INFLUENCE OF VESICULAR ARBUSCULAR MYCORRHIZA ON PHYTOCHEMICALS, PRODUCTIVITY, AND SALINE STRESS ALLEVIATION OF *MORINGA OLEIFERA*

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DECLARATION

I, Thamia Laka, declare that the mini dissertation submitted to the University of Mpumalanga for the degree of Master of Science in Agriculture has not been previously submitted for any degree at this or any other institution. This dissertation in design and execution is my work and does not contain other persons' graphs, pictures or data unless duly acknowledged.

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DEDICATION

I dedicate this work to my supportive parents, Khomotso Laka and Koena Laka, and my lovely siblings, Goodness and Thato Laka. Their love, encouragement, and belief in me have been a constant source of strength throughout my academic journey. Not forgetting Atlegang Laka, whose presence gave me courage to persevere and inspired to become stronger and better.

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ABSTRACT

Moringa oleifera Lam, valued for its medicinal properties, faces productivity challenges due to soil salinity caused by global warming. The objectives of the study were to: (1) determine the effect of Vesicular Arbuscular Mycorrhizal fungi on the growth of *M. oleifera* and saline stress alleviation (2) evaluating whether biosynthesis and accumulation of phytochemicals of *M. oleifera* will be influenced using VAM fungi in reducing salinity. The two objectives were separately undertaken at the University of Mpumalanga using four salinity levels (0, 0.25, 0.5, and 0.75 dS m^{-1}) of combined ratios of NaCl and $CaCl_2$ (3:1) and four VAM (0, 10, 20, and 30 g) treatments arranged in a factorial experiment using a randomized complete block design under microplot and shadenet conditions in 2024. Results showed significant impacts of salinity ($p \leq 0.05$) on plant height, branches, chlorophyll, and stem diameter, with relative impacts (RI) ranging from 2.44% to 53.83%. VAM and salinity interaction notably improved plant height and chlorophyll concentration, enhancing soil organic matter, pH, and electrical conductivity.

The results of the study indicate that the interaction of VAM and salinity treatments did have a significant effect ($p \leq 0.05$) on TFC and TPC in both experiments. Relative to the control the interaction of VAM and salinity treatments had increased the TFC in Experiment 1 with RI of 4.40 to 5.50 mg QE/g and in Experiment 2 with a RI of 5.00 to 6.10 mg QE/g. In both Experiment 1 and 2, relative to the control TPC was enhanced by the interaction of VAM and salinity treatments with RI of 21.00 to 39.68 mg QE/g and 15.00 to 41.71 mg QE/g, respectively. The findings underscore the importance of VAM in improving *Moringa oleifera*'s productivity in saline environments.

Keywords: *Moringa oleifera Lam.,* global warming, saline stress, productivity, Vesicular Arbuscular Mycorrhiza.

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LIST OF ABBREVIATIONS

VAM - Vesicular Arbuscular Mycorrhiza

VAMF - Vesicular Arbuscular Mycorrhizal Fungi

AMF - Arbuscular Mycorrhizal Fungi

NaCl - Sodium Chloride

$CaCl_2$ - Calcium Chloride

CO_2 - Carbon dioxide

P - Phosphorus

OH - Hydroxide

NH_2 - Nitrate

N - Nitrogen

K^+ - Potassium Ion

Na^+ - Sodium Ion

Cl^- - Chlorine Ion

AD - Alzheimer’s disease

WHO - World Health Organization

FAO - Food and Agriculture Organization

RDF - Recommended Dose of Fertiliser

S.Em+ - Standard Error of Mean Plus

CD - Critical Difference

TLC - Thin Layer Chromatography

HPLC - High Performance Liquid Chromatography

MS - Mass Spectrometry

NMR - Nuclear Magnetic Resonance

FTIR - Fourier Transform Infrared Spectrometry

Pas - Pyrrolizidine alkaloids

mg- milligram

g - gram

EC - Electrical Conductivity

RI - Relative Impact

TTV - Total Treatment Variation

CC - Chlorophyll Content

NB - Number of Branches

PH - Plant Height

Reps - Replications

SD - Stem Diameter

MS - Meas Separation

P - Probability

TFC - Total Flavonoid Content

TPC - Total Phenolic Content

GAE - Gallic Acid Equivalent

dS m^{-1} - desi Siemens per meter

QE - Quercetin Equivalent

DAFF- Department of Agriculture, Forestry and Fisheries

RESEARCH OUTPUT

1. **Laka, T., Masenya, T.A., Mabila, S.W., Mabuela, A. and Khanyile, N. 2024**. Exploring the impact of Vesicular Arbuscular Mycorrhiza on phytochemical profiles, productivity enhancement and saline stress alleviation in *Moringa oleifera*. *Reviews in Agricultural Science,* 12: 213-236.

CHAPTER 1

BACKGROUND

1.1 Introduction

Moringa oleifera Lam., holds medicinal significance due to its utilisation in the treatment of chronic ailments, such as cancer (Rani *et al.,* 2018; Ashfaq *et al.,* 2012). This has necessitated heightened cultivation and international commerce (Rani *et al.,* 2018; Ashfaq *et al.,* 2012). According to Rani *et al.* (2018), the plant has historically been employed for its therapeutic properties in the treatment of diabetes, colds, wounds, and water purification. The plant exhibits a notable nutritional composition, as evidenced by the presence of calcium, vitamin C, potassium, protein, beta-carotene, and organic antioxidants in its leaves (Gokila *et al.,* 2017). Moringa plant has been praised by anti-hunger organisations due to its ability to be consumed fresh without undergoing long-term preservation, without compromising its nutritional value (McGee, 2004). According to Farooq *et al.* (2022) and Zulfiqar *et al.* (2020), the human population will promptly increase, with estimates suggesting that it would reach 9.6 billion by the year 2050. The rising prevalence of chronic diseases and the emergence of new illnesses, coupled with the escalating cost of western medications, have created a growing demand for alternative treatments, with medicinal plants including moringa emerging as a promising option. The growing utilisation of traditional medicinal products as a means of identifying new drugs through their secondary metabolites has had a substantial impact on primary healthcare (Zulfiqar *et al.,* 2020).

Moringa, like most medicinal plants, naturally produce phytochemicals as secondary metabolites, which play a crucial role in their ability to adapt to environmental stressors such as drought, heat, inadequate drainage, low-fertility soil, and saline. Additionally, phytochemicals have been found to have diverse pharmacological applications, including anti-fertility and anti-diabetic properties (Mishra *et al.,* 2011). Both biotic and abiotic environmental conditions have impact on the production and content of secondary metabolites (Pavarini *et al.,* 2012). Various genetic, ontogenetic, morphogenetic, and environmental factors influence the generation and buildup of secondary metabolites (Carr *et al.*, 2016).

According to Mishra *et al*. (2011), moringa is rich in a variety of phytochemicals, including flavonoids, tannins, glycosides, saponins, alkaloids, and phenols. These naturally occurring compounds make the plant highly beneficial to human health and help it adapt to its environment. Phytochemicals, known for their antioxidant, antibacterial, and anti-inflammatory properties, have attracted significant attention, particularly in the study of medicinal plants like moringa (Mohammed & Manan, 2015).

The increased cultivation of important medicinal plants like moringa are also affected by environmental stressors like drought and salinity at high concentrations. The phenomenon of global warming leading to climate change has resulted in temperature and rainfall changes, as well as an expected increase in CO_2 levels. These changes including the poor farming practices such as poor-quality irrigation water have caused a rise in saline stress, which has exerted detrimental impacts on agriculture worldwide (Corwin, 2021). Consequently, it is anticipated that agricultural productivity will undergo adverse transformations. According to Farooq *et al.* (2022), the scarcity of land areas suitable for cultivating crucial crops in regions with low saline concentrations is a significant concern. Inadequate drainage, insufficient precipitation, ineffective irrigation techniques, and high transpiration rates in irrigated regions all contribute to the problem of soil salinity, which is a common concern (Farooq *et al.*, 2022). Salinity has been estimated to harm approximately six percent of the earth's landmass (Cao *et al.*, 2020). Leading to a loss of over 20% of agricultural output annually (Eswar *et al.*,2021). Additionally, it is anticipated that an additional 0.3–1.5 million hectares of cropland will be added each year. According to Sarri *et al.* (2021), the presence of elevated salt concentrations in soils hinders the growth and developmental progression of crops, thereby impacting crop productivity. Furthermore, the activities of enzymes are diminished as a result of the osmotic and ionic constraints that affect significant biochemical and physiological events (Ahmad, 2010). According to El-Massry *et al.* (2013), the moringa plant has a moderate level of tolerance to salt, which can be attributed to the high antioxidant activity rate present in its leaves. Despite the moderate tolerance of moringa to salt concentration, the current rise in global salt variability is expected to have an impact on crop output including moringa (Islam *et al.,* 2023).

It is crucial to promptly adopt climate-smart agriculture practices, which involve implementing efficient management techniques and innovations to adapt selected crops to expected extremes, such as salt stress (Carr *et al.,* 2016). The use of Vesicular Arbuscular Mycorrhiza (VAM) fungi is a good example of this approach because it changes the roots to make it easier for nutrients to get to the plant and be absorbed (Marwa and Mohamed, 2021). Empirical evidence suggests that VAM fungi play a crucial role in fostering advantageous symbiotic relationships with a diverse array of plants (Marwa and Mohamed, 2021). Hence facilitating plant growth across many ecological contexts. Furthermore, studies have demonstrated that VAM fungi can enhance plant growth and increase yields in various saline soils (Masenya *et al.,* 2023; Hildebrandt *et al.,* 2007).

1.2 Justification of study

The implementation of environmentally sustainable techniques to enhance crop yield considering the worldwide scarcity of cultivable land, agricultural inefficiencies, and excessive soil salinity is crucial in addressing the challenges posed by climate change (Masenya *et al.,* 2023). Extensive documentations exist regarding the adverse effects of salt on plant development and growth (Masenya *et al.,* 2023; Corwin, 2021). However, there is a dearth of evidence pertaining to the enhancement of growth and development in saline soil (Corwin, 2021). Moringa plant possesses a diverse array of phytochemicals, rendering it valuable in the field of medicine and possessing significant economic worth. The expanded cultivation of this plant is expected to yield benefits for both the formal and informal sectors of the economy (Masenya, 2022). Numerous literatures indicate that VAM fungi has resulted in enhanced agricultural productivity in underdeveloped regions where saline concentrations are high (Masenya *et al.,* 2023; Hildebrandt *et al.,* 2007). When crop plants are exposed to VAM fungi symbiosis, they grow faster and produce more because they are better able to absorb nutrients, handle drought and saltiness, and fight off diseases (Pal and Pandey, 2014). The study by Masenya *et al.* (2023) has demonstrated that VAM fungi can enhance plant growth and increase yields in various saline soils thereby constituting as a potential biofertilizer. The efficacy and success of use of VAM fungi as a bio-fertilizer will enable moringa growers residing on impoverished salt-affected soils throughout South Africa to enhance their productivity, thus, contributing to the sustainability of their livelihoods.

1.3 Problem statement

Climate change is expected to have various detrimental impacts on the environment, including alterations in soil properties, depletion of organic matter, leaching of soil nutrients, salinisation, alterations in insect patterns, changes in weather patterns, fluctuations in temperature, and erosion (Corwin, 2021). Soil saline-alkaline stress is a prominent environmental issue that contributes to substantial reductions in agricultural productivity, terrestrial biodiversity, and ecological well-being (Cao *et al.,* 2020). According to Imadi *et al.* (2016), moringa exhibit restricted expression of their inherent genetic capacity for growth, development, and yield when exposed to elevated levels of salt in the soil. Consequently, this leads to a decrease in their commercial and economic worth. Furthermore, soil salinity causes the land it affects to change from a fruitful state to an unproductive state, which lowers economic productivity (Imadi *et al.*, 2016). A significant portion of the global land area, over 800 million hectares, has experienced adverse impacts due to salinity or alkalinity (Cao *et al.,* 2020). According to the South African SOTER database, approximately 776,131 hectares of land in South Africa are classified as severely saline, whereas around 54,000 hectares of cultivated land are classified as extremely waterlogged and alkaline (Eswar *et al.,* 2021). Saline stress has significantly reduced the cultivation of most crops including moringa by adversely affecting its growth, yield, and overall health. This stress has led to a restriction in suitable land for moringa cultivation, particularly in arid and semi-arid regions where soil salinity is naturally high or exacerbated by irrigation practices and climate change. The effects of saline stress not only decrease productivity but potentially also render moringa cultivation less economically viable in salt-affected areas. Therefore, it is imperative to identify novel alternative approaches to enhance its productivity in soil environments with elevated salt content, with the aim of ameliorating the negative impact of salt on the growth and productivity of moringa plant as a valuable medicinal plant.

1.4 Purpose of study

1.4.1 Aim

To establish protocols for enhanced growth of moringa and phytochemicals enrichment under saline stress in the context of climate-smart agriculture.

1.4.2 Objectives

i. To determine the effect of Vesicular Arbuscular Mycorrhiza (VAM) fungi on the growth and yield of *Moringa oleifera* and saline stress alleviation.

ii. To evaluate the possible effect of VAM fungi on the biosynthesis and accumulation of phytochemicals in *Moringa oleifera* in soil with varying salinity levels.

1.4.3 Hypotheses

i. Vesicular Arbuscular Mycorrhizal fungi will improve the growth and yield of *Moringa oleifera* and alleviate saline stress.

ii. Biosynthesis and accumulation of phytochemicals of *Moringa oleifera* will be enhanced using VAM fungi in reducing salinity.

1.5 Reliability, validity, and objectivity

Statistical analysis with a significance threshold of 5% evaluated the data's dependability. The data's validity was established by replicating the experimental treatments. The attainment of objectivity was accomplished through the comprehensive consideration of empirical evidence, thereby eliminating any elements of subjectivity from the analysis.

1.6 Bias

To mitigate bias, it was ensured that each experiment was conducted with sufficient replications and randomization to account for potential errors (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The rising prevalence of chronic diseases and the emergence of new illnesses, coupled with the escalating cost of Western medications, have created a growing demand for alternative treatments, with medicinal plants including moringa emerging as a promising option. These plants offer a more affordable and accessible solution, particularly in low-income communities, while also aligning with traditional knowledge and cultural practices that have been relied upon for centuries. Additionally, medicinal plants are often associated with fewer side effects when used appropriately and contribute to sustainability by promoting biodiversity and eco-friendly practices. Nevertheless, arable land is scanty, while the available land has high ionic concentrations of NaCl and $CaCl_2$ salts. Saline stress is among the factors that limit production throughout the world. Hence, the exploration of strategies to augment the cultivation of medicinal plants in saline soil while simultaneously safeguarding the integrity and richness of secondary metabolites is of utmost importance. The knowledge acquired will contribute to the enhancement of moringa production in soils with low fertility, ensuring a plentiful supply of moringa therapeutic products to meet the rising demand. This, in turn, has a direct positive influence on the economy which also indirectly influence food security.

1.8 Structure of the dissertation

Chapter 1 provides a detailed background, problem statement, justification, aims and Objectives, while Chapter 2 presents a comprehensive evaluation of the existing literature related to the research subject. Chapters 3 and 4 focus on the attainment of objectives 1 and 2, respectively. Chapter 5 consolidates and combines the findings from all the chapters to provide a comprehensive overview of their significance and offer recommendations for future research. The presentation of conclusions follows this. The University of Mpumalanga Senate approved the utilisation of the Harvard referencing style for both in-text citations and reference lists.

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CHAPTER 2

LITERATURE REVIEW

2.1.1 Introduction

Moringa oleifera Lam., a member of the Moringaceaea family, is widely recognised as the most extensively utilised plant within this botanical group (Stohs and Hartman, 2015). The plant is indigenous to Asia and has been cultivated and acclimated to Africa. It has ability to thrive in tropical and subtropical regions (Gopalakrishnan *et al.,* 2016). According to Oyeyinka and Oyeyinka (2018), the maturation period of moringa typically spans approximately four to five months. Singh *et al.* (2018) reported that in nations characterized by a high prevalence of malnutrition, there is a notable increase in the cultivation of moringa. The value of the moringa plant has been recognised by the World Health Organization (WHO) as a feasible substitute for imported dietary supplements in addressing malnutrition in developing nations (Mushtaq *et al.,* 2021). The introduction of moringa as a farmed crop in rural populations, specifically in Limpopo Province, South Africa, occurred in 2006 (Lekgau, 2011). Since that time, there has been an increase in the production and utilisation of it in different agro-ecological zones across the country (Ekesa, 2017; Department of Agriculture, Forestry and Fisheries, 2013). In response to the increasing national interest in the moringa tree, several stakeholders, including the South African government, farmers, and higher education institutions, have initiated flagship initiatives focused on moringa (Mashamaite *et al.,* 2021). The reason for this is attributed to its capacity to enhance the nutritional status, financial resources, and overall well-being of marginalised populations (Mashamaite *et al.,* 2021).

Constant consumption of moringa in various forms, such as juice, fresh leaves, or dried leaf powder, can significantly aid in fulfilling nutritional requirements and reducing the likelihood of malnutrition among pregnant women, breastfeeding mothers, and young children (Mushtaq *et al.,* 2021). The extensive utilisation of various medicinal products derived from different crops has been associated with adverse effects in humans (Ekor, 2014). This would typically be the result of improper utilisation of medicinal products. Nevertheless, no such detrimental effects have been identified in relation to the consumption of moringa products (Laka *et al.,* 2024). The diverse nutritional benefits of moringa were also highlighted by Sujatha and Patel (2017), who reported that moringa possesses the potential to mitigate malnutrition due to its substantial protein and vitamin composition. This medicinal plant has also been praised by food organisations such as the Food and Agriculture Organisation (FAO) of the United Nations (Prajapati *et al.,* 2022), who highlighted moringa`s leaves' ability to be consumed in their fresh state without undergoing preservation for extended periods while retaining their nutritional value intact, underscoring the value of the crop. Singh *et al.* (2018) asserts that the discovery of moringa's beneficial properties dates back to several centuries; in recent times, the plant has gained recognition as a highly notable herbal supplement within the holistic health industry.

Moringa has gained recognition as a remarkable botanical specimen due to its noteworthy nutritional properties, therapeutic attributes, and capacity for environmental preservation (Yang *et al.,* 2006). The plant is regarded as a highly valuable and distinctive botanical specimen due to its multifaceted utility. Virtually every component of the plant as shown in Figure 2.1, including its flowers, seeds, leaves, stem, and roots, can be effectively employed in various domains such as nutrition, healthcare, and industrial applications (Tshabalala *et al.,* 2019). According to Rode *et al.* (2022), this particular plant possesses the capacity to enhance nutritional value, food security, and facilitate the advancement of rural communities. Throughout history, humans have extensively utilised all parts of this plant for a diverse range of domestic applications, including but not limited to domestic water purification, biogas production, animal feed, fertiliser, plant nutrient spraying, and green manure utilisation (Biswas and Sinha, 2021).

Moreover, the study conducted by Abdel-Latif *et al.* (2022) discovered that moringa antioxidants reduce blood pressure and promote fat burning processes. According to Alpern (2008), the plant has a reputation as a panacea because it has therapeutic benefits for more than 300 diseases. Padayachee and Baijnath (2020) stated that moringa has demonstrated efficacy as an anti-neoproliferative agent, effectively inhibiting the proliferation of cancer cells. The potential of moringa as a potent neuroprotectant is evident (Alpern, 2008). Furthermore, Padayachee and Baijnath (2020) demonstrated that the antioxidants found in moringa can potentially reduce the levels of reactive oxygen species, thereby providing protection to the brain. This problem is caused by cerebral ischemia, a condition characterised by the obstruction of blood flow to the brain, which has been identified as a contributing factor (Padayachee and Baijnath, 2020).

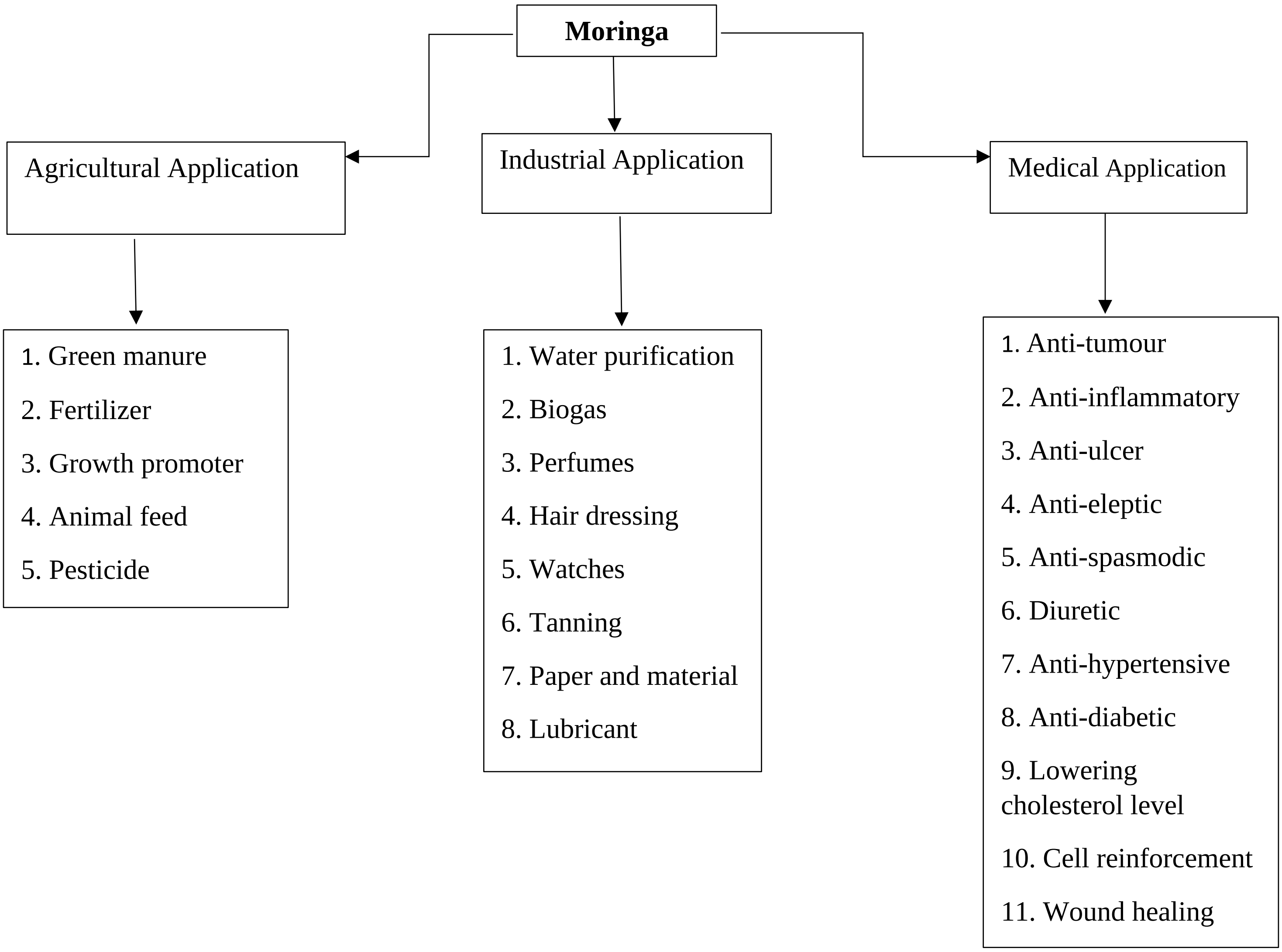


Figure 2.1: The application of moringa plant.

