Physiology, yield, and nutritional contribution of Indian Hemp (*Cannabis sativa* L) grown under different fertilizer types and environment.

A dissertation

submitted

by

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Abstract

The first three Sustainable Development Goals (SDG 1, 2 and 3) set by the United Nations focus on ending poverty, promoting good agriculture to eradicate malnutrition, and ensuring good health and well-being, respectively. Humans have traditionally used *Cannabis sativa* L. for a variety of uses, including medicine and as a raw ingredient for goods with added value including drinks, cakes, cookies, and oil. In 2020, the Constitutional Court of South Africa ruled that laws prohibiting cultivation and consumption of *Cannabis* in private were unconstitutional. In addition, the South African government suggested that the country's *Cannabis* production, including its cultivation, processing, and commercialization, could provide billions in revenue annually, and could lead to about 10 000 to 25 000 employment opportunities in the industry. The legalisation of *Cannabis* has generated interest in *Cannabis* farming, which has associated challenges regarding the production of high-quality plant material because currently there is insufficient scientifically validated cultivation protocols, particularly in respect to the South African climate. Thus, the goals of the study were to find out how different fertilizer types and growing conditions (in the open or under shade netting) affected the yield and biochemical components of *Cannabis sativa* L. in order to help growers produce high-quality *Cannabis sativa* crops for commercialisation. Fresh and freeze-dried plant Cannabis bud samples were used to measure yield and biochemical constituents. Treatment combinations of plant growth under shade netting and the addition of chemical fertilizer resulted in superior Cannabis bud water content (40.2 g) and total phenols (14.7 GAE/100 g DW) compared to other treatments. Therefore, growers should consider the shade netting and chemical fertilizer combination for yield and quality maximisation.

Keywords: Macronutrients. Recommended daily intake. Vitamins. Yield

Kakaretšo

Maikemišetšo a Mokgatlo wa Dinagakopano tša Lefase go Leano la Tlhabollo la go ya go ile (SDG 1, 2 le 3) ke go fediša bodidi, phepompe le go kaonafatša tša maphelo le boiketlo. Go tloga kgale batho ba šomiša *Cannabis sativa L*. (Patše) bjalo ka sehlare, go dira dino, dikhekhe, dikuku le makhura. Ka 2020 Kgorotsheko ya Molaotheo ya Afrika Borwa e file kahlolo ya gore ga go molaong go thibela tšhomišo ya phoraebete ya *cannabis*. Go tlaleletša se, mmušo wa Afrika Borwa o laeditše gore go tšweletša *Cannabis* ka nageng, go akaretšwa go e bjala, tšhomišo le thekišo ya yona go ka tsentšha letseno la dibilione ngwaga ka ngwaga, gomme se se ka hlola mešomo ye e balelwago go 10 000 go fihla go 25 000 ka intastering. Se se hlotše kgahlego ye kgolo go bolemi le tšweletšo ya *Cannabis.* Nyakišišo ye e utullotše gore go hloka mokgwa wo o netefaditšwego wa saense wa go bjala *Cannabis* go amile khwalithi ya dibjalo tšeo di tšweleditšwego ka lebaka la maemo a boso ka Afrika Borwa. Maikemišetšo a nyakišišo ke go sekaseka ka moo mehuta ye e fapanego ya menontšha e ka šomišwago le dikarolwana tša payokhemikhale go *Cannabis sativa* mafelong a go fapana (mo go nago le nete le mo go se nago nete), go thuša balemi go tšweletša dibjalwa tša *Cannabis sativa* tša khwalithi ya godimo tšeo di ka rekišwago. Nyakišišo e šomišitše disampole tše di omišitšwego le tše tala go lekanyetša puno le dikarolwana tša payokhemikhale. Dikutullo tša nyakišišo di laeditše gore mo go šomišitšwego nete le monontšha wa dikhemikhale go hlogile mahlogedi a go ba le monola kudu (40.2g) le difenolo (14.7 GAE/100g DW) ge di bapetšwa le mekgwa ye mengwe. Ka gona, nyakišišo e fa tšhišinyo ya gore balemi ba šomiše nete le monontšha wa dikhemikhale go tšweletša dibjalo tša khwalithi ka bontši.

Mantšu a bohlokwa: Dijophepo. Tšhomišo ye e laeditšwego ya letšatši ka letšatši. Divithamini. Puno.

Nkomiso

Ku hava vusweti, ku herisiwa ka ku pfumaleka ka swakudya, rihanyu lerinene na vuhlayiseki i swin'wana swa swikongomelokulu leswi vekiweke hi Nhlangano wa Matiko hi ku tirhisa Swikongomelo swa wona swa Nhluvukiso wa Nkarhi Woleha (SDG 1, 2 na 3). Vanhu hi ndzhavuko a va tirhisa *Cannabis sativa* L. eka mitirhiso yo hambanahambana, ku katsa tanihi murhi na tanihi xichelana ximbisi eka nhundzu leyi nga na nkoka lowu engetelekeke ku katsa swo nwa, makhekhe, makukisi, na oyili. Hi 2020, Khoto ya Vumbiwa yi bohile leswaku milawu leyi yirisaka ku byala na ku tirhisiwa ka khanabisi exihundleni a yi lwisana na vumbiwa. Hi ku tlhandlekela, mfumo wa Afrika Dzonga wu tlhele wu kumbetela leswaku vuhumelerisi bya khanabisi bya tiko , ku katsa ku byariwa ka yona, ku purosesiwa, na ku xavisiwa swi nga nyika mabiliyoni ya mali leyi nghenaka lembe na lembe, naswona swi nga yisa eka kwolomu ka swivandlanene swa mitirho yo ringana 10 000 ku ya fika eka 25 000 eka vumaki. Leswi swi endlile leswaku ku va na ku tsakela swinene eka ku rima khanabisi, leswi vangeke mitlhontlho eka ku humesa matheriyali ya xiyimo xa le henhla ya swimilana hikuva sweswi ku hava milawu yo byala leyi ringaneleke leyi tiyisisiweke hi sayense, ngopfungopfu ehansi ka tlimeti ya Afrika Dzonga. Hikwalaho, xikongomelo xa dyondzo a ku ri ku lavisisa mbuyelo lowu hlanganisiweke wa tinxaka to hambana ta manyoro, swiphemu swa bayokhemikali swa khanabisi sativa ehansi ka tindhawu to hambanahambana to byala (nete ya ndzhuti na ndhawu leyi pfulekeke), leswaku nxopanxopo wo fananisa wu ta kota ku endliwa ku pfuna vabyari ku humesa swimila swa xiyimo xa le henhla swa Khanabisi sativa hi xikongomelo xo xavisa. Tisampulu ta furexe na leti omisiweke hi ku firiziwa ti tirhisiwile ku pima mbuyelo xikan'we na swiphemu swa bayokhemikali. Nhlanganiso wa maendlelo ya nete wa ndzhuti na manyoro lama nga na tikhemikali wu endlile leswaku ku va na mati ya le henhla ya ximila (40.2g) na nhlayo ya tifenolo (14.7 GAE/100g DW) loko ku fananisiwa na maendlelo man'wana. Hikwalaho, vabyari va fanele va tekela enhlokweni nhlangano wo endla ndzhuti/wo sirhelela na manyoro ya khemikali ku kuma mbuyelo na ku kurisa khwaliti

Maritoyankoka: Macronutrients. Mhumambhumelo wa madyelo ya siku na siku. Tivhitamini. Mbuyelo.

DECLARATION

I, Kgaogelo Ignatius Thobejane, hereby declare that this dissertation, which I am submitting for the Master of Science in Horticulture degree at the University of South Africa, under the heading "Physiology, yield, and nutritional contribution of Indian Hemp (*Cannabis sativa* L) grown under different fertilizer types and environment," is entirely original with no previous work that I have submitted to this or any other university.

I certify that this dissertation is free of any written work—pictures, graphs, data, or other information—that has been submitted by someone else without giving proper credit to the original author. The researcher and the Unisa library did a literature review before registering this project to make sure no previous studies of a comparable nature had been carried out in South Africa or overseas.

I thus certify that all instances in which written language has been utilized have been paraphrased and cited, and all instances in which exact language from a source has been used have been enclosed in quote marks and cited.

I affirm that I have properly cited all sources I have used in the dissertation's reference section and have not just copied and pasted content from the Internet without giving due credit to the original author.

I thus certify that I followed the University of South Africa's Research ethical Policy during my work, obtained ethical approval before starting data collection, and did not act outside the parameters of my approval.

I certify that, prior to the final submission for review, the content of my dissertation or thesis was checked for plagiarism using an electronic tool.

________________________ 05 August 2024

Signature Date

Student number : 39086259

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ABBREVIATIONS

AGB	Above-ground biomass
ARC	Agricultural Research Council
CE	Catechin equivalents
cm	Centimeter
EC	Electrical conductivity
g	Gram
DW	Dry weight
GAE	Garlic acid equivelant
HPLC	High-performance liquid chromatography
$\mu mol/m^2$	Mass per area of leaf surface/micromoles per square meter
$mmol\ m^{-2}\ s^{-1}$	Millimoles per square meter per second

GLOSSARY

Chlorophyll	The pigment chlorophyll is responsible for the distinctive green color of plants and for promoting photosynthesis, which enables plants to make their own food and energy (Maluleke, 2022).
Harvest index	Harvest index is a metric for measuring crop productivity that represents the physiological capacity of crop plants to transform total plant dry matter into product; it is calculated as the product yield divided by the total plant biomass (Sharifi et al. 2009; Davis & Brema, 2017).
Total biomass	Biomass, as defined by Burgel et al. (2020), is the total weight of all plant parts, including above and below plant materials.
Bud water content	One of the most crucial variables in yield calculation is the bud water content, which is primarily determined by subtracting the dry weight from the initial fresh weight (Tuckeldoe et al. 2023).
Flavonoids	According to Lekoba et al. (2024), total flavonoids are naturally occurring chemicals that have antiviral, anti-inflammatory, and antioxidant properties. They are generally found in most plants. Furthermore, they have been described as critical compounds that support the human body's cardio- and neuroprotective functions, both of which are essential for the process of chemical coordination.
Phenols	Maluleke et al. (2021) describe total phenols as families of compounds derived from aromatic amino acids. These materials primarily serve as a barrier against UV rays and a means of defense against potential predators (Tuckeldoe et al. 2023).

Stomatal conductance	Tuckeldoe et al. (2023) defines stomatal conductance as the rate at which carbon dioxide enters a leaf and the amount of water vapor that is released through the stomata.
Macro-nutrients	The nutrients, which are required in greater amounts by both plants and animals, which are significant sources of energy (Maluleke et al. 2024).
Micro-nutrients	These are the minerals that are needed in trace amounts for the steady growth and development of living organisms (Maluleke et al. 2023).

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Ending poverty and eradication of hunger and malnutrition are the first two Sustainable Development Goals set by the United Nations (UN, 2024). Humans have traditionally used *Cannabis sativa* L for a variety of uses, including medicine and as a raw ingredient for goods with added value including drinks, cakes, cookies, and oil. In 2020, the Constitutional Court of South Africa ruled that laws prohibiting cultivation and consumption of *Cannabis* in private were unconstitutional. In addition, the government of South Africa also projected that the country's *Cannabis* production, including its cultivation, processing, and commercialisation, could provide billions of rands in revenue annually, and could provide 10 000 to 25 000 employment opportunities. This has generated interest in *Cannabis* farming in South Africa, which led to the need to produce high-quality plant materials particularly as there are currently insufficient scientifically validated *Cannabis* cultivation protocols optimised according to the country’s climate.

A major problem in *Cannabis* cultivation is finding a way to produce enough plant material while meeting quality standards (De Prato et al. 2022). The potential yield and nutrient quality of many crops, like *Cannabis sativa* L., are directly influenced by a number of factors, including soil fertility and growth environment. The ideal growth, productivity, and quality of *Cannabis* crops are influenced by these elements, either separately or in combination (Maluleke, 2022).

The raw materials and processed value-added products derived from *Cannabis sativa* L have been found to have the following benefits, according to De Prato et al. (2022) and Lyu et al. (2023): (i) stimulating appetite; (ii) lowering stress; (iii) relieving pain and reducing nausea; and (iv) offering a rich source of macro- and micronutrients, which are necessary for human daily health, such as calcium, magnesium, potassium, sodium, iron, and zinc. The South African government's decision to decriminalize *Cannabis sativa* L to facilitate its commercialization could lead to the emergence of small, medium, and large-scale Cannabis growers (Gwala, 2023).

Few studies have been done on the best growing conditions and fertilizer combinations to increase the quantity and quality of *Cannabis sativa* L., despite the fact that most horticultural and medicinal crops thrive in South Africa. Consequently, the goal of the current study was to close this gap in the literature by examining the combined effects of various fertilizers—both organic and inorganic/chemical—on the physiology, growth, yield, and nutritional quality of *Cannabis sativa* L plants grown in a variety of settings, including open spaces and under shade netting.

1.2 Hypothesis

This research study addressed the hypothesis below:

- The growth parameters of *Cannabis sativa* L plants cultivated in various conditions are impacted by different types of fertilizer.
- Yield components of *Cannabis sativa* L are affected by different types of fertilizer and growth environments.
- The biochemical composition of *Cannabis sativa* L. buds is influenced by the type of fertilizer used and the growing conditions.

1.3 Research problem

The decriminalisation of *Cannabis sativa* L and opening of commercialisation opportunities by the South African government has attracted a lot of interest in Cannabis farming from small, medium, and large-scale level (Gwala, 2023). While Lyu et al. (2023) pointed out that intensive farming methods can yield higher quantities of *Cannabis sativa* L, more work needs to be done to strike a balance between yield enhancement and the provision of high-quality plant material that can satisfy medicinal and nutritional needs. Various authors such as Rightford (2020) and Maluleke et al. (2021) suggested that most crops find it easy to adapt and grow under South African climates. However, baseline credible data are lacking as to the optimal combination of factors to maximize *Cannabis sativa* L growth, production, and quality under South African conditions.

The absence of such cultivation data could indirectly have a detrimental impact on economic growth since it may deter prospective farmers from participating in Cannabis production out of concern for possible financial loss, which in turn limits the opportunity to generate employment, particularly in South Africa that has been flagged as one of the countries with a high unemployment of 32.9% (StatsSA, 2024). Concerns about Cannabis cultivation's high costs and preference for corporate growers over start-up farmers were raised by joint research from the Universities of Bristol and Cape Town (The Conversation, 2022). Thus, research that will provide pertinent data is therefore crucial to address these problems in a constructive manner. Such reliable baseline data generated from the current study will assist farmers to gain interest in *Cannabis* farming since they will have a better comprehension of the ideal combination of factors needed to maximize their crop yield and quality. This reliable information will boost their assurance that they will be able to recover their investment. Additionally, this will lead to a rise in employment, which will aid in the accomplishment of the Sustainable Development Goals (SDGs 1 and 2: Zero hunger and poverty, respectively).

1.4 Research gap

According to Maluleke et al. (2024), the UN Sustainable Development Goal (SDG) 1 is to end poverty because poverty is not only becoming more widespread and unfair, endangering the integrity of millions of people, but it also makes inequality worse, which weakens social cohesion and significantly slows down economic growth. South Africa is one of the countries which is currently facing high unemployment, particularly involving young people (Banerjee et al. 2008). According to Mosikari (2013), there could have an adverse effect on social stability, poverty, and economic growth due to the high percentage of unemployment. Additionally, there could be a loss of potential and productivity, which could further exacerbate social instability. Due to the South African government's decision to decriminalise *Cannabis sativa* L and opening of commercialization opportunities, there may be the potential for investment and a high level of interest in small, medium, and large-scale Cannabis farming, which could increase employment opportunities (Gwala, 2023).

Therefore, scientific data acquired from this study will help establish the baseline cultivation procedure for *Cannabis sativa* to maximize production and quality of these plants, which will reassure investors that their investment would provide financial returns and encourage them to employ more farm labourers, and so address poverty reduction in SDG 1, while producing highly nutrient-dense plant material that may, subsequently, address hunger reduction in SDG 2.

1.5 Aim and objectives of the study

1.5.1 Study aim

The aim of the study was to examine the combined effects of various fertilizer types on yield and the biochemical components of *Cannabis sativa* grown in two different growing environments (open-space and shade net), in order to conduct a comparative analysis that will help growers in South Africa produce high-quality *Cannabis sativa* crops with greater potential for commercialization.

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1.5.2 Study objectives

The objectives of the study are:

- **Objective 1:** To evaluate the impact of distinct fertilizer types on the growth and productivity of *Cannabis sativa* L plants grown in various conditions (such as in open spaces or under shade netting), dependent plant variables including water content in plant buds, stomatal conductance, plant height, stem diameter, total biomass, and harvest index were measured and compared across all treatments.
- **Objective 2:** To determine the most efficient combination for growing nutrient-dense *Cannabis sativa* L buds including (i) types of fertilizers (organic or inorganic/chemical) and (ii) growing environments (under shade netting or in the open). Biochemical constituents (vitamin C, vitamin E, total flavonoids, total phenols, macro and micronutrients) were compared across all treatments and their potential role in human nutrition were evaluated by comparing them with recommended daily intake (RDI).

1.6 Reliability and validity

A study's validity is established by the methods and tools used to gather and assess data in order to address relevant research issues. Thus, it is essential that the study employ genuine, credible, and appropriate methodologies and execute a control experiment to improve the accuracy of the results (Tuckeldoe et al., 2023). Reliability, according to Creswell (2014), is the accuracy and consistency of the tools and techniques used to measure the variables and parameters in a scientific investigation. In order to measure and generate reliable data on the biochemical constituents, growth, and yield of *Cannabis sativa* L plants grown on various types of fertilizers (both inorganic and inorganic/chemical) and environments (open field and shade-netting), this study used (triplicate samples) for analysis, randomised block designs, and appropriate statistical techniques.

