# Comprehensive Research Plan: Optimizing Nutrient Ratios for Cannabis Cultivation

## 1. Introduction

### 1.1 Rationale and Significance

The global landscape surrounding *Cannabis sativa* L. is undergoing a significant transformation, marked by increasing legalization for medicinal and recreational purposes. This shift has catalyzed rapid growth in the cannabis industry, creating substantial economic opportunities but also demanding scientifically optimized cultivation practices to ensure product quality, consistency, and yield. Central to achieving these goals is nutrient management. The supply of essential mineral nutrients profoundly influences cannabis plant growth, development, overall biomass yield, and, critically, the biosynthesis and accumulation of secondary metabolites, including cannabinoids (e.g., tetrahydrocannabinol - THC, cannabidiol - CBD) and terpenes, which dictate the plant's therapeutic efficacy and market value.

Cultivation within Controlled Environment Agriculture (CEA) systems, particularly hydroponics and soilless media, offers unparalleled opportunities for precision nutrient management. CEA allows growers to meticulously control environmental variables (light, temperature, humidity, CO\_2) and root-zone conditions (pH, electrical conductivity - EC, nutrient solution composition), thereby optimizing resource use efficiency for water and fertilizers compared to traditional field agriculture. This precision is vital not only for maximizing productivity but also for minimizing the environmental footprint associated with nutrient runoff and resource depletion.

### 1.2 Current Knowledge Gaps and Challenges

Despite the critical importance of nutrition, the scientific understanding of optimal fertilizer nutrient ratios for cannabis remains surprisingly limited and often contradictory. Much of the current commercial cultivation practice relies heavily on anecdotal evidence, grower experience derived from previously clandestine operations, or generic recipes provided by fertilizer companies, rather than data derived from rigorous, controlled scientific investigation.

Several factors contribute to this knowledge gap. Historical prohibition severely restricted research, particularly on "drug-type" cannabis (phenotypes I and II, cultivated for cannabinoids) as opposed to "fiber-type" hemp (phenotype III). These types possess distinct genetic backgrounds and have been selected for different traits (e.g., biomass vs. flower/cannabinoid production), leading to demonstrably different nutrient requirements and utilization efficiencies, particularly for nitrogen (N) and phosphorus (P). Furthermore, the existing scientific literature presents conflicting data on optimal nutrient levels, especially for P and potassium (K). Reported optima vary significantly depending on the specific cultivar studied, the growing system employed (e.g., deep water culture (DWC), nutrient film technique (NFT), coco coir, peat mixes), environmental conditions (light intensity and spectrum, CO\_2 concentration, root zone temperature), and the experimental methodologies used. This high degree of context-dependency suggests that a single, universal "optimal" nutrient recipe for all cannabis cultivation scenarios is improbable. Research must therefore be highly specific regarding cultivar, system, and environment to yield meaningful and applicable results.

Adding to the complexity is the relationship between nutrient supply and the production of secondary metabolites. There is considerable evidence suggesting a potential trade-off: nutrient regimes maximizing biomass yield may not necessarily maximize the *concentration* of cannabinoids and terpenes. Several studies report higher cannabinoid or terpene concentrations under conditions of slight nutrient stress or deficiency (particularly for N, P, and K), potentially indicating that secondary metabolism is, in part, a plant stress response. Conversely, high nutrient levels that promote vigorous growth may lead to a "dilution effect," where the total amount of secondary metabolites increases due to greater biomass, but their concentration within the tissue decreases. The specific form in which nutrients are supplied, such as the ratio of ammonium (NH\_4^+) to nitrate (NO\_3^-) for nitrogen, can also significantly influence plant function, yield, and secondary metabolite profiles. Practices common among growers, such as late-stage nutrient manipulation or "flushing" (irrigating with plain water before harvest), also lack robust scientific validation regarding their purported effects on improving final product quality (e.g., taste, smoothness, ash color). Existing studies on flushing have shown minimal impact on final bud nutrient content or cannabinoid/terpene levels, and sometimes negative impacts on taste panel preferences.

Finally, the lack of standardized research protocols and reporting in many past studies hinders direct comparison and synthesis of findings. Variations in experimental design, environmental controls, measurement techniques, and statistical analyses make it difficult to discern whether conflicting results stem from true biological differences or methodological inconsistencies.

### 1.3 Research Scope Overview

This research plan addresses these knowledge gaps by outlining a rigorous, systematic investigation into the effects of varying macronutrient ratios—specifically Nitrogen (N), Phosphorus (P), and Potassium (K)—on cannabis growth, yield, and quality within a precisely controlled hydroponic system. The study will encompass both the vegetative and flowering stages of development, utilizing a selected, commercially relevant cannabis cultivar propagated from clones to ensure genetic uniformity.

Key parameters to be quantified include vegetative growth metrics (height, stem diameter, biomass accumulation rate), final yield (inflorescence dry weight), nutrient uptake and partitioning within the plant, and the concentration profiles of major cannabinoids (e.g., THCA, CBDA, CBGA, THC, CBD, CBG) and representative terpenes (e.g., myrcene, limonene, α-pinene, β-pinene, β-caryophyllene, α-humulene) in mature inflorescences.

The experimental design emphasizes scientific best practices, including stringent environmental control within a CEA setting, adequate replication, randomization, precise and validated measurement techniques (e.g., HPLC for cannabinoids, GC-MS for terpenes, ICP for tissue nutrients), and appropriate statistical analysis methods (ANOVA, regression, potentially RSM). The ultimate goal is to generate reliable, quantitative data that can inform evidence-based nutrient management strategies, enhancing both the fundamental understanding of cannabis physiology and the optimization of commercial cultivation practices for specific outcomes (yield vs. quality). The detailed reporting of all methods and conditions aims to improve the comparability and utility of the findings for the broader research and cultivation community.

## 2. Research Objectives

### 2.1 Reasoning

Clear, specific, and measurable objectives are essential to guide the research process, ensuring that the experimental design and data collection directly address the identified knowledge gaps and contribute meaningfully to the field. The objectives are formulated based on the overarching goal of optimizing NPK ratios for cannabis cultivation in CEA, considering impacts on growth, yield, and chemical profile. They are designed to be SMART (Specific, Measurable, Achievable, Relevant, Time-bound).

### 2.2 Primary Objective

The primary objective of this research is to quantitatively determine the optimal N:P:K nutrient ratios, expressed as concentrations (e.g., mg L^{-1} or ppm) in a hydroponic nutrient solution, required to maximize distinct key performance indicators for a selected cannabis cultivar under controlled environmental conditions. Specifically, the research will identify the NPK ratios that optimize:

* a) **Vegetative Growth:** Maximizing growth rate (e.g., height increase per day, biomass accumulation rate) and total vegetative biomass (dry weight of leaves and stems) at the end of a defined vegetative period.
* b) **Inflorescence Yield:** Maximizing the dry weight yield of trimmed inflorescences (buds) per plant or per unit area (g/plant or g/m^2) at the time of harvest.
* c) **Cannabinoid Potency and Yield:** Maximizing both the *concentration* (percentage by dry weight, %) and the *total yield* (mg/plant or g/m^2) of key phytocannabinoids (specifically THCA, CBDA, CBGA, and their neutral counterparts THC, CBD, CBG) within the mature, harvested inflorescences.
* d) **Terpene Profile and Yield:** Maximizing both the *concentration* (%) and the *total yield* (mg/plant or g/m^2) of representative and commercially important monoterpenes (e.g., myrcene, limonene, α-pinene, β-pinene) and sesquiterpenes (e.g., β-caryophyllene, α-humulene) within the mature, harvested inflorescences.

Recognizing the potential trade-offs between maximizing biomass and maximizing the concentration of secondary metabolites is fundamental. Therefore, a critical component of this primary objective is to define potentially *different* optimal NPK ratios for maximizing yield (Objective 2.2b) versus maximizing cannabinoid or terpene *concentration* (Objectives 2.2c and 2.2d). The research aims to quantify these potentially divergent optima and the associated trade-offs.

### 2.3 Secondary Objectives

To provide a more comprehensive understanding of the effects of NPK ratios, the following secondary objectives will be pursued:

* To systematically evaluate and document the effect of varying N:P:K ratios on plant health indicators throughout the growth cycle. This includes monitoring and recording the onset and progression of visual nutrient deficiency or toxicity symptoms using standardized descriptors and photographic evidence , and quantifying leaf chlorophyll content using non-destructive methods (e.g., SPAD/CCI meter readings) as a proxy for N status and overall photosynthetic potential.
* To assess the impact of different N:P:K supply ratios on nutrient uptake dynamics and allocation within the plant. This involves analyzing the elemental concentrations (N, P, K, and potentially key secondary macronutrients like Ca, Mg, S, and micronutrients) in various plant tissues (representative leaves, stems, roots, and inflorescences) sampled at critical developmental stages (e.g., end of vegetative phase, mid-flowering, final harvest). This analysis provides insight into how nutrient supply affects actual nutrient accumulation and partitioning, helping to explain observed growth and chemotype responses and identify phenomena like luxury consumption or nutrient antagonism.
* To investigate and quantify potential interactive effects between N, P, and K supply on the primary and secondary outcome measures (growth, yield, cannabinoid/terpene profiles, nutrient uptake). This acknowledges that the optimal level of one nutrient may depend on the availability of others.
* *(Optional Extension)* To evaluate the implications of optimized NPK ratios for resource use efficiency. This could involve calculating Nutrient Use Efficiency (NUE) metrics (e.g., biomass produced per unit nutrient supplied or taken up) for both biomass and cannabinoid production. Depending on resources, this could be extended to compare mineral versus organic nutrient sources or to scientifically evaluate the impact of late-flower nutrient manipulation or flushing strategies on NUE and final product quality.

## 3. Literature Review Synthesis

### 3.1 Reasoning

A comprehensive synthesis of the existing literature is foundational to this research plan. It establishes the current state of scientific knowledge regarding cannabis nutrition, identifies areas of consensus and controversy, pinpoints critical knowledge gaps, and provides the scientific rationale for the proposed hypotheses and experimental approach. This review focuses on the roles of macronutrients (N, P, K) and key micronutrients, their interactions, the influence of environmental factors prevalent in CEA, and their specific effects on cannabis secondary metabolite production.

