou daného prvku. Z těchto výsledků pramení optimální hladina dusíku pro vegetativní fázi růstu konopí určeného k léčebným účelům 160 mg/l. Stejná koncentrace se jevila jako optimální i v navazujících pokusech ve fázi kvetení (Saloner & Bernstein 2021). Rozdílné výsledky jednotlivých studií poukazují na fakt, že výživa léčebného konopí a její vliv na růst a případnou produkci sekundárních metabolitů jsou odrůdově specifické.

Publikace navazující na výše zmíněnou práci (Caplan et al. 2017a) pokračuje ve sledování vlivu dusíkaté výživy na výnos a obsah kanabinoidů ve fázi generativní (květové),

kdy konopí bylo pěstováno ve stejném substrátu na bázi kokosových vláken. Generativní cyklus započal 19. den od transplantace zakořeněných klonů. Vegetativní režim probíhal dle schématu Caplan et al. (2017a) s tím, že ve fázi generativní bylo hnojení dusíkem prováděno v následujících obsazích: 57, 113, 170, 226, and 283 mg N/l (Caplan et al. 2017b). Z výsledků vyplývá, že výnos rostlin stoupal spolu se stoupající dávkou dusíku. Na druhé straně zvýšený výnos byl spojen se zřeďovacím efektem u obsahu THC, THCA a CBGA. Pro optimalizaci výnosu kanabinoidů autoři doporučují snížit dusíkaté hnojení v průběhu generativní fáze a naopak dávku navýšit v průběhu fáze vegetativní. Jako optimální dávku uvádějí autoři 212 - 261 mg N/l. Jedná se ovšem o údaj, který je platný pouze při použití organického hnojiva rozpustného ve vodě, pro danou odrůdu ve dvou použitých substrátech na bázi kokosového vlákna. Sami autoři dodávají, že je nutné brát v potaz proměnlivost způsobenou odlišnou odrůdou a pěstební technologií (zvolený substrát, technologie, závlaha atd.) (Caplan et al. 2017b, 2017a).

Saloner et al. (2019) se ve své studii zaměřují na vliv různých úrovní výživy draslíkem na příjem této živiny během vegetativní fáze růstu dvou odrůd konopí a na další vlivy s tím spojené. Celkem 5 úrovní výživy bylo aplikováno po dobu 30 dní v následujících koncentracích: 15, 60, 100, 175, and 240 mg K/l.. Z výsledků v první řadě vyplývá, že vliv K je odrůdově závislý. Obecně lze konstatovat, že hladina 15 mg K/l je nedostatečná pro optimální vývoj konopí s tím, že obě varianty vykazovaly viditelné příznaky deficience. Maximální dávka 240 mg/l naopak vykazovala toxicitu pouze u jedné z odrůd. U odrůdy druhé tato hladina K stimulovala růst výhonů a kořenů. Rozdíly mezi genotypy konopí se projevily v rozdílech příjmu, transportu a kumulace živin v rostlině. Srovnatelný trend obou odrůd byl pozorován ve vzájemné kompetici příjmu K ve vztahu k Ca, Mg. Naopak žádný negativní vliv neměla hladina draslíku na příjem N a P s výjimkou varianty s deficitním množstvím K, kdy při nedostatku této živiny došlo i ke snížení příjmu N a P. U mikroprvků byl pozorován kompetitivní vztah mezi příjmem K a Mn, Zn a Fe, kdy se projevila snížená koncentrace těchto mikroživin se zvyšující se hladinou K. Většina mikroelementů byla kumulována v kořenech. Jako optimální koncentrace K v živném roztoku uvádí Saloner et al. (2019) 175 mg K/l pro první odrůdu s tím, že vyšší dávky již negativně ovlivňovaly morfologii a růst rostlin. Naopak druhý genotyp vykazoval pozitivní stimulaci výživou K až do hodnoty 240 mg K/l. Ze studie vyplývá, že nejnižší dávka, kterou lze u zkoumaných genotypů považovat za optimální ve smyslu nepřítomnosti negativních symptomů, je 60 mg K/l.

Pokud se jedná o mikroprvky, dosavadní literatura nabízí obdobné výsledky jako pro výše zmíněné makroprvky, co se týče vlivu jednotlivých mikroživin na produkci kanabinoidů. Obsah Δ^9-THC a CBN je možné v rostlině ovlivnit množstvím dostupného manganu v živném roztoku. Jako možné vysvětlení se nabízí hypotéza, že kataláza rozkládající peroxid vodíku, který je pro rostliny toxický, vyžaduje mangan jako kofaktor. Během rozkladu peroxidu vodíku na vodu a kyslík se Mn s oxidačním číslem III redukuje na Mn s oxidačním číslem II, který se znovu oxiduje z Mn (II) na Mn (III) následnou reakcí s peroxidem (Wu et al. 2004). Další z majoritně zastoupených kanabinoidů, konkrétně CBD, je naopak závislý na koncentraci železa (Fe). Železo je ovšem v negativní korelaci s chromem (Cr), co se týče obsahu CBD v listech konopí. Přestože chrom není pro růst rostliny esenciální, vyskytuje se v přírodě jako komplexní oxid právě se železem. Obsah železa je tedy v pozitivní korelaci s obsahem CBD, naopak chrom je v korelaci negativní. (Pate 1994; Radosavljevic-Stevanovic et al. 2014). Kromě již zmíněného byla zaznamenána negativní korelace poměru iontů vápníku a zinku (Ca/Zn) a hořčíku ku mědi (Mg/Cu) v půdě s obsahem CBD v listech. Naopak poměr Ca/Mg v listech je v pozitivní korelaci s obsahem Δ^9-THC v listech rostlin konopí (Shibuya et al. 2007). Pozitivní korelace byla publikována také pro obsah Δ^8-THC u hořčíku a železa. Pravděpodobně z důvodu, že se tyto prvky vyskytují jako kofaktory v enzymech zodpovědných za produkci tohoto kanabinoidu (Coffman & Gentner 1975; Pate 1994; Radosavljevic-Stevanovic et al. 2014).

2.5.6 Hodnota pH

Tato hodnota je důležitá z hlediska vlivu na dostupnost živin pro rostliny. Obecně doporučovaný rozsah pH je 5,5 – 6,5 s tím, že pro hydroponický systém se jedná o rozpětí 5,5 – 6,0 s maximální absorpcí živin při pH 5,8 (Velazquez et al. 2013). Při využití půdního substrátu, či substrátu na bázi kokosu je doporučené optimum pH upraveno na rozmezí 5,8 – 7,2. Při poklesu mimo toto rozpětí může docházet k projevům nedostatku makroživin v důsledku jejich špatné dostupnosti. Naopak při překročení tohoto rozpětí může docházet k znepřístupnění některých mikroživin. Tolerance jednotlivých odrůd k výkyvům pH může být ovšem rozdílná (Caplan et al. 2017b).

2.5.7 Rostliné biostimulanty

Za biostimulant je podle du Jardin (2015) považována jakákoliv látka, druh mikroorganismu, nebo jejich směs, která je na rostliny aplikována za účelem zvýšení nutriční efektivity, zlepšení kvalitativních parametrů rostliny, či zvýšení odolnosti vůči abiotickému stresu. Tyto všechny aspekty mohou být ovlivněny bez ohledu na obsah živin v daném biostimulantu. Tento autor popisuje 7 hlavních kategorií biostimulantů: huminové a fulvo kyseliny, proteinové hydrolyzáty, mořské řasy a botanické extrakty, chitosan a biopolymery, prospěšné bakterie, prospěšné houby a minerály.

V současné době jsou pouze omezené literární zdroje věnující se vlivu biostimulantů při pěstování konopí. Při polním pěstování se při pokusu Da Cunha Leme Filho et al. (2020) projevil pozitivní vliv přídavku huminové kyseliny na fotosyntézu, obsah chlorofylu a výšku rostlin. Tyto výsledky se projevily v období, kdy rostliny byly vystaveny suboptimálním podmínkám a trpěly nedostatkem vláhy. Vliv huminové kyseliny jako doplňku při pěstování léčebného konopí je podle dostupných zdrojů spíše negativní. Prostorová variabilita distribuce kanabinoidů napříč rostlinou popsaná ve studii (Bernstein et al. 2019a) je v důsledku aplikace huminové kyseliny snížena. V nejvyšších částech rostlin byl zjištěn pokles koncentrace THC až o 37 % a pokles CBD až o 39 % (Bernstein et al. 2019b).

3. HYPOTÉZY A CÍLE PRÁCE

3.1 Hypotézy práce

Z literatury vyplývá, že vliv pěstebních podmínek spolu s výživou má dopad na produkci nadzemní biomasy, květu a biosyntézu sekundárních metabolitů v konopí – konkrétně kanabinoidů. Při polním pěstování lze výživu rostlin řešit pomocí minerálních i organických hnojiv, včetně využití vedlejších surovin z bioplynových stanic. Předpokládáme, že vzhledem k jejich povaze a složení, bude dosaženo srovnatelného, či lepšího výnosu biomasy konopí v plné zralosti rostlin. Předpokládáme, že při indoor pěstování léčebného konopí dojde při porovnání rozdílných živných roztoků či jejich koncentrací ke změně výnosu a produkce sekundárních metabolitů.

3.2 Cíle práce

Tato práce si klade za cíl experimentálně porovnat a vyhodnotit výnos nadzemní biomasy a kumulaci vybraných makro a mikro živin porovnáním rozdílných způsobů výživy konopí ve dvouletém polním experimentu. Dále je cílem studium vlivu dvou hydroponických systémů s rozdílným hospodařením s živným roztokem a současně porovnání vlivu rozdílných výživových schémat v nezávislých hydroponických experimentálních cyklech na tvorbu a výnos květenství, na biosyntézu dominantních kanabinoidů v rostlinách léčebného konopí a na jejich celkovou produkci.

4. PUBLIKOVANÉ PRÁCE

Doktorská disertační práce předkládaná formou svázaných vědeckých článků vznikla na základě níže uvedených čtyř publikovaných prací v časopisech databáze Web of Knowledge s Impact Factor indexem. Ostatní publikace, uveřejněné mimo rozsah práce, jsou uvedené v kapitole 8 na konci této práce.

4.1 Application of Individual Digestate Forms for the Improvement of Hemp Production

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Article

Application of Individual Digestate Forms for the Improvement of Hemp Production

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Abstract: In a two-year vegetation field experiment, the fertilizing effects of by-products from the agricultural biogas plant—a solid phase of digestate (SPD) and a liquid phase of digestate (LPD)—were studied and compared with mineral fertilization (NPK) on the biomass yield, content and nutrient uptake by *Cannabis sativa* L. plants. Furthermore, the agrochemical properties of the soil were evaluated at the end of the experiment. In all variants of the experiment, a uniform nitrogen dose of 150 kg/ha was applied. The dose of other nutrients corresponded to the fertilizer used. The biggest fertilizing effect, and therefore the greatest hemp biomass yield and nutrient uptake, was demonstrated when combining SPD and LPD fertilization in one treatment. However, the differences were statically insignificant ($p \leq 0.05$). The applied amount appeared to be sufficient for the nutrition of hemp plants and was comparable to mineral fertilization. The distribution of nutrients between leaves and stems varied depending on the nutrient monitored. Analyses after the end of the experiment did not show different contents of accessible nutrients in the soil on the studied variants. The content of accessible risk elements in the soil was not affected by the application of the SPD and the LPD. The experiment showed that cannabis plants are able to achieve equivalent biomass yields (8.68 t/ha) using the combination of LPD and SPD by-products from a biogas plan compared to commercial mineral fertilizer (7.43 t/ha). Therefore, we can recommend a split application of LPD and SPD as a suitable alternative to mineral fertilization. Due to prolonged nutrient release from SPD, we can expect a smaller negative environmental impact than current fertilization practices.

Keywords: *Cannabis sativa* L.; fertilization; biogas plants; solid phase of digestate; liquid phase of digestate

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1. Introduction

As a result of ever-increasing greenhouse gas emissions from burning fossil fuels, there has been a great boom in the development and use of biogas in recent years, especially as a source of environmentally friendly energy in the production of heat and electricity [1–3]. It is estimated that the consumption of biogas in Europe in the coming years will double, from 14.5 gigawatts in 2012 to 29.5 gigawatts in 2022 [4]. From the perspective of farmers, agricultural biogas plants also offer the possibility of stable extra income [5].

Biogas is produced by the anaerobic microbiological degradation of organic compounds, and it usually contains two major components: methane and carbon dioxide; it can also include other gases such as hydrogen sulfide and nitrogen (N_2) [6]. The positive aspect of anaerobic digestion is the fact that it reduces pathogens, kills viruses, fungi, bacteria of the genus *Listeria*, *Salmonella* and *Escherichia coli* and inactivates plant seeds [7–11]. The secondary product of this wet fermentation is digestate [12]. Hemp appears to be a suitable alternative crop for biogas production due to high biomass yields. It also has low adverse environmental impacts compared to other crops commonly used in Europe for biogas production (corn, sugar and beet) [13–15]. Considering soil ecology and sustainable soil use

in the Czech Republic, *Cannabis sativa* L. could work as a plant that alternates commonly grown plants used for biogas production. The aim is to reach a closed feedstock circle in which *Cannabis sativa* L. is grown due to using biogas station outputs as a fertilizer. In addition, soil conditions are improved by *Cannabis sativa* L. growth, and the consequently gained biomass might be used for further biogas production.

1.1. Composition of Digestate

Digestate is a heterogeneous liquid formed as a by-product of biogas production from organic matter with a significant proportion of undecomposed solid organic fraction (60–80% in dry matter). The dry matter of digestate is in the range of 7–12% and is comparable to slurry. It has a similar nitrogen content in fresh matter as manure (0.2–1%) but a higher pH value ranging from 7–8 [16–18]. The nutrient content in the dry mass (DM) of digestate is reported by Möller and Müller as follows: total N: 3.1–14%, P: 0.6–1.7% and K: 1.9–4.3% [19]. The application of digestate as an organic fertilizer to agricultural land is already considered as a standard method of its use [20,21]. The use of biogas residue as a substitute for mineral fertilizers has also been mentioned by other authors. Studies show that the use of digestate from agricultural biogas plants reduces the environmental risks that are generally associated with the use of mineral fertilizers and, at the same time, achieves comparable yield parameters for agricultural crops such as *Medicago sativa* L. and *Triticum aestivum* L. At the same time, the availability of nutrients depends on the input of raw materials, and it is not possible to say that, in general, we achieve better field crop yields by using by-products of anaerobic digestion [19,22–24].

The solid phase (SPD) and the liquid phase of digestate (LPD) are formed by the mechanical separation of the digestate in order to obtain two homogeneous materials. The composition of the SPD and the content of macronutrients and micronutrients is influenced by the composition of the input raw materials into the fermentation process and the retention time of the raw materials in the fermenter [25,26]. Slurry, silage corn, grass silage, cereals, sorghum and sugar beet pulp are produced as input materials [27]. The dry matter content in the SPD is in the range of 21–30% [16]. Möller and Müller present a content of nitrogen in the range of 2.2–3%, a content of phosphorus of 1.9% and a potassium content of 3.6% in the dry matter (DM) of the solid part of the digestate [19]. Due to the chemical composition and physical features, the applied SPD can positively affect biomass yield and soil structure [28,29].

Kolář et al. [26] refer to the LPD as a dilute solution containing a wide range of nutrients in a form acceptable to plants. The values given for the liquid part of the digestate (DM) are 7.7–9.2% for nitrogen, 0.4–0.7% for phosphorus and 3.9% for potassium [19]. The LPD appears to be a suitable raw material for application to arable land during vegetation, and its fertilizing and irrigation effects can be used [16,26]. Schievano et al. [30] characterize the LPD as an organic fertilizer that contains mineral nutrients along with organic matter. The dry matter of the LPD is in the range of 0.8–4%. Nitrogen is mainly present in mineral form, which means that it is easily accessible to plants. Its concentration is in the range of 0.15–0.30%, which is comparable to the potassium content. A study presented by Coelho et al. showed concentrations of plant essential nutrients as follows: N (6.6–24.1%, average 11.7%), P (0.81–3.28 % DW, average 1.74%) and K (0.81–17.35 % DW, average 6.15%). Because the proportions of N–P–K are variable in each digestate, it is necessary to provide an analysis of the specific digestates before actual application on the field [31]. Other nutrients are present in significantly lower concentrations [32].

1.2. Hemp (Cannabis sativa L.) Plants

Hemp (*Cannabis sativa* L.) comes from western Asia and is one of the earliest domesticated agricultural crops. According to Chinese historical records and archaeological findings, its cultivation for fiber and seeds dates back to the period of approximately 3000–4000 years BCE [33,34]. Over the centuries, hemp fibers have been used to manufacture

fabrics, sails, ropes and paper, while hemp seeds have been used as protein-rich food and feed [35].

According to European regulations, industrial hemp may contain no more than 0.3% of tetrahydrocannabinol (THC). In several European countries (e.g., the Netherlands and Belgium), a maximum THC content of 0.2% is allowed. In the European Union, only hemp cultivars approved by the European Commission—i.e., industrial hemp cultivars with a THC content below 0.2%—are permitted for industrial hemp cultivation [36–38].

Hemp biomass has been used for energy purposes for centuries. However, the energetic use of hemp has traditionally been limited to the use of oil pressed from hemp seeds; e.g., for lighting purposes. To date, the commercial use of industrial hemp biomass for energy purposes has been proposed in many countries. Hemp can be used to produce heat and energy by directly burning biomass from whole plants, or it can be converted to biomass-bound energy into liquid or gaseous transport biofuels such as bioethanol and biogas [39–41].

According to van der Werf et al. [42], the maximum yield of industrial hemp stems in field cultivation can be reached at a plant density of 90 plants/m^2. Amaducci et al. [43] reached an average yield of 14 tons/ha in a three-year field hemp cultivation experiment. The fertilization of hemp plants with nitrogen at a rate of 150 kg N/ha ensures optimal plant height, higher seed yield, higher stem strength [44] and overall high biomass production [44–47]. Nitrogen uptake is, according to Landi [48], greatest in the early stages of growth. An adequate nitrogen supply is ensured if the nitrogen content of the plant in dry matter is in the range of 5–6% [49]. The need for nitrogen by plants depends on the variety, as stated by Finnan and Burke [46]. Alaru et al. [50] compared the use of nitrogen by plants in variants where ammonium nitrate, waste sludge and beef manure were applied. The nitrogen dose for all variants was chosen identically, at 100 kg N/ha. Their results showed the suitability of using sludge as an organic fertilizer in the cultivation of cannabis for energy purposes. On the contrary, the application of beef manure failed to ensure the maximum yield of biomass. Malceva et al. [51] demonstrated the negative effects of increasing the nitrogen dose on the fiber content of the hemp stem. During a growing season with a higher level of precipitation, an application of 60 kg N/ha is recommended. According to the authors, this dose can be considered optimal. Regarding nitrogen cannabis nutrition, application rates vary from country to country. In Canada, a rate of 60–90 kg N/ha is used, while in EU countries, higher rates are used, ranging from 80–160 kg/ha and depending on soil properties and climatic conditions.

Phosphorus from the soil is taken up evenly by the plant, and its consumption is increased during the flowering and ripening period of the seeds [44]. Phosphorus uptake by cannabis plants ranges from 25–38 kg/ha, depending on the yield [45]. Ivanyi [49] states that a sufficient supply of phosphorus is at a content of 0.5–0.6% P in young fully developed leaves. The required amount of phosphorus to form one ton of dry plant matter is 1.7 kg. If the soil is rich in phosphorus, fertilization with this nutrient can be omitted [52].

Potassium is an important nutrient for the formation of cannabis stems and fibers. Interactions between nitrogen and phosphorus increase the quality of the fiber and the yield of hemp stalks. Potassium is mostly absorbed by cannabis plants during periods of intensive growth [48]. According to Barron et al. [47], potassium requirements for cannabis plants are high. They range from 75 to 100 K kg/ha, and in extreme cases up to 300 kg/ha. However, hemp is able to use potassium from the deeper layers of the soil profile. Cannabis concentrates most of the potassium in the stem, at up to 70–75% [46]. Iványi and Izsáki [53] state that the optimal potassium content in the plant is 2.7–3.0%.

According to Johnson [44] and Landi [48], calcium is also necessary for soils with a neutral pH due to its high consumption in the growth of the root system, stems and seeds. Landi [48] states that the need for calcium, together with nitrogen and potassium, is dominant for cannabis in terms of macronutrients. Depending on the yield, calcium intake is in the range of 151–227 kg/ha at yields of 8–10 tons/ha. In soils with a deficiency,

compensatory fertilization is necessary. Cannabis plants absorb calcium mainly at the end of the growing season.

Magnesium is involved in ensuring the good health of the plant [44]. Landi [48] indicates a magnesium uptake by cannabis plants in the range of 36–54 kg/ha, depending on the yield.

From micronutrients, hemp initially accumulates zinc and copper into the vegetative organs of the plant; later, it transports them to the generative organs, while iron, boron and manganese accumulate mainly in the vegetative organs.

For the field study of hemp growth, we hypothesized that the application of byproducts from a biogas station can sufficiently saturate plants with nutrients to achieve a comparable yield to hemp fertilized by mineral NPK fertilizers. The goal of our experiment was to verify our formulated hypotheses.

2. Materials and Methods

2.1. Vegetation Experiment Establishment

Cannabis plants were grown in a precise two-year vegetation field experiment. The experiment occurred on a demonstration and experimental site of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences in Prague (GPS 50°7′40″ N and 14°22′33″ E). The land is located at an altitude of 286 m above sea level, with an average annual temperature of 9.1 °C and an average total annual precipitation of 495 mm [54]. The soil type is a partly degraded Chernozem—slightly agglomerated on loess and loess clays.

2.2. Description of the Used Hemp Variety

Cannabis sativa L. variety "Tiborszállási", native to Hungary, was used in a precise field experiment. It is a dioecious variety with a proportion of sex individuals in the stand of approximately 1:1. In the case of cultivation, in order to achieve the maximum biomass yield, the growing season is in the range of 105–110 days; in our case, the plants were harvested after 101 days for both years of the experiment. This variety is specific for its early ripening and provides a high yield of stems as well as green biomass. The THC content is declared to be below 0.2% [36].

2.3. Origin of the Digestate and Its Separation into Liquid and Solid Parts

Within the experiment, a digestate originated from the agricultural biogas station Krásná Hora nad Vltavou with an energy output of 526 kWh. Corn silage, grass silage and livestock manure from local stables were used as energy sources. On average, over 20 tons of silage and 44 tons of cattle manure served as daily input. The solid and liquid phases were obtained by the mechanical separation of the digestate on the principle of a centrifuge and a press. All used raw materials were taken in the required amount before starting the experiment directly from the mentioned biogas plant. Some of the raw materials, which were applied only during the experiment, were stored in closed containers in a cooling box at a constant temperature of 4 °C.

2.4. Layout of Individual Plots and Sowing of Plants

The area in which the experiment took place was divided into 12 separate sub-plots measuring 2.5 m × 5 m. Eighty-five grams of seed were sown on each plot, up to 12.5 cm distance per row at a depth of 3 cm. Thus, a total of 1050 g of seed was sown for the entire area of the experiment. The seed rate was calculated from the seeding amount value (70 kg seed/ha).

In the experiment, four variants were established. Each variant was realized in three repetitions arranged such that two identical fertilization variants were not adjacent.

2.5. Amount of Applied Fertilizers in Individual Variants

In the first (NPK) variant, a mineral fertilizer was used in which nitrogen, phosphorus and potassium were added to the soil. Nitrogen was supplied in the form of ammonium nitrate with limestone containing 27% nitrogen (50% NH_4^+, 50% NO_3^-). The amount of nitrogen determined for the field experiment was 150 kg/ha. The dose was chosen depending on the intention to use the cannabis (biomass yield), according to the authors Sausserde and Adamovičs [55], Vera, Malhi, Phelps, May and Johnson [45] and Finnan and Burke [46]. Phosphorus was supplied at a rate of 20 kg/ha in the form of triple superphosphate with a phosphorus content of 21% (48% P_2O_5). Potassium was supplied at a rate of 150 kg/ha in the form of a potassium salt (60% K_2O).

The second variant included a corresponding dose of the SPD converted to a nitrogen content corresponding to 150 kg N/ha, with respect to the first control variant. The analysis of the SPD itself revealed a dry matter of 21.71% and a nitrogen content of 2.49% in the dry matter. For the delivery of 150 kg N/ha, it was necessary to deliver 34.80 kg of SPD on a partial plot with an area of 12.5 m^2. The application of the SPD took place 14 days before sowing, with simultaneous incorporation to a depth of about 8 cm.

The third variant was fertilized with a divided dose of SPD and LPD in a ratio of N (1:1), such that the total dose corresponded to 150 kg N/ha. A corresponding dose of 17.4 kg/12.5 m^2 was separated into the soil two weeks before sowing. The LPD fertilization took place three times during the vegetation, at two-week intervals, with the first application taking place 32 days after sowing. Later application was impossible in practice due to the involvement of cannabis. The LPD at 6% dry mass contained 5.78% N in dry matter. For the purposes of the experiment, it was necessary to supply 27.01 kg of the LPD divided into three equal batches, weighing 9 kg per sub-plot. For each of the three applications, the LPD was applied using a can.

The fourth variant was fertilized only with the LPD, divided into four doses. In the fourth variant, it was necessary to supply a quantity of 54 kg of the LPD per sub-plot. The application was identical to the previous variant. The amount of nitrogen supplied in individual variants during the vegetation is shown in Table 1.

Table 1. The amount of nitrogen supplied in individual variants during vegetation.

The Amount of N Supplied (kg/ha)					
Variant	**Basic Fertilization**	**1. Additional Fertilization**	**2. Additional Fertilization**	**3. Additional Fertilization**	**Total**
NPK	150	0	0	0	150
SPD	150	0	0	0	150
SPD + LPD	75	25	25	25	150
LPD	75	25	25	25	150

LPD fertilization in the third and fourth variants was performed using a watering can 3 times in an interval of 14 days during the phase of intensive cannabis growth. Prior to the actual application, a groove was formed, into which the LPD was applied and then covered with soil to prevent volatilization of the ammonium. The purpose of this method of application was to simulate a hose applicator commonly used in agricultural practice. During the vegetation, the plants were not treated against diseases or pests. The inter-row treatment against weeds was performed using a hand hoe as needed. The amount of individual biogas by-product for the delivery of 150 kg N/ha was as follows: SPD—27.8 t/ha, LPD—43.2 t/ha, combined dose of SPD + LPD—13.9 + 21.6 t/ha, respectively.

2.6. Harvesting and Plants Sampling, Soil Sampling

Cannabis plants were harvested by hand by plucking, including the root, from an area of 1 m^2, separately from each plot of all variants. After washing and drying the roots, the whole biomass from 1 m^2 was weighed, and the yield was subsequently evaluated.

Individual plant parts (root, stem and leaf) were separated from the harvested plant sample. These samples for analysis were then dried and homogenized. After the plants were harvested, soil samples were taken from individual plots. Samples were taken with a soil probe (20 punctures) to a soil profile depth of 20 cm. The samples were used to determine agrochemical properties. Analyses of all samples took place at the Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague. All plant samples, samples of the SPD and the LPD, were dried at 35 °C and then homogenized in a 1 mm sieve grinder.

2.7. Determination of pH Value and Content of Soluble Salts in Soil Samples

From the chemical features, the pH value and the content of soluble salts in the aqueous extract of the sample and demineralized water were determined in a ratio of 1:10 (volume:weight). A 10 g sample of dried soil was weighed at 25 °C into plastic PE bottles with lids and poured into 100 mL of distilled water. The samples were shaken for 5 min and then stood still for 5 min. The measurement was performed with a calibrated pH meter and a calibrated conductometer marked HI 991,300 Hanna Instruments.

2.8. Determination of Individual Nitrogen Forms in Soil Samples

For the purpose of the analysis, 10 g of fresh homogenized soil (sieved through a mesh size of 5 mm) was weighed into 250 mL polyethylene bottles and filled with 100 mL of a 0.01 mol/L $CaCl_2$ solution. The solution was then shaken for two hours. The samples were then removed and filtered. The total contents of mineral nitrogen, ammonium and nitrate form were determined in fresh soil by the colorimetric method on the SKALAR SAN^{PLUS} SYSTEM analyzer (Breda, The Netherlands).

2.9. Determination of Acceptable Nutrients from Soil Samples According to Mehlich 3

The soil samples were dried at 35 °C and then sieved through a sieve with a mesh diameter of 2 mm. A 10 g sample soil was weighed into plastic PE bottles, which was extracted with 100 mL of Mehlich 3 reagent [56]. Shaking was performed for 10 min, and then the obtained solution was filtered. Individual extracts were analyzed for phosphate content by the colorimetric method with ICP OES. The extracts were also measured for potassium, magnesium and calcium using an atomic absorption spectrometer (ASS), type Varian Vista Pro (Mulgrave, Australia).

2.10. Determination of Nitrogen Content in Samples of Plant Material

The Kjeldahl method was used to determine the total nitrogen content of the plant material. Total nitrogen includes both organic and ammoniacal nitrogen. For the determination, 0.50 g of a dry homogenized sample of plant material was weighed and mineralized. Mineralization took place in glass cuvettes. To the sample in the cuvette, 2 g of catalyst (mixture of 100 g K_2SO_4, 1 g $CuSO_4$, 0.1 g Se) and 10 mL of concentrated sulfuric acid (H_2SO_4) were added. Decomposition was performed for 90 min at 420 °C. After mineralization, the samples were prepared for distillation by adding 20 mL of distilled water to the cuvette, which was placed in the Gerhardt Vapodest 30s (Königswinter, Germany). Then, distillation into H_3BO_3 took place, and the total nitrogen content in the sample was determined.

2.11. Determination of Macronutrients, Micronutrients and Hazardous Substances Using an Absorption Spectrometer

The decomposition of the samples was carried out in a microwave system in cuvettes, into which 0.5 g of dry plant material, SPD and LPD, ground to a fraction size of 1 mm, was weighed. Then, 8 mL of HNO_3 (65% p.a.) and 2 mL of H_2O_2 (30% H_2O p.a.) were added to the sample. The resulting mineralizate was evaporated after 20 min. Internal reference material (IRM) analysis was performed on each series of samples. The contents

of macroelements, microelements and hazardous elements were determined by ICP-OES (Varian Vista Pro, Mulgrave, Australia).

2.12. Statistical Evaluation

As part of the statistical evaluation, the average yields of fresh and dry biomass, nutrient content and nutrient intake of individual cannabis variants were statistically evaluated by the Statistica 12 program (Statsoft) by a test of homogeneity of variance and then by an analysis of variance ($p \leq 0.05$). More detailed differences between individual averages were evaluated by Tukey's HSD test ($p \leq 0.05$).

3. Results and Discussion

The SPD and the LPD used for fertilization were characterized by a pH value ranging from 8.3–8.6, which matches the approach of Makádi, Tomócsik and Orosz [16]. The LPD obtained on the pressure production separator contained, on average, 6.04% of dry matter, which was significantly more than stated by Kolář et al. [26]. This was probably due to the meshes in the sieve, which, due to their size, allowed the passage of small solid particles into the LPD. Tables 2 and 3 show the different contents of soluble salts. In the SPD, the content of dry matter was more than twice as much as in the LPD, which coincides with the approach of Makádi et al. [16].

Table 2. Specifications of applied SPD in dry mass.

Dry Matter (%)	pH (H_2O)	EC (mS/cm)	Total N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
21.71 ± 0.261	8.6 ± 0.141	2.749 ± 0.072	24,900 ± 452	3127 ± 129	29,419 ± 632	40,358 ± 772	4364 ± 518	2793 ± 516
Fe (mg/kg)	**Zn (mg/kg)**	**Cu (mg/kg)**	**B (mg/kg)**	**Mn (mg/kg)**	**Pb (mg/kg)**	**Cd (mg/kg)**	**Cr (mg/kg)**	**As (mg/kg)**
296 ± 23.3	90 ± 9.33	5.65 ± 0.919	70.1 ± 19.5	144 ± 10.8	0.065 ± 0.001	0.085 ± 0.002	1.425 ± 0.13	0.07 ± 0.001

Table 3. Specifications of the LPD applied in dry mass.

Dry Matter (%)	pH (H_2O)	EC (mS/cm)	Total N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
6.04 ± 0.127	8.35 ± 0.353	>4000 ± 0	57,800 ± 1265	12,912 ± 562	42,988 ± 1214	39,996 ± 2586	4268 ± 272	3228 ± 342
Fe (mg/kg)	**Zn (mg/kg)**	**Cu (mg/kg)**	**B (mg/kg)**	**Mn (mg/kg)**	**Pb (mg/kg)**	**Cd (mg/kg)**	**Cr (mg/kg)**	**As (mg/kg)**
267 ± 96	251 ± 68	6.1 ± 0.52	76.5 ± 12.5	189 ± 15.7	0.9 ± 0.02	0.09 ± 0.001	1.01 ± 0.02	3.79 ± 0.12

Furthermore, the LPD was characterized by an approximately twice greater content of total N compared to the SPD, even when taking into account errors according to the standard deviation. The total contents of other macronutrients were similar for both applied raw materials. Of the macronutrients, the greatest content was found for calcium in the applied SPD. The contents of macroelements and microelements in the SPD and the LPD coincide with the approaches of Makádi et al. [16], Kolář et al. [26] and Dubský et al. [29]. Analyses of the SPD and LPD confirmed the findings of Makadi et al. [28], in which these raw materials possess features suitable for plant nutrition. Of the micronutrients, the greatest content was found for iron and zinc. The lowest content in both materials analyzed found was for copper. Both components, SPD and the LPD, were characterized by a low content of hazardous substances. Therefore, neither the SPD nor the LPD posed a risk of soil contamination and transport of these substances to the plant parts of cannabis.

Tables 4 and 5 show the individual nutritional variants of the experiment, recalculated for the application of kg of a nutrient per hectare.

Table 4. Specifications of applied NPK in relation to the application of nutrients per hectare of soil.

Total N	P	K
(kg/ha)	(kg/ha)	(kg/ha)
150.0	20.0	150.0

Table 5. Specifications of the applied SPD, SPD + LPD and LPD in relation to the application of nutrients per hectare of soil.

	SPD	SPD + LPD	LPD
		(kg/ha)	
Total N	150.50	150.70	150.8
P	18.90	26.30	33.7
K	177.80	145.00	112.2
Ca	243.90	174.20	104.4
Mg	26.40	18.80	11.1
S	16.90	12.70	8.4
Fe	1.80	1.20	0.7
B	0.40	0.30	0.2
Mn	0.90	0.70	0.5

Table 6 presents the analysis of soils before sowing cannabis seeds according to nutritional variants, two weeks after fertilizer application.

Table 6. Soils before sowing.

