https://dx.doi.org/10.4314/dujopas.v10i2a.37
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 2a June 2024*

Phytostimulatory Effects of *Moringa oleifera* leaf Extract on the Growth and Chlorophyll Composition of *Amaranthus hybridus* using Phytopriming and Foliar Spraying Techniques

***Keshinro, O. M.[1], Sharaibi, O. J.[1], Oluwa, O. K.[1] Giwa, A. O.[1] and Mohammed, O. E.[1]**

[1]Department of Botany,
Lagos State University (LASU),
Ojo,
Lagos State.

Email: olajide.keshinro@lasu.edu.ng

ORCID: 0000-0002-7333-4795

Abstract

The consistent dependence of agriculturalist on inorganic fertilizer not only continues to pose an environmental threat, but also remains unsafe for human consumption. In this study, Moringa oleifera leaf extract (MLE) was used as a plant growth stimulator for Amaranthus hybridus, using two different application techniques (priming and foliar spraying). The treatments used for the experiment were 10%, 20%, and 30% MLE solutions, and 10%, 20%, and 30% mg/L gibberellic acid (GA_3) solutions, and distilled water. Viable seeds of A. hybridus were primed with MLE treatments and sown in 21 different polythene bags filled with top soils. Similarly, seeds of A. hybridus were sown in another 21 polythene bags and sprayed foliarly every two days for four weeks. All the 42 bags were arranged in a Completely Randomized Design at the screened house of the Botanical Garden, Lagos State University. The results from the growth analysis showed that although 20% GA_3 had the highest value of 14.37 cm for shoot height ($p<0.05$), 30% MLE was the closest among the MLE treatments with 9.12 cm. While there was no significant difference across the samples for stem girth and number of leaves ($p>0.05$), there was a standout performer for chlorophylls. 30% MLE recorded the highest values of 9.33 mg/100g, 4.67 mg/100g, 14.00 mg/100g for Chlorophyll a, b, and total chlorophyll, and all with significant differences. Interestingly, all the best performing treatments were from A. hybridus plants cultivated using the foliar spraying techniques, thereby making it a better application technique than the phytopriming. Generally, the 30% MLE displayed the best potential in the stimulation of growth and enhancement of chlorophyll contents of A. hybridus which could be of great value to those in the agriculture, food and pharmaceutical industries.

Keywords: Phytopriming, *Amaranthus hybridus*, Foliar, Chlorophyll, *Moringa oleifera*

INTRODUCTION

Amaranthus hybridus, commonly known as green amaranth is an annual herbaceous vegetable belonging to the family *Amaranthaceae* (Aderibigbe *et al*., 2022). It is widely cultivated and consumed for its nutritional and medicinal properties (Baraniak & Kania-Dobrowolska, 2022). It is highly rich in iron, carbohydrates, fats, carbohydrates, vitamins, proteins, essential amino

Author for Correspondence

acids and fibre (Baraniak & Kania-Dobrowolska, 2022; Janmohammadi *et al*., 2023). *A. hybridus* is a dynamic plant that can easily adapt to harsh environmental conditions (Martínez-Núñez *et al*., 2019), a fundamental criterion in a country like Nigeria, where arable lands are getting scarce by the day due to increase in population and infrastructural developments. According to World bank (2019), Nigeria's agricultural sector employs about 70% of its workforce, yet there is still low productivity, and this is largely due to inaccessibility of yield-enhancing and sustainable agricultural inputs (Balana & Fasoranti, 2022). In Nigeria, inorganic fertilizers are often used to enhance the growth of herbaceous plants such as vegetables, and the constant application of chemical fertilizers over a period of time often damages the soil structure and disrupts microbial activities in the soil (Kakar *et al*., 2020; Sukyankij *et al*., 2024). More recently, excessive use of inorganic fertilizers on crops such as fruits and vegetables, which are often eaten raw or blanched, have been linked to several health problems such as Alzheimer's disease, lipid peroxidation, diabetes mellitus, DNA damage and oxidative stress, which can collectively lead to rapid cellular degeneration and death (De la Monte *et al*. 2009). Not only are chemical fertilizers hazardous to humans and the environment, they are also becoming a scarce and expensive commodity (Action-aid, 2023). In lieu of this, there is a growing advocacy for green economy where organic fertilizers and biostimulants replace chemical fertilizers for the safety of the environment and human health. More recently, plant-based products (compost and extracts) have been used to enhance plant growth and productivity (Saa *et al*., 2015; Sadar *et al*., 2021; Keshinro *et al*., 2023). A typical example is the extract of *Moringa oleifera* which is gaining a global attention for its use in promoting yield and productivity in agricultural crops like Buckwheat (*Fagopyrum esculentum*), Almonds (*Prunus dulcis* [Mill.] D. A. Webb) and so on (Saa *et al*., 2015; Baloch *et al*., 2024).

