USE OF BIOSTIMULANTS TO ASSESS THE PRODUCTION OF THE UNDERUTILISED LEGUME, BAMBARA GROUNDNUT

by

ZIDUBULE SIPHIWO

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Sustainable Agriculture)

at
Stellenbosch University

Sustainable Agriculture, Faculty of AgriSciences

Pectora roborant cultus recti

Supervisor: Dr EE Phiri

Co-supervisor: Dr PN Mothapo

March 2021

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2021

Abstract

Underutilised crops are an important part of local diets but have not been fully adopted in modern agriculture. Often, they are cultivated in marginal lands with low, if any, nutrients. The need for underutilised crop research becomes apparent not only because a big portion of plant based human food is mainly derived from a limited number of staple crops. With the threat of global warming and associated more extreme weather, and indirect impacts such as crop diseases, reliance on a few crops is a major challenge to food and nutritional security. Moreover, existing deficiencies of key vitamins and minerals pose a serious constraint to livelihoods, more especially in rural areas. Bambara groundnut (*Vigna subterranea* (L) Verdc.) is one of the crops that could be used to increase food production. With a reported high drought tolerance, Bambara groundnut is cultivated across Africa, but its full potential has not been realised and its agronomic performance scantily documented. Therefore, two greenhouse experiments, using two landraces of Bambara groundnut, were conducted at the Welgevallen experimental farm, Stellenbosch University, Stellenbosch. The aim was to investigate the effect of different biostimulants on the growth performance of Bambara groundnut. The specific objectives were to: (1) Evaluate the effect of different biostimulants on agronomic plant performance, (2) Assess the effects of biostimulants on the nutritional composition of Bambara groundnut seeds. The study consisted of five treatments (untreated control, chicken manure fertiliser, Kelpak®, humic substance and Moringa leaf extract, n=6 replicates each), conducted in a tunnel production system. Biostimulant treated plants reached 50% flowering significantly earlier that the control and fertiliser treated plant. Plants treated with Kelpak® showed significantly higher plant height, larger leaf area, and higher leaf dry matter relative to control and other treatments. Treatment with humic substance and Moringa leaf extracts resulted in higher yield parameters, number of pods per plant, 100-seed weight, and total seed yield were the highest in humic substance treated plants followed by Moringa leaf extract. Therefore, the humic substance showed the highest harvest index percentage relative to the other treatments. Of the three biostimulants treatments, Kelpak® treated plants produced the lowest seed yield. Using multiple linear regression we found that variation in total seed yield could be explained by the input of treatment, particularly humic substance and Moringa. Kelpak® was found to not have a significant effect on seed yield but rather on plant height and dry shoot mass, indicating better suitability to growing leafy vegetables than grain. The nutrient composition was improved under Moringa and humic substance treatment, meaning that these two factors not only improved plant growth and productivity but maintained the nutritional composition of Bambara. Therefore, biostimulant application positively influenced Bambara groundnut growth, development, and yield without compromising the nutritional content of the crop seeds. Therefore, biostimulants alongside appropriate levels of fertilization may be used to enhance the production of Bambara groundnut crops, and other underutilised and neglected crops. However, the results need to be validated by investigating the remaining landraces of Bambara groundnut.

Keywords: Bambara groundnut, biostimulants, underutilised crops, landraces.

Opsomming

Alhoewel onderbenutte gewasse ŉ belangrike deel van plaaslike diëte uitmaak, word dit nie ten volle in die hedendaagse landbou opgeneem nie. Dit word dikwels op marginale grond met min, indien enige, voedingstowwe verbou. Daar is ŉ duidelike behoefte aan navorsing oor onderbenutte gewasse, nie net omdat ŉ groot deel van plantgebaseerde voedsel vir mensgebruik hoofsaaklik van ŉ beperkte getal stapelgewasse afkomstig is nie. In die lig van die gevaar van aardverhitting en verwante meer ekstreme weer, en indirekte impakte soos gewassiektes, is die afhanklikheid van enkele gewasse ŉ groot uitdaging vir voedsel- en voedingsekerheid. Origens plaas die bestaande gebrek aan belangrike vitamiene en minerale ŉ ernstige stremming op lewensbestaan, veral in landelike gebiede. Die Bambara-grondboontjie (*Vigna subterranea* (L) Verdc.) is een van die gewasse wat gebruik kan word om voedselproduksie te verhoog. Na berig word, is die Bambara-grondboontjie, wat regoor Afrika verbou word, hoogs weerstandig teen droogte, maar die volle potensiaal daarvan is nog nie verwesenlik nie en die agronomiese prestasie daarvan is karig gedokumenteer. Daarom is twee kweekhuisproewe met twee landrasse van die Bambara-grondboontjie by die Welgevallen-proefplaas van die Universiteit Stellenbosch gedoen. Die doel was om die effek van verskillende biostimulante op die groeiprestasie van die Bambara-grondboontjie te ondersoek. Die spesifieke doelstellings was: (1) om die effek van verskillende biostimulante op agronomiese plantprestasie te evalueer, en (2) om die effekte van biostimulante op die voedingsamestelling van Bambara-grondboontjiesaad te bepaal. Die studie het bestaan uit vyf behandelings (onbehandelde kontrole, hoendermisbemesting, Kelpak®, humusstof en moringablaar-ekstrak; n=6 replikate elk), wat in ŉ tonnelproduksiestelsel uitgevoer is. Plante wat met biostimulante behandel is, het 50% blomvorming aansienlik vroeër as die kontrole- en messtofbehandelde plant bereik. Plante wat met Kelpak® behandel is, het aansienlik groter plantgrootte, groter blaaroppervlak en meer droë blaarmaterie in vergelyking met die kontrole en ander behandelings getoon. Behandeling met humusstof en moringablaar-ekstrakte het hoër opbrengsparameters, getal peule per plant en 100-saadgewig tot gevolg gehad, en totale saadopbrengs was die hoogste in plante wat met humusstof behandel is, gevolg deur moringablaar-ekstrak. Die humusstof het dus die hoogste oesindekspersentasie in vergelyking met die ander behandelings getoon. Van die drie biostimulant-behandelings het Kelpak®-behandelde plante die laagste saadopbrengs gelewer. Met behulp van meervoudige lineêre regressie is bepaal dat variasie in totale saadopbrengs deur behandelingsinset, veral humusstof en moringa, verklaar kon word. Daar is bevind dat Kelpak® nie ʼn noemenswaardige effek op saadopbrengs het nie, maar wel op plantgrootte en droë lootmassa, wat op beter geskiktheid vir blaargroente as graan dui. Die voedingsamestelling het verbeter met moringa- en humusstofbehandeling, wat beteken dat hierdie twee faktore nie net plantgroei en produktiwiteit verbeter het nie, maar ook die voedingstofsamestelling van die Bambara-grondboontjie gehandhaaf het. Die gebruik van biostimulante het dus die groei, ontwikkeling en opbrengs van die Bambara-grondboontjie positief beïnvloed sonder om die voedingsinhoud van die gewassade in die gedrang te bring. Biostimulante kan dus saam met geskikte vlakke bemesting gebruik word om die produksie van Bambara-grondboontjiegewasse en ander onderbenutte en afgeskeepte gewasse te verbeter. Die geldigheid van die resultate moet egter bepaal word deur die res van die landrasse van die Bambara-grondboontjie te ondersoek.

Sleutelwoorde: Bambara-grondboontjie, biostimulante, onderbenutte gewasse, landrasse.

DEDICATION

This thesis is dedicated to my late partner Ms Wendy Fafa Chingono. Thank you for pushing me to go further and encouraging me to chase my dreams. Thank you for being my biggest support. Thank you for all the wonderful memories that you allowed me to be a part of; thank you for all the love and care.

YOU ARE DEARLY MISSED!!!

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- My supervisor: Dr E.E. Phiri, for her unwavering support, encouragement, guidance, insight, and mentorship.

- My co-supervisor: Dr N.P. Mothapo for her guidance and input especially with statistical analysis.

- Mr A.W. Baloyi for all his assistance with experimental work.

- My friends and family for their prayers, support, and encouragement, especially my mother (Mirriam Zidubule) and sister (Zine Zidubule), for their deep desire to see me succeed in life.

- United Exports® for the bursary.

- Mr Joubert Bosman for Moringa extract.

- Mr Kevin Hodgeson and Mr Kevin Goodrem (Vigoland) for the humic substance.

- Mr Heino Papenfus for Kelpak® (seaweed extract).

- Almighty God for his enduring love that gave me strength, courage, and hope to complete this study.

Table of Contents

Chapter 3

The effects of biostimulants on plant growth, yield, and seed nutritional content of Bambara groundnut

Chapter 4

Synopsis

List of Tables

List of Figures

CHAPTER 1: INTRODUCTION

1.1. Introduction

Climate and environmental changes accompanied by ever increasing environmental ameliorations are predicted to pose significant threats to the production of well-known cash crops and staple food crops in the future (Aliyu, Massawe, & Mayes, 2014). Subsequently, there is a need for diversification of cultivated crops across the world. Inclusion of underutilised crops into mainstream or commercial agriculture can relieve pressure and over reliance on staple crops for food and nutritional security (Azam-Ali et al., 2001; Bamshaiye et al., 2011). Out of about 7000 crop species reported to have been cultivated or collected in the past, only 20 species provide about 90% of the world food and nutritional requirement (Collins & Hawtin, 1999). Of the 20 most consumed crop species, maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) account for 60% of world population diets (Collins & Hawtin, 1999), thus, leaving an abundance of crops unutilised which could contribute food and nutritional requirements of the world population.

Underutilized crops are species that are currently not classified as major crops (Chivenge et al., 2015), with little utilisation at the worldwide scale, and yet assume a crucial role in the food and nutritional security of rural farmers and consumers. Most underutilized crops are adaptable to extreme ecological conditions and perform well in marginal and low input farming lands (Mkandawire, 2007; Mabhaudhi & Modi, 2013). These crops also play a crucial role in the economy of developing countries by providing a source of income to small-scale farmers (Nedumaran et al., 2015). The African Orphan Crop Consortium (AOCC, 2011) have highlighted 101 underutilized crop species. Many of these are indigenous to the African continent including finger millet (*Eleusine coracana*), tef (*Eragrostis tef*), and Bambara groundnut (AOCC, 2011; Hendre et al., 2019). Underutilised species that are found and grown in South Africa include taro (*Colocasia esculenta*) spider plant (*Cleome gynandra*), kei apple (*Dovyalis caffra*), and marula (*Sclerocarya birrea*) (AOCC, 2011; Hendre et al., 2019).

This study focuses on Bambara groundnut (*Vigna subterranean (*L) Verdec) – an underutilised grain legume mainly produced by small-holder farmers in many African countries, including South Africa (Mabhaudhi & Modi, 2013). The crop has several advantages over preferred species as far as health benefits and tolerance to unfavourable environmental conditions is concerned (Mkandawire, 2007). In most African countries, Bambara groundnut is the third most consumed grain legume after peanuts (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Koné, Paice, & Touré, 2011). Nigeria is presumed as the highest producer of Bambara groundnut with around 100,000 tonnes of grain yield a year (Hillocks et al., 2012). In South Africa, Bambara groundnut is mostly produced at small-scale by women farmers for subsistence and major production areas are in the Limpopo, Mpumalanga, North West, Gauteng, and KwaZulu-Natal provinces (Swanevelder, 1998; DAFF, 2016). Bambara groundnut is advantageous in that it can be produced in poor soils with little precipitation and is normally used in intercropping systems with maize or taro (*Colocasia esculenta*) (Mabhaudhi & Modi, 2014). Moreover, Bambara groundnut is a good source of biological fixation of nitrogen, important for supplying nitrogen for crop production, thus modifying the soil for increased crop productivity (Mkandawire, 2007; Hillocks et al., 2012; Mabhaudhi et al., 2013). However, regardless of the significant characteristics of Bambara groundnut, its agro-ecological capabilities have not yet been fully realized nor its full economic significance met. Adebooye et al. (2005) reported major constraints that limit the productivity of Bambara groundnut including non-availability of improved seeds and poor agronomic practices which lead to inadequate yield. Attempts to increase crop yields in agricultural systems without production land expansion is currently dependent on the large- scale use of chemical fertilisers (Adesemoye et al., 2009). The enormous use of chemical fertiliser in crop production is influenced by growing food demand impacted by factors such as worldwide population growth, economic growth, and local effects of climate change affecting agricultural production (Alves et al., 2008). Chemical fertilisers have become fundamental a part of present-day agriculture since they provide basic plant supplements, for example, nitrogen, phosphorus, and potassium. However, overuse of these fertilisers has unforeseen ecological and environmental effects, and its prices are so high that subsistence farmers in most developing countries cannot afford them (Adesemoye et al., 2009).

To accomplish extreme advantages as far as fertiliser savings and better crop development, natural-product based biostimulants ought to be used with appropriate levels of chemical fertilisation. Du Jardin (2015) defined a biostimulant as any substance applied to plants to improve or enhance nutritional efficiency, stress resilience, and quality (Du Jardin, 2015). Biostimulants are not fertilisers as they do not contain supplements intended to be directly delivered to plants, rather, they may encourage mineral procurement (European Biostimulants Industry Council, 2012). Their fundamental capacity is centred on plant growth enhancement while assisting with improved abiotic stress handling by the crop and promoting harvest yield and quality. The utilisation of biostimulants is an important procedure for sustainable farm management, lessening ecological and environmental problems by reducing the use of chemical fertilisers (Hungria et al., 2010), and increasing production and economic potential of the farm.

Considering the beneficial effects of biostimulants in enhancing crop growth and yield, the present study investigated the effect of Moringa leaf extracts, Kelpak® (seaweed extract) and a humic substance on Bambara groundnut performance with the aim to investigate the agronomic productivity of Bambara groundnut. Moringa leaf extracts, Kelpak®, and humic substance contain nutritional substances such that when applied to plants they stimulate its growth and yield making them a natural plant growth enhancer. This aim was achieved by the following objectives:

i) Evaluate the effect of different biostimulants on agronomic plant performance and soil chemical properties.
ii) Assess the effect of different biostimulants on Bambara groundnut seed nutritional content.

1.2. References

Adebooye, O.C., Ajayi, S.A., Baidu-Forson J.J., & Opabode, J.T. (2005). Seed constraint to cultivation and productivity of African indigenous leaf vegetables. African Journal of Biotechnology, 13,1480–1484.

Adesemoye, A.O., Torbert, H. A., & Kloepper, J. W. (2009). Plant growth-promoting *rhizobacteria* allow reduced application rates of chemical fertilisers. Microbial Ecology, 58(4), 921–929.

African Orphan Crops Consortium: http://www.africanorphancrops.org.(2018). Accessed 08 Dec 2020.

Aliyu, S., Massawe, F., & Mayes, S. (2014) Beyond landraces: developing improved germplasm resources for underutilized species – a case for Bambara groundnut, Biotechnology and Genetic Engineering Reviews, 30, 2,127–141.

Alves, A., Crous, P. W., Correia, A., & Phillips, A. J. L. (2008). Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity, 28,1–13.

Azam-Ali, S.N., Sesay, A., Karikari, S.K., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M., & Hampson, K.J. (2001). Assessing the potential of an underutilised crop—a case study using Bambara groundnut. Experimental Agriculture, 37,433–472.

Bamshaiye, O.M., Adegbola, J.A., & Bamishaiye, E.I. (2011). Bambara groundnut: an under-utilised nut in Africa. Advances in Agricultural Biotechnology, 1,60–72.

Chivenge, P., Mabhaudhi, T., Modi, A.T M., & Mafongoya, P. (2015). The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. International Journal of Environmental Research and Public Health, 12, 5685–5711.

Collins, W.W. & Hawtin, G.C., (1999). Conserving and using crop plant biodiversity in agroecosystems. pp 267-281. In Collins, W.W. and Qualset, C.O. (Eds.), Biodiversity in agro-ecosytems. CRC Press, Boca Raton, Washington.

Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae, 196, 3–14.

European Biostimulants Industry Council. (2012). EBIC and Biostimulants in Brief. http:// www.biostimulants.eu/. Accessed 14 Dec 2019

Hendre, P.S, Muthemba, S., Kariba, R., Muchugi, A., Fu, Y., Chang, Y., Song, B., Liu, H., Liu, M., Liao, X., Sahu, S. K., Wang, S., Li, L., & Lu, H. (2019). African Orphan Crops Consortium (AOCC): status of developing genomic resources for African orphan crops. Planta, 250(3), 989–1003.

Hillocks, R.J., Bennett, C., & Mponda, O.M. (2012). Bambara nut: a review of utilisation, market potential and crop improvement. African Crop Science Journal, 20(1),1–16.

Hungria, M., & Kaschuk, G. (2014). Regulation of N_2 fixation and NO_3^- / NH_4^+ assimilation in nodulated and N – fertilized *Phaseolus vulgaris* L. exposed to high temperature stress. (2014). Environmental and Experimental Botany, 98,32–39.

Koné, M., Paice, A. G., & Touré, Y. (2011). Bambara Groundnut [*Vigna subterranea* (L.) Verdc: (Fabaceae)] Usage in Human Health. Nuts and Seeds in Health and Disease Prevention,189–196.

Mabhaudhi, T. & Modi, A.T. (2014). Intercropping Taro and Bambara Groundnut. Sustainable Agriculture Reviews, 13.

Mabhaudhi, T., Modi, A. T., & Beletse, Y. G. (2013). Growth, phenological and yield responses of a Bambara groundnut (*Vigna subterranea* L. Verdc) landrace to imposed water stress: II. Rain shelter conditions. Water South Africa, 39(2),191–198.

Mkandawire, C. H. (2007). Review of Bambara groundnut (*Vigna subterranea* (L.) verdc.) production in sub-Saharan Africa. Agricultural Journal, 2, 464–470.

Nedumaran, S., Abinaya, P., Jyosthnaa, P., Shraavya, B., Rao, P., & Bantilan, C. (2015). Grain legumes production, consumption and trade trends in developing countries. Working paper series no. 60. ICRISAT Research Program, Markets, Institutions and Policies. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India.

Republic of South Africa, Department of Agriculture, Forestry & Fisheries (DAFF). (2016). Bambara Groundnuts: Production guideline. Department of Agriculture, Forestry & Fisheries, Pretoria. http://ProdguideBambara.pdf.

Swanevelder, C.J., (1998). Bambara – food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa.

CHAPTER 2: LITERATURE REVIEW

2.1. Bambara groundnut as a food source

Bambara groundnut, which is popularly known as jugo beans in South Africa, originated in West Africa (Linnemann & Azam-Ali, 1992). Their name originates from Bambara, a district on the Upper Niger. Bambara groundnut has been grown for centuries and contributes to the food security of many people (Azam-Ali et al., 2001; Mwale et al., 2007). Bambara groundnut seeds are grown for human consumption but can also be used as a livestock feed (Ntundu et al., 2006; Shegro et al., 2013). All cultivated Bambara groundnut genotypes are landraces that have evolved directly from their wild relatives (Massawe et al., 2005, Mabhaudhi & Modi, 2013). They have adapted to many environments and are popular among farmers for their stability under different environmental conditions. Several scientists have collected Bambara groundnut landraces from different regions of Africa and beyond; the International Institute of Tropical Agriculture (IITA) in Nigeria is one of the major collectors of Bambara groundnut germplasm (Massawe et al., 2005) for breeding purposes. In South Africa, Shegro et al. (2013) looked at the phenotypic differences among 20 Bambara groundnut landraces. Similarly, Unigwe et al. (2018) assessed the nutritional value of 30 Bambara groundnut landraces. They found highly significant differences among the landraces for all phenotypic traits studied and that the contents of the four anti-nutritional compounds varied widely among the Bambara groundnut landraces.

2.1.1. Processing and utilization of Bambara groundnut

Bambara groundnut seeds can be eaten in several ways, fresh or roasted and can also be boiled or milled into flour and combined with maize to prepare porridge (Bamshaiye et al., 2011; DAFF, 2016). The use of Bambara groundnut seeds also varies among nations. In the western and northern parts of Cote d'Ivoire seeds are eaten as a snack (Hillocks et al., 2012). In Zimbabwe, freshly harvested seeds are eaten as snacks after boiling for approximately an hour (Plahar et al., 2002; Hillocks et al., 2012). The seeds can also be milled into flour and used to make cakes or bread (Mubaiwa et al., 2017). In Nigeria, the seeds are made into a local pudding called Moi Moi or Okpa (Okpuzor et al., 2010), whilst in South Africa, the seeds are consumed similar to Cote d'Ivoire and Botswana, in addition to being prepared into a flour to make cakes or to blend in with maize to make porridge, especially in the Limpopo Province (DAFF, 2016).

Several studies have looked at the utilization of Bambara groundnut in the fortification process of different foods. In Nigeria, a quality assessment of hamburger patties formulated with Bambara groundnut seed flour has been tried (Alakali et al., 2010). In another study, a puree was formulated with Bambara groundnut seeds and banana (Ijarotimi et al., 2009). The authors reported that the utilization of the complementary puree was sufficient to meet the prescribed dietary allowance for vitality, protein, and minerals for babies and that the puree had a fundamental amino acid profile. Bankole (2013), also reported an improvement in the nutritional content of a traditional dish (Garri), a fermented cassava fortified with Bambara groundnut. Composite flours have additionally been made with Bambara groundnut seeds and wheat flour in the baking of biscuits (Nwosu, 2013). As expected, the biscuits had a higher protein content than those produced using unmodified wheat flour. Nonetheless, studies on the utilisation of Bambara groundnut in formulating complementary nourishments in South Africa are very limited.

Bambara groundnut fits well into the socio-economic potential of many in developing nations. However, because of disregard by the research establishment in the past, little agronomic data is recorded about this crop. A combination of factors, *inter alia*, the absence of better agronomic practices, seed improvement, and nutritional inputs comprises a significant limitation in its production, which result in poor or low yield. Frequently, poor yield profitability of underutilized crops is due to natural limitations and the utilization of wasteful agricultural techniques which start from land planning, poor germination and plant development, harvesting, and storage (Denning, 2009). Furthermore, since most rich croplands are utilised to grow staple harvests, native crops are generally grown on degraded soils.

2.1.2. Nutritional profile

The seeds of Bambara groundnut are a complete food source containing about 18 – 24% protein with high lysine and methionine content, 51 – 70% carbohydrates, 4 – 12% crude oil and 3 – 12% of dietary fibre (Mayes et al., 2019; Bamshaiye et al., 2011). They also contain major micronutrients such as iron, potassium, sodium, and calcium (Amarteifio et al., 2006; Fasoyiro et al., 2006). Table 1 shows the chemical composition of some of the commonly consumed legumes in Africa. It is evident that carbohydrates and protein are the major nutrients in Bambara groundnut and, thus, is a cheap source of protein especially in areas where animal protein is not affordable. Bambara groundnut protein content compares well with other legumes including pigeon pea, African yam bean, cowpea, and lima bean, and more so with groundnut and soybean in terms of mineral composition (Table 1). Fasoyiro et al. (2006) reported a comparable sodium, calcium, potassium, phosphorus, and iron quantities in soybean, groundnut, and Bambara groundnut. The levels of nutritional content are reported to differ according to landrace and growth conditions (Nti et al., 2009).

