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Original Research Article

Bio-Stimulant and Nitrogen Fixating Efficacy of Leaf Extract on the Early Growth of *Adansonia digitata* Linneus for Nursery Development

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ABSTRACT
Slow initial growth of baobab seedlings presents a significant challenge for widespread cultivation and productive plantation establishment. Synthetic growth promoters often carry environmental risks, prompting the exploration of eco-friendly alternatives. This study evaluates the bio-stimulant and nitrogen-fixing efficacy of leaf powders derived from locally available plants to enhance the growth of *Adansonia digitata* seedlings during nursery development. This research focused on the application of Moringa (*Moringa oleifera*), Bitter Leaf (*Vernonia amygdalina*), Senna (*Senna semia*), and Tamarind (*Tamarindus indica*) leaf powders as growth promoter. A Completely Randomized Design (CRD) with three replications were used to assess the impact of these treatments on shoot height, stem diameter, leaf number, and leaf area. Results revealed that Moringa leaf powder significantly enhanced all growth parameters. Seedlings treated with Moringa showed the highest increase in shoot height, reaching 6.23 cm, compared to 3.6 cm in the control group. Similarly, Moringa-treated seedlings exhibited the most substantial leaf production, with up to five leaves per seedling. Bitter Leaf and Tamarind treatments also demonstrated moderate effects with Bitter Leaf improving shoot height and leaf number, while Tamarind slightly increased leaf area and stem diameter 2.43 cm and 20.27 mm respectively. The findings emphasize the potential of plant-derived bio-stimulants as sustainable alternative to synthetic growth enhancers. The use of these bio-stimulants not only improves seedling vigor but also aligns with environmental sustainability goals by reducing dependency on chemical fertilizers.

INTRODUCTION

The use of bio-stimulants derived from plant extracts has garnered significant attention in recent years as a sustainable and eco-friendly approach to enhancing plant growth and productivity. Bio-stimulants are natural substances or microorganisms that stimulate plant growth and development processes, leading to improved nutrient uptake, stress tolerance, and overall plant health (Calvo *et al.,* 2014). Among the various sources of bio-stimulants, plant extracts have emerged as a promising alternative to synthetic chemicals, offering a range of benefits while minimizing environmental impact. One of the critical aspects of bio-stimulant efficacy is their ability to facilitate nitrogen fixation, a process crucial for plant growth and development. Nitrogen is an essential macronutrient required for the synthesis of amino acids, proteins, nucleic acids, and chlorophyll (Marschner, 2011). However, atmospheric nitrogen is unavailable to plants in its gaseous form, necessitating the action of nitrogen-fixing bacteria or specialized root nodules in leguminous plants Peoples *et al., (*1995). The application of bio-stimulants derived from plant extracts have shown promising results in enhancing nitrogen fixation, thereby improving plant growth and yield.

Adansonia digitata (baobab) is an important multipurpose tree species in many tropical and subtropical countries. The African baobab (*Adansonia digitata*) has edible nutrient-rich fruits and leaves that are used as food and feed. Its flowers and bark also have medicinal value. It is called the "tree of life" and has cultural significance. It also has cultural and medicinal significance. Promoting cultivation of baobab can support food security, nutrition, and livelihoods. However, slow initial growth of seedlings poses a challenge (Narayan *et al.,* 2018). Use of plant extracts as growth promoters represents a sustainable way to stimulate robust seedling growth for quality plantation stock. Various plant extracts have growth influencing properties due to presence of phytochemicals such as auxins, gibberellins, and other bioactive compounds Hussain *et al.,* (2020). Studies have demonstrated beneficial effects of various plant extracts at low concentrations on seed germination and seedling vigour parameters like shoot height, root length, stem diameter and leaf area, etc. Aquatic plants like *Spirodela polyrrhiza, Azolla pinnata, Eichhornia crassipes* and terrestrial plants like *Tithonia diversifolia, Moringa oleifera*, *Vernonia amygdalina* etc. have shown promise as source of growth promoters (Sanchez *et al*., 2011). However, research is inadequate on use of locally available plant resources to promote early seedlings growth of baobab.