Moringa oleifera Linn. commonly known as a miracle plant, is a deciduous tree belonging to the family *Moringaceae*. It is a rapid growing plant that can grow as high as 10 to 15 m tall (Abd El-Hack, 2018). *M. oleifera* Linn. is a multi-functional plant that have been utilized for its medicinal, nutritional, nutraceuticals, and pharmacological values (Ali *et al*., 2004; Adline and Devi, 2014; Gopalakrishnan *et al*., 2016; Patil *et al*., 2022). More recently, it has been widely adopted for its use in agriculture to stimulate plant growth and increase crop yield (Abd El-Hack, 2018; Sarda *et al*., 2021). According to Sarda *et al*. (2021) *M. oleifera* contains natural cytokinins such as dihydrozeatin, isopentyl adenine and zeatin which helps in root stimulation and growth, breaking of dormant seeds and buds, inhibition of leaf senescence, promotion of bud formation in leaf cuttings and also improve chlorophyll content and nutrient uptake in plants. Despite all the recent reports on the potency of *Moringa* leaf extracts (MLE) as a plant growth stimulator (Saa *et al*., 2015; Sadar *et al*., 2021; Baloch *et al*., 2024), there have been little or no reports on Nigerian grown vegetables and fruits. Therefore, in this study, MLE was used as a biofertilizer and bio-stimulant in the cultivation of *A. hybridus* using phytopriming and foliar spraying techniques.

MATERIALS AND METHODS

Collection of Experimental Samples

The seeds of *Amaranthus hybridus* was obtained from Lusada market in Ogun State, Nigeria. Soil samples was collected behind the screened house at the Botanical Garden, Lagos State University (LASU), Ojo, Lagos State. Moringa leaves were collected from a *Moringa oleifera* tree beside MBA lecture hall, LASU, while gibberellic acid (GA_3) was obtained from the Chemical store of the Department of Botany, LASU. The seeds of *Amaranthus hybridus* and *Moringa* leaf samples were identified by a Taxonomist at the herbarium unit of the aforementioned school.

Preparation of Plant Treatments

Moringa oleifera leaf extract (MLE) was prepared according to the method described by Yasmeen *et al*. (2013) for both foliar spraying and phytopriming treatments of *A. hybridus*. Briefly, 50 g of *Moringa oleifera* leaves were weighed, thoroughly washed with distilled water and blended with 1 L of distilled water in an electronic blending machine (Philips HR 2056, China). The extract from the blended leaves was centrifuged at 8000 rpm for 20 min and the supernatant was collected. Different concentrations of MLE (10%, 20%, and 30%) were prepared and used as treatments in this study. The same series of concentrations were prepared for GA_3 to serve as control in the experimental set-up.

Phytopriming of *A. hybridus* Seeds

A. hybridus seeds were carefully rinsed in three regimes of distilled water, after which the seeds were soaked in 10%, 20%, and 30% of MLE, together with the same concentrations of GA_3 and distilled water as controls (Plate 1). The treatments were kept in a cupboard under aseptic conditions for 24 hours. The seeds were then carefully collected, rinsed with distilled water, and planted in perforated polythene bags containing top soils to ensure optimal growing conditions. The treated seeds were planted in duplicates and the bags arranged using a completely randomized design.

Foliar Spraying of *Moringa oleifera* Leaf Extracts on *A. hybridus*

Viable seeds of *A. hybridus* were sown in 21 planting bags containing planting soils and arranged in a completely randomized design according to the treatments listed in section 2.2. Foliar spraying of *A. hybridus* commenced at two weeks after planting, and each treatment was sprayed on the foliar part of the plants every two days for four weeks.