1.7 Bias

Mouton (2013) argued that it is essential to employ useful strategies that reduce biases when performing experimental research. Creswell (2014) defines a bias as a mistake made during the planning or execution of an experimental investigation that causes a distortion in one direction due to non-random circumstances. By increasing replications and implementing randomization, bias and experimental error were minimized in this study.

1.8 Significance of the study

For many years, humans have utilized *Cannabis sativa* L for various purposes, namely; (i) medical purposes such as treatment of pain, asthma, sleeplessness, depression and appetite loss, (ii) nutritionally, it can be utilised as a fresh material for the creation of value-added products including cakes, cookies, beverages, and oil. Since the decriminalization of *Cannabis sativa* L by the South African government and opening up of opportunities surrounding *Cannabis* cultivation and associated product commercialization, there could be interest in small, medium, and large-scale *Cannabis* farming as well as potential investment, which could lead to an increase in employment opportunities (Gwala, 2023). Hence, the scientific information gathered from this study (chlorophyll content, stomatal conductance, plant height, total biomass, water content, harvest index, total flavonoids, total phenols, vitamin C, vitamin E, macro and micro-nutrients) will aid in the establishment of the reliable baseline protocol for *Cannabis sativa* in order to maximize production and quality. By producing highly nutrient-dense plant material, this will help to achieve UN Sustainable Development Goals, namely (poverty and hunger eradication) and reassure investors that their investments will provide financial returns.

1.9 Overview of a dissertation

The dissertation format outlines the procedures and study process, and it presents the research findings in an organised and thorough manner. Below are the chapter headings and a summary of each of the contents:

Chapter 1: Introduction

The purpose, objectives, and importance of the study are covered in this chapter, after a brief review of the history of *Cannabis sativa* L plant production under various conditions.

Chapter 2: Review of the literature

Comprehensive background information on the effects of various factors on the growth, yield, development, and biochemical composition of the *Cannabis sativa* L crop is provided by the literature review. Additionally, it draws attention to the variables that have an impact on the growth, development, yield, and biochemical makeup of the crop both directly and indirectly. Furthermore, it underscores the need for additional research on the impacts of fertilizers and growing conditions on agricultural crops quality. In summary, this chapter closes with highlighting gaps in the literature and offering recommendations for more research.

Chapter 3: Impact of various fertiliser types on *Cannabis sativa* L (Indian hemp) growth and yield in differing growing conditions

The yield and growth of *Cannabis sativa* L. grown in a range of growing settings were examined in this experimental chapter in relation to varying forms of fertilisers. Statistics were used to analyse the gathered data and factual conclusion were reached.

Chapter 4: Biochemical constituents of *Cannabis sativa* L (Indian Hemp) grown under different fertiliser types and environment

The macro- and micronutrients of *Cannabis sativa* buds grown in different conditions, as well as the biochemical components, including vitamin C, vitamin E, total flavonoids, total phenols, and others, are examined in this chapter, in relation to different types of fertilizers. Statistical analysis of the gathered data was employed to reach factual conclusion.

Chapter 5: Overall findings, suggestions, and further research

This final chapter of the dissertation summarizes the main research findings and the key conclusions from this study. Additionally, it suggests the best combination of factors, including growing conditions and fertilizer types, to help establish baseline protocols for the production of *Cannabis sativa* L. plants that meet daily dietary requirements while producing a higher yield and quality. In addition, this chapter includes suggestions for additional research.

1.10 References

Banerjee, A., Galiani, S., Levinsohn, J., McLaren, Z., & Woolard, I. (2008). Why has unemployment risen in the new South Africa? 1. Economics of Transition, 16(4), 715-740.

Creswell, J. W. (2014). Research design: Qualitative, quantitative, and mixed methods approaches (4th ed.). Thousand Oaks, California: Sage Publications.

Davis, J., & Brema, J. (2017). Increasing the plant metabolism in polyhouse farming using colour filters. International Journal of Innovative Research and Advanced Studies, 4, 2394-2404.

De Prato, L., Ansari, O., Hardy, G. E. S. J., Howieson, J., O'Hara, G., & Ruthrof, K. X. (2022). Morpho-physiology and cannabinoid concentrations of hemp (*Cannabis sativa* L.) are affected by potassium fertilisers and microbes under tropical conditions. Industrial Crops and Products, 182, 1-13.

Gwala, R. S. (2023). The legalisation of Cannabis in South Africa: Proposing an economic value chain model for South Africa. In Rapid Innovation and Development in the Global *Cannabis* Market, pp. 98-122. IGI Global.

Maluleke, M. K., Moja, S. J., Nyathi, M., & Modise, D. M. (2021). Nutrient concentration of African horned cucumber (*Cucumis metuliferus* L) fruit under different soil types, environments, and varying irrigation water levels. Horticulturae, 7(4),1-16.

Maluleke, M. K. (2022). Metabolite profile of African horned cucumber (*Cucumis metuliferus* E. May. Ex Naudin) fruit grown under differing environmental conditions. Scientific Reports, 12(1), 1-18.

Maluleke, M. K., Boitumelo, L., & Marokane-Radebe, C. K. (2024). Exploring the biochemical constituents, medicinal properties and potential commercialization of *Ximenia caffra* Sond. for enhancing human health: A comprehensive review. Discover Applied Sciences, 6(1), 1-13.

Mosikari, T. J. (2013). The effect of unemployment rate on gross domestic product: Case of South Africa. Mediterranean Journal of Social Sciences, 4(6), 429-434.

Mouton, J. (2001). How to succeed in your masters' and doctoral studies. Pretoria: Van Schaik.

Tuckeldoe, R. B., Maluleke, M. K., & Adriaanse, P. J. S. R. (2023). The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (*Capsicum annuum*) varieties. Scientific Reports, 13(1), 1-13.

Lyu, D., Berrué, F., Martinez-Farina, C., Huii, J. P., & Smith, D. L. (2023). Plant growth-promoting rhizobacteria (PGPR) with microbial growth broth improve biomass and secondary metabolite accumulation of *Cannabis sativa* L. Journal of Agricultural and Food Chemistry, 71(19), 7268–7277.

Rightford, B. W. B. (2020). Exploring hemp farming as a sustainable agriculture in South Africa, using a social-ecological systems approach (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Sharifi, R. S., Sedghi, M., & Gholipouri, A. (2009). Effect of population density on yield and yield attributes of maize hybrids. Research Journal of Biological Sciences, 4, 375-379.

StatsSA (2024). SA unemployment rate increases. Available at: https://www.sanews.gov.za/south-africa/sa-unemployment-rate-increases#:~:text=According%20to%20Stats%20SA%2C%20the,2023%20(Q4%3A%202023).

The Conversation. (2022). *Cannabis* policy changes in Africa are welcome. But small producers are the losers. Available at: https://theconversation.com/cannabis-policy-changes-in-africa-are-welcome-but-small-producers-are-the-losers-179681.

United Nations (UN). 2024. Progress towards the Sustainable Development Goals. Report of the Secretary- General. Available at: https://hlpf.un.org/sites/default/files/2024-05/SG%20SDG%20Progress%20Report%202024.pdf.

CHAPTER 2: LITERATURE REVIEW

This literature review's main objective was to look at earlier research on how different fertilizers and growing conditions affected crop growth, yield, and the biochemical makeup of *Cannabis sativa* plants. In addition, it evaluated several approaches and indicators for estimating the nutritional benefit of *Cannabis sativa* and its potential role in human nutrition.

One of the primary goals of the Sustainable Development Goals (SDGs) adopted by the United Nations (UN) is to end poverty and ensure that all people have year-round access to safe medication and food, according to Maluleke et al. (2024). A lot of interest in small to medium, as well as large-scale Cannabis farming may result from the South African government decision to decriminalize *Cannabis sativa* L and to open up its commercialisation prospects (Gwala, 2023).

The move could put producers under a lot of strain, which could lead to higher quantity but lower quality production. However, growers may be able to establish cultivation settings that will help them produce sufficient plant material of a higher caliber by looking into factors such the types of fertilisers to use and the ideal growing environment. The findings from the current investigation described in this dissertation will describe suitable cultivation conditions that will help growers to produce high-quality plant material and which will help attract investments to the *Cannabis* industry. Furthermore, the availability of trustworthy data from the study might boost confidence in the production of *Cannabis sativa* crops, which could lead to the creation of more agricultural jobs and the eventual eradication of poverty, especially in South Africa and other parts, globally. The gap in the literature, especially regarding the production of *Cannabis sativa* L under South African climatic conditions, will be addressed by the investigation of different fertiliser types under varying growing environments which, in turn, assist in yield increment. In addition, a chemical analysis of plant compounds will contribute to our biochemical knowledge of compounds from *Cannabis sativa* plants that have both food and medicinal application.

2.1 *Cannabis sativa L* as a research crop

Cannabis sativa, as depicted in Figure 2.1*,* the almanac herbaceous flowering crop in the Cannabaceae (Kornpointner et al. 2021). The plant is native to Central Asia, but because of its resilience to a variety of environmental circumstances, it has spread around the globe (Hourfane et al. 2023). According to Kepe (2003) and Hourfane et al. (2023), it is an annual herb that grows to a height of four meters and has hollow, ribbed internodes on its green, hairy stems. There are three to eleven sessile leaflets with serrated edges on the compound palmately and alternately arranged leaves of the plant, which grows up to up to 50 mm (Rupasinghe et al. 2020). The seeds have netted veins, with smooth texture, which is approximately 4 mm in diameter (Morello et al., 2022). The crop has been cultivated for food, industrial fiber, medicine, and seed oil throughout recorded history (Vanhove et al. 2011; Rupasinghe et al. 2020). The crop is utilised worldwide for variety of purpose, including recreational drug, religious and spiritual purposes, according to Xu et al. (2022).

Figure 2.1: *Cannabis sativa* L.

2.2 Various uses of *Cannabis sativa* L

Fresh raw industrial hemp seeds are an important food source that can be juiced or added to other foods because of its high content of essential amino acids, which is available in a digestible form and provide immediate energy (Farinon et al. 2020). It has also been reported to play a pivotal role in enhancing learning capacities, while improving emotional stability and cognitive functioning (Farinon et al. 2020; Vastolo et al. 2021). Additionally, Krüger et al. (2022) have reported on a number of innovative delicacies that comprise plant parts of *Cannabis sativa*.

2.3 *Cannabis sativa* L. cultivation

As reported by Żuk-Gołaszewska et al. (2018), *Cannabis* seeds germinate quickly, and the success of germination depends on the surrounding conditions. In South Africa, the crops thrive at temperatures between 20°C and 30°C from spring through summer (Potter, 2013). According to Rehman et al. (2021), the successful growth of Cannabis plants should start with the careful selection of agronomic elements including appropriate soil type and fertilisers. The crop will likely adapt to most soil types, although Amaducci et al. (2008) and Papastylianou et al. (2018) contend that the climate and the types of fertilizers applied will ultimately determine the crop's growth and yield. The present study investigated the effects of several types of fertilizers on the growth, physiological performance, yield, and quality of *Cannabis sativa* grown in various settings (e.g., in open spaces or beneath shade netting).

2.4 Impact of fertilisers on the growth, physiology and yield of *Cannabis sativa* L in different environments

Fertilisers, whether chemical or organic, are substances that include ingredients known to enhance plant growth and productivity (De Prato et al. 2022). Scholars such as Backer et al. (2019), stated that fertilizers provide plants with essential nutrients in the form of soluble salt compounds, which have a direct effect on plant development, physiology, and yield. Crop growers should choose the type of fertilisers that are in line with their objectives. For example, authors such as Caplan et al. (2017) proposed that improving soil health and sustainable agriculture requires the use of organic fertilisers. They added that employing organic fertiliser has several benefits, including being widely available and reasonably priced. These benefits could be crucial in boosting crop yields without endangering the soil, while maximising profits. Yet, according to authors like Papastylianou et al. (2018), the usage of chemical fertilizers has increased food yield, which has eliminated hunger. Chemical fertilisers also provide a quick solution to many agronomical problems, as they dissolve rapidly and become available to plant in a very short period, resulting in yield maximisation (Massuela et al. 2023).

Good selection of the growing environment is essential for optimal growth and development of plants, including *C. sativa* L (Bevan et al., 2021). As a result, cultivating the crop in an ideal environment may increase production, but growing the crop in an unfavourable environment may reduce crop growth and hence negatively impact overall yield (Łochyńskaa & Frankowski, 2021). On the other hand, a more comprehensive perspective holds that the growing environment cannot determine the growth and output of crops alone. In order to boost agricultural yield, Campiglia et al. (2017), for instance, proposed that choosing the ideal growing environment and using the right fertilizer should greatly accelerate plant growth.

Researchers Bevan et al. (2021) and Łochyńskaa & Frankowski (2021) appear to have contrasting views on the use of a suitable fertiliser type to enhance the growth, physiology, and yield of *Cannabis sativa*. In addition, there seems to be limited information on the most suitable fertiliser type to improve their growth, physiology, and yield in South Africa.

Since the government has legalised the growing of *Cannabis sativa*, for commercial purpose, potential large-to-medium scale farmers would need reliable scientific data as a benchmark in maximising crop yield and growth. As a result, the current study closes a gap in the literature by exposing the *Cannabis sativa* L plants to different types of fertilizer (organic and chemical/inorganic) and growing conditions (in the open or under shade netting). The goal was to compare the growth and yield parameters and identify the best combination (fertiliser and growing environment) for maximum commercial production, especially in South Africa.

2.4.1 Chlorophyll content of *Cannabis sativa* L plants

Green pigments called chlorophyll enable plants to absorb light and use it to produce their own energy source through the process of photosynthesis (Maluleke et al. 2022). Light energy is gathered by chloroplasts and then transferred to two distinct kinds of molecules that store energy. Using its stored energy, the plant converts carbon dioxide from the air and water into glucose, which is what it needs to survive (Tuckeldoe et al., 2023). Additionally, according to Olle et al. (2012), minerals including iron and magnesium are necessary to produce chlorophyll in plants and a deficiency in these elements may lead to chlorotic patches, which normally cause yellowing in plant leaves (Maluleke et al. 2021). Using an integrated plant nutrition system, Da Cunha Leme Filho et al. (2020) examined the physiological responses of *Cannabis sativa*. Authors reported that the amount of chlorophyll in the plants varied, and that the plants treated with chemical fertilizers had a higher chlorophyll content than the ones treated with organic fertilizers or with no fertilizer at all.

Campiglia et al. (2017) evaluated the impact of fertilizer on *Cannabis sativa* physiological performance in an open field scenario. These researchers observed that the plants' chlorophyll level increased, whether fertiliser was applied, which is an interesting conclusion from the study. Even though plants treated with fertiliser depicted superior chlorophyll concentration compared to plants not treated with any fertiliser still showed a reasonable chlorophyll level, indicating that temperature could have had some influence on plant chlorophyll content.

There is little information available on how these plants respond to these factors in terms of chlorophyll content as, according to the literature review, most studies on the chlorophyll content of *Cannabis sativa* did not evaluate the combined effect of fertiliser types (organic and inorganic) under varying growing environments (under shade netting or in the open space). This research addressed a gap in the literature by performing a comparative analysis to determine the ideal combination of growing environment and fertiliser type for commercially cultivating *Cannabis sativa* in South Africa.

2.4.2 Stomatal conductance of *Cannabis sativa* L plants

The diffusion of gases through the plant stomatal opening, including carbon dioxide, water vapor, and oxygen, is known as stomatal conductance (Evans & von Caemmerer, 1996). It also serves as a gauge for stomatal opening in reaction to external factors, which directly influence a plant's physiological functioning and, in turn, effect the plant's growth, yield, and quality (De Prato et al. 2022). The stomatal opening of a crop is strongly correlated with its nutrient supply and the climate in which it grows, therefore factors like the types of fertilisers used and the growing environment are critical to the crop's physiological performance (Tang et al. 2018).

Sheldon et al. (2021) reported that *Cannabis sativa* L. plants were grown in a controlled environment or in an open field and subjected to physiological performance screening. The authors observed differences in the stomatal conductance of the crops, with crops grown in the controlled environments exhibiting higher stomatal conductance than crops grown in the open space. These authors proposed that the adaptability by the plants to the environment was unstable because of the continuous temperature changes brought on by the growing environment. This resulted in a variance in stomatal opening, which was determined by temperature, and may affect the net photosynthesis, subsequently affecting the overall yield of the crop.

Regarding the impact of fertilisers on crop stomatal conductance, Yep and Zheng (2021) assessed the efficacy of applying a potassium- and micronutrient-rich fertiliser to *Cannabis sativa* plants that were cultivated aquaponically. According to the scientists, plants treated with fertilisers deficient in potassium and micronutrients displayed a decrease in stomatal conductance, whereas crops fed with fertilisers rich in potassium and micronutrients shown an increase in stomatal conductance.

Few studies appear to have examined the combined effects of different types of fertilisers (organic as well as inorganic) and open spaces or under shade netting on *Cannabis sativa* L. stomatal conductance. In order to close this gap in the literature, the current study examined the effects of several fertilizer types on the stomatal conductance of *Cannabis sativa* L cultivated in a variety of conditions (shade-netting and open field setting). This allowed for a comparative analysis to be conducted to identify the best combination of fertilizer or environmental growth conditions for possible mass production under the climate of South Africa.

2.4.3 Height of *Cannabis sativa* L plant

An important morphological and developmental feature that directly reflects a plant's overall growth is plant height, which is widely used to evaluate a crop's performance in a particular environment (Wielgusz et al., 2022). Plant morphology, particularly plant height, is known to be influenced by mineral components such as phosphorus (Da Cunha Filho et al. 2020). On the other hand, plants cultivated in conditions with strong sunshine exhibit greater heights than plants grown in lower light levels, according to Maluleke (2022).

Research on the effects of nitrogen fertilizer and plant density on the agronomic performance of *Cannabis sativa* in the Mediterranean environment was conducted by Campiglia et al. (2017). Plant height was shown to be significantly reduced in greater density plants treated with nitrogen fertiliser, but plant height was found to be increased in combinations of lower density and nitrogen fertiliser. Conversely, Vera et al. (2004) investigated how nitrogen- and phosphorus-rich fertilisers affected *Cannabis sativa* L. growth, particularly plant height. Nitrogen-rich fertilisers markedly boosted the height of *Cannabis sativa* L plants, but more phosphorus-rich fertilisers resulted in shorter plants relative to other treatments.