Table 2.1: Different traditional uses of different parts of *Moringa oleifera* plant

Moringa plant parts	**Traditional uses**
Leaves	Rashes, sores, skin infection, anaemia, cuts, sign of aging, diarrhoea, malaria, fever, swellings, boost immune system, cardiac stimulant, arthritis, elicit lactation
Bark	Aiding digestion, stomach pain, poor vision, ulcer, hypertension, joint pain, anaemia, diabetes
Oil	Acute rheumatism, gout
Flowers	Tumour, inflammation, hysteria, muscle disease, aphrodisiac substances
Seeds	Warts, antibacterial, inflammation, hypertension, diabetes, antitumor, immune booster
Roots	Toothache, anthelmintic, ant paralytic

The multipurpose use of moringa includes the extraction of oil from moringa seeds, commonly referred to as Ben oil (Sreeja *et al.,* 2021), as shown in Table 2.1. The oil exhibits a notable abundance of oleic acid, tocopherols, and sterols. According to Sreeja *et al.* (2021), it has been observed that the oil also exhibits resistance to oxidative rancidity. The oil possesses versatile applications, including its use as a culinary alternative to olive oil, as a as a fragrance, and as a lubricant (Sreeja *et al.,* 2021). The pods can absorb organic contaminants and pesticides (Sreeja *et al.,* 2021). According to Shija *et al.* (2019), moringa seeds hold exceptional coagulation properties, enabling them to effectively separate organic and mineral particles from various solutions. Seed extracts have antimicrobial properties that inhibit bacterial growth, thereby potentially serving as a preventive measure against waterborne diseases (Shija *et al.,* 2019). Given the abundance of plant seeds, they offer diverse applications in disease prevention and have the potential to enhance the quality of life in rural regions (Seifu and Teketay, 2020). The comprehensive significance of the entire plant to human existence necessitates its preservation in order to guarantee ample availability and provision. Okorie *et al.* (2019) have reported that the pharmacological properties of the plant have resulted in a rise in its cultivation and international trade. With the changing climate, agriculture faces increasing problems with extreme weather events leading to considerable crop yield losses (Laka *et al.,* 2024). Alternative strategies to mitigate or reduce the impact of abiotic stress factors are necessary to ensure stability in yields while ensuring sustainability for the environment (Okorie *et al.,* 2019). Salinity as a stress factor has a deleterious impact on crop production and continues to threaten the availability of arable land and sustained crop productivity worldwide (Okorie *et al.,* 2019). The use of alternative strategies, such as the use of Vesicular Arbuscular Mycorrhiza (VAM) fungi, in adapting plants to stress is yet to be extensively exploited on medicinal plants such as moringa. This study presents a comprehensive analysis of VAM fungi’s influence on growth and yield, saline stress alleviation, and the biosynthesis and accumulation of phytochemicals in *Moringa oleifera*.

2.2.2 The Vesicular Arbuscular Mycorrhiza (VAM) Fungi

Mycorrhiza is the relationship between plant roots and fungi that is mutually beneficial and not harmful to the plant (Parihar *et al.,* 2024; Marwa and Mohamed, 2021). This association is known to contribute significantly to soil fertility and the promotion of sustainable crop production. Mycorrhizal fungi dominate the vast assemblage of soil organisms, engaging in symbiotic relationships with plant roots and playing a crucial role in facilitating plant development (Hildebrandt *et al.,* 2007). Among the various relationships, VAM fungi stand out as the most prevalent and widely recognised symbiotic association within the taxonomic order Glomales (Xie *et al.*, 2020). In addition, VAM fungi contained in Table 2.2 below have a variety of spores from the rhizosphere of different host species, mainly belonging to *Glomus*, *Gigospora, Acaulospora, Entrophospora,* and *Sclerocystis* (Xie *et al.,* 2020).

Table 2.2: The most common type of VAM isolates (Xie *et al.*, 2020)

Genus	**Example of species**
Glomus	*G. fassiculatum, G. mosseae*
Gigaspora	*Gigaspora nigra*
Acaulospora	*A. scrobiculata*
Sclerocystis	*S. clavispora*
Endogone	*E. increseta*

Mycorrhizal symbiosis plays a crucial role in determining the productivity and biodiversity of the natural plant ecosystem. Consequently, the potential loss or disruption of this connection could potentially yield adverse consequences for the well-being, efficiency, or botanical ecosystem (Marwa and Mohamed, 2021). In various scenarios, a need may arise to manipulate or regulate the mycorrhizal symbiosis to enhance plant productivity, reinstate plant cover, or ameliorate plant health (Xie *et al.*, 2020). While it is possible for natural phenomena, such as volcanic activity and climate variations, to play a role in these situations, they are generally attributed to human activities (Xie *et al.*, 2020). In instances where subterranean soils are brought to the surface, such as through mining activities, tunnelling operations, or volcanic depositions, it is possible to identify soils lacking the appropriate mycorrhizal propagules (Marwa and Mohamed, 2021).

Arbuscular Mycorrhiza Fungi (AMF) have a long history, dating back millions of years, and is known to occur naturally in soil ecosystems (Lau *et al.*, 2019). In agreement with Lau *et al.* (2019), Hepper (2018) asserts that the occurrence of AM fungi can be traced back to the early stages of terrestrial plant evolution. The establishment of mycorrhizae occurs when plant roots release a chemical signal that promotes the colonisation of the root system by fungi (Lau *et al.*, 2019). For spores and/or propagules to effectively colonize the roots, a sufficient quantity of them must be present in the soil. Abiala *et al.* (2013) have observed that while AM fungi are present in certain plant species, other plants do not establish this symbiotic association. However, the underlying cause of this absence remains unknown. Hussein *et al.* (2020) postulate that this phenomenon may be attributed to the presence of toxic fungi within the cortical tissue of the roots, or the exudates released by the roots. Snoeck *et al.* (2010) suggested that the lack of mycorrhizal associations in some plants might be because they have high levels of salicylic acid, which stops mycorrhizal colonisation. This implies that plant species possessing a genetic predisposition for increased salicylic acid production have undergone evolutionary adaptations to avoid mycorrhizal symbiosis.

When crop plants are exposed to VAM fungi symbiosis, they grow faster and produce more because they are better able to absorb nutrients, handle drought and saltiness, and fight off diseases (Pal and Pandey, 2014). Furthermore, Abbasi *et al.* (2015) supports this assertion that mycorrhizae possess significant potential for practical implementation in agricultural settings. Their research demonstrated that mycorrhizae effectively enhanced the productivity of cereal, fruit, and vegetable crops while simultaneously mitigating the detrimental effects of nematodes and fungal infections. According to Lupatini *et al.* (2017), sustainable agricultural soil conditions may be more conducive to the growth and development of VAM fungi compared to conventional agricultural soil conditions. In intensive agriculture, mycorrhizosphere organisms may not be given enough attention because microbial populations are usually changed by practices like tilling the soil and using inorganic fertilisers, herbicides, and pesticides (Xia *et al.*, 2019). This suggests that while VAM fungi have the potential to increase crop growth and yield, its effectiveness depends on a variety of environmental factors, such as soil conditions with saline stress and soil conditions without saline stress. However, the extent to which salinity influences the efficacy of VAM fungi is not yet known (Laka *et al.,* 2024). Moreover, Itelima *et al.* (2018) assert that despite the numerous advantages of VAM fungi to the host plant, its commercialisation to tap into these beneficial effects has posed significant challenges. Therefore, further optimisation through empirical data is necessary to enhance its potential in agriculture.

Various agricultural techniques, including the implementation of resistant cultivars, the utilisation of chemical fungicides, the adoption of crop rotation practices, and the application of soil fumigation, have been employed to mitigate the prevalence of soil-borne diseases (Itelima *et al.*, 2018). Scientists are currently investigating the utilisation of beneficial microorganisms, such as antagonistic bacteria and fungi, to enhance plant resistance against diseases (Laka *et al.*, 2024). These bacterial organisms engage in competitive interactions for resources and habitat by producing antibiotics, parasitising diseases, or evolving resistance in host plants. The potential use of VAM fungi as a biofertilizer has been demonstrated (Abbasi *et al.*, 2015). This alternative has the capability to substitute the fertiliser requirements of trees in regions characterised by low fertility, thereby diminishing the necessity for current levels of synthetic fertilisers (Laka *et al.*, 2024). Therefore, employing VAM fungi as an agricultural method can effectively reduce soil-borne diseases. This is because VAM fungi can thrive without competitive interactions for resources and habitat with host plant, allowing them to maintain soil health and suppress pathogens independently (Hepper, 2018).

2.2 Root colonization by Arbuscular Mycorrhiza fungi

Root colonization refers to the growth and proliferation of microorganisms in, on, and around the root as it develops (Romano *et al.,* 2020). The location of these microorganisms often depends on the root's age. Colonization begins with the dispersal phase where microorganisms either move towards the root or encounter as it grows (Romano *et al.,* 2020). This is followed by a growth phase, where the microorganisms multiply (Romano *et al.,* 2020). As the root grows, cell division at the apical meristem produces root cap cells and mucilage, which are utilized by microorganisms that degrade cellulose and pectin (Romano *et al.,* 2020). Although the root cap and elongation zones are generally free from microbial colonization due to rapid root growth, the root hair zone is a hub of intense microbial activity (Romano *et al.,* 2020). Mycorrhizal fungi colonize the root cortex, forming structures like arbuscules and vesicles, or ensheathing short roots, depending on the type of mycorrhizae (Kaur and Kaur, 2018). As the root ages, its epidermal and cortical cells are invaded by bacteria and fungi (Kaur and Kaur, 2018). Indigenous microorganisms colonize roots as they grow, encountering organic matter and colloidal material in the soil (Kaur and Kaur, 2018). In contrast, introduced organisms must spread from a single inoculum source along the growing root, competing with already established native microorganisms (Mallon *et al.,* 2018).

Mycorrhizal fungi can colonize plant roots from three main sources of inoculum: spores, colonized root fragments, and vegetative hyphae, collectively known as propagules (Sobat, 2022). For colonization to occur, these propagules must be present in the substrate near actively growing roots of a compatible plant (Sobat, 2022). Root exudates emitted by growing root tips signal the fungi to initiate colonization and establish symbiosis. Once colonized, the process becomes self-sustaining as the mycelia grow with the plant's root system, producing additional spores and hyphae (Sobat, 2022). Versicular Arbuscular Mycorrhiza fungi propagules can be introduced into the substrate before or during planting, top-dressed and watered into a porous substrate, or applied as a dip or slurry during cutting, seeding, or transplanting (Panda, 2011). They can also be applied as a drench, to the rootball surface before transplanting or in the transplant hole and backfill soil (Panda, 2011).

Chao *et al.* (1986) found that fungi and bacteria, such as *Trichoderma harzianum*, *T. koningii*, *Gliocladium virens*, *Penicillium funicuiosum*, *Enterobacter cloacae*, *Pseudomonas fluorescens*, and *P. putida*, introduced on pea seeds, failed to colonize roots more than 3 cm below the seed unless water was applied to the soil. Similarly, *Bradyrhizobium japonicum* and *P. putida* were limited to 0-2.7 cm below the seed on soybean and snap bean roots without water infiltration (Madsen and Alexander, 1982). Parke *et al.* (1986) found that *P. fluorescens* strain 2-79 was initially carried on the root tip but was left behind after 4 days, suggesting that passive carriage by the root is not always an effective means of dispersal. In a screening of legumes, significant variation in mycorrhizal colonization was observed among different species (Ghosh and Dutta, 2016). For example, root colonization in *Vigna radiata* was 90% while in *V. mungo* it was 38%, and in *Vigna unguiculata* it was 80% (Ghosh and Dutta, 2016). This indicates that mycorrhizal colonization can vary significantly even among species within the same genus (Ghosh and Dutta, 2016).

The ability of VAM fungi to colonize the roots of *Moringa oleifera* is influenced by various factors, including environmental conditions, soil properties, and the specific fungal species present in the rhizosphere (Adedayo and Babalola, 2023). Vesicular Arbuscular Mycorrhiza fungi form a symbiotic relationship with moringa by penetrating the plant roots and establishing structures like arbuscules and vesicles within the root cortex (Adedayo and Babalola, 2023). This colonization enhances the plant's nutrient uptake, particularly phosphorus, and improves its overall stress tolerance (Adedayo and Babalola, 2023). Studies have shown that *Moringa oleifera* roots can be successfully colonized by a diverse range of VAM fungi, with genera like *Glomus* and *Acaulospora* being particularly prominent (Madawala, 2024; Aggangan *et al.*, 2015). The extent of colonization can vary significantly based on the site-specific conditions, such as soil pH, available nutrients, and other environmental factors (Aggangan *et al.*, 2015). For example, Dessai and Rodrigue (2020) investigated the VAM fungal species associated with moringa at four different sites in Goa, highlighting variations in root colonization and spore density. The study observed maximum VAM fungi root colonization at Majorda (65%) with the highest density of spores (919.67 spores per 100g of soil), while the minimum root colonization was at Shiroda (37.33%) with lowest spore density (119 spores per 100g of soil) (Dessai and Rodrigue, 2020). The study further emphasized that the highest number of VAM fungal propagules was observed at the Old Goa site (Majorda), where the available phosphorus (P) levels were low (Dessai and Rodrigue, 2020). This low phosphorus environment likely encouraged the colonization of VAM fungi on moringa roots, as the fungi assist the plant in acquiring sufficient P under such conditions (Laka *et al.,* 2024). This symbiotic relationship enables the plant to thrive in nutrient-deficient soils by enhancing its phosphorus uptake through the fungal network.

2.3 Effect of salinity on Arbuscular Mycorrhiza fungi colonization of roots

Salinity not only negatively affects the host plant but also hampers the colonization capacity, spore germination, and hyphal growth of Arbuscular Mycorrhiza fungi (Evelin *et al.,* 2009). Numerous studies (Sachan *et al.,* 2023; Jahromi *et al.,* 2008; McMillen *et al.,* 1998; Estaun, 1990) have documented the detrimental effects of salinity on these fungi. Specifically, the presence of NaCl has been shown to reduce root colonization by AM fungi (Hernández-Dorta *et al.,* 2024; Zhang *et al.,* 2014; Sheng *et al.,* 2008; Juniper and Abbott, 2006; Ojala *et al.,* 1983), likely due to the direct impact of NaCl on the fungi (Juniper and Abbott, 2006). This suppression of arbuscular mycorrhiza formation suggests that salinity inhibits early colonization stages (Tian *et al.,* 2004; Sheng *et al.,* 2008).

For successful primary colonization of plant roots by AM fungi, fungal propagules in the soil must first become hydrated, activated, and produce a germ tube (Parihar *et al.,* 2024). Hyphal growth, which is typically slow in the absence of host roots, accelerates in response to host exudates, leading to extensive branching (Adame-Garnica *et al.,* 2023). However, this hyphal growth is not always directional toward roots unless the roots are in close proximity (Steinberg *et al.,* 2017). Branching occurs upon contact with the root surface, but not on non-host roots, indicating that directional attraction might be specific to certain hosts (Adame-Garnica *et al.,* 2023). The degree to which NaCl inhibits AM fungi colonization depends on the timing of observation, with more significant inhibition occurring in the early stages of symbiosis suggesting that the primary infection stages are most affected by salinity (Gupta *et al.,* 2021).

The success of AM fungi symbiosis with plant roots can also depend on various factors, such as topographical or biochemical signals on the root surface and the phenology of host plants (Cusack *et al.,* 2021). Carvalho *et al.* (2001) found that in *Aster tripolium* and *Inula crithmoides*, the highest levels of AM fungi colonization occurred during periods of peak plant growth and flowering summer and autumn, respectively. The varying levels of AM fungi colonization may also be influenced by the specific behaviour of each AM fungal species, even within similar ecosystems, or by different environmental conditions (Tedersoo *et al.,* 2020).

Contrary to previous reports, some studies have found that AM fungi colonization is not reduced in the presence of NaCl (Porras-Soriano *et al.,* 2009; Hartmond *et al.,* 1987; Levy *et al.,* 1983). In fact, increased AM fungi sporulation and colonization under salt-stress conditions have also been observed (Aliasgharzadeh *et al.,* 2001). Yamato *et al.* (2008) reported that colonization rates were unaffected in all AM fungi present in coastal vegetation on Okinawa Island, Japan, even when exposed to high salinity levels of 200 mM. These discrepancies suggest the potential existence of salt-tolerant species of AM fungi. While NaCl delays spore germination rather than preventing it (Juniper and Abbott, 2006; Cantrell and Lindermann, 2001), pre-inoculating transplants with VAM fungi can bypass the inhibitory effects of salt on spore germination (Al-Karaki, 2006; Cantrell and Lindermann, 2001).

2.4 The effect of AM fungi on growth and yield

The research conducted by Manjulatha (2015) demonstrated that the utilisation of VAM fungi significantly enhanced both the cob and grain yield of maize, as shown in Table 2.3. The study further concluded that the application of VAM on crops can serve as a viable alternative from both an economic and soil health perspective (Manjulatha, 2015). Additionally, it offers the potential for achieving a greater benefit-to-cost ratio. Similarly, Kazadi *et al.* (2022) revealed that the growth of maize seedlings inoculated with VAM fungi exhibited a significant improvement compared to the control group without VAM fungi associations when transplanted into the field. The efficacy of VAM fungi is also reported to improve multiple terrestrial plants (Bagyaraj, 2018).

Table 2.3: Effect of VAM on cob and crop yield of maize (Manjulatha, 2015)

Treatments	**Cob yield (kg/ha)**	**Grain yield (kg/ha)**	**B:C ratio**
T1: VAM seed treatment + RDF	10836	8564	2.80
T2: VAM seed treatment + 75% RDF	10382	8295	2.75
T3: VAM seed treatment + 50% RDF	9115	7021	2.38
T4: VAM Topdressing + RDF	11278	8958	2.91
T5: VAM Topdressing + 75% RDF	10450	8542	2.81
T6: VAM Topdressing + 50% RDF	9392	7440	2.48
T7: Control (RDF)	10612	8388	2.78
C.D (0.05)	873	648	-
S.Em+	280	208	-
C.V (%)	4.7	4.4	-

RDF= Recommended Dose of Fertilizer, S.Em+=Standard Error of Mean Plus, CD= Critical Difference.

The use of VAM fungi is cited to also improve acidic and low-fertility soils (Suri and Choudhary, 2013). The growth of soybean plants in acidic and low-fertility soils was enhanced by their interaction with VAM organisms (Suri and Choudhary, 2013). This symbiotic relationship enables the plants to acquire immobile phosphorus and other essential nutrients. According to Gao *et al.*

(2020), plants engaged in the VAM fungi symbiosis exhibit a higher phosphorus uptake than plants that do not form VAM fungi associations. Additionally, VAM fungi are crucial in enhancing plant growth in phosphorus-limited soil conditions, where the growth of non-mycorrhizal plants is hindered (Laka *et al.,* 2024).

Plants engaged in VAM fungi symbiosis exhibit enhanced nutrient uptake, particularly with regards to phosphate, when supplied with low-soluble phosphate sources such as rock phosphate (Suri and Choudhary, 2013). This observation was supported by Gao *et al.* (2020), who observed that the symbiotic relationship between VAM fungi and cotton plants led to a significant increase in the expression of specific genes belonging to the phosphate transporter family. Additionally, there was a notable enhancement in the phosphorus content within the biomass of cotton plants (Gao *et al.*, 2020). According to Gao *et al.* (2020), the mutualistic association between cotton and VAM fungi resulted in enhanced photosynthetic activity, growth, boll production per plant, and fibre maturation. Saia *et al.* (2020) reported similar findings in the context of tomatoes. The study found that VAM fungi improved the growth of tomato under Triple Superphosphate.

Basak *et al.* (2011) found that the application of AM fungi inoculation to tomato seedlings cultivated under saline conditions did not lead to a significant increase in seedling growth. However, it did induce changes in growth and development, resulting in higher fresh stem and root weight as well as increased chlorophyll content (Basak *et al.,* 2011). These findings suggest that VAM fungi inoculation could positively impact tomato growth and yield. It is arguable that the application of VAM fungi did not result in a significant increase in seedling growth as both the inoculated and non-inoculated seedbeds had a slight similar seedling growth. However, it did facilitate the acquisition of essential nutrients by the seeds, thereby enabling their emergence and subsequent growth, ultimately leading to the development of robust plants compared to the control. In line with this result Masenya *et al*. (2023) observed the improvement of growth on the cancer bush under salinity stress when VAM fungi were applied. Other studies have also indicated that inoculating seedlings with VAM fungi formulations provides significant growth and development benefits to crops (Abbott and Robson, 2018; Candido *et al.*, 2015; Maya *et al.*, 2012; Premsekhar, and Rajashree, 2009). Allsop and Stock (1995) observed that non-mycorrhizal plants faced difficulties acquiring soil phosphorus unless they underwent alterations in their root system to acquire nutrients from alternative sources. The study revealed a negative correlation between the logarithm of seed mass and phosphorus content, and the mycorrhizal responses of inoculated plants in terms of mass and phosphorus content (Allsop and Stock, 1995). This implies that the introduction of VAM fungi during seed planting enhances the growth and development of plants, albeit without directly affecting seedling growth. Nonetheless, it does contribute to increased overall plant growth and yield (Laka *et al.,* 2024). Indriani *et al.* (2016) observed that the interaction of VAM fungi with other plant growth enhancers improves plant growth as shown in Table 2.4. Insufficient data exists regarding the impact of VAM fungi inoculation on the growth and yield of moringa. However, existing studies primarily concentrate on the combined application of VAM fungi with other plant growth enhancers, rather than solely examining the effects of VAM fungi inoculation with the intention to improve plant growth and development under abiotic and biotic stress factors.