### 3.2 Macronutrient Roles (N, P, K)

* **Nitrogen (N):** Universally recognized as critical for plant growth, N is a core component of proteins, enzymes (including those in photosynthetic pathways), and chlorophyll. Cannabis exhibits high N demand during the vegetative stage to support rapid development of leaves and stems, establishing the plant's structural framework. Recommended N concentrations often decrease during the flowering stage. Excessive N supply during flowering has been frequently linked to reduced concentrations of cannabinoids (THCA, CBDA) and terpenes, potentially due to a dilution effect associated with increased biomass or direct interference with secondary metabolite pathways. Studies using response surface methodology (RSM) or varying concentrations in hydroponics often find optimal N for *flowering yield* around 160-200 mg L^{-1} , though vegetative optima might be similar or slightly higher. The chemical form of N supplied (NH\_4^+ vs. NO\_3^-) is also a significant factor; high NH\_4^+/NO\_3^- ratios can impair growth, reduce yield, negatively affect cannabinoid/terpene profiles, and potentially lead to toxicity, while exclusive NO\_3^- nutrition often yields the best results for secondary metabolites. Furthermore, hemp-type cannabis may reach optimal N levels at lower concentrations and exhibit higher N use efficiency compared to drug-type cannabis.
* **Phosphorus (P):** P plays a fundamental role in energy transfer (ATP), nucleic acid synthesis (DNA, RNA), membrane structure, and is particularly crucial for root development and the initiation and development of flowers and seeds. Consequently, P demand typically increases during the transition to and throughout the flowering stage. However, the optimal P concentration for cannabis, especially during flowering, is a subject of considerable debate in the scientific literature. Reported optima range widely: some studies suggest maximal yield or quality is achieved at relatively low concentrations, potentially as low as 11-15 mg L^{-1} , while others indicate optima around 30 mg L^{-1} , 60 mg L^{-1} , or even up to 90 mg L^{-1} for certain cultivars. Several studies have found no significant increase in flower yield or cannabinoid concentration when P supply exceeds approximately 11-25 mg L^{-1}. This discrepancy highlights a critical need for standardized research methodologies, as variations in cultivars, experimental systems (e.g., DWC vs. substrate culture), measurement timing, and statistical power likely contribute to the conflicting findings. There is evidence that low P supply, potentially bordering on deficiency, can increase the *concentration* of certain cannabinoids, although this often comes at the expense of overall biomass yield. Cannabis inflorescences act as strong sinks for P, accumulating significantly higher concentrations than leaves. Over-fertilization with P is a major environmental concern due to its contribution to eutrophication via runoff, making optimization crucial for sustainability.
* **Potassium (K):** K is involved in numerous physiological processes, including osmotic regulation (stomatal control), enzyme activation, protein synthesis, photosynthesis, and carbohydrate transport. It also plays a role in plant stress resistance. K demand generally increases during the flowering stage to support bud development and potentially resin production. Similar to P, optimal K levels are debated. Some rigorous studies using RSM in hydroponics found no significant inflorescence yield response to K concentrations ranging from 60 to 340 mg L^{-1} , suggesting the lower end of this range might be sufficient. Other research suggests optimal ranges between 60-175 mg L^{-1} for flowering. Similar to N and P, low K supply (e.g., 15 mg L^{-1}) can impair growth and yield but may increase the concentration of cannabinoids and terpenes, while levels above ~60 mg L^{-1} often lead to decreased secondary metabolite concentrations without further yield benefits. High K levels can also interfere with the uptake of other cations, particularly Calcium (Ca) and Magnesium (Mg), due to competitive inhibition at root uptake sites.

### 3.3 Micronutrient Roles (Brief Overview)

While NPK are the primary focus, other nutrients are essential and can influence outcomes:

* **Calcium (Ca) & Magnesium (Mg):** Ca is vital for cell wall structure and membrane function, while Mg is the central component of chlorophyll and essential for photosynthesis and enzyme activation. Increased demand for Ca and Mg has been noted under LED lighting or in environments with high K supply. Deficiencies manifest visually: Mg deficiency typically causes interveinal chlorosis (yellowing between veins) on older or mid-plant leaves, while Ca deficiency often appears as necrotic spots, distorted new growth, or tip burn. Unlike N, P, and K, optimal (not deficient) Mg levels (e.g., 35 mg L^{-1}) may be required for maximizing cannabinoid concentrations. Cation competition, particularly high K or NH\_4^+, can induce Ca or Mg deficiencies even if supply seems adequate.
* **Sulfur (S):** A component of essential amino acids (methionine, cysteine) and involved in protein and enzyme structure. It may also play a role in supporting terpene synthesis. Deficiency typically causes general yellowing, often starting in younger leaves.
* **Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu):** These micronutrients primarily function as cofactors in various enzymatic reactions, including those involved in chlorophyll synthesis and photosynthesis. Deficiencies can lead to various symptoms, often including interveinal chlorosis on younger leaves, stunted growth, or necrosis. Their availability is highly pH-dependent, decreasing at higher pH levels. Mn toxicity is a risk at low pH (<5.5) in hydroponics. Fe is particularly crucial for chlorophyll synthesis; one study linked increased Fe supply (along with P and K) to higher THC yield.
* **Boron (B):** B is essential for cell wall synthesis and structural integrity, membrane function, and potentially carbohydrate metabolism and reproductive development. It is taken up primarily as uncharged boric acid. B is relatively immobile within the plant, so deficiency symptoms appear first on new growth, causing distorted, stunted, or necrotic growing tips, brittle stems, and poor flower/pollen development. B toxicity typically manifests as marginal yellowing and necrosis on older leaves. While direct links to secondary metabolism are less studied, B's role in cell wall integrity and potential signaling could indirectly influence pathways.
* **Silicon (Si):** While not strictly essential for most plants, Si provides numerous benefits, particularly under stress conditions and in hydroponic systems where it is often lacking. Si deposits in cell walls (as phytoliths), strengthening tissues, improving plant architecture, and increasing resistance to pests and diseases. It can also alleviate abiotic stresses like drought, salinity, or heavy metal toxicity. Studies in cannabis (hemp) have shown that supplemental Si (as silicate) is bioavailable, increases leaf Si content, and can potentially increase inflorescence yield without negatively impacting cannabinoid or terpene profiles. Si may also limit the excessive uptake of certain micronutrients (Fe, Mn, Zn, Cu) or heavy metals.

### 3.4 Nutrient Interactions and Environmental Factors

Optimizing cannabis nutrition requires considering more than just individual nutrient levels.

* **Nutrient Interactions:** The balance between nutrients is critical. Studies using RSM have demonstrated significant interactions between N, P, and K affecting various growth parameters like root, stem, and leaf mass, as well as leaf area and chlorophyll content. Antagonistic relationships exist, such as high K inhibiting Ca and Mg uptake , or high P potentially affecting micronutrient availability. The form of nitrogen (NH\_4^+ vs. NO\_3^-) also influences the uptake of other cations.
* **Environmental Factors (CEA Context):** Nutrient requirements are tightly linked to environmental conditions.
  + *Light:* Higher light intensity (PPFD) generally increases photosynthetic rates and growth, thereby increasing nutrient demand. Light spectrum can also influence specific metabolite production.
  + *CO2 Enrichment:* Elevated CO\_2 levels boost photosynthesis and growth, leading to significantly higher demand for N, P, K, and micronutrients. Optimal nutrient levels must be matched to CO\_2 levels to realize yield benefits.
  + *Root Zone Temperature (RZT):* RZT directly impacts root metabolism, respiration, and the rate of water and nutrient uptake. Optimal RZT for cannabis is often cited as 20-24°C (68-75°F). Temperatures outside this range can significantly reduce nutrient uptake and growth, regardless of nutrient availability in the solution.
  + *Growing Medium:* In soilless culture, the substrate's properties are crucial. Inert media like rockwool have minimal impact on nutrient availability, requiring complete nutrient delivery via the solution. Organic-based media like peat or coco coir possess Cation Exchange Capacity (CEC), which allows them to retain and release cationic nutrients (K+, Ca2+, Mg2+, etc.). Coco coir has a notable CEC and can preferentially bind Ca and Mg, potentially leading to deficiencies if not accounted for with specialized coco-specific nutrient formulations and proper buffering. Substrate choice dictates irrigation and fertilization strategies.
  + *Hydroponic Solution Management:* Precise control of the nutrient solution is paramount in hydroponics. Maintaining pH within the optimal range (typically 5.5-6.5 for soilless media/hydroponics) is critical for ensuring the availability of all essential nutrients. Electrical Conductivity (EC) is used to monitor the total concentration of dissolved salts (nutrients). Monitoring EC of both the input solution and the runoff/leachate helps manage nutrient delivery and prevent excessive salt buildup in the root zone. Dissolved Oxygen (DO) in the nutrient solution is essential for root respiration and nutrient uptake, particularly in DWC systems where roots are fully submerged. Low DO levels (<5 mg L^{-1}) promote anaerobic conditions, favoring pathogens like *Pythium* (root rot) and impairing root function.

### 3.5 Nutrient Effects on Secondary Metabolites

The influence of mineral nutrition extends beyond growth and yield to significantly impact the chemical profile of cannabis, particularly the valuable cannabinoids and terpenes.

* **General Trend & Potential Mechanisms:** A recurring theme in the literature is an inverse relationship between the supply of major macronutrients (N, P, K) and the *concentration* (but not necessarily total yield per plant) of cannabinoids and terpenes in the inflorescences. This phenomenon could be explained by a simple "dilution effect," where rapid biomass accumulation under high fertility dilutes the concentration of secondary metabolites within the plant tissues. Alternatively, or perhaps concurrently, moderate nutrient stress may actively trigger or enhance secondary metabolite biosynthesis pathways as a defense or adaptation mechanism. Secondary metabolites often play roles in plant defense and response to environmental cues. Nutrients themselves can act as signals influencing these pathways or affect the availability of precursors (e.g., from primary metabolism) required for cannabinoid and terpene synthesis. The biosynthesis pathways for cannabinoids and terpenes share common precursors derived from primary metabolism (e.g., geranyl diphosphate from the MEP pathway) , suggesting that shifts in nutrient availability could impact the flow of carbon into these pathways.
* **Specific Nutrient Effects:**
  + *Nitrogen:* High N supply consistently correlates with lower concentrations of THCA, CBDA, and various terpenes. The form of N (NH\_4^+ vs. NO\_3^-) also significantly impacts these profiles, with high NH\_4^+ generally reducing concentrations compared to NO\_3^- dominant nutrition.
  + *Phosphorus:* The effect of P on secondary metabolites is less consistent. Some studies report increased cannabinoid concentrations under P deficiency , while others find little or no effect on major cannabinoid concentrations once P levels are above deficiency thresholds. One study noted P deficiency increased geraniol concentration.
  + *Potassium:* Low K supply often leads to higher concentrations of acidic cannabinoids and most terpenes, with concentrations decreasing as K supply increases into the optimal range for growth. Effects on terpenes can be genotype-specific.
* **Other Influencing Factors:** Beyond NPK, other factors modulate secondary metabolite profiles. Biostimulants, such as amino acid supplements, have been shown to alter terpene profiles, potentially increasing monoterpenes like limonene and myrcene while decreasing certain cannabinoids like CBNA. The cultivation environment itself plays a major role; for instance, outdoor cultivation in living soil can result in significantly different terpene profiles (often higher sesquiterpenes) and lower levels of oxidized/degraded cannabinoids compared to indoor cultivation under artificial lights. Light spectrum and intensity are also known modulators of cannabinoid and terpene synthesis.