Variant	Nitrate N (mg/kg)	Ammonia N (mg/kg)	Carbon (mg/kg)	Total N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
NPK	21.25	29.96	287.79	57.50	534.56	502.78	8170.00	221.89	24.44
SPD	17.18	25.62	291.63	50.35	542.89	545.56	8233.33	234.44	25.56
SPD + LPD	9.31	17.83	303.57	39.62	548.89	543.33	8190.00	246.89	26.26
LPD	8.97	15.35	294.41	33.24	530.50	523.63	8443.75	234.75	25.23

The average dry mass yield of cannabis plants is shown in Figure 1. There were no significant differences between variants caused by the high non-uniformity of harvested plants in the field experiment. Similar results were reported by Tsachidou et al., who claim that applications of anaerobic digestion residues as a nitrogen source have shown the ability to maintain forage yields at a similar level as when using mineral NPK fertilizer. At the same time, the environmental risk associated with nitrogen leaching is reduced in this practice [23]. In both years, the average biomass yield was greatest in the variant fertilized by a divided dose of SPD and LPD (8.68 tons/ha), while the lowest dry mass yield was found in the variant where NPK was applied (7.45 tons/ha). The yield differences between the variants were statistically insignificant. The greatest yield in the variant with pre-sown-applied SPD and with fertilization with LPD was probably caused by the sufficient development of the root system in soil fertilized with a lower dose of SPD and regular fertilization with LPD during vegetation, which coincides with the findings of Alaru et al. [50] and Landi [48].

Table 4. Specifications of applied NPK in relation to the application of nutrients per hectare of soil.

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(kg/ha)	(kg/ha)	(kg/ha)
150.0	20.0	150.0

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	SPD	SPD + LPD	LPD
		(kg/ha)	
Total N	150.50	150.70	150.8
P	18.90	26.30	33.7
K	177.80	145.00	112.2
Ca	243.90	174.20	104.4
Mg	26.40	18.80	11.1
S	16.90	12.70	8.4
Fe	1.80	1.20	0.7
B	0.40	0.30	0.2
Mn	0.90	0.70	0.5

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NPK	21.25	29.96	287.79	57.50	534.56	502.78	8170.00	221.89	24.44
SPD	17.18	25.62	291.63	50.35	542.89	545.56	8233.33	234.44	25.56
SPD + LPD	9.31	17.83	303.57	39.62	548.89	543.33	8190.00	246.89	26.26
LPD	8.97	15.35	294.41	33.24	530.50	523.63	8443.75	234.75	25.23

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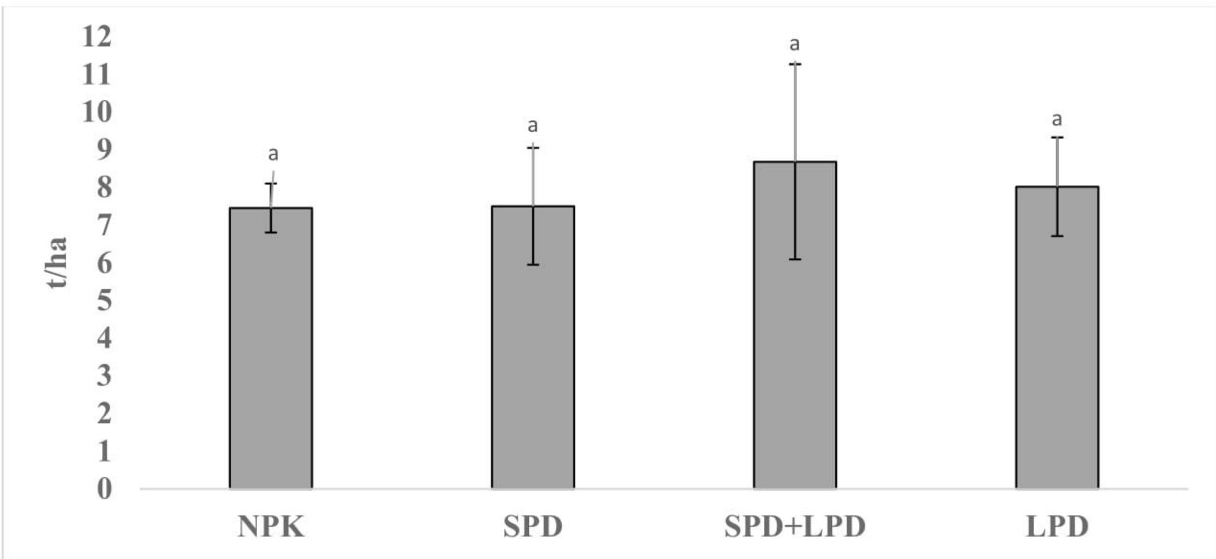

Figure 1. Average dry biomass yield of cannabis with individual variants.

The divided application of the LPD in the phase of intensive cannabis growth ensured a sufficient increase in biomass, which was reflected in the second greatest yield in the experiment. The results agree with the statements of Landi [48] and Barron et al. [47], who both state that the greatest nitrogen intake by cannabis plants is in the phase of their intensive growth. Nitrogen is also a key factor that influences the quantity and quality of cannabis production [57].

The contents of macronutrients, micronutrients and hazardous substances in dry matter in individual parts of cannabis plants are shown in Tables 7 and 8. The differences between the individual variants were not statistically significant for any of the analyzed plant parts. Overall, the greatest N content was found in cannabis leaves and decreased in the order of stem and root.

In the SPD + LPD variant, the greatest content of N (3.5%) was found in the leaves and stems (2.15%) of cannabis. The value found is slightly lower than that indicated by Iványi and Izsáki [53]. In contrast, the lowest nitrogen concentration in the leaves was found after the application of a divided dose of the LPD (3.2%) in the stems (1.95%) and roots (0.57%) of the NPK variant. The nitrogen concentration in the stems of all variants was lower than indicated by Hakala et al. [58], except for in the roots, in which it was relatively similar (0.61%).

Similar to N, other macronutrients were found in higher amounts in the leaves and in significantly lower amounts in the stems and roots, where they did not differ statistically. The greatest content in the leaves was found for calcium in the variant fertilized with NPK, which could have been caused by the release of a significant amount of calcium after a single application of water-soluble fertilizers. Our assumptions are also confirmed by the fact that the lowest calcium content was measured in cannabis leaves in the variant fertilized SPD, where, on the contrary, the share of available nutrients was clearly the lowest. In the case of other evaluated macronutrients, their content in leaves or in other parts of the plant did not significantly statistically differ between individual variants. Phosphorus and potassium contents in cannabis leaves were, on average, lower than 0.5–0.6% P and 2.7–3% K, which Ivanyi [49] had stated regarding young leaves.

This was probably due to the fact that, in our case, the leaves of the whole plant were analyzed, including old and dried leaves, which both contain significantly fewer nutrients.

When evaluating the content of micronutrients, the trend of their accumulation in individual parts of plants was far from unambiguous, as in the case of macronutrients (Table 8). Again, most of the elements accumulated in the highest concentrations in the leaves; only iron was found in the greatest concentrations in the roots, thus confirming its

limited mobility [59]. Despite its high accumulation in the roots, its content in the leaves was also the greatest of all monitored microelements. Lower contents were determined for boron and manganese and the lowest were found for zinc and copper. Overall, the contents of micronutrients in cannabis plants corresponded to the values reported by Ivanyi [49]. Differences in the contents of microelements in individual parts of plants were not statistically affected by the fertilizer used. The contents of molybdenum and risk elements were low in all plant parts, specifically below the detection limit of the analytical technique used.

Table 7. Macronutrient contents in individual parts of cannabis in dry biomass.

Variant	Root	Stem	Leaf
		Calcium (%)	
NPK	0.59 [a]	0.89 [a]	3.40 [a]
SPD	0.60 [a]	0.80 [a]	2.07 [a]
SPD + LPD	0.61 [a]	0.47 [a]	2.81 [a]
LPD	0.57 [a]	0.68 [a]	2.85 [a]
Variant		**Magnesium (%)**	
NPK	0.079 [a]	0.100 [a]	0.32 [a]
SPD	0.076 [a]	0.089 [a]	0.30 [a]
SPD + LPD	0.130 [b]	0.060 [a]	0.23 [a]
LPD	0.083 [a]	0.093 [a]	0.24 [a]
Variant		**Sulfur (%)**	
NPK	0.047 [a]	0.036 [a]	0.14 [a]
SPD	0.034 [a]	0.034 [a]	0.12 [a]
SPD + LPD	0.055 [a]	0.030 [a]	0.10 [a]
LPD	0.049 [a]	0.032 [a]	0.12 [a]
Variant	**Root**	**Stem**	**Leaf**
		Nitrogen (%)	
NPK	0.57 [a]	1.95 [a]	3.36 [a]
SPD	0.73 [a]	1.98 [a]	3.30 [a]
SPD + LPD	0.73 [a]	2.15 [a]	3.53 [a]
LPD	0.75 [a]	2.01 [a]	3.22 [a]
Variant		**Phosphorus (%)**	
NPK	0.11 [a]	0.11 [a]	0.24 [a]
SPD	0.096 [a]	0.12 [a]	0.25 [a]
SPD + LPD	0.14 [a]	0.089 [a]	0.21 [a]
LPD	0.11 [a]	0.11 [a]	0.21 [a]
Variant		**Potassium (%)**	
NPK	1.31 [a]	1.27 [a]	1.98 [a]
SPD	1.10 [a]	1.18 [a]	1.95 [a]
SPD + LPD	1.20 [a]	1.37 [a]	1.75 [a]
LPD	1.28 [a]	1.21 [a]	1.92 [a]

Different superscript letters indicate statistical significance.

Figure 2 shows the average macronutrient total uptake of cannabis plants in kg/ha. Consumption by plants was calculated on the basis of the yield of individual parts of dry biomass on the plot and the content of individual nutrients in these parts of cannabis plants. The calculated samples confirmed that cannabis plants have a high uptake capacity and that nutrient samples exceeded their application rates in all cases. For this ability, cannabis is also commonly used in soil phytoremediation [60,61]. Overall, the greatest average consumption, at a level of about 300 kg/ha, was determined to be for nitrogen, only slightly lower for potassium, and at a level of 200 kg/ha for calcium. LPD and SPD fertilization led to higher N and K uptake on all variants in comparison with the variant

fertilized with NPK. The greatest nitrogen uptake was found in plants in the variant with a divided dose of SPD and LPD. The high nitrogen uptake was probably caused by a sufficient supply of accessible nitrogen during the growing season. This effect can only be expected with the direct application of digestates immediately incorporated into the soil to minimize losses of ammonia N present in high portions in both components of the digestate [19]. Nitrogen uptake in the mentioned variants exceeds the values presented by Ivanyi and Izsaki [52], who both reported an average sampling over a four-year trial of 213 kg N/ha. On the contrary, the plants in the variant with the application of NPK achieved the lowest nitrogen uptake. In addition, the results of Sogn et al. showed that digestates are a promising alternative to NPK mineral fertilizers, although the levels of K and P in particular may fluctuate in these raw materials. When evaluating wheat yields using anaerobic digestion residues, comparable yields were achieved when using NPK fertilizer as a control [24].The greatest potassium uptake was again determined in the variant with the divided dose of SPD and LPD (345 kg/ha) and the lowest was in the variant with NPK (179 kg/ha). The observed values of potassium uptake by cannabis plants in the experiment are higher than indicated in Barron, Coutinho, English, Gergely, Lidouren and Haugaard-Nielsen [47]. The authors stated the range of potassium intake to be in the range of 75–300 kg/ha.

Table 8. Contents of micronutrients (mg/kg) in individual parts of cannabis in dry mass.

Variant	Root	Stem	Leaf
		Iron (ppm)	
NPK	182 [a]	28.84 [a]	94.61 [a]
SPD	280 [a]	50.72 [a]	77.38 [a]
SPD + LPD	236 [a]	33.90 [a]	67.52 [a]
LPD	125 [a]	49.07 [a]	73.01 [a]
Variant		**Manganese (ppm)**	
NPK	21.29 [a]	26.53 [a]	54.14 [a]
SPD	20.96 [a]	23.11 [a]	36.27 [a]
SPD + LPD	22.76 [a]	14.31 [a]	26.20 [a]
LPD	17.51 [a]	25.30 [a]	37.93 [a]
Variant		**Boron (ppm)**	
NPK	11.39 [a]	12.50 [a]	45.73 [a]
SPD	12.79 [a]	15.65 [a]	41.82 [a]
SPD + LPD	24.50 [a]	8.36 [a]	35.77 [a]
LPD	14.54 [a]	12.27 [a]	35.04 [a]
Variant	**Root**	**Stem**	**Leaf**
		Zinc (ppm)	
NPK	7.37 [a]	6.49 [a]	19.24 [a]
SPD	7.18 [a]	6.07 [a]	13.62 [a]
SPD + LPD	11.45 [a]	5.03 [a]	8.91 [a]
LPD	6.54 [a]	6.34 [a]	15.71 [a]
Variant		**Copper (ppm)**	
NPK	2.68 [a]	2.31 [a]	4.72 [a]
SPD	2.67 [a]	2.68 [a]	4.62 [a]
SPD + LPD	3.25 [b]	2.28 [a]	3.80 [a]
LPD	2.64 [a]	2.64 [a]	4.45 [a]

Different superscript letters indicate statistical significance.

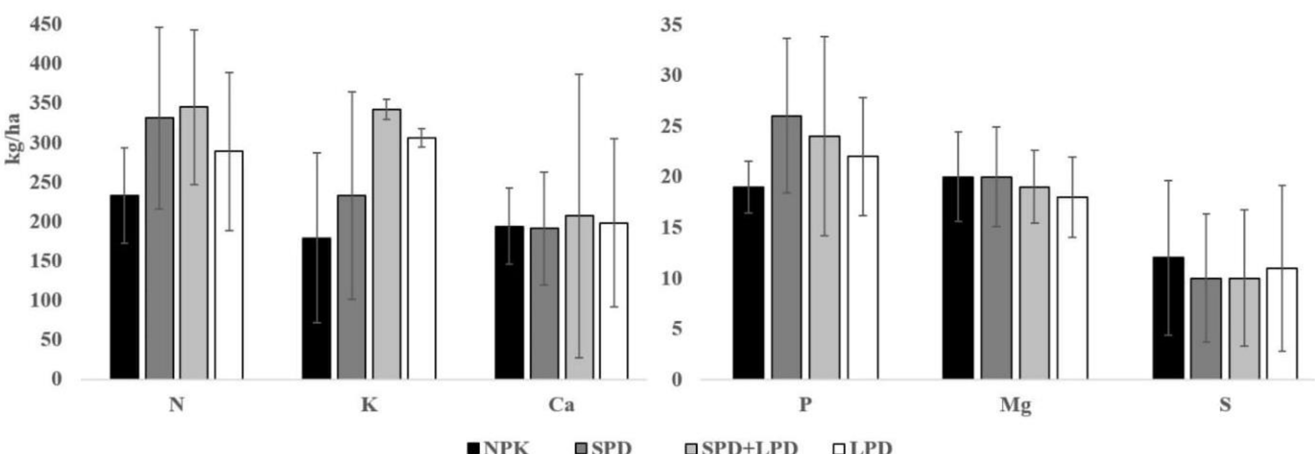

Figure 2. The average total uptake of cannabis macronutrients of individual experimental variants.

Calcium uptake by cannabis was high, not significantly affected by the fertilizer used and averaged at 191–207 kg/ha. These values correspond to the calcium samples given by Landi [48], at 150–227 kg Ca/ha.

The average phosphorus uptake ranged from 19–26 kg/ha and was higher for the variants fertilized with SPD and LPD than for the variant fertilized with NPK. These values match those of Landi [48], who reported P withdrawals by cannabis plants from 12 to 35 kg/ha. In addition, they are in line with the values stated by Ivanyi and Izsaki [52].

The average intakes of magnesium and sulfur were similar in all variants. The consumption of magnesium ranged from 18–20 kg/ha and that of sulfur ranged from 10–12 kg/ha.

Micronutrient uptake by plants was significantly reduced (Figure 3). The greatest consumption was found for iron; the consumption was lower for manganese and boron and lowest for zinc and copper. In the case of iron and boron, fertilization by SPD and LPD had a favorable effect. Especially in the variant with a divided dose of SPD and the LPD, cannabis plants took up most of these microelements. The lowest consumption by plants was for manganese and boron in the variant fertilized with LPD and NPK. For other nutrients, no significant differences were found between the individual variants. A higher uptake of iron and boron by plants on variants with SPD and LPD was probably due to their higher content in these materials and easier accessibility (Tables 2 and 3).

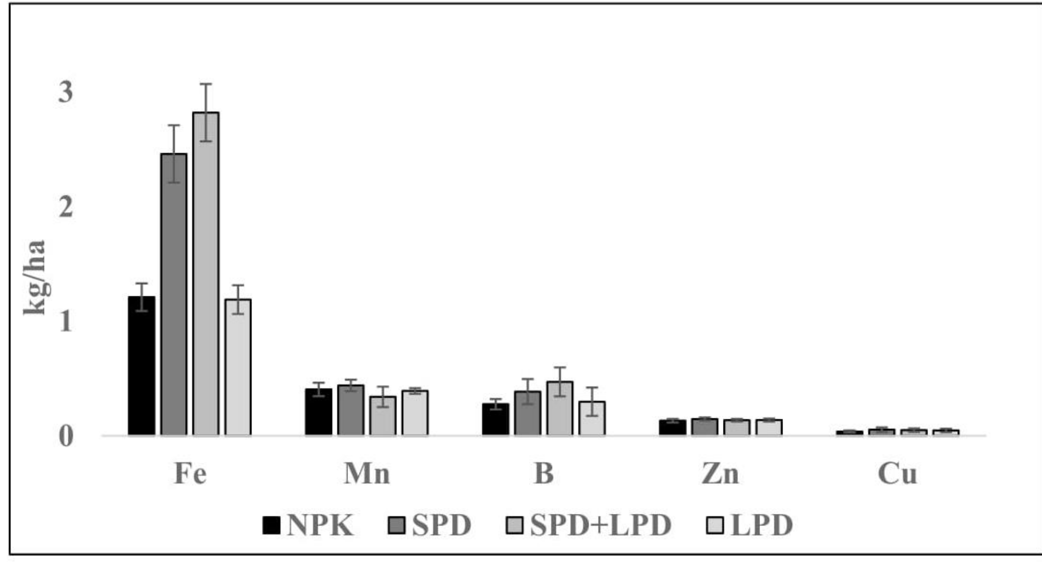

Figure 3. The average intakes of cannabis micronutrients of individual experimental variants (kg/ha).

After each harvest, the basic agrochemical features were determined (Table 9). The obtained values confirmed that the experiment was based on fertile soil; therefore, the application of NPK, SPD and LPD did not have a statistically significant effect on the evaluated parameters. The pH value determined in the aqueous extract was similar for all variants and corresponded to a pH of 8.7. The content of dissolved salts in the soil was the same in all variants. Slight differences were found in the content of individual forms of mineral nitrogen. The nitrogen supplied by the LPD oxidized more rapidly, and the ammonium N content was the lowest for these variants. The improvement of the soil structure led to the greatest overall consumption of N on the SPD + LPD variant, which was reflected in the lowest content of nitrate N after the cannabis harvest on this variant.

Table 9. Dry matter content, pH value, soluble salt value and mineral nitrogen content in soil samples after plant harvest.

Variant	Dry Matter (%)	pH (H_2O)	EC (mS/cm)	N (mg/kg)	
				NO_3^-	NH_4^+
NPK	90.8 ± 0.07	8.71 ± 0.00	0.114 ± 0.008	21.55 ± 0.49	18.35 ± 6.33
SPD	90.3 ± 0.14	8.74 ± 0.04	0.105 ± 0.001	21.12 ± 5.53	21.13 ± 6.32
SPD + LPD	89.7 ± 0.07	8.76 ± 0.08	0.110 ± 0.013	18.23 ± 2.60	15.54 ± 3.34
LPD	90.3 ± 0.02	8.73 ± 0.02	0.108 ± 0.003	23.09 ± 2.06	11.84 ± 5.04

The contents of accessible nutrients and risk elements in the soil after the cannabis harvest confirmed that the application of LPD and SPD did not significantly affect their accumulation in the topsoil layer (Table 10). The high pH value was confirmed by the high content of accessible calcium, which did not differ between the individual variants of the experiment. The contents of other cations were also high. The K content was not affected by the applied fertilizer, and the Mg content was slightly increased on the variants fertilized with the LPD and SPD, which could be caused by its supply in organic fertilizers. This trend was reflected on a smaller scale in the case of the evaluation of the available sulfur content in the soil.

Table 10. Macronutrient content in soil samples after harvest of plants in dry matter.

Variant	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
NPK	614 ± 113	460 ± 59	7328 ± 1190	240 ± 27.6	29.0 ± 7.1
SPD	628 ± 122	468 ± 108	7637 ± 843	252 ± 26.2	30.5 ± 7.8
SPD + LPD	629 ± 114	479 ± 90	7367 ± 1163	253 ± 10.6	32.5 ± 9.2
LPD	613 ± 117	476 ± 66	7374 ± 1512	259 ± 35.4	34.5 ± 13.4

Similar to the contents of the macroelements, the accessible content of microelements depended mainly on their amount in the soil and was only slightly affected by the applied SPD and LPD (Table 11). Only in the case of Cu can we assume that its high affinity for organic matter meant a slight decrease in its accessible forms in the soil. In the case of the micronutrients, their content depended on the habitat when assessing the accessible proportion of risk elements, and the applied fertilization did not lead to any significant changes in their content.

Table 11. Microelements content in soil samples after harvest of plants in dry matter.

Sample	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	B (mg/kg)	Mn (mg/kg)	Mo (mg/kg)
NPK	62.6 ± 19.1	16.4 ± 3.1	9.87 ± 1.59	21.9 ± 5.45	291 ± 78	<0.005
SPD	63.9 ± 16.1	16.9 ± 3.2	8.68 ± 0.40	20.6 ± 3.33	302 ± 83	<0.005
SPD + LPD	64.5 ± 15.1	17.2 ± 2.4	8.82 ± 0.31	21.5 ± 4.60	299 ± 74	<0.005
LPD	64.0 ± 21.9	17.0 ± 3.6	8.59 ± 0.27	21.4 ± 4.81	290 ± 77	<0.005

In conclusion, the data obtained from this experiment suggest that the by-products from anaerobic digestion can be used as an alternative to mineral NPK fertilizers. Comparable yield parameters were achieved by cannabis plants and were supported by a greater degree of nutrient accumulation in individual plant tissues. However, these materials are variable both in the composition of specific nutrients and in their accessibility to plants. This variability is due to differences of the input raw materials into the anaerobic digestion process, and this factor must be taken into account.

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4.2 The Oerview of Existing Knowledge on Medicinal Cannabis Plants Growing

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The overview of existing knowledge on medical cannabis plants growing

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Abstract: The use of cannabis for medicinal purposes dates back well before the era of modern medicine, but in recent years research into the use of medical cannabis in the medical and pharmaceutical sciences has grown significantly. In European countries, most cannabis plants have been and still are grown for industrial purposes. For this reason, hemp cultivation technology is relatively well researched, while little is known about the key factors affecting cannabis cultivation for medical purposes. The active substances of cannabis plant targeted by this review are called phytocannabinoids. The biosynthesis of phytocannabinoids is relatively well understood, but the specific environmental factors that influence the type and number of phytocannabinoids have been much less studied. Indoor or greenhouse cultivation, which uses automated lighting, ventilation, irrigation systems and complex plant nutrition has become much more sophisticated and appears to be the most effective method for producing medical cannabis. There are many different cultivation systems for cannabis plants, but one of the essential elements of the process is an optimal plant nutrition and selection of fertilisers to achieve it. This review summarises the existing knowledge about phytocannabinoid biosynthesis and the conditions suitable for growing plants as sources of medical cannabis. This review also attempts to delineate how nutrient type and bioavailability influences the synthesis and accumulation of specific phytocannabinoids based on contemporary knowledge of the topic.

Keywords: *Cannabis sativa* L.; tetrahydrocannabinol; cannabidiol; chemical profile; growing conditions

Cannabis is one of the earliest of domesticated crops. According to Chinese historical records and archaeological findings, its cultivation and utilisation can be traced back to 3 000 to 4 000 years BCE (Yu 1987, Jiang et al. 2006). The first use of cannabis for therapeutic purposes, directly evidenced by the finding of the stable cannabis compound, Δ^6-tetrahydrocannabinol (Δ^6-THC), has been dated to around 400 CE in a carbonised material discovered in a tomb at Beit Shemesh near Jerusalem (Zlas et al. 1993). Recent years have seen a boom in research on medical cannabis in the biomedical and pharmaceutical sectors. The applicability and acceptability of medical cannabis is expanding, as seen by the growing number of countries that allow its use for specific therapeutic indications (Shelef et al. 2011, Troutt and Didonato 2015, Balneaves and Alraja 2019). The number of active phytocannabinoids under investigation continues to increase and their effects on a variety of diseases such as chronic pain (Lynch and Campbell 2011, Portenoy et al. 2012, Wilsey et al. 2013), nausea and vomiting (Lane et al. 1991, Duran et al. 2010), spasticity (Pooyania et al. 2010, Corey-Bloom et al. 2012), depression (Wade et al. 2004, Selvarajah et al. 2010, Portenoy et al. 2012), glaucoma (Järvinen et al. 2002), inflammatory bowel disease (Ravikoff Allegretti et al. 2013), psychosis, motor and non-motor symptoms of Parkinson disease (Lotan et al. 2014), anxiety and sleep disorder (Russo et al. 2007, Bonn-Miller et al. 2014, Babson et al. 2017) are being studied (Doyle and Spence 1995, Järvinen et al. 2002, Lynch and Campbell 2011, Grotenhermen and

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Muller-Vahl 2012, Ravikoff Allegretti et al. 2013, Lotan et al. 2014). Nearly 150 different phytocannabinoid compounds are currently known (Hanuš et al. 2016).

TAXONOMY

History

The genetic plasticity of cannabis makes it difficult to catalog, and there is still a debate about its proper botanical classification. Linnaeus (1753) described *Cannabis sativa* as a single species. Based on comparative analyses of the psychoactive effects, leaf size, shape and structure of Indian and European varieties, Jean-Baptiste Lamarck (1786) classified the Indian cultivars as a separate species, *Cannabis indica*. At the beginning of the 20th century, the Russian botanist Janischevsky (1924) found that the local Russian plants possessed different characteristics from both *C. sativa* and *C. indica* yet still belonged to the cannabis taxon. These small, wild-growing, auto-flowering plants have been classified as a separate species named *Cannabis ruderalis* (Figure 1).

Current nomenclature

Small and Cronquist (1976) utilised a biphasic approach combining morphological and chemical characteristics to divide the *Cannabis* genus into the following four groups:

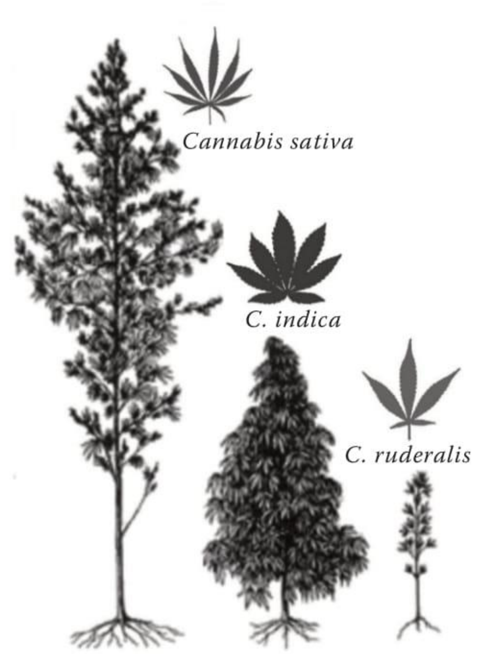

Figure 1. Species of cannabis (Hartsel et al. 2016)

1. *Cannabis sativa* L. subsp. *sativa* var. *sativa*,
2. *Cannabis sativa* L. subsp. *sativa* var. *spontanea* Vavilov,
3. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *indica* (Lam) Wehmer,
4. *Cannabis sativa* L. subsp. *indica* Small & Cronquist var. *kafiristanica* (Vavilov) Small & Cronquist (Figure 2).

Hillig (2005) concluded from his genomic study of the classification of *C. sativa* that none of the previous taxonomic concepts sufficiently defined the *sativa* and *indica* genes. He analysed different genotypes from various geographical origins and was therefore inclined to a multi-species classification including *C. sativa*, *C. indica* and *C. ruderalis*. Small (2015) has recently proposed two possible cannabis taxonomic classifications. The first is consistent with an earlier division (Small and Cronquist 1976) and is in accordance with the International Code of Nomenclature for Algae, Fungi, and Plants (McNeill et al. 2012). The second, for domesticated cannabis, follows the guidelines of the International Code of Nomenclature for Cultivated Plants (Brickell et al. 2009):

Non-narcotic plants, domesticated for stem fiber and/or oilseeds in West Asia and Europe. Low Δ^9-tetrahydrocannabinol (THC) content and high cannabidiol (CBD) content (Hillig and Mahlberg (2004) *Cannabis sativa* "hemp biotype").

Non-narcotic plants, domesticated for stem fiber and/or oilseeds in East Asia, mainly China. From low to moderate THC content and high CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "hemp biotype").

Psychoactive plants, domesticated in Southern and Central Asia. High THC content and low or absent CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "narrow-leaflet drug (NLD) biotype").

Psychoactive plants, domesticated in Southern Asia (Afghanistan and neighboring countries). From moderate to high THC and CBD content (Hillig and Mahlberg (2004) *Cannabis indica* "wide-leaflet drug (WLD) biotype").

In addition, two hybrid classes have also been generated:

5. Non-narcotic plants, hybrid cultivars between two fiber (hemp) groups (1 and 2).
6. Psychoactive plants, hybrid cultivars between two narcotic groups (3 and 4).

Hillig and Mahlberg (2004) analysed the content of cannabinoids in various cannabis plants and based on geographical origins, morphological features and

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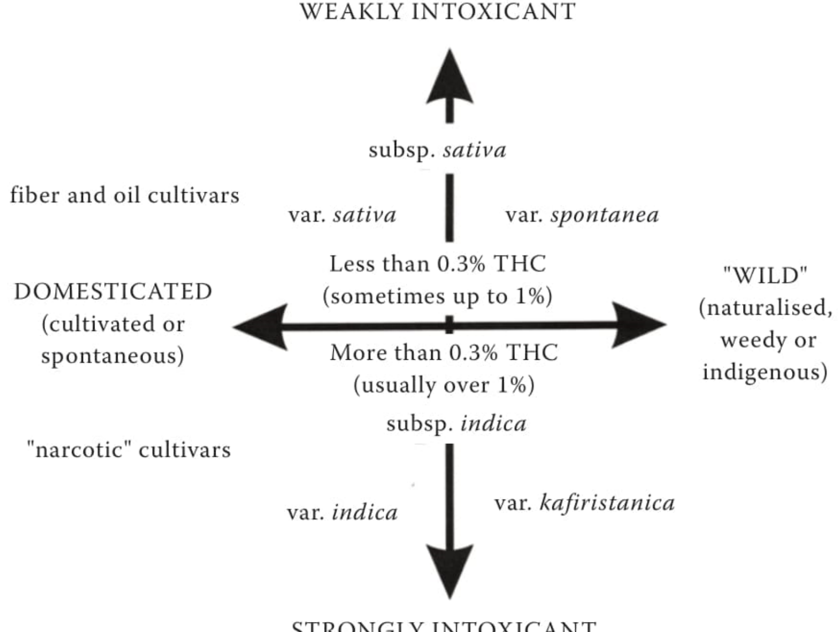

Figure 2. Cannabis chemotypes (Small and Cronquist 1976). THC – Δ^9-tetrahydrocannabinol

the supposed purpose of cultivation assigned them to the intraspecific taxa (biotypes):

Cannabis sativa "hemp biotype" – 62 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–11.5% and CBD were measured 0.0–13.6%.

Cannabis indica "hemp biotype" – 45 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–9.3% and CBD were measured 0.0–8.5%.

Cannabis indica "narrow-leaflet drug (NLD) biotype" – 68 plants were analysed, ranges of the dry-weight percentages of THC were measured 1.4–12.4% and CBD were measured 0.0–0.1%.

Cannabis indica "wide-leaflet drug (WLD) biotype" – 40 plants were analysed, ranges of the dry-weight percentages of THC were measured 0.1–14.7% and CBD were measured 0.0–11.0%.

All cannabis species successfully cross and produce fertile hybrids (Beutler and Marderosian 1978). *Indica* and *sativa* plants have also been found to differ in terpene and cannabinoid profiles. Thus, these chemotaxonomic markers are a promising tool for screening hybrids (Hillig 2004, Hillig and Mahlberg 2004, Fischedick et al. 2010, Elzinga et al. 2015). Zhang et al. (2018) are recommending that Cannabis should be recognised as a monotypic species typified by *Cannabis sativa* L., containing three subspecies: subsp. *sativa*, subsp. *indica*, and subsp. *ruderalis*. This proposal is based on their study focused on DNA sequence variations of cannabis plants. Also, McPartland (2018) in his work mentions that DNA barcode analysis supports the separation cannabis at a subspecies level and recognising the nomenclature of *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica*.

BIOSYNTHESIS OF CANNABINOIDS

History

Actual cannabinoid research is based on a number of major discoveries made by Professor Raphael Mechoulam and Professor Yechiel Gaoni. In the 1960's they identified the psychoactive component in *Cannabis sativa*, Δ^9-tetrahydrocannabinol, determined and described its chemical structure (Gaoni and Mechoulam 1964, Mechoulam and Gaoni 1967) and synthesised it (Mechoulam et al. 1967). Endogenous cannabinoid receptor ligands, called endocannabinoids, were identified in mammalian tissues in the 1990s. The best-known examples are anandamide (Devane et al. 1992) and 2-arachidonoylglycerol (Mechoulam et al. 1995). Endocannabinoids are derived from arachidonic acid, and membrane lipids serve as a potential source of this fatty acid (Giuffrida et al. 2001). For this reason, cannabinoids from cannabis are often referred to as phytocannabinoids to differentiate them from endocannabinoids.

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Biosynthesis of phytocannabinoids

Phytocannabinoids can be divided into two groups, neutral cannabinoids and cannabinoid acids. Diversification is based on how many carboxyl groups the molecule has, but non-enzymatic decarboxylation can occur during storage and especially at elevated temperatures when cannabis is smoked (Kimura and Okamoto 1970, Shoyama et al. 1970). Phytocannabinoids, prenylated polyketides of mixed biosynthetic origin, are synthesised from fatty acid precursors and isoprenoids. All phytocannabinoid structures contain a monoterpenic unit attached to the phenolic ring having the C3 alkylated carbon (Dewick 2002). The alkyl side chain can vary in length from one to five carbons (Figure 3) and n-pentyl is the most abundant (Elsohly and Slade 2005). Phytocannabinoids containing an n-propyl side chain are referred to as cannabivarins. Tetrahydrocannabivarin (THCV), the THC analogue with an n-propyl side chain, often occurs in *C. indica* (Hillig and Mahlberg 2004).