Table 2.4: Interaction effect between Rock phosphate and VAM fungi on plant height (cm) of *Centrosema pubescens (*Indriani *et al.*, 2016)

Rock Phosphate (kg ha^{-1})	Rock Phosphate and VAM (10 g)
0	40.67 abB
100	44.67 cA
200	46.33 cB
300	30.33 aA

2.5 The effect of salinity stress on growth and yield

Salinity is the degree of saltiness or quantity of dissolved salt in a body of water (Rybak, 2018). Soil salinization is the process by which salt builds up in the soil, rendering it unfit for cultivating crops (Kumar *et al.,* 2023). The occurrence of this phenomenon can be attributed to the accumulation of naturally occurring salts or the entry of irrigation water containing high levels of salt (Laka *et al.,* 2024). Salinisation may also arise due to insufficient drainage, resulting in the accumulation of water inside the soil, leading to the dissolution and subsequent accumulation of salts from the soil (Kumar *et al.,* 2023). Soil salinisation has become a major global environmental and socioeconomic issue, and it is expected to worsen due to projected climate changes (Kumar *et al.,* 2023). It impacts the supply of food, the accessibility of water, and the well-being of humans (Laka *et al.,* 2024). The impact of salinity on plant growth and development is significant, making it a prominent environmental stressor. Salinity is one of the harshest environmental variables, limiting crop plant productivity. Elevated concentrations of salt have the potential to induce the destruction of soil structure. It can result in the development of a hardpan layer, which can impede the penetration of roots into the soil (Kumar *et al.,* 2023). Therefore, a substantial decrease in crop yields is observed. Eswar *et al.* (2021) asserted that crop productivity is diminished by a magnitude exceeding 20%, resulting in an anticipated yearly expansion of the saline environment by approximately 0.3–1.5 million hectares. Consequently, there is a decrease in the overall output capacity by a range of 20 to 46 million acres (Farooq *et al.,* 2022). The alterations in salinity levels have been observed to impact metabolic processes, resulting in impaired growth and reduced enzymatic activity. The phenomenon of high salinity has a significant impact on approximately 7% of the earth's land, resulting in its unsuitability for human habitation (Eswar *et al.,* 2021). According to the World Bank, salinisation results in an annual loss of up to $20 billion (Dewi *et al.,* 2022). As a result of intricate natural processes, abiotic stress hampers plant growth, diminishes agricultural output, and exacerbates soil degradation. The revenues of farmers and other local economies experience a significant deceleration (Khamidov *et al.,* 2022).

Saline stress induces a range of physiological disturbances, including nutritional and hormonal imbalances, ion toxicity, oxidative and osmotic stress, and heightened vulnerability to diseases (Eswar *et al.*, 2021). Plants are susceptible to salt stress, which can lead to detrimental effects such as impaired soil porosity and hydraulic conductivity, ultimately reducing soil moisture capacity (Kumar *et al.*, 2020). Consequently, this induces water stress, resulting in physiological drought conditions. Additionally, the presence of excessive ions, primarily sodium ions (Na^+), can disrupt the stability of cell membranes and cause protein degradation, further exacerbating the toxic effects on plants (Kumar *et al.*, 2020). Kumar *et al.* (2023) have identified multiple mechanisms via which plants respond to salt stress. Figure 2.2 below illustrates the classification of these reactions into two categories: physiological and biochemical (Kumar *et al.*, 2023).

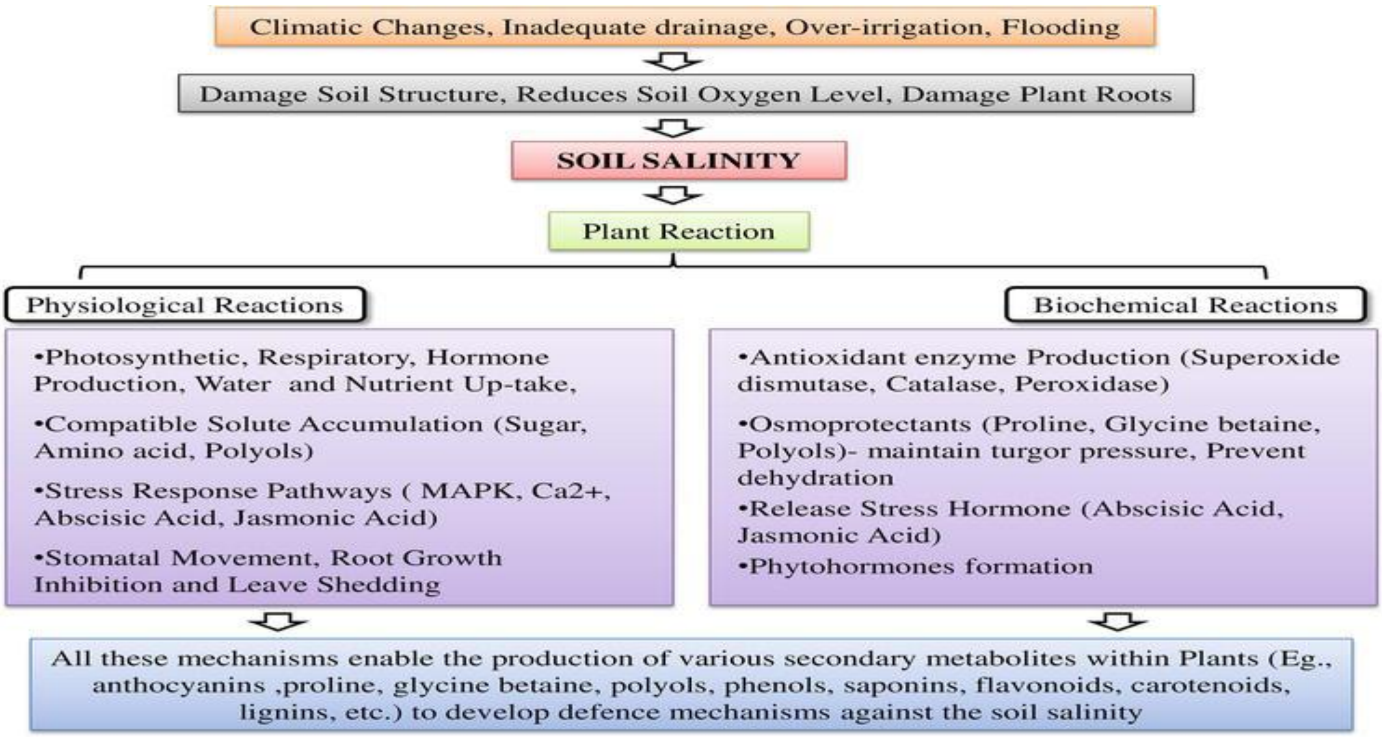


Figure 2.2: The physiological and biochemical effects of salt stress on secondary metabolite accumulation in plants subjected to salt stress.

Salt stress induces an elevation in osmotic potential, a reduction in water potential, an augmentation in photosynthetic activity, and a decrease in stomatal conductance. Salt stress enhances the activity of antioxidant enzymes, the production of stress-related genes, the accumulation of proline, and the building of soluble sugars. Plants require these reactions to maintain their health and prevent salt-induced stress (Kumar *et al.*, 2023).

Changes in the pace of plant growth represent a specific category of physiological responses (Kumar *et al.*, 2023). According to Kumar *et al.* (2023), additional physiological processes encompass alterations in photosynthesis, respiration, transpiration, water absorption, nutrition absorption, and hormone synthesis. Plants respond to salt stress by increasing the amount of solutes inside their cells. This makes it easier for them to keep their turgor pressure and lowers their risk of drying out (Irshad *et al*., 2021). Compatible solutes refer to tiny molecules that have the ability to accumulate within a cell without causing any disturbance to its osmotic equilibrium (Kumar *et al.*, 2023). Sugars, amino acids, and polyols are among the solutes that exhibit compatibility (Kumar *et al.*, 2023). Plants exhibit a reaction to salt stress by initiating multiple stress-response pathways, including the mitogen-activated protein kinase, calcium, and abscisic acid pathways (Laka *et al.*, 2024). These pathways aid in the plant's ability to manage stress and safeguard against harm (Hao *et al.*, 2021). Salt stress also influences the mobility of stomata (Kumar *et al*., 2023). Plants stomatally close to minimise water loss via transpiration. This aids in water conservation and minimizes the influx of salt into the plant (Kumar *et al.*, 2023). According to Kumar *et al.* (2023), stomata are minute apertures located on the outer layer of leaves, responsible for the regulation of gas and water vapor exchange through their opening and closing mechanisms. When plants close their stomata, they emit less water vapor into the environment (Kumar *et al.*, 2023). This phenomenon aids in water conservation and salt absorption by the plant (Kiani-Pouya *et al.*, 2020; Kumar and Akhtar, 2019). Additionally, certain plant species, such as specific grass species, exhibit the ability to withstand elevated salinity levels (Kumar *et al.*, 2023). Some plants may exhibit short-term survival but experience long-term harm. In the most severe instances, the plants may perish. Plants utilise metabolites for several functions. The utilisation of these metabolites by plants facilitates the regulation of their internal water balance, hence enabling them to effectively manage salt stress (Gul *et al.,* 2022; Kumar, 2021). These molecules facilitate the process of detoxification and the removal of surplus salt from plant cells. They serve the purpose of safeguarding the plant's cells against the detrimental impacts of salt (Kumar *et al.,* 2023). They serve as an energy source, facilitate growth and development regulation, manufacture hormones, provide protection against environmental stress, generate pigments, aid in pathogen defence, and contribute to the production of other molecules (Kumari, 2021).

Azeem *et al*. (2023) indicated moringa can mitigate mild salinity (50 mM NaCl) through the preservation of succulence, weight ratios, and biomass distribution patterns in both the shoot and root. Azeem *et al.* (2023) achieved this effect with only a minor reduction in dry biomass. Nevertheless, it is worth noting that the growth metrics in moringa exhibited a significant decrease under conditions of high salinity (100 mM NaCl), particularly when compared to the control group (Laka *et al.,* 2024). Azeem *et al.* (2023) also found that the plant responded to the salt stress by accumulating more Na^+, Cl^-, and K^+ ions. Similarly, Elhag and Abdalla (2014) conducted a study on moringa and arrived at a similar finding. According to Nouman *et al.* (2012), the survival of moringa under abiotic stress can be attributed to its stronger antioxidant defences in comparison to other salinity-tolerant plants. These findings suggest that while the plant exhibits some tolerance to moderate salinity, it is still unable to achieve optimal growth and development compared to plants grown in soil without any salinity. One could propose that the observed phenomenon is attributable to the plant's activation of survival mechanisms, wherein it allocates its resources towards facilitating growth in the face of stress rather than adhering to its typical growth patterns (Laka *et al.,* 2024).

2.6 The influence of Arbuscular Mycorrhiza fungi on saline stress alleviation

Arbuscular Mycorrhiza (AM) fungi play a vital role in the natural ecosystem, particularly in saline environments, by enhancing early plant growth and promoting resistance to salinity (Tahat and

Sijam, 2012). Numerous studies have demonstrated that the utilization of Versicular Arbuscular Mycorrhiza (VAM) fungi can effectively mitigate the detrimental effects of salt stress on plants (Frahat and Shehata, 2021; Oztekin *et al.,* 2012). This is achieved through various mechanisms, including the facilitation of nutrient absorption, the preservation of enzyme functionality, and the facilitation of water absorption (Bhardwaj and Kumar, 2020). Arbuscular Mycorrhiza (AM) fungi have been observed to enhance the transportation of mineral nutrients to plants in soil that is subjected to salt-induced stress (Laka *et al.,* 2024). This is particularly evident in the case of phosphorus, which tends to precipitate in the form of phosphate salts. The fungi play a crucial role in ameliorating the detrimental consequences of salt stress on plants, leading to enhanced plant growth (Evelin *et al.,* 2009). Additionally, they provide protection to host plants against the adverse effects of stressors by augmenting antioxidant responses and/or inducing acquired systemic tolerance (Evelin *et al.,* 2009).

The study by Ebrahim and Saleem (2017) on tomatoes found that the application of VAM fungus to control plants resulted in enhanced plant growth and development as shown in Table 5. Consequently, the presence of VAM fungi enabled the plants to effectively withstand the adverse consequences of salinity, even at relatively low NaCl concentrations (Ebrahim and Saleem, 2017). Masenya *et al.* (2023) research on the cancer bush's response to saline alleviation using VAM fungi produced similar results. Nevertheless, definitive conclusions regarding moringa cannot be drawn due to the fact that the efficacy of VAM fungi in improving a crop's adaptability to the environment is crop specific.

Table 2.5: Interactive effects of NaCl (mM) and Vesicular Arbuscular Mycorrhizal Fungus (VAMF) on leaf metabolite content [mg/g (dm)] and shoot biomass [g. (dm)/plant] yielded by 3-month-old tomato plants (cv. Super Strain-B) (Ebrahim and Saleem, 2017).

NaCl level (mM)	Mycorrhizal treatment	Total sol. Sugars (TSS)	Polysacch.	Total carbohyd.	Total sol. Proteins (TSP)	Shoot biomass	Root/Shoot (DW basis)
0	n-M	187	328	515	121	22	0.29
	M	200	339	539	131	25	0.32
50	n-M	199	276	475	96	16	0.41
	M	216	286	502	111	19	0.37
100	n-M	222	214	436	56	8	0.64
	M	237	227	464	78	11	0.52
Factor							
NaCl level (A)		12	26	39	11.60	1.7	0.031
Mycorrhizae (B)		8	23	35	7.91	1.3	0. 022
Interaction (Ax B)		19	32	47	18.20	2.3	0.043

2.7 The phytochemicals of moringa

Phytochemicals are secondary metabolites that are abundantly present in plants, yet they have minimal or negligible involvement in the growth and development of plants (Ji *et al.,* 2022). Various plant species, including fruits, vegetables, legumes, and grains, synthesise phytochemicals (Ji *et al.,* 2022). Endophytic microorganisms play a crucial role in the plant's immune response by providing protection against a wide range of pathogens, including viruses, bacteria, fungi, and parasites (Shree *et al.,* 2022). Phytochemicals exhibit protective effects in both plants and humans. Ma *et al.* (2020) assert that humans have used phytochemicals as medicinal agents across various historical periods to alleviate and protect against a diverse range of ailments. Moreover, Li *et al.* (2023) assert that these cells protect against environmental contaminants and the body's inherent metabolic processes.

Moringa species are characterised by a diverse range of phytoconstituents, and the presence of these phytochemicals contributes to the manifold medicinal applications of the plant (Wang *et al.,* 2022). Furthermore, it is important to note that no singular type of phytochemical confers a solitary benefit (Laka *et al.,* 2024). Various food sources, such as soybeans, flaxseed, peaches, and garlic, contain phytoestrogens that may potentially provide protective effects against conditions like bone loss, breast cancer, uterine cancer, and cardiovascular disease (Kasolo *et al.*, 2010). According to Kasolo *et al.* (2010), the identification of phytochemicals in moringa leaves indicates the potential for preventive and therapeutic properties. Kasolo *et al.* (2010) further asserted that additional pharmacological research is necessary to substantiate the use of moringa as a medicinal plant. Rabizadeh *et al*. (2022) have similarly argued for the need for further investigation to confirm the connection between traditional uses and the biological characteristics of medicinal plants. This suggests that despite the existence of numerous studies on the medicinal properties of moringa, there remains a necessity for additional research on its therapeutic applications.

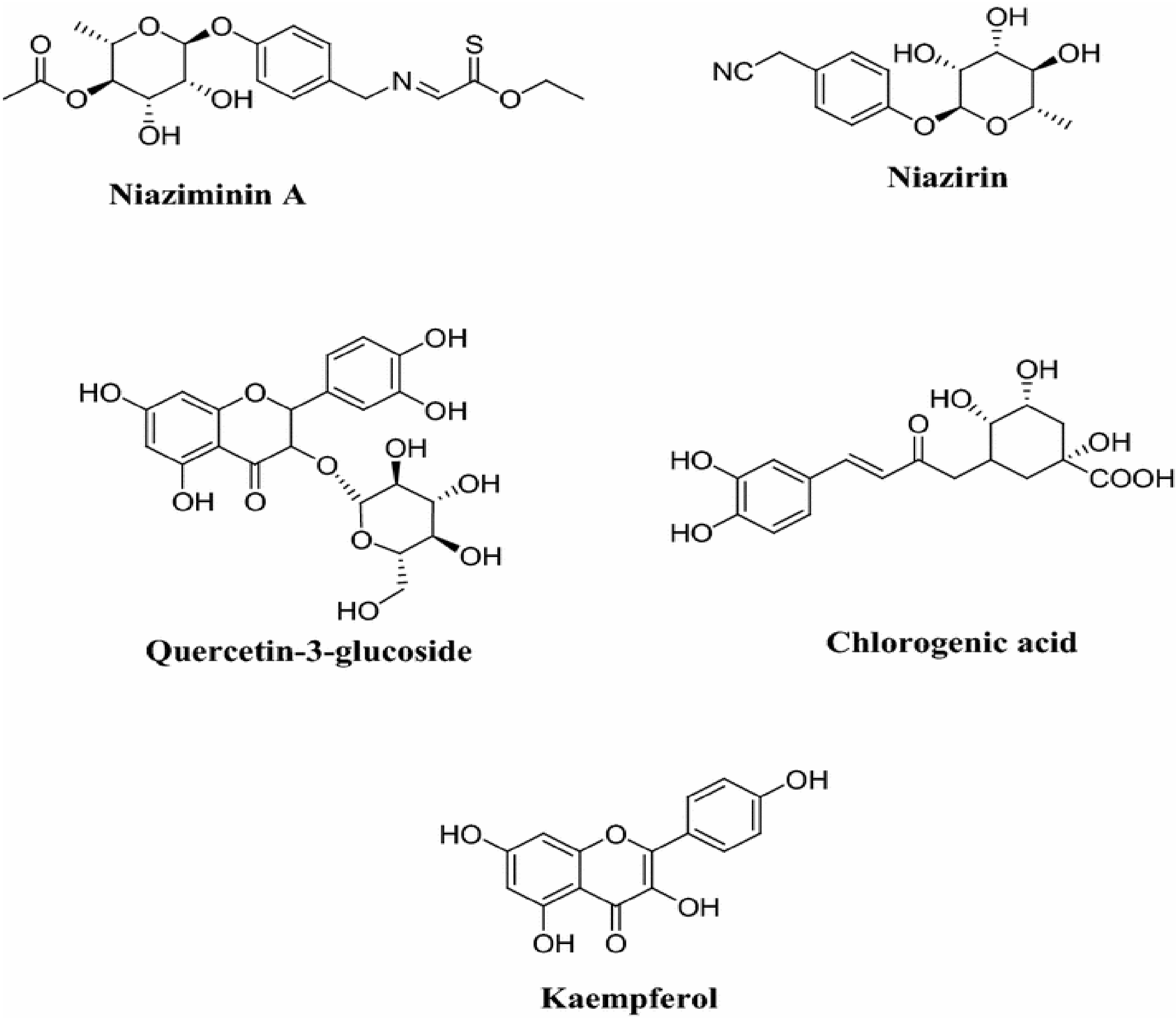


Figure 2.3: Structures of some phytoconstituents isolated from moringa plant (Cornelius, 2019).

The phytochemicals in the moringa plant were identified in a study conducted by Cornelius (2019), including cyanogenic glycosides, saponins, flavonoids, and alkaloids (Figure 2.3). In line with the results by Cornelius (2019) and Ma *et al.* (2020) reported that an abundance of flavonoids, phenolic acids, and tannins, all belonging to the class of polyphenols, has been identified in moringa. Hence, the moringa plant is regarded as a significant botanical resource in the field of medicine due to its diverse array of phytoconstituents. Even though moringa plant possesses a wide range of phytochemicals that contribute to its medicinal properties, the distribution and abundance of these phytochemicals vary throughout the plant (Laka *et al.*, 2024). Polyphenols have been identified in both flowers and seeds (Ma *et al.*, 2020). However, their concentration in these plant parts is significantly lower compared to that in leaves (Table 2.6). Based on the research conducted by Cornelius (2019), moringa leaves contain a greater abundance of phytochemicals such as saponin, alkaloid, and cyanogenic glycosides compared to flowers as shown in Table 2.6. However, the concentration or proportion of flavonoids was found to be higher in flowers as opposed to leaves. Furthermore, the abundance and accessibility of phytochemicals in plants are contingent upon environmental factors and geographical positioning.

Table 2.6: Analyses of some phytochemicals in moringa leaves and flowers (Cornelius, 2019)

	Saponin	Flavonoid	Alkaloid	Cyanogenic Glycoside
Percentages	%	%	%	mg/10g
Moringa leaves	5.00	5.42	5.36	0.20
Moringa flowers	3.20	7.12	1.56	0.16

Martínez *et al.* (2022) conducted a study that demonstrated significant variability across geographical regions in terms of antioxidant activity. Specifically, the findings indicated that cold conditions were associated with heightened antioxidant activity, potentially attributable to elevated thermal stress (Laka *et al.*, 2024). Additionally, Martínez *et al.* (2022) found that variation in the antioxidant properties, as well as the levels of phenolic and flavonoid compounds in chestnuts, are not solely determined by the specific variety but are also influenced by environmental factors. The active phytoconstituents such as alkaloid, saponin, tannin, glycoside, flavonoid, and phenols have both positive and negative influences on plants, humans, and animals, as briefed below.

2.7.1 Alkaloid

Alkaloids represent a category of nitrogenous compounds that are synthesised by plants in response to various biotic or abiotic factors (Othman *et al.,* 2019). This synthesis results in alkaloids possessing notable biological efficacy and a diverse range of structural characteristics (Othman *et al.,* 2019). Alkaloids are a class of naturally occurring toxic amines synthesised by plants, primarily as a means of defence against herbivores (Mondal *et al.,* 2019). The primary detrimental effects of alkaloids encompass disturbances in the central nervous system, digestive functions, reproductive processes, and the immune system (Mondal *et al.,* 2019). Despite the potential adverse effects associated with alkaloids, it is important to acknowledge their advantageous influence on human physiology (Laka *et al.,* 2024). Alkaloids possess the ability to directly impact the human brain and stimulate vital organs such as the central nervous system (Laka *et al.,* 2024). The toxic alkaloids in honey are obtained through ingesting pollen contaminated with pyrrolizidine alkaloids (PAs) (Kempf *et al.,* 2010). Several plant species that contain PAs are known to contribute to the potential toxicity of honey (Kempf *et al.,* 2010).

