PHYSIOLOGICAL AND BIOCHEMICAL EFFECT OF BIOSTIMULANTS ON *ABELMOSCHUS ESCULENTUS* (L.) AND C*LEOME GYNANDRA* (L.)

By

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Submitted in fulfilment of the requirement for the degree of Master of Science (Agriculture), Horticultural Science

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Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science, Higher Degrees Office for examination by the University appointed Examiners.

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Conference Contributions from this Thesis

1. Gugulethu Makhaye, Stephen O. Amoo, Abe S. Gerrano, Adeyemi O. Aremu., Samson Tesfay. Effect of biostimulants on germination of okra (*Abelmoschus esculentus* L.) genotypes. Combined Congress 2020. 20 - 23 January 2020, University of the Free State, Bloemfontein, South Africa.

Publications from this Thesis

1. Gugulethu Makhaye, Motiki M Mofokeng, Samson Tesfay, Adeyemi O. Aremu, Johannes Van Staden, Stephen O. Amoo. 2021. Influence of plant biostimulant application on seed germination. *In:* Biostimulants for crops from seed germination to plant development. Shubhpriya Gupta and Johannes Van Staden (eds). Elsevier. (Accepted).

Potential Publications from this Thesis

1. Effects of biostimulants on the germination of *Abelmoschus esculentus* and *Cleome gynandra* genotypes.

2. Effects of biostimulants on the growth, yield, biochemical and mineral elements content on *Abelmoschus esculentus* genotypes.

3. Effects of biostimulants on growth, yield, biochemical and mineral elements content of *Cleome gynandra* genotypes.

Acknowledgements

I would like to express my appreciation and gratitude to:

- My supervisor, Prof S. Tesfay, for providing institutional support and guidance.
- My co-supervisor, Prof S.O Amoo, for his guidance and support throughout the completion of this study.
- My co-supervisor, Prof A.O. Aremu, for constructive criticism, encouragement and support throughout the thesis write-up.
- Agricultural Research Council-VOPI staff for their support and assistance when conducting my experimental work and Miss Liesl Morey from ARC-Biometry for assisting with statistical analysis.
- The National Research Foundation (grant UID: 114065), AgriSeta and University of KwaZulu-Natal scholarship for financial support.
- My mom and late dad, for their support and prayers and my son for being a constant source of my motivation.
- I thank God for his grace, strength, blessings and love.

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List of Abbreviations

ABA	Abscisic acid
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
As	Arsenic
ATP	Adenosine triphosphate
Ca	Calcium
Cu	Copper
CVG	Coefficient of velocity of germination
EC	Electrical conductivity
Fe	Iron
FGP	Final germination percentage
GA	Gibberellic acid
GI	Germination Index
GRI	Germination rate index
HCl	Hydrochloric acid
HM	Humic material
HPLC	High-performance liquid chromatography
HS	Humic substances
IAA	Indole-3-acetic acid
ICP-OES	Inductively coupled plasma - optical emission spectrometry
K	Potassium
LSD	Least significant difference
Mg	Magnesium
MGT	Mean germination time

N	Nitrogen
Na	Sodium
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
Pb	Lead
PGPR	Plant growth promoting rhizobacteria
TCA	Tricarboxylic acid
TFC	Total flavonoid content
TPC	Total phenolic content
TSG	Time spread of germination
VOC	Volatile organic compounds
Zn	Zinc

Abstract

Abelmoschus esculentus (L.) and *Cleome gynandra* (L.) are neglected plants, often collected from the wild, with dual benefits of nutritional and medicinal values, especially in rural communities. Biostimulants are well-known for their stimulatory effect on plant physiological processes, from germination to full maturity. In the current study, the effect of biostimulant application was investigated on the germination, growth, yield and biochemical quality of selected *A. esculentus* and *C. gynandra* genotypes, as a tool for improving their physiological and biochemical aspects. The study involved two biostimulants [Kelpak® (1:100, 1:40 and 1:20, dilutions)] and plant growth promoting rhizobacteria = PGPR (1:5, 1:10 and 1:15, dilutions)] as well as their interaction effect on the different genotypes of *A. esculentus* (Okra PB1, PB2, PB3, PB4 and PB5) and *C. gynandra* (TOT10212, TOT8420, *Cleome* 3, *Cleome* Maseno and *Cleome* Arusha). The parameters evaluated were seed germination, vegetative growth, yield, biochemical (β-carotene, vitamin C, total phenolic, flavonoids and condensed tannins) and mineral elements content (Ca, Fe, K, Mg, Na and Zn).

Germination of *A. esculentus* and *C. gynandra* was influenced by different genotypes and biostimulants. Okra PB2 and Okra PB4 had significantly enhanced Final Germination Percentage (FGP), Germination index (GI) and Germination Rate Index (GRI). Similarly, genotype TOT10212 had significantly increased FGP, GI and GRI while *Cleome* 3 had least FGP, GI, GRI and Coefficient of Velocity of Germination (CVG). The effect of Kelpak® treatments on FGP, GI, Mean Germination Time (MGT) and GRI was significantly comparable to that of control. The effect of PGPR treatments on FGP, GI and GRI significantly increased with increasing PGPR dilutions. In *A. esculentus*, the interaction of Kelpak® (1:100) and genotype OkraPB1 significantly improved germination parameters (FGP, GI and GRI) while no stimulatory effect was observed on the interaction of biostimulants and Okra PB2, PB3, PB4 and PB5. In *C. gynandra*, the biostimulants especially PGPR (1:5, 1:10 and 1:15), inhibited germination parameters (FGP, GI and GRI) of genotype TOT10212.

A. esculentus genotypes showed different growth parameters. For instance, Okra PB5 had significantly higher plant height while Okra PB4 had least plant height. Biostimulants further influenced the vegetative growth and yield of *A. esculentus* and *C. gynandra* genotypes. Plant height, chlorophyll content and stem diameter of *A. esculentus* genotypes was significantly enhanced by PGPR (1:5, 1:10 and 1:15) application. The yield (number of pods, total fresh weight and total dry weight) of *A. esculentus* was enhanced by PGPR (1:5, 1:10 and 1:15) application. Plant growth promoting rhizobacteria (1:5, and 1:10) enhanced the chlorophyll content, stem diameter and yield (total fresh and total dry weight of leaves) of *C. gynandra* genotypes. No inhibitory effect was observed on the growth and yield of *A. esculentus* and *C. gynandra* genotypes

following biostimulant treatments. Interaction of biostimulants with *A. esculentus* and *C. gynandra* genotypes had no significant effect on growth and yield parameters.

The biochemical and mineral elements content of *A. esculentus* and *C. gynandra* genotypes was influenced by genotype and biostimulant (both Kelpak® and PGPR dilutions) application. In *C. gynandra*, biostimulants enhanced the β-carotene, total flavonoid and total phenolic content. Okra PB4 had significantly enhanced vitamin C and total phenolic content while Okra PB5 had significantly higher total flavonoid content. Genotype TOT10212 had significantly increased Ca, Fe, Mg and Na content. However, the content of condensed tannins together with Fe and Mg of *C. gynandra* genotypes was inhibited by biostimulants application. Application of PGPR-1:5, Kelpak®-1:40 and Kelpak®-1:20 significantly enhanced total phenolic, total flavonoid and condensed tannins of *A. esculentus* genotypes. Furthermore, biostimulants had varying effects on the mineral element content. A significant increase was observed on Fe content when *A. esculentus* genotypes were treated PGPR (1:10). Application of Kelpak® (1:100 and 1:40) caused a significant decrease on the Ca content of *A. esculentus* genotypes. The interaction effect of biostimulants application and genotypes significantly inhibited the mineral elements of *C. gynandra* genotypes while significantly enhancing the vitamin C and condensed tannins of Okra PB3.

The current study demonstrated the differential effect of biostimulants application (Kelpak® and PGPR) on *A. esculentus* and *C. gynandra* genotypes. The application of biostimulants can therefore, be used to enhance germination, growth, yield, biochemical content and mineral elements, depending on the crop genotype, and hence assist in combatting food insecurity in food insecure communities.

Chapter 1: General introduction

1.1. Background

In developing countries, approximately 805 million people are undernourished, in addition to an estimated 60% childhood deaths attributed to malnutrition (Fawole et al., 2015). During the period 1990 to 2014, hunger in Sub-Saharan Africa has increased at a rate of 9.3% (Fawole et al., 2015, Ilaboya et al., 2012). The major causes for this increase include climate change, increasing population, and poor agricultural sector development leading to insufficient agricultural outputs (Garrity et al., 2010, Fawole et al., 2015).

In Africa, the population growth is estimated to reach 2.4 billion by 2050, which will result in an approximately one in four people subjected to food insecurity and nutritional deficiencies (Garrity et al., 2010, Meerman, 2012, Hall et al., 2017). This has led to an increase in agricultural-related activities in an attempt to address hunger. Agricultural intensification and expansion have played a major role in yield increment and have conversely led to land degradation (Hartemink, 2007, Garrity et al., 2010). In Sub-Saharan Africa, approximately 65% of land used for agricultural production is subjected to land degradation leading to low soil fertility, thus affecting 65% of livelihoods and food production (Garrity et al., 2010, Bot and Benites, 2005).

Soil fertility is the ability of the soil to sustain good agricultural plant production through the provision of essential nutrients while causing the least environmental degradation (Chakraborty and Mistri, 2015). Soil fertility has been declining at an alarming rate and has led to various agricultural setbacks, including nutrient depletion, acidification, loss of organic matter, and an increase in toxic elements (Hartemink, 2007). Several strategies have been implemented to mitigate this challenge including the use of manure, inorganic fertilizer, lime, organic materials (compost, mulch, and biostimulants), and inclusion of legumes in the cropping systems (Hartemink, 2007, ITPS, 2015).

1.1. Potential of biostimulants on plant growth

Biostimulants are organic material, other than organic fertilizers, that when applied to the plant, growth media or seeds, positively alter physiological processes of the plant and promote plant growth (Du Jardin, 2015). Biostimulants can affect plants both internally and externally (Roberts et al., 2015). Internally, they promote various biological activities including photosynthesis, nucleic acid synthesis and respiration, antioxidant and chlorophyll production, and increased metabolism (Sharma et al., 2013). Externally, they interact with the environment by promoting soil microbial activity and soil enzymes through the promotion of phytohormones activity (Duan-yin et al., 2014). Furthermore, some

biostimulants promote growth of endophytic and non-endophytic organisms that interact with phytohormones (Brown and Saa, 2015). Thus, biostimulants can increase yield and enhance quality, promote plant tolerance to and recovery from abiotic stress, promote nutrient assimilation, translocation, and use, and promote efficient water use (Calvo et al., 2014, Bulgari et al., 2019). Biostimulants promote plant growth and development in all growth stages of plant's life cycle, from germination to full maturity (Calvo et al., 2014).

1.2. Underutilized multipurpose plants

Plants have, for centuries, been consumed for nutritional purposes and used for primary healthcare in Africa. Some of these plants are multipurpose, and are mainly used as medicine and food security crops. Two examples of such plants are described below.

1.2.1. *Cleome gynandra* L.

Cleome gynandra L. is an erect annual plant that originated from tropical Africa and South-East Asia and belongs to the Cleomaceae family (Kiebre et al., 2015, Omondi et al., 2017). *Cleome gynandra* is one of the most important and common leafy vegetables in Africa because of its natural, voluntary occurrences (Kwarteng et al., 2018) and nutritional content. The plant serves as a dietary supplement during the dry season, providing health benefits to the rural communities where nutrient deficiencies are a common occurrence (Kiebre et al., 2015, Kwarteng et al., 2018). As a leafy vegetable, *C. gynandra* is predominantly high in vitamin A, iron, and iodine (Kujeke et al., 2017) while as a medicinal plant, it is rich in bioactive secondary metabolites including glucosinolates and flavonoids (Omondi et al., 2017).