The starting materials for aromatic ring synthesis, including the alkyl on the third carbon (Hanuš et al. 2016), are three molecules of malonyl-CoA and one molecule of hexanoyl-CoA derived from hexanoic (caproic) acid (Dewick 2002). The hexanoyl-CoA acts as a primer for the type III polyketide synthase enzyme, also known as tetraketide synthase (TKS), which also requires the olivetolic acid cyclase enzyme (OAC) catalysing a C2–C7 intramolecular aldol condensation with carboxyl group retention to produce olivetolic acid (Taura et al. 2009, Gagne et al. 2012). These transformations can give rise to by-products such as 4-hydroxy-6-pentylpyran-2-one (PDAL), 4-hydroxy-6-(2-oxoheptyl)pyran-2-one (HTAL) and olivetol. Cannabigerolic acid (CBGA) is further derived from olivetolic acid after alkylation with a monoterpene unit, geranylpyrophosphate, with the participation of geranylpyrophosphate:olivetolate geranyltransferase (GOT) (Figure 4) (Fellermeier and Zenk 1998). Also, the (Z)-isomer of cannabigerolic acid, cannabinerolic acid (CBNRA), is synthesised to a small extent when neryl pyrophosphate is used by the GOT enzyme instead of geranyl pyrophosphate (Taura et al. 1995a). There are three acids that can be formed from CBGA and CBNRA.

Figure 3. Structure of cannabinoids

Tetrahydrocannabinolic acid (THCA) is produced during the formation of the heterocyclic ring by the THCA synthase enzyme, which can convert CBGA or CBNRA to THCA (Figure 5) (Taura et al. 1995b). However, the low THCA synthase specificity for CBNRA compared to CBGA suggested that THCA was predominantly synthesised from CBGA. The course of this reaction is similar to that of other reactions catalysed by monoterpenic cyclases. Most of the cyclases require divalent ions such as Mg^{2+} or Mn^{2+} for their activity, but this is not the case with THCA synthase (Taura 2009). The presence of a carboxyl group in the substrate molecule is essential for the reaction because THCA synthase does not recognize neutral phytocannabinoids such as cannabigerol (CBG) as substrates (Taura et al. 2007a).

The structure of cannabidiolic acid (CBDA) is the result of a pericyclic reaction involving loss of a proton (Figure 6) (Dewick 2002). The modification is catalysed by the intramolecular oxidoreductase, CBDA synthase, which selectively favours the formation of CBDA from CBGA over its (Z)-isomer, CBNRA (Taura et al. 1996). The effects of various metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+} and Cu^{2+}) on its activity were investigated, but they did not alter the rate of catalysis. In contrast, the Hg^{2+} ion completely inhibited enzyme activity at a concentration of 2 mmol, and the chelating agent, ethylenediaminetetraacetic acid (EDTA), at concentrations up to 5 mmol showed a low positive effect on enzyme activity. Thus, CBDA synthase does not appear to require metal ions for CBGA oxidocyclization (Taura et al. 1996). CBDA synthase and THCA synthase catalyse the formation of single optical isomers at a purity of greater than 95% (Taura et al. 2007b).

Cannabichromenic acid (CBCA) is derived from CBGA by oxidation and cyclisation by cannabichromenic acid synthase (CBCA synthase) (Figure 7). CBCA is synthesised as a 5:1 enantiomeric mixture, probably because of the partial release of intermediates from the CBCA synthase active site prior to completion of the reaction (Morimoto et al. 1997). Tests of the metal ions, Mg^{2+}, Zn^{2+}, Ca^{2+} and Cu^{2+}, showed that none of them stimulated enzyme activity. Hg^{2+}, however, completely inhibited the reaction at a concentration of 1 mmol. EDTA slightly increased enzyme activity suggesting that the CBCA synthase reaction does not require metal ions (Morimoto et al. 1998).

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hexanoyl-CoA

TKS 2 x malonyl-CoA

By-products

hydrolysis

PDAL

TKS malonyl-CoA

hydrolysis

HTAL

OAC

TKS

olivetolic acid

olivetol

GOT geranyl pyrophosphate

cannabigerolic acid

Figure 4. Biosynthesis of phytocannabinoids 1/2. TKS – tetraketide synthase; PDAL – 4-hydroxy-6-pentylpyran-2-one; OAC – olivetolic acid cyclase enzyme; HTAL – 4-hydroxy-6-(2-oxoheptyl)pyran-2-one; GOT – geranylpyrophosphate: olivetolate geranyltransferase

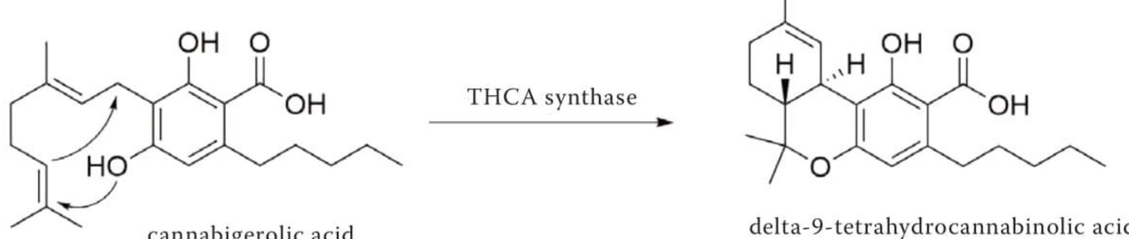

Figure 5. Δ^9-THCA (tetrahydrocannabinolic acid) synthesis

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Figure 6. Cannabidiolic acid (CBDA) synthesis

Croteau (1987) discovered that all terpene cyclases require bivalent cations for their function because these metal ions are able to neutralise the negative charge on the diphosphate groups on the terpene molecules and ionise the allyl diphosphate substrate. Since CBGA does not contain a diphosphate group it is to be expected that CBCA synthase, CBDA synthase and THCA synthase have no requirement for bivalent cations. The most of the cannabinoids present in *C. sativa* can be categorised as Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD, CBC, CBG, cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE) and cannabitriol (CBT) (Turner et al. 1980, Razdan 1986, Ross and Elsohly 1995). Δ^9-THCA, CBDA and CBCA are also sometimes called primary phytocannabinoids because other phytocannabinoids are generated from these three precursors predominantly by nonenzymatic degradative pathways.

Primary phytocannabinoids can either be decarboxylated to their neutral form (Figure 8) or converted to CBE, CBN, CBT, Δ^8-tetrahydrocannabinol (Δ^8-THC) or CBL *via* exposure to light, heat and oxygen (Figure 9). CBD can undergo photooxidation or pyrolysis to form CBE. Δ^9-THC is converted to the thermodynamically more stable Δ^8-THC when exposed to heat, or it may be degraded to CBT or CBN in the presence of oxygen (Elsohly and Slade 2005). The presence of CBT and CBN together with high levels of decarboxylated phytocannabinoids, are the chemical indicators of lengthy storage under poor conditions (Shoyama et al. 1970). The degradation rate of primary phytocannabinoids to these secondary phytocannabinoids increases with higher temperature, higher initial concentrations of primary phytocannabinoids, and with an increase in the inflorescence surface, and thus greater surface exposure to air (Milay et al. 2020). CBC in the presence of light converts to CBL-type phytocannabinoids (Elsohly and Slade 2005). Cannabivarins are generated by the same biosynthetic pathways from cannabigerovarinic acid (CBGVA), a homologous CBGA precursor (Shoyama et al. 1984). The cannabinoid profile in Cannabis undergoes rapid changes in the early stages of growth (Potter 2014). CBDA and THCA synthases have very similar catalytic rates (k_{cat} = 0.19/s and 0.20/s) and affinity (K_M = 134 µmol and 137 µmol) for cannabigerolic acid (Taura et al. 1995b, 1996). The CBCA synthase, however, shows a lower Michaelis constant (K_M = 23 µmol) as well as a higher catalytic rate (k_{cat} = 0.04/s). In the early stages of cultivation, where CBGA is still present at low concentrations, CBCA synthesis predominates (Morimoto et al. 1998). However, as the CBGA concentration increases over time, the efficacy of THCA and CBDA biosynthesis increases, and these molecules soon outweigh the CBCA concentration. At later stages of growth, CBGA synthesis slows and its relative proportion in the phytocannabinoid profile is gradually reduced (Potter 2014).

CULTIVATION

In European countries, most cannabis is grown for industrial purposes (Zuk-Golaszewska and Golaszewski

Figure 7. Cannabichromenic acid (CBCA) synthesis

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Figure 8. Decarboxylation of primary phytocannabinoids

2018) such as hemp fibers (Pickering et al. 2007), seeds as a source of oil (Mölleken and Theimer 1997, Kriese et al. 2004) and protein (Patel et al. 1994). For this reason, the procedures for hemp cultivation are well known, while the growth factors affecting cannabis cultivation for medical purposes are poorly understood (Zuk-Golaszewska and Golaszewski 2018).

Indoor or outdoor medical cannabis cultivation?

The conditions under which cannabis plants are grown for drug production is subject to more stringent protocols relating to the content and type of the active phytocannabinoids. Among the factors influencing the composition and yield of phytocannabinoids are the genotype of the plant, the growing conditions, maturity at harvest time, storage and handling (Potter 2014).

It is much more efficient to grow medical cannabis plants in a greenhouse where light, temperature and humidity can be controlled. Until recently, this method of cultivation was used mainly by illegal cannabis growers (Drugs 2009). Outdoor cultivation is less expensive, but the variability of the environment makes it almost impossible to obtain a high-potency, homogeneous product. Cannabis that is grown outdoors is also at greater risk from pests and plant diseases (Potter 2014). Cannabis entrepreneurs now use sophisticated indoor cultivation methods with automated control of lighting and photoperiod, temperature, ventilation and irrigation, and complex systems for providing nutrients. However, much of the information on indoor cannabis production is still obtained from anecdotal sources (Vanhove et al. 2011). Current data on the influence of photoperiod and even light spectrum allow indoor growers to regulate such aspects as leaf and shoot growth and time of vegetation cycle and thus achieve several growth cycles per year (Farag and Kayser 2015). Three to six harvests per year (six harvests per year is the maximum, and in this case, you have to skip the vegetative phase) can be attained by applying modern controlled growing practices (Leggett 2006).

Hydroponics *versus* soil

Indoor cannabis cultivation can be accomplished in several ways, but primarily either in soil or in soilless culture using hydroponic media. Hydroponic

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Figure 9. Biosynthesis of phytocannabinoids 2/2. CBGA – cannabigerolic acid; CBGVA – cannabigerovarinic acid; CBDA – cannabidiolic acid; CBDVA – cannabidivarinic acid; CBCA – cannabichromenic acid; CBCVA – cannabichromevarinic acid; THCA – tetrahydrocannabinolic acid; THCVA – tetrahydrocannabivarinic acid; CBEA – cannabielsoin acid; CBEVA – cannabielsovarinic acid acid; CBLA – cannabicyclolic acid; CBLVA – cannabicyclolvarinic acid; CBTA – cannabitriolic acid; CBNA – cannabinolic acid

cultivation has become increasingly popular among growers. A soilless media such as mineral wool, coconut fibers, perlite or expanded clay are used while nutrients provided by solutions are applied directly

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to the roots (Vanhove et al. 2011). The conventional type of cultivation is in soil with fertilisers applied through irrigation or by mixing with the soil substrates. Potter (2014) found that there was no increase in phytocannabinoid potency or in biomass under hydroponic conditions compared to standard soil cultivation, and the hydroponic system was more complicated and difficult to operate and maintain.

Vegetation cycle of cannabis

Cannabis is a short-day plant, naturally blooming in autumn, and the induction of flowering is regulated by specialised photoreceptor proteins called phytochromes. Therefore, the effect of photoperiod must be taken into an account in indoor cultivation (Halliday and Fankhauser 2003). The vegetative phase lasts from 2–4 weeks after rooting the clones or germinating the seeds (De Backer et al. 2012). The relative humidity in this phase should be from 70% to 80% with a temperature from 21 °C to 28 °C (Chandra et al. 2008). The generative phase is induced by shortening the photoperiod to 12 h light and 12 h dark. The first flowers should appear about one week after the reduction of the light period. The development of stems and leaves gradually slows down and stops after three weeks of this photoperiod, while the flowers continue to develop over the next 8 weeks (De Backer et al. 2012). The monitoring of 200 high THC cannabis varieties showed that the average flowering time with 12 h light period was 57 days, and 88% of the plants flowered between 7 and 9 weeks (Carpentier et al. 2012). The recommended temperatures are similar to the previous phase from 21 °C to 28 °C. However, humidity should be lowered to 40% over the generative phase to reduce the risk of fungal diseases (Vanhove et al. 2011, 2012).

Effect of CO_2 concentration

In order to prevent mold, a dry environment and constant air circulation should be ensured in indoor cannabis growing rooms, either from outdoor ventilation with filters or by indoor fans. It is also recommended to increase the concentration of CO_2 during the light phase of the day (cycle) to improve photosynthesis, plant growth and thus increase biomass yield (Kimball 1983, Wheeler et al. 1996, Chandra et al. 2008, 2011). Elevated CO_2 concentration can improve the assimilation of carbon, thereby accelerate plant growth and potentially improve productivity (Kimball 1983). There is a close correlation between plant yield and photosynthesis rate because more than 90% of plant dry matter is derived from photosynthetic CO_2 assimilation (Zelitch 1975). However, the improved level of plant photosynthesis and growth appear to be species- and variety-specific (Minorsky 2002).

Wang et al. (2008) investigated the effects of standard (370 ppm) and high (700 ppm) CO_2 concentrations on photosynthesis tolerance to acute heat stress (daily growth temperature was increased by 15 °C every day for 4 h) in cool-season and warm-season of C3 plants. High CO_2 concentration increased the cool-season and warm-season C3 plants tolerance of photosynthesis to acute heat stress. Hamilton et al. (2008) further elaborated the previous idea and concluded that the effects of growth temperature on photosynthetic thermotolerance between C3 and C4 plants are different and affected by the state of acclimatisation of the plants. A high concentration of CO_2 (700 ppm) increases the thermotolerance of C3 plants photosynthesis, except for C3 plants grown at the supra-optimal (5 °C above optimal) growth temperature, then increased CO_2 may provide no advantage or even reduce photosynthesis. On the other hand, increased CO_2 often reduces the photosynthetic thermotolerance of C4 plants at both optimal and supra-optimal growth temperatures.

Chandra et al. (2011) performed experiments directly on cannabis and showed that increasing CO_2 concentration from 390 ppm to 700 ppm increased the rate of photosynthesis in different varieties of *Cannabis sativa* by 38–48% and improved efficiency of water uptake.

Artificial light

To achieve optimal biomass and phytocannabinoid production, artificial lighting must meet certain parameters. These include light intensity in lumens per m^2 (lux units) and radiation intensity in watts per m^2 and the wavelength. Wavelength is particularly important because plants require different wavelengths of light during the growth. In the vegetative (roots and shoots) phase, the light should be 420–460 nm which corresponds to blue light, which promotes phototropism and growth hormone production in the plants. In the flowering phase, a red spectrum (600–680 nm) that is well absorbed by chlorophyll is best (Mahlberg and Hemphill 1983). For indoor cannabis cultivation, fluorescent T-5 lighting, metal-halide lamps (MH), high-pressure sodium lamps (HPS) for the growth and light-emitting diodes (LED),

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high-pressure sodium lamps (HPS) for the generative phase are most commonly used (Sweet 2016). These lamps differ in the composition of the inside gases, and they produce the light of different wavelengths.

The optimal intensity of illumination. The experiments of Potter and Duncombe (2012) showed positive relationship between the intensity of illumination and amount of biomass harvested. They determined three zones with elevated illumination energy 270, 400 and 600 W/m^2. Five plants of each variety were placed in each of the three zones at a density of 10 $plants/m^2$. In the growth rooms, daily average temperatures were maintained at 25 ± 2 °C. A constant supply of fresh air kept CO_2 concentration in the environment between 350 ppm and 390 ppm. Irradiance levels at the surface of the plant canopy were measured using a hand-held light meter determined the photosynthetically active radiation 80, 120 and 180 W/m^2 according to variants. Within plants growth, the lamps were kept at a constant distance from the cannabis canopy. The greatest harvest was achieved at 600 W/m^2 of the illumination intensity. Furthermore, the THC contents in the leaves and inflorescences of the mentioned variants were measured, but no significant increase in the concentration of THC was recorded with an increase of light intensity. Toonen et al. (2006) also reported that plants grown under 600 W lamps achieved higher yields than plants grown under 400 W lamps.

Decreasing tendency of plants to convert light energy into biomass with increasing levels of radiation is probably due to the fact, that plants have a limited ability to use light for photosynthesis. Under low light conditions, plants normally show an initial linear increase in the rate of photosynthesis and thus a tendency to convert light energy into biomass in response to increasing irradiation. However, under brighter conditions, the growth rate slows as chloroplasts become more and more saturated with light (Evans et al. 1993, Ögren and Evans 1993). This has also been proven on cannabis. The rate of increase in photosynthetic activity went down rapidly when irradiation levels rose above 100 W/m^2 of photosynthetically active radiation. Since 300 W/m^2 of photosynthetically active radiation, almost no increase in photosynthetic activity has been observed (Lydon et al. 1987).

HPS lamps *versus* LED. Magagnini et al. (2018) concluded that HPS-lit plants were higher and had a larger amount of dry matter than LED-lit plants. Conversely, plants under LED fixtures contained higher levels of CBD and THC than under the HPS. Namdar et al. (2019) also found out significant increase in concentration of CBGA in the inflorescences that flowered under LED illumination, with CBGA:THCA ratio of 1:2 as opposed to 1:16 when grown under HPS. Because of the high level of illumination, it was necessary to install a ventilation fan for cooling to the optimum temperature for photosynthesis of 25 °C to 30 °C (Bazzaz et al. 1975). A more efficient alternative is to use banks of LEDs that produce relatively little heat (Bessho and Shimizu 2012). LEDs do not consume much energy, do not require ballasts, and produce only a small amount of heat compared to high intensity discharge lamps. LEDs are compact, have long lives, very good wavelength specificity, relatively cool radiating surfaces, and linear photon output with electrical input current (Massa et al. 2008).

NUTRITION

In the area of plant nutrition for medical cannabis production, there is currently a lack of experimental data in the literature (Caplan et al. 2017a). It is known that the content of cannabinoids in leaves gradually decreases from top to bottom of the hemp plant (Hemphill et al. 1980) and from the literature about hemp cultivation can be deduced that nutrient application can affect the final cannabinoid content of the plants as well as their total yield. This suggests that nutrition could play a similar role for medical cannabis grown under controlled conditions. However, cannabis for hemp production has been selectively bred to produce fiber and is therefore likely to have slightly different nutrient needs than cannabis grown for medicinal purposes. The hemp crop is also grown in the field and not indoors (Hillig and Mahlberg 2004, Van Bakel et al. 2011, Amaducci et al. 2015).

Acceptable forms of individual essential nutrients are divided by Barker and Pilbeam (2015) into two groups according to plant needs, namely macronutrients: nitrogen (NO_3^-, NH_4^+), phosphorus ($H_2PO_4^-$, HPO_4^{2-}), potassium (K^+), calcium (Ca^{2+}), sulfur (SO_4^{2-}), magnesium (Mg^{2+}), and micronutrients: iron (Fe^{2+}, Fe^{3+}), chlorine (Cl^-), manganese (Mn^{2+}), zinc (Zn^{2+}), copper (Cu^+, Cu^{2+}), boron (H_3BO_3, $H_2BO_3^-$), molybdenum (MoO_4^{2-}) and nickel (Ni^{2+}).

Macronutrients

Nitrogen, phosphorus and potassium (NPK). It is assumed that the nitrogen content in the vegetative parts of

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the hemp plant positively correlates with the THC content (Haney and Kutscheid 1973). Thus, older leaves contain less THC than younger leaves because they contain less nitrogen. In contrast, high nitrogen levels in applied nitrogen fertilisers reduce the THC content of the hemp leaves (Bócsa et al. 1997). For example, good hemp production requires optimum soil nitrogen levels in the range of 50–200 kg/ha (Vera et al. 2004, Aubin et al. 2015), but these recommendations are not applicable for hydroponic or soil cultivation where studies on indoor cannabis cultivation indicated nitrogen fertilisation should be provided in the range of 190–400 mg N/L. This value has also been reported for nitrogen supplementation of organic, greenhouse-grown tomatoes (Zhai et al. 2009, Surrage et al. 2010).

Hemp growth and an increase in THC content were positively correlated with soil P content (Coffman and Gentner 1977). A negative relationship has been reported for CBD content in leaf tissue relative to available P. Hemp grown on soils depleted of P showed an increased CBD content (Coffman and Gentner 1975). Conversely, phosphorus enhancement did not show any positive effect on THC, CBD, CBN or CBG concentrations in buds from the top of the medical cannabis plants (Bernstein et al. 2019b).

Saloner et al. (2019) investigated response of medical cannabis to different potassium supply in vegetative growing phase. The results show that the response to nutrition is highly dependent on the genotype. Plants in this study were exposed to five different levels of K supply (15–240 ppm). Generally, both cultivars showed increased K concentration in all plant parts with increased K supply. Insufficient K dose for optimal growth and function was the lowest tested supply 15 ppm of K. Also, the highest dose proved excessive and damaging effect to development for one of the two tested genotypes. Similarities proven at both genotypes were in trends of accumulation and uptake. Results demonstrated competition between K and Ca with Mg uptake and no effect on P and N uptake except in the K deficiency range. Potassium supply showed only little effect on micronutrient accumulation in the plant shoot which was similar for both cultivars.

In contrast, no significant effect on hemp biomass and THC was observed in relation to different doses of N and K (Coffman and Gentner 1977). According to Hanuš and Dostálová (1994), various combinations of selected macroelements (N, P, K) in hemp culture can significantly affect the type of phytocannabinoids present and their individual contents. One of a few available sources of scientific literature dealing directly with this issue is the article by Caplan et al. (2017a, b), who reported a concentration of 389 mg N/L as optimal during the growth phase for maximum yield. The ratio of the basic macroelements (N, P, and K) in the vegetative period was 4:1.3:1.7. After making the calculations for P and K, we obtained values of 126 mg P/L and 165 mg K/L. In the generative phase, 212–261 mg N/L was the optimal amount. A nitrogen concentration of 283 mg N/L gave the maximum yield of inflorescence and biomass, but the concentrations of phytocannabinoids in the dried product was lower. The ratio of N, P, and K in the generative period was set at 2:0.87:3.32. Therefore, an initial concentration of 283 mg N/L, would require 123 mg P/L and 470 mg K/L. The plants tested were propagated from 17 day-old cuttings, which were fertilised with a solution of the indicated concentration for the following 21 days of vegetative growth. Another study has proved sensitivity of phytocannabinoids metabolism to mineral nutrition. The results presented by Bernstein et al. (2019b) show that increased treatment of inorganic NPK increased levels of CBG in flowers by 71% and decreased levels of CBN in flowers by 38% compared to a control treatment. Plants in the control variant were cultivated in potting mixture with fertigation. Concentration of dissolved nutrients in the control variant was as follows: 65 ppm N (1:2 ratio of NH_4^+/NO_3^-), 17 ppm P (40 ppm P_2O_5), 90 ppm K (108 ppm K_2O). Micronutrients were supplied chelated with EDTA at concentration of 0.4 ppm Fe, 0.2 ppm Mn, and 0.06 ppm Zn.

The rest of macronutrients. The magnesium cation content in soils is relatively mobile and its concentration in plants, especially in leaves, is high because it is a component of chlorophyll. The negative correlation between this metal and copper results from the fact that the radii of their ions are similar and both ions can compete for the same binding sites. The content of Δ^9-THC and CBD in hemp leaves decreases with increasing Mg concentration in the soil. The Δ^9-THC content in leaves is positively correlated with the ratio of accessible Ca/Mg in soil. CBD is negatively correlated with available Ca/Zn and Mg/Cu ratios. Positive correlations of magnesium with Δ^8-THC have been reported with the hypothesis that this nutrient may be cofactor in the enzyme responsible for its production (Coffman and Gentner 1975, Pate 1994, Radosavljevic-Stevanovic et al. 2014).

Micronutrients

Similar results have been seen for micronutrient requirements. Positive correlations of iron with

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Δ^8-THC have been reported with the hypothesis that this nutrient may be cofactor in the enzyme responsible for its production (Pate 1994, Radosavljevic-Stevanovic et al. 2014). CBD content in hemp plants is decreasing with increasing iron concentration (Radosavljevic-Stevanovic et al. 2014). The negative correlation of iron (Fe) and chromium (Cr) with CBD can be explained because the catalase responsible for the decomposition of hydrogen peroxide from the CBDA synthase reaction is a member of the class that contains four heme iron groups. Hydrogen peroxide is strongly sterically hindered upon entry into the heme cavity where the first step of catalysis takes place. Transferring a proton from an oxygen atom to a hydrogen peroxide molecule, and then to a second oxygen atom extends and polarises the O-O bond, which eventually decays heterolytically. The first oxygen atom of the hydrogen peroxide molecule is coordinated with a heme center, which releases water and creates an $O=Fe^{(IV)}$-enzym$^{(+)}$ heme radical. The radical then quickly breaks down by electron transfer, removing the radical electron from the porphyrin ring, which remains unchanged. During the second step, in a similar two-electron transmission reaction, the $O=Fe^{(IV)}$-enzym$^{(+)}$ reacts with a second molecule of hydrogen peroxide to form the parent molecule $Fe^{(III)}$ – enzym, water, and molecular oxygen (Boon et al. 2007, Vlasits et al. 2010).

Proposed reaction mechanism:

$$H_2O_2 + Fe^{(III)}\text{-enzyme} \leftrightarrow H_2O + O=Fe^{(IV)}\text{-enzyme}^{(+)}$$

$$H_2O_2 + O=Fe^{(IV)}\text{-enzyme}^{(+)} \leftrightarrow H_2O + Fe^{(III)}\text{-enzyme} + O_2$$

Fe-enzyme represents the center of heme iron attached to the rest of the enzyme.

The transition state, $O=Fe^{(IV)}$-enzyme$^{(+)}$ is energetically unstable, so these reactions are disadvantageous (Boon et al. 2007, Vlasits et al. 2010). Although chromium is not important for plant growth, its negative correlation with CBD is explained by the fact that Fe and Cr occur together in nature as a complex oxide (Radosavljevic-Stevanovic et al. 2014).

The concentration of CBN and Δ^9-THC in hemp plants can be influenced by the amount of manganese (Radosavljevic-Stevanovic et al. 2014). A positive correlation of manganese with CBN has been reported (Pate 1994, Radosavljevic-Stevanovic et al. 2014). THCA synthase, which catalyses the oxidative cyclisation of CBGA to THCA, contains a flavin adenine dinucleotide (FAD) prosthetic group that is reduced to $FADH_2$. Molecular oxygen is required to re-oxidise the $FADH_2$ to FAD, with the formation of hydrogen peroxide in a 1:1 molar ratio to the resulting THCA as a by-product of the reaction (Flores-Sanchez and Verpoorte 2008, Shoyama et al. 2012). CBDA synthase also contains FAD that is reduced to $FADH_2$ with release of H_2O_2, but the reaction differs from THCA synthase in the proton transfer step (Figure 10) (Taura et al. 2007a). It is estimated that about 1% of oxygen in plants is used to form reactive oxygen species in different subcellular locations with hydrogen peroxide being the most abundant. Hydrogen peroxide causes oxidative damage to cells that can lead to apoptosis (Quan et al. 2008), and plants have evolved efficient ways of eliminating toxic levels of H_2O_2. Catalase is a peroxidase enzyme found in all oxygen-using organisms that rapidly converts H_2O_2 to water and oxygen. There are three types of catalase and the non-heme form utilises manganese (Mn^{3+}) in its catalytic center that is reduced to Mn^{2+} during the decomposition of H_2O_2 to water and oxygen. Mn^{2+} can then react with more peroxide and be converted back to Mn^{3+} according to the following equations:

$$2Mn^{3+} + H_2O_2 \leftrightarrow 2Mn^{2+} + O_2 + 2H^+$$

$$2Mn^{2+} + H_2O_2 + 2H^+ \leftrightarrow 2H_2O + 2Mn^{3+}.$$

Both reactions are energetically advantageous ($\Delta G < 0$). The correlation between manganese and CBN is also positive since CBN is the primary THC degradation product (Wu et al. 2004).

Bernstein et al. (2019a) describes translocation of individual macro and microelements in relation to individual plant parts' age. The work also describes, inter alia, the distribution of cannabinoids in the plant. The research shows that the concentration of cannabinoids increases with the height of the plant and the highest concentration can be found in flowers and inflorescence leaves. The concentration found in fan leaves is about 1/10 the concentration found in flowers. The distribution of mineral nutrients between plant organs shows a typical uptake and translocation in the plant. Lower concentrations of N, P, K, and higher Ca in fan leaves compared to inflorescence supports physiological findings that the fan leaves are older than the inflorescence leaves.

pH value

Suggested optimal pH range of nutrient solution is between 5.5–6.5. pH is important because it affects the availability and absorption of nutrients needed

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Figure 10. Reaction mechanism of tetrahydrocannabinolic acid (THCA) synthase and cannabidiolic acid (CBDA) synthase. CBGA – cannabigerolic acid; FAD – flavin adenine dinucleotide

for plant growth. In hydroponic culture, the recommended pH range is 5.5–6.0 and the maximum absorption of nutrients is usually at pH 5.8 (Velazquez et al. 2013). In growing substrate, a pH range of 5.8–7.2 is recommended and the maximum absorption of essential nutrients is typically at pH 6.5. When the pH falls below this range, many macronutrients are less available and macronutrient deficiencies can be developed. When pH values rise above this range, many micronutrients will not be available for the plant uptake causing micronutrient deficiencies (Caplan et al. 2017a). These authors also mention the need for further research to confirm the optimal range of pH for multiple cannabis varieties.

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Plant biostimulants

A plant biostimulant is any substance, micro-organism strain or mixture of both applied to plants to increase tolerance to abiotic stress, nutritional efficiency or crop quality characteristics, regardless of its nutrient content. Seven main biostimulant categories were proposed: humic and fulvic acids, protein hydrolysates, seaweed and botanical extracts, chitosan and biopolymers, beneficial bacteria, beneficial fungi and beneficial minerals (Du Jardin 2015).

Humic and fulvic acids in cannabis nutrition. Humic substances are natural components of soil organic matter. It is a mixture of heterogeneous compounds originally classified according to their molecular weights and solubility into humins, humic acids and fulvic acids (Du Jardin 2015).

Humic acid supplementation had a positive effect on cannabis in the case of the height of cannabis plants, the chlorophyll content and the efficiency of photosynthesis, especially immediately after the period of water stress (Da Cunha Leme Filho et al. 2020).

According to the current literature, the effect on phytocannabinoids is rather negative. Bernstein et al. (2019b) mentioned that nutritional supplements such as humic acids significantly reduced spatial variability of cannabinoids throughout the plant parts. This increased uniformity came at the expenses of THC and CBD content which was reduced by 37% and 39% respectively in the top parts of plants. The decrease of THC has been associated with an additional trend of CBN increasing. This was probably due to the accelerated degradation of cannabinoids in the plant parts with their high concentration.

Other biostimulants in cannabis nutrition. Conant et al. (2017) demonstrated that microbial biostimulant Mammoth P™ promoted cannabis growth during the blooming phase. Lyu et al. (2019) hypothesised that future research will show that plant growth-promoting bacteria can affect the accumulation of phytocannabinoids, increase inflorescence yields, protect against plant pathogens by producing antimicrobial compounds and reduce the impact of abiotic stresses.

CONCLUSIONS AND FUTURE PERSPECTIVE

Based on the above information, it can be stated that quality of medical cannabis biomass, spectrum and concentration of phytocannabinoids can be influenced by cultivation conditions as well as nutrition during cultivation.

For the cultivation of medical cannabis, due to safety reasons, unpredictable environmental influences and required homogenity of harvest, indoor cultivation is definitely a better option because optimal growing conditions can be set and cannabis can be harvested from three to six times per year. Of the growing conditions, artificial light, the level of CO_2 concentration and the humidity of the surrounding environment influence the harvest quantity and quality the most. It is very important to choose the right combination of all mentioned conditions because they affect each other.

There is currently only a few experimental data on the medical cannabis nutrition, so most of this information is based on the hemp cultivation, which was bred for fiber production rather than inflorescence. However, it can be concluded from the current literature the concentration and spectrum of individual macronutrients, micronutrients and plant biostimulants in plant nutrition has a fundamental impact on biomass formation, spectrum and amount of medical cannabis cannabinoids.

In the future, the effects of nutrient ratios and availability can reasonably be expected to be one of the main factors influencing the content and type of cannabinoids in medical cannabis plants, separate from genotype and microclimate. These issues should be explored through further experiments, which will certainly be beneficial because of growing interest in the phytocannabinoids development in public and commercial spheres. Future technical research in this area should focus on possible new indoor medical cannabis cultivation techniques or the automation of existing cultivation technologies to facilitate work.

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4.3 Amino Acid Supplementation as a Biostimulant in Medical Cannabis (*Cannabis sativa* L.) Plant Nutrition

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Amino Acid Supplementation as a Biostimulant in Medical Cannabis (*Cannabis sativa* L.) Plant Nutrition

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There is growing evidence to support the involvement of nutrients and biostimulants in plant secondary metabolism. Therefore, this study evaluated the potential of amino acid-based supplements that can influence different hydroponic nutrient cycles (systems) to enhance the cannabinoid and terpene profiles of medical cannabis plants. The results demonstrate that amino acid biostimulation significantly affected ion levels in different plant tissues (the "ionome"), increasing nitrogen and sulfur content but reducing calcium and iron content in both nutrient cycles. A significantly higher accumulation of nitrogen and sulfur was observed during the recirculation cycle, but the calcium level was lower in the whole plant. Medical cannabis plants in the drain-to-waste cycle matured 4 weeks earlier, but at the expense of a 196% lower maximum tetrahydrocannabinolic acid yield from flowers and a significantly lower concentration of monoterpene compounds than in the recirculation cycle. The amino acid treatments reduced the cannabinolic acid content in flowers by 44% compared to control in both nutritional cycles and increased the monoterpene content (limonene) up to 81% in the recirculation cycle and up to 123% in the drain-to-waste cycle; β-myrcene content was increased up to 139% in the recirculation cycle and up to 167% in the drain-to-waste cycle. Our results suggest that amino acid biostimulant supplements may help standardize the content of secondary metabolites in medical cannabis. Further experiments are needed to identify the optimal nutrient dosage and method of administration for various cannabis chemotypes grown in different media.