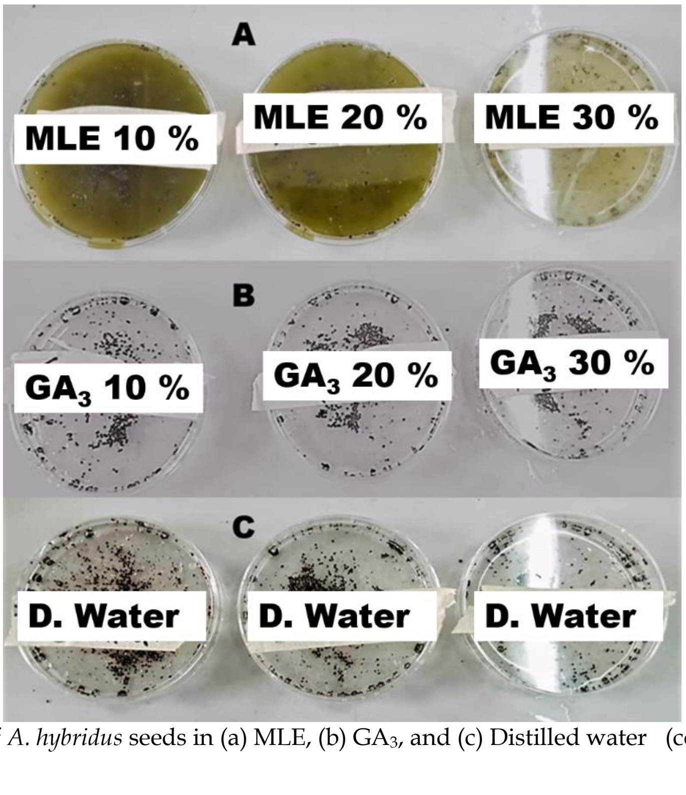

Plate 1: Phytopriming of *A. hybridus* seeds in (a) MLE, (b) GA_3, and (c) Distilled water (control).

Growth Parameters
The shoot height was measured from ground to the terminal bud using a meter rule, while the stem diameter was measured using a vernier caliper. The total number of leaves on each of the treated and control plants were also counted and recorded.

Determination of Chlorophyll Content
The extraction of chlorophyll from the leaves of *A. hybridus* was carried out according to the method described by Arnon (1949) and Huang *et al*. (2022). The following equations were used to determine the chlorophyll concentrations:
Chlorophyll A (mg/mL) = 12.7 (A_{663}) - 2.69 (A_{645})
Chlorophyll B (mg/mL) = 22.9 (A_{645}) – 4.68 (A_{663})
Total Chlorophyll (mg/mL) = Chlorophyll A + Chlorophyll B.
Where: A_{645} = absorbance at a wavelength of 645 nm and,
A_{663} = absorbance at a wavelength of 663 nm.

Statistical Analysis
Data obtained from this study were computed in Microsoft Excel and imported into R software for analysis. Analysis of Variance (ANOVA) was employed to estimate the mean and standard deviations in each experimental group, while the Tukey HSD Post Hoc Test was used to estimate the significant differences between the groups, and the variations within the group. The former was reported as probability value, and the latter as F-value. Significant associations were established when $p<0.05$.

RESULTS

Growth Parameters of Treated Plants
The shoot height after four weeks of planting showed that *A. hybridus* plants treated with GA_3 had the highest shoot height across all the treatments, which could be attributed to the function of gibberellic acid as a growth and shoot height promoter (Figure 1). However, the 30 % MLE treatments administered using both the phytopriming and foliar spraying methods resulted in a higher shoot height of 8.45±0.92 cm and 9.12±1.60 cm respectively when compared to the lower concentrations. Consequently, there was no significant difference between the 10% and 20% MLE treatments and the distilled water controls irrespective of the method of administration (Figure 1).

The stem girth after four weeks of planting showed that 30 % GA_3 and 20 % GA_3 had the highest stem girths with 9.9±2.88 mm and 9.00±2.30 mm respectively (Figure 2). However, plants treated with 10 % GA_3 showed similar performance with the 30 % MLE treatment with both having 8.5±1.10 mm and 8.0±1.15 mm stem girth respectively (Figure 2).

Although there are no significant differences in the number of leaves across all treatments, the highest number of leaves were recorded in 20%, 30% and 10% GA_3 with 12.50±1.73, 12.50±1.73 and 11.25±1.60 respectively (Figure 3). This was followed by the distilled water and 20% MLE treatments with 10.83±1.12 and 9.75±1.10 respectively. Interestingly, all the aforementioned treatments were recorded from the phytopriming method of cultivation (Figure 3).