### 3.6 Research Gaps Identified

Based on the reviewed literature, several key research gaps persist:

* **Conflicting Optima:** Lack of consensus on optimal P and K concentrations for flowering yield and quality.
* **Cultivar Specificity:** Insufficient comparative studies examining diverse, commercially relevant cultivars under identical, controlled conditions to parse genetic vs. environmental effects on nutrient response.
* **Micronutrient Roles:** Limited understanding of the specific roles and optimal levels of micronutrients (especially B, Si, Fe, Mn, Zn) in cannabis, particularly concerning their influence on secondary metabolism and interactions with macronutrients.
* **Integrated Systems Approach:** Need for studies that integrate nutrient management strategies with other critical CEA variables like light spectrum/intensity, CO\_2 concentration, root zone temperature, and dissolved oxygen levels.
* **Validation of Grower Practices:** Lack of rigorous scientific validation for common practices such as the use of high-P "bloom boosters" and pre-harvest flushing.
* **Terpene Focus:** While cannabinoid responses are more frequently studied, research specifically targeting the effects of nutrition on the full terpene profile, underlying biosynthesis pathways, and terpene synthase (TPS) gene expression is limited.
* **Nutrient Use Efficiency (NUE):** More research is needed to quantify and optimize NUE and Water Use Efficiency (WUE) in cannabis CEA systems for improved sustainability and reduced production costs.

### 3.7 Proposed Table: Summary of Reported Nutrient Optima

To consolidate the varied findings, Table 1 summarizes reported optimal nutrient concentrations from key studies, highlighting the context dependency.

**Table 1:** Summary of Reported Optimal Nutrient Concentrations (mg L^{-1}) for Cannabis Growth Stages from Key Literature.

| Study Reference | Cultivar Type / Chemotype | Growth Stage | Growing System | Optimal N (mg L^{-1}) | Optimal P (mg L^{-1}) | Optimal K (mg L^{-1}) | Outcome Measured / Key Finding | Notes |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Saloner & Bernstein, 2021 | Drug-type (Medical) | Flowering | Pots (Soilless) | 160 | - | - | Max Inflorescence Yield & reasonable cannabinoid/terpene conc. Higher N decreased cannabinoids/terpenes. | N range tested: 30-320 |
| Saloner & Bernstein, 2022 | Drug-type (Medical, 2 cultivars) | Flowering | Pots (Soilless) | - | - | 60 | Max yield maintained, highest cannabinoid/terpene conc. at low K (15), decreasing >60. | K range tested: 15-240 |
| Shiponi & Bernstein, 2021 | Drug-type (Medical, 2 cultivars) | Flowering | Pots (Soilless) | - | 30-90 | - | Max yield at 30-90. Highest cannabinoid conc. at <30. | P range tested: 5-90 |
| Bevan et al., 2021 | Drug-type (High THC) | Flowering | DWC Hydroponics | 194 (predicted) | 59 (predicted) | No response (60-340 tested) | Max Inflorescence Yield (RSM prediction) | N range: 70-290; P range: 20-100; K range: 60-340 |
| Hershkowitz et al., 2025 | Drug-type (High CBD, 'T1') | Flowering | DWC Hydroponics | - | ≥15 | - | No yield/cannabinoid increase above 15. Tolerated high EC (4 mS/cm). | P range tested: 15-90 |
| Westmoreland & Bugbee, 2022 | Drug-type (High CBD, 'Trump') | Flowering | Peat/Vermiculite/Rice Hulls | - | ≥25 | - | No yield/cannabinoid increase above 25. Increased P leaching >25. | P range tested: 25-75 |
| Cockson et al., 2020 | Drug-type (High CBD) | Vegetative / Flowering | Modified Hoagland's | - | 11.25 | - | Max yield/cannabinoids at 11.25. No increase above this level. | P range tested: 3.75-30 |
| Caplan et al., 2017a | Drug-type (Medical) | Vegetative | Coir Substrates | 389 (interpolated) | (Implied by NPK ratio: ~126) | (Implied by NPK ratio: ~165) | Max Vegetative Yield & THC conc. | Used organic 4.0N–1.3P–1.7K fertilizer. N range tested: 117-585 |
| Caplan et al., 2017b | Drug-type ('Wappa') | Flowering | Coir Substrates | 212-261 (U2-HP substrate) / 283 (U2 substrate) | (Implied by NPK ratio: ~92-114 / ~123) | (Implied by NPK ratio: ~352-433 / ~469) | Max Yield (higher N) vs. Max Cannabinoid Yield (lower N in range for U2-HP). Dilution effect observed. | Used organic 2.0N–0.87P–3.32K fertilizer. N range tested: 57-283 |
| Zheng et al., 2024 | Drug-type ('The New') | Vegetative | DWC Hydroponics | 160-200 | 30 | 60 | Max desirability & NUE (RSM prediction). Significant NPK interactions observed. | N range: 133-267; P range: 10-111; K range: 21-214 |

*Note: This table synthesizes findings but direct comparison is limited by differing methodologies, cultivars, and conditions.*

## 4. Hypotheses

### 4.1 Reasoning

Based on the synthesis of existing literature and the defined research objectives, specific, testable hypotheses are formulated. These hypotheses translate the research questions into predictable relationships between the independent variables (NPK concentrations) and the dependent variables (growth, yield, quality metrics). They provide a clear framework for the experimental design and statistical analysis, allowing for the formal testing of expected outcomes derived from prior knowledge and identified gaps.

### 4.2 Primary Hypotheses

The following primary hypotheses will be tested regarding the effects of varying N, P, and K concentrations in a hydroponic solution on the selected cannabis cultivar:

* **H1 (Vegetative Growth):** Supplying nitrogen at concentrations within the upper range identified as potentially optimal for vegetative growth (e.g., 160-240 mg L^{-1}) , while maintaining non-limiting P and K levels, will result in significantly greater vegetative biomass accumulation (total dry weight of leaves and stems) compared to treatments receiving lower N concentrations (e.g., <120 mg L^{-1}). This reflects N's primary role in vegetative development.
* **H2 (Flowering Yield):** Inflorescence dry weight yield at harvest will exhibit a quadratic response to both N and P concentrations, being maximized at specific intermediate levels (predicted optima around N: 160-200 mg L^{-1} and P: 40-60 mg L^{-1} based on DWC studies ). Yield will be significantly lower at N and P concentrations substantially below or above these optima. Within a sufficient range (e.g., 60-200 mg L^{-1}), variations in K concentration will not significantly affect inflorescence yield.
* **H3 (Cannabinoid Concentration):** The percentage concentration of the primary acidic cannabinoids (THCA and CBDA) in mature, dried inflorescences will be significantly higher in treatments receiving the lowest non-deficient levels of N and P compared to treatments receiving higher N and P levels known to maximize biomass. This reflects the commonly observed inverse correlation or dilution effect.
* **H4 (Terpene Concentration):** The percentage concentration of key representative monoterpenes (e.g., myrcene, limonene) and sesquiterpenes (e.g., β-caryophyllene) in mature, dried inflorescences will be significantly higher in treatments receiving lower (but non-deficient) levels of N and K compared to treatments receiving higher N and K levels.
* **H5 (Total Cannabinoid/Terpene Yield):** Despite potentially lower concentrations at yield-optimizing nutrient levels (per H3 & H4), the total yield of cannabinoids and terpenes per plant or per unit area (mg/plant or g/m^2) will be maximized under the same intermediate N and P conditions that maximize inflorescence biomass yield (per H2). This is because the increase in flower mass at optimal N/P levels will outweigh the decrease in percentage concentration.

### 4.3 Secondary Hypotheses

Additionally, the following secondary hypotheses related to plant health and nutrient interactions will be tested:

* **H6 (Plant Health Indicators):** The appearance of specific visual nutrient deficiency symptoms (e.g., lower leaf chlorosis for N, interveinal chlorosis for Mg, tip burn/spotting for K/Ca/P) will correlate with significantly reduced concentrations of the corresponding element in foliar tissue analysis compared to control plants. However, the onset of visual symptoms may occur only after tissue concentrations have already fallen below critical levels. Leaf chlorophyll meter readings (SPAD/CCI) will show a strong positive correlation with foliar N concentration.
* **H7 (Nutrient Interactions):** Statistically significant interaction effects between N, P, and K supply levels will be detected for key response variables, including biomass yield and potentially cannabinoid/terpene concentrations. This indicates that the response to changing the level of one nutrient is dependent on the levels of the other nutrients supplied.

These hypotheses are structured to directly test the core questions about optimizing cannabis nutrition, particularly the critical balance between yield and quality. H2 and H5 versus H3 and H4 explicitly frame the yield-versus-concentration trade-off, allowing the research to quantify this relationship. Furthermore, the hypotheses are formulated to facilitate quantitative modeling through regression analysis (Section 7), enabling prediction of optimal nutrient levels beyond simple comparison of discrete treatment means.

## 5. Experimental Design

### 5.1 Reasoning

A robust and meticulously controlled experimental design is paramount to isolate the effects of varying NPK ratios and test the proposed hypotheses accurately. This section details the methodology, emphasizing practices that minimize experimental error and ensure the validity and reproducibility of the results within a CEA context. The design choices prioritize precision in nutrient delivery, environmental control, genetic uniformity, and statistical power.