1.2.2. *Abelmoschus esculentus* (L.) Moench

Abelmoschus esculentus (L.) Moench is a warm-season flowering plant belonging to the Malvaceae family which originated from Africa (DAFF, 2012, Poorva and Sunita, 2017). In international markets, *A. esculentus* plays a role as a food security crop and for its medicinal value (Tian et al., 2015). It is a rich source of carbohydrates, fats, proteins, vitamins, and minerals, all of which make it a valuable crop for combatting human nutrient deficiencies (Adekiya et al., 2017). Furthermore, *A. esculentus* is highly valued in pharmaceutical industries for its high biopolymers and bioactive compounds including β-carotene, pectins, carotenoids, and flavonoids (Kumar et al., 2018, Petropoulos et al., 2018).

1.3. Problem statement

The increasing world population is expected to impose a 70% increase in global demand for agricultural production (FAO., 2011, FAO, 2017). With agricultural expansion and intensification being potential tools for combatting world hunger, a further decline in soil fertility is inevitable (FAO., 2011). Various factors contribute to decreasing soil fertility, including climate change and anthropogenic activities such as production and use of inorganic fertilizers (Smith et al., 2016). During the cultivation of multipurpose plants, the use of inorganic fertilisers is often employed.

Multipurpose plants play a vital role in various communities as they ensure food security and also serve as medicine. About 80% of the population in developing countries depend on traditional medicine for primary healthcare (Jamshidi-Kia et al., 2018). In Ethiopia, *A. esculentus* is known as a perfect villagers crop because of its contribution in rural communities, serving as food and holding pharmaceutical value (Kumar et al., 2018, Gemede, 2015). On the other hand, *C. gynandra* has played an important role over the years in rural communities as evident in the Ayurvedic pharmacopeia of India indicating that the consumption of *C. gynandra* date back to 3 000 years (Seethapathy et al., 2019). However, more research on *C. gynandra* including crop improvement, out-of-season cultivation, and fertilizer regimes to ensure successful cultivation of this plant remains essential (Chweya and Mnzava, 1997, Motsa et al., 2015). In Africa, these two plants remain amongst the most consumed vegetables in rural communities while being undervalued in urban communities (Mokganya and Tshisikhawe, 2019, Chagomoka et al., 2015).

However, climate change and declining soil fertility resulting from modern agricultural expansion and intensification makes the domestication of multipurpose plants a challenge (El-Naggar et al., 2019). This, therefore, heightens the need for improved cultivation techniques of multipurpose plants, including *A. esculentus* and *C. gynandra*. However, there is insufficient information on cultivation inputs with the potential to positively affect the physiology and biochemistry of these plants and with minimum negative impact on the environment.

1.4. Aim and objectives

This study aims to determine the physiological and biochemical effects of two biostimulants [Kelpak® (KLP) and plant growth promoting rhizobacteria (PGPR)] on the cultivation of *Abelmoschus esculentus* and *Cleome gynandra*.

The objectives of this study are to:

- Determine the effect of the biostimulants on the germination, seedling establishment, growth and yield of *A. esculentus* and *C. gynandra* genotypes.

• Assess the effect of the biostimulants on the biochemical and mineral elements content of *A. esculentus* and *C. gynandra* genotypes.

1.5. Research questions

The current research is guided by the following questions:

• What are the effect of biostimulants on seed germination, seedling establishment, seedling growth and yield of *A. esculentus* and *C. gynandra*?

• How does the application of biostimulants affect the biochemical and mineral elements content of *A. esculentus* and *C. gynandra*?

1.6. Hypothesis

Biostimulant application will not improve the germination rate, growth, yield, biochemical content and mineral elements of *A. esculentus* and *C. gynandra* genotypes.

1.7. Overview of chapters in this thesis

Chapter 1 provides the background, problem statement, aim and objectives, and research questions of the current study.

Chapter 2 entails a critical appraisal of the nutritional and pharmacological potential of the two selected multipurpose plants (*Abelmoschus esculentus* and *Cleome gynandra*). In addition, the chapter provides a detailed overview on the potential of biostimulants on crop production (seed germination, plant growth, yield, biochemical and mineral elements content).

Chapter 3 presents an evaluation of the effect of biostimulant application on the germination parameters of *Abelmoschus esculentus* and *Cleome gynandra* genotypes.

Chapter 4 focusses on the physiological (growth and yield) influence of biostimulant application on *A. esculentus* and *C. gynandra* genotypes.

Chapter 5 details the effect of biostimulants on the phytochemical and nutritional value of *A. esculentus* and *C. gynandra* genotypes.

Chapter 6 presents a summary of the main findings of the study.

The section 'References' is a list of all the literature cited in this thesis.

Chapter 2: Literature review

2.1. Introduction

Globally, soil fertility has been declining at an alarming rate, which poses a challenge in agricultural production (Hartemink, 2007). Various strategies have been employed to address soil infertility, and the use of chemical fertilizers has proven to be beneficial in maximizing the yield. Even though chemical fertilizers increase agricultural production, and enhance the nutritional and biochemical content in plants, their indiscriminate use deteriorates the environment over a long period. Biostimulant application can enhance crop production with reduced dependency on chemical fertilizers due to their effect on the physiology and biochemistry of plants. Because of their positive effect on crop production, biostimulants are used by growers to promote plant growth, especially in less fertile soils (Halpern et al., 2015).

In a food-insecure society, the cultivation of multipurpose plants is often neglected because their nutritional and pharmacological potentials are poorly documented. Genus *Abelmoschus* consists of up to approximately 14 species, of which only four are cultivated (Patil et al., 2015b, Werner et al., 2015). *Abelmoschus* species are predominantly annual, biennial, or perennial herbs with often tomentose or hispid trichomes (Yadav et al., 2014). Cultivated species of *Abelmoschus* genus are consumed as food and also explored for their medicinal value. The genus *Cleome* was first described under the family Capparidaceae by Linnaeus in 1753 and was later elevated to the family Cleomaceae by Airy Shaw in 1965 (Riaz and Abid, 2018). However, phylogenetic studies show that Cleomaceae species are closer to Brassicaceae as compared to Capparaceae (Aparadh et al., 2012). *Cleome* genus comprises of over 200 species, generally characterized by glandular pubescent or glabrous herbs lacking spines (Zhang et al., 2018, Castro et al., 2014). This genus is well-documented for its medicinal properties and its value in food security and nutrition. This chapter documents the potential effect of biostimulants on crop production and provides an appraisal of the nutritional and pharmacological potential of the two selected multipurpose plants.

2.2. Approaches to improve soil fertility

Soil is a dynamic living system that is capable of providing many ecosystem services such as water regulation, nutrient cycling, and controlling pests and diseases (Kumar et al., 2018). It is a non-renewable loose material found on the surface of the earth, consisting of both inorganic and organic matter, and microorganisms, that degrades rapidly but extremely slow in the formation and regeneration process (Hartemink, 2007). Soil amendments are often used to assist in the regeneration process, sustaining and increasing the productivity of the soil.

Soil fertility refers to the ability of the soil to sustain good agricultural plant production through the provision of essential nutrients while causing negligible environmental degradation (Chakraborty and Mistri, 2015). Generally, soil fertility is a term used to describe the physical, chemical, and biological properties of the soil (Voltr, 2012). In a fertile soil, the biological parameters (soil organisms) effectively turn organic matter and nutrients to plant yields, protect plants from biotic stress, build-up organic material, and improve the physical properties of soils (ORC, 2016). Soil is considered fertile when it yields healthy crops over a long period with minimal inputs especially fertilizers (ORC, 2016).

Soil infertility negatively affects the physical and chemical properties of soil, which is coupled with a decrease in soil organic matter, pH, available plant nutrients, and cation exchange capacity (CEC) (Hartemink, 2007). This infertility further includes a decrease in soil available nutrients, nutrient mining, and acidification (caused by an increase in exchangeable Al, Mn) (Hartemink, 2007). Soil fertility is important in maintaining agricultural homeostasis. Soil degradation (due to industrialization and intensive agricultural practice) is a major contributor to soil fertility decline along with salinization, desertification, erosion, poor organic matter management, overgrazing, and continuous cultivation (Yebo, 2015, El-Naggar et al., 2019). In a continually cultivated and unsustained soil, an average of 22 kg of nitrogen, 2.5 kg of phosphorus, and 15 kg of potassium are lost per hectare per season (Agwe et al., 2007). This, therefore, raises the need for soil fertility management.

Soil fertility management refers to the application of the knowledge of agricultural practices, which focus on maximizing nutrient use efficiency to increase agricultural production (El-Naggar et al., 2019). Furthermore, soil fertility management combines technologies and strategies that preserve soil quality while promoting its productivity (Nguemezi et al., 2020, Sanginga and Woomer, 2009). This practice includes the use of inorganic fertilizers (pre-plant and top-dressing), application of organic resources coupled with enhancement and maintenance of soil organisms and biological processes over a long period (Krah et al., 2019). Agricultural amendments have been used as a soil fertility management strategy to correct soil infertility and secure food security for humanity (Hue and Silva, 2000).

Agricultural amendments refer to any material or substance that when added to the soil improves the physical properties to provide a better environment for plant growth (Davis and Whiting, 2013). Soil amendments can either be organic or inorganic, and the difference between the two is based on their origin. Inorganic amendments are usually mined or artificial. The major agricultural amendment that is largely used is inorganic fertilizers. However, inorganic fertilizers are not a sustainable measure for the restoration and rehabilitation of the soil in the long-term (El-Naggar et al., 2019). In certain parts of the world, especially in Africa where soils are extremely degraded, the sole use of inorganic fertilizers has proven to be inadequate in improving and sustaining soil fertility even though they provide nutrients that are readily available to plants (García-Carmona et al., 2020, Stewart et al., 2020). The use of inorganic fertilizers does not only improve crop yields but also increases the number of available crop

residues which are useful for organic inputs to the soil (Sanginga and Woomer, 2009). Typical examples of inorganic amendments include but are not limited to perlite, tire chunks, sand, vermiculite, and pea gravel (Davis and Whiting, 2013). However, the continual use of inorganic fertilizers do not sustain the environment but rather deteriorates it. This is because of the observed effects of the leaching of nitrogen (and volatilization) and phosphorus into water bodies, thus causing water contamination and eutrophication (Fairhurst, 2012).

Organic amendments are sourced from materials that originate from living organisms and include sphagnum, wood chips, peat, straw, grass clippings, manure, compost, wood ash, sawdust, and biosolids (Davis and Whiting, 2013). Soil organic amendments increase soil organic matter while providing various benefits to the soil including improving soil aeration, water and nutrient holding capacities, water infiltration, pH and EC, porosity, and biological activity and composition (Stewart-Wade, 2020). Organic amendments are less concentrated, thus, insufficient in providing required nutrient levels because their nutrients are often not readily available and are released slowly into the soil through decomposition and mineralization (Buckwalter and Fake, 2003). These amendments can tie up nitrogen in the soil causing nitrogen deficiency (Davis and Whiting, 2013). Risks associated with organic amendments include poorly made products with unacceptable levels of impurities and contamination (heavy metals, pathogens from livestock manure), and inappropriate matching of a compost product for the intended use (maturity and application timing) (Wealth and Protection, 2018). The need for sustainable and environmentally friendly strategies of agricultural production other than the use of soil amendments remain high. This is because of the limitations that come with both organic and inorganic soil amendments (soil conditioners). However, the use of biostimulants in crop production has recently gained more attention due to their positive effects (sustainable and environmentally friendly).

2.1.1. Plant biostimulants and agricultural production

Biostimulants are substances/micro-organisms other than fertilizers, pesticides, soil conditioners, and phytohormones that when applied to the plant, seed, or growth substance, positively alter the plant's physiological processes to increase growth, mitigate stress-induced limitations and increase the yield (Yakhin et al., 2016, Du Jardin, 2015). Biostimulants are sometimes referred to as plant conditioners, metabolic enhancers, or phytostimulators (Yakhin et al., 2016). These materials are often concoctions of one or more materials such as plant growth promoting rhizobacteria (PGPR), enzymes, seaweed extracts, humic acid and trace elements, micro-organism, and yeast (Abbas, 2013). Biostimulants are derived from complex sources that contain various bioactive compounds that can potentially benefit plants (Nardi et al., 2016, Brown and Saa, 2015). Major groups of biostimulants include beneficial fungi and bacteria, chitosan and other biopolymers, protein hydrolysates, and other N-containing compounds, seaweed extracts and botanicals, and humic and fulvic acids (Du Jardin, 2015).