The slow initial growth of *Adansonia digitata* (baobab) seedlings hinders its effective propagation and wider cultivation, despite its economic and nutritional potential. Synthetic chemicals used to promote seedling vigor have ecological drawbacks. Therefore, exploring sustainable, eco-friendly solutions like plant extracts as bio-stimulants is necessary to produce quality nursery stock and unlock the tree's full potential.

Plant extracts offer a low-cost, easily adoptable solution for enhancing germination and early seedling vigor in crops. They contain plant hormones, nutrients, and antioxidants, making them effective bio-stimulants (Kurmar *et al.,* 2020). The findings are expected to provide accessible and farmer-friendly growth promoting options for quality stock production. The propagation protocols developed can facilitate wider cultivation of baobab to enhance incomes and nutrition. In summary, promoting use of plant extracts to stimulate early seedling growth in baobab and tamarind has tremendous potential for income generation and sustainable livelihoods. This study aims to evaluate the response of *Adansonia digitata* to treatments with leaf extracts from selected locally available leaf plants for nursery development.

MATERIALS AND METHODS

Description of Study Area

The study site is Federal University Dutse, Jigawa State on latitude 11°46'04 "N and longitude 9° 41'94"E with an elevation of 435 m above the sea level. The area falls within Sudan savannah agro-ecological zone (Jibo *et al*., 2021b; Salami *et al*., 2020; Bidoli *et al.,* 2012). The climate of Jigawa State falls within wet and dry season, generally hot semi-arid tropics. The rainy season starts at the end of May and lasts till September with maximum rainfall in August. The average annual rainfall is 650mm (Jibo *et al.,* 2018; Bidoli *et al.,* 2012) and the minimum and maximum rainfall range between 600 to 1000mm (Salami *et al*, 2018) whereas the mean minimum and the mean maximum temperature are 32 to 41°C. The soil is well known to be fertile ranging sandy to loamy, pH ranges from 6.07 to 6.72. Nitrogen content ranges from 0.63 to 1.64 gkg^{-1} phosphorus ranges from 6.25 to 12.04 $mgkg^{-1}$ and potassium ranges from 0.18 to 0.63$cmolkg^{-1}$ (Jibo *et al.,* 2021; Garba *et al.,* 2021a; Salami *et al.,* 2021; Salami *et al*, 2019).