### 5.2 Growing System and Environment (CEA)

* **System Type:** A Deep Water Culture (DWC) hydroponic system will be employed. This choice allows for maximum control over the root-zone environment, enabling precise manipulation and monitoring of nutrient solution composition (pH, EC, DO, temperature) without the confounding chemical and physical buffering effects of substrates like coco coir or peat. DWC facilitates straightforward nutrient solution sampling, analysis, and complete replacement, ensuring accurate maintenance of target treatment concentrations throughout the experiment. Individual DWC units (e.g., 15-20 L opaque plastic containers to prevent algal growth ) will house each experimental unit (plant or replicate group).
* **Environmental Setting:** The experiment will be conducted within a dedicated, controlled environment growth chamber(s) or a partitioned grow room designed for research purposes. This ensures consistent environmental conditions across all treatments, minimizing variability not related to nutrient supply.
* **Environmental Controls and Monitoring:** Strict control and continuous monitoring of key environmental parameters are essential:
  + *Lighting:* Full-spectrum LED luminaires with adjustable intensity will be used. PPFD will be maintained uniformly across the canopy for all treatments, targeting levels appropriate for each growth stage (e.g., 400-600 µmol·m⁻²·s⁻¹ during vegetative phase, potentially increasing to 800-1000 µmol·m⁻²·s⁻¹ during flowering, especially if CO\_2 is supplemented). Light intensity will be measured regularly at canopy level using a quantum sensor and adjusted as needed. Photoperiod will be strictly maintained at 18 hours light / 6 hours dark for the vegetative stage and 12 hours light / 12 hours dark to induce and maintain flowering.
  + *Temperature:* Air temperature will be controlled to 25±1°C during the light period and 21±1°C during the dark period. Root Zone Temperature (RZT) within the DWC reservoirs will be actively managed using submersible heaters and/or chillers to maintain an optimal range of 20-24°C, critical for root function and nutrient uptake. Both air and RZT will be continuously monitored with calibrated sensors.
  + *Humidity:* Relative humidity (RH) will be controlled using humidifiers/dehumidifiers integrated with the environmental control system, targeting 60-70% RH during the vegetative stage and 40-50% RH during the flowering stage to optimize growth and minimize disease risk. Vapor Pressure Deficit (VPD) will be monitored and maintained within a target range (e.g., 1.0-1.5 kPa) appropriate for the growth stage.
  + *Carbon Dioxide (CO\_2):* CO\_2 levels will be monitored continuously. If resources permit, CO\_2 will be supplemented to maintain a consistent, elevated level (e.g., 800-1000 ppm) across all treatments to maximize photosynthetic potential, as nutrient demand is linked to CO\_2 availability. If supplementation is not feasible, ambient levels (~400-500 ppm) will be maintained and recorded.
  + *Air Circulation:* Internal fans will ensure adequate air movement within the growth chamber/room to prevent stratification of temperature, humidity, and CO\_2, and to promote transpiration.
  + *Dissolved Oxygen (DO):* DO levels in each DWC reservoir will be maintained above a minimum threshold (e.g., >5 mg L^{-1}, ideally near saturation for the given water temperature) using appropriately sized air pumps and air stones to ensure adequate oxygen supply for root respiration and nutrient uptake. DO levels will be monitored regularly. The critical nature of DO in DWC necessitates this active management, as stagnant, warm water quickly becomes hypoxic, impairing root health and nutrient absorption.

### 5.3 Plant Material

* **Cultivar Selection:** A single, commercially relevant and well-documented cannabis cultivar will be selected for the study. The choice will depend on the specific research focus (e.g., high-THC Type I, balanced Type II, or high-CBD Type III) but consistency is key. The cultivar 'T1' (Trump) , 'Wappa' , or 'The New' are examples used in previous nutrient studies.
* **Propagation Method:** The experiment will exclusively use genetically identical vegetative clones derived from a single, healthy, virus-indexed mother plant. This is non-negotiable to eliminate the confounding effects of genetic variation inherent in seed-propagated populations. Clones will be rooted under standardized conditions in an inert medium (e.g., rockwool starter plugs ) using rooting hormone. Only healthy, uniformly sized rooted cuttings (e.g., ~15 cm tall with 4-5 nodes ) will be selected and transplanted into the DWC systems at the start of the experiment.

### 5.4 Treatments and Nutrient Solutions

* **Experimental Design:** A Response Surface Methodology (RSM) approach, specifically a Central Composite Rotatable Design (CCRD), will be utilized to investigate the effects of N, P, and K concentrations and their interactions. RSM is chosen over a standard factorial design because it allows for the modeling of quadratic responses and the efficient estimation of optimal nutrient combinations, even those lying between the tested levels, while requiring fewer treatment combinations for three factors. The design will include axial points and center points to assess curvature and estimate experimental error.
* **Nutrient Concentration Levels:** Based on the literature synthesis (Table 1) and previous RSM studies , five levels for each factor (N, P, K), coded as -α, -1, 0, +1, +α (where α ≈ 1.682 for a rotatable design with 3 factors), will be established. Example concentration ranges (mg L^{-1}) might be:
  + *Vegetative Stage (e.g., 2-3 weeks):* N (133, 160, 200, 240, 267); P (10, 30, 60, 90, 110); K (21, 60, 117.5, 175, 214) - adapted from.
  + *Flowering Stage (e.g., 8-10 weeks):* N (70, 120, 180, 250, 290); P (20, 40, 60, 80, 100); K (60, 120, 200, 280, 340) - adapted from.
  + The exact levels will be finalized based on the chosen cultivar's known characteristics and preliminary range-finding if necessary.
* **Control Group:** The center point of the CCRD (coded level 0 for N, P, and K) will serve as a control or reference point within the design. Its replication provides a robust estimate of experimental error.
* **Nutrient Solution Formulation:** All nutrient solutions will be prepared using high-purity reagent-grade salts dissolved in deionized (DI) or reverse osmosis (RO) water to minimize background contaminants. A base nutrient solution providing all other essential macro- (Ca, Mg, S) and micro-nutrients (Fe, Mn, Zn, Cu, B, Mo) at consistent, sufficient levels (e.g., based on modified Hoagland or established cannabis hydroponic recipes ) will be used for all treatments. The concentrations of N, P, and K will be adjusted according to the experimental design by varying the amounts of specific salts (e.g., Ca(NO\_3)\_2, KNO\_3, KH\_2PO\_4, K\_2SO\_4, etc.), while carefully balancing accompanying ions to maintain consistency where possible. The complete ionic composition of each treatment solution will be calculated and documented.
* **Nutrient Solution Management:** To maintain target nutrient concentrations and prevent significant depletion or imbalance due to differential plant uptake, the entire nutrient solution volume in each DWC unit will be completely replaced on a regular basis, at least weekly. Solution pH will be monitored daily (or continuously with sensors) and adjusted as needed to maintain the target range (e.g., 5.8-6.2) using dilute acids (e.g., phosphoric acid, nitric acid) or bases (e.g., potassium hydroxide) suitable for hydroponics. Solution EC will be monitored daily as an indicator of total dissolved solids. DO levels will be checked regularly (e.g., daily or multiple times per week) to ensure they remain above 5 mg L^{-1}.

### 5.5 Replication and Randomization

* **Experimental Unit:** A single cannabis plant grown in an individual DWC container will constitute one experimental unit.
* **Replication:** Each unique NPK treatment combination defined by the CCRD (including center points) will be replicated a minimum of 4 to 6 times (i.e., 4-6 plants per treatment combination). This level of replication is standard for plant science field/greenhouse trials and provides sufficient statistical power to detect meaningful treatment differences and estimate experimental error accurately.
* **Randomization:** Treatments will be assigned to the experimental units (DWC containers/plant positions) completely at random within the controlled environment growth space. If significant environmental gradients (e.g., light intensity variation from edges to center) are known or suspected within the growth chamber/room, a Randomized Complete Block Design (RCBD) will be implemented. In an RCBD, the experimental area is divided into blocks (representing areas with similar environmental conditions), and each treatment combination is randomly assigned to one unit within each block. This helps to account for spatial variability and reduces experimental error. The physical position of DWC units may be re-randomized periodically (e.g., weekly) if feasible, to further minimize potential location effects.

### 5.6 Duration

* **Acclimation:** Following transplanting, all plants will receive a standard, balanced nutrient solution (e.g., the control/center point solution) for a brief acclimation period (e.g., 3-7 days) before initiating the specific NPK treatments.
* **Vegetative Stage:** The vegetative growth phase under treatment conditions will last for a defined period, typically 2 to 4 weeks, consistent with commercial practices and previous research, allowing sufficient time for vegetative growth differences to manifest.
* **Flowering Stage:** Following the vegetative period, the photoperiod will be switched to 12/12 hours to induce flowering. Plants will remain under their assigned NPK treatments throughout the flowering stage until physiological maturity is reached. Harvest timing will be standardized based on consistent visual indicators, typically trichome coloration (e.g., transition from clear to milky/amber, aiming for ~50% amber) or pistil senescence, usually occurring 8-10 weeks after the photoperiod switch, depending on the cultivar. All plants will be harvested within a narrow time window to minimize developmental variation.

