Cape Peninsula
University of Technology

Effect of different nitrogen sources and growth media on water use of blueberry cultivated under shade net.

by

Asemahle Mshweshwe

Thesis submitted in fulfilment of the requirements for the degree Master of Agriculture

in the Faculty of Agriculture

at the Cape Peninsula University of Technology

Supervisor: Prof FB Lewu
Co-supervisor: Ms N Mfeka

Wellington
October 2025

DECLARATION

I, Asemahle Mshweshwe, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

October 2025

Signed **Date**

ABSTRACT

Globally blueberry (*Vaccinium* spp.) production has rapidly increased in recent years, driven by consumers' increased demand for this nutritious fruit. This study investigated the interactive effects of different N sources and soilless growth media on water use, growth, yield, and quality of blueberry. A pot experiment was carried out in polythene bags under a white shade net. Three nitrogen sources: ammonium sulphate [$(NH_4)_2SO_4$] (N 21.1%), calcium nitrate [$Ca\ (NO_3)_2$] (N 16.6%), and urea (H_2NCONH_2) (N 46%) were applied to two soilless substrates growth media (100% coir and a combination of 80% coir: 20% zeolite) in a randomized complete block design. Growth, fruit yield, and berry quality parameters were measured. Our results showed that treatment with NH_4^+ and 100% coir yielded higher values for most growth parameters, chlorophyll content index, proanthocyanidin, as well as the availability of macro and micronutrients in the soilless substrate. The 100% coir treatment favours early shoot expansion due to superaeration and root expansion, and the addition of zeolite to coir reduced water demand by the test plants. The retention of NH_4^+ by the 20% zeolite treatment was not beneficial to the blueberry plants, hindering chlorophyll content accumulation and photosynthesis. These results suggest that a further investigation into the influence of different zeolite levels on blueberry production cultivated using pots. The application of NH_4^+ enhances fruit size and weight, primarily by enhancing the process of photosynthesis through higher N and chlorophyll content in leaves, and ultimately improving nutrient assimilation and nutrient use efficiency.

Keywords: Blueberries, Growth media, Fertilizer, Nitrogen sources, Secondary metabolites

ACKNOWLEDGEMENTS

I am sincerely grateful to my co-supervisor, Ms Mfeka, my supervisor, Prof. Lewu, and Dr Tanga for their valuable input and guidance throughout this journey. I am especially thankful to Dr Lewu for the opportunity to pursue this research.

I wish to express my appreciation to the Cape Peninsula University of Technology (CPUT), an institution that I was registered with for providing the necessary support and research facilities.

I extend my gratitude to Dr Nel, our lab technician, Sandisiwe Buyabuye, Ms Andiswa Bolilitye and Muhle Ndimande for their kind assistance and technical support during the course of this study

Finally, I wish to express my profound gratitude to my family for their love, which sustained and motivated me throughout this journey.

The research study was funded by the Water Research Commission (WRC), project number 2024/2025-00838.

The financial assistance of the National Research Foundation towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at are those of the author and are not necessarily to be attributed to the National Research Foundation.

DEDICATION

This work is lovingly dedicated to my cute son, **Hlalumi Mshweshwe**, whose presence brings me endless joy. Although distance has kept us apart during parts of this journey, my love for you has never wavered. Everything I do is with you in my heart, and it is my greatest hope that this achievement will one day remind you that, with love, perseverance, and faith, anything is possible.

TABLE OF CONTENTS

Contents

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

Abbreviations	**Explanation**
N	Nitrogen
NUE	Nitrogen use efficiency
NR	Nitrate reductase
NiR	Nitrite reductase
NH_4^+	Ammonium
NO_3^-	Nitrate
C	Carbon
TPC	Total phenolic content
TFC	Total flavonoid content
PAC	Proanthocyanins
CEC	Cation exchange capacity
WUE	Water use efficiency

CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 Introduction

The blueberry (*Vaccinium* spp.), a perennial shrub of the family Ericaceae, is widely recognised as an economically valuable crop in many regions worldwide (Yang *et al*., 2021). It is classified as a calcifuge (acid-loving) plant, adapted to acidic soils with a pH range of 4.2 to 5.5 (Retameales & Hancock, 2018) and requires sandy loam enriched with high organic matter (>3%) (Rosen *et al*., 2019). Given the limited availability of nutrients in acidic soils (Imler *et al*., 2019), blueberry have evolved to thrive under low nutrient conditions, albeit with slower growth rates (Doyle *et al*., 2021). Under commercial cultivation, an effective fertilization regime, primarily consisting of nitrogen (N), is required to ensure optimal fruit yield (Bryla & Strik, 2015). Blueberry plants are shallow-rooted crops with no root hairs, making them highly susceptible to water deficits as the root zone can rapidly become depleted of water during periods of high demand; therefore, commercial production depends on frequent irrigation (Bryla & Strik, 2007; Bryla, 2011). Fruits are rich in phenolic compounds and possess antioxidant properties, offering significant nutritional, medicinal, and therapeutic benefits (Zahra *et al*., 2023)

The global cultivation yield has increased from 151,000 tons in 2001 to over 1.5 million tons by 2021 (Pienaar *et al*., 2022), reaching approximately 1.86 million tons by 2024 (International Blueberry Organisation, 2024). The domestication of many seedless fruits has rapidly increased in recent years, driven by consumers' preferences and demand for nutritious fruits (Osorio *et al*., 2020). However, the blueberry industry in South Africa (SA) is in the emerging stage compared to the United States, Canada, and Chile, which have dominated global production for decades (Retamales *et al*., 2014). The first South African blueberry cultivation practice occurred during the

1970 s and at a commercial scale during the 1990s (Retamales & Hancock, 2012). Despite its late start in commercial production, the South African blueberry industry has shown notable growth, with production increasing from approximately 1,700 tons in 2011 to 27,700 tons in 2021, projections indicating expansion to nearly 56 000 tons by 2031, representing an annual growth rate of 6.6% (Pienaar *et al*., 2022). The increasing international demand, high profitability, and substantial job creation have contributed to the rapid growth of this industry, which has led to a doubling of industry value during each successive season in recent years (Pienaar *et al*., 2019).

The economic importance of blueberries has called for attention to developing cultivation practices from factors that can negatively impact productivity, with particular emphasis on effective nutritional and water management practices. The World Economic Forum (WEF) reported that global risks, including the global water demand, are rising due to the development of water use for domestic, industrial, agricultural and livestock purposes (WEF, 2015). The report also stated that 40% of the world's population experiences water shortages for at least a month per year. In sub-Saharan Africa, the agricultural challenge is one of the most pressing demands to enhance crop productivity and reduce water usage during farming operations, known as the "more crop per drop" approach (Fan *et al*., 2012). In SA, the scarcity of water is an issue of major concern recently; Cape Town was classified as the city having the highest water crisis (Department of Water and Sanitation, 2018). Improving water use efficiency (WUE) in agriculture is crucial for meeting future global demand (Howell, 2001). In recent years, the use of soilless culture systems has gained attention as a sustainable method for enhancing WUE (Gruda *et al*., 2016). Within these systems, the integration of coir and zeolite as growth media has shown promising improvement in water and nutrient management (Mfeka *et al*., 2023).

In blueberry cultivation, water and nutrient supply are key interacting factors influencing growth, yield and physiological responses (Guo *et al*., 2021). Among essential nutrients, N is particularly critical, making up about 1.5-2.1% of blueberry leaf dry weight (Alt *et al*., 2017). Blueberries can take up N, primarily in the ammonium (NH_4^+) and nitrate (NO_3^-) forms (Bryla *et al*., 2010). However, unlike many crops, they show a distinct preference for NH_4^+ due to their limited nitrate reductase activity (Merhaut & Darnell, 1996; Poonnachit & Darnell, 2004). Although NH_4^+ is generally regarded as the more suitable N source, its continuous or excessive supply can result in NH_4^+ toxicity which inhibits plant growth and development (Britto & Kronzucker, 2002). Urea, as an inexpensive source of nitrogen, could be useful in reducing potential overacidification caused by the long-term application of ammonium sulphate in substrate production (Kingston *et al*., 2017). Moreover, studies have also reported the beneficial effects of NO_3^-, including increased biomass, N content and enhanced bud and root development (Merhaut & Darnell, 1996; Crisóstomo *et al*., 2014; Alt *et al*., 2017). Taking these findings into account, the response of blueberries to different N sources varies depending on various factors. There is a minimal amount of scientific data, particularly within the South African context, on the impact of various N sources on the growth and yield attributes of blueberry. This study aimed to investigate the influence of growth media combinations and different N sources on growth and yield attributes of blueberries in the Cape Wineland region of the Western Cape Province, South Africa.

1.2 Motivation of the research

The worldwide challenges on water scarcity (Beck *et al*., 2016) and N depletion due to soil overuse are identified as a threatening factor in agricultural productivity and food security. These factors negatively impact crop yield and quality. The blueberry industry in SA has experienced remarkable growth during recent years and has established itself as a significant economic sector for the nation (Bureau for Food and Agricultural Policy 2018). Water conservation strategies have become critical in mitigating the impacts of water scarcity and the use of alternative growth media has shown promise in improving water use while supporting plant development (del Amor *et al*., 2009; Nichols & Savidov, 2009; Méndez Argüello *et al*., 2018). As a N-demanding crop, blueberry requires a sufficient and well-balanced N supply for proper growth fruit development, and yield (Bryla *et al*., 2010). However, the effectiveness of different N forms remains insufficiently studied in combination with water efficient substrates under SA climatic conditions.

Given the increasing demand for high quality, antioxidant-rich blueberries and the species unique N uptake preferences (Doyle *et al*., 2021; Zahra *et al*., 2023), it is crucial to investigate how different N sources affect not only growth and yield but also the biosynthesis of secondary metabolites like phenolic compounds. With the global population expected to reach 10 billion by 2050 (Nguyen & Wang, 2024) food production must double to ensure food security. Improving crop water productivity could significantly impact food security and water sustainability.

1.3 Significance of the study

There is limited information that addresses resource optimization in blueberry production in South Africa. The study investigated the combined effects of different nitrogen sources and growth media influenced blueberry production. By focusing on the two growth media, coir and zeolite, the research provided insights into the growth media performance and their influence on nutrient availability and water use. Emphasizing optimizing N management in combination with growth media selection to enhance blueberry production under Mediterrenean climate conditions.

The use of different N sources in combination with growth media has not been fully explored under the South African blueberry production. The findings generated primary field data, providing evidence on how resource optimization could improve blueberry growth, yield, and fruit quality. This research will also contribute to the sustainable use of inputs such as fertilizers and water, offering practical strategies for efficient and commercially viable blueberry production.

1.4 Aim and Objectives

The research aimed to investigate the interactive effects of different N sources and soilless growth media on water use, growth, yield, and quality of blueberry.

- To investigate the effect of different growth media on water use of blueberry production.
- To assess the effect of different nitrogen sources on blueberry production.
- To evaluate the effect of different nitrogen sources on the mineral composition of blueberry.

1.5 Chapter outline

This thesis consists of five chapters. Chapter 1 provides the background and introduction of the study. Chapter 2 presents a comprehensive review with background on blueberry cultivation under different nitrogen sources. Chapter 3 presents the results and discussion on the influence of different nitrogen sources and growth media on water use, growth, and yield attributes of blueberry. Chapter 4 presents the results and discussion on the influence of different nitrogen sources and growth media on mineral composition and blueberry phenolic constituents. Chapter 5 provides the general conclusions and recommendations of the study.

Publications Arising from this Study

As part of this study, a review paper on ‘Blueberry cultivation under different nitrogen sources’ has been accepted for publication in the Journal of Medicinal Plants for Economic Development. In addition, two research papers based on the experimental results are currently in preparation for submission to peer-reviewed journals.

Findings from this study were also presented at the $U6^{+}$ International Conference, with a title ‘Influence of different nitrogen sources and growth media on blueberry yield and phenolic constituents’. The conference presentation provided an opportunity to share preliminary results with the broader scientific community and receive constructive feedback by providing guidance on the technical aspects. The conference was held from September 10-12, 2024, at Cape Peninsula University of Technology, D6 campus.

References

Alt, D. S., Doyle, J. W., & Malladi, A., 2017. Nitrogen-source preference in blueberry (Vaccinium sp.): Enhanced shoot nitrogen assimilation in response to direct supply of nitrate. *Journal of Plant Physiology*, *216*, 79–87. https://doi.org/10.1016/j.jplph.2017.05.014

Arenas, M., Vavrina, C. S., Cornell, J. A., Hanlon, J. A., & Hochmuth, G. J., 2002. Coir as an alternative to peat in media for tomato transplant production. *HortScience*, *37*(2). https://doi.org/10.21273/hortsci.37.2.309

Aslan, M. U., & Arslan, H., 2024. The Effect of Zeolites on Soil and Plant: A Review. *Communications in Soil Science and Plant Analysis*, *55*(14), 2197–2216. https://doi.org/10.1080/00103624.2024.2346211

Beck, T., Rodina, L., Luker, E., & Harris, L., 2016. Institutional and Policy Mapping of the Water Sector in South Africa. In *Program on Water Governance* (Issue 1996).

BFAP, 2018. The potential impact of the national minimum wage on the agricultural sector. Pretoria: Bureau for Food and Agricultural Policy. (www.bfap.co.za)

Bryla, D. R., Shireman, A. D., & MacHado, R. M. A., 2010. Effects of method and level of nitrogen fertilizer application on soil pH, electrical conductivity, and availability of ammonium and nitrate in Blueberry. *Acta Horticulturae*, *868*, 95–101. https://doi.org/10.17660/actahortic.2010.868.8

Crisóstomo, Crisóstomo Natividad, M., Hernández Rodríguez, O. A., Medina, J. L., Manjarrez- Domínguez, C., & Pinedo-Alvárez, A., 2014. Relaciones amonio/nitrato en soluciones nutritivas ácidas y alcalinas para arándano* Ammonium/nitrate ratios in acid and alkaline solutions nutritious blueberry. *Revista Mexicana de Ciencias Agrícolas*, *5*(3).

Darnell, R. L., and Cruz-Huerta, N., 2011. Uptake and assimilation of nitrate and iron in cultivated and wild Vaccinium species. *International Journal of Fruit Science*, *11*(2), 136–150. https://doi.org/10.1080/15538362.2011.578518

del Amor, F. M., & Gómez-López, M. D., 2009. Agronomical Response and Water Use Efficiency of Sweet Pepper Plants Grown in Different Greenhouse Substrates. *HortScience*, *44*(3), 810–814. https://doi.org/10.21273/HORTSCI.44.3.810

Department of Water and Sanitation., 2018. National water and sanitation master plan (NWSMP). Vol. 1, "Call to Action", version 10.1. Republic of South Africa. [Accessed 10 June 2025].

Dev, V., Ali, N., & Tech Scholar, M., 2020. Influence of coconut fibres on the properties of soil. In *International Journal For Technological Research In Engineering* (Vol. 8, Issue 1). www.ijtre.comwww.ijtre.com

Doyle, J. W., Nambeesan, S. U., & Malladi, A., 2021. Physiology of Nitrogen and Calcium Nutrition in Blueberry (Vaccinium sp.). *Agronomy*, *11*(4), 765. https://doi.org/10.3390/agronomy11040765

Fan M, Shen J, Yuan L, Jiang R, Chen X, Davies WJ, Zhang F., 2012. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. J Exp Bot 63(1):13–24. https://doi.org/10.1093/jxb/err248

Gruda, N.; Gianquinto, G.; Tüzel, Y.; Savvas, D. 2016. Soilless Culture. In Encyclopedia of Soil Sciences, 3rd ed.; Lal, R., Ed.; CRC Press Taylor & Francis Group: Boca Raton, FL, USA; pp. 533–537

Guo, X., Li, S., Wang, D., Huang, Z., Sarwar, N., Mubeen, K., Shakeel, M., & Hussain, M., 2021. Effects of water and fertilizer coupling on the physiological characteristics and growth of rabbiteye blueberry. *PLOS ONE*, *16*(7), e0254013. https://doi.org/10.1371/journal.pone.0254013

Heil, J., Vereecken, H., & Brüggemann, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. In *European Journal of Soil Science* (Vol. 67, Issue 1). https://doi.org/10.1111/ejss.12306

Hongpakdee, P., & Ruamrungsri, S., 2015. Water use efficiency, nutrient leaching, and growth in potted marigolds affected by coconut coir dust amended in substrate media. *Horticulture Environment and Biotechnology*, *56*(1). https://doi.org/10.1007/s13580-015-0064-7

Howell, T. A., 2001. Enhancing Water Use Efficiency in Irrigated Agriculture. *Agronomy Journal*, *93*(2), 281–289. https://doi.org/10.2134/agronj2001.932281x

International Blueberry Organization., 2024, July 10. *Global Blueberry Supply Falling Short of Growing Demand*. Retrieved from https://www.internationalblueberry.org/2024/07/10/global-blueberry-supply-falling-short-of-growing-demand/

Ippolito, J. A., Tarkalson, D. D., & Lehrsch, G. A., 2011. Zeolite Soil Application Method Affects Inorganic Nitrogen, Moisture, and Corn Growth. *Soil Science*, *176*(3), 136–142. https://doi.org/10.1097/SS.0b013e31820e4063

Jayasinghe, G. Y., Tokashiki, Y., Kitou, M., & Kinjo, K., 2008. Oil palm waste and synthetic zeolite: an alternative soil-less growth substrate for lettuce production as a waste management practice. *Waste Management & Research: The Journal for a Sustainable Circular Economy*, *26*(6), 559–565. https://doi.org/10.1177/0734242X08090046

Karlovich, P. T., & Fonteno, W. C., 1986. Effect of Soil Moisture Tension and Soil Water Content on the Growth of Chrysanthemum in 3 Container Media. *Journal of the American Society for Horticultural Science*, *111*(2), 191–195. https://doi.org/10.21273/JASHS.111.2.191

Kritzinger, H., 2014. Seasonal Patterns in Carbohydrates and Macronutrients in Southern Highbush Blueberry Plants. Faculty of AgriSciences, University of Stellenbosch, South Africa. MSc Thesis.

Méndez Argüello, B., Vera Reyes, I., Cárdenas Flores, A., de los Santos Villarreal, G., Ibarra Jiménez, L., & Lira Saldivar, R., 2018. Water holding capacity of substrates containing zeolite and its effect on growth, biomass production, and chlorophyll content of Solanum lycopersicum Mill. *Nova Scientia*, *10*(21), 45–60. https://doi.org/10.21640/ns.v10i21.1413

Merhaut, D. J., & Darnell, R. L., 1996. Vegetative growth and nitrogen/carbon partitioning in blueberry as influenced by nitrogen fertilization. *Journal of the American Society for Horticultural Science*, *121*(5). https://doi.org/10.21273/jashs.121.5.875

Mfeka, N., Tanga, M., Mulidzi, A.R., & Lewu, F.B., 2023 'Water Requirement of Blueberry Cultivated in Different Growth Media', *39th Johannesburg International Conference on Chemical, Biological and Environmental Engineering (JCBEE-23)*, 16-17 November, Johannesburg, South Africa. https://doi.org/10.17758/iicbe5.c1123050

Nguyen, H. P., & Wang, C. M., 2024. Advances in Offshore Aquaculture and Renewable Energy Production. *Journal of Marine Science and Engineering*, *12*(9), 1679. https://doi.org/10.3390/jmse12091679

Nichols, M. A., & Savidov, N. A., 2009. Recent advances in coir as a growing medium. *Acta Horticulturae*, *843*, 333–336. https://doi.org/10.17660/ActaHortic.2009.843.44

Osorio, R., Cáceres, C., & Covarrubias, J. I., 2020. Vegetative and Physiological Responses of "Emerald" Blueberry to Ammoniacal Sources with a Nitrification Inhibitor. *Journal of Soil Science and Plant Nutrition*, *20*(2), 507–515. https://doi.org/10.1007/s42729-019-00135-7

Pienaar L, Lingani M, Swart P., 2019. The economic contribution of the South African blueberry industry. Berries ZA. https://www. saberries.co.za/economic-contribution-of-the-south-african-blueberry-industry

Pienaar, L., Smit, P., Hattingh, J. & Cloete, K., 2022. Developing economic models for South African blueberry industry: Outlook 2022-2031. Report Commissioned by BerriesZA. Paarl: Bureau for Food and Agricultural Policy (BFAP)

Poonnachit, U., & Darnell, R., 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in Vaccinium species. *Annals of Botany*, *93*(4). https://doi.org/10.1093/aob/mch053

Rafiee, G., & Saad, C. R., 2006. The Effect of Natural Zeolite (Clinoptiolite) on Aquaponic Production of Red Tilapia (Oreochromis sp.) and Lettuce (Lactuca sativa var. longifolia), and Improvement of Water Quality. In *J. Agric. Sci. Technol* (Vol. 8).

Raviv, M., 2017. Can compost improve sustainability of plant production in growing media? Acta Hortic., 1168, 119–133.

Retamales, J. B., & Hancock, J. F., 2012. Blueberries. In *Blueberries*. https://doi.org/10.2307/j.ctvthhbxx.20

Retamales, J. B., Palma, M. J., Morales, Y. A., Lobos, G. A., Moggia, C. E., & Mena, C. A., 2014. Blueberry production in Chile: current status and future developments. *Revista Brasileira de Fruticultura*, *36*(1), 58–67. https://doi.org/10.1590/0100-2945-446/13

Sandeep Jakkula, V., & Wani, S. P., 2018. Zeolites: Potential soil amendments for improving nutrient and water use efficiency and agriculture productivity. *Sci Revs Chem Commun.*, *8*(1).

Sastiono, A., Pengajar, S., Tanah, J., Pertanian, F., Pertanian, I., Meranti, B. J., & Darmaga, K., 2004. Pemanfaatan Zeolit di Bidang Pertanian. In *Journal of Indonesian Zeolites* (Vol. 3, Issue 1).

World Economic Forum, Global Risks 2015, 10th Edition (World Economic Forum, Geneva, Switzerland., 2015. [10 June 2025].

Zahra, N., Saeed, M. K., Hamid, H., Qamar, A., & Saeed, A., 2023. Nutritional composition, health benefits, and potential applications of blueberry: a comprehensive review. *Innovare Journal of Agricultural Sciences*, 7–13. https://doi.org/10.22159/ijags.2023.v11i5.48548

CHAPTER TWO

BLUEBERRY CULTIVATION UNDER DIFFERENT NITROGEN SOURCES: A REVIEW

This section is based on a review paper that has been accepted for publication on the peer review Journal of Medicinal Plant for Economic Development (Annexure A). It provides a comprehensive background on blueberry cultivation under different nitrogen sources and serve as part of the foundation and guide context for the experimental work presented in this thesis.

2.1 General Introduction

The global blueberry (*Vaccinium* spp.) production has proliferated in recent years owing to consumers increased demand for this nutritious fruit (Osorio *et al*., 2020). Driven by increasing consumer awareness of nutritional benefits the worldwide blueberry cultivation area increased significantly from 151,000 tons in 2001 to over 1.5 million tons in 2021 (Pienaar *et al*., 2022).

Fresh blueberries are famous for delaying human ageing while providing various health benefits. The antioxidant properties of blueberries protect human health by neutralising free radicals that cause ageing and various diseases including cancer and cardiovascular disease, as well as immune system deterioration, brain dysfunction and cataracts (Tarkanyi *et al*., 2019). Nitrogen (N) fertilization has been shown to influence the accumulation of bioactive compounds such as phenolics, carotenoids, and glucosinolates, in crops which determines the nutritional value and health benefits of the fruit (Kishorekumar *et al*., 2020).

Nitrogen is an essential nutrient for plant growth and development accounting for approximately 50% of yield performance. It is a key component of various metabolic processes in plant physiology involving shoot biomass root development, and N use efficiency (NUE) (Li *et al*., 2021).

N in blueberry production promotes vegetative growth as a result, it is important for the production of strong leaves, stems, branches, and flower bud differentiation (Leitzke *et al.*, 2015). Yuan-Yuan *et al.* (2021) indicated that optimal N levels increase the photosynthetic rate of blueberry plants by serving as an critical constituent of chlorophyll pigment which captures light energy and contributes to fruit development by improving the seed setting rate for the quality and yield of fruits.

Blueberry plants obtain N through ammonium ion (NH_4^+) and nitrate ion (NO_3^-) absorption which leads to specific genetic and metabolic responses in plants (Peterson *et al.*, 2022). Blueberries show a preference for NH_4^+ as their N source, while most plants prefer NO_3^-, although NH_4^+ is less available in soil than NO_3^- (Yuan-Yuan *et al.*, 2021). Plant growth responses to different N sources are influenced by NH_4^+ or NO_3^- uptake and environmental factors such as temperature, soil pH, and nutrient availability (Ye *et al.*, 2022). This makes the selection of N sources a critical aspect in blueberry production, which influences plant growth, yield, and physiology. N has been noted to be essential for many physiological processes including biomass production, root development, and enzymatic activity (Alt *et al.*, 2017; Osorio *et al.*, 2020). The effect of N on the complex synthesis of phenolic compounds which are important for blueberry antioxidant properties, nutritional value, and health benefits, remains sufficient investigation.

Nitrogen is one of the growth limiting nutrients in plants. In blueberries different sources of N stimulate vegetative growth however, this is usually at the expense of secondary metabolite synthesis (González *et al.*, 2018). Because of increasing global demand for high-quality blueberries and their unique preference for nitrogen sources a comprehensive understanding of how different nitrogen sources affect blueberry growth, yield, and secondary metabolites is essential. Studies on the preferred N sources for blueberry plants will assist in enhancing production while using low N fertiliser rates which will reduce production cost and harsh environmental impacts. This review explores the role of various N sources in blueberry growth, yield and physiology. It further suggests areas for future research for sustainable N application in blueberry production.

2.2 Methods

The search was conducted for relevant literature using various platforms to ensure all the sources were reliable and credible. The Cape Peninsula University of Technology library database, where we accessed this information, included online platforms such as ProQuest Agriculture Journals, ScienceDirect, Springer Nature Link, Scopus, Wiley, and Google Scholar. Frontiers, ResearchGate, and AI tools like Connected Papers and Lit maps were used to find relevant articles linked to the information of interest. The review employed an extensive search using a combination of the following key words: (i) blueberry, (ii) nitrogen sources, (iii) fertiliser, and (iv) phenolic compounds. Boolean operators were applied to refine searches in the databases accessed. The search covered published research articles from 2014 to 2024 and only articles published in English were selected. Grammarly was used to correct grammar to improve readability, Turnitin for the similarity index, and Mendeley as a reference management tool. Proper attribution to all original authors and sources was maintained throughout the review process, and findings were reported transparently.

2.3 Findings

The flowchart with the number of selected and excluded criteria in each stage was built using PRISMA guidelines (Figure 1). The initial search produced 1408 articles from the five databases; 1050 duplicates were excluded, thereafter, 291 articles were excluded were excluded after reading the titles and abstracts. 67 articles were imported into the reference manager software (Mendeley) for further eligibility; finally, 47 studies were included in this review.