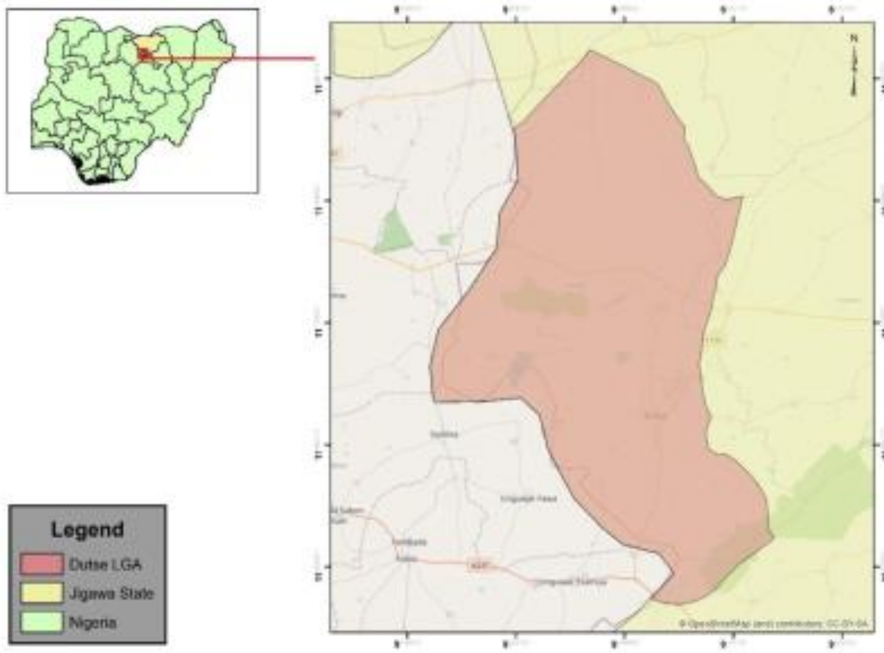

Figure 1: Map of the study area
Source: Garba *et al.,* 2021

Data Collection

Seeds were collected from mother trees in Federal University Dutse, Jigawa State to obtain genetically pure genetic materials. Healthy, uniform sized seeds were selected and subjected to pre-sowing treatments. After germination, two-week-old uniform seedlings was selected and transplanted into polybags. Non-destructive sampling was done at 15 days interval. Primary data regarding effects of different plant extracts on various vegetative growth parameters was collected through establishment of pot experiments under nursery conditions following standard procedures. Observations on parameters like shoot height, stem girth, leaf number, was recorded at regular intervals through non-destructive methods on sample plants from each treatment and replicate. Emergence, and growth was compiled, tabulated and subjected to statistical analysis. All findings were presented as mean values ± standard error and significance tested to draw valid interpretations.

Sampling Design and Experimental Layout

Sampling design

A Completely Randomized Design (CRD) was employed with five (3) replications for each treatment. Therefore, nine treatments were examined (control, two bio-stimulants and two nitrogen fixing leaf powder at different levels 0.5 g and 1.0 g). Twenty-seven sampling pot was used for study.

Experimental Layout

Table 1: Experimental layout for Nitrogen fixing plant

TREATMENT	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_9	C
R1	T_1R_1	T_2R_1	T_3R_1	T_4R_1	T_5R_1	T_6R_1	T_7R_1	T_8R_1	CoR_1
R2	T_1R_2	T_2R_2	T_3R_2	T_4R_2	T_5R_2	T_6R_2	T_7R_2	T_8R_2	CoR_2
R3	T_1R_3	T_2R_3	T_3R_3	T_4R_3	T_5R_3	T_6R_3	T_7R_3	T_8R_3	CoR_3

Key: T_1: Moringa 0.5g; T_2: Moringa 1.0g; T_3: Bitter leaf 0.5g; T_4:Bitter leaf 1.0g; T_5: *Senna semia* 0.5g; T_6: *Senna semia* 1.0g T_7: Tamarind 0.5g T_8: Tamarind 1.0g C:Control

Data Analysis

The data collected on various vegetative growth parameters under different plant extract treatments was compiled, organized and subjected to appropriate tools as follows:

RESULTS AND DISCUSSION

Results

Figure 2 showed the influence of bio-stimulant and nitrogen fixing leave powder on the shoot height of *Adansonia digitata* seedlings, The control of 0g (no treatment applied) had the lowest shoot height of 3.6 cm, T1 and T2 which are 0.5g and 1.0g of Moringa leaf powder on Baobab showed the height of 5.93cm and 6.23cm respectively, T3 and T4 which are 0.5g and 1.0g *Senna semia* on Baobab shows the height of 4.43 cm and 4.56 cm. T5 and T6 which are 0.5g and 1.0g of *Vernonia amygdalina (Bitter leaf powder)* on Baobab shows 4.46 and 4.56 cm respectively, with T7 and T8 which are *Tamarindus indica* at 0.5g and 1.0g showing 5.4 cm and 5.4 cm respectively. It can be seen from the result that, among the bio-stimulants, which are T1, T2, T3 and T4, T1 and T2 shows the highest growth in height.