Keywords: medical cannabis, phytocannabinoids, amino acids, *Cannabis sativa* L., terpenoids, biostimulant, hydroponics

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INTRODUCTION

Medical cannabis research has developed dramatically in recent years (Grotenhermen and Muller-Vahl, 2012). The use of these plants in healthcare and pharmaceutics places rigorous demands on the growing environment for optimal production of the desired active compounds (Potter, 2014). For these reasons, and because it is now legal, many growers have opted to use indoor facilities as a more efficient way to grow medical cannabis, a method used mainly by illegal growers until recently (Drugs and Crime, 2009). Consequently, indoor cultivation has become

more sophisticated with automated lighting, ventilation, and irrigation systems being commonly in use. It can be implemented in several ways, but always comes down to two basic methods—cultivation in soil substrates or hydroponically. The nutrients are dissolved in the irrigation water, or already fertilized soil substrates can be used. Hydroponics is currently one of the fastest developing methods in the horticultural industry (Vanhove et al., 2011) and cannabis growers have already started to use it extensively. In hydroponic cultivation the nutrients are supplied in the form of an aqueous solution directly in contact with the plant's root system. Thanks to the possibility of year-round growth in a controlled environment, this method has the potential to produce high yields of homogeneous plant material of excellent quality (Bouchard and Dion, 2009).

At present, basic research information about regulating the biosynthesis of secondary metabolites of medical cannabis is lacking because of legal restrictions in most countries (Aguilar et al., 2018). With respect to the internal and external factors influencing the secondary metabolite content and spectrum of cannabinoids, the main determining internal factor is the genetics of *Cannabis sativa* L. subsp. *sativa* and subsp. *indica* (Janatová et al., 2018; Mcpartland, 2018). This directly impacts the chemotype, habitus, cannabinoid, and terpene profile of the cultivated cannabis plant (Aizpurua-Olaizola et al., 2016). However, the genetics and the plant phenotypes are strongly influenced by external factors, with growing conditions playing a crucial role in productivity and quality. The main external parameters include light (Danziger and Bernstein, 2021), irrigation (Caplan et al., 2019), carbon dioxide concentration (Chandra et al., 2011), and nutrition (Malík et al., 2021). Nutrients play a central role in many aspects of plant metabolism. There is a wealth of experimental evidence to support the effects of nutrients, especially nitrogen (Saloner and Bernstein, 2021), phosphorus (Shiponi and Bernstein, 2021a), and potassium (Yep et al., 2021), on secondary metabolites of medical cannabis plants. The cannabinoid and terpene profile of medical cannabis can be influenced by the concentration and ratio of these major nutrients (Caplan et al., 2017a; Bernstein et al., 2019). Although emphasis is placed on the availability of sufficient amounts of these major plant nutrients, the potential effects of micronutrients and plant biostimulants must also be considered (Bernstein et al., 2019).

Several studies have used protein hydrolysates and amino acids (AAs) as plant biostimulants. The mechanism of their action on plants is thought to involve modulating nitrogen absorption and assimilation by regulating the enzymes and structural proteins involved in these processes. AA biostimulants also affect nitrogen uptake by the roots through modulation of specific signaling pathways. By controlling the enzymes of the Krebs (citric acid) cycle, they contribute to crosstalk between carbon and nitrogen metabolites (Colla et al., 2014; Du Jardin, 2015). The beneficial effect of chelation by some AAs has also been reported. In this way, certain AAs can protect plants from heavy metals, but they also contribute to the mobility and acquisition of micronutrients by the roots. AAs can also reduce environmental stress by scavenging free oxygen radicals, thereby contributing to antioxidant activity (Calvo et al., 2014). The stem and leaves of cannabis, like other plants, contain various concentrations of incorporated AAs (Audu et al., 2014). Plants can absorb and incorporate nitrogen in the form of intact AAs (Persson and Nasholm, 2001; Sauheitl et al., 2009), and thus, solutions of protein hydrolysates and AAs can increase plant growth (El-Ghamry et al., 2009; Talukder et al., 2018) and the nitrogen content of above-ground biomass (Matsumoto et al., 1999). Supplementing plants with environmentally friendly AA biostimulants can reduce the use of inorganic fertilizers (Ugolini et al., 2015).

Several commercial products derived from protein hydrolysates of plant and animal origin have already been marketed. Various results have been reported for agricultural and horticultural crops, but their application has led to significant improvements in yield and quality parameters (Calvo et al., 2014). So far, however, there have been no publications about their effects on plant secondary metabolism. Therefore, in this study, we focused on the physiological and chemical responses of medical cannabis plants to supplementation with a spectrum of AAs in a nutrient solution and subsequently compare the outcomes with two different hydroponic nutritional cycles. We proposed the following hypotheses: (1) the nutritional AA supplement causes a change in the amount of above-ground biomass and affects the inflorescence yield of medical cannabis plants; (2) the nutritional AA supplement causes a change in the medical cannabis plants cannabinoid and terpene profile; (3) the induced changes will be correlated with the contents of macro- and micro-elements in plant organs (leaves, stems, flowers); and (4) the induced changes will differ in each nutrition systems (recirculation vs. drain-to-waste). To test the hypotheses, we monitored the effects of AA supplementation in the nutrient solution of both systems on the amount of above-ground biomass and growth of leaves, stems, and flowers, the concentration of cannabinoids and terpenes, and the tissue ionome of the medical cannabis plant.

MATERIALS AND METHODS

Basic Parameters of the Growing Space

Cannabis plants were grown on tables in a room with controlled conditions. Each 2 m^2 (1×2m) table supported a separate experiment with an independent 100l tank for the nutrient solution. The container was made of inert plastic certified for food industry use. Each table held a maximum of 55 black conical square pots made of polypropylene (PP), each with a volume of 3.45l with dimensions: TOP - 15cm x 15cm, BASE - 11.5cm x 11.5cm, HEIGHT –20cm. Irrigation was provided by capillaries, which were placed in each pot to reach every plant separately using a needle applicator. The pump's timer was set for nine irrigation cycles, each lasting 60s. During one cycle, 94ml of nutrient solution was supplied to each plant (846ml per plant per day). The growing tables allowed us to choose the irrigation method--either recirculation of the nutrient solution or drain-to-waste system, where the spent solution went to a separate waste tank and was no longer mixed with the original solution. Microclimatic parameters were provided by an air ventilation unit that maintained and recorded the set parameters (relative

humidity, temperature, CO_2 level). Enrichment of the atmosphere of the growing space with CO_2 was made possible by a generator that burned methane. Six double-ended high-pressure sodium lamps provided a suitable spectrum of light at a power of 1,000 W. Based on the photosynthetic photon flux density (PPFD), the lamps provided 1,029 µmol/m^2 s at a power of 6,000 W. The light mode was also recorded every minute using a data logger.

Plants and Growing Conditions

The plants used in the experiments came from the mother plants of the medical cannabis genotype with the working name "McLove." Plants are classified as chemotype I - high Δ^9-tetrahydrocannabinolic acid/cannabidiolic acid (THCA/CBDA) ratio (>>1.0). Appropriate mother plants were kept in a separate growing room with controlled conditions. A total of 220 cuttings were made (110 cuttings per cycle) and cultivated for 21 days in a rock-wool cube (4×4 cm). Rooted clones were moved to a growing room, where they were placed in 3.45-liter pots filled with three liters of Euro Pebbles (expanded clay) growing medium. The light mode was set to 18 h of light and 6 h of darkness, temperature in the light phase was 25°C, the relative humidity was 60%, and the CO_2 concentration was 550 ppm (1,065 mg/m^3). The dark phase temperature was reduced to 22°C with the same humidity. The vegetative phase lasted 7 days, after which the cultivation regime was adjusted to the generative phase. The light period was set at 12 h light and 12 h dark, the temperature and CO_2 concentration was left the same as the vegetative phase, and the relative humidity was reduced to 40%. From the 10th week, plants were irrigated with demineralized water (DMW). Plant density was 27.5 plants per m^2 (55 plants/table/treatment).

Treatments

Compared to the controls (CN), the experimental plants (ET) were exposed to one enhanced nutrition treatment with two separate nutritional cycles. The first cycle (1C) was performed with recirculated nutrient solution, and the second cycle (2C) used the drain-to-waste system. The enhanced treatments were set up for both nutritional cycles and received the AA biostimulant (**Table 1**) added from the 2nd week for the last 24 h at a volume 2 ml/l before changing the nutrient solution. The new nutrient solution was prepared from reverse osmosis water every 7 days from the first day of the experiment. The pH of the nutrient solution was adjusted to 5.9 (Velazquez et al., 2013). In the recirculation system the nutrient solution was adjusted to this value every day. The pH and electrical conductivity (EC) were recorded when mixing the new solution and on the last day before changing it. After preparing the fresh nutrient solution, a sample was taken from each treatment for analysis. The measured composition of the control treatment (CN) nutrient solution is shown in **Table 2**, and the composition of the enhanced treatment (ET) nutrient solution with the addition of AAs is shown in **Table 3**.

Sampling Plant Material

Three plants were harvested from each treatment, one plant randomly selected from each highlighted sector 1–3 (**Figure 1**), every 7 days during the entire vegetation cycle. Subsequently,

TABLE 1 | Amino acid content in biostimulant.

AA	mg/L
Lys	0.071
His	0.00483
Arg	0.04615
Asp	0.0327
Thr	0.00954
Ser	0.0175
Glu	0.062
Pro	0.0828
Gly	0.1449
Ala	0.05569
Cys	0.036
Val	0.01401
Met	0.0039
Ile	0.00966
Leu	0.01836
Tyr	0.0016
Phe	0.01305

a random plant from the edge (outside the sectors) was transferred to an empty space in each sector. Plants were uprooted, weighed whole fresh, and divided into leaves, stems, and flowers, which were weighed fresh separately for all plants. The materials were then dried at 25°C to constant moisture (8–10%) and re-weighed. To determine the dry matter, a reference amount of each part of the plant was dried at 105°C to constant weight. The plant parts were homogenized just before analysis. The flowers (including the leaves until the 4th week) were frozen in liquid nitrogen and then ground in a mortar and pestle. The dried leaves (from the 5th week) and stems were ground in a grinder.

Dry Decomposition and Elemental Analysis

To determine the content of macroelements (except nitrogen), microelements, and trace elements in the plant, the leaves, stems, and flowers were analyzed separately. Weighed and homogenized plant biomass in a beaker was covered with a watch glass, placed on a hotplate 160°C, and the temperature was raised to 350°C over 4 h during which the samples gradually decomposed. The samples were next transferred to a muffle furnace, where they remained at 450–500°C for 12 h (Miholová et al., 1993). One ml of 65% HNO_3 was then added to the cooled beakers, which were placed on a 120°C hot plate for 60 min. The samples were then annealed for 90 min in an oven at 500°C and suspended in 1.5% HNO_3 with stirring in an ultrasonic bath. Elemental analysis of the samples was performed by flame atomic absorption spectrometry (FAAS) on a Varian 280FS with inductively coupled plasma optical emission spectrometry (ICP-OES) by Varian Vista-PRO instrument (Varian, Mulgrave, Australia; Hoenig, 2003).

Determination of Nitrogen in Plant Material by the Kjeldahl Method

For nitrogen determination, 0.5 g of plant material was weighed and put into a distillation tube. The samples were then mineralized by boiling with 95% H_2SO_4. After alkalization with sodium

TABLE 2 | Composition of control treatment (CN) nutrient solution (mg/L).

Elements	Weeks				
	1	2	3, 5	4, 6–9	10–13
N	100.85 ± 1.64	116.00 ± 1.85	130.00 ± 1.75	150.00 ± 1.92	DMW[a]
P	32.01 ± 0.75	39.40 ± 0.82	43.88 ± 0.59	51.73 ± 0.79	DMW[a]
K	124.93 ± 1.85	151.00 ± 1.38	173.11 ± 1.92	193.25 ± 1.58	DMW[a]
Ca	98.53 ± 1.32	119.00 ± 1.35	132.38 ± 1.42	146.00 ± 1.28	DMW[a]
Mg	25.17 ± 0.38	30.50 ± 0.42	34.94 ± 0.48	39.13 ± 0.45	DMW[a]
S	21.75 ± 0.25	26.72 ± 0.29	31.34 ± 0.34	34.53 ± 0.38	DMW[a]
Fe	0.91 ± 0.09	1.11 ± 0.09	1.21 ± 0.11	1.44 ± 0.08	DMW[a]
Mn	0.66 ± 0.07	0.74 ± 0.05	0.83 ± 0.08	0.99 ± 0.07	DMW[a]
Zn	0.21 ± 0.03	0.27 ± 0.03	0.28 ± 0.04	0.33 ± 0.03	DMW[a]
Cu	0.07 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.13 ± 0.02	DMW[a]
B	0.14 ± 0.02	0.19 ± 0.01	0.22 ± 0.02	0.25 ± 0.02	DMW[a]
Mo	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	DMW[a]
EC	0.97 ± 0.01	1.19 ± 0.01	1.46 ± 0.01	1.74 ± 0.01	DMW[a]

[a]*demineralized water.*

TABLE 3 | Composition of enhanced treatment (ET) nutrient solution with the addition of AAs (mg/L).

Elements	Weeks				
	1	2	3, 5	4, 6–9	10–13
N	100.00 ± 1.59	300.00 ± 2.94	331.00 ± 3.01	353.00 ± 3.52	DMW[a]
P	32.20 ± 0.49	40.17 ± 0.52	44.18 ± 0.92	52.09 ± 0.57	DMW[a]
K	125.00 ± 1.56	151.51 ± 1.27	174.17 ± 1.38	194.26 ± 1.95	DMW[a]
Ca	98.50 ± 1.32	120.58 ± 1.24	133.15 ± 1.49	146.83 ± 1.56	DMW[a]
Mg	25.30 ± 0.34	31.00 ± 0.38	34.06 ± 0.43	40.03 ± 0.37	DMW[a]
S	21.49 ± 0.31	51.80 ± 0.52	56.27 ± 0.61	61.84 ± 0.85	DMW[a]
Fe	0.93 ± 0.08	1.14 ± 0.07	1.19 ± 0.09	1.47 ± 0.07	DMW[a]
Mn	0.64 ± 0.06	0.75 ± 0.03	0.81 ± 0.04	1.01 ± 0.07	DMW[a]
Zn	0.22 ± 0.04	0.27 ± 0.01	0.29 ± 0.02	0.36 ± 0.03	DMW[a]
Cu	0.07 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.13 ± 0.01	DMW[a]
B	0.15 ± 0.01	0.20 ± 0.02	0.22 ± 0.02	0.26 ± 0.01	DMW[a]
Mo	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	DMW[a]
EC	0.97 ± 0.01	1.38 ± 0.01	1.71 ± 0.01	2.14 ± 0.01	DMW[a]

[a]*demineralized water.*

hydroxide, the free ammonia was distilled with water steam into H_3BO_3. Its content was determined by titration with HCl (0.5 mol/l) and then measured by Gerhardt Vapodest 30s (Königswinter, Germany; Baker and Thompson, 1992; Velechovský et al., 2021).

Phytocannabinoid Extraction, Identification, and Quantification

Phytocannabinoids from ground homogenized flowers (including the leaves until the 4th week) were extracted by the optimized method of dynamic maceration (Brighenti et al., 2017). Samples (0.30 g) from each experiment group were mixed with 10 ml of 96% ethanol and macerated for 60 min with constant stirring at 300 rpm. Mixtures were then filtered under vacuum using a Morton filter device (porosity S4/P16), and the filtrate was collected. The flowers were removed from the filter and mixed with another 10 ml of solvent. This step was repeated twice, and the filtrates were pooled. Aliquots of 0.5 ml of each sample were diluted to 10 ml with 96% ethanol and filtered once more through nylon syringe filters (0.22 μm) into vials. Samples of the extracts were injected into high-performance liquid chromatography system equipped with diode array detection (HPLC-DAD; Agilent 1,260, Agilent Technologies Inc., United States) and a Luna® C18 column (2) 250 × 3 mm, particle size 3 μm (Phenomenex, United States). The isocratic mobile phase consisted of acetonitrile/H_2O (31:9, v/v) with 0.1% HCOOH (v/v) and 0.1 mol/l NH_4COOH (without pH adjustment), flow rate was 0.55 ml/min, temperature 37°C, sample injection volume 8 μl, and UV detection at 275 nm (Križman, 2020). The instrument was externally calibrated using cannabinolic acid (CBNA) in the range of 0.3–10 mg/l and THCA, 0.3–100 mg/l, (Sigma-Aldrich, Czech Republic) as standards. Data were analyzed using OpenLAB CDS software, ChemStation Edition, Rev. C.01.5.

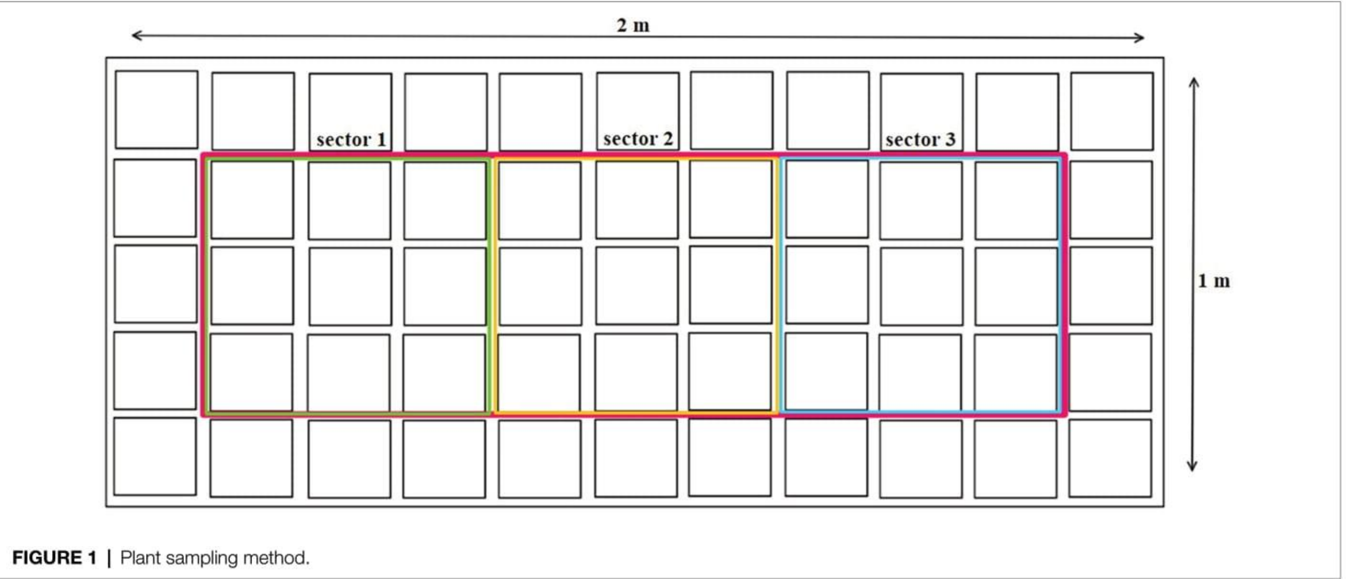

FIGURE 1 | Plant sampling method.

Terpene Extraction, Identification, and Quantification

Terpenes from ground and homogenized mature flowers (week 8–10, vegetation) were extracted with hexane. Plant samples (0.1 g) were mixed with 1 ml of hexane and pentadecane was added to a final concentration of 1 mg/ml as an internal standard. The samples were vortexed and placed in an ultrasonic bath for 30 min. Subsequently, the samples were centrifuged and filtered through polytetrafluoroethylene (PTFE) syringe filters (0.22 μm) into vials. Filtered samples (1.5 μl) were first injected into a gas chromatograph with a flame-ionization detector (GC-FID; Agilent Technologies 7890A, Palo Alto, CA). The GC-FID conditions were: column DB5 30 m × 0.25 mm × 0.25 μm film thickness, inlet temperature 230°C, detector temperature 300°C, and nitrogen flow rate of 1 ml/min. The initial temperature was 60°C, which was increased at the rate of 3.5°C/min until a temperature of 150°C was reached, and then at a rate of 30°C/min until a final temperature of 300°C was reached. Samples were also injected into a gas chromatograph connected to a mass spectrometer (GC–MS; Agilent Technologies 5975C, Palo Alto, CA). The GC–MS conditions were: column HP-5MS 30 m × 0.25 mm × 0.25 μm film thickness, inlet temperature 230°C, detector temperature 300°C, and helium flow rate of 1 ml/min. The initial temperature was 60°C, which was increased at the rate of 3.5°C/min until a temperature of 150°C was reached, and then at a rate of 30°C/min until a final temperature of 300°C was reached. Compounds detected by GC–MS were identified by comparing the mass spectrum and relative retention index with the published values of the National Institute of Standards and Technology (NIST) database, and the values for the standards, β-myrcene and limonene (Sigma-Aldrich, Czech Republic). The GC-FID data revealed the relative concentration of the identified substances, based on the peak area of the monitored substance relative to the total area of all detected substances.

Statistical Analyses

Data were subjected to ANOVA followed by Tukey's HSD test. The analysis was performed using IBM SPSS Statistics software (version 25, 2017, Chicago, Illinois, United States).

RESULTS

The AA nutritional supplement and the variable nutritional cycles (1C and 2C) induced changes in the tissue ionome of medical cannabis plants. The content of nitrogenous compounds was lowest in the stems and highest in the flowers (**Figure 2**). The concentrations of N in the leaves and flowers of control (CN) and enhanced treatment (ET) plants with AA supplement in the recirculation (1C) cycle began to differ significantly from the 5th week. The most significant differences in N concentrations between control and AA treatment were 34% for flowers at week 6 (CN, 44.26 mg/g; ET, 59.19 mg/g; **Figure 2A**). In contrast to 1C, the concentration of N in the stems and leaves of CN and ET began to differ significantly from week 2 to 4 in the drain-to-waste (2C) nutritional cycle; but, from week 5 to 13, fewer significant differences were observed with 2C than 1C. The most significant differences in N concentrations between nutritional treatments were 7% for flowers at week 7 (CN, 43.02 mg/g; ET, 45.85 mg/g; **Figure 2B**). The N concentration also differed between 1C and 2C of ETs with AA supplement, and the differences were evident beginning at week 2. The most significant differences in N concentrations in ETs between nutritional cycles were 31% (6% between CNs) for flowers at week 5 (1C, 61.63 mg/g; 2C, 47.18 mg/g; **Figure 2C**).

The calcium content was lowest in the stems and highest in the leaves, and showed a cumulative trend over time (**Figure 3**). The Ca concentration in the leaves of CN and ET in 1C began to differ significantly from the third week. The most significant differences in Ca concentration between nutritional treatments were 60% for leaves at week 11 (CN,

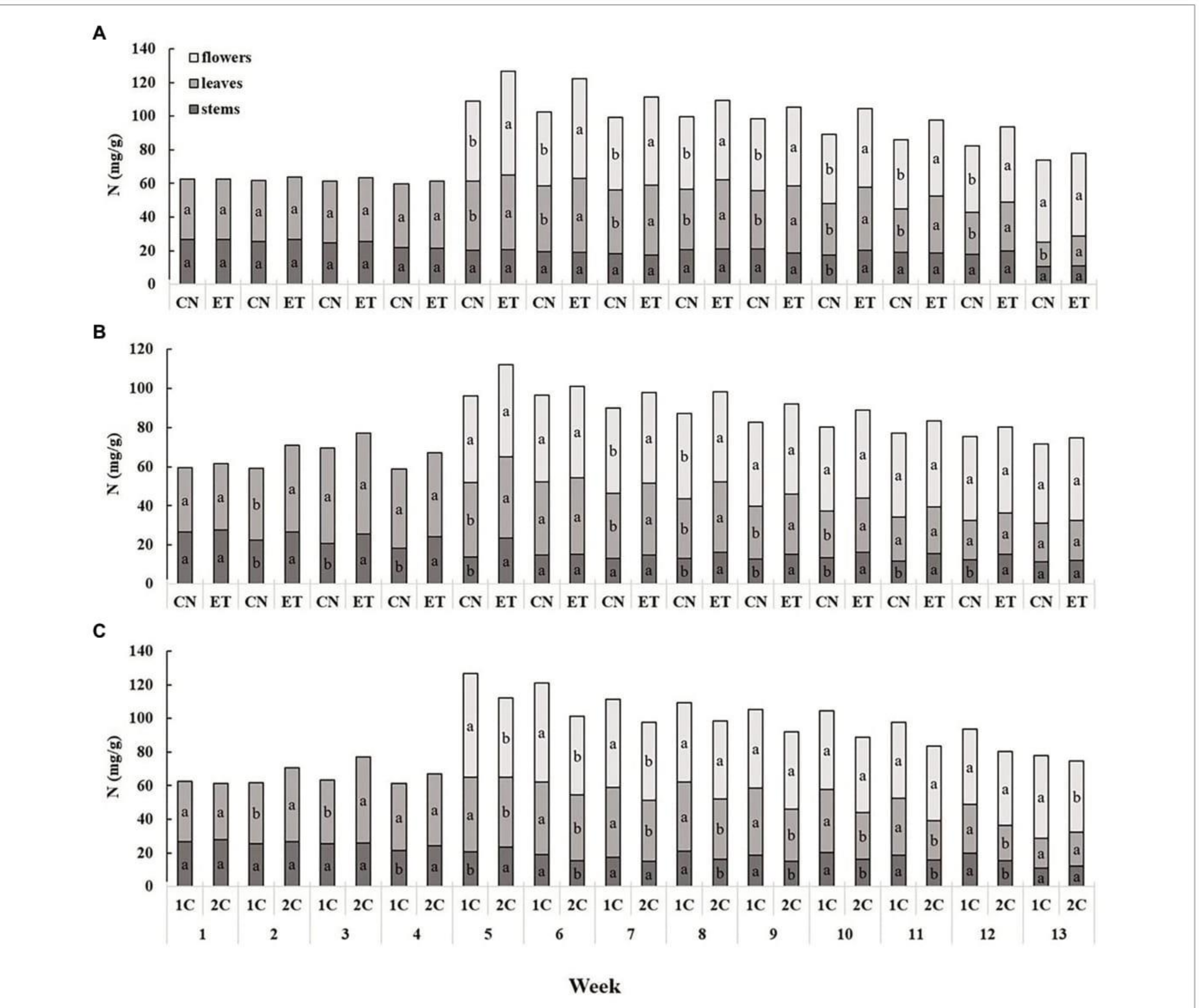

FIGURE 2 | Distribution of nitrogen among the organs of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. N concentration of control (CN) and enhanced treatment (ET) with AAs nutritional supplement in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means ± SE ($n=3$). The different small letters inside the bars represent significant differences within the plant organs (stems, leaves, and flowers) between the variants in a particular week according to Tukey's HSD test at $\alpha=0.05$.

85.17 mg/g; ET, 53.13 mg/g; **Figure 3A**). In contrast to 1C, the Ca concentration in the CN and ET leaves differed significantly as early as week 2 in 2C. The most significant differences in Ca concentrations between CN and ET were 32% for leaves at week 7 (CN, 79.60 mg/g; ET, 60.13 mg/g; **Figure 3B**). The Ca concentration in ET also varied between 1C and 2C, beginning at week 1. The most significant differences in Ca concentrations in ETs between 1C and 2C in the last weeks of vegetation growth were 67% (11% between CNs) for leaves at week 11 (1C, 53.13 mg/g; 2C, 88.87 mg/g; **Figure 3C**).

The content of sulfur compounds was the lowest in the stems and the highest in the leaves (**Figure 4**). The concentration of S in the stems and leaves for CN and ET with 1C began to differ significantly from the third week. The most significant differences in S between CN and ET were 28% for leaves in week 8 (CN, 2375 mg/kg; ET, 3029 mg/kg; **Figure 4A**). In contrast to 1C, the concentration of S in the stems and leaves of CN and ET began to differ significantly from the second week for 2C; however, fewer significant differences than in 1C were observed. The most significant differences in S concentrations between nutritional treatments were 23% for leaves at week 5 (CN, 1834 mg/kg; ET - 2,260 mg/kg; **Figure 4B**). The S concentration also varied between 1C and 2C of ETs but was almost identical till the 3rd week. The most significant differences in S concentrations in ETs between 1C and 2C were 46% (27% between CNs) for leaves at week 8 (1C, 3,029 mg/kg; 2C, 2068 mg/kg; **Figure 4C**).

FIGURE 3 | Distribution of calcium among the organs of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. Ca concentration in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplement in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means ± SE (n=3). The different small letters inside the bars represent significant differences within the plant organs (stems, leaves, and flowers) between the variants in a particular week according to Tukey's HSD test at α=0.05.

The iron content was the lowest in leaves and highest in stems and showed a cumulative trend over time (**Figure 5**). The concentration of Fe in the stems for CN and ET in 1C began to differ significantly from week 6 to 13. The most significant differences in Fe between CN and ET were 79% for stems at week 8 (CN, 609.5 mg/kg; ET, 340.6 mg/kg; **Figure 5A**). In contrast to 1C, the concentration of Fe in the stems of the CN and ET began to differ significantly from week 3 to 13 in 2C. The most significant difference in Fe concentrations between CN and ET was 139% for stems at week 8 (CN, 666.4 mg/kg; ET, 279.3 mg/kg; **Figure 5B**). The Fe concentration also varied between 1C and 2C of ETs by the first week. The most significant difference in Fe concentrations in ETs between 1C and 2C in the last weeks of vegetation growth was 45% (40% between CNs) for stems at week 13 (1C, 844.2 mg/kg; 2C, 584.4 mg/kg; **Figure 5C**).

Nutritional supplementation with AAs in the two different nutritional cycles caused some change in growth of medical cannabis plants. Up to week 5, the increase in biomass was relatively slow, but was sharply increased from week 6. The largest weekly dry weight gain was recorded for flowers (**Figure 6**). The increase in biomass of stems, leaves, and flowers for CNs and ETs in 1C was almost identical until week 7. From week 8 to 12, leaf and flower biomass differed somewhat (**Figure 6A**). In contrast to 1C, stems, leaves, and flowers of CN and ET plants in 2C increased significantly from week 9. ET reached maximum dry plant biomass at week 11, and CN by week 12 (**Figure 6B**). Biomass also

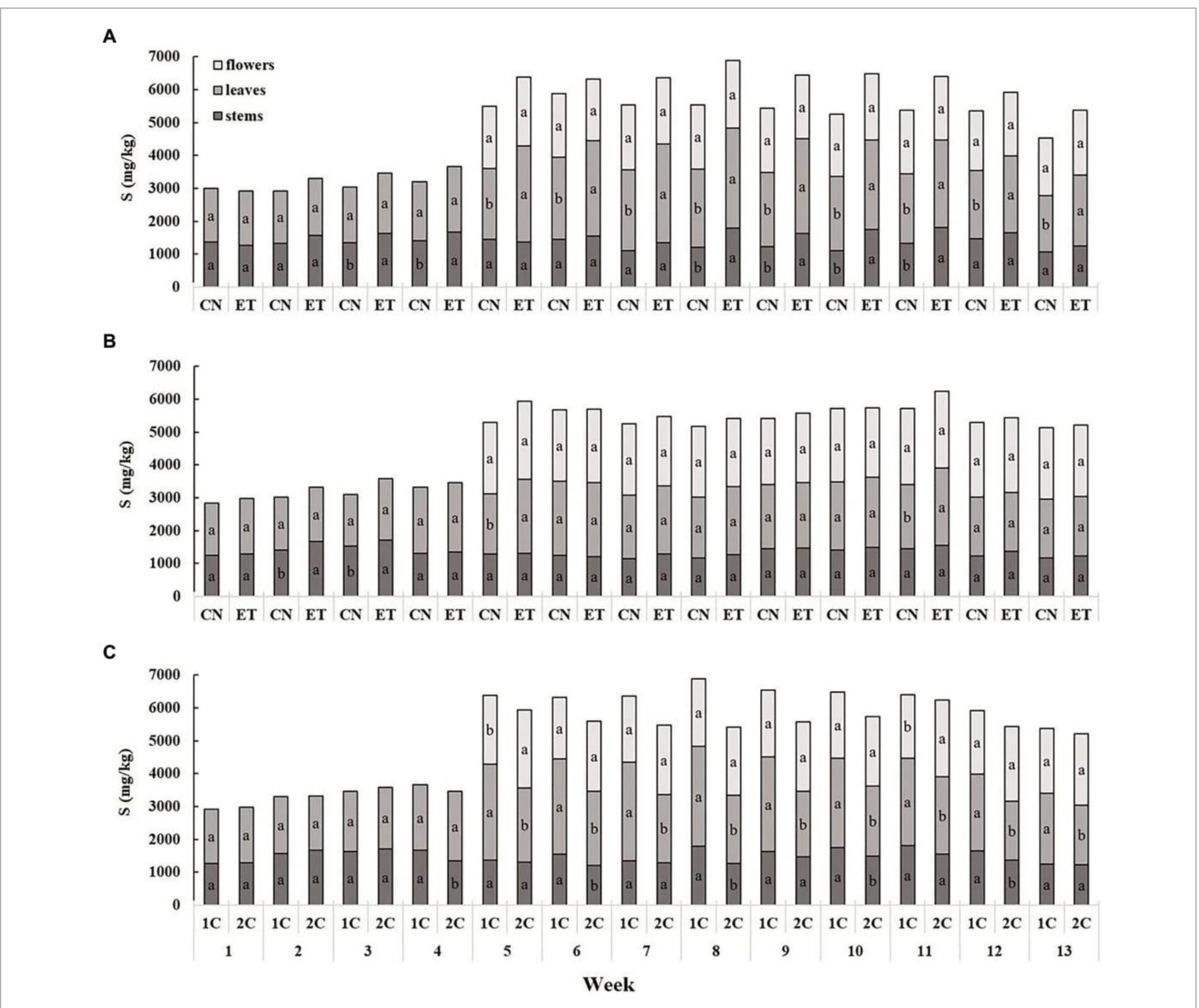

FIGURE 4 | Distribution of sulfur among the organs of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. S concentration in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplement in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means ± SE ($n=3$). The different small letters inside the bars represent significant differences within the medical cannabis plant organs (stems, leaves, and flowers) between the variants in a particular week according to Tukey's HSD test at $\alpha=0.05$.

varied between 1C and 2C of ETs, but from week 7 (**Figure 6C**).

