## **REVIEWS**



**OPEN ACCESS** 

# Exploring the Impact of Vesicular Arbuscular Mycorrhiza on Phytochemical Profiles, Productivity Enhancement and Saline Stress Alleviation in *Moringa Oleifera*

Thamia Laka<sup>1</sup>, Tsobedi A. Masenya<sup>1\*</sup>, Wandile S. Mabila<sup>1</sup>, Amogelang Mabuela<sup>1</sup> and Khanyile Nokuthula<sup>2</sup>

<sup>1</sup> School of Agriculture, University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa

<sup>2</sup> School of Chemical and Physical Sciences, University of Mpumalanga, Private Bag X11283, Mbombela, 1200, South Africa

#### ABSTRACT

*Moringa oleifera* Lam. is a widely used plant in the Moringaceaea family. It thrives in tropical and subtropical regions and has a maturation period of four to five months. The cultivation of moringa is swiftly gaining popularity and has entered both formal and informal world economies due to its pharmacological and ethnomedicinal benefits. Moringa has been found to tolerate mild salinity (50mM NaCl) by preserving succulence and biomass distribution, but its growth is still significantly affected by high salinity. Crop production worldwide is highly affected by salinity, and with the increasing instability in weather patterns due to climate change, abiotic stress factors such as salinity are expected to increase due to rise in temperature. Saline stress induces physiological disturbances and impairs soil moisture capacity, resulting in water stress and reduced plant growth. With the unpredictability and expected rise in temperatures due to climate change; there is a need to find alternative strategies to adapt plants to stress factors. Several studies have shown that the application of Versicular Arbuscular Mycorrhiza (VAM) fungi plays a vital role in alleviating salt stress by enhancing nutrient absorption and promoting resistance to salinity. Vesicular Arbuscular Mycorrhizal is a fungus that can dissolve the phosphates found in abundance in the soil. They enhance plant growth and protect against stressors. The use of VAM fungi not only helps the plant adapt during stress but can influence the accumulation of phytochemicals, which are highly utilized in medicinal plants. The distribution and abundance of phytochemicals vary throughout the plant and are influenced by environmental factors. The present review discusses and analyses the impact of VAM on moring development and productivity, as well as its potential in mitigating saline stress.

#### Keywords

moringa oleifera Lam, saline stress, Versicular Arbuscular Mycorrhiza (VAM), phytochemicals, climate.

## 1. Introduction

*Moringa oleifera* Lam., a member of the Moringaceaea family, is widely recognized as the most extensively utilized plant within this botanical group [1]. The plant is indigenous to Asia and has been cultivated and acclimated to Africa. It has the ability to thrive in tropical and subtropical regions [2]. According to Oyeyinka and Oyeyinka [3], the maturation period of moringa typically spans approximately four to five months. Singh *et al.* [4] reported that in nations characterized by a high prevalence of malnutrition, there is a notable increase in the cultivation of moringa.

Received November 21, 2023, Revised June 05, 2024, Accepted June 18, 2024

Published online: September 15, 2024. \*Correspondence to T.M: tamasenya@gmail.com



The value of 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 [5].

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 [5]. The extensive utilization of various medicinal products derived from different crops has been associated with adverse effects in humans [6]. This would typically be the result of improper utilization of medicinal products. Nevertheless, no such detrimental effects have been identified in relation to the consumption of moringa products. The diverse nutritional benefits of moringa, were also highlighted by Sujatha and Patel [7], 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 organizations such as the Food and Agriculture Organization (FAO) of the United Nations and FAO in India [8], 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, underlines the value of the crop. Singh *et al.* [4] assert 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 [9].

The plant is regarded as a highly valuable and distinctive botanical specimen due to its multifaceted utility. Virtually every component of the plant, including its flowers, seeds, leaves, stem, and roots, can be effectively employed in various domains such as nutrition, healthcare, and industrial applications [10] (Fig.1). According to Rode *et al.* [11], this plant possesses the capacity to enhance nutritional value, enhance food security, and facilitate the advancement of rural communities. Throughout history, humans have extensively utilized all parts of this plant for a diverse range of domestic applications, including but not limited to domestic water purification, biogas production, animal feed, fertilizer, plant nutrient spraying, and green manure utilization [12] (Fig.1).



Figure 1: The application of moringa plant



Moreover, the study conducted by Abdel-Latif *et al.* [13] discovered that moringa antioxidants contribute to reducing blood pressure and promotion of fat burning processes. The plant is known as a panacea due to its purported ability to provide therapeutic benefits for more than 300 diseases, as documented by Alpern [14]. According to a study by Padayachee and Baijnath [15], moringa has satisfactory efficacy as an anti-neo proliferative agent, effectively inhibiting the proliferation of cancer cells. The potential of moringa as a potent neuroprotectant is evident [14]. According to Padayachee and Baijnath [15], 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 characterized by the obstruction of blood flow to the brain, which has been identified as a contributing factor [15].

The multipurpose use of moringa includes the extraction of oil from moringa seeds, commonly referred to as Ben oil [16] as shown in (Table 1). The oil exhibits a notable abundance of oleic acid, tocopherols, and sterols. According to Sreeja *et al.* [16], 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, fragrance, and as a lubricant [16]. The pods can absorb organic contaminants and pesticides [16]. According to Shija *et al.* [17], 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 [17]. 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 [18]. The comprehensive significance of the entire plant to human existence necessitates its preservation in order to guarantee ample availability and provision. Okorie *et al.* [19] have reported that the pharmacological properties of the plant have resulted in a rise in its cultivation and international trade.

| 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  |
|                     |   |

Table 1: Different traditional uses of different parts of Moringa oleifera plant

The limiting factor to constant availability of this medicinal plants and its products, is the lack of arable land and the resulted changing climate which has led to increasing problems with extreme weather events leading to considerable yield losses of crops. Alternative strategies to mitigate or reduce the impact of abiotic stress factors are necessary to ensure stability in yields, while ensuring sustainability to the environment [19]. One of the common and threatening abiotic stressors in the world is salinity. Salinity as a stress factor has results in deleterious impact on crop production and continue to threaten availability of arable land and sustained crop productivity worldwide [19]. The solutions to this problem are scanty, however recent studies have shown how the utilization of mycorrhiza fungi is advancing as the best strategy to alleviate salinity in high order plants. The use of alternative strategies such as the use of Vesicular Arbuscular Mycorrhiza (VAM) fungi in adapting plant to stress, is yet to be exploited extensively on medicinal plants such as moringa. This study presents a comprehensive analysis of VAM's influence on growth of crops, saline stress alleviation, and the possible biosynthesis and accumulation of phytochemicals in *Moringa oleifera*.



## 2. Vesicular Arbuscular Mycorrhiza (VAM) fungi

Mycorrhiza refers to the mutualistic symbiotic association between angiosperm root systems and fungi, characterized by a non-pathogenic nature [20]. 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 [21]. Among the various relationships, Vesicular Arbuscular Mycorrhiza (VAM) fungi stand out as the most prevalent and widely recognized symbiotic association within the taxonomic order *Glomales* [22]. Vesicular Arbuscular Mycorrhiza (VAM) as the most popular endomycorrhiza is a kind of mycorrhizae whose hyphae penetrate the cells of plant roots by producing balloon-shape structures (vesicles) or dichotomously branching invaginations (arbuscules). In addition, VAM fungi have a variety of spores from rhizosphere of different host species mainly belonging to *Glomus, Gigospora, Acaulospora, Entrophospora*, and *Sclerocystis* as outlined in (Table 2) [22].

| Table 2: The most common | type of VAM isolates | [22] |
|--------------------------|----------------------|------|
|--------------------------|----------------------|------|

| Genus        | Example of species          |
|--------------|-----------------------------|
| Glomus       | G. fassiculatum, G. mosseae |
| Gigaspora    | Gigaspora nigra             |
| Acaulospora  | A. scrobiculata             |
| Sclerocystis | S. clavispora               |
| Endogone     | <i>E. increseta</i>         |

The 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 [20]. 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 [22]. 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 [22]. 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 [20].

Quilambo [23] has argued that the reliance on investigations of a limited number of taxa in studies has led to the formulation of general theories and conclusions. This emphasis on a narrow range of taxa highlights the extensive diversity of VAM and also indicates that our understanding of mycorrhizas may be less comprehensive than previously assumed [23]. Vesicular Arbuscular Mycorrhiza (VAM) has a long history, dating back millions of years, and is known to occur naturally in soil ecosystems [24]. In agreement with Lau *et al.* [24], Hepper [25] asserts that the occurrence of VAM 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 colonization of the root system by fungi [24]. In order for spores and/or propagules to effectively colonize the roots, a sufficient quantity of them must be present in the soil. Abiala *et al.* [26] have observed that while VAM is present in certain plant species, other plants do not establish this symbiotic association. However, the underlying cause for this absence remains unknown. Hussein *et al.* [27] postulate that this phenomenon may be attributed to the presence of fungi toxic compounds within the cortical tissue of the roots, or the exudates released by the roots. Snoeck *et al.* [28] proposed that the absence of mycorrhizal associations in certain plants may be attributed to elevated levels of salicylic acid, which have been found to impede mycorrhizal colonization. This implies that plant species possessing a genetic predisposition for increased salicylic acid production have undergone evolutionary adaptations to avoid mycorrhizal symbiosis.