Table 1. Chemical composition of some commonly consumed legumes

Legume	Carbohydrate (%)	Protein (%)	Ash (%)	Crude oil (%)	Crude fibre (%)
African yam bean	50.02	24.19	3.31	5.04	7.20
Bambara groundnut	**55.63**	**22.06**	**3.97**	**2.02**	**21.31**
Lima bean	50.44	24.19	5.64	2.92	2.07
Marama bean	24.10	34.10	3.70	33.50	4.40
Mung bean	59.30	26.37	4.30	1.10	4.30
Pigeon pea	48.31	25.98	4.06	1.91	4.62
Soybean	25.19	37.27	4.86	17.79	5.05
Benniseed	16.60	18.10	11.20	36.10	14.10
Cowpea	55.93	23.87	3.80	1.50	3.93
Groundnut	13.74	27.01	5.80	45.81	3.03

(Fasoyiro, Ajibade, Omole, Adeniyan & Farinde 2006; Amarteifio, Tibe & Njogu, 2006).

2.2. Role of mineral nutrition to successful plant growth and development

The two significant nutrientss that, to a great extent, limit plant development particularly in smallholder cultivation in Africa, are nitrogen (N) and phosphorus (P) (Kelly, 2005; Ulzen et al., 2016). This has been to a great extent credited to the deficient use of these supplements and poor soil management. Nitrogen is a significant constituent of amino acids, nucleic acids, and other compounds and is accessible to plants as nitrate and ammonium molecules (Uchida, 2000; Reeve et al., 2016). Amino acids are vital to plant growth and development as they are key in forming protoplast which is additionally the site for cell division. Moreover, N is a significant auxiliary segment of the chlorophyll molecule and thus bolsters photosynthesis by improving the quality and quantity of leaves (Uchida, 2000). In legumes, N is likewise required for development, flower formation, seed set, and to advance seed size. The combination of every one of these variables decides the total harvest yield.

Phosphorus, together with N, is one of the most significant nutritional components concerning the nourishment of the plants (Sharma et al., 2013) and is also a fundamental macronutrient for plant development and improvement. It is an important constituent for vitality change and guideline of different enzymatic reactions (Schulze et al., 2006). This component assumes a role in numerous metabolic processes identified with the above-ground organs, including energy generation, photosynthesis, respiration, and nucleic acid synthesis (Vance et al., 2003; Suliman et al., 2013). Therefore, low accessibility of P in soil would restrict plant growth and improvement.

Potassium (K^+) is another significant macronutrient required by plants and has numerous functions in physiological, biochemical, and metabolic processes. It is responsible for the upkeep of osmotic potential, charge balance, cytoplasmic pH homeostasis, enzyme activation, stomatal regulation, protein actuation, photosynthates translocation and water uptake (Oosterhuis et al., 2014). Photosynthetic metabolism requires adequate levels of K^+ for proper functioning (Marschner & Marschner, 2012). With fertilisers not being affordable to most small-scale farmers (Sanginga & Woomer, 2009), the average fertiliser application in Sub-Saharan Africa is 9 $kg.ha^{-1}$, with the lowest use in Sudan and Central Africa with 4 $kg.ha^{-1}$ and 3 kg per hectare, respectively. Instead of chemical fertiliser inputs, many low-income farmers generally intercrop with legumes to supplement inputs for nitrogen fixation. Under such conditions, legumes rely upon normal nitrogen fixation through beneficial interaction with rhizobia to meet their N prerequisite (Hungria & Kaschuk, 2014). However, the majority of the regular rhizobia cannot meet all the N necessities (Sanginga et al., 1996). In their study, Fening & Danso (2002), looked at the adequacy of local rhizobia in nodulating cowpea. They found out that only about 32% of the local rhizobia that nodulated cowpea was effective. Therefore, nutrient application in the form of fertilisers or biostimulants is needed to supply plants with all necessary nutrients for growth.

Regardless of affordability of chemical fertiliser, there are concerns over environmental pollution coming about because of high nitrate draining into natural systems (Dong et al., 2005). When overused, fertilisers are washed away from the fields because of runoff and become inaccessible to the crops (Halpern et al., 2015). To make up for the misfortune, farmers generally apply more fertilisers than the plant needs, and these huge amounts of synthetic fertilisers bring about significant expenses and can result in extreme natural contamination (Vance, 2016). The chemical process of manufacturing synthetic fertilisers is also an intense procedure that has serious implications given the huge worldwide carbon discharges (Chen, 2006; Vance, 2016).

2.3. Sustainable intensification: An alternative approach in promoting plant growth and yield

Food production must be expanded to meet the food needs of the rapidly growing world population. Further expansion of the production area is probably not going to be the solution because of the negative ecological and environmental effect of changing indigenous habitats to croplands (Ramankutty et al., 2018). Therefore, food needs should be fulfilled by increased efficiency per unit land area, as opposed to growing the farming outskirts. The aim is to search for a methodology to balance crop production with environmental and ecological impacts. Bambara groundnut is traditionally cultivated without any fertilizers (Mabhaudhi & Modi, 2013; Meena & Massawe, 2013). A few studies have made use of manure in the production of Bambara groundnut. Oyiga & Uguru (2011) applied pig dung before planting. Several studies have also used organic fertilisers (Mabhaudhi & Modi, 2013) and chemical fertilizers (Mwale et al., 2007; Jonah et al., 2010; Ilyas & Sopian, 2013; Makanda et al., 2014). Chen (2006) proposed the utilization of biofertiliser and organic farming practices as an elective technique in advancing harvest development while maintaining the fertility of the soil and preventing ecological contamination. Introduction of biostimulants to crops via leaves as a foliar spray, seed soaking or soil can fill in as a method for invigorating development and keeping up agrarian yields in an environmentally friendly manner (Halpern et al., 2015). Current research demonstrates the utilization of biostimulants to be the best elective methodology for increased crop productivity and yield.

2.4. Natural product-based biostimulants

Plant biostimulants incorporate different substances and microorganisms that enhance plant development. They contain substances as well as microorganisms whose work, when applied to plants or in the rhizosphere, is to stimulate natural processes to enhance nutrient uptake and effectiveness, resistance to biotic and abiotic stress and, harvest quality (Calvo et al., 2014). Biostimulants have no immediate activity against pests or placing nutrients into the soil, therefore, cannot be classified as pesticides or fertiliser (Parrado et al., 2008; European Biostimulants Industry Council, 2012). Calvo et al. (2014) and European Biostimulants Industry Council (2012), characterized biostimulants impacts on plants as to cultivate plant development and advancement throughout the plant life cycle from seed germination to harvest. There are many different types of biostimulants with varying components depending on the natural source but are generally grouped into four classifications: In particular, (1) seaweed and plant leaf extract, (2) fulvic and humic substances, (3) microbial inoculants, and (4) protein hydrolysates and amino acids (Du Jardin, 2015). This study focused on one commercial biostimulant product (Kelpak®) that is currently in the South African market and two products that are under field trial investigation (a humic substance and Moringa leaf extract), both are developed and produced in South Africa.

2.4.1. Seaweed extract (Kelpak®)

Seaweed extracts are a plant-based growth enhancer used as a non-polluting source of nutrients in sustainable and organic farming. Seaweeds constitute a vast group of species that are classified into three different phyla, including brown (Phaeophyta), red (Rhodophyta) and green algae (Chlorophyta) (Tandon & Dubey, 2015). *Ascophyllum nodosum*, which belongs to the Phaeophyta phylum, is generally utilised in horticulture as biostimulants (Khan et al., 2009). Seaweeds contain plant hormones, for example, auxins, cytokinin, abscisic acid and amino acids (Khan et al., 2009; Lötze & Hoffman, 2016), in addition to growth stimulating constituents including micro- and macronutrients and sterols, all of which are known to act on soils as well as on the plant (Halpern et al., 2015; Lötze & Hoffman, 2016). Seaweed concentrate could be applied either by seed priming, seedling root drench preceding transplant, irrigation, or foliar spray. The technique for application, times of use and the rate of utilization vary as per development targets, plant variety, and development stages (Papenfus et al., 2013). Matysiak (2011) uncovered that the stimulatory impacts of seaweeds application were increasingly apparent when applied at the beginning period of plant development. Seaweed extracts contain organically dynamic compounds, some of which have been affirmed to positively affect soil health (soil structure, moisture retention and impact on microorganisms in the plant rhizosphere), plant development (root improvement and mineral ingestion, shoot development and photosynthesis, and harvest yield) and resistance to environmental stresses (mitigating biotic and abiotic stress) (Khan et al., 2009). These effects are ascribed to the presence of organic molecules which chelate mineral supplements making them progressively bioavailable (Schmidt et al., 2003).

The commercial product Kelpak® is acquired from the seaweed algal species *Ecklonia maxima*. It is a rich source of cytokinin and auxin precursors, enzymes, chelating agents, minerals, betaines, organic acids, amino acids, and hydrolysed proteins (Khan et al., 2009; Lötze & Hoffman, 2016; Tandon & Dubey, 2015). Kelpak® is broadly utilised in horticultural research (Featonby-Smith & Van Staden, 1987, Van Staden, 2015), with significant evidence of plant development and enhancement reported. The use of Kelpak® in wheat plants increased root to shoot dry mass proportion, which showed that the components in the seaweed considerably affected the root development of the plants (Van Staden, 2015). The root advancing action was seen when the extracts were applied to the plant rhizosphere or as a foliar spray. In another study, Featonby-Smith & Van Staden (1987) indicated that the concentration and number of applications of the seaweed extract is a critical factor to its viability. It was observed that when foliar was applied to groundnut plants at a dilution of two dm^3 per hectare twice a season, the overall seed mass increased by sixty-five percent than when a similar dilution was applied just a single time (forty-one percentage increase). Moreover, a maximum yield from plants that received two applications, also yielded the greatest number of seeds that were categorised as the large size category.

2.4.2. Moringa leaf extract

Moringa oleifera is one of thirteen species of the genus Moringa and family Moringaceae, with *Moringa oleifera* being the most known and utilised species (Murro et al. 2003; Arora et al. 2013). It is notable over the globe including Africa, Arabia, and

India; the Moringa tree is widely commercialised across the world as super food due to the high nutrient content found in its leaves (Moyo et al., 2011, Ekesa, 2017). Moringa leaf extract is considered as one of the most promising plants biostimulants and can be applied as a natural and elective source of mineral sustenance to plants (Phiri & Mbewe, 2010; Abdalla, 2013). Leaves of the Moringa trees are rich in growth-promoting substances including phytohormones, cytokinin (zeatin) and auxins and other compounds such as ascorbate, phenolics, and minerals (calcium, potassium, zinc, and iron) (Abdalla, 2013). Supplements, antioxidants, and phytohormones reported in Moringa leaf extract makes it an excellent natural plant growth enhancer. Even though the use of Moringa leaf extract as a biostimulant is on the rise, however, little work on its application has been reported on other crops including maize, sugarcane, black lentils, cantaloupe, melon, and soybean (Makkar & Becker, 1997; Foidl et al., 2001; Biswas, Hoque, & Abedin, 2016). A study conducted by Prabhu (2010) on Moringa leaf extract in basil revealed a noteworthy increment in various agronomic and physiological parameters including plant length, leaf number, leaf area, and biomass yield. In a correlation study by Latif & Mohamed (2016), Moringa leaf extract application increased shoot, root length and their biomass weights, photosynthetic pigments concentration, and phytohormone content in common bean (*Phaseolus vulgaris*).

2.4.3. Humic substances

Humic substances are heterogeneous natural particles that structure in the soil, as a result of microbial digestion of dead organic matter, including soils, municipal waste, and vermicompost (Nardi et al., 2002). It is applied in a few different ways, including foliar spray or direct application into the plant rhizosphere (Halpern et al., 2015; Katkat, 2014). The humic substance is one of the most widely recognized natural substances, making up sixty percent of the organic matter in the soils (Sutton & Sposito, 2005; Muscolo et al., 2007). A humic substance is reported to elicit different morphological changes in plants, prompting changes in plant development and improvement (Trevisan et al., 2010). They have also been used to promote growth and yield in many crops such as soybean, wheat, rice, maize, potato, cucumber, and peppers (Calvo et al., 2014). Additionally, it is known to influence soil health by improving the structure of the soil and nutrient solubility (Ayuso et al., 1996).

2.5. Sustainability

Endeavours to increase worldwide food security face several complex and interlinking difficulties. A lesson from the 2008 global food price crisis, which started revolts in excess of two dozen nations, is that essentially producing enough food is not in itself enough when the poor are not in a situation to have the buying capacity to access that food source (Christiaensen, 2009; Rosset, 2014). The need to provide food to the large and increasing human population by 2050 will require an escalation of cultivating frameworks, but indigenous knowledge, for example, nutritional diversity of traditional diets will need more attention to accomplish the fight against food insecurity. Underutilized crops have the potential to play a pivotal role in ensuring food security. This incorporates the economic development of developing countries by providing the poor with subsistence and source of income (Mabhaudhi, Modi & Beletse, 2013). Hence, an intervention is expected to improve the yield of these crops to get a higher income for the farmers and nutritious foods for the consumers. Perhaps the greatest test is the advancement of these crops into a profitable, feasible, and ecologically well-disposed production system. One of the most imaginative and promising answers to address this comprises the utilization of natural-based biostimulants. Biostimulants have been used as elective method to improve soil richness by keeping up microbial biodiversity in agricultural soils and ultimately increase yield (Du Jardin, 2015). As such, as we advocate for the advancement of underutilised crops and alternative cultivating practices, such conversations ought to likewise concentrate on sustainability.

2.6. Summary

Underutilised crops have the potential to contribute to attaining food security by providing subsistence and source of income to many rural communities. Bambara groundnut is dubbed as a "complete food" due to its high and diverse nutritional content. In addition, it is resilient to extreme environmental conditions and adaptable to marginal and low input environments. However, low yields hinder the full potential and economic worth of this crop. The use of biostimulants along with appropriate levels of chemical fertilisation could play a significant role in improving the production of Bambara groundnut in a sustainable manner.

2.7. References

Abdalla, M.M. (2013). The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. sativa) plants. International Journal of Plant Physiology and Biochemistry, 5 (3),42–49.

Adebooye, O.C., Ajayi, S.A., Baidu-Forson J.J., & Opabode, J.T. (2005). Seed constraint to cultivation and productivity of African indigenous leaf vegetables. African Journal of Biotechnology, 13,1480–1484.

Adesemoye, A.O., Torbert, H. A., & Kloepper, J. W. (2009). Plant growth-promoting *rhizobacteria* allow reduced application rates of chemical fertilisers. Microbial Ecology, 58(4), 921–929.

Adu-Dapaah, H., & Sangwan, R.S. (2004). Improving Bambara groundnut productivity using gamma irradiation and in vitro techniques. African Journal Biotechnology, 3 (5),260–265.

Alakali, J.S., Irtwange, S. V., & Mzer, M. T. (2010). Quality evaluation of beef patties formulated with Bambara groundnut (*Vigna subterranean* L.) seed flour. Meat Science, 85(2), 215–223.

Aliyu, S., Massawe, F., & Mayes, S. (2014) Beyond landraces: developing improved germplasm resources for underutilized species – a case for Bambara groundnut, Biotechnology and Genetic Engineering Reviews, 30, 2,127–141.

Alves, A., Crous, P. W., Correia, A., & Phillips, A. J. L. (2008). Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity, 28,1–13.

Amarteifio, J.O., Tibe, O., & Njogu, R.M. (2006). The mineral composition of Bambara groundnut (*Vigna subterranea* (L) Verdc) grown in Southern Africa. African Journal Biotechnology, 5,2408–2411.

Amin, A., & Nasim, W. (2017). Optimizing the phosphorus use in cotton by using CSM-CROPGRO-cotton model for semi-arid climate of Vehari-Punjab, Pakistan. Enviromental Science and Pollution Research (International), 24 (6),5811–5823.

Arora, D. S., Onsare, J. G., & Kaur, H. (2013). Bioprospecting of Moringa (*Moringaceae*): microbiological perspective. Journal of pharmacognosy and phytochemistry, 1(6), 193–215.

Ayuso, M., Hernández, T. & Garcia, C. (1996). Effect of humic fractions from urban wastes and other more evolved organic materials on seed germination. Journal of Science of Food and Agriculture, 72,461–468.

Azam-Ali, S.N., Sesay, A., Karikari, S.K., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M., & Hampson, K.J. (2001). Assessing the potential of an underutilised crop—a case study using Bambara groundnut. Experimental Agriculture, 37,433–472.

Bamshaiye, O.M., Adegbola, J.A., & Bamishaiye, E.I. (2011). Bambara groundnut: an under-utilised nut in Africa. Advances in Agricultural Biotechnology, 1,60–72.

Bankole, Y.O. Tanimola, O.A. Odunukan, R.O., & Samuel, D. O. (2013). An Assessment of the Functional Properties, Proximate Composition, Sensory Evaluation and Rheological Value of Gari Fortified with Bambara Groundnut Flour (*Voandzeia Subterranean Thouars*). Academic Journal of Interdisciplinary Studies, 2(10),165–174.

Biswas, A., Hoque, T., & Abedin, M. (2016). Effects of moringa leaf extract on growth and yield of maize. Progressive Agriculture, 27(2), 136–143.

Calvo, P., Nelson, L. & Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. Plant and Soil, 38(3),3–41.

Chen, J.H. (2006). The combined use of chemical and organic fertilisers and/or biofertiliser for crop growth and soil fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertiliser Use, 16 (20),1–11.

Chivenge, P., Mabhaudhi, T., Modi, A.T M., & Mafongoya, P. (2015). The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. International Journal of Environmental Research and Public Health, 12, 5685–5711.

Christiaensen, L. (2009). Revisiting the Global Food Architecture: Lessons from the 2008 Food Crisis.

Collins, W.W. & Hawtin, G.C., (1999). Conserving and using crop plant biodiversity in agroecosystems. pp 267-281. In Collins, W.W. and Qualset, C.O. (Eds.), Biodiversity in agro-ecosytems. CRC Press, Boca Raton, Washington.

Denning, G., Kabambe, P., Sanchez, P., Malik, A., Flor, R., Harawa, R., Nkhoma, P., Zamba, C., Banda, C., Magombo, C., Keating, M., Wangila, J., & Sachs, J. (2009). Input subsidies to improve smallholder maize productivity in Malawi: toward an african green revolution. PLoS biology, 7(1), 23.

Dong, S., Neilsen, D., Neilsen, G.H. & Fuchigami, L.H. (2005). Foliar N application reduces soil NO-3 N leaching loss in apple orchards. Plant and Soil, 268,357–366.

Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae, 196, 3–14.

Ekesa, B. N. (2017). Selected superfoods and their derived super diets. In Superfood and Functional Food-The Development of Superfoods and Their Roles as Medicine. IntechOpen.

European Biostimulants Industry Council. (2012). EBIC and Biostimulants in Brief. http:// www.biostimulants.eu/. Accessed 14 Dec 2019

Fasoyiro, S.B., Ajibade, S.R., Omole, A.J., Adeniyan, O.N., & Farinde, E.O. (2006). Proximate, minerals and antinutritional factors of some underutilised grain legumes in south-western Nigeria. Nutrition and Food Science, 36,18–23.

Featonby-Smith, B. C., & van Staden, J. (1987). Effect of seaweed concentrate on yield and seed quality of *Arachis hypogaea*. South African Journal of Botany, 53,190–193.

Fening, J. O., & Danso, S. K. A. (2002). Variation in symbiotic effectiveness of cowpea *bradyrhizobia* indigenous to Ghanaian soils. Applied Soil Ecology, 21, 23–29.

Foidl, N., Makkar, H.P, & Becker, K. (2001). The potential of Moringa oleifera for agricultural and industrial uses. Proceedings of International Workshop "What development potential for Moringa product" Dares –Salaam, Tanzania. pp.47–67.

Halimi, A.R., Mayes, S., Barkla, B., & King, G. (2019). The potential of the underutilised pulse Bambara groundnut (*Vigna subterranea* (L.) Verdc.) for nutritional food security. Journal of Food Composition and Analysis, 77,47–59

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T. & Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. Advances in Agronomy, 130,141–174.

Hillocks, R.J., Bennett, C., & Mponda, O.M. (2012). Bambara nut: a review of utilisation, market potential and crop improvement. African Crop Science Journal, 20(1),1–16.

Hungria, M., & Kaschuk, G. (2014). Regulation ofN2 fixation and NO3- / NH4+ assimilation in nodulated and N – fertilized *Phaseolus vulgaris* L. exposed to high temperature stress. (2014). Environmental and Experimental Botany, 98,32–39.

Idowu, O.O., (2009). Contribution of neglected and underutilised crops to household food security and health among rural dwellers in Oyo State, Nigeria. In Janicke et al. (Eds.) Proc. IS on Underutilised Plants. Acta Horticulturae. 806, 49–56.

Ijarotimi, O.S., & Esho, T.R. (2009). Comparison of nutritional composition and anti-nutrient status of fermented, germinated and roasted bambara groundnut seeds (*Vigna subterranea*). British Food Journal, 111, (4),376–386.

Ilyas, S., & Sopian, O. (2013). Effect of Seed Maturity and Invigoration on Seed Viability and Vigor, Plant Growth, and Yield of Bambara Groundnut (*Vigna subterranea* (L.) Verdc). Acta Horticulturae, 979,695–702.

Jonah, P.M., Adeniji, O.T., & Wammanda, D.T. (2010). Genetic correlations and path analysis in Bambara groundnut (*Vigna subterranea*). Journal of Agriculture and Social Sciences, 6,1–5.

Katkat, A. V. (2014). Effects of soil and foliar applications of humic substances on dry weight and mineral nutrients uptake of wheat under calcareous soil conditions Effects of Soil and Foliar Applications of Humic Substances on Dry Weight and Mineral Nutrients Uptake of Wheat under Calcareous Soil Conditions. Australian Journal of Basic and Applied Sciences, 3(2), 1266–1273

Kelly, V., (2005). Farmers' demand for fertilizer in Sub-Saharan Africa, Department of Agricultural Economics. Michigan State University, East Lansing, Michigan.

Khan, W., Rayirath, U.P., Subramanian, Sowmya-Lakshmi, Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J. & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth: Review. Journal of Plant Growth Regulation, 28, 386–399.

Koné, M., Paice, A. G., & Touré, Y. (2011). Bambara Groundnut [*Vigna subterranea* (L.) Verdc: (Fabaceae)] Usage in Human Health. Nuts and Seeds in Health and Disease Prevention,189–196.

Laker, M. C. (2007). Introduction to the special edition of Water SA on indigenous crops, water and human nutrition. Water South Africa, 33(3).

Latif, H. H., & Mohamed, H. I. (2016). South African Journal of Botany Exogenous applications of Moringa leaf extract effect on retrotransposon, ultrastructural and biochemical contents of common bean plants under environmental stresses. South African Journal of Botany, 106, 221–231.

Linnemann, A.R., Azam-Ali, S.N. (1992). Bambara groundnut (*Vigna subterranea*) literature review: a revised and updated bibliography. Department of Tropical Agriculture, Wageningen Agricultural University.

Lötze, E. & Hoffman, E.W. (2016). Nutrient composition and content of various biological active compounds of three South African-based commercial seaweed biostimulants. Journal of Applied Phycology, 28,1379–1386.

Mabhaudhi, T. & Modi, A.T. (2014). Intercropping Taro and Bambara Groundnut. Sustainable Agriculture Reviews, 13.

Mabhaudhi, T., Modi, A. T., & Beletse, Y. G. (2013). Growth, phenological and yield responses of a Bambara groundnut (*Vigna subterranea* L. Verdc) landrace to imposed water stress: II. Rain shelter conditions. Water South Africa, 39(2),191–198.