AA supplementation and nutritional cycle changed the concentration of THCA and CBNA in the flowers of cannabis plants, but concentration curves of both cannabinoid acids were similar for the same nutritional cycle and treatment (**Figure 7**). THCA in leaves and flowers slowly increased in both treatments until week 4, but from week 5, the concentration of THCA began to differ significantly because only flowers were analyzed (**Figures 7A–C**). The CN and ET concentrations of THCA began to differ significantly from week 5 to 13 in 1C, and CN (18.2%) and ET (16.0%) reached maximum at week 11 (**Figure 7A**). In contrast to 1C, the concentration of THCA in CN and ET (2C) differed significantly by the third week, but the differences were smaller. THCA peaked at week 9 for CN (15.4%) and week 7 for ET (15.4%; **Figure 7B**). The THCA levels in 1C and 2C of ETs differed significantly from week 5–13 (**Figure 7C**). The CBNA concentration in CN and ET in 1C began to vary significantly between week 5 and 13. CBNA peaked at week 11 in both treatments and differed significantly by 44% (**Figure 7D**). In contrast to 1C, the CBNA concentration in CN and ET did not differ significantly in 2C until weeks 5 and 10. CBNA in CN reached two maxima in 2C: at week 9, where it differed significantly by 41%, and at week 11 where it differed significantly by 44%. The CBNA for ET also reached two maxima in 2C: at week 7 where it differed significantly by 17%, and at week 11, the same as CN (**Figure 7E**). CBNA concentrations between 1C and 2C

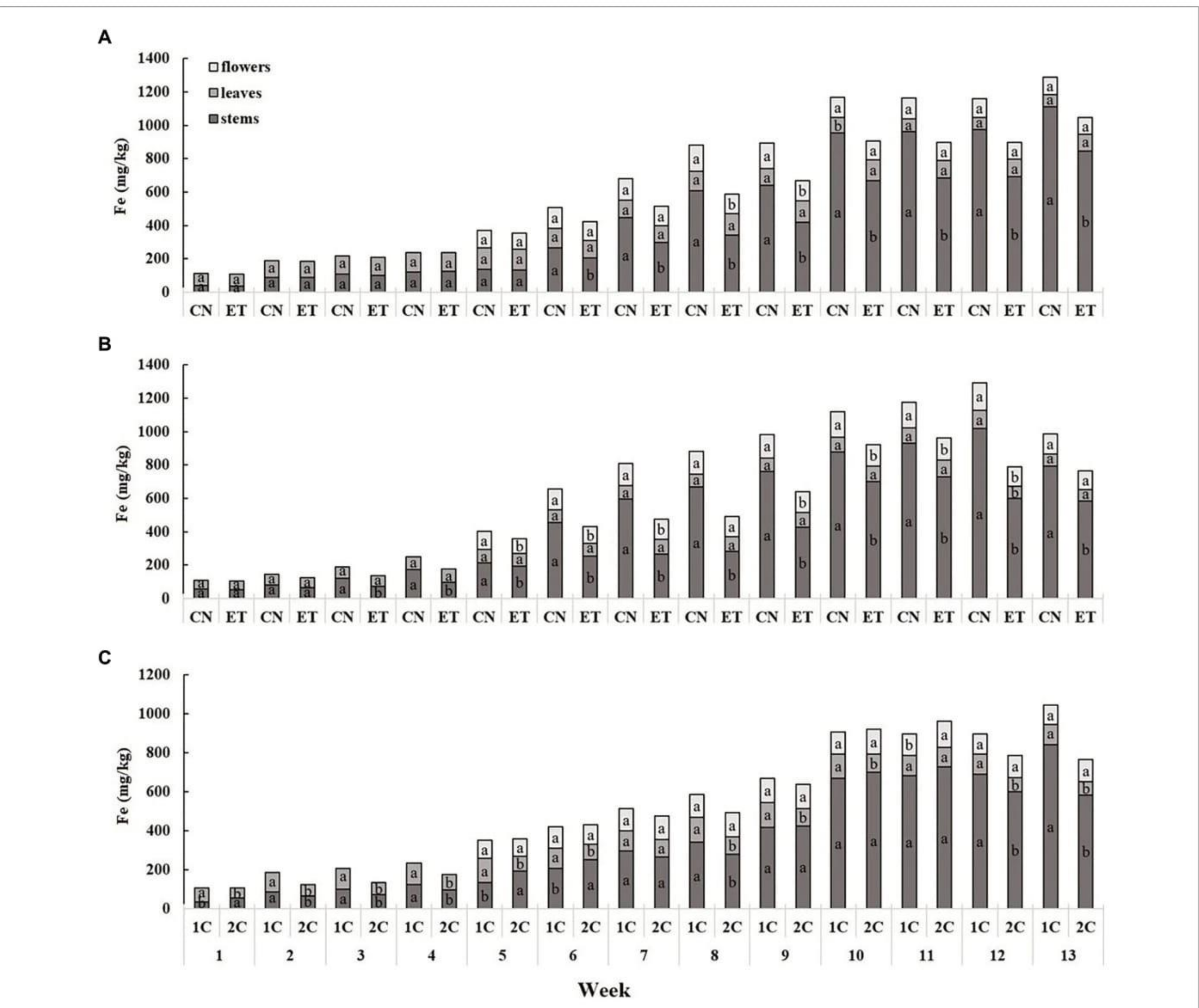

FIGURE 5 | Distribution of iron among the organs of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. Fe concentration in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplements in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means ± SE (n=3). The different small letters inside the bars represent significant differences within the medical cannabis plant organs (stems, leaves, and flowers) between the variants in a particular week according to Tukey's HSD test at α=0.05.

of ETs were almost identical until weeks 5 and 9. As stated above, the CBNA concentration of ET reached maximum at week 11 in 1C, when it differed significantly by 33% (also 33% for CNs) and at week 7 in 2C, when it differed significantly by 83% (7% for CNs; **Figure 7F**).

THCA is the most concentrated cannabinoid in our medical cannabis plant chemotype. The THCA yield per plant from dried flowers over time and the effect of the AA supplement and variable nutritional cycle was measured (**Figure 8**). THCA yields were almost identical for CN and ET with 1C until week 6 but differed significantly from week 7–13. The largest significant difference (46%) between the nutritional treatments was achieved at week 11, but the highest yield for both treatments was at week 13 (**Figure 8A**). The THCA yield for CN with 2C compared to 1C reached its maximum at week 12 (significant difference, 34%) and for ET at week 11 (significant difference, 10%; **Figure 8B**). As stated above, the THCA yield for ET with 1C reached a maximum at week 13 (significant difference between ETs, 279%) and at week 11 for ET with 2C (difference between ETs, 28%; **Figure 8C**).

The concentrations of limonene and β-myrcene in the flowers were also affected by AA supplementation and nutrient cycle (**Figure 9**). Limonene peaked at week 9 for CN (1.33 mg/g) and at week 10 for ET (2.12 mg/g). The most significant difference in limonene concentration between these two treatments was 81% reached at week 8 in 1C (**Figure 9A**). As in 1C, limonene concentration peaked at week 9 for CN (0.94 mg/g) but at week 8 for ET (1.58 mg/g) in 2C. The largest

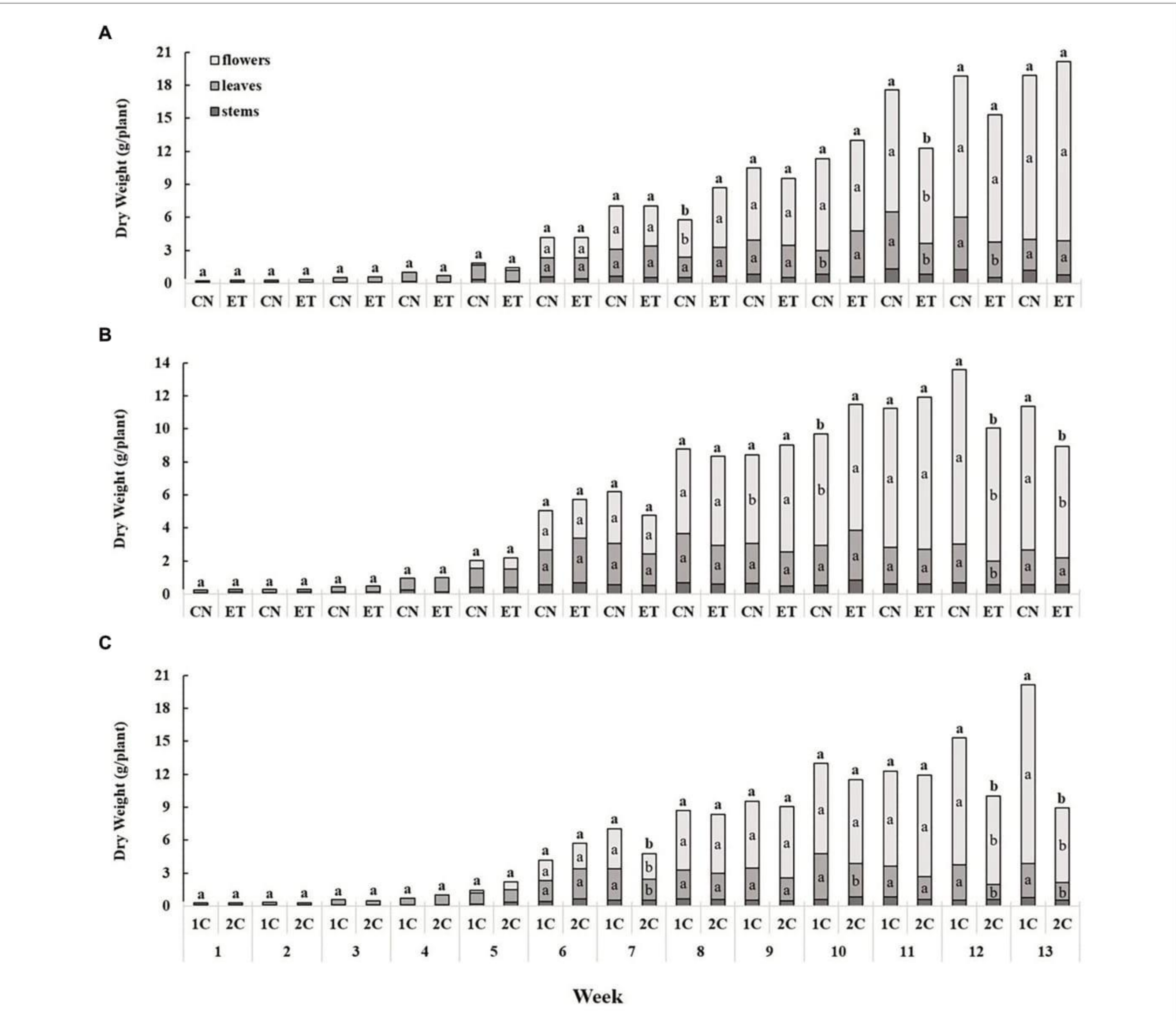

FIGURE 6 | The effect of amino acid supplementation (AAs) and growing nutritional cycle on medical cannabis plant biomass. Dry biomasses of stems, leaves, and flowers in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplements in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)**. Data are means ± SE (n=3). The different small letters inside the bars and small bold letters above the bars represent significant differences within the medical cannabis plant organs (leaves and flowers) and the whole plant biomass between the variants in a particular week according to Tukey's HSD test at α=0.05.

significant difference in limonene concentration between these two treatments was 123% reached at week 10 (**Figure 9B**). Comparing limonene concentrations of ETs for 1C and 2C, the largest significant difference between these two cycles was 37% at week 10 (**Figure 9C**). β-myrcene levels peaked at week 9 for CN (0.89 mg/g) and at week 10 for ET (1.46 mg/g). The largest significant difference in β-myrcene concentration between these two treatments was 139% at week 8 in 1C (**Figure 9D**). As in 1C, β-myrcene peaked at week 9 for CN (0.61 mg/g), but at week 8 for ET (1.38 mg/g) in 2C. The largest significant difference in β-myrcene concentration between these two treatments was 167% at week 8 (**Figure 9E**). Comparing β-myrcene concentration in ETs for 1C and 2C, the most significant difference between these two cycles was 28% reached at week 10 (**Figure 9F**).

DISCUSSION

Nutrition is undoubtedly an important factor in the development, function, and metabolism of all plant organs and tissues. Data are already known regarding the optimal levels of individual macronutrients, such as N, P, and K, for normal function and development of the root system and above-ground biomass (Saloner et al., 2019; Saloner and Bernstein, 2020; Shiponi and Bernstein, 2021b) and formation of the desirable secondary

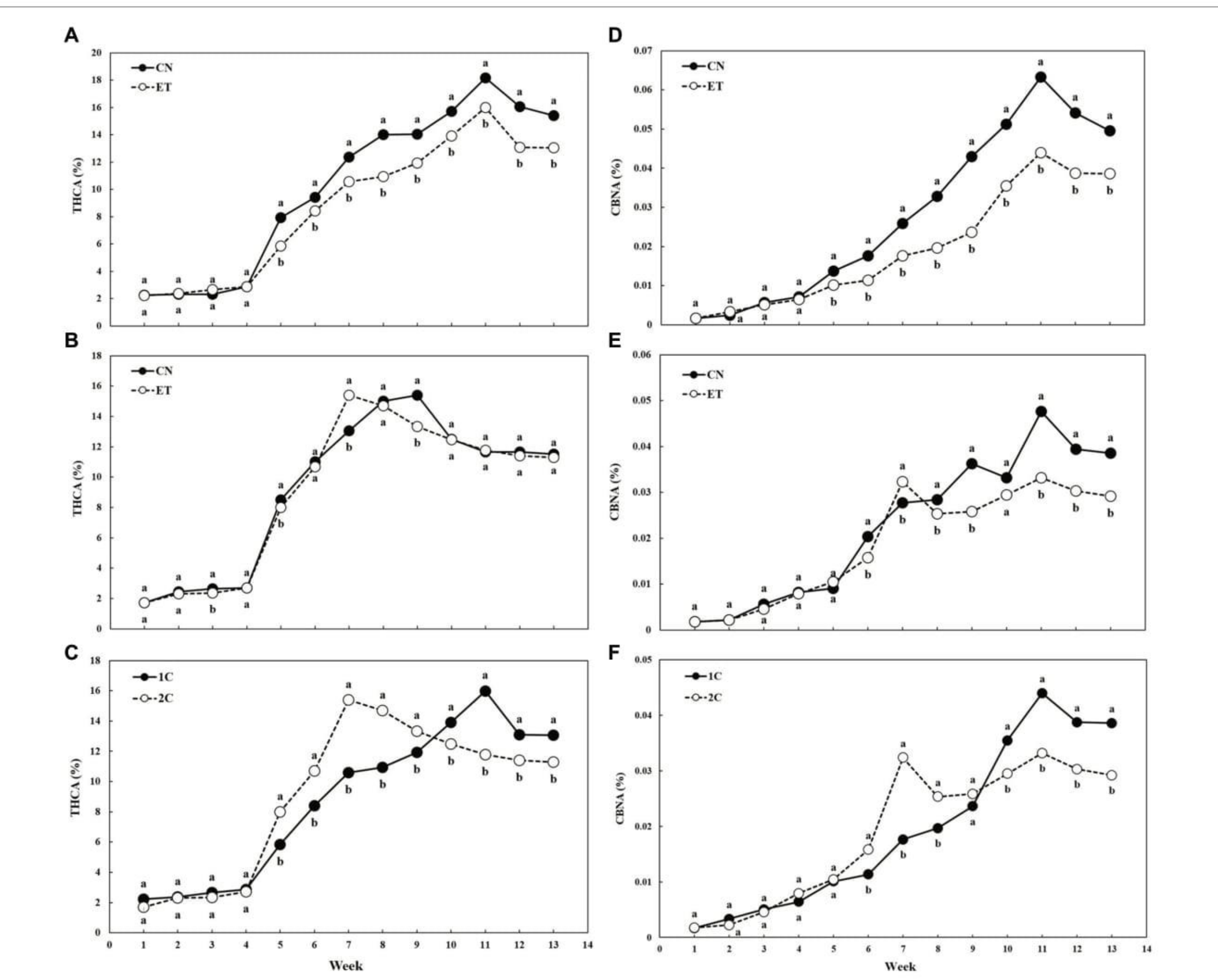

FIGURE 7 | Concentrations of tetrahydrocannabinolic acid (THCA) and cannabinolic acid (CBNA) in the flowers of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. THCA concentration in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplements in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)**. CBNA concentration of control (CN) and enhanced treatment (ET) with AAs nutritional supplements in recirculation (1C) growing cycle **(D)**, in drain-to-waste (2C) growing cycle **(E)**, ETs in 1C and 2C **(F)**. The whole inflorescence of the plant was analyzed. Data are means ± SE ($n=3$). Different bold small letters represent significant differences in cannabinoid concentration between the variants in a particular week according to Tukey's HSD test at $\alpha=0.05$.

metabolites of medical cannabis plants (Caplan et al., 2017a; Bernstein et al., 2019; Saloner and Bernstein, 2021; Yep et al., 2021; Shiponi and Bernstein, 2021a). However, there is still emphasis on the availability of sufficient quantities of these major plant macronutrients in an optimal ratio. The effects of micronutrients (Yep et al., 2021) and plant biostimulants (Bernstein et al., 2019) must also be considered.

Nutritional treatment with AA supplements in different nutrient cycles clearly affected the concentrations of macro- and micro-elements in cannabis plants. Antagonistic and synergistic interactions between nutrient anions and cations during root cell membrane transport have been relatively well reported. However, the timing of replenishment of AAs and variations in pH, as in the case of the recirculation cycle, 1C, could affect their accessibility from the nutrient solution and thus the subsequent physiological and metabolic response of plants. The enhanced treatment (ET) with AA supplementation resulted in significantly greater nitrogen accumulation (**Figures 2A,B**) in all three plant organs, but mostly in flowers and leaves. This finding is consistent with claims that plants can absorb and incorporate intact amino acids directly (Matsumoto et al., 1999; Persson and Nasholm, 2001; Jämtgård et al., 2008). AAs can also modulate the assimilation and absorption of N in plants by regulating the enzymes and structural proteins involved in these processes. AAs also affect N uptake signaling pathways in roots and promote transfer between nitrogen and carbon metabolites by controlling enzymes of the tricarboxylic acid cycle (Colla et al., 2014; Du Jardin, 2015). When comparing nutritional cycles (**Figure 2C**), higher N concentrations were observed in the above-ground organs of plants, especially in leaves and flowers from ET plants in 1C. This was probably due

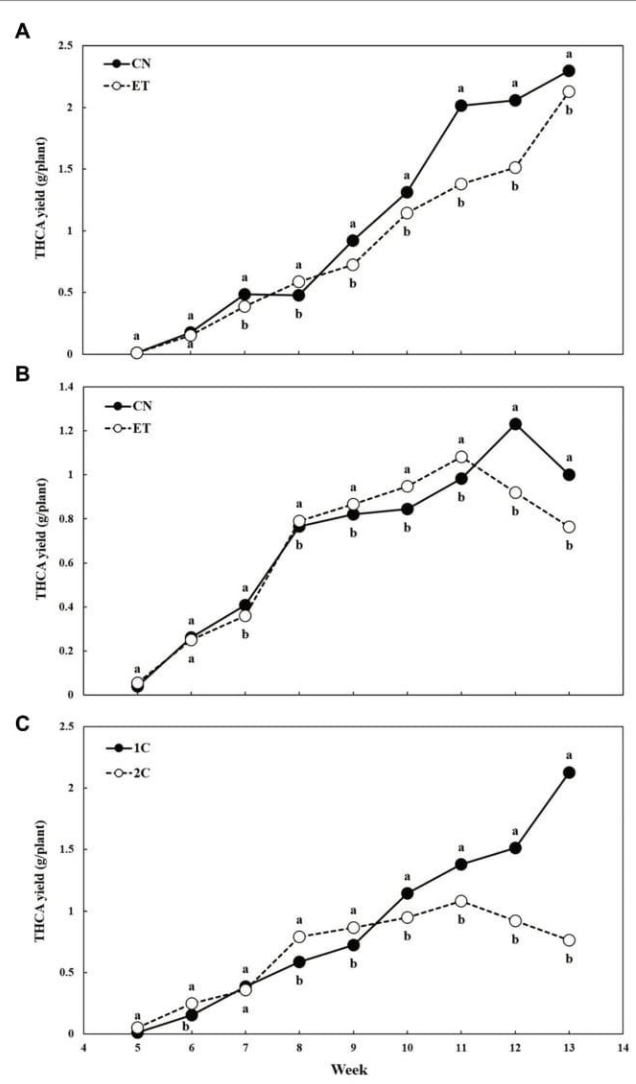

FIGURE 8 | Tetrahydrocannabinolic acid (THCA) yield per plant as affected by amino acid (AA) supplementation and nutrient cycle. THCA yield per plant in control (CN) and enhanced treatment (ET) with AAs nutritional supplements in recirculation (1C) nutrient cycle **(A)**, in drain-to-waste (2C) nutrient cycle **(B)**, ETs in 1C and 2C **(C)**. The whole inflorescence of the plant was analyzed. Data are means ± SE ($n = 3$). Different bold small letters represent significant differences in cannabinoid concentration between the variants in a particular week according to Tukey's HSD test at $\alpha = 0.05$.

to fluctuations in the pH of the 1C nutrient solution from addition of AAs, which increased the pH to 8.05 after 24 h. The initial pH of the nutrient solution, 5.9 (the constant pH of the 2C nutrient solution), was close to the isoelectric point of most AAs (Pogliani, 1992), but recirculation may have resulted in the formation of a partial charge on some AA molecules. At pH 5.9, most AAs were in the neutral zwitterionic form, making them less able to enter plant cells because of lipophilic interactions during membrane transport (Trapp, 2004). Sulfur showed an accumulation trend similar to N, but at a lower concentration (**Figure 4**), because of the sulfur-containing AAs, cysteine and methionine (**Table 1**). In 2C (**Figure 4B**), the S concentrations were almost identical in both treatments, probably because of lower solubility of the sulfur AAs at pH 5.9 and reduced absorption.

Calcium accumulation followed an opposite trend (**Figure 3**). In the ET group, the AA supplementation significantly lowered calcium accumulation (**Figures 3A,B**) in all three plant organs, but mostly in leaves and flowers. The same trend was observed for magnesium accumulation (*data not shown*), but with minor differences because of lower concentration. This was probably due to the coordination of calcium with the carboxyl, hydroxyl, thiol, and amino groups of the AAs to form complexes with limited accessibility (Maeda et al., 1990). When comparing nutritional cycles (**Figure 3C**), higher calcium concentrations were observed in above-ground parts, especially leaves and flowers, from ET plants in 2C. This was probably due to the stable 5.9 pH of the 2C nutrient solution, in which the AAs were in the form of zwitterions that did not complex with *Ca*. The increased formation of root exudates containing negatively charged or free electron pair groups capable of coordinating and binding Ca from the nutrient solution might also have contributed to this process. It is probable that more exudates were excreted in 1C because of the pH change in the cytosol and also from the increase in TCA cycle function after uptake of negatively charged AAs (Ryan et al., 2001). In the case of 2C, replenishment with fresh nutrient solution also contributed to increased calcium ions. Iron showed an accumulation trend similar to calcium, only at lower concentrations, where it occurred mainly in the stem due to low mobility (**Figure 5**). However, when comparing nutritional cycles (**Figure 5C**), a higher Fe concentration was observed at some weeks in above-ground organs, especially leaves and stems, of ET plants in 1C. This may have resulted from the Fe levels of ET plants in 2C reaching a maximum at week 11 compared to week 13 in 1C, and also, from the chelating effects of some AAs, which could contribute to mobility and micronutrient acquisition by roots (Calvo et al., 2014). The levels of phosphorus and potassium (*data not shown*) did not differ in nutrient solutions, nor did they show many significant differences in accumulation in the above-ground organs of both treatments, so they were not discussed.

The changed accessibility and supply of individual nutrients within CN and ET plants during different nutritional cycles also affected the yield of dry biomass of stems, leaves, and flowers (**Figure 6**). In CN with 1C, only a slight increase in the weight of above-ground biomass was observed from week 11 to 13, whereas in ET we saw a sharp increase in total dry matter, especially in flowers, in the last weeks (**Figure 6A**). This was probably caused by an increased supply of nitrogenous and possibly other compounds in the root cells of ET plants and their subsequent transport to flowers during the so-called rinsing period (watering only with DMW; **Table 3**) from week 10–13 (Pratelli and Pilot, 2014; Yao et al., 2020). In CN plants with 2C, the maximum increase in biomass was reached at week 12, and in ET a week earlier (**Figure 6B**). This probably resulted from earlier maturation of the plants with 2C compared to 1C. The differences in dry biomass in the CN and ET groups in

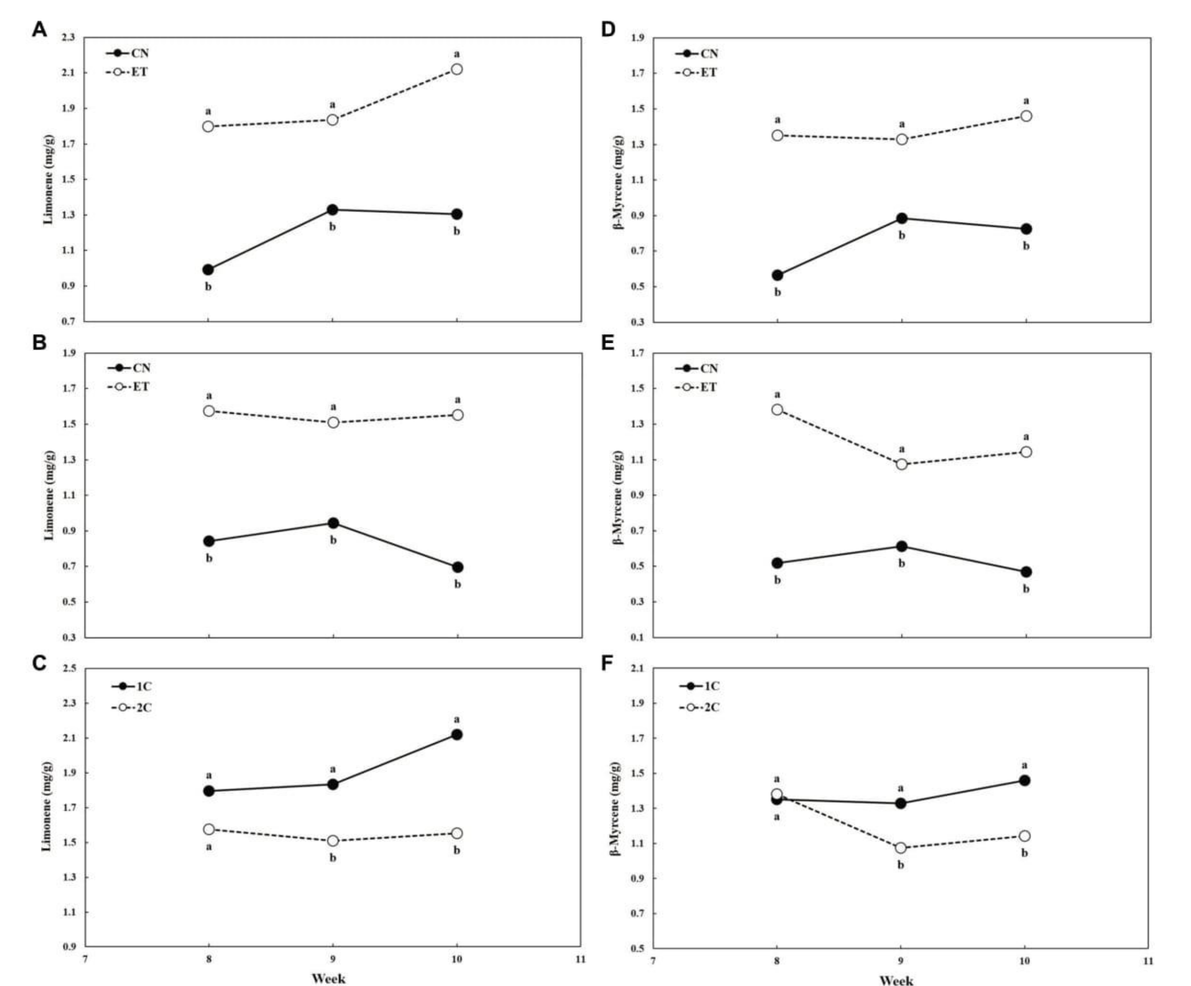

FIGURE 9 | Concentration of limonene and β-myrcene in flowers of medical cannabis plants as affected by amino acid (AA) supplementation and nutrient cycle. Limonene concentration in control (CN) and enhanced treatment (ET) plants with AAs nutritional supplements in recirculation (1C) nutrient cycle **(A)**, in drain-to-waste (2C) nutrient cycle **(B)**, ETs in 1C and 2C **(C)**, the β-myrcene concentration of control (CN) and enhanced treatment (ET) with AAs nutritional supplements in recirculation (1C) growing cycle **(D)**, in drain-to-waste (2C) growing cycle **(E)**, ETs in 1C and 2C **(F)**. The whole inflorescence of the plant was analyzed. Data are means ± SE ($n=3$). Different bold small letters represent significant differences in cannabinoid concentration between the variants in a particular week according to Tukey's HSD test at $\alpha=0.05$.

both cycles were mainly due to the different N doses from AAs delivered to ET plants from the second (first blooming) week (**Tables 2** and **3**). According to Saloner and Bernstein (2021), the optimal dose of mineral N for medical cannabis in bloom is 160 mg/l. In our experiments, the amount of mineral N in the nutrient solution was gradually increased from 116 mg/l (week 2) to 150 mg/l (weeks 4 and 6–9) in both CN and ET. Caplan et al. (2017a) stated that the optimal dose of N in organic fertilizers for maximum biomass of medical cannabis plants in bloom was 283 mg/l. In our experiments, the amount of organic N in the nutrient solution for ET plants was gradually increased from 184 mg/l (week 2) to 203 mg/l (weeks 4 and 6–9). However, the amount of total N supplied in the nutrient solution for ET ranged from 300 mg/l (week 2) to 353 mg/l (weeks 4 and 6–9; **Table 3**). Therefore, this amount of total nitrogen in the nutrient solution may already have exceeded the optimal dosage for medical cannabis plants, especially with 2C (Albornoz, 2016).

This hypothesis was partially supported by the premature ripening of plants based on the concentration of THCA in ET in 2C (**Figure 7B**), but this could also be caused by increased abiotic stress from high N doses (Gepstein and Glick, 2013). Conversely, the higher dose of nutrients in 2C compared to 1C ensured optimal fertigation, which can shorten the ripening time of cannabis (Caplan et al., 2017b). This hypothesis was supported by the nearly identical trend of increasing THCA concentration with 2C in both treatments, although ET peaked at week 7 compared to CN at week 9 (**Figure 7B**). When comparing ET results at 1C and 2C, the difference was 4 weeks because the ET plants with 1C did not reach their maximum THCA concentrations until the 11th week (**Figure 7C**). Differences in THCA concentrations in both treatments and cycles, but especially in 1C, could

be explained by the previously discovered positive correlation of calcium with Δ^9-tetrahydrocannabinol (Δ^9-THC), which is a decarboxylation product of THCA (**Figures 3**, **7**; Pate, 1994). Its oxidation product, CBNA, had a similar course and maxima as THCA, but reversed (**Figures 7D–F**), probably because of the antioxidant activity of AAs, which reduced environmental stress by scavenging free oxygen radicals (Calvo et al., 2014).

The combination of the dry weight of flowers and the concentration of THCA was reflected in the yield of THCA. In 1C, an almost linear dependence of THCA yield on time could be seen for both treatments (**Figure 8A**) because of the lower amount of total nutrients supplied in 1C compared to 2C, and thus the delay in ripening time. However, when comparing ETs from both cycles at the weeks of their maximum THCA yield (week 13 for 1C and week 11for 2C), the THCA yield with 1C was more than twice as high (**Figure 8C**). This may have been a result of the increased production of abscisic acid (ABA) in response to stress, which slows plant growth and increases THCA production (Mansouri et al., 2009). It was also likely to cause oxidative stress (Jiang and Zhang, 2001), thus indirectly increasing CBNA production (**Figure 7F**).

The final concentration of monoterpenes showed the same trend in the respective weeks in both cycles and treatments as the concentration of THCA (**Figure 9**). This was consistent with Aizpurua-Olaizola et al. (2016) who claimed that this could be explained by the fact that monoterpenes were synthesized in the same glandular trichomes as cannabinoids (Meier and Mediavilla, 1998). Similar to Saloner and Bernstein (2021), our results showed that the increased N in the nutrient solution decreased THCA concentration proportionally. But conversely when exceeding a specific limit of nitrogen fertilization, as 160 mg N/L in the case of Saloner and Bernstein (2021), a reversible increase in limonene and myrcene concentration was observed. This was in agreement with studies showing a positive dependence of isoprene unit formation on N fertilization (Mccullough and Kulman, 1991; Close et al., 2004). High N concentrations in leaves promoted photosynthetic activity, which increased the availability of assimilated carbon used to generate metabolites *via* the methylerythritol pyrophosphate (MEP) pathway (Ormeno and Fernandez, 2012). Two biosynthetic pathways contributed to the early steps in the production of plant terpenes. The first is the cytosolic mevalonic acid (MVA) pathway, which is involved in the biosynthesis of sesquiterpenes and triterpenes. The second, plastid-localized methylerythritol phosphate (MEP) pathway, is involved in the biosynthesis of monoterpenes, diterpenes, and tetraterpenes (Bouvier et al., 2005). Phytocannabinoids are synthesized from isoprenoid precursors combined with fatty acids (Dewick, 2002). However, the geranyl pyrophosphate necessary for the production of the terpenoid part of cannabinoids is predominantly (>98%) synthesized by the MEP pathway in plastids (Fellermeier et al., 2001). Because limonene, β-myrcene, and the terpenoid part of THCA are synthesized *via* the same MEP pathway and exhibit a concentration response in medical cannabis flowers opposite to that from addition of AAs to the nutrient solution, which increases N levels, it can be concluded that the biosynthesis of the ketide (fatty acid) part of the THCA molecule may be affected (Tedesco and Duerr, 1989). However, further research will be needed to draw relevant conclusions.

CONCLUSION

This study investigated the effects of amino acid supplementation and two different nutritional cycles (systems) on medical cannabis growth. The exact relationship between the content of secondary metabolites and the nutritional supplements remains unclear. This connection is complex and involves several parameters, including nutrient availability, biosynthetic conditions, and physiological signals. The amino acid-based nutritional supplement significantly increased the nitrogen and sulfur content and reduced the accumulation of calcium and iron in both cycles throughout the plant. It caused earlier maturation in plants as reflected in the THCA concentration in the drain-to-waste cycle and reduced the CBNA content in flowers. Furthermore, in both nutritional cycles, it significantly increased the content of monoterpenes, limonene and β-myrcene. When comparing the nutritional cycles of treatments with the amino acid supplement, it can be seen that a significantly higher content of nitrogen and sulfur was achieved in the recirculation cycle, but a lower content of calcium in the whole plant. In the drain-to-waste cycle, medical cannabis plants matured about a month earlier, based on THCA concentration, but at the expense of half-maximal THCA yield in flowers and significantly lower concentrations of limonene and β-myrcene than with the recirculation cycle. This study clearly shows the advantages and disadvantages of the amino acid-based biostimulant and of the different nutritional cycles. In the recirculation cycle, higher yields of secondary metabolites were achieved with much lower total nutrient consumption, but over a more extended time. On the contrary, the drain-to-waste cycle allowed better control of the nutrient solution, stable supply of accurate nutrient concentration, and accelerated plant ripening, but with higher fertilizer consumption and lower overall yield of secondary metabolites. This study examined a high-yield THCA variety classified as chemotype I grown hydroponically in Euro Pebbles (expanded clay) medium. Therefore, it would be interesting to carry out these studies on cannabis varieties of different chemotypes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MM designed the study, wrote the manuscript, controlled the cultivation scheme, and performed physiological, chemical, and data analyses. JV designed the study, controlled the cultivation scheme, and performed physiological and chemical analyses. LP and AJ performed chemical analyses. ZK controlled the cultivation scheme. PK supervised the study. PT designed and supervised the study. All authors contributed to the article and approved the submitted version.