Biostimulants are widely used by growers throughout the growth cycle of plants (Albrecht, 2017). Biostimulants promote plant growth and development throughout life cycle of the plant, from germination to full physiological maturity (Calvo et al., 2014). Biostimulants can either affect the plant biochemical cascade or stimulate endophytic and non-endophytic fungi, and bacteria to facilitate the production of molecules that will benefit the plant (Brown and Saa, 2015).

These substances and organisms promote plant growth, production of hormones or growth regulators, the activity of rhizosphere microbes and soil enzymes, and biological processes including photosynthesis (Nardi et al., 2016). Biostimulants improve the soil physical-chemical properties, water, and nutrient use holding capacity, lateral root growth and architecture, crop quality, and tolerance to biotic and abiotic stress (Brown and Saa, 2015). These substances further facilitate nutrient assimilation, translocation and use, and quality attributes (including nutrition and sugar content) (Calvo et al., 2014). **Table 2.1** outlines the role of biostimulants on seed germination.

Table 2.1: Effect of different biostimulants on seed germination.

Biostimulant type	Tested biostimulant	Plant species	Tested dose/ Concentration	Method of application	Germination response(s)	References
Plant growth promoting rhizobacteria	*Azospirillum brasilense* Sp7, Sp7-S and Sp245	*Lycopersicon esculentum* L.	100 µL	Soaking and drenching	Increased germination value	(Mangmang et al., 2016)
		Lactuca sativa L.	100 µL	Drenching in filter paper	Increased germination value	(Mangmang et al., 2016)
	Rhizobacteria-PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2	*Oryza sativa* L.	Not specified	Not specified	Germination (%)	(Ashrafuzzaman et al., 2009)
	A. lipoferum, P. fluorescens, and P. putida	*Zea mays* L.	1×10^8 cfu/mL	Inoculated into filter paper	No significant effect on germination (%)	(Agbodjato et al., 2016)
	Pseudomonas fluorescens	*Triticum aestivum*	1×10^8 cfu/mL	Seed coating	Increased germination (%) and germination rate	(Sirohi et al., 2015)
	Panax schinseng	*Lactuca sativa*	1×10^6 cfu/mL	Dipping/ soaking	Decreased germination rate	(Hussein and Joo, 2018)
		Raphanus sativus	1×10^6 cfu/mL	Dipping/ soaking	No effect on germination rate	(Hussein and Joo, 2018)
	Bacillus subtilis	*Sorghum bicolor* L.	$1x10^8$ cfu/mL	Soaking	Increased germination (%)	(Prathibha and Siddalingeshwara, 2013)
	Pseudomonas fluorescence	*Sorghum bicolor* L.	$1x10^8$ cfu/mL	Soaking	Increased germination (%)	(Prathibha and Siddalingeshwara, 2013)

	Brevibacillus brevis	*Gossypium hirsutum*	$1x10^8$ cfu/mL	Not specified	Increased germination (%) and germination speed	(Nehra et al., 2016)
Seaweed extracts	*Ascophyllum nodosum*	*Phaseolus vulgaris*	0.8 mL/L	Soaking	Increased germination (%), speed index of germination and seedling emergence (%)	(Carvalho et al., 2013)
	Rosenvingea intricate	*Abelmoschus esculentus*	10, 20, 30, 40, 50 and 100% (v/v)	Soaking	20 and 30% significantly increased germination (%)	(Thirumaran et al., 2009)
	Sargassum liebmannii	*Solanum lycopersicum* L.	0.2, 0.4 and 1% (v/v)	Soaking	Increased germination (%), germination index, mean germination time and germination energy	(Hernández-Herrera et al., 2013)
	Sargassum liebmannii	*Trigonella foenum-graecum* L.	5, 10 and 15% (v/v)	Soaking	Increased germination (%)	(El-Sheekh et al., 2016)
	Ascophylum nodosum	*Allium cepa*	3500, 6500 and 7500 mg/L	Soaking	All concentrations increased germination (%)	(Hidangmayum and Sharma, 2015)
	Sargassum vulgare	*Phaseolus vulgaris* L.	0.2 and 0.5% (v/v)	Soaking	Concentrations increased germination rate	(Salma et al., 2014)

Humic-substance	Humic acid	*Sesamum indicum* L.	1000 mg/L	Imbibition of seeds	Increased germination index and coefficient of velocity of germination	(Souguir and Hannachi, 2017)
	Humic acid	*Zea mays* L.	100, 200, 300, 400 and 500 mL/kg	Not specified	No significant effect on germination (%)	(Rodrigues et al., 2017)
	Humic acid	*Medicago sativa* L.	0.009 mg/kg	Inoculated on petri-dishes	Increased germination (%) and rate	(Sofi et al., 2018)
	Humic acid	*Borago officinalis*	15 and 30 g/L	Inoculated into petri-dishes	All concentrations increased germination rate and mean germination time	(Ebrahimi and Miri, 2016)
	Humic acid	*Cichorium intybus*	15 and 30 g/L	Inoculated into petri-dishes	Both concentrations had no significant effect on the germination rate	(Ebrahimi and Miri, 2016)
	Humic acid	*Chenopodium album* agg.	15 and 30 g/L	Inoculated into petri-dishes	Both concentrations increased germination (%)	(Šerá and Novák, 2011)
	Humic acid	*Capsicum frutescens* L.	15 and 30 g/L	Inoculated into substrate	Increased germination percentage and mean germination time	(Vieira et al., 2018)
	Humic acid	*Raphanus sativus* L.	15 and 30 g/L	Soaking	Germination (%) was increased	(Pandurangan et al., 2014)
Chitosan	Chitosan	*Carum copticum*	0.05, 0.1, 0.2 and 0.5% (v/v)	Soaking	0.05, 0.01; 0.2 and 0.5 % increased germination (%) while 0.2 and 0.5% increased germination rate	(Mahdavi and Rahimi, 2013),

Chitosan	*Capsicum annuum*	0.001, 0.01 and 0.05% (v/v)	Soaking	All concentrations had no significant effect on germination (%) and they increased mean germination time	(Mahdavi and Rahimi, 2013)

2.1.1.1. Seaweed extracts

Seaweed extracts are extracts of quintessential members of inshore, marine ecosystems which provide shelter and food to numerous marine biota and can even contribute to the modification of physicochemical properties of seawater (Khan et al., 2009). The biochemical content and functional properties of these products are complex and affected by the preparation method (EL Boukhari et al., 2020). In general, because of their potential effects against seasonal stress, the benefits of seaweed extracts are likely to be seasonally and concentration-dependent.

These extracts act as chelators, improving the utilization of mineral nutrients by plants, and improving soil structure and aeration, which may stimulate root growth (EL Boukhari et al., 2020). Seaweed extracts are a rich source of amino acids, bioactive secondary metabolites, vitamins, vitamin precursors, polysaccharides, phytohormones, macro- and microelements (Battacharyya et al., 2015). Bioactive secondary metabolites, vitamins, and vitamin precursors interact synergistically to improve plant growth by various mechanisms. Polysaccharides improve growth, play a role in plant defence against fungal and bacterial pathogens, and are involved in the induction of genes encoding various pathogenesis-related proteins with antimicrobial properties (Battacharyya et al., 2015).

The efficacy of seaweed extracts is dependent on the growth stage of the plant and sometimes the method of application (Du Jardin, 2015). Seaweed extracts can be applied in one of the two ways: soil drenching and foliar application. Seaweed extracts alter physical, biochemical, and biological properties of the soil and may also affect the architecture of plant roots facilitating efficient uptake of nutrients (Calvo et al., 2014) Seaweed extracts are rich sources of phytohormones such as cytokinins, polyamines, indole acetic acid (IAA), gibberellic acid (GA), and abscisic acid (ABA) (EL Boukhari et al., 2020). The presence of phytohormones in seaweed extracts was confirmed using high-pressure liquid chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry (Yakhin et al., 2016). Some phytohormones can improve leaf chlorophyll content and regulate the growth and development of higher plants (Di Mola et al., 2019). Seaweed extracts generally improve plant growth at low concentrations (diluted as 1:1000 or more) and inhibit growth at high concentrations (Hidangmayum and Sharma, 2015). Seaweed extracts can affect plant physiology (Castro et al., 2014) and cause changes to the metabolome of treated plants (Sangha et al., 2014). They may also affect the quality, phytochemistry, and nutritional content of the treated plants (Rathore et al., 2009).

2.1.1.2. Humic substances

Humic substances are end products of chemical and biological transformations of plant and animal matter, and from microbial metabolism that represents a major pool of organic carbon at the earth's surface (Calvo et al., 2014). Humic substances include humic acid (soluble in basic media), fulvic acid

(soluble in both alkali and acidic media), and humins (not extractable from the soil) (Souguir and Hannachi, 2017). These substances are considered to be the most abundant naturally occurring organic molecules on earth and contribute to the regulation of many crucial ecological and environmental processes as they regulate the global carbon and nitrogen cycles, the growth of plants and microorganisms (Canellas et al., 2015). Attempts to use humic substances for promoting plant growth and crop yield show positive results globally. This is because these substances positively contribute to soil fertility, influencing the physical, physicochemical, chemical, and biological properties of the soil (Souguir and Hannachi, 2017). In addition to the regulation of both soil carbon and nitrogen cycling, humic substances further regulate the fate and transport of anthropogenic-derived compounds and heavy metals, and the stabilization of soil structure (Lipczynska-Kochany, 2018). Biostimulant effect of HM has resulted in improved seed germination, root and plant growth development, and are major constituents of organic fertilizers (Rouphael and Colla, 2018).

Humic substances supply nutrients through various mechanisms. These substances chelate minerals and release readily available nutrients through their degradation (Canellas et al., 2015). Humic substances increase the availability of phosphorus by interfering with calcium phosphate precipitation (Nardi et al., 2016). They increase the uptake of both macro- and micronutrients by increasing the cation exchange capacity of the soil containing polyanionic constituents (Lipczynska-Kochany, 2018). The H^+-ATPase activity can be induced by humic material (HM) and can energize secondary ion transporters and promote nutrient uptake (Sofi et al., 2018). This activity, therefore, converts the free energy released by ATP hydrolysis into a trans- membrane electrochemical potential used for the import of nitrate and other nutrients (Canellas et al., 2015).

Humic substances affect both primary and secondary plant metabolisms. HM may promote primary plant metabolism stimulation of enzymes linked to N assimilation (Wadas and Dziugieł, 2020). Canellas et al. (2015) illustrated that HS enhanced the expression of the phenylalanine (tyrosine) ammonialyase (PAL/TAL) that catalyses the first main step in the biosynthesis of phenolics, by converting phenylalanine to trans-cinnamic acid and tyrosine to *p*-coumaric acid. The positive effects of HS on plants could be due to hormone-like activity, as several hormones enclosed in the humus structure have been identified. HS displays auxin, cytokinin, and gibberellic-like activities (Nardi et al., 2016). The enhanced lateral root development by HS is attributed to auxin-like activity while its promotion of germination is due to its gibberellin-like activity (Lipczynska-Kochany, 2018).

2.1.1.3. Microbial inoculants

Microbial inoculants are living microorganisms that act as ‘biofertilizers’ or biocontrol agents and mainly include free-living bacteria, fungi, and arbuscular mycorrhizal fungi isolated from a variety of environments including composted manure, plant residues, soil, plants, and water (Nehra et al., 2016).