Makanda, I., Tongoona, P, Madamba, R., Icishahayo, D., & Derera, J. (2014). Path Coefficient Analysis of Bambara Groundnut Pod Yield Components at Four Planting Dates. Research Journal of Agriculture and Biological Sciences, 5(3),287–292.

Makkar, H.P.S., & Becker, K. (1997). Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. J ournal of Agricultural Science, 128,311–322.

Marschner, H., & Marschner, P., (2012). Marschner's Mineral Nutrition of Higher

Plants. Elsevier, London, UK.

Massawe, F. J., Mwale, S. S., Azam-Ali, S. N., & Roberts, J. A. (2005). Breeding in Bambara groundnut (*Vigna subterranea* (L.) Verdc.): Strategic considerations. African Journal of Biotechnology, 4(6),463–471.

Matysiak, K., Kaczmarek, S., & Krawczyk, R. (2011). Influence of seaweed extracts and mixture of humic acid fulvic acids on germination and growth of *Zea mays* L. Acta Scientiarum Poloronum Agricultura, 10,33–45.

Mayes, S., Ho, W. K., Chai, H. H., Gao, X., Kundy, A. C., Mateva, K. I., & Azam, S. N. (2019). Bambara groundnut: an exemplar underutilised legume for resilience under climate change. Planta.

Meena, S. L., & Massawe, F. J. (2013). Evaluation of Bambara groundnut (*Vigna subterranea*) landraces for their agronomic and physiological traits. Indian Journal of Agricultural Sciences, 83(5), 579–581.

Mkandawire, C. H. (2007). Review of Bambara groundnut (*Vigna subterranea* (L.) verdc.) production in sub-Saharan Africa. Agricultural Journal, 2, 464–470.

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechnology, 10 (60),12925–12933.

Mubaiwa, J., Fogliano, V., Chidewe, C., & Linnemann, A.R. (2017). Hard-to-cook phenomenon in bambara groundnut (*Vigna subterranea* (L.) Verdc.) processing: Options to improve its role in providing food security, Food Reviews International, 33,2,167–194, DOI: 10.1080/87559129.2016.1149864.

Murro, J. K., Muhikambele, V. R. M., & Sarwatt, S. V. (2003). *Moringa oleifera* leaf meal can replace cottonseed cake in the concentrate mix fed with Rhodes grass (*Chloris gayana*) hay for growing sheep. Livestock Research for Rural Development, 15(11),1–4.

Muscolo A, Sidari M, Francioso O, Tugnoli V, & Nardi S (2007) The auxin-like activity of humic substances is related to membrane interactions in carrot cell cultures. Journal of Chemical Ecology, 33,115–129.

Mwale, S.S., Azam-Ali, S.N. & Massawe, F.J. (2007). Growth and development of Bambara groundnut (*Vigna subterranea*) in response to soil moisture I. Dry mater and yield. European Journal of Agronomy, 26,345–353.

Nardi, S., Pizzeghello, D., Muscolo, A. & Vianello, A. (2002). Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry, 34, 1527–1536.

Nedumaran, S., Abinaya, P., Jyosthnaa, P., Shraavya, B., Rao, P., & Bantilan, C. (2015). Grain legumes production, consumption and trade trends in developing countries. Working paper series no. 60. ICRISAT Research Program, Markets, Institutions and Policies. International Crops Research Institute for the Semi-Arid

Tropics, Patancheru, Telangana, India.

Nnam, N. M. (2001). Comparison of the protein nutritional value of food blends based on Sorghum, Bambara groundnut and sweet potatoes. International Journal of Food Sciences and Nutrition, 52,25–29.

Nti, C.A. (2009). Effects of Bambara groundnut (*Vigna subterranea*) variety and processing on the quality and consumer appeal for its products. International Journal of Food Science and Technology, 44,2234–2242.

Ntundu, W. H., Shillah, S. A., Marandu, W. Y. F., & Christiansen, J. L. (2006). Morphological diversity of bambara groundnut [Vigna subterranea (L.) Verdc.] landraces in Tanzania. Genetic Resources and Crop Evolution, 53(2),367–378.

Nwosu, N.A. (2013). Production and Evaluation of Biscuits from Blends of Bambara Groundnut (*Vigna Subterranae*) and Wheat (*Triticum Eastrum*) Flours. International Journal of Food Science and Technology, 2(1),4–9.

Okpuzor, J., Ogbunugafor, H.A., Okafor, U., & Sofidiya, M.O. (2010). Identification of protein types in Bambara nut seeds: perspectives for dietary protein supply in developing countries. EXCLI Journal, 9,17–28.

Oosterhuis, D.M., Loka, D.A., Kawakami, E.M., & Pettigrew, W.T. (2014). The physiology of potassium in crop production. Advances in Agronomy,126, 203–233.

Oyeyinka, A.T., Pillay, K., Tesfay, S., & Siwela. (2017). Physical, nutritional and antioxidant properties of Zimbabwean Bambara groundnut and effects of processing methods on their chemical properties. International Journal of Food Science and Technology, 52(10),2238–2247.

Oyiga, B.C., & Uguru, M.I. (2011). Interrelationships among Pod and Seed Yield Traits in Bambara Groundnut (*Vigna subterranea* L. Verdc) in the Derived Savanna Agro-Ecology of South – Eastern Nigeria under Two Planting Dates. International Journal of Plant breeding, 5, 106–111.

Papenfus, H.B., Kulkarni, M.G., Stirk, W.A., Finnie, J.F. & Van Staden, J. (2013). Effect of a commercial seaweed extract (Kelpak®) and polyamines on nutrient-deprived (N, P and K) okra seedlings. Scientia Horticulturae, 151,142–146.

Parrado, J., Bautista, J., & Romero, E.F. (2008). Production of a carob enzymatic extract: Potential use as a biofertiliser. Bioresour Technology. 99,2312–2318.

Phiri, C., & Mbewe, D. N. (2010). Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes. International Journal of agriculture and Biology, 12(2),315–317.

Plahar, W.A., Annan, N.T., Larweh, P.M., Golob, P., Swetman, T., Greenhaulgh, P., Coote, C. & Hodges, R. (2002). Marketing and processing of Bambara groundnuts (W. Africa). Crop Post Harvest Programme. Food Research Institute (FRI): Accra, Ghana.

Prabhu, M., Kumar, A.R., & Rajamani, K. (2010). Influence of Different Organic Substances on Growth and Herb Yield of Sacred Basil (*Ocimum Sanctum* L.). Indian Journal of Agricultural Research, 4(1),48–51.

Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., & Rieseberg, L. H. (2018). Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. Annual Review of Plant Biology, 69(1),789–815.

Reeve, J. R., Hoagland, L. A., Villalba, J. J., Carr, P. M., Atucha, A., Cambardella, C., & Delate, K. (2016). Organic farming, soil health, and food quality: Considering possible links. In Advances in Agronomy, 137.

Republic of South Africa, Department of Agriculture, Forestry & Fisheries (DAFF). (2016). Bambara Groundnuts: Production guideline. Department of Agriculture, Forestry & Fisheries, Pretoria. http://ProdguideBambara.pdf.

Rosset, P. (2008). Food sovereignty and the contemporary food crisis. Development, 51(4), 460–463.

Sanginga, N., & Woomer, P. L. (Eds.). (2009). Integrated soil fertility management in Africa: principles, practices, and developmental process. CIAT.

Sanginga, N., Abaidoo, R., Dashiell, K., Carsky, R., & Okogun, A. (1996). Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. Applied Soil Ecology, 3,215–224.

Schmidt, R.E., Ervin, E.H., & Zhang, X. (2003). Questions and answers about biostimulants. Golf Course Management, 71(6),91–94.

Schulze, J., Temple, G., Temple, S.J., Beschow, H., & Vance, C.P. (2006). Nitrogen fixation by white lupin under phosphorus deficiency. Annals of Botany, 98, 731–740.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., & Gobi, T.A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus, 2,587.

Shegro, A.W., van Rensburg, J., & Adebola, P. O. (2013). Assessment of genetic variability in Bambara groundnut (*Vigna subterrenea* L. Verdc.) using morphological quantitative traits. Academia journal of agricultural research,1(3),45–51.

Sulieman, S., Van Ha, C., Schulze, J., & Tran, L.S.P. (2013) Growth and nodulation of symbiotic *Medicago truncatula* at different levels of phosphorus availability, Journal of Experimental Botany, 64,2701–2712.

Sutton, R. & Sposito, G. (2005). Molecular structure in soil humic substances: the new view. Environtal Science and Technology, 39,9009–9015.

Swanevelder, C.J., (1998). Bambara – food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa.

Tandon, S. & Dubey, A. (2015). Effects of biozyme (*Ascophyllum nodosum*) biostimulant on growth and development of soybean [*Glycine Max* (L.) Merill]. Communications in Soil Science and Plant Analysis, 46,845–858.

Trevisan, S., Francioso, O., Quaggiotti, S. & Nardi, S. (2010). Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signaling & Behavior, 5(6),35–43.

Uchida, R. (2000). Essential nutrients for plant growth: nutrient functions and deficiency symptoms. Plant nutrient management in Hawaii's soils,31–55.

Ulzen, J., Abaidoo, R. C., Mensah, N. E., Masso, C., & Abdelgadir, A. H. (2016). *Bradyrhizobium* Inoculants Enhance Grain Yields of Soybean and Cowpea in Northern Ghana. Frontiers in Plant Science, 7,1–9.

Unigwe, A.E., Doria, E., Adebola, P., Gerrano, A. S., & Pillay, M. (2018). Anti-nutrient analysis of 30 Bambara groundnut (*Vigna subterranea*) accessions in South Africa. Journal of Crop Improvement, 32,2,208-224, DOI: 10.1080/15427528.2017.1405857.

Van Staden, J. (2015). Kelp products international (Pty) Ltd. Simon's Town, South Africa. 4

Vance, C.P. (2016). Update on the state of nitrogen and phosphorus nutrition symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiology, 127,390–397.

Vance, C.P., Uhde-Stone, C., & Allan, D.L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist, 157 (3),423–447.

CHAPTER 3: THE EFFECT OF BIOSTIMULANTS ON GROWTH, YIELD, AND NUTRIONAL CONTENT OF BAMBARA GROUNDNUT

Introduction

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is a grain legume mostly grown by subsistence farmers in several regions across Africa (Mkandawire, 2007). It is commonly known as Jugo beans or Izindlubu in South Africa and believed to have originated in Northern Africa and migrated with indigenous people to Southern Africa (Mabhaudhi & Modi, 2013). For centuries, Bambara groundnut has contributed to the food and nutritional security of African people (FAO, 2001; Azam-Ali et al., 2001; Mwale et al., 2007). However, introduction of more popular leguminous crops such as peanuts (*Arachis hypogaea*) has relegated Bambara groundnut to its current underutilised status (Swanevelder, 1998). Bambara groundnut plants can grow under harsh climatic and drought conditions (Gregory et al., 2019). The crop is perfect for a water-scarce country like South Africa, with approximately 80% of the country classified as hyper-arid to semi-arid (Bennie & Hensley, 2001). In most African countries, Bambara groundnut is the third most consumed grain legume after peanuts and cowpea (*Vigna unguiculata*) (Koné, Paice, & Touré, 2011). Its production is estimated to be approximately 300 000 tonnes per year. Almost a half of this amount are produced in West Africa, with Nigeria being the world leading producer with around 100 000 tonnes per year (Bamshaiye et al., 2011). It is mostly utilised as an intercrop with maize or taro but also does well as a sole crop (Mabhaudhi & Modi, 2014). Previous studies shown that Bambara groundnut predominantly exist as landraces with hardly any varieties created through controlled breeding (Aliyu, Massawe & Mayes, 2014; Massawe et al., 2005). Research activities on establishing reproducing strategies to encourage production of higher yielding varieties of Bambara groundnut are gaining momentum (AOCC, 2011; Oyeyinka et al., 2015; Shegro et al., 2013).

Bambara groundnut landraces come in various sizes, shapes, and colour shading (Oyeyinka et al., 2017). According to DAFF (2016), it has seven landraces separated by their shading that range from black, red, and creamy white. As an annual plant, it takes around a quarter to half a year to develop, depending on environmental conditions and landrace. Flowering, which determines maturity and pod development, typically begins at 30 to 35 days after planting depending on the landrace and ecological conditions (Mkandawire, 2007; DAFF, 2016). During this process, the flower stem lengthens, sepal expands, and the pod develops just above or underneath the soil surface. Bambara groundnut is an unconventional legume of the genus *Vigna* because it develops pods underground. Pod development is influenced by internal and external variables, including photoperiod, hormonal parity, and physiological development (Linnemann & Azam-ali, 1993). Bambara groundnut is mainly grown for its edible seeds; therefore, the economically important part of the Bambara groundnut plant is the pod which encloses the seed grain. The size of the seeds and the quantity of the seeds per unit are significant standards that influence the market estimation of grain legume (Adu-Dapaah & Sangwan, 2004). The yield of the Bambara groundnut is typically dictated by the quantity of fertile flowers produced per plant, which determines pod and seed development. The yield components, namely the quantity of pods per plant, number of seeds per unit and seed weight are also of importance. The highest seed yields are expected when all yield components are maximized. In addition, growth parameters, for example, the quantity of leaves, leaf area, and leaf area index also influence crop yield (Liu & Stutzel, 2004).

Bambara groundnut grains have a comparable nutritional composition to that of cowpea (Oyeyinka et al., 2017). Because of lower cultivation and utilization levels globally, Bambara groundnut contribute less to worldwide caloric intake and protein diet requirements than other legumes. However, this crop does assume a major role in worldwide food and nutritional security by providing rural populations with an affordable and resilient plant-based source of fibre, fat, protein, and other minerals (Graham & Vance, 2003; Mubaiwa et al., 2016). Moreover, they fill in as an important source of protein for many in developing regions that cannot afford animal protein (Ho et al., 2017). Bambara groundnut protein stands out in its moderately higher lysine and methionine content in contrast with other commonly consumed legumes including soybean (Oyeyinka et al., 2018).

In addition to a high protein content, Bambara groundnuts also contain a significant amount of carbohydrates (Kaptso et al., 2014, 2016; Oyeyinka et al., 2015) and major micronutrients such as iron, potassium, sodium, and calcium (Amarteifio et al. 2006; Fasoyiro et al., 2006). Due to its balanced amino acid and micronutrient profile, the high protein content in Bambara groundnut seed grain may be utilised as a useful ingredient in the food industry (Bamshaiye et al., 2011), together with the antioxidant properties of its protein hydrolysate and peptide fractions (Arise et al., 2015). Many studies have reported on the important use of this legume in foods for improved protein and energy contents. Egounlety & Aworh (2003) reported a higher protein and energy content of a cereal-based weaning food fortified with soybean, cowpea, and Bambara groundnut compared to a plain cereal. A study by Nnam (2001) also showed an appreciable nutrient density in a composite blend of germinated sorghum and Bambara groundnut flour with fermented sweet potato flour. This study showed a similar result as that of Ijarotimi & Esho (2009), who reported a simultaneous increase in energy, protein, fat, and fiber content of a banana-based complementary supplement upon the addition of Bambara groundnut flour.

Despite these important dietary properties, Bambara groundnut remains an underutilised crop, for the most part, grown for subsistence. Regardless of the potential of Bambara groundnut, the crop stays a customary harvest with constrained use. Due to neglect by the research community in the past, little is documented about the ideal agronomic practices for this crop. Non-availability of improved seeds and poor agronomic practices also present a major constraint to the cultivation and productivity of the Bambara groundnut, leading to large yield gaps (Adebooye et al., 2005). In addition, like most underutilized crops it is grown at a small scale with minimal inputs (Mabhaudhi, Modi & Beletse, 2013).

Hence, there is a need to explore different innovative and sustainable methods for improving the growth and yield parameters of Bambara groundnut. A set of physio-chemical, biological, and integrated approaches have been used to improve crop yield (Bedada et al., 2016; Gaudin et al., 2015). Among them, the use of natural plant based biostimulants as growth stimulants are considered a viable approach to improve yield. Biostimulants are natural growth enhancers that stimulate crop yield via enhanced nutrient uptake and efficiency, improved tolerance to biotic and abiotic factors and enhancement of the rhizosphere activities (Jardin, 2015). They include natural sources like seaweed extracts, humic substances, microbial inoculants, and plant leaf extracts (European Biostimulants Industry Council, 2012; Jardin, 2015; Glodowska et al., 2016). *Moringa oleifera* leaf extracts, humic substances, and seaweed extracts are commonly used growth enhancers, applied as a seed priming agent, foliar spray and/or directly into the plant rhizosphere via irrigation. It has been shown that these substances positively modify plant growth and production with alterations in metabolic processes (Rady et al., 2013; Yasmeen et al., 2013; Khan et al., 2017a, 2017b).

Moringa, among all the natural occurring plant growth stimulants, is starting to receive enormous attention from the scientific community because of its rich source of growth hormones, antioxidants, vitamins, and mineral nutrients in the leaves (Foidl et al., 2001; Yasmeen et al., 2013). In literature, application of Moringa leaf extract via a seed, foliar spray, or plant rhizosphere has been demonstrated to enhance emergence, seedling, and plant growth as well as economical yield (Basra et al., 2011; Khan et al., 2017a). Khan et al. (2017a) reported that Moringa leaf extract has a higher biostimulant potential regarding seed emergence and plant vigour.

Seaweed extracts are a plant-based growth enhancer used as a good agricultural practice in sustainable and organic farming. Seaweeds contain plant hormones, auxins, cytokinin, abscisic acid and amino acids (Khan et al., 2009; Lötze & Hoffman, 2016), in addition to plant advancing constituents including micro and macronutrients, all of which are known to act on soils as well as on the plants (Halpern et al., 2015; Lötze & Hoffman, 2016). Seaweed extracts can be applied either by seed priming prior to germination, seedling root drench preceding transplant or/and foliar spray. Several studies have reported the influence of seaweed extracts on plant growth and production yield.

Humic substance has been reported to elicit different morphological changes in plants, prompting changes in plant growth and development (Trevisan et al., 2010). They have been shown to provide growth in many plant species, including soybean (*Glycine max*), wheat (*Triticum aestivum*), rice (*Oryza sativa*) maize (*Zea mays*), and vegetables crops such as potato (*Solanum tuberosum*), cucumber (*Cucumis sativus*), and peppers (*Capsicum annuum*) (Calvo et al., 2014). Similar to Moringa leaf extract and seaweed, humic substances can also be applied in few different ways including foliar spray or direct application into the plant rhizosphere (Halpern et al., 2015).

To further increase the utilisation of Bambara groundnut beyond traditional usage, it may be important to explore nutrient management strategies mainly focusing on supplying optimum levels of nutrients to plants for high or economically optimum seed yield. Also, it may be important to explore the nutritional quality of the Bambara groundnut following application of different biostimulats. Therefore, the present study aimed at investigating the influence of biostimulants, Kelpak®, a humic substance, and Moringa leaf extract on the performance of two Bambara groundnut landraces in a controlled greenhouse environment. This was achieved by determining the effect of the above-mentioned biostimulants on agronomic plant performance in comparison to an untreated control and fertiliser application. Moreover, the seed protein, carbohydrate, and lipid composition of the landraces was evaluated for each treatment.

3.1. Methods and Materials

Two independent experimental trials were conducted to assess the effects of biostimulant application on the growth, yield, and nutritional content of two Bambara groundnut landraces.

3.1.1. Plant growth conditions and preparation of materials

The experiments were conducted under tunnel conditions (between August 2019 and February 2020) at Welgevallen experimental farm, Stellenbosch University, South Africa (33°56'33"S; 18°51'56"E). Polythene plastic bags (10 L capacity) were filled with a 1:1 river sand to potting soil growth medium. The bags were perforated at the bases to reduce waterlogging. Seeds from the two landraces (cream with a black and white eye (BG1) and red with white eye (BG2) (Figure 1) were germinated in a closed room with temperature of about 22°C in seedling trays filled with Hygromix and vermiculite growing mixture.

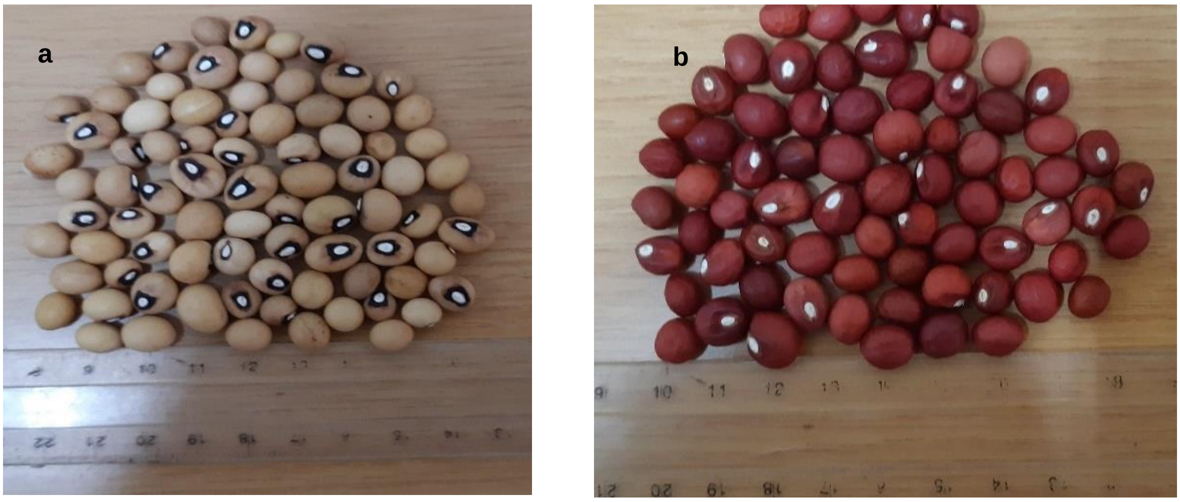


Figure 1: Two Bambara groundnut landraces (a) BG1 and (b) BG2

The seeds were checked daily and irrigated. Landrace BG1's first emergence was after ten days and reached 50% emergence thirteen days after planting, whereas BG2's first emergence and 50% emergence were fourteen and eighteen days after planting, respectively. Also, 93% of seed germination was recorded in BG1, and 72% was recorded for BG2 (Figure 2a and b).

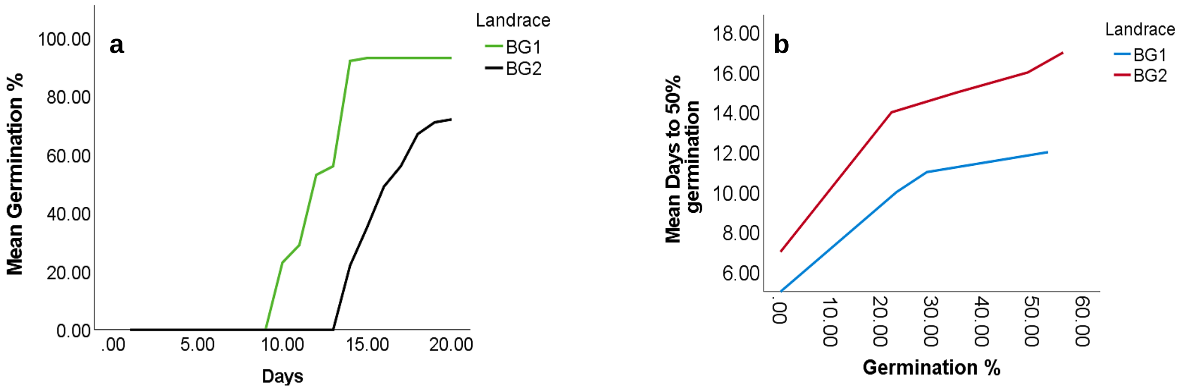


Figure 2: Germination percentage (a) and number of days to 50% germination (b) of two Bambara groundnut landraces (BG1 and BG2).