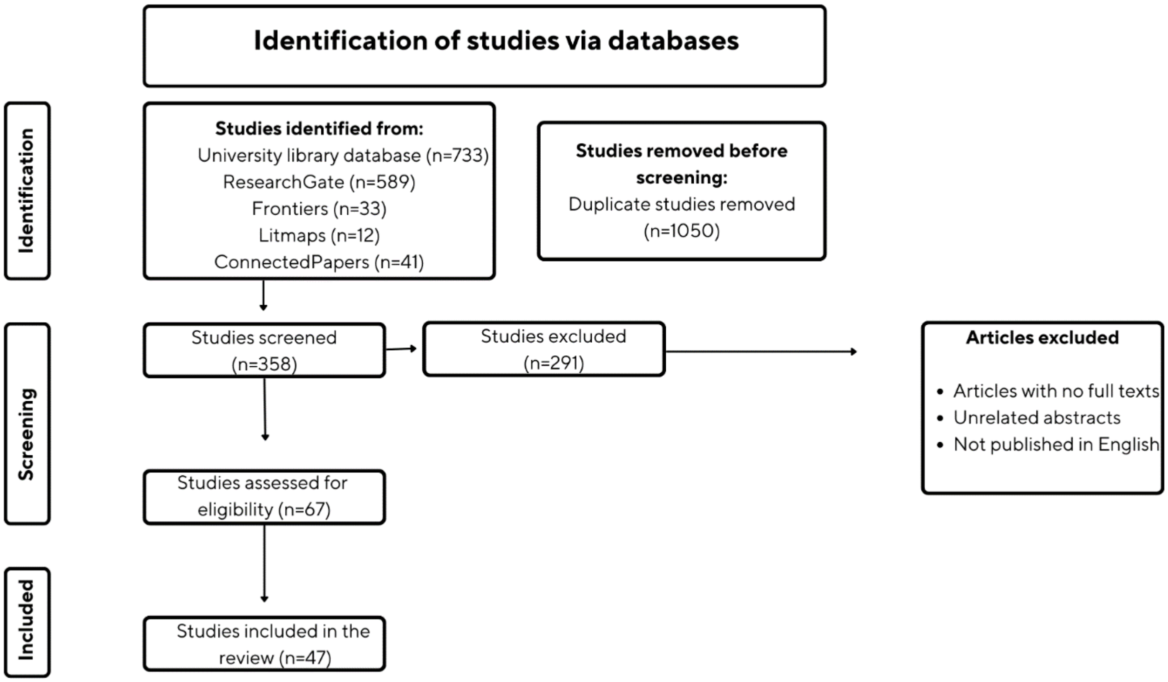


Figure 2.1. PRISMA flow diagram illustrating the process of searching and selecting studies based on the established inclusion and exclusion criteria

2.4 Nitrogen Sources and Overview

Plants primarily rely on two N forms, NH_4^+ and NO_3^-, which are derived from various soil processes as mineralisation and nitrification (Zhang *et al*., 2018). Nitrogen is available in the atmosphere, primarily in its gaseous form (N_2), which constitutes about 78% of the Earth's atmosphere (Glass & Rousk, 2024). Nitrogen fixation occurs through a symbiotic relationship between root nodule-dwelling N-fixing bacteria (rhizobia) and plants, where the plant provides the bacteria with carbohydrates while bacteria fix N_2 into a form that the plant can use (Ahmadi, 2023). Another way that some plants may obtain nitrogen for their nutrition is through nitrite (NO_2^-) from the atmosphere (Bashir *et al*., 2024). NO_2^- is a significant air pollutant produced in the soil when N-containing substances break down under low oxygen conditions (Ye *et al*. 2022). However, most of it is produced through the combustion of fossil fuels (vehicles, power plants, and industrial processes). In soil, NO_2^- availability is generally low, and at high concentrations, it becomes toxic to plants (Bashir *et al*., 2024).

2.4.1 Ammonium (NH_4^+) as a Nitrogen source

Ammonium N (NH_4^+) is present in soils through mineralization of soil organic N and applied as a product of urea hydrolysis. NH_4^+ uptake is mediated by both high- and low-affinity transport systems, possibly via an NH_4^+ uniport or K^+ channel (Jose *et al*., 2023). NH_4^+ is the preferred form of N uptake when plants grow under N deficiency. It is rapidly assimilated into amino acids within the roots via glutamine synthetase/glutamate synthase (GS/GOGAT) pathway (Figure 2), which requires less energy than NO_3^- assimilation (Zhang *et al*., 2018). Due to its positive charge, NH_4^+ is adsorbed by negatively charged soil colloids (clay and organic matter), and thus is less prone to leaching. Uptake of NH_4^+ causes rhizosphere acidification due to H^+ exchange (Imler *et al*., 2019). The most used single N (NH_4^+) is ammonium sulphate, containing 21% N and 24% sulphur (S).

2.4.2 Nitrate (NO_3^-) as a Nitrogen source

Most agricultural soils allow plant roots to absorb N mainly through NO_3^- even though NH_4^+ might be more accessible in certain soil types. This is mainly due to the higher concentration of NO_3^- in soils as compared to NO_2^- and NH_4^+. Additionally, due to its (NO_3^-) negative charge, it remains in the soil solution rather than binding to negatively charged soil particles, allowing for high mobility and plant uptake (Pinheiro *et al.,* 2020). NO_3^- is absorbed via a NO_3^-/H^+ symport (Figure 2), involving three transport systems (Muratore *et al*., 2021), and the uptake of NO_3^- leads to rhizosphere alkalinization (Imler *et al*., 2019).

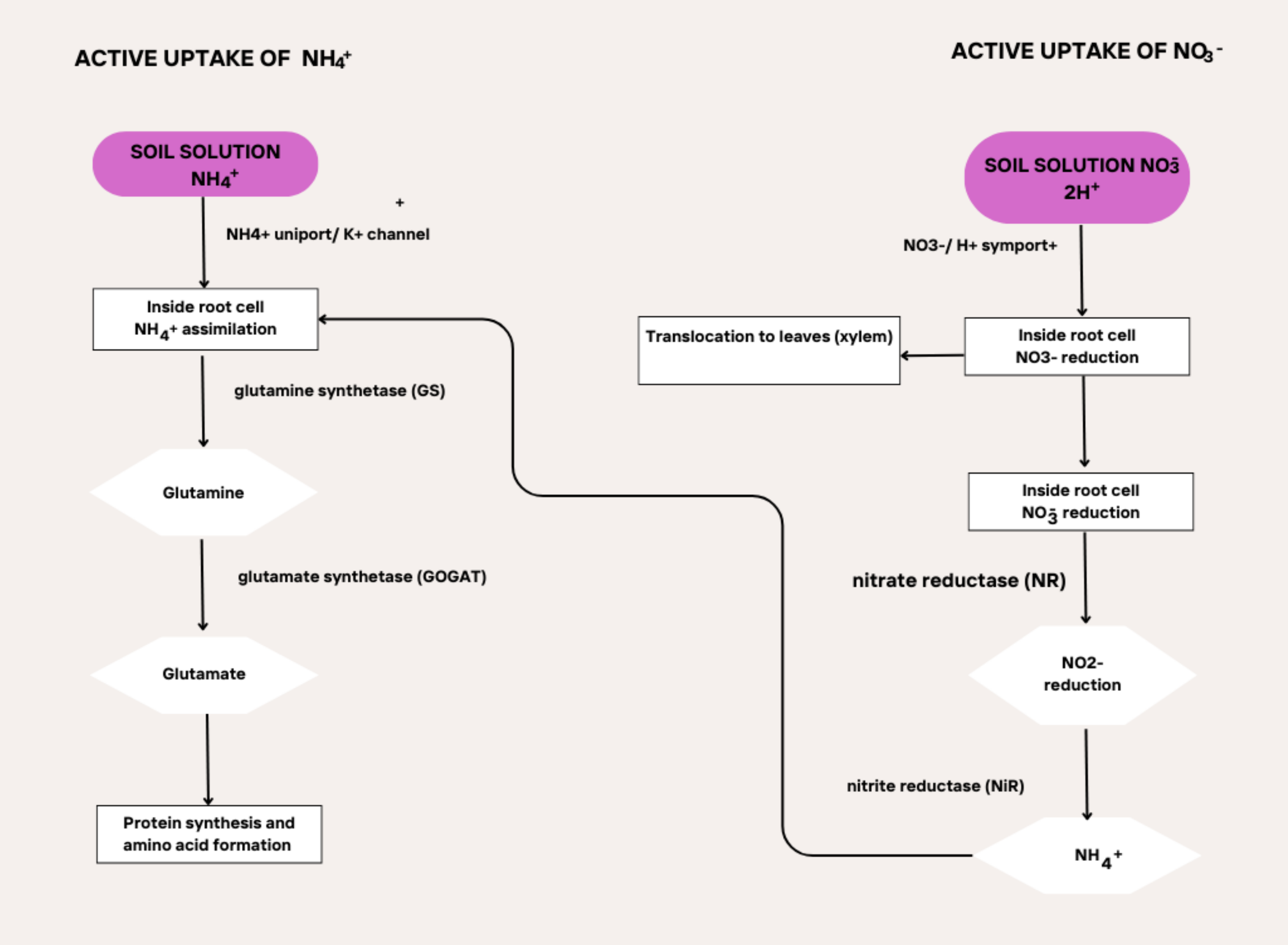


Figure 2.2. Shows the assimilation of the two nitrogen sources, as NH_4^+ undergoes glutamine synthetase and NO_3^- through a reduction process by nitrate reductase (Adapted from Imler *et al*., 2019; Muratore et al., 2021; Jose *et al*., 2023).

The conversion of NO_3^- to NH_4^+ and amino acid synthesis for protein synthesis depends on nitrate reductase enzyme activity, which is inefficient in blueberries (Kishorekumar *et al*., 2020). Blueberry plants demonstrate N form and concentration sensitivity in acidic NH_4^+-dominant soils; however, they thrive best at pH 4.0 to 5.5, which supports acidic soil conditions that favour NH_4^+

uptake as their preferred N source (Yang *et al*., 2022). Sensitivity of young blueberry plants to high ammonium sulphate applications may be due to ammonium toxicity, which is linked to increased electrical conductivity (EC) in the soil solution, with growth suppression observed at EC levels above 1.5 $dS \cdot m^{-1}$ (Machado *et al*., 2014). Table 1 below shows that N form and soil acidity are important, with most studies indicating a preference for NH_4^+ over NO_3^- as a N source.

Table 2.1 The Effect of Different Nitrogen Sources on Growth and Nutrient Accumulation in Blueberry Species Across Varied pH Levels

Blueberry species	**N forms**	**pH**	**Key Findings (Notes)**	**Reference**
'Northblue ('V. *corymbosum* x V.*angustifolium)*	NH_4^+, NO_3^- NH_4NO_3	4.5 and 6.5	More vegetative growth at pH 4.5 vs. 6.5, regardless of N form. No effect of N form at the given pH.	Rosen *et al*. (2019)
'Climax' and 'Chaoyue No. 1' (*V. corymbosum* L.)	NH_4^+ vs NO_3^-	4.5, 5.3 & 6	Low pH (4.5) enhanced growth, yield, photosynthesis, and micronutrient uptake; high pH (6.0) reduced growth and fruit quality. NH_4^+ alleviated high pH stress more effectively than NO_3^-	Jiang *et al.* (2019)
Andean blueberry (V. *meridionale Swartz*)	100%NH_4^+, 100%NO_3^- 50%NH_4^+: 50%NO_3^-	6.0	NH_4^+ fertilisation led to higher dry matter accumulation, shoots and leaves. NO_3^- fertilization increased anthocyanin production due to stress from N deficiency and low chlorophyll synthesis.	Gonzalez *et al*. (2018)
'Tifblue' rabbiteye (V. *ashei* Reade)	NH_4^+ vs NO_3^-	3.5-7.5	Higher fruit yield, greater shoot growth, and higher leaf nutrient concentration with NH_4^+ compared to NO_3^-	Spiers (2022)
'Emerald' (*V. corymbosum)*	NH_4^+ vs NO_3^-	5. & 75	Plants grew better at pH 5.0 than at pH 7.5, and the plant growth was the best with NH_4^+: NO_3^- ratio of 2:1 at pH 5.0	Xu *et al*. (2021)

N- nitrogen, NH_4^+- ammonium, NO_3^- -nitrate, and *V- Vaccinium*

2.5 Blueberry Growth and Yield Response to Different Nitrogen Sources

Nitrogen form plays a critical role in determining blueberry growth and yield responses. As shown in Table 2 below, numerous studies have investigated the effects of different N sources, including NH_4^+, NO_3^-, and combinations thereof, on various blueberry cultivars and developmental parameters. Overall, NH_4^+-N tends to be more favourable than nitrate-N in most studies (Yañez-Mansilla *et al.*,2015; Vargas & Bryla 2015; Alt *et al.*, 2017; González *et al.*, 2018; Rosen *et al.*, 2019; Imler *et al.*, 2019; Osorio *et al.*, 2020; Messiga *et al.*, 2021; Xu *et al.*, 2021; Yuan-Yuan *et al.*, 2021; Peterson *et al.*, 2022; Arias *et al.*, 2024; Anwar *et al.*, 2024), with consistent improvements in shoot growth chlorophyll content, leaf dry mass, and yield. This trend may be linked to the limited nitrate reductase activity in *Vaccinium* species, as well as their preference for acidic soils which complements the acidifying effect of NH_4^+ nutrition.

Additionally, a combination of N sources particularly NH_4^+: NO_3^- ratios of 2:1 or 1:1, has demonstrated synergistic effects on physiological and yield-related traits (Anwar *et al.*, 2024). These ratios often outperform singular forms by enhancing N recovery leaf area index and net assimilation rate without the adverse effects seen with high NO_3^- concentrations (Xu *et al.*, 2021). Table 2.2 below summarises these findings, offering insight into the understanding of blueberry N nutrition. However, recent studies seem to be placing increased emphasis on physiological responses, such as N uptake efficiency and photosynthetic activity, in addition to yield attributes. While cultivar-specific responses and environmental factors (such as soil pH and substrate) can modulate outcomes, the preference for NH_4^+-dominated nutrition or a combination of forms remains a consistent recommendation for optimising blueberry production.

Table 2.2 Blueberry Growth and Yield Responses to Different Nitrogen Sources

Study/Source	N Source	Blueberry Cultivar/ Species	Growth/Yield Response	Key Observations
Rosen *et al*. (2019)	NH_4^+	'Northblue'	Shoot length ↑ from 38.4 cm to 127.3 cm (pH 4.5)	Significant shoot elongation under NH_4^+
Osorio *et al*. (2020)	NH_4^+ vs NO_3^-	'Emerald'	Leaf dry mass: NH_4^+ (24.8 g) > NO_3^- (17.4 g); Chlorophyll: NH_4^+ (20 µg/cm²) > NO_3^- (16 µg/cm²)	NH_4^+ improves leaf growth and chlorophyll content
Peterson *et al*. (2022)	NH_4^+ vs NO_3^-	*V. corymbosum* L.	Higher NH_4^+ uptake in hydroponic systems	NH_4^+ is preferred in hydroponics
Arias *et al*. (2024)	$^{15}NH_4^+$ vs $^{15}NO_3^-$	'Blue Ribbon'	N accumulation: NH_4^+ (243.5 mg/plant) > NO_3^- (213.6 mg/plant); ^{15}N recovery rate ↑ 10.7% with NH_4^+	Greater N use efficiency with NH_4^+
González *et al*. (2018)	NH_4^+ vs NO_3^-	*V. meridionale.* Swartz	Shoots/plant: NH_4^+ (22) > 50:50 (20); Higher N% with NH_4^+ (1.72%), ↑ Net assimilation rate (NAR), LAI, dry matter	NH_4^+ improves shoot development, N accumulation, and better photosynthetic performance
Alt *et al*. (2017)	NO_3^-	'Alapaha' & 'Sweetcrisp'	Growth ↓ by 30–60% with NO_3^-; Low nitrate reductase activity	NO_3^- assimilation is limited due to enzyme inefficiency

Rosen *et al*. (2019)	NO_3^-	‘Northblue’	Higher dry weight of plant parts at pH 6.5	NO_3^- can be effective at neutral pH
Messiga *et al*. (2021)	High NO_3^-	‘Duke’	↓ Fruit set and quality	High NO_3^- can negatively affect reproductive traits
Imler *et al*. (2019)	NH_4^+ vs NO_3^-	‘Emerald’	NH_4^+ acidifies rhizosphere; NO_3^- increases pH	pH shifts affect nutrient availability and uptake
Xu *et al*. (2021); Anwar *et al*. (2024)	2:1 NH_4^+: NO_3^-	‘Emerald’ & ‘Nangao Z9’	↑ Chlorophyll (1.2 mg/g FW), crown width ↑ 11%	A 2:1 ratio is optimal for vegetative growth
Yañez-Mansilla *et al*. (2015)	$NH_4^+NO_3^-$	‘Legacy’ & ‘Bluegold’	Root N: Legacy (15 g/kg) > Bluegold (8 g/kg)	Cultivar-specific N responses
Yuan-Yuan *et al*. (2021)	Various NH_4^+:NO_3^- ratios	‘Northsky’	Improved bud, root development, and photosynthesis	Balanced ratios support overall plant health
Vargas & Bryla (2015)	NH_4^+ vs urea	‘Bluecrop’	Berry weight: NH_4^+ (2.22 g) > urea (2.17 g)	NH_4^+ is linked to better cellular growth when a fertigation system with a split application method is used

N- nitrogen, NH_4^+- ammonium, NO_3^- -nitrate, *V*- *Vaccinium*, µg - microgram, cm – centimeters, mg - milligram, g – grams, FW – fresh weight, kg – kilograms, and LAI – leaf area index.

2.6 Nitrogen Sources on Berry Phenolic Compounds

The antioxidant compounds anthocyanins, phenolic acids, and polyphenols, which are present in blueberry plants, provide multiple health advantages (Krishna *et al*., 2023). Anthocyanin accumulation serves as a protective response for N-deficient plants by making leaves more light-sensitive through chlorophyll reduction. The presence of anthocyanins in plants enhances their ability to withstand N deficiency stress (Liang & He,2018). The accumulation of anthocyanin is triggered by N deficiency but also results from different nutritional imbalances, making it a useful crop nutrient status indicator (Jezek *et al*., 2023). Low N availability has been shown to enhance secondary metabolite production in plants by redirecting excess carbon (C) energy toward biosynthesis pathways, including flavonoid synthesis (Li *et al*., 2021).

High N availability can lead to decreased anthocyanin levels and reduced reproductive development. In blueberries findings vary while high N may reduce anthocyanin accumulation Gonzalez *et al*. (2018) observed increased anthocyanin levels in specific N treatments such as a balanced 50:50 NH_4^+: NO_3^- ratio, as shown on (Table 2.3.). NO_3^--based sources generally favour C allocation toward flavonoid production, whereas NH_4^+ sources tend to enhance N assimilation, potentially at the expense of flavonoid synthesis. In a study that investigated blackberries, show that distinct N forms impact the expression of genes involved in flavonoid biosynthesis, specifically Dihydroflavonol 4-reductase (DFR) and Chalcone synthase (CHS). Ammonium (NH_4^+) increases gene activity related to phenolic compound production (Duan *et al*., 2023). As research specifically investigating the effect of different N sources on phenolic compound accumulation in blueberries is limited, data from studies on related species have been included to provide a broad context. These trends are summarised in Table 2.3, presenting several studies on how different N sources and conditions influence phenolic compound accumulation across various plants.

Table 2.3 Effects of Nitrogen Forms on Phenolic Compound Biosynthesis

Source	**Plant**	**N Source / Condition**	**Key Findings**	**Implications**
Gonzalez *et al*. (2018)	Blueberry	100% NO_3^-, 100% NH_4^+, 50:50 NH_4^+:NO_3^-	100% NH_4^+: 1.90 mg/100 g FW, 100% NO_3^-: 11.68 mg/100 g FW anthocyanin, and 50:50 mix: 12.79 mg/100 g FW (highest)	NO_3^- favours anthocyanin synthesis over NH_4^+; balanced N form is most effective.
Liang & He (2018)	General	N deficiency	Anthocyanin accumulation increases under N deficiency as a stress response; it reduces chlorophyll, increases light sensitivity	Anthocyanins serve as protective metabolites under N stress.
Jezek *et al*. (2023)	General	Nutritional imbalances, including N deficiency	Anthocyanin accumulation reflects multiple nutrient imbalances, not just N deficiency	Anthocyanins are effective biomarkers for nutrient status.
Leitzke *et al*. (2015)	Blueberry ('O'Neal')	High N availability	Increased anthocyanin production observed alongside a pH drop and toxic Al accumulation	Excess N can stimulate anthocyanin synthesis but also cause soil acidification.
Arias *et al*. (2024)	Blueberry	NO_3^- treatment	Lower biomass (leaves, stems, roots) and reduced secondary metabolite production, including anthocyanins	NO_3^- may suppress overall plant growth, negatively impacting secondary metabolites.
Duan *et al*. (2023)	Blackberry	Urea, ammonium sulphate, calcium nitrate	NH_4^+ and urea: ↑ anthocyanins, ellagic acid $Ca(NO_3)_2$: ↑ flavonoid biosynthesis & antioxidant capacity	N-form affects specific bioactive compound production differently. NO_3^- promotes carbon reallocation to secondary metabolism.

Huang *et al*. (2022)	General	N deficiency	N deficiency triggers C metabolism activation and energy accumulation	Explains why secondary metabolite biosynthesis increases under N deficiency.
Li *et al*. (2021)	General	N deficiency	Energy surplus from C metabolism promotes flavonoid synthesis to rebalance C/N metabolism	Flavonoids help regulate energy balance under nutrient stress.
Kishorekumar *et al*. (2020)	General	NH_4^+ vs. NO_3^- assimilation pathways	NH_4^+: directly assimilated; NO_3^-: energy-costly reduction to NH_4^+; influences phenolic synthesis differently	NH_4^+ may supply more precursors but less energy, whereas NO_3^- impacts resource allocation more strongly.

N- nitrogen, NH_4^+- ammonium, NO_3^- -nitrate, FW – fresh weight, mg – milligram, g – grams, Ca $(NO_3)_2$ - calcim nitrate, C – carbon, Al – aluminum,

2.7 Plant Physiological Responses to Different N Sources

The response of blueberry plants to different N forms shows N availability as a critical factor that affects both growth and photosynthesis (González *et al*., 2018; Osorio *et al*., 2020; Yuan-Yuan *et al.,* 2021). Adequate N supply remains essential because chlorophyll synthesis depends on N to enable light absorption and photosynthetic efficiency. The use of NH_4^+ as a nutrient source has been shown to increase stomatal conductance in blueberries, which leads to better gas exchange and supports photosynthesis (Osorio *et al*., 2022). The application of NH_4^+ resulted in better gaseous exchange parameters than NO_3^-, (Yuan-Yuan *et al*. (2021) demonstrated that a 5:1 NH_4^+: NO_3^- ratio produced the best photosynthetic products and stomatal performance.

However, the advantage of this depends on concentration because excessive NH_4^+ leads to metabolic imbalance and oxidative stress, and impaired photosynthetic functions (Yañez-Mansilla *et al*., 2014). Excessive NH_4^+ stress disrupts electron transport and reduces carboxylation efficiency this decreasing CO_2 assimilation (Wang *et al*., 2019). The assimilation of NO_3^- requires more energy than NH_4^+ but allows sustained photosynthesis through its ability to generate ATP and NADPH needed for the Calvin cycle (Kishorekumar *et al*., 2020). The study by Cárdenas-Navarro *et al*. (2024) demonstrated that blueberry plants supplied with NO_3^- nutrition showed better carbon fixation rates and electron transport activity.

Urea-based fertilizers which are hydrolyzed into NH_4^+ in the soil have shown photosynthetic outcomes like NH_4^+ sources (Nasraoui-Hajaji & Gouia, 2014). The controlled N release from urea leads to higher chlorophyll content and better C assimilation (Kozos & Ochmian, 2016). The photosynthetic response extend longer because N from urea becomes available more gradually than from NH_4^+ or NO_3^- (Smolander *et al*., 2022). The most successful approach to maximize photosynthetic efficiency while preventing N-related stress in blueberries involves maintaining balanced NH_4^+: NO_3^- inputs.

2.8 Effects of Nitrogen Sources on Water-Use Efficiency and Drought Tolerance in Plants

The different N sources influence water-use efficiency (WUE) transpiration, and osmotic adjustment in blueberry plants these are key processes for maintaining water status under drought (Ruiz-Romero *et al.*, 2024). NH_4^+ nutrition enhance blueberry plant drought resistance through multiple physiological processes. The increased root abscisic acid content in drought-stressed NH_4^+-fed plants leads to better WUE (Ding *et al.*, 2016). The accumulation of osmolytes such as proline and soluble sugars helps sustain root development to reach deeper soil water (Zaher-Ara *et al.*, 2016). Highbush blueberry cultivars showed different levels of drought resistance after drought stress reduced and how their photochemical efficiency and increased proline content (Balboa *et al.*, 2020).

Under water-limited conditions NH_4^+ nutrition controls stomatal conductance to minimise excessive water loss through transpiration while allowing sufficient CO_2 uptake for photosynthesis to support plant development (Torralbo *et al.*, 2019). The drought resistance of *Malus prunifolia* increased with higher NH_4^+ uptake but lower NO_3^- uptake indicating the importance of NH_4^+ in drought tolerance (Huang *et al.*, 2018). Likewise, in other crops high NH_4^+ concentrations cause ion imbalances which lead to toxicity and damage the plant's water stress tolerance (Shilpha *et al.*, 2023). Research conducted by Faralli *et al.* (2023) demonstrated that NO_3^--based fertilisation enhances plant development under sufficient irrigation by improving transpiration efficiency. The positive effects of NO_3^--nutrition on transpiration reached their peak when water availability was sufficient yet NO_3^- does not provide drought tolerance at the same level as NH_4^+. Plants that received NH_4^+ nutrition demonstrated superior drought tolerance compared to those receiving NO_3^- under water-stressed conditions (Ding *et al.*, 2016). However, plants treated with NO_3^- still maintained positive hydration status because NO_3^- enabled appropriate stomatal conductance for efficient CO_2 uptake and reduced water loss during photosynthesis (Ding *et al*., 2016).

2.9 Conclusions and Future Research Directions

The selection of N sources along with application methods determines the most effective method to promote sustainable blueberry production while maintaining environmental sustainability. The combination of NH_4^+ with NO_3^- or NH_4^+ alone results in superior plant growth and fruit quality compared to NO_3^- alone particularly when the soil conditions are acidic which are favourable for blueberry cultivation. Based form these findings further research should investigate how different blueberry cultivars respond to the combination of N forms under varying acidic conditions. The practice of split fertiliser applications and fertigation systems enhancing nutrient utilisation efficiency while reducing nutrient loss. However, the long-term effects of continuous NH_4^+ fertilisation on soil acidification and associated changes in nutrient dynamics under blueberry production remain under investigated highlighting the need for further research.

Future research should also invistigate the interactions between N sources and secondary metabolite production especially phenolic compounds which are important for blueberry quality and human health benefits. Knowledge of the mechanisms through which N influences phenolic synthesis could provide new ways of improving fruit quality through fertilisation practices. Selection of N source is the main factor in improving the plant growth and physiological performance; hence, it is important to explore this area of research, particularly in blueberry secondary metabolite accumulation, which is relatively scarce in the current available literature.