The commonly documented group found in the rhizosphere where they interact with plant roots and influence plant growth are generally referred to as plant growth promoting rhizobacteria. Several factors need to be considered during the development of microbial inoculants such as the species of microorganisms (Hashem et al., 2019). This is because different plant varieties and cultivars produce different types of root exudates which can either support or reject the activity of the inoculated microorganisms during substrate development of biologically active substances (Hassan and Dinesh, 2018). This type of biostimulant is considered to be multipurpose because of its various effects and mechanisms in plants. Microbial inoculants stimulate plant growth through the production of volatile organic compounds, sequestering of iron by the production of siderophores, asymbiotic nitrogen fixation, and solubilization of nutrients (Mahmood et al., 2016).

Several plant growth promoting rhizobacteria (PGPR) produce volatile organic compounds (VOCs) which promote plant growth (Gowtham et al., 2018). Volatile organic compounds produced by biocontrol strains can induce systematic resistance against pathogens and inhibit nematodes, fungal, and bacteria pathogens; and can further promote leaf surface area, biomass, lateral root number and yield (Asghari et al., 2020, Hashem et al., 2019). Siderophores are molecules that bind and transports iron under iron-limiting conditions, and enhance iron (Fe) uptake capacity in microorganisms (Sirohi et al., 2015). Plant growth promoting rhizobacteria produce and utilize the siderophores produced by other microbes present in the rhizosphere for fulfilling their iron requirement (Gouda et al., 2018, Orhan et al., 2006)). Plant growth promoting rhizobacteria can increase the concentration and accessibility of nutrients by either locking or fixing their supply for plant growth and productivity (Gouda et al., 2018). Plant growth promoting rhizobacteria can fix nitrogen either through symbiotic or non-symbiotic interactions between plants and microbes (Bukhat et al., 2020). Inoculation with PGPR can enhance phosphorus availability in plants through solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria. Furthermore, PGPR can increase the availability of potassium by solubilizing potassium rock through the production of organic acids that can release inaccessible potassium (Kumari et al., 2018). Microbial inoculants can also modify plant hormone status through synthesis, localization, and signalling of phytohormones (Hassan and Dinesh, 2018). Plant growth promoting rhizobacteria can alter the localization, signalling and concentration of phytohormones including gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, and auxins, which are responsible for various actions including root and shoot invigoration (Tsukanova et al., 2017).

2.1.1.4. Protein hydrolysates

Protein hydrolysates are biostimulants obtained from enzymatic and/or chemical hydrolysis of proteins from agro-industrial by-products from plant sources, animal waste, and biomass of dedicated legumes (Colla et al., 2015). These biostimulants are recommended for foliar applications since they have a short

half-life in soil (Abbas, 2013). The production of protein hydrolysates from by-products of agro-industry provides an environmental and economically friendly solution for disposing of waste (Colla et al., 2015). These products do not only contain amino acids and proteins/peptides but also consist of other non-protein components, which also contribute to their stimulating effect on plants (Yakhin et al., 2016). For example, non-protein carob germ extracts in addition to proteins and amino acids, contain carbohydrates, macro- and micronutrient elements, and phytohormones (triacontanol and indole-3-acetic acid) (D'Addabbo et al., 2019).

This group of biostimulants plays various roles in plant growth and development. Protein hydrolysates play a major role in the assimilation and modulation of N uptake (Caruso et al., 2020). This is achieved through regulating enzymes that aid in the assimilation of N and their structural genes and by acting on the signaling pathway of N acquisition in roots (Colla et al., 2015). These products further regulate enzymes of the tricarboxylic acid cycle (TCA), which plays a significant role in the cross-talk between carbon and nitrogen metabolism (Du Jardin, 2015). Protein hydrolysate mode of action extends to influencing soil chemical and physical properties. In soil, they increase the respiration together with microbial biomass and activity (Du Jardin, 2015). These products further improve the solubility and mobility of micronutrients, especially Fe, Zn, Mn, and Cu (Abbas, 2013).

2.1.1.5. Effect of biostimulants on seed germination

Seed germination is the initial step in the life cycle of plants, which begins when the inactive dry seed imbibes water and is completed with the protrusion of the radicle from the seed coat (Nonogaki et al., 2010). Seed germination is a complex process, which involves several signals and is influenced by both intrinsic and extrinsic factors (Miransari and Smith, 2014). Intrinsic factors include seed dormancy and available food stores while water, temperature, oxygen, light, relative humidity, chemicals in the seed surrounding environment, and substrate used constitute extrinsic factors (Makena et al., 2018, Bhardwaj, 2014, Savaedi et al., 2019). The germination process plays a key role in the domestication of crops as lack of uniform seed germination can result in poor stand establishment, which affects overall crop yield. Germination is largely affected by the balance of phytohormones, especially abscisic acid (ABA) and gibberellin ratios (Miransari and Smith, 2014). The process of seed germination is comprised of three prominent stages (Nonogaki et al., 2010):

- Phase I, rapid imbibition of water by the dry seed;
- Phase II, metabolism reactivation, including mobilization of food reserves and protein synthesis; and
- Phase III, radicle protrusion.

Water imbibition by the seeds hydrate matrices including reserve polymers and cell walls within the cell (Miransari and Smith, 2014). Water uptake by dry seeds during the first phase of germination is rapid, while resumption of phase II is more gradual (Miransari and Smith, 2014). Rapid water uptake stimulates the embryo to produce phytohormones, especially gibberellins, which disseminate to the aleurone layer in order to resume a biochemical cascade leading to the synthesis of hydrolytic enzymes including α-amylase (Miransari and Smith, 2014). During metabolic reactivation (phase II), hydrolytic enzymes are activated with a concomitant decrease in ABA endogenous content (Wang et al., 2015). These enzymes then hydrolyze the endosperm food reserves into metabolizable sugars, which in turn provide energy for the growth of radicle and plumule, leading to the protrusion of the radicle (phase III) (Farooq et al., 2017).

Exogenous application of gibberellic acid (GA) have been demonstrated to promote seed germination by supplementing the endogenous GA content (Mahmood et al., 2016), resulting in increased germination rate, and decreased germination time spread (Ali and Elozeiri, 2017). Nitrogen-containing compounds can also stimulate germination, even under salinity stress, by enhancing α-amylase activities, and increasing adenosine triphosphate (ATP) production and seed respiration through K^+/Na^+ ratio adjustment (Miransari and Smith, 2014). Poor seed germination rate, inadequate seedling emergence, and poor stand establishment are amongst the major challenges facing global crop production (Nonogaki et al., 2010). This situation has led to several strategies being employed to synchronize radicle emergence and subsequent seedling to mature plant growth. Two of the strategies widely used include: priming and exogenous application of phytohormones.

Biostimulants have been widely used to improve seed germination either as a priming agent or through direct application to seeds. Biostimulants are sometimes referred to as plant conditioners, metabolic enhancers, or phytostimulators (Yakhin et al., 2016), and they are widely used by growers throughout the growth cycle of various plants in order to promote and enhance growth, production of phytohormones or growth regulators, the activity of rhizosphere microbes and soil enzymes, and biological processes (Albrecht, 2017). Biostimulants such as seaweed extracts can be a source of important phytohormones including gibberellins, auxins, and cytokinins (Stirk et al., 2020). These phytohormones enhance crop productivity and yield by modulating plant metabolism under both favorable and unfavorable conditions (Bulgari et al., 2019) and they play an important role in plant growth and development, including during seed germination.

Biostimulants are a rich source of various phytohormones such as cytokinins, auxins, and gibberellin. These phytohormones play a role in plant growth and development in a plant life cycle, especially during germination. Humic substances promote seed germination due to their hormone content. These substances have been found to exhibit gibberellin, cytokinin, and auxin-like activities (Rouphael and Colla, 2018). The presence of phytohormones in humic substances was confirmed by enhanced

metabolism, activation of the auxin synthetic reporter DR5: GUS, and transcription of the early auxin-responsive gene (*IAA19*) (Nardi et al., 2016). Seaweed extracts improve the physiology of plants through the provision of phytohormones, especially gibberellins. Plant growth promoting rhizobacteria does not contain phytohormones but rather produces them as secondary metabolites (Grobelak et al., 2018). One of the hormones produced by this group is gibberellins (Calvo et al., 2014).

Various studies have been conducted to assess the role of biostimulants on seed germination. Plant growth promoting rhizobacteria effect was studied on chili seed germination and was found to significantly enhance the final germination percentage and seedling vigour in comparison with the control (Gowtham et al., 2018). PGPR improved the germination of *Brassica nigra* (Román-Ponce et al., 2017). Furthermore, PGPR inoculation significantly increased the germination rate and viability index of *Oryza sativa* L. in both normal and arsenic exposure conditions (Xiao et al., 2020). In tomato, PGPR (*Azospirillum brasilense* -all strains) inoculated seeds achieved a significantly higher germination percentage in comparison with the control (uninoculated) (Mangmang et al., 2016). In lettuce seeds, only *A. brasilenseI* Sp7 significantly enhanced germination percentage while strain Sp245 inhibited germination (Mangmang et al., 2016). The authors also discovered that the method of inoculation has a significant role in seed germination. This is because of the observed promotion of tomato seed germination when soaked than when soil drenched. Furthermore, a significant promotion of cucumber seed germination was observed when inoculated by soil drenching compared to soaking (Mangmang et al., 2016). In *Allium cepa*, PGPR (*Azotobacter* sp., *Bacillus subtillis,* and *Pseudomonas* sp.) had no significant effect on seed germination but rather inhibited the germination (Stamenov et al., 2018). *In vitro* germination of field dodder seeds were significantly inhibited by *B. amyloliquefaciens, B. megatherium* ZP6, and *Pseudomonas fluorescens* while *Bacillus licheniformis, B. pumilus,* and *B. megatherium* ZP6 had no significant effect when measured against the control (Sarić-Krsmanović et al., 2017). Plant growth promoting rhizobacteria significantly increased germination percentage and germination rate index of *Cicer arietinum* L. salt stress with reference to the control (Hossain et al., 2016). The germination percentage of wheat was significantly enhanced by *Azospirillum lipoferum* in comparison to the untreated control (Mohammad, 2014). However, *Azospirillum lipoferum* extended the germination rate when compared with *Azotobacter chroococcum* and control in wheat (Mohammad, 2014). This study further illustrated that PGPR efficacy differs with cultivar because of the observed effect of different PGPR on different wheat cultivars. *Azotobacter chroococcum* significantly improved the germination of milan and shanghai cultivars while it had no significant influence on zhagros and tajan against the control (Mohammad, 2014).

Kelpak®, a common seaweed extract in South Africa significantly increased the germination percentage of *Ceratotheca triloba* seeds under low temperature and osmotic potential conditions, when compared to the control (Masondo et al., 2018). Treating *Solanum lycopersicum* with the brown alga *Sargassum tenerrimum* extract had a significant effect on germination (%) as compared to the control (Sasikala et

al., 2016). Up to 100% germination was obtained with *S. tenerrimum* extract at 0.8% (v/v) concentration while 0.6% (v/v) extract concentration increased germination up to 90% (Sasikala et al., 2016). The germination of *Allium cepa* L. was significantly influenced by the extract of another brown seaweed, *Ascophyllum nodosum*, at various concentrations (i.e. 3.5, 4.5, 5.5, 6.5, and 7.5 mL/L) when compared with the control (water) (Hidangmayum and Sharma, 2015). Furthermore, seaweed extracts of the green *Ulva lactuca* and brown *Padina gymnospora* at 0.2, 0.4 or 1.0% (v/v) significantly improved germination percentage and mean germination time of *Solanum lycopersicum* while treatments with extracts of *Caulerpa sertularioides* (G) and *Sargassum liebmannii* (B) inhibited germination at all tested concentrations [0.2, 0.4 and 1% (v/v) (Hernández-Herrera et al., 2013). However, an increase in extract concentration of *U. lactuca* and *P. gymnospora* significantly reduced germination, as evidenced in reduced germination percentages and indices, as well as increased germination time (Hernández-Herrera et al., 2013). Even though the effect of biostimulants on seed germination has been widely studied, specific focus on the effect of biostimulants on the germination of *Abelmoschus esculentus* and *Cleome gynandra* are limited.