According to Kurek (2019), alkaloids are a fascinating group of compounds that exhibit a diverse array of both adverse and advantageous impacts on the physiological systems of animals and humans. Previously, the general consensus viewed them as mere by-products of plant metabolism (Laka *et al.,* 2024). However, recent empirical evidence has unveiled their indispensable role in the biological processes of plants (Laka *et al.,* 2024). Alkaloids have been found to exhibit a wide range of physiological effects, including antibacterial, antimitotic, anti-inflammatory, analgesic, local anaesthetic, hypnotic, psychotropic, and anticancer properties (Nett *et al.,* 2020).

Empirical studies have established numerous, albeit atypical, alkaloids in moringa. Nevertheless, the precise quantity of these alkaloids present in moringa leaves remains undetermined (Leone *et al.,* 2015). The concentration of alkaloids in plants is contingent upon environmental conditions. Young and actively proliferating tissues predominantly synthesis alkaloids; therefore, any factors influencing the growth of these tissues will subsequently affect the synthesis of alkaloids (Roddan *et al.,* 2020). Environmental factors can influence the production and degradation of various alkaloids, potentially leading to differential production levels between species (Roddan *et al.,* 2020).

Alkaloids typically exhibit the characteristics of being colourless and odourless crystalline solids, although in certain instances, they may manifest as yellowish liquids (Adamski *et al.,* 2020). These compounds frequently taste bitter, rendering them effective as natural inhibitors of herbivorous organisms (Adamski *et al.,* 2020). Consequently, certain plants use them as natural pesticides (Laka *et al.,* 2024).

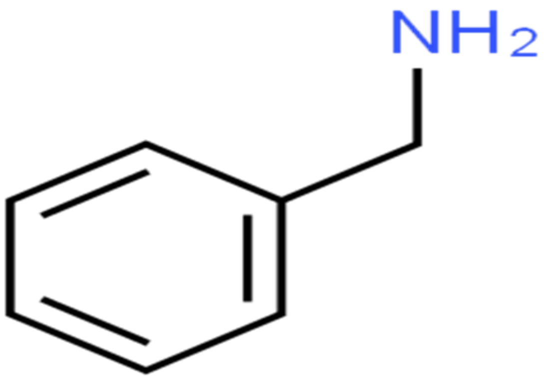


Figure 2. 4: Chemical structure of alkaloid moringine isolated from moringa plant material (Adamski *et al*., 2020).

Furthermore, the phenolic extract from moringa leaves inhibits cell proliferation and induces death in human melanoma cells (Nett *et al.,* 2020). Numerous investigations have demonstrated that alkaloids are the primary mediators of moringa's anticancer action (Wang *et al.,* 2022; Nett *et al.,* 2020; Kasolo *et al.,* 2010). According to Sreelatha and Padma (2009), the moringa plant produces moringine, an alkaloid, by a sequence of chemical reactions illustrated in Figure 2.4. This series of tasks involves the collection and preparation of plant parts, extraction of alkaloids, concentration of the raw extract, division of the mixture into fractions, study of these fractions using spectroscopic techniques, cleaning of the fraction containing moringine, confirmation of the identity of the isolated moringine by comparing its spectroscopic data and physical properties with those reported in literature or real standards, and description of the isolated moringine through further analysis (Sreelatha and Padma, 2009). Additionally, Sreelatha and Padma (2009) propose that the details of each stage may vary according on the equipment and expertise at hand, and it is crucial to strictly follow safety protocols while dealing with solvents and chemicals. A precise balance of chromatographic techniques, spectroscopic analysis, purification, and confirmation of the isolated moringine's identification is required in this procedure (Sreelatha and Padma, 2009). The detection of moringine requires the execution of a series of techniques (Sreelatha and Padma, 2009). Firstly, a sample of moringa plant material that contains alkaloids is obtained and subjected to the process of purification and drying (Sreelatha and Padma, 2009). Following that, the alkaloids are removed from the plant material using a suitable solvent. To increase the alkaloid concentration, the raw extract is evaporated. Initial analysis for alkaloids is performed using conventional chemical assays (Sreelatha and Padma, 2009). An examination of Thin-Layer Chromatography (TLC) is conducted to separate and identify compounds, such moringine, based on their Retention factor (Rf) value and the development of spots (Sreelatha and Padma, 2009). High-Performance Liquid Chromatography (HPLC) is used to confirm the presence of moringine and quantify its concentration levels (Sreelatha and Padma, 2009). Molecular weight verification and identification of fragment ions specific to moringine are achieved by mass spectrometry (MS) analysis (Sreelatha and Padma, 2009). Finally, the chemical composition of the separated product is determined using Nuclear Magnetic Resonance (NMR) spectroscopy (Sreelatha and Padma, 2009). Effective identification of moringine in the extract of the moringa plant can be achieved by following these procedures and using several analytical methods.

2.7.2 Cyanogenic glycosides

Cyanogenic glycosides are plant constituents derived from amino acids, present in more than 2500 plant species, and widely distributed across 100 groups of flowering plants (Cressey and Reeve, 2019). The component possesses the capacity to generate hydrogen cyanide, a highly toxic substance, upon degradation by plant enzymes (Cressey and Reeve, 2019). Cyanogenic glycosides are naturally occurring toxic compounds present in a diverse range of plant species, with a predominant occurrence in those plants that are commonly consumed by humans (Bolarinwa *et al.*, 2013). Cyanide poisoning may arise from the inadvertent or deliberate ingestion of cyanogenic glycosides, leading to acute intoxication characterized by stunted growth and neurological manifestations stemming from the destruction of central nervous system tissues (Bolarinwa *et al.*, 2013). Processing procedures can effectively detoxify cyanogenic glycosides, thereby reducing the risk of cyanide poisoning. However, the effectiveness of cyanide removal depends on the specific processing technology employed and the extent of processing applied (Laka *et al.*, 2024). According to Møller (2010), in isolation, cyanogenic glycosides do not possess any detrimental effects.

The presence of cyanogenic glycosides in plants serves as a defensive mechanism against herbivory. However, certain herbivores have developed diverse metabolic mechanisms to overcome the toxicity (Nyirenda, 2020). The existence of cyanogenic glycosides in food and animal feed can pose significant social and economic challenges in numerous global regions. The consumption of cassava as a dietary staple in Africa has been associated with tropical neuropathy illness, known as konzo, as well as cyanide poisoning (Mosayyebi *et al.*, 2020). According to Bolarinwa (2013), farmers persist in cultivating crops containing significant levels of cyanogenic glycosides, despite their known harmful effects. This is because these compounds serve as inherent pesticides, providing protection against animal pests that threaten crop yield.

Cyanogenic glycosides in plants are subject to variation based on factors such as plant age, genetic variety, and prevailing environmental conditions. According to Oluwole *et al.* (2007), there is a documented observation that crops cultivated in regions with lower altitudes exhibit elevated concentrations of cyanogenic glycosides, while crops grown in higher-altitude regions display lower levels of cyanogenic glycosides .

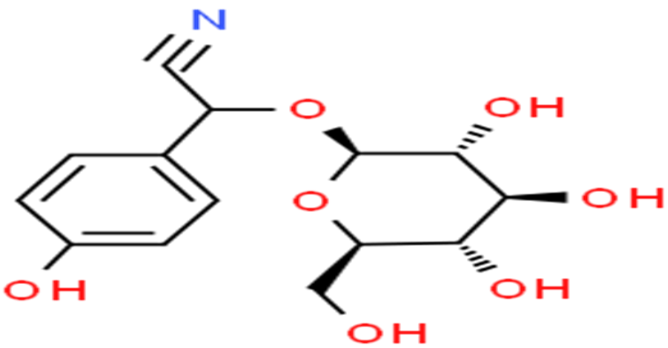


Figure 2.5: Chemical structure of dhurrin, a type of cyanogenic glycoside isolated from moringa plant material (Oluwole *et al*., 2007).

The concentrations of cyanogenic glycosides report in moringa were 3.2%, 2.4%, and 1.0%, respectively in the roots, leaves, and stem barks (Chioma and Okah, 2019). The roots and leaves of the plant are the best sources of these phytoconstituents (Chioma and Okah, 2019). These concentration percentages in the plant show that it is less toxic and produces a minor quantity of hydrogen cyanide, which can easily be detoxified (Chioma and Okah, 2019).

Using a suitable solvent, such as methanol or ethanol, Chioma and Okah (2019) described the procedure of extracting the plant material, often leaves or seeds, from moringa in order to isolate cyanogenic glycosides. After completing the extraction procedure, the solvent undergoes evaporation to obtain a pure extract (Chioma and Okah, 2019). Furthermore, it is possible to use several chromatographic techniques, such as column chromatography or HPLC, to separate and isolate the glycosides based on their unique properties, such as polarity (Chioma and Okah, 2019). The identification of specific cyanogenic glycosides can be achieved by employing spectroscopic protocols such as NMR or mass spectrometry (Chioma and Okah, 2019).

2.7.3 Saponin

Saponins are naturally derived compounds that are abundantly present in the cellular composition of leguminous plants (Sun *et al.,* 2009). Saponins are a group of chemical compounds that are very different and complicated. They got their name from the way they naturally foam up in water, making a soap-like foam (Silva *et al.,* 2021). Furthermore, saponins exhibit several noteworthy medicinal properties, including anti-inflammatory, anti-fungal, anti-bacterial, anti-parasitic, anti-cancer, and antiviral effects (Liao *et al.,* 2021; Yi, 2021).

Surfactants are commonly employed in various industries, such as food, pharmaceuticals, and cosmetics, due to the emulsifying properties exhibited by saponins (Sharma *et al.,* 2021). This enables the creation and maintenance of emulsion-based products. The adsorption of amphiphilic surfactants at the oil-water interfaces during homogenisation lowers the interfacial tension, which leads to the formation of stable emulsions (Elekofehinti *et al.,* 2021). Prior research has indicated that the utilisation of natural emulsifiers has the capacity to generate an emulsion that is both efficacious and enduring in its stability.

Environmental factors influence the variation in saponin concentration in plants. Pecetti *et al.* (2006) conducted a study in which they observed a notable disparity in the levels of saponins found in plants harvested during the summer and winter seasons. Specifically, the researchers noted that the concentration of saponins was comparatively low during the winter months, while it reached its highest point during the middle of the summer. Yu *et al.* (2022) found that environmental stimuli influence the production of saponins in plants. Nevertheless, according to the Pecetti *et al.* (2006) study, it can be inferred that chemicals are not the sole determinant of the summer conditions. Hence, the impact of environmental factors on saponin concentration remains uncertain.

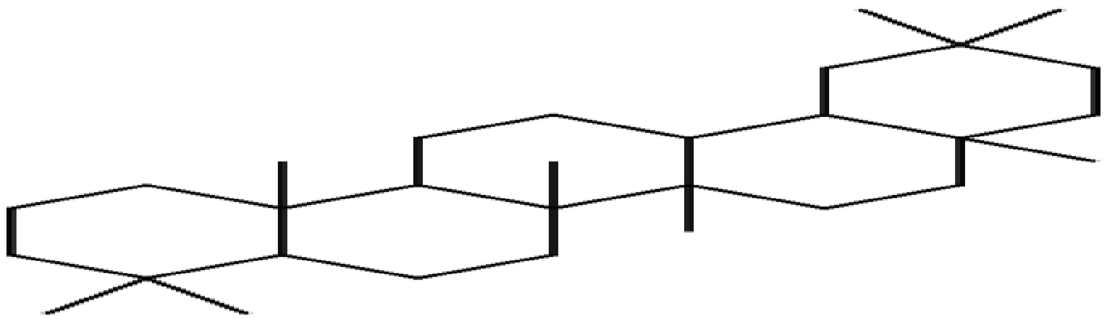


Figure 2.6: Chemical structure of oleanane, a type of saponin isolated from moringa plant material (Pecetti *et al.*, 2006).

Chioma and Okah (2019) found the saponin concentration in moringa leaves to be 5% and 3.2% in flowers. They then concluded that both the leaves and flowers of moringa are good sources of saponin, which contains high amounts of lipids, and the high caloric value was due to the high lipids (Chioma and Okah, 2019). Moreover, the phytoconstituents assist in protecting the plant from microbes and fungi, enhance the absorption of nutrients, and aid in the digestion of animals (Chioma and Okah, 2019).

Oleanane isolation from moringa involves several steps, including extraction, purification, and characterisation (Pasha *et al.*, 2020). Moringa leaves are pulverised into a fine powder and the oleanane component is isolated by solvent extraction (Pasha *et al.*, 2020). The removal of impurities is achieved by filtration, and the unprocessed extract is compressed at reduced pressure (Pasha *et al.*, 2020). The process of liquid-liquid partitioning selectively separates the oleanane from the organic layer (Pasha *et al.*, 2020). To improve the purity of the organic layer, further purification techniques such as column chromatography or preparative TLC are used (Pasha *et al.*, 2020). Finally, the purified chemical is subjected to investigation using spectroscopic methods including NMR, IR, and mass spectrometry to confirm its identification as oleanane (Pasha *et al.*, 2020). The ultimate stage in obtaining the pure form of oleanane is recrystallization or, if required, further chromatographic purification (Pasha *et al.*, 2020).

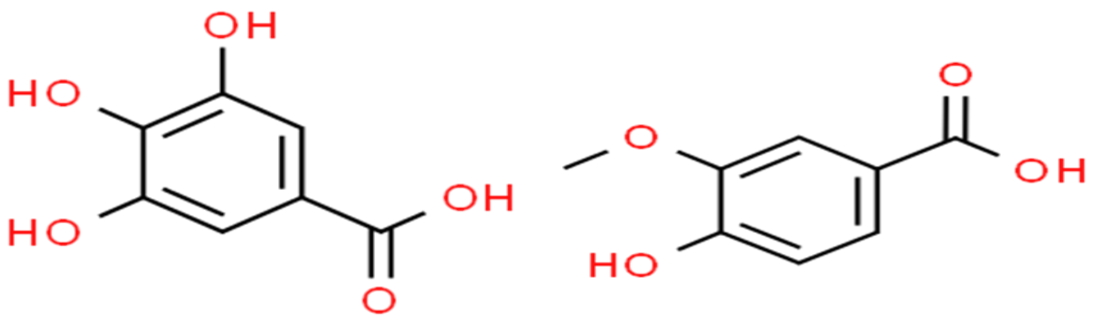
2.7.4 Phenolic Acid

Phenol is considered one of the principal classifications of plant phenolic compounds (Yu *et al.*, 2022). A diverse array of plant-based meals contains these compounds, with the highest concentrations observed in seeds, fruit and vegetable skins, and leafy greens (Ghasemzadeh and Ghasemzadeh, 2011). Phenolic acids, a diverse group of plant polyphenols, have garnered significant research interest (Laka *et al.*, 2024). The shikimic acid pathway synthesises phenylpropanoids, specifically as by-products in the monolignol pathway (Chen *et al.*, 2020). Additionally, the degradation of cell wall polymers like lignin generates these compounds (Chen *et al.*, 2020). In certain instances, microbes also contribute to their production (Laka *et al.*, 2024).

Bioavailability, defined as the proportion of these compounds absorbed, digested, and metabolised upon entering the circulatory system, primarily determines the biological activity of phenolic acids (Rashmi and Negi, 2020). A large amount of epidemiological and experimental research has consistently shown that phenolic acids may be able to protect against degenerative diseases like heart disease, cancer, diabetes, and inflammation (Marchiosi *et al.*, 2020).

According to Kiokias *et al.* (2020), phenolic acids serve a variety of functions in plants, including protein synthesis, allelopathy, nutrient absorption, structural integrity, enzyme catalysis, and photosynthetic processes. While this part does have some benefits, phenolic compounds can build up in water and sediment, where aquatic organisms look for food (Liu *et al.*, 2020). This is because they don't break down and stay there for a long time (Laka *et al.*, 2024). When present at certain levels, exposure to phenolic compounds through water and sediment can potentially endanger aquatic ecosystems and human health (Abedi *et al.,* 2020).

In their study, Mpofu *et al.* (2006) observed significant variations in the total phenolic content, antioxidant activities, and concentrations of various phenolic acids in wheat across different genotypes and environmental conditions. The study by Fernandez-Orozco *et al.* (2010) on wheat corroborated the aforementioned results. However, there exists a dearth of knowledge regarding the diverse genotypes and environmental factors influencing the presence of phenolic acids in moringa (Laka *et al.,* 2024).



(A) Gallic acid **(B) Vanillic acid**

Figure 2.7: Chemical structures of phenolic acid types isolated from moringa plant materials (A) Gallic acid and (B) Vanillic acid (Mpofu *et al*., 2006).

One of the most dominant phenolic acids in moringa is gallic acid, which is most abundant in dried leaves at a concentration of 1.034 mg/g (Vergara-Jimenez *et al.,* 2017). The chlorogenic acid, a major phenolic acid in moringa, plays a significant role in glucose metabolism, whereby it inhibits the glucose-6-phosphate translocate in the rat liver and reduces hepatic gluconeogenesis (Vergara-Jimenez *et al.,* 2017). Moreover, Hassan *et al.* (2021) discovered that the phenolic acids of the moringa leaves are responsible for many biological activities, for example, plant growth, development, and defence, which prevent and mitigate complications of diseases such as hypertension.

The extraction of phenolic acid from moringa plant materials involves several procedures (Saucedo-Pompa *et al.,* 2018). According to Saucedo-Pompa *et al.* (2018), the initial stage involves the pulverization of the plant material followed by its extraction using a solvent such as ethanol or methanol. The extract undergoes further filtration to remove any remaining solid particles. The compounds are then separated into fractions by techniques including liquid-liquid extraction or column chromatography (Saucedo-Pompa *et al.,* 2018). To further refine the fraction containing phenolic acids, crystallization or preparative chromatography techniques are employed (Saucedo-Pompa *et al.,* 2018). The purified molecule is analyzed using spectroscopic techniques such as NMR and mass spectrometry to verify its classification as a phenolic acid (Saucedo-Pompa *et al.,* 2018). Quantification of phenolic acids in the extract can be achieved using either HPLC or spectrophotometry (Saucedo-Pompa *et al.,* 2018).

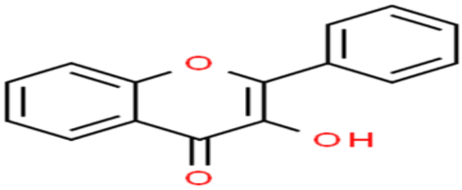
To differentiate moringa phenolic acids, several analytical techniques can be employed (Saucedo-Pompa *et al.*, 2018). High-performance liquid chromatography employs absorbance spectra and retention durations for the detection and separation of phenolic acids, whereas gas chromatography-mass spectrometry (GC-MS) separates substances according to their molecular weight and volatility (Saucedo-Pompa *et al.*, 2018). Nuclear Magnetic Resonance Spectroscopy provides information on the structures of phenolic acids, while UV-Vis Spectroscopy involves the measurement and quantification of these compounds at certain wavelengths (Saucedo-Pompa *et al.,* 2018). Fourier Transform Infrared Spectroscopy (FTIR) quantifies the functional groups present in phenolic acids. These techniques, when used in conjunction with the phenolic acid concentration of moringa and reference standards, enable a more accurate determination of these chemicals (Saucedo-Pompa *et al.,* 2018).

2.7.5 Flavonoid

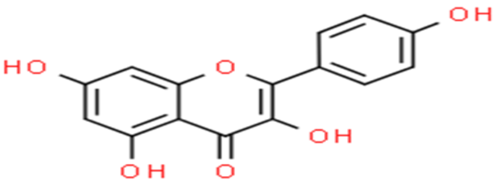
Flavonoids are a big group of naturally occurring chemicals that are mostly found as secondary metabolites in plants (Laka *et al.,* 2024). They are known for having a polyphenolic structure (Maleki *et al.,* 2019). Various fruits, vegetables, and certain beverages widely distribute these compounds (Maleki *et al.,* 2019). A diverse range of advantageous biochemical and antioxidant characteristics have been associated with numerous diseases, including cancer, Alzheimer's disease (AD), and atherosclerosis, among others (Aryal *et al.*, 2019). Flavonoids are essential for the growth and defense mechanisms of vegetables against harmful plaques (Karak, 2019). Numerous flavonoids commonly recognise floral pigments in majority of angiosperm families (Karak, 2019). However, their presence extends beyond flowers to various other components of plants (Laka *et al.,* 2024). According to Cornelius (2019), the flower of moringa exhibits a significant abundance of flavonoids, which consequently contributes to its distinct coloration. This factor is the origin of the flower's inherent vibrant hue and fragrance (Dias *et al.*, 2021).

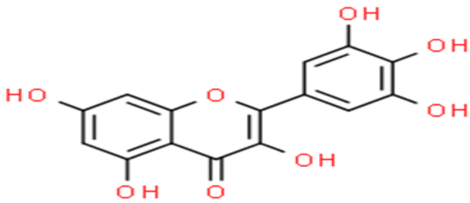
Flavonoids possess a variety of biological roles within the domains of plants, animals, and microbes (Laka *et al.*, 2024). Flavonoids fulfil various roles in enhancing plant resilience against frost and drought, and they may additionally contribute to plant adaptation to high temperatures and tolerance to freezing conditions (Yeh *et al.,* 2021). Numerous investigations have been undertaken to examine the antioxidant properties of various flavonoids, revealing their potential therapeutic applications in mitigating oxidative stress (Li *et al.,* 2021; Yeh *et al.,* 2021). The moringa genus exhibits notable antioxidant properties due to its elevated concentration of flavonoids (Rode *et al.,* 2022). Despite the recent increase in scholarly attention to the functions of flavonols, certain functional aspects continue to be a subject of controversy.

According to Laoué *et al.* (2022), the exact function of polyphenols in plants remains challenging to ascertain. However, ample evidence suggests that flavonoids, specifically flavonols, play a significant role in providing protection and indirectly modulating plant growth in the presence of abiotic stressors (Laoué *et al.*, 2022; Li *et al.*, 2021). We can interpret the enhancement of the plant's chemical defence mechanism as an advancement in the metabolic process of flavonoids, leading to their production in response to climatic stress conditions (Laoué *et al*., 2022).