Crop plants demonstrate improved growth and productivity when subjected to VAM symbiosis, as a result of enhanced nutrient absorption, increased resistance to salinity and drought, and heightened tolerance to diseases [29]. Furthermore, Abbasi *et al.* [30] support 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.* [31], sustainable agricultural soil conditions may be more conducive to the growth and development of VAM fungi compared to conventional agricultural soil conditions. The neglect of mycorrhizosphere organisms in intensive agriculture may be attributed to the alteration of microbial populations in typical agricultural systems through practices such as tillage and the application of inorganic fertilizers, herbicides, and pesticides [32]. This suggests that while VAM has the potential to improve crop growth and yield, its effectiveness is influenced by different 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 is not yet known. Moreover, Itelima *et al.* [33] assert that despite the numerous advantages of VAM to the host plant, its commercialization to tap into these beneficial effects has posed significant challenges. Therefore, further optimization through empirical data is necessary to enhance its potential in agriculture.

Various agricultural techniques, including the implementation of resistant cultivars, utilization of mulching and adoption of crop rotation practices have been employed to mitigate the severity of saline stress [33]. However, the management of long-term persistent survival structures presents challenges as a result of declining inoculum levels and limited plant resistance [33]. Scientists are currently investigating the utilization of beneficial microorganisms, such as antagonistic bacteria and fungi, in order to enhance plant resistance against saline stress. This alternative has the capability to substitute the fertilizer requirements of trees in regions characterized by low fertility, thereby diminishing the necessity for current levels of synthetic fertilizers. Hence, the utilization of VAM as an agricultural technique may effectively mitigate saline incidences by virtue of its ability to operate independently from the host plant in terms of nutrient and space requirements [25].

## 3. The effect of VAM on plant development

The research conducted by Manjulatha [34] has demonstrated that the utilization of VAM has significantly enhanced both the cob and grain yield of maize (*Zea Mays* L.), as shown in (Table 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 [34]. Additionally, it offers the potential for achieving a greater benefit-to-cost ratio. Similarly, Kazadi *et al.* [35] revealed that the growth of maize seedlings inoculated with VAM exhibited a significant improvement compared to the control group without VAM associations when transplanted into the field. The efficacy of VAM is said to improve multiple terrestrial plants.

The growth of soybean (*Glycine max* (L.) Merr) plants in acidic and low-fertility soils was enhanced by their interaction with VAM organisms [36]. This symbiotic relationship enables the plants to acquire immobile phosphorus and other essential nutrients. According to Gao *et al.* [37], plants engaged in the VAM symbiosis exhibit a higher phosphorus uptake than plants that do not form VAM associations. Additionally, VAM fungi are said to be crucial in enhancing plant growth in phosphorus-limited soil conditions, where the growth of non-mycorrhizal plants is hindered.



| 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                 | -         |

Table 3: Effect of VAM on cob and crop yield maize [34]

Notes: RDF= Recommended Dose of Fertilizer, C.D(0.05)= Critical difference, S.Em+= Standard Error of Mean, C.V(%)= Coefficient of Variation.

Basak et al. [39] found that VAM inoculation to tomato seedlings cultivated under saline conditions did not lead to a significant increase in seedling growth. However, it did induce positive changes in growth and development, resulting in higher fresh stem and root weight as well as increased chlorophyll content [39]. These findings suggest that VAM inoculation could positively impact tomato growth and yield. However, it is arguable that the application of VAM did not result in a significant increase in seedling growth as both the inoculated and non-inoculated seedbeds had a slight similar seedling growth. Nevertheless, the observation that VAM enhance development was supported by the acquisition of essential nutrients in 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. [40] observed the improvement of growth on the cancer bush under salinity stress when VAM was applied. The study showed that relative to uninoculated plants, Versicular Arbuscular Mycorrhiza (VAM) fungi application significantly improved plants. Other studies have also indicated that inoculating seedlings with VAM formulations provides significant growth and development benefits to crops [41, 42, 43, 44]. Allsop and Stock [45] 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 [45]. This implies that the introduction of VAM fungi during seed planting enhances the growth and development of plants, albeit without directly affecting seedling growth. Indriani et al. [46] observed that the interaction of VAM with other plant growth enhancers improved centro legumes (Centrosema pubescens) (Table 4). Insufficient data exists regarding the impact of VAM inoculation on the growth and yield of moringa. Existing studies primarily concentrate on the combined application of VAM with other plant growth enhancers on various studies than moringa, rather than solely examining the effects of VAM inoculation with the intention to improve plant growth and development under abiotic and biotic stress factors.



| Table 4. The interaction effect between Rock phosphate and VANI on plant height (enf) of centrosenia publications [40] |                              |  |  |  |
|--|------------------------------|--|--|--|
| <b>Rock Phosphate</b> (kg ha <sup>-1</sup> )   | Rock Phosphate and VAM (10g) |  |  |  |
| 0  | 40.67 <sup>ab</sup>          |  |  |  |
| 100  | 44.67 <sup>ca</sup>          |  |  |  |
| 200  | 46.33 <sup>cb</sup>          |  |  |  |
| 300  | 30.33 <sup>cb</sup>          |  |  |  |

Table 4: The interaction effect between Rock phosphate and VAM on plant height (cm) of Centrosema pubescens [46]

Notes: Values followed with same small letter in the same column and values followed with same capital letter in the same row were not significantly different according to 5 % Duncan's Multiple Range Test

#### 4. Salinity stress

Salinity is the degree of saltiness or quantity of dissolved salt in a body of water [47]. 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. Eswar *et al.* [48] asserted that, crop productivity is diminished by a magnitude exceeding 20%, resulting in an anticipated yearly expansion of saline environment by approximately 0.3-1.5 million hectares. As a consequence, there is a decrease in the overall output capacity by a range of 20 to 46 million acres [49]. 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 [48]. Saline stress induces a range of physiological disturbances, including nutritional and hormonal imbalances, ion toxicity, oxidative and osmotic stress, and heightened vulnerability to diseases [48]. 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 [50]. Consequently, this induces water stress, resulting in physiological drought conditions. Additionally, the presence of excessive ions, primarily Sodium ion (Na<sup>+</sup>), can disrupt the stability of cell membranes and cause protein degradation, further exacerbating the toxic effects on plants [50].

Azeem *et al.* [51] 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. This effect was achieved with only a minor reduction in dry biomass [51]. 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. Additionally, the plant demonstrated an increased accumulation of Na<sup>+</sup>, chlorine ion (Cl<sup>-</sup>), and potassium ion (K<sup>+</sup>) ions in response to the salinity stress [51]. Similarly, Elhag and Abdalla [52] conducted a study on moringa and arrived at a similar finding. According to Nouman *et al.* [53], 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 of slight tolerance is more 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.

#### 5. The influence of VAM on saline stress alleviation

Vesicular Arbuscular Mycorrhiza (VAM) fungi plays a vital role in the natural ecosystem, particularly in saline environments, by enhancing early plant growth and promoting resistance to salinity [54]. Numerous studies have demonstrated that the utilization of VAM fungi can effectively mitigate the detrimental effects of salt stress on plants [55, 56, 57]. This is achieved through various mechanisms, including the facilitation of nutrient absorption,



preservation of enzyme functionality, and facilitation of water absorption [58]. Vesicular Arbuscular Mycorrhiza fungi have been observed to enhance the transportation of mineral nutrients to plants in soil that is subjected to salt-induced stress. 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 [59]. Additionally, they provide protection to host plants against the adverse effects of stressors by augmenting antioxidant responses and/or inducing acquired systemic tolerance [59].