2.1.1.6. Effect of biostimulants on vegetative growth and yield

After seeds have germinated, the next development step is seedling emergence and growth. Seedling emergence and stand establishment are dependent on environmental conditions as well as soil physical properties. However, soil infertility leads to poor soil physical properties and thus poor soil functions and characteristics including nutrient holding capacity, available plant nutrients, water filtration, water holding capacity, and aggregation (porosity). Biostimulants can modify root morphology directly, ameliorate nutrient transport in plants, or change soil structure and nutrient solubility to facilitate increased nutrient uptake (Halpern et al., 2015). Biostimulants can enhance abiotic stress tolerance, nutrition efficiency, and crop quality traits.

Protein hydrolysates promote the uptake of nutrients by increasing the absorptive surface area via stimulating root and leaf biomass (Colla et al., 2015).

Seaweed extracts affect both plants and soil. In soil, they promote gel formation (which enhances uptake of trace elements), water retention, and soil aeration due to their high polysaccharide content (Battacharyya et al., 2015, Beckett and van Staden, 1990). Polyanionic compounds of seaweed extracts fix and exchange cations which play a major role in soil remediation (Khan et al., 2009). Furthermore, seaweed extract's hormone content promotes different plant development and growth processes (Stirk and Van Staden, 2014). Seaweed extracts enhance seedling establishment, improve growth, yield, flower set, fruit production, postharvest shelf life, and increase resistance to biotic and abiotic stress (Yakhin et al., 2016).

Microbial inoculants are a group of microorganism which include beneficial fungi and beneficial bacteria. Beneficial fungi promote nutrition efficiency, water balance, and tolerance to abiotic stress (Pagnani et al., 2018). Beneficial bacteria (PGPR) are multipurpose and affect all aspects of the plant's life cycle including interaction with other organisms in the agroecosystem, nutrition, growth, morphogenesis and development, and response to biotic and abiotic stress (Grobelak et al., 2018). Microbial inoculants alter root architecture via the degradation or production of major groups of plant hormones (Ahemad and Kibret, 2014). Plant growth promoting rhzobacteria inoculants are viewed as plant probiotics or major contributors to plant immunity and nutrition (Naeem et al., 2018). Microbial inoculants increase root biomass and nutrient uptake capacity (Asghari et al., 2020)..

Plant growth promoting rhizobacteria significantly increased vegetative growth parameters of chili seeds including plant height, the number of leaves, shoot fresh weight as well as dry weight (Gowtham et al., 2018). Roman Ponce et al. (2017) observed an increase in root architecture (secondary roots and hair generation) of *Brassica nigra* under heavy metal (As, Cu, Pb, and Zn) conditions following the application of PGPR. The application of PGPR significantly increased plant height, chlorophyll content, plant biomass, and leaf surface area of mung beans when compared to the control (Kumari et al., 2018). PGPR inoculation significantly increased shoot fresh weight, leaf area, and the number of leaves of *Mentha piperita* (Chiappero et al., 2019). PGPR also increased the yield of *Oryza sativa* L. under arsenic exposure (Xiao et al., 2020). On the other hand, PGPR (*P. aeruginosai*, *P. putida*, *B. subtillis*, *P. polymyxa*, and *B. boronophillus*) significantly decreased shoot and root length, and shoot and root dry length of *Cicer arietinum* L. (Yadav et al., 2010). Application of *A. lipoferum*, *P. fluorescens*, and *P. putida* had no significant effect on plant height, number of leaves and stem diameter of *Zea mays* while *A. lipoferum* significantly increased leaf area in comparison with the control (Agbodjato et al., 2016).

Seaweed extract (Kelpak® product) treatments significantly enhanced the yield of two common bean cultivars (Aura and Toska) (Kocira et al., 2018). Kelpak® treatments significantly stimulated plant height, stem diameter, and leaf weight of energy willow plants (Digruber et al., 2018). Application of Kelpak® using the soil drenching method significantly increased the shoot fresh weight of *Amaranthus hybridus* L. while the foliar application had no observable effects (Ngoroyemoto et al., 2019). However, a combination of foliar and soil drenching methods significantly improved the number of leaves, number of roots, root length, stem diameter, leaf area, and shoot fresh weight of *Amaranthus hybridus* (Ngoroyemoto et al., 2019). Under no nutrient deficiency, Kelpak® did not improve the growth parameters of okra seedlings (Papenfus et al., 2013). However, under phosphorus deficiency, Kelpak® significantly increased shoot length, number of leaves, stem diameter, and shoot fresh weight (Papenfus et al., 2013). The authors further observed that Kelpak® application under potassium deficiency enhanced shoot length, number of roots, stem diameter, and fresh biomass of okra seedlings. When applied to *Spinacia oleracea* L., Kelpak® had no visible effect on growth parameters but significantly improved photosynthetic pigments, proteins, and proline content (Kulkarni et al., 2019). Seaweed

extract (*Sargassum tenerrimum*) significantly increased shoot length, number of leaves, leaf area, and plant height of *Solanum lycopersicum* in a pot study after 40 days of planting (Sasikala et al., 2016). Root length was reduced at a concentration of 0.2% (v/v) but was significantly stimulated at 0.4, 0.6, 0.8, and 1% (v/v) when measured against the control (Sasikala et al., 2016). At low concentrations [(2.5 and 7.5% (v/v)], *Kappaphycus alvarezii* had no significant effect on plant height, number of pods and harvest index of *Glycine max* while at high concentrations [7.5, 10, 12.5 and 15% (v/v)], plant height, number of pods and harvest index were significantly enhanced in comparison with the control (Rathore et al., 2009). Foliar application of *U. lactuca* at 0.2% (v/v), *C. sertularioides* and *P. gymnospora* [1% (v/v)] had no significant effect on shoot length of *Solanum lycopersicum* L. while *U. lactuca* [0.4 and 1% (v/v)] and *P. gymnospora* [0.2 and 0.4% (v/v)] enhanced shoot length (Hernández-Herrera et al., 2013). Furthermore, both foliar and soil drench application of *S. liebmannii* significantly reduced growth parameters (plant height, shoot and root length) of *Solanum lycopersicum* L. when measured against the control (Hernández-Herrera et al., 2013). Studies on biostimulant effect on *Abelmoschus esculentus* growth and yield are limited, while there are none on *Cleome gynandra*.

2.1.1.7. Effect of biostimulants on phytochemicals

Plants with medicinal potential can cure different diseases, infections, and conditions (Ahmad and Aslam, 2016). These plants often possess specific compounds (known as phytochemicals) occurring in various plant parts that can neutralize or treat diseases and infections. These phytochemicals have a physiological effect on humans (Kia et al., 2018) and tend to function differently within the human body.

Few biostimulants have been reported to influence the phytochemical content of plants. Protein hydrolysates improve the production of secondary metabolites including phenols and antioxidants and can further increase flavonoid biosynthesis (Nardi et al., 2016). Seaweed extract application enhances nutritional quality through plant provision of both macro- and micronutrients (Du Jardin, 2015). The nutritional content (nitrogen, phosphorus, and potassium) of *Glycine max* grains was significantly enhanced in the presence of seaweed extract (*Kappaphycus alvarezii*) at varying dilutions [10, 12.5, and 15% (v/v)] when compared to the control (Rathore et al., 2009). The mineral elements concentration (nitrogen and potassium) of *Glycine max* straw remained unaffected by seaweed extract application while phosphorus content was significantly enhanced at 5, 7.5, and 10% (v/v) when measured against the control (Rathore et al., 2009). Brown seaweed extracts (*Sargassum vulgare*, *Colpomenia sinuosa*, and *Padina pavonica*) significantly improved the protein content of *Trigonella foenum-graecum* L. (El-Sheekh et al., 2016)

Plant growth promoting rhizobacteria treatments significantly reduced the proline content of *Mentha piperita* while increasing total phenolic content when compared to the control (Chiappero et al., 2019).

A significant increase was observed in the phenolic content of *Phaseolus vulgaris* cultivar Toska treated with Kelpak® while cultivar Aura had no increase in phenolic content (Kocira et al., 2018). However, flavonoid content was significantly enhanced in both cultivars. Furthermore, Kelpak® had no significant effect on the main nutrients (starch, free sugars, albumins and globulins) of *Phaseolus vulgaris* (Kocira et al., 2020). The polyphenol content of potato tubers was significantly increased by Kelpak® application (Ramírez et al., 2014). Research on the effect of biostimulants on the phytochemistry of plants has been conducted, however, limited research has been conducted on *Abelmoschus esculentus* and *Cleome gynandra*.

2.3. Multipurpose plants

2.3.1. Distribution and general morphology of *Abelmoschus* and *Cleome* species

Abelmoschus species have diverse origins and some studies have suggested that they originated from Asia while some indicated Ethiopian and Egyptian origin (Ogwu et al., 2016). The distribution of *Abelmoschus* species has spread to the Middle East and North Africa (Patil et al., 2015a). Cultivated species of *Abelmoschus* are distributed throughout tropical and subtropical regions of the world, excluding *A. caillei* whose cultivation is restricted to West Africa (Werner et al., 2015). *Abelmoschus* species are mostly grown in Mali, Ghana, Pakistan, Mexico, Egypt, Malaysia, Nigeria, and India (Ali et al., 2017).

Species of this genus are generally annual, biennial, or perennial herbs with either entire or palmately lobed leaves (Ya et al., 1984). Flowers of *Abelmoschus* genus are funnel-shaped, with five petals, often with a red or purple corolla that when matured bears smooth, glabrous and globose or reniform seeds (Ya et al., 1984).

Cleome species are reported to have originated from Africa but are widespread through tropical and subtropical regions (Ahouansinkpo et al., 2016). This genus consist of herbaceous plants, bearing seeds that are enclosed in a capsule with two membranous valves separating from replum with woolly, asperulous, or reniform seeds (Kamel et al., 2010). Fruits of *Cleome* species are generally linear-oblong with many seeds while leaves are simple and composed of 3-7 leaflets with flowers that are either purple, white, or yellow (Kamel et al., 2010). These species are one of the most important and common leafy vegetables in Africa because of its natural and voluntary occurrences ranging from wastelands to roadsides and household gardens as weeds (Kwarteng et al., 2018).

2.3.1.1. *Abelmoschus esculentus*

Abelmoschus esculentus is an annual herb that is cultivated worldwide (Ya et al., 1984). This species is commonly known as lady's finger or gumbo (Bhat et al., 2015). *Abelmoschus esculentus* is native to Africa, mostly cultivated in tropical to subtropical regions for its delicious tender fruits (Molfetta et al., 2013, Werner et al., 2015, Yadav et al., 2014). *Abelmoschus esculentus* is considered a summer crop, however, in India it has been reported to flower throughout the year (Das and Islam, 2019, Kumar et al., 2018). **Figure 2.1** presents the different parts of *A. esculentus* plant.

Figure 2.1: Morphology of *Abelmoschus esculentus* A-whole plant, B-pods, and C-seeds (source: https://avrdc.org/give/).

2.3.1.2. *Cleome gynandra*

Cleome gynandra syn. *Gynandropsis gynandra* (L.) Briq. is an annual crop that originated from Africa and southeast Asia (Wu et al., 2017, Adhikari and Paul, 2018). This species is most famous in southern Africa as a herb and is distributed in South Africa (Limpopo, North West, Mpumalanga, Gauteng, the Northern Cape and Eastern Cape) and Namibia (Adhikari and Paul, 2018, Kiebre et al., 2015, Wu et al., 2017). *Cleome gynandra* is commonly known as cat's whisker, African cabbage, and spider plant (Omondi et al., 2017, Kiebre et al., 2015). It is generally consumed as a leafy vegetable and is traditionally harvested from the wild, but because of its high content of nutrients, domestication of this plant has been introduced in certain regions of Africa (Kiebre et al., 2015). Furthermore, *C. gynandra* is a C4 species and hence has a high production potential (Wu et al., 2017). The C4 photosynthetic

pathway allows a higher photosynthetic rate and efficient use of nitrogen (Kujeke et al., 2017). **Figure 2.2** presents different plant parts of *C. gynadra*.