(A) Flavanols





(B) Kaempferol **(C) Myricetin**

Figure 2.8: Chemical structure of types of flavonoids isolated from moringa plant materials (A) Flavonols (B) Kaempferol and (C) Myricetin (Li *et al.*, 2021).

The main flavonoids found in moringa leaves are myrecytin, quercetin, and kaempferol in concentrations of 5.8, 0.207, and 7.57 mg/g, respectively (Vergara-Jimenez *et al.*, 2017). Quercetin is found in the dried leaves of moringa and is a strong antioxidant (Vergara-Jimenez *et al.*, 2017). The extraction of flavonoids from moringa involves several technical processes. The first step is collecting and meticulously disinfecting recently obtained moringa leaves (Yerena-Prieto *et al.*, 2022). Furthermore, the leaves are obtained via the process of solvent extraction utilizing ethanol, methanol, or acetone (Yerena-Prieto *et al*., 2022). A second filtration is performed on the extract to remove any solid particles (Yerena-Prieto *et al.*, 2022). The filtrate is then concentrated using techniques such as rotary evaporation or vacuum distillation (Yerena-Prieto *et al.*, 2022). Following this, the concentrated extract is purified using techniques such as chromatography to separate particular flavonoids from other compounds (Yerena-Prieto *et al*., 2022). Further analysis of the extracted flavonoids is conducted using spectroscopic techniques including UV-Vis spectroscopy, mass spectrometry, and NMR to confirm their identification and purity (Yerena-Prieto *et al.*, 2022). The individual flavonoids are strategically stored to maintain their stability under ideal conditions. Rigorous compliance with safety measures is of utmost importance, and it is important to consult extensive recommendations for direction (Yerena-Prieto *et al.*, 2022).

2.7.6 Tannin

Tannins are a class of polyphenolic compounds that are inherent constituents of numerous plant species (Huang *et al.*, 2018). The aforementioned chemicals are present in the dermal, seed, and stem tissues of grapes, functioning as a means of protection against various animal and insect threats (Falcão and Araújo, 2018). Tannins possess a tactile quality and sensation, as opposed to a distinct gustatory attribute (Ghahri and Pizzi, 2018). The intensity of tannins can elicit a transient sensation of puckering or dryness, akin to consuming an unripe fruit or imbibing a robust infusion of black tea (Pizzi, 2019).

Previous studies have established a correlation between tannins and negative effects on various aspects of animal experimentation, including reduced feed intake, growth rate, feed efficiency, net metabolisable energy, and protein digestibility (Pizzi, 2019). Consequently, it is widely believed that meals containing high levels of tannins exhibit diminished nutritional content (Pizzi, 2019). According to recent scholarly investigations, the primary impact of tannins does not appear to be centered on the reduction of food consumption or digestion (Pizzi, 2019; de Hoyos-Martínez *et al.*, 2019). Instead, it is observed that tannins lead to a diminished efficacy in the conversion of ingested nutrients into new bodily constituents (de Hoyos-Martínez *et al.*, 2019). Tannins play a crucial role in the conservation of distinct plant species (Laka *et al.,* 2024). The accumulation of tannins within the bark of trees serves as a protective mechanism against bacterial and fungal infections (Frahat and Shehata, 2021). In this particular scenario, tannins effectively separate enzymes and other protein secretions originating from bacteria and fungi, thereby impeding their ability to cause infection in the tree.

Moringa leaves are characterised by a substantial presence of tannins. Complex polyphenol compounds have the ability to bind to and cause the precipitation of proteins, amino acids, alkaloids, and various other chemical molecules in aqueous solutions (Xu *et al.,* 2019). The concentration of tannins exhibits variation across different sections of the moringa tree, with the dried leaves demonstrating the highest concentration (Xu *et al.,* 2019). Tannins are present in seeds in limited quantities as well (Xu *et al.,* 2019).

Tannins can mess up nutrient cycling in several ways, including slowing down the rate of decomposition, forming complexes with proteins, making microbial communities toxic, and decreasing enzyme activity (Maisetta *et al.,* 2019). Consequently, tannins have the potential to mitigate nutrient losses in infertile environments and alter the process of nitrogen cycling, leading to an increase in the relative abundance of organic nitrogen compared to mineral nitrogen (Muhoza *et al.,* 2019). Increased levels of tannins can lead to allelopathic reactions, alterations in soil characteristics, and reduced productivity within ecosystems (Du Toit *et al.,* 2020). The aforementioned effects possess the capacity to alter or impact successional processes. Researchers have conducted extensive research to understand the role of tannins in nutrient dynamics, but many aspects of tannin biogeochemistry are still unknown.

The study by Du Toit and Vorster (2020) discovered that the leaves of moringa across all months had a higher content of tannin, and they increased as the season progressed from September to

May. They further stated that the winter season triggered an increase in tannin that could be due to the plants initiating ways of defense against the cold stress (Du Toit *et al.,* 2020).

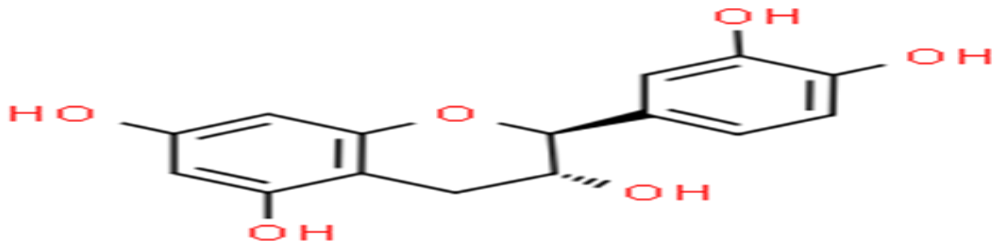


Figure 2. 9: Chemical structure of catechin, a type of tannic acid isolated from moringa plant material (Du Toit *et al.*, 2020).

Cathechin is a tannic acid found in moringa trees, as depicted in Figure 2.9. It is produced by crushing leaves and extracting them using solvents like ethanol or methanol (Budaraga and Putra, 2020). After achieving cleanliness, the extract undergoes particle removal by filtration, condensation by evaporation or rotary evaporation, and partitioning into its constituent parts by column chromatography or liquid-liquid extraction (Budaraga and Putra, 2020). The refined catechin undergoes further purification techniques, including recrystallization and preparative HPLC (Budaraga and Putra, 2020). Subsequently, the purified catechin is analysed for its distinct properties and degree of purity by methods such as NMR spectroscopy or mass spectrometry (Budaraga and Putra, 2020). Nevertheless, this process requires expertise in organic chemistry and strict compliance with safety protocols while handling solvents and chemicals (Budaraga and Putra, 2020).

2.8 The influence of VAM fungi on the biosynthesis and accumulation of phytochemicals

One advantage of VAM fungi is its ability to regulate the synthesis of primary and secondary compounds derived from plant metabolism (Mohammadi *et al.*, 2017). Researchers conducted studies on medicinally significant plants, revealing an increase in the production of biomolecules with medicinal properties (Abdelhalim *et al.,* 2022; Kumar *et al.,* 2017). Plants can synthesise compounds through secondary metabolism, which can imbue them with medicinal attributes (Laka *et al.,* 2024). Terpenes, nitrogen compounds, and phenolic compounds are the three primary classes from which these compounds originate (Kumar *et al.,* 2017). Vesicular Arbuscular Mycorrhizal (VAM) fungi inoculation is a potential alternative for augmenting the production of these compounds and enhancing the pytomass (del Rosario Cappellari *et al.,* 2015).

Abdelhalim *et al.* (2022) conducted a study that demonstrated that sorghum cultivars inoculated with VAM fungi exhibited a notably elevated concentration of phytochemicals (156.97%) in comparison to those that were not subjected to inoculation (74.48%). The study additionally concludes that the increase in the accumulation of phenolic compounds and antioxidant activity in sorghum grains following VAM fungi inoculation is a promising indication of the efficacy of this treatment and may serve as a viable approach to enhance the presence of health-promoting compounds in sorghum. Multiple studies have demonstrated that the establishment of symbiotic VAM fungi associations with plant roots has yielded notable improvements in the synthesis of various secondary compounds, including phenolic compounds (Kumar *et al.,* 2020; Santos *et al*., 2017; Jaleel *et al.*, 2009).

Kumar *et al.* (2020) observed in a recent study that the introduction of mycorrhiza to plant species altered their biochemical and molecular pathways. This, in turn, led to an increased accumulation of secondary metabolite compounds in various plant tissues (Laka *et al.,* 2024). This suggests that there is potential for commercial exploration of their application to enhance the presence of health-promoting phytochemical compounds in moringa (Laka *et al.,* 2024).

Furthermore, Silva *et al.* (2018) and Santis *et al.* (2017) have observed that mycorrhization not only promotes the synthesis of secondary compounds in various groups, but also enhances the biosynthesis of specific compounds that hold significant importance in the pharmaceutical sector.

Oliveira *et al.* (2019) observed an elevation in the foliar vitexinin concentration in yellow passion fruit. Thangavel *et al.* (2009) found that seedlings cultivated in the presence of VAM fungi exhibited enhanced growth, elevated levels of nutritional components (such as sugars, nitrogen, phosphorus, potassium, zinc, calcium, and manganese), increased total chlorophyll content, and higher concentrations of secondary metabolites in the leaves of patchouli plants. Notably, the magnitude of these enhancements varied depending on the specific species of VAM fungi utilized. Moreover, a positive correlation was observed between the concentration of phosphorus and all growth parameters as well as the content of phytochemical constituents except for essential oil . Another advantage of VAM fungi is their capacity to promote plant nutrient absorption, altering the content of secondary metabolites directly or indirectly (Chen *et al.*, 2017). For example, the study by Chen *et al.* (2017) discovered that root and shoot biomass, root system architecture, and flavonoid were enhanced by VAM fungi in Glycyrrhiza *uralensis* grown in phosphorus-deficient conditions. In summary, the application of VAM to medicinal plants presents a viable approach to enhancing both the quantity and quality of secondary metabolites that hold significance in the fields of pharmacology, medicine, and cosmetics. The study by Zhao *et al.* (2022) found that the application of VAM fungi to medicinal plants represents a viable strategy for enhancing the production of secondary metabolites with pharmacological, medical, and cosmetic significance.

2.9 The influence of VAM fungi on the biosynthesis and accumulation of phytochemicals while alleviating salinity stress.

According to Kumar *et al.* (2023), the secondary metabolic profile of plants in soils subjected to saline conditions exhibits an elevation in the enzymatic activity associated with the production of secondary metabolites. The activity of these enzymes tends to increase in response to elevated salt concentrations. Furthermore, when exposed to salt stress, plants can increase the expression of genes that encode additional enzymes involved in secondary metabolite production (Kumar *et al.*, 2023). Plants experience several stresses, including the manifestation of certain elicitors or signal molecules, which lead to the buildup of secondary metabolites (Jan *et al.,* 2021; Kumari, 2021). Kumar *et al.* (2023) state that several stimuli, such as injury, intense cold or heat, dehydration, and exposure to salt or light, can synthesise secondary metabolites. The plant's metabolic response to stress can also synthesise these chemicals (Kumar *et al.,* 2023). Secondary metabolites frequently contribute to a plant's defensive mechanism, encompassing both herbivore and pathogen defenses (Kumar *et al.,* 2023). Additionally, they possess the capability to facilitate communication among plants, both among themselves and with their immediate surroundings (Kumar *et al.,* 2023).

According to Kumar *et al.* (2023), alterations in a single environmental factor, while keeping all other factors unchanged, have the potential to impact the concentrations of secondary metabolites in a wide range of plant species. Kumar *et al.* (2023) asserts that salinity-induced ionic or osmotic pressure in plants can either positively or negatively influence the accumulation of specific secondary metabolites. Secondary metabolites protect the plant cells from oxidative damage resulting from ion accumulation at the cellular and sub-cellular levels. Salt stress can trigger the production of secondary metabolites, which help reduce the negative effects of salinity (Hossain *et al.,* 2017). According to Kumar *et al.* (2023), there is a decline in secondary metabolic profiles across several species when salinity levels increase. Various factors, including a reduction in cellular count and compromised metabolic enzyme activity, contribute to the decline in metabolic activity (Kumar *et al.,* 2023). Abiotic variables like elevated soil salinity and ion deposition negatively impact the growth and output of cultivated plants (Kumar *et al*., 2023). Excessive concentrations of sodium chloride (NaCl) hinder the growth of plants as a result of reduced hydraulic conductivity, leading to hypererosmotic stress and the accumulation of ions to levels that are detrimental to their proliferation, causing hypoteric stress (Kumar *et al.,* 2023). According to Kumar *et al.* (2023), plants undergo alterations in their biochemical and physiological processes as a result of these stressors.

According to Kumar *et al.* (2023), a number of studies have documented that salt stress has the potential to induce a reduction in the synthesis of secondary metabolites in plants. Chowdhary *et al.* (2021) did a study examining the impact of salt stress on basil plants. The findings revealed a reduction in the concentrations of specific secondary metabolites, such as β-carotene, cryptoxanthin, lutein flavonoids, and phenolic acids, in both the leaves and flowers. Yang *et al.* (2018) conducted a separate investigation that demonstrated a detrimental effect of salt stress on the concentrations of secondary metabolites, specifically stilbenoids and flavonoids, in grapevine leaves. Nevertheless, it is crucial to acknowledge that the impact of salt stress on secondary metabolism may differ based on the particular plant species and the relevant metabolites. Previous research has also documented a rise in the synthesis of specific secondary metabolites as a result of salt-induced stress, indicating that the association between salinity stress and secondary metabolism is intricate and remains incompletely comprehended. Plants generate secondary metabolites in response to salt stress, which aid in their adaptation to the altered environment. According to Kumar *et al.* (2023), these metabolites have the ability to assist plants in minimising water loss, enhancing their resistance against pests and diseases, and improving their tolerance to salt. Despite the fact that the synthesis of secondary metabolites, including phenols, saponins, flavonoids, carotenoids, and lignins, tends to increase in plants subjected to salt stress, previous research has only extensively examined a limited number of specific chemicals (Lucini *et al.,* 2016; Ramalingam *et al.,* 2015). Numerous studies have demonstrated the validity of the association between antioxidant activity and the accumulation of phenolic compounds in plants subjected to salt stress. Nevertheless, it has been observed that certain plant species exhibit greater resilience to the oxidative harm induced by salt stress. These plants are commonly characterised by their elevated levels of antioxidant activity (Kumar *et al.,* 2023). An example of a plant that exhibits notable antioxidant activity and resistance to salt stress is rosemary, as demonstrated by Abdal *et al.* (2022). Abdal *et al*. (2022) attributes the observed phenomenon to the elevated concentrations of polyphenols in rosemary, which possess potent antioxidant properties capable of shielding cells against oxidative stress-induced harm. Researchers have identified grapefruit, black tea, and green tea as additional plant species that exhibit resistance to salt stress and possess notable antioxidant activity (Abdal *et al.,* 2022).

According to Ghanem *et al.* (2021), significant quantities of secondary metabolites such as flavonoids, polyphenols, tannins, and anthocyanins have been identified. These compounds have the potential to enhance plants' salt tolerance by improving the efficacy of their antioxidant mechanisms. Flavonoids, a class of secondary metabolites, are abundantly present in plants and has the ability to enhance plants' salt tolerance (Kumar *et al.*, 2023). According to Kumar *et al.* (2023), polyphenols have the potential to enhance plants' tolerance to salt, whilst tannins and anthocyanine can aid in the plants' ability to withstand salt-induced damage. In their study, Yang *et al.* (2017) employed saponin as a priming agent to facilitate the growth of quinoa plants under saline conditions. This phenomenon can be attributed to the ability of saponins to eliminate reactive oxygen species (Yang *et al.*, 2017). By examining alterations in metabolites at the entire metabolome level and incorporating these changes in metabolic profiles with other "omic" investigations like genome, transcriptome, and proteome analysis, it is possible to decipher the regulatory networks and identify biomarkers that govern stress responses. These biomarkers can then be utilised to enhance plant performance (Sarri *et al.*, 2021; Kumar *et al.,* 2017).

When plants experience salt stress, applying VAM fungi can be the most effective approach to alleviating the stress. Researchers have reported that VAM fungi alleviate salt stress in plants through diverse pathways. According to Sherstha *et al.* (1995), the colonization of VAM fungi in citrus plants resulted in an increase in leaf area, photosynthesis, and phosphorus uptake by the plants. Furthermore, Hasheem *et al.* (2015)'s research revealed that the introduction of VAM fungi augmented antioxidant enzyme activity, facilitating the rapid removal of reactive oxygen species and minimising its impact on metabolic processes. Qun *et al.* (2007) further documented that seedling inoculated with VAM fungi and subjected to salinity stress exhibited elevated levels of antioxidant enzyme activities compared to seedlings not infected and exposed to salt stress. In accordance with previous research, Mehr *et al.* (2012) provided evidence of increased phenol buildup in Anethum graveolens when exposed to salinity-stressed soil through the inoculation of VAM fungi. Hasheem *et al.* (2015) conducted a study that further supports the idea that VAM fungi technology can mitigate the negative effects of salt stress on *P. turgidum's* growth, antioxidant system, and mineral nutrients. Consequently, the implementation of VAM fungi technology emerges as a viable approach to enhance crop production in stressful environments.

Moringa plants exhibit an increase in root length when grown in saline soil (Wassif *et al.*, 2012). These findings indicate that moringa roots exhibit a propensity to expand and elongate when subjected to stressful conditions. Wassif *et al.* (2012) found that moringa demonstrates enhanced survival at a concentration of 8 dS m^{-1}, despite a negligible decrease in its nutritional value. The antioxidant system of moringa exhibits a higher level of strength compared to other plants that are tolerant to salt, hence enabling its survival in the face of abiotic stress conditions (Wassif *et al.*, 2012). Hence, it can be posited that an elevation in salt content leads to an augmentation in specific secondary metabolites of moringa, thereby serving as a means to alleviate stress, similar to that observed in other plant species. Implementing VAM fungi can help mitigate salt stress and enhance the production of secondary metabolites while preserving the nutritional value of plants, as observed in other plant species. However, it is not possible to form a definitive conclusion due to the scarcity of studies conducted on the plant.

2.10 Conclusion

Moringa oleifera has been found to have no detrimental effects and can significantly aid in fulfilling nutritional requirements and reducing the likelihood of malnutrition. However, production is limited by abiotic factors such as high salinity levels. The mycorrhizal symbiosis, particularly VAM, plays a crucial role in soil fertility and sustainable crop production. However, there is still much to learn about mycorrhizas, and further research is needed to understand their diversity and the factors influencing their occurrence. Effective communication of information regarding the application of VAM fungi is crucial to encourage farmers to adopt its usage and promote sustainable agricultural practices. The research conducted on VAM associations in various crops has consistently shown positive effects on plant growth, nutrient uptake, and crop yield. However, the effectiveness of VAM fungi inoculation may vary depending on the specific plant species and environmental conditions, such as salinity stress. Research on tomatoes and cancer bush shows that VAM fungi enhance plant growth and development, even at low NaCl concentrations. However, definitive conclusions on moringa plants are needed due to concurrent VAM inoculation and Rock phosphate application.

The application of VAM inoculation holds significant potential to enhance the production of phytochemical compounds in medicinal plants, including moringa, under salinity stress. Vesicular-Arbuscular Mycorrhizal symbiosis can play a crucial role in improving the accumulation and biosynthesis of phytochemicals in moringa by mitigating the adverse effects of salinity and promoting metabolic activity. Salinity stress itself may trigger increased phytochemical accumulation as part of the plant's defense mechanisms, and this response could be further amplified through symbiosis with VAM. Exploring this interaction offers a promising opportunity to optimize the medicinal value of moringa and other plants grown under stressful environmental conditions.

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CHAPTER 3

VESICULAR ARBUSCULAR MYCORRHIZA (VAM) FUNGI EFFECT ON THE GROWTH OF *MORINGA OLEIFERA* UNDER SALINE STRESS CONDITIONS

3.1 Introduction

The demand for synthetic fertilizers to sustain agricultural output has been amplified by saline stress, as this stress adversely affects plant development and soil fertility, therefore diminishing the accessibility and absorption of vital nutrients by plants (Syed *et al.,* 2021). The challenges of increasing population necessitated increased crop production under global climate change with limited arable land. Nevertheless, the ongoing utilization of artificial synthetic fertilizers is not a viable long-term resolution. Although they may provide a short-term increase in agricultural productivity, in the long run, they might deteriorate the health of the soil, the quality of water, and the general equilibrium of the ecosystem (El-Ramady *et al.,* 2018). By contrast, the use of Vesicular Arbuscular Mycorrhizal (VAM) fungus as a biofertilizer presents a more environmentally reliable method. Vesicular Arbuscular Mycorrhizal fungus improves the accessibility of trace elements and stabilises phosphates in the soil without producing any negative impact on the environment (Ram *et al.,* 2007).

This characteristic renders VAM fungi especially advantageous in the restoration of deteriorated and abandoned areas. Extensive research has demonstrated that VAM fungus can augment plant growth and productivity by helping the plant adapt to adverse abiotic and biotic factors, thus fostering robust plant development (Kumari *et al.,* 2022; Hashem *et al.,* 2015). The increased utilisation of mycorrhizal fungi in South Africa needs more empirical data and practical applications. Vesicular Arbuscular Mycorrhiza fungus offers a feasible opportunity to enhance the economy and food security by increasing crop productivity, decreasing input expenses, and encouraging sustainable agricultural methods (Benami *et al.,* 2020). Hence, the objective of the study was to determine the effect of VAM fungi on the growth of *Moringa oleifera* and saline stress alleviation.

3.2 Material and methods

3.2.1 Study area

Two simultaneous studies were conducted at the Mbombela campus farm at the University of Mpumalanga (27°26’14.9’’S, 30°59’53.2’’E), Nelspruit, South Africa. The place receives an average annual precipitation of less than 867 mm and has mean temperatures ranging from 10°C to 38°C. Under the shade-net settings, the daytime temperatures measured between 20°C and 29°C, while the nighttime temperatures fluctuated between 14°C and 18°C, contingent upon the prevailing meteorological condition.