References

Ahmadi, A. Y., 2023, ‘Relations of Legumes with Soil Health and Succeeding Crops’, *Open Access Journal of Agricultural Research*, *8*(4), 1–9. https://doi.org/10.23880/oajar-16000333

Alt, D. S., Doyle, J. W., & Malladi, A., 2017, ‘Nitrogen-source preference in blueberry (Vaccinium sp.): Enhanced shoot nitrogen assimilation in response to direct supply of nitrate’, *Journal of Plant Physiology*, *216*, 79–87. https://doi.org/10.1016/j.jplph.2017.05.014

Anwar, A., Zheng, J., Chen, C., Chen, M., Xue, Y., Wang, J., Su, W., Chen, R., & Song, S., 2024, ‘Effects of NH_4^+-N: NO_3^--N ratio on growth, nutrient uptake and production of blueberry (*Vaccinium spp.*) under soilless culture’, *Frontiers in Plant Science*, *15,1438811.* https://doi.org/10.3389/fpls.2024.1438811

Arias, M. I., Nario, A., Rojas, K., Blanc, P., & Bonomelli, C., 2024, ‘Newly Established Blueberry Plants: The Role of Inorganic Nitrogen Forms in Nitrogen and Calcium Absorption’, *Horticulturae*, *10*(11), 1168. https://doi.org/10.3390/horticulturae10111168

Balboa, K., Ballesteros, G. I., & Molina-Montenegro, M. A., 2020, ‘Integration of Physiological and Molecular Traits Would Help to Improve the Insights of Drought Resistance in Highbush Blueberry Cultivars’, *Plants*, *9*(11), 1457. https://doi.org/10.3390/plants9111457

Bashir, M. R., Alomran, M., Gill, A. N., Tufail Khan, Q. A., Jameel, S., Rafiq, M. U., & Khan, F., 2024, ‘Rising Nitrogen Dioxide (NO_2): A Growing Threat to Environment and Plant Health’, *Journal of Agriculture and Biology*, *2*(1), 76–83. https://doi.org/10.55627/agribiol.002.01.01023

Cárdenas-Navarro, R., Luna-Béjar, J. A., Castellanos-Morales, V. del C., Bravo-Hernández, N. L., & López-Pérez, L., 2024, ‘Effect of the concentration and ionic form of nitrogen (N) on photosynthesis, growth and fruit production of blueberry (Vaccinium corymbosum L.)’, *Biotecnia*, *26*, e2325. https://doi.org/10.18633/biotecnia.v26.2325

Ding, L., Li, Y., Wang, Y., Gao, L., Wang, M., Chaumont, F., Shen, Q., & Guo, S., 2016, ‘Root ABA Accumulation Enhances Rice Seedling Drought Tolerance under Ammonium Supply: Interaction with Aquaporins’, Frontiers *in Plant Science*, *7*, 5–9.. https://doi.org/10.3389/fpls.2016.01206

Duan, Y., Yang, H., Yang, H., Wu, Y., Fan, S., Wu, W., Lyu, L., & Li, W., 2023, ‘Integrative physiological, metabolomic and transcriptomic analysis reveals nitrogen preference and carbon and nitrogen metabolism in blackberry plants’, *Journal of Plant Physiology*, *280*, 4–14. 153888. https://doi.org/10.1016/j.jplph.2022.153888

Faralli, M., Bianchedi, P. L., Moser, C., Bontempo, L., & Bertamini, M., 2023, ‘Nitrogen control of transpiration in grapevine’, *Physiologia Plantarum*, *175*(2), 13906. https://doi.org/10.1111/ppl.13906

Glass, J. B., & Rousk, K., 2024, ‘Microbial nitrogen transformation processes across environments: more than just a cycle’ *Trends in Microbiology*, *32*(6), 519–521. https://doi.org/10.1016/j.tim.2024.04.006

González, L. K., Rugeles, L. N., & Magnitskiy, S., 2018, ‘Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (Vaccinium *meridionale swartz*)’, *Agronomia Colombiana*, *36*(1), 58–67. https://doi.org/10.15446/agron.colomb.v36n1.69304

Huang, L., Li, M., Zhou, K., Sun, T., Hu, L., Li, C., & Ma, F., 2018, ‘Uptake and metabolism of ammonium and nitrate in response to drought stress in Malus prunifolia’, *Plant Physiology and Biochemistry*, *127*, 185–193. https://doi.org/10.1016/j.plaphy.2018.03.031

Huang, W.-T., Zheng, Z.-C., Hua, D., Chen, X.-F., Zhang, J., Chen, H.-H., Ye, X., Guo, J.-X., Yang, L.-T., & Chen, L.-S. 2022, ‘Adaptive responses of carbon and nitrogen metabolisms to nitrogen-deficiency in Citrus sinensis seedlings’, BMC Plant Biology, 22(1), 370. https://doi.org/10.1186/s12870-022-03759-7

Imler, C. S., Arzola, C. I., & Nunez, G. H., 2019, ‘Ammonium Uptake Is the Main Driver of Rhizosphere pH in Southern Highbush Blueberry’, *HortScience*, *54*(5), 955–959. https://doi.org/10.21273/HORTSCI13764-18

Jezek, M., Allan, A. C., Jones, J. J., & Geilfus, C., 2023, ‘Why do plants blush when they are hungry?’, *New Phytologist*, *239*(2), 494–505. https://doi.org/10.1111/nph.18833

Jiang, Y., Zeng, Q., Wei, J., Jiang, J., Li, Y., Chen, J., & Yu, H., 2019, ‘Growth, Fruit Yield, Photosynthetic Characteristics, and Leaf Microelement Concentration of Two Blueberry Cultivars under Different Long-Term Soil pH Treatments’, *Agronomy*, *9*(7), 357. https://doi.org/10.3390/agronomy9070357

Jose, E., Soni K. B., Alex, S., Shalini Pillai P., Beena R., & Stephen, R., 2023, ‘Molecular Frameworks of Nitrogen Response in Plants: A Review’, *International Journal of*

Environment and Climate Change, *13*(12), 380–390. https://doi.org/10.9734/ijecc/2023/v13i123694

Kishorekumar, R., Bulle, M., Wany, A., & Gupta, K. J., 2020, ‘An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants’, *National Library of Medicine* (pp. 1–13). https://doi.org/10.1007/978-1-4939-9790-9_1

Kozos, K., & Ochmian, I., 2016, ‘The influence of fertilisation urea phosphate on growth and yielding bush of two highbush blueberry cultivars (v. Corymbosum)’, *Folia Pomeranae Universitatis Technologiae Stetinensis Agricultura, Alimentaria, Piscaria et Zootechnica*, *325*(37), 29–38. https://doi.org/10.21005/AAPZ2016.37.1.04

Krishna, P., Pandey, G., Thomas, R., & Parks, S., 2023, ‘Improving Blueberry Fruit Nutritional Quality through Physiological and Genetic Interventions: A Review of Current Research and Future Directions’, *Antioxidants*, *12*(4), 810. https://doi.org/10.3390/antiox12040810

Leitzke, L. N., Picolotto, L., Pereira, I. D. S., Vignolo, G. K., Schmitz, J. D., Vizzotto, M., & Antunes, L. E. C., 2015, ‘Nitrogen fertilizer affects the chemical composition of the substrate, the foliar nutrient content, the vegetative growth, the production and fruit quality of blueberry’, *Científica*, *43*(4), 316. https://doi.org/10.15361/1984-5529.2015v43n4p316-324

Li, S., Huang, Y. Z., Liu, X. Y., & Fu, X. D., 2021, ‘Genetic improvement of nitrogen use efficiency in crops’, In *Yi chuan = Hereditas*, 43(7),21–064. https://doi.org/10.16288/j.yczz.21- 064

Liang, J., & He, J., 2018, ‘Protective role of anthocyanins in plants under low nitrogen stress’, *Biochemical and Biophysical Research Communications*, *498*(4), 946–953. https://doi.org/10.1016/j.bbrc.2018.03.087

Machado, R. M. A., Bryla, D. R., & Vargas, O., 2014, ‘Effects of salinity induced by ammonium sulphate fertilizer on root and shoot growth of highbush blueberry’, *Acta Horticulturae*, *1017*, 407–414. https://doi.org/10.17660/ActaHortic.2014.1017.49

Messiga, A. J., Nyamaizi, S., Yu, S., & Dorais, M., 2021, ‘Blueberry yield and soil mineral nitrogen response to nitrogen fertilizer and nitrification inhibitors under drip-fertigation systems’, Agronomy, *11*(11), 2144 https://doi.org/10.3390/agronomy11112144

Muratore, C., Espen, L., & Prinsi, B., 2021, ‘Nitrogen Uptake in Plants: The Plasma Membrane Root Transport Systems from a Physiological and Proteomic Perspective’, *Plants*, *10*(4), 681. https://doi.org/10.3390/plants10040681

Nasraoui-Hajaji, A., & Gouia, H., 2014, ‘Photosynthesis sensitivity to NH_4^+-N change with nitrogen fertilizer type’, *Plant, Soil and Environment*, *60*(6), 274–279. https://doi.org/10.17221/7418-PSE

Osorio, R., Cáceres, C., & Covarrubias, J. I., 2020, ‘Vegetative and Physiological Responses of “Emerald” Blueberry to Ammoniacal Sources with a Nitrification Inhibitor’, *Journal of Soil Science and Plant Nutrition*, *20*(2), 507–515. https://doi.org/10.1007/s42729-019-00135-7

Peterson, L. A., Stang, E. J., & Dana, M. N., 2022, ‘Blueberry Response to NH_4-N and NO_3-N’, *Journal of the American Society for Horticultural Science*, *113*(1), 9–12. https://doi.org/10.21273/jashs.113.1.9

Pienaar, L., Smit, P., Hattingh, J. & Cloete, K., 2022, ‘Developing economic models for South African blueberry industry: Outlook 2022-2031’, Report Commissioned by BerriesZA, Paarl: Bureau for Food and Agricultural Policy (BFAP), viewed 25 June 2025, from http://dx.doi.org/10.13140/RG.2.2.27846.75845

Pinheiro, R. B., Cantarutti, R. B., Villani, E. M. de A., Cavalcante, V. S., & Fontes, M. P. F., 2020, ‘Nitrate Mobility in Variable-Charge Tropical Soils’, *Communications in Soil Science and Plant Analysis*, *51*(16), 2163–2173. https://doi.org/10.1080/00103624.2020.1820025

Rosen, C. J., Allan, D. L., & Luby, J. J., 2019, ‘Nitrogen form and solution pH influence growth and nutrition of two Vaccinium clones’, *Journal of the American Society for Horticultural Science*, *115*(1), 83–89. https://doi.org/10.21273/jashs.115.1.83

Ruiz-Romero, R., de la Peña, M., Ayala-Díaz, I., Montoya, C., & Romero, H. M., 2024, ‘Genotype and Nitrogen Source Influence Drought Stress Response in Oil Palm Seedlings’, *Agronomy*, *14*(9), 2082. https://doi.org/10.3390/agronomy14092082

Smolander, A., Martikainen, P. J., & Henttonen, H. M., 2022, ‘Half-a-century effects of a slow-release nitrogen fertilizer, ureaformaldehyde, on stand growth and soil processes in a Scots pine stand’, *Forest Ecology and Management*, *519*, 120320. https://doi.org/10.1016/j.foreco.2022.120320

Spiers, J. M., 2022, ‘Effects of pH Level and Nitrogen Source on Elemental Leaf Content of ‘Tifblue’ Rabbiteye Blueberry’, *Journal of the American Society for Horticultural Science*, *103*(6), 523–525. https://doi.org/10.21273/jashs.103.6.705

Tarkanyi, P. C., Bordean, D. M., Catargiu, A., & Poiana, M. A., 2019, ‘Blueberries - pleading for health’, Journal *of Agroalimentary Processes and Technologies*, *25*(4), 187–194.

Torralbo, F., González-Moro, M. B., Baroja-Fernández, E., Aranjuelo, I., & González-Murua, C., 2019, ‘Differential Regulation of Stomatal Conductance as a Strategy to Cope With Ammonium Fertilizer Under Ambient Versus Elevated CO_2’, Frontiers *in Plant Science*, *10,* 597. https://doi.org/10.3389/fpls.2019.00597

Vargas, O. L., & Bryla, D. R., 2015, ‘Growth and Fruit Production of Highbush Blueberry Fertilized with Ammonium Sulfate and Urea Applied by Fertigation or as Granular Fertilizer’, *HortScience*, *50*(3), 479–485. https://doi.org/10.21273/HORTSCI.50.3.479

Wang, F., Gao, J., Shi, S., He, X., & Dai, T., 2019, ‘Impaired electron transfer accounts for the photosynthesis inhibition in wheat seedlings (*Triticum aestivum* L.) subjected to ammonium stress’, *Physiologia Plantarum*, *167*(2), 159–172. https://doi.org/10.1111/ppl.12878

Xu, J., Fang, Y., Tavakkoli, E., Pan, X., Liao, F., Chen, W., & Guo, W., 2021, ‘Preferential ammonium: Nitrate ratio of blueberry is regulated by nitrogen transport and reduction systems’, *Scientia Horticulturae*, *288,* 11034. https://doi.org/10.1016/j.scienta.2021.110345

Yañez-Mansilla, E., Cartes, P., Reyes-Díaz, M., Ribera-Fonseca, A., Rengel, Z., Lobos, W., & Alberdi, M., 2015, ‘Leaf nitrogen thresholds ensuring high antioxidant features of Vaccinium corymbosum cultivars’, *Journal of Soil Science and Plant Nutrition*, *15*(3),574– 586. https://doi.org/10.4067/S0718-95162015005000025

Yang, H., Wu, Y., Zhang, C., Wu, W., Lyu, L., & Li, W., 2022, ‘Growth and physiological characteristics of four blueberry cultivars under different high soil pH treatments’, *Environmental and Experimental Botany*, *197*, 104842. https://doi.org/10.1016/j.envexpbot.2022.104842

Ye, J. Y., Tian, W. H., & Jin, C. W., 2022, ‘Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation’, In *Stress Biology*, 2(1), 4. https://doi.org/10.1007/s44154-021-00030-1

Yuan-Yuan, Z., Jun-Ping, T., Jun, C., Yu-Hui, H., & Yu-Shi, L., 2021, ‘Effects of different NH_4^+/NO_3^- ratios on the photosynthetic and physiology responses of blueberry (*Vaccinium spp.*) seedlings growth’, Journal *of Plant Nutrition*, *44*(6), 854–864. https://doi.org/10.1080/01904167.2021.1871754

Zaher-Ara, T., Boroomand, N., & Sadat-Hosseini, M., 2016, ‘Physiological and morphological response to drought stress in seedlings of ten citrus’, *Trees*, *30*(3), 985–993. https://doi.org/10.1007/s00468-016-1372-y

Zhang, J., Cai, Z., & Müller, C., 2018, 'Terrestrial N cycling associated with climate and plant-specific N preferences: a review', *European Journal of Soil Science*, *69*(3), 488–501. https://doi.org/10.1111/ejss.12533

CHAPTER THREE

INFLUENCE OF DIFFERENT NITROGEN SOURCES AND GROWTH MEDIA ON WATER USE, GROWTH, AND YIELD ATTRIBUTES OF BLUEBERRY

3.1 Abstract

Blueberry (*Vaccinium* spp.) is an economically important berry fruit that is widely distributed geographically and has attracted increasing attention in recent years as a third-generation fruit. The understanding of how coir and zeolite media interact with nitrogen (N) sources under blueberry cultivation still lacks depth and comprehensiveness, particularly in the South African climatic conditions. This study aimed to investigate how different N sources and growth media affect water use, growth, and yield of blueberry. Two growth media were used with three N sources: ammonium sulphate (N 21.2%), calcium nitrate (N 15.5%) and urea (N 46%), resulting in six treatment combinations with three biological replicates per treatment and three plants per replicate. The growth parameters investigated in this study were influenced by N sources and growth media. Ammonium sulphate with 100% coir surpassed other treatments recording the largest fruit diameter, with an average of (16 mm) and individual berry weight (1.81 g). Overall, the blueberry fruit diameter, weight per berry and total fresh yield increased gradually during the early harvest time reached a peak at mid harvest and subsequently declined towards the end of the harvest period.

Keywords: Substrate, Water Use Efficiency, Chlorophyll content, Blueberry, Nitrogen sources

3.2 Introduction

Blueberry (*Vaccinium* spp.) is an economically important berry fruit that is widely distributed geographically and has attracted increasing attention in recent years as a third-generation fruit (Akšić *et al*., 2019). South Africa’s (SA) blueberry production has grown from around 1,700 tons in 2011 to 27,700 tons in 2021, with projections for an increase to 56,000 tons by 2031, which translates to a 6.6% growth rate (Pienaar *et al*., 2022). However, the South African blueberry industry is a small player producing 2-5% of the world share, whilst leading producers, including China, the United States, Peru, Chile, and Mexico, globally supply around 68% of commercial production (IBO, 2023). Despite the low production share, South African exports have been significantly increasing due to being well-positioned to supply fresh blueberries to the market during the counter season of traditional blueberry-producing countries situated in the Northern Hemisphere (Pienaar *et al*., 2019). To cope with the increasing market demand, it is necessary to further improve the production and quality of blueberries in SA.

Blueberries are quite sensitive to fertilization, growers often apply large amount of nitrogen (N) to improve both yield and fruit quality (Duan *et al*., 2023); however, improper fertilization not only affects production but can harm the plant, causing the leaves wilt and physiological dysfunction, in extreme cases, the entire plant can wither and die (Alt *et al*., 2017). N use efficiency is very low, less than 40% of the N applied can be directly taken up by crops, and the rest is wasted (Yousaf *et al*., 2017). Simply increasing the use of N fertilizer will not boost crop production, but it will raise agricultural expenses and lead to plant nutrition imbalances, destroying soil and water resources. N fertilizers are primarily classified into three forms according to the N-containing group: ammonium (NH_4^+), nitrate (NO_3^-), and amide-N, such as urea (Hachiya *et al*., 2021). Urea is an organic N source, mostly used worldwide due to its advantages of high N content and easy transportation (Witte, 2011). Calcifuge plants, including blueberries, mostly prefer to absorb NH_4^+

rather than NO_3^-; this preference has been associated with the low capacity to assimilate the NO_3^- form. NO_3^- assimilation is mediated by nitrate reductase (NR) and nitrite reductase (NiR) enzymes, which are ineffective in blueberries, especially within the shoot tissue (Guo *et al*., 2007). Alt *et al*. (2017) showed that NO_3^- growing media supplementation directly to the shoots increased the biomass and N content of blueberry plants. Other authors have claimed that applying NO_3^- instead of NH_4^+ has a positive impact on the development of buds and roots along with the process of photosynthesis (Merhaut & Darnell, 1996; Crisóstomo *et al*., 2014). Blueberry response to the N source applied may be influenced by various factors including the growing media, as it inherently prefers acidic organic-rich substrates (Ortiz-Delvasto *et al*., 2023).

Blueberries are naturally low nutrient demanding plants with shallow roots (Messiga *et al*., 2018) due to these specific requirements growers are increasingly investigating alternative methods to grow the plants in regions with suboptimal conditions. One approach gaining popularity involves cultivation in containers with soilless substrates and highly controlled fertilization systems (Kingston *et al*., 2017). In the absence of soil amendments, many *Vaccinium* species cultivated in less favourable soils display stunted shoot growth and decreased yield, often associated with a decreased ability to uptake and assimilate N (Poonnachit & Darnell, 2004). The use of coir as growing media has rapidly increased. It has favourable hydrological properties with desirable levels of physical and chemical properties, which make it the best medium for soilless systems (Mariyappillai & Arumugam, 2021). However, the primary disadvantages of coir are low cation exchange capacity (CEC) and low-pressure heads due to a low percentage of large particles, creating a problem in the water-air balance and gas exchange under different watering regimes (Londra *et al*., 2012). To mitigate these disadvantages different materials characterized by large particle sizes, such as zeolite, are added in the growing media (Mfeka *et al*., 2023). Zeolite incorporated with othe growth media is proposed to not only retain water but also improve crop performance minimizing the rate of nutrient release from both organic and inorganic fertilizers and enabling better nutrient availability throughout the crop growth stages (Perez-Caballero *et al*., 2008). The understanding of how these growing media interact with N source under blueberry cultivation still lacks depth and comprehensiveness particularly in the South African climatic conditions. This study aimed to investigate how different N sources and growth media affect water use, growth and yield of blueberry. The findings of this study can provide scientific and realistic fertilization solutions for production practices with significant implications for enhancing nitrogen and water use efficiency reducing production cost and mitigating environmental pollution.

3.3 Materials and Methods

3.3.1 Experimental Site

The experiment was conducted at the Agri Hub, Department of Agriculture, Cape Peninsula University of Technology, Wellington campus, South Africa (33° 38′ 21″ S, 19° 00′ 21″ E). The site is characterized by a Mediterranean climate with an average annual temperature and precipitation of 16.9 °C and 821 mm, respectively. The experimental setup was established under a polythene white shade net.

3.3.2 Plant material and experimental design

Blueberry seedlings were obtained from De Fynne, a commercial nursery in the Western Cape Province, South Africa. The seedlings were two-year-old blueberry cv. ‘Legacy’ approximately 20 cm tall in pots with a capacity of one liter (L). Two types of substrates were evaluated, and the plants were selected by similar height and transplanted in 45L bags (width 23cm, height 15.6 cm, length 124.8 cm), with a commercial substrate composed of coir, which is 100% coir (4.2kg per bag substrate, provided by Glencairns, Paarl, South Africa). In the same way, plants were selected and transplanted into bags with 80% coir (3.6 kg) mixed with 20% zeolite (6.8 kg) (zeolite: AristaLite Granular 1-3 mm 25 kg, provided by Agring Consultants, Heidelberg, South Africa). Plants were spaced 70 cm apart between rows and grown from August 2023 to December 2024. The experiment was arranged in a factorial design to assess the interaction between N sources and growth media. After 30 days of adaptive pre-culture, each growth medium was combined with three N sources: ammonium sulphate (N 21.2%), calcium nitrate (N 15.5%), and urea (N 46%) (provided by Agrimark, Simondium), resulting in six treatment combinations, with three replicates per treatment and three plants per replicate (Table 3.1). Giving a total of 54 experimental units (2 growth media × 3 N sources × 3 replicates ×3 plants). During the experiment, fertilizer was applied biweekly at a rate of 1 L of diluted solution per bag. The total N supply concentration was kept constant across the three treatments. N was applied using a split application method, with 6 g supplied in the first split and 9 g in the second split, calculated based on the available N (%) of each fertilizer source.

Table 3.1 Experimental Layout

Replicates	**Treatment Layout**					
Rep 1	T_1	T_2	T_3	T_4	T_5	T_6
	T_1	T_2	T_3	T_4	T_5	T_6
	T_1	T_2	T_3	T_4	T_5	T_6
Rep 2	T_2	T_3	T_4	T_5	T_6	T_1
	T_2	T_3	T_4	T_5	T_6	T_1
	T_2	T_3	T_4	T_5	T_6	T_1
Rep 3	T_3	T_4	T_5	T_6	T_1	T_2
	T_3	T_4	T_5	T_6	T_1	T_2
	T_3	T_4	T_5	T_6	T_1	T_2

T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

3.3.3 Cultivation and Irrigation Management

The concentrations of other major nutrient elements used in this experiment were adopted from the nutrient formulation reported by Yang et al. (2025) for blueberry cultivation in a soilless system. The initial pH was 3.8 for 100% coir and 4.2 for 80%coir: 20% zeolite. Following fertilization application, pH and electrical conductivity (EC) of the growth media were monitored biweekly using laboratory procedures with a Milwaukee pocket-size pH meter and EC meter (Leal-Ayala *et al*., 2021). The irrigation management was guided by the continuous moisture monitoring using DFM© soil moisture probes inserted to a depth of 30 cm, connected to a data logger. Irrigation was triggered when the moisture content dropped to 75% of bag field capacity or below at which point water was applied to replenish the moisture back to bag capacity. This was achieved by applying 4.25 L of water to 100% coir bags and 4 L of water to bags containing a combination of 80% coir and 20% zeolite (v/v) (Mfeka *et al*., 2023). All other cultivation practices including irrigation, pruning and weeding were thoroughly observed and kept uniform across all treatments.

Figure 3.1. (**A**) Blueberry plants obtained from the nursery before transplanting, (**B**) plants transplanted into 45 L growth bags containing the designated media treatments, (**C**) installation of a soil moisture probe at 30 cm depth for irrigation monitoring, (**D**)measurement of plant height using a measuring ruler, (**E**) assessment of vegetative growth parameters, including the number of leaves and branches, (**F**) leaf chlorophyll content index (CCI) measurement with an Opti-Sciences CCM-200 chlorophyll content meter, and (**G**) a vernier caliper used for stem diameter.

3.3.4 Data Collection

Growth parameters were measured at the beginning of the experiment during seedling transplants at four weeks, and subsequently at two weeks after transplanting from October 2023 to December 2024.

a. Plant height

The height of plants was measured from the base of the stems to the apex of the last leaf using a transparent ruler (cm). The measurements of all the tagged plants for data collection were averaged, providing a value per plant.

b. Main stem diameter

The stem diameter was measured using a manual vernier caliper (mm), which was set to zero before use. The measurements were taken at 5cm above the soil surface by placing the caliper jaws around the stem without applying pressure.

c. Number of leaves

The total number of blueberry plants with both fully developed and emerging leaves was counted and recorded.

d. Number of branches

The number of branches from primary and secondary branches was counted and recorded for each treatment.

e. Chlorophyll content index (CCI)

CCI on leaves was measured using a Opti-Sciences CCM-200 chlorophyll content meter. Readings were taken from five points along the upper edge of each fully expanded leaf avoiding the main veins and the average was calculated to obtain a representative measurement. The instrument was calibrated according to the manufacturer’s guidelines before each measurement session.

f. Water application (L)

Moisture content was continuously monitored using DFM© soil moisture probes with readings logged automatically via a data logger in real time. Irrigation data was recorded whenever the moisture content dropped to 75% or below to replenish to field capacity.

g. Fruit production

The harvest was done manually, at the peak of the ripening period, berries were harvested weekly between October to December in both the 2023 and 2024 growing seasons. After each harvest, the berries per shrub were weighed using a weighing scale, measured in diameter (mm) using a fruit measuring gauge, counted per diameter, counted per shrub, and placed in properly labelled bags. They were stored at –80 °C for further analysis. Nitrogen use efficiency (NUE) was determined from berry yield per unit of nitrogen applied and was calculated using the following formula (Fixen *et al*., 2012): NUE = $\frac{Yield\ (g)}{Quanitity\ of\ N\ applied\ (g)}$

Figure 3.2. (**A**) pH and electrical conductivity were monitored using a Milwaukee pocket-sized pH meter and EC meter, (**B**) observation of berry colour change at the onset of ripening, (**C**) fruit harvested at maturity, (**D-E**) berry diameter measured with a sizing gauge, (**F**) yield determined by weighing fruit per shrub at each harvest, (**G**) berries packed, labelled, and stored in a freezer, and (**H**) all data recorded for analysis.

3.3.5 Statistical Analysis

The data of the different experimental variables were captured in an Excel spreadsheet for analysis. Analysis of variance ANOVA at a 95% confidence level was carried out using IBM SPSS software version 30.0. Means separated were done using Duncan`s Multiple Range Test (DMRT). The analysis of results was presented as the mean ± standard deviation across all experiments.

3.4 Results

3.4.1 Influence of nitrogen sources and growth media on the growth parameters of blueberry.

The growth parameters investigated in this study were influenced by nitrogen (N) sources and growth media (Table 3.2). During the 2023 cultivation season, plant height, number of leaves, and stem diameter had significant ($P<0.05$) differences among the different treatment applications. However, the number of branches and chlorophyll content had no significant difference ($P > 0.05$) among the different treatment applications. T_3 (100% coir + calcium nitrate) had the most impact on the number of leaves and number of branches, with an 80% and 14% increase, compared to the control (T_1=100% Coir + ammonium sulphate). However, T_5 (100% coir + urea) decreased the number of leaves and branches by 11.7% and 8.3%, respectively (Figure 3.3B-C). Notwithstanding, T_1 outperformed the most on plant height, followed by T_5, and the least by T_4 (80% coir: 20% zeolite+ calcium nitrate). Furthermore, T_4 recorded the most effect across the different treatments in stem diameter and chlorophyll content index (CCI), with a 46% and 29% increase compared to control (T_1).

Interestingly, during the 2024 cultivation season, the growth parameters investigated had a significant ($P< 0.05$) difference among all treatment applications.T_2 (80% coir: 20% zeolite+ ammonium sulphate) achieved the highest leaf number, with a 19% increase compared to $T_{1,}$ and accumulated the greatest CCI, which was 33% higher than T_3 (Figure 3.3A-B). Additionally, T_2 also increased plant height by 35% compared to the lowest-performing treatment, T_4. However, T_1 maintained the highest overall performance in terms of plant height, CCI, and number of branches, producing 52.78 branches per plant, compared to 48.78 branches in T_2. Stem diameter was the thickest under T_4 application, which exceeded T_1 by 8%.

Despite seasonal variation in treatments, the control treatment, T_1, consistently promoted plant height and the number of branches. In 2023, T_3 had the strongest effect on leaf number and branching, whereas in 2024, T_2 emerged as the most effective for leaf number. Stem diameter was improved under T_4 treatment applications in both cultivation seasons, while T_5 generally had a suppressive effect on the growth.

Table 3.2 Influence of nitrogen sources and growth media on growth parameters of blueberry during two cultivation seasons.