### 5.7 Proposed Table: Experimental Design Parameters

**Table 2:** Experimental Design Parameters for Cannabis NPK Optimization Study.

| Parameter Category | Specific Parameter | Value/Setting | Rationale / Reference |
| --- | --- | --- | --- |
| **Growing System** | System Type | Deep Water Culture (DWC) | Precise nutrient/root zone control |
|  | Container Size | 15-20 L opaque containers | Sufficient volume, prevents algae |
|  | Aeration | Air pump + air stone per container | Maintain DO > 5 mg L^{-1} |
| **Environment (CEA)** | Location | Controlled growth chamber/room | Minimize environmental variability |
|  | Lighting | Full-spectrum LED | Energy efficient, controllable spectrum |
|  | PPFD (Veg) | 400-600 µmol·m⁻²·s⁻¹ | Standard vegetative light level |
|  | PPFD (Flower) | 800-1000 µmol·m⁻²·s⁻¹ | Higher intensity for flowering |
|  | Photoperiod (Veg) | 18 hr light / 6 hr dark | Standard vegetative photoperiod |
|  | Photoperiod (Flower) | 12 hr light / 12 hr dark | Standard flowering induction |
|  | Air Temperature (Day/Night) | 25±1°C / 21±1°C | Optimal range for cannabis growth |
|  | Root Zone Temp (RZT) | 20-24°C (Actively controlled) | Optimal for root function/uptake |
|  | Relative Humidity (Veg) | 60-70% | Appropriate for vegetative stage |
|  | Relative Humidity (Flower) | 40-50% | Lower RH reduces mold risk in flower |
|  | CO2 Level | Monitor ambient or supplement to 800-1000 ppm | Enhance growth potential, document level |
| **Plant Material** | Cultivar | Selected commercial cultivar (Specify name, e.g., 'T1') | Relevance and consistency |
|  | Propagation | Vegetative clones from single mother plant | Ensure genetic uniformity |
|  | Initial Size | Uniform rooted clones (~15 cm, 4-5 nodes) | Minimize starting variability |
| **Treatments** | Design | Response Surface Methodology (RSM) - CCRD | Efficient optimization & interaction analysis |
|  | Factors | N, P, K concentrations (mg L^{-1}) | Primary macronutrients of interest |
|  | Levels (Example - Flower) | N: 70-290; P: 20-100; K: 60-340 (5 levels each) | Cover range from deficiency to excess based on literature |
|  | Base Nutrients | Consistent supply of Ca, Mg, S, micronutrients | Ensure only NPK are limiting/varying |
|  | Solution Management | Weekly replacement, daily pH (5.8-6.2) & EC monitoring | Maintain target concentrations & availability |
| **Design & Duration** | Experimental Unit | Single plant in DWC container | Basic unit receiving treatment |
|  | Replication | Minimum 4-6 biological replicates per treatment | Statistical power |
|  | Randomization | Completely Randomized or RCBD (if gradients exist) | Minimize bias |
|  | Vegetative Phase Duration | 2-4 weeks post-acclimation | Allow treatment effects on veg growth |
|  | Flowering Phase Duration | ~8-10 weeks (until maturity) | Allow full flower development |

## 6. Measurement and Data Collection

### 6.1 Reasoning

Precise, consistent, and comprehensive data collection is fundamental to achieving the research objectives and testing the hypotheses. This section outlines the specific parameters to be measured, the standardized methodologies and instrumentation to be employed, and the frequency of data collection throughout the experiment. Adherence to these protocols ensures data quality, reliability, and suitability for statistical analysis. Combining multiple types of measurements (visual, physiological, biomass, chemical) provides a holistic view of the plant's response to the nutrient treatments.

### 6.2 Growth and Morphological Parameters

* **Parameters:**
  + Plant Height: Measured from the base of the stem (at the DWC lid level) to the apical meristem.
  + Stem Diameter: Measured at a consistent point above the base (e.g., 5 cm above lid) using digital calipers.
  + Number of Nodes/Primary Branches: Counted along the main stem.
  + Canopy Area / Growth Index: Calculated from perpendicular width measurements at the widest point and height (Growth Index = Height × Width1 × Width2) or using overhead imaging and analysis software.
  + Leaf Area (Optional): Measured destructively on representative leaves using a leaf area meter or non-destructively using image analysis at specific time points.
* **Methods:** Standard measuring tools (rulers, calipers). Consistent measurement points and techniques across all plants and time points. Photographic documentation alongside measurements.
* **Frequency:** Weekly measurements during the vegetative stage to capture rapid growth. Bi-weekly or tri-weekly measurements during the flowering stage may suffice as vertical growth slows. Final measurements recorded immediately before destructive harvest.

### 6.3 Biomass Yield

* **Parameters:**
  + Fresh Weight (FW): Recorded for separated plant components (inflorescences, leaves, stems, roots) immediately after harvest.
  + Dry Weight (DW): Recorded for separated plant components after drying to constant weight. Inflorescence DW is the primary yield metric.
  + Harvest Index (HI): Calculated as (Inflorescence DW) / (Total Aboveground DW).
* **Methods:** At the end of the flowering period (determined by maturity indicators), plants will be destructively harvested. The main stem will be cut at the DWC lid level. Roots will be carefully removed, rinsed gently with DI water if necessary, and blotted dry. Aboveground biomass will be separated into stems, fan leaves, and inflorescences. Inflorescences will be trimmed according to standard commercial practice (removal of large sugar leaves) before weighing. Fresh weights of each component will be recorded immediately using an analytical balance. Components will be placed in labeled paper bags and dried in a forced-air oven set at a consistent temperature (e.g., 60-70°C) until constant weight is achieved (verified by repeated weighing, typically 48-72 hours). Dry weights will be recorded. Dried, trimmed inflorescences and representative samples of other tissues will be ground into a homogeneous powder using a sample mill for subsequent chemical analyses.
* **Frequency:** Final destructive harvest at the end of the flowering stage.

### 6.4 Plant Health and Physiology

* **Parameters:**
  + Visual Symptoms: Qualitative assessment of any signs of nutrient deficiency (e.g., chlorosis, necrosis, stunting, leaf deformation) or toxicity (e.g., leaf tip burn, severe necrosis).
  + Leaf Chlorophyll Content: Quantitative, non-destructive measurement using a portable chlorophyll meter (e.g., Konica Minolta SPAD-502 or Apogee MC-100) providing relative indices (SPAD) or estimated concentration (CCI, µmol m^{-2}).
  + *(Optional)* Gas Exchange: Photosynthesis rate (A), stomatal conductance (g\_s), transpiration rate (E) measured using a portable infrared gas analyzer (IRGA) system (e.g., LI-COR LI-6800).
* **Methods:** Visual assessments will be conducted systematically using a standardized rating scale (e.g., 1-5 scale for severity) and detailed notes, focusing on symptom location (upper/lower leaves, new/old growth) and type. High-resolution photographs of representative symptoms and whole plants will be taken weekly. Chlorophyll meter readings will be taken on the mid-leaflet of several (e.g., 3-5) uppermost, recently fully expanded, mature fan leaves per plant, avoiding major veins. The meter will be calibrated and used according to the manufacturer's instructions. Gas exchange measurements (if performed) would follow standard protocols under controlled chamber conditions (light, CO\_2, temperature, humidity).
* **Frequency:** Visual assessment performed weekly throughout the experiment. Chlorophyll meter readings taken weekly or bi-weekly. Gas exchange measurements (if included) might be taken at key stages (e.g., mid-vegetative, mid-flowering).

### 6.5 Chemical Analysis (Quality Parameters)

* **Parameters:**
  + Cannabinoid Profile: Quantitative analysis of the concentration (% dry weight) of major acidic cannabinoids (THCA, CBDA, CBGA) and their corresponding neutral forms (THC, CBD, CBG), as well as potentially other relevant cannabinoids like CBN, CBC, THCV, CBDV. Total THC and Total CBD calculated using standard conversion factors.
  + Terpene Profile: Quantitative analysis of the concentration (% or µg/g dry weight) of a target list of volatile terpenes, including key monoterpenes (e.g., α-pinene, β-pinene, myrcene, limonene, linalool, terpinolene) and sesquiterpenes (e.g., β-caryophyllene, α-humulene) known to be prevalent in cannabis and contribute to aroma and potential entourage effects.
* **Methods:**
  + *Sample Preparation:* Analysis will be performed on representative samples of dried, cured, and homogenized inflorescence material from each experimental unit. A standardized extraction protocol will be followed, typically involving solvent extraction (e.g., methanol, ethanol, or acetonitrile/methanol mixture) often aided by vortexing or sonication. Extracts will be filtered (e.g., 0.2 or 0.45 µm PTFE or nylon filters) prior to injection. Appropriate internal standards (e.g., 4-androstene-3,17-dione for cannabinoids , n-tridecane for terpenes ) will be added during extraction or before analysis for accurate quantification.
  + *Cannabinoid Analysis:* High-Performance Liquid Chromatography (HPLC) coupled with a Photodiode Array (PDA) or UV detector is the preferred method as it allows quantification of both acidic and neutral cannabinoids without thermal degradation (decarboxylation) that occurs in GC. A validated Standard Operating Procedure (SOP), potentially based on regulatory methods or established protocols , will be strictly followed using a suitable reversed-phase column (e.g., C18 ). Calibration curves will be generated using certified reference materials (CRMs) for each target cannabinoid. Quality control measures (blanks, calibration verification standards, matrix spikes if needed) will be included in each analytical batch. Results will be reported on a dry weight basis.
  + *Terpene Analysis:* Gas Chromatography (GC) coupled with either Mass Spectrometry (GC-MS) or Flame Ionization Detection (GC-FID) is the standard technique for analyzing volatile terpenes. GC-MS provides definitive identification based on mass spectra and retention time, making it preferable for comprehensive profiling. Either liquid injection of the solvent extract or headspace sampling (static or dynamic HS-GC-MS) can be used, with headspace often preferred for cleaner analysis and potentially higher sensitivity for volatiles. A suitable capillary column (e.g., DB-5MS or SLB-5ms ) and optimized temperature program will be used for separation. Quantification will be performed using external calibration curves generated from terpene standard mixtures (CRMs) and an internal standard.
* **Frequency:** Chemical analyses will be performed on the final harvested and processed (dried, cured, homogenized) inflorescence samples from each replicate plant.

### 6.6 Nutrient Analysis

* **Parameters:**
  + *Plant Tissue:* Concentration (e.g., % or mg kg^{-1} dry weight) of macro- (N, P, K, Ca, Mg, S) and micro-nutrients (Fe, Mn, Zn, Cu, B, Mo, potentially Si) in dried, ground samples of different tissues (representative leaves, stems, roots, inflorescences).
  + *Nutrient Solution:* pH, EC, DO, and temperature of the hydroponic solution in each DWC unit. Potentially, concentrations of specific ions (N, P, K, Ca, Mg) in the input solution and runoff/leachate using appropriate methods.
* **Methods:**
  + *Tissue Preparation:* Tissue samples collected at specified time points will be dried to constant weight and finely ground into a homogenous powder. Subsamples will undergo acid digestion (e.g., nitric-perchloric acid digestion or microwave digestion) to bring elements into solution for analysis.
  + *Tissue Elemental Analysis:* Concentrations of P, K, Ca, Mg, S, and micronutrients will be determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) or Mass Spectrometry (ICP-MS) for higher sensitivity, especially for micronutrients. Total Nitrogen (N) and potentially Total Carbon (C) will be determined by dry combustion analysis using an elemental analyzer (e.g., LECO CN analyzer). All analyses will follow validated laboratory SOPs and include appropriate standards and quality controls. Plant sap analysis using portable ion-selective meters could be explored for real-time monitoring but requires careful calibration and validation against standard lab methods.
  + *Solution Analysis:* pH will be measured using a calibrated pH meter. EC will be measured using a calibrated EC meter. DO will be measured using a calibrated DO probe/meter. Temperature will be measured using calibrated thermometers or integrated sensors. Analysis of specific ion concentrations in solution samples would typically require laboratory methods like ion chromatography or ICP. Runoff/leachate samples should be collected carefully after irrigation events allow for equilibration within the root zone (if applicable in substrate systems) or directly from DWC systems during solution changes.
* **Frequency:** Nutrient solution parameters (pH, EC, DO, Temp) will be monitored daily to ensure stability and adherence to target ranges. Runoff/leachate analysis (pH, EC) may be conducted weekly or bi-weekly if applicable. Plant tissue nutrient analysis will be conducted on samples collected at key developmental points, such as the end of the vegetative stage, mid-flowering, and at final harvest, to understand nutrient uptake and partitioning dynamics over time.