3.2.2 Experimental design and cultural practices

Experimental trials were conducted in two distinct settings: micro-plot (Experiment 1) and shade-net (Experiment 2). The trials were carried out in 2024 (May to June) using 4 x 4 complete factorial arrangement in a randomized complete block design (RCBD) with 5 replications. The experiments included four salinity levels (0, 0.25, 0.5, and 0.75 dS m^{+1}) of combined ratio of NaCl and $CaCl_2$ at a ratio of (3:1), as well as four levels of VAM fungus (0, 10 g, 20 g, and 30 g). A micro-plot experiment was conducted by placing 30 cm square plastic bags into 15 cm deep holes spaced 0.5 m inter and intra spacing. The shade nets were implemented using a 40% alunet shade cloth that was strung 2 metres above the ground. A shade-net experiment was conducted by arranging 30 cm plastic bags at intervals of 0.5 m inter × 0.5 cm intra spacing. Moringa seeds were planted in a 200-cell seeding tray using Hygromix (Hygrotech, Pretoria, South Africa) as the propagating medium. Two weeks after emerging, the seedlings were hardened off for one week by intermittently withdrawal from irrigation and then transplanted into 30 cm pots containing 3000 ml of steam-pasteurized loam and sandy soil in a 3:1 (v/v) proportion. The fungus employed was RRHIZ-UP, and the isolates identified in the product were *Funneliformis coronatum, Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *rhizophagus* irregularis species. Scouting and surveillance for insect pests were conducted biweekly, and hand hoes were used to manually weed among pots as needed. Throughout the experiment, plants were watered with 300 ml of water free of chlorine every alternate day, replacing treatments with irrigation once a week. Initially, the treatment was administered seven days after transplantation to enable the plants to endure transplanting stress.

(A) (B)

Figure 3.1: Experiment in a (A) shade net (B) microplot

3.2.3 Data collection

The plant parameters were measured on day 110 after initiating treatments. The monitored plant parameters included plant height, stem diameter, number of branches, root length, fresh weight, dry matter content and chlorophyll content. The height of the plants was measured from their crowns to the tips of their flag leaves, the stem diameter was measured using the Vernier calliper, number of branches were manually counted, and chlorophyll content was measured using the chlorophyll meter. The root length was measured using the measuring tape and fresh weight was determined using the weighing scale. Subsequently, the roots and shoots samples were dried at a temperature of 55°C in an oven for a duration of 72 hours for dry matter determination. Following the removal of roots from the shoots, the roots were cleansed to eliminate any residual soil particles, dried using a laboratory towel to remove any extra water, and subsequently weighed to ascertain their fresh weight before estimating their dry weight. The soil in each container was well blended, and subsequently, a 250 ml sample was extracted for the purpose of analysing the pH and electrical conductivity (EC). After undergoing air-drying and screening through a 2-mm mesh, a soil subsample weighing 10 grams was introduced into a glass beaker containing 25 ml of deionised water. The resulting mixture was vigorously spun for a duration of 5 seconds using a glass rod, followed by incubation for a period of 50 minutes (Rayment and Higginson, 1992). Following a 50-minute period, the contents were agitated once more and allowed to rest for 10 minutes. Subsequently, pH and EC measurements were obtained from the supernatant using a HANNA Smart Bench-Top pH/EC/DO Metre. The remaining 10 g ± 0.1g of airdried and screened soil was used to measure organic content. The 10 g ± 0.1 g soil sample were put inside a known weight dry crucible, the sample were then heat slowly in a muffle furnace (temperature raised in steps 100, 200, and 550°C). The final temperature of 550°C was maintained for 8 hours. After 8 hours the crucible containing greyish white ash was removed from the muffle furnace and cooled in a desiccator then weight was measured (Ball, 1964; Davies, 1974).

$$\text{Ash \%} = (W3 - W1)/ (W2 – W1)$$

$$\text{Organic matter \%} = 100 - \text{Ash \%}$$

W1 = the weight of the empty dry crucible; W2 = the weight of the dry crucible containing soil sample; and W3 = the weight of the dry crucible containing soil after ignition.

3.2.4 Data analyses

This study employed the Shapiro-Wilk test to assess the adherence of the data to a normal distribution (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). The analysis of the data was conducted using Statistix 10.0. The determination of the total treatment variation (TTV) involved the division of the mean sum of squares (MSS) for each variable. A Tukey's HSD test was employed to distinguish the means with a significance level of 5% (Mashela *et al.*, 2015). The results discussed only parameters that were significant at the probability level of $p \leq 0.05$, unless otherwise stated. The quadratic equation relations ($Y = ax^2 + bx + c$) were estimated using regression curves to model the relationships between the independent and dependent variables. These estimates were computed using Excel, with $x = -b/2a$, to determine the optimal values of accumulation of secondary metabolites (Mashela *et al.*, 2015).

3.3 Results

3.3.1 Plant Variables

Among the applied treatments salt, VAM, and Salt*VAM had a significant impact ($p \leq 0.05$) on the measured plant variables. Salts had an impact on plant height, number of branches, chlorophyll concentration, and stem diameter with a total treatment variation (TTV) of 47.66%, 36.67%, 42.72%, and 64.00% respectively, in Experiment 1 (Table 3.1). Relative to the control, the study showed that plant height, number of branches, stem diameter, and chlorophyll content exhibited a decline from 38.87% to 47.88%, 2.44% to 34.21%, 8.51% to 23.40%, and 45.44% to 53.83%, of the respective variables (Table 3.2). In Experiment 2, the application of salt had a substantial effect ($p \leq 0.05$) on stem diameter, chlorophyll concentration, and number of branches, resulting in a TTV of 14.56%, 56.08%, and 44.40%, respectively (Table 3.3). Relative to the control, salt treatments resulted in the decline of the stem diameter, chlorophyll content, and number of branches by 7.50% to 22.50%, 13.39% to 54.96%, and 14.56% to 27.19%, respectively ($p \leq 0.05$).

Table 3.1: Source of variation affecting plant height, number of branches, chlorophyll content, and stem diameter of *Moringa oleifera* under microplot conditions (Experiment 1).

		Plant height		Number of branches		Chlorophyl content		Stem diameter	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	4	18.85	2.94^{ns}	11.62	30.86^{ns}	22.68	3.21 ^{ns}	0.018	18.00 ^{ns}
Salt	3	306.09	47.66***	13.81	36.67**	301.83	42.72***	0.064	64.00***
VAM	3	291.78	45.43***	4.08	10.38^{ns}	310.43	43.94***	0.002	2.00^{ns}
Salt*VAM	9	17.31	2.70**	3.66	9.72^{ns}	65.68	9.30***	0.007	7.00^{ns}
Error	60	8.18	1.27^{ns}	4.49	11.92^{ns}	5.84	0.83 ^{ns}	0.004	4.00^{ns}
Total	79	642.21	100	37.66	100	706.46	100	0.10	100

***Highly significant at $p \leq 0.001$, **significant at $p \leq 0.05$, ^{ns} not significant, TTV (%) Total treatment Variation

Table 3.2: The effect of salt on plant height (PH), number of branches (NB), chlorophyll content (CC) and stem diameter (SD) of *Moringa oleifera* at 110 days after initiation of treatment application under microplot conditions (Experiment 1).

Salt	PH	RI (%)	NB	RI (%)	CC	RI (%)	SD	RI (%)
0	17.65^{a}	0	5.32^{a}	0	15.01^{a}	0	0.47^{a}	0
0.25	10.79^{b}	-38.87	5.19^{ab}	-2.44	8.19^{b}	-45.44	0.43^{a}	-8.51
0.5	10.13^{b}	-42.61	4.58^{ab}	-13.91	7.20^{b}	-52.03	0.36^{b}	-23.40
0.75	9.20^{b}	-47.88	3.50^{b}	-34.21	6.93^{b}	-53.83	0.36^{b}	-23.40

[y]Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 3. 3: Source of variation affecting stem diameter, chlorophyll content and number of branches of *Moringa oleifera* under shade net conditions (Experiment 2).

	Stem Diameter			Chlorophyll content		Number of branches	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	4	0.093	13.68^{ns}	3.91	4.24^{ns}	3.20	16.60^{ns}
Salt	3	0.099	14.56***	51.70	56.08**	8.56	44.40***
VAM	3	0.006	0.88^{ns}	5.46	5.92^{ns}	4.73	24.53**
Salt*VAM	9	0.039	5.74^{ns}	17.00	18.44^{ns}	1.18	6.12^{ns}
Error	60	0.445	65.44^{ns}	14.12	15.32^{ns}	1.61	8.35^{ns}
Total	79	0.68	100	92.19	100	19.28	100

***Highly significant at p≤0.001, **significant at p≤0.05, ^{ns} not significant, TTV (%) Total treatment Variation.

Table 3.4: The effect of salt on stem diameter, chlorophyll content and number of branches of *Moringa oleifera* at 110 days after initiation of treatment application under shade net conditions (Experiment 2).

Salt (dS m^{-1})	Stem Diameter	RI (%)	Chlorophyll Content	RI (%)	Number of branches	RI (%)
0	0.40^{a}	0	6.35^{a}	0	5.70^{a}	0
0.25	0.37^{ab}	-7.50	5.50^{ab}	-13.39	4.87^{ab}	-14.56
0.5	0.35^{ab}	-12.50	3.66^{ab}	-42.36	4.65^{ab}	-18.42
0.75	0.31^{b}	-22.50	2.86^{b}	-54.96	4.15^{b}	-27.19

^{y}Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

In Experiment 1, the VAM fungi had a substantial impact ($p \leq 0.05$) on both plant height and chlorophyl concentration, with a TTV of 45.43% and 43.94% respectively (Table 3.1). Relative to the control the presence of VAM fungus had a favourable impact on both the plant height and chlorophyl content, increasing both respective variables by 16.04% to 102.65% and from 9.09% to 141.58% correspondingly (Table 3.5). Experiment 2 showed that VAM fungus had a substantial impact ($p \leq 0.05$) on the number of branches, resulting in a TTV of 24.53% (Table 3.3). The effect of VAM did not increase the number of branches, number of branches declined by a relative effect of 5.74% to 17.78% (Table 3.7). The computed optimum is below the expected range between 10 to 30 g VAM but ranged between 0 to 10 g VAM (Table 3.6).

Table 3.5: The effect of Vesicular Arbuscular Mycorrhiza (VAM) on plant height and chlorophyll content of *Moringa oleifera* at 110 days after initiation of treatment application under microplot conditions (Experiment 1).

VAM (g)	Plant Height	RI (%)	Chlorophyll content	RI (%)
0	8.29^{a}	0	5.94^{a}	0
10	9.62^{b}	16.04	6.48^{b}	9.09
20	13.05^{bc}	57.42	10.48^{bc}	76.43
30	16.80^{c}	102.65	14.35^{c}	141.58

^{y}Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

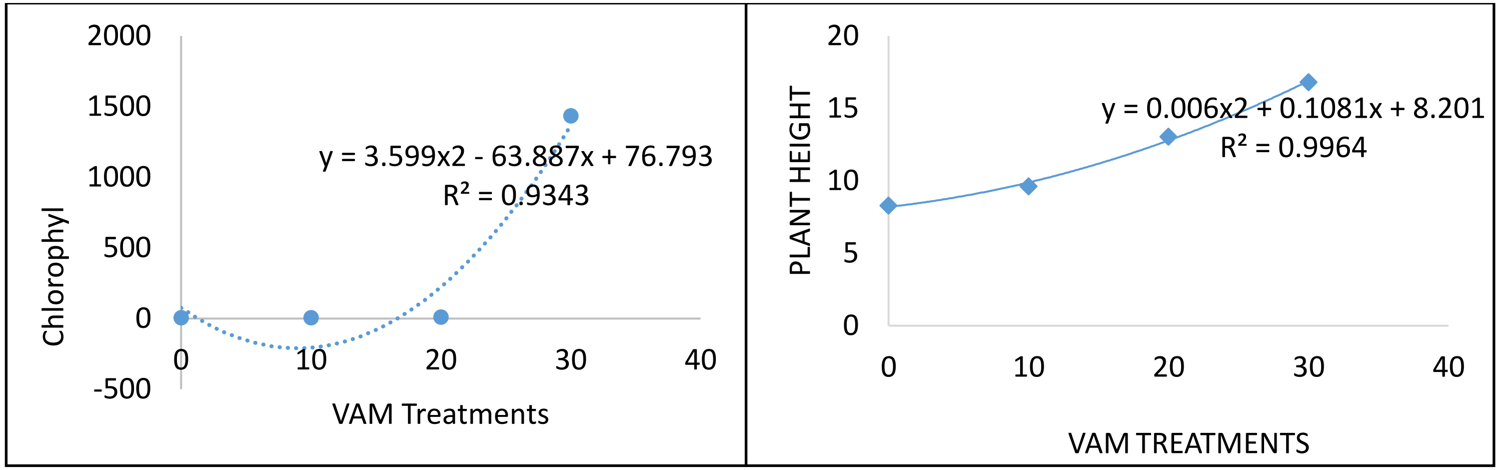

Figure 3.2: The effect of Vesicular Arbuscular Mycorrhiza (VAM) on chlorophyll and plant height of moringa.

Table 3.6: Quadratic relationship, coefficient of determination and computed optimum Vesicular Arbuscular Mycorrhiza (VAM) concentration for chlorophyll and plant height of moringa.

Variable	Quadratic relation	R_2	xz
Chlorophyll	$y = 3.5599x^2 - 63.887x + 76.793$	0.93	0.028
Plant height	$y = 0.006x^2 + 0.1081x + 8.201$	0.97	0.028
Mean		0.056	

zx = $b1/2b_2$, where x is optimum concentration.

Table 3.7: The effect of Vesicular Arbuscular Mycorrhiza (VAM) on number of branches of *Moringa oleifera* at 110 days after initiation of treatment application under shade net conditions (Experiment 2)

VAM (g)	Number of branches	RI (%)
0	5.40^{a}	0
0.25	5.09^{a}	-5.74
0.5	4.45^{b}	-17.59
0.75	4.44^{b}	-17.78

^{y}Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

In Experiment 1, results showed that the interaction between salt and VAM fungus had a significant effect ($p \leq 0.05$) on plant height and chlorophyl concentration, with a TTV of 2.70% and 9.30%, respectively (Table 3.3). While in Experiment 2, the interaction of VAM and salinity didn't have a significant effect ($p > 0.05$) on the measured plant variables. The interaction between 0 g of VAM fungus and various levels of salt treatment resulted in a notable reduction in plant height. Conversely, when 10 g, 20 g, and 30 g of VAM fungus were combined with different levels of salt treatment, plant height increased. The greatest increase of 42.86% was observed at 30 g VAM and 0 dS m^{-1} of salt interacted (Table 3.8). The chlorophyll content exhibited a similar rise by 5.26% when 30g of VAM fungi and 0 dS m^{-1} of salt interacted (Table 3.8).

Table 3.8: The effect of Vesicular Arbuscular Mycorrhiza (VAM) and salt interaction on plant height and chlorophyll content of *Moringa oleifera* at 110 days after initiation of treatment application under microplot conditions (Experiment 1).

VAM (g)	Salt (dS $m^{-1)}$	Plant Height	RI (%)	Chlorophyll content	RI (%)
0	0	16.8^{a}	0	19.00^{a}	0
0	0.25	7.20^{b}	-57.14	2.28^{b}	-88.00
0	0.5	4.56^{c}	-72.86	1.18^{c}	-93.79
0	0.75	4.60^{c}	-72.62	1.13^{c}	-94.05
10	0	13.62^{d}	-18.93	7.44^{c}	-60.84
10	0.25	9.00^{e}	-46.43	7.80^{d}	-58.95
10	0.5	8.88^{ef}	-47.14	5.28^{e}	-72.21
10	0.75	7.00^{f}	-58.33	5.40^{e}	-71.58
20	0	16.20^{g}	-3.57	13.60^{f}	-28.42
20	0.25	12.56^{h}	-25.24	9.10^{fg}	-52.11
20	0.5	12.86^{h}	-23.45	10.22^{fg}	-42.21
20	0.75	10.60^{i}	-36.9	9.00^{g}	-52.63
30	0	24.00^{j}	42.86	20.00^{h}	5.26
30	0.25	14.40^{k}	-14.29	13.60^{i}	-28.42
30	0.5	14.20^{k}	-15.48	11.80^{jk}	-37.89
30	0.75	14.60^{k}	-13.10	12.00^{k}	-36.84

[y]Column means ± standard error followed by the same superscripts were not different ($P \leq 0.05$) according to Turkey's HSD test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

3.3.2 Soil variables

The implementation of salt treatment had a significant effect ($p \leq 0.05$) on both the pH and electrical conductivity in both Experiment 1 and 2. As observed in Experiment 1, salts treatments had a TTV of 56.42% and 82.37%, of the respective soil variables (Table 3.9). In Experiment 2, the TTV was 62.30% and 28.39% on pH and EC, respectively (Table 3.9). Relative to the control, the salts increased the pH by 13.22% to 25.74% in Experiment 1, while the EC fell from 5.56% to 33.33 (Table 16). By contrast, in Experiment 2, the pH and EC both declined from 2.04% to 20.92% and 5.56% to 44.44%, respectively (Table 3.10).

Table 3.9: Source of variation affecting pH and EC of *Moringa oleifera* in Experiment 1 and 2.

		Experiment 1				Experiment 2			
		pH		EC		pH		EC	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	4	0.22	1.14^{ns}	0.001	2.22^{ns}	1.38	7.86^{ns}	0.001	0.32^{ns}
Salt	3	10.86	56.42***	0.030	66.67***	10.94	62.30***	0.088	28.39***
VAM	3	4.81	24.99***	0.009	20.00**	4.13	23.52***	0.027	8.71**
Salt*VAM	9	2.24	11.64**	0.002	4.44***	0.57	3.25^{ns}	0.022	7.10^{ns}
Error	60	1.12	5.82^{ns}	0.003	6.67^{ns}	0.54	3.08^{ns}	0.168	54.19^{ns}
Total	79	19.25	100	0.045	100	17.56	100	0.31	100

***Highly significant at p≤0.001, **significant at p≤0.01, ^{ns} not significant, TTV (%) Total treatment Variation

Table 3.10: The effect of salt on pH and EC of *Moringa oleifera* on the 110th day after initiation of treatment application in Experiment 1 and 2.

Salt (dS m^{-1})	Experiment 1				Experiment 2			
Salt	pH	RI (%)	EC	RI (%)	pH	RI (%)	EC	RI (%)
0	5.75^{a}	0	0.18^{a}	0	7.36^{a}	0	0.18^{a}	0
0.25	6.51^{b}	13.22	0.17^{b}	-5.56	7.21^{a}	-2.04	0.17^{a}	-5.56
0.5	7.32^{bc}	27.30	0.10^{bc}	-44.44	6.32^{b}	-14.13	0.12^{b}	-33.33
0.75	7.23^{c}	25.74	0.12^{c}	-33.33	5.82^{b}	-20.92	0.10^{b}	-44.44

^{y}Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

The utilization of VAM fungi had a significant effect ($p \leq 0.05$) on both the pH and electrical conductivity in both trials. In Experiment 1, VAM fungi had a TTV of 24.99% and 20.00% on pH and EC, respectively (Table 3.9). In Experiment 2, the TTV was 23.52% and 8.71% on pH and EC, respectively (Table 3.9). The pH dropped from 3.87% to 10.22% relative to the control while the electrical conductivity (EC) rose from 8.33% to 33.33% as the application of VAM fungus increased in Experiment 1 (Table 3.11). By contrast, in Experiment 2, both pH and EC relative to the control declined from 4.98% to 13.69% and 5.88% to 29.41%, respectively (Table 3.11).

The interaction between VAM fungus and salt treatment had a notable impact ($p \leq 0.05$) on pH and EC, particularly in Experiment 1. While in Experiment 2, VAM fungus and salt treatment had no notable impact ($P > 0.05$) on pH and EC. The TTV was 11.64% for pH and 8.68% for EC (Table 15). A rise in pH from 13.39% to 24.74% was accompanied by a decline in EC from 64.70 to 79.41% relative to the control (Table 3.12). The increase was observed at 20 and 30g when the salt content was at 0.

Among the treatments, the application of salt treatment had substantial influence on soil organic matter in Experiment 1 and 2. Organic matter was recorded higher in non-saline soils compared to saline soils (Table 3.13). In both experiments when salt treatment increased, the soil organic matter drastically decreased from 0.52 to 0.22% in Experiment 1 and 0.29 to 0.06% in Experiment 2 (Table 3.12). The application of VAM fungi alone increased the soil organic matter in Experiment 1 and 2 from 0.85 to 0.98% and 0.30 to 0.41% respectively (Table 3.13). Similarly, the interaction of VAM fungi and salt increased soil organic matter in both experiments. In Experiment 1, organic matter content increased from 0.85 to 0.92% and increased from 0.18 to 0.84% in Experiment 2 (Table 3.12).

Table 3.11: The effect of VAM on pH of *Moringa oleifera* at 110 days after initiation of treatment application in Experiment 1 and 2.

	Experiment 1				Experiment 2			
VAM	pH	RI (%)	EC	RI (%)	pH	RI (%)	EC	RI (%)
0	7.24^{a}	0	0.12^{a}	0	7.23^{a}	0	0.17^{a}	0
0.25	6.96^{ab}	-3.87	0.13^{b}	8.33	6.87^{ab}	-4.98	0.16^{ab}	-5.88
0.5	6.10^{ab}	-15.75	0.15^{c}	25.00	6.37^{bc}	-11.89	$0.13a^{b}$	-23.53
0.75	6.50^{b}	-10.22	0.16^{c}	33.33	6.24^{c}	-13.69	0.12^{b}	-29.41

^{y}Column means ± standard error followed by the same superscripts were not different (p≤ 0. 05) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 3.12: The effect of VAM and salt interaction on pH and EC of *Moringa oleifera* at 110 days after initiation of treatment application under microplot conditions (Experiment 1).