Uniform and hardened-off four weeks old Bambara groundnut seedlings were transplanted into polythene bags. Overall, the two landraces differed significantly in seedling height (Kruskal-Wallis $H_{(4,40)} = 23.268$; $p < 0.000$) and leaf number ((Kruskal-Wallis $H_{(4,40)} = 23.268$; $p < 0.000$).

3.1.2. Experimental design and treatment application

The three biostimulants were donated by the respective companies. *Trial 1*: The biostimulants were applied in combination with a fertiliser (chicken manure), excluding humic substance and were arranged in a randomised complete block design (RCBD) with six replications (n=6). Chicken manure was used because most Bambara groundnut growers are small scale farmers and used chicken manure as a source of nutrients. The treatments were as follows: T1 = Control (1L of untreated tap water per plant); T2= Fertiliser (1L of untreated water plus 100 ml chicken fertiliser per plant); T3 = Humic substance (1L solution per plant); T4 = Moringa leaf extract (1L solution plus 100 ml fertiliser per plant); T5 = Kelpak® (1L solution plus 100 mL fertiliser per plant). T3 and T4 was applied according to protocol which required an application of 3%v/v once a week during the growing season. For T5, an application of 3%v/v twice a week during the growing season was required. T1 and T2 was also applied twice a week until the end of the season. 630g of chicken manure was soaked in 5L of water for 24 hours. Recommended fertiliser application concentrations (100 ml per plant) and rates were followed, which was twice a week after transplant and again after ninety days. All treatments were soil applied via hand irrigation. The exact same protocols were followed for *Trial 2* and was carried out three months after *trial 1* commenced.

3.1.3. Data collection

Trial 1: Number of days to 50% flowering, leaf area, leaf dry weight, plant height, number of pods per plant, 100 seed weight, total seed yield, harvest index, seed nutritional composition and soil chemical properties were measured. Following a sampling method (Peksen, 2007), leaves of the same age were sampled from the same level of the canopy (2nd leaf) during the full-foliage period (100 days after initiation of treatments). The leaf area was recorded using a (LI-COR 3100) leaf area meter. The leaf samples were oven-dried at 60° C for five days after which the dry weight was recorded. Days to 50% flowering were monitored and counts were carried out on all the plants. The plants were checked twice weekly for the duration of the flowering period (20-80 days). The number of days it took each plant in a pot to show a fully opened flower was recorded.

Plant height was measured from the ground level to the highest tip of the leaves. This was done with the use of a metre ruler at hundred days after transplant. All the plants were harvested at hundred and fifty days after initiation of treatments. For number of pods per plant, plants were harvested per treatment by carefully removing the plant from the soil using a garden shovel and all the pods plucked. These were then counted manually. The grain yield was determined by threshing the harvested pods after they have dried to a constant weight. These were put in labelled envelopes by treatment and then weighed. The resulting weights, in grams (g) were then presented. The 100-seed weight was determined by counting 100 seeds of the same size per treatment. These were weighed to represent the mean seed weight per treatment. The harvest index was recorded as the ratio of grain yield to total above-ground biomass. The same variables were measured in *Trial 2* in the same sequence.

3.1.4. Soil chemical properties and seeds nutritional content

Soil samples were taken before planting (initial) and after plant harvest by treatment. The soil samples were then sent to BemLab (Somerset West, Western Cape) for total nutrient analysis. The seeds were cleaned manually, and any foreign objects removed, the whole seeds of the Bambara groundnut landraces were ground to a fine powder in a mixer mill. Three replicates, obtained by pooling five-gram powder from treatment samples, were used for each essay. The powder was then sent to the Central Analytical Facilities-ICP-MS &XRF Unit, Stellenbosch University, for nutrient analysis (amino acids, fatty acids, and carbohydrates in the form of sugars).

3.2. Statistical analysis

A normality test (Shapiro Wilk test) was used to determine if a data set had been drawn from a normally distributed population. When our data followed a normal distribution (p>0.05), parametric tests were employed, otherwise non-parametric methods were used. One-way analysis of variance (ANOVA) with Tukey HSD test (at 5% probability) was performed to determine which treatment had an influence on the 100 seed weight. The impact of treatment on plant height, leaf area, days to 50% flowering, pods per plants, grain yield and harvest index were compared using Kruskal-Wallis test followed by Multiple Mann-Whitney U test as Posthoc with a Bonferroni correction applied to correct for multiple pairwise comparisons. The Bonferroni corrections gives a new alpha value to have a more conserved interpretation of the output and to avoid making type two errors. It is calculated as follows: *Bonferroni corrected p value=(α)current p value÷(n) number of pairwise comparisons*; the new p value used was 0.005. Multiple linear regression analysis was used to determine which of the growth parameters had an influence on total seed yield under the different treatments. For seed nutrient composition data, Multivariate analysis of variance (MANOVA) with Tukey HSD test for multiple comparisons were used to compare differences in nutrient concentrations based on treatment. Kruskal-Wallis test followed by Multiple Mann-Whitney test U test and One-way ANOVA with Tukey HSD test was used to test if there are any differences on soil chemical properties between the treatments. Data were analysed using SPSS 27 (IBM® SPSS Statistics 2020), and statistical significance was accepted at $p = 0.05$.

3.3. Results and Discussion

3.3.1. Effects of different biostimulants on plant growth parameters

Our results showed that the application of biostimulants had a significant influence on leaf dry weight (Kruskal-Wallis $H_{(4,60)} = 30.010$; $p < 0.00$), plant height (Kruskal-Wallis $H_{(4,60)} = 34.326$; $p < 0.00$), and number of days to 50% flowering (Kruskal-Wallis $H_{(4,60)} = 26.704$, $p < 0.00$). Additionally, findings showed that the effect of treatment had no significant influence on leaf area (Kruskal-Wallis $H_{(4,60)}$; $p > 0.463$).

Figure 3a-c present the variation in Bambara groundnut growth parameters according to treatment. Plants supplied with humic substance had a significantly lower number of days to 50% flowering than the control and fertiliser, respectively (Figure 3a). Moringa leaf extract and Kelpak® also had a relatively lower average number of days to 50% flowering than control and fertiliser, even though there were not statistically significant (Figure 3a). Kelpak® produced higher dry leaf weight than all other treatments (Figure 3c) and had a significantly higher plant height than the control and Moringa leaf extract (Figure 3b). Relative to control and fertilizer, the humic substance had a higher plant height (Figure 3b).

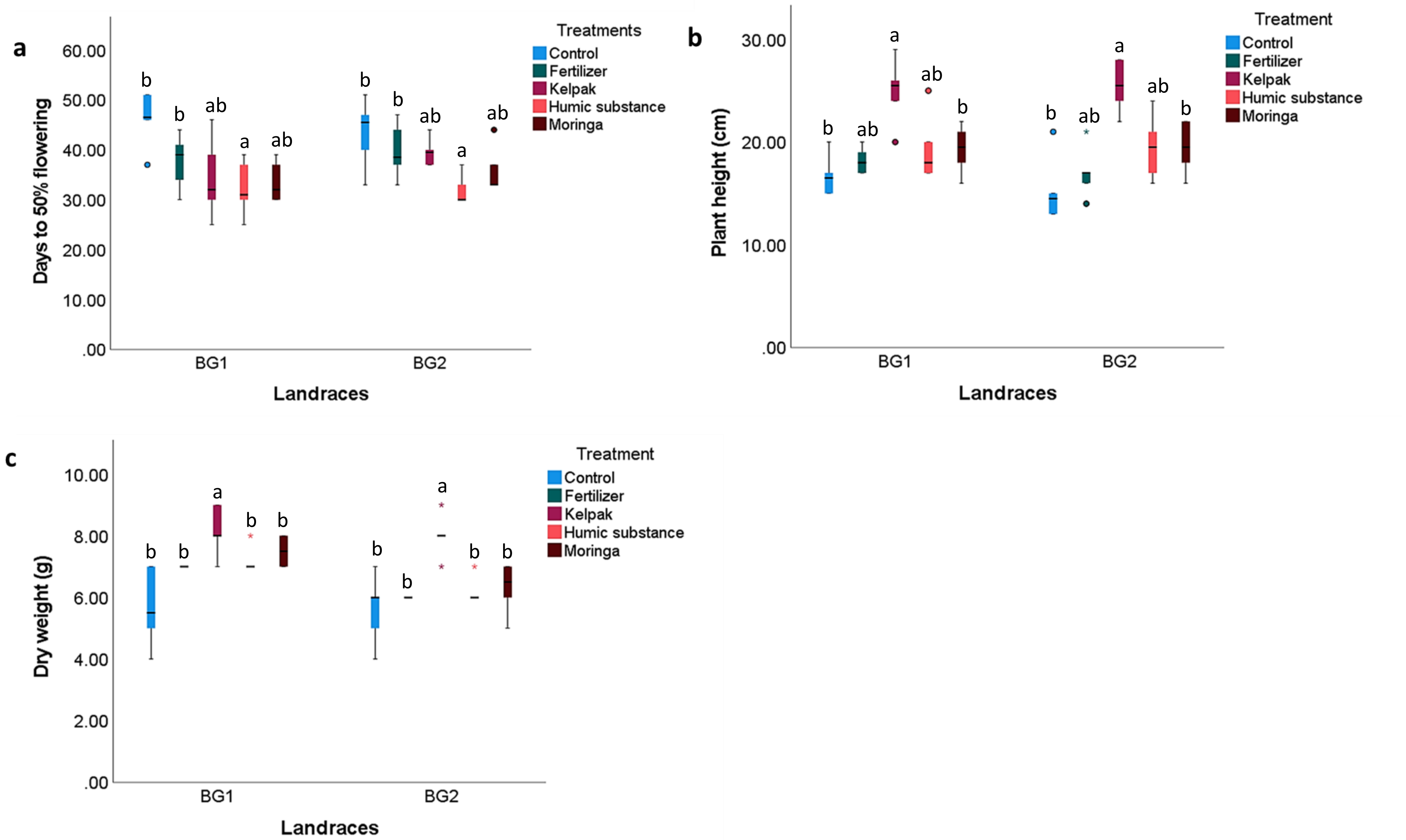


Figure 3: Effect of biostimulants on (a) number of days to 50% flowering, and (b) plant height, (c) dry leaf weight of two bambara groundnut landraces (BG1 and BG2). Boxplots showing the median, min and max, 25% and 75% interquartile range for the variables days to 50% flowering,

plant height, and dry leaf weight. Stars indicate outliers. Vertical error bars indicate standard error (SE). Statistical tests used were the Kruskal Wallis and Mann-Whitney test.

These results corroborate previous studies reported by several authors. For example, Halpern et al. (2015) have reported the effect of biostimulants from different sources in increasing plant growth and yield. Likewise, Rathore et al. (2009) showed that shoot yield of soybean treated with biostimulants increased by 6% when compared to the control. Additionally, Van Staden et al. (1994) reported an increase in shoot mass of marigold (*Tagetes patula*) treated with biostimulants compared to an untreated control. Kowalczyk & Zielony (2008) also reported that lettuce (*Lactuca sativa*) sprayed with a biostimulant had a higher head mass and increased leaf dry matter content. An increase in plant height due to biostimulant application have been reported in several crops including soybean (Rathore et al., 2009), common bean (*Phaseolus vulgaris*) (Latique et al., 2016) and chickpea (*Cicer arietinum*) (Boghdady et al., 2016). Kocira et al. (2018) have also reported a significant increase in plant height in soybean due to seaweed extract application.

The observed differences between the treatments can be attributed to different growth stimulating effect of different biostimulants. Biostimulants contain phytohormones and macro and micronutrients (Khan et al., 2009; Halpern et al., 2015; Latif & Mohammed, 2016, Khan et al., 2020, Zulfiqar et al., 2020). Yasmeen et al. (2013) have reported the presence of both macro- and micronutrients, such as N, P, K, Ca, B, Mg, Cu, Zn, Mn, Na and Fe in Moringa leaf extracts. Several studies have also reported the presence of zeatin, ascorbic acid, vitamin E, phenolic compounds, and mineral nutrients in different biostimulant sources (Foidl et al., 2001; Yasmeen et al., 2012; Yasmeen et al., 2013). The presence of phytohormones (i.e., auxins, cytokinin, and gibberellins) have also been reported from different biostimulants sources (Featonby-Smith & van Staden ,1987; Foidl et al, 2001; Khan et al, 2003; 2009; 2020; Abdalla, 2013; Halpern et al., 2015). Similar to a study by Rathore et al. (2009) in soybean the plant height of Bambara groundnut was increased by Kelpak® application. This was attributed to the role of phytohormones (cytokinin) in promoting cell division and elongation. Several studies reported the stimulating effect of cytokinins in plants. Cytokinins stimulate cell division and growth of cell tissues in growing plants (Taiz et al., 2015)..

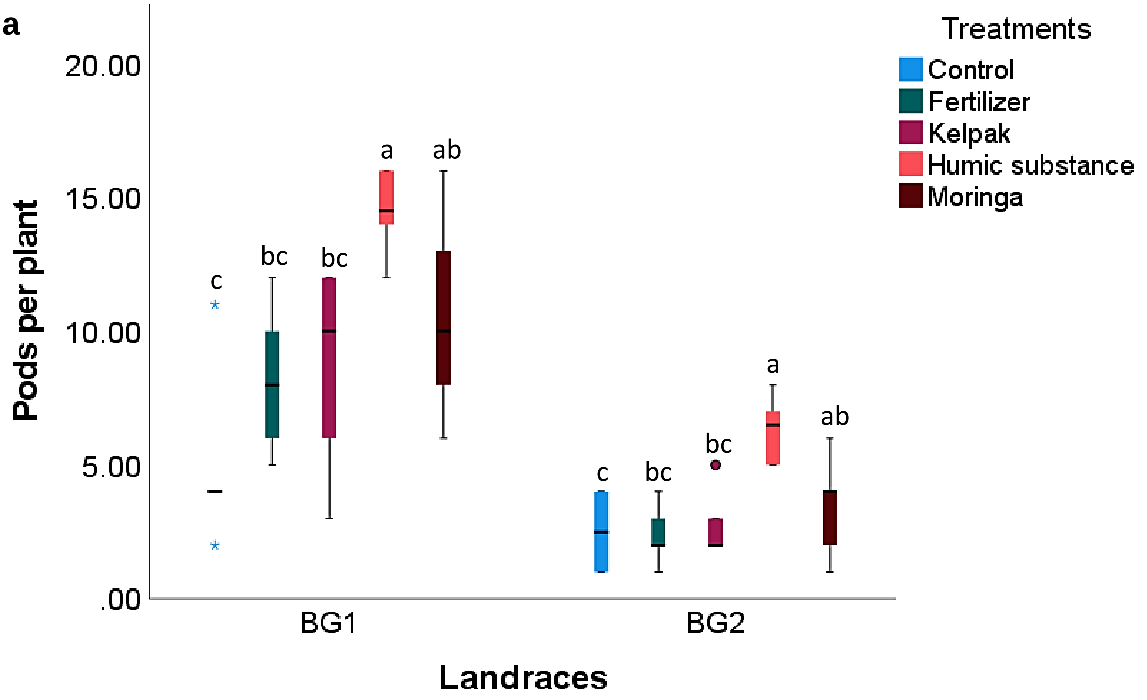
Early flowering is viewed to infer early plant development and maturity (Shegro et al., 2013), and it is a significant trait in crop production as early maturity can be selected for early season finish and rotation. Also, early plant development may help facilitate avoidance of stressful environmental conditions and may enable selection for adaptation to drought prone regions. Generally, flowering in Bambara groundnut occurs about 30 to 40-45 days after planting and might continue until the crop reaches maturity depending on a landrace (Unigwe et al., 2016). Massawe et al. (2005), Masindeni (2006), and Shegro et al. (2013) reported that days to flowering in Bambara groundnut ranged from 43 to 80 days after planting depending on the landrace. In the current study, days to 50% flowering was quite variable among the treatments ranging from 25 to 50 days after planting. The effect of biostimulants on flowering was clear in the current study as all three biostimulants had a lower number of days to 50% flowering relative to the control and fertiliser treatments on both landraces. Although only the humic substance was significantly different from the control and fertiliser, in general the average number of days to 50% flowering was lower in Moringa leaf extract and Kelpak® relative to the control and fertiliser, respectively. Early flower initiation in biostimulant treated plants has been reported in previous studies. In their study, Taylor & Wilkinson (1977) reported early flowering in plants that received low dosage of seaweed extract. Similarly, Kumar et al. (2012) also reported early flowering in mung bean (*Vigna radiata*) treated with seaweed extract. This is attributed to the ability of biostimulants to promote hormonal activity in plants resulting in the initiation of flowers at an early stage. Furthermore, these results can be accredited to the accelerating effect of biostimulants in plants leading to fast growth and maturity. Harris et al. (2002) have reported that early crop establishment leads to rapid growth and early completion of phenological events such as flowering.

3.3.2. Effect of different biostimulants on Bambara groundnut yield

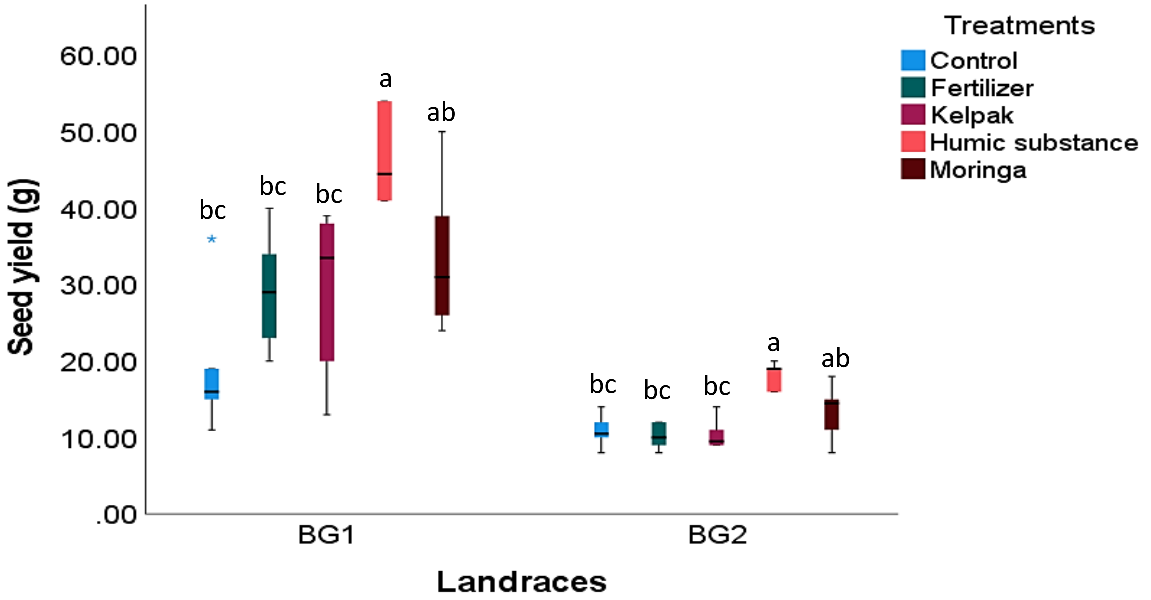
Our findings showed that the effect of treatment had a significant influence on the number of pods per plant (Kruskal-Wallis $H_{(4,60)} = 16.157$, $p < 0.00$), seed yield (Kruskal-Wallis $H_{(4,60)} = 12.80$, $p < 0.00$), and harvest index (Kruskal-Wallis $H_{(4,60)} = 11.507$, $p < 0.00$). Hundred-seed weight ($F_{(4,60)} = 10.01$, $p < 0.000$) was also significantly influenced by treatment.

Figure 4 showed the differences in yield parameters between the treatments. The humic substance had a significantly higher number of pods per plant than all treatments except Moringa leaf extract (Figure 4a), whereas Moringa leaf extract was only significantly different from the control treatment. Humic substance had a significantly higher seed yield than all the treatments, except Moringa leaf extract (Figure 4b). The harvest index percentage was highest in humic substance treated plant (Figure 4c).

a



b



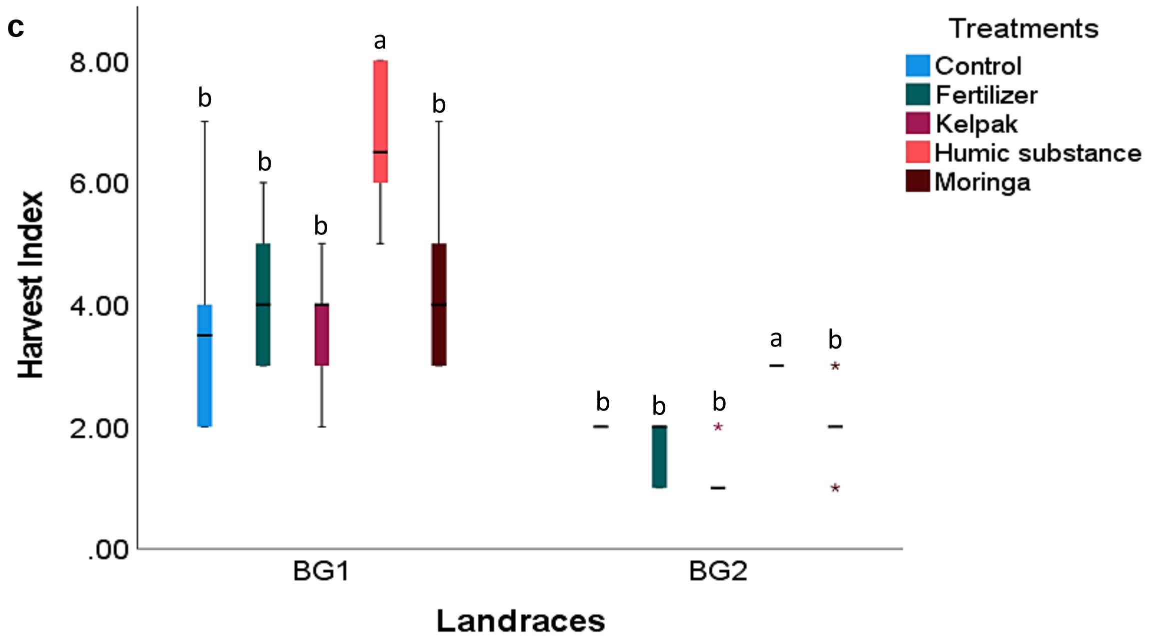


Figure 4: Effect of biostimulants on (a) number of pods per plant, and (b) seed yield, and (c) harvest index of two bambara groundnut landraces (BG1 and BG2). Boxplots showing the median, min and max, 25% and 75% interquartile range for the variables pods per plant and seed yield. Stars indicate outliers. Vertical error bars indicate standard error (SE). (Kruskal Wallis test and Mann-Whitney test).

The humic substance had a significantly higher 100 seed weight than all treatments except the Moringa leaf extract (Figure 5).