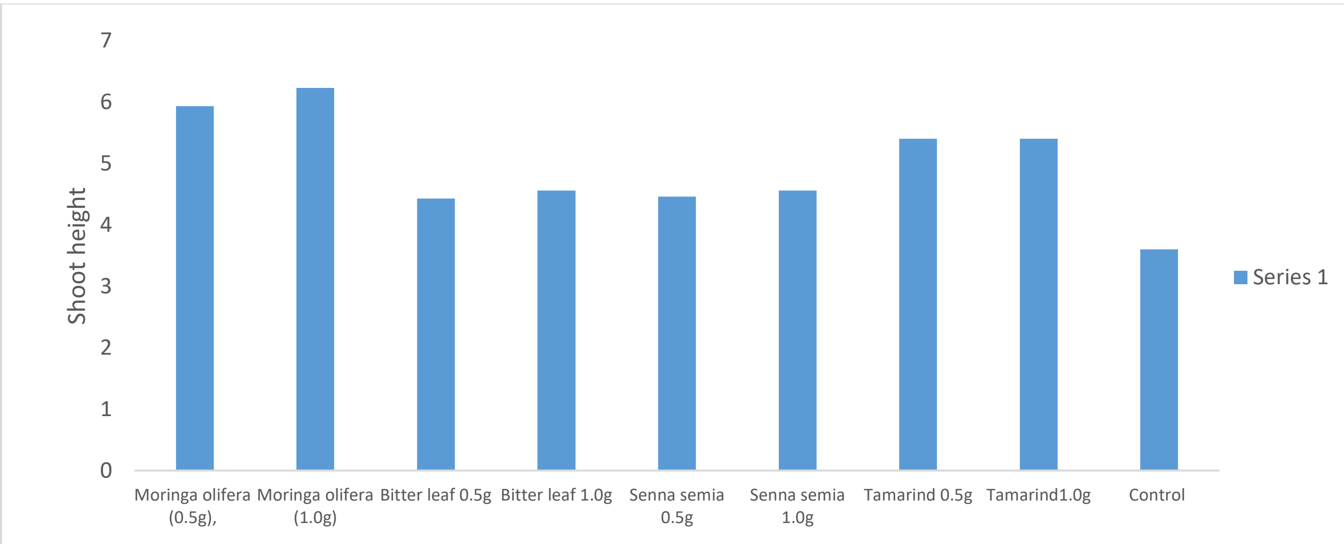

Figure 2: Showing the effect of bio-stimulant and Nitrogen fixing leave powder on plant height of *Adansonia digitata* seedlings

Figure 3 below shows the influence of bio-stimulant and nitrogen fixing leave powder on the stem diameter of *Adansonia digitata* seedlings, The control of 0g (no treatment applied) has a stem diameter of 2.23cm, T1 and T2 which are 0.5g and 1.0g of Moringa leaf powder on Baobab shows diameter of 2.43 and 2.16 cm respectively, T3 and T4 which are 0.5 g and 1.0 g *Senna semia* on Baobab shows a diameter 1.93 cm and 1.63cm. T5 and T6 which are 0.5 g and 1.0 g of *Vernonia amygdalina (Bitter leaf powder)* on Baobab shows 2.26 and 1.93 cm respectively, with T7 and T8 which are *Tamarindus indica* at 0.5 g and 1.0 g showing 1.63 cm and 1.85 cm respectively.