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4.4 Effect of Augmented Nutrient Composition and Fertigation System on Biomass Yield and Cannabinoid Content of Medicinal Cannabis (*Cannabis Sativa* L.) Cultivation

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Effect of augmented nutrient composition and fertigation system on biomass yield and cannabinoid content of medicinal cannabis (*Cannabis sativa* L.) cultivation

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Growing evidence underscores the role of nutrients and fertigation systems in soilless production, influencing medicinal cannabis biomass and secondary metabolite content. This study delves into the impact of enhanced nutrient regimes on the 'ionome' and its ramifications for biomass and cannabinoid production in medicinal cannabis, comparing two distinct fertigation systems: recirculation and drain-to-waste. Notably, we assess the optimal harvest time for maximizing profitability. In comparing the experimental variant with elevated levels of phosphorus (P), potassium (K), and iron (Fe) in the nutrient solution to the control variant, we observe distinct patterns in element composition across stems, leaves, and flowers, with significant differences between fertigation systems. Total nitrogen content was determined through the Kjeldahl method. Flame atomic absorption spectrometry (FAAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) were employed for elemental analysis. Cannabinoid identification and quantification used high-performance liquid chromatography with a diode-array detector (HPLC/DAD). Followed statistical analyses included ANOVA and Tukey's HSD test. Although the augmented nutrient regimen does not substantially increase plant biomass, interesting differences emerge between the two fertigation systems. The recirculation fertigation system proves more profitable during the recommended harvest period. Nonetheless, the altered nutrient regime does not yield statistically significant differences in final inflorescence harvest mass or cannabinoid concentrations in medicinal cannabis. The choice of fertigation system influences the quantity and quality of harvested inflorescence. To optimize the balance between the dry biomass yield of flowers and cannabinoid concentration, primarily total THC yield (sum of tetrahydrocannabinolic acid, Δ^9-tetrahydrocannabinol, and Δ^8-tetrahydrocannabinol), we propose the 11th week of cultivation as the suitable harvest time for the recirculation system. Importantly, the recirculation system consistently outperformed the drain-to-

waste system, especially after the ninth week, resulting in significantly higher total THC yields. Enriched nutrition, when compared with control, increased THC yield up to 50.7%, with a remarkable 182% surge in the recirculation system when compared with the drain-to-waste system.

KEYWORDS

indoor-cultivation, cannabinoids, THC, *Cannabis sativa* L., soilless-cultivation, fertigation

1 Introduction

The pharmaceutical industry faces unique challenges in large-scale cultivation and quality control of plant-based medications, especially with *Cannabis sativa* L., which is subject to varying international regulations (Chandra et al., 2017; Malík et al., 2021). Indoor cultivation has evolved through techniques like 'sinsemilla' (cultivating non-pollinated female plants), cuttings from superior mother plants, and hydroponic systems (Moeller and Lindholst, 2014). *In vitro* plant tissue culture techniques have emerged as a helpful approach (Hesami et al., 2021; Král et al., 2022; Šenkyřík et al., 2023), allowing for large-scale production of genetically identical plants in controlled laboratory conditions (Lata et al., 2016). These methods, combined with intensive breeding, have increased yields of female inflorescences and their THC (Δ^9-tetrahydrocannabinol) levels (Toonen et al., 2006) and improved cannabinoid profile uniformity by altering plant architecture (Danziger and Bernstein, 2021). Indoor cultivation now incorporates automated systems (Malík et al., 2022), with hydroponics gaining popularity for its potential to yield high-quality plant material (Bouchard and Dion, 2009; Vanhove et al., 2011).

Environmental factors, particularly nutrient availability, significantly affect the quantity and quality of secondary metabolites such as cannabinoids and terpenes in cannabis (Janatová et al., 2018). Nutrients like nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) play pivotal roles in plant growth and secondary metabolism (Bernstein et al., 2019b; Bevan et al., 2021; Kumar et al., 2021). Phosphorus, for instance, influences root growth, flower and seed production, and stem strength (Zheng, 2022) and affects terpene profiles in aromatic plants (Rioba et al., 2015). While inadequate P supplementation can lead to deficiency symptoms, excessive supply often accumulates in roots (Shiponi and Bernstein, 2021a; Llewellyn et al., 2023). The impact of increased phosphorus supplementation on medicinal cannabis varies across organs and compounds, with key cannabinoids remaining unaffected in upper flowers (Bernstein et al., 2019b; Shiponi and Bernstein, 2021b).

Potassium regulates water and nutrient transport, cell turgor pressure, disease resistance, stem strength, and inflorescence quality and yield (Oosterhuis et al., 2014; Johnson et al., 2022). It influences various secondary metabolites, such as phenolic compounds (Nguyen et al., 2010; Varo et al., 2022), flavonoids (Chrysargyris et al., 2017; Gaaliche et al., 2019), carotenoids (San Martín-Hernández et al., 2021), and organic acids (Naumann et al., 2020), but excessive potassium can reduce secondary metabolites like cannabinoids and terpenoids (Saloner and Bernstein, 2022). Optimizing K supply is crucial to maintaining medicinal cannabis desired functionality, yield, and secondary metabolite profiles. Iron facilitates oxidation-reduction and electron transfer reactions, activates enzymes, and is essential for photosynthesis and respiration (Briat et al., 2015; Zheng, 2022). Fe deficiency leads to chlorosis in young leaves, impaired root development, yield reduction, and compromised nutritional quality in crops (Kumar et al., 2021; Llewellyn et al., 2023). Fe concentrations in plant nutrition may have varying effects on secondary metabolite production (Shi et al., 2018; Chaouqi et al., 2023), but no specific study has explored the influence of Fe nutrition in indoor medicinal cannabis cultivation. Maintaining appropriate rootzone pH is crucial for nutrient availability, microorganism activity, and root development, affecting water and nutrient uptake (Zheng, 2021). The recommended pH range in hydroponic culture is typically between 5.5–6.0 (Velazquez, 2013), while soilless production suggests 5.5–6.5 (Zheng, 2022). If the pH in the soilless production drops below 5.5, there is a risk of toxicity due to excessive levels of manganese (Mn), while a pH higher than 6.5 can result in limited availability of essential elements such as P, Fe, and Mn for plant uptake (Balliu et al., 2021). pH levels affect nutrient availability and variations between recirculation and drain-to-waste systems, with recirculation systems exhibiting fluctuations (Malík et al., 2023).

This investigation examines the physiological and chemical reactions of medicinal cannabis plants when exposed to varying concentrations of phosphorus (P), potassium (K), and iron (Fe) in the nutrient solution. The study compares the results obtained under two distinct fertigation systems. In light of these objectives, the following hypotheses were put forth: (1) Alterations in the concentrations of P, K, and Fe in the nutrient solution can lead to different inflorescence yields of medicinal cannabis plants; (2) changes in the cannabinoid profile of indoor-cultivated cannabis plants; (3) these induced changes will exhibit a correlation with the content of macro-elements and micro-element (Fe) within different plant organs, including leaves, stems, and flowers; (4) furthermore, the induced

changes will display variability between different nutrition systems, namely recirculation and drain-to-waste approaches.

2 Materials and methods

2.1 Parameters of the cultivation room

Cannabis plants were cultivated in a controlled environment within growing tables in a soilless system. A cultivation chamber has an area of 15 m^2 (3 × 5 m), with the actual cultivation space delineated by four cultivation tables, covering a total area of 8 m^2. The tables, measuring 2 m^2 (1 × 2 m), were designated for individual experiments and featured independent 100-litre tanks for nutrient solutions. These tanks were constructed using food-grade inert plastic materials. Each table accommodated a maximum of 55 black conical, square pots made of polypropylene (PP). These pots had a volume of 3.45 liters and dimensions: top – 15 cm × 15 cm, base – 11.5 cm × 11.5 cm, height – 20 cm. Capillaries were used for fertigation, ensuring each plant received individualized watering through a needle applicator. The fertigation system was programmed to provide nine cycles per day, with each cycle lasting 60 seconds. During each cycle, 94 mL of the nutrient solution was delivered to each plant (equivalent to 846 mL per plant per day). In the recirculation system, the plant drainage was piped back to the storage tank. In the drain-to-waste system, the used nutrient solution was directed to a separate waste tank and not mixed with the original solution. Microclimate parameters, including relative humidity, temperature, and CO_2 levels, were regulated and monitored by an air ventilation unit. A methane-burning generator facilitated CO_2 enrichment in the growth environment (550 ppm). Six double-ended high-pressure sodium lamps provided illumination with a power output of 1,000 W, delivering a suitable light spectrum for plant growth. The lamps generated a photosynthetic photon flux density (PPFD) of 1,029 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a total power of 6,000 W. The light conditions were continuously recorded using a data logger, capturing measurements every minute.

2.2 Plant material and cultivation conditions

The plant material utilized in this study consisted of cuttings (apical parts with at least 3 fully expanded leaves) obtained from *C. sativa* L. 'McLove' mother plants (Atherton and Li, 2023). These plants belong to chemotype I, characterized by a high ratio of Δ^9-tetrahydrocannabinolic acid:cannabidiolic acid (THCA : CBDA > 1.0) (Handbook of cannabis, 2016). The mother plants were carefully maintained in a dedicated separate growth chamber under controlled conditions. A total of 220 cuttings were prepared. These cuttings were then cultivated for a period of 21 days in rock-wool cubes measuring 4 × 4 cm. Once the cuttings had developed roots, they were transferred to a separate cultivation room and placed in 3.45-liter pots filled with three liters of expanded clay growing medium. During the vegetative phase, the light regime consisted of 18 hours of light and 6 hours of darkness, with a temperature of 25°C, a relative humidity of 60%, and a CO_2 concentration of 550 ppm (1.065 $mg \cdot L^{-1}$). During the dark phase, the temperature was lowered to 22°C while maintaining the same humidity level. The vegetative phase lasted 7 days, after which the cultivation conditions were adjusted for the generative phase. During the generative phase, the light period was set to 12 hours of light and 12 hours of darkness. The temperature and CO_2 concentration remained the same as during the vegetative phase, while the relative humidity was reduced to 40%. Starting from the 10th week, the plants were irrigated only with demineralized (DM) water.

2.3 Treatments

In the cultivation room, the plants were divided into 4 tables, each containing 55 plants (27.5 plants/m^2), as shown in Figure 1. Plants were grown in two fertigation systems. The first cycle (1C) involved the recirculation of the nutrient solution, while the second cycle (2C) employed the drain-to-waste system. At the same time, the plants were divided into two groups: control treatment (CN) and enhanced treatment (ET). Compared to CN, the ET underwent

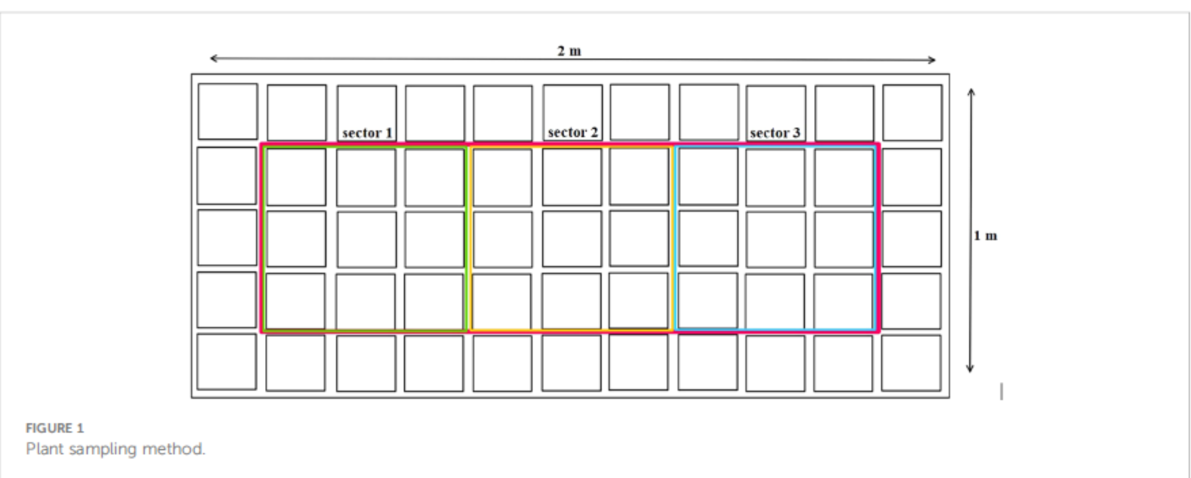

FIGURE 1
Plant sampling method.

an augmented nutritional regime. For the 1C, the nutrient solution was prepared by mixing DM water every 7 days, starting from the experiment's first day. Since the 10th week, plants were irrigated solely with DM. Solution pH was adjusted to 5.9, and EC values were recorded immediately after preparing the fresh solution and remeasured on the last day before its replacement. A sample of the nutrient solution was collected after each preparation. For the 2C, everything remained the same as for 1C, except the stock solution was prepared after depleting the stock tank, approximately every 2nd to 3rd day. Nutrient content was increased according to the age of plants. Table 1 presents the composition of the nutrient solution used for the CN as determined through measurement, and descriptions of the provided nutrients for ET can be found in Table 2. In contrast to the CN variant, the content of P, K, and Fe increased by an average of 83% P, 39% K, and 860% Fe from the 5th week to the 9th week in the ET variant.

2.4 Plant material sampling

For each treatment and cycle, three plants were harvested every 7 days throughout the entire vegetation period. One plant was randomly selected from each highlighted sector (1–3), as shown in Figure 1. Additionally, a random plant from the edge (outside the sectors) was transferred to an empty space within each sector. The sampled plants were weighed fresh as a whole and then divided into leaves, stems, and flowers, which were weighed separately. Subsequently, the materials were dried at a constant moisture level of 8–10% at 25°C and reweighed. To determine the dry matter, a reference amount of each plant part was dried at 105°C until a constant weight was achieved. Just before analysis, the plant parts were homogenized. The flowers (including leaves until the 4th week) were frozen using liquid nitrogen and then ground using a mortar and pestle. The dried leaves (from the 5th week) and stems were ground using a grinder.

2.5 Dry decomposition and elemental analysis

The leaves, stems, and flowers were individually analyzed to determine the content of macroelements (excluding nitrogen), microelements, and trace elements in the plant samples. The plant biomass, which had been weighed and homogenized, was placed in a beaker and covered with a watch glass. The beaker was then placed on a hotplate set at 160°C, and the temperature was gradually increased to 350°C over a period of 4 hours to facilitate decomposition of the samples. Subsequently, the samples were transferred to a muffle furnace and maintained at a temperature of 450–500°C for 12 hours, as described in previous studies (Miholová et al., 1993). After cooling, 1 mL of 65% HNO_3 was added to the beakers, which were then placed on a hot plate set at 120°C for 60 minutes. Following this step, the samples were annealed for 90 minutes in an oven at 500°C and suspended in 1.5% HNO_3 with stirring using an ultrasonic bath. Elemental analysis of the samples was conducted using flame atomic absorption spectrometry (FAAS) on a Varian 280FS instrument (Varian, Australia), coupled with inductively coupled plasma optical emission spectrometry (ICP-OES) performed on a Varian Vista-PRO (Varian, Australia), (Mester and Sturgeon, 2003).

2.6 Determination of nitrogen content in plant material

The Kjeldahl method was employed to determine the total nitrogen content in the plant material, encompassing both organic

TABLE 1 Composition of control treatment (CN) nutrient solution ($mg \cdot L^{-1}$).

Week	1	2	3	4	5	6–9	10–13
N	101 ± 1.6	116 ± 1.9	130 ± 1.8	150 ± 1.9	130 ± 1.8	150 ± 1.9	DM
P	32. ± 0.8	39 ± 0.8	44 ± 0.6	52 ± 0.8	44 ± 0.6	52 ± 0.8	DM
K	125 ± 1.9	151 ± 1.4	173 ± 1.9	193 ± 1.6	173 ± 1.9	193 ± 1.6	DM
Ca	99 ± 1.3	119 ± 1.4	132 ± 1.4	146 ± 1.2	132 ± 1.4	146 ± 1.3	DM
Mg	25 ± 0.4	31 ± 0.4	35 ± 0.5	39 ± 0.5	35 ± 0.5	39 ± 0.5	DM
S	22 ± 0.3	27 ± 0.3	31 ± 0.3	35 ± 0.4	31 ± 0.3	35 ± 0.4	DM
Fe	0.9 ± 0.09	1.1 ± 0.09	1.2 ± 0.11	1.4 ± 0.08	1.2 ± 0.11	1.4 ± 0.08	DM
Mn	0.7 ± 0.07	0.7 ± 0.05	0.8 ± 0.08	0.9 ± 0.07	0.8 ± 0.08	0.9 ± 0.07	DM
Zn	0.2 ± 0.03	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.03	DM
Cu	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	DM
B	0.1 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	DM
Mo	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	DM
EC	0.97 ± 0.01	1.19 ± 0.01	1.46 ± 0.01	1.74 ± 0.01	1.46 ± 0.01	1.74 ± 0.01	DM

DM, demineralized water.

TABLE 2 Composition of enhanced treatment (ET) nutrient solution with the elevated P, K, and Fe (mg·L^{-1}).

Week	1	2	3	4	5	6–9	10–13
N	100 ± 0.9	115 ± 1.0	129 ± 0.1	150 ± 1.8	129 ± 1.7	150 ± 1.8	DM
P	32 ± 0.5	39 ± 0.4	42 ± 0.5	51 ± 0.9	92 ± 1.9	93 ± 1.9	DM
K	125 ± 1.6	143 ± 1.2	172 ± 1.9	194 ± 1.4	258 ± 2.5	266 ± 2.7	DM
Ca	98 ± 0.9	118 ± 1.2	132 ± 1.	146 ± 1.2	133 ± 1.1	144 ± 1.4	DM
Mg	25 ± 0.2	29 ± 0.4	35 ± 0.4	39 ± 0.4	33 ± 0.4	39 ± 0.7	DM
S	22 ± 0.1	26 ± 0.9	31 ± 0.4	34 ± 0.3	31 ± 0.4	35 ± 0.3	DM
Fe	0.9 ± 0.01	1.1 ± 0.06	1.3 ± 0.05	1.3 ± 0.08	12.3 ± 0.4	13.8 ± 0.9	DM
Mn	0.7 ± 0.06	0.7 ± 0.04	0.7 ± 0.09	0.9 ± 0.04	0.8 ± 0.06	0.9 ± 0.07	DM
Zn	0.2 ± 0.04	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.04	0.3 ± 0.03	DM
Cu	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	DM
B	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.02	DM
Mo	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	DM
EC	0.96 ± 0.01	1.20 ± 0.01	1.45 ± 0.02	1.54 ± 0.01	2.05 ± 0.02	2.34 ± 0.06	DM

DM, demineralized water.

and ammonia nitrogen. Approximately 0.50 g of the sample was weighed for analysis, followed by mineralization. The mineralization process was conducted in glass vials, where 2 g of catalyst (a mixture of 100 g K_2SO_4, 1 g $CuSO_4$, 0.1 g Se) and 10 mL of concentrated sulfuric acid (H_2SO_4) were added to the sample. Decomposition took place for 90 minutes at a temperature of 420°C. After mineralization, the samples were prepared for distillation. Within the apparatus, 20 mL of distilled water was automatically added to the vial, followed by distillation into H_3BO_3, allowing for the determination of the total nitrogen content in the sample. The content was ascertained through titration using HCl (0.5 mol·L^{-1}) and subsequently measured using the Gerhardt Vapodest 30s instrument (Königswinter, Germany) (Baker and Thompson, 1992).

2.7 Identification and quantification of cannabinoids

An optimized method of dynamic maceration was employed to identify and quantify cannabinoid content using HPLC/DAD (high-performance liquid chromatography with a diode-array detector), as described by Patel et al. (2017). Subsequently, 0.150 g of the sample was weighed into a 50 mL beaker, and 5 mL of solvent (96% ethanol) was added. The sample was then dynamically macerated for 60 minutes, filtered using a Morton filtration device (P16 porosity), and the filtrate was transferred to 50 mL vials. The same solvent in the same volume was added again to the initial 0.150 g of plant material, and the sample was dynamically macerated for another 60 minutes. This process was repeated three times, resulting in a composite sample derived from the three-phase dynamic maceration process in a 1:100 (V:W) ratio. This sample was diluted 20 times (using 96% ethanol), filtered through a syringe filter (0.22 µm), transferred to vials, and prepared for analysis in this form. Samples of the extracts were introduced into a high-performance liquid chromatography system equipped with diode array detection (HPLC-DAD; Agilent 1,260, Agilent Technologies Inc., United States), utilizing a Luna® 1C8 column (2) with dimensions of 250 × 3 mm and a particle size of 3 µm (Phenomenex, United States). The mobile phase employed was an isocratic mixture of acetonitrile/H_2O (31:9, v/v) containing 0.1% HCOOH (v/v) and 0.1 mol/L NH_4COOH (without pH adjustment). The flow rate was set at 0.55 mL/min, the temperature at 37°C, the sample injection volume at 8 µl, and UV detection was performed at 275 nm (Križman, 2020). To ensure accuracy, the instrument was externally calibrated using Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabigerolic acid (CBGA), cannabigerol (CBG) and cannabinolic acid (CBNA) ranging from 0.3 to 10 mg·L^{-1}, and THCA ranging from 0.3 to 100 mg·L^{-1} (Sigma-Aldrich, Czech Republic) as reference standards. Data analysis was carried out using OpenLAB CDS software, ChemStation Edition, Rev. C.01.5.

2.8 Statistical analyses

The data were subjected to analysis of variance (one-way ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test using IBM SPSS Statistics software (version 25, 2017, Chicago, Illinois, United States).

3 Results

Implementing an enhanced nutritional regime and using different fertigation systems (1C and 2C) resulted in alterations in the plant nutrient composition of cannabis plants. Simultaneously, the utilization of two distinct systems resulted in variations in the

pH levels of the nutrient solution. In the 2C system, a consistent pH value of 5.9 was upheld throughout the cycle, whereas in the 1C system, pH exhibited fluctuations within the range of 5.9–6.95. The nitrogenous compounds exhibited the lowest concentration in the stems, while the flowers displayed the highest concentration (Figure 2). Comparing the leaves and flowers of CN and ET plants under the augmented nutritional regime in the 1C cycle, statistically significant differences in N concentrations were observed only twice in the leaves (6th and 13th week) and twice in the stems (8th and 13th week). Notably, the most significant variation in N concentrations between CN and ET within the 1C system occurred in the stems during the 13th week, with a difference of 28% (CN: 10.44 mg·g^{-1}, ET: 7.5 mg·g^{-1}; Figure 2A). In contrast, N concentrations in the stems and leaves of CN and ET plants in 2C did not significantly differ in any of the tested weeks. Nevertheless, the concentration of N in flowers was significantly different in

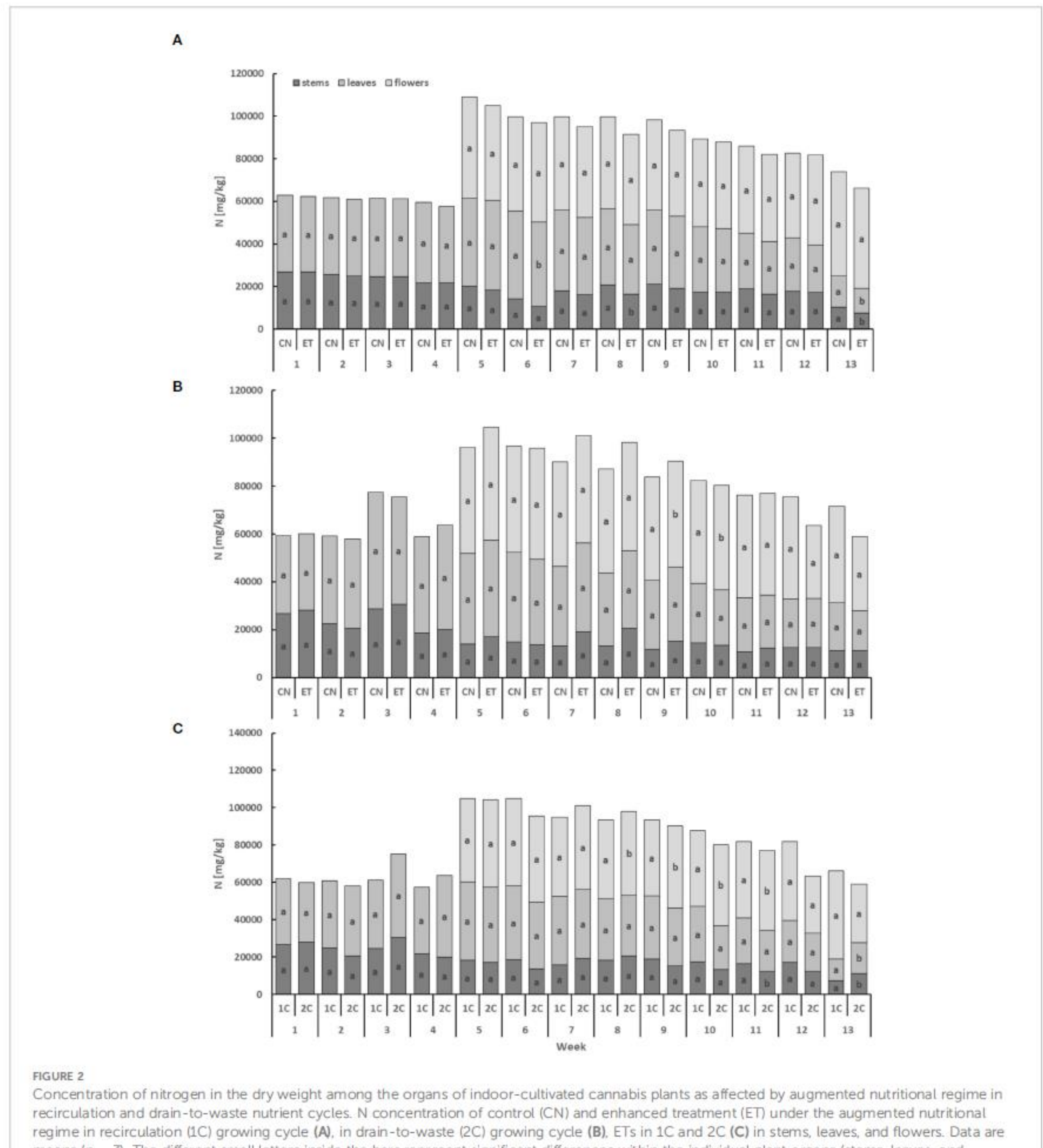

FIGURE 2
Concentration of nitrogen in the dry weight among the organs of indoor-cultivated cannabis plants as affected by augmented nutritional regime in recirculation and drain-to-waste nutrient cycles. N concentration of control (CN) and enhanced treatment (ET) under the augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means (n = 3). The different small letters inside the bars represent significant differences within the individual plant organs (stems, leaves, and flowers) between the variants in a particular week, according to Tukey's HSD test at α = 0.05.

weeks 9 and 10 in the 2C regime. N concentrations exhibited over all the highest differences between 1C and 2C of ETs under the augmented nutritional regime, primarily noticeable in the flowers from week 8 to 11. During the last week, significant differences were observed only in the stems and leaves. The most significant variation in N concentrations between fertigation systems in ETs was observed during the 13th week, with a difference of 42% (21% between CNs) in the leaves (1C: 42.18 $mg \cdot g^{-1}$, 2C: 45.28 $mg \cdot g^{-1}$; Figure 2C).

The phosphorus (P) content exhibited lower levels in the leaves and stems compared to the flowers, displaying a cumulative pattern over time (Figure 3). In the stems, the P concentration of CN and ET in the 1C cycle significantly differed only in weeks 7 and 13. Significant differences were observed in the leaves in weeks 8, 9, and 13, while in the flowers, differences were found in weeks 8 and 10. Notably, the most significant variation in P concentration between nutritional treatments was observed in the leaves during the 13th week, with a difference of 63% (CN: 63.48 $mg \cdot g^{-1}$, ET: 38.9 $mg \cdot g^{-1}$; Figure 3A). In contrast to the 1C cycle, the P concentration of CN and ET in 2C plants showed significant differences only in the 11th week in the stems, with a difference of 108% (CN: 33.3 $mg \cdot g^{-1}$, ET: 69.15 $mg \cdot g^{-1}$; Figure 3B). Furthermore, the P concentration in the leaves and flowers of ET plants also varied between the 1C and 2C cycles. Significant differences were observed in the leaves in weeks 7, 9, 10, 11, and 12, while in the flowers, differences were found in weeks 7, 9, and 10. However, when comparing these two regimes, no statistically significant differences were observed in the P concentration of stems. The most notable differences in P concentrations between the 1C and 2C cycles in ETs were observed during the 11th week, with a difference of 31.6% (36% between CNs) in the leaves (1C: 64.87 $mg \cdot g^{-1}$, 2C: 49.31 $mg \cdot g^{-1}$; Figure 3C).

The potassium (K) content displayed the lowest levels in the stems and the highest levels in the leaves (Figure 4). In the leaves, the concentration of K for CN and ET with the 1C cycle started to exhibit significant differences from the 6th week until the 12th week. Significant differences were observed in the flowers in weeks 8, 10, and 11, while in the stems, differences were found only in week 9. Notably, the most significant variation in K concentration between CN and ET was observed in the stems during the 9th week, with a difference of 45.5% (CN: 16.12 $mg \cdot g^{-1}$, ET: 29.57 $mg \cdot g^{-1}$; Figure 4A). In contrast to the 1C cycle, the concentration of K in the 2C cycle of CN and ET plants showed significant differences only in the stems in weeks 10, 11, and 13. The most substantial differences in K concentrations between nutritional treatments were observed in the stems during the 10th week, with a difference of 77% (CN: 18.02 $mg \cdot g^{-1}$, ET: 31.88 $mg \cdot g^{-1}$; Figure 4B). Likewise, the K concentration also varied between the 1C and 2C cycles of ETs, with significant differences occurring in weeks 6, 7, 9, and 10. The most notable differences in K concentrations between 1C and 2C in ETs were observed in the stems during the 10th week, with a difference of 38.8% (20% between CNs) (1C: 23.03 $mg \cdot g^{-1}$, 2C: 31.88 $mg \cdot g^{-1}$; Figure 4C).

The iron (Fe) content exhibited the lowest levels in leaves and the highest levels in stems, displaying a cumulative trend over time (Figure 5). In the stems, the concentration of Fe for CN and ET with the 1C cycle showed significant differences in weeks 9, 11, and 13. Notably, the most significant variation in Fe concentration between CN and ET was observed in the stems during the 11th week, with a difference of 188.7% (CN: 337.2 $mg \cdot kg^{-1}$, ET: 955.8 $mg \cdot kg^{-1}$; Figure 5A). In contrast to the 1C cycle, the concentration of Fe in the stems of CN and ET plants in the 2C cycle exhibited significant differences only in week 10, in the leaves merely in weeks 6 and 10, and in the flowers from the 7th until the 8th week. The most substantial difference in Fe concentrations between CN and ET of 2C plants was observed in the flowers during the 8th week, with a difference of 55% (CN: 167.3 $mg \cdot kg^{-1}$, ET: 108.2 $mg \cdot kg^{-1}$; Figure 5B). Besides, the Fe concentration varied between the 1C and 2C cycles of ETs, with significant differences observed in the stems during weeks 6 and 11, in the leaves during weeks 11 and 12, and in the flowers during week 8. The most notable difference in Fe concentrations between 1C and 2C in ETs was observed in the stems during the 11th week, with a difference of 90.6% (11% between CNs) (1C: 337.2 $mg \cdot kg^{-1}$, 2C: 642.6 $mg \cdot kg^{-1}$; Figure 5C).

Implementing an augmented nutritional regime in two distinct nutritional cycles led to noticeable effects on the growth of cannabis plants. The biomass increase was relatively slow during the initial four weeks, but a significant acceleration was observed from week 5 onwards. Notably, the highest weekly dry weight gain was observed in the flowers (Figure 6). In the 1C cycle, the biomass increases in stems, leaves, and flowers for both CN and ET plants was nearly identical until week 4. However, from week 5 to 8, some differences in leaf and flower biomass emerged (Figure 6A), and in the 13th week, a statistically significant disparity in flower dry weight was noted. In contrast, the dry weight of stems, leaves, and flowers in the 2C cycle did not exhibit significant increases for CN and ET plants, except for the dry weight of leaves in the 3rd week and the dry weight of flowers in the 11th week. ET plants reached their maximum dry biomass at week 11, while CN plants achieved it by week 12 (Figure 6B). Biomass variations were also observed between the 1C and 2C cycles in ET plants, commencing from week 6 (Figure 6C).

The implementation of an augmented nutritional regime had statistically significant impact on the concentration of total THC (sum of Δ^9-tetrahydrocannabinol, Δ^8-tetrahydrocannabinol, and tetrahydrocannabinolic acid) total CBG (sum of cannabigerolic acid and cannabigerol), and CBNA in the flowers of medicinal cannabis plants, with distinct patterns observed for different fertigation systems and nutrition treatments (Figure 7). The total THC and CBNA concentration curves exhibited similar trends within the same growing cycle and treatment. At the same time, total THC yield (g/m^2) was evaluated, where there was a clear trend in favor of ET across all comparisons.