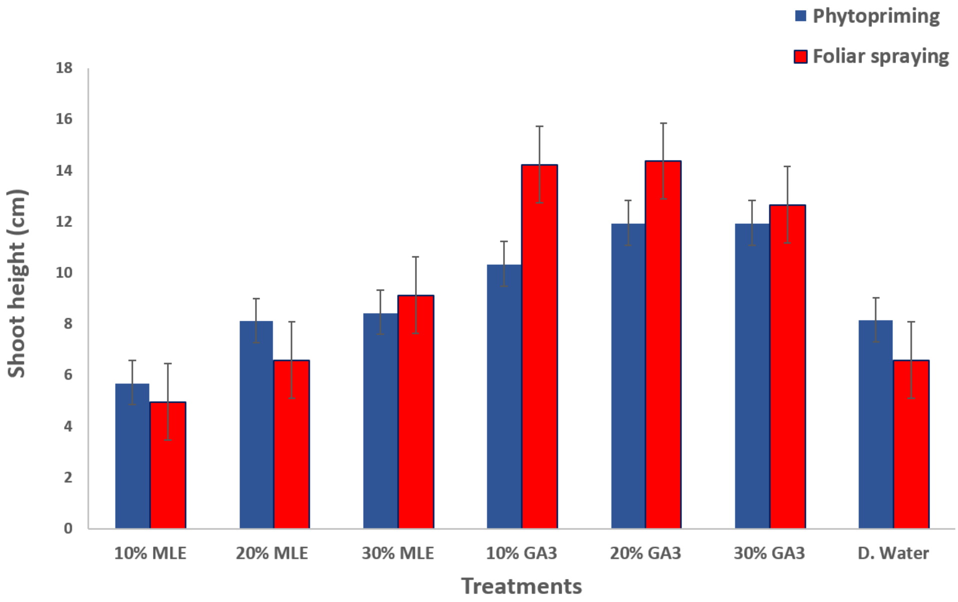

Figure 1: Shoot height analysis of *A. hybridus* treated with MLE and GA_3 using phytopriming and foliar spraying techniques.

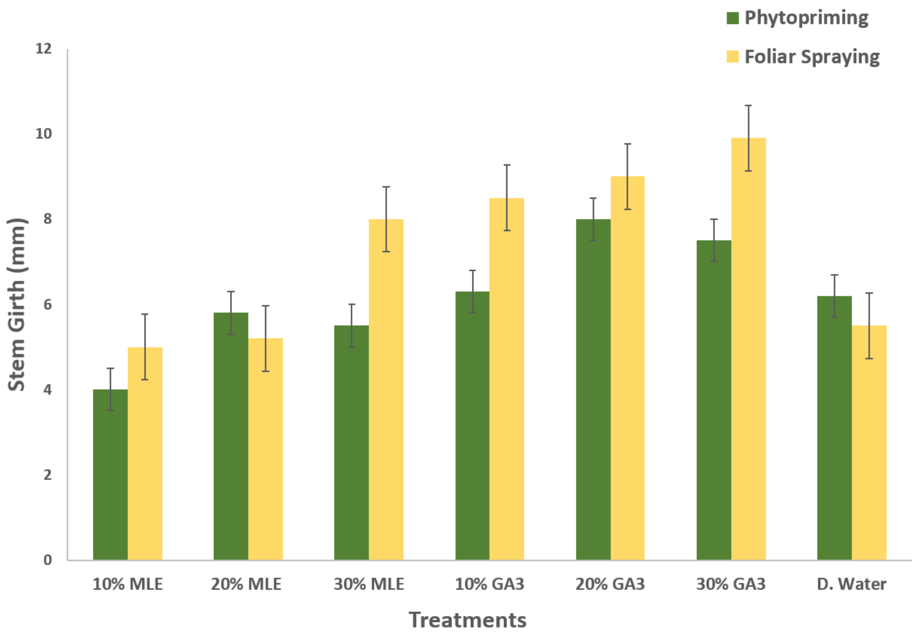

Figure 2: Stem girth analysis of *A. hybridus* treated with MLE and GA_3 using phytopriming and foliar spraying techniques