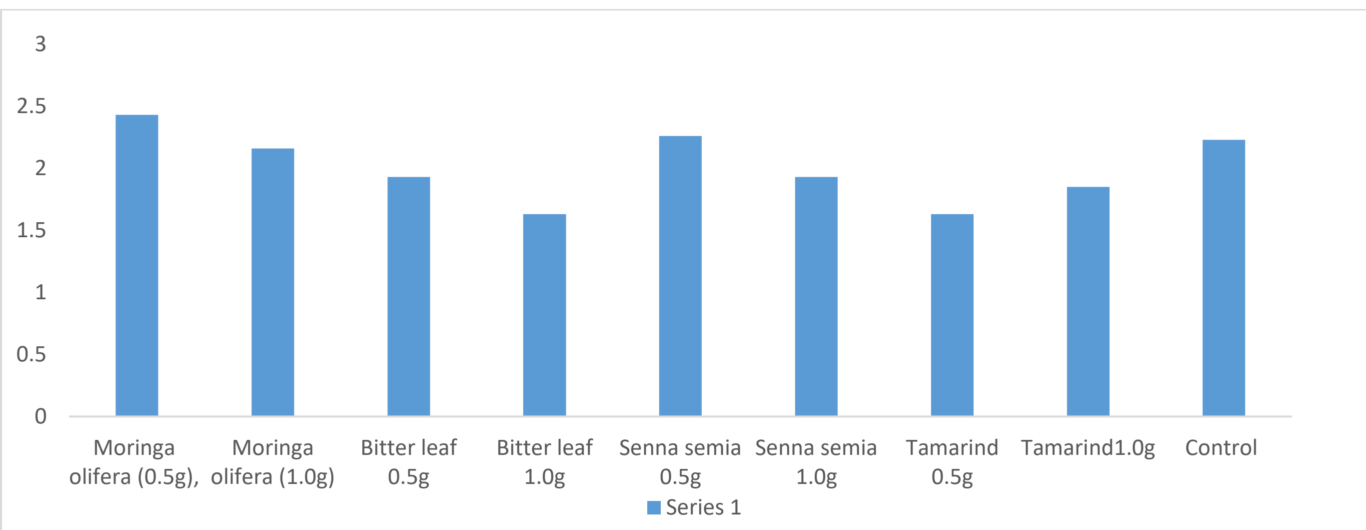

Figure 3: Showing the effect of bio-stimulant and Nitrogen fixing leave powder on stem diameter of *Adansonia digitata* seedling

Figure 4 below shows the influence of bio-stimulant and nitrogen fixing leave powder on the leaf number of *Adansonia digitata* seedlings, The control of 0g (no treatment applied) has a leaf number of 2, T1 and T2 which are 0.5 g and 1.0 g of Moringa leaf powder on Baobab leaf number of 5 and 4 respectively, T3 and T4 which are 0.5g and 1.0g *Senna semia* on Baobab shows a leaf number of 3 and 2, T5 and T6 which are 0.5 g and 1.0 g of *Vernonia amygdalina (Bitter leaf powder)* on Baobab shows 4 and 3 respectively, with T7 and T8 which are *Tamarindus indica* at 0.5g and 1.0g showing 3 and 3 respectively.

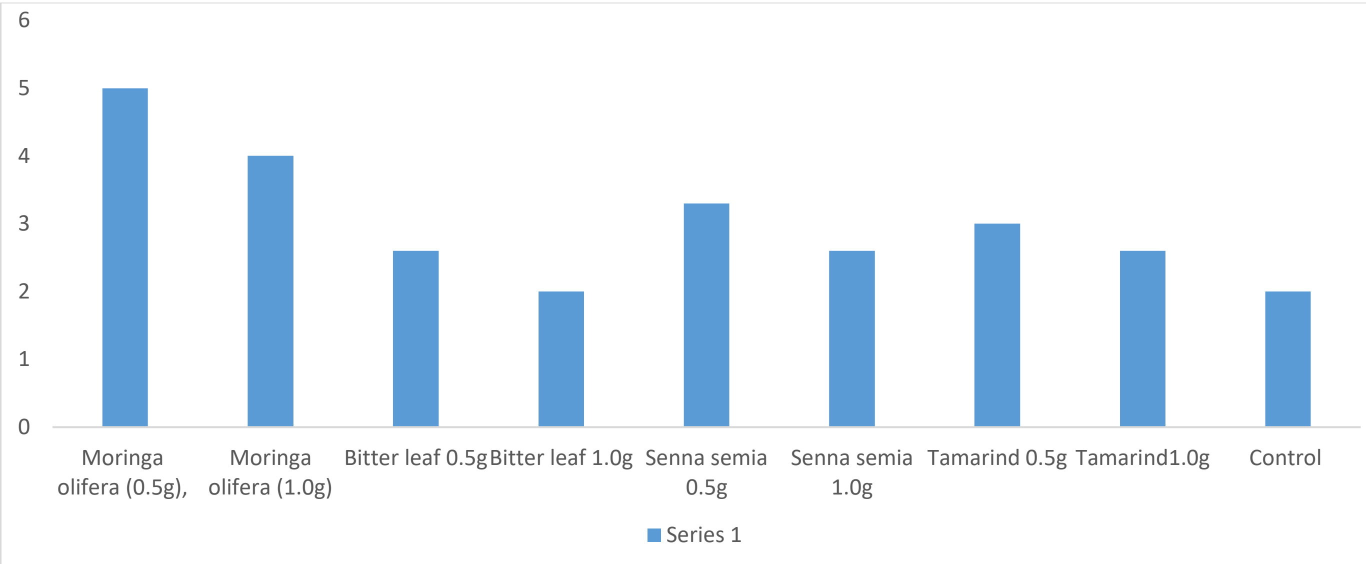

Figure 4: Showing the effect of bio-stimulant and Nitrogen fixing leave powder on Leave number of *Adansonia digitata* seedling