Initially, the concentration of total THC in leaves and flowers almost stagnated in both treatments until week 4. However, from week 5 onwards, substantial growth with significant differences in total THC concentration was observed because only flowers were analyzed (Figures 7A-C). In the 1C cycle, significant differences in total THC concentration between CN and ET treatments occurred in weeks 5, 6, 9, and 10. The maximum concentrations were reached at week 11 for both CN (18.5%) and ET (19.4%) (Figure 7A). In contrast, in the 2C cycle, significant differences in total THC

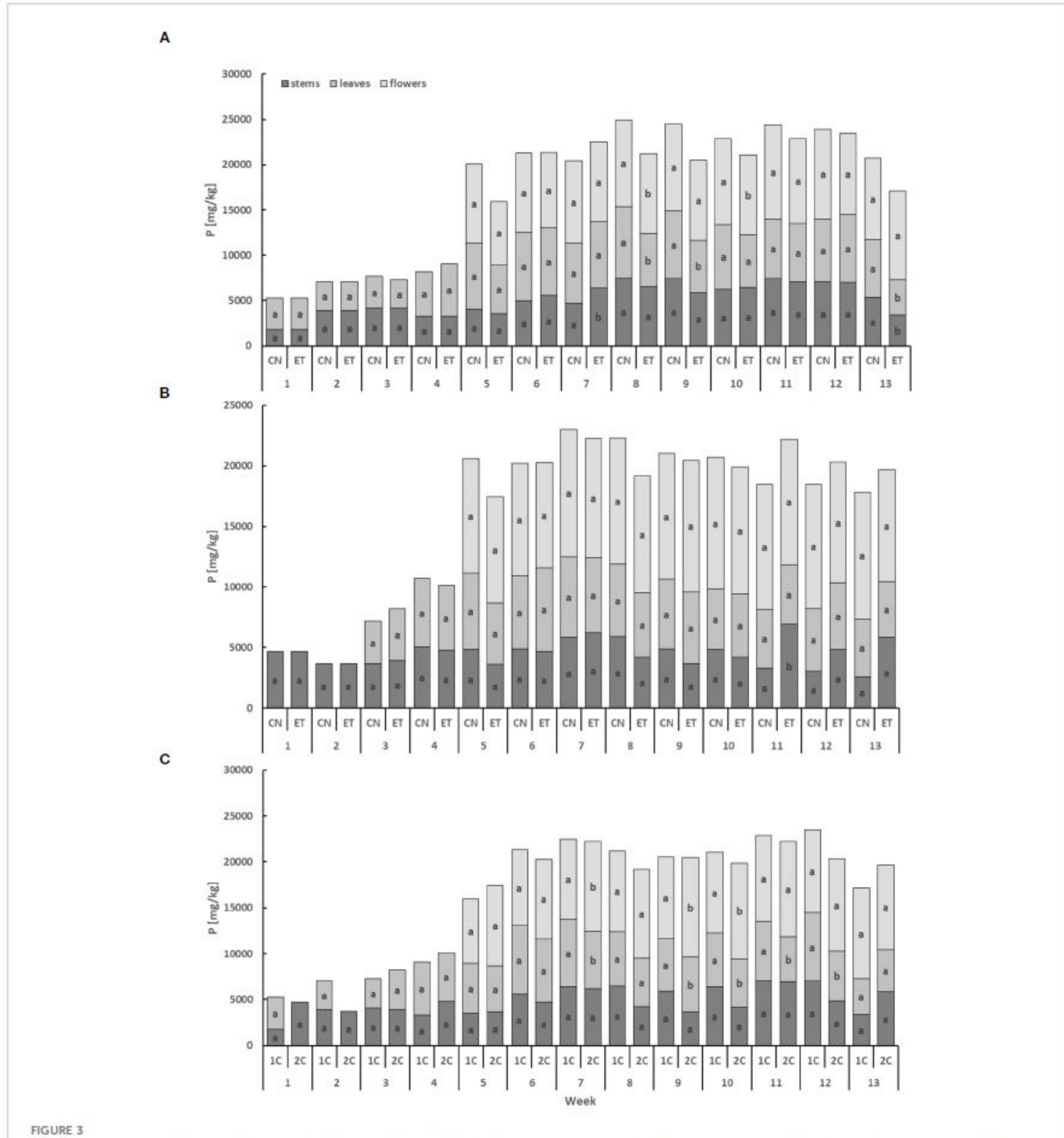

FIGURE 3
Phosphorus concentration in the dry weight among the organs of indoor-cultivated cannabis plants as affected by the augmented nutritional regime in recirculation and drain-to-waste nutrient cycles. P concentration of control (CN) and enhanced treatment (ET) under the augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means (n = 3). The small letters inside the bars represent significant differences within the plant organs (stems, leaves, and flowers) between the variants in a particular week, according to Tukey's HSD test at α = 0.05.

concentration between CN and ET treatments were observed only in the 6th week. CN reached its peak concentration at week 8 (15.9%), while ET reached its peak at week 9 (15.7%) (Figure 7B). Significant differences in total THC levels between 1C and 2C of ET treatments were observed from week 10 until the end of the experiment, where the 1C overperformed 2C (Figure 7C). The yield of this dominant cannabinoid (THC) showed significant differences in the 1C regime in favor of the ET treatment. Specifically, there was an increase in yield per square meter in weeks 5, 6 and 10 by 402%, 315% and 46%, respectively (Figure 8A). In drain to waste system (2C), this trend was similar. Still, a statistically significant difference was found only in the eleventh week, when there was an increase in THC yield in the ET variant by 50.7% (Figure 8B). An interesting finding is that when comparing

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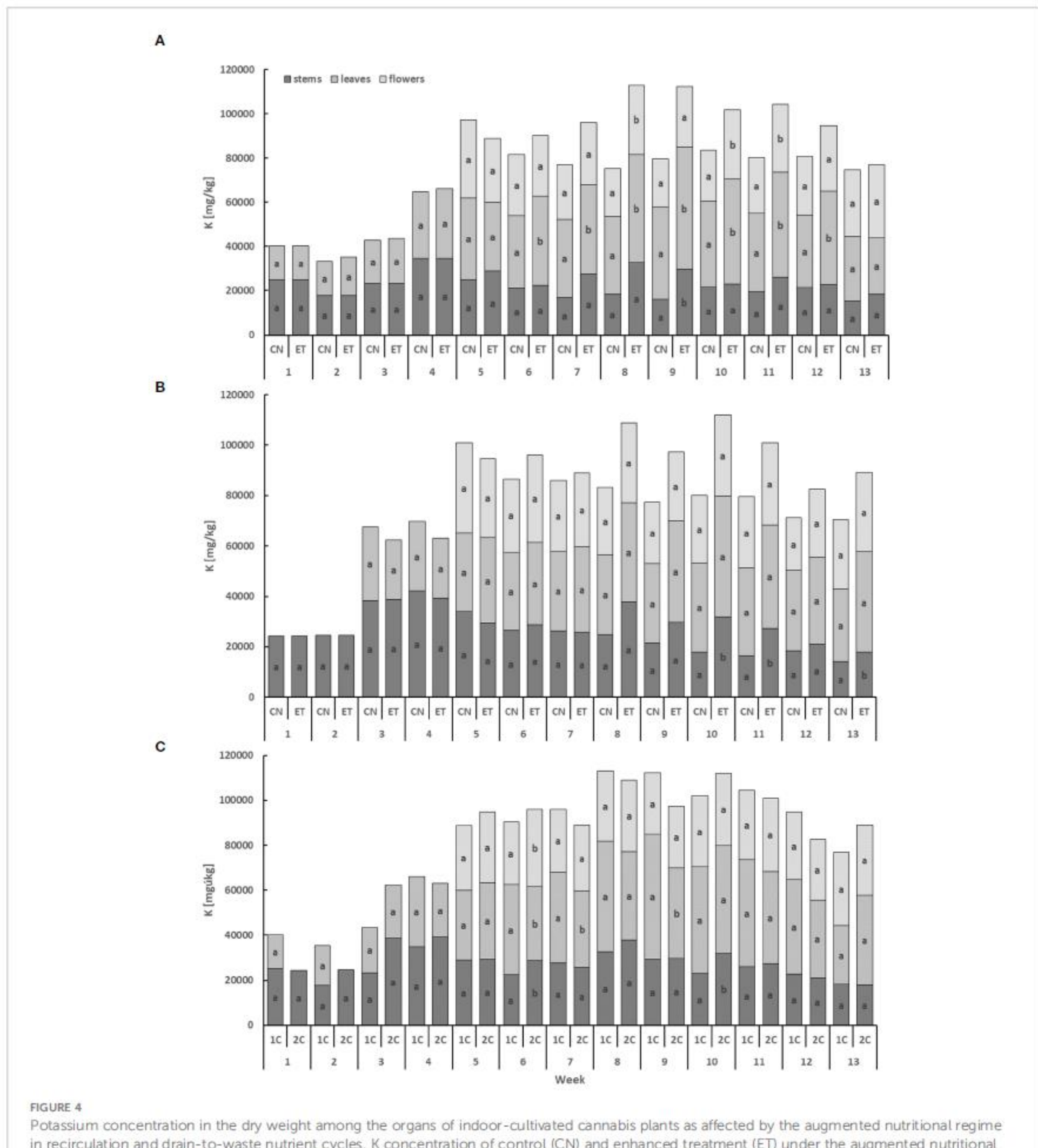

FIGURE 4
Potassium concentration in the dry weight among the organs of indoor-cultivated cannabis plants as affected by the augmented nutritional regime in recirculation and drain-to-waste nutrient cycles. K concentration of control (CN) and enhanced treatment (ET) under the augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means (n = 3). The small letters inside the bars represent significant differences within the plant organs (stems, leaves, and flowers) between the variants in a particular week, according to Tukey's HSD test at $\alpha = 0.05$.

the 1C and 2C regimes with the ET variant, there was an overall lower THC yield with the drain-to-waste system (2C). Specifically, there was a statistically significant difference in weeks 6, 10, 12 and 13 when there was a decrease in the THC yield of the 2C system to 6.6%, 25.3%, 41.5% and 31.1% of the THC yield value of the 1C recirculation system (Figure 8C).

For total CBG, the concentration in leaves and flowers nearly remained stagnant until week 4, with a notably lower concentration compared to THC by more than an order of magnitude. However, a significant increase in total CBG concentration was observed in week 5, focusing only on the flowers (Figures 7D-F). In the 1C cycle, the concentrations of total CBG in CN and ET treatments began to differ

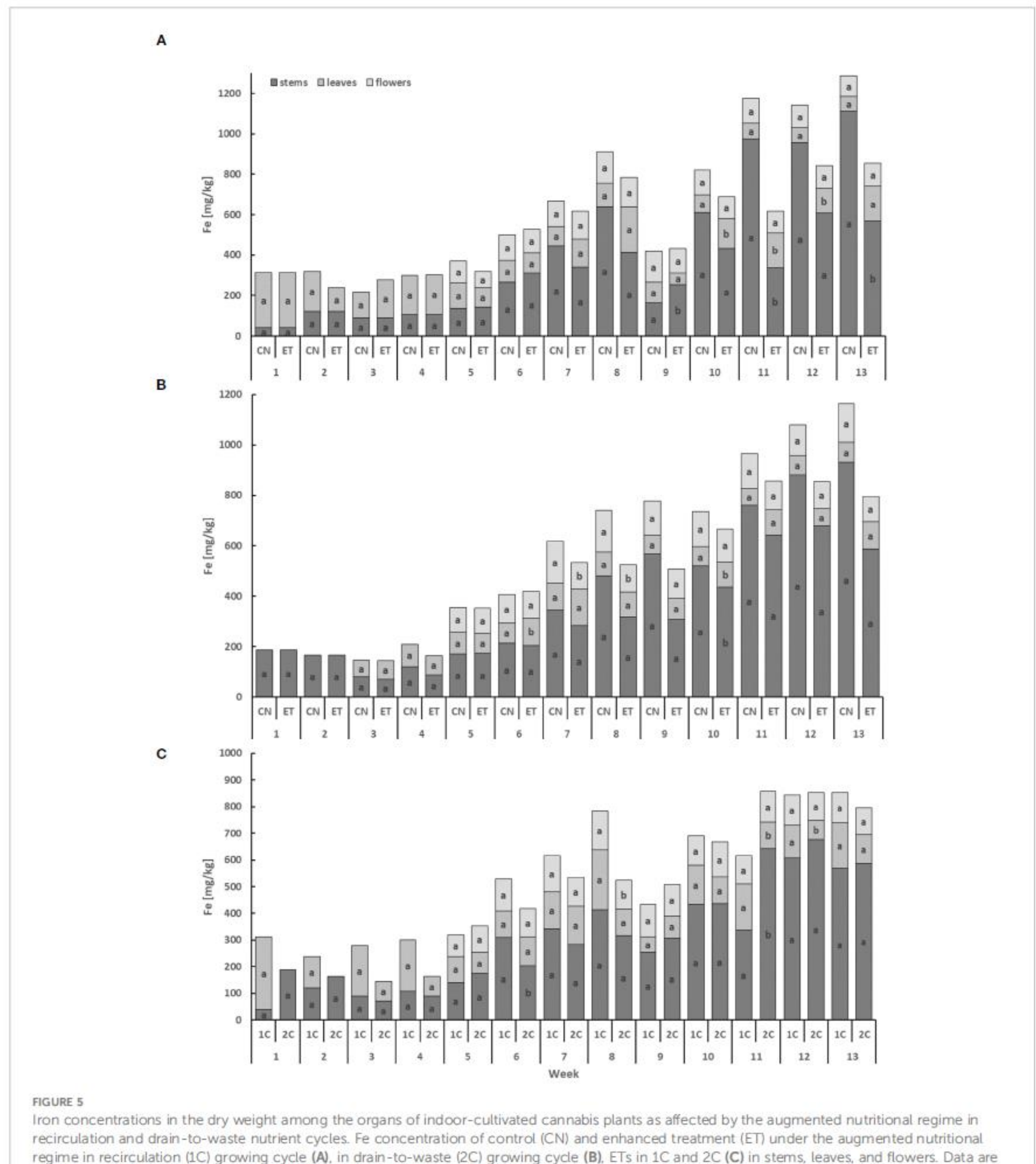

FIGURE 5
Iron concentrations in the dry weight among the organs of indoor-cultivated cannabis plants as affected by the augmented nutritional regime in recirculation and drain-to-waste nutrient cycles. Fe concentration of control (CN) and enhanced treatment (ET) under the augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)** in stems, leaves, and flowers. Data are means (n = 3). The small letters inside the bars represent significant differences within the plant organs (stems, leaves, and flowers) between the variants in a particular week, according to Tukey's HSD test at α = 0.05.

significantly from week 7 until week 11 and peaked at week 10 for CN (1.1%) and week 6 for ET (0.9%). The most significant differentiation between CN and ET occurred at week 10, with a difference of 218% in favor of CN. Following the peak concentrations, a foretellable decrease in concentration was observed, followed by a slight increase in the last three weeks. In the 2C cycle, significant differences in total CBG concentration between CN and ET treatments were observed only in weeks 6, 8, and 11. The maximum concentrations were reached at week 9 for both CN (1.3%) and ET (0.8%) (Figure 7E). Significant differences in total CBG levels between 1C and 2C of ET treatments were observed in weeks 1, 2, and from week 6 except the 10th week until the end of the experiment (Figure 7F).

Regarding CBNA concentration, significant variations between CN and ET treatments in the 1C cycle were observed in weeks 5 and 6.

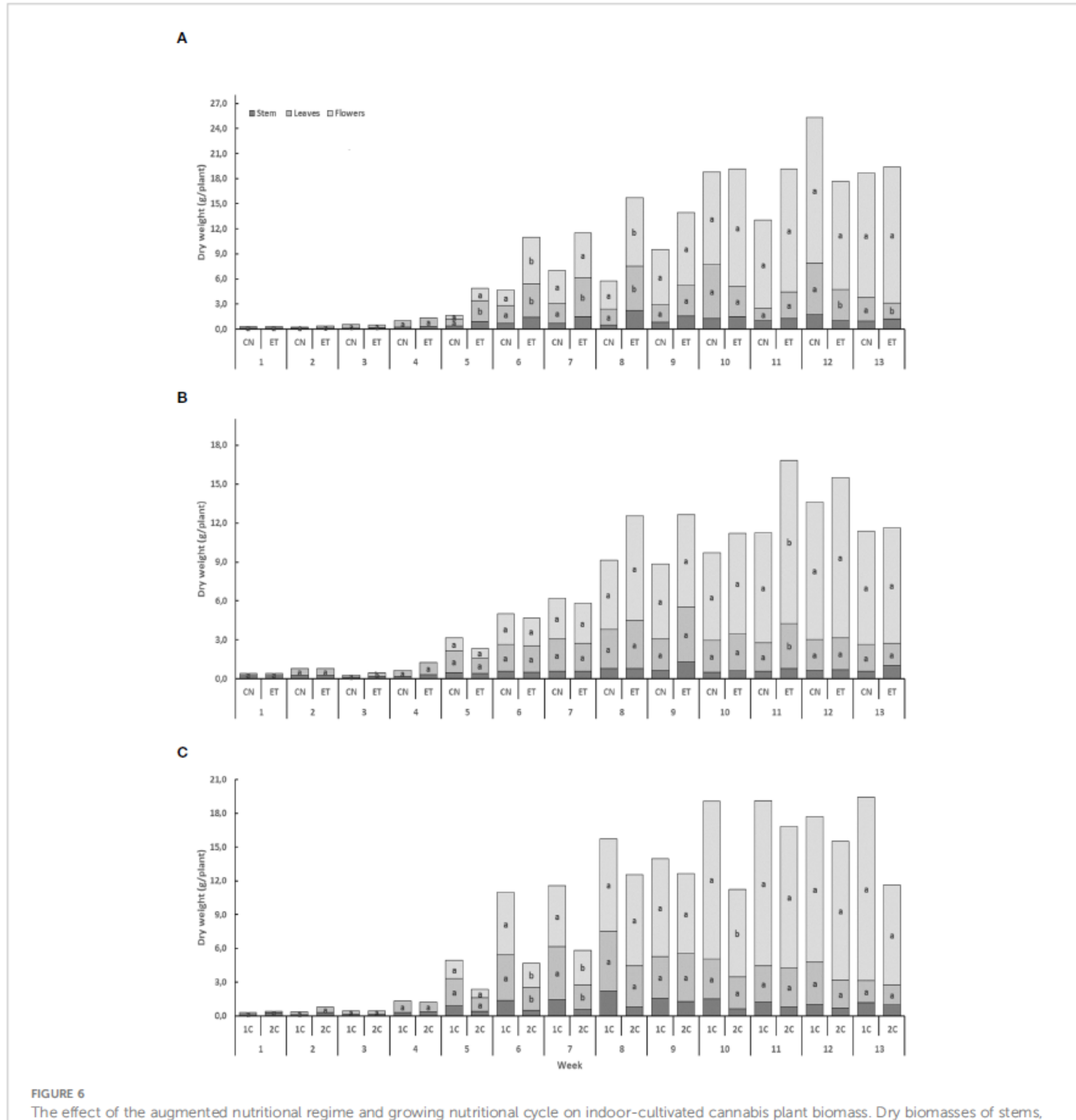

FIGURE 6
The effect of the augmented nutritional regime and growing nutritional cycle on indoor-cultivated cannabis plant biomass. Dry biomasses of stems, leaves, and flowers in control (CN) and enhanced treatment (ET) plants with augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)**. Data are means (n = 3). The different small letters inside the bars and small bold letters above the bars represent significant differences within the indoor-cultivated cannabis plant organs (leaves and flowers) and the whole plant biomass between the variants in a particular week according to Tukey's HSD test at $\alpha = 0.05$.

CBNA concentration peaked at week 10 for CN (0.06%) and week 13 for ET (0.06%) (Figure 7G). In the 2C cycle, significant differences in CBNA concentration between CN and ET treatments were observed in weeks 6, 7, and 11. CN reached its maximum concentration (0.04%) in 2C at week 11, with a significant difference of 55%, while ET reached its maximum in the 13th week (Figure 7H). CBNA concentrations between 1C and 2C of ET treatments showed significant differences in the first two weeks, followed by nearly identical concentration levels in weeks 3 and 4. From week 5 onwards, a steady growth trend was observed, with 1C outperforming 2C. Significant differences were observed in weeks 1, 2, 5, and 6 and from week 10 until the end of the experiment. As mentioned, the CBNA concentrations in both ET variants reached their maximum at week 13, with a significant difference of 34% (Figure 7I).

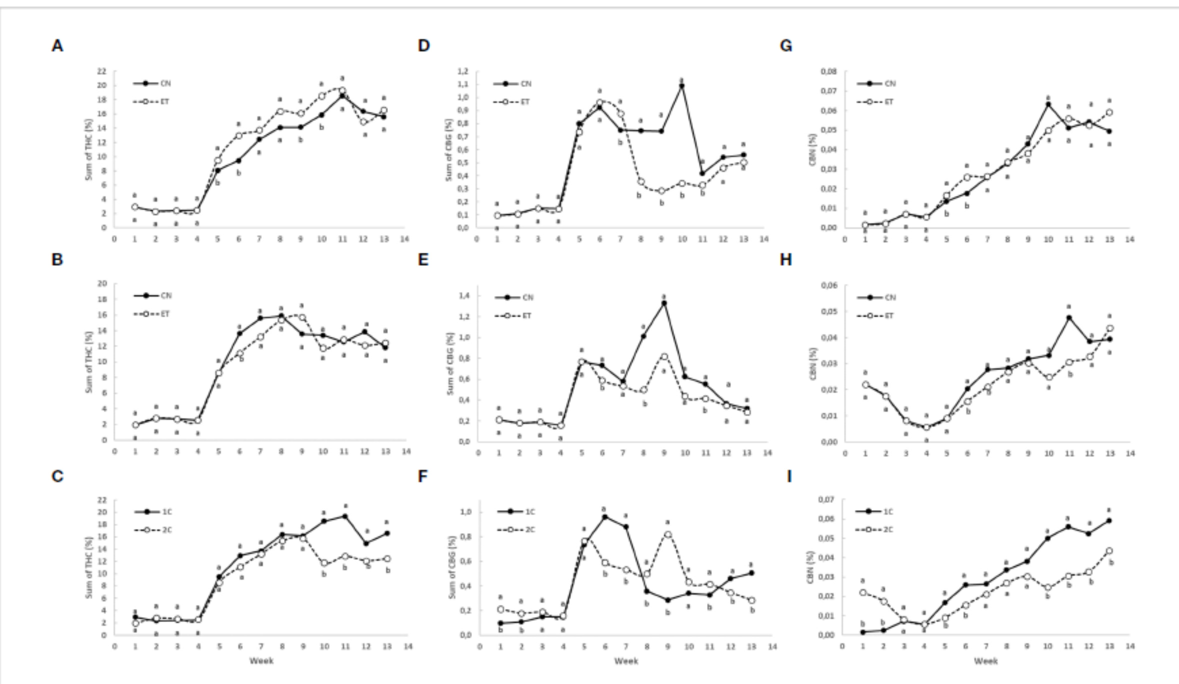

FIGURE 7
Concentrations of total THC (sum of tetrahydrocannabinolic acid, Δ^9-tetrahydrocannabinol, and Δ^8-tetrahydrocannabinol), total CBG (sum of cannabigerolic acid and cannabigerol), and cannabinolic acid (CBNA) in the flowers of indoor-cultivated cannabis plants as affected by augmented nutritional regime and nutrient cycle. The sum of THC concentration in control (CN) and enhanced treatment (ET) plants with augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)**. The sum of CBG concentration of control (CN) and enhanced treatment (ET) with augmented nutritional regime in recirculation (1C) growing cycle **(D)**, in drain-to-waste (2C) growing cycle **(E)**, ETs in 1C and 2C **(F)**. CBNA concentration of control (CN) and enhanced treatment (ET) with augmented nutritional regime in recirculation (1C) growing cycle **(G)**, in drain-to-waste (2C) growing cycle **(H)**, ETs in 1C and 2C **(I)**. The whole inflorescence of the plant was analyzed. Data are means (n = 3). Different bold small letters represent significant differences in cannabinoid concentration between the variants in a particular week according to Tukey's HSD test at $\alpha = 0.05$.

4 Discussion

The present study focused on investigating the effects of an enhanced nutritional regime and different fertigation systems on the ionome composition, biomass, and cannabinoid content of indoor-cultivated cannabis plants. The results indicate substantial variations in N, P, K, and Fe concentrations in different plant parts under the augmented nutritional regime. These alterations could be attributed to nutrient uptake, translocation, and allocation modifications resulting from the introduced nutritional variations. Undoubtedly, nutrition plays a pivotal role in shaping the development, functionality, and metabolic processes of various plant organs and tissues (Shiponi and Bernstein, 2021b; De Prato et al., 2022). Existing knowledge has extensively documented the ideal thresholds of specific macronutrients, including N, P and K, along with micronutrients like Fe, necessary for ensuring the normal growth and functioning of both root systems and above-ground biomass (Shiponi and Bernstein, 2021a; Shiponi and Bernstein, 2021b; Malík et al., 2022; Saloner and Bernstein, 2022; Llewellyn et al., 2023), as well as the production of valuable secondary metabolites in medicinal cannabis plants (Caplan et al., 2017; Bernstein et al., 2019b; Shiponi and Bernstein, 2021b; Saloner and Bernstein, 2022). It is crucial to emphasize that nutrient solution fertigation was provided to the plants only up to the ninth week of cultivation. Subsequently, from the ninth week onwards, the plants received fertigation solely with deionized (DM) water. Consequently, the plants relied solely on their accumulated nutrient reserves during this period. Modifications in the nutrient solution's P, K, and Fe concentrations within two distinct nutrient cycles undeniably exerted discernible impacts on the levels of macro and micro-elements in cannabis plants' above-ground organs. While the N concentration remained unchanged between the CN and ET systems, some statistically significant differences in N distribution among the above-ground organs of cannabis plants were observed within each fertigation system. The composition and proportional disbalance of nutrients in nutrient solutions have been recognized as factors capable of influencing the profiles of cannabinoids and terpenes in cannabis. These observed variations are likely linked to the complex interplay between nutrient levels in the solution and the growth of the plant. The different fertigation systems employed in this study also exhibited discernible effects on the concentrations of both macro- and microelements within the tissues of cannabis plants. It should be emphasized that the interactions between cations and anions of nutrients during root cell membrane transport have been extensively documented in the scientific literature (Meychik and Yermakov, 2001; Roberts, 2006; Langenfeld et al., 2022).

Nevertheless, variations in the pH of the nutrient solution, such as those observed in the RS (1C), and the increased nutrient

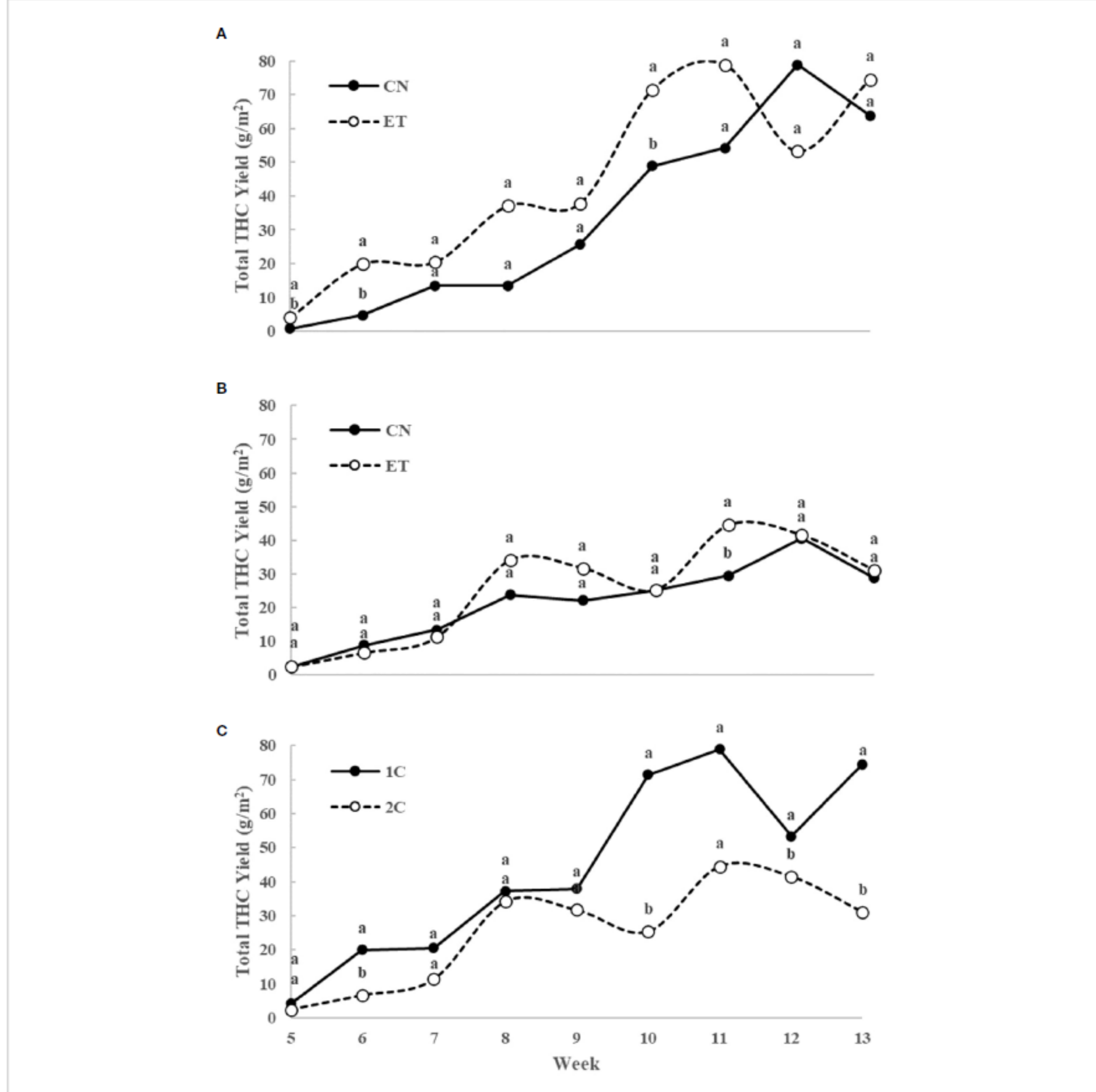

FIGURE 8
Total yield of THC (sum of tetrahydrocannabinolic acid, Δ^9-tetrahydrocannabinol, and Δ^8-tetrahydrocannabinol) per one square meter of indoor-cultivated cannabis plants as affected by augmented nutritional regime and nutrient cycle. Total THC yield in control (CN) and enhanced treatment (ET) plants with augmented nutritional regime in recirculation (1C) growing cycle **(A)**, in drain-to-waste (2C) growing cycle **(B)**, ETs in 1C and 2C **(C)**. Data are means (n = 3). Different bold small letters represent significant differences in cannabinoid concentration between the variants in a particular week according to Tukey's HSD test at α = 0.05.

quantity provided in the DS (2C), have the potential to influence the availability of specific nutrients and, consequently, the physiological and metabolic reactions within the plants (Malik et al., 2023). Furthermore, a more pronounced variation in N concentrations was evident when comparing the two distinct fertigation systems, which aligns with our study. Nitrogen (N) plays a vital role in organs with high metabolic activity, such as leaves and flowers, where it is a crucial component of proteins and compounds related to energy metabolism. As a result, N tends to accumulate in smaller quantities in structural compartments with lower metabolic activity, such as stems (Marschner and Marschner, 2012). In our investigation comparing the 2C and 1C systems, the 2C system consistently exhibited higher N concentrations in flowers during weeks 8 to 11 (an average 8.7% increase in favor of the 2C system,

with values ranging from 6.3% to 11.6%). This contrasts with the findings of Malík et al. (2023), where they observed higher levels of N in plant tissues in the recirculation systems. In our case, we presume that the more frequent nutrient replenishment (2–3 times a week) in the drain-to-waste system resulted in more significant nutrient accumulation in both the above-ground biomass and the roots. Subsequently, when nutrition was stopped, and only DM water was irrigated, there was translocation from the roots to the above-ground biomass. Also, another hypothesis could be that some of the nutrients that were supplied in addition to the ET variant (P, K, or Fe) could be limiting in the recirculation system. Therefore, when it was supplied more in drain-to-waste, other nutrients (in this case, N) could subsequently be more incorporated into tissues (Ågren et al., 2012; Asao, 2012).

As expected, alterations in the concentrations of P, K, and Fe in the nutrient solution in some observations significantly impacted the concentrations of these elements in the above-ground organs of medicinal cannabis plants. Notably, in the 1C regime, differences between CN and ET were more pronounced than in the 2C regimen. This observation can be attributed to the fluctuation and pH increase in the 1C compared with the stable pH in the 2C regime (Malík et al., 2023). Surprisingly, despite doubling the concentration of P in the nutrient solution in ET compared to CN within the 1C fertigation system, P accumulation was notably higher in CN than in ET. This observation might be attributed to the fact that root analysis was not conducted; therefore, it remains plausible that any excess P is encased in the roots. As indicated by the accumulation data, P content within the plant tissues did not exhibit a decline following the cessation of nutrient supplementation. This phenomenon suggests a potential release of P from vacuoles (Shiponi and Bernstein, 2021a; Shiponi and Bernstein, 2021b), serving as a defense mechanism against P toxicity in the shoot (Hawkesford et al., 2012). This mechanism prevents the exposure of shoot cells to damaging P concentrations by compartmentalizing excessive P supply. The compartmentalization of P is recognized as a fundamental mechanism for averting the accumulation of cytoplasmatic P to toxic levels. Under adequate P nutrition, approximately 70–95% of intercellular phosphate (Pi) is sequestered within vacuoles. Consequently, the regulation of transporters under varying P conditions facilitates the maintenance of Pi cellular homeostasis (Liu et al., 2016). So far, there is no information about P transporters in *Cannabis sativa* L. This information is needed to understand better P remobilization and translocation in the cannabis plant (Shiponi and Bernstein, 2021b). An alternative explanation for this unexpected result could be a potential nutrient lockout effect in the ET due to the elevated P concentration in the solution. Phosphorus has an inclination to form complexes with soil minerals, such as iron Fe, potentially diminishing its overall bioavailability. However, it is essential to note that such an effect was observed by Mardamootoo et al. (2021) exclusively in soil-based cultivation systems. When soluble phosphatic fertilizers are applied to soils, they initially dissolve, causing an immediate increase in soil solution P concentration. Subsequently, P primarily engages in adsorption and precipitation processes (Prasad and Power, 1997). The reactions that transpire among phosphate ions in the soil solution, soil constituents, and non-phosphatic components in the fertilizers primarily sequester P from the solution phase, rendering phosphate less soluble over time (Sample et al., 2015). This phenomenon is commonly referred to as P fixation, adsorption, or retention. Consequently, P becomes markedly immobile in soils and tends to remain in proximity to the point of application (Prasad and Power, 1997; Mardamootoo et al., 2021). In plant cells, P serves as a critical component of nucleic acids, membrane lipids, and phosphorylated intermediates involved in energy metabolism. As a result, maintaining cellular phosphorus homeostasis is essential for ensuring the normal function of various physiological and biochemical processes (Raghothama, 2015). The elevated P concentration observed in the 2C system compared to the 1C system may likely be attributed to an increase and fluctuations in pH within the 1C regime, a phenomenon in line with the findings of Lefever et al. (2017). The higher pH levels, reaching up to 6.95 in the nutrient solution, could lead to the partial precipitation of phosphates by calcium (Ca^{2+}) and magnesium (Mg^{2+}). This precipitation could result in the formation of insoluble and consequently unavailable salts within the hydroponics solution (Lee et al., 2017).