The study by Ebrahim and Saleem [60] on tomatoes, found that the application of VAM fungus enabled the plants to effectively withstand the adverse consequences of salinity, even at relatively low NaCl concentrations (Table 5) [60]. The findings of the research conducted by Masenya *et al.* [40] regarding the cancer bush on saline alleviation using VAM yielded similar outcomes. Nevertheless, definitive conclusions regarding moringa cannot be drawn due to the fact that the efficacy of VAM in improving crop's adaptability to the environment is crop specific, due to the symbiotic relation needed with the crop.

| NaCl level (mM)   | Mycorrhizal | Total sol. | Polysacch. | Total     | Total sol. | Shoot   | <b>Root/Shoot</b> |
|-------------------|-------------|------------|------------|-----------|------------|---------|-------------------|
|                   | treatment   | Sugars     |            | carbohyd. | Proteins   | biomass | (DW basis)        |
|                   |             | (TSS)      |            |           | (TSP)      |         |                   |
| 0                 | n-M         | 187        | 328        | 515       | 121        | 22      | 0.29              |
| 0                 | М           | 200        | 339        | 539       | 131        | 25      | 0.32              |
| 50                | n-M         | 199        | 276        | 475       | 96         | 16      | 0.41              |
|                   | М           | 216        | 286        | 502       | 111        | 19      | 0.37              |
| 100               | n-M         | 222        | 214        | 436       | 56         | 8       | 0.64              |
| 100               | М           | 237        | 227        | 464       | 78         | 11      | 0.52              |
| Factor            |             |            |            |           |            |         |                   |
| NaCl level (A)    |             | 12         | 26         | 39        | 11.60      | 1.7     | 0.0031            |
| Mycorrhizae (B)   |             | 8          | 23         | 35        | 7.91       | 1.3     | 0.022             |
| Interaction (A×B) |             | 19         | 32         | 47        | 18.20      | 2.3     | 0.043             |

Table 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) [60]

# 6. 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 [61]. Phytochemicals are synthesized by various plant species, encompassing fruits, vegetables, legumes, and grains [61]. 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 [62].

Moringa species are characterized by a diverse range of phytoconstituents, and the presence of these phytochemicals which contributes to the manifold medicinal applications of the plant [65]. Furthermore, it is important to note that no singular type of phytochemical confers a solitary benefit. Phytoestrogens present in various food sources, including soybeans, flaxseed, peaches, and garlic, have been suggested to potentially confer protective effects against conditions such as bone loss, breast cancer, uterine cancer, and cardiovascular disease [66]. According to Kasolo *et al.* [66], the identification of phytochemicals in moringa leaves indicates the potential for preventive and therapeutic properties. Kasolo *et al.* [66] further asserted that additional pharmacological research is necessary to substantiate the use of moringa as a medicinal plant. Rabizadeh *et al.* [67] have similarly posited that additional



investigation is warranted to validate the correlation between conventional uses and the biological attributes 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.

The phytochemicals in the moringa plant were identified in a study conducted by Cornelius [68] including cyanogenic glycosides, saponin, flavonoid, and alkaloid (Fig.2). In line with the results by Cornelius [68], Ma et al. [63] 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. Despite the fact that the 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. Polyphenols have been identified in both flowers and seeds [63]. However, their concentration in these plant parts is significantly lower compared to that in leaves (Table 6). Based on the research conducted by Cornelius [68], moringa leaves contain a greater abundance of phytochemicals such as saponin, alkaloid, and cyanogenic glycoside compared to flowers. 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.

Martínez et al. [69] 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. Additionally, Martínez et al. [69], found that variations 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               | 1 2         | e             |              |                               |
|-----------------|-------------|---------------|--------------|-------------------------------|
|                 | Saponin (%) | Flavonoid (%) | Alkaloid (%) | Cyanogenic Glycoside (mg/10g) |
| Moringa leaves  | 5.00        | 5.42          | 5.36         | 0.20                          |
| Moringa flowers | 3.20        | 7.12          | 1.56         | 0.16                          |

Table 6: Analyses of some phytochemicals in moringa leaves and flowers [68]



Kaempferol Figure 2: Structures of some phytoconstituents isolated from moringa plant [68]



## 6.1 Alkaloid

Alkaloids represent a category of nitrogenous compounds that are synthesized by plants in response to various biotic or abiotic factors [70]. This synthesis results in alkaloids possessing notable biological efficacy and a diverse range of structural characteristics [70]. Alkaloids are a class of naturally occurring toxic amines synthesized by plants primarily as a means of defense against herbivores [71]. The primary detrimental effects of alkaloids encompass disturbances in the central nervous system, digestive functions, reproductive processes, and the immune system [71]. Despite the potential adverse effects associated with alkaloids, it is important to acknowledge their advantageous influence on human physiology. Alkaloids possess the ability to directly impact the human brain and stimulate vital organs such as the central nervous system. The toxic alkaloids in honey are obtained through ingesting pollen contaminated with pyrrolizidine alkaloids (PAs) [72]. Several plant species that contain PAs are known to contribute to the potential toxicity of honey [72].

According to Kurek [73], 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, it was widely believed that they were regarded as mere by- products of plant metabolism. However, recent empirical evidence has unveiled their indispensable role in the biological processes of plants. 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 [74].

Numerous alkaloids in moringa have been empirically established, albeit atypical. Nevertheless, the precise quantity of these alkaloids present in moringa leaves remains undetermined [75]. The concentration of alkaloids in plants is contingent upon environmental conditions. The synthesis of alkaloids is predominantly observed in youthful and actively proliferating tissues; thus, any factors that influence the growth of such tissues will consequently affect the synthesis of alkaloids [76]. The production and degradation of various alkaloids can be influenced by environmental factors, potentially leading to differential production levels between species [76]. Alkaloids typically exhibit the characteristics of being colorless and odorless crystalline solids, although in certain instances, they may manifest as yellowish liquids [77]. These compounds frequently taste bitter, rendering them effective as natural inhibitors of herbivorous organisms [77]. Consequently, certain plants utilize them as natural pesticides.

Furthermore, the phenolic extract from moringa leaves inhibits cell proliferation and induces death in human melanoma cells [74]. Numerous investigations have demonstrated that alkaloids are the primary mediators of moringa's anticancer action [65, 64, 74]. Sreelatha and Padma [78] asserted that the moringa plant yields moringine, an alkaloid, through a series of procedures. In this set of tasks, plant parts are gathered, prepared, alkaloids are extracted, the raw extract is concentrated, the mixture is split into fractions, the fractions are studied using spectroscopic techniques, the fraction containing moringine is cleaned, the identity of the isolated moringine is confirmed by comparing its spectroscopic data and physical characteristics with those described in literature or with real standards, and the isolated moringine is described through additional analyses [78] (Fig.3). Sreelatha and Padma [78] further suggests that the specifics of each stage may differ depending on the available equipment and skill, and it is imperative to adhere to safety measures when handling solvents and chemicals. The process entails a meticulous equilibrium of chromatographic methodologies, spectroscopic examination, purification, and verification of the isolated moringine's identification [78].





Figure 3: Chemical structure of alkaloid moringine isolated from moringa plant material [78]

To detect the presence of moringine, a sequence of procedures should be performed [78]. Initially, a specimen of moringa plant material containing alkaloids is acquired and processed by the procedure of cleansing and desiccation [78]. The alkaloids are subsequently extracted from the plant material with an appropriate solvent. The raw extract is condensed to enhance the alkaloid concentration. Standard chemical assays are used to conduct a first examination for alkaloids [78]. Thin-Layer Chromatography (TLC) analysis is performed to separate and identify chemicals, such as moringine, by using their Retention factor (Rf) value and spot appearance [78]. High-Performance Liquid Chromatography (HPLC) is employed to validate the existence of moringine and measure its concentration [78]. Mass spectrometry (MS) analysis is conducted to verify the molecular weight and identify fragment ions specific to moringine [78]. Ultimately, Nuclear Magnetic Resonance (NMR) spectroscopy is employed to examine the separated product and ascertain its chemical composition [78]. The identification of moringine in the moringa plant extract can be successfully accomplished by following these steps and employing diverse analytical techniques.

## 6.2 Cyanogenic glycosides

Cyanogenic glycosides are plant constituents derived from amino acids, present in more than 2500 plant species and are widely distributed across 100 groups of flowering plants [79]. The component possesses the capacity to generate hydrogen cyanide, a highly toxic substance, upon degradation by plant enzymes [79]. Cyanogenic glycosides are naturally occurring toxic compounds present in a diverse range of plant species (Fig.4), with a predominant occurrence in those plants that are commonly consumed by humans [80,82]. 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 [80]. Processing procedures can effectively detoxify cyanogenic glycosides, thereby reducing the risk of cyanide poisoning. However, the effectiveness of cyanide removal relies on the specific processing technology employed and the extent of processing applied. According to Møller [81], in isolation cyanogenic glycoside does not possess any detrimental effects.



Figure 4: Chemical structure of dhurrin, a type of cyanogenic glycoside isolated from Moringa plant material



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 [82]. 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 [83]. According to Bolarinwa [84], farmers persist in cultivating crops containing significant levels of cyanogenic glycosides, despite their known harmful effects. This is due to the fact that 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.* [85], 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.

The concentration of cyanogenic glycosides in moringa were found to be 3.2%, 2.4%, and 1.0% in the roots, leaves, and stem barks, respectively [86]. The roots and leaves of the plant are the best sources of these phytoconstituent [86]. These concentration percentages in the plant shows that, it is less toxic and produces minor quantity of hydrogen cyanide which can easily be detoxified [86].

Chioma and Okah [86] indicated that the process of isolating cyanogenic glycosides from moringa entails extracting the plant material, usually leaves or seeds, using an appropriate solvent, such as methanol or ethanol. Following the extraction process, the solvent is subjected to evaporation in order to get a raw extract [86]. Subsequently, one can employ diverse chromatographic methodologies, such as column chromatography or HPLC, to segregate and extract the glycosides according to their distinctive characteristics, such as polarity [86]. Ultimately, the individual cyanogenic glycosides can be identified through the utilization of spectroscopic techniques such as NMR or mass spectrometry [86].