Figure 2.2: Morphology of *Cleome gynandra* A-whole plant, B-flowers, and C-seeds (source: https://avrdc.org/give/).

2.4. Nutritional value of *Abelmoschus* and *Cleome* species

Inadequate consumption of fruits and vegetables is associated with various health risks including cardiovascular diseases and cancer. In South Africa, about 25.6% of the population has low intake of fruits and vegetables and this leads to the development of various diseases and malnutrition (Ronquest-Ross et al., 2015). In 2000, this trend resulted in about 3.2% of deaths in South Africa (Peltzer and Phaswana-Mafuya, 2012).

Furthermore, the increasing world population threatens food security, hence, finding alternative nutritious diet supplements to be implemented in a regular diet has become a priority. For a well-balanced diet, a minimum of 400 g of fruits and vegetables is recommended daily, which is currently not attained in most rural communities (Peltzer and Phaswana-Mafuya, 2012). As a result, researchers have studied several African indigenous plants for their nutritional content to mitigate food insecurity, especially at the household level. *Abelmoschus* and *Cleome* species have been documented as plants with potential for food and nutritional security.

2.4.1. *Abelmoschus esculentus*

Abelmoschus species are nutritious plants because of their high fat, carbohydrate, protein, dietary fiber, minerals, and vitamin C contents, playing a major role in combatting nutritional deficiencies (Ghori et al., 2014). *Abelmoschus esculentus* is used as a supplement to cereal because of its high protein content

qualifying it to be an alternative to soybean (Adekiya et al., 2017). Some *Abelmoschus* species including *A. esculentus* are widely known as 'perfect villager's vegetable' because of their role in human diets (Gemede, 2015). *Abelmoschus esculentus* is a well-known nutraceutical that is rich in proteins and tryptophan amino acids, vitamins (A, C, E, and K), thiamine (B_1), riboflavin (B_2), calcium, iron, magnesium, potassium, and zinc (Kumar et al., 2013). *A. esculentus* is also a rich source of oil which consists of up to 47.2% linoleic acid (Fekadu Gemede, 2015).

2.4.2. *Cleome gynandra*

Cleome gynandra is a leafy vegetables with medicinal properties (DAFF, 2014). As vegetables, *C. gynandra* is a nutritious supplement of proteins, carbohydrates, vitamins, minerals, phenols, and essential oils (Kujeke et al., 2017). In some communities, it is used as a nutritious meal for lactating and pregnant women because of its claimed ability to limit dizzy spells and ease childbirth (Singh et al., 2018).

Even though *C. gynandra* is a late-season crop, it plays a major role in food security as a nutraceutical crop, especially in rural communities. This is due to its high content of phytonutrients, it is a rich source of mineral elements including iron, magnesium, calcium, and zinc (Omondi et al., 2017). *Cleome gynandra* contains high levels of vitamins (provitamin A and vitamin C), lipids, and crude protein (Lokesha, 2018, Poorva and Sunita, 2017, Sogbohossou et al., 2018). Amino acid profile of *C. gynandra* is high when in comparison with that of groundnut (Lokesha, 2018).

Cleome gynandra and *Abelmoschus esculentus* can play a vital role as a nutritional additive in diets especially in food insecure communities in Africa since they can be easily cultivated. Concerns of contaminants from chemical fertilizers and pesticides in conventionally produced agricultural products has resulted in consumer paradigm shift towards organic agricultural production. Therefore, further studies are of importance to evaluate the effect of biostimulants on the nutritional value of *A. esculentus* and *C. gynandra*.

2.5. Medicinal properties of *Abelmoschus* and *Cleome* species

The use of plants as medicine dates to ancient times with cultivation traced back to approximately 6 0000 years ago (Jamshidi-Kia et al., 2018). Medicinal plants are most popular in the developing countries since approximately 3.4 billion individuals in these countries depend on such plants for medicine (Doughari, 2010). Furthermore, industrialized countries show interest in the use of traditional medicinal plants to process them into 'alternative or complementary medicine' and medicinal drugs. As of 2018, about 50% of drugs available in the market consist of components of medicinal plants (Jamshidi-Kia et al., 2018). *Cleome* and *Abelmoschus* species have been reported to have medicinal properties.

2.5.1. *Abelmoschus esculentus*

Cultivated species of the *Abelmoschus* genus possess components that are valued in the treatment and curing of various diseases. *Abelmoschus esculentus* is used in Ayurveda's traditional system and is prepared as an edible infusion for its diuretic effect (Roy et al., 2014). It relieves hemorrhoids and is used in the treatment of ulcers (Messing et al., 2014). *Abelmoschus esculentus* is further renowned for its demulscent, anodyne, emollient properties while being an effective in dysentery and diaphoretic treatments (Onakpa, 2013, Kumar et al., 2013). It has been widely used as an antidiabetic, anticancer and antimicrobial agents (Onakpa, 2013). In Indian ethnomedicine *A. esculentus* is used as antipyretic and plasma replacement (Roy et al., 2014).

2.5.2. *Cleome gynandra*

Besides being a nutraceutical, *C. gynandra* is used as medicine in most developing countries. *Cleome gynandra* is useful in strengthening the immune system, curing inflammations, wounds, epileptic fits, malaria, and digestive disorders (Adhikari and Paul, 2018, Sogbohossou et al., 2018). In India, *C. gynandra* is used to treat various conditions including earaches, boils, headaches, bronchitis and nasal congestion (Lokesha, 2018). *Cleome gynandra* is further used in the treatment of migraines, diarrhoea, uterine complaints, and stomach ache (Adhikari and Paul, 2018). The leaves of *C. gynandra* have been widely used to reduce the severity of stomach-ache and constipation, thread-worm infections and arthritis (Mishra et al., 2011). *Cleome gynandra* plant is further used to relieve recurrent malaria, anaemia, pneumonia and coughing (Chweya and Mnzava, 1997).

2.6. Phytochemistry of *Abelmoschus esculentus* and *Cleome gynandra*

Phytochemicals are naturally occurring biologically active chemical compounds of medicinal plants and are often used in the development of synthetic drugs (Jamshidi-Kia et al., 2018). About 75% of drugs in the United States market are derivatives of medicinal plants (Inda et al., 2008).

The phytochemicals from *A. esculentus* and *C. gynandra* are summarised in **Table 2.2**. *Abelmoschus* species are a rich source of phytochemicals. *Abelmoschus esculentus* is rich in flavonoids, pectin, oxalic acid, tannins, phenolic compounds, and carotenoids (Roy et al., 2014, Ahmad and Aslam, 2016). Species of this genus are a source of volatile compounds α-humulene and β-elemene (Molfetta et al., 2013).

Cleome gynandra is a rich source of tannins, steroids, flavonoids, and leucoanthocyanidin (Ahouansinkpo et al., 2016). Leaves of *C. gynandra* are predominantly high in lectins, glycosides, flavonoids, steroids, and phenolic compounds (Lokesha, 2018, Sogbohossou et al., 2018, Singh et al.,

2018). Its leaves consist of free radical scavengers (glutathione and superoxide dismutase) while seeds are a rich source of glucosinolates, cleomin, and glycocapparin as well as acrid volatile oil which is similar to that of custard oil (Lokesha, 2018).

Table 2.2: Phytochemical content of *Abelmoschus esculentus* and *Cleome gynandra*

Plant species	Compound	IUPAC name	Reference
Abelmoschus esculentus	Hyperoside/Hyperin	dihydroxyphenyl)-3-[(3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4Hchromene-4,5,7-triol	(Onakpa, 2013)
Abelmoschus esculentus	Coumarin scopoletin	7-hydroxy-6-methoxychromen-2-one	(Onakpa, 2013)
Abelmoschus esculentus	Flavonoid glycoside	5,7,3′,4′-tetrahydroxy flavonol -3-O-[β-D-glucopyranosyl-(1→6)]-β-Dglucopyranoside	(Onakpa, 2013)
Abelmoschus esculentus	Flavonoid glycoside	5,7,3′,4′-tetrahydroxy-4″-O-methyl flavonol-3-O-β-D-glucopyranoside	(Onakpa, 2013)
Cleome gynandra	Flavonoid glycoside	Quercetin-3-diglucoside; Quercetin-3-rutinoside; Isorhamnetin-3-diglucoside; Kaempferol-3-diglucoside; sorhamnetin-3-rutinoside; Kaempferol-3-rutinoside	(Omondi et al., 2017, Neugart et al., 2017)
Cleome gynandra	Glucosinolates	3-hydroxypropyl glucosinolate, glucocappari); 2-hydroxy-2-methylbutyl	(Omondi et al., 2017)
Cleome gynandra	methylated flavonoids	5,7,4′-trihydroxy-3-methoxyflavone; 5,7,4′-trihydroxy-3,3′-dimethyoxyflavone, 5,7,4′-trihydroxy-6,3′- dimethoxyflavone, 5,4′-dihydroxy-3,6,7-trimethoxyflavone; 5,7,3′,4′-tetrahydroxy-3,6-dimethoxyflavone; 5,7,4′-trihydroxy-6,3′-5′-trimethoxyflavone	(Lokesha, 2018)

Cleome gynandra	Carotenoids	β-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin	(Neugart et al., 2017)

The impact of different fertilizers on the production of secondary metabolites in plants is widely studied. The use of potassium fertilizer in banana increased ascorbic acid while decreasing overall fruit acidity (Institute and Association, 2012). The combination of NPK fertilizer and compost had an increasing effect on the biochemical content of *Moringa oleifera* leaves (carbohydrates, phenolics, and flavonoids) (Sarwar et al., 2019). While the use of fertilizers may improve phytochemistry, their use is not environmentally friendly. Therefore, biostimulants can be used as agents to improve plants biochemical content. Biostimulants have the potential to improve phytochemistry, however, there are inadequate studies that support this theory. For example, in *T. foenum-graecum* L., seaweed extracts significantly increased total chlorophyll, carotenoids, carbohydrates and protein, amino acids, total polyphenols, total nitrogen, and total ash content (Pise and Sabale, 2010). Bioactive compounds and hormones exuded by seaweed extracts promotes the host plant's production of bioactive compounds through internal metabolic pathways (Ashour et al., 2020). More studies of this regard need to be undertaken.

2.7. Conclusion

Various studies have been conducted that evaluate the impact of biostimulants on seed germination, plant growth, yield, nutrition, and phytochemistry of multipurpose plants. Hence, the current review revealed the potential of biostimulants in agricultural production and food security. Even though biostimulants promote plant growth and nutrition, their efficacy differs with plant species and environmental conditions.

Abelmoschus esculentus and *Cleome gynandra* are among underutilised species of important value in food security, especially in developing communities. With the increasing global malnutrition, optimizing yield, biochemical content and mineral elements of *A. esculentus* and *C. gynandra* remains of utmost importance, especially in food insecure regions. This, therefore, demands more studies that will highlight the biochemical and physiological effect of biostimulants on *A. esculentus* and *C. gynandra*.

Chapter 3: Effects of biostimulants on the germination of *Abelmoschus esculentus* and *Cleome gynandra* genotypes

3.1. Introduction

The increasing world population has caused food demand to exceed the current food supply, especially in developing countries. This increase has therefore led to the promotion of the cultivation of multipurpose plants. However, the cultivation of these plants is largely dependent on seed germination, which in turn is influenced by several factors (Makena et al., 2018). Seed germination is the initial step in a plant life and refers to the protrusion of the radicle from the seed coat (Kader, 2005). Germination is an internal process that is facilitated by signalling pathways required to activate α-amylase and commence the breakdown of the starchy endosperm to provide energy for the growing embryo (Wang et al., 2015)..