The combined effects of various fertilisers (organic and inorganic) on the plant height of *Cannabis sativa* L grown in various environments (e.g., in open spaces or shade netting) have not been extensively studied. Therefore, the current study filled the gap in the literature by evaluating the cumulative influence of the parameters on the plant height of *Cannabis sativa* L. by comparing the data obtained across all treatments.

2.4.4 The impact of various fertiliser types and growing environment on *C. sativa* L. stem diameter

Water, nutrients, and sugars are carried up through the stem from the roots to the entire plant canopy (McElrone et al. 2013). Carrying water from the roots to the leaves is one of its primary purposes, since it allows the plant to use the nutrients it has ingested for growth, development, and overall quality (Deng et al. 2021). The response of various varieties of pepper plants to coconut coir substrate in terms of stem diameter was investigated by Tuckeldoe et al. (2023). The plants' stem diameters varied, as the authors discovered. The role of genotype on plant stem growth, especially stem diameter was also emphasized by these authors, particularly the plant development and productivity.

Less research appears to have been done on the combined effects of various fertilizer types (both organic and inorganic) on the stem diameter of *Cannabis sativa* L plants cultivated in various growing environments. The current study addressed a gap in the literature and optimized the combinations, for future mass production of *Cannabis sativa* L under South African climate. This was achieved by analysing the stem diameter of plants subjected to different types of fertiliser and growing environment.

2.5 Yield component of *Cannabis sativa* L

For a farmer, the quantity of plant material gathered per unit of harvested produce, is of utmost importance (Fischer, 2015). Crop yield statistics are typically calculated by dividing production data by harvested area data (Demura and Ye, 2010). The three most important factors to examine for assessing agricultural yield are water content, harvest index, and total biomass, according to Wang et al. (2020) and Rahil and Qanadillo (2015).

2.5.1 Biomass of *Cannabis sativa* L plants

As stated by Burgel et al. (2020), biomass is the total weight of all plant parts, including roots and above-ground parts like leaves, stems, and fruits. The process of photosynthetic energy conversion into vegetative tissues and increased plant biomass production is influenced by solar energy, carbon dioxide, soil type, and water (Tang et al. 2018). However, after cultivating *Cannabis sativa* L at different temperatures and with different types of fertilisers, Kakabouk et al. (2021) found differences in biomass productivity.

Deng et al. (2019) also studied the effect of fertilizers on the biomass of *Cannabis sativa* L. Their findings indicated that plants treated with chemical fertilizers generated more biomass than plants grown under control conditions and with organic fertilizers. The authors also observed that balanced fertilizers with high potassium and phosphorus levels seemed to be more successful in raising crop biomass when compared to nitrogen-rich fertilizers.

Nonetheless, it appears that there is little research on how different fertilizer kinds impact the total biomass of *Cannabis sativa* L cultivated in various conditions. The current study filled a vacuum in the literature by measuring the total biomass of the *Cannabis sativa* L crop grown with various types of fertilisers in different cultivation environments. This allowed researchers to determine the optimal combination for potential mass crop production, particularly given South Africa's climate.

2.5.2 Water content of *Cannabis sativa* L plant buds

According to Tuckeldoe et al. (2023), a crucial element in evaluating a crop's yield is its water content, which is mostly measured by subtracting the dry weight from the initial fresh weight. In the food industry, moisture content is classified as a global metric that is primarily used to determine the quality, shelf life in storage, and processability of raw materials, intermediate products, and finished goods (Mokoena et al. 2011). Tuckeldoe et al. (2023), looked at the water content of fruits harvested from various pepper varieties grown on coconut coir. Based on the study results, the variation in water content was shown to be caused by the genotype of the crop rather than any other treatment.

An investigation by Kakabouki et al. (2021) evaluated the impact of using urea and growth inhibitors to improve the yield of *Cannabis sativa* L, taking into account the water content of the plant buds. In the buds of *Cannabis sativa* L, the authors observed a considerable variance in water content so that when urea fertiliser and growth inhibitors were applied to plants, the plants' bud water content was higher than those of other treatments.

There seem to be scanty literature, combined effect of both (organic and inorganic) and growing environment on the water content of *Cannabis sativa* L crops. By measuring the water content of *Cannabis sativa* L buds in plants grown in differing environment and subjected to different types of fertilisers, the current study closed the gap in the literature by determining the optimal combinations,especially witin the South African climate.

2.5.3 Harvest index of *Cannabis sativa* L plants

The harvest index (HI) measures the ratio of plant dry biomass to total bud dry matter biomass and is a valid metric for assessing how well bud development has occurred in a given environmental setting (Yang et al., 2021). The interplay of agronomic variables, such as fertiliser and growing conditions, determines the harvest index (Caplan et al. 2017).

Kakabouki et al. (2021) assessed the combined impact of growth inhibitors and fertilisers on the harvest index of *Cannabis sativa* L. In addition to a variation in the growth response of plants exposed to different fertilisers and growth inhibitors, plants subjected to urea fertilisers appeared to show an improved harvest index when compared to plants produced under other treatments. Leleh et al. (2021) also reported varying results when comparing the yield response of *Cannabis sativa* L cultivated under inorganic and organic fertilisers. In this study, *Cannabis sativa* L treated with organic fertilisers (manure) showed a greater harvest index, whereas *Cannabis sativa* L plants treated to inorganic fertilisers showed higher compacted buds but a lower harvest index.

Research on how different cultivating environments and types of fertilizer impact the harvest index of *Cannabis sativa* L appears to be lacking. To determine the crop's harvest index, the current study looked at data across all treatments. This was one in order to provide baseline data, particularly for the South African agriculture sector, that could help farmers maximize their profitability.

2.6 Biochemical constituents of *Cannabis sativa* L

Although there are many reasons why people eat various plant foods and their value-added products, Lekoba et al. (2024) pointed out that the main traditional motivation for consumption is the potential supply of energy, minerals, and medicine. Since plants like *Cannabis sativa* L. provide humans with nutrients like flavonoids, phenols, vitamins, and macro- and micronutrients, it is critical to assess the crop's biochemical makeup to ascertain its potential contribution to meeting the daily intake recommendations and eradicating issues like malnutrition. This will help to address SDG 2, which aims to achieve food security, improved nutrition, and the promotion of sustainable agriculture (UN, 2024).

Scholars such as Bernstein et al. (2019); Da Cunha Leme Filho et al. (2020) and De Prato et al. 2022) have examined the impact of various fertilisers on the quality of *Cannabis sativa* L. However, little is understood about how various fertiliser types interrelate with the growing environment and their influence on the biochemical elements like vitamins, flavanoids, phenols, and macro- and micronutrients, particularly in light of the climate in South Africa. In the current study, the biochemical components of *Cannabis sativa* buds grown under different types of fertilizer and growing settings were evaluated in order to determine the optimal combination that can assist address the quality issues linked to *Cannabis sativa* L buds.

2.6.1 Total flavanoids

Dias et al. (2021), defines total flavonoids as compounds that are found naturally throughout the plant and are responsible for many qualities such as color, flavor, and aroma. They are also known to regulate cell growth and provide protection against stress. The majority of plants usually contain these chemicals (Lekoba et al. 2024). Low molecular weight polyphenols called flavonoids have a wide range of therapeutic properties, such as anti-inflammatory, anti-cancer, antiviral, and antioxidant effects. They also protect the human body's cardiovascular and neurological systems and help prevent diseases like diabetes and asthma (Tungmunnithum et al. 2018; Karak, 2019; Dias et al. 2021).

As to the findings of Fenech et al. (2019), plants like *Cannabis sativa* L are essential components of the daily diet of humans, since they contain total flavonoids. Due to its nutritional advantages, *Cannabis sativa* L is more likely to be included in the diets of people whose lives depend on making sensible food choices (Maluleke et al. 2021).

Research has assessed the effect that different fertilisers have on different types of crops, including leafy vegetables. For instance, Chibueze and Akubugwo (2011) evaluated the effect of varying fertilers on the nutritional and phytochemical contents of African water leaf crops. These authors found that the flavanoids content of the crop did not vary based on the type of fertiliser applied, but rather that this varied based on the application rate. They suggested that fertiliser types combined with the growing environment should be given more attention by growers if they hope to maximise the quality of their crop.

Research on the combined effects of several types of fertilizers (both organic and inorganic) on the flavanoids content of *Cannabis sativa* L grown in different conditions (under shade netting or in open spaces) seem to be scanty. The present study filled a vacuum in the literature on the flavanoid content of *Cannabis sativa* L by comparing the data obtained across all treatments, providing reliable scientific solution to address quality elements of the crop in the South African market.

2.6.2 Total phenols

Total phenols are defined as classes of chemicals that arise from aromatic amino acids (Maluleke et al. 2021). These substances mainly function as a UV radiation shield and a defense mechanism against possible predators (Tuckeldoe et al. 2023). When phenolic compounds are combined with vitamin C and vitamin E, they can act as antioxidants to shield different body tissues from oxidative damage (Lekoba et al. 2024). In fruit and vegetable-based diets, polyphenols have been reported as the most prevalent antioxidants (Vastolo et al. 2021; Mashau et al. 2022).

The phenolic content of *Cannabis sativa* L cultivars have been extracted and analysed using a variety of procedures in previous investigations. For instance, the phenolic components in Finola cultivars were extracted, which showed that the presence of relatively high concentrations of phenolic compounds in the cultivar significantly influenced the amount and quality of its oil, in relation to other cultivars (Smeriglio et al. 2016). There also appears to be little in the way of publication describing the combined effects of various fertiliser types and growing environments on the phenolic content of *Cannabis sativa* L buds. This further validates the current study.

2.6.3 Vitamin C

Maluleke et al. (2024) defined vitamin C as a water-soluble substance that can be found in fruits, berries, and vegetables, and which is included in a general diet where it is typically recommended as a supplements or in natural form to improve human nutrition and health. Vitamin C is essential because it promotes tissue growth and repair throughout the body (Achaglinkame et al. 2019). Additionally, according to Maluleke et al. (2021), it creates collagen, a vital protein required for the development of blood vessels, tendons, ligaments, and skin. It has also been characterised as a necessary component that is important for the formation of scar tissue and the healing of wounds (Tuckeldoe et al. 2023). Farinon et al. (2022), assessed how malting impact the nutritional qualities of *Cannabis sativa* seeds. Their research revealed that, in comparison to the control, malting, which modifies the processes of seed matabolism, germination, and product storage, enhanced the vitamin C content of the seed.

Notwithstanding the intriguing findings regarding the vitamin C content of *Cannabis sative* L seeds, that study only focused at the vitamin C content of the seeds, not the buds. In order to produce high-quality *Cannabis sativa* L bud material in large quantities, the current study supplied baseline data regarding the proper agronomic combination by evaluating the impact of the above indicated parameters on the vitamin C content of *Cannabis sativa* L buds.

2.6.4 Vitamin E

Vitamin E, a fat-soluble substance that functions as an antioxidant in the human body, is abundant in leafy greens, fruits, and medicinal crops (Tuckeldoe et al., 2023). It protects cells from potential damage caused by free radicals as an antioxidant (Maluleke et al. 2021). Free radicals, which are chemicals that are produced when the body transform food into energy have the potential to increase risk of illness (Al Khoury et al. 2021). Consequently, vitamin E acts as an antioxidant to protect the body from free radicals, which have been linked to several diseases, including heart disease and some types of cancer (Achaglinkame et al. 2019).

A study conducted by Tuckeldoe et al. (2023), found that the genotype has a contributing impact on the vitamin E content of varying pepper cultivars that were grown on organic soil. Other investigation by Lekoba et al. (2024), found that the vitamin E content of wild sour plum fruit is influenced by environmental elements including temperature and radiation.

By comparing data on the vitamin E content of *Cannabis sativa* L plants grown under differing treatments, such as varying types of fertilizer and growing environments, the current study closed a gap in the knowledge, by establishing baseline knowledge that could assist potential growers in producing high-quality *Cannabis sativa* L plant material in South Africa.

2.6.5 Macro and micro-nutrients

A set of components known as macro- and micronutrients are needed on a daily basis to sustain physiological functions, according to Barrett et al. (2010). Additionally, authors noted that although a greater amount of macronutrients is needed, an acceptable lesser amount of micronutrients is also required daily. According to Wang et al. (2008); Maluleke et al. (2024) and Lekoba et al. (2024), the availability of macro and micronutrients in fruits and leafy crops is mostly stable. However, the content of both elements in crops can be significantly affected by the growing environment and types of fertiliser (Maluleke et al. 2021).

The effect of cultivar type and fertilisers on various micronutrients (iron, manganese, copper, boron, and zinc) and macronutrients (calcium, magnesium, phosphorus, and potassium) was investigated by scholars such as Yep and Zheng (2021) and Angelini et al.(2014). These authors reported that the primary cause of the notable fluctuations in minerals such as magnesium and potassium was fertiliser application (Yep & Zheng, 2021). In contrast, cultivar type was found to have a significant impact on the concentration of micronutrients such iron, manganese, copper, zinc, and boron (Angerini et al. 2014).They eventually came to the conclusion that, in order to maximize quality and positively impact their profit margins, growers should choose cultivars according to the reason for planting a particular crop, such as food or medicine.

Thus, the current study added to the literature by comparing data across all treatments to identify the best combination that will help growers produce high-quality *Cannabis sativa* L plant material that will contribute to an increased profit return in South Africa.

2.7 References

Achaglinkame, M. A., Aderibigbe, R. O., Hensel, O., Sturm, B., & Korese, J. K. (2019). Nutritional characteristics of four underutilized edible wild fruits of dietary interest in Ghana. Foods, 8(3), 1-12.

Al Khoury, A., Sleiman, R., Atoui, A., Hindieh, P., Maroun, R. G., Bailly, J. D., & El Khoury, A. (2021). Antifungal and anti-aflatoxigenic properties of organs of *Cannabis sativa* L.: Relation to phenolic content and antioxidant capacities. Archives of Microbiology, 203(7), 4485-4492.

Angelini, L. G., Tavarini, S., Cestone, B., & Beni, C. (2014). Variation in mineral composition in three different plant organs of five fibre hemp (*Cannabis sativa* L.) cultivars. Agrochimica, 58(1), 1-18.

Amaducci, S., Zatta, A., Raffanini, M., & Venturi, G. (2008). Characterisation of hemp (*Cannabis sativa* L.) roots under different growing conditions. Plant and Soil, 313, 227-235.

Backer, R., Schwinghamer, T., Rosenbaum, P., McCarty, V., Eichhorn Bilodeau, S., Lyu, D., & Smith, D. L. (2019). Closing the yield gap for cannabis: A meta-analysis of factors determining *Cannabis* yield. Frontiers in Plant Science, 10(495), 1-15.

Bernstein, N., Gorelick, J., Zerahia, R., & Koch, S. (2019). Impact of N, P, K, and humic acid supplementation on the chemical profile of medical Cannabis (*Cannabis sativa* L). Frontiers in Plant Science, 10(736), 1-13.

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. Food Science and Nutrition, 50(5), 369-389.

Bevan, L., Jones, M., & Zheng, Y. (2021). Optimisation of nitrogen, phosphorus, and potassium for soilless production of *Cannabis sativa* in the flowering stage using response surface analysis. Frontiers in Plant Science, 12, 1-10.

Burgel, L., Hartung, J., Schibano, D., & Graeff-Hönninger, S. (2020). Impact of different phytohormones on morphology, yield and cannabinoid content of *Cannabis sativa* L. Plants, 9(6), 1-16.

Caplan, D., Dixon, M., & Zheng, Y. (2017). Optimal rate of organic fertilizer during the flowering stage for Cannabis grown in two coir-based substrates. Horticultural Science, 52(12), 1796-1803.

Chibueze, U., & Akubugwo, E. I. (2011). Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agriculture and Biology Journal of North America, 2(12), 1437-1444.

Campiglia, E., Radicetti, E., & Mancinelli, R. (2017). Plant density and nitrogen fertilization affect agronomic performance of industrial hemp (*Cannabis sativa* L.) in Mediterranean environment. Industrial Crops and Products, 100, 246-254.

Da Cunha Leme Filho, J. F., Chim, B. K., Bermand, C., Diatta, A. A., & Thomason, W. E. (2024). Effect of organic biostimulants on *Cannabi*s productivity and soil microbial activity under outdoor conditions. Journal of Cannabis Research, 6(1), 1-13.

Deng, G., Yang, M., Saleem, M. H., Rehman, M., Fahad, S., Yang, Y., Elshikh, M., Alkahtani, J., Ali, S., & Khan, S. M. (2021). Nitrogen fertilizer ameliorate the remedial capacity of industrial hemp (*Cannabis sativa* L.) grown in lead contaminated soil. Journal of Plant Nutrition, 44(12), 1770-1778.

De Prato, L., Ansari, O., Hardy, G. E. S. J., Howieson, J., O'Hara, G., & Ruthrof, K. X. (2022). Morpho-physiology and cannabinoid concentrations of hemp (*Cannabis sativa* L.) are affected by potassium fertilisers and microbes under tropical conditions. Industrial Crops and Products, 182, 1-13.

Dias, M. C., Pinto, D., & Silva, A. (2021). Plant flavonoids: Chemical characteristics and biological activity. Molecules, 26(17), 5377.

Evans, J., & von Caemmerer, S. (1996). Carbon dioxide diffusion inside leaves. Plant Physiology, 110, 339.

Farinon, B., Molinari, R., Costantini, L., & Merendino, N. (2020). The seed of industrial hemp (*Cannabis sativa* L.): Nutritional quality and potential functionality for human health and nutrition. Nutrients, 12(7), 1-59.

Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C content in fruits: Biosynthesis and regulation. Frontiers in Plant Science, 9, 1-21.