### 6.7 Data Recording

All collected data, including environmental parameters, plant measurements, visual observations, solution management logs (pH/EC adjustments, solution changes), and analytical results, will be meticulously recorded in standardized digital spreadsheets (e.g., Microsoft Excel, Google Sheets) or a dedicated laboratory information management system (LIMS). Each data point will be associated with the specific date, time, experimental unit ID, treatment, and replicate number. Digital photographs will be labeled and archived systematically. Maintaining accurate, organized, and detailed records is crucial for data integrity, analysis, and reporting.

### 6.8 Proposed Table: Measurement Parameters, Methods, and Frequency

**Table 3:** Measurement Parameters, Methods, and Frequency for Cannabis NPK Optimization Study.

| Parameter Category | Specific Parameter | Method/Instrument | Sample Type/Location | Frequency | Reference(s) |
| --- | --- | --- | --- | --- | --- |
| **Growth & Morphology** | Plant Height | Ruler/Measuring Tape | Apical Meristem | Weekly (Veg), Bi-weekly (Flower), Final Harvest |  |
|  | Stem Diameter | Digital Caliper | Base Stem (consistent height) | Weekly (Veg), Bi-weekly (Flower), Final Harvest |  |
|  | Node/Branch Count | Visual Count | Main Stem | Weekly (Veg), Final Harvest |  |
|  | Growth Index / Canopy Area | Caliper/Imaging + Calculation | Whole Plant | Weekly (Veg), Bi-weekly (Flower), Final Harvest |  |
| **Biomass Yield** | Fresh Weight (FW) | Analytical Balance | Separated Inflorescence, Leaf, Stem, Root | Final Harvest |  |
|  | Dry Weight (DW) | Dried @ 60-70°C to constant weight + Analytical Balance | Separated Inflorescence, Leaf, Stem, Root | Final Harvest |  |
|  | Harvest Index (HI) | Calculation (Flower DW / Total Aboveground DW) | Final DW Data | Final Harvest |  |
| **Plant Health & Physiology** | Visual Symptoms (Deficiency/Toxicity) | Standardized Rating Scale + Photography | Whole Plant, Specific Leaves | Weekly |  |
|  | Chlorophyll Content Index | SPAD Meter (e.g., SPAD-502) or CCI Meter (e.g., Apogee MC-100) | Uppermost Mature Full Expanded Leaf | Weekly or Bi-weekly |  |
| **Cannabinoids** | THCA, CBDA, CBGA, THC, CBD, CBG, etc. (% DW) | HPLC-PDA/UV | Dried, Homogenized Inflorescence | Final Harvest |  |
| **Terpenes** | Myrcene, Limonene, Pinene, Caryophyllene, etc. (% or µg/g DW) | GC-MS (preferred) or GC-FID | Dried, Homogenized Inflorescence (Solvent Extract or Headspace) | Final Harvest |  |
| **Tissue Nutrients** | N, C (%) | Combustion Analyzer (e.g., LECO) | Dried, Ground Tissue (Leaf, Stem, Root, Flower) | Key Stages (End Veg, Mid-Flower, Final Harvest) |  |
|  | P, K, Ca, Mg, S, Micronutrients (mg kg^{-1} DW) | ICP-OES / ICP-MS | Dried, Ground Tissue (Leaf, Stem, Root, Flower) | Key Stages (End Veg, Mid-Flower, Final Harvest) |  |
| **Solution Chemistry** | pH | Calibrated pH Meter | DWC Reservoir | Daily |  |
|  | Electrical Conductivity (EC) | Calibrated EC Meter | DWC Reservoir | Daily |  |
|  | Dissolved Oxygen (DO) | Calibrated DO Meter | DWC Reservoir | Daily or Regular Checks |  |
|  | Temperature (Solution) | Calibrated Thermometer/Sensor | DWC Reservoir | Continuous/Daily |  |
|  | Runoff/Leachate pH, EC (Optional/System Dependent) | pH Meter, EC Meter | Collected Runoff | Weekly or Bi-weekly |  |

## 7. Data Analysis Plan

### 7.1 Reasoning

A predefined data analysis plan is crucial for ensuring objectivity, selecting appropriate statistical tools to address the research hypotheses, and drawing statistically valid conclusions from the collected data. This section outlines the steps for data preparation, the specific statistical methods to be employed, the software tools, and how results will be presented. The chosen methods are standard in agricultural and plant science research and are suitable for the experimental design and data types generated.

### 7.2 Data Preparation

Prior to formal statistical analysis, the raw data collected will undergo rigorous preparation:

1. **Data Entry and Verification:** All data from field logs, instrument outputs, and laboratory analyses will be entered into a structured digital format (e.g., Microsoft Excel, CSV files, or a database). Data entry will be double-checked for accuracy.
2. **Data Cleaning:** The dataset will be screened for potential errors, inconsistencies, or biologically implausible values. Outliers will be identified using standard methods (e.g., box plots, standardized residuals) and investigated. Decisions regarding outlier removal or correction will be documented and justified based on experimental notes or clear evidence of error. Missing data points will be noted and handled appropriately during analysis (e.g., using methods robust to missing data if possible, or excluding units if critical data is missing).
3. **Assumption Checking:** Before applying parametric statistical tests like ANOVA and regression, the assumptions underlying these tests will be checked for key response variables.
   * *Normality:* The distribution of residuals (the difference between observed values and model predictions) will be assessed for normality using graphical methods (e.g., Q-Q plots, histograms) and statistical tests (e.g., Shapiro-Wilk test ).
   * *Homogeneity of Variances (Homoscedasticity):* The assumption that the variance of residuals is equal across different treatment groups will be checked using graphical methods (e.g., residual vs. fitted plots) and statistical tests (e.g., Levene's test or Bartlett's test).
4. **Data Transformation:** If the assumptions of normality or homogeneity of variances are significantly violated, appropriate data transformations (e.g., logarithmic, square root, arcsine for percentage data) may be applied to the response variable to better meet the assumptions. If transformations are unsuccessful, non-parametric statistical methods will be considered as alternatives.

### 7.3 Statistical Methods

The following statistical methods will be employed to analyze the data and test the hypotheses:

1. **Analysis of Variance (ANOVA):** Appropriate ANOVA models will be used to determine the overall statistical significance of the N, P, and K treatments (main effects) and their interactions on the measured response variables (e.g., biomass components, cannabinoid concentrations, terpene concentrations, tissue nutrient levels, chlorophyll readings).
   * For analyzing main effects at specific time points or for single-factor comparisons, one-way ANOVA will be used.
   * For the core analysis of N, P, and K effects and interactions, a multi-way ANOVA corresponding to the experimental design (factorial or RSM model) will be applied. The model will include terms for the main effects of N, P, K, their two-way interactions (N×P, N×K, P×K), and the three-way interaction (N×P×K).
   * If a Randomized Complete Block Design (RCBD) is used, 'Block' will be included as a factor in the ANOVA model to account for spatial variation.
   * The significance level (alpha, α) for determining statistical significance will be set at p ≤ 0.05. The F-statistic and corresponding p-value for each factor and interaction term will be reported.
2. **Mean Separation Tests (Post-Hoc Analysis):** When the ANOVA indicates a statistically significant effect for a main factor or interaction (p ≤ 0.05), post-hoc tests will be conducted to determine which specific treatment level means differ significantly from each other. Tukey's Honestly Significant Difference (HSD) test will be the primary method used for all pairwise comparisons among treatment means. Tukey's HSD is chosen because it controls the family-wise error rate (maintaining the overall α at 0.05 across all comparisons), is suitable for comparing all pairs of means, and is robust to slightly unequal sample sizes if minor data loss occurs. Results will be presented using letter groupings or significance indicators on tables and graphs.
3. **Regression Analysis:** To model the quantitative relationship between the nutrient concentrations (independent variables N, P, K) and the key continuous response variables (e.g., yield, cannabinoid/terpene concentration), linear and non-linear regression analyses will be performed.
   * Polynomial regression (e.g., quadratic models) will be used to test for curvilinear responses and estimate optimal nutrient levels where yield or concentration peaks and then potentially declines (as hypothesized for H2, H5).
   * Linear regression will be used to model potentially linear relationships (e.g., inverse correlation hypothesized for H3, H4).
   * Model fit will be assessed using the coefficient of determination (R^2), residual analysis, and statistical significance of the regression coefficients (p ≤ 0.05). The resulting regression equations can be used to predict responses at intermediate nutrient levels.
4. **Correlation Analysis:** Pearson correlation coefficients (for normally distributed data) or Spearman rank correlation coefficients (for non-normally distributed data or monotonic relationships) will be calculated to quantify the strength and direction of linear or monotonic associations between pairs of variables. This will be used to explore relationships such as: nutrient supply vs. tissue nutrient concentration, yield vs. cannabinoid concentration (testing the dilution effect), SPAD readings vs. foliar N concentration, etc.
5. **Response Surface Methodology (RSM) Analysis:** If the CCRD design is used, specific RSM analysis techniques will be applied to the data. This involves fitting a second-order polynomial model to the response variables as a function of the coded levels of N, P, and K. The analysis will:
   * Determine the statistical significance of linear, quadratic, and interaction terms in the model.
   * Generate predictive equations for each response variable.
   * Identify the specific combination of N, P, and K levels predicted to result in the maximum (or minimum) response for each outcome (e.g., maximizing yield, maximizing THCA concentration).
   * Create contour plots and 3D surface plots to visualize the response surface and the influence of nutrient interactions on the outcomes.

The combination of ANOVA/HSD for identifying significant differences between tested levels and regression/RSM for modeling the dose-response relationship provides a comprehensive analytical approach. Explicitly testing for interactions is vital, as the effect of one nutrient often depends on the levels of others , and ignoring interactions can lead to misleading conclusions. Rigorous checking of statistical assumptions ensures the validity of the chosen parametric tests.