Figure 5 below shows the influence of bio-stimulant and nitrogen fixing leave powder on the leaf area of *Adansonia digitata* seedlings, The control of 0g (no treatment applied) has a leaf area of 15.49, T1 and T2 which are 0.5g and 1.0g of Moringa leaf powder on Baobab leaf area of 20.27 and 16.77 respectively, T3 and T4 which are 0.5g and 1.0g *Senna semia* on Baobab shows a leaf area of 16.56 and 12.96, T5 and T6 which are 0.5g and 1.0g of *Vernonia amygdalina (Bitter leaf powder)* on Baobab shows 10.61 and 9.78 respectively, with T7 and T8 which are *Tamarindus indica* at 0.5g and 1.0g showing 14.01 and 14.45 respectively.

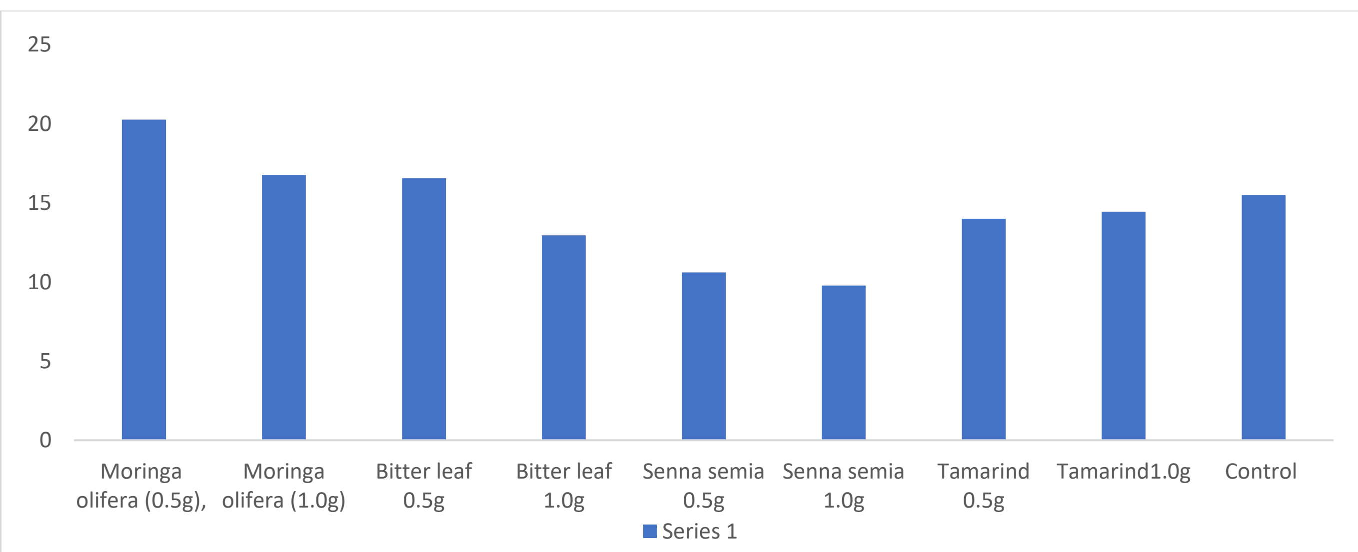

Figure 5: Showing the effect of bio-stimulant and Nitrogen fixing leave powder on Leave area of *Adansonia digitata* seedling