Fischer, R. (2015). Definitions and determination of crop yield, yield gaps, and of rates of change. Field Crops Research, 182, 9-18.

Gwala, R. S. (2023). The legalisation of Cannabis in South Africa: Proposing an economic value chain model for South Africa. In Rapid Innovation and Development in the Global Cannabis Market, 98-122.

Hourfane, S., Mechqoq, H., Bekkali, A. Y., Rocha, J. M., & El Aouad, N. (2023). A comprehensive review on *Cannabis sativa* ethnobotany, phytochemistry, molecular docking and biological activities. Plants, 12(6), 1-43.

Kakabouki, I., Kousta, A., Folina, A., Karydogianni, S., Zisi, C., Kouneli, V., & Papastylianou, P. (2021). Effect of fertilization with urea and inhibitors on growth, yield and CBD concentration of hemp (*Cannabis sativa* L.). Sustainability, 13(4), 1-13.

Karak, P. (2019). Biological activities of flavonoids: An overview. International Journal of Pharmaceutical Sciences and Research, 10(4), 1567-1574.

Kepe, T. (2003). *Cannabis sativa* and rural livelihoods in South Africa: Politics of cultivation, trade and value in Pondoland. Development Southern Africa, 20(5), 605-615.

Kornpointner, C., Martinez, A. S., Marinovic, S., Haselmair-Gosch, C., Jamnik, P., Schröder, K., & Halbwirth, H. (2021). Chemical composition and antioxidant potential of *Cannabis sativa* L. roots. Industrial Crops and Products, 165, 1-8.

Krüger, M., van Eeden, T., & Beswa, D. (2022). *Cannabis sativa* cannabinoids as functional ingredients in snack foods - historical and developmental aspects. Plants, 11(23),1-19.

Laleh, S., Al-Ahmadi, M. J., & Parsa, S. (2021). Response of hemp (*Cannabis sativa* L.) to integrated application of chemical and manure fertilizers. Acta Agriculture Slovenica, 117(2), 1-15.

Lekoba, B., Maluleke, M. K., & Mphahlele, R. (2024). Nutritional composition of wild sour plum (*Ximenia caffra* subs caffra) fruit harvested in varying geographical regions and its potential role in human nutrition. Discover Applied Sciences, 6(5), 1-11.

Łochyńska, M., & Frankowski, J. (2022). The effects of silkworm excrement organic fertilizer on the hemp yield. Journal of Natural Fibers, 19(3), 847-857.

Magagnini, G., Grassi, G., & Kotiranta, S. (2018). The effect of light spectrum on the morphology and cannabinoid content of *Cannabis sativa* L. *Medical Cannabis and Cannabinoids*, *1*(1), 19-27.

Maluleke, M. K. (2022). Metabolite profile of African horned cucumber (*Cucumis metuliferus* E. May. Ex Naudin) fruit grown under differing environmental conditions. Scientific Reports, 12(1), 1-18.

Maluleke, M. K., Moja, S. J., Nyathi, M., & Modise, D. M. (2021). Nutrient concentration of African horned cucumber (*Cucumis metuliferus* L) fruit under different soil types, environments, and varying irrigation water levels. Horticulturae, 7(4),1-16.

Maluleke, M. K., Ralulimi, T. S., & Machete, M. (2024). Biochemical constituents and the role of African wild medlar (*Vangueria infausta*) in human nutrition: A review. Discover Sustainability, 5(1), 1-10.

Mashau, M. E., Kgatla, T. E., Makhado, M. V., Mikasi, M. S., & Ramashia, S. E. (2022). Nutritional composition, polyphenolic compounds and biological activities of marula fruit (*Sclerocarya birrea*) with its potential food applications: A review. International Journal of Food Properties, 25(1), 1549-1575.

Massuela, D. C., Munz, S., Hartung, J., Nkebiwe, P. M., & Graeff-Hönninger, S. (2023). Cannabis Hunger Games: Nutrient stress induction in flowering stage -impact of organic and mineral fertilizer levels on biomass, cannabidiol (CBD) yield and nutrient use efficiency. Frontiers in Plant Science, 14, 1-19.

McElrone, A. J., Choat, B., Gambetta, G. A., & Brodersen, C. R. (2013). Water uptake and transport in vascular plants. Nature Education Knowledge, 4(5), 1-12.

Mokoena, M. L., Funnah, S. M., & Ngobeni, N. D. (2011). Performance of hemp cultivars under different nitrogen levels in Eastern Cape Province of South Africa. African Crop Science Conference Proceedings, 10, 569 - 574

Olle, M., Ngouajio, M., & Siomos, A. (2012). Vegetable quality and productivity as influenced by growing medium: Žemdirbystė=Agriculture, 99(4), 399-408.

Papastylianou, P., Kakabouki, I., & Travlos, I. (2018). Effect of nitrogen fertilization on growth and yield of industrial hemp (*Cannabis sativa* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46(1), 197-201.

Potter, D. J. (2014). A review of the cultivation and processing of Cannabis (*Cannabis sativa* L.) for production of prescription medicines in the UK. Drug Testing and Analysis, 6(1-2), 31-38.

Rehman, M., Fahad, S., Du, G., Cheng, X., Yang, Y., Tang, K. & Deng, G. (2021). Evaluation of hemp (*Cannabis sativa* L.) as an industrial crop: A review. Environmental Science and Pollution Research, 28(38), 52832-52843.

Rupasinghe, H. V., Davis, A., Kumar, S. K., Murray, B., & Zheljazkov, V. D. (2020). Industrial hemp (*Cannabis sativa* subsp. sativa) as an emerging source for value-added functional food ingredients and nutraceuticals. Molecules, 25(18), 1-24.

Sheldon, K., Shekoofa, A., Walker, E., & Kelly, H. (2021). Physiological screening for drought-tolerance traits among hemp (*Cannabis sativa* L.) cultivars in controlled environments and in field. Journal of Crop Improvement, 35(6), 816-831.

Smeriglio, A., Galati, E. M., Monforte, M. T., Lanuzza, F., D'Angelo, V., & Circosta, C. (2016). Polyphenolic compounds and antioxidant activity of cold-pressed seed oil from Finola cultivar of *Cannabis sativa* L. Phytotherapy Research, 30(8), 1298-1307.

Tang, K., Fracasso, A., Struik, P. C., Yin, X., & Amaducci, S. (2018). Water- and nitrogen-use efficiencies of hemp (*Cannabis sativa* L.) based on whole-canopy measurements and modeling. Frontiers in Plant Science, 9, 1-14.

Tuckeldoe, R. B., Maluleke, M. K., & Adriaanse, P. J. S. R. (2023). The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (*Capsicum annuum*) varieties. Scientific Reports, 13(1), 1-13.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines, 5(3), 1-93.

Vanhove, W., Van Damme, P., & Meert, N. (2011). Factors determining yield and quality of illicit indoor *Cannabis* (Cannabis spp.) production. Forensic Science International, 212(1-3), 158-163.

Vastolo, A., Calabrò, S., Pacifico, S., Koura, B. I., & Cutrignelli, M. I. (2021). Chemical and nutritional characteristics of *Cannabis sativa* L. co-products. Journal of Animal Physiology and Animal Nutrition, 105, 1-9.

Vera, C. L., Malhi, S. S., Raney, J. P., & Wang, Z. H. (2004). The effect of N and P fertilization on growth, seed yield and quality of industrial hemp in the Parkland region of Saskatchewan. Canadian Journal of Plant Science, 84(4), 939-947.

Wang, X. X., Zhao, F., Zhang, G., Zhang, Y., & Yang, L. (2017). Vermicompost improves tomato yield and quality and the biochemical properties of soils with different tomato planting history in a greenhouse study. Frontiers in Plant Science, 8(1978), 1-11.

Wielgusz, K., Praczyk, M., Irzykowska, L., & Świerk, D. (2022). Fertilization and soil pH affect seed and biomass yield, plant morphology, and cadmium uptake in hemp (*Cannabis sativa* L.). Industrial Crops and Products, 175, 1-9.

Xu, H., Owens, M. M., Farncombe, T., Noseworthy, M., & MacKillop, J. (2023). Molecular brain differences and Cannabis involvement: A systematic review of positron emission tomography studies. Journal of Psychiatric Research, 162,1-13.

Yang, Y., Zha, W., Tang, K., Deng, G., Du, G., & Liu, F. (2021). Effect of nitrogen supply on growth and nitrogen utilization in hemp (*Cannabis sativa* L.). Agronomy, 11(11), 1-12.

Yep, B., & Zheng, Y. (2020). Potassium and micronutrient fertilizer addition in a mock aquaponic system for drug-type *Cannabis sativa* L. cultivation. Canadian Journal of Plant Science, 101(3), 341-352.

Zuk-Golaszewska, K., & Golaszewski, J. (2018). *Cannabis sativa* L.–cultivation and quality of raw material. Journal of Elementology, 23(3),1-14.

CHAPTER 3: Impact of various fertiliser types on *Cannabis sativa* L (Indian hemp) growth and yield in differing growing conditions

3.1 Abstract

Most industrial crops, including *Cannabis sativa* L, which is known for its nutritional and medicinal qualities, rely on a variety of variables to support their growth, physiological function, and yield. Most industrial crops' physiological performance, and yield are influenced by several factors, including an optimal growing environment and a balanced, nutrient-rich substrate. The goal for which the crop is grown determines which of these factors should be chosen. Some producers prefer using inorganic fertilisers because they can speed up plant growth and yield in a short amount of time, while others prefer using organic fertilisers because they can release nutrients to plants gradually and have less of an environmental impact because they easily decompose into the soil. Despite their strength to assist growers to maximise yield of crops, a suitable combination of fertiliser type and growing environment plays a pivotal role on the growth, physiological performance, and yield of crops. A factorial experiment, namely (i) different fertiliser types (organic and inorganic) and (ii) different growing environment (under shade netting or in the open space), was carried out at Unisa Florida Science Campus (26°09'31.8"S 27°54'14.4"E) during summer (December 2023 to February 2024). The study's goal was to ascertain how different fertilisers and growing conditions combined, affect *Cannabis sativa* L., physiological performance, and yield. In comparison to other treatments, the study showed that growing the crop under shade netting and the application of inorganic fertilisers demonstrated a higher chlorophyll content (85.7 $\mu mol \cdot m^{-2}$). Similar treatments exhibited a superior harvest index (0.57) compared to other treatments. Therefore, it can be concluded that growing *Cannabis sativa* L under the treatment combination involving inorganic/chemical fertiliser and under shade netting has the potential to boost the yield of the crop. Therefore, growers are encouraged to grow their *Cannabis sativa* plants crop using these treatments for higher yield for profit maximisation.

Keywords: *Cannabis sativa* L. Organic fertilisers. Chemical fertilisers. Harvest index.

3.2 Introduction

The productivity of Indian hemp (*Cannabis sativa* L), herbaceous plant in the Cannabaceae family, is dependent on several variables, including the type of fertilizer used and the growing environment (Kornpointner et al. 2021). Although native to Central Asia, this plant has spread over the globe because of its adaptability to a variety of environments (Kepe 2003; Hourfane et al. 2023). In 2020, the South African Constitutional Court declared that laws that forbade the private cultivation and consumption of Cannabis were unconstitutional (Gwala 2023). In addition, the South African government also projected that the country's Cannabis production, including its cultivation, processing, and commercialisation, could provide billions in revenue annually, and may lead to about 10 000 to 25 000 employments in the industry (Riley et al. 2020). Viviers et al. (2022) claim that this has caused a sharp rise in the production of Cannabis for a variety of uses, including consumption, as well as processed value-added goods like drinks, oils, and pharmaceuticals, which will help achieve several Sustainable Development Goals (SDGs), including zero hunger and no poverty. Quality plant growth in the agricultural and horticulture sectors is dependent on several variables, including (i) the growing environment and (ii) the types of fertilisers used (Maluleke et al. 2021). According to researchers such as Vivek and Duraisamy (2017), a healthy soil is advantageous since it retains a stable environment and provides plants with vital nutrients. Paucek et al. (2020) claim that climatic conditions influence account for more than half of crop productivity. The three main climatic variables that affect crop growth, development, and yield are rainfall, temperature, and sun radiation (Wang et al., 2018). While other authors such a Campiglia et al. (2017) and Frankowski et al. (2023) reported the benefit of fertilisers in crop production as being enhancement of plant health, improvement of crop yield, and profit maximisation. Hatfield et al. (2011), among other authors, emphasized that optimal crop production is more likely when appropriate climatic conditions, such as temperature and light, are provided. Even though every author presented ample evidence about the advantages of individual factors such as fertiliser types and growing environment, there appears to be little data regarding the combined impact of these factors on the growth, physiological performance, and yield of industrial crops, particularly *Cannabis sativa* L. Consequently, the study's goal was to ascertain the combined impact of various fertiliser types (organic and inorganic/chemical) on the growth, physiological performance, and yield of *Cannabis sativa* L grown under various environments in order to do a comparative analysis and establish baseline data that could expedite mass production of the crop while optimizing yield under the South African climate.

3.3 Materials and methods

3.3.1 Study site and growing conditions

This study was carried out in 2023 in a shade-net and open-space environment, Figure 1, at the University of South Africa, Florida Campus, Johannesburg, Gauteng province, South Africa (lat. 26º10'30"S, long. 27º55'22.8"E). The minimum and maximum temperatures in shade-net (13-30°C) and open-space environment (14-34°C) was recorded during the experimental period shown on Table 4. Plants were exposed to natural light for a minimum of 10 to 12 hours every day during the trial period (Table 5).

3.3.2 Growth media and fertiliser treatment of *Cannabis sativa* L plants

With two independent variables, the experiment was completely randomised and included: (i) different fertilisers (both organic and inorganic/chemical) under different growing settings (shade-net and open-space). For the experiment, seedlings were sown using certified seeds that were acquired from the research facility in Cape Town, South Africa. Using certified seeds obtained from the research institution in Cape Town, South Africa, uniform seedlings germinated from peat growth media were transferred to 30 cm depth × 30 cm width pots filled with, (i) loam soil under differing environments (shade -net and open-space) during spring to summer of 2023 as shown in Figures 3.1 and 3.2. Ten (10) replicates were used per treatment. After the establishment of seedlings, plants were supplied with five (5) grams of granule fertilisers of different types, namely (i) organic fertiliser (GuanoBoost, Pretoria, South Africa) with Nitrogen (N), Phosphorus (P) and Potassium (K) [N-3, P-2, K-5] and inorganic/chemical fertiliser (Nitrogreen KAN/LAN, Protek, Pretoria, South Africa) with

Nitrogen (N), Phosphorus (P) and Potassium (K) [N-2, P-3, K-2] (Table 3.1). Plants grown on loam soil with fertiliser treatment was classified as control.

The treatments were imposed two (2) weeks later, after establishment. Plants were well irrigated throughout the experiment period. Briefly the area (depth × width) 30cm × 30cm = 900cm^2, A =$\pi \left(\frac{d}{2}\right) \times 2$ d =286.5 cm^2 planting pots. Soil mineral analysis was carried out at the Agricultural Research Council (25° 44' 19.4" S 28° 12' 26.4" E), Arcadia, Pretoria (Table 3.1). Throughout the experiment, 2 litres of water were used to irrigate the plants thoroughly. The next watering schedule was determined using a three-way moisture meter. Three moisture indications are available on the meter: dry, moist, and wet. Only after the gauge indicated that the soil was dry were plants watered.

Table 3.1: Different fertiliser types used for the experimental treatment.

	Organic fertiliser (ratio)
Nitrogen (N)=3	6
Phosphorus (P)=2	4
Potassium (K)=5	10
Total= 20%	
	Inorganic/chemical fertiliser (ratio)
Nitrogen (N)=2	4.9
Phosphorus (P)=3	7.3
Potassium (K)=2	4.9
Total= 17%	

3.3.3 Soil mineral analysis

The soil samples depicted in Table 3.2 were tested for mineral and/or chemical content at the Institute for Soil, Climate, and Water (ARC-ISWC) of the Agricultural Research Council in Arcadia, Pretoria (25° 44′ 19.4″ S28° 12′ 26.4″ E) prior to planting, using methods outlined by Tuckeldoe et al. (2023).

Table 3.2: Mineral content of experimental soil.

Mineral content analysis								
Fe	**Mn**	**Cu**	**Zn**	**P**	**Ca**	**Mg**	**K**	**Na**
38	17	14	15	25	1528	223	223	58

3.3.4 Meteorological data of shade-net and open-space environment

Following the method of Gul et al. (2020), meteorological data was collected throughout the experimental period as evinced in Table 3.3.

Table 3.3: Meteorological data of the experimental sites.

	Open space (L1)		
	T_{max} (˚C)	**T_{min}**	**Rainfall (mm)**
Month			
September	30˚C	14˚C	27.2
October	31˚C	16˚C	97.1
November	32˚C	19˚C	79.3
December	33˚C	20˚C	140.3
			516.4
	Shade-net (L2)		
September	28˚C	13˚C	10
October	29˚C	15˚C	22
November	27˚C	15˚C	68
December	30˚C	16˚C	119
			337

L1 = means open-space environment. L2 = means shade-net environment.

3.3.5 Light intensity of shade-net and open space environment

Following the method of Tuckeldoe et al. (2023), light intensity data were collected throughout the experimental period, as shown in Table 3.4.

Table 3.4: Light intensity (Lux) data of the experimental sites.

	Light intensity (Lux)	
Environment (2023)	**Shade-net**	**Open-space**
September	411	823
October	245	477
November	210	599
December	270	1156
January	324	1147
	1460	**4202**

3.3.6 Measurement of plant height

As followed by Tuckeldoe et al. (2023), plant height was recorded on day five of every week during the study period. Using a measuring tape, the height of the plants was measured in centimeters (Webco Tool, South Africa). Figures 3.1 and 3.2 indicate the relative growth of plants under shade netting or in the open, respectively.

Figure 3.1: Growth of *Cannabis sativa* plants grown under shade netting.