# 6.3 Saponin

Saponins are naturally derived compounds that are abundantly present in the cellular composition of leguminous plants [87]. Saponins encompass a diverse and intricate assemblage of chemical compounds, deriving their nomenclature from their inherent ability to generate enduring, soap-like froths when dissolved in aqueous solutions [88]. Furthermore, saponins exhibit several noteworthy medicinal properties, including anti-inflammatory, anti-fungal, anti-bacterial, anti-parasitic, anti-cancer, and antiviral effects [89, 90].

Surfactants are commonly employed in various industries, such as food, pharmaceuticals, and cosmetics, due to the emulsifying properties exhibited by saponins [91]. This enables the creation and maintenance of emulsionbased products. The phenomenon of amphiphilic surfactants undergoing adsorption at oil-water interfaces during the process of homogenization leads to a reduction in interfacial tension, thereby resulting in the formation of stable emulsions [92]. Prior research has indicated that the utilization of natural emulsifiers has the capacity to generate an emulsion that is both efficacious and enduring in its stability.

The variation in saponin concentration in plants is influenced by environmental factors. Pecetti *et al.* [93] 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. According to the findings of Yu *et al.* [94], the production of saponins in plants is influenced by environmental stimuli. Nevertheless, according to Pecetti *et al.*'s study, it can be inferred that the chemicals are not the sole



influenced summer conditions [93]. Hence, the impact of environmental factors on saponin concentration remains uncertain.

Chioma and Okah [86] found that 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 source of saponin which contain high amounts of lipids and the high caloric value was due to the high lipids [86]. Moreover, the phytoconstituent assist in protecting the plant from microbes and fungi and enhances the absorption of nutrients and aid in the digestion of animals [86].

The procedure for isolating oleanane from moringa entails multiple stages, which include extraction, purification, and characterization [95] (Fig.5). The procedure commences by pulverizing moringa leaves into a refined powder and isolating the oleanane compound using solvent extraction [95]. Impurities are eliminated through filtration, and the raw extract is condensed using reduced pressure [95]. Liquid-liquid partitioning separates the oleanane into the organic layer [95]. Additional purification methods, such as column chromatography or preparative thin-layer chromatography (TLC) are employed to enhance the purity of the organic layer [95]. Subsequently, the purified chemical undergoes analysis utilizing spectroscopic techniques such as NMR, IR, and mass spectrometry to validate its identity as oleanane [95]. To achieve the pure form of oleanane, the final step involves recrystallization or, if necessary, further chromatographic purification [95].



Figure 5: Chemical structure of oleanane, a type of saponin isolated from Moringa plant material [95]

#### 6.4 Phenolic Acid

Phenol is considered one of the principal classifications of plant phenolic compounds [96]. A diverse array of plant-based meals contains these compounds, with the highest concentrations observed in seeds, fruit and vegetable skins, and leafy greens [96]. Phenolic acids, a diverse group of plant polyphenols, have garnered significant research interest. Phenylpropanoids are synthesized via the shikimic acid pathway, specifically as by-products in the monolignol pathway [97]. Additionally, they are generated during the degradation of cell wall polymers such as lignin [97]. In certain instances, microbes also contribute to their production.

The precise role of phenolic acids in plants remains uncertain; however, they have been identified as having diverse functions such as protein synthesis, allelopathy, nutrient absorption, structural integrity, enzyme catalysis, and photosynthetic processes [98]. However, despite the advantages offered by this component, the non-biodegradable and persistent nature of phenolic compounds can result in their accumulation in water and sediment, which are habitats where aquatic organisms search for food [99]. When present at certain levels, exposure to phenolic compounds through water and sediment can potentially endanger aquatic ecosystems and human health [100].

In their study, Mpofu *et al.* [101] observed significant variations in the total phenolic content, antioxidant activities, and concentrations of various phenolic acids in wheat across different genotypes and environmental conditions. In the study by Fernandez-Orozco *et al.* [102] 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.

One of the most dominant phenolic acids in moringa is the gallic acid and is most abundant in dried leaves at concentration of 1.034 mg/g [103]. The chlorogenic acid, major phenolic acid in moringa, plays a significant role in glucose metabolism whereby it inhibits the glucose-6-phosphate translocate in rat liver and reducing hepatic gluconeogenesis [103]. Moreover, Hassan *et al.* [104] discovered that, phenolic acids of the moringa leaves are responsible for many biological activities for example plant growth, development, and defense, which prevent and mitigate complications of diseases such as hypertension.

Phenolic acid isolation from moringa plant materials requires multiple processes [104] (Fig.6). The first step is to grind up the plant material and extract it using a solvent like ethanol or methanol [104]. The extract is further subjected to filtration to eliminate any solid particles. Subsequently, the chemicals are separated into fractions using methods such as liquid-liquid extraction or column chromatography [104]. Crystallization or preparative chromatography methods further refine the fraction containing phenolic acids [104]. Spectroscopic techniques like NMR and mass spectrometry analyze the purified molecule to confirm its classification as a phenolic acid [104]. Either High Performance Liquid Chromatography (HPLC) or spectrophotometry perform the quantification of phenolic acids in the extract [104].

Moringa phenolic acids can be distinguished using a variety of analytical methods [104]. High-performance liquid chromatography (HPLC) uses absorbance spectra and retention times to detect and separate phenolic acids, while GC-MS separates compounds based on molecular weight and volatility [104]. Regarding phenolic acids, NMR Spectroscopy reveals their structures, whereas UV-Vis Spectroscopy measures and counts them at particular wave lengths [104]. Fourier Transform Infrared Spectroscopy (FTIR) provides information about the functional groups in phenolic acids [104]. These methods, when combined with the phenolic acid content of moringa and reference standards, allow for a more precise identification of these compounds [104].



Figure 6: Chemical structures of phenolic acid types isolated from Moringa plant materials (A) Gallic acid and (B) Vanillic acid [104]

#### 6.5 Flavonoid

Flavonoids represent a prominent category of naturally occurring compounds, primarily found as plant secondary metabolites, characterized by their polyphenolic structure [105]. These compounds are widely distributed in various fruits, vegetables, and certain beverages [105]. 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 [106]. Flavonoids are essential for the growth and defense mechanisms of



vegetables against harmful plaques [107]. Floral pigments in the majority of angiosperm families are commonly recognized as numerous flavonoids [107]. However, their presence is not restricted solely to flowers and can be observed in various other components of plants. According to Cornelius [68], the flower of moringa exhibits a significant abundance of flavonoids, which consequently contributes to its distinct coloration. The origin of the flower's inherent vibrant hue and fragrance is attributed to this factor [108].

Flavonoids possess a variety of biological roles within the domains of plants, animals, and microbes. 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 [109]. Numerous investigations have been undertaken to examine the antioxidant properties of various flavonoids, revealing their potential therapeutic applications in mitigating oxidative stress [109, 110]. The Moringa genus exhibits notable antioxidant properties due to its elevated concentration of flavonoids [11]. Despite the recent increase in scholarly attention towards the functions of flavonols, certain functional aspects continue to be a subject of controversy.

According to Laoué *et al* [111], the exact function of polyphenols in plants remains challenging to ascertain. However, ample evidence suggesting that flavonoids, specifically flavonols, play a significant role in providing protection and indirectly modulating plant growth in the presence of abiotic stressors [110, 111]. The enhancement of the plant's chemical defense mechanism can be interpreted as an advancement in the metabolic process of flavonoids and, consequently, their production in response to climatic stress conditions [111]. The main flavonoids found in moringa leaves are myricetin, quercetin, and kaempferol in concentrations of 5.8, 0.207, and 7.57 mg/g, respectively [103]. The quercetin is found in dried leaves of moringa and is a strong antioxidant [103].

The extraction of flavonoids from moringa include multiple of procedures. Firstly, freshly harvested moringa leaves are gathered and thoroughly sanitized [112] (Fig.7). Next, the leaves are extracted using a solvent such as ethanol, methanol, or acetone [112]. The extract is further strained to eliminate any solid particulates [112]. Subsequently, the filtrate is concentrated by methods such as rotary evaporation or vacuum distillation [112]. Subsequently, the concentrated extract undergoes purification through procedures such as chromatography to isolate specific flavonoids from other substances [112]. The extracted flavonoids are further examined using spectroscopic methods such as UV-Vis spectroscopy, mass spectrometry, and nuclear magnetic resonance (NMR) to verify their identification and purity [112]. The individual flavonoids are stored under optimal conditions to preserve their stability. Strict adherence to safety precautions is crucial, and it is essential to refer to comprehensive guidelines for guidance [112].