K exhibits a clear accumulation advantage in the ET variant in 1C as a highly bioavailable plant element. In accordance with (Bernstein et al., 2019a), it is noteworthy that cannabis plant stems tend to exhibit a preference for K accumulation, as evidenced by concentrations similar to those found in fan leaves. Typically, this nutrient's temporary storage occurs in the stem's xylem or phloem parenchyma. The K concentration in cannabis stems remained notably high at the end of the developmental period, a phase characterized by high nutrient demand, indicating active accumulation. While our study did not measure sugar leaves but entire inflorescences, where K accumulation was slightly higher than in the stem, the results for K suggest that the nutrient concentration does not significantly decrease in tissues after nutrient cessation, indicating its excessive accumulation. This phenomenon, known as the luxury consumption of K and its temporary storage, has been observed in various plant species (Marschner and Marschner, 2012). It is pertinent to add that, based on the experiments conducted by Saloner et al. (2019) and Saloner and Bernstein (2022), there appears to be no competition for nutrient uptake between K and the uptake of N and P at suitable concentrations. Yep and Zheng (2021) studied the yield of *Cannabis sativa* inflorescence with K fertilizer in aquaponic solutions, similar to hydroponic systems. The added K and micronutrient fertilizers did not affect vegetative growth or leaf physiology. However, a positive linear correlation was observed between K concentration in the nutrient solution and both apical inflorescence yield (g/plant) and total inflorescence yield. Additionally, K fertilizer enhanced the harvest index.

Similarly, Fe demonstrates higher accumulation in the ET in 1C, except for the end of the cultivation, where the trend reverses. In contrast, interestingly, within the 2C fertigation system, significant differences in element accumulation between CN and ET treatments for any of the elements were absent. Generally, the differences in nutrient accumulation were predominantly insignificant when comparing CN and ET. These distinctions can be considered negligible, except for a few isolated instances. Furthermore, the disparities between the 1C and 2C regimens can be attributed to the 2C system's superior control over nutrient

solutions, ensuring stable and precise nutrient concentrations (Malík et al., 2023). Analyzing these variations in nutrient accumulation between the 1C and 2C fertigation systems provides valuable insights into how fertigation systems shape the 'ionome' composition of cannabis plants. We found that N concentrations favored the 2C system during later flowering stages, notably in weeks 8 through 11. P concentrations in flowers also showed advantages for the 2C system, particularly in weeks 7, 9, and 10. Conversely, P concentrations in leaves favored the 1C regimen, especially in weeks 9 through 12. K concentrations in leaves followed a similar trend, favoring the 1C system in weeks 6, 7, and 9. Fe concentrations exhibited variations favoring both systems, with Fe in flowers favoring the 1C system at week 8, whereas Fe in leaves and stems favored the 1C system in weeks 11, 12, and 6, respectively. These findings underscore the complexity of nutrient dynamics in fertigation, with the 2C regimen's precise control over nutrient concentrations offering valuable insights for optimizing nutrient uptake and enhancing plant development. Considering the results regarding all concentrations of the studied elements in all the plant organs examined, our third hypothesis concerning the influence of the changed nutritional regime on the composition of the 'ionome' was confirmed.

Regarding the dry weight of above-ground organs, the ET had minimal effects compared to the CN. When comparing CN with ET in the 1C fertigation system, statistically significant changes were primarily observed in the dry weight of leaves. The supplementation of P did not induce any significant alterations in cannabinoid concentration within the flowers, but it led to a decrease in concentration within the inflorescence leaves. It is noteworthy that studies conducted with industrial hemp have demonstrated varying responses to P fertilization depending on growing conditions and cultivars (Bernstein et al., 2019b). In instances where the initial P concentration in the soil is relatively high, the additional P application tends to have limited effects. Phosphorus is indeed a critical nutrient required in relatively substantial amounts by plants. However, conventional practices in the medicinal cannabis industry involve the application of higher P concentrations than those typically used for most other crops. This practice is rooted in the belief that cannabis plants necessitate elevated P concentrations to optimize their functionality and enhance yield (Bevan et al., 2021). It is worth noting that mineral nutrition significantly influences plant morpho-development, and our findings indicate that supplemental P beyond optimal requirements does not contribute to further increases in plant biomass. This outcome aligns with prior research on hemp (Vera et al., 2010). It reinforces our conclusion that medicinal cannabis exhibits a broad optimal P-level range (Shiponi and Bernstein, 2021a). Interestingly, in the ET, plants exhibited greater leaf biomass during the early generative phase of flowering. However, their leaf biomass declined as flower maturation progressed and became statistically more minuscule than the CN. These findings intriguingly suggest that ET group plants in the 1C system displayed a more foliated profile at the onset of flowering, a phenomenon in line with the work of Hawkesford et al. (2012). Shiponi and Bernstein (2021a) recommend a minimum P requirement of 15 $mg \cdot L^{-1}$ P, with a suggested application rate of 30 $mg \cdot L^{-1}$, based on the functional physiology and ionome profiling revealing genotypic variability in P sensitivity. Notably, our tested P application rates, ranging from a minimum of 32 $mg \cdot L^{-1}$ to a maximum of 93 $mg \cdot L^{-1}$, significantly exceed those employed in the reference study. Nevertheless, fewer leaves were present in the inflorescence during the last two weeks of cultivation in the 1C system. This phenomenon could simplify post-harvest inflorescence arrangement and contribute to a higher quality of harvested inflorescence. In conclusion, when comparing CN and ET at 1C, it becomes evident that ET produces noticeably more biomass in flowers only at week 8, potentially suggesting this is an optimal harvesting time. However, in the subsequent weeks, flower biomass continued to increase. As we will explore in the following discussion, cannabinoid concentrations, mainly THC, were also found to be higher in the later stages of cultivation. Therefore, a later harvest time beyond week 8 seems preferable to optimize economic efficiency while maximizing yield. When comparing CN with ET in the 2C fertigation system, statistically significant differences were observed only during the 11th week of cultivation. Comparing the two fertigation systems, 1C and 2C, statistically significant differences in the dry weight of leaves and flowers favored 1C at weeks 6 and 7 and favored 1C only for flowers at week 10. However, no statistically significant differences were observed at the end of cultivation. In summary, the results of the dry weight yield of biomass suggest that neither the altered nutritional regime of the ET variant nor the type of fertigation system had a statistically conclusive effect on the final inflorescence harvest mass of medicinal cannabis. Therefore, our first hypothesis about the inflorescence yield was only partially confirmed because we recorded a statistically significant difference between CN and ET in the harvest period only at week 11 in the 2C system.

Cannabinoid levels represent a critical aspect in assessing the excellence of medicinal cannabis. Subjects purchasing the resulting cultivated product – the dry female inflorescence of medicinal cannabis – when selecting a supplier are oriented largely according to THC concentrations and, eventually, CBD in dry matter (Zhu et al., 2021). Since cannabinoids are the constituents conferring exceptional value to cannabis, it is imperative to conduct additional research into the influence of mineral nourishment on cannabis productivity and the association between yield and potency (Bevan et al., 2021). Prior research suggests that plants' mineral nutrition can influence the production of secondary metabolites in cannabis (Caplan et al., 2017; Saloner and Bernstein, 2021; Saloner and Bernstein, 2022). An inverse correlation between cannabis yield and cannabinoid concentrations has been observed in previous studies. These studies have consistently reported a linear decrease in cannabinoid concentrations with increasing yield (the dilution effect) (Caplan et al., 2017; Yep et al., 2020; Shiponi and Bernstein, 2021a). This effect was not observed to a significant extent in our case. Additionally, future investigations should encompass the examination of other compounds acknowledged for their influence on product quality. Treatments involving metals such as Fe and Cu have demonstrated the potential to enhance secondary metabolite production in numerous plant species (Gorelick and Bernstein, 2014). The impact of metals, including

Ni, Ag, Fe, and Co, on bioactive compound production is attributed to their ability to modulate various aspects of secondary metabolism Zhao et al., 2001). In the context of hemp cultivation, it has been observed that N supplementation leads to increased plant height and biomass (Papastylianou et al., 2018). Conversely, it is noteworthy that P or K fertigation treatments have shown limited efficacy in eliciting substantial responses (Aubin et al., 2015). It is important to acknowledge that while these findings provide valuable insights into plant growth dynamics, their direct relevance to cannabis cultivation is somewhat limited. In the context of cannabis, the paramount consideration is the concentration of therapeutic cannabinoids, a factor that far outweighs concerns related to overall biomass.

This study correspondingly delved into the intricate relationship between fertigation systems and cannabinoid concentrations in medicinal cannabis, providing insights contributing to the growing body of knowledge in this field. Our findings elucidate critical factors influencing both yield and cannabinoid potency and yield.

These findings, confirming our 4th hypothesis, align with prior research, particularly studies exploring the impact of fertigation systems on cannabis cultivation. For example, a study comparing recirculation and drain-to-waste systems found that recirculation led to higher yields of THCA and CBNA, the prominent cannabinoids in medical cannabis chemotype I, but resulted in lower sesquiterpene concentrations. Drain-to-waste, however, allowed for better control over nutrient delivery but consumed more resources and yielded fewer monoterpenes and THCA (Malík et al., 2023). In our case, there was also a significant increase in THC yield in the recirculation system (up to 182% more in the 10th week).

A previous investigation (Bernstein et al., 2019b) noted that employing mild nutritional treatments, which closely adhered to the optimal range for plant growth, resulted in subtle changes in plant development. However, there emerged an observable influence on the cannabinoid profile. These findings suggest the intriguing possibility that slight adjustments in nutritional status could play a role in modulating secondary metabolism in cannabis. This statement is also confirmed by our results, where the combination of changes in the yield and concentrations of the dominant cannabinoid (THC) resulted in an increase in the yield of total THC ($g{\cdot}m^2$) by an average of 48% during harvest period for the variant with enriched nutrition.

In accordance with prior research, our findings align with established knowledge, indicating that the lowest recommended P supply for optimal yield is 30 $mg{\cdot}L^{-1}$, and optimal yields are maintained at concentrations up to 90 $mg{\cdot}L^{-1}$ (Shiponi and Bernstein, 2021b). An earlier study also observed that the impact of increased P supplementation on medicinal cannabis is dependent upon the specific organ and compound. Specifically, the concentrations of key cannabinoids such as THC, CBD, CBN, and CBG remained unaffected by the treatment of enhanced P supplementation. The addition of P did not significantly affect cannabinoid concentrations within the flowers; however, it did lead to a reduction in cannabinoid concentration in the leaves of the inflorescence (Bernstein et al., 2019b). It is worth noting that in our study, the experimental ET variants were exposed to elevated P levels, reaching as high as 93 $mg{\cdot}L^{-1}$ P toward the end of cultivation. Importantly, similar to existing research, we did not observe any significant impact of elevated P levels in the nutrient solution on the production of cannabinoids. In contrast, interestingly, an older study from Coffman and Gentner (1977) reported a notable increase in total cannabinoid content per plant with higher P supply. This is consistent with our finding that the nutrient-enhanced variant achieved higher THC yields.

In line with previous research, our findings confirm that increasing K supply beyond 60 $mg{\cdot}L^{-1}$ K does not yield beneficial effects. It is noteworthy that variations in K supply had a relatively modest impact on both cannabinoid and terpenoid levels within the plant (Saloner and Bernstein, 2022). Notably, the decrease observed in these compounds was generally mild and less pronounced compared to the significant influence of N on secondary metabolism (Saloner and Bernstein, 2021). It is worth noting that in our study, the ET variant had elevated K levels up to 265 $mg{\cdot}L^{-1}$. Similarly, we did not observe any significant impact of increased K levels on the augmentation of cannabinoid concentrations. However, it is necessary to point out again that there was a significant increase in THC yield per square meter in ET variant.

To the best of our knowledge, no prior studies have explored the influence of Fe concentration in the nutrient solution on the content of secondary metabolites in the harvested inflorescence of medicinal cannabis. Our investigation revealed a notable outcome, which examined the effects of elevated macro- and micronutrient levels, including iron, in the ET variant. Contrary to some expectations, the increased iron content did not substantially impact the final cannabinoid content of the harvested medicinal cannabis inflorescence.

Exploring additional variables, such as specific nutrient formulations and variations in environmental conditions, will be essential to comprehensively understand their influence on cannabinoid production in indoor-cultivated cannabis plants.

The complexity of the interactions between various nutrients, environmental factors, and cannabinoid biosynthesis remains a rich area for investigation. Refining our understanding of these dynamics can unlock even more precise methods for optimizing cannabinoid production in indoor-cultivated cannabis. Such advancements hold great promise for both enhancing the therapeutic potential of indoor-cultivated cannabis products and improving their overall cultivation efficiency. This knowledge is valuable for cultivators seeking to maximize both yield and cannabinoid potency in a sustainable and resource-efficient manner.

5 Conclusion

In this study, we explored the intricate relationship between enhanced nutrition, different fertigation systems, and the ionome composition, biomass, and cannabinoid content of indoor-cultivated cannabis plants, comparing the yield of THC in the harvested inflorescence per square meter. Our research reveals multifaceted connections between nutrient supply, plant development, and valuable secondary metabolites production in medicinal cannabis. Significant nutrient variations, including N, P, K, and Fe concentrations in various plant organs under the augmented nutritional regime, result from shifts in nutrient uptake,

translocation, and allocation due to changes in nutrient supply. This underscores the pivotal role of nutrition in shaping plant development, functionality, and metabolic processes, aligning with prior studies. Our findings emphasize the need for precise nutrient management strategies in cannabis cultivation. Adjusting nutrient regimens in different fertigation systems yielded significant outcomes. Notably, increased iron content in the ET variant did not substantially impact cannabinoid content, challenging assumptions. The impact of different fertigation systems on nutrient concentrations within plant tissues was evident. Adjusting the nutrient regimen in the 2C system led to a significant 108% increase in P accumulation in the stems in the 11th week, and there was a preference for K accumulation in cannabis stems, with up to a 77% increase in K levels in the ET. The ET increased the Fe concentrations, mainly in stems, up to 189%. The yield of THC in flowers per square meter with enriched nutrition increased by up to 50.7% compared to the control variant. There was even an 182% increase in THC yield in the recirculation system in the case of the ET comparison. As we conclude this study, we invite further investigations into the nuanced relationship between mineral nutrition and cannabis productivity, considering the complex interplay between yield and potency. Our research resonates beyond the scientific realm, offering actionable guidance for cultivators striving to maximize both yield and cannabinoid potency. While our study has yielded valuable insights into the relationship between fertigation systems and cannabinoid concentrations in indoor-cultivated cannabis, it is essential to acknowledge that further investigations should specifically delve deeper into the impact of enhanced nutritional compositions within the nutrient solution.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JV: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. MM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. JŠ: Data curation, Formal analysis, Validation, Visualization, Writing – original draft. PT: Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1322824/full#supplementary-material

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5. SOUHRNNÁ DISKUSE

Tato kapitola je rozdělena do jednotlivých podkapitol podle tematických celků publikovaných článků a v souvislosti s tím, jak a proč jednotlivé práce vznikaly. V podkapitole 5.1 jsou diskutovány výsledky vzniklé při polním pěstování rostlin technického konopí a vlivu výživy na produkci biomasy a kumulaci živin spolu s jejich odběrem. V podkapitole 5.2 je diskutována produkce léčebného konopí, zejména vhodné kultivační podmínky v řízeném prostředí s přihlédnutím na výnos biomasy sušeného květenství, kumulaci živin v rostlinných tkáních a produkci, případně výtěžnost kanabinoidů.

5.1 Výživa technického konopí, výnos a kumulace živin

S ohledem na stále rostoucí potřebu obnovitelných zdrojů energie je část zemědělské produkce zaměřena na produkci zemědělských plodin určených k produkci bioplynu. Převládající plodinou pěstovanou za tímto účelem je kukuřice, která se vyznačuje vysokou produkcí energie, ale současně celou řadou negativních environmentálních dopadů (riziko půdní eroze, snížení biodiverzity) (Michal et al. 2023). Konopí se nabízí jako vhodná alternativní plodina, jejíž pěstování má naopak benefitní dopad na prostředí. Je zlepšující plodinou v osevním postupu a navíc ji lze, včetně zbytků z jejího zpracování, kromě jiného, využít k produkci bioplynu (Struik et al. 2000; Kreuger et al. 2011; Prade et al. 2011; Michal et al. 2023). Vzhledem k dostupným znalostem o výživě technického konopí se jako alternativní způsob hnojení nabízí využití vedlejších surovin ze zemědělských bioplynových stanic, digestátů, konkrétně separátu a fugátu. Mnoho autorů uvádí vhodnost použití těchto surovin pro hnojení polních plodin (Kolář et al. 2010; Makdi et al. 2012; Coelho et al. 2018).

Srovnání konvenčně používaného minerálního NPK hnojiva s variantami, kdy byl k hnojení použit fugát, separát, nebo jejich kombinace, přineslo výsledky, které potvrzují předešlé tvrzení. Všechny varianty hnojení dodaly shodně 150 kg N/ha, což je dle vědecké literatury vhodná dávka, která by měla zajistit optimální růst rostlin, pevnost stonku, výnos semen a obecně vysoký výnos nadzemní biomasy (Barron et al. 2003; Vera et al. 2010; Finnan & Burke 2013; Finnan & Styles 2013; Michal et al. 2023).

Výnos suché nadzemní biomasy konopí byl srovnatelný ve všech hodnocených variantách a byla tak prokázaná vhodnost použití fugátu i separátu ke hnojení technického konopí s tím, že se touto agrotechnickou praxí snižuje environmentální riziko vyplavování dusíku (Makdi et al. 2012; Tsachidou et al. 2019; Velechovský et al. 2021).

Ve všech variantách dvouletého polního experimentu došlo i ke srovnatelné kumulaci makro živin, která vzhledem k výnosu poukazuje na to, že rostliny netrpěly deficitem při srovnání výživových variant, navzdory tomu, že naměřené hodnoty koncentrace živin v listech a stoncích byly mírně nižší, než uvádějí někteří autoři (Iványi 2005; Hakala et al. 2009; Iványi & Izsáki 2009). Tento fakt je ale možné vysvětlit tím, že v případě našeho experimentu byly analyzovány všechny listy, včetně senescentních, na rozdíl od uvedených studií, kde byly analyzovány pouze mladé, plně vyvinuté listy. V největším množství se živiny kumulovaly v listech konopí. U mikroprvků byl trend obdobný, s výjimkou vysoké kumulace železa v kořenech. To potvrzuje jeho špatnou pohyblivost v rostlině (Mengel 1994). Obecně kumulace mikroprvků je v souladu s hodnotami, které uvádí (Iványi 2005). Hodnoty odběru jednotlivých živin jsou rovněž v souladu s dalšími autory a to opět potvrzuje, že rostliny konopí prosperovaly ve všech testovaných výživových variantách (Landi 1997). Výživové potřeby rostlin byly tedy uspokojeny. V praxi to znamená, že je možné dosáhnout optimálního výnosu biomasy s využitím alternativních zdrojů živin, bez nutnosti výraznějších změn v zemědělské praxi. Tento přístup je nejen účinný, ale také ekonomicky výhodný pro farmáře a přispívá k celkové udržitelnosti zemědělské produkce.

5.2 Výživa léčebného konopí, kumulace živin, výnos květu, produkce kanabinoidů

Na základě předchozích pokusů s polním pěstováním spolu s rozvojem tématu léčebného konopí v posledních letech, se jako logické navázání jevilo pokračovat ve zkoumání chování této rostliny v kontrolovaných podmínkách s důrazem nejen na samotnou výživu, ale i její vliv na kumulaci sekundárních metabolitů v rostlinách léčebného konopí.

Výživa je jedním z dominantních faktorů, které ovlivňují vývoj rostlin, jednotlivých tkání a orgánů, jejich metabolismus a tím i konečný výnos, nejen biomasy ale i rostlinných metabolitů (Caplan et al. 2017a; Saloner et al. 2019; Saloner & Bernstein 2020; Shiponi & Bernstein 2021b). Toto tvrzení platí nejen pro rostliny, které jsou pěstovány tradičně na poli (Coffman & Gentner 1975; Bócsa et al. 1997), ale výzkumy zabývající se pěstováním v řízených podmínkách za účelem produkce rostlin konopí k léčebnému použití tento fakt nejen potvrzují, ale ještě zdůrazňují a poukazují i na významný vliv biostimulantů (Bernstein et al. 2019a; Bevan et al. 2021; De Prato et al. 2022; Malík et al. 2023).

Pokud jde o kumulaci makroživin, ukazují se rozdíly způsobené nejen rozdílnou minerální výživou, přídavkem biostimulantů ve formě aminokyselin (AMK), ale také díky rozdílné použité hydroponické technologii.

Jedním z dominantních faktorů při pěstování v hydroponických systémech je dostupnost živin, která přímo souvisí s hodnotou pH živného roztoku, jeho stabilitou a se stanoveným poměrem a dostupností živin (Velazquez et al. 2013).

Součástí této práce bylo mimo jiné srovnání dvou hydroponických systémů – recirkulačního (RS) a průtočného (PS), kdy v prvním zmíněném dochází k cirkulaci roztoku do doby, než je jeho objem většinově vyčerpán, a naopak v průtočném, kdy rostliny dostávají pokaždé stejný poměr živin, čerstvě připraveného roztoku hnojiva. Navíc byly porovnávány dvě úrovně minerální výživy.

Pokud jde o zastoupení makroprvků a jejich vzájemné poměry v nadzemní biomase rostlin konopí, které byly pěstovány ve dvou různých hydroponických systémech - RS a PS, a za nesourodých podmínek výživy, byly v některých případech zaznamenány statisticky významně rozdílné koncentrační hladiny. V případě dusíku byla v rostlinách pěstovaných v systému RS zaznamenána výraznější akumulace ve všech nadzemních orgánech (květech, listech a stoncích). Tento rozdíl byl dále zesílen v případě výživy s přídavkem AMK, což je v souladu s dřívějšími poznatky a schopnostmi rostlin přijímat a inkorporovat aminokyseliny (Matsumoto et al. 1999; Persson & Näsholm 2001; Jämtgård et al. 2008). Analogický trend byl pozorován i u kumulace fosforu v RS systému, kde docházelo ke kolísání pH mezi 5,9 – 8,05. Vyšší koncentrace P v RS byla také pravděpodobně způsobena zvýšeným pH, podobně jako výsledky Lefever et al. (2017). V rostlinných buňkách je P hlavní složkou nukleových kyselin, membránových lipidů a fosforylovaných meziproduktů energetického metabolismu. Buněčná homeostáza fosforu je tedy nezbytná pro normální fyziologické a biochemické procesy (Raghothama, 2005). Celkově nižší hodnoty vápníku a hořčíku v rostlinách konopí pěstovaných v recirkulačním systému mohly souviset rovněž s vyšším pH v živném roztoku ve srovnání s průtočným systémem (konstantní pH 5,8). Při vyšším pH může dojít k vysrážení vápníku a hořčíku s fosforečnany na nerozpustné soli nepřístupné rostlinám (Lee et al. 2017). Tato odlišnost mezi hydroponickými systémy byla ještě zvýrazněna v případě výživy s AMK, kde bylo dosaženo statistických rozdílů v obsazích zmíněných prvků (Ca a Mg) mezi variantami v nadzemních orgánech již od 3. vegetačního týdne rostlin. Pravděpodobně toto bylo způsobeno tím, že vápník a hořčík je schopen se koordinovat s karboxylovými, thiolovými a aminoskupinami dodaných AMK za vzniku komplexů, jejichž dostupnost pro rostliny je

omezená (Maeda et al. 1990). Zvýšení pH živného roztoku na 6,5 taktéž podporuje lepší absorpci dusíku v amonné formě (NH_4^+), jak bylo zdůrazněno (Dyhr-Jensen & Brix 1996). Tato forma dusíku je pro rostliny v hydroponickém prostředí snadněji dostupná a rychleji přijímána než nitrátová forma (NO_3^-), jak uvádí Langenfeld et al. (2022). Výzkumy Schortemeyer et al. (1993) podpořily myšlenku, že s rostoucím pH se zvyšuje preferenční absorpce NH_4^+ oproti NO_3^-. Pro optimální poměr NH_4^+/NO_3^- v hydroponickém roztoku bylo doporučeno 10 - 30% zastoupení NH_4^+ při koncentraci 200 mg celkového N/l. Překročení tohoto optimálního poměru může zvýšit riziko toxického působení NH_4^+, jak naznačují Saloner & Bernstein (2022b). V kontextu nárůstu nadzemní biomasy a koncentrace sekundárních metabolitů u rostlin pěstovaných v různých systémech lze usuzovat, že stabilní pH v PS mohlo přispět k optimálnímu příjmu dusíku ve formě NH_4^+. Dále, jak podotýká (Langenfeld et al. 2022), vyšší absorpce NH_4^+ může bránit vstřebávání důležitých kationtů, jako jsou Ca^{2+} a Mg^{2+}.

Při porovnání dvou různých hydroponických systémů, recirkulačního (RS) a průtokového (PS), z hlediska zastoupení a vzájemného poměru prvků v rostlinách, lze dospět k závěru, že kromě kolísání pH živného roztoku má na toto rovněž výrazný vliv způsob hospodaření s živným roztokem. V RS systému dochází k recyklaci živného roztoku a tím pádem ke změnám poměru a koncentrace živin v živném roztoku. To se děje zejména v důsledku primárního příjmu vody rostlinami, kdy se zbývající roztok v nádrži koncentruje. Tento stav může vést k nerovnováze živin v důsledku selektivity přijímání některých živin z roztoku (Ho & Adams 1995; Bugbee 2004; Sambo et al. 2019; Malík et al. 2023).

Z výsledků části této práce rovněž vyplývá, že vyšší koncentrace živin v roztoku nutně nevede k navýšení tvorby suché biomasy květenství léčebného konopí, může ale vést k alternacím v kumulaci jednotlivých živin v rostlinných tkáních, a dochází i k výrazným změnám ve smyslu výtěžnosti kanabinoidů. Z prací jiných autorů vyplývá, že pokud dochází k navýšení výnosu biomasy květů, je tento jev často doprovázen i tzv. zřeďovacím efektem, kdy dochází k poklesu koncentrace kanabinoidů v květenství na úkor jejich výnosu (Caplan et al. 2017b; Yep et al. 2020). Při porovnání dvou úrovní minerální výživy – kontrolní (CN) a obohacené (OV) o P, K a Fe ve dvou hydroponických systémech (RS a PS), nedošlo k významným změnám ve výnosu květů. Pravděpodobným vysvětlením je, že nedošlo ke změně, či navýšení hodnot dodaného dusíku, který je označován jako nejvýznamnější prvek pro tvorbu biomasy (Papastylianou et al. 2018), a že koncentrace P a K v kontrolní variantě nebyla limitující pro tvorbu biomasy. Navýšení koncentrací P a K v živném roztoku nevedlo k změněným výnosům biomasy ani u pokusů jiných autorů (Aubin et al. 2015). Ve studii

(Bernstein et al. 2019b) bylo zjištěno, že použití mírných nutričních úprav, které jsou v rozmezí optimálních hodnot pro růst rostlin, vede pouze k jemným změnám ve vývoji rostlin a tvorbě biomasy. Byl však pozorován vliv na kanabinoidní profil, což naznačuje možný vliv výživy na modulaci sekundárního metabolismu konopí. Toto tvrzení potvrzují i naše výsledky, kdy obohacená výživa vedla ke zvýšení výnosu celkového THC v období sklizně v průměru o 48 %. Pokud jde o výživovou variabilitu s AMK, naše výsledky korespondují s poznatky Saloner & Bernstein (2021). Bylo zjištěno, že nadbytečný dusík v roztoku může snížit koncentraci THC. Avšak, při určitých koncentracích dusíku, například 160 mg N/l, může dojít k reverzibilnímu zvýšení koncentrace terpenických sloučenin jako je limonen a myrcen. Naše výsledky jsou v souladu s dřívějšími studiemi, které dokumentovaly pozitivní vztah mezi hnojením dusíkem a tvorbou monoterpenů (McCullough & Kulman 1991; Close et al. 2004).

Dopad zvýšeného přísunu P na léčivé konopí závisí na konkrétním orgánu a sledované sloučenině (metabolitu). Konkrétně koncentrace klíčových kanabinoidů v květech, jako jsou THC, CBD, CBN a CBG, zůstaly ošetřením zvýšenou suplementací P neovlivněny (Bernstein et al. 2019b). Naopak je zajímavé, že starší studie zaznamenala výrazné zvýšení celkového obsahu kanabinoidů v rostlině při vyšším přísunu P (Coffman & Gentner 1977). To je v souladu s naším zjištěním, že varianta se zvýšeným obsahem živin, včetně P, dosahovala vyšších výnosů THC.

Ve studii (Saloner & Bernstein 2022a) měla zvýšená dávka K nízký vliv na hladinu kanabinoidů i terpenoidů v rostlině. Efekt draslíku na sekundární metabolismus je ve srovnání s výrazným vlivem N nízký (Saloner & Bernstein 2021). Zajímavým faktem je, že z části výsledků této práce vyplývá, že obohacená varianta o K se rovněž projevila zvýšením výnosu THC na pěstební plochu. Toto je v souladu se zjištěním Yep & Zheng (2021), kteří studovali výnos konopného květenství s přídavkem K v akvaponických a hydroponických živných roztocích. Přídavek K a Fe neovlivnil vegetativní růst ani fyziologii listů. Byla však pozorována pozitivní korelace mezi koncentrací K v živném roztoku a výnosem apikálního a celkového květenství (g/rostlinu).

Ve variantách s obohacenou výživou byla zaznamenána výrazná preference akumulace K ve stoncích konopí, což koresponduje s předchozími výzkumy o luxusní spotřebě K a jeho dočasném ukládání (Marschner 2011). Zvýšení hladiny K v OV vedlo k markantnímu 77% nárůstu akumulace K ve stoncích rostlin. Současně se v OV projevil vzrůst koncentrace železa (Fe) ve stoncích až o 189 %. V oblasti hodnocení léčebného konopí hrají klíčovou roli hladiny kanabinoidů, zejména THC a CBD, přičemž subjekty při výběru dodavatele upřednostňují

produkty s vyšší koncentrací těchto látek v sušině (Zhu et al., 2021). Kombinace koncentrace zmíněných fytokanabinoidů a suché hmotnosti květů se odrazila ve výnosu kanabinoidů z rostliny a pěstební plochy. Při analýze sušených květů byl výtěžek THC sledován týdně v obou hydroponických systémech a různých výživových podmínkách. V systému RS vykazovaly rostliny téměř lineární nárůst výnosu THC v průběhu času ve všech výživových variantách. V kontrastu s tím dosáhly rostliny v systému PS maximálního výnosu THC dříve, což bylo ještě zesíleno při použití výživové varianty s AMK. V terapeutickém kontextu pěstování konopí koncentrace kanabinoidů tedy převažuje nad obavami týkajícími se výnosu celkové biomasy. Výsledky naší studie podtrhuje skutečnost, že absolutní výnos THC v květech na plochu při obohacené výživě dosáhl zvýšení až o 50,7 % oproti kontrolní variantě.

6. ZÁVĚR

Tato disertační práce se zaměřuje na konkrétní aspekty výživy rostlin konopí (*Cannabis sativa L)* s cílem posoudit vliv na výnos nadzemní biomasy, výnos květenství a produkci sekundárních metabolitů v případě rostlin léčebného konopí. Přestože polní pěstování rostlin konopí bylo již dříve poměrně dobře prozkoumáno, nový přístup k výživě rostlin technického konopí se stal důležitým směrem v souvislosti se stále vyšším využíváním obnovitelné energie i s ekologickými hledisky zemědělské produkce. V první části se proto tato práce zaobírá možností optimalizace využití vedlejších produktů zemědělských bioplynových stanic jakožto alternativního hnojiva pro pěstování technického konopí. Srovnáním výsledků dvouletého experimentu lze konstatovat, že zmíněné produkty anaerobní digesce jsou vhodnou alternativou pro polní produkci konopí. Rostliny dosahovaly srovnatelných výnosů biomasy a míra kumulace živin byla srovnatelná. Kvalita těchto vedlejších surovin je závislá na vstupních materiálech do procesu anaerobní digesce a může tak být ovlivněna kvantita a dostupnost živin pro rostliny z produkovaných digestátů.

Další část práce se věnuje rostlinám léčebného konopí. Pěstování rostlin za účelem využití ve farmacii a zdravotnictví, klade na samotnou produkci výrazné nároky z hlediska kontroly kvality veškerých procesů a zpravidla se tudíž odehrává v řízených podmínkách vnitřních pěstebních zařízení. Vzhledem k nedostatku relevantních vědeckých zdrojů na toto téma byly provedeny dvě navazující studie, sledující vliv výživy na produkci sušeného květenství a sekundárních metabolitů, zejména THC. Experimenty sledovaly vliv dvou různých hydroponických systémů spolu s vlivem upravených výživových variant, které mezi sebou byly porovnávány. Průtokový hydroponický systém nabídl precizní kontrolu nad živným roztokem,

což pravděpodobně urychlilo růst a následné dozrávání rostlin. Toto urychlení však vedlo k vyšší spotřebě vody a hnojiv a zároveň k nižšímu celkovému výnosu monoterpenů a THC. Přidání biostimulantu na bázi aminokyselin významně zvýšilo obsah dusíku, avšak současně byla omezena kumulace vápníku napříč nadzemními orgány rostlin konopí v obou použitých hydroponických systémech. V současné době je stále nedostatek poznatků, ze kterých by bylo možné vyvodit jednoznačné závěry, které jdou vztáhnout ke konkrétní živině a platily by za obecný princip. Samotná literatura i vlastní experimenty často vedou k různým výsledkům. Společným jmenovatelem je ovšem to, že minerální výživa spolu s hydroponickým systémem, potažmo způsob hospodaření s živným roztokem, hrají významnou roli při ovlivňování produkce sekundárních metabolitů v rostlinách konopí a mohou mít vliv na výnosové charakteristiky.

Celkově lze konstatovat, že tato práce přináší důležité poznatky, které mohou ovlivnit pěstitelskou praxi i výzkum v oblasti konopí. Je zjevné, že při navrhování agrotechniky pro konkrétní druhy konopí je nezbytné brát v úvahu specifické požadavky na výživu, aby bylo dosaženo nejen optimálních výnosů biomasy, ale i kvalitativně vysoce hodnotných rostlinných metabolitů. Výzkum se soustředil především na konopný chemotyp I s vysokým obsahem THC pěstovaný v keramzitu. Návazně by bylo zajímavé prověřit tyto metody s různými chemotypy konopí pěstovanými na dalších různých pěstebních médiích. Tímto směrem lze v budoucnu upřít další výzkumné snahy s cílem plně porozumět interakcím mezi výživou, genetikou a prostředím, které formují vlastnosti konopí.

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8. PUBLIKOVANÉ PRÁCE MIMO ROZSAH DISERTACE

8.1 Články ve vědeckých časopisech

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