Table 2: Mean of Morphological Parameters of *Adansonia digitata*

Variables	T1	T2	T3	T4	T5	T6	T7	T8	T9(Control)
Shoot Height(cm)	17.8	18.7	13.3	14.7	13.4	13.7	16.5	16.2	11.8
Stem Diameter (mm)	2.43	2.16	1.93	1.63	2.26	1.93	1.63	1.85	2.23
Leaf Number	5	4	2.6	2	3.3	2.6	3	2.6	2
Leaf Area	20.27	16.7	16.56	12.96	10.61	9.78	14.01	14.45	15.49

Table 3: Showing the Analysis of Variance of selected morphological variables of the species

Source of Variation	Degree of Freedom	Sum of squares	Mean square	Variables	Fcal	F tab
Treatment	8	1.94	0.22	Shoot height	0.18 ns	2.46
Error	18	21.5	1.19	Stem Diameter	1.86 ns	
				Leaf number	2.02 ns	
				Leaf area	2.10 ns	
Total	26					

Note: not significant at 0.05 % (ns)

Discussion

Morphological Responses of Baobab

The use of bio-stimulants and nitrogen-fixing leaf extracts has gained attention in recent years as a sustainable approach to enhance tree growth. Studies have shown that leaf extracts from certain plant species can stimulate seed germination, seedling growth, and nutrient uptake in tree species. For instance, a study by Koffi *et al*. (2020) found that *Moringa oleifera* leaf extract significantly enhanced the growth and development of *Acacia senegal* seedlings. Similarly, a study by Ogbonnaya *et al*. (2022), reported that *Vernonia amygdalina* leaf extract improved the germination and seedling growth of *Parkia biglobosa*. Moringa leaf powder demonstrated superior performance in enhancing shoot height, likely due to its rich composition of bioactive compounds, including phytohormones like cytokinins and gibberellins. Du Jardin, (2015) highlighted similar findings where bio-stimulants were noted to promote growth by improving nutrient uptake and stress tolerance. The dose-dependence observed in this study aligns with earlier research conducted by (Hussain *et al.,* 2020) on optimal concentration ranges for maximum efficacy. The results indicated that bio-stimulants have limited impact on stem diameter compared to shoot height. Moringa treatments performed slightly better than other bio-stimulants, suggesting their potential to enhance structural growth. Khan *et al.,* (2020) supported the bio-stimulants improve mechanical properties and stem rigidity by influencing hormonal activity and nutrient assimilation. The relatively low response in stem diameter here underscores the variation in bio-stimulant effects.

The findings further validate earlier research highlighting Moringa's bioactive properties as particularly effective in stimulating vegetative growth. Moringa treatments demonstrated superior performance across all growth parameters, making it the most promising bio-stimulant in this study. (Hussain *et al.,* 2020) similarly identified plant-based bio-stimulants like Moringa as effective growth promoters due to their rich phytochemical content. Higher doses (1.0 g) generally yielded better results, although the improvements were less pronounced at higher concentrations. This suggests the existence of an optimal dose range for maximizing growth benefits without wastage of resources, as also noted by Yakhins *et al*., (2016).