Cultivation seasons	Treatment	Plant height (cm)	Stem diameter (mm)	No. of leaves/ plant	No. of branches
2023	T_1	42.44±14.38^{a}	9.4±4.4^{b}	80.67±49.48^{b}	33.67±21.57^{a}
	T_2	28.72±13.16^{b}	12.5±2.1^{ab}	85.56±64.33^{b}	34.22±6.08^{a}
	T_3	22.51±4.12^{b}	9.6±3.6^{b}	145.56±65.57^{a}	38.44±6.58^{a}
	T_4	31.67±9.51^{b}	13.7±3.6^{a}	91.33±46.11^{ab}	32.22±9.37^{a}
	T_5	32.00±8.63^{b}	13.7±4.4^{a}	71.22±59.23^{b}	30.87±4.35^{a}
	T_6	29.78±7.29^{b}	11.8±3.9^{ab}	79.33±66.37^{b}	31.67±4.61^{a}
	LSD	6.21	0.2	8.11	NS
2024	T_1	75.57±12.17^{a}	18.4±6.7^{ab}	148.61±87.92^{ab}	52.78±18.86^{a}
	T_2	62.32±17.38^{b}	19.6±5.8^{a}	176.39±106.36^{a}	48.78±24.69^{a}
	T_3	46.47±5.63^{de}	17.0±1.6^{b}	133.78±66.64^{bc}	34.31±11.57^{b}
	T_4	46.02±10.38^{e}	19.9±1.0^{a}	98.17±44.54^{d}	31.42±10.99^{b}
	T_5	52.60±8.32^{c}	16.9±3.8^{b}	77.86±52.43^{d}	30.33±13.58^{b}
	T_6	51.82±12.10^{cd}	19.1±3.9^{a}	104.44±49.97^{cd}	34.72±9.88^{b}
	LSD	0.45	0.1	20.31	1.09

Values are means ± standard deviation at ($P<0.05$). Means with different letters in the same column are statistically significant at $P < 0.05$, *P*– probability, LSD– Least Significant Difference, S.D– standard deviation, cm– centimeters, mm- millimeters, No.–number, NS– Not significant, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

3.4.2 Influence of nitrogen sources and growth media on blueberry water use.

Water application had a significant difference ($p<0.05$) among the different treatments used in the study, across the different seasons (Table 3.3). The treatments with 100% coir (T_1, T_3, and T_5)

had a higher water application compared to the treatments with 80% coir: 20% zeolite (T_2, T_4, and T_6) across the different seasons.

Table 3.3 Influence of nitrogen sources and growth media on blueberry water use during two cultivation seasons.

Treatment	2023 Water application (L)	2024 Water application (L)
T_1	85 ± 3.68^{a}	76.85 ± 16.74^{a}
T_2	50.67 ± 8.72^{b}	46.67 ± 16.26^{b}
T_3	85 ± 3.68^{a}	76.85 ± 16.74^{a}
T_4	50.67 ± 8.72^{b}	46.67 ± 16.26^{b}
T_5	85 ± 3.68^{a}	76.85 ± 16.74^{a}
T_6	50.67 ± 8.72^{b}	46.67 ± 16.26^{b}
LSD	34.33	30.18

Values are means ± standard deviation at ($P<0.05$). Means with different letters in the same column are statistically significant at $P < 0.05$, *P*– probability, LSD– Least Significant Difference, S.D– standard deviation, L– liters, NS– Not significant, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

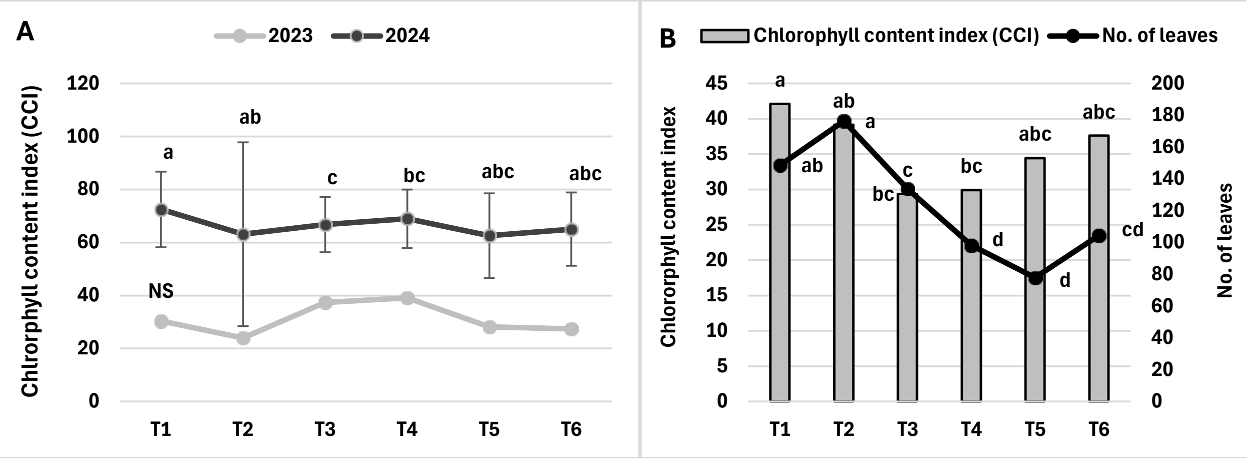


Figure 3.3. The effect of nitrogen sources and growth media on (**A**) chlorophyll content index (CCI) during 2023 and 2024 cultivation seasons and (**B**) the interactive trend between the number of leaves and CCI during 2024 cultivation season. The different letters above the columns and line markers indicate significant differences among the treatments at p<0.05. T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

3.4.3 Influence of nitrogen sources and growth media on yield and nitrogen use efficiency (NUE) of blueberry.

The yield attributes measured in this study were influenced differently ($p<0.05$) by N source and growth medium across the two cultivation seasons (Table 3.4). In 2023, N sources and growth media had no significant effect on most yield parameters, except for fruit diameter and fruit weight per diameter. The control treatment (T_1) had the largest fruit diameter and fruit weight per diameter compared to all the different treatments (Figure 3.4A). There was a decrease in average fruit weight by 28% for T_4 and 19% for T_3 and T_6 relative to T_1. A similar pattern was observed for total fresh yield, where T_1 (25.47 g) exceeded T_3, T_4, and T_6 by 59-69%. Furthermore, nitrogen use efficiency (NUE) was highest under T_1, nearly double that recorded for T_3, T_4, and $T_{6,}$ despite no statistical differences in most yield attributes (Figure 3.4B).

In the year 2024, treatment effects became more evident with varied differences observed in fruit diameter, total fresh yield, and NUE (Table 3.4). Notwithstanding T_1 outperformed all other treatments had the largest berries (16mm) (Figure 3.4A) with the greatest single fruit weight (1.81 g). This represented a 20-40% increase compared to T_3, T_4, and T_6. Total fresh yield under T_1 treatment was substantially higher exceeding T_3 by more than 117% and T_4 by over 300%. NUE followed a similar trend, with T_1 achieving the greatest efficiency (20.56 g yield per g N applied). Although T_2 produced yields that were statistically comparable to T_1, its NUE value was 42% lower, indicating less efficient N utilization despite relatively high yields (Figure 3.4B).

Seasonal variation was also evident in both cultivation seasons during the harvest period; yield peaked in November, while in December number of fruits per diameter continued to increase, but with reduced fruit diameter and weight, resulting in lower overall yield and NUE (Figure 3.4C-D).

Table 3.4 Influence of N sources and growth media on the number of fruits per plant, average fruit diameter, average weight per fruit, number of fruit diameters per plant, total fresh yield, and NUE during two growing seasons

Cultivation Season	Treatment	No. of fruits per plant	Average weight/fruit (g)	Mean fruit diameter/plant	Total fresh yield (g)/ plant	NUE (g yield/ g N applied)
2023	T$_1$	18.78±13.59^{a}	1.34±0.41^{a}	6.63±7.31^{ab}	25.47±21.57^{a}	5.66±4.79^{a}
	T$_2$	15.22±6.70^{a}	1.20±0.30^{ab}	5.91±4.23^{b}	17.05±9.15^{ab}	3.79±2.03^{ab}
	T$_3$	14.11±6.23^{a}	1.08±0.22^{bc}	7.47±6.10^{ab}	13.58±6.14^{b}	3.02±1.36^{b}
	T$_4$	13.56±6.21^{a}	0.97±0.15^{c}	10.17±6.45^{a}	12.38±5.67^{b}	2.75±1.26^{b}
	T$_5$	13.67±8.60^{a}	1.18±0.29^{abc}	5.59±3.54^{b}	15.23±11.43^{ab}	3.38±2.54^{ab}
	T$_6$	10.44±5.32^{a}	1.08±0.22^{bc}	5.53±3.69^{b}	10.79±6.65^{b}	2.39±1.48^{b}
	LSD	NS	0.11	NS	NS	NS
2024	T$_1$	107.78±93.58^{a}	1.81±0.80^{a}	20.25±16.86^{a}	185.08±187.3^{a}	20.56±20.81^{a}
	T$_2$	67±43.93^{ab}	1.69±0.78^{a}	13.38±9.75^{a}	107.58±95.2^{ab}	11.95±10.57^{ab}
	T$_3$	72.89±46.05^{ab}	1.56±0.67^{ab}	17.26±23.58^{a}	85.23±64.56^{b}	9.47±7.17^{b}
	T$_4$	48.89±33.78^{b}	1.29±0.47^{b}	20±30.34^{a}	45.64±29.15^{b}	5.07±3.24^{b}
	T$_5$	67.56±25.82^{ab}	1.70±0.77^{a}	14.90±18.08^{a}	79.43±44.31^{b}	8.83±4.92^{b}
	T$_6$	52.22±24.81^{b}	1.51±0.59^{ab}	13.43±17.05^{a}	58.07±30.49^{b}	6.45±3.39^{b}
	LSD	NS	NS	NS	12.43	1.38

Means with different letters in the same column are statistically significant at $P < 0.05$, P– probability, LSD– Least Values are means ± standard deviation at ($P<0.05$). Significant Difference, S.D– standard deviation, mm– millimeters, g– grams, No.–number, NUE– Nitrogen Use Efficiency, N– Nitrogen, NS– Not significant, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

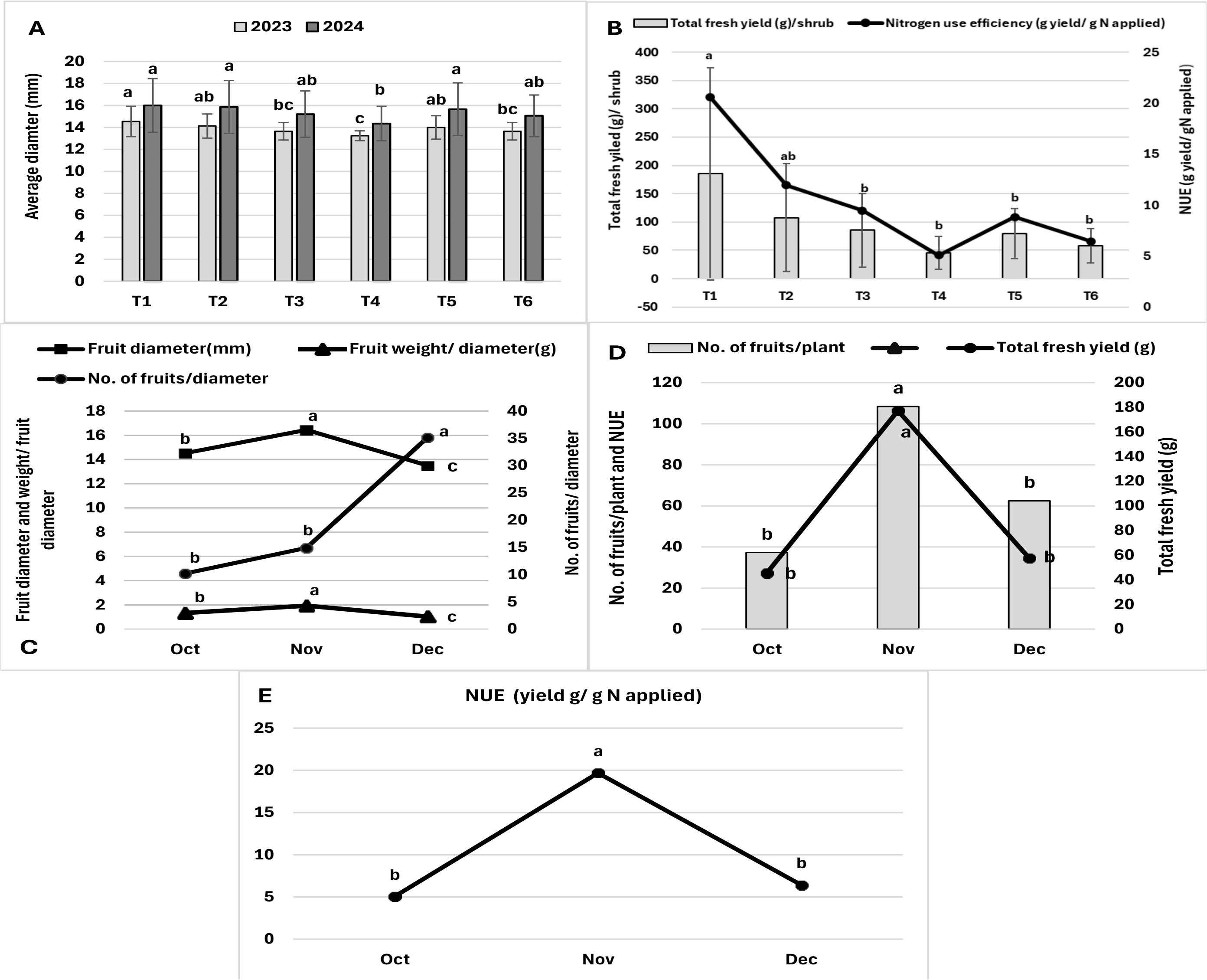


Figure 3.4. Effect of different nitrogen sources and growth media on (**A**) average fruit diameter during 2023 and 2024 cultivation seasons, (**B**) interactive trend between total fresh yield and Nitrogen use efficiency (NUE) during 2024 cultivation. Influence of harvest period on (**C**) Fruit diameter, fruit weight per diameter, and number of fruits per diameter, (**D**) number of fruits per plant, total fresh yield, and (**E**) nitrogen use efficiency (NUE). The different letters above the columns and line markers indicate significant differences among the treatments at $p<0.05$. T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

3.5 Discussion

3.5.1 Influence of nitrogen sources and growth media on the growth parameters of blueberry.

Nitrogen (N) is an indispensable element in blueberry fertilization (Smolarz, 2009). In this study, during the 2023 cultivation period, T_1 had the best results in most growth parameters, confirming its use as the industry standard in the Western Cape blueberry production. Plants treated with T_1 (42.44 cm) and T_5 (32 cm) had a higher plant height compared to T_3 (22.51 cm) treatments during the 2023 cultivation season. This may be attributed to the highly acidic nature of ammonium sulphate and urea, which lowers soil pH and enhances nutrient uptake (Vargas & Bryla, 2015; Kozos & Ochimian, 2016). Ammonium (NH_4^+) can be available in the soil as a product of urea hydrolysis, and urea increases the content of mineral forms of N, especially NH_4^+; however, it is less effective than ammonium sulphate (Grata, 2013) The greatest plant height observed under T_1 and T_5 agrees with Osorio *et al*. (2020), who reported enhanced shoot length when NH_4^+ was the dominant N source, with similar trends observed in *V. corymbosum* cv. ‘Anna’ (Yang *et al*., 2024).

These results further confirm the preference of blueberry for NH_4^+ over NO_3^- (Alt *et al*., 2017). This preference is linked to the inefficient nitrate reductase (NR) and nitrite reductase (NiR) activity, the enzymes responsible for the conversion of NO_3^- to NH_4^+ and amino acid synthesis for protein synthesis (Kishorekumar *et al*., 2020). However, other studies have suggested that the balance between NH_4^+ and NO_3^- may also influence growth. Anwar *et al*. (2024) reported that cv. ‘Nangao Z9’ showed the greatest plant height at a 50:50 NH_4^+: NO_3^- ratio, followed by 75:25 treatments, indicating that an appropriate NH_4^+: NO_3^- ratio may enhance the morphological growth of blueberry. NH_4^+ based fertilizers promoted shoot elongation in the ‘Legacy’ cultivar used in this study and in other studied cultivars, thereby demonstrating the species' physiological preferences for NH_4^+ nutrition.

During the 2024 cultivation period, a slightly different trend was observed for the plant height. Although the control (T_1) consistently had the tallest plants across both seasons, the second-highest values were recorded under T_2 (62.32 cm), in contrast to 2023 cultivation, where T_5 followed T_1 (Table 3.2). T_1 and T_2 were both supplied with the same NH_4^+ based fertilizer; however, T_2 showed a decrease, demonstrating the influence of different growth media.

Coir has been demonstrated to be suitable for the container cultivation of many Ericaceae species due to its aeration, drainage, and low bulk density (Scagel, 2003; Kingston *et al*., 2017). Ortiz-Delvasto *et al*. (2023) reported the superiority of pure coir for the blueberry cv. 'Legacy', supporting the present finding for T_1 treatments. The depression of plant height in T_2 can be attributed to the incorporation of zeolite into the growing medium; however, studies for cucumber seedlings and Swiss chard showed enhanced shoot height under zeolite-amended substrates (Jankauskienė *et al*., 2019; Sindesi *et al*., 2025). Blueberry is shallow rooted with fibrous root systems (Holzapfel, 2009), which require substrate with good aeration and low bulk density, conditions that are typically provided by coir. Zeolite additions to coir increase the bulk density, which may lead to a reduction in air-filled porosity, restrict oxygen (O_2) diffusion to the root zone, and increase compaction (Khan *et al*., 2024), which can limit root penetration for water and nutrient uptake, thus restricting shoot elongation and resulting in reduced plant height relative to 100% coir.

Similarly, during 2024, the number of primary and secondary branches was considerably greater in T_1 compared to T_2 (Table 3.2). Interestingly, T_2 had a higher leaf number (176.39) than T_1 (148.61), indicating that NH_4^+ based fertilizers in zeolite-amended media may support vigorous blueberry leaf growth. This may have resulted from the slow, gradual release of nutrients by zeolite (Kavoosi, 2007). Previous studies reported that 100% NH_4^+ sources promote the production of primary branches and reached a maximum of 254 leaves per plant compared to 100% NO_3^- based fertilization (González *et al*., 2018). A high N concentration in the shoots of blueberry plants was recorded when NH_4^+ was applied which could be as the results to the formation of new shoots (Takamizo & Sugiyama, 1991). However, Anwar *et al*. (2024) found that blueberry branches reached maximal production in a 50:50 NH_4^+: NO_3^- ratio. Similarly in *V. corymbosum* x *V. angustifolium* 'Northsky' the NH_4^+: NO_3^- ratio improved the number of leaves per plant compared to the initial increase then a gradual decrease under NH_4^+ (Yuan-Yuan *et al*., 2021) demonstrating that the NH_4^+: NO_3^- ratios may further optimize blueberry growth. While T_1 promoted branch development, T_2 stimulated greater leaf production, suggesting the zeolite amendment may shift biomass allocation (Chatzistathis *et al*., 2021).

Stem diameter was the greatest under T_4 (19.9 mm) compared to T_1 (18.4 mm) treatment. These results indicate that NO_3^- based fertilizers can increase stem thickness, while NH_4^+ treatments promote leaf and branch development. NO_3^- taken up by blueberry roots is assimilated within the roots or stored for later use, which may enhance the diameter of the main stem, causing nutrient imbalances to affect branch development and restrict NO_3^- translocation to the shoots (Alt *et al*., 2017). This is in concurrence with the results of Yang *et al*. (2025) in blueberry cv. 'Anna' found that the main stem diameter was considerably greater in NO_3^- based treatments than in NH_4^+ application. The NO_3^- application supports structural development like stem diameter through carbohydrate allocation and calcium (Ca^{2+}) uptake, while NH_4^+ application promotes iron (Fe^{2+}) uptake by inducing protons (H^+) release from the cell, which acidifies the rhizosphere, but this process may limit the structural growth relative to NO_3^- nutrition (Fernandes & Rossiello, 1995). However, Hachiya and Sakakibara (2016), in their review, concluded that the NH_4^+: NO_3^- ratio is widely accepted and maximizes plant growth at a certain ratio depending on the crop species.

There is limited information on the influence of zeolite-amended substrates on the growth and development of blueberries. However, several studies have been conducted to investigate the effect of zeolite on vegetables and other crops. Cattivello (1995) reported that adding 3-7% zeolite levels into the substrate for growing lettuce, tomato, melon, and certain flower seedlings resulted in better growth performance compared to the addition of 15% zeolite. Further studies observed a positive influence on stem thickness, leaf area, and plant dry mass of cucumber and tomato seedlings with increasing zeolite levels (5-20%) (Rydenheim, 2007). Moreover, Karami *et al*. (2011) reported that 10% and 50% zeolite levels increased *Dieffenbachia Amoena* number of leaves, and the largest stem was observed in 10% zeolite. Our current results showed that adding 20% zeolite to 80% coir decreased plant height and number of branches; however, it significantly enhanced the number of leaves and stem diameter. In Solanum *lycopersicum* Mill cultivation, substrate with 30% zeolite addition produced a greater number of leaves (92%) and increased stem diameter (Méndez Argüello *et al*., 2018). These findings highlight the variations in species-specific responses to zeolite amendments and warrant further investigation in blueberry cultivation. Given the unique preference of NH_4^+ over NO_3^-, identifying the optimal zeolite level is therefore essential to maximize blueberry production when integrated with NH_4^+ based fertilizers and the NH_4^+:NO_3^- ratios.

3.5.2 Influence of nitrogen sources and growth media on the Chlorophyll content index (CCI) of blueberry.

Chlorophyll is an essential pigment that plays a crucial role in photosynthesis, a photochemical process that is indispensable for plant growth and development (Kim *et al*., 2020). In 2023, T_3 (39.08 CCI) accumulated the highest chlorophyll content index (CCI), whereas during the 2024 cultivation season, T_1 was superior (42.11 CCI), followed by T_2 (39.17 CCI) and T_6 (37.61 CCI) treatments (Figure 3.3A). This year-to-year variation suggests that the N sources and environmental conditions had an impact on chlorophyll accumulation as the plants were still acclimatizing in 2023, whereas by 2024, they had developed resilience and shown enhanced growth.

In 2024, leaf CCI was significantly lower under T_3 and T_4 (NO_3^-) compared to NH_4^+ based treatments. These results align with the findings in blueberry (Imler *et al.*, 2019) and other crops (Duan *et al.*, 2023; Guo *et al.*, 2002), where NH_4^+ fertilization enhanced the photosynthetic pigments in blueberry leaves and improved plant productivity. *Vaccinium* species can take up both NH_4^+ and NO_3^- forms of N; however, Alt (2015) reported that *V. corymbosum* showed approximately twice the uptake of NH_4^+ compared to NO_3^-, emphasizing the preference for NH_4^+ based fertilizers. In plants, most absorbed N is assimilated and stored within the chloroplast (Mu *et al.*, 2016); therefore, reduced uptake efficiency of NO_3^- relative to NH_4^+ may result in lower N availability. This limited availability could be attributed to the observed decrease in CCI under T_3 and T_4 treatments. This agrees with the findings of Merhaut and Darnell (1996), who affirm that although *Vaccinium* sp. can absorb both N forms under different pH levels for optimal growth, assimilation of NO_3^- by blueberry plants is limited compared to NH_4^+. Similar findings were reported in canola (Bybordi, 2011) and eggplant (Savvas *et al.*, 2010), where a higher proportion of NH_4^+ in NH_4^+ based fertilizer promoted greater accumulation of total N in plant tissue.

The optimization of N allocation in leaves is an adaptive mechanism in response to different N sources. Plants exposed to N deficiency tend to allocate more N to sustain electron transport, while relatively less N is directed towards chlorophyll and photosynthetic proteins (Mu *et al.*, 2016) Duan *et al.* (2023) in blackberry found the genes related to chloroplast regulation were mostly downregulated under NO_3^- compared to NH_4^+ and urea-based fertilizers, which was likely to be the cause of chlorophyll decrease in leaves.

In contrast, several studies in other crops have demonstrated that in NH_4^+ fed plants, only a small fraction of N is translocated from the roots to shoots (Ramirez-Builes *et al*., 2024), when NO_3^- was used as N source, however, the proportion of N compounds transported in the xylem as NO_3^- ion was significantly higher (Liu *et a*l., 2019; Pilbeam & Kirkby, 2023), suggesting that NO_3^- is primarily assimilated in the shoots of crops with active NR and NiR enzymes. This process varies among plant species and justifies the unique preference of *Vaccinium* sp. and for NH_4^+ nutrition and highlighting the importance of ongoing investigations into the optimal NH_4^+:NO_3^- ratio. Previous studies have shown that the NH_4^+:NO_3^- ratio can help provide a steady supply of nutrition, supporting the accumulation of chlorophyll content and photosynthesis capacity. The mixture of N forms, 2:1 NH_4^+:NO_3^-, significantly increased chlorophyll content in blueberry seedlings (Xu *et al*., 2021). In Andean blueberry, chlorophyll content in leaves for 100%NH_4^+ and 50%NH_4^+:50%NO_3^- treatments increased significantly compared to the considerably lower chlorophyll content levels under 0%NH_4^+ and 100%NO_3^- treatments (González *et al*., 2018).

Furthermore, Hao *et al*. (2023) reported that a 50:50 NH_4^+:NO_3^- was the most effective treatment, as it enhanced chlorophyll content accumulation and photosynthetic activity in centipedegrass. These findings suggest that a balanced supply of N forms may optimise physiological processes, although the extent of this response may vary with different crop species and growing conditions. Herein, in this study, the number of leaves were greater in T_2; however, T_1 had accumulated the highest CCI (Figure 3.3A-B), the competition for nutrients may be attributed to the imbalance between the parameters, previous study reported that low N concentration causes stunted growth by reducing leaf expansion, subsequently reducing the chlorophyll content due to reduced leaf area for photosynthesis (Mu *et al*., 2016). However, restricted leaf expansion is often associated with higher chlorophyll and enzyme density per unit leaf area, potentially enhancing assimilation rates (Guo *et al*., 2002), although this response may vary with stress severity. Moreover, coir and zeolite may have influenced the nutrient availability and allocation.

Ortiz-Delvasto and Carvajal (2025) reported that ‘Legacy’ blueberry plants grown in 100% coir and 90% coir: 10% peat(v/v) presented the highest values of photosynthetic rates, chlorophyll, which is a critical pigment for plant photosynthesis, can be partially indicated by the level of photosynthetic rates. These results are in agreement with the current study where CCI under T_1 is relatively higher than T_2. Previous findings by Gómez-Bellot *et al*. (2020) further revealed that tomatoes grown using coir as a substrate increased nutrients and water availability improving physiological parameters such as leaf water and chlorophyll fluorescence. These results can be attributed to the coir’s capacity to retain water and provide greater aeration however 20% zeolite incorporation to coir has been shown to have greater water retention capacity compared to 100% coir (Mfeka *et al*., 2023). Zeolite plays an important role in regulating plant water and N uptake due to its porous nature and strong affinity for NH_4^+ ions (Gül *et al*., 2005). In contrast excessive zeolite application may reduce nutrient availability to plants. Due to its high cation exchange capacity (CEC) zeolite can bind cations including NH_4^+ reducing nutrient leaching (Rodríguez Valdivia *et al*., 2021). This it can be suggested that the retention of NH_4^+ by the 20% zeolite addition may not be beneficial in this case and hence causes a slight decrease in blueberry growth through hindering chlorophyll content accumulation and photosynthesis. However, zeolite was reported to show better CCI in Swiss chard under 20% zeolite and 30% zeolite applications (Sindesi *et al*., 2025). These findings suggest a further investigation into the influence of different zeolite levels in blueberry production under container cultivation.

3.5.3 Influence of N sources and growth media on blueberry water use

Water availability is among the most limiting factors that directly influence the blueberry plants grown in soilless substrates (Muñoz *et al*., 2022). In a pot experiment, Mfeka *et al*. (2023) reported that the combination of 80% coir: 20% zeolite (v/v) growth media significantly enhanced water retention. This seems to resemble the present study; it was observed that water application was consistently reduced in zeolite-amended treatments (T_2, T_4, and T_6) compared to 100% coir (T_1, T_3, and T_5), confirming the ability of zeolite to reduce water demand in plants through its high cation exchange capacity (CEC) (Sindesi *et al*., 2023; Jabbar, 2025). Despite zeolite reducing water demand, plants grown in T_1 had greater growth and development (Table 3.3 and 3.4), suggesting that 100% coir is more conducive to early shoot expansion due to super aeration and root expansion, these results are similar to those of Evans and Stamps (1996), who showed that plants grown in coir flowered sooner compared to other substrates.