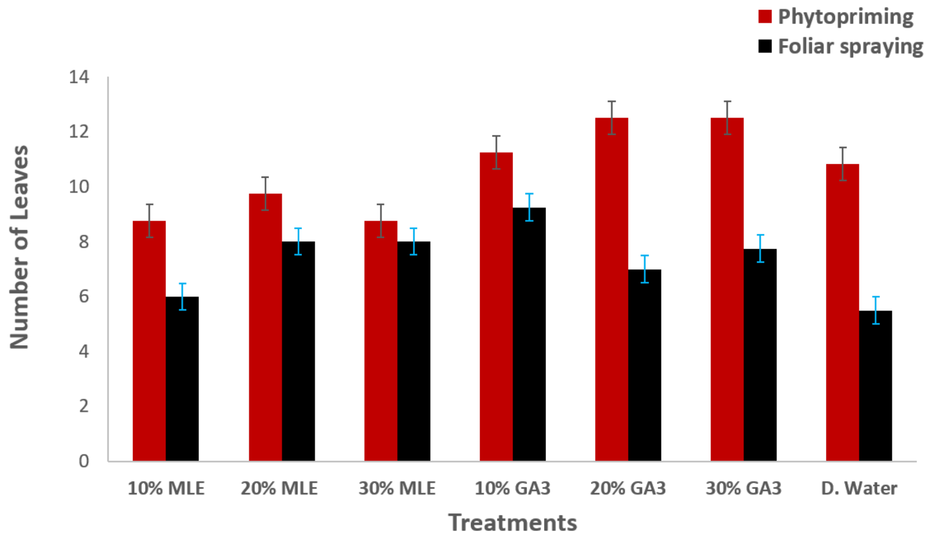

Figure 3: Analysis of number of leaves of *A. hybridus* treated with MLE and GA_3 using phytopriming and foliar spraying techniques.

Chlorophyll Contents of Treated *A. hybridus*

The results from the chlorophyll content analysis of the *A. hybridus* plants cultivated using phytopriming cultivation method showed that the highest amount of Chlorophyll A and Total chlorophyll were recorded in 10% MLE (1.98±1.42 mg/100g and 2.97±2.01 mg/100g), and 10 % GA_3 (2.01±1.36 mg/100g and 3.14±2.10 mg/100g) respectively (Table 1). However, there was no significant difference ($p<0.05$) in the chlorophyll content of both treatments. (Table 1). Furthermore, the chlorophyll content of the *A. hybridus* plants cultivated using foliar spraying method showed that 30% MLE had the highest values of 9.33± 0.58 mg/100g, 4.67±0.57 mg/100g, and 14.00±1.00 mg/100g for Chlorophyll A, B, and Total chlorophyll respectively (Table 2). These values were significantly different at $p<0.05$ when compared across the treatments (Table 2).

Table 1: Mean value of chlorophyll contents of *A. hybridus* cultivated using phytopriming

	Chlorophyll Content (Mean± S.D.)		
Plant treatment	**Chlorophyll a (mg/100g)**	**Chlorophyll b (mg/100g)**	**Total chlorophyll (mg/100g)**
10 % MLE	$1.98±1.42^{ab}$	$0.99±0.69^{a}$	$2.97±2.01^{b}$
20 % MLE	$0.50±0.41^{a}$	$0.27±0.21^{a}$	$0.74±0.61^{a}$
30 % MLE	$0.74±0.55^{a}$	$0.42±0.30^{a}$	$1.15±0.85^{a}$
10 % GA_3	$2.01±1.36^{b}$	$1.13±0.73^{a}$	$3.14±2.10^{b}$
20 % GA_3	$1.15±0.78^{a}$	$0.68±0.40^{a}$	$1.82±1.20^{a}$
30 % GA_3	$1.42±0.82^{ab}$	$0.96±0.55^{a}$	$2.38±1.38^{b}$
D. Water	$0.24±0.15^{a}$	$0.05±0.05^{a}$	$0.28±0.16^{a}$

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability.