### 7.4 Software

Data analysis will be performed using established statistical software packages known for their capabilities in ANOVA, regression, RSM, and graphical representation. Potential options include R (with relevant packages like agricolae, emmeans, rsm), SAS (Statistical Analysis System), JMP (SAS Institute), or SPSS (IBM Statistics).

### 7.5 Data Presentation

Results will be presented clearly and concisely using a combination of tables and figures in the final report and publications.

* **Tables:** Will include summary statistics (means, standard errors or standard deviations) for each treatment group, results of ANOVA tests (F-values, p-values, degrees of freedom), results of mean separation tests (e.g., letter groupings from Tukey's HSD), correlation matrices, and coefficients from regression models.
* **Figures:** Will include bar charts with error bars to visualize mean comparisons, scatter plots with fitted regression lines to show dose-response relationships, and contour or 3D surface plots generated from RSM analysis to illustrate interactions and optimal regions.

## 8. Expected Outcomes and Implications

### 8.1 Reasoning

Anticipating the potential outcomes of the research and considering their broader implications is essential for understanding the study's potential impact. This section outlines the likely results based on the hypotheses and existing literature, and discusses their significance for both scientific understanding and practical cannabis cultivation.

### 8.2 Anticipated Results

Based on the experimental design and the hypotheses derived from the literature review, the following outcomes are anticipated:

* **Distinct Optima:** The study is expected to confirm that the optimal NPK ratios differ depending on the growth stage (vegetative vs. flowering) and the desired outcome (biomass yield vs. cannabinoid/terpene concentration). Specifically, intermediate N levels (e.g., ~160-200 mg L^{-1}) combined with intermediate P levels (e.g., ~40-60 mg L^{-1}) are expected to maximize inflorescence dry weight yield. The effect of K on yield within the tested sufficient range (e.g., 60-200 mg L^{-1}) is anticipated to be minimal or non-significant, supporting findings from some DWC studies.
* **Yield vs. Concentration Trade-off:** A clear inverse relationship is expected between the supply concentration of N, P, and potentially K, and the percentage concentration of major cannabinoids (THCA, CBDA) and key terpenes in the harvested inflorescences. The highest *concentrations* are likely to be observed at the lowest non-deficient nutrient levels tested.
* **Quantification of Total Metabolite Yield:** Despite lower concentrations at yield-optimizing nutrient levels, the *total yield* of cannabinoids and terpenes per plant or per unit area (mg/plant or g/m^2) is expected to peak under the same intermediate N and P conditions that maximize biomass yield. The greater flower mass produced under these conditions is likely to compensate for the lower percentage concentration. This quantifies the practical trade-off for cultivators.
* **Nutrient Interactions:** Significant interaction effects, particularly N×P, N×K, and potentially N×P×K, are anticipated to influence biomass production and possibly the chemotype profile. This would demonstrate that the optimal level for one nutrient is contingent upon the levels of the others, emphasizing the need for balanced fertilization.
* **Diagnostic Indicators:** Correlations are expected between visual deficiency/toxicity symptoms, non-destructive measurements like SPAD/CCI readings, and laboratory-determined foliar nutrient concentrations. This could help establish practical diagnostic thresholds for the specific cultivar under the tested conditions, although symptom onset might lag behind actual tissue deficiency.

### 8.3 Scientific Implications

The successful execution of this research plan is expected to yield several significant scientific contributions:

* **Resolving Literature Conflicts:** By providing robust, quantitative data on NPK optima for a specific cultivar under precisely controlled DWC conditions, the study will contribute valuable data points to help clarify the conflicting findings prevalent in the current literature, particularly regarding P and K requirements during flowering.
* **Mechanistic Understanding:** The integration of yield data, chemical profiles (cannabinoids, terpenes), and tissue nutrient analysis will provide insights into nutrient uptake, partitioning, and utilization efficiency, shedding light on the physiological mechanisms underlying the observed responses, including the yield-concentration trade-off and potential nutrient interactions. This moves beyond simple input-output correlations.
* **Secondary Metabolism Regulation:** The results will enhance understanding of how macronutrient availability influences secondary metabolite biosynthesis pathways in cannabis. Quantifying the relationship between nutrient stress and metabolite concentration provides empirical data to evaluate hypotheses about stress-induced synthesis versus dilution effects.
* **Foundation for Future Work:** The detailed methodology and findings will serve as a foundation for future research exploring micronutrient roles, environmental interactions, cultivar comparisons, and the effects of biostimulants or microbes.

### 8.4 Practical Applications for Cultivation

The findings from this research are expected to have direct and significant practical applications for commercial cannabis cultivators, particularly those operating in CEA settings:

* **Evidence-Based Fertilizer Recipes:** The primary outcome will be the development of data-driven, stage-specific NPK recommendations tailored for the tested cultivar and DWC system. Cultivators can use this information to formulate or adjust their nutrient solutions to specifically target either maximum biomass yield or maximum cannabinoid/terpene concentration, depending on their market goals. The quantitative data on the yield vs. concentration trade-off will allow for informed decisions.
* **Improved Diagnostics:** Correlated data between visual symptoms, SPAD/CCI readings, and tissue nutrient analysis can lead to more accurate and timely diagnosis of nutrient imbalances in the specific cultivar, enabling faster corrective actions.
* **Resource Optimization and Sustainability:** The identification of precise optimal nutrient levels, particularly the potential finding that current P and K rates may be excessive for yield , offers opportunities to reduce fertilizer inputs. This translates to direct cost savings for cultivators and significantly reduces the environmental impact associated with nutrient manufacturing and runoff pollution, a key benefit of precision CEA.
* **Informed Late-Flower Strategies:** The data on nutrient uptake dynamics towards the end of the flowering cycle and the effects of varying nutrient levels on final quality will provide a scientific basis for evaluating or refining late-flower nutrient manipulation or flushing strategies. This research could challenge common practices lacking scientific support, potentially leading to more efficient protocols.
* **Support for Precision Fertigation:** The detailed understanding of stage-specific nutrient requirements and uptake patterns generated by this study provides essential data for programming and optimizing automated precision fertigation systems in CEA. These systems rely on accurate models of plant needs to adjust nutrient delivery in real-time, maximizing efficiency and consistency.
* **Cultivar-Specific Management Emphasis:** While providing specific data for one cultivar, the results will underscore the broader principle that nutrient management should be tailored to specific genetics. This encourages larger operations to conduct in-house trials or seek specific data for their chosen cultivars, moving away from generic feeding schedules.

## 9. Timeline and Resources

### 9.1 Reasoning

A realistic projection of the project timeline and required resources is essential for effective planning, management, and securing necessary funding. This section outlines a potential schedule for completing the research phases and details the personnel, facilities, equipment, consumables, and estimated budget required for successful execution.

### 9.2 Proposed Timeline

A projected timeline for this comprehensive research project is 24 months. The major phases and estimated durations are as follows (a detailed Gantt chart would accompany a formal proposal):

* **Months 1-3: Setup and Preparation (3 months)**
  + Finalize detailed experimental protocols.
  + Complete comprehensive literature review and synthesis.
  + Acquire and propagate selected cannabis cultivar clones; establish mother plants.
  + Set up and calibrate DWC systems and CEA growth chambers/room.
  + Procure all necessary nutrient salts, chemicals, standards, and lab consumables.
  + Establish data logging and recording systems.
* **Months 4-9: Experimental Run 1 (Vegetative & Flowering) (6 months)**
  + Transplant uniform clones, acclimate.
  + Execute the experiment applying NPK treatments through vegetative and flowering stages (~4 months growth).
  + Conduct regular monitoring and data collection (environmental, growth, physiological).
  + Perform final harvest and sample processing (drying, weighing, grinding).
* **Months 10-12: Experimental Run 2 (Replicate Run) (3 months)**
  + Execute a replicate run of the full experiment to ensure reproducibility and increase statistical power. This run can overlap with sample analysis from Run 1.
  + Regular monitoring, data collection, harvest, and sample processing.
* **Months 10-18: Sample Analysis and Preliminary Data Analysis (9 months overlap)**
  + Submit processed samples (inflorescence, leaf, stem, root) for chemical analysis (HPLC, GC-MS, ICP). This phase runs concurrently with experimental execution due to analytical lab turnaround times.
  + Receive and collate analytical data.
  + Perform initial statistical analysis (ANOVA, regression) on data from Run 1 and Run 2.
  + Prepare preliminary reports and identify key findings or areas needing further investigation.
* **Months 19-21: Potential Extension Study / Final Data Collation (3 months)**
  + Based on preliminary results, potentially conduct a smaller, focused extension study (e.g., refining optima, testing flushing, exploring micronutrients). Alternatively, use this time for final data collation, verification, and addressing any analytical issues.
* **Months 22-24: Final Analysis and Dissemination (3 months)**
  + Complete all statistical analyses, including RSM modeling if applicable.
  + Interpret results and draw conclusions.
  + Write final comprehensive research report.
  + Prepare manuscript(s) for submission to peer-reviewed scientific journals (e.g., *HortScience*, *Frontiers in Plant Science*, *Agronomy Journal*).
  + Prepare presentations for scientific conferences (e.g., ASHS Annual Conference ).