3.5.4 Influence of nitrogen sources and growth media on the yield and nitrogen use efficiency (NUE) of blueberry.

3.5.4.1 Effect of N sources on fruit yield attributes

Different N forms can affect crop yield; these effects are directly observed in the external physical traits of the fruit. Consumer preference studies showed that larger berries are more desirable (Safner *et al*., 2008) and reduce harvest costs (Strick *et al*., 2003). From an industry perspective smaller berries present a higher surface area to volume ratio which leads to greater losses of water making larger fruits more desirable (Undurranga & Vargas, 2013). In this study, blueberry fruits exhibited the largest fruit diameter, weight per fruit, total fresh yield, and nutrient use efficiency (NUE) under T_1 ($p < 0.05$) compared to the other treatments (Table 3.4). This suggested that NH_4^+ based treatments contribute to enhancing the size and weight of berries. In contrast, the value of these physical indicators was lowest when NO_3^- was applied as the N source. The number of fruits per plant and the number of fruits per diameter remained consistent across treatments, indicating that enhanced yield under NH_4^+ treatments was driven primarily by increased berry mass rather than berry number.

The evident increase in total fresh yield likely resulted from an increase initiation of flowers per surface and enhanced allocation of assimilates to individual berries. These results are supported by a previous study, which found that flower bud number was increased by N fertilization (Lanfond & Ziadi, 2011). Anwar *et al*. (2024) reported an earlier initiation of flowering under 50:50 NH_4^+:NO_3^-, suggesting that a balance of N forms was conducive for early bloom and the application of higher NH_4^+ in the 75:25 ratio hastens the flowering stage and shortens its duration, indicating that plants rely heavily on NH_4^+ as their main N source, thus the most effective in enhancing fruit set and later yield of the fruit. Additionally, fruit set and quality decreased in high application of NO_3^-, negatively affecting reproductive traits and yield (Messiga *et al*., 2021). However, in apples, increasing the NH_4^+:NO_3^- ratio did not affect fruit size (Mohammad *et al*., 2015), highlighting the varying responses of species to NH_4^+:NO_3^- ratios. Previous studies have shown that the physical traits of blackberry fruits were the largest under NH_4^+ or urea-based treatments (Duan *et al*., 2023). Furthermore, in 'Bluecrop' blueberry, application of NH_4^+ and urea through the fertigation system by the split method significantly increased berry weight (2.22 g and 2.17 g, respectively) and total yield of blueberry plants (Vargas & Bryla, 2015).

3.5.4.2 N forms, physiological responses, and NUE

Carbon (C) and N metabolism in plants are closely interconnected, with each influencing and regulating the other (Lu *et al*., 2011). Zhang *et al*. (2020) noted that the application of NH_4^+ led to greater carbohydrate accumulation in fruits, largely due to its assimilation requiring a large C skeleton. This explains the larger fruit diameter and weight per berry observed under NH_4^+ fed plants (Figure 3.4A). Enhanced leaf chlorophyll and photosynthetic activity under NH_4^+ improve nutrient uptake and NUE (Xu *et al*., 2021; Hao *et al*., 2023). NUE is an important index, determined from crop yield per unit of nutrient (Fixen *et al*., 2012). In this study, NUE was the highest in T_1 (20.56 g yield/ g N applied), followed by T_2 (11.95 g yield/ g N applied) treatments (Figure 3.4B), suggesting that N uptake was more efficient under NH_4^+ treatments. Our results align with Arias *et al*. (2024), who showed that NH_4^+ increased N accumulation and ^{15}N rate in 'Blue Ribbon' blueberry, and Kingston *et al*. (2017), who found higher NUE in ‘Snowchaser’ grown in coir under NH_4^+. These results demonstrate that NH_4^+ fertilization in coir substrates enhances both fruit yield and NUE in blueberry.

3.5.4.3 Interactive effects of N source and growth media

The interaction between N sources and growth media is essential for determining the growth and development of crops. Coir is one of the most abundant organic wastes of plant origin, and is characterized by high porosity, good aeration, and high WHC (van Gerrewey *et al*., 2020), often lacking CEC, making it less effective in retaining NH_4^+ and NO_3^- ions. According to Ortiz-Delvasto and Carvajal (2025), a 90% coir:10% peat substrate presented a higher NUE, indicating that in blueberries, coir can balance C assimilation to a higher extent compared to other substrates. These findings are concurrent with the present study, where T_1 had the greatest NUE, fruit diameter and total fresh yield compared to T_2. Furthermore, the coir substrate resulted in higher fresh weight and significantly larger fruit diameter of ‘Legacy’ blueberry (*Ortiz-Delvasto* et al., 2023), the greatest yield of the ‘Bianca’ rose variety (Fascella & Zizzo, 2005), and the highest individual fruit weight and total fruit weight in tomatoes (Xiong *et al*., 2017).

In contrast, zeolite ammendment reduced blueberry yield in all treatments (Table 3.4). This response is unexpected as it has been shown that zeolite-amended amended increases the growth and development of vegetables and other crops by retaining and reducing nutrient leaching (Cattivello, 1995; Rydenheim, 2007; Méndez Argüello *et al*., 2018; Sindesi *et al*., 2025). Herein, this study, T_1 consistently produced greater results compared to T_2; thereafter, T_5 compared to T_6 in yield attributes (Table 3.4). This contrasts the results of Latifah *et al*. (2017), who demonstrated that mixing clinoptilolite zeolite with urea significantly reduced NH_4^+ leaching from urea compared to using urea alone in the soil. This was attributed to the high surface area of the zeolite to adsorb NH_4^+; furthermore, they observed that NO_3^- treatments without zeolite had leachates, suggesting the zeolite's potential to improve NUE in crops.

Blueberry has been established to be an ammoniophic plant due to its inactive NR and NiR enzymes, thus showing a preference for NH_4^+ uptake leading to rhizosphere acidification (Alt *et al*., 2017). The zeolites particles are high in negative charges that attract NH_4^+ and other metal cations depending on the rhizosphere (Jorgensen & Weatherley, 2008) therefore, the coir: zeolite combination could provide a conductive environment where NH_4^+ is retained for gradual uptake whereas coir maintains high porosity and good aeration.

The opposite effect observed in this study could be due to the blueberry's shallow root system with no root hairs (Bryla & Strik, 2015) reducing their ability to exploit the nutrient-holding capacity of zeolite effectively. Although zeolite has been observed to retain nutrients particularly NH_4^+ from leaching (Latifah *et al*., 2017) its physical properties may pose challenges in container production systems. The addition of high zeolite levels tends to compact the medium restricting root penetration and aeration. Additionally during the experiment the granular material of zeolite was observed to sink into the bottom of the plant bags used placing the retained nutrients beyond the reach of the active upper root zone. These structural dynamics likely diminished the expected benefits of zeolite, particularly with the young blueberry plants used in this study. However, Sindesi *et al*. (2025), when using the field, suggested that zeolite may need more time to fully integrate into the soil system and provide a positive impact on crop growth. Moreover, a previous study indicated that applying NH_4^+ charged clinoptilolite zeolite to highly productive agricultural land did not adversely affect crop growth or nutrition and could potentially provide significant agronomic benefits with lasting positive impact on soil properties (Campisi *et al*., 2016).

3.5.4.4 Seasonal variation and phenological cycles

Greater NUE is observed in T_3 (8.83 g yield per g N applied) relative to T_5 (6.45 g yield per g N applied) (Table 3.4), despite reports of urea or NH_4^+ enhancing crop performance (Hart *et al*., 2006; Duan *et al*., 2023). In young or establishing plantings, treatments that increase the number of fruits per plant can depress average berry weight, particularly when N supply or sink number is high, a pattern reported across years of N fertilization experiments where higher N rates reduced average berry size and diameter (Bryla & Strik, 2015; Vargas & Bryla, 2015). T_3 likely promoted greater fruit set than T_5; however, the consequent nutrient partitioning would explain the smaller diameter and lower berry weight, while T_5 produced fewer berries but larger diameter and berry weight. This phenomenon was also reported in the results of *V. corymbosum* varieties, where there was an increase in the number of viable seeds per berry, resulting in an increased berry mass, demonstrating the mechanistic relationship between seed set (assimilate sink strength via pollination) and berry mass (Doi *et al*., 2018).

During the vegetative stage, T_3 produced a greater number of leaves and branches compared with T_5 (Table 3.3), which may reflect increased competition for available nutrients and water. This likely contributed to the production of smaller berries, although in greater numbers. These findings are consistent with a study that observed a higher number of shoots could generate smaller fruits and a greater individual fruit weight (T_3= 1.56 g and T_5= 1.70 g) is achieved with fewer fruits as well as noting that excessive vegetative growth can reduce water and carbohydrate availability per fruit, decreasing the single fruit size and weight, however the total yield could increase (Romero-Bravo *et al*., 2024) as demonstrated in T_3 (85.23 g) and T_5 (79.43 g) (Table 3.4).

In the 2023 cultivation season, blueberry production was low in all treatments; however, plants grown under T_1 treatments showed better fruiting potential and produced a larger fruit diameter (Figure 3.4A). Low production rates are common at the development stage, as the formation of inflorescence is low due to the limitations of prioritizing vegetative development (Álvarez Robledo *et al*., 2020). Variation of fruiting in relation to the phenological cycle has also been observed in the Highbush and Rabbiteye cultivars (Gündüz *et al*., 2015). By the second year (2024), plants have stabilized within the root system and developed resistance, resulting in improved yield and NUE (Figure 3.4B). This progressive increase in yield across seasons agrees with the findings of Retamale and Hancock (2012), who reported that blueberry plants tend to reach the peak of production after a few years of acclimatization.

Overall, the blueberry fruit diameter, weight per berry, and total fresh yield increased gradually during the early harvest time, reached a peak at mid harvest, and subsequently declined towards the end of the harvest period (Figure 3.4C-D). In most cases, it is common for fruit volume to decline towards the end of the harvesting season (Godara *et al*., 2025). Mallik and Hamilton (2017) reported that across three wild blueberry genotypes, the lowest fresh weight and size were recorded towards the end of the harvest period when investigating fruit quality characteristics at harvest date and storage effect. The decrease in berry size and weight at the end of harvest season may be attributed to increased fruit transpiration combined with reduced efficiency of nutrient transport through the phloem (McCarthy & Coombe, 1999), thus, the low NUE in December (Figure 3.4C-D).

3.6 Conclusions and Recommendations

Different nitrogen sources treatments were applied during blueberry growth and development. It was found that ammonium sulphate (NH_4^+) in 100% coir (T_1) produced the best results in both years of cultivation, confirming its role as the blueberry industry standard in the Western Cape. Urea in 100% coir (T_5) also enhanced plant height, supporting the use of NH_4^+ derived N from hydrolysis. In contrast, calcium nitrate (T_3 and T_4) enlarged stem diameter but resulted in fewer branches and a low chlorophyll content index (CCI), suggesting that NO_3^- treatments promoted structural growth at the expense of photosynthetic capacity, which resulted in reduced production. NH_4^+ also had a strong impact on blueberry diameter and weight, rather than the number of berries per plant, which was not significantly influenced by N sources.

The growing medium used influenced the results blueberry plants grown in 100% coir outperformed those in the mixture of coir and zeolite. The zeolite amended medium significantly improved water holding capacity leaf number, and stem thickness but reduced plant height, branching and CCI. This response is likely to be influenced by the shallow-rooted blueberry system with no root hairs which requires a well-aerated growing medium and the addition of zeolite increases bulk density, limiting nutrient uptake and thus restricting shoot elongation. T_1 produced the greatest fruit diameter, weight per fruit, total fresh weight and NUE, followed by T_2. T_5 also performed well but the incorporation of zeolite reduced yields across all treatments. Fruit production was relatively low in the first year of cultivation (2023) as young plants prioritized vegetative growth over fruiting, or there was a lack of reproductive buds. By the second year (2024) plants had established strong root systems resulting in greater yield and enhanced nitrogen use efficiency (NUE). During both cultivation years, fruit size and weight gradually increased at the beginning of the harvest season, peaked mid-season, and declined at the end, consistent with patterns reported in other studies.

Although zeolite-amended substrate reduced water use in blueberry plants, the granular material of zeolite was observed to sink into the bottom of the bags used, placing the retained nutrients beyond the reach of the active upper root zone. These structural dynamics likely diminished the expected benefits of zeolite, particularly with the young blueberry plants used in this study. Further experiment is needed to modify less than 20% zeolite levels for enhanced blueberry production; furthermore, to clarify the use of zeolite in container production, as it is thought to excrete toxins over time. Further research on urea’s stability in substrate production is warranted, as urea is an inexpensive source of N and could be useful to reduce potential over-acidification with ammonium sulphate in long-term container production. Moreover, NO_3^- should be applied in a balanced ratio with NH_4^+ to obtain optimal blueberry growth and development, depending on the species requirements.

References

Akšić, M. F., Tosti, T., Sredojević, M., Milivojević, J., Meland, M., & Natić, M., 2019. Comparison of Sugar Profile between Leaves and Fruits of Blueberry and Strawberry Cultivars Grown in Organic and Integrated Production System. *Plants*, *8*(7), 205. https://doi.org/10.3390/plants8070205

Alt, D. S., Doyle, J. W., & Malladi, A., 2017. Nitrogen-source preference in blueberry (Vaccinium sp.): Enhanced shoot nitrogen assimilation in response to direct supply of nitrate. *Journal of Plant Physiology*, *216*, 79–87. https://doi.org/10.1016/j.jplph.2017.05.014

Alt, D.S., 2015. *Physiological and molecular basis for low nitrate (NO_3^-) assimilation in blueberry*. MSc thesis. University of Georgia, Athens.

Álvarez Robledo, Y. A., Oliva Cruz, M., Collazos Silva, R., Vilca Valqui, N. C., & Huaman Huaman, E., 2020. Desempeño agronómico de cuatro variedades de arándano (*vaccinium corymbosum* L.) cultivadas en diferentes sustratos y pisos altitudinales. In *Bioagro* (Vol. 32, Issue 3).

Anwar, A., Zheng, J., Chen, C., Chen, M., Xue, Y., Wang, J., Su, W., Chen, R., & Song, S., 2024. Effects of NH4+-N: NO3−-N ratio on growth, nutrient uptake and production of blueberry (Vaccinium spp.) under soilless culture. *Frontiers in Plant Science*, *15*. https://doi.org/10.3389/fpls.2024.1438811

Arias, M. I., Nario, A., Rojas, K., Blanc, P., & Bonomelli, C., 2024, 'Newly Established Blueberry Plants: The Role of Inorganic Nitrogen Forms in Nitrogen and Calcium Absorption', *Horticulturae*, *10*(11), 1168. https://doi.org/10.3390/horticulturae10111168

Bryla, D. R., & Strik, B. C. ,2015. Nutrient Requirements, Leaf Tissue Standards, and New Options for Fertigation of Northern Highbush Blueberry. *HortTechnology*, *25*(4), 464–470. https://doi.org/10.21273/HORTTECH.25.4.464

Bybordi, A. 2011. Effect of ammonium: Nitrate ratio on fatty acid composition and proline accumulation of canola cultivars grown under salinity stress. *African Journal of Biotechnology*, *10*(74). https://doi.org/10.5897/AJB11.2425

Campisi, T., Abbondanzi, F., Faccini, B., di Giuseppe, D., Malferrari, D., Coltorti, M., Laurora, A., & Passaglia, E., 2016. Ammonium-charged zeolitite effects on crop growth and nutrient leaching: greenhouse experiments on maize (Zea mays). *CATENA*, *140*, 66–76. https://doi.org/10.1016/j.catena.2016.01.019

Cattivello, C.,1995. Use of substrates with zeolites for seedling vegetables and pot plant production. *Acta Horticulturae*, *401*, 251–258. https://doi.org/10.17660/ActaHortic.1995.401.30

Crisóstomo Natividad, M., Hernández Rodríguez, O.A., Medina, J.L., Manjarrez-Domínguez, C. and Pinedo-Alvárez, A., 2014. ‘Relaciones amonio/nitrato en soluciones nutritivas ácidas y alcalinas para arándano: Ammonium/nitrate ratios in acid and alkaline nutrient solutions for blueberry’, *Revista Mexicana de Ciencias Agrícolas*, 5(3).

Doi, K., Nozaki, R., Takahashi, K., & Iwasaki, N., 2018. Effects of the Number of Seeds per Berry on Fruit Growth Characteristics, Especially on the Duration of Stage II in Blueberry. *Plants*, *7*(4), 96. https://doi.org/10.3390/plants7040096

Duan, Y., Yang, H., Wei, Z., Yang, H., Fan, S., Wu, W., Lyu, L., & Li, W., 2023. Effects of Different Nitrogen Forms on Blackberry Fruit Quality. *Foods*, *12*(12), 2318. https://doi.org/10.3390/foods12122318

Duan, Y., Yang, H., Yang, H., Wu, Y., Fan, S., Wu, W., Lyu, L., & Li, W., 2023. Integrative physiological, metabolomic and transcriptomic analysis reveals nitrogen preference and carbon and nitrogen metabolism in blackberry plants. *Journal of Plant Physiology*, *280*, 153888. https://doi.org/10.1016/j.jplph.2022.153888

Evans, M. R., & Stamps, R. H., 1996. Growth of Bedding Plants in Sphagnum Peat and Coir Dust-Based Substrates. *Journal of Environmental Horticulture*, *14*(4). https://doi.org/10.24266/0738-2898-14.4.187

Fascella, G., & Zizzo, G. V., 2005. Effect of growing media on yield and quality of soilless cultivated rose. *Acta Horticulturae*, *697*, 133–138. https://doi.org/10.17660/ActaHortic.2005.697.15

Fernandes, M. S., & Rossiello, R. O. P., 1995. Mineral Nitrogen in Plant Physiology and Plant Nutrition. *Critical Reviews in Plant Sciences*, *14*(2), 111–148. https://doi.org/10.1080/07352689509701924

Fixen, P., Brentrup, F., Bruulsema, T., F, G., Norton, R., & Zingore, S., 2012. Chapter 1. Nutrient/fertilizer use efficiency (N/FUE): Measurement, Current Situation and Trends. *Draft*, *3400*.

Godara, A., Rubio Ames, Z., & Deltsidis, A., 2025. Delayed Harvest Reduces Postharvest Quality and Storability of Southern Highbush cv. Meadowlark and Rabbiteye Blueberry cv. Brightwell. *HortScience*, *60*(2), 182–190. https://doi.org/10.21273/HORTSCI18301-24

Gómez-Bellot, M. J., Lorente, B., Sánchez-Blanco, M. J., Ortuño, M. F., Nortes, P. A., & Alarcón, J. J., 2020. Influence of Mixed Substrate and Arbuscular Mycorrhizal Fungi on Photosynthetic Efficiency, Nutrient and Water Status and Yield in Tomato Plants Irrigated with Saline Reclaimed Waters. *Water*, *12*(2), 438. https://doi.org/10.3390/w12020438

González, L. K., Rugeles, L. N., & Magnitskiy, S., 2018, 'Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (Vaccinium *meridionale swartz*)', *Agronomia Colombiana*, *36*(1), 58–67. https://doi.org/10.15446/agron.colomb.v36n1.69304

Grata K. 2013. Ecological effects of urea phosphate on soil diazotrophs in spring. Part II. Ecol. Chem. Eng. A 20(7–8), 819–825.

Gül, A., Eroğul, D., & Ongun, A. R., 2005. Comparison of the use of zeolite and perlite as substrate for crisp-head lettuce. *Scientia Horticulturae*, *106*(4), 464–471. https://doi.org/10.1016/j.scienta.2005.03.015

Gündüz, K., Serçe, S., & Hancock, J. F., 2015. Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics. *Journal of Food Composition and Analysis*, *38*, 69–79. https://doi.org/10.1016/j.jfca.2014.09.007

Guo, S., Brück, H., & Sattelmacher, B., 2002. Effects of supplied nitrogen form on growth and water uptake of French bean (Phaseolus vulgaris L.) plants: Nitrogen form and water uptake. *Plant and Soil*, *239*(2). https://doi.org/10.1023/A:1015014417018

Guo, S., Zhou, Y., Shen, Q., & Zhang, F., 2007. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants - Growth, photosynthesis, photorespiration, and water relations. In *Plant Biology* (Vol. 9, Issue 1). https://doi.org/10.1055/s-2006-924541

Hachiya, T., & Sakakibara, H., 2016. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany*, erw449. https://doi.org/10.1093/jxb/erw449

Hachiya, T., Inaba, J., Wakazaki, M., Sato, M., Toyooka, K., Miyagi, A., Kawai-Yamada, M., Sugiura, D., Nakagawa, T., Kiba, T., Gojon, A., & Sakakibara, H., 2021. Excessive ammonium assimilation by plastidic glutamine synthetase causes ammonium toxicity in Arabidopsis thaliana. *Nature Communications*, *12*(1), 4944. https://doi.org/10.1038/s41467-021-25238-7

Hao, D.-L., Zhou, J.-Y., Li, L., Qu, J., Li, X.-H., Chen, R.-R., Kong, W.-Y., Li, D.-D., Li, J.-J., Guo, H.-L., Liu, J.-X., Zong, J.-Q., & Chen, J.-B., 2023. An appropriate ammonium: nitrate ratio promotes the growth of centipedegrass: insight from physiological and micromorphological analyses. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1324820

Hart, J & Strik, B & White, L & Yang, W & Hart, John., 2006. Nutrient management for blueberries in Oregon. 8918.

Holzapfel, E. A., 2009. Selection and management of irrigation systems for blueberry. *Acta Horticulturae*, *810*, 641–648. https://doi.org/10.17660/ActaHortic.2009.810.85

Imler, C. S., Arzola, C. I., & Nunez, G. H., 2019. Ammonium Uptake Is the Main Driver of Rhizosphere pH in Southern Highbush Blueberry. *HortScience*, *54*(5), 955–959. https://doi.org/10.21273/HORTSCI13764-18

International Blueberry Organization (IBO), 2023. Global State of the Blueberry Industry Report 2023*. IBO*. https://www.internationalblueberry.org [Accessed 11 July 2025]

Jabbar, H. A., 2025. Zeolite Application Improves Soil Structure and Water Retention in Agriculture. *Academia Open*, *10*(1). https://doi.org/10.21070/acopen.10.2025.11393

Jankauskienė, J., Brazaitytė, A., Kairienė, V. V., & Zalatorius, V., 2019. Effects of peat and peat-zeolite substrates on quality, growth indices of cucumber seedlings and crop productivity. *Acta Scientiarum Polonorum Hortorum Cultus*, *18*(5), 161–170. https://doi.org/10.24326/asphc.2019.5.16

Jorgensen, T. C., & Weatherley, L. R., 2008. Continuous ion-exchange removal of ammonium ion onto clinoptilolite in the presence of contaminants. *Asia-Pacific Journal of Chemical Engineering*, *3*(1), 57–62. https://doi.org/10.1002/apj.103

Karami, A., Torkashvand, M., & Khomami, M., 2011. The Effect of Medium Containing Zeolite and Nutrient Solution on the Growth of Dieffenbachia Amoena. *Scholars Research Library Annals of Biological Research*, *2*(6), 378–383. www.scholarsresearchlibrary.com

Kavoosi, M., 2007. Effects of zeolite application on rice yield, nitrogen recovery, and nitrogen use efficiency. *Communications in Soil Science and Plant Analysis*, *38*(1–2). https://doi.org/10.1080/00103620601093652

Khan, M. R., Tarafder, M. A., & Priti, M. S., 2024. Potential Use Of Zeolites In Agriculture: A Review. *SAARC Journal of Agriculture*, *22*(1), 17–30. https://doi.org/10.3329/sja.v22i1.72433

Kim, D.-H., Yang, J.-H., Kim, H.-J., Rhee, J., Lee, J.-Y., & Lim, S.-H., 2020. Recent Advances in Genetic Regulation of Chlorophyll Metabolism in Plants. *Korean Journal of Breeding Science*, *52*(4), 281–297. https://doi.org/10.9787/KJBS.2020.52.4.281

Kingston, P. H., Scagel, C. F., Bryla, D. R., & Strik, B., 2017. Suitability of Sphagnum Moss, Coir, and Douglas Fir Bark as Soilless Substrates for Container Production of Highbush Blueberry. *HortScience*, *52*(12), 1692–1699. https://doi.org/10.21273/HORTSCI12374-17

Kishorekumar, R., Bulle, M., Wany, A., & Gupta, K. J., 2020, 'An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants', *National Library of Medicine* (pp. 1–13). https://doi.org/10.1007/978-1-4939-9790-9_1

Kithome, M., Paul, J. W., Lavkulich, L. M., Bomke, A. A., Canada, A.-F., & Agri, P.-C. 1998. Kinetics of Ammonium Adsorption and Desorption by the Natural Zeolite Clinoptilolite.*Soil Sci Soc Am J*.62:622–629

Kozos, K., & Ochmian, I., 2016. The influence of fertilisation urea phosphate on growth and yielding bush of two highbush blueberry cultivars (v. Corymbosum). *Folia Pomeranae Universitatis Technologiae Stetinensis Agricultura, Alimentaria, Piscaria et Zootechnica*, *325*(37), 29–38. https://doi.org/10.21005/AAPZ2016.37.1.04

Lafond, J., & Ziadi, N., 2011. Fertilisation azotée et phosphatée dans la production du bleuet nain sauvage au Québec. *Canadian Journal of Plant Science*, *91*(3), 535–544. https://doi.org/10.4141/cjps10133

Latifah, O., Ahmed, O. H., & Majid, N. M. A., 2017. Enhancing nitrogen availability from urea using clinoptilolite zeolite. *Geoderma*, *306*, 152–159. https://doi.org/10.1016/j.geoderma.2017.07.012

Liu, S., Chi, Q., Cheng, Y., Zhu, B., Li, W., Zhang, X., Huang, Y., Müller, C., Cai, Z., & Zhang, J. 2019. Importance of matching soil N transformations, crop N form preference, and climate to enhance crop yield and reducing N loss. *Science of The Total Environment*, *657*, 1265–1273. https://doi.org/10.1016/j.scitotenv.2018.12.100

Londra, P. A., Paraskevopoulou, A. T., & Psychoyou, M., 2012. Evaluation of water-air balance of various substrates on Begonia growth. *HortScience*, *47*(8). https://doi.org/10.21273/hortsci.47.8.1153

Lu, Y., Luo, F., Yang, M., Li, X., & Lian, X., 2011. Suppression of glutamate synthase genes significantly affects carbon and nitrogen metabolism in rice (Oryza sativa L.). *Science China Life Sciences*, *54*(7), 651–663. https://doi.org/10.1007/s11427-011-4191-9

Mallik, A. U., & Hamilton, J., 2017. Harvest date and storage effect on fruit size, phenolic content and antioxidant capacity of wild blueberries of NW Ontario, Canada. *Journal of Food Science and Technology*, *54*(6), 1545–1554. https://doi.org/10.1007/s13197-017-2586-8

Mariyappillai, A., & Arumugam, G., 2021. Physico-chemical and hydrological properties of soilless substrates. *Journal of Environmental Biology*, *42*(3), 700–704. https://doi.org/10.22438/jeb/42/3/MRN-1504

Marschner, H., Römheld, V. and Cakmak, I., 1987. ‘Root-induced changes of nutrientavailability in the rhizosphere’, *Journal of Plant Nutrition*, 10(9–16), pp. 1175–1184. doi: 10.1080/01904168709363645.

Marty, C., Lévesque, J.-A., Bradley, R. L., Lafond, J., & Paré, M. C., 2019. Lowbush blueberry fruit yield and growth response to inorganic and organic N-fertilization when competing with two common weed species. *PLOS ONE*, *14*(12), e0226619. https://doi.org/10.1371/journal.pone.0226619

McCarthy, M. G., & Coombe, B. G., 1999. Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? *Australian Journal of Grape and Wine Research*, *5*(1), 17–21. https://doi.org/10.1111/j.1755-0238.1999.tb00146.x

Méndez Argüello, B., Vera Reyes, I., Cárdenas Flores, A., de los Santos Villarreal, G., Ibarra Jiménez, L., & Lira Saldivar, R., 2018. Water holding capacity of substrates containing zeolite and its effect on growth, biomass production and chlorophyll content of Solanum lycopersicum Mill. *Nova Scientia*, *10*(21), 45–60. https://doi.org/10.21640/ns.v10i21.1413

Merhaut, D. J., & Darnell, R. L., 1996. Vegetative growth and nitrogen/carbon partitioning in blueberry as influenced by nitrogen fertilization. *Journal of the American Society for Horticultural Science*, *121*(5). https://doi.org/10.21273/jashs.121.5.875

Messiga, A. J., Haak, D., & Dorais, M., 2018. Blueberry yield and soil properties response to long-term fertigation and broadcast nitrogen. *Scientia Horticulturae*, *230*. https://doi.org/10.1016/j.scienta.2017.11.019

Messiga, A. J., Nyamaizi, S., Yu, S., & Dorais, M., 2021, 'Blueberry yield and soil mineral nitrogen response to nitrogen fertilizer and nitrification inhibitors under drip-fertigation systems', Agronomy, *11*(11), 2144 https://doi.org/10.3390/agronomy11112144

Mfeka, N., Tanga, M., Mulidzi, A.R., & Lewu, F.B., 2023 'Water Requirement of Blueberry Cultivated in Different Growth Media', *39th Johannesburg International Conference on Chemical, Biological and Environmental Engineering (JCBEE-23)*, 16-17 November, Johannesburg, South Africa. https://doi.org/10.17758/iicbe5.c1123050

MIing DW, Allen ER.1999., Zeoponic substrates for space applications: Advances in the use of natural zeolites for plant growth. Natural Microporous Materials in Environmental Technology. Misaelides, P. (Ed). Kluwer Academic Publishers, Netherlands.157-76 *(PDF) Zeolites: Potential soil amendments for improving nutrient and water use efficiency and agriculture productivity*. Available from: https://www.researchgate.net/publication/325544295_Zeolites_Potential_soil_amendments_for_improving_nutrient_and_water_use_efficiency_and_agriculture_productivity [accessed Aug 24 2025].