Figure 7: Chemical structure of types of flavonoids isolated from Moringa plant materials (A) Flavonols, (B) Kaempferol and (C) Myricetin [112]

## 6.6 Tannins

Tannins are a class of polyphenolic compounds that are inherent constituents of numerous plant species [113]. 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 [114]. Tannins possess a tactile quality and sensation as opposed to a distinct gustatory attribute [115]. 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 [116].

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 metabolizable energy, and protein digestibility [116]. Consequently, it is widely believed that meals containing high levels of tannins exhibit diminished nutritional content [116].

According to recent scholarly investigations, the primary impact of tannins does not appear to be centred on the reduction of food consumption or digestion [116, 117]. Instead, it is observed that tannins lead to a diminished efficacy in the conversion of ingested nutrients into new bodily constituents [117]. Tannins play a crucial role in the conservation of distinct plant species. The accumulation of tannins within the bark of trees serves as a protective mechanism against bacterial and fungal infections [118]. 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 characterized 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 [118]. The concentration of tannins exhibits variation across different sections of the moringa tree, with the dried leaves demonstrating the highest concentration [118]. Tannins are present in seeds in limited quantities as well [118].



Tannins have the potential to disrupt nutrient cycling through various mechanisms, such as impeding the rate of decomposition, forming complexes with proteins, inducing toxicity in microbial communities, and diminishing enzyme activity [119]. 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 [120]. Increased levels of tannins can lead to allelopathic reactions, alterations in soil characteristics, and reduced productivity within ecosystems [121]. The aforementioned effects possess the capacity to alter or impact successional processes. The study by Toit and Vorster [122] discovered that the leaves of moringa across all months had higher content of tannin and they increased as the season progressed from September to May. They further stated that winter season triggered an increase in tannin, that could be due to the plants initiating ways of defense against the cold stress [122].

Catechin is a type of tannic acid present in moringa plants (Fig.8), obtained by pulverizing leaves and extracting them with solvents such as ethanol or methanol [123]. Once the extract is clean, solid particles are removed using filtration, it is condensed using evaporation or rotary evaporation, and it is split into its different parts using column chromatography or liquid-liquid extraction [123]. The purified catechin is subjected to additional purification methods, such as recrystallization or preparative high-performance liquid chromatography (HPLC) [123]. Following that, the purified catechin is examined for its specific characteristics and level of purity using techniques such as NMR spectroscopy or mass spectrometry [123]. Nevertheless, this procedure necessitates proficiency in organic chemistry and adherence to safety measures while dealing with solvents and chemicals [123].



Figure 8: Chemical structure of catechin, a type of tannic acid isolated from Moringa plant material [123]

#### 7. The influence of VAM on the biosynthesis and accumulation of phytochemicals

One advantage of VAM is its ability to regulate the synthesis of primary and secondary compounds derived from plant metabolism [124]. In this context, research was carried out involving plants of medicinal significance, documenting the increase in the production of biomolecules that possess medicinal properties [125, 126]. Plants can synthesize compounds through secondary metabolism, which can imbue them with medicinal attributes. These compounds are derived from precursors of primary metabolism and can be categorized into three primary classes: terpenes, nitrogen compounds, and phenolic compounds [126]. Vesicular Arbuscular Mycorrhizal inoculation is a potential alternative for augmenting the production of these compounds and enhancing the pytomass [127].

Abdelhalim *et al.* [125] conducted a study which 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 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 associations with plant roots has yielded notable improvements in the synthesis of various secondary compounds, including phenolic compounds [50, 128, 129].

In a recent study conducted by Kumar *et al.* [50], it was observed that the introduction of mycorrhiza to plant species resulted in alterations to their biochemical and molecular pathways. This, in turn, led to an increased accumulation of secondary metabolite compounds in various plant tissues. This suggests that there is potential for commercial exploration of their application to enhance the presence of health-promoting phytochemical compounds in moringa.

Furthermore, it has been observed that mycorrhization not only promotes the synthesis of secondary compounds in various groups [129, 130], but also enhances the biosynthesis of specific compounds that hold significant importance in the pharmaceutical sector. Oliveira et al. [131] observed an elevation in the foliar vitexinin concentration in yellow passion fruit. In the study conducted by Thangavel et al. [132], it was 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, with the exception of essential oil. Another advantage of VAM is their capacity to promote plant nutrient absorption, altering the content of secondary metabolites directly or indirectly [133]. For example, the study by Chen et al. [133] discovered that root and shoot biomass, root system architecture, and flavonoid were enhanced by VAM in glycyrrhiza uralensis grown in phosphorus deficient conditions. In summary, the application of VAM to medicinal plants presents a viable approach to enhance 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. [134] found that the application of VAM to medicinal plants represents a viable strategy for enhancing the production of secondary metabolites with pharmacological, medical, and cosmetic significance.

## 8. Conclusion

Moringa production is limited by abiotic factors such as high salinity levels, the need to enhance its cultivation is driven by the increasing population, which results in increased demand for food and medicinal plants. Vesicular Arbuscular Mycorrhiza (VAM) has been evidenced to play a crucial role in soil fertility and sustainable crop production. Research conducted on VAM associations in various crops has consistently shown positive effects on plant growth, nutrient uptake, and crop yield. With well recorded evidence of soil degradation, poor soil fertility and lack of arable land due to climate variability, the study recommends the application of VAM as a viable solution in the cultivation of moringa for increase production. However, there is still much to learn about mycorrhiza fungi, and further research is needed to understand their diversity and the factors influencing their occurrence. Effective communication of information regarding the application VAM is crucial to encourage farmers to adopt its usage and promote sustainable agricultural practices.

Versicular Arbuscular Mycorrhiza fungi are essential in natural ecosystems, particularly in saline environments, as they enhance plant growth and promote resistance to salt stress. They aid in transporting mineral nutrients, particularly phosphorus, to plants in salt-induced soil. The fungus enhances plant growth and development, even at



low NaCl concentrations. However, definitive conclusions on moringa plants need to be investigated on a crop basis as the mycorrhiza symbiosis is crop specific. Additionally, VAM fungi provide protection against stressors by augmenting antioxidant responses and inducing acquired systemic tolerance.

The phytochemicals in moringa contribute to its medicinal properties, and the distribution and abundance of these phytochemicals vary throughout the plant. The application of VAM inoculation has the potential to enhance the production of phytochemical compounds in medicinal plants such as moringa as evident in other crops. There is a need to investigate the impact of VAM symbiosis on the accumulation and biosynthesis of phytochemicals in moringa plants under salinity stress. Where the accumulation of phytochemicals under salinity stress and symbiosis with VAM can be evaluated.

#### REFERENCES

- [1] Stohs SJ and Hartman MJ (2015) Review of the safety and efficacy of Moringa oleifera. Phytother. Res., 29(6): 796-804.
- [2] Gopalakrishnan L, Doriya K and Kumar DS (2016) *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food. Sci. Hum. Wellness., 5(2): 49–56.
- [3] Oyeyinka AT and Oyeyinka SA (2018) Moringa oleifera as a food fortificant: Recent trends and prospects. J. Saudi. Soc. Agric. Sci., 17(2): 127–136.
- [4] Singh V, Arulanantham A, Parisipogula V, Arulanantham S and Biswas A (2018) Moringa olifera: nutrient dense food source and world's most useful plant to ensure nutritional security, good health and eradication of malnutrition. European. J. Nutr. Food. Saf., 8(4): 204–214.
- [5] Mushtaq BS, Hussain MB, Omer R, Toor HA, Waheed M, Shariati MA. Sergey P and Heydari M (2021). *Moringa oleifera* in malnutrition: A comprehens. Rev. Curr. Drug. Discov. Technol.Title., 18(2): 235–243.
- [6] Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. pharmacol., 4: 177.
- [7] Sujatha BK and Patel P (2017) Moringa oleifera-nature's gold. Imp. J. Interdiscip. Res., 3(5): 1175-1179.
- [8] Prajapati C, Ankola M, Upadhyay TK, Sharangi AB, Alabdallah NM, Al-Saeed FA, Muzammil K and Saeed M (2022) *Moringa oleifera*: Miracle plant with a plethora of medicinal, therapeutic, and economic importance. Hortic., 8(6): 492.
- [9] Yang RY, Chang LC, Hsu JC, Weng BB, Palada MC, Chadha ML and Levasseur V (2006) Nutritional and functional properties of moringa leaves-from germplasm, and to plant, to food, to health. Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU. Paris., 1–9.
- [10] Tshabalala T, Ncube B, Madala NE, Nyakudya TT, Moyo HP, Sibanda M and Ndhlala AR (2019) Scribbling the cat: a case of the "miracle" plant, *Moringa oleifera*. Plant. J., 8(11): 510.
- [11] Rode SB, Dadmal A, Salankar HV and Dadmal Jr AA (2022). Nature's gold (Moringa oleifera): miracle properties. Cureus J., 14(7).
- [12] Biswas K and Sinha SN (2021) The Miracle Plant-Moringa. Bioresource Utilization and Management: Applications in Therapeutics, Biofuels, Agriculture, and Environmental Science.
- [13] Abdel-Latif HM, Abdel-Daim MM, Shukry M, Nowosad J and Kucharczyk D (2022) Benefits and applications of *Moringa oleifera* as a plant protein source in Aquafeed. A Rev. Aquac., 547: 737369.
- [14] Alpern SB (2008) Exotic plants of western Africa: Where they came from and when. J. Afr. Hist., 35:63–102.
- [15] Padayachee B and Baijnath HJSAJOB (2020) An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. S. Afr. J. Bot., 129: 304–316.
- [16] Sreeja M, Jayasri P, Keerthi N, Yeshashwini J and Praveen J (2021) Moringa oleifera: A review on nutritive importance and its potential use as nutraceutical plant. J. Med. Plant. Res., 9(2): 15–7.
- [17] Shija AE, Rumisha SF, Oriyo NM, Kilima SP and Massaga JJ (2019) Effect of *Moringa oleifera* leaf powder supplementation on reducing anemia in children below two years in Kisarawe District, Tanzania. Food Sci. Nutr., 7(8): 2584–2594.
- [18] Seifu E and Teketay D (2020) Introduction and expansion of *Moringa oleifera* Lam. in Botswana: Current status and potential for commercialization. S. Afr. J. Bot., 129: 471–479.