Figure 3.2: *Cannabis sativa* grown in an open environment

3.3.7 Stem diameter measurement

Every fifth day of the week, the stem diameter of the plants undergoing treatment was measured in millimetres (mm) using a Vernier Caliper (Digimatic 150Mm, Epacon supplies Pty, Edenvale, South Africa). In short, the caliper was set up on aluminum and "invar" (a nickel and iron alloy with low thermal expansion) holders. Elastic straps were utilised to secure the holders to the plant stems, and a sealing paste was applied to the plant stem surface to connect the sensor needle (Yang et al., 2021). Results were continually recorded.

3.3.8 Total biomass and bud water content measurement

Following the procedure of Tuckeldoe et al. (2023), an electronic scale (Uni-Bioc, China) was used to weigh the fresh biomass of the fruits (in grams) at the conclusion of the experiment. The fruits were first weighed, then moved to the covered cud box, and to the oven that was set to 80°C for 72 hours. The water content of *Cannabis sativa* buds was then quantified using the fruit dry weight (Table 3.5).

Total biomass and water content was determined using the formula below:

<u>Total biomass</u> = above-ground biomass (dry) + fruit biomass (dry) **(Equation 1)**

<u>Bud water content</u> = bud fresh weight (fresh) – bud dry weight (dry) **(Equation 2)**

3.3.9 Harvest index measurement

Tuckeldoe et al. (2023) procedure depicted in Table 3.5, was adopted for harvest index measurement.

Table 3.5: Equations used to measure certain variables.

Variant	Equation	Number
Total biomass	Total biomass=above-ground biomass (dry) + fruit biomass (dry)	1
Harvest index	HI=Bud dry biomass(dry)/total biomass	2
Water content	Fresh fruit biomass-dry fruit biomass	3

3.3.10 Ethical consideration

It is important to note that the study experiments were carried out in strict accordance with the guidelines and specifications set forth by the UNISA College of Agriculture and Environmental Sciences' Research and Higher Degree Committee. Furthermore, the study was granted ethical approval by the Department of Health of the Republic of South Africa **(Permit no: POS 093/2023/2024)** and the UNISA-CAES Health Research Ethics Committee **(reference number 2022/CAES_HREC/006)**.

3.4 Statistical analysis

The growth and yield of *Cannabis sativa* L grown in two distinct fertilisers (organic and inorganic/chemical fertilizers), under two different growing environments (shade netting or in an open space) were analysed using a two-way analysis of variance (ANOVA). Data analysis was performed using generalised linear mixed model procedures using (GenStat, version 22.1, 2023, UK). The model was utilised to evaluate the fixed variables (fertiliser types and growing environments) impact on the dependent variables (plant height, stem diameter, bud water content, harvest index, stomatal conductance, total biomass, and chlorophyll content). For each variable, the least significant difference (LSD) of the means was expressed.

3.5 Results and discussion

3.5.1 Physiological components

3.5.1.1 Chlorophyll content and stomatal conductance

Combined impact of varying fertiliser types on the chlorophyll content and stomatal conductance of *Cannabis sativa* L grown under differing environment. is displayed in Table 3.6. It was discovered that there was a substantial (P≤0.05) variation between the stomatal conductance and chlorophyll concentration. The findings indicated that the range of the chlorophyll concentration varied from 31.8 to 85.7 µmol·m^{-2}. Results of the study also indicated that plants grown in an open-space environment on loam soil (control) had a decrease in chlorophyll content from 85.7 to 31.8 µmol·m^{-}2, whereas plants cultivated in a shade-net environment with chemical fertiliser had an increase in chlorophyll content from 31.8 to 85.7 µmol·m^{-2}. There was a 53.9 µmol·m^{-2} difference in chlorophyll content between the highest (85.7) and lowest (31.8). According to Tuckeldoe et al. (2023), light reactions in plants occur in the chloroplast thylakoids, which contain the chlorophyll pigments. Light energy makes the electrons in the pigment molecules active, and these electrons are subsequently transmitted along the electron transport chain found in the thylakoid membrane (Maluleke 2022).

The variation between the highest (85.7) and lowest (31.8) chlorophyll content was 53.9 µmol·m-^{2}. The chloroplast thylakoids, which house the chlorophyll pigments, are where the light reactions in plants take place (Tuckeldoe et al. 2023). The pigment molecules' electrons become energetic when light energy enters them, and these electrons are then transferred through electron transport chain located in the thylakoid membrane (Maluleke 2022). Plants constantly manufacture chlorophyll to maintain the net photosynthesis rate in their leaves (Shu et al. 2013). A contributing element to the change in chlorophyll content was found to have temperature and light intensity, open-space had maximum temperature of (32), while shade-net was (28.6), which varied by 3.4°C (Table 3.3). Regarding light intensity, the shade-net light intensity (1460) and open-space environment (4202) varied by 2742 lux (Table 3.4). These findings are compatible with those of Maluleke (2022), who found variations in the chlorophyll concentration of plants cultivated under varying environmental conditions.

For stomatal conductance, results illustrated that it varied between 34.5 and 75.3 $mmol \cdot m^{-2} \cdot s^{-1}$. Furthermore, it showed that in comparison to other treatments, plants grown on loam soil (control) in a shade-net environment had the lowest conductance (34.5 $mmol \cdot m^{-2} \cdot s^{-1}$), whereas plants grown in an open-space environment with chemical fertiliser treatment showed the highest conductivity (75.3 $mmol \cdot m^{-2} \cdot s^{-1}$). The variation between the highest (75.3) and lowest (34.5) stomatal conductance was 40.8 $mmol \cdot m^{-2} \cdot s^{-1}$. Stomatal pores normally open when guard cells expand because of water absorption and close when guard cells shrink (Maluleke et al., 2021).

Light intensity, temperature, humidity, and carbon dioxide levels are a few variables that affect stomatal opening and closing according to Shu et al. (2013). With adjustments to the stomatal pore size, stomata govern both the exchange of gases between the plant and its surroundings as well as the loss of water, subsequently affecting the net photosynthesis rate, causing an increase or decrease in the plant overall yield (Tuckeldoe et al. 2023). The open-space environment's light intensity (4202 lux), which was (2742) lux higher than the shade-net environment, proved to be the main contributing factor to the variation in the stomatal conductance performance amongst plants (Table 3.4). These observations are in line with those of Maluleke (2022), who reported a variation in plants grown under different light intensities and environmental conditions.

Table 3.6: Combined impact of varying fertiliser types on the chlorophyll content and stomatal conductance of *Cannabis sativa* L grown under differing environment.

Treatments	**Chlorophyll content (µmol·m-2)**	**Stomatal conductance (mmol·m-2·s-1)**
L1F1	47.2(7)	48(5.6)
L1F2	80.7(13)	75.3(8.7)
L1C	31.8(6.3)	40.8(6.7)
L2F1	40.2(2.6)	35(16.7)
L2F2	85.7(30.2)	62.7(12.2)
L2C	40.8(7.5)	34.5(7.6)
Grand mean	54.4	49.4
LSD0.05	11.31	9.95
Pvalue	**0.001**	**0.001**

L1 = means open-space environment. **L2** = means shade-net environment. **F1** = means organic fertiliser. **F2** = means chemical fertiliser. **C** = means control. The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. The least significant difference between means, or LSD0.05, is used.

3.5.1.2 Plant height and stem diameter

Combined impact of varying fertiliser types on the plant height and stem diameter of *Cannabis sativa* L grown under differing environment is shown in Figure 3.3 (A and B). The study findings showed that there were substantial ($P \leq 0.05$) differences on the impact of various fertiliser types on the plant height and stem diameter of *Cannabis sativa* grown in various environments. The results indicated that the plant height, as shown in Figure 3.3A, varied between 41.2 and 61.3 cm. Furthermore, the results showed that plants cultivated in loam soil (control) decreased in height from 61.3 to 41.2 cm, while plants treated with a combination of chemical fertiliser and open-space environment increased in height from 41.2 to 61.3 cm.

Plant growth and development are significantly influenced by light (Reichel et al. 2021). It is critical to many processes, including photosynthesis, which yields carbohydrates, a substance that plants need for respiration, and to several other processes, including plant growth, development, and hormone distribution control (Tang et al. 2017). According to the study findings, there was a 3.4°C difference in temperature between the open-space environment (4202) and the shade-net (1460), while there was only a 2742 lux variation in light (Table 3.4). The results of this study showed that, in comparison to other treatments, the main cause of variations in plant height was a combination of high light intensity, temperature, and phosphorus content of chemical/inorganic fertilizer in an open-space environment. The present study's results are consistent with those of Saloner et al. (2019) and Danziger and Berstein (2021), who observed fluctuations in plant growth response to distinct photoperiod regimes.

The study findings regarding stem diameter (Figure 3.3B), indicated that it varied between 0.95 and 9.55 mm. Additionally, study findings showed that the stem diameter was reduced from 9.55 to 0.95 mm under the treatment combination of organic fertiliser and shade-net. The combination of chemical fertiliser treatment and open-space habitat resulted in the maximum stem diameter of 9.55 mm. There was an 8.6 mm difference in stem diameter between the lowest (0.95) and highest (9.55) values. Phosphorus is a nutrient that is present in every cell of plants and is necessary for their growth and development (Moher et al. 2022). Furthermore, according to Tuckeldoe et al. (2023), it is vital to several plant functions, including photosynthesis, energy transmission, nutrient transport inside plant cells, and sugar transformation.

While the phosphorus level of fertilisers ranged from 3.3% for inorganic/chemical fertilizers, to 4% for organic fertilizers, the difference in plant height between the lowest (41.2) and maximum (61.3) was 20.1 cm. When compared to other treatments, the values from this study indicate that the mineral phosphorus from chemical/inorganic fertiliser and the greater light intensity (4202) from the open-space environment were the contributing factors for the difference in stem diameter of *Cannabis sativa* L.

Furthermore, findings suggests that the high phosphorus content of chemical fertilisers allowed the plant to easily promote the passage of nutrients, water, and air, all of which are predominantly transported from the roots to the stems and leaves. These discoveries are consistent with those of Kakabouk et al. (2021), who observed height and stem variation in *Cannabis sativa* plants treated with different doses of fertiliser.

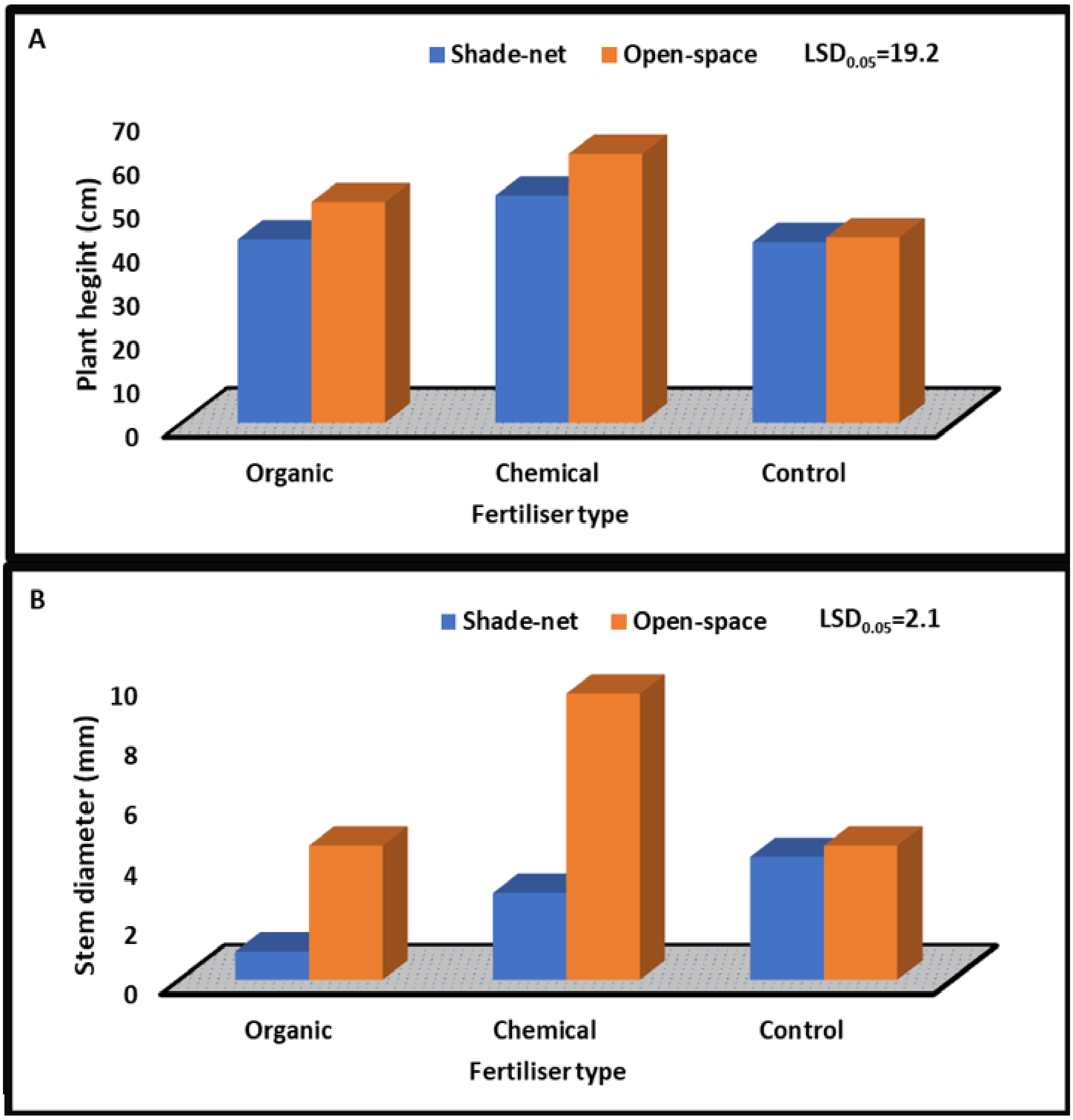

Figure 3.3: Impact of varying fertiliser types on the plant height (**Figure A**) and stem diameter (**Figure B**) of *Cannabis sativa* grown under shade-net and open-space environment.

3.5.2 Yield components

3.5.2.1 Total biomass

The impact of diverse fertiliser types on the yield components (total biomass) of *Cannabis sativa* L. cultivated in varied environmental settings is shown in Table 3.5. The study findings showed that there was a substantial (P≤0.05) variation in the total biomass of *Cannabis sativa* growing in different settings across treatments. The total biomass varied from 0.9 to 0.58 kg, according to study data. Furthermore, the study's findings demonstrated that the total biomass decreased from 0.58 to 0.9 kg when organic fertilizers and a shade net were used as a treatment combination, but the total biomass increased noticeably from 0.9 to 0.58 kg when chemical fertilizers and an open-space environment were used as a treatment combination. Minerals like phosphorus have been shown to have affect the overall biomass of plants (Kakabouk et al. 2021). Based on the study findings, the phosphorus content ratio amongst fertiliser types (organic-4%) and (inorganic/chemical-7.3%), which varied by 3.3%, proved to be a contribution factor in total biomass variation of *Cannabis sativa* grown under varying environment (Table 3.3). The results of Forrest and Young (2008), and Bernstein et al. (2019), who observed variations in biomass of plants grown under various fertiliser types and ratios, are consistent with our findings.

3.5.2.2 Harvest index

Table 3.5 depict the impact of different types of fertilisers on the harvest index of *Cannabis sativa* L cultivated in differing locations. Study results evinced that there was a substantial (P≤0.05) variation in the harvesting index of *Cannabis sativa* under treatment combination of varying fertiliser types and environment. Harvest index varied from 0.04 to 0.57, according to study data.

The harvest index also shown to be reduced from 0.57 to 0.04 by the treatment combination of an open-space environment and organic fertilizer, whereas it was increased from 0.04 to 0.57 by the combination of a shade-net environment and chemical fertilizer. There was a difference of 0.53 between the superior harvest index (0.57) and the lowest (0.04).

The superior harvest index proved to have been caused by the varying potassium content from fertilisers (organic-10%) and (inorganic/chemical-4.9%), which varied by 5.1% (Table 3.1), with shade-net proving to be the favoring environment. Potassium is a macronutrient that improves the transportation of water and nutrients inside plants, increases the amount of carbohydrates in plant tissue, and improves the synthesis of ATP, which controls the pace of photosynthesis (Maluleke 2022). These findings concur with those of Ramadan et al. (2014) and Lustosa-Filho et al. (2020), who observed differences in the harvest index of plants treated with various fertilizers.

3.5.2.3 Bud water content

The impact of distinct fertilizer types on the bud water content of *Cannabis sativa* L grown in varied conditions is shown in Table 3.7. The results showed that there was a substantial ($P \leq 0.05$) variation between treatments when it came to thebud water content of *Cannabis sativa* L growing in different conditions. Bud water content, according to the results showed that it ranged from 5.8 to 40.2 g. Moreover, results showed that treatment combination of shade-net environment and organic fertilisers resulted in lower water content (5.8 g), while treatment combination of shade-net environment and chemical fertiliser obtained the highest water content (40.2 g), compared to other treatments. The variation between the lowest (5.8) and highest (40.2) water content was 34.4 g. The availability of crop water and fertilizers, particularly potassium, have been shown to have an impact on bud size (Tuckeldoe et al., 2023). In Addition, potassium is the primary nutrient that aids in the management of biomass buildup in plants. Potassium concentration ratios in fertilizers (organic 10% and inorganic/chemical 4.9) varied by 5.1%, which was found to be the source of the difference in bud water content. These findings align with those of El-Mageed et al. (2018), who discovered that plant competition for resources like light and water leads to variations in fruit weight and size.

Table 3.7: The impact of various fertilizer types on the water content, harvest index, and total biomass of *Cannabis sativa* produced in various environments.