Mohammad Sokri, S., Babalar, M., Barker, A. v., Lesani, H., & Asgari, M. A., 2015. Fruit Quality and Nitrogen, Potassium, and Calcium Content of Apple as Influenced by Nitrate: Ammonium Ratios in Tree Nutrition. *Journal of Plant Nutrition*, *38*(10), 1619–1627. https://doi.org/10.1080/01904167.2014.964364

Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G., 2016. Within-Leaf Nitrogen Allocation in Adaptation to Low Nitrogen Supply in Maize during Grain-Filling Stage. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00699

Muñoz, V., France, A., Uribe, H., & Hirzel, J., 2022. Effect of nitrogen rate and water replacement level on leaf biomass production and leaf nitrogen concentration of ten pot-grown blueberry cultivars. *Chilean Journal of Agricultural Research*, *82*(2), 294–308. https://doi.org/10.4067/S0718-58392022000200294

Orha, C., Lăzău, C., & Bandas, C., 2015. Structural and fertilizer properties of potassium doped natural zeolite. *J Agroaliment Proc Technol*, *21*.

Ortiz-Delvasto, N., & Carvajal, M., 2025. Elevated carbon dioxide and substrate composition affect growth, nutrient uptake, and water use efficiency in blueberry cultivation under greenhouse. *Journal of Plant Nutrition*, *48*(12), 2137–2154. https://doi.org/10.1080/01904167.2025.2470388

Ortiz-Delvasto, N., Garcia-Ibañez, P., Olmos-Ruiz, R., Bárzana, G., & Carvajal, M., 2022. Substrate Composition Affecting Nutrient and Water Availability in Blueberry Soilless Culture Determines Their Growth and Quality. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.4121271

Ortiz-Delvasto, N., Garcia-Ibañez, P., Olmos-Ruiz, R., Bárzana, G., & Carvajal, M., 2023. Substrate composition affects growth and physiological parameters of blueberry. *Scientia Horticulturae*, *308*, 111528. https://doi.org/10.1016/j.scienta.2022.111528

Osorio, R.; Cáceres, C.; Covarrubias, J.I. Vegetative and Physiological Responses of “Emerald” Blueberry to Ammoniacal Sources with a Nitrification Inhibitor. J. Soil Sci. Plant Nutr. 2020, 20, 507–515

Penney, B. G., McRae, K. B., & Bishop, G. A., 2003. Second-crop N fertilization improves lowbush blueberry (*Vaccinium angustifolium* Ait.) production. *Canadian Journal of Plant Science*, *83*(1), 149–155. https://doi.org/10.4141/P02-057

Perez-Caballero, R., Gil, J., Benitez, C., & Gonzalez, J. L., 2008. The effect of adding zeolite to soils in order to improve the N-K nutrition of olive trees. Preliminary results. *American Journal of Agricultural and Biological Science*, *3*(1). https://doi.org/10.3844/ajabssp.2008.321.324

Perrin, T. S., Drost, D. T., Boettinger, J. L., & Norton, J. M.,1998. Ammonium-loaded clinoptilolite: a slow-release nitrogen fertilizer for sweet corn. *Journal of Plant Nutrition*, *21*(3), 515–530. https://doi.org/10.1080/01904169809365421

Pienaar L, Lingani M, Swart P., 2019. The economic contribution of the South African blueberry industry. Berries ZA. https://www. saberries.co.za/economic-contribution-of-the-south-african-blueberry-industry

Pienaar, L., Smit, P., Hattingh, J. & Cloete, K., 2022. Developing economic models for South African blueberry industry: Outlook 2022-2031. Report Commissioned by BerriesZA. Paarl: Bureau for Food and Agricultural Policy (BFAP) https://doi.org/10.2307/j.ctvthhbxx.20

Pilbeam, D. J., & Kirkby, E. A., 2023. Some aspects of the utilization of nitrate and ammonium by plants. In *Nitrogen Metabolism of Plants*. https://doi.org/10.1093/oso/9780198577522.003.0005

Poonnachit, U., & Darnell, R., 2004. Effect of ammonium and nitrate on ferric chelate reductase and nitrate reductase in Vaccinium species. *Annals of Botany*, *93*(4). https://doi.org/10.1093/aob/mch053

Ramirez-Builes, V. H., Küsters, J., Thiele, E., & Lopez-Ruiz, J. C., 2024. Physiological and Agronomical Response of Coffee to Different Nitrogen Forms with and without Water Stress. *Plants*, *13*(10), 1387. https://doi.org/10.3390/plants13101387

Retamales, J.B. and J.F. Hancock. 2012. Blueberries. CABI, Cambridge, MA.

Rodríguez Valdivia, M., Urday Urday, E., & Ocharán Velásquez, G. 2021. Evaluation of the cation exchange capacity, CEC, of natural zeolite or of zeolite exchanged with sodium from ignimbritic formations in Puno, peru, by measuring the removal of ammonium and heavy metals. *Revista Boliviana de Química*, *38*(2), 25-103. https://doi.org/10.34098/2078-3949.38.2.5

Romero-Bravo, S., Araya-Alman, M., Moggia, C., Lobos, G. A., Calderon, F., & Espinoza, S., 2024. Plant Morphology and Fruit Quality Traits Affecting Yield and Post-Harvest Behavior of Two Highbush Blueberry Cultivars in Central Chile. *Horticulturae*, *10*(12), 1380. https://doi.org/10.3390/horticulturae10121380

Rydenheim, L., 2007. Effects of zeolites on the growth of cucumber and tomato seedlings by Effects of zeolites on the growth of cucumber and tomato seedlings. *Department of Horticulture Swedish University of Agriculture (SLU)*, *4*.

Savvas, D., Leneti, H., Mantzos, N., Kakarantza, L., & Barouchas, P., 2010. Effects of enhanced NH_4^+-N supply and concomitant changes in the concentrations of other nutrients needed for ion balance on the growth, yield, and nutrient status of eggplants grown on rockwool. *The Journal of Horticultural Science and Biotechnology*, *85*(4), 355–361. https://doi.org/10.1080/14620316.2010.11512680

Scagel, C. F., 2003. Growth and Nutrient Use of Ericaceous Plants Grown in Media Amended with Sphagnum Moss Peat or Coir Dust. *HortScience*, *38*(1), 46–54. https://doi.org/10.21273/HORTSCI.38.1.46

Sindesi, O.A., Ncube, B., Lewu, M.N., Mulidzi, A.R. & Lewu, F.B., 2025. Growth and fresh yield responses of Swiss chard (*Beta vulgaris* L. var. *cicla*) cv. Ford Hook Giant to zeolite soil amendment. *Malaysian Journal of Sustainable Agriculture*, 9(2), pp.85–90.

Sindesi, O.A., Ncube, B., Lewu, M.N., Mulidzi, A.R., Lewu, F.B. 2023., Cabbage and Swiss chard yield, irrigation requirement and soil chemical responses in zeolite-amended sandy soil. 2022. *Asian Journal of Agriculture and Biology*, *Online First*. https://doi.org/10.35495/ajab.2021.11.387

Smolarz K., 2009. Short information about the history of the commercial cultivation highbush blueberry in Poland. Agronomijas Vēstis. 12, 119-122.

Takamizo, T. and n. Sugiyama. 1991. Growth responses to n forms in rabbiteye and highbush blueberries. j. jpn. Soc. Hort. Sci. 60, 41-45

Urban, A., Rogowski, P., Wasilewska-Dębowska, W., & Romanowska, E., 2021. Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants*, *10*(9), 1932. https://doi.org/10.3390/plants10091932

van Gerrewey, T., Ameloot, N., Navarrete, O., Vandecruys, M., Perneel, M., Boon, N., & Geelen, D., 2020. Microbial activity in peat-reduced plant growing media: Identifying influential growing medium constituents and physicochemical properties using fractional factorial design of experiments. *Journal of Cleaner Production*, *256*, 120323. https://doi.org/10.1016/j.jclepro.2020.120323

Vargas, O. L., & Bryla, D. R., 2015. Growth and Fruit Production of Highbush Blueberry Fertilized with Ammonium Sulfate and Urea Applied by Fertigation or as Granular Fertilizer. *HortScience*, *50*(3), 479–485. https://doi.org/10.21273/HORTSCI.50.3.479

Witte, C. P., 2011. Urea metabolism in plants. *Plant Science*, *180*(3), 431–438. https://doi.org/10.1016/j.plantsci.2010.11.010

Xiong, J., Tian, Y., Wang, J., Liu, W., & Chen, Q., 2017. Comparison of Coconut Coir, Rockwool, and Peat Cultivations for Tomato Production: Nutrient Balance, Plant Growth and Fruit Quality. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01327

Xu, J., Fang, Y., Tavakkoli, E., Pan, X., Liao, F., Chen, W., & Guo, W., 2021. Preferential ammonium: nitrate ratio of blueberry is regulated by nitrogen transport and reduction systems. *Scientia Horticulturae*, *288*, 110345. https://doi.org/10.1016/j.scienta.2021.110345

Yang, H., Wu, Y., Zhang, C., Lyu, L., Wu, W., Huang, Z., & Li, W., 2025. Growth and Physiological Traits of Blueberry Seedlings in Response to Different Nitrogen Forms. *Plants*, *14*(10), 1444. https://doi.org/10.3390/plants14101444

Yousaf, M., Li, J., Lu, J., Ren, T., Cong, R., Fahad, S., & Li, X., 2017. Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-01412-0

Yuan-Yuan, Z., Jun-Ping, T., Jun, C., Yu-Hui, H., & Yu-Shi, L., 2021. Effects of different NH4+/NO3- ratios on the photosynthetic and physiology responses of blueberry (Vaccinium spp.) seedlings growth. *Journal of Plant Nutrition*, *44*(6). https://doi.org/10.1080/01904167.2021.1871754

Zhang, J., Lv, J., Xie, J., Gan, Y., Coulter, J. A., Yu, J., Li, J., Wang, J., & Zhang, X., 2020. Nitrogen Source Affects the Composition of Metabolites in Pepper (Capsicum annuum L.) and Regulates the Synthesis of Capsaicinoids through the GOGAT–GS Pathway. *Foods*, *9*(2), 150. https://doi.org/10.3390/foods9020150

CHAPTER FOUR

INFLUENCE OF DIFFERENT NITROGEN SOURCES AND GROWTH MEDIA ON MINERAL COMPOSITION AND BLUEBERRY PHENOLIC CONSTITUENTS

4.1 Abstract

Blueberry (*Vaccinium* spp.) is an economically important fruit, increasingly popular worldwide due to consumer interest in health-improving crops. Despite rising in production little research has examined how blueberry phenolic constituents respond to mineral nutrition this knowledge gap limits the development of the crop as a functional food or medicinal resource. This study evaluated the influence of nitrogen (N) sources and growth media on mineral compositions, total phenolic, flavonoid, and proanthocyanin contents in blueberries. The experiment was conducted under a polythene white shade net at Cape Peninsula University of Technology, Wellington campus, South Africa, using two-year-old plants. Growth media were 100% coir and 80% coir: 20% zeolite with three N source fertilizers. Healthy plants were transplanted into 45L pots. The six treatment combinations were replicated three times in a randomized complete block design. The experiment was conducted over a period of two years and the yield for each season was measured. The growth media mineral composition was analyzed at BemLab and the phenolic compounds were determined using the Folin-Ciocalteu assay. Results showed T_1 (100% coir + ammonium sulphate) improved yield, nitrogen use efficiency (NUE) and total phenolic content (TPC), T_2 (80% coir: 20% zeolite + ammonium sulphate) enhanced proanthocyanidin content (PAC) while T_5 (100% coir + urea) increased total flavonoid content (TFC). Treatment 6 (80% coir: 20% zeolite + urea) showed higher NO_3^-, NH_4^+, Fe and Mg with lower pH and cation exchange capacity. Phenolic compounds increased with plant age regardless of treatment. Findings then suggests that optimized fertilization can balance yield with biosynthesis of phenolic constituents improving fruit quality and nutritional value.

Keywords: blueberry, secondary metabolites, N sources, mineral composition, growth media

4.2 Introduction

Blueberry (*Vaccinium* spp.), an economically important member of the Ericaceae family is increasing in popularity worldwide due to growing consumer interest in health-improving crops (Brazelton, 2015). High amounts of phenolic compounds in blueberry extracts have been related to antioxidant activity in human cells (Bornsek *et al*., 2012) and contribute to reducing inflammation, high blood pressure, and preventing cardiovascular disease (Johnson *et al*., 2015). Driven by consumer demand the global cultivated area was 126,144 ha in 2020 a significant increase from 1980 when the cultivated area was 24,460 ha (International Blueberry Organization (IBO), 2025). The production of blueberries and their consumption are predicted to expand to over 1 million tons by 2030 (IBO, 2025). However, despite the increasing production of blueberries little research has been carried out to investigate how blueberry phenolic constituents respond to mineral nutrition (Jasminka *et al*., 2025) this knowledge gap hinders the potential development of this crop as a functional food or medicinal resource.

Blueberry plants are classified as calcifuge plants limited to acidic soils and usually show optimal growth at a lower pH of 4.0 to 5.5 (Kingston *et al*., 2017). Compared to most crops, blueberries require less nutrient input; however, an effective fertilization plan is necessary to achieve rapid plant growth and production of high-quality fruit (Korcak, 1988). Nitrogen (N) is the most required element and can be absorbed by plants in cationic (NH_4^+) and anionic (NO_3^-) forms, which not only affect plant growth and development (Savvas *et al*., 2006), but also the nutritional quality of higher plants (Sun *et al*., 2018). Blueberry leaves contain about 1.5-2.1% of N by dry weight (Alt *et al*., 2017) and have been shown to prefer NH_4^+ ion over NO_3^- ion due to their inefficient nitrate reductase (NR) and nitrite reductase (NiR) enzymes for conversion of NO_3^- to NH_4^+ (Alt *et al*., 2017). These ions represent 80% of the total cations and anions absorbed by plants; therefore, the form of N has a significant impact on the uptake of other essential elements (Neumann & Römheld, 2012), subsequently affecting the nutritional quality of the fruit (Leal-Ayala *et al*., 2021). Although blueberry prefers NH_4^+ based fertilizers, the sole application of NH_4^+ tends to reduce calcium (Ca) uptake, whereas NO_3^- nutrition can improve Ca uptake, but blueberry utilizes NO_3^- less efficiently (Doyle *et al*., 2021). Malladi and Cabrera (2020) also found that the application of NH_4^+ nutrition promoted iron (Fe), manganese (Mn), and zinc (Zn) solubility by acidifying the rhizosphere; however, it often lowers the uptake of base cations such as Ca and magnesium (Mg). Moreover, the application of NH_4^+: NO_3^- 50:50 ratio treatments increased chlorophyll content, photosynthetic capacity, and phosphorus (P), Ca, and Mg in blueberry leaves (Anwar *et al*., 2024).

High N application rates above 120 kg N ha^{-1} are believed to reduce antioxidant concentrations in blueberries by favouring vegetative growth over reproductive development, thereby reducing the allocation of nutrients and water towards secondary metabolite synthesis in the fruit (Lee & Kader, 2000). González *et al*. (2018) reported that application of 100% NH_4^+ as N source increased chlorophyll content in leaves, favouring vegetative growth; however, 100% NO_3^- or 0%N significantly resulted in higher concentration of anthocyanin in Andean blueberry leaves, indicating that N deficiency may stimulate antioxidant synthesis. In contrast, Zhang *et al*. (2023) found that rational application of fertilizer significantly increased anthocyanin content and total phenol content (TPC) in rabbiteye blueberry plants by 9.5% and 62.96%, respectively, compared to non-fertilized plants. Furthermore, Chatzigianni *et al*. (2018) reported that the application of NH_4^+ in *Cichorium spinosum* had an important role in promoting the accumulation of TPC.

Blueberries have been increasingly cultivated in containers with soilless substrate due to their unusual requirements caused by a poor fibrous root system (Heller & Nunes, 2022). The key reason for using a soilless cultivation system is that it can control the growing environment through the amount and nutrient concentrations, moisture content, and limiting soil stress, therefore improving the crop productivity (Engler & Krarti, 2021). Determining the physical and chemical properties of the substrates is crucial for their efficient use, as they significantly impact plant growth and development (Lemaire, 1995). Despite reports of effective soilless culture with bark source on different vegetables and ornamental crop production (Cantliffe *et al*., 2003), phytotoxicity may occur due to phenolic compounds extracted from the substrates composed of peat, sawdust, and pine bark (Politycka *et al*., 1985). Currently, the type of raw materials used for soilless growing media is standardized and utilized in various ways (Gruda *et al*., 2022). However, each substrate has unique characteristics and typically varies from one another; hence, these variations in growing media must be considered.

Zeolites have several unique properties, such as high-water absorption, high cation exchange capacity (CEC), and high buffering ability of pH; they are widely used as soil conditioners to improve soil physio-chemical properties (Sangeetha & Baskar, 2016). The high CEC and ability to hold and gradually release nutrients have been found to improve yield and quality in tomato and cucumber (Rydenheim, 2007). Similarly, coconut coir, which is in great demand in the ornamental crops and greenhouse industries, has been marketed as a substitute for rockwool due to its suitable physical and chemical properties (He *et al*., 2022). Previous research showed that 80% coir mixed with 20% zeolite, or 100% coir, can influence blueberry performance, with the 80% coir: 20% zeolite mixture improving water use efficiency compared to 100% coir (Mfeka *et al*., 2023). Scagel (2003), reported that the growth of many ericaceous species could be promoted when plants were cultured in media mixed with coir; however, the volume of coir in media never contained more than 20%. The effects of different N forms on the regulation mechanisms of secondary metabolites in blueberry fruits are still unclear. The present study aimed to evaluate the effects of N sources and growth media on mineral compositions and total phenolic, flavonoid, and proanthocyanin contents in blueberries. The results of this research are of great significance for improving blueberry fruit quality and reducing production costs, environmental pollution, and the optimum derivation of these vital therapeutic components

4.3 Materials and Methods

4.3.1 Plant material and Experimental design

Blueberry plants cultivar ‘Legacy’ (De Fynne Nursery, Paarl, South Africa) were used as the experimental material and cultivated under a shade net in AgriHub at Cape Peninsula University of Technology, South Africa. In the experiment, 100% coir and 80% coir: 20% zeolite were selected as the growing media (Glencairns, Paarl, and Agring Consultants, Heidelberg, South Africa). Uniformly healthy plants with no pests or sickness were transplanted into 45L bags (23 cm upper diameter, 23 cm lower diameter, 15.6 cm height, and 124.8 cm length), with one plant per pot. After 30 days of adaptive pre-culture, the plants were fed with three N sources, including [$(NH_4)_2SO_4$] (N 21.1%), [$Ca\ (NO_3)_2$] (N 16.6%), and (H_2NCONH_2) (N 46%). Each N source was applied to both growth media treatments, resulting in six treatment combinations. Each treatment was replicated three times, with three pots per replicate, and the experiment was conducted following a randomized complete block design as shown in Table 1. During cultivation, fertilizer was applied once a week, with 1 L applied to each bag. The total amount of N applied to each blueberry plant was kept consistent. A 500 g dry sample of growth media from each treatment was submitted to a commercial facility (BemLab, Strand, South Africa) for initial and post-harvest analysis of its physical and chemical properties. The analyses included pH, Cation exchange capacity (CEC), organic matter content, organic carbon, available nitrogen forms (NH_4^+ and NO_3^-), as well as other macro and micronutrients (P, K, Ca, Mg, Na, Fe, Zn, Mn), as shown in Tables 4.1, 4.3 and 4.4.

4.3.2 Cultivation and Irrigation Management

The pH and electrical conductivity (EC) of the growing media were assessed every two weeks following fertilizer application, using standard laboratory methods and a Milwaukee handheld pH meter and an EC meter (Leal-Ayala *et al*., 2021). All other cultivation practices, including pruning, irrigation, and weed control, were carried out uniformly across all treatments.

Table 4.1 Initial chemical characteristics of growth media used to grow potted plants of 'Legacy' blueberry

Medium	%Total N	%Total Ca	%Total K	% Total Mg	CEC cmol/kg	pH
100% coir	2.13	27.91	5.32	13.16	7.53	3.8
80% coir: 20% zeolite	14.86	28.94	14.05	14.86	10.09	4.2

N- nitrogen, Ca – calcium, K – potassium, Mg – magnesium, CEC – cation exchange, cmol - centimoles , and kg – kilograms.

4.3.3 Sample extraction

The harvest was done manually, at the peak of the ripening period, with berries being harvested weekly between October to December in both the 2023 and 2024 growing seasons.

To preserve the berries, they were immediately frozen and stored at −80 °C after harvesting. Berry samples were freeze-dried (−40 °C; 0.050 mbar vacuum) in a VirTis SP Scientific Wizard 2.0 freeze-dryer (SP Industries, Warminster, PA, USA) and ground into powder form using a mortar and pestle. Thereafter, the phenols were extracted from the blueberry powdered samples by adding 5 mL of 80% methanol with glacial acetic acid (1 mL of 100% acetic acid per 1 L of 80% methanol) to 0.5 g of ground samples, vortexing for 30 seconds, and then leaving the mixture for 24 hours. After 24 hours, the mixtures were centrifuged at 2000 rpm for 15 min (Centrifuge Lasec).

The final supernatants were transferred to 10 mL vessels, properly labeled, and stored at −80 °C for the determination of phenolic compounds.

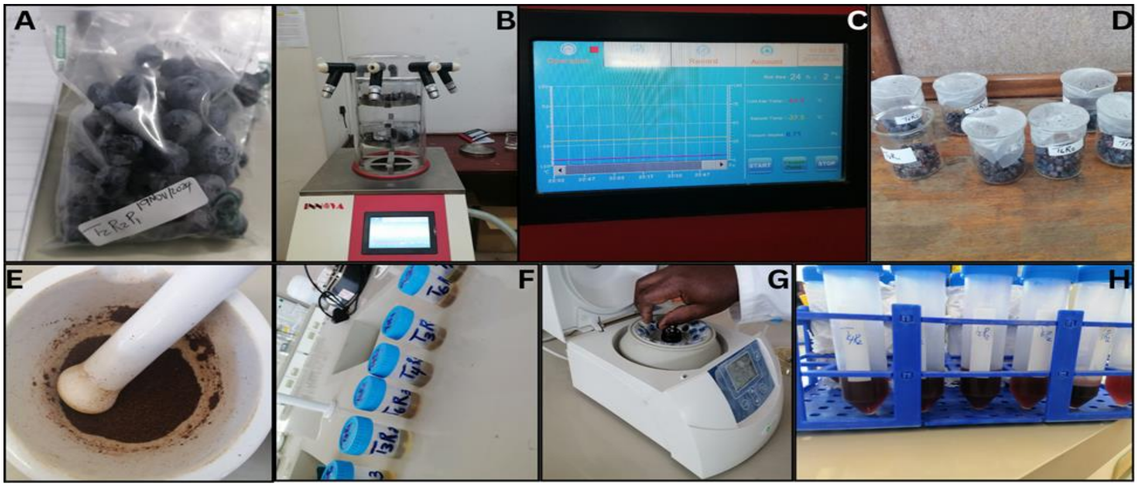


Figure 4.1. (**A**) Properly labeled samples after harvest, (**B-C**) freeze-drying of samples at -40 °C; 0.050 mbar vacuum, (**D-E**) grinding of dried samples into finer powder using a mortar, (**F**) extraction over 24 hours using 80% MeOH with acetic acid, (**G**) centrifugation of mixtures at 2000 rpm for 15 min, and (**H**) collection of supernatants for phenolic compound determination.

4.3.4 Determination of Total Phenolic Content (TPC)

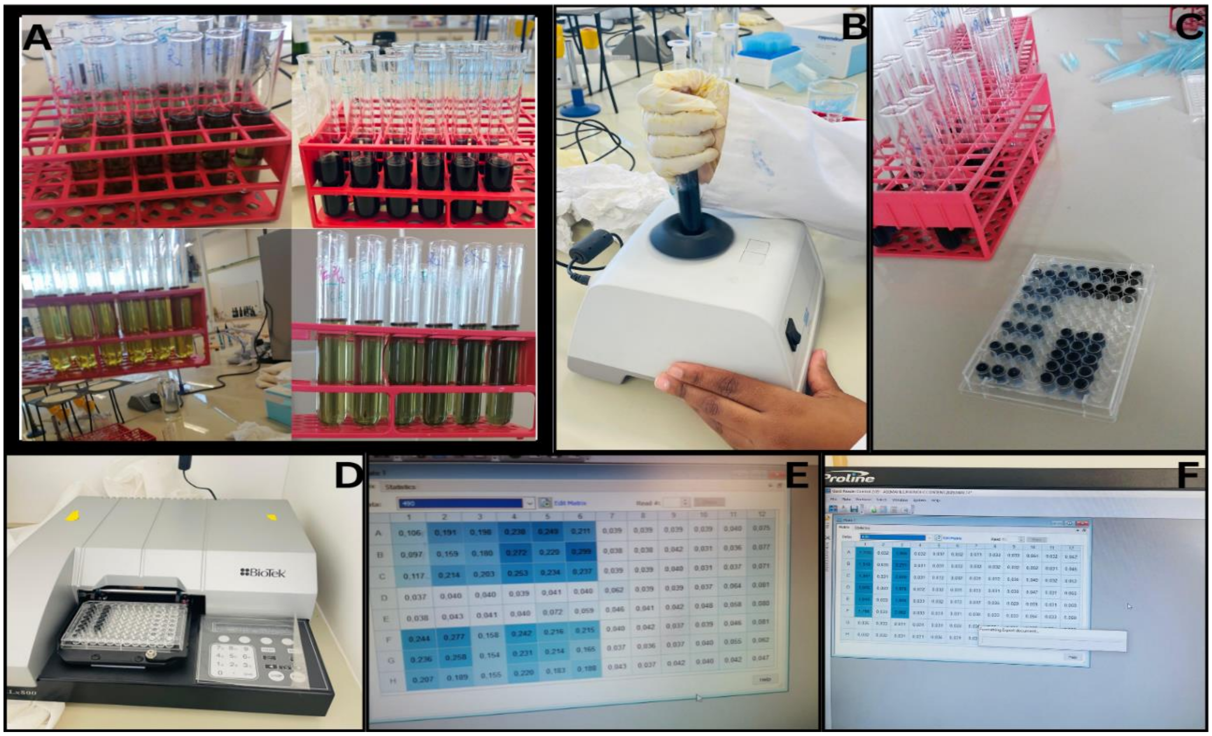
The TPC for the different treatments was determined using the Folin-Ciocalteu assay method (Jimoh *et al*., 2019) with slight modifications. The supernatants of 0.5 mL were mixed with 2.5 mL of 10% Folin–Ciocalteu`s reagent in 10 mL test tubes. Next, 2 mL of a 7.5% saturated sodium carbonate solution was added, vortexed, and shaken for 5 min, followed by incubation at 40 °C for 30 min. The absorbance values were measured at 750 nm using a microplate reader spectrophotometer (Model 680, Bio-Rad, USA). A blank was prepared with distilled water. A standard curve was prepared to estimate the total phenolic content using gallic acid at a concentration range (0.063-2.0 mg/ml). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry matter from the standard curve $y=0.0179x +0.0473$, $R^2=0.9852$. All analyses were carried out in triplicate.