- [19] Okorie C, Ajibesin K, Sanyaolu A, Islam A, Lamech S, Mupepi K, Mupepi T, Oseni A, Oyeleke O and Abioye A (2019) A review of the therapeutic benefits of *Moringa oleifera* in controlling high blood pressure (hypertension). Curr. Tradit. Med., 5(3): 232–245.
- [20] Marwa GSF and Mohamed RAS (2021) Effect of esicular Arbuscular Mycorrhizal (VAM) fungus and rock-phosphate application on the growth and biomass of *Moringa oleifera Lam: Seedlings under Salinity Stress*. Alex. Sci. Exch. J., 42: 307– 325.
- [21] Hildebrandt U, Regvar M and Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochem., 68: 139146.
- [22] Xie MM, Zou YN, Wu QS, Zhang ZZ and Kuča K (2020) Single or dual inoculation of arbuscular mycorrhizal fungi and rhizobia regulates plant growth and nitrogen acquisition in white clover. Plant Soil. Environ., 66(6): 287–294.
- [23] Quilambo OA (2003) The vesicular-arbuscular mycorrhizal symbiosis. Afr. J. Biotechnol., 2(12): 539-546.
- [24] Lau CK, Chui CFR and Au N (2019) Examination of the adoption of augmented reality: A VAM approach. Asia Pac. J. Tour. Res., 24(10): 1005–1020.
- [25] Hepper CM (2018) Isolation and culture of VA mycorrhizal (VAM) fungi. In VA mycorrhiza. CRC press, pp. 95-112.
- [26] Abiala MA, Popoola OO, Olawuyi OJ, Oyelude OJ, Akanmu AO, Killani AS, Osonubi O and Odebode AC (2013) Harnessing the potentials of Vesicular Arbuscular Mycorrhizal (VAM) fungi to plant growth–a review.
- [27] Hussein HA, Shiker MA and Zabiba M.S (2020) A new revised efficient of VAM to find the initial solution for the transportation problem. J. Phys. Conf: Confer Series., 1591(1): 012032.
- [28] Snoeck D, Abolo D and Jagoret P (2010) Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. Agrofor Syst., 78: 323–328.
- [29] Pal A and Pandey S (2014) Role of glomalin in improving soil fertility. Int. J. Plant Soil. Sci., 3: 112-129.
- [30] Abbasi H, Akhtar A and Sharf R (2015) Vesicular Arbuscular Mycorrhizal (VAM) fungi: a tool for sustainable agriculture. Amer. J. Plant Nutr. Fertil. Tec., 5(2): 40–49.
- [31] Lupatini M, Korthals GW, De Hollander M, Janssens TK and Kuramae EE (2017) Soil microbiome is more heterogeneous in organic than in conventional farming system. Front. Microbiol., 7:2064.
- [32] Xia Y, Sahib MR, Amna A, Opiyo SO, Zhao Z and Gao YG (2019) Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. Sci. Rep., 9(1): 1669.
- [33] Itelima JU, Bang WJ, Onyimba IA, Sila MD and Egbere OJ (2018) Bio-fertilizers as key player in enhancing soil fertility and crop productivity: A review. Direct Res. J. Agric. and Food Sci., 6(3): 73–83.
- [34] Manjulatha G (2015) Effect of vesicular arbuscular mycorrhiza on maize growth and yield.
- [35] Kazadi AT, Lwalaba JLW, Ansey BK, Muzulukwau JM, Katabe GM, Karul MI, Baert G, Haesaert G and Mundende RM (2022) Effect of phosphorus and Arbuscular Mycorrhizal Fungi (AMF) inoculation on growth and productivity of maize (*Zea mays* L.) in a tropical ferralsol. Gesunde Pflanz., 74: 159–165.
- [36] Suri VK and Choudhary AK (2013) Effect of vesicular arbuscular-mycorrhizal fungi and phosphorus application through soiltest crop response precision model on crop productivity, nutrient dynamics, and soil fertility in soybean-wheat-soybean crop sequence in an acidic alfisol. Commun. Soil Sci. Plant. Anal, 44(13): 2032–2041.
- [37] Gao X, Guo H, Zhang Q, Guo H, Zhang L, Zhang C, Gou Z, LiuY, Wei J, Chen A and Chu Z (2020) Arbuscular Mycorrhizal Fungi (AMF) enhanced the growth, yield, fiber quality and phosphorus regulation in upland cotton (*Gossypium hirsutum* L.). Sci. Rep., 10(1): 2084.
- [38] Saia S, Aissa E, Luziatelli F, Ruzzi M, Colla G, Ficca AG, Cardarelli M and Rouphael Y (2020) Growth-promoting bacteria and arbuscular mycorrhizal fungi differentially benefit tomato and corn depending upon the supplied form of phosphorus. Mycorrhiza., 30: 133–147.
- [39] Basak H, Demir K, Kasim R and Okay FY (2011) The effect of endo-mycorrhiza (VAM) treatment on growth of tomato seedling grown under saline conditions. Afri. J. Agric. Res., 6(11): 2532–2538.
- [40] Masenya TA, Mabila SW, Hlophe T and Letsoalo ML (2023) Vesicular Arbuscular Mycorrhizal influence on growth of cancer bush (*Sutherlandia frutescens*) and alleviation of saline stress. Res. Crop., 24: 179–184.
- [41] Abbott LK and Robson AD (2018) The effect of VA mycorrhizae on plant growth. In VA mycorrhiza. CRC Press., 113–130.
- [42] Candido V, Campanelli G. D'Addabbo T, Castronuovo D, Perniola M and Camele I (2015) Growth and yield promoting effect of artificial mycorrhization on field tomato at different irrigation regimes. Sci. Hortic., 187:35–43.