VAM (g)	Salt (dS m^{-1})	pH	RI (%)	EC	RI (%)
0	0	5.90^{a}	0	0.34^{a}	0
0	0.25	6.69^{b}	13.39	0.12^{b}	-64.70
0	0.5	8.49^{c}	43.90	0.06^{c}	-82.35
0	0.75	7.91^{cd}	34.07	0.15^{d}	-55.88
10	0	6.49^{de}	10	0.27^{e}	-20.59
10	0.25	6.08^{de}	3.05	0.26^{e}	-23.53
10	0.5	7.25^{ef}	22.88	0.11^{f}	-67.65
10	0.75	8.02^{f}	35.93	0.06^{g}	-82.35
20	0	5.49^{gh}	-6.95	0.41^{h}	20.59
20	0.25	6.89^{hi}	16.78	0.11^{i}	-67.65
20	0.5	6.38^{hi}	8.14	0.25^{j}	-26.47
20	0.75	5.65^{ij}	-4.24	0.08^{k}	-76.47
30	0	5.13^{ij}	-13.05	0.46^{l}	35.29
30	0.25	6.37^{jk}	7.97	0.29^{m}	-14.70
30	0.5	7.17^{k}	21.52	0.09^{n}	-73.53
30	0.75	7.36^{k}	24.74	0.07^{n}	-79.41

[y]Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 3.13: The effect of Vesicular Arbuscular Mycorrhiza (VAM) and salt interaction on soil organic matter at 110 days after the initiation of treatments under Shade net (Experiment 2) and Microplot (Experiment 1) conditions.

VAM	Salt (dS m^{-1})	Organic Matter (%) -		Intensity	
		Shade net	Microplot	Shade net	Microplot
0	0	0.76	0.91	High	High
0	0.25	0.29	0.52	Low	Moderate
0	0.5	0.10	0.30	Low	Moderate
0	0.75	0.06	0.22	Low	Low
10	0	0.30	0.85	Moderate	High
10	0.25	0.18	0.71	Low	High
10	0.5	0.24	0.66	Low	High
10	0.75	1.10	0.42	High	Moderate
20	0	0.20	0.98	Low	High
20	0.25	0.37	0.92	Moderate	High
20	0.5	0.61	0.87	High	High
20	0.75	0.12	0.82	Low	High
30	0	0.41	0.78	Moderate	High
30	0.25	0.05	0.76	Low	High
30	0.5	0.30	0.69	Moderate	High
30	0.75	0.84	0.66	High	High

3.4 Discussion

3.4.1 Plant variables

The results of the study showed that salinity had a negative effect on moringa plant growth. Salinity exerts a substantial impact on plant growth by modifying the absorption of water and nutrients, therefore inducing stress and contributing to plant toxicity. Masenya *et al*. (2023) reported similar observations when salts were applied on cancer bush (*Sutherlandia frutescens*). The increased concentration of salt in the soil has adverse effects on the physiological and biochemical characteristics of plants, resulting in a reduction in plant biomass and economic productivity (Seeda *et al.*, 2022). *Moringa oleifera* has been recognized as exceptionally resistant to the effects of salt stress. Results of the current study indicate a negative correlation between salt stress and growth indices including plant height, branch count, stem diameter, and chlorophyll concentration. A modest drop in growth parameters was seen at 0.5 dS m^{-1}, while a significant reduction in plant parameters was reported when salt concentration reached 0.75 dS m^{-1}. The present findings support the conclusions of prior investigations that the primary consequences of salt stress include a decrease in the rate of growth (Seeda *et al.*, 2022; Safdar *et al.*, 2019). The first effect is a decrease in the plant's water absorption capacity, resulting in a deceleration of growth. This is the manifestation of osmotic or water shortage caused by salinity. Furthermore, it can infiltrate the transpiration stream and ultimately damage cells in the transpiring leaves, hence further constraining growth. Ali *et al.* (2023) likewise noted that the plant characteristics of *Vigna radiata* L. decreased under conditions of extreme salt stress (6 dS m^{-1}).

The current study showed that Vesicular-Arbuscular Mycorrhizae (VAM) fungus had a positive influence on plant growth in both trials. Consistent with the findings of Alikamanoglu and Sen (2011) and Abou El-Leel *et al.* (2018) in their studies, where VAM fungus promoted plant growth by improving nutrient absorption, stress tolerance, and disease resistance. Consistent with this research, Bokhari *et al.* (2023) found that the use of VAM fungus effectively in both potted and field studies, and significantly improved plant development. Several studies have also emphasized the beneficial influence of VAM fungus on the physiological growth and development of plants (Koshariya *et al.*, 2023; Chandrasekaran, 2022).

In the current work, the combined application of salt and VAM fungus revealed a more significant positive effect on the growth and development of *Moringa oleifera*. Elevated salt concentration has been shown to inhibit the growth of moringa plants. However, the negative impacts of salt were mitigated when VAM fungus interacted with the salt treatment. The study conducted by Ebrahim and Saleem (2017) revealed that the introduction of VAM fungus to control plants (non-saline) resulted in enhanced plant development. However, the introduction of VAM-fungus to NaCl-stressed plants mitigated the negative impacts of salt. Moderate sodium chloride (NaCl) concentrations allowed the plant to effectively withstand the detrimental impacts of salinity (Ebrahim and Saleem, 2017). Moreover, Masenya *et al.* (2023) demonstrated that the presence of VAM fungi enhanced plant growth under saline stress, suggesting a potential symbiotic relationship that mitigates the detrimental effects of salinity on plant development. The findings presented here are consistent with the research conducted by Heikham *et al.* (2019) and Frahat and Shehata (2021).

3.4.2 Soil variables

In Experiment 1, the application of salt to the soil resulted in a little increase in the pH range from 5.75 to 7.232 and a decrease in the EC from 5.56 to 33.33. However, in Experiment 2, the pH and EC values declined as the salt concentration increased. An investigation conducted by Tan and Thanh (2021) in the laboratory found that the pH range of soil dropped from 5.14-5.72 to 4.08-5.14 as the soil salinity rose from 0 to 25%. Additionally, the EC of saline soils also showed a substantial increase. Although the pH of the soil does not have a direct impact on its electrical conductivity, it is likely to influence the solubility of salts and the moisture content of the soil, which subsequently influences the EC of the soil (Aizat *et al.,* 2014). Salt content in soil or water plays a crucial role in determining both pH and EC. The application of saline water for irrigation might result in the buildup of salts (Aizat *et al.,* 2014). This can result in either acidic or alkaline soil conditions, depending on the specific salts employed. As the salt concentrations grow, the EC of the soil or water likewise increases due to the dissociation of salts into ions, which are highly efficient conductors of electricity.

The current findings from both trials indicated a disparity in pH and EC. In Experiment 1, the pH and EC both reached higher values, but in Experiment 2, both the EC and pH declined. The observed pH and EC values differences between Experiment 1 and Experiment 2 may be attributed to variations in environmental conditions such as temperature, moisture, and precipitation. Temperature, in particular, plays a significant role in the dissociation of salts in the soil (Wang *et al.,* 2024). As temperature increases, the solubility of salts also rises, leading to greater ion mobility and availability in the soil solution (Wang *et al.,* 2024). This increased ion concentration can result in higher EC and a shift in pH, depending on the nature of the ions involved (Wang *et al.,* 2024). Therefore, the differences in pH and EC values between the two experiments are likely due to the distinct environmental conditions in each case.

The treatment of VAM fungus resulted in a reduction in soil pH in both trials. In Experiments 1 and 2, the pH range declined from 7.249 to 6.50 and 7.23 to 6.24, respectively. In contrast, Mulyadi and Jiang (2023) reported a rise in pH levels upon the application of VAM fungus. These findings are comparable to those reported in the study conducted by Maighal e*t al.* (2016). The pH range of typical soils found in landscape planting is between 4.5 and about 8.0, and VAM fungus are generally present over this whole range (Tedersoo *et al.,* 2020). Nevertheless, there seemingly exist some pH preferences among both plants and mycorrhizal fungus. The impact of soil pH on plant growth and establishment is not a decisive determinant of the success of mycorrhizal inoculation (Tedersoo *et al.,* 2020). Although it may influence plant development and establishment, both plants and their fungal partners have the ability to actively modify this crucial factor for the advantage of the host plant and their fungal partners (Tedersoo *et al.,* 2020).

While EC has traditionally been used to quantify soil salinity, it can also serve as an approximation for other soil characteristics, such as soil depth, in non-saline soils. Electrical conductivity is affected by the concentration of soluble salts in a solution and the temperature of the soil. Although it does not directly impact plant growth, soil EC has been employed as an indirect measure of nutrient availability for plant absorption and salinity levels (Bañón *et al.,* 2021). The present work found that the EC marginally improved with the application of VAM fungi in Experiment 1. However, in Experiment 2, the EC decreased with the increase in VAM fungal application. The two trials were conducted in different atmospheric conditions, one subjected to direct sunlight and the other being not. Experiment 1 was subjected to precipitation on the experimental site and direct sunlight, while Experiment 2 was shielded from both rainfall and sunlight by an aluminet shade cloth. The disparity in EC can be attributed to the two distinct surroundings. Doane *et al.* (2019) argued that sunshine should be classified as one of the variables that affect dynamic soil properties, along with soil pH and EC. The soil temperature is subject to daily fluctuations due to the sun, whereby as the sun warms, the soil also warms (Onwuka and Mang, 2018). When the temperature of soil water is below the freezing point, electrical conductivity drops by approximately 2.2% per degree centigrade (Onwuka and Mang, 2018). This decrease is caused by the increased viscosity of water and reduced mobility of ions. Conversely, EC increases when the water table is high (Smagin *et al.,* 2018). Hence, the EC of both experiments exhibits variation. The work of Alkobaisy *et al.* (2020) conducted in pots at the field supports Experiment 2 but contradicts Experiment 1 by showing a reduction in both pH and EC on the application of VAM fungus.

The concurrent use of VAM fungus and salt treatment has resulted in a rise in soil pH and a decrease in soil EC. The greatest soil pH was reported at 0g VAM fungus with a salt treatment of

0.5 dS m^{-1} (43.90%) and at 20g VAM fungus with a salt treatment of 0 dS m^{-1} (-13.05%). The present investigation is in direct opposition to the research conducted by Garg and Manchanda (2008). The research demonstrated an increased electrical conductivity in the presence of VAM fungus (Garg and Manchanda, 2008). The study conducted by Yan *et al.* (2023) revealed a positive correlation between pH and EC. The findings of Hu *et al.* (2019) provide evidence that the use of VAM fungus has a notable impact on soil condition through the elevation of pH of the soil.

Soil organic matter is an interconnected combination of plant, animal, and microbial remains at different stages of decomposition (Benbow *et al.,* 2019). Plants and microbial populations are often the most significant contributors to soil organic matter (Benbow *et al.,* 2019). Soil salinization affects different soil properties that directly also influence organic content of the soil (Benbow *et al.,* 2019). Saline soils accelerate the decomposition of organic materials and lowers soil microbial biomass and activity, which affects carbon dioxide flows. The present results highlighted the direct impact salt has on organic matter (Benbow *et al.,* 2019). The results showed a decrease in organic matter when salt treatment increased in both experiments. The results are supported by the study of Zhang *et al.* (2019), the research demonstrated a decrease in organic matter when salt increased. Increasing Na+ concentrations harm soil structure, permeability, and plant development by reducing the exchangeable capacity of organic molecules and nutrient bioavailability (Mohamed, 2017; Horneck *et al*., 2011).

The application of VAM fungi plays a crucial role in the amendments of soil organic matter (Frey, 2019). The rhizosphere is a natural environment with diverse interactions between soil fungi and plants (Frey, 2019). Beneficial plant-microbe interactions in the rhizosphere are key factors in plant health and soil fertility (Frey, 2019). Plant inoculation with microbial symbionts, such as mycorrhizal fungi, aids in plant establishment while also improving soil physicochemical and biological qualities (Frey, 2019). The present study has shown an increase in soil organic matter when VAM fungi was applied in both experiments. The study is corroborated by the study of

Sandhya *et al.* (2013), which discovered that when VAM fungi were applied to the soil, organic matter content increased. The interaction between VAM fungi and salt treatment has shown to alleviate the detrimental effects of salt on organic matter. The organic matter content increased in the presence of both VAM fungi and salt treatment. The result of the current study is supported by the study of Medina and Azcón (2010). Their study highlighted that VAM fungi alleviates salt impact on soil organic matter.

3.5 Conclusion

Elevated salinity levels impaired plant growth and development. Nevertheless, the use of VAM fungus has demonstrated the potential to alleviate the negative effects of salinity stress on plant growth. Research findings indicate that the introduction of VAM fungus into soil without saline stress greatly enhances the productivity of moringa. Furthermore, inoculating VAM fungus on soil under salt stress mitigates the detrimental impacts of salinity. This was shown by improved soil organic matter and reduced soil pH. The study revealed that Moringa *oleifera* had limited tolerance to salt stress, as it was significantly impacted by even a moderate amount of salt. Nevertheless, it is not possible to definitively show that moringa is not tolerant to mild salt, as the salt tolerance feature of the plant is mostly influenced by the environment and season to which it is exposed.

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CHAPTER 4

BIOSYNTHESIS AND ACCUMULATION OF PHYTOCHEMICALS OF MORINGA INFLUENCED BY THE APPLICATION OF VAM IN REDUCING SALINITY

4.1 Introduction

Moringa *oleifera* is acknowledged as a promising and cost-effective reservoir of phytochemicals. One of its notable pharmacological features is its anti-cancer activity (Singh *et al.,* 2020). The plant's pharmacological characteristics are intricately linked to the existence of its bioactive components, such as flavonoids (Singh *et al.,* 2020). Moringa is a highly abundant source of polyphenols, including flavonoids, phenolic acids, and tannins (Cornelius, 2019). These compounds constitute a significant group of phytochemicals that possess either one (phenolic acids) or many phenol rings (flavonoids) in their chemical composition (Ma *et al.*, 2020). The concentration of phytoconstituents in the moringa plant varies. For instance, Sankhalkar and Vernekar (2016) conducted a study that revealed that the total flavonoid content in moringa leaf and flower differed, with values of 4.44 mg/mL and 4.41 mg/mL, respectively. In addition, the phytochemical composition found in plants is contingent upon the specific geographical location and prevailing environmental circumstances (Sankhalkar and Vernekar, 2016). Plant metabolism can be influenced by abiotic environmental stressors, resulting in the suppression or enhancement of secondary metabolites (Sankhalkar and Vernekar, 2016).

Abiotic stressors encompass the process of soil salinization. Salt-stressed plants typically produce several secondary metabolites such as polyamines, polyphenols, and flavonoids (Nouman *et al.,* 2018). These metabolites function as antioxidants and metal ion chelators, safeguarding the plants against oxidative damage induced by salt stress (Nouman *et al.,* 2018). Furthermore, the buildup of these chemicals can assist in augmenting osmotic pressure, enabling plants to effectively manage the osmotic stress induced by salt (Nouman *et al.,* 2018). Conversely, plants experiencing salt stress may also reduce the production of specific secondary metabolites, such as terpenes and alkaloids, which can be harmful to plants when found in excessive amounts (Nouman *et al.*, 2018). Hence, the magnitude of salt stress significantly influences the accumulation or reduction of secondary metabolites in plants subjected to salt stress. Research has indicated that the utilization of VAM has the potential to increase water relations and bolster resilience to environmental pressures, such as salinity (Ostadi *et al.*, 2022; Al-Arjani *et al.*, 2020; Li *et al.*, 2019; Moradtalab *et al.*, 2019; Eulenstein *et al.*, 2016). In addition, multiple studies have indicated that VAM aids plants in mitigating salt stress by augmenting the functions of antioxidant enzymes. These studies have observed greater activity of antioxidant enzymes in plants inoculated with VAM compared to plants not inoculated with VAM (Garg and Manchanda, 2008; Alguacil *et al.*, 2003; Feng *et al.*, 2002). The study's objective was to determine whether the biosynthesis and accumulation of phytochemicals in moringa would be influenced by VAM fungi and salinity.

Methods and materials

4.2.1 Study area

The study was conducted as previously described (Chapter 3) also in (May to June) 2024 under microplot and shade net conditions.

4.2.2 Experimental design and cultural practices

The experimental design and cultural practices are similar to the previously described (Chapter 3). The experiment had 3 replications.

4.2.3 Data collection

Following a 110-day growth period, *Moringa oleifera* plants were harvested and processed for analysis to assess their phenolic and flavonoid content. The harvested plants were transported to the University of Mpumalanga laboratory, where the shoots and roots were dried at a temperature of 55°C in an oven for a duration of 72 hours, ground, and passed through a 500 µm sieve to produce a fine powder suitable for extraction. Methanol was used as the solvent to extract both phenolic compounds and flavonoids from the powdered samples. The quantification of total phenolics was achieved using the Folin–Ciocalteu colorimetric method, slightly modified for this experiment. In this process, 0.50 mL of the methanol extract (1 mg/mL) was mixed with Folin–Ciocalteu reagent and sodium carbonate, incubated for 2 hours, and the absorbance was measured at 760 nm using a UV–vis spectrophotometer. A gallic acid calibration curve provided the standard for calculating phenolic content, expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g), with values averaged over three replicates. For flavonoid quantification, the aluminum chloride colorimetric method (also modified) was used. Extracts (400 μL) were mixed with sodium nitrite, aluminum chloride, and sodium hydroxide, then diluted to a final volume of 1 mL with distilled water. After a 15-minute dark incubation, the absorbance was measured at 510 nm. A quercetin-based calibration curve was used to express the flavonoid content as milligrams of quercetin equivalent per gram of dry weight (mg QE/g), with standard deviation values calculated from three replicates. This thorough methodology allowed for precise assessment of the phytochemical properties of *Moringa oleifera* under study conditions (Singh *et al.,* 2022).

4.2.4 Data analyses

This study employed the Shapiro-Wilk test to assess the normality of the distribution (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). The analysis of the data was conducted using Statistix 10.0 after assessing normality. Absorbance of quercetin at 760nm was achieved using concentration of standard quercetin. A Tukey's HSD test was employed to distinguish the means with a significance level of 5% (Mashela *et al.,* 2015). The results discussed only parameters that were significant at the probability level of $p \leq 0.05$, unless otherwise stated.

4.3 Results

Using concentrations derived from the calibration curve, the study quantified the Total Phenolic (TPC) and Total Flavonoid Content (TFC) in *Moringa oleifera* extract, revealing that TPC was consistently higher than TFC in both experiments, as shown in Table 4.4. Among the applied treatments, salts had a significant effect ($p \leq 0.05$) on TPC and TFC in both experiments (Table 4.1). Salts treatments had a significant effect on TFC in Experiment 1 and 2 with Total Treatment Variation (TTV) of 80.90 and 37.52%, respectively (Table 4.1). Relative to the control, TFC was lower in plants treated with salt alone with a relative impact (RI) of 3.10 to 4.00 mg GAE/g in Experiment 1 compared to the 4.10 mg GAE/g of the control. While in Experiment 2, TFC was lower in plants treated with salt, however plants treated with moderate salinity at 0.25 dS m^{-1} had high TFC relative to the control with a RI of 5.30 mg GAE/g compared to the 5.00 mg GAE/g (Table 4.3). Similarly, salts treatments had a significant effect on TPC in Experiment 1 and 2 with TTV of 77.26 and 66.58%, respectively. Correspondingly, with the TPC in both experiments, plants treated with salinity ($p \leq 0.05$) had a low TPC relative to the control. However, plants treated with moderate salt at 0.25 dS m^{-1} had high TPC relative to the control as shown in Table 4.3.

The application of VAM without salt had a significant effect ($p \leq 0.05$) on TFC and TPC with TTV of 2.39 and 15.17% of TFC and in Experiment 1and 2, also TTV of 13.84 and 28.32% of TPC in Experiment 1and 2, respectively (Table 4.1). The application of VAM in the absence of salt lead to increased TFC relative to the control in Experiment 1, with a RI of 4.30 to 5.50 mg QE/g compared to the 4.10 mg QE/g of the control. Similarly in Experiment 2, TFC relative to the control was increased when plants were treated with VAM in the absence of salt with a RI of 5.10 to 6.00 mg QE/g compared to 5.00 mg QE/g of the control. The results revealed that VAM greatly enhanced TPC in the absence of salinity, with RI of 21.25 to 28.00 mg QE/g relative to the control (15.00 mg QE/g) in Experiment 1. In Experiment 2, TPC was increased in the absence of salinity when treated with VAM, with a relative impact of 13.00 to 20.00 mg QE/g compared to the control (10.00 mg QE/g).

The results of the study indicate that the interaction of VAM and saline treatments did have a significant effect ($p \leq 0.05$) on TFC and TPC in both experiments (Table 4.1). The interaction of VAM and salinity treatments did influence TFC in Experiment 1 and 2, with a TTV of 4.07 and 1.40%, respectively. While the TPC was influenced by a TTV of 6.25 and 4.60 in in Experiment 1 and 2, respectively. Relative to the control the interaction of VAM and salinity treatments had increased the TFC in Experiment 1 with RI of 4.40 to 5.50 mg QE/g. Likewise in Experiment 2, except the interaction of 20g and 0.75 dS m^{-1}, comparable to the control the TFC was increased by the interaction with a RI of 5.00 to 6.10 mg QE/g. In both Experiment 1 and 2, relative to the control TPC was enhanced by the interaction of VAM and salinity treatments with RI of 21.00 to 39.68 mg QE/g and 15.00 to 41.71 mg QE/g.

Compared to the control, salt treatment increased both the total phenolic and total flavonoid content in both Experiment 1 and Experiment 2 (Table 4.2). In Experiment 1, the total flavonoid content rose from 24.62% to 42.21%, while in Experiment 2, it increased from 22.90% to 35.02% (Table 4.2). The total phenolic content in Experiment 1 increased from 63.07% to 109%, and in Experiment 2, it grew from 211.87% to 292.12% (Table 4.2). On the other hand, Vesicular Arbuscular Mycorrhiza (VAM), when compared to the control, reduced both the total flavonoid and total phenolic content in both experiments (Table 4.3). In Experiment 1, the total flavonoid content dropped from 0.81% to 4.71%, and in Experiment 2, it decreased from 4.56% to 12.96% (Table 4.3). The total phenolic content in Experiment 1 decreased from 29.94% to 3.11%, and in Experiment 2, it fell from 117.66% to 92.47% (Table 4.3).

Table 4.1: Source of variation affecting Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of *Moringa oleifera* in Experiment 1 and 2.

	Experiment 1					Experiment 2			
	Total Flavonoid content			Total Phenolic Content		Total Flavonoid Content		Total Phenolic Content	
Source	**DF**	**MS**	**TTV (%)**	**MS**	**TTV**	**MS**	**TTV (%)**	**MS**	**TTV (%)**
Rep	2	0.88	12.36	23,70	2.56	0,28	5.59	10,48	0.37
Salt	3	5.76	80.90***	713,68	77.26***	1,88	37.52***	1885,10	66.58***
VAM	3	0.17	2.39***	127,85	13.84***	2,02	40.32***	801,76	28.32***
Salt*VAM	9	0.29	4.07***	57,77	6.25***	0,76	15.17***	130,38	4.60***
Error	30	0.02	0.28	0,74	0.08	0,07	1.40	3,60	0.13
Total	47	7.12	100	923.74	100	5.01	100	2831.32	100

***Highly significant at p≤0.001, **significant at p≤0.05, ns not significant, TTV (%) Total treatment Variation

Table 4.2: The estimation of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of *Moringa oleifera* in Experiment 1 and 2 under salt treatment.