Table 2: Mean value of chlorophyll contents of *A. hybridus* cultivated using foliar spraying methods

Treatments	Chlorophyll content (Mean ± S.D.)		
	Chlorophyll a (mg/100g)	**Chlorophyll b (mg/100g)**	**Total Chlorophyll (mg/100g)**
10 % MLE	6.00± 4.00^{b}	3.67±1.53^{ab}	9.67±5.50^{b}
20 % MLE	3.33± 1.54^{a}	3.67±1.53^{ab}	6.67±3.51^{ab}
30 % MLE	9.33± 0.58^{c}	4.67±0.57^{b}	14.00±1.00^{c}
10 % GA_3	4.33±1.53^{ab}	2.00±0.00^{a}	6.67±3.61^{ab}
20 % GA_3	4.00±2.00^{ab}	2.33±1.53^{a}	6.67±1.53^{ab}
30 % GA_3	4.00±4.00^{ab}	2.67±2.51^{ab}	6.67±6.51^{ab}
D. Water	1.00±1.00^{a}	0.67±0.58^{a}	1.67±1.52^{a}

*Means followed by the same letters on the same column are not significantly (p>0.05) different according to Duncan Multiple Range Test at 5% probability.

DISCUSSION

As the quest to finding a sustainable organic fertilizer for agricultural crops continue to be an iterative process, it is pertinent to consider the diversity of plants, which is an important criterion to how they adapt to the physiological and biochemical changes in their immediate environment. The same way chemical fertilizers are administered to crops based on their nutrient requirement, the response of plants to organic fertilizers could also differ between plants. In this study, aqueous *Moringa* leaf extracts (MLE) were administered to *A. hybridus* plant by phytopriming and foliar spraying techniques. The results of the growth parameters showed that 30% MLE treatment, which had the closest performance to the positive controls (gibberellic acid treatments), looks promising in terms of its potential in enhancing the shoot height and stem girth of *A. hybridus*. Interestingly, all the samples cultivated by foliar spraying performed better than those by phytopriming technique. This result conforms with the studies of Saa *et al*. (2015) and Sardar *et al*. (2021) which showed that foliar application of MLE has the potential to enhance the growth and nutritional composition of *Prunus dulcis* [Mill.] D. A. Webb and *Stevia rebaudiana* Bertoni respectively. The 30 % MLE applied to the leaves of *A. hybridus* using foliar spraying techniques was also a standout best, recording the highest amount of 14.00 mg/100g (approximately 0.14 mg/g) total chlorophyll. This result shows the capability of MLE in enhancing the chlorophyll content of *A. hybridus* which is of great value to those in the food and pharmaceutical companies, where chlorophylls are being harvested and used as colorants and as a remedy to some chronic diseases respectively (Mishra *et al*., 2011; Sun *et al*., 2024).

CONCLUSION

This study has shown that foliar application of *Moringa oleifera* leaf extract (MLE) to *A. hybridus* has a better performance than phytopriming, which implies that selection of an appropriate technique is crucial in the application of phyto-extracts. Therefore, MLE can be a sustainable biological product for the cultivation and stimulation of growth and chlorophyll contents in plants. Finally, the utilization of such phyto-extracts can serve as a healthy alternative to inorganic fertilizers, which pose health risks to human health.

REFERENCES

Abd El-Hack, M. E., Alagawany, M., Elrys, A. S., Desoky, E. M., Tolba, H. M. N., et al. (2018). Effect of forage *Moringa oleifera* L. (*Moringa*) on animal health and nutrition and its beneficial applications in soil, plants and water purification. *Agriculture,* 8:45.

Aderibigbe, O. R., Ezekiel, O. O., Owolade, S. O., Korese, J. K., Sturm, B. et al. (2022).

Exploring the potentials of underutilized grain amaranth (*Amaranthus* spp.) along the value chain for food and nutrition security: A review. *Critical Reviews in Food Science and Nutrition,* 62:656-669

Adline, J. and Devi, J. A. (2014). Study on phytochemical screening and antibacterial activity of *Moringa oleifera*. *International Journal* of *Applied Research,* 2:169-176.

Ali, G. H., El-Taweel, G. E. and Ali, M. A. (2004). The cytotoxicity and antimicrobial efficiency of Moringa oleifera seeds extracts. *International Journal of Environmental Studies,* 61:699-708.

Arnon, D. (1949). Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiology,* 24:1-15.

Balana, B. B. and Fasoranti, A. S. (2022). A historical review of fertilizer policies in Nigeria. *The International Food Policy Research Institute.* IFPRI Discussion Paper 02145

Baloch, H., Sabir, I. A., Leghari, S. K., Saddiq, M. S., Alam, P., et al. (2024). Moringa leaf extract enhances the growth and yield characteristics of buckwheat genotypes by modulating the biochemical and physiological activities. *Asian Journal of Agriculture and Biology,* DOI: 10.35495/ajab.2023.328.