- [43] Maya C, Roopa B, Makari HK and Nagaraj K (2012) The synergistic effect of VAM fungi with *Rhizobium* on the growth and yield of Cicer arietinum L. Int. Multidiscip. Res. J., 2(1): 16–20.
- [44] Premsekhar M and Rajashree V (2009) Influence of bio-fertilizers on the growth characters, yield attributes, yield and quality of tomato. Am Eurasian J. Sustain. Agric., 3(1): 68–70.
- [45] Allsopp N and Stock W (1995) Relationships between seed reserves, seedling growth and mycorrhizal responses in 14 related shrubs (*Rosidae*) from a low-nutrient environment. Funct. Ecol., 248–254.
- [46] Indriani NP, Yuwariah Y, Rochana A, Susilawati I and Khairani L (2016) The role of Vesicular Arbuscular Mycorrhiza (VAM) and rock phosphate application on production and nutritional value of centro legumes (*Centrosema pubescens*). Legum, Res. Intern J., 39(6): 987–990.
- [47] Rybak AS (2018) Species of Ulva (Ulvophyceae, Chlorophyta) as indicators of salinity. Ecol. Indic., 85: 253-261.
- [48] Eswar D, Karuppusamy R and Chellamuthu S (2021) Drivers of soil salinity and their correlation with climate change. Curr. Opin. Environ. Sustain., 50: 310–318.
- [49] Farooq F, Rashid N, Ibrar D, Hasnain Z, Ullah R, Nawaz M, Irshad S, Basra SM, Alwahibi MS, Elshikh MS and Dvorackova H (2022) Impact of varying levels of soil salinity on emergence, growth, and biochemical attributes of four *Moringa oleifera* landraces. PLoS One, 17: 0263978.
- [50] Kumar A, Singh S, Gaurav AK, Srivastava S and Verma JP (2020) Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front. Microbiol., 11: 1216.
- [51] Azeem M, Pirjan K, Qasim M, Mahmood A, Javed T, Muhammad H, Yang S, Dong R, Ali B and Rahimi M (2023) Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. Sci. Rep., 13(1): 2895.
- [52] Elhag AZ and Abdalla MH (2014) Investigation of sodium chloride tolerance of moringa (*Moringa Oleifera* Lam.) Transplants. Univl. J. Agric. Res., 2: 45–49.
- [53] Nouman W, Siddiqui MT, Basra SMA, Khan RA, Gull T, Olson ME and Hassan M (2012) Response of *Moringa oleifera* to saline conditions. Int. J. Agric. Biol., 14(5): 757–762.
- [54] Tahat MM and Sijam K (2012) Mycorrhizal fungi and abiotic environmental conditions relationship. Res J. Env. Sci., 6(4): 125– 133.
- [55] Oztekin GB, Tuzel Y and Tuzel IH (2013) Does mycorrhiza improve salinity tolerance in grafted plants?. Sci. Hortic., 49: 55-60.
- [56] Frahat MG and Shehata MR (2021) Effect of vesicular arbuscular mycorrhizal (VAM) Fungus and rock-phosphate application on the growth and biomass of *Moringa oleifera* Lam. seedlings under salinity stress. Alex. Sci. Exch. J., 42(2): 307–325.
- [57] Giri B, Kapoor R and Mukerji KG (2002) Versicular Arbuscular Mycorrhizal techniques/VAM technology in establishment of plants under salinity stress conditions. Techni. in mycorrhiza stud., pp.313–327.
- [58] Bhardwaj S and Kumar P (2020) Salinity stress, its physiological response and mitigating effects of microbial bio inoculants and organic compounds. J. pharmacogn. Phytochem., 9(4): 1297–1303.
- [59] Evelin H, Kapoor R and Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot., 104(7): 1263–1280.
- [60] Ebrahim MK and Saleem AR (2017) Alleviating salt stress in tomato inoculated with mycorrhizae: Photosynthetic performance and enzymatic antioxidants. J. Taibah Univ. Sci., 11(6): 850–860.
- [61] Ji D, Wang Q, Lu T, Ma H and Chen X (2022) The effects of ultrasonication on the phytochemicals, antioxidant, and polyphenol oxidase and peroxidase activities in coffee leaves. Food Chem., 373: 131480.
- [62] Shree P, Mishra P, Selvaraj C, Singh SK, Chaube R, Garg N and Tripathi YB (2022) Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants–Withania somnifera (Ashwagandha), Tinospora cordifolia (Giloy) and Ocimum sanctum (Tulsi)–a molecular docking study. J. Biomol. Struct., 40(1): 190–203.
- 63] Ma ZF, Ahmad J, Zhang H, Khan I and Muhammad S (2020) Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera* L.) as a potential functional food. S. Afr. J. Bot., 129: 40–46.
- [64] Li J, Liu H, Mazhar MS, Quddus S, Agar OT and Suleria HAR (2023) Australian Native Plum: A Review of the Phytochemical and Health Effects. Food Rev. Int., pp.1–29.
- [65] Wang F, Bao Y, Zhang C, Zhan L, Khan W, Siddiqua S, Ahmad S, Capanoglu E. Skalicka- Woźniak K, Zou L and Simal-Gandara J (2022) Bioactive components and anti-diabetic properties of *Moringa oleifera* Lam. Crit. Rev. Food Sci. Nutr., 62(14): 3873–3897.



- [66] Kasolo JN, Bimenya GS, Ojok L, Ochieng J and Ogwal-Okeng J (2010) Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. Journal of Medicinal Plants Research, 4(9): 753–757.
- [67] Rabizadeh F, Mirian MS, Doosti R, Kiani-Anbouhi R and Eftekhari E (2022) Phytochemical classification of medicinal plants used in the treatment of Kidney disease based on traditional persian medicine. Evidence-Based Complementary and Alternative Medicine. Evidence-Based Complementary and Alternative Medicine, Volume 2022, Article ID 8022599, 13pages. https://doi.org/10.1155/2022/8022599
- [68] Cornelius W (2019) Phytochemical Analysis of *Moringa oleifera* (leaves and flowers) and the functional group. Glob. Sci. J., 7: 41–51.
- [69] Martínez S, Fuentes C and Carballo J (2022) Antioxidant activity, total phenolic content and total flavonoid content in sweet chestnut (*Castanea sativa* Mill.) cultivars grown in northwest Spain under different environmental conditions. Foods, 11(21): 3519.
- [70] Othman L, Sleiman A and Abdel-Massih RM (2019) Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Front. Microbiol., 10: 911.
- [71] Mondal A, Gandhi A, Fimognari C, Atanasov AG and Bishayee A (2019) Alkaloids for cancer prevention and therapy: Current progress and future perspectives. Eur. J. Pharmacol., 858: 172472.
- [72] Kempf M, Reinhard A and Beuerle T (2010) Pyrrolizidine alkaloids (PAs) in honey and Pollen legal regulation of PA levels in food and animal feed required. Mol. Nutr. Food Res., 54(1): 158–168.
- [73] Kurek, J. (2019) Alkaloids: their importance in nature and human life. BoD-Books on Demand.
- [74] Nett RS, Lau W and Sattely ES (2020) Discovery and engineering of colchicine alkaloid biosynthesis. Nature, 584(7819): 148– 153.
- [75] Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J and Bertoli S (2015) Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. Int. J. Mol. Sci., 16(6): 12791–12835.
- [76] Roddan R, Ward JM, Keep NH and Hailes HC (2020) Pictet–Spenglerases in alkaloid biosynthesis: Future applications in biocatalysis. Curr. Opin. Chem. Biol., 55: 69–76.
- [77] Adamski Z, Blythe LL, Milella L and Bufo SA (2020) Biological activities of alkaloids: from toxicology to pharmacology. Toxins., 12(4): 210.
- [78] Sreelatha S and Padma PR (2009) Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum. Nutr., 64: 303-311.
- [79] Cressey P and Reeve J (2019) Metabolism of cyanogenic glycosides: Rev. Food Chem. Toxicol., 125: 225-232.
- [80] Bolarinwa IF, Oke MO, Olaniyan SA and Ajala AS (2016) A review of cyanogenic glycosides in edible plants. In Toxicology: New Aspects to This Scientific Conundrum; Soloneski, S., Larramendy, M., Eds.; Intech Open: London, UK.
- [81] Møller BL (2010) Functional diversifications of cyanogenic glucosides. Curr. Opin. Plant Biol., 13(3): 337-346.
- [82] Nyirenda KK (2020) Toxicity potential of cyanogenic glycosides in edible plants. Medic. Toxicol., 1-19.
- [83] Mosayyebi B, Imani M, Mohammadi L, Akbarzadeh A, Zarghami N, Edalati M, Alizadeh E and Rahmati M (2020). An update on the toxicity of cyanogenic glycosides bioactive compounds:Possible clinical application in targeted cancer therapy. Mater. Chem. Phys., 246: 122841.
- [84] Bolarinwa I (2013) Cyanogenic glycosides in plant foods (Doctoral dissertation, University of Leeds).
- [85] Oluwole OSA, Onabolu AO, Mtunda K and Mlingi N (2007) Characterization of cassava (*Manihot esculenta* Crantz) varieties in Nigeria and Tanzania, and farmers' perception of toxicity of cassava. J. Food Compos. Anal., 20(7): 559–567.
- [86] Chioma D and Okah R (2019) Analysis of *Moringa oleifera* (leaves and flowers) to determine some phytochemicals and functional groups. Direct Res. J. Chem. Mater. Sci., 6(3): 45–49.
- [87] Sun HX, Xie Y and Ye YP (2009) Advances in saponin-based adjuvants. Vaccine, 27(12): 1787–1796.
- [88] Silva M, Kato Y, Melo MB, Phung I, Freeman BL, Li Z, Roh K, Van Wijnbergen JW, Watkins H, Enemuo CA and Hartwell BL (2021) A particulate saponin/TLR agonist vaccine adjuvant alters lymph flow and modulates adaptive immunity. Sci. Immunol., 6(66): 1152.
- [89] Liao Y, Li Z, Zhou Q, Sheng M, Qu Q, Shi Y, Yang J, Lv L, Dai X and Shi X, (2021) Saponin surfactants used in drug delivery systems: A new application for natural medicine components. Intern. J. Pharm., 603: 120709.
- [90] Yi YS (2021) New mechanisms of ginseng saponin-mediated anti-inflammatory action via targeting canonical inflammasome signaling pathways. J. Ethnopharmacol., 278: 114292.