Treatments	Total biomass (kg)	Harvest index	Bud Water content (g)
L1F1	0.126(6.1)	0.04(0.16)	9.8(1.2)
L1F2	0.14(2.5)	0.05(0.02)	18.8(2)
L1C	0.33(3.5)	0.12(0.09)	6.6(4.7)
L2F1	0.9(2.1)	0.35(0.1)	5.8(3.6)
L2F2	0.58(10.5)	0.57(0.42)	40.2(4.5)
L2C	0.27(15.1)	0.26(0.03)	9(1.4)
Grand mean	0.3	0.23	15
LSD0.05	0.11	0.17	14.12
Pvalue	**0.004**	**0.001**	**0.005**

L1 = means open-space environment. **L2** = means shade-net environment. **F1** = means organic fertiliser. **F2** = means chemical fertiliser. **C** = means control. The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. The least significant difference between means, or LSD0.05, is used.

3.5.2.4 Conclusion

Determining the effect of both the type of fertilizer and the growing environment on the physiology and yield of *Cannabis sativa* L plants was the aim of this study. Study findings revealed that the chemical/inorganic fertiliser was the main contributor in terms of physiological (chlorophyll and stomatal conductance) performance of the plant, regardless of the growing environment. Likewise, regarding the plant height, yield components such as total biomass, harvest index and water content, a treatment combination of chemical/inorganic fertiliser and plant growth under shade-net environment proved to be the best combination when compared to other treatments.

3.*6* References

Abdelraouf, R. E., El Habbasha, S. F., Taha, M. H., & Refaie, K. M. (2013). Effect of irrigation water requirements and fertigation levels on growth, yield and water use efficiency in wheat. Middle-East Journal of Scientific Research, 16(4), 441-450.

Bernstein, N., Gorelick, J., Zerahia, R., & Koch, S. (2019). Impact of N, P, K, and humic acid supplementation on the chemical profile of medical Cannabis (*Cannabis sativa* L). Frontiers in Plant Science, 10, 1-13.

Campiglia, E., Radicetti, E., & Mancinelli, R. (2017). Plant density and nitrogen fertilization affect agronomic performance of industrial hemp (*Cannabis sativa* L.) in Mediterranean environment. Industrial Crops and Products, 100, 246-254.

Danziger, N., & Bernstein, N. (2022). Too dense or not too dense: Higher planting density reduces cannabinoid uniformity but increases yield/area in drug-type medical cannabis. Frontiers in Plant Science, 13, 1-18.

El-Mageed, T. A., & Semida, W. M. (2015). Effect of deficit irrigation and growing seasons on plant water status, fruit yield and water use efficiency of squash under saline soil. Scientia Horticulturae, 186, 89-100.

Frankowski, J., Przybylska-Balcerek, A., Graczyk, M., Niedziela, G., Sieracka, D., & Stuper-Szablewska, K. (2023). The effect of mineral fertilization on the content of bioactive compounds in hemp seeds and oil. Molecules, 28(12), 1-23.

Forrest, C., & Young, J. P. (2006). The effects of organic and inorganic nitrogen fertilizer on the morphology and anatomy of *Cannabis sativa* “Fédrina” (industrial fibre hemp) grown in Northern British Columbia, Canada. Journal of Industrial Hemp, 11(2), 3-24.

Gwala RS. (2023). The legalisation of Cannabis in South Africa: Proposing an economic value chain model for South Africa. In Rapid Innovation and Development in the Global Cannabis Market, 98-122.

Gul, F., Ahmed, I., Ashfaq, M., Jan, D., Fahad, S., Li, X., & Shah, S. A. (2020). Use of crop growth model to simulate the impact of climate change on yield of various wheat cultivars under different agro-environmental conditions in Khyber Pakhtunkhwa, Pakistan. Arabian Journal of Geosciences, 13, 1-14.

Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thompson, A., & Wolfe, D. (2011). Climate impacts on agriculture: Implications for crop production. Agronomy Journal, 103(2), 351-370.

Hourfane, S., Mechqoq, H., Bekkali, A. Y., Rocha, J. M., & El Aouad, N. (2023). A comprehensive review on *Cannabis sativa* ethnobotany, phytochemistry, molecular docking and biological activities. Plants, 12(6), 1-43.

Kakabouki, I., Kousta, A., Folina, A., Karydogianni, S., Zisi, C., Kouneli, V., & Papastylianou, P. (2021). Effect of fertilization with urea and inhibitors on growth, yield, and CBD concentration of hemp (*Cannabis sativa* l.). Sustainability, 13(4), 1-13.

Kepe, T. (2003). *Cannabis sativa* and rural livelihoods in South Africa: Politics of cultivation, trade and value in Pondoland. Development Southern Africa, 20(5), 605-615.

Kornpointner, C., Martinez, A. S., Marinovic, S., Haselmair-Gosch, C., Jamnik, P., Schröder, K., & Halbwirth, H. (2021). Chemical composition and antioxidant potential of *Cannabis sativa* L. roots. Industrial Crops and Products, 165, 1-8.

Lustosa-Filho, J. F., da Silva Carneiro, J. S., Barbosa, C. F., de Lima, K. P., do Amaral Leite, A., & Melo, L. C. A. (2020). Aging of biochar-based fertilizers in soil: Effects on phosphorus pools and availability to Urochloa brizantha grass. Science of the Total Environment, 709,1-10.

Maluleke, M. K., Moja, S. J., Nyathi, M., & Modise, D. M. (2021). Nutrient concentration of African horned cucumber (*Cucumis metuliferus* L) fruit under different soil types, environments, and varying irrigation water levels. Horticulturae, 7(4), 1-16.

Maluleke, M. K. (2022). Metabolite profile of African horned cucumber (*Cucumis metuliferus* E. May. Ex Naudin) fruit grown under differing environmental conditions. Scientific Reports, 12(1), 1-18.

Moher, M., Llewellyn, D., Jones, M., & Zheng, Y. (2022). Light intensity can be used to modify the growth and morphological characteristics of *Cannabis* during the vegetative stage of indoor production. Industrial Crops and Products, 183, 1-8.

Paucek, I., Appolloni, E., Pennisi, G., Quaini, S., Gianquinto, G., & Orsini, F. (2020). LED lighting systems for horticulture: Business growth and global distribution. Sustainability, 12(18), 1-19.

Reichel, P., Munz, S., Hartung, J., Präger, A., Kotiranta, S., Burgel, L., & Graeff-Hönninger, S. (2021). Impact of three different light spectra on the yield, morphology and growth trajectory of three different *Cannabis sativa* L. strains. Plants, 10(9), 1-24.

Riley, E. D., Vittinghoff, E., Wu, A. H., Coffin, P. O., Hsue, P. Y., Kazi, D. S., & Lynch, K. L. (2020). Impact of polysubstance use on high-sensitivity cardiac troponin I over time in homeless and unstably housed women. Drug and Alcohol Dependence, 217, 1-8.

Saloner, A., Sacks, M. M., & Bernstein, N. (2019). Response of medical Cannabis (*Cannabis sativa* L.) genotypes to K supply under long photoperiod. Frontiers in Plant Science, 10, 1-16.

Shu, S., Yuan, L. Y., Guo, S. R., Sun, J., & Yuan, Y. H. (2013). Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in *Cucumis sativus* L. under salt stress. Plant Physiology and Biochemistry, 63, 209-216.

Tang, K., Fracasso, A., Struik, P. C., Yin, X., & Amaducci, S. (2018). Water-and nitrogen-use efficiencies of hemp (*Cannabis sativa* L.) based on whole-canopy measurements and modeling. Frontiers in Plant Science, 9, 1-14.

Tuckeldoe, R. B., Maluleke, M. K., & Adriaanse, P. J. S. R. (2023). The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (*Capsicum annuum*) varieties. Scientific Reports, 13(1), 1-13.

Vivek, P., & Duraisamy, V. M. (2017). Study of growth parameters and germination on tomato seedlings with different growth media. International Journal of Agricultural Sciences Research, 7(3), 461-470.

Viviers, H. J., Petzer, A., & Gordon, R. (2022). An assessment of solvent residue contaminants related to cannabis-based products in the South African market. Journal of Cannabis Research, 4(1), 1-10.

Wang, J., Vanga, S. K., Saxena, R., Orsat, V., & Raghavan, V. (2018). Effect of climate change on the yield of cereal crops: A review. Climate, 6(2),1-19.

Yang, Y., Zha, W., Tang, K., Deng, G., Du, G., & Liu, F. (2021). Effect of nitrogen supply on growth and nitrogen utilization in hemp (*Cannabis sativa* L.). Agronomy, 11(11), 1-12.

CHAPTER 4 : Biochemical constituents of *Cannabis sativa* L grown under varying fertiliser types and environment: its potential role in human nutrition

4.1 Abstract

Most industrial crops, including *Cannabis sativa* L., an Asian native plant with both medical and nutritional properties, have biochemical components that are impacted by variables like climate and fertilisers. Growers can maximize crop quality through the careful combination of these parameters, potentially leading to the maximization of profits. Humans have traditionally used *Cannabis sativa* L for a variety of uses, including medicine, as well as a raw ingredient for goods with added value including drinks, cakes, cookies, and oil. This has generated a lot of interest in Cannabis farming, which led to challenges in producing high-quality plant materials because there is currently insufficient scientifically validated cultivation protocol, especially under South African climates. The investigation was carried out in 2023 in a shade-net and open-space environment at the University of South Africa, Florida Campus, Johannesburg, Gauteng province, South Africa (lat. 26°10'30"S, long. 27°55'22.8"E). The goal of the study was to assess how various fertiliser types affect the biochemical components of *Cannabis sativa* L grown in different environments (open-space or shade-net), to conduct a comparative analysis that would help growers produce high-quality *Cannabis sativa* L crops for commercial market. Fresh and freeze-dried samples were used to measure biochemical constituents. Combination of shade-net environment and chemical fertiliser resulted in superior total phenols (14.7 GAE/100g DW), vitamin C (66 mg/100g DW) and calcium (96.7 mg/100g DW) compared to other treatments. As a result, growers should consider the shade-net environment and chemical fertiliser combination for yield and quality maximisation.

Keywords: Macro-nutrients. Micro-nutrients. Recommended daily intake. Vitamins.

4.2 Introduction

Many factors directly affect the biochemical components of crops, including the kind of fertilizer applied and the growth environment (Sheldon et al. 2021). Instead of using a natural setup, most crop growers choose to employ various types of fertilisers and conditions in order to maximize quality (Maluleke et al. 2022). Very little is known about the interactions between growing conditions and different types of fertilizers, particularly with regard to the biochemical components of *Cannabis sativa* L., as opposed to the numerous studies on the effects of different types of fertilizers on crop development, growth, and yield (Vera et al. 2004; Yep and Zheng 2020; Wielgusz et al. 2022). The medicinal benefits of *Cannabis sativa* have been widely reported by various scholas as being, (i) important in lowering blood pressure, (ii) reducing inflammation, (iii) treatment of anxiety disorders, (iv) fighting cancer (Salami et al. 2020; Salehi et al. 2022; Malabadi et al. 2023). In the food industry, humans have traditionally used *Cannabis sativa* L for a variety of uses such as a raw ingredient for goods with added value including drinks, cakes, cookies, and oil (Smeriglio et al. 2016; Sorrentino 2021; Rasera et al. 2021; Spano et al. 2022). Laws that forbade the private cultivation and consumption of Cannabis were declared unlawful by the South African Constitutional Court in 2020. In addition, the South African government also projected that the country's Cannabis production, including its cultivation, processing, and commercialisation, could provide billions in revenue annually, and may lead to about 10 000 to 25 000 employments in the industry. This has generated a lot of interest in Cannabis farming, which led to challenges in producing high-quality plant materials because there is currently insufficient scientifically validated cultivation protocol, especially under South African climates. As a result, the goal of the study was to assess how various fertilizer types affect the biochemical components of *Cannabis sativa* L grown in two different growing environments (open-space and shade netting), in order to conduct a comparative analysis that would help growers produce high-quality *Cannabis sativa* L crops for the purpose of commercialisation.

4.3 Materials and procedures

The investigation was carried out in 2023 in a shade-net and open-space environment, Figures 3.1 and 3.2, at the University of South Africa, Florida Campus, Johannesburg, Gauteng province, South Africa (lat. 26º10’30”S, long. 27º55’22.8”E). The minimum and maximum temperatures in shade-net (13-30°C) and open-space environment (14-34°C) was recorded during the experimental period shown on Table 3.3. Plants were exposed to natural light for a minimum of 10 to 12 hours every day during the trial period (Table 3.4). Consequently, the trial was a fully randomised design with two independent variables: (i) different fertilisers (organic and inorganic/chemical) in deferring environments (shade-net and open-space). Certified seeds obtained from the research institution (Cape Town, South Africa) were used to germinate seedlings for the experiment. Healthy, uniform seedlings of *Cannabis sativa*, that were 30 days old and germinated from peat substrate were transplanted to 30 cm depth × 30c m width pots filled with, (i) loam soil under differing environments (shade -net and open-space) during spring to summer of 2023. Ten (10) replicates were used per treatment. After the establishment of seedlings, plants were supplied with five (5) grams of granule fertilisers of different types, namely (i) organic fertiliser (GuanoBoost, Pretoria, South Africa) with Nitrogen (N), Phosphorus (P) and Potassium (K) [N-3, P-2, K-5] and inorganic/chemical fertiliser (Nitrogreen KAN/LAN, Protek, Pretoria, South Africa) with Nitrogen (N), Phosphorus (P) and Potassium (K) [N-2, P-3, K-2] (Table 1). Plants grown on loam soil with fertiliser treatment was classified as control. The treatments were imposed two (2) weeks later, after establishment. Plants were well irrigated throughout the experiment period. Briefly the area (depth × width) 30cm × 30cm = 900cm^2, A =$\pi\left(\frac{d}{2}\right)\times 2$ d =286.5 cm^2 planting pots. Soil mineral analysis was carried out at the Agricultural Research Council (25° 44' 19.4" S 28° 12' 26.4" E), Arcadia, Pretoria (Table 2). Notably, the experiment was conducted strictly in compliance with the protocols and requirements issued by the Research and Higher Degree Committee of the UNISA College of Agriculture and Environmental Sciences. The Department of Health of the Republic of South Africa **(Permit no: POS 093/2023/2024)** and the UNISA-CAES Health Research Ethics Committee **(reference number 2022/CAES_HREC/006)** have also granted ethical authorization for the study.

4.4 Data collection

4.4.1 Vitamin C measurement

The *Cannabis sativa* L. bud samples were freeze-dried for 72-hour period using a freeze-drier (HARVEST-RIGHT, Barcelona). In triplicates, buds samples were analysed using a slightly modified version of the protocol described by Moyo et al. (2018). Sterile food blender was used to properly homogenize freeze-dried Cannabis buds before nutritional analysis. 10 mL of 5% metaphosphoric acid were added to each sample after it had been weighed (1 gram) into a tube. After sonicating for 15 minutes, each tube was placed in an ice-cold water bath for centrifugation and filtering. The model system Prominence-i HLCP-PDA was used for the analysis. To accomplish chromatographic separation, a C18 Luna ® column (150/4.6 mm, 5 µl) was kept at 25 µC. A 1 ml/min flow rate of an acetonitrile:formic acid (99:0.9:0.1) water-based isocratic mobile phase was employed. The detector was calibrated at 245 nm, and each injected sample had a volume of 20 µl. A comparison of each sample to the L-ascorbic acid calibration curve allowed quantification of vitamin C in each sample. Samples of vitamin C were measured in milligrams per 100 grams of dry weight (mg/100g DW).

4.4.2 Vitamin E measurement

The Martin et al. (2000) method, which Tuckeldoe et al. (2023) adapted, was used to assess the levels of vitamin E (a- and g-tocopherol). Reverse-phase high performance liquid chromatography (HPLC) was used, in short, to assess the vitamin E content of *Cannabis sativa* L. bud samples. 100 mL of the homogenized material were mixed with 100 mL of ethanol. After vertexing, tocopherols were extracted into 500 mL of hexane that contained 0.002% butylated hydroxyl toluene (BHT, Sigma Chemical Co., St Louis, MO). The combination was supplemented with Tocol, an internal standard (Hoffmann-La Roche, Nutley, NJ). The materials were centrifuged for five (5) minutes at 800 rpm. The supernatant was reconstituted in 100 mL of methanol following collection and drying in a nitrogen gas stream. Tocopherols were separated by HPLC using a 3 mm C18 reverse-phase column (Perkin-Elmer, Norwalk, CT).

The mobile phase was supplied at a flow rate of 1.2 mL/min and consisted of 1% water in methanol with 20 mM lithium perchlorate. The samples were injected using an autosampler (1100 series, Hewlett Packard Co., Wilmington, DE). An LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN) operating at an applied potential of 10.6 V was used to identify eluted peaks. Tocopherols eluted in separate peaks with a retention duration of two to six minutes. The amount of vitamin E was expressed as milligrams per 100 g dry weight (mg/100 g DW).

4.4.3 Total flavonoids measurement

Triplicate *Cannabis sativa* bud samples were quantified using the aluminum chloride colorimetric method with slight modification as described by Maluleke et al. (2021) and Tuckeldoe et al. (2023). Briefly, 1 mL methanol was used to dissolve 50 mg of Cannabis bud powder (1 mg/mL ethanol), which was then combined with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution. After five (5) minutes of incubation, 0.3 mL of 10% AlCl3 solution was added to the mixture, and it was allowed to stand for six minutes. After adding 2 mL of NaOH solution, double-distilled water was added to the combination until its total volume reached 10 mL. The mixture was allowed to settle for fifteen (15) minutes, and then the absorbance at 510 nm was measured. The total flavonoid concentration was reported in catechin equivalents per hundred grams dry weight (CE/100g DW).

4.4.4 Total phenols measurement

With minor adjustments, the method utilized by Maluleke et al. (2021), adopted by Tuckeldoe et al. (2023) was used to determine the total phenolic content from triplicate samples of *Cannabis sativa*. Using gallic acid as a reference, the total phenol concentration of freeze-dried *Cannabis sativa* plant buds was used to extract the total phenolic content (Sigma, St. Louis, MO). A portion of the extract was oxidized using the Folin Ciocalteu reagent (2 N, Sigma, St. Louis, MO) at a volume/volume ratio of 10:1. A microplate reader (Synergy HT, Bio-Tek, Winooski, VT) was used to measure the absorbance at 750 nm after the samples were incubated in 96-well microplates for 20 minutes at room temperature. The total phenolic content was measured in milligrams per hundred-gram dry weight using gallic acid equivalents (GAE/100g DW).