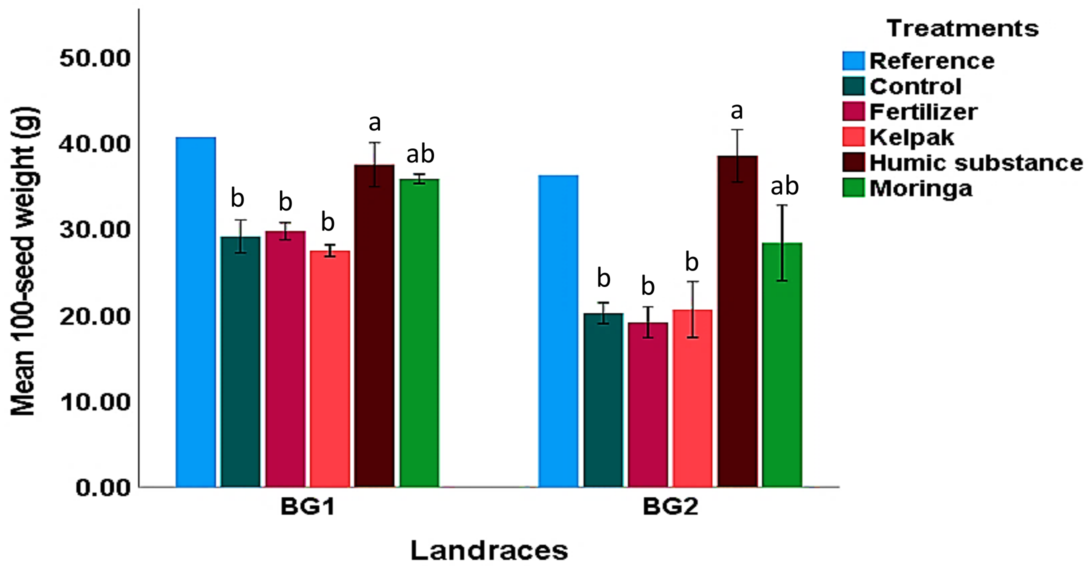


Figure 5: Effect of biostimulants on 100-seed weight of two Bambara groundnut landraces. Bar charts showing mean and standard error (SE) for the variable 100-seed weight. (One-way ANOVA was used to compare the means of the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another).

The observed increase in yield parameters, and ultimately grain yield in the current study affirms past investigations depicting the positive impact of biostimulants on the yield of different crops (Khan et al., 2009; European Biostimulants Industry Council, 2012; Halpern et al., 2015). Foliar spraying of biostimulants have been reported to improve the yield of harvests, including maize (*Zea mays*), lupine (*Lupinus polyphyllus*), potatoes (*Solanum tuberosum*), common bean (*Phaseolus vulgaris*), and groundnut (*Arachis hypogaea*) (Featonby-Smith and van Staden, 1987; Matysiak et al., 2012; Kocira, Korna, & Kocira, 2013). Our findings are in line with those of Featonby-Smith and van Staden (1987), who reported a significant effect of biostimulants on yield and yield parameters of peanuts (*Arachis hypogaea*). Moreover, biostimulant application was found to have improved the production and average weight of tepary bean (*Phaseolus acutifolius*) seeds (Beckett et al., 1994). Among various traits contributing to final yield of a crop, number of pods per plant and 100-seed weight are of prime importance in legumes. In the current study humic substance had the highest number of pods per plant and 100-seed weight followed by Moringa leaf extract for both landraces. These results confirmed previous findings by other authors. In their study, Featonby-Smith & Van Staden (1987) reported a maximum of 41% seed weight increase in peanut plants treated with biostimulants when compared to the control. Our findings were further supported by Emengor (2015) who reported an increase in pod length and pod yield per plant of green beans (*Phaseolus vulgaris*) treated with Moringa leaf extract as a biostimulant. The explanation behind the observed yield increment can be ascribed to the growth stimulating effect of biostimulants.

The growth enhancing potential of the biostimulants is attributed to the presence of phytohormones, and micro- and macronutrients in different biostimulants sources (Khan et al., 2009; Halpern et al., 2015). Andrews (2006) reported the presence of zeatin, dihydrozeatin, and isopentyladenine in Moringa leaf extracts which are endogenous cytokinins. Phytohormones are known to affect cellular metabolism in treated plants, prompting enhanced growth and yield (Crouch et al., 1992; Crouch &Van Staden 1993; Stirk et al., 2006; Stirk et al., 2009). In vegetative plant organs cytokinin are associated with nutrient partitioning whereas in reproductive organs, high levels of cytokinin are linked with nutrient mobilization. This view was supported by several studies, e.g. Stirk et al. (2009), and Ciura & Kruk (2018), who observed that plants treated with seaweed extracts had a higher concentration of cytokinin contrasted with untreated fruits. Abdalla (2013) also found that biostimulant foliar application in Rocket (*Eruca vesicaria subsp. sativa*) plants at 2% and 3% enhanced endogenous auxins, gibberellins and cytokinin levels compared to untreated plants.

These responses show that biostimulants might be involved either in enhancing the mobilization of phytohormones from the roots to the developing vegetative growth, fruit, grain, or, by improving the quantity or synthesis of endogenous plant phytohormones (Takei et al., 2002). In addition, the growth enhancing potential of biostimulants is also attributed to the ability of biostimulants to improve and facilitate micro and macronutrients uptake by the plants (Khan et al., 2009; Halpern et al., 2015; Latif & Mohammed, 2016, Khan et al., 2020, Zulfiqar et al., 2020), a process that has been reported in several literature studies. In their study, Rathore et al. (2009) reported a significant increase of N, P, and K uptake by soybean plants treated with a biostimulant compared to an untreated control. Crouch et al. (1990) also noted an increased uptake of Mg, K, and Ca in lettuce with biostimulant application. In a separate study, Turan & Kose (2004) and Mancuso et al. (2006) reported increased uptake of N, P, K, and Mg in grapevines (*Vitis vinifera*) and cucumber (*Cucumis sativus*) plants treated with a biostimulant.

3.3.3. The prediction of growth parameters influence on Bambara groundnut seed yield under different treatments.

A multiple linear regression was run to predict which of the growth parameters influence Bambara groundnut seed yield under the different treatments. We ran individual multiple linear regression analysis using the plant growth parameters (leaf area, days to 50% flowering, plant height, pods per plant, 100-seed weight, and harvest index) as predictors of seed yield which is the independent variable (Table 2). The model showed that the growth factors significantly explained the variation in seed yield under control ($F_{(5,11)}$ = 27.81, p < 0.00), fertiliser ($F_{(5,11)}$ = 75.10, p < 0.00), Kelpak® ($F_{(5,11)}$ = 116.28, p < 0.00), humic substance ($F_{(5,11)}$ = 81.95, p < 0.00) and Moringa leaf extract ($F_{(5,11)}$ = 94.61, p < 0.00) treatments and explained 94% (adjusted r^2=0.94), 98% (adjusted r^2=0.98), 98% (adjusted r^2=0.98), 98% (adjusted r^2=0.98) and 98% (adjusted r^2=0.98) of the variation in yield, respectively. With fertiliser and Moringa leaf extract treatments, the combination of all the predictor variables significantly influenced seed yield but individually none of the variables had a significant effect on seed yield (Figure 4 b). The variation in seed yield was largely explained by the number of pods per plant (β = 0.69, t= 2.60, p < 0.05) in the control plants and Kelpak® (β = 0.87, t = 6.32, p < 0.00) treatment, respectively. However, both the control and Kelpak® produced the lowest seed yield, and thus did not differ in their influence on Bambara groundnut yield. Under treatment with humic substance the variation in seed yield was largely explained by harvest index (β = 0.63, t = 3.12; p < 0.03), which is the ratio of grain yield and biomass. Therefore, increased seed yield under humic substance treatment can be attributed to the effects of the treatment on variation in growth parameters that result in higher biomass, and subsequent translocation into grain yield (Figure 4c).

It has been reported in literature that these characters (pods per plant, seed weight, and harvest index) are closely associated with legume grain yield (Ayaz et al., 2001). In their study, Ouedraogo et al. (2008) have reported that the number of pods per plant is strongly associated with grain yield in Bambara groundnut. Associations of pods per plant, seed weight and harvest index with grain yield have also been reported in common vetch (*Vicia sativa*) (Albayrak, 2006). Moreover, Misangu et al. (2012) have reported that number of pods per plant are highly associated with seed yield in Bambara groundnut.

Table 2: Individual multiple linear regression analysis using plant growth parameters as predictors of seed yield under different treatments

Predictor variables	Control			Fertiliser			Kelpak			Humic substance			Moringa extract		
	β	**t**	**P**	**β**	**t**	**P**	**β**	**t**	**P**	**β**	**t**	**P**	**β**	**t**	**P**
(Constant)		0,36	0,734		0,371	0,726		2,556	0,051		-1,184	0,290		-0,052	0,961
Leaf area (cm^2)	-0,056	-0,436	0,681	0,315	1,718	0,146	0,011	0,139	0,895	0,130	1,140	0,306	0,120	1,125	0,312
100-seed weight (g)	0,176	1,183	0,29	-0,046	-0,496	0,641	-0,102	-1,990	0,103	0,049	0,579	0,588	-0,057	-1,108	0,318
Plant height (cm)	-0,051	-0,566	0,596	-0,004	-0,078	0,941	-0,023	-0,379	0,72	-0,013	-0,236	0,822	-0,002	-0,044	0,967
Harvest Index	0,306	1,200	0,284	0,488	2,238	0,075	0,167	1,198	0,285	**0,628**	**3,118**	**0,026**	0,390	2,091	0,091
Pods per plant	**0,635**	**2,603**	**0,048**	0,242	0,87	0,424	**0,869**	**6,317**	**0,001**	0,276	1,828	0,127	0,551	2,278	0,072
Days to 50% flowering	-0,005	-0,037	0,972	-0,057	-0,897	0,411	-0,062	-0,992	0,367	0,012	0,109	0,918	0,030	0,577	0,589

3.3.4. Effect of different biostimulants on Bambara groundnut seed nutritional content

3.3.4.1. Fatty acid content

The major fatty acid content found in all our samples were myristic acid, pentadecylic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, linoleic acid, arachidic acid, α-linolenic acid, behenic acid, erucic acid, docosadienoic acid, and lignoceric acid. Our results show that the fatty acids myristic acid ($F_{(4,30)} = 7.48$, $p < 0.00$), linoleic acid ($F_{(4,30)} = 3.92$, $p < 0.02$), and α-linolenic acid ($F_{(4,30)} = 18.46$, $p < 0.00$) were significant influenced by treatment (Table 3).

Table 3: Fatty acid composition (mg/g) of Bambara groundnut seeds grown under different treatment conditions. (Multivariate ANOVA was used to compare the means of the different samples).

Fatty acids	Common name	DF	F	P	Partial Eta Squared
C14	Myristic acid	**4**	**7,482**	**0,001***	**0,599**
C15	Pentadecylic acid	4	1,747	$0,179^{ns}$	0,259
C16	palmitic acid	4	0,976	$0,443^{ns}$	0,163
C16:1	palmitoleic acid	4	0,965	$0,448^{ns}$	0,162
C17	margaric acid	4	1,505	$0,239^{ns}$	0,231
C 18	stearic acid	4	2,03	$0,129^{ns}$	0,289
C18:1(cis)	oleic acid	4	0,943	$0,46^{ns}$	0,159
C18:2 (cis)	**linoleic acid**	**4**	**3,916**	**0,017***	**0,439**
C20	arachidic acid	4	1,274	$0,313^{ns}$	0,203
C18:3n3	**α-linolenic acid**	**4**	**18,458**	**0,000***	**0,787**
C22	behenic acid	4	2,227	$0,103^{ns}$	0,308
C22:1	erucic acid	4	0,637	$0,642^{ns}$	0,113
C22:2	Docosadienoic acid	4	1,817	$0,165^{ns}$	0,267
C24	lignoceric acid	4	1,309	$0,300^{ns}$	0,207

*indicates significant differences (new p value <0.005), ^{ns} indicate no significant differences.

The differences in fatty acid content of bambara groundnut seeds based on treatment are presented in Figure 6. Moringa leaf extract had a significantly higher myristic acid content than both the fertiliser and humic substance for both landraces. The fertiliser had the lowest myristic acid content of all the treatments in both landraces (Figure 6a). The linoleic acid concentration was highest in humic substance treatment followed by the fertiliser and control for BG1, while in BG2 fertiliser had the highest linoleic acid content followed by Kelpak® and humic substance (Figure 6b). Only the fertiliser and humic substance treatments resulted in a significantly higher α-linolenic acid content than all other treatments for BG1, while the fertiliser, Kelpak®, and humic substance treatments resulted in significantly higher α-linolenic acid content than all other treatments for BG2 (Figure c).

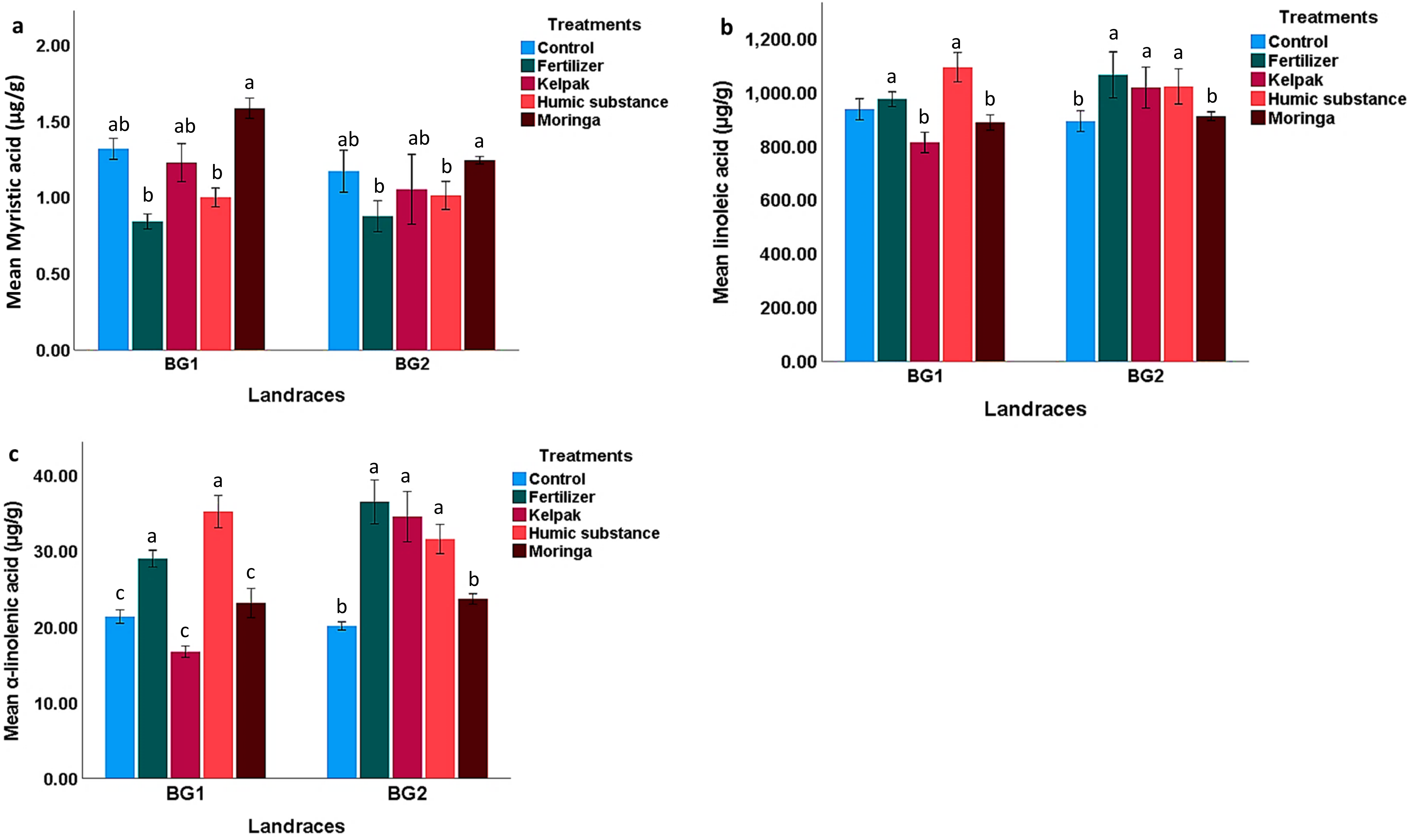


Figure 6: Effect of biostimulants on (a) Myristic acid, (b) linoleic acid, and (c) α-linolenic acid of two Bambara groundnut landraces. Bar charts showing mean and standard error (SE) for the variable Myristic acid, linoleic acid, and α-linolenic acid. (MANOVA was used to compare the means of

the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another).

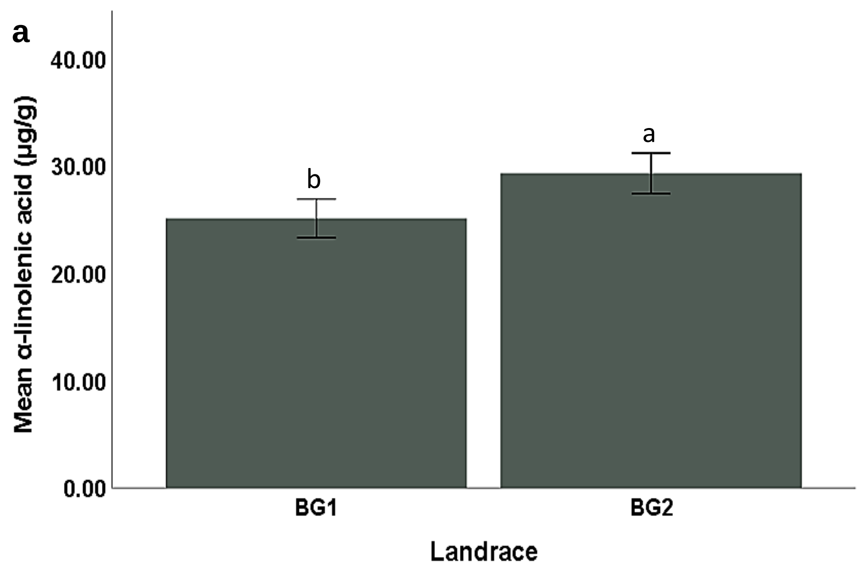
Landrace was also found to be a significant factor in Bambara groundnut fatty acid content. Our results showed that the fatty acids, α-linolenic acid ($F_{(4,30)}$ = 12,75, p < 0.00, eta=0,389), and docosadienoic acid ($F_{(4,30)}$ = 5,07, p < 0.04, eta=0,202) concentrations differed between the two landraces (Table 4).

Table 4: Fatty acid composition (mg/g) of Bambara groundnut seeds based on landrace. (Multivariate ANOVA was used to compare the means of different samples).

Fatty acids	Common name	DF	F	P	Partial Eta Squared
C14	Myristic acid	1	3,114	0,093^{ns}	0,135
C15	Pentadecylic acid	1	0,17	0,684^{ns}	0,008
C16	palmitic acid	1	0,022	0,883^{ns}	0,001
C16:1	palmitoleic acid	1	0,773	0,39^{ns}	0,037
C17	margaric acid	1	0,806	0,38^{ns}	0,039
C18	stearic acid	1	3,49	0,076^{ns}	0,149
C18:1(cis)	oleic acid	1	2,784	0,111^{ns}	0,122
C18:2 (cis)	linoleic acid	1	1,542	0,229^{ns}	0,072
C20	arachidic acid	1	0,761	0,393^{ns}	0,037
C18:3n3	α-linolenic acid	**1**	**12,745**	**0,002***	**0,389**
C22	behenic acid	1	0,873	0,361^{ns}	0,042
C22:1	erucic acid	1	1,646	0,214^{ns}	0,076
C22:2	Docosadienoic acid	**1**	**5,069**	**0,036***	**0,202**
C24	lignoceric acid	1	0,004	0,953^{ns}	0,000

*indicate significant differences (p value <0.05), ns indicate no significant differences.

As can be seen in Figure 7, BG2 had a significantly higher α-linolenic acid concentration than BG1 (Figure 7a), whereas BG1 had a significantly higher Docosadienoic acid concentration than BG2 (Figure 7b).



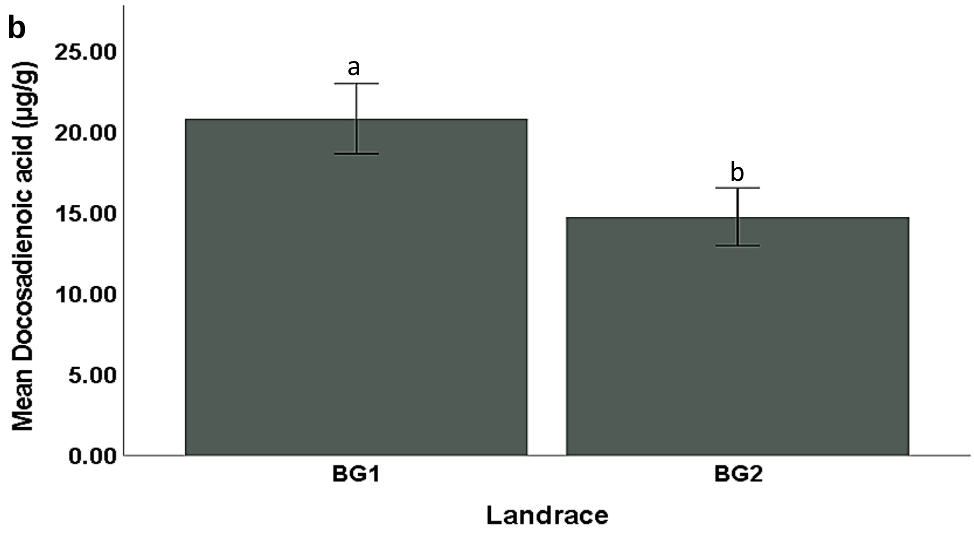


Figure 7: Difference in seed (a) α-linolenic acid, and (b) Docosadienoic acid between BG1 and BG2. Bar charts showing mean and standard error (SE) for the variables α-linolenic acid, and Docosadienoic acid. (Multivariate ANOVA was used to compare the means of the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another).

The current study confirmed the abundance of fatty acids in Bambara groundnut seeds. The concentrations of palmitic acid, margaric acid, stearic acid, oleic acid, linoleic acid, and α-linolenic acid were found in large amounts in all our samples. Our results agree with those of Minka & Brunetean (2002), who reported that palmitic acid, linoleic acid, and α-linolenic acid are found in large amounts in Bambara groundnut seeds. Similarly, Halimia et al. (2019) have reported high concentrations of palmitic acid, stearic acid, oleic acid, linoleic acid, and α-linolenic acid in Bambara groundnut seeds. The essential dietary fatty acids linoleic acid and α-linolenic acid are of particular interest to human health. Previous reports suggest that intake of these fatty acids can reduce the likelihood of hypercholesterolemia and improve cardiovascular function (Mensink et al., 2003; Wanders et al., 2010).

Previous studies on the effect of biostimulants on individual fatty acid concentrations of legume is limited. However, the use of growth regulators auxins (Nawaz et al., 2016), gibberellins (Saleem et al., 2008), and cytokinins (Khalid et al., 2012) have been reported to improve plant growth, productivity, as well as fruit nutritional quality. Biostimulants including humic substance and Moringa leaf extract contain traces of these growth hormones in addition to micro and macronutrients (Khan et al., 2009; Halpern et al., 2015; Khan et al., 2020), and can be the result of the observed differences in treatments in the current study. In their study, Nasir et al. (2016), reported an increase in nutrient content of a citrus Kinnow mandarin (*Citrus reticulate*) after application of Moringa leaf extract. Similar findings were reported by Yasmeen et al. (2013), and this was ascribed to the rich nutritional content of Moringa leaf extracts, which when applied as foliar spray directly or indirectly affected the plant metabolism. Our results showed that Moringa leaf extract and humic substance increased the fatty acid content of Bambara groundnut, however, these results were not consistent across the two landraces. This suggests the need to conduct research on each landrace to determine differences in landraces.