Seeds of various plant species have different chemical responses that are entirely based on the genetic makeup of that species (Wakjira and Negash, 2013, Nonogaki et al., 2010). The ability of a seed to germinate is environment-dependent, amongst other factors, especially temperature and photoperiod (Nwoke, 1982). Currently, plant cultivation is faced with poor seed germination rate, inadequate seedling emergence, and poor stand establishment, which negatively affects the yield (Nonogaki et al., 2010). As a result, it is pertinent to explore strategies to improve and synchronize seed germination. Researchers have investigated the effect of seed soaking and priming with various compounds that can promote germination (Tian et al., 2015). The stimulatory effect of biostimulants such as seaweed extracts and plant growth promoting rhizobacteria (PGPR) on seed germination has been widely recognized (Du Jardin, 2015). Seaweed extracts are predominantly high in phytohormones which tend to play a major role in seed germination (Battacharyya et al., 2015). On the other hand, PGPR can synthesize phytohormones through their secondary metabolism (Mahmood et al., 2016).

The current study aimed to determine the effect of Kelpak® (seaweed-based biostimulants) and PGPR on the various germination parameters of *Abelmoschus esculentus* and *Cleome gynandra* genotypes under laboratory conditions.

3.2. Materials and methods

3.2.1. Source of biostimulants and seeds

Kelpak® was obtained from Kelp Products (Pty) Ltd, Simon's Town, South Africa. Plant growth promoting rhizobacteria commercial solution (a mixture of organic acids, *Bacillus* sp., amino/fulvic

acid, and soil bacteria) was purchased from Agriman (Pty) Ltd, South Africa. Seeds of *A. esculentus* and *C. gynandra* were obtained from the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants, Pretoria, South Africa.

3.2.2. Soaking duration

Both *A. esculentus* (genotype OkraPB1) and *C. gynandra* (genotype *Cleome* Maseno) seeds were surface sterilized with a 1% sodium hypochlorite for 5 min and rinsed thoroughly with distilled water. For the imbibition test, *A. esculentus* seeds were soaked in distilled water for 0, 6, 12, and 24 h while *C. gynandra* seeds were soaked for 0, 6, 12, 24, and 48 h. From each soaking duration, 25 seeds of each species were placed in 90 mm Petri dishes lined with two layers of filter paper (Whatman No.1) moistened with 10 ml distilled water. This was replicated three times. Petri dishes were then transferred into growth chambers set at 25 ℃ and 12/12 h light and dark regime for *A. esculentus*, and alternating temperature and photoperiod of 30/20 ℃ and 16/8 light and dark regime, respectively for *C. gynandra*. Germination activity was monitored and recorded daily and germination parameters were calculated thereafter.

3.2.3. Seed germination using biostimulants

This study involves two factors (genotypes and biostimulant treatments). Based on the imbibition results (**Table 3.1 and 3.2**) , seeds were soaked in biostimulants (*A. esculentus* for 24 h and *C. gynandra* for 48 h) at varying concentrations [Kelpak® solution (1:100, 1:40 and 1:20 v/v) and plant growth promoting rhizobacteria (PGPR) (1:5, 1:10 and 1:15 v/v)] while distilled water was used as the control. For each treatment, 25 seeds were placed in 90 mm Petri dishes lined with two layers of Whatman No. 1 filter paper.

Five genotypes for each plant were used for the study (*A. esculentus*- Okra PB1, PB2, PB3, PB4 and PB5; *C. gynandra*- TOT10212, TOT8420, *Cleome* 3, *Cleome* Maseno and *Cleome* Arusha). *Abelmoschus esculentus* seeds were incubated at 25℃ in a 12/12 h light and dark regime while *C. gynandra* seeds were incubated at alternating temperatures and photoperiod, 30/20 ℃ in a 16/8 light and dark regime, respectively. *Abelmoschus esculentus* seeds were incubated for 14 days and *C. gynandra* seeds were incubated for 21 days. The petri-dishes were laid out in a completely randomised design and replicated three times. Seed germination was monitored and recorded daily, while moisture was maintained with distilled water. Germination was considered to be complete when the radicle had protruded at least 2 mm. Germination parameters [final germination percentage (FGP), mean germination time (MGT), germination index (GI), coefficient of the velocity of germination (CVG),

germination rate index (GRI), and time spread of germination (TSG)] were calculated according to Kader (2005) with modifications.

FGP = (Final no. of seeds germinated in a seed lot/ total number of seeds in a lot) × 100

MGT =$\sum f.x/\sum f$

CVG =$N_1 + N_2 + \cdots + Nx/100 \times N_1T_1 + \cdots + NxTx$

GRI =G1/1 + G2/2 +· · ·+ Gx/x

GI = (10×n1) + (9×n2) + · · · + (1×n10)

TSG =the time in days between the first and last germination events occurring in a seed lot

Where f =Seeds germinated on day x, N=no. of seeds germinated each day, T=no. of days from seeding corresponding to N, G1 =germination percentage × 100 at the first day after sowing, G2=Germination percentage × 100 at the second day after sowing, n1, n2 . . . n10 = No. of germinated seeds on the first, second and subsequent days until the 10th day, 10, 9 . . . and 1 are weights given to the number of germinated seeds on the first, second, and subsequent days, respectively

3.2.4. Data analysis

Data were analyzed using analysis of variance (ANOVA) following a completely randomized design using Genstat 64-bit Release 18.2 (PC/Windows 8). For statistical significance ($p \leq 0.05$), mean values were separated using Fischer's (least significant difference) LSD test.

3.3. Results

3.3.1. Effect of soaking period on *Abelmoschus esculentus* and *Cleome gynandra* seeds germination parameters.

In *A. esculentus* (**Table 3.1)** FGP, GI and GRI increased with increasing soaking period. Soaking period of 24 h had significantly higher FGP, FI, CVG, GRI and least MGT whem compared to control, and hence qualifying it to be the optimum soaking period for *A. esculentus* seeds.

Saoking period affected germination parameters of *C. gynandra* (**Table 3.2)** at varying levels. Soaking *C. gynandra* seeds for 12 h achieved significantly reduced FGP, GI, CVG and GRI when compared to

48 h. *C. gynanrda* seeds soaked for 48 h had significantly enhanced FGP, GI, CVG, GRI and had least MGT when measured against control, 6, 12 and 24 h.

Table 3.1: Imbibition period of *Abelmoschus esculentus* seeds incubated at 25℃. FGP= final germination percentage, MGT= mean germination time, GI= germination index, CVG= coefficient of velocity of germination, GRI = germination rate index, TSG =time spread of germination. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences, n.s= not significant

Soaking period (h)	FGP (%)	MGT (day)	GI	CVG	GRI (%/day)	TSG (day)
0	37.33^{b}	3.730 a	58.7^{c}	3.447^{b}	12.25^{c}	4.000
6	62.67^{a}	3.610 a	99.7^{b}	9.050^{a}	19.25^{bc}	4.667
12	60.00^{a}	2.657 b	110.0^{b}	6.037^{ab}	26.20^{b}	3.000
24	72.00^{a}	2.523 b	134.7^{a}	8.197^{a}	38.09^{a}	5.000
LSD ($p \leq 0.05$)	14.91	0.6243	24.60	3.655	10.68	n.s

Table 3.2: Imbibition period of *Cleome gynandra* seeds incubated at alternating temperatures of 30/20℃. FGP= final germination percentage, MGT= mean germination time, GI= germination index, CVG= coefficient of velocity of germination, GRI = germination rate index, TSG =time spread of germination. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences, n.s= not significant.

Soaking period (h)	FGP (%)	MGT (day)	GI	CVG	GRI (%/day)	TSG (day)
0	50.67^{b}	1.977^{bc}	165.0^{b}	3.243^{bc}	31.45^{b}	2.33
6	20.00^{c}	3.663^{a}	56.3^{c}	1.047^{c}	6.19^{c}	3.67
12	21.33^{c}	3.783^{a}	59.3^{c}	1.330^{c}	6.30^{c}	3.33

24	56.00[b]	2.200[b]	179.0[b]	4.420[ab]	32.9[b]	3.00
48	76.00[a]	1.643[c]	253.3[a]	6.180[a]	57.19[a]	3.33
LSD ($p \leq 0.05$)	17.73	0.5534	52.09	2.879	8.79	n.s

3.3.3. Effect of biostimulant treatments on *Abelmoschus esculentus* and *Cleome gynandra* genotypes germination parameters.

Biostimulant treatments affected the germination parameters of *A. esculentus* at varying levels (**Figure 3.3**). Kelpak® (all dilutions) and PGPR (1:15) treatments had no significant effect on FGP while PGPR (1:5 and 1:10) had a negative effect compared to the control. Likewise, Kelpak® (all dilutions) and PGPR (1:15) had no significant effect on GRI and GI while PGPR (1:5 and 1:10) reduced these parameters. The MGT was not significantly affected by Kelpak® (all dilutions) and PGPR (1:10 and 1:15) treatments. However, PGPR (1:5) had the highest MGT, and hence, delayed germination. PGPR (all dilutions) and Kelpak® (1:40) had a negative effect on CVG while Kelpak® (1:100 and 1:20) had no significant difference compared to control. Kelpak® (1:100) treatment had the lowest TSG.

Even though varying concentrations of biostimulant treatments affected germination parameters of *C. gynandra*, no positive effect by the treatments was observed except in a case of CVG (**Figure 3.4**). Kelpak® (all dilutions) and PGPR (1:10 and 1:15) had no significant effect on FGP when compared to the control. However, PGPR (1:5) significantly reduced the FGP of *C. gynandra* seeds. PGPR (1:5 and 1:15) extended MGT and significantly reduced GI and GRI. Relative to the control, the application of Kelpak® (all dilutions) and PGPR (1:10) had no positive effect on MGT, GI, and GRI while PGPR (1:5 and 1:15) had a negative effect on these parameters. Kelpak® (1:40) significantly increased CVG of *C. gynandra* seeds.