4.3.5 Determination of Total Flavonoid Content (TFC)

The TPF was determined using the colometric assay (Elufioye *et al.*, 2019) with slight modifications. 0.5 mL of supernatant was mixed with 3 mL of distilled water, 0.3 mL of 5% sodium nitrite, and after 5 min, 0.3 mL of 10% aluminium chloride was added. In 6 minutes, 2 mL of 1 M sodium hydroxide was added, and finally, 5 mL of water was added, and the mixtures were vortexed after each addition. The absorbance values were measured at 510 nm using a microplate reader spectrophotometer (Model 680, Bio-Rad, USA). A standard curve was prepared using graded concentrations of quercetin (0.063-2.0 mg/ml) to estimate the total flavonoid content, and the results were expressed as mg quercetin equivalent per gram dry weight from the calibration curve $y=0.0141x + 0.0473$, $R^2= 0.9591$. All analyses were carried out in triplicate.

4.3.6 Determination of Proanthocyanidin content (condensed tannin)

The proanthocyanidin content was determined according to the previously reported procedure (Elufioye *et al.*, 2019) with slight modifications. 0.5 ml of berry extracts (mg/ml) was mixed with 3 ml of vanillin (4%) in a methanol solution. Thereafter, 1.5 ml of hydrochloric acid (1.5%) was added, mixed thoroughly, and incubated for 15 minutes at room temperature. 250 microliters of each of the extract solutions and graded concentrations of the gallic acid were pipetted into wells of a 96-well microplate, and absorbance was measured at 490 nm with the aid of the microplate reader model 680, BIORAD, made in the USA. A blank was prepared with distilled water. A standard curve of gallic acid at a concentration range (0.063-2.0 mg/ml) was used to determine the proanthocyanidin content expressed as mg gallic acid equivalent per gram dry weight from the calibration curve $y=0.011x + 0.0403$, $R^2=0.9689$. All the analyses were carried out in triplicate



	1	2	3	4	5	6	7	8	9	10	11	12
A	0,106	0,191	0,198	0,238	0,249	0,211	0,039	0,039	0,039	0,039	0,040	0,075
B	0,097	0,159	0,180	0,272	0,220	0,299	0,038	0,038	0,042	0,031	0,036	0,077
C	0,117	0,214	0,203	0,253	0,234	0,237	0,039	0,039	0,040	0,031	0,037	0,071
D	0,037	0,040	0,040	0,039	0,041	0,040	0,062	0,039	0,039	0,037	0,064	0,081
E	0,038	0,043	0,041	0,040	0,072	0,059	0,046	0,041	0,042	0,048	0,058	0,080
F	0,244	0,277	0,158	0,242	0,216	0,215	0,040	0,042	0,037	0,039	0,046	0,081
G	0,236	0,258	0,154	0,231	0,214	0,165	0,037	0,036	0,037	0,040	0,055	0,062
H	0,207	0,189	0,155	0,220	0,183	0,188	0,043	0,037	0,042	0,040	0,042	0,047

Figure 4.2. (**A**) Variation in colour among different extract solutions, (**B**) thorough mixing of the extracts using a vortex mixer, (**C**) arrangement of extract solutions in the microplate with treatment labels, (**D**) measurement of absorbance using a Microplate Reader, and (**E-F**) representative results obtained in comparison with the respective standards.

4.3.7 Statistical Analysis

The reported results and data are presented as averages for the main effect of N sources and growth media composition on phenolic constituents of blueberry. Analysis of variance ANOVA at a 95% confidence level was carried out using IBM SPSS version 30.0 software. Means separated were done using Duncan`s Multiple Range Test (DMRT).

4.4 Results

4.4.1 Effect of nitrogen sources and growth media on blueberry phenolic constituents

The response of total phenolic content (TPC), total flavonoid content (TFC), and proanthocyanidin content (PAC) to different treatment combinations varied between two harvest seasons (Table 4.2). In the 2023 harvest season, treatment applications influenced ($p< 0.05$) TFC; however, the data remained consistent ($p>0.05$) in TPC and PAC. The results revealed a high stimulatory effect of T_5 (100% coir+ urea) on berry TFC (11.54 mg/ g FW) accumulation, with an increase of more than 280% compared to the lowest TFC (3.03 mg/ g FW) under T_1 (Control), followed by T_2 and T_3. Application of T_4 and T_6 treatments also enhanced TFC relative to T_1; however, their effects were less pronounced than that of T_5 treatments.

In 2024, overall concentrations of the phenolic compounds were considerably higher than in the previous season. Remarkably, the treatment applications caused notable changes in blueberry PAC ($p< 0.05$); however, TFC showed no significant variation among treatments (Table 4.2). PAC was greatest under T_2, exceeding T_1 by 9.89%. In contrast, the lowest PAC levels were recorded under T_4, T_5, and T_6 treatments. Although the mean TPC values tended to be higher in T_2 and T_1, differences among treatments remained consistent.

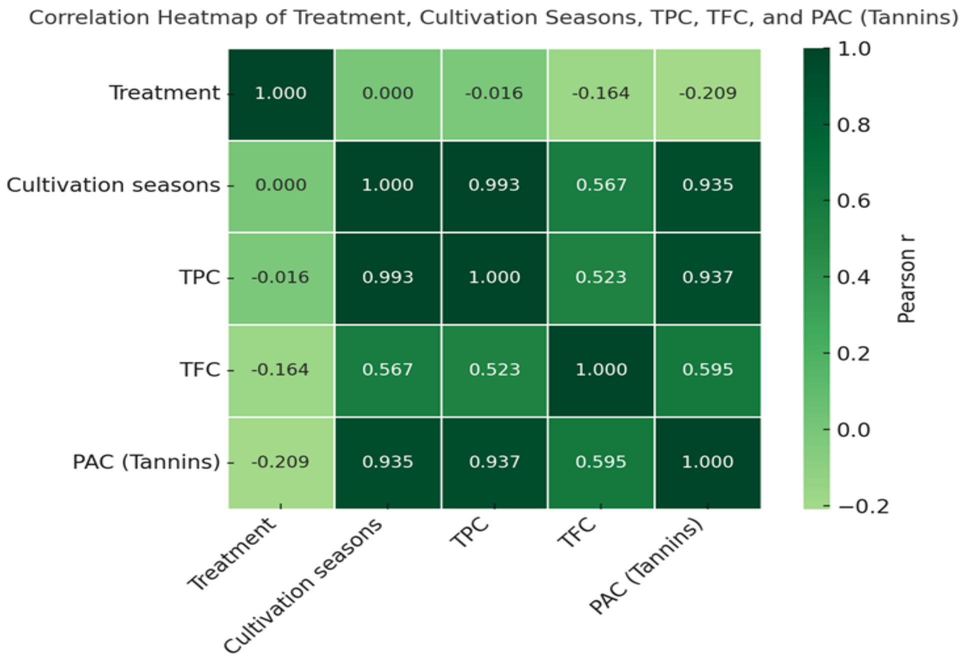
Table 4.2 Influence of different nitrogen sources and growth media on the total phenolic content (TPC), total flavonoid content (TFC), and proanthocyanidin content (PAC) in the extract of blueberry during two harvest seasons

Harvest Season	Treatment	TPC (mg GAE/g FW)	TFC (mg QE/g FW)	PAC (mg GAE/g FW)
2023	T_1	23.27 ± 0.93^{a}	3.03 ± 1.42^{c}	2.02 ± 1.12^{b}
	T_2	21.63 ± 1.52^{ab}	3.97 ± 1.78^{c}	2.88 ± 0.43^{ab}
	T_3	18.02 ± 2.12^{b}	5.16 ± 3.55^{c}	2.19 ± 0.26^{b}
	T_4	18.59 ± 2.88^{ab}	9.88 ± 3.91^{ab}	3.29 ± 0.17^{a}
	T_5	19.54 ± 4.32^{ab}	11.54 ± 0.00^{a}	2.88 ± 0.26^{ab}
	T_6	20.28 ± 0.51^{ab}	6.81 ± 1.08^{bc}	2.94 ± 0.52^{ab}
	LSD	NS	0.94	NS
2024	T_1	102.89 ± 7.43^{b}	51.73 ± 51.19^{a}	126.38 ± 8.77^{b}
	T_2	118.50 ± 11.26^{a}	25.16 ± 0.07^{a}	138.88 ± 3.82^{a}
	T_3	109.98 ± 3.99^{ab}	23.24 ± 0.85^{a}	113.56 ± 3.32^{c}
	T_4	110.85 ± 0.69^{ab}	18.35 ± 0.57^{a}	71.97 ± 5.18^{d}
	T_5	109.98 ± 1.73^{ab}	21.82 ± 0.92^{a}	76.79 ± 0.73^{d}
	T_6	106.10 ± 6.56^{ab}	21.47 ± 3.12^{a}	80.25 ± 7.27^{d}
	LSD	NS	NS	4.82

Values are means ± standard deviation at ($P<0.05$). Means with different letters in the same column are statistically significant at $P < 0.05$, *P*– probability, LSD– Least Significant Difference, S.D– standard deviation, GAE- gallic acid equivalence, mg- milligrams, g-grams, FW- fresh weight, QE- quercetin equivalence, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

4.4.2 Correlation of blueberry phenolic constituents

Pearson correlations were determined to analyse the relationship of blueberry fruit phenolic constituents (Figure 4.3). The statistical analysis revealed a highly positive correlation of TPC with cultivation years ($r = 0.993$, $p < 0.001$), PAC ($r = 0.937$, $p < 0.001$) and a moderately significant correlation with TFC ($r = 0.523$, $p = 0.001$). Cultivation year displayed highly significant correlations with PAC ($r = 0.935$, $p < 0.001$) and moderate correlation with TFC ($r = 0.567$, $p < 0.001$). The treatments did not show significant correlations with the phenolic constituents ($p > 0.05$).



	Treatment	Cultivation seasons	TPC	TFC	PAC (Tannins)
Treatment	1.000	0.000	-0.016	-0.164	-0.209
Cultivation seasons	0.000	1.000	0.993	0.567	0.935
TPC	-0.016	0.993	1.000	0.523	0.937
TFC	-0.164	0.567	0.523	1.000	0.595
PAC (Tannins)	-0.209	0.935	0.937	0.595	1.000

Figure 4.3. Correlation matrix of different physiological indexes in blueberry plants. A significant correlation is represented by the strong green colour for highly significant correlation, and light green for moderately significant correlation. TPC, Total Phenolic Content; TFC, Total Flavonoid Content; PAC, Proanthocyanidin Content. Heatmap of phenolic constituents in blueberry fruits in response to different N sources and growth media.

4.4.2 The physicochemical parameters of blueberry cultivation substrates

The post-harvest analysis of substrates' physicochemical parameters differed significantly ($p< 0.05$) depending on the N source and growth media used (Table 4.3). Compared to T_1 (control), T_5 treatment greatly reduced ($p< 0.05$) the pH value of the cultivated substrates, followed by the T_6 treatment group. However, T_4 greatly increased the pH value of the substrates. The CEC value of growth media under T_2 treatments was more than three times that of T_1. The T_6 treatments had the lowest organic matter and organic carbon content, a decrease of 51% compared to T_1, followed by the T_2 treatment. There were no significant differences observed in electrical resistance among the treatments.

Table 4.3 Physiological parameters of blueberry cultivation substrates in response to different sources of N treatments

Treatment	pH	Organic Matter (%)	Organic Carbon (%)	T-value/CEC (cmol/kg)	Electrical Resistance (Ohm)
T_1	$3.43^{\pm}0.15^{a}$	$30.99^{\pm}2.69^{a}$	$18.02^{\pm}1.57^{a}$	$15.64^{\pm}0.67^{c}$	$420^{\pm}200^{a}$
T_2	$3.4^{\pm}0.60^{b}$	$15.44^{\pm}0.66^{c}$	$8.98^{\pm}0.39^{c}$	$53.46^{\pm}3.69^{a}$	$335^{\pm}145^{a}$
T_3	$3.37^{\pm}0.06^{b}$	$19.49^{\pm}0.20^{bc}$	$11.33^{\pm}0.12^{bc}$	$20.42^{\pm}2.0^{b}$	$355^{\pm}35^{a}$
T_4	$4.07^{\pm}0.25^{a}$	$15.97^{\pm}2.69^{c}$	$9.29^{\pm}1.57^{c}$	$9.29^{\pm}1.57^{d}$	$330^{\pm}30^{a}$
T_5	$3.27^{\pm}0.25^{b}$	$22.14^{\pm}3.29^{b}$	$12.87^{\pm}1.91^{b}$	$12.87^{\pm}1.91^{cd}$	$225^{\pm}25^{a}$
T_6	$3.3^{\pm}0^{b}$	$15.34^{\pm}5.53^{c}$	$8.92^{\pm}3.21^{c}$	$8.92^{\pm}3.21^{d}$	$230^{\pm}10^{a}$
LSD	0.03	0.1	0.06	0.37	NS

Values are means ± standard deviation at ($P<0.05$). Means with different letters in the same column are statistically significant at $P < 0.05$, cmol- centimoles, kg- kilograms, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea

4.4.3 The influence of nitrogen sources and substrates on the availability of macro- and micronutrients during blueberry cultivation

Post-harvest nutrient concentrations in the substrates differed significantly ($p < 0.05$) according to the nitrogen source and growth medium used (Table 4.4). Ammonium (NH_4^+) treatments (T_1, T_2, T_5, and T_6) retained higher levels (104 –136 mg/kg), with the highest values in T_2 and T_6 (zeolite mixtures), while nitrate (NO_3^-) treatments (T_3 and T_4) had lower NH_4^+ concentrations (7.55 –19.85 mg/kg). Oddly, NO_3^- concentrations were highest in T_5 and T_6 treatments; however, there were no statistically significant differences observed.

Cation accumulation was strongly influenced by zeolite. T_2 treatment had the highest ($p<0.05$) concentrations of K, Mg, and Na compared to T_1; furthermore, T_4 treatment had the greatest effect on Ca availability, recording values four times higher than T_1 treatments. However, Fe and Zn concentrations were the highest under T_5 treatments compared to T_1, with an increase of 29% and 24%, respectively, and T_6 treatments had the most influence on P concentration compared to T_1. In contrast, Mn concentrations were increased by T_1 treatment compared to T_3 and T_6 treatment groups.

Table 4.4 Concentrations of macronutrients and micronutrients in the substrates under different nitrogen source treatments.

Treatment	NO_3^-	NH_4^+	Ca (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	P (mg/kg)
T_1	72.5 ± 50.5^{ab}	120.1 ± 51.9^{a}	6.7 ± 0.2^{d}	237 ± 3^{c}	2.05 ± 0.35^{c}	0.55 ± 0.45^{c}	39.45 ± 12.2^{b}	2.50 ± 0.3^{ab}	4.2 ± 0.6^{a}	2.3 ± 0.1^{c}
T_2	49.9 ± 39.2^{b}	125.2 ± 47.8^{a}	15.75 ± 0.35^{b}	4265 ± 315^{a}	7.6 ± 1.7^{a}	13 ± 2.8^{a}	23.65 ± 2.25^{c}	0.66 ± 0.33^{e}	2.35 ± 1.35^{bc}	13.6 ± 5.8^{bc}
T_3	73.6 ± 15.9^{ab}	19.85 ± 1.65^{b}	12.1 ± 1.7^{c}	120.8 ± 26.2^{c}	0.71 ± 0.6^{c}	0.2 ± 0^{c}	51.05 ± 0.65^{a}	1.9 ± 0.2^{bc}	1.70 ± 0.7^{c}	4 ± 0^{bc}
T_4	58.8 ± 15.2^{b}	7.55 ± 4.25^{b}	25.6 ± 0.5^{a}	3200 ± 480^{b}	4.85 ± 2.05^{b}	6.5 ± 2.7^{b}	27.25 ± 4.75^{c}	1.37 ± 0.63^{cd}	1.95 ± 0.65^{bc}	10.2 ± 8^{bc}
T_5	132.7 ± 41.3^{a}	104 ± 47.7^{a}	6.55 ± 0.25^{d}	245 ± 16^{c}	2.35 ± 0.15^{c}	0.53 ± 0.1^{c}	51.05 ± 3.05^{a}	3.10 ± 0.2^{a}	3.30 ± 0.5^{ab}	17.95 ± 15.75^{b}
T_6	76.95 ± 2.95^{ab}	136 ± 0^{a}	14.85 ± 0.45^{b}	2990 ± 100^{b}	5.83 ± 0.45^{ab}	5.3 ± 1.5^{b}	38.20 ± 2.7^{b}	0.99 ± 0.11^{de}	1.60 ± 0^{c}	36.57 ± 0.35^{a}
LSD	NS	12.3	0.15	116.2	1.34	0.35	3.6	0.33	0.10	1.7

Values are means ± standard deviation at ($P<0.05$). Values are shown as means ± SD. Means followed by different letters were significantly different according to Duncan's test ($p<0.05$), NO_3^- –nitrate, NH_4^+ –ammonium, Ca–calcium, K –potassium, Mg – magnesium, Na – sodium, Fe –iron, Zn –zinc, Mn – manganese, mg – milligrams, kg– kilograms, T1 –100% Coir + ammonium sulphate (Control), T2 – 80% coir: 20% zeolite+ ammonium sulphate, T3– 100% coir + calcium nitrate, T4– 80% coir: 20% zeolite + calcium nitrate T5 – 100% coir + urea, and T6 – 80% coir: 20% zeolite + urea.

4.5. Discussion

The environmental factors, the form of fertilizer applied, and the harvest period influence the transport and allocation of nutrients and water within plants, thus affecting the concentration and profile of secondary metabolites in fruits, such as proanthocyanidins, flavonoids, and phenolic compounds (Dyukaryeva & Mallik, 2023). These secondary metabolites enable blueberries to exhibit extremely high antioxidant activity, particularly in polyphenols such as anthocyanins, which, unlike endogenous enzyme systems, are readily available dietary antioxidants to the human body (Yang *et al*., 2023).

4.5.1 Effect of nitrogen sources on blueberry phenolic constituents

Total Phenolic Content (TPC)

Excessive N application is believed to reduce antioxidant concentrations, such as anthocyanins and vitamin C, in blueberries by favouring vegetative growth over reproductive development, thereby reducing the allocation of nutrients and water towards secondary metabolite synthesis in the fruit (Lee & Kader, 2000). Herein, this study, the effect of different N sources did not significantly differ in terms of total phenolic content (TPC), which remained within the range of approximately 17.3-26.59 mg/g FW, values observed in the pulp (17.3) and whole ripe (26.59) of the Southern highbush blueberries (Sun *et al*., 2018), in agreement with the observed results (18.02-23.27 mg/g FW) during 2023 harvest season (Table 4.2).

Although there were no significant differences, NH_4^+ based treatments (T_1 and T_2) appeared to increase the accumulation of TPC, which is consistent with previous findings that reported NH_4^+ plays an important role in promoting TPC accumulation (Chatzigianni *et al*., 2018). However, the non-significant response observed indicates that TPC may be relatively stable under the tested conditions, suggesting that the plant tissues were not subjected to sufficient nutrient limitations to stimulate enhanced phenolic synthesis, a response that is often observed when there is N deficiency (Ibrahim *et al*., 2011). These findings are supported by previous studies which reported that the accumulation of excess carbon (C) in response to N deficiency leads to increased production of carbon based secondary metabolites (Suh *et al*., 2020) thereby increasing TPC.

Total Flavonoid Content (TFC)

Flavonoids serve not only as nutritional compounds but also play a role in regulating plant defence systems against adverse conditions and mediating signal transduction in response to both biotic and abiotic stresses (Agati *et al*., 2020). In this study, T_5 treatment (11.54 mg QE/ g FW) significantly enhanced the total flavonoid content (TFC) accumulation in blueberry fruits compared to other treatments during the 2023 harvest season (Table 4.2). It has been reported that the supply of urea-based treatments in grapes promoted the accumulation of flavonoids compared to ammonium sulphate and calcium nitrate treatments (Hui *et al*., 2021). Portu *et al*. (2015) similarly found that the total flavanol content was significantly higher only under low concentration urea application compared to the control. These findings suggest that urea-based fertilizers enhance the accumulation of flavonoids in berries and improve the quality.

Urea hydrolysis not only supplies N in the form of NH_4^+ but also carbon dioxide (CO_2), which can potentially contribute to the maintenance of photosynthesis under abiotic stress, suggesting reallocation of C skeleton and a higher assimilation of NH_4^+ by shifting C and N allocation towards secondary metabolites such as phenylpropanoid and flavonoid synthesis (Gonçalves & Mercier, 2021). Furthermore, Hui *et al*. (2021) reported that ammonium sulphate, calcium ammonium nitrate, phenylalanine, glutamate and clear water (control) did not increase or decrease flavonoid content except for urea. This is consistent with the findings of the present study, where TFC accumulation did not differ significantly among most treatments, whereas T_5 exhibited a significantly higher TFC except for T_4 (9.88 mg QE/ g FW), suggesting that the addition of zeolite affected TFC accumulation. In contrast, Duan *et al*. (2023) found that blackberries supplied with NO_3^- and no N treatments were associated with enhanced flavonoid content compared to NH_4^+ based treatments, suggesting that NH_4^+ promotes flavonoid accumulation in blueberries and grapes, whereas NO_3^- nutrition appears to favour flavonoid synthesis in blackberries, highlighting the crop-specific influence of N form on secondary metabolite production.

Proanthocyanidin content (PAC)

Proanthocyanidins (PAC) also known as condensed tannins are natural polyphenolic compounds widely distributed in different crops which are mixtures of oligomers and polymers formed by the condensation of flavan-3-ols (Rauf *et al.*, 2019) found in high proportions in skins of grapes and berries, among other fruits (Wang *et al*., 2019). Results from this study found that PAC tended to increase with the maturity of blueberries with high levels in the 2024 harvest season compared with those recorded in 2023 suggesting an interannual increase possibly due to differences in the cultivation conditions. On blackberries, the highest concentration of PAC was recorded in early unripe fruit which then decreased with the ripening process (Chen *et al*., 2012) a pattern that was also observed in strawberries and persimmons (Salvatierra *et al*., 2010). However, the pattern was completely different in grape berries where PAC levels increased progressively from the unripe stage to ripening (Downey *et al*., 2003). These contrasting patterns in the availability and concentration of PAC highlight the need for further investigation into factors influencing the significant increase in blueberry PAC during fruit development stages.

The variation of PAC observed in the 2024 harvest season ($p<0.05$) indicates that N fertilization significantly influenced PAC synthesis in blueberries (Table 4.2). This is in concurrence with the findings of Narvekar and Tharayil (2021), who reported that with PAC, the less abundant class of phenolic oligomers exhibited higher levels of concentration in response to supplied N treatments compared to the most abundant class of phenolic, such as ellagitannins. The synthesis of PAC or condensed tannins shares a similar step in the flavonoid pathway with the synthesis of anthocyanin and flavonols (Narvekar & Tharayil, 2021). In blackberries, NO_3^- fertilization increased the flavonoid content compared to NH_4^+ based treatments (Duan *et al*., 2023). These findings contrast with our results, where NH_4^+ promoted PAC accumulation in the 2024 harvest season compared with NO_3^-. Such varied responses may be attributed to differences in the physiological and metabolic processes specific to each species.

4.2 Interactive effect of N sources and growth media on blueberry phenolic constituents

There is limited literature on how the interactive effect of N sources and coir: zeolite influences the biosynthesis and accumulation of various secondary metabolites, including phenolics, and most mechanisms involved are still unclear (Duan *et al*., 2023). Coir was found to significantly increase the contents of total phenols in cucumber (He *et al*., 2022). In this study, it was found that TFC in the T_5 group treatments was highest, indicating that plants had the strongest antioxidant activity under this treatment. The acidification from urea hydrolysis (Yang *et al*., 2025) and the low buffering capacity of coir properties (Kozos & Ochmian, 2016) may have stimulated the blueberry roots and regulated genes to enhance the total phenols in blueberry fruits as a stress signal (He et *al*., 2022). Plants grown in T_2 had accumulated higher PAC than T_1 and T_5 (Table 4.2). Due to the blueberry shallow fibrous root system (Ortiz-Delvasto *et al*., 2023), the zeolite's capacity to retain NH_4^+ may have been excessive, potentially inducing stress signals and leading to an increased accumulation of phenolic acids as a defence response (Goncharuk & Zagoskina, 2023).

Additionally, correlation analysis showed a strong correlation of phenolic constituents with cultivation years regardless of the treatments applied (Figure 4.3). The strong correlations between TPC and PAC indicate the presence of tannins and the overall phenolic content of berry samples. This correlation of TPC and PAC was also observed by Vo *et al*. (2022) with grapes. These findings suggest that the phenolic contents of blueberries mainly consisted of condensed tannins and the accumulation of secondary metabolites in blueberries increases with plant age or is determined by maturity.

4.5.2 Influence of N sources and growth media on growth media analysis

Soil conditions directly influence the availability and uptake of nutrients affecting plant growth and development (Philippot *et al*., 2013). In this study blueberry plants exhibited improved growth under T_1, T_2, and T_5 treatments. Analysis of the physicochemical properties of growth media under T_1 and T_5 showed lower pH values, higher organic matter, and carbon (C) (Table 4.3). Additionally, T_6 also had a lower pH value; these results indicate that the treatments supplied greater amounts of mineral nutrients particularly NH_4^+ while the lower pH creates an acidic environment favourable for blueberry growth. These findings are supported by previous studies which reported that the NH_4^+ acidifies the rhizosphere by inducing protons (H^+) release from the cell (Fernandes & Rossiello, 1995). The results further demonstrate that N forms significantly influenced growth media CEC with T_2 showing the highest followed by T_3, highlighting their critical role in blueberry growth and development. The NH_4^+ based treatment (T_1 and T_5) likely increased organic matter and organic C content by stimulating both plant growth and microbial activity leading to a greater accumulation of microbial residues. Previous studies indicate that N provides energy for microorganisms, thereby accelerating the decomposition of organic matter, while also enhancing microbial activity through root exudates, which promotes organic C accumulation (Kallenbach *et al*., 2016).

The mineral composition of growth media plays a vital role in plant growth and development. Growers must also monitor these properties in the drainage solution to adjust fertilization and irrigation practices (He *et al*., 2022). Coir is generally characterized by its relatively high concentration of potassium (K), sodium (Na), and chlorine (Cl), while containing low content in phosphorus (P) (Ross *et al*., 2012). This is in agreement with the present study results, where high P concentration was observed in T_6 and T_2 compared to T_1 (Table 4.4). This can be attributed to the zeolite's high CEC under T_2 (53.46 cmol/kg) compared to T_1 (15.64 cmol/kg) and its ability to retain nutrients and gradually release them (Latifah *et al*., 2017).

The comparison between initial and post-harvest growth media properties showed a significant increase from before transplanting to after harvest analyses. In all treatments pH was within a range of 4.5-5.5 which is recommended for southern and northern highbush blueberry (Retamales & Hancock, 2012). Moreover, the higher nitrogen use efficiency (NUE) observed in T_1 and T_2 treatments (Chapter 3, Table 3.4) suggests that NH_4^+ minimized nutrient losses likely due to the greater retention of zeolite and the cation of NH_4^+. Research shows that 100%N and zeolite significantly influenced NUE in wheat farming minimizing N losses through leaching and emissions (Ahmad *et al*., 2025). However, further research should investigate how coir and zeolite combinations affect the uptake of N and NUE while maintaining the optimum yield and fruit quality of blueberry plants.

4.6 Conclusions and Recommendations

Irrespective of the form of N and growth media used, N supply had no significant effect on TPC; however, T_5 promoted the accumulation of TFC, while T_1 and T_2 maintained greater PAC compared to other treatments. The phenolic compounds greatly increased from 2023 cultivation to the 2024 harvest season; this discrepancy might be related to the age of the crop. Total fresh yields under T_1, T_2, and T_5 were considerably higher and the phenolic compounds correspond with the yield responses suggesting that these treatment combinations might be favourable for both yield and antioxidant activity in blueberry fruits. However, there is insufficient literature on how these treatment combinations influence the various secondary metabolites biosynthesis and further research is needed to investigate the physiological and biochemical mechanisms. Moreover, compared to T_1, T_6 showed higher NO_3^-, NH_4^+, Fe, and Mg content in the substrate with lower pH and EC. These findings suggest an optimized fertilization strategy for blueberry cultivation, with a promising balance between yield produced and the biosynthesis of phenolic constituents protecting the plants against biotic and abiotic stresses.