Our results further showed that the effect of treatment did not significantly influence the fatty acid content of pentadecylic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, arachidic acid, behenic acid, erucic acid, docosadienoic acid, and lignoceric acid concentrations (Table 3). Moreover, no significant differences were found between the landraces on the fatty acid content with regard to myristic acid, pentadecylic acid, palmitic acid, palmitoleic acid, margaric acid, oleic acid, linoleic acid, arachidic acid, behenic acid, erucic acid, and lignoceric acid concentrations (Table 4). Our findings are not directly comparable to previous work, as there are few evaluations on the association between individual fatty acid composition and biostimulant application. In general, these results can be viewed to mean that biostimulants can be used to better the growth and total yield of Bambara groundnut without compromising/negatively affecting the nutritional composition of the crop. Additionally, the comparability of the control treatment with the fertiliser and the biostimulants treatments observed in most of the studied amino acids validate previous reports on the ability of Bambara groundnut to grow and produce on low input soils (Massawe et al. 2005; Kone et al. 2011; Mabhaudhi and Modi 2013).

3.3.4.2. Amino acid content

The essential and non-essential amino acids found in all our samples were, valine, leucine, isoleucine, methionine, threonine, phenylalanine, lysine, histidine, tyrosine, tryptophan, and cysteine, glutamic acid, aspartic acid, serine, proline, respectively. The effect of treatment had a significant influence on amino acid content of Bambara groundnut seeds with regard to: glycine ($F_{(4,30)} = 3.03$, $p < 0.04$), leucine ($F_{(4,30)} = 4.02$, $p < 0.02$) lysine ($F_{(4,30)} = 5.09$, $p < 0.01$), histidine ($F_{(4,30)} = 4.51$, $p < 0.01$), and tryptophan ($F_{(4,30)} = 5.54$, $p < 0.00$) (Table 5). Our results also showed that the effect of treatment did not significantly influence the amino acid concentrations of alanine, valine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, cysteine, and tyrosine (*p*>0.05) (Table 5).

Table 5: Amino acid composition (mg/g) of Bambara groundnut seeds grown under different treatment conditions. (Multivariate ANOVA was used to compare the means of different samples).

Amino acids	DF	F	P	Partial Eta Squared
Alanine	4	2,458	0,079^{ns}	0,33
Glycine	**4**	**3,033**	**0,042***	**0,378**
Valine	4	2,309	0,093^{ns}	0,316
Leucine	**4**	**4,018**	**0,015***	**0,446**
Isoleucine	4	1,158	0,359^{ns}	0,188
Proline	4	1,633	0,205^{ns}	0,246
Methionine	4	2,637	0,064^{ns}	0,345
Serine	4	1,297	0,305^{ns}	0,206
Threonine	4	1,511	0,237^{ns}	0,232
Phenylalanine	4	1,500	0,240^{ns}	0,231
Aspartic acid	4	1,108	0,38^{ns}	0,181
Cysteine	4	0,802	0,538^{ns}	0,138
Glutamic acid	4	1,130	0,371^{ns}	0,184
Lysine	**4**	**5,09**	**0,005***	**0,504**
Histidine	**4**	**4,508**	**0,009***	**0,474**
Tyrosine	4	0,941	0,461^{ns}	0,158
Tryptophan	**4**	**5,541**	**0,004***	**0,526**

*indicate significant differences (p value <0.05), ^{ns} indicate no significant differences.

Figure 8 shows the differences in amino acid concentrations of Bambara groundnut seed based on treatment. The three biostimulants had a significantly higher leucine concentration than the control and fertiliser treatments for BG1. The Moringa leaf extract treatment had a significantly higher leucine concentration followed by fertiliser and humic substance for BG2 (Figure 8a). The control, Kelpak®, Moringa leaf extract treated Bambara groundnut had a significantly higher glycine concentration for BG1 compared to the fertiliser and humic substance treatments. For BG2, fertiliser and Moringa leaf extract had a significantly higher glycine concentrations relative to the other treatments (Figure 8b). Similarly, the fertiliser treatment had an extremely high lysine concentration for BG2 compared to other treatments. For BG1, the control and humic substance had a relatively higher lysine to the other treatments (Figure 8c). The control and Moringa leaf extract treatments had a higher histidine concentration for BG1. Whereas the fertiliser had a significantly higher histidine content than all other treatments for BG2 (Figure 8d). The Moringa leaf extract treatment had the highest tryptophan concentration which was significantly different from humic substance and fertiliser treatments, respectively (Figure 8e).

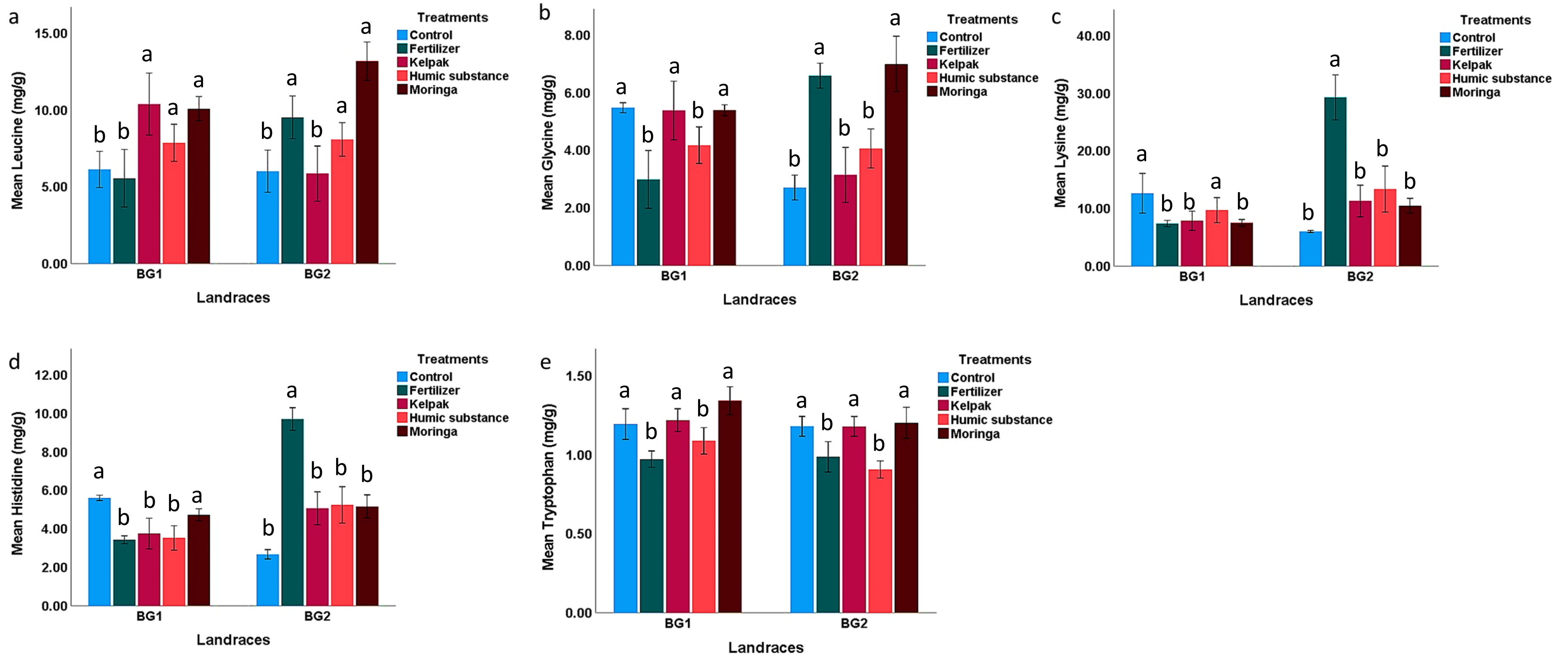


Figure 8: Effect of biostimulants on (a) leucine, (b) glycine, (c) lysine, (d) histidine, and (e) tryptophan of two Bambara groundnut landraces. Bar charts showing mean and standard error (SE) for the variables leucine, glycine, lysine, histidine, and tryptophan. (MANOVA was used to compare the means of the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another.

Our results further revealed that there was a significant difference between the two Bambara groundnut landraces with regards to: methionine ($F_{(4,30)}$ = 6.63, p < 0.02), threonine ($F_{(4,30)}$ = 6.29, p < 0.02), phenylalanine ($F_{(4,30)}$ = 6.78, p < 0.02), lysine ($F_{(4,30)}$ = 10.60, p < 0.00), histidine ($F_{(4,30)}$ = 9.39, p < 0.01), and tyrosine ($F_{(4,30)}$ = 6.22, p < 0.02) (Table 6). Moreover, no significant differences were found between the landraces with regard to the amino acid concentrations of alanine, glycine, valine, leucine, isoleucine, proline, serine, aspartic acid, glutamic acid, and tryptophan (p> 0.05) (Table 6).

Table 6: Amino acid composition (mg/g) of Bambara groundnut seeds based on landrace. (Multivariate ANOVA was used to compare the means of the different samples).

Amino acids	DF	F	P	Partial Eta Squared
Alanine	1	0,154	0,699^{ns}	0,008
Glycine	1	0,001	0,970^{ns}	0,000
Valine	1	0,436	0,517^{ns}	0,021
Leucine	1	0,33	0,572^{ns}	0,016
Isoleucine	1	0,029	0,867^{ns}	0,001
Proline	1	0,007	0,933^{ns}	0,00
Methionine	**1**	**6,634**	**0,018***	**0,249**
Serine	1	2,031	0,170^{ns}	0,092
Threonine	**1**	**6,288**	**0,021***	**0,239**
Phenylalanine	**1**	**6,783**	**0,017***	**0,253**
Aspartic acid	1	0,904	0,353^{ns}	0,043
Cysteine	1	3,51	0,076^{ns}	0,149
Glutamic acid	1	0,524	0,478^{ns}	0,026
Lysine	**1**	**10,6**	**0,004***	**0,346**
Histidine	**1**	**9,394**	**0,006***	**0,32**
Tyrosine	**1**	**6,216**	**0,022***	**0,237**
Tryptophan	1	2,111	0,162^{ns}	0,095

*indicate significant differences (p value <0.05), ns indicate no significant differences

Figure 9a-f shows that BG2 had a significantly higher methionine, threonine, phenylalanine, lysine, histidine, and tyrosine concentrations than BG1.

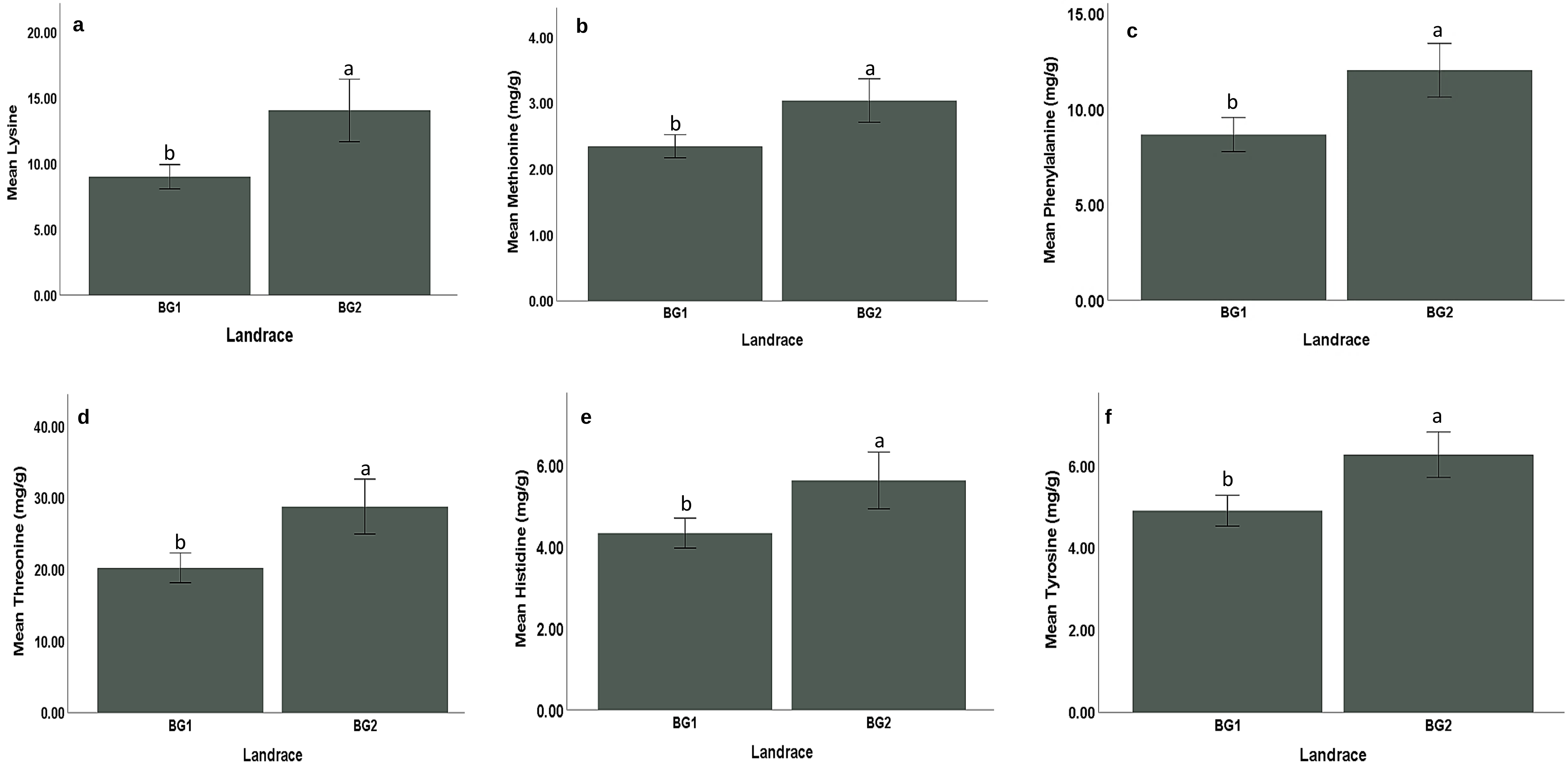


Figure 9: Difference in seed (a) lysine, (b) methionine, (c) phenylalanine, (d) threonine, (e) histidine, and (f) tyrosine between BG1 and BG2. Bar charts showing mean and standard error (SE) for the variables lysine, methionine, phenylalanine, threonine, histidine, and tyrosine. (Multivariate ANOVA with used to compare the means of the different landraces and Tukey HSD test was used to find out which of the means are significantly different from one another).

The quantification of essential amino acids concentration relative to nutritional requirements are typically used to assess the protein quality of foods. Our results agree with those of Glew et al. (1997), who reported that Bambara groundnut seeds contain all the essential amino acid. Among the essential amino acids found in the current study, lysine, threonine, phenylalanine, leucine, were found to be predominant followed by valine, tyrosine, histidine, and isoleucine. The sulphur-containing amino acids, cysteine, and methionine, as well as tryptophan were found to be the most limiting in all studied samples. These results confirm previous findings by other researchers on legumes being poor sources of sulphur containing amino acids (Iqbal et al., 2005). In their study, Yao et al. (2015) reported that Bambara groundnut landraces had a very limited amount of tryptophan. However, despite this limiting factor, Bambara groundnut could still play an important role in meeting people's protein needs. Very high lysine levels were observed in all studied samples, a very important nutritional attribute that makes Bambara groundnut a good supplementary protein to lysine deficient foods. In addition to lysine, glutamic acid, aspartic acid and threonine were the most abundant of the studied amino acids. These results confirm findings from previous studies; in their study, Adebowale et al. (2011), Mune et al. (2011), and Arise et al. (2017) reported that Bambara groundnut protein contain higher levels of aspartic acid and glutamic acid concentrations than any other amino acids.

The biostimulants influenced amino acids, with the Moringa leaf extract treatment showing a more consistent increase in concentration compared to to all studied amino acids. There is limited information on the effect of biostimulants on individual amino acid content in legumes. However, several studies have previously reported a positive influence of biostimulants on plants nutrient content. In their study, Zulfiqar et al. (2020) reported that spinach plants supplemented with Moringa leaf extract showed an increase in concentration of total soluble protein and other bioactive compounds. Similarly, Fan et al. (2013) reported an increase in total soluble protein content in spinach treated with a biostimulant application. The varietal effect on proximate composition of legumes have previously been reported (Nti, 2009, Yao et al., 2015; Unigwe et al., 2018). The current study also showed that BG2 had a higher methionine, threonine, phenylalanine, lysine, histidine, and tyrosine concentrations than BG1. Our results agree with previous studies by other authors. Kaptso et al. (2007) reported a significant difference on the protein and carbohydrates content of Bambara groundnut landraces. Additionally, Nti (2009) observed that dark seeded landraces (black and red) had a higher nutrient and mineral contents than light seeded ones (cream) which was also observed in the current study. The effect of treatment did not significantly influence the amino acid concentrations of alanine, valine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, cysteine, and tyrosine nor did the landraces with regard to the amino acid concentrations of alanine, glycine, valine, leucine, isoleucine, proline, serine, aspartic acid, glutamic acid, and tryptophan.

Our findings are not directly comparable to previous work. Although there are many studies on the effect of biostimulant application on nutritional value and quality of plant products such as leaves and grain legumes (Matysiak et al., 2012; Kocira, 2018), however, there are limited studies demonstrating the effect of different biostimulants on specific amino acid concentrations. In the current study biostimulant application mostly increased or had comparable amino acid content to that of fertiliser and control treatments. As such, biostimulant application can be presumed to improve the growth and yield of Bambara groundnut without compromising the nutritional value of the crop. Our findings also indicated that the control treatment in some cases showed higher nutritional content than both the fertiliser and the biostimulant treatments.

3.3.4.3. Sugar content

The major sugars found in all our samples were, D-Fructose, D-Glucose, Mannitol, Sorbitol, Myo-inositol, and sucrose. Research looking at sugar content of Bambara groundnut is limited. However, there has been previous results stating that fructose, glucose, mannitol, sorbitol, myo-inositol, and sucrose are the most dominant sugars in legume species (Onweluzo et al., 2002; Zouari et al., 2020). The sugar sucrose was the predominant in all our samples. This result support previous work by other authors. In their study, Onweluzo et al. (2002) reported that sucrose is present in several of the commonly consumed legumes. The effect of treatment had a significant influence on sugar content of Bambara groundnut seed with regards to sucrose $F_{(4,30)} = 14.14$, $p< 0.00$) (Table 7).

Table 7: Sugar contents (mean ± S.D., n = 3) in Bambara groundnut seeds grown under different treatment conditions. (Multivariate ANOVA was used to compare the different samples).

Sugars	DF	F	P	Partial Eta Squared
D-Fructose	4	1,082	0,392^{ns}	0,178
D-Glucose	4	0,591	0,673^{ns}	0,106
Mannitol	4	0,763	0,562^{ns}	0,132
Sorbitol	4	0,609	0,661^{ns}	0,109
Myo-inositol	4	2,251	0,100^{ns}	0,31
Sucrose	**4**	**14,139**	**0,000***	**0,739**

*indicate significant differences (p value < 0.05), ^{ns} indicate no significant differences.

The fertiliser and humic substance had the highest sucrose concentrations for both landraces, which was significantly higher than Moringa leaf extract, control, and Kelpak®, respectively (Figure 10).

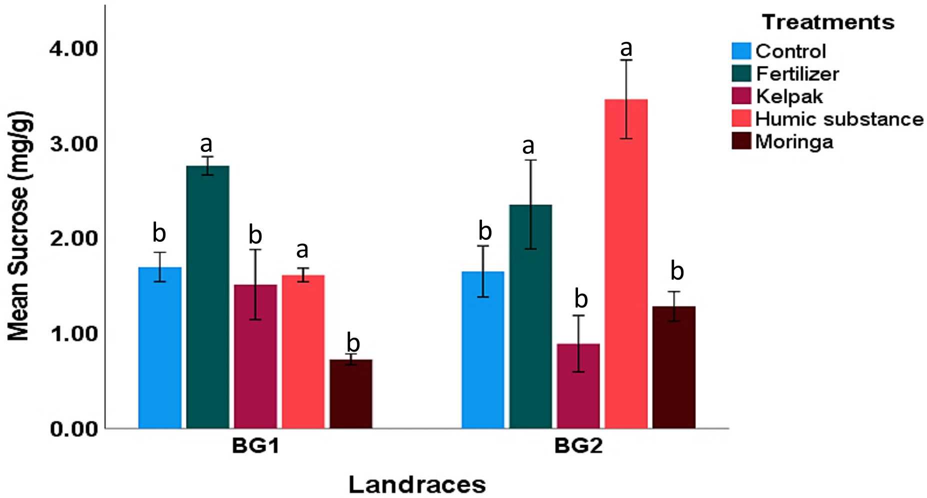


Figure 10: Effect of biostimulants on sucrose of two Bambara groundnut landraces. Bar charts showing mean and standard error (SE) for the variable sucrose. (One-way ANOVA and was used to compare the means of the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another).

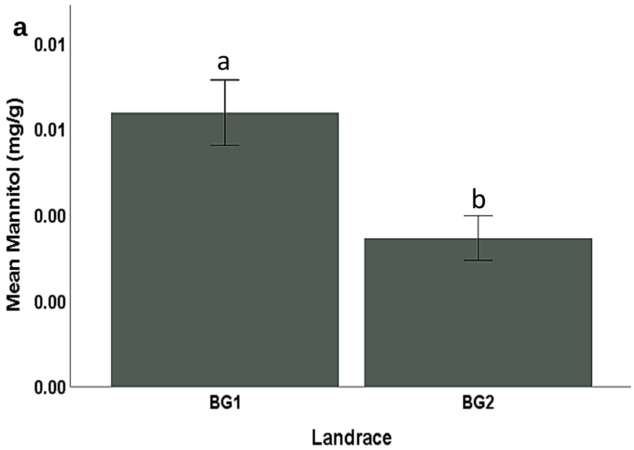
Our results also showed that there was a significant difference in seed sugar content of the two Bambara groundnut landraces with regards to: Mannitol $F_{(4,30)} = 10.99$, $p = 0.00$) and Sorbitol $F_{(4,30)} = 9.43$, $p = 0.01$) (Table 8).

Table 8: Sugar contents (mean ± S.D., n = 3) in Bambara groundnut seeds based on landrace. (Multivariate ANOVA was used to compare the means of the different samples).

Sugars	DF	F	P	Partial Eta Squared
D-Fructose	1	2,116	0,161^{ns}	0,096
D-Glucose	1	1,106	0,305^{ns}	0,052
Mannitol	**1**	**10,99**	**0,003***	**0,355**
Sorbitol	**1**	**9,434**	**0,006***	**0,321**
Myo-inositol	1	0,541	0,47^{ns}	0,026
Sucrose	1	2,345	0,141^{ns}	0,105

*indicate significant differences (p value <0.05), ^{ns} indicate no significant differences.

BG1 had a significantly higher mannitol, and sorbitol concentrations relative to BG2 Figure 11a-b.