### 9.3 Resource Requirements

* **Personnel:**
  + Principal Investigator (PI): PhD-level Plant Scientist/Horticulturist with expertise in plant nutrition, CEA, and experimental design. (Time commitment: e.g., 25-50% FTE).
  + Research Technician(s): 1-2 full-time equivalents (FTE) with experience in hydroponics, plant care, data collection, sample processing, and basic lab techniques.
  + Analytical Support: Collaboration with or access to analytical chemists experienced in HPLC, GC-MS, and ICP analysis and method validation.
  + Student Involvement: Potential for undergraduate or graduate student participation in data collection and analysis, providing training opportunities.
* **Facilities:**
  + Controlled Environment Growth Chamber(s) / Grow Room(s): Sufficient space to accommodate all experimental units with required environmental controls (lighting, HVAC, humidity, CO2). Must allow for proper randomization and blocking if necessary.
  + Laboratory Space: Bench space for nutrient solution preparation, sample processing (weighing, drying, grinding), and basic measurements (pH, EC, DO).
  + Analytical Laboratory Access: Access to facilities equipped with HPLC-PDA, GC-MS, ICP-OES/MS, elemental analyzer, and associated sample preparation equipment. This may be in-house or through a certified external testing laboratory.
* **Equipment:**
  + *Growing System:* Complete DWC units (containers, lids, net pots), air pumps, air stones, water pumps (for solution changes/mixing), reservoir heaters/chillers.
  + *Environmental Control & Monitoring:* Programmable LED grow lights, HVAC system, humidifier/dehumidifier, CO2 controller/monitor (if supplementing), environmental sensors (temperature, RH, PAR, CO2), RZT probes, DO meters, pH meters, EC meters.
  + *Measurement & Processing:* Calipers, rulers, analytical balance (0.1 mg readability), top-loading balance, drying ovens (forced air), sample grinder/mill, chlorophyll meter (SPAD/CCI), cameras for documentation.
  + *Analytical (Access Required):* HPLC-PDA system with appropriate column, GC-MS system with appropriate column, ICP-OES or ICP-MS system, Combustion elemental analyzer.
  + *General Lab:* Glassware (beakers, flasks), plasticware (tubes, vials), pipettes and tips, filters (syringe, vacuum), wash bottles, DI/RO water source, safety equipment (gloves, glasses, lab coats, fume hood access).
* **Consumables:**
  + Plant Material: Verified cannabis clones of the selected cultivar.
  + Growing Media: Rockwool starter plugs.
  + Nutrients: Reagent-grade salts for preparing all macro- and micro-nutrient stock solutions (e.g., KNO\_3, Ca(NO\_3)\_2, KH\_2PO\_4, MgSO\_4, K\_2SO\_4, chelated micronutrients, etc.).
  + Chemicals: pH up/down solutions (e.g., H\_3PO\_4, KOH), acids for digestion (e.g., trace metal grade HNO\_3), solvents for extraction (HPLC/GC grade methanol, ethanol, acetonitrile, ethyl acetate), calibration standard solutions (pH, EC, DO).
  + Analytical Standards: Certified Reference Materials (CRMs) for all target cannabinoids and terpenes. Multi-element standards for ICP calibration.
  + Gases: High-purity Helium for GC-MS , Argon for ICP , Nitrogen for LC-MS (if used) and general lab use.
  + Labware: Sample bags (paper), storage vials (glass/plastic), centrifuge tubes, pipette tips, syringe filters, HPLC/GC vials and caps.

### 9.4 Budget Outline

The following provides an illustrative budget outline. Actual costs will vary significantly based on location, institutional rates, specific equipment choices, scale, and whether analytical services are performed in-house or outsourced. Costs are estimated for a 2-year project duration.

**Table 4:** Estimated Budget Breakdown for Cannabis NPK Optimization Study (2 Years).

| Budget Category | Item Description | Estimated Cost (Total 2 Years) | Justification/Notes |
| --- | --- | --- | --- |
| **Personnel** | PI Salary & Benefits (e.g., 30% FTE) | $60,000 - $120,000 | Based on institutional rates |
|  | Research Technician(s) (1.5 FTE) | $100,000 - $180,000 | Based on institutional rates |
|  | Student Stipends (Optional) | $10,000 - $20,000 | Support for data collection/processing |
| **Subtotal Personnel** |  | **$170,000 - $320,000** |  |
| **Equipment Access/Rental** | Growth Chamber/Room Usage Fees | $20,000 - $60,000 | Assuming use of existing facility; covers energy, maintenance |
|  | Analytical Instrument Access Fees (HPLC, GC-MS, ICP, Elemental Analyzer) | $15,000 - $50,000 | If using core facilities; based on hourly/sample rates |
| **Subtotal Equipment** |  | **$35,000 - $110,000** |  |
| **Consumables** | Plant Material (Clones) | $2,000 - $5,000 | Initial purchase and mother plant maintenance |
|  | Nutrient Salts & Chemicals | $5,000 - $10,000 | Reagent grade salts, pH adjusters, acids |
|  | Solvents (HPLC/GC Grade) | $4,000 - $8,000 | Methanol, Ethanol, Acetonitrile, etc. |
|  | Analytical Standards (CRMs) | $5,000 - $15,000 | Cannabinoids, Terpenes, ICP standards |
|  | Gases (He, Ar, N2) | $3,000 - $7,000 | For GC-MS, ICP, etc. |
|  | Labware & General Supplies | $5,000 - $10,000 | Vials, filters, tubes, bags, pipettes, etc. |
| **Subtotal Consumables** |  | **$24,000 - $55,000** |  |
| **Analytical Services (If Outsourced)** | Cannabinoid Analysis (HPLC) | $20,000 - $50,000 | Est. 2 runs x ~100 samples/run @ $100/sample |
|  | Terpene Analysis (GC-MS) | $20,000 - $60,000 | Est. 2 runs x ~100 samples/run @ $100-300/sample |
|  | Tissue Nutrient Analysis (ICP) | $10,000 - $30,000 | Est. 2 runs x ~100 samples/run @ $50/sample |
| **Subtotal Analytical Services** | *(Alternative to Equipment Access Fees)* | ***($50,000 - $140,000)*** | *Major cost driver if outsourced* |
| **Travel** | Conference Presentation (e.g., ASHS) | $3,000 - $6,000 | PI and/or Technician/Student travel |
| **Publications** | Journal Page Charges/Open Access Fees | $2,000 - $5,000 | For peer-reviewed publication |
| **Subtotal Dissemination** |  | **$5,000 - $11,000** |  |
| **Direct Costs Subtotal** | *(Assuming In-house Analysis)* | **$234,000 - $496,000** |  |
| **Indirect Costs (Overhead)** | (e.g., 15% - 50% of Modified Total Direct Costs) | $35,100 - $248,000 | Based on institutional/funding agency rates |
| **Total Estimated Cost** | *(Assuming In-house Analysis)* | **$269,100 - $744,000** |  |
| **Contingency** | (~10-15% of Direct Costs) | $23,400 - $74,400 | For unforeseen expenses |
| **GRAND TOTAL ESTIMATED RANGE** | *(Highly Variable)* | **~$292,500 - $818,400** | *Outsourcing analysis could shift costs significantly* |

*Note: This budget is illustrative. Detailed quotes for equipment, consumables, and analytical services are required for accurate budgeting. Personnel costs are highly dependent on institutional salary scales and benefits.* The significant cost drivers are personnel, CEA facility operation/access, and analytical testing. The cost of analytical testing, whether through in-house equipment maintenance/operation or outsourcing fees, represents a substantial portion of the budget and requires careful planning regarding sample numbers and analytical scope.

## 10. Future Directions / Potential Extensions

While this research plan focuses on the foundational effects of NPK ratios, the findings will inevitably open avenues for further investigation. Potential future directions and extensions include:

* **Micronutrient Optimization:** Building on the NPK optima, investigate the specific roles and optimal levels of key micronutrients (e.g., B, Si, Fe, Mn, Zn, Cu) and their interactions with the optimized macronutrient regime, particularly focusing on their influence on secondary metabolite profiles and plant resilience.
* **Biostimulants and Microbial Inoculants:** Explore how the application of plant biostimulants (e.g., humic acids, seaweed extracts, amino acids ) or beneficial microbial inoculants (e.g., PGPR, mycorrhizal fungi ) interacts with nutrient supply to affect nutrient uptake efficiency, secondary metabolism, and overall plant performance under optimized or potentially reduced NPK levels.
* **System and Substrate Comparisons:** Compare the optimized NPK ratios found in DWC with other common CEA systems (e.g., NFT, aeroponics) or substrates (e.g., rockwool, coco coir) to determine if optima are system-dependent.
* **Environmental Interactions:** Investigate the interplay between optimized nutrient ratios and other critical CEA environmental factors, such as varying light spectra/intensities , elevated CO\_2 concentrations , different root zone temperatures , or varying dissolved oxygen levels.
* **Cultivar Diversity:** Replicate key experiments using a wider range of commercially important cannabis cultivars with diverse genetic backgrounds and chemotypes (Type I, II, III) to assess the generality of the findings and develop cultivar-specific recommendations.
* **Late-Flower and Post-Harvest Quality:** Conduct focused studies on late-flower nutrient manipulation (e.g., targeted reduction of specific nutrients like N ) and flushing protocols, extending measurements beyond yield and basic chemotype to include detailed sensory analysis, drying/curing characteristics, and shelf-life assessments of the final product.
* **Gene Expression Analysis:** Correlate nutrient treatments with the expression levels of key genes involved in nutrient transport and assimilation, as well as those in the cannabinoid and terpene biosynthesis pathways (e.g., synthases like THCAS, CBDAS, CBGAS; terpene synthases - TPS genes) to gain deeper mechanistic insights.
* **Nutrient and Water Use Efficiency (NUE/WUE):** Explicitly design experiments to quantify and optimize NUE and WUE under different nutrient regimes and CEA conditions, aiming for more sustainable and cost-effective production practices.

## 11. Conclusions

This research plan outlines a comprehensive, scientifically rigorous approach to address critical knowledge gaps in cannabis nutrient management, specifically focusing on the optimization of NPK ratios in controlled environment agriculture. By employing a precise DWC hydroponic system, utilizing genetically uniform clones, implementing a robust experimental design (RSM), and conducting detailed measurements of growth, yield, plant health, nutrient uptake, and chemical profiles (cannabinoids and terpenes) using validated methods, this study aims to generate reliable and actionable data.

The plan acknowledges the complexity of cannabis nutrition, recognizing the influence of growth stage, cultivar genetics, environmental factors, and the potential trade-offs between maximizing biomass yield and optimizing secondary metabolite concentrations. The objectives and hypotheses are structured to directly investigate these complexities, moving beyond anecdotal practices towards evidence-based recommendations.

The expected outcomes include the determination of stage-specific and potentially outcome-specific optimal NPK ratios for the tested cultivar, a quantitative understanding of nutrient interactions and the yield-versus-quality trade-off, and validated diagnostic indicators for nutrient status. These findings hold significant implications for both fundamental plant science—elucidating nutrient effects on secondary metabolism—and practical cannabis cultivation. The results can inform the development of precision fertigation strategies, improve resource use efficiency (reducing costs and environmental impact), challenge unsubstantiated grower practices like excessive P use or flushing, and ultimately contribute to producing more consistent, high-quality cannabis products for medicinal and recreational markets. The detailed methodology also aims to set a standard for future research in this rapidly evolving field, facilitating better comparison and synthesis of knowledge. Successful execution of this plan will provide valuable insights essential for the sustainable and optimized production of *Cannabis sativa* in modern CEA systems.

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