The study's findings highlight a notable improvement in the growth performance of seedlings treated with bio-stimulants and nitrogen-fixing leaf powders compared to the control group. Among the treatments, the application of *Moringa oleifera* (T1 and T2) showed the most pronounced effect. Moringa's well-documented bioactive compounds, such as cytokinins, vitamins, and essential amino acids, likely contributed to improved nutrient uptake and stress resilience, particularly in higher dosages (1.0g). These findings align with prior research emphasizing Moringa's role in enhancing plant metabolism and growth. Similarly, treatments involving *Senna semia* (T3 and T4) and Bitter leaf (*Vernonia amygdalina*, T5 and T6) demonstrated significant growth improvements, though to a lesser degree than Moringa. The effectiveness of *Senna semia* may stem from its known allelopathic effects, which can positively influence nutrient availability and root development. Bitter leaf treatments, which showed moderate growth stimulation, could owe their efficacy to the plant's bioactive compounds, known to enhance microbial activity in the rhizosphere. *Tamarindus indica* (T7 and T8) also positively influenced growth, especially in the higher dosage treatment (1.0 g). Tamarind is known for its rich nutrient profile and its potential to act as a nitrogen-fixing agent, which may explain the observed growth enhancements. This suggests a promising avenue for exploring Tamarind's use as a bio-stimulant in sustainable agriculture. The mechanisms underlying the observed growth enhancements likely involve several interconnected processes. Bio-stimulants are known to modulate physiological processes such as chlorophyll synthesis, enzymatic activity, and nutrient assimilation. The application of bio-stimulants, particularly those derived from Moringa and Tamarind, likely enhanced root development and increased the seedlings' capacity to absorb essential nutrients like nitrogen, phosphorus, and potassium. Additionally, the nitrogen-fixing properties of the leaf powders contributed to improved soil nitrogen levels, fostering better seedling growth and reducing reliance on synthetic fertilizers. These findings resonate with the insights from prior research, which highlighted the mechanisms of bio-stimulant action, including improved chlorophyll synthesis, enhanced nutrient assimilation, and stress tolerance. For example, Du Jardin, (2015) emphasized that bio-stimulants can reduce fertilizer needs and improve crop productivity through the modulation of vital plant processes. Similarly, Yakhin *et al.* (2016) and Lumini, (2019) underscored the role of bio-stimulants in increasing photosynthetic activity and enhancing metabolic functions- outcomes that are consistent with the observed growth improvements in this study. Additionally, the allelopathic effects mentioned by El-Khawas *et al.* (2011) align with the potential influence of *Senna semia* in promoting nutrient availability and growth. The nitrogen-fixing properties of the treatments also find support in the literature reviewed, where Berger *et al.* (2013) and Pii *et al.* (2015), discussed the critical role of nitrogen-fixing bacteria in sustaining plant growth in nutrient-deficient soils. The potential nitrogen-fixing associations observed in treatments such as *Tamarindus indica* parallel findings by Bada, (2015) and Adeoti *et al*. (2016), who explored similar relationships in other non-leguminous species. This convergence of findings reinforces the practical value of integrating bio-stimulants and nitrogen-fixing agents into sustainable agricultural practices.

The findings of this study have significant implications for the development of sustainable forestry practices, particularly in tropical regions where Adansonia digitata is native. The use of bio-stimulants and nitrogen-fixing leaf powders as an alternative to synthetic fertilizers can promote healthier seedling growth, improve soil fertility, and reduce environmental pollution. This approach can also enhance the resilience of tree seedlings to climate change and other environmental stresses. As noted by Khan *et al*. (2020), the integration of bio-stimulants and nitrogen-fixing agents into forestry practices can contribute to the development of more sustainable and climate-resilient forest ecosystems. By adopting these eco-friendly approaches, forestry practitioners and conservationists can promote the health and productivity of Adansonia digitata and other valuable tree species.

CONCLUSION

The findings of this study underscore the effectiveness of bio-stimulants and nitrogen-fixing leaf powders in addressing the critical challenge of slow seedling growth in *Adansonia digitata*. Among the treatments evaluated, Moringa leaf powder emerged as the most effective bio-stimulant, significantly enhancing shoot height, stem diameter, leaf number and leaf area. Its rich composition of phytohormones and nutrients makes it a viable solution for promoting early seedling vigor. Bitter Leaf and Tamarind powders also demonstrated potential, albeit with less pronounced effects compared to Moringa. These results align with existing literature that highlights the importance of plant-derived bio-stimulants in sustainable agriculture. They confirm the capacity of these treatments to improve nutrient uptake, enhance stress tolerance, and promote vegetative growth. The observed dose-dependent responses further emphasize the need to optimize application rates for maximum efficacy. Moringa should be prioritized as the primary bio-stimulant for nursery development of *Adansonia digitata* due to its superior performance across all growth parameters. Also, additional studies should explore the synergistic effects of combining these bio-stimulants with Moringa to optimize their efficacy. Developing standardized protocols for the preparation and application of leaf powders will ensure consistent results and scalability for large-scale use.

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