	Experiment 1				Experiment 2			
Salt	**TFC**	**RI (%)**	**TPC**	**RI (%)**	**TFC**	**RI (%)**	**TPC**	**RI (%)**
0	3.98^{a}	0	16.11^{a}	0	4.82^{a}	0	9.27^{a}	0
0.25	4.96^{bc}	24.62	30.89^{bc}	91.74	5.26^{bc}	22.90	28.91^{b}	211.87
0.5	5.66^{bc}	42.21	26.27^{bc}	63.07	5.78^{bc}	35.05	35.17^{cd}	279.39
0.75	4.70^{bc}	18.09	33.69^{c}	109.12	5.41^{c}	26.40	36.35^{d}	292.12

[y]Column means ± standard error followed by the same superscripts were not different (p≤ 0. 05) according to Turkey's HSD test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 4.3: The estimation of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of *Moringa oleifera* in Experiment 1 and 2 under VAM treatment.

	Experiment 1				Experiment 2			
VAM	TFC	RI (%)	TPC	RI (%)	TFC	RI (%)	TPC	RI (%)
0	4.92^{a}	0	23.78^{a}	0	5.48^{a}	0	15.40^{a}	0
10	4.96^{b}	0.81	30.90^{b}	29.94	5.73^{b}	4.56	33.52^{bc}	117.66
20	4.75^{bc}	-3.45	27.73^{cd}	16.61	5.29^{cd}	-3.47	31.14^{bc}	102.21
30	4.70^{c}	-4.71	24.52^{d}	3.11	4.77^{d}	-12.96	29.64^{c}	92.47

^{y}Column means ± standard error followed by the same superscripts were not different (p≤ 0. 05) according to Turkey's HSD test. ^{z}Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 4.4: The estimation of Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of *Moringa oleifera* in Experiment 1 and 2.

VAM (g)	Salt (dS m^{-1})	Total Flavonoid Content (mg QE/g)		Total Phenolic Content (mg QE/g)	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	0	$4.10^{a} \pm 0.1$	$5.00^{a} \pm 0.13$	$15.00^{a} \pm 2.15$	$10.00^{a} \pm 2.52$
0	0.25	$4.00^{ab} \pm 0.15$	$5.30^{a} \pm 0.16$	$20.00^{b} \pm 1.20$	$11.00^{a} \pm 2.36$
0	0.50	$3.30^{b} \pm 1.56$	$4.70^{b} \pm 0.32$	$14.00^{bc} \pm 2.01$	$9.00^{ab} \pm 2.65$
0	0.75	$3.10^{b} \pm 1$	$4.20^{b} \pm 0.25$	$13.55^{c} \pm 1.32$	$7.00^{ab} \pm 2.59$
10	0	$4.30^{bc} \pm 0.14$	$5.10^{c} \pm 0.11$	$21.25^{d} \pm 1.62$	$13.00^{b} \pm 1.25$
10	0.25	$4.80^{bc} \pm 0.20$	$5.20^{c} \pm 0.16$	$39.68^{de} \pm 0.23$	$38.16^{c} \pm 0.20$
10	0.50	$4.70^{bc} \pm 0.25$	$5.00^{c} \pm 0.18$	$32.00^{e} \pm 0.18$	$30.10^{c} \pm 0.96$
10	0.75	$5.00^{c} \pm 0.64$	$5.10^{c} \pm 0.12$	$26.00^{fg} \pm 0.96$	$28.00^{c} \pm 0.58$
20	0	$5.50^{c} \pm 0.73$	$6.00^{de} \pm 0.53$	$25.00^{fg} \pm 0.64$	$15.00^{d} \pm 1.23$
20	0.25	$5.30^{c} \pm 0.52$	$6.10^{de} \pm 0.58$	$29.00^{fg} \pm 0.25$	39.60 ± 0.12^{ef}
20	0.50	$5.40^{c} \pm 0.42$	$5.90^{e} \pm 0.19$	$24.00^{fg} \pm 0.86$	$39.90^{ef} \pm 0.09$
20	0.75	$5.10^{cd} \pm 0.30$	$4.10^{fg} \pm 0.82$	$21.00^{fg} \pm 0.75$	$41.00^{f} \pm 1.12$
30	0	$4.70^{d} \pm 0.18$	$5.30^{g} \pm 0.14$	$28.00^{g} \pm 0.87$	$20.62^{g} \pm 2.10$
30	0.25	$4.70^{d} \pm 0.17$	$5.40^{g} \pm 0.16$	$30.00^{h} \pm 0.27$	$37.30^{h} \pm 0.26$
30	0.50	$4.50^{d} \pm 0.15$	$5.20^{g} \pm 0.51$	$36.00^{h} \pm 0.19$	$41.71^{i} \pm 1.13$
30	0.75	$4.40^{d} \pm 0.21$	$5.10^{g} \pm 0.23$	$34.00^{h} \pm 0.12$	$41.63^{i} \pm 1.41$

[y]Column means ± standard error followed by the same superscripts were not different ($p \leq 0.05$) according to Turkey's HSD test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

4.4 Discussion

Moringa oleifera is recognized for its elevated levels of bioactive chemicals, encompassing total phenols and total flavonoids (Lin *et al.,* 2018). These chemicals substantially enhance its therapeutic attributes and nutritional worth. The pharmaceutical industry uses the elevated levels of phenols and flavonoids in moringa for their therapeutic properties (Lin *et al*., 2018). They are often included in diverse health supplements, herbal teas, and functional meals because of their capacity to enhance overall well-being, combat inflammation, bolster metabolic health, and diminish the chance of chronic ailments such as diabetes and heart disease (Lin *et al.,* 2018). The current study has identified variations in Total Phenol (TPC) and Total Flavonoid Content (TFC) under different production environments.

The study revealed that a rise in salt content resulted in a rise in the examined phytochemicals (TPC and TFC). The research conducted by Hassan *et al.* (2020) showed an elevation in total phenol levels with an increase in salt treatment. Furthermore, consistent with Hassa *et al.* (2020), the research conducted by Hoang and Rehman (2022) showed that a rise in salt concentration corresponded with elevated levels of total phenolic content, total flavonoid content, and antioxidant activities. Hoang and Rehman (2020) demonstrated that the elevation of total flavonoids and total phenols results from plants evolving mechanisms to mitigate the impacts of salinity through the production of suitable metabolites and various antioxidants. This results from variations in salt content, plant variety, and exposure length. Elevated salt stress results in decreased phenol and flavonoid levels, potentially leading to osmotic stress, nutritional imbalances, or stunted development (Bistgani *et al.,* 2019). Excessive salt might cause ion toxicity, potentially hindering the formation of these molecules (Bistgani *et al.,* 2019). Salt may disrupt enzymatic pathways essential for the production of phenols and flavonoids (Bistgani *et al.,* 2019). Salt stress can either elevate or diminish phenolic and flavonoid levels in plants, contingent upon the severity of the stress and the plant's adaptive capacity (Bistgani *et al.*, 2019). Moderate salinity may elevate these chemicals owing to the plant's stress response, but elevated salt concentrations often diminish their levels due to adverse effects on plant metabolism and development (Bistgani *et al.*, 2019). Nonetheless, in the current investigation, the examined phytochemicals increased with the escalation of salt therapy. The research conducted by Duc *et al.* (2021) revealed that moderate salinity enhanced phenolic compound synthesis in non-AM plants at 4 weeks; however, after 8 weeks, a reduction in phenolic compound content was seen in uncolonized plants exposed to both saline environments.

The investigation demonstrated a demotion in both TFC and TPC with the augmentation of VAM fungus treatment was observed in both Experiment 1 and 2. The research is contradicted by Liu *et al.* (2022), which emphasized that VAM fungus enhanced TPC production. Moreover, Dos Santos *et al.* (2017) corroborated the results of Liu *et al.* (2022) by demonstrating that the treatment of VAM fungus enhanced TPC in *Libedibia Ferrea*. Arbuscular Mycorrhizae fungi are advantageous symbiotic microorganisms that establish associations with the roots of several plants, including moringa (Frahat and Shehata, 2021). This symbiosis can augment the plant's capacity to assimilate nutrients (especially phosphorus), promote water absorption, and bolster its resilience to diverse stressors, potentially affecting the synthesis of secondary metabolites, such as phenolics and flavonoids (Frahat and Shehata, 2021). This symbiosis seemed beneficial in the current study were TFC and TPC were greatly influenced when plants were treated with VAM because excessive accumulation of certain phytochemicals such as the ones examined in the paper can make a plant toxic (Gololo, 2018).

Furthermore, the interaction between salt and VAM fungus has demonstrated an increase in the phytochemicals examined. The interaction between salt and VAM fungus resulted in a considerably elevated concentration of TFC and TPC compared to plants treated with VAM alone and those treated with salt alone. The VAM fungus mitigated the stress induced by salt on plants by enhancing the TFC and TPC. The research aligns with the findings of Mehr *et al.* (2012), which demonstrated that salinity-stressed soil, when inoculated with VAM fungus, resulted in heightened phenol accumulation in *Anethum graveolens*. Vesicular Arbuscular Mycorrhizal fungi may markedly augment the TFC and TPC in plants subjected to salt stress by enhancing nutrient absorption, alleviating oxidative stress, and activating the plant's defense systems (Alizadeh *et al.,* 2021). Vesicular Arbuscular Mycorrhizal fungi can stimulate the plant's systemic defense systems, resulting in the synthesis of secondary metabolites such as phenolic compounds, flavonoids, and other phytochemicals (Alizadeh *et al.,* 2021). These chemicals function as components of the plant's comprehensive defense mechanism, offering protection against infections, herbivores, and environmental challenges, including salinity (Alizadeh *et al.,* 2021). Mycorrhizal fungi frequently elicit systemic resistance (ISR) in plants, enhancing their resilience to stress and perhaps elevating secondary metabolite levels (Alizadeh *et al.,* 2021).

4.5 Conclusion

Salt adversely affected the overall TFC and TPC in moringa; however, the treatment of VAM fungus alleviated this influence and progressively enhanced the phytochemical levels. The utilization of VAM fungus on non-saline soil slightly decreased the overall concentration of flavonoids and phenols. Moringa possesses elevated levels of bioactive chemicals, such as phenols and flavonoids. The symbiotic relationship with mycorrhizal fungus may augment the plant's capacity to synthesize these chemicals for defense or growth regulatory purposes. VAM fungi enhance the plant's tolerance to salt stress and stimulate the synthesis of antioxidant chemicals, resulting in elevated phenol and flavonoid levels, so aiding the plant in managing environmental stress and augmenting its nutritional and therapeutic properties.

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CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH, AND CONCLUSIONS

5.1 Summary

Moringa oleifera Lam. is a medicinal plant with significant medicinal properties, including treating chronic ailments like cancer and diabetes. As the human population grows, so does the need for herbal drugs and natural health products. Moringa plants produce secondary metabolites, which are highly useful for health purpose. These phytochemicals have diverse pharmacological applications, including anti-fertility and anti-diabetic properties. However, global warming has led to increase in abiotic stress such as saline stress, affecting agricultural productivity. Climate-smart agriculture practices, such as Vesicular Arbuscular Mycorrhiza (VAM) fungi, can help adapt crops to extremes like salt stress, enhancing plant growth and yields in various saline soils.

The study investigated the effect of Vesicular Arbuscular Mycorrhiza (VAM) fungi on the growth and yield of *M. oleifera* and saline stress alleviation and whether biosynthesis and accumulation of phytochemicals of *M. oleifera* will be influenced using VAM fungi in reducing salinity. The results showed that elevated salinity levels impaired plant growth and development. Nevertheless, the use of VAM fungus has demonstrated the potential to alleviate the negative effects of salinity stress on plant growth. Research findings indicate that the introduction of VAM fungus into soil without saline stress greatly enhances the productivity of *M. oleifera*. Salt had a negative impact on the total phenols and flavonoid content on moringa however the application of VAM fungi mitigated the effect and gradually increased the phytochemicals. The application of VAM fungi on non-saline soil increased the total content of Total Flavonoid and Total Phenols Content.

5.2 Significance of findings

The results underscore numerous essential elements of employing climate smart agriculture regenerative practices in the cultivation of *M. oleifera* in the face of environmental stressors under climate change. *Moringa oleifera* is a significant medicinal plant recognized for its efficacy in addressing several chronic conditions, including cancer and diabetes, attributable to its phytochemical constituents. With the rising demand for herbal treatments, moringa's importance in natural health products is amplified, especially in the development of sustainable supplies of natural medicines. Moringa synthesizes secondary metabolites (phytochemicals) that enhance its therapeutic attributes and enable the plant to withstand environmental stresses such as drought, elevated temperatures, and saltwater environments. These phytochemicals, including phenols and flavonoids, possess pharmacological properties are advantageous to human health, although they can be influenced by stressors, particularly salt.

Global warming has resulted in heightened salinity stress, which presents problems to agricultural output. Saline environments inhibit the growth and development of moringa, hence affecting the production and quality of its phytochemicals, which are essential for medical applications and plant resilience. The significance of Vesicular Arbuscular Mycorrhiza (VAM) fungus in climate-smart agriculture is emphasized as a promising resource. The incorporation of VAM fungus alleviates the detrimental impacts of salt stress on moringa by augmenting nutrient absorption and promoting growth under stressful circumstances. This enhances moringa's production in saline soils while also maintaining and elevating the concentrations of beneficial phytochemicals such as phenols and flavonoids as seen in the current study.

5.3 Recommendation and Future Research

Considering the inconsistencies noted in *Moringa oleifera*'s reaction to saline stress, the following study recommendations are proposed: Examine seasonal and environmental fluctuations in salt tolerance. The present study indicates that moringa exhibits lower tolerance to salt stress than previously reported; hence, more research should be conducted across various seasons and environmental circumstances. This can elucidate the impact of external influences on moringa's salt tolerance and guarantee that the findings are relevant to various agricultural contexts.

Measure salt tolerance intensity under diverse conditions. Propose trials that investigate moringa's growth, yield, and survival rates across various salt concentrations. Standardize "moderate" and "high" salt levels across various situations to create a uniform baseline for assessing tolerance and potential threshold effects.

Investigate phytochemical reactions to salinity stress thoroughly. Due to the inconsistent results regarding phytochemical concentration under salt stress, further investigation should concentrate on the effects of different salinity levels on the manufacture of essential phytochemicals (e.g., phenols and flavonoids). This may involve biochemical tests conducted in both controlled and field settings to ascertain whether moringa's phytochemical profile may be augmented or is typically reduced under stress.

Assess the function of VAM fungi in alleviating salt stress impacts. Given that VAM fungus has beneficial benefits in alleviating saline stress, subsequent investigations should evaluate the influence of VAM inoculation on the salt tolerance and phytochemical composition of moringa across various environmental circumstances. Evaluating VAM fungi alongside other biotic and abiotic stress mitigators may facilitate the identification of optimal strategies for improving moringa resilience and phytochemical synthesis. Investigate the genetic and molecular mechanisms underlying salt tolerance and phytochemical synthesis.

Future research may explore the molecular mechanisms that regulate salt tolerance and phytochemical production in moringa. Comprehending these systems may yield insights into selective breeding or genetic engineering initiatives aimed at enhancing moringa's resistance and nutritional quality in the face of environmental challenges. Execute Longitudinal and multi-site trials conducting extensive trials in several geographic areas and soil types can provide a more thorough understanding of the variations in moringa's salt tolerance and phytochemical profiles across geographies and temporal contexts, facilitating worldwide farming recommendations. This study may yield significant insights for sustainable moringa cultivation in saline-affected areas, augment the plant's application as a therapeutic crop, and support climate-resilient agriculture.

5.4 Conclusions

This work underscores the substantial influence of salinity on the growth and development of *Moringa oleifera*, since increased salt concentrations hinder both plant production and the synthesis of essential phytochemicals such as phenols and flavonoids. The incorporation of VAM fungus has shown significant potential to alleviate these adverse effects. Vesicular Arbuscular Mycorrhizal fungus not only promoted moringa's growth under salt stress but also improved the plant's phytochemical profile, augmenting antioxidant chemicals that enhance its therapeutic potential. Vesicular Arbuscular Mycorrhizal fungus inoculation enhanced moringa's production and phytochemical content even under non-saline settings, highlighting the advantages of this symbiotic relationship. Although moringa's salt tolerance may be constrained and affected by seasonal and environmental variables, our results underscore the significance of VAM fungus as a valuable asset in enhancing plant resilience, especially in saline-prone regions. This study indicates that VAM fungus may serve as a beneficial component of sustainable farming techniques, improving the nutritional and therapeutic properties of moringa, particularly in adverse environmental situation.

APPENDICES

Appendix 1: Analysis of variance for soil pH in Experiment 1

Source	DF	SS	MS	F	P
Rep	4	0.8868	0.2217		
VAM	3	14.4424	4.8141	4.28	0.0084
Salt	3	32.5778	10.8593	9.65	0.0000
VAM*Salt	9	20.1596	2.2400	1.99	0.0561
Error	60	67.5234	1.1254		
Total	79				

Appendix 2: Analysis of variance for number of branches in Experiment 1

Source	DF	SS	MS	F	P
Rep	4	46.476	11,6189		
VAM	3	12.256	4.0852	0.91	0.4414
Salt	3	41.437	13.8123	3.08	0.0342
VAM*Salt	9	32.955	3.6617	0.82	0.6034
Error	60	269.191	4.4865		
Total	79				

Appendix 3: Analysis of variance for chlorophyl content in Experiment 1

Source	DF	SS	MS	F	P
Rep	4	90.731	22.683		
VAM	3	931.290	310.430	53.15	0.0000
Salt	3	905.491	301.830	51.68	0.0000
VAM*Salt	9	591.099	65.678	11.24	0.0000
Error	60	350.438	5.841		
Total	79				

Appendix 4: Analysis of variance for stem diameter in Experiment 1

Source	DF	SS	MS	F	P
Rep	4	0.07279	0.01820		
VAM	3	0.00493	0.00164	0.38	0.7711
Salt	3	0.19195	0.06398	14.61	0.0000
VAM*Salt	9	0.06215	0.00691	1.58	0.1428
Error	60	0.26271	0.00438		
Total	79				

Appendix 5: Analysis of variance for plant height in Experiment 1

Source	DF	SS	MS	F	P
Rep	4	75.407	18.852		
VAM	3	875.338	291.779	35.68	0.0000
Salt	3	918.273	306.091	37.43	0.0000
VAM*Salt	9	155.781	17.309	2.12	0.0418
Error	60	490.614	8.177		
Total	79				

Appendix 6: Analysis of variance for soil pH in Experiment 2

Source	DF	SS	MS	F	P
Rep	4	5.5237	1.3809		
VAM	3	12.3925	4.1308	7.68	0.0002
Salt	3	32.8093	10.9364	20.33	0.0000
VAM*Salt	9	5.1704	0.5745	1.07	0.3995
Error	60	32.2844	0.5381		
Total	79				

Appendix 7: Analysis of variance for soil EC in Experiment 2

Source	DF	SS	MS	F	P
Rep	4	0.00571	0.00143		
VAM	3	0.02710	0.00903	3.21	0.0291
Salt	3	0.08794	0.02931	10.43	0.0000
VAM*Salt	9	0.02247	0.00250	0.89	0.5406
Error	60	0.16858	0.00281		
Total	79				

Appendix 8: Analysis of variance for chlorophyl content in Experiment 2

Source	DF	SS	MS	F	P
Rep	4	15.629	3.9074		
VAM	3	16.394	5.4646	0.39	0.7628
Salt	3	155.103	51.7011	3.66	0.0172
VAM*Salt	9	153.015	17.0017	1.20	0.3097
Error	60	847.409	14.1235		
Total	79				

Appendix 9: Analysis of variance for number of branches in Experiment 2

Source	DF	SS	MS	F	P
Rep	4	12.800	3.200		
VAM	3	14.194	4.731	2.93	0.0407
Salt	3	25.676	8.559	5.30	0.0026
VAM*Salt	9	10.588	1.176	0.73	0.6810
Error	60	96.875	1.615		
Total	79				

Appendix 10: Analysis of variance for stem diameter in Experiment 2

Source	DF	SS	MS	F	P
Rep	4	0.093	0.024		
VAM	3	0.006	0.002	0.26	0.850
Salt	3	0.099	0.03	4.44	0.007
VAM*Salt	9	0.039	0.004	0.58	0.808
Error	60	0.445	0.007		
Total	79				

Appendix 11: Analysis of variance for Total Flavonoid Content in Experiment 1

Source	DF	SS	MS	F	P
Rep	2	1.764	0.882		
Salt	3	17.274	5.758	233.93	0.0000
VAM	3	0.5233	0.174	7.09	0.0010
Salt*VAM	9	2.584	0.287	11.66	0.0000
Error	30	0.738	0.025		
Total	47	22.883			

Appendix 12: Analysis of variance for Total Flavonoid Content in Experiment 2

Source	DF	SS	MS	F	P
Rep	2	0.560	0.28000		
Salt	3	5.656	1.885	26.35	0.0000
VAM	3	6.054	2.018	28.20	0.0000
Salt*VAM	9	6.817	0.757	10.59	0.0000
Error	30	2.147	0.71		
Total	47	21.233			

Appendix 13: Analysis of variance for Total Phenolic Content in Experiment 1

Source	DF	SS	MS	F	P
Rep	2	47.41	23.705		
Salt	3	2141.05	713.683	967.30	0.0000
VAM	3	383.55	127.851	173.29	0.0000
Salt*VAM	9	519.96	57.773	78.30	0.0000
Error	30	22.13	0.738		
Total	47	3114.10			

Appendix 14: Analysis of variance for Total Phenolic Content in Experiment 2

Source	DF	SS	MS	F	P
Rep	2	20.96	10.48		
Salt	3	5655.30	1885.10	523.75	0.0000
VAM	3	2405.27	801.76	222.76	0.0000
Salt*VAM	9	1173.38	130.38	36.22	0.0000
Error	30	107.98	3.60		
Total	47	9362.89			