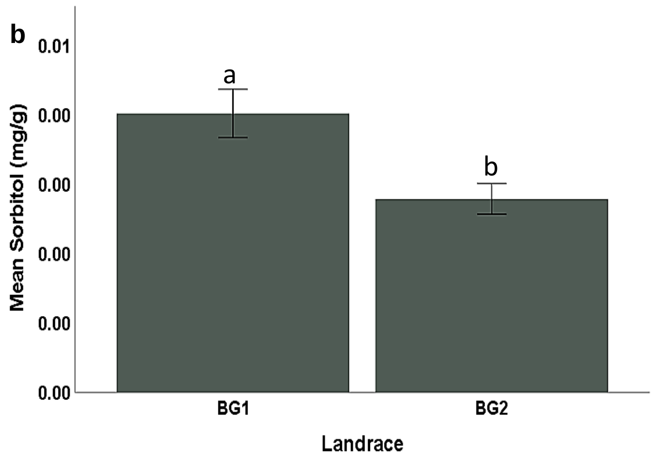


Figure 11: Difference in seed (a) mannitol, and (b) sorbitol between BG1 and BG2. Bar charts showing mean and standard error (SE) for the variables mannitol, and sorbitol. (Multivariate ANOVA with Tukey HSD test. Multivariate AVOVA was used to compare the

means of the different landraces and Tukey HSD test was used to find out which of the means are significantly different from one another).

Several studies have reported the effect of biostimulants on plant sugar levels. Foidl et al. (2001), reported that biostimulant foliar application to cantaloupe (*Cucumis melo var. cantalupensis*) and sugarcane (*Saccharum officinarum*) increased sugar and mineral levels, respectively. Similarly, Nasir et al. (2016) reported that Moringa leaf extract applied as a biostimulant improved fruit nutrient quality, such as sugars and total antioxidants in mandarin orange. The improvement could be ascribed to the fact that most biostimulants contain minerals, growth hormones, phenolics, antioxidants, amino acids and vitamins which are linked to better fruit growth and development process (Abdalla, 2013; Halpern et al. 2015; Khan et al., 2009; Khan et al., 2020).

Our results further showed that the effect of treatment did not significant impact the sugar content of D-Fructose ($F_{(4,30)}$ = 1,08, $p < 0.39$), D-Glucose ($F_{(4,30)}$ = 0,59, $p < 0.67$), Mannitol ($F_{(4,30)}$ = 0,76, $p < 0.56$), Sorbitol ($F_{(4,30)}$ = 0,61, $p < 0.66$), and Myo-inositol ($F_{(4,30)}$ = 2,25, $p < 0.10$) (Table 7). Moreover, no significant differences were found between the landraces with regards to: D-Fructose ($F(_{4,30)}$ = 2,12, $p < 0.16$), D-Glucose ($F_{(4,30)}$ = 1,11, $p < 0.31$), Myo-inositol ($F_{(4,30)}$ = 0,54, $p < 0.47$), and Sucrose ($F_{(4,30)}$ = 02,35, $p < 0.14$) concentrations (Table 8). Our results are not directly comparable to previous findings; there are few evaluations of associations between biostimulant application and individual sugar concentrations. In general, sugar content in legumes have not received much attention in the research community. Historically, the primary concern has been on oil or protein content (Teixeira et.al., 2012). Of particular interest is the fact that the control treatment had a comparable concentration with the other treatments for almost all the sugars. These results support previous findings stating that Bambara groundnut are able to growth and produce on low input soils. High nutritional value and the ability to yield on low input soils are few of Bambara groundnut great attributes (Massawe et al., 2005; Mkandawire, 2007).

3.3.5. Effect of biostimulants on soil chemical and physical properties

Our results show that the effect of treatment had a significant influence on chemical nutrients with regards to: Ca ($F_{(4,62)}$ = 12.63, $p < 0.00$), Cu ($F_{(4,62)}$ = 3.66, $p < 0.01$), B

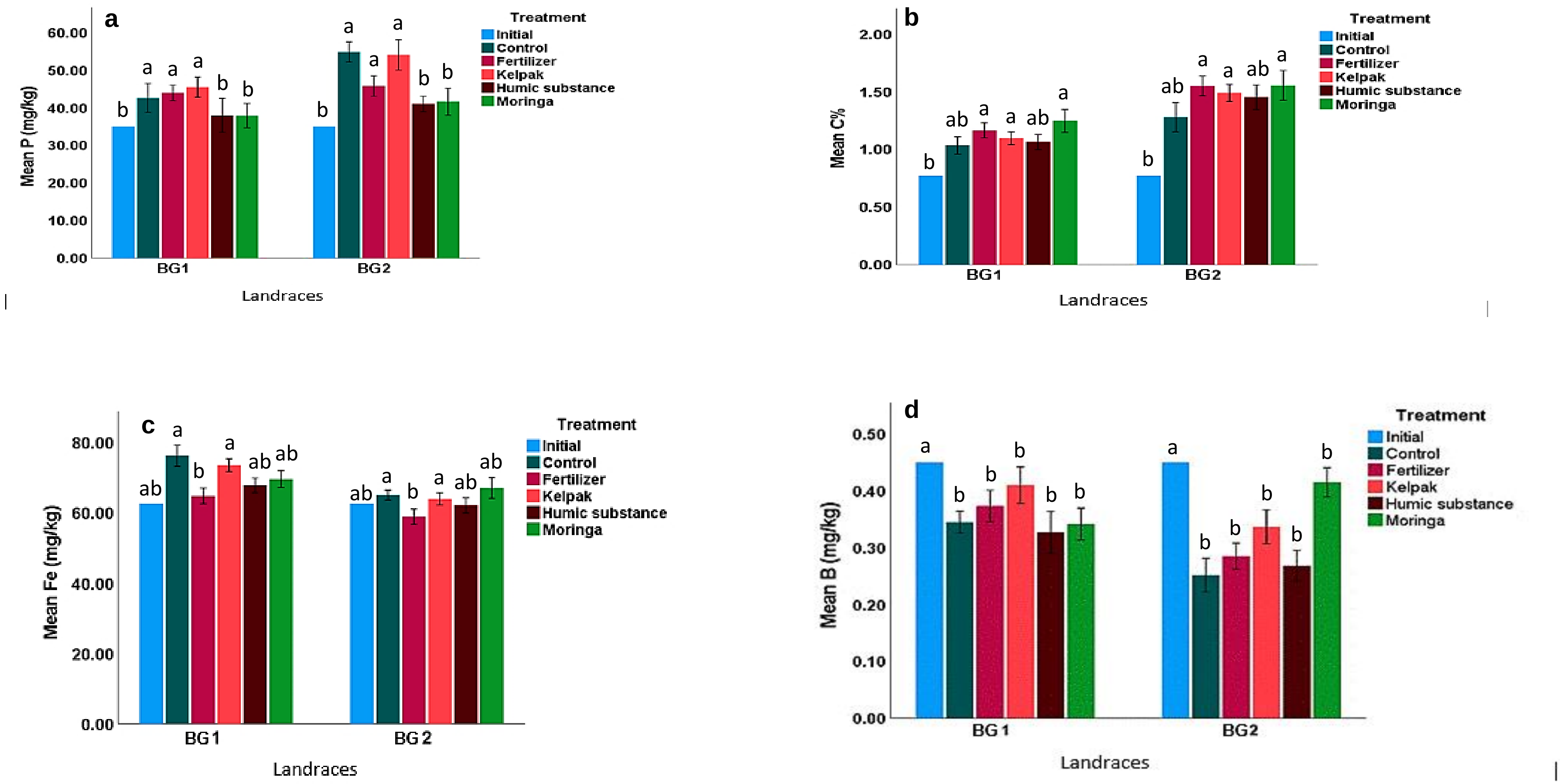
$(F_{(4,62)}$ = 3.35, $p < 0.01$), Fe ($F_{(4,62)}$ = 2.93, $p < 0.02$), C $(F_{(4,62)}$ = 2.46, $p < 0.04$), and P($F_{(4,62)}$ = 3.72, $p < 0.01$) (Table 9).

Table 9: Effect of biostimulants on soil chemical properties. One-way analysis of variance (ANOVA) was performed for each of the variables.

Variable	DF	F	P
Ca	4	**12.634**	**0.000***
Cu	4	**3.658**	**0.006***
B	4	**3.345**	**0.010***
Fe	4	**2.932**	**0.020***
C	4	**2.461**	**0.044***
P	4	**3.721**	**0.006***

*indicate significant differences (*p* value < 0.05), ^{ns} indicate no significant differences

The phosphorus (P) level for the Kelpak® was significantly higher than Moringa and humic substance, respectively, but comparable with the other treatments including the initial soil (Figure 12a). The initial soil sample had a significantly lower carbon (C) percentage than all treatments except the control and humic substance, and no significant differences were observed between the treatments (Figure 12b). The only notable differences in iron (Fe) were between the fertiliser with control and Kelpak®, respectively. The control had a significantly higher Fe level than the fertiliser. Similarly, the Kelpak® treatment had a significantly higher Fe than the fertiliser (Figure 12c). The initial, humic substance, and Moringa leaf extract had a non-significant boron (B) levels compared to Kelpak®, control and fertilizer (Figure 12d). The treatments had a significantly higher calcium (Ca) levels when compared to the initial soil. Kelpak® had a significantly higher Ca levels than all treatments, except the control. The humic substance had a significantly lower Ca levels than almost all the treatments (Figure 12e). The copper (Cu) levels from all the treatments were significantly higher than the initial soil but no significant differences were found between the treatments (Figure 12 f).


a
Mean P (mg/kg)
0.00
10.00
20.00
30.00
40.00
50.00
60.00
BG1
BG2
Landraces
Treatment
Initial
Control
Fertilizer
Kelpak
Humic substance
Moringa
b
Mean C%
0.00
0.50
1.00
1.50
2.00
BG1
BG2
Landraces
Treatment
Initial
Control
Fertilizer
Kelpak
Humic substance
Moringa
c
Mean Fe (mg/kg)
0.00
20.00
40.00
60.00
80.00
BG1
BG 2
Landraces
Treatment
Initial
Control
Fertilizer
Kelpak
Humic substance
Moringa
d
Mean B (mg/kg)
0.00
0.10
0.20
0.30
0.40
0.50
BG 1
BG2
Landraces
Treatment
Initial
Control
Fertilizer
Kelpak
Humic substance
Moringa

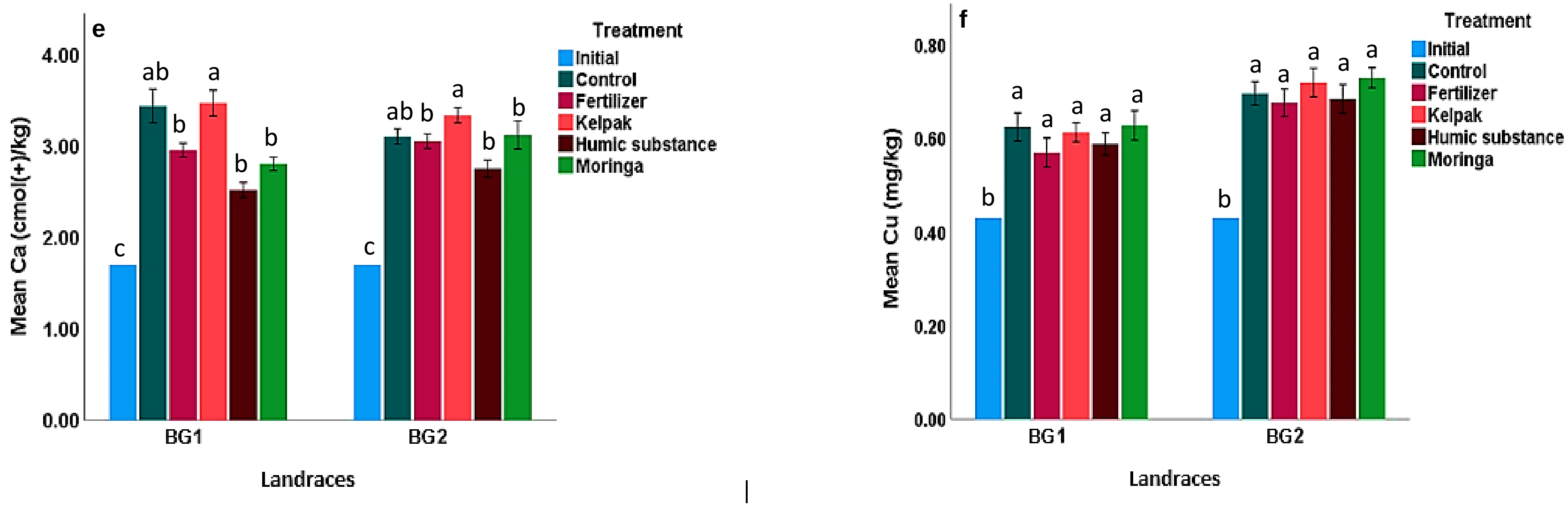


Figure 12: Effect of different biostimulants on (a) Phosphorus, (b) Carbon, (c) Iron, (d) Boron, (e) Calcium, and (f) Copper on soil chemical properties. Bar charts showing mean and standard error (SE) for the variables Calcium, and Copper. (One-way ANOVA and was used to compare the means of the different landraces according to treatment and Tukey HSD test was used to find out which of the means are significantly different from one another).

Our findings are not directly comparable to previous work, as there few studies looking at the association between biostimulants and soil chemical properties. However, biostimulants have been reported to affect the physical, biological, and chemical properties of the soil. For example, Du Jardin (2015) reported the use of humic substance as biostimulant as an essential contributor to soil fertility, acting on physical, and chemical and biological properties of the soil.

Our results further showed that the effect of treatment did not have a significant influence on soil chemical nutrients with regard to: N, P, K, Na, Zn, Mn, S, Mg, and Na (*p*> 0.05) (Table 10).

Table 10: Effect of biostimulants on soil chemical properties. (Kruskal-Wallis test was used to assess for significant differences of the variables).

Variable	N	P	K	Na	Zn	Mn	S	Mg
DF	4	4	4	4	4	4	4	4
Kruskal-Wallis H	12.629	11.117	7.797	10.165	12.870	14.969	12.802	18.813
P	0.027^{ns}	0.049^{ns}	0.168^{ns}	0.071^{ns}	0.025^{ns}	0.010^{ns}	0.025^{ns}	0.02^{ns}

*indicate significant differences (p value < 0.005), ^{ns} indicate no significant differences

It is important to keep in mind that biostimulants are not fertilisers meant to correct soil nutrient deficiency but are chemicals that when used in small amount promote and improve plant growth through their direct effects on metabolic processes (Gallant, 2004). Du Jardin (2015) and Schmidt et al. (2003) have emphasised the point that biostimulants unlike chemical fertilisers do not directly supply any nutrients to crops but may enhance growth possible by facilitating important plant cellular metabolisms as well as nutrient utilization from the soil.

3.4. Conclusion

Our results showed that Bambara groundnut exhibited significant changes during growth, with plant height, leaf area, number of days to 50% flowering, number of pods per plant, seeds yield, and harvest index varied between the treatments. The current study demonstrated that plants treated with the humic substance had the shortest number of days to 50% flowering followed by moringa leaf extract and Kelpak® for both landraces. The control and fertiliser took the longest to reach 50% flowering. Plants treated with humic substance and moringa leaf extract produced the highest number of pods per plants, total seed yield and 100-seed weight for both landraces. Kelpak® produced the lowest seed yield of the three biostimulants but had the highest plant height and shoot dry weight. As such, it is evident that Kelpak® might be more suitable for production of leafy vegetables than is to grain. The results of the regression analysis revealed that, under fertiliser and Moringa leaf extract treatments, the combination of all the predictor variables significantly influenced seed yield but individually none of the variables had a significant effect on Bambara groundnut seed yield. Under the control and Kelpak® treatments, the variation in Bambara groundnut seed yield was found to be largely explained by the number of pods per plant. Moreover, under treatment with humic substance the variation in seed yield was largely explained by harvest index. Our results further revealed that the effect of treatment had a significant influence on some individual fatty acids, amino acids, and sugars, respectively. Out of the three biostimulants, Moringa leaf extract showed more consistent results with regards to the above-mentioned nutrient compositions. Our results further revealed that the untreated control was comparable to the other treatments for most of the individual seed nutrients analysed. Our results showed observable differences between the initial soil and treatments with regards to phosphorus, carbon, iron, boron, calcium, and copper. In the light of the achieved data, it may be concluded that humic substance and Moringa leaf extract increased the seed yield of Bambara groundnut without compromising or/and negatively affecting the nutritional value of the crop. Therefore, biostimulants can be used as a sustainable and effective tool to improve Bambara groundnut crop yield. However, this study only focused on two of the seven Bambara groundnut landraces that are classified by DAFF in South Africa. Therefore, further research is required to validate the results on the five identified Bambara groundnut landraces.

In conclusion, the unique characteristics of Bambara groundnut differentiate it from many other legume plants. With its extreme tolerance to harsh conditions, high nutritional content, and ability to fix nitrogen, Bambara groundnut is well suited for widespread productivity, use, and profitability. However, in order for Bambara groundnut crop production to meet burgeoning demands, a multipronged approach will be necessary. Successfully improvement of Bambara groundnut can positively impact the profitability of small-scale production systems by enhancing returns, as well as contribute to the economic development of rural communities. Effective and sustainable crop management strategies are needed to increase the yields of established crops without further land use changes, as demonstrated in this study.

3.5. References

Abdalla, M. M. (2013). The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria subsp. sativa*) plants. International Journal of Plant Physiology and Biochemistry, 5 (3),42–49.5.

Adebooye, O.O., Ajayi, S.A., Baidu-Forson, J.J., & Opabode, J.T. (2005). Seed constraint to cultivation and productivity of African indigenous leaf vegetables. African Journal of Biotechnology, 4(13),1480–1484.

Adebowale, Y.A., Schwarzenbolz, U., & Henle, T. (2011). Protein isolates from Bambara groundnut (*Vigna subterranean* L.): chemical characterization and functional properties. International Journal of Food Properties, 14,758–775.

Adu-Dapaah, H., & Sangwan, R.S. (2004). Improving Bambara groundnut productivity using gamma irradiation and in vitro techniques. African Journal of Biotechnology, 3(5), 260–265.

Albayrak, S., & Tongel, Ö. (2006). Path analyses of yield and yield-related traits of common vetch (*Vicia sativa* L.) under different rainfall conditions. Journal of Agricultural Faculty, OMU, 21(1),27–32.

Aliyu, S., Massawe, F., & Mayes, S. (2014). Beyond landraces: developing improved germplasm resources for underutilised species - a case for Bambara groundnut. Biotechnology & Genetic Engineering Reviews, 30(1–2),127–141.

Amarteifio, J.O., Tibe, O., & Njogu, R.M. (2006). The mineral composition of Bambara groundnut (*Vigna subterranea* (L) Verdc) grown in Southern Africa. African Journal of Biotechnology, 5,2408–2411.

Andrews, D. (2006). Nutraceutical Moringa composition. Retrieved from http://www.gooogle.com/patents/ US20060222882.

Arise, A. K., Alashi, A. M., Nwachukwu, I. D., Malomo, S. A., Aluko, R. E., & Amonsou, E. O. (2017). Inhibitory properties of Bambara groundnut protein hydrolysate and peptide fractions against angiotensin converting enzymes, renin and free radicals. Journal of the Science of Food and Agriculture, 97,2834–2841.

Arise, A. K., Amonsou, E. O., & Ijabadeniyi, O. A. (2015). Original article Influence of extraction methods on functional properties of protein concentrates prepared from South African Bambara groundnut landraces. International Journal of Food Science and Technology, 50, 1095–1101.

Ashok Kumar, N., Vanlalzarzova, B., Sridhar, S., & Baluswami, M. (2012). Effect of liquid seaweed fertiliser of *Sargassum wightii* Grev. on the growth and biochemical content of green gram (*Vigna radiata* (L.) R.). Recent Research in Science and Technology, 4,40–45.

Ayaz, S., McNeil, D.L., McKenzie, B.A. & Hill, G.D. (2001). Population and sowing date effect on yield components of grain legumes. Proceedings of the 10th Australian Agronomy conference. Hobart, Australia, pp. 125.

Azam-Ali, S.N., Sesay, A., Karikari, S.K., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M., & Hampson, K.J. (2001). Assessing the potential of an underutilised crop—a case study using Bambara groundnut. Experimental Agriculture, 37,433–472.

Bamshaiye, O.M., Adegbola, J.A., & Bamishaiye, E.I. (2011). Bambara groundnut: an under-utilised nut in Africa. Advances in Agricultural Biotechnology, 1,60–72.

Basra, S.M.A., Iftikhar, M.N., & Afzal, I., (2011). Potential of Moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. International Journal of Agriculture and Biology, 13, 1006–1010.

Beckett, R. P., Mathegka, A. D. M., & Van Staden, J. (1994). Effect of seaweed concentrate on yield of nutrient-stressed tepary bean (*Phaseolus acutifolius Gray*). Journal of applied phycology, 6(4), 429-430.

Bedada, W., Lemenih, M., & Karltun, E. (2016). Soil nutrient build-up, input interaction effects and plot level N and P balances under long-term addition of compost and NP fertiliser. Agriculture, Ecosystems & Environment, 218, 220–231.

Bennie, A.T.P. & Hensley, M. (2001). Maximizing precipitation utilization in dryland agriculture in South Africa – a review. Journal of Hydrology, 241,124–139.

Boghdady, M. S., Selim, D. A. H., Nassar, R. M. A., & Salama, A. M. (2016). Influence of foliar spray with seaweed extract on growth, yield and its quality, profile of protein pattern and anatomical structure of chickpea plant (*Cicer arietinum* L.). Middle East Journal of Applied Science and Technology, 6,207–221.

Calvo, P., Nelson, L., & Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. Journal of Plant and Soil, 383, 3–41.

Ciura, J., & Kruk, J. (2018). Phytohormones as targets for improving plant productivity and stress tolerance. Journal of plant physiology, 229,32–40.

Crouch, I.J., & van Staden, J. (1993). Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regulators, 13,21–29.

Crouch, I.J., Smith, M.T., Van Staden, J., Lewis, M.J. & Hoad, G.V. (1992). Identification of auxins in a commercial seaweed concentrate. Journal of plant physiology, 139(5),590–594.

Crough, I. J., Beckett, R. P., & van Staden, J. (1990). Effect of seaweed concentrate on the growth and mineral nutrition of nutrient-stressed lettuce. Journal of Applied Phycology, 2,269 – 272.

Republic of South Africa, Department of Agriculture, Forestry & Fisheries (DAFF). (2016). Bambara Groundnuts: Production guideline. Department of Agriculture, Forestry & Fisheries, Pretoria. http://ProdguideBambara.pdf.

European Biostimulants Industry Council. (2012). EBIC and Biostimulants in Brief. http://www.biostimulants.eu/. Accessed 14 Dec 2019

Egounlety, M., & Aworh, O. (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max Merr*.), cowpea (*Vigna unguiculata* L.) and groundbean (*Macrotyloma geocarpa Harms*). Journal of Food Engineering, 56, 249–254.

Emongor, V. E. (2015). Effects of Moringa (*Moringa Oleifera*) Leaf Extract on Growth, Yield and Yield Components of Snap Beans (*Phaseolus vulgaris*). British Journal of Applied Science and Technology, 6(2),114–122.

Fan, D., Hodges, D.M., Critchley, A.T., & Prithiviraj, B. (2013). A commercial extract of brown macroalga (*Ascophyllum nodosum*) affects yield and the nutritional quality of spinach in vitro. Communications in Soil Science and Plant Analysis, 44, 1873–1884.

Food and Agricultural Organization (FAO). (2001). A global mapping system for bambara groundnut production. FAO, Rome, Italy.