Baraniak, J. and Kania-Dobrowolsk, M. (2022). The dual nature of amaranth-functional food and potential medicine. *Foods,* 11:618.

De la Monte, S. M., Neusner, A., Chu, J., and Lawton, M. (2009). Epidemiological trends strongly suggest exposures as etiologic agents in the pathogenesis of sporadic alzheimer's disease, diabetes mellitus, and non-alcoholic steatohepatitis. *Journal of Alzheimer's Disease,* 17(3):519-529

Gopalakrishnan, L., Doriya, K. and Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness,* 5:49-56.

Huang, Y., Ma, Q., Wu, X., Li, H., Xu, K. et al., (2022). Estimation of chlorophyll content in *Brassica napus* based on unmanned aerial vehicle images. *Oil Crop Science,* 7:149-155.

Janmohammadi, H., Hosseintabar-Ghasemabad, B., Oliyai, M, Alijani, S., Gorlov, I. F., et al. (2023). Effect of dietary Amaranth (*Amaranthus hybridus chlorostachys*) supplemented with enzyme blend on egg quality, serum biochemistry and antioxidant status in laying hens. *Antioxidants* 12:456.

Kakar, K., Xuan, T. D., Noori, Z., Aryan, S. and Gulab, G. (2020). Effects of organic and inorganic fertilizer application on growth, yield, and grain quality of rice. *Agriculture* 10:544.

Keshinro, O. M., Ogbe, A. A., Oreshile, K. Y. and Omolokun, K. T. (2023). The effects of foliar application of some selected phytohormones on the proximate, phytochemical, and mineral compositions of roselle (*Hibiscus sabdariffa* l.) leaves. *FUDMA Journal of Sciences (FJS)* 7(3):249-256.

Martínez-Núñez, M., Ruiz-Rivas, M., Vera-Hernández, P. F., Bernal-Muñoz, R., Luna-Suárez, S., et al. (2019). The phenological growth stages of different amaranth species grown in restricted spaces based in BBCH code. *South African journal of Botany* 124:436-443.

Mishra, V. K., Bacheti, R. K. and Husen, A. (2011). Medicinal Uses of Chlorophyll: a critical overview. In: *Chlorophyll: Structure, Function and Medicinal Uses*, Le, H. and Salcedo, E. Eds., Nova Science Publishers, Inc., Hauppauge, NY 11788 (ISBN 978-1-62100-015-0), pp.177-196.

Patil, S. V., Mohite, B. V., Marathe, K. R., Salunkhe, N. S., Marathe, V., et al. (2022). Moringa tree, gift of nature: a review on nutritional and industrial potential. *Current Pharmacology Reports,* 8:262-280

Saa, S., Olivos-Del Rio, A., Castro, S. and Brown, P. H. (2015). Foliar application of microbial and plant based biostimulants increases growth and potassium uptake in almond (*Prunus dulcis* [Mill.] D. A. Webb). *Frontiers in plant science,* 6: 87

Sardar, H., Nisar, A., Anjum, M. A., Naz, S., Ejaz, S., et al. (2021) Foliar spray of moringa leaf extract improves growth and concentration of pigment, minerals and *Stevioside* in stevia (*Stevia rebaudiana* Bertoni). *Industrial Crops & Products,* 166:113485

Sukyankij, S., Phun-iam, M. and Panich-pat, T. (2024). Response of green chiretta to different bio-fertilizers and their effect on phosphorus availability in the soil. *Journal of degraded and mining lands management,* 11(3):2502-2458

Sun, D., Wu, S., Li, X., Ge, B., Zhou, C., et al. (2024). The structure, functions and potential medicinal effects of chlorophylls derived from microalgae. *Marine Drugs,* 22:65.

World Bank (2019). Moving Toward a Middle-Class Society - Nigeria on the Move: A Journey to Inclusive Growth, Nigeria Systematic Country Diagnostic. World Bank.

Yasmeen, A., Basra, S. M. A., Rehman, H., Hussain, N., Shahid, M., and Afzal, I. (2013). *Moringa oleifera* as a potential fertilizer for sunflower (*Helianthus annuus* L.) production. *Journal of Agricultural Science and Technology*, 15(3):559-569.