- [91] Sharma P, Tyagi A, Bhansali P, Pareek S, Singh V, Ilyas A, Mishra R and Poddar NK (2021) Saponins: Extraction, biomedicinal properties and way forward to anti-viral representatives. Food Chem. Toxicol., 150: 112075.
- [92] Elekofehinti OO, Iwaloye O, Olawale F and Ariyo EO (2021) Saponins in cancer treatment: Current progress and future prospects. Pathophysiology., 28(2): 250–272.
- [93] Pecetti L, Tava A, Romani M, De Benedetto MG and Corsi P (2006) Variety and environment effects on the dynamics of saponins in lucerne (*Medicago sativa* L.). Eur. J. Agron., 25(3): 187–192.
- [94] Yu B, Patterson N and Zaharia LI (2022) Saponin biosynthesis in pulses. Plant., 11(24): 3505.
- [95] Pasha SN, Shafi KM, Joshi AG, Meenakshi I, Harini K, Mahita J, Sajeevan RS, Karpe SD, Ghosh P, Nitish S and Gandhimathi A (2020) The transcriptome enables the identification of candidate genes behind medicinal value of Drumstick tree (*Moringa oleifera*). Genomics, 112(1): 621-628.
- [96] Ghasemzadeh A and Ghasemzadeh N (2011) Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plant Res., 5(31): 6697–6703.
- [97] Chen J, Yang J, Ma L, Li J, Shahzad N and Kim CK (2020) Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Sci Rep., 10(1): 2611.
- [98] Rashmi HB and Negi PS (2020) Phenolic acids from vegetables: A review on processing stability and health benefits. Food Res. Int., 136: 109298.
- [99] Marchiosi R, dos Santos WD, Constantin RP, de Lima RB, Soares AR, Finger-Teixeira A, Mota TR, de Oliveira DM, Foletto-Felipe MDP, Abrahão J and Ferrarese-Filho O (2020) Biosynthesis and metabolic actions of simple phenolic acids in plants. Phytochem. Rev., 19: 865–906.
- [100] Kiokias S, Proestos C and Oreopoulou V (2020) Phenolic acids of plant origin-A review on their antioxidant activity in vitro (o/w emulsion systems) along with their in vivo health biochemical properties. Foods, 9(4): 534.
- [101] Liu J, Du C, Beaman HT and Monroe MBB (2020) Characterization of phenolic acid antimicrobial and antioxidant structure– property relationships. Pharm., 12(5): 419.
- [102] Abedi F, Razavi BM and Hosseinzadeh H (2020) A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: Comprehensive pharmacology, toxicology, and some pharmaceutical aspects. Phytother Res., 34(4): 729–741.
- [103] Zhou M, Zhang J and Sun C (2017) Occurrence, ecological and human health risks, and seasonal variations of phenolic compounds in surface water and sediment of a potential polluted river basin in China. Int. J. Environ. Res. Public Health., 14(10): 1140.
- [104] Saucedo-Pompa S, Torres-Castillo JA, Castro-López C, Rojas R, Sánchez-Alejo EJ, Ngangyo-Heya M and Martínez-Ávila GCG (2018) Moringa plants: Bioactive compounds and promising applications in food products. Food Res. Inter., 111: 438–450.
- [105] Mpofu A, Sapirstein HD and Beta T (2006) Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem., 54(4): 1265–1270.
- [106] Fernandez-Orozco R, Li L, Harflett C, Shewry PR and Ward JL (2010) Effects of environment and genotype on phenolic acids in wheat in the Health Grain diversity screen. J. Agric. Food Chem., 58(17):.9341–9352.
- [107] Vergara-Jimenez M, Almatrafi MM and Fernandez ML (2017) Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxid., 6(4): 91.
- [108] Hassan M.A, Xu T, TianY, Zhong Y, Ali FAZ, Yang X and Lu B (2021) Health benefits and phenolic compounds of *Moringa oleifera* leaves. A Comprehensive Rev. Phytomed., 93: 153771.
- [109] Maleki SJ, Crespo JF and Cabanillas B (2019) Anti-inflammatory effects of flavonoids. Food Chem., 299: 125124.
- [110] Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R and Koirala N (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plant., 8(4): 96.
- [111] Karak P (2019) Biological activities of flavonoids: An overview. Inter J. Pharm Sci. Res., 10(4): 1567–1574.
- [112] Yerena-Prieto BJ, Gonzalez-Gonzalez M, Vázquez-Espinosa M, González-de-Peredo AV, García-Alvarado MÁ, Palma M, Rodríguez-Jimenes GDC and Barbero GF (2022) Optimization of an ultrasound-assisted extraction method applied to the extraction of flavonoids from Moringa leaves (*Moringa oleífera* Lam.). Agronomy, 12(2): 261.
- [113] Dias MC, Pinto DC and Silva AM (2021) Plant flavonoids: Chemical characteristics and biological activity. Mol., 26(17): 5377.
- [114] Yeh TS, Yuan C, Ascherio A, Rosner BA, Willett WC and Blacker D (2021) Long-term dietary flavonoid intake and subjective cognitive decline in US men and women. Neurology, 97(10): 1041–1056.



- [115] Li P, Ruan Z, Fei Z, Yan J and Tang G (2021) Integrated transcriptome and metabolome analysis revealed that flavonoid biosynthesis may dominate the resistance of Zanthoxylum bungeanum against stem canker. J. Agric. Food Chem., 69(22): 6360– 6378.
- [116] Laoué J, Fernandez C and Ormeño E (2022) Plant flavonoids in mediterranean species: A focus on flavonols as protective metabolites under climate stress. Plant, 11(2): 172.
- [117] Huang Q, Liu X, Zhao G, Hu T and Wang Y (2018) Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim Nutri., 4(2): 137–150.
- [118] Falcão L and Araújo MEM (2018) Vegetable tannins used in the manufacture of historic leathers. Mol., 23(5): 1081.
- [119] Ghahri S and Pizzi A (2018) Improving soy-based adhesives for wood particleboard by tannins addition. Wood Sci. Technol., 52: 261–279.
- [120] Pizzi A (2019) Tannins: Prospectives and actual industrial applications. Biomolecules, 9(8): 344.
- [121] de Hoyos-Martínez PL, Merle J, Labidi J and Charrier–El Bouhtoury F (2019) Tannins extraction: A key point for their valorization and cleaner production. J Cleaner. Prod., 206: 1138–1155.
- [122] Frahat MG and Shehata MR (2021) Effect of vesicular arbuscular mycorrhizal (VAM) Fungus and rock-phosphate application on the growth and biomass of *Moringa oleifera* Lam. seedlings under salinity stress. Alex. Sci. Exch J., 42(2): 307–325.
- [123] Budaraga IK and Putra DP (2020) Study of Green Tea Catechin Dipped with Moringa Leaves. In IOP Conf. Ser: Earth Env. Sci. 515(1): 012027.
- [124] Maisetta G, Batoni G, Caboni P, Esin S, Rinaldi AC and Zucca P (2019) Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant Cytinus. BMC Complement Altern. Med., 19: 1–11.
- [125] Abdelhalim TS, Tia NA, Elkhatim KAS, Othman MH, Joergensen RG, Almaiman SA and Hassan AB (2022) Exploring the potential of arbuscular mycorrhizal fungi (AMF) for improving health-promoting phytochemicals in sorghum. Rhizos., 24: 100596.
- [126] Kumar A, Singh DR, Lata S and Johri RM (2017) Effect of arbuscular mycorrhizal fungi on plant growth, essential oil and Artemisinin content of *Artemisia annua* L. Int. J. Pharmacol. Biol. Sci., 11(1): 33.
- [127] del Rosario Cappellari L, Santoro MV, Reinoso H, Travaglia C, Giordano W and Banchio E (2015) Anatomical, morphological, and phytochemical effects of inoculation with plant growth-promoting rhizobacteria on peppermint (*Mentha piperita*). J. Chem. Ecol., 41: 149–158.
- [128] Jaleel CA, Gopi R, Gomathinayagam M and Panneerselvam R (2009) Traditional and non-traditional plant growth regulators alters phytochemical constituents in Catharanthus roseus. Process Biochem., 44(2): 205–209.
- [129] Santos EL, Silva FA and Silva FSB (2017) Arbuscular Mycorrhizal Fungi increase the phenolic compounds concentration in the bark of the stem of Libidibia Ferrea in field conditions. Open Microbiol J., 11: 283–291.
- [130] Silva FA, Maia LC and Silva FSB (2018) Arbuscular mycorrhizal fungi as biotechnology alternative to increase concentrate of secondary metabolites in Zea mays L. Braz. J. Bot., 42: 189–193.
- [131] Oliveira JSF, Xavier LP, Lins A, Andrade EHA, Maia JGS, Mello AH, Setzer WN, Ramos AR and Silva JKR (2019) Effects of inoculation by arbuscular mycorrhizal fungi on the composition of the essential oil, plant growth, and lipoxygenase activity of *Piper aduncum* L. AMB Express, 9: 29.
- [132] Thangavel S, Nisha MC and Sevanan R (2009) Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of Pogostemon patchouli Pellet. Maejo Int. J. Sci. Technol., 3(1): 222–234.
- [133] Chen M, Yang G, Sheng Y, Li P, Qiu H, Zhou X, Huang L and Chao Z (2017) Glomus mosseae inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of liquorice under nutrient stress. Front. Plant Sci., 8: 931.
- [134] Zhao Y, Cartabia A, Lalaymia I and Declerck S (2022) Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. Mycorrhiza., 32(3-4): 221–256.