Fasoyiro, S.B., Ajibade, S.R., Omole, A.J., Adeniyan, O.N., & Farinde, E.O. (2006). Proximate, minerals and antinutritional factors of some underutilised grain legumes in south-western Nigeria. Nutrition and Food Science, 36,18–23.

Featonby-Smith, B. C., & van Staden, J. (1987). Effect of seaweed concentrate on yield and seed quality of *Arachis hypogaea*. South African Journal of Botany, 53(3),190–193.

Foidl, N., Makkar, H.P.S., & Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. The miracle tree: The multipurpose attributes of Moringa. CTA publications, Wageningen, the Netherlands,45–76.

Gallant, A., 2004. Biostimulants: What they Are and how they Work. TURF and Recreation, Canada.

Gaudin, A.C.M., Tolhurst, T.N., Ker, A.P., Janovicek, K., Tortora, C., Martin, R.C., & Deen, W. (2015). Increasing crop diversity mitigates weather variations and improves yield stability. PLoS ONE, 10 (2),1–20.

Glew, R.H., VanderJagt, D.J., Lockett, C., Grivetti, L.E., Smith, G.C., Pastuszyn, A., & Millson, M. (1997). Amino Acid, Fatty Acid, and Mineral Composition of 24 Indigenous Plants of Burkina Faso. Journal of Food Composition and Analysis, 10,205–217.

Glodowska, M., Husk, B., Schwinghamer, T., & Smith, D. (2016). Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a *pseudomonad*. Agronomy for Sustainable Development 36, 21.

Graham, P.H., & Vance, C.P. (2003). Legumes: importance and constraints to greater use. Plant Physiology. 131, 872–877.

Gregory, P. J., Mayes, S., Hui, C., Ebrahim, H., Advina, J., & Giva, J. (2019). Crops For the Future (CFF): an overview of research efforts in the adoption of underutilised species. Planta.

Halimia, R.A., Barklaa, B.J., Mayesb, S., Graham J., & King, G.J. (2019). The potential of the underutilised pulse bambara groundnut (*Vigna subterranea* (L.) Verdc) for nutritional food security, Journal of Food Composition and Analysis., 77, 47–59.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. In *Advances in agronomy*,130,141–174. Academic Press.

Harris, D, Rashid, A, Miraj G, Arif, M, & Shah, H. (2002). On-farm' seed priming with zinc sulphate solution-A cost-effective way to increase the maize yields of resource-poor farmers. Field Crops Research, 102,119–127.

Ho, W.K., Chai, H.H., Kendabie, P., Ahmad, N.S., Jani, J., Massawe, F., Kilian, A., & Mayes, S. (2017). Integrating genetic maps in Bambara groundnut [*Vigna subterranea* (L.) Verdc.] and their syntenic relation- ships among closely related legumes. BMC Genome, (18) 1,192.

Ijarotimi, O.S., & Esho, T.R. (2009). Comparison of nutritional composition and anti-nutrient status of fermented, germinated, and roasted Bambara groundnut seeds (*Vigna subterranea*). British Food Journal ,111(4),376–386.

Iqbal, A., Khalil, I.A., Ateeq, N., & Khan, M.S. (2005). Nutritional quality of important food legumes. Food Chemistry, 97 (2), 331–335.

Jardin, P.D. (2015). Plant biostimulants: definition, concept, main categories and regulation. Scientia Horticulturae, 196, 3–14.

Kaptso, G. K., Njintang, N. Y., Nguemtchouin, M. G. M., Amungwa, A. F., Scher, J., & Hounhouigan, J. (2016). Characterization of morphology and structural and thermal properties of legume Flours: Cowpea (*Vigna unguiculata* L. Walp) and Bambara groundnut (*Vigna subterranea* L. Verdc.) varieties. International Journal of Food Engineering, 12,139–152.

Kaptso, G.K., Njintang, N.Y., Hounhouigan, J.D., Scher, J. & Mbofung, C.M.F. (2007). Production of Bambara groundnut (*Vigna subterranean*) flour for use in the preparation of koki (a steamed cooked paste): effect of pH and salt concentration on the physicochemical properties of flour. International Journal of Food Engineering, 3, (5).

Kaptso, K., Njintang, Y., Nguemtchouin, M., Scher, J., Hounhouigan, J., & Mbofung, C. (2014). Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: Bambara groundnut (*Vigna subterranea*). Journal of Food Science and Technology, 52,4915–4924.

Khalid, S., Malik, A.U., Khan, A.S., & Jamil, A. (2012). Influence of exogenous applications of plant growth regulators on fruit quality of young 'Kinnow' mandarin (*Citrus nobilis*) trees. International Journal of Agriculture and Biology, 14,229–234.

Khan, N.A., & Samiullah, M. (2003). Comparative effect of modes of gibberellic acid application on photosynthetic biomass distribution and productivity of rapeseed-mustard. Physiology and Molecular Biology of Plants, 9,141–145.

Khan, S., Basra, S. M. A., Nawaz, M., Hussain, I., & Foidl, N. (2020). Combined application of Moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.). South African Journal of Botany, 129,74–81.

Khan, S., Basra, S.M.A., Afzal, I., & Wahid, A. (2017a). Screening of Moringa landraces for leaf extract as biostimulant in wheat. International Journal of Agriculture and Biology, 19, 999–1006.

Khan, S., Basra, S.M.A., Afzal, I., Nawaz, M., & Rehman, H.U. (2017b). Growth promoting potential of fresh and stored *Moringa oleifera* leaf extracts in improving seedling vigor, growth and productivity of wheat crop. Environmental Science and Pollution Research, 24,27601–27612.

Khan, W., Rayirath, U.P., Subramanian, Sowmya-Lakshmi, Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J. & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth: Review. Journal of Plant Growth Regulation, 28, 386–399.

Kocira, A., Kornas, R., & Kocira, S. (2013). Effect Assessment Of Kelpak® SL on the Bean Yield (*Phaseolus vulgaris* L.). Journal of Central European Agriculture, 14(2),67–76.

Kocira, S., Szparaga, A., Kocira, A., Czerwińska, E., Wójtowicz, A., Bronowicka-Mielniczuk, U., & Findura, P. (2018). Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. Frontiers in plant science, 9, 388.

Koné, M., Paice, A. G., & Touré, Y. (2011). Bambara Groundnut [*Vigna subterranea* (L.) Verdc: (Fabaceae)] Usage in Human Health. Nuts and Seeds in Health and Disease Prevention. Academic press, 189–196.

Kowalczyk, K., & Zielony, T. (2008). Effect of amino plant and asahi on yield and quality of lettuce grown on rockwool. Proc. Conf. of Biostimulators in Modern Agriculture,7–8.

Latif, H. H., & Mohamed, H. I. (2016). South African Journal of Botany Exogenous applications of Moringa leaf extract effect on retrotransposon, ultrastructural and biochemical contents of common bean plants under environmental stresses. South African Journal of Botany, 106 ,221–231.

Linnemann, A.R., & Azam-Ali, S.N. (1993). Bambara groundnut (*Vigna subterranea* L. Verdc). In: Williams JT (ed.) Underutilised Crops Series. II. Vegetable and Pulses. Chapman and Hall, London, UK. 13–58.

Liu, F., & Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. Scientia Horticulturae, 102,15–27.

Lötze, E. & Hoffman, E.W. (2016). Nutrient composition and content of various biological active compounds of three South African-based commercial seaweed biostimulants. Journal of Applied Phycology 28,1379–1386.

Mabhaudhi, T., & Modi, A. T. (2013). Growth, phenological and yield responses of a bambara groundnut (*Vigna subterranea* (L.) Verdc.) landrace to imposed water stress under field conditions. South African Journal of Plant and Soil, 30(2),69–79.

Mabhaudhi, T., Modi, A.T., Beletse, Y.G. (2013). Growth, Phenological and Yield Responses of a Bambara Groundnut (*Vigna subterranea* l. Verdc) Landrace to Imposed Water Stress: II. Rain Shelter Conditions. Water South Africa, 39.

Mabhaudhi, T. & Modi, A.T. (2014). Intercropping Taro and Bambara Groundnut. Sustainable Agriculture Reviews, 13.

Mancuso, S., Azzarello, E., Mugnai, S., & Briand, X. (2006). Marine bioactive substances (IPA extract) improve foliar ion uptake and water stress tolerance in potted *Vitis vinifera* plants. Advances in Horticultural Science, 20,156–161.

Masindeni, D.R. (2006). Evaluation of Bambara groundnut (*Vigna subterranea*) for yield stability and yield related characteristics (Doctoral dissertation, University of the Free State).

Massawe, F. J., Mwale, S. S., Azam-Ali, S. N., & Roberts, J. A. (2005). Breeding in Bambara groundnut (*Vigna subterranea* (L.) Verdc.): Strategic considerations. African Journal of Biotechnology, 4(6), 463–471.

Matysiak, K., Kaczmarek, S., & Kierzek, R. (2012). Effect of algae *Ecklonia maxima* (Kelpak® SL) on winter oilseed rape. Oilseed Crops, 33(1),81–88.

Matysiak, K., Kaczmarek, S., & Krawczyk, R. (2011). Influence of seaweed extracts and mixture of humic acid fulvic acids on germination and growth of *Zea mays* L. Acta Scientiarum Polonorum Agricultura, 10,33–45.

Mensink, R.P., Zock, P.L., Kester, A.D.M., & Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. The American Journal of Clinical Nutrition, 77,1146–1155.

Minka, S.R., Bruneteau, M. (2000). Partial chemical composition of Bambara pea [*Vigna subterranea* (L.) Verde]. Food Chemistry, 68,273–276.

Misangu, R. N., Azmio, A., Reuben, S. O. W. M., Kusolwa, P. M., & Mulungu, L. S. (2007). Path coefficient analysis among components of yield in Bambara groundnut (*Vigna subterranea* L. Verdc) landraces under screen house conditions. Journal of Agronomy.

Mkandawire, C. H. (2007). Review of Bambara groundnut (*Vigna subterranea* (L.) verdc.) production in sub-Saharan Africa. Agricultural Journal, 2,464–470.

Mubaiwa, J., Fogliano, V., Chidewe, C., & Linnemann, A.R. (2016). Hard-to-cook phenomenon in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) processing: options to improve its role in providing food security. Food Reviews International, 33, 167–194.

Mune, M.A.M., Minka, S.R., Mbome, I.L., & Etoa, F.X. (2011). Nutritional potential of Bambara bean protein concentrate. Pakistan Journal of Nutrition, 10,112–119.

Mwale, S.S., Azam-Ali, S.N. & Massawe, F.J. (2007). Growth and development of Bambara groundnut (*Vigna subterranea*) in response to soil moisture I. Dry mater and yield. European Journal of Agronomy, 26,345–353.

Nasir, M., Khan, A.S., Basra, S.M.A., & Malik, A.U. (2016). Foliar application of Moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow' mandarin. Scientia Horticulturae, 210, 227–235

Nawaz, H., Yasmeen, A., Anjum, M.A., & Hussain, N. (2016). Exogenous Application of Growth Enhancers Mitigate Water Stress in Wheat by Antioxidant Elevation. Frontiers in Plant Science,7.

Nnam, N. M. (2001). Comparison of the protein nutritional value of food blends based on sorghum, Bambara groundnut and sweet potatoes. International Journal of Food Sciences and Nutrition, 52,25–29.

Nti, C. A. (2009). Original article Effects of bambara groundnut (*Vigna subterranea*) variety and processing on the quality and consumer appeal for its products. International of Food Science and Technology, 44, 2234–2242.

Onweluzo, J. C., Ramesh, H. P., & Tharanathan, R. N. (2002). Characterization of free sugars and xyloglucan-type polysaccharides of two tropical legumes. Carbohydrates and Polymers, 47,253–257.

Ouedraogo, M., Ouedraogo, J. T., Tignere, J. B., Balma, D., Dabire, C. B., & Konate, G. (2008). Characterisation and evaluation of accessions of Bambara groundnuts (*Vigna subterranea* (L.) Verdc) from Burkina Faso. Science and Nature Journal, 5, 191–197.

Oyeyinka, A.S., Tijani, T.S., Oyeyinka, A.T., Arise, A.K., Balogun, M.A., Kolawo, F.L, Obalowu, M.A., & Joseph, J.K. (2018). Value added snacks produced from Bambara groundnut (*Vigna subterranea*) paste or flour. LWT - Food Science and Technology, 88,126–131.

Oyeyinka, A.T., Pillay, K., Tesfay, S., & Siwela. (2017). Physical, nutritional and antioxidant properties of Zimbabwean Bambara groundnut and effects of processing methods on their chemical properties. International Journal of Food Science and Technology, 52(10),2238–2247.

Oyeyinka, S.A., Singh, S., Adebola, P.O., Gerrano, A.S., & Amonsou, E.O. (2015). Physicochemical properties of starches with variable amylose contents extracted from Bambara groundnut genotypes. Carbohydrate Polymers, 133,171–178.

Peksen, E. (2007). Non-destructive leaf area estimation model for faba bean (*Vicia faba* L.). Scientia Horticulturae, 113(4),322–328.

Rady, M.A., Varma, B.C., & Howladar, S.M. (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of pre-soaking in *Moringa oleifera* leaf extract. Scientia Horticulturae, 162, 63–70.

Rathore, S. S., Chaudhary, D. R., Boricha, G. N., & Ghosh, A. (2009). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. South African Journal of Botany, 75(2),351–355.

Saleem, B. A., Malik, A. U., Pervez, M. A., Khan, A. S. & Khan, M. N. (2008). Spring Application of Growth Regulators Affects Fruit Quality Of 'Blood Red' Sweet Orange. Pakistan Journal of Botany, 40(3),1013–1023.

Shegro, A.W., van Rensburg, J., & Adebola, P. O. (2013). Assessment of genetic variability in Bambara groundnut (*Vigna subterrenea* L. Verdc.) using morphological quantitative traits. Academia journal of agricultural research,1(3),45–51.

Stirk, W. A., & van Staden, J. (2006). Seaweed products as biostimulants in agriculture. World seaweed resources [DVD-ROM]: ETI Information Services Lts, Univ. Amesterdam. ISBN, 9075000, 80–4.

Stirk, W. A., Novák, O., Hradecká, V., Pěnčík, A., Rolčík, J., Strnad, M., & Van Staden, J. (2009). Endogenous cytokinins, auxins and abscisic acid in *Ulva fasciata* (Chlorophyta) and *Dictyota humifusa* (Phaeophyta): towards understanding their biosynthesis and homoeostasis. European Journal of Phycology, 44(2),231–240.

Swanevelder, C.J., (1998). Bambara – food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development. Sinauer Associates, Inc., Sunderland, MA.

Takei, K.; Takahashi, T.; Sugiyama, T.; Yamaya, T., & Sakakibara, H. (2002). Multiple routes communicating nitrogen availability from roots to shoots: A signal transduction pathway mediated by cytokinin. Journal of Experimental Botany, 53,971–977.

Taylor, I. E. P. & Wilkinson, A. J. (1977). The occurrence of gibberellins and gibberellins like substance in algae. Journal of Applied Phycology, 16,37–42.

Teixeira, A. I., Ribeiro, L. F., Rezende, S. T., Barros, E. G., & Moreira, M. A. (2012). Development of a method to quantify sucrose in soybean grains. Food Chemistry, 130(4), 1134–1136.

Trevisan, S., Francioso, O., Quaggiotti, S. & Nardi, S. (2010). Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signaling & Behavior, 5,635–43.

Turan, M., & Köse, C. (2004). Seaweed extracts improve copper uptake of grapevine. Acta Agriculturae Scandinavica B. soil and plant Science, 54, 213–220.

Unigwe, A.E., Gerrano, A.S., Adebola, P., & Pillay, M. (2016). Morphological Variation in Selected Accessions of Bambara Groundnut (*Vigna subterranea* L. Verdc) in South Africa. Journal of Agricultural Science, 8(11), 69.

Unigwe, A. E., Doria, E., Adebola, P, Gerrano, A.S., & Pillay, M. (2018). Anti-nutrient analysis of 30 Bambara groundnut (*Vigna subterranea*) accessions in South Africa, Journal of Crop Improvement. Taylor & Francis, 32(2), 208–224.

Van Staden, J., Upfold, S. J., & Drewes, F. E. (1994). Effect of seaweed concentrate on growth and development of the marigold (*Tagetes patula*). Journal of applied phycology, 6(4), 427–428.

Wanders, A.J., Brouwer, I.A., Siebelink, E., & Katan, M.B. (2010). Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. PLoS One, 5, e9000.

Yao, D.N., Kouassi, K.N., Erba, D., Scazzina, F., Pellegrini, N., & Casiraghi, M.C. (2015). Nutritive evaluation of the Bambara groundnut Ci12 landrace [*Vigna subterranea* (L.) verdc. (Fabaceae)] Produced in cote d'Ivoire. International Journal of Molecular Sciences,16, 21428–21441.

Yasmeen, A., Basra, S. M. A., Ahmad, R., & Wahid, A. (2012). Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. Chilean Journal of Agricultural Research, 72, 92–97.

Yasmeen, A., Basra, S.M.A., Farooq, M., Rehman, H., Hussain, N., & Athar, H.R. (2013). Exogenous application of Moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. Plant Growth Regulation, 69,225–233.

Zhang, X., Ervin, E. H., & Schmidt, R. E. (2003). Physiological effects of liquid applications of a seaweed extract and a humic acid on creeping bent grass. Journal of the American Society for Horticultural Science, 128(4), 492–496.

Zouari, I., Mechri, B., Attia, F., Cheraief, I., & Mguidiche, A. (2020). Mineral and carbohydrates changes in leaves and roots of olive trees receiving biostimulants and foliar fertilisers. South African Journal of Botany, 135, 18–28.

Zulfiqar, F., Casadesús, A., Brockman, H., & Munnébosch, S. (2020). Plant Science An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on Moringa leaf extracts. Plant Science, 295, 110–194.

CHAPTER 4: SYNOPSIS

In this study, the effects of biostimulants (humic substance, Kelpak® and Moringa leaf extract) on the growth, yield, and nutritional content of Bambara groundnut was investigated. The experimental results showed statistically significant differences between the treatments regarding Bambara groundnut growth, yield, and nutritional composition. The plants treated with the humic substance and Moringa leaf extract produced the highest number of pods per plant, 100-seed weight, total seed yield, and harvest index percentage. Kelpak® had the highest above-ground yield (leaf area, shoot dry matter and plant height) but lowest seed yield of the three biostimulants. The results of the regression analysis revealed that under fertiliser and moringa leaf extract treatments, the combination of all the predictor variables significantly influenced seed yield but individually none of the variables had a significant effect on seed yield. Under the control and Kelpak® treatments, the variation in Bambara groundnut seed yield was found to be largely explained by the number of pods per plant. Moreover, under treatment with humic substance the variation in seed yield was largely explained by the harvest index.

Our results also showed that the different treatments had a significant influence on fatty acids, amino acids, and sugars of Bambara groundnut seeds. The Moringa leaf extract treatment had a significantly higher myristic acid content than both the fertiliser and humic substance for both landraces. The fertiliser had the lowest myristic acid content compared to all the treatments in both landraces. The Moringa had the lowest linoleic acid concentration than all other treatments. The fertiliser and humic substance treatments had a significantly higher α-linolenic acid content than all other treatments in BG1. Whereas, fertiliser, Kelpak®, and humic substance treatments had a significantly higher α-linolenic acid content than all other treatments in BG2. Our study revealed that BG2 had a significantly higher α-linolenic acid concentration than BG1, whereas BG1 had a significantly higher Docosadienoic acid concentration than BG2. The results further showed that the effect of treatment did not influence pentadecylic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, arachidic acid, behenic acid, erucic acid, docosadienoic acid, and lignoceric acid concentrations.

Moreover, no significant differences were found on myristic acid, pentadecylic acid, palmitic acid, palmitoleic acid, margaric acid, oleic acid, linoleic acid, arachidic acid, behenic acid, erucic acid, and lignoceric acid concentrations between the landraces. The three biostimulants resulted in a significantly higher leucine concentration than the control and fertiliser treatments for BG1. Moringa leaf extract had a significantly higher leucine concentration followed by fertiliser and humic substance in BG2. The control, Kelpak®, Moringa leaf extract had a significantly higher glycine concentration in BG1 compared to the fertiliser and humic substance treatments. In BG2, fertiliser and moringa leaf extract had a significantly higher glycine concentrations relative to the other treatments. In BG1, the control and humic substance had a relatively higher lysine to the other treatments. The fertiliser treatment had an extremely high lysine concentration in BG2 compared to other treatments. The control and Moringa leaf extract treatments had a higher histidine concentration in BG1. In BG2, the fertiliser had a significantly higher histidine content than all other treatments. Whereas the Moringa leaf extract treatment had the highest tryptophan concentration which was significantly different from humic substance and fertiliser treatments, respectively. Furthermore, our results showed that BG2 had a significantly higher methionine, threonine, phenylalanine, lysine, histidine, and tyrosine concentrations than BG1. There were no significant differences between the amino acid concentrations of alanine, valine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, cysteine, glutamic acid, and tyrosine across the treatments. Moreover, no significant differences were found between the landraces with regard alanine, glycine, valine, leucine, isoleucine, proline, serine, aspartic acid, cysteine, glutamic acid, and tryptophan concentrations.

The major sugars D-fructose, D-glucose, mannitol, sorbitol, myo-inositol, and sucrose were found in all the samples. Sucrose was the dominant sugar in all our samples. Our results showed that fertiliser and humic substance had the highest sucrose concentrations for both landraces, which was significantly higher than Moringa leaf extract, control, and Kelpak®, respectively. Our results also showed that there was a significant difference in seed sugar content of the two Bambara groundnut landraces with regards to mannitol and sorbitol. BG1 had a significantly higher mannitol, and sorbitol concentrations compared to BG2. Our results further showed that there were no significant differences in D-fructose, D-glucose, mannitol, sorbitol, and myo-inositol concentrations across the treatments. Moreover, no significant differences were found between the landraces with regards to D-fructose, D-glucose, myo-inositol, and sucrose concentrations.

The results revealed that the phosphorus (P) level for the Kelpak® treatment was significantly higher than Moringa and humic substance treatments, respectively, but comparable with the other treatments including the initial soil sample. All the treatments had a significantly higher calcium (Ca) levels when compared to the initial soil sample, whereas, the Kelpak had a significantly higher Ca levels than all treatments, except the control. The copper (Cu) levels from all the treatments were significantly higher than the initial soil but no significant differences were found between the treatments. The control had a significantly higher iron (Fe) level than the fertiliser. The initial soil sample had a significantly lower carbon (C) percentage than all treatments except the control and humic substance, and no significant differences were observed between the treatments. Finally, our results showed that there were no significant differences on soil chemical nutrients with regard to N, P, K, Na, Zn, Mn, and S across all treatments.

As a future study it would be worth looking at the effect of these biostimulants on Bambara groundnut at a field level. Moreover, only one concentration level (manufacturers' protocols) was used in the current study, three or four levels of concentration could help to give a better view of the biostimulants mode of action. Due to the induced increase in growth and yield parameters of Bambara groundnut plants by biostimulant application it can be concluded that biostimulants (humic substance and Moringa leaf extract) has the potential to be used as a cheap source of plant growth hormones and nutrient elements to help improve the growth and development of Bambara groundnut plants.

4.1. Limitations

This study was conducted only two landraces. In a larger study conducted by a current PhD student in our research group, at least 42 landraces were identified as occurring in South Africa (contrary to DAFF's seven landraces). The two landraces in this study were the most abundant seeds collected by the farmers. From these results, we will further analyse the nutrition profile of the additional landraces, which was beyond the scope of the MSc.