4.4.5 Macro and micro-nutrients measurement

A diffused microwave system (MLS 1200 Mega; Milestone S.r. L, Sorisole, Italy) was used to digest samples of freeze-dried *Cannabis sativa* L buds. The process of congelating and drying the samples was then followed, with some slight adjustments, as outlined in the method described by Moyo et al. (2018), followed by Tuckeldoe et al. (2023). Aliquots of 2 mL of HNO3 (67% analphur) and 1 mL of H_20_2 (30% analytical grade) were introduced into polytetrafluoroethylene vessels containing three (3) replicates of 25 mg each. Upon digestion, each solution was diluted to 15 mL in a test tube filled with deionized water, and then it was subjected to Inductively Coupled Plasma-Mass Spectrometry analysis (ICP-MS). The analysis was performed using an octapole reaction system (ORS 3) and quadrupole mass analyzer (Agilent 7700; Agilent Technologies, Tokyo, Japan). Analysis was done on several nutrient elements, including iron (Fe), calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P). The single element certified reference material (Analytika Ltd, Czech Republic) was diluted accordingly with deionized water (18.2 MΏ.cm, Direct-Q; Millipore, France) at 1.000 ± 0.002 g/l for each element to create the calibration solution. The accuracy of the measurement was verified using certified reference material of water TM-15.2 (National Water Research Institution, Ontario, Canada). Micro- and macronutrients were reported as milligrams of dry weight per 100 grams (mg/100g DW).

4.4.8 Statistical evaluation

Data on the biochemical components and nutritional content of *Cannabis sativa* L buds grown in distinct growing settings (shade-net and open-space) and treated to different types of fertilisers (organic and inorganic/chemical) were analyzed using a two-way analysis of variance (ANOVA). Vitamins C and E, total flavonoids, total phenols, and macro- and micronutrients were among the dependent variables that were measured. For every variable under study, the least significant difference (LSD) was taken into account. Version 10 of StatSoft (USA) was used for all statistical analysis.

4.5 Results and discussion

4.5.1 Vitamin C, vitamin E, total flavonoids, and total phenols

The impact of various fertilizer types on the biochemical components (vitamins C, E, total flavonoids, and total phenols) of *C. sativa* L. cultivated in various settings is shown in Table 4.1. The findings of the study showed a substantial ($P \leq 0.05$) variation in the biochemical components between treatments. The results showed that the range of vitamin C was 26–66 mg/100g DW. Moreover, the vitamin C content was shown to decrease from 66 to 26 mg/100g DW in loamy soil (control) and shade-net environments, while increasing from 26 to 66 mg/100g DW in the treatment combination of shade-net environment and chemical fertilizer. The variation between the highest vitamin C content (66) and recommended daily intake (95) was 29 mg.

Findings from this study may indicate that *Cannabis sativa* L provides about 69% of the daily vitamin C needed by humans. For growth and repair, vitamin C is necessary for every tissue in the human body (Tuckeldoe et al. 2023). Furthermore, it has a role in the synthesis of collagen, an essential protein that builds skin, tendons, ligaments, and blood vessels (Maluleke et al. 2024). Moreover, it is necessary for wound healing and the production of scar tissue (Uusiku et al. 2010). Results from this investigation may suggest that consuming *Cannabis sativa* and value-added products cultivated in a shade-net environment with chemical fertilisers may help prevent conditions like excessive bleeding, swollen joints, skin problems, and poor wound healing, all of which are associated with low vitamin C intake in the human diet (Achanglinkame et al. 2019; Tuckeldoe et al. 2023; Maluleke et al. 2024).

Regarding vitamin E, findings of the study indicated that the range was 8.7 to 32 mg/100g DW. Furthermore, the treatment combination of an open-space environment and chemical fertiliser raised vitamin E content from 8.7 to 32.3 mg/100g DW, whereas loam soil (control) paired with a shade-net environment decreased it from 32.3 to 8.7 mg/100g DW. There was (10.3 mg) variation between the highest vitamin E (32.3) and recommended daily intake (22). Findings obtained from this study suggest that the *Cannabis sativa* vitamin is higher than the recommended daily intake by 10.3 mg. Vitamin E has demonstrated significant benefits in the prevention and treatment of several diseases in the human body, owing to its anti-inflammatory, antioxidant, and immune-boosting capabilities (Achanglinkame et al. 2019; Tuckeldoe et al. 2023). Thus, consumption of *Cannabis sativa* L buds and its value-added products created from buds harvested from crops cultivated in an open-space setting subjected with organic fertilizer may help prevent conditions like muscle pains and constant fatigue, which are all associated with inadequate vitamin intake in human diet (Goulas and Manganaris, 2012).

In terms of total flavonoids, it was found that it ranged between 1.02 and 2.9 CE/100g DW, according to the study results. Loam soil (control) and that shade-net environment reduces total flavonoids from 2.9 to 1.02 CE/100g DW, meanwhile treatment combination of shade-net and chemical fertiliser increate it from 1.02 to 2.9 CE/100g DW. The difference between the highest total flavonoids (2.9) and recommended daily intake (225) was 252.5mg. This means that *Cannabis sativa* grown under the treatment combination of shade-net environment and chemical fertiliser, which obtained the highest concentration could contribute about 1.3% of total flavonoids required in human daily diet. Biological substances called flavonoids provide several health advantages, such as antiviral, anticancer, and antioxidant qualities (Maluleke et al. 2024). Additionally, they also have cardio- and neuroprotective properties which aid in enhancement of human health (Tuckeldoe et al. 2023).

Although the study's values were below the recommended daily intake, the consumption of *Cannabis sativa* buds and its value-added products created from buds material harvested from plants grown under combined interaction of chemical fertiliser and shade-net could help prevent conditions like diabetes and cardiovascular disease, which are linked to low flavonoid intake in the diet (Maluleke et al. 2021).

Total phenols, according to the study findings ranged from 1.6 to 14.7 GAE/100g DW. Furthermore, loam soil (control) in conjunction with shade-net decreased total phenols from 14.7 to 1.6 GAE/100g DW. The highest total phenolic content (14.7 GAE/100g DW) was observed under the treatment combination of shade-net environment and chemical fertiliser. There was a 985.3 mg discrepancy between the maximum total phenolic content (14.7) and the recommended daily consumption (1000). Phenolic compounds are essential for defensive mechanisms including anti-ageing, anti-inflammatory, antioxidant, and anti-proliferative actions from a physiological perspective (Smeriglio et al. 2016; Al Khoury et al. 2021).

The results of this study suggest that *Cannabis sativa* cultivated in a shade-net setting with organic fertiliser contributes roughly 1.5% of the daily phenols that humans need. Although values reported were lower, in comparison to the recommended daily intake, consuming *Cannabis sativa* buds and its value-added products harvested from plants grown from treatment combination of shade net and chemical fertiliser may help reduce the symptoms of illnesses like skin burns and kidney damage, which are associated with low phenol levels in the human diet, even if the values achieved are lower (Maluleke et al. 2024).

Table 4.1: Impact of different fertiliser types on the biochemical constituents of *Cannabis sativa* L grown under varying environment.

Treatments	Vitamin C (mg/100g DW)	Vitamin E (mg/100g DW)	Total flavonoids (CE DW)	Total phenols (GAE DW)
L1F1	27.5(3.6)	12.7(2.3)	1.47(0.9)	3.5(1.4)
L1F2	51.5(8.5)	32.3(8.5)	2.8(1.5)	11.2(2.0)
L1C	26.8(6.6)	30.5(1.5)	1.5(0.3)	3.6(0.2)
L2F1	37(4.6)	13.1(2)	1.9(0.4)	4.1(1.5)
L2F2	66(4.6)	26.5(5.9)	2.9(0.8)	14.7(2.7)
L2C	26(7.8)	8.7(8)	1.02(1.0)	1.6(1.2)
Grand mean	39.1	20.62	1.93	6.45
LSD0.05	8.71	7.03	0.939	2.088
Pvalue	**0.001**	**0.001**	**0.001**	**0.001**

L1 = means open-space environment. **L2** = means shade-net environment. **F1** = means organic fertiliser. **F2**= means chemical fertiliser. **C** = means control. The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. The least significant difference between means, or LSD0.05, is used.

4.5.2 Macro and micro-nutrients

Table 4.2 depict the impact of various fertiliser types and growing environments on the macro and micronutrients of *Cannabis sativa* L. The study findings showed that there were substantial (P≤0.05) variations in the macro- and micronutrients content (calcium, magnesium, phosphorus, potassium, and iron) in *Cannabis sativa* L. grown in various growing settings and treated with different types of fertilisers.

4.5.2.1 Calcium

The findings of the study indicated that the range of calcium was 32.8 to 96.7 mg/100g DW. The results also showed that while the combination of chemical fertiliser and shade-net treatment increased the calcium content from 32.8 to 96.7 mg/100g DW, the combination of loam soil and shade-net reduced it from 96.7 to 32.8 mg/100g DW. According to Maluleke et al. (2024), calcium is necessary for the human body to contract its muscles and for its nerves to transmit information from the brain to every area of the body. Moreover, calcium maintains the blood arteries that carry blood throughout the body and promotes the production of hormones that have an impact on a variety of physiological functions (Tuckeldoe et al. 2023). There was a 1053 mg discrepancy between the highest calcium content (96.7) and the required daily requirement (1150).

According to study values gathered from this study, *Cannabis sativa* buds may provide roughly 8.3% of the daily calcium needs in human daily diet. Furthermore, even though *Cannabis sativa* had a lower calcium content compared to the recommended daily intake, consuming *Cannabis sativa* L and its value-added products derived from chemical fertilisers and shade-net harvesting may help lessen symptoms like loss of appetite, stomach pains, cramping in the muscles, and frequent urination, of which are align to insufficient calcium intake in the human diet (Achanglinkame et al. 2019). These findings are in harmony with those of Lekhuleni et al (2024), who are of a view that plant-based products should be full maximised to prevent certain alignments, which may result in human health and nutrition enhancement.

4.5.2.2 Magnesium

The findings of the study indicated that the range of magnesium was 157 to 597 mg/100g DW. Furthermore, the results showed that magnesium content decreased from 597 to 157 mg/100g/DW when the plant was to shade-net environment and chemical fertiliser treatment, while it raised from 157 to 597 mg/100g DW when subjected to open-space environment and chemical fertiliser. There was a 232 mg discrepancy between the maximum magnesium content (597) and the required daily consumption (365). The magnesium concentration of *Cannabis sativa* buds was found to be about three times higher in this study when compared to the recommended daily intake.

According to Usiku et al. (2010), magnesium is a necessary cofactor for most enzyme systems that regulate a variety of biochemical processes in the body, such as blood glucose regulation, muscle and neuron function, protein synthesis, and blood pressure regulation. Study findings may indicate that consuming *Cannabis sativa* buds along with value-added goods derived from chemical fertiliser and open-space environments may help prevent diseases like diabetes, hypertension, and heart-related disorders (Tuckeldoe et al. 2023; Maluleke et al. 2024). These findings agree with those of Lekoba et al. (2024), who recommended the inclusion of plant-based diet as a source of nutrients need for fulfilment of required daily nutrients.

4.5.2.3 Phosphorus

The study findings indicated that the range of phosphorus content was 198 to 609 mg/100g DW. According to study results, phosphorus concentration in loam soil (control) under shade-net conditions decreased from 609 to 198 mg/100g DW, whereas it increased in plants subjected to an open-space environment combined with chemical fertiliser, from 198 to 609 mg/100g DW. The maximum phosphorus level (609) differed by 366 mg from the recommended daily requirement (975 mg). This suggests that value-added products and *Cannabis sativa* buds could provide almost 62% of the phosphorus that people need each day.

Phosphorus is mostly needed for the synthesis of bones and teeth, but it also has a significant impact on how the body utilises fats and carbohydrates (Maluleke et al. 2024). In addition, the body needs it to produce protein for tissue and cell growth, maintenance, and repair (Akbari et al. 2022). As a result, consumption of *Cannabis sativa* and its value-added products created from buds harvested in an open space setting along with chemical fertiliser may help prevent ailments like fatigue, loss of appetite, and respiratory difficulties, of which are symptoms associated with low phosphorus in the diet (Maluleke et al. 2021).

4.5.2.4 Potassium

The findings of the study indicated that the range of potassium was 349 to 989 mg/100g DW. The results also showed that, where the treatment of an open-space environment and chemical fertiliser were combined, there was increased in the potassium content from 349 to 989 mg/100g DW, while the combination of loam soil (control) and shade-net environment decreased it from 989 to 349 mg/100g DW. The difference between the highest potassium concentration (989) and the recommended daily requirement (2850) was 1861 mg. This suggests that *Cannabis* sativa may provide almost 35% of the phosphorus that humans need each day. According to the results of this study suggest that conditions like fatigue, cramping in the muscles, and irregular heartbeat, all which are linked to low potassium intake in the diet may be prevented by consuming *Cannabis sativa* buds and value-added products harvested from plants grown under an open-space environment and chemical fertiliser. These findings are in consistent with those of Tuckeldoe et al. (2023), who suggested that plant-based phosphorus could assist in curbing malnutrition, which are a threat to human health and well-being.

4.5.2.5 Iron

The study results showed that the iron concentration varied between 4.17 and 11.7 mg/100g DW. Furthermore, the study findings showed that while the iron content rose from 4.17 to 11.7 mg/100g DW in the shade-net environment when combined with chemical fertiliser, it decreased from 11.7 to 4.17 mg/100g DW in the open space and environment combined organic fertiliser. The difference of 13.3 mg was found between the daily required consumption (17.5) and the highest iron level (4.17). In other words, *Cannabis sativa* may provide roughly 23% of the daily iron that humans require. Although the iron content value is low, consuming *Cannabis sativa* buds and its value-added products created from buds harvested from plants grown under the treatment combination of chemical fertiliser and shade-net may help reduce conditions like inflammation, abnormal heartbeat, chest pain, and persistent fatigue, which all are associated with low iron intake in the human diet (Borochov-Neori et al. 2008; Uusiku et al. 2010; Achaglinkame et al. 2019; Zhang et al. 2021).

Table 4.2: The effect of different fertiliser types on macro and micro-nutrients (mg/100g DW) of *Cannabis sativa* L. grown under varying environment.

Treatments	Calcium	magnesium	Phosphorus	Pottasium	Iron
L1F1	33.7(8.3)	378(30.3)	351(62.2)	467(50.9)	4.17(3.1)
L1F2	79.3(13.5)	597(42.5)	609(34.1)	989(29.3)	12.7(2)
L1C	39.7(6.5)	325(66.7)	346(80.5)	378(28.1)	8.5(1.5)
L2F1	36.2(7.2)	222(22.8)	239(40.8)	399(49.4)	4.7(1.4)
L2F2	96.7(4.0)	157(36.3)	597(39.7)	956(40.9)	11.7(3.1)
L2C	32.8(4.2)	553(57.5)	198(27.5)	349(37.2)	4.2(1.2)
Grand mean	53.1	372	390	590	7.64
LSD0.05	11.24	74.3	90.9	223.9	2.66
Pvalue	**0.001**	**0.001**	**0.001**	**0.001**	**0.004**

L1 = means open-space environment. L2 = means shade-net environment. F1 = means organic fertiliser. F2= means chemical fertiliser. C = means control. The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. The least significant difference between means, or LSD0.05, is used.

4.6 Conclusion

The purpose of this study was to ascertain how the growing environment and type of fertiliser combination affect the biochemical components of *Cannabis sativa* L. Study findings revealed that biochemical constituents such as vitamin C, phenols, and flavonoids were superior in the treatment combination of shade netting and supplementation with chemical/inorganic fertiliser, while macro-nutrients (magnesium, phosphorus, potassium) were higher in the treatment combination of an open-space environment and chemical/inorganic fertiliser. Micro-nutrient (iron) was superior under treatment combination of a shade netting environment and chemical fertiliser. This study suggests that growers should be mindful of plant growth factors such as the environment and fertiliser types, which aid positively in the production of enough plant material with high quality for both the medical and food industry, and creation of value-added products. As a result, the commercialisation of *Cannabis sativa* will promote stability and sustainability, which helps to achieve Sustainable Development Goals 1) and 2, which are to eradicate poverty and hunger, through the production of adequate high-quality plant material.

4.7 References

Achaglinkame, M. A., Aderibigbe, R. O., Hensel, O., Sturm, B., & Korese, J. K. (2019). Nutritional characteristics of four underutilized edible wild fruits of dietary interest in Ghana. Foods, 8(3), 1-12.

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors, 48(3), 611-633.

Al Khoury, A., Sleiman, R., Atoui, A., Hindieh, P., Maroun, R. G., Bailly, J. D., & El Khoury, A. (2021). Antifungal and anti-aflatoxigenic properties of organs of *Cannabis sativa* L.: Relation to phenolic content and antioxidant capacities. Archives of Microbiology, 203(7), 4485-4492.

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Journal of Taibah University for Science, 9(4), 449-454.

Borochov-Neori, H., Judeinstein, S., Greenberg, A., Fuhrman, B., Attias, J., Volkova, N., & Aviram, M. (2008). Phenolic antioxidants and antiatherogenic effects of Marula (*Sclerocarrya birrea subsp. caffra*) fruit juice in healthy humans. Journal of Agricultural and Food Chemistry, 56(21), 9884-9891.

Goulas, V., & Manganaris, G. A. (2012). Exploring the phytochemical content and the antioxidant potential of Citrus fruits grown in Cyprus. Food Chemistry, 131(1), 39-47.