As mentioned the phenolic compounds accumulate naturally with plant age, regardless of treatment. Future management practices should aim to balance stress-induced phenolic accumulation with yield to optimize fruit quality and nutritional value.

The promising results under T_6 treatments warrant further studies in exploring the modifications of the coir: zeolite ratio with urea, as urea is an inexpensive source of N and could be useful to reduce potential over-acidification with ammonium sulphate in long-term container production.

References

Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., Sebastiani, F., & Tattini, M., 2020. Are Flavonoids Effective Antioxidants in Plants? Twenty Years of Our Investigation. *Antioxidants*, *9*(11), 1098. https://doi.org/10.3390/antiox9111098

Ahmad, A., Ijaz, S. S., Zhao, F., Rafa, H. U., & Farid, G.2025. The Effect of Zeolitic Fertilizer on Nitrogen Retention in Soil and Its Availability to Plants. *Nitrogen*, *6*(2), 46. https://doi.org/10.3390/nitrogen6020046

Alt, D. S., Doyle, J. W., & Malladi, A., 2017. Nitrogen-source preference in blueberry (Vaccinium sp.): Enhanced shoot nitrogen assimilation in response to direct supply of nitrate. Journal of Plant Physiology, 216, 79–87. https://doi.org/10.1016/j.jplph.2017.05.014

Anwar, A., Zheng, J., Chen, C., Chen, M., Xue, Y., Wang, J., Su, W., Chen, R., & Song, S., 2024, 'Effects of NH_4^+-N: NO_3^--N ratio on growth, nutrient uptake and production of blueberry (*Vaccinium spp.*) under soilless culture', *Frontiers in Plant Science*, *15,1438811.* https://doi.org/10.3389/fpls.2024.1438811

Arias, M. I., Nario, A., Rojas, K., Blanc, P., & Bonomelli, C., 2024, 'Newly Established Blueberry Plants: The Role of Inorganic Nitrogen Forms in Nitrogen and Calcium Absorption', *Horticulturae*, *10*(11), 1168. https://doi.org/10.3390/horticulturae10111168

Bornsek, S. M., Ziberna, L., Polak, T., Vanzo, A., Ulrih, N. P., Abram, V., Tramer, F., & Passamonti, S., 2012. Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells. *Food Chemistry*, *134*(4), 1878–1884. https://doi.org/10.1016/j.foodchem.2012.03.092

Brazelton, C., 2015. 2014 World Blueberry Statistics & Global Market Analysis. U.S. Highbush Blueberry Council

Cantliffe, D. J., Funes, J., Jovicich, E., Paranjpe, A., Rodriguez, J., & Shaw, N., 2003. Media and containers for greenhouse soilless grown cucumbers, melons, peppers, and strawberries. *Acta Horticulturae*, *614*, 199–203. https://doi.org/10.17660/ActaHortic.2003.614.28

Chatzigianni, M., Alkhaled, B., Livieratos, I., Stamatakis, A., Ntatsi, G., & Savvas, D., 2018. Impact of nitrogen source and supply level on growth, yield and nutritional value of two contrasting ecotypes of *Cichorium spinosum* L. grown hydroponically. *Journal of the Science of Food and Agriculture*, *98*(4), 1615–1624. https://doi.org/10.1002/jsfa.8636

Chen, Q., Zhang, X. N., Yu, H. wei, Yan-Wang, & Tang, H. R., 2012. Changes of total anthocyanins and proanthocyanidins in the developing blackberry fruits. *International Journal of ChemTech Research*, *4*(1).

Downey, M. O., Harvey, J. S., & Robinson, S. P., 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*, *9*(1), 15–27. https://doi.org/10.1111/j.1755-0238.2003.tb00228.x

Doyle, J. W., Nambeesan, S. U., & Malladi, A., 2021. Physiology of Nitrogen and Calcium Nutrition in Blueberry (Vaccinium sp.). *Agronomy*, *11*(4), 765. https://doi.org/10.3390/agronomy11040765

Duan, Y., Yang, H., Wei, Z., Yang, H., Fan, S., Wu, W., Lyu, L., & Li, W., 2023. Effects of Different Nitrogen Forms on Blackberry Fruit Quality. *Foods*, *12*(12), 2318. https://doi.org/10.3390/foods12122318

Dyukaryeva, V., & Mallik, A. U., 2023. Shade Effect on Phenology, Fruit Yield, and Phenolic Content of Two Wild Blueberry Species in Northwestern Ontario, Canada. *Plants*, *12*(24), 4099. https://doi.org/10.3390/plants12244099

Elufioye, T. O., Olusola, D. M., & Oyedeji, A. O., 2019. Correlation of Total Phenolic, Flavonoid and Tannin Content of Bryophyllum pinnatum (Lam.) (Crassulaceae) Extract with the Antioxidant and Anticholinesterase Activities. *Pharmacognosy Journal*, *11*(5), 1003– 1009. https://doi.org/10.5530/pj.2019.11.158

Engler, N., & Krarti, M., 2021. Review of energy efficiency in controlled environment agriculture. *Renewable and Sustainable Energy Reviews*, *141*, 110786. https://doi.org/10.1016/j.rser.2021.110786

Fernandes, M. S., & Rossiello, R. O. P., 1995. Mineral Nitrogen in Plant Physiology and Plant Nutrition. *Critical Reviews in Plant Sciences*, *14*(2), 111–148. https://doi.org/10.1080/07352689509701924

Fixen, P., Brentrup, F., Bruulsema, T., F, G., Norton, R., & Zingore, S., 2012. Chapter 1. Nutrient/fertilizer use efficiency (N/FUE): Measurement, Current Situation and Trends. *Draft*, *3400*.

Gonçalves, A. Z., & Mercier, H., 2021. Transcriptomic and Biochemical Analysis Reveal Integrative Pathways Between Carbon and Nitrogen Metabolism in Guzmania monostachia (Bromeliaceae) Under Drought. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.715289

Goncharuk, E. A., & Zagoskina, N. V., 2023. Heavy Metals, Their Phytotoxicity, and the Role of Phenolic Antioxidants in Plant Stress Responses with Focus on Cadmium: Review. *Molecules*, *28*(9), 3921. https://doi.org/10.3390/molecules28093921

González, L. K., Rugeles, L. N., & Magnitskiy, S., 2018. Effect of different sources of nitrogen on the vegetative growth of andean blueberry (Vaccinium meridionale Swartz). *Agronomia Colombiana*, *36*(1). https://doi.org/10.15446/agron.colomb.v36n1.69304

Gruda, N. S., 2022. Advances in Soilless Culture and Growing Media in Today's Horticulture—An Editorial. In *Agronomy* (Vol. 12, Issue 11). https://doi.org/10.3390/agronomy12112773

He, L., Ding, X., Jin, H., Zhang, H., Cui, J., Chu, J., Li, R., Zhou, Q., & Yu, J., 2022. Comparison of rockwool and coir for greenhouse cucumber production: chemical element, plant growth, and fruit quality. *Heliyon*, *8*(10), e10930. https://doi.org/10.1016/j.heliyon.2022.e10930

Heller, C. R., & Nunez, G. H., 2022. Preplant Fertilization Increases Substrate Microbial Respiration but Does Not Affect Southern Highbush Blueberry Establishment in a Coconut Coir-based Substrate. *HortScience*, *57*(1). https://doi.org/10.21273/HORTSCI16220-21

Hui, Y., Wang, J., Jiang, T., Ma, T., & Wang, R., 2021. Effect of nitrogen regulation on berry quality and flavonoids during veraison stage. *Food Science & Nutrition*, *9*(10), 5448–5456. https://doi.org/10.1002/fsn3.2503

Ibrahim, M. H., Jaafar, H. Z. E., Rahmat, A., & Rahman, Z. A., 2011. Effects of Nitrogen Fertilization on Synthesis of Primary and Secondary Metabolites in Three Varieties of Kacip Fatimah (Labisia Pumila Blume). *International Journal of Molecular Sciences*, *12*(8), 5238–5254. https://doi.org/10.3390/ijms12085238

International Blueberry Organization (IBO), 2023. Global State of the Blueberry Industry Report 2023*. IBO*. https://www.internationalblueberry.org [Accessed 11 July 2025]

Jasminka, M., Vuk, M., Dragan, R., Slavica, S., & Dragišić Maksimović, J., 2025. How does fertilizer management strategy in soilless blueberry cultivation change the phytochemical profile and phenoloxidases activity during fruit ripening? *Journal of the Science of Food and Agriculture*, *105*(5), 3084–3096. https://doi.org/10.1002/jsfa.14072

Jimoh, M. O., Afolayan, A. J., & Lewu, F. B., 2019. Antioxidant and phytochemical activities of Amaranthus caudatus L. harvested from different soils at various growth stages. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-49276-w

Johnson, S. A., Figueroa, A., Navaei, N., Wong, A., Kalfon, R., Ormsbee, L. T., Feresin, R. G., Elam, M. L., Hooshmand, S., Payton, M. E., & Arjmandi, B. H., 2015. Daily Blueberry Consumption Improves Blood Pressure and Arterial Stiffness in Postmenopausal Women with Pre- and Stage 1-Hypertension: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Journal of the Academy of Nutrition and Dietetics*, *115*(3), 369–377. https://doi.org/10.1016/j.jand.2014.11.001

Kallenbach, C. M., Frey, S. D., & Grandy, A. S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms13630

Kingston, P. H., Scagel, C. F., Bryla, D. R., & Strik, B., 2017. Suitability of Sphagnum Moss, Coir, and Douglas Fir Bark as Soilless Substrates for Container Production of Highbush Blueberry. *HortScience*, *52*(12), 1692–1699. https://doi.org/10.21273/HORTSCI12374-17

Korcak, R. F., 1988. Nutrition of Blueberry and Other Calcifuges. In *Horticultural Reviews*. https://doi.org/10.1002/9781118060834.ch6

Kozos, K., & Ochmian, I., 2016. The influence of fertilisation urea phosphate on growth and yielding bush of two highbush blueberry cultivars (V. Corymbosum). *Folia Pomeranae Universitatis Technologiae Stetinensis Agricultura, Alimentaria, Piscaria et Zootechnica*, *325*(37), 29–38. https://doi.org/10.21005/AAPZ2016.37.1.04

Latifah, O., Ahmed, O. H., & Majid, N. M. A., 2017. Enhancing nitrogen availability from urea using clinoptilolite zeolite. *Geoderma*, *306*, 152–159. https://doi.org/10.1016/j.geoderma.2017.07.012

Leal-Ayala, O. G., Sandoval-Villa, M., Trejo-Téllez, L. I., Sandoval-Rangel, A., de La Fuente, M. C., & Benavides-Mendoza, A., 2021. Nitrogen form and root division modifies the nutrimental and biomolecules concentration in blueberry (Vaccinium corymbosum L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *49*(1). https://doi.org/10.15835/nbha49111998

Lee, S. K., & Kader, A. A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207–220. https://doi.org/10.1016/S0925-5214(00)00133-2

Lemaire, F., 1995. Physical, chemical and biological properties of growing medium. *Acta Horticulturae*, *396*, 273–284. https://doi.org/10.17660/ActaHortic.1995.396.33

Lu, Y., Luo, F., Yang, M., Li, X., & Lian, X., 2011. Suppression of glutamate synthase genes significantly affects carbon and nitrogen metabolism in rice (Oryza sativa L.). *Science China Life Sciences*, *54*(7), 651–663. https://doi.org/10.1007/s11427-011-4191-9

Malladi, A. and Cabrera, M., 2020. *Determining uptake of nitrate vs ammonium nitrogen in blueberry*. 2020-R-13 Progress Report. University of Georgia, Athens, GA.

Mfeka, N., Tanga, M., Mulidzi, A.R., & Lewu, F.B., 2023 'Water Requirement of Blueberry Cultivated in Different Growth Media', *39th Johannesburg International Conference on Chemical, Biological and Environmental Engineering (JCBEE-23)*, 16-17 November, Johannesburg, South Africa. https://doi.org/10.17758/iicbe5.c1123050

Narvekar, A. S., & Tharayil, N., 2021. Nitrogen Fertilization Influences the Quantity, Composition, and Tissue Association of Foliar Phenolics in Strawberries. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.613839

Neumann, G., & Römheld, V., 2012. Rhizosphere Chemistry in Relation to Plant Nutrition. In *Marschner's Mineral Nutrition of Higher Plants* (pp. 347–368). Elsevier. https://doi.org/10.1016/B978-0-12-384905-2.00014-5

Ortiz-Delvasto, N., Garcia-Ibañez, P., Olmos-Ruiz, R., Bárzana, G., & Carvajal, M., 2023. Substrate composition affects growth and physiological parameters of blueberry. *Scientia Horticulturae*, *308*, 111528. https://doi.org/10.1016/j.scienta.2022.111528

Philippot, L., Raaijmakers, J. M., Lemanceau, P., & van der Putten, W. H., 2013. Going back to the roots: The microbial ecology of the rhizosphere. In *Nature Reviews Microbiology* (Vol. 11, Issue 11). https://doi.org/10.1038/nrmicro3109

Politycka, B., Wójcik-Wojtkowiak, D., & Pudelski, T., 1985. Phenolic compounds as a cause of phytotoxicity in greenhouse substrates repeatedly used in cucumber growing. *Acta Horticulturae*, *156*, 89–94. https://doi.org/10.17660/ActaHortic.1985.156.13

Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., & Garde-Cerdán, T., 2015. Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. *Food Chemistry*, *180*, 171–180. https://doi.org/10.1016/j.foodchem.2015.02.042

Rauf, A., Imran, M., Abu-Izneid, T., Iahtisham-Ul-Haq, Patel, S., Pan, X., Naz, S., Sanches Silva, A., Saeed, F., & Rasul Suleria, H. A., 2019. Proanthocyanidins: A comprehensive review. In *Biomedicine and Pharmacotherapy* (Vol. 116). https://doi.org/10.1016/j.biopha.2019.108999

Retamales, J. B., & Hancock, J. F., 2012. Blueberries. In *Blueberries*. https://doi.org/10.2307/j.ctvthhbxx.20

Ross, P. R., Paramanandham, J., Thenmozhi, P., Abbiramy, K. S., & Muthulingam, M., 2012. Determination of physico-chemical properties of coir pith in relation to particle size suitable for potting medium. *International Journal of Research in Environmental Science and Technology*, *2*(2).

Rydenheim, L., 2007. Effects of zeolites on the growth of cucumber and tomato seedlings by Effects of zeolites on the growth of cucumber and tomato seedlings. *Department of Horticulture Swedish University of Agriculture (SLU)*, *4*.

Salvatierra, A., Pimentel, P., Moya-Leon, M. A., Caligari, P. D. S., & Herrera, R., 2010. Comparison of transcriptional profiles of flavonoid genes and anthocyanin contents during fruit development of two botanical forms of Fragaria chiloensis ssp. chiloensis. *Phytochemistry*, *71*(16), 1839–1847. https://doi.org/10.1016/j.phytochem.2010.08.005

Sangeetha, C., & Baskar, P., 2016. Zeolite and its potential uses in agriculture: A critical review. *Agricultural Reviews*, *of*. https://doi.org/10.18805/ar.v0iof.9627

Savvas, D., Passam, H. C., Olympios, C., Nasi, E., Moustaka, E., Mantzos, N., & Barouchas, P., 2006. Effects of ammonium nitrogen on lettuce grown on pumice in a closed hydroponic system. *HortScience*, *41*(7). https://doi.org/10.21273/hortsci.41.7.1667

Scagel, C. F., 2003. Growth and Nutrient Use of Ericaceous Plants Grown in Media Amended with Sphagnum Moss Peat or Coir Dust. *HortScience*, *38*(1), 46–54. https://doi.org/10.21273/HORTSCI.38.1.46

Suh, D. H., Kim, Y. X., Jung, E. S., Lee, S., Park, J., Lee, C. H., & Sung, J., 2020. Characterization of Metabolic Changes under Low Mineral Supply (N, K, or Mg) and Supplemental LED Lighting (Red, Blue, or Red–Blue Combination) in Perilla frutescens Using a Metabolomics Approach. *Molecules*, *25*(20), 4714. https://doi.org/10.3390/molecules25204714

Sun, Y., Li, M., Mitra, S., Hafiz Muhammad, R., Debnath, B., Lu, X., Jian, H., & Qiu, D., 2018. Comparative Phytochemical Profiles and Antioxidant Enzyme Activity Analyses of the Southern Highbush Blueberry (Vaccinium corymbosum) at Different Developmental Stages. *Molecules*, *23*(9), 2209. https://doi.org/10.3390/molecules23092209

Vo, G. T., Liu, Z., Chou, O., Zhong, B., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R., 2022. Screening of phenolic compounds in Australian-grown grapes and their potential antioxidant activities. *Food Bioscience*, *47*, 101644. https://doi.org/10.1016/j.fbio.2022.101644

Wang, Y., Fong, S. K., Singh, A. P., Vorsa, N., & Johnson-Cicalese, J., 2019. Variation of Anthocyanins, Proanthocyanidins, Flavonols, and Organic Acids in Cultivated and Wild Diploid Blueberry Species. *HortScience*, *54*(3), 576–585. https://doi.org/10.21273/HORTSCI13491-18

Yang, H., Han, T., Wu, Y., Lyu, L., Wu, W., & Li, W., 2023. Quality analysis and metabolomic profiling of the effects of exogenous abscisic acid on rabbiteye blueberry. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1224245

Yang, H., Wu, Y., Zhang, C., Lyu, L., Wu, W., Huang, Z., & Li, W., 2025. Growth and Physiological Traits of Blueberry Seedlings in Response to Different Nitrogen Forms. *Plants*, *14*(10), 1444. https://doi.org/10.3390/plants14101444

Zhang, J., Lv, J., Xie, J., Gan, Y., Coulter, J. A., Yu, J., Li, J., Wang, J., & Zhang, X., 2020. Nitrogen Source Affects the Composition of Metabolites in Pepper (Capsicum annuum L.) and Regulates the Synthesis of Capsaicinoids through the GOGAT–GS Pathway. *Foods*, *9*(2), 150. https://doi.org/10.3390/foods9020150

Zhang, X., Li, S., An, X., Song, Z., Zhu, Y., Tan, Y., Guo, X., & Wang, D., 2023. Effects of nitrogen, phosphorus and potassium formula fertilization on the yield and berry quality of blueberry. *PLoS ONE*, *18*(3 March). https://doi.org/10.1371/journal.pone.0283137

CHAPTER FIVE

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study investigated the effect of different nitrogen (N) sources and growth media on the water use, growth, yield, phenolic constituents and mineral composition of 'Legacy' blueberry cultivated under shade net. This study was conducted as part of a bigger research project focused on water conservation in blueberry production, where preliminary findings showed 80% coir + 20% zeolite combination growth media improve water retention. Building on this, the current research examined the integration of different N sources into these water conserving growth media to optimize blueberry productivity while reducing inputs such as water and fertilizer. The supply of ammonium sulphate (T_2), calcium nitrate (T_4) and urea (T_6) in coir: zeolite based media demonstrated the complexity of N uptake and assimilation in blueberry cultivation, highlighting that N absorption and efficiency depend strongly on the growth medium, pH, plant maturity and environmental conditions.

The interaction between N sources and growth media showed that the addition of zeolite to coir had varying effects depending on the N source applied. Across the six treatments studied, 100% coir when supplied with ammonium sulphate (T_1) had the highest growth parameters and yield attributes during both cultivation seasons, validating its suitability as the standard for blueberry cultivation in the Western Cape. Urea applied in 100% coir (T_5) also increased plant height, indicating the effective use of NH_4^+ derived from urea hydrolysis. In contrast, calcium nitrate treatments (T_3 and T_4) increased stem diameter but reduced lateral branching and accumulation of chlorophyll content index (CCI), suggesting that NO_3^- supported structural growth over photosynthetic capacity, resulting to lower overall yield.

The growth medium notably influenced plant growth. Plants grown in 100% coir outperformed those in 80% coir: 20% zeolite combination according to the plant height, branching and CCI regardless of the N source treatment used. The addition of zeolite into coir improved water holding capacity, number of leaves and stem diameter; however, it reduced plant height and number of branches, likely due to increased bulk density and nutrient retention beyond the shallow root zone of blueberry plants. Although zeolite reduced water use, the fibrous roots of blueberry limited their ability to uptake the retained nutrients, contracting the expected benefits of zeolite. Moreover, the zeolite granular structure was noticed to gradually sink to the bottom of the bags used for cultivation, placing retained nutrients beyond the reach of the active upper root zone.

Yield patterns during the two cultivation seasons reflected plant establishment. In the first year (2023) of cultivation, young plants prioritised vegetative growth over fruiting, causing lower yield. By the second year (2024), established root systems allocated higher N use efficiency (NUE) and improved fruit diameter and weight, which peaked mid-harvest season and dropped towards the end of the season, consistent with established blueberry growth patterns.

Blueberry phenolic constituents, as stress response metabolites, their accumulation varied with N sources, growth media and plant maturity. The findings indicated that moderate nutrient stress, attained through careful selection of N source and growth medium, may improve phenolic compounds synthesis without severely compromising yield. Furthermore, the literature review chapter concluded that a combination of NH_4^+ plus NO_3^- or NH_4^+ alone, consistently promoted superior growth and fruit quality compared to NO_3^- alone, predominantly in acidic medium, which is favourable for blueberry cultivation.

5.2 Recommendations

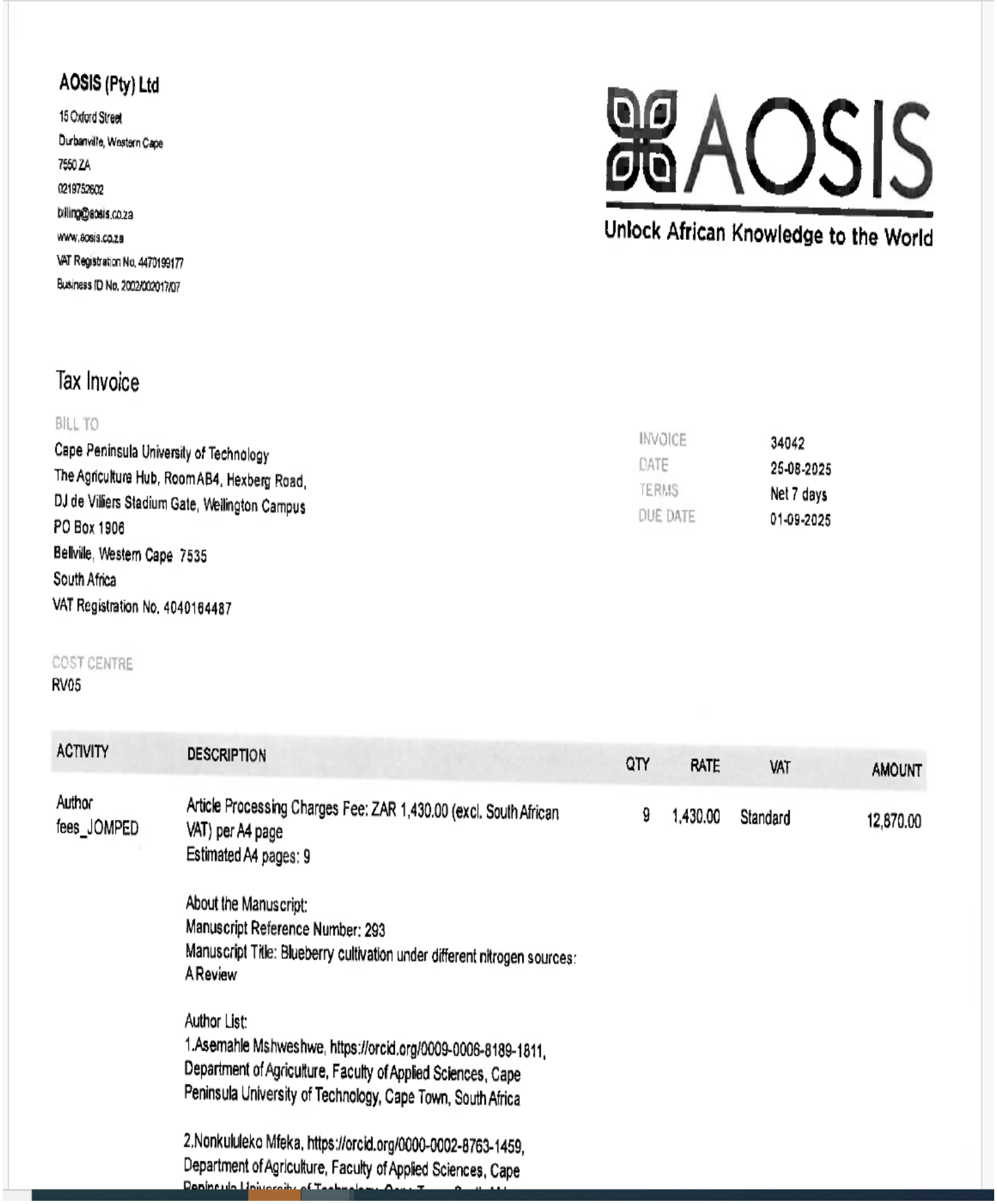
Although this study provides valuable insight into N sources and growth media dynamics in blueberry cultivation, various research gaps remain unexplored. Zeolite can improve water holding capacity, however, further research should be done to investigate the quantities of coir: zeolite for optimal production, as the 20% granular zeolite seems to sink to the bottom of the pots used over time, limiting nutrient availability to the shallow fibrous-structured blueberry root system. Moreover, NH_4^+ based fertilisers such as ammonium sulphate or urea should be prioritised for blueberry cultivation as they acidify the soil to favour blueberry preference, however, the combination of both NH_4^+: NO_3^- with mostly a higher ratio of NH_4^+ has been proven to achieve balanced growth and fruit quality.

Incorporation of different N sources into water conserving growth media can reduce resource inputs, such as water and fertiliser without severely compromising yield. The slight yield reduction observed with T_2 (80% coir: 20% zeolite + ammonium sulphate) may be compensated by the reduction in water costs during cultivation compared to T_1, highlighting potential economic advantages.

Phenolic constituents accumulate naturally with plant maturity, regardless of the treatment applied. Future management practices should aim to balance stress induced phenolic synthesis with yield to optimise fruit quality with nutritional value. Furthermore, future studies should explore the suitability of urea in blueberry container production, as the application of ammonium sulphate may have long term negative impacts on soil toxicity due to acidification.

Further studies on how various blueberry cultivars respond to NH_4^+, NO_3^- and different ratios of NH_4^+: NO_3^- would be beneficial. To conclude, the strong influence of year to year variation on the treatment’s dynamics highlight the need for further research on the plant age and environmental interactions. Understanding how factors such as temperature and light affect the accumulation of secondary metabolites, growth and yield would be crucial for optimising cultivation management strategies in different growth media under container production.

ANNEXURE A



AOSIS (Pty) Ltd
15 Oxford Street
Durbanville, Western Cape
7550 ZA
0219752602
billing@aosis.co.za
www.aosis.co.za
VAT Registration No. 4470199177
Business ID No. 2002/002017/07

AOSIS
Unlock African Knowledge to the World

Tax Invoice

BILL TO
Cape Peninsula University of Technology
The Agriculture Hub, Room AB4, Hexberg Road,
DJ de Villiers Stadium Gate, Wellington Campus
PO Box 1906
Bellville, Western Cape 7535
South Africa
VAT Registration No. 4040164487

INVOICE	34042
DATE	25-08-2025
TERMS	Net 7 days
DUE DATE	01-09-2025

COST CENTRE
RV05

ACTIVITY	DESCRIPTION	QTY	RATE	VAT	AMOUNT
Author fees_JOMPED	Article Processing Charges Fee: ZAR 1,430.00 (excl. South African VAT) per A4 page Estimated A4 pages: 9 About the Manuscript: Manuscript Reference Number: 293 Manuscript Title: Blueberry cultivation under different nitrogen sources: A Review Author List: 1.Asemahle Mshweshwe, https://orcid.org/0009-0006-8189-1811, Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa 2.Nonkululeko Mfeka, https://orcid.org/0000-0002-8763-1459, Department of Agriculture, Faculty of Applied Sciences, Cape	9	1,430.00	Standard	12,870.00