Malabadi, R. B., Kolkar, K. P., Chalannavar, R. K., & Baijnath, H. (2023). *Cannabis sativa*: Difference between medical *Cannabis* (marijuana or drug type) and industrial hemp. GSC Biological and Pharmaceutical Sciences, 24(3), 377-381.

Maluleke, M. K., Moja, S. J., Nyathi, M., & Modise, D. M. (2021). Nutrient concentration of African horned cucumber (*Cucumis metuliferus* L) fruit under different soil types, environments, and varying irrigation water levels. Horticulturae, 7(4), 1-16.

Maluleke, M. K. (2022). Metabolite profile of African horned cucumber (*Cucumis metuliferus* E. May. Ex Naudin) fruit grown under differing environmental conditions. *Scientific Reports*, *12*(1), 1-18.

Maluleke, M. K., Ralulimi, T. S., & Machete, M. (2024). Biochemical constituents and the role of African wild medlar (*Vangueria infausta*) in human nutrition: A review. Discover Sustainability, 5(1), 1-10.

Martin, A., Prior, R., Shukitt-Hale, B., Cao, G., & Joseph, J. A. (2000). Effect of fruits, vegetables, or vitamin E–rich diet on vitamins E and C distribution in peripheral and brain tissues: Implications for brain function. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 55(3), 144-151.

Moyo, M., Amoo, S. O., Aremu, A. O., Gruz, J., Šubrtová, M., Jarošová, M., Tarkowski, P., & Doležal, K. (2018). Determination of mineral constituents, phytochemicals and antioxidant qualities of Cleome gynandra, compared to Brassica oleracea and Beta vulgaris. Frontiers in Chemistry, 5, 1-9.

Santos-Zea, L., Gutiérrez-Uribe, J. A., & Serna-Saldivar, S. O. (2011). Comparative analyses of total phenols, antioxidant activity, and flavonol glycoside profile of cladode flours from different varieties of Opuntia spp. Journal of Agricultural and Food Chemistry, 59(13), 7054-7061.

Salehi, A., Puchalski, K., & Asgary, S. (2022). Differentiating *Cannabis* products: Drugs, food, and supplements. Frontiers in Pharmacology, 13, 1-19.

Salami, S. A., Martinelli, F., Giovino, A., Bachari, A., Arad, N., & Mantri, N. (2020). It is our turn to get Cannabis high: Put cannabinoids in food and health baskets. Molecules, 25(18), 1-24.

Sheldon, K., Shekoofa, A., Walker, E., & Kelly, H. (2021). Physiological screening for drought-tolerance traits among hemp (*Cannabis sativa* L.) cultivars in controlled environments and in field. Journal of Crop Improvement, 35(6), 816-831.

Smeriglio, A., Galati, E. M., Monforte, M. T., Lanuzza, F., D'Angelo, V., & Circosta, C. (2016). Polyphenolic compounds and antioxidant activity of cold-pressed seed oil from Finola cultivar of *Cannabis sativa* L. Phytotherapy Research, 30(8), 1298-1307.

Sorrentino, G. (2021). Introduction to emerging industrial applications of Cannabis (*Cannabis sativa* L.). Rendiconti Lincei. Scienze fisiche e naturali, 32(2), 233-243.

Spano, M., Di Matteo, G., Ingallina, C., Sobolev, A. P., Giusti, A. M., Vinci, G., & Mannina, L. (2022). Industrial hemp (*Cannabis sativa* L.) inflorescences as novel food: The effect of different agronomical practices on chemical profile. Foods, 11(22), 1-19.

Tuckeldoe, R. B., Maluleke, M. K., & Adriaanse, P. J. S. R. (2023). The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (Capsicum annuum) varieties. Scientific Reports, 13(1), 1-13.

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. Journal of Food Composition and Analysis, 23(6), 499-509.

Vera, C. L., Malhi, S. S., Raney, J. P., & Wang, Z. H. (2004). The effect of N and P fertilization on growth, seed yield and quality of industrial hemp in the Parkland region of Saskatchewan. Canadian Journal of Plant Science, 84(4), 939-947.

Wielgusz, K., Praczyk, M., Irzykowska, L., & Świerk, D. (2022). Fertilization and soil pH affect seed and biomass yield, plant morphology, and cadmium uptake in hemp (*Cannabis sativa* L.). Industrial Crops and Products, 175, 1-9.

Yep, B., & Zheng, Y. (2020). Potassium and micronutrient fertilizer addition in a mock aquaponic system for drug-type *Cannabis sativa* L. cultivation. Canadian Journal of Plant Science, 101(3), 341-352.

CHAPTER 5: SUMMARY AND FUTURE WORK

5.1 General summary

Regardless of the growing environment, the study's findings showed that the chemical/inorganic fertilizer was the primary factor influencing the physiological (chlorophyll and stomatal conductance) performance of the *Cannabis sativa* plant.

The best combination of plant treatment was growth under shade netting and chemical/inorganic fertilizer for yield components like harvest index and water content. While the concentration of macronutrients (magnesium, phosphorus, and potassium) was higher in the treatment combination of open-space environment and chemical/inorganic fertiliser, biochemical constituents like vitamin C, phenols, and flavonoids were superior in the shade netting and chemical/inorganic fertiliser treatment. The concentration of the micronutrient (iron) was superior under a treatment combination of growth under shade netting and treatment with chemical fertiliser. This study suggests that growers should be mindful of the many plant growth factors, particularly the environment in which the plants are grown and the fertiliser types, which aid positively in the production of important plant compounds that can be purified at high quality for the preparation of value-added products for both the medical and food industries, Moreover, the production of sufficient quality plant material of *Cannabis sativa* creates stability and sustainability in the commercialisation of *Cannabis sativa*, subsequently achieving Sustainable Development Goals 1 and 2 that focus on ending poverty and hunger.

5.1.1 Conclusion 1

By measuring and comparing dependent variables like chlorophyll content, stomatal conductance, plant height, stem diameter, total biomass, harvest index, and bud water content across all treatments, this conclusion addressed **study Objective 1**, which was to evaluate the impact of different fertiliser types on the growth and yield of *Cannabis sativa* L plants grown under varying environments (shade-net and open space).

Chlorophyll content, stomatal conductance, plant height and stem diameter

The findings of the study showed that when plants were grown under shade netting and subjected to inorganic fertiliser, there was a discernible rise in the amount of chlorophyll. Plants cultivated in the open space and treated with chemical fertiliser had improved stomatal conductance when compared to other treatments. Plants cultivated in open spaces and with chemical fertiliser were shown to have thicker stem diameters and taller plant than plants produced with other combinations of treatments.

Harvest index, total biomass, and water content

According to study findings, total biomass increased when plants were cultivated under the open space and treated with chemical fertiliser. When plants were subjected to shade netting and received chemical fertiliser treatment, the harvest index was superior compared to other treatments. Plants cultivated under shade netting and fertilised with chemical fertiliser showed higher plant bud water contents than other plants subjected to different treatments.

The assertion that research objective 1 was suitably addressed is supported by this succinct findings of the study, which detail plant development, growth, and yield under differing treatments.

5.1.2 Conclusion 2

This conclusion addressed **Objective 2** that was to assess the most effective combination, namely (i) fertiliser types (organic or inorganic/chemical) and (ii) plant growth environments (under shade netting and in the open) for growing nutrient dense *Cannabis sativa* buds and compare all measured biochemical constituents (vitamin C, vitamin E, total flavonoids, total phenols, macro- and micronutrients) across all treatments, while comparing them with recommended daily intake (RDI).

Vitamin C, vitamin E

The findings of the study demonstrated that applying different types of fertilisers under varying growth environment to *Cannabis sativa* plant substantially altered the vitamin content. When inorganic fertiliser and shade netting were used together as a treatment, the content of vitamin C was higher than with other treatments. When plants were treated with inorganic fertiliser under open space, the amount of vitamin E content, also content increased.

Total flavonoids and total phenols

There was a notable variation found in the total flavonoids and total phenols. When plants were grown under shade netting and treated with an inorganic fertiliser, the content of total flavonoids and total phenols was much greater than those of other treatments.

Macro and micro-nutrients

The concentrations of macronutrients (calcium, magnesium, phosphorus, and potassium) and micronutrients (iron) varied significantly, according to the study findings. *Cannabis sativa* plants subjected to the combination of open space and inorganic fertiliser had higher concentrations of magnesium, phosphorus, and potassium other treatments. When growing under shade netting and inorganic fertiliser combined, the concentration of calcium and iron in *Cannabis sativa* plants increased.

According to the study findings, there was a statistically variation in the concentrations of several biochemical elements. These elements included calcium, phosphorus, magnesium, potassium, iron, vitamin C, and vitamin E. This indicates a relationship between the dependent variables and the observed independent variables (fertiliser types and growing environment). Therefore, conclusions regarding both independed and dependent elements are acknowledged.

The claim that study objective 2 was suitably addressed is supported by this succinct presentation of study data that describes the biochemical results of *Cannabis sativa* buds that developed in plants that were grown in different fertiliser types and growing environments.

5.2 Future work

The results of the study were the firstly to illustrate the combined effect of different types of fertilisers and growing environments on the growth, physiological performance, yield, and biochemical constituents of *Cannabis sativa* L. Future research should take the following into account:

- The combined effect of various soil types, irrigation schedules, and fertilizer types on the metabolite profile of *Cannabis sativa* L cultivated in a range of environmental conditions.

5.3 Contribution to agriculture research and knowledge base

The results of this experiment have shown conclusively that growing plants under shade netting and using inorganic fertilisers combined can boost the output of *Cannabis sativa* L crops. Additionally, it showed that when inorganic fertilisers were combined with shade netting, it promotes plant development, biochemical components like vitamin C, total flavonoids, and total phenols rose. This investigation has provided solid evidence that combinations of inorganic fertilisers and growth of plants under shade netting contribute towards an increase in the yield of *Cannabis sativa* L crops. Moreover, it also demonstrated that biochemical constituents such as vitamin C, total flavonoids and total phenols increased under the treatment combination of inorganic fertilisers and plant growth under shade netting.

Nevertheless, regardless of the type of fertiliser used, crops that are grown under open space seem to perform better physiologically. In terms of stomatal conductance and chlorophyll content, plants that were grown under open space subjected to inorganic fertiliser had higher concentrations of macronutrients such potassium, phosphorus, and magnesium.

This knowledge is helpful because it gives growers a basic understanding of the various components that work together to maximise crop yield while maintaining quality. Additionally, it accelerates the UN SDGs 1 and 2, which aim to eradicate poverty and hunger, while providing crucial information about the nutritional contribution of *Cannabis sativa* L in terms of fulfilling the recommended daily intake (RDI).

SUPPORTING DOCUMENTS

SUPPORTING DOCUMENT I: Ethical clearance approval

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 25/04/2022

NHREC Registration # : REC-170616-051
REC Reference # : 2022/CAES_HREC/006
Name : Mr KI Thobejane
Student #: 39086259

Dear Mr Thobejane

Decision: Ethics Approval from 25/04/2022 to 30/04/2025

Researcher(s): Mr KI Thobejane
kgaogelokg@gmail.com; 011-999-7559

Supervisor (s): Dr MK Maluleke
malulm@unisa.ac.za; 011-471-3838

Mr KG Koopa
koopakg@unisa.ac.za; 011-471-3725

Working title of research:

The effect of irrigation levels on the growth, yield and quality of *Cannabis indica* under different growing environments conditions

Qualification: MSc Ornamental Horticulture

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to submission of the relevant permit and yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 30 April 2025
The progress report is available on the college ethics webpage: https://w2.unisa.ac.za/www.unisa.ac.za/sites/corporate/default/Colleges/Agriculture-%26-Environmental-Sciences/Research/Research-Ethics.html

Please note the points below for further action:

1. The committee notes that the researchers have begun the process to obtain the permit to do research on Cannabis indica. Please note that the field trials may only commence once the permit has been obtained and submitted to the committee. Should the research begin before the permit has been obtained, the ethics clearance will be revoked.

*The **high risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 25 April 2022 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.

8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2022/CAES_HREC/006** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof MA Antwi
Chair of UNISA-CAES Health REC
E-mail: antwima@unisa.ac.za
Tel: (011) 670-9391

Prof M Ntwasa
Acting Executive Dean: CAES
E-mail: ntwasmm@unisa.ac.za
Tel: (011) 471-2272

SUPPORTING DOCUMENT II: Study permit from department of health

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH
Private Bag X828
PRETORIA, 0001
Republic of South Africa

UMNYANGO WEZEMPILO
LEFAPHA LA MAPHELO

PERMIT IN TERMS OF SECTION 22A(9)(a)(i) OF THE MEDICINES AND RELATED SUBSTANCES ACT, 1965 TO ACQUIRE, POSSESS AND USE SCHEDULE 6 AND 7 SUBSTANCES FOR THE ANALYTICAL & RESEARCH PURPOSES.

Date of Issue: 19 April 2023	**Expiry Date: 18 April 2024**	**Permit No: POS 093/2023/2024**

Authority is hereby granted in terms of Section 22A (9)(a)(i) of the above-mentioned Act to **Mr KI Thobejane** of **UNISA Greenhouse, Florida Campus, 28 Pioneer Ave, Florida Park, Roodepoort, 1709, South Africa** acquire, possess and use, subject to the conditions stated, the under-mentioned Schedule 6 and Schedule 7 substances in respect of which the quoted quantity should not be exceeded during the period **19 April 2023** to **18 April 2024**.

Name of Scheduled Substance(s)	Schedule	Total quantity of substance(s) and/or preparation(s) allocated per calendar year
Cannabis plants	Schedule-6	108 plants [one hundred and eight plants]

Total Items: **1**

The acquisition, possess and use the relevant substances are subject to the following conditions:

1. The substances shall be used for **Research Purpose** only.

2. The control over the substances shall be the responsibility of:

   Full Name & Surname: Dr MK Maluleke

   ID Number: 840120 5413 083

3. Complete details of the substances acquired and used shall be recorded in registers designed specifically for this purpose in accordance with the provisions of the relevant regulations to the Medicines and Related Substance Act, 1965.

4. Other people assisting in Research:

Name	ID Number
Mr KG Koopa	**880907 5706 081**

5. When the substances are acquired, the name and address of the supplier, the date supplied, the quantity supplied and the number of the relevant invoice shall be recorded **on this permit.**

6. The register referred to in paragraph 3, as well as copies of orders and invoices pertaining to the supply of the substances, shall be available at the offices of the **UNISA Greenhouse, Florida Campus, 28 Pioneer Ave, Florida Park, Roodepoort, 1709, South Africa** for a period of at least three years and shall be subject to inspection by Inspectors appointed in terms of the Medicines and Related Substances Act, 1965.

7. This permit expires on **18 April 2024** and shall on expiry be returned to the Department of Health for cancellation and shall be accompanied by a statement reflecting the quantity of substances on stock at expiry.

Digitally Signed by:
Sandile Buthelezi

DIRECTOR-GENERAL OF HEALTH:
DR SANDILE BUTHELEZI
DATE:
24/04/2023 03:00:59 PM

SUPPORTING DOCUMENT III: Manuscript under review

Confirmation of your submission to Journal of Cannabis Research - JCAN-D-24-00043 - [EMID:381c77dfa181e3d6]

JO em.jcan.0.8a96f2.95cf1fd8@editorialmanager.com on behalf of Journal of Cannabis Research Editorial Office <em@editorialmanager.com>

To Maluleke, Mdungazi

Reply | Reply All | Forward | ...

Tue 2024/04/09 00:21

We removed extra line breaks from this message.

JCAN-D-24-00043

Physiology, Yield, and Nutritional Contribution of Indian Hemp (Cannabis sativa L) Grown Under Different Fertiliser Types and Environment Dr. Mdungazi Knox Knox Maluleke; Kgaogelo R Thobejane Journal of Cannabis Research

Dear Dr Maluleke,

Thank you for submitting your manuscript 'Physiology, Yield, and Nutritional Contribution of Indian Hemp (Cannabis sativa L) Grown Under Different Fertiliser Types and Environment' to Journal of Cannabis Research.

The submission id is: JCAN-D-24-00043

Please refer to this number in any future correspondence.

em Journal of Cannabis Research

Dr. Mdungazi Knox Maluleke | Logout

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Submissions Being Processed for Author

Page: 1 of 1 (1 total submissions) Results per page 10

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links	JCAN-D-24-00043	Physiology, Yield, and Nutritional Contribution of Indian Hemp (Cannabis sativa L) Grown Under Different Fertiliser Types and Environment	08 Apr 2024	09 May 2024	Reviewers Assigned

Page: 1 of 1 (1 total submissions) Results per page 10

SUPPORTING DOCUMENT IV: Turnitin outcome

PAPER NAME	AUTHOR
K.I. Thobejane-Final Dissertation _August_2024.docx	KGAOGELO IGNATIUS THOBEJANE

WORD COUNT	CHARACTER COUNT
18254 Words	103023 Characters
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101 Pages	4.9MB
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SUPPORTING DOCUMENT V: Proof of language editing

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4 June 2024

Dear Dr Maluleke,

This letter is to confirm that I completed a language and content edit of a dissertation entitled: **Physiology, yield, and nutritional contribution of Indian Hemp (*Cannabis sativa* L) grown under different fertilizer types and environment.**

This dissertation describes a research study under your supervision and will be presented to the Department of Environmental Sciences, University of South Africa in fulfilment for the requirements for the degree MSc in Horticulture. The dissertation was prepared by Mr Kgaogelo Rodney Thobejane.

My edit included the following:

- Including US spelling for words such as fertilizer, commercialization, maximize, etc.
- Correcting the pagination of the dissertation.
- Checking the references according to the APA format.

Content formatting included:

- Suggesting that the introductory chapters could include details of differences between *Cannabis sativa* subspecies such as sativa and indica.
- Suggesting that the section on medicinal and nutritional uses of cannabis could be boosted by including the preparation of nutritious meals/drinks containing preparations of health-giving cannabis plant buds.
- Suggesting a relook at the analysis and discussion of results in Chapters 4 and 5.

Yours sincerely,

John Dewar