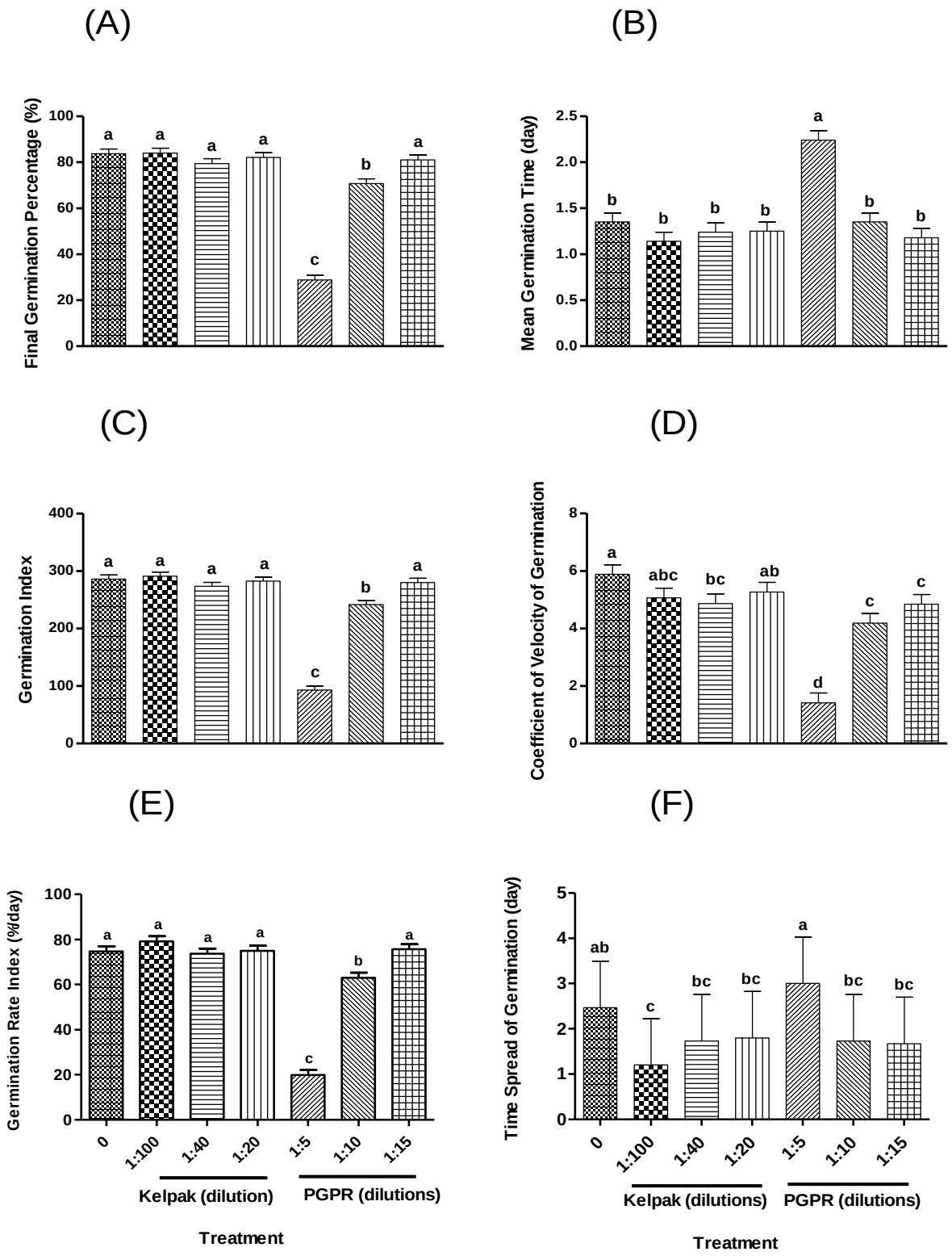

Figure 3.1: Effect of biostimulant treatments on germination parameters of *Abelmoschus esculentus* genotypes (A) final germination percentage, (B) mean germination time, (C) germination index, (D) co-efficient of the velocity of germination, (E) germination rate index and (F) time spread of germination. Bars with a different letter(s) are significantly different ($p \leq 0.05$).

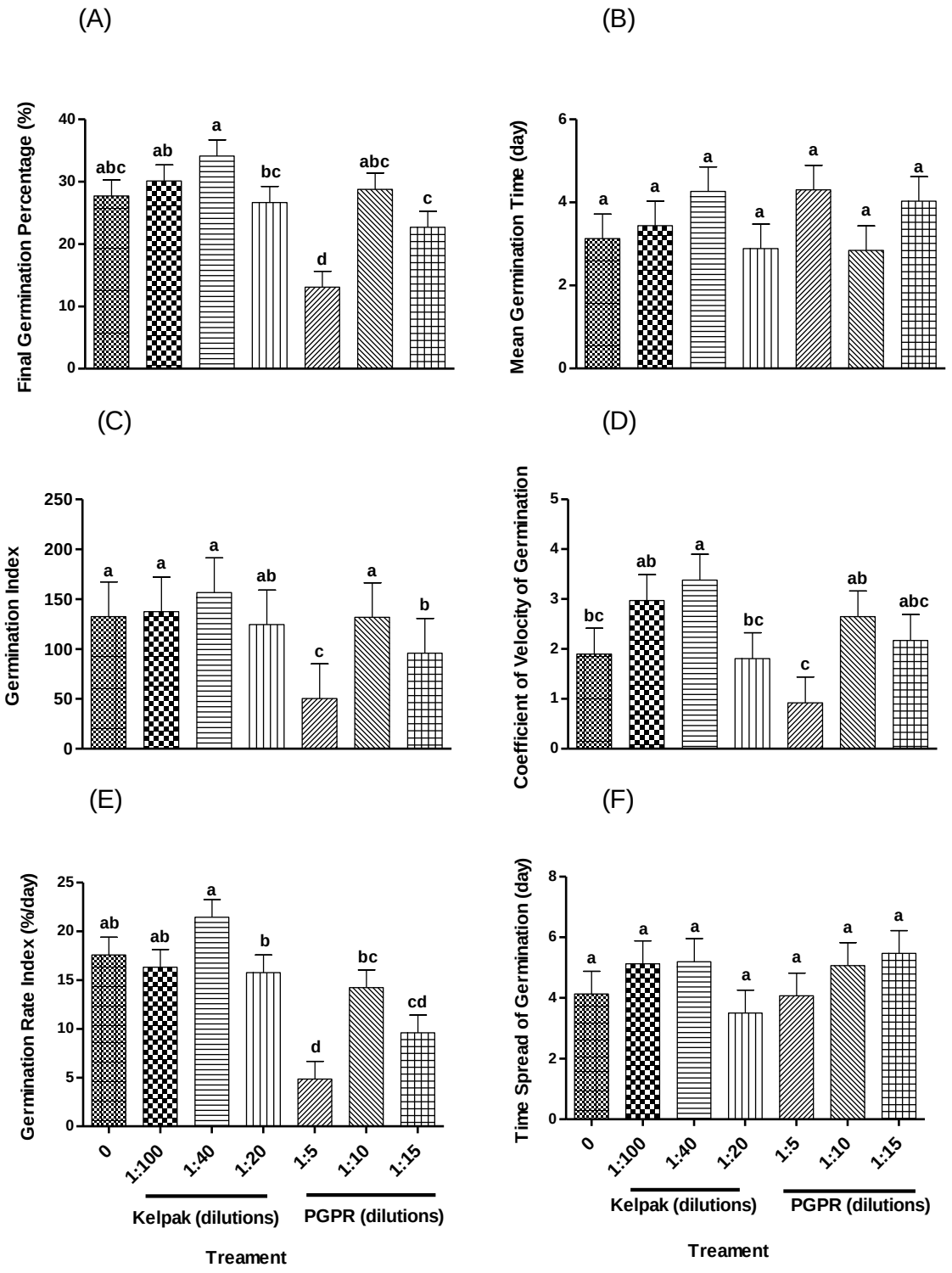

Figure 3.2: Effect of biostimulant treatments on germination parameters of *Cleome gynandra* genotypes (A) final germination percentage, (B) mean germination time, (C) germination index, (D) coefficient of the velocity of germination, (E) germination rate index and (F) time spread of germination. Bars with a different letter(s) are significantly different ($p \leq 0.05$).

3.3.4. Interaction effect of genotype and treatment of *Abelmoschus esculentus* and *Cleome gynandra* germination parameters.

There was no significant interaction effect for MGT, CVG and TSG as per the ANOVA **Table 1** (Appendix) in *A. esculentus* genotypes while there was also no interaction effect (Appendix: **Table 2**) on CVG and TSG in *C. gynandra* genotypes. However, there were significant interaction effect of biostimulant application and *A. esculentus* genotypes on FGP, GI and GRI (**Table 3.3**) while there were significant interaction effect on FGP, MGT, GI and GRI for *C. gynandra* (**Table 3.4**). The interaction effect OkraPB1 with Kelpak® (1:100) significantly increased FGP, GI, and GRI when compared to the control. However, there was a decrease in FGP with decreasing Kelpak® dilution treatment in Okra PB1. Although not significantly higher than control, the FGP, GI and GRI increased with increasing PGPR dilution in all *A. esculentus* genotypes.

In *C. gynandra*, no significant stimulatory effect was observed in TOT10212, TOT8420, and *Cleome* 3 in response to biostimulant application. However, biostimulant treatments including Kelpak® (all dilutions) and PGPR (1:10 and 1:15) applied to *Cleome* Maseno genotype seeds significantly enhanced GI, GRI and FGP.

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Table 3.3: Interaction effect of *Abelmoschus esculentus* genotypes and biostimulant (KLP =Kelpak®, PGPR =plant growth promoting rhizobacteria) treatments. FGP= final germination percentage, MGT= mean germination time, GI= germination index, CVG= coefficient of velocity of germination, GRI = germination rate index, TSG =time spread of germination. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Treatment	FGP (%)	GI	CVG
Okra PB1	Control	69.33^{g-j}	235.00^{g-k}	4.46^{b-f}
	KLP 1:100	82.67^{a-f}	286.30^{a-f}	4.94^{a-f}
	KLP 1:40	78.67^{c-h}	266.30^{d-h}	5.75^{a-e}
	KLP 1:20	74.67^{e-i}	250.30^{f-j}	5.52^{a-e}
	PGPR 1:5	64.00^{ij}	214.70^{ijk}	4.25^{b-f}
	PGPR 1:10	72.00^{f-j}	248.30^{f-j}	3.96^{c-f}
	PGPR 1:15	72.00^{f-j}	249.30^{f-j}	3.73^{ef}
Okra PB2	Control	94.67^{a}	326.70^{a}	6.72^{a}
	KLP 1:100	88.00^{a-d}	304.70^{a-d}	5.53^{a-e}
	KLP 1:40	82.67^{a-f}	288.00^{a-f}	4.55^{b-f}
	KLP 1:20	90.67^{abc}	315.70^{abc}	5.52^{a-e}
	PGPR 1:5	17.33^{k}	54.70^{l}	0.453^{h}
	PGPR 1:10	78.67^{c-h}	270.70^{d-h}	4.83^{a-f}
	PGPR 1:15	92.00^{ab}	320.30^{ab}	5.68^{a-e}
Okra PB3	Control	77.33^{d-h}	259.30^{e-i}	5.99^{abc}
	KLP 1:100	74.67^{e-i}	258.30^{e-i}	4.07^{c-f}
	KLP 1:40	81.33^{b-g}	279.30^{b-g}	5.21^{a-e}
	KLP 1:20	78.67^{c-h}	268.30^{d-h}	5.51^{a-e}
	PGPR 1:5	25.33^{k}	78.70^{l}	1.06^{gh}
	PGPR 1:10	61.33^{j}	199.70^{k}	4.67^{a-f}
	PGPR 1:15	78.67^{c-h}	270.00^{d-h}	4.99^{a-e}
Okra PB4	Control	94.67^{a}	330.00^{a}	5.93^{a-d}

	KLP 1:100	85.33^{a-e}	298.30^{a-e}	4.62^{a-f}
	KLP 1:40	88.00^{a-d}	307.30^{a-d}	4.99^{a-e}
	KLP 1:20	86.67^{a-e}	303.00^{a-e}	4.78^{a-f}
	PGPR 1:5	16.00^{k}	51.00^{l}	0.41^{h}
	PGPR 1:10	80.00^{b-g}	277.00^{b-g}	4.63^{a-f}
	PGPR 1:15	82.67^{a-f}	286.70^{a-f}	4.81^{a-f}
Okra PB5	Control	82.67^{a-f}	279.70^{c-g}	6.27^{ab}
	KLP 1:100	89.33^{a-d}	307.30^{a-d}	6.21^{ab}
	KLP 1:40	66.67^{hij}	226.70^{h-k}	3.85^{def}
	KLP 1:20	80.00^{b-g}	275.00^{c-g}	5.00^{a-e}
	PGPR 1:5	21.33^{k}	66.70^{l}	0.91^{gh}
	PGPR 1:10	61.33^{j}	212.70^{jk}	2.847^{fg}
	PGPR 1:15	80.00^{b-g}	275.00^{c-g}	5.02^{a-e}
LSD ($p\leq0.05$)		13.23	44.83	2.11

Table 3.4: Interaction effect of *Cleome gynandra* genotypes and biostimulant (KLP =Kelpak®, PGPR =plant growth promoting rhizobacteria) treatments. FGP= final germination percentage, MGT= mean germination time, GI= germination index, CVG= coefficient of velocity of germination, GRI = germination rate index, TSG =time spread of germination. In each column, values followed by different letters indicate statistically significant ($p\leq0.05$) differences.

Genotype	Treatment	FGP (%)	MGT (day)	GI	GRI (%/day)
TOT10212	Control	62.67^{a}	1.53^{hij}	320,70^{a}	52.03^{a}
	KLP 1:100	56.00^{ab}	2.53^{f-j}	272,30^{ab}	41.60^{abc}
	KLP 1:40	56.00^{ab}	2.67^{f-j}	269,30^{ab}	43.47^{ab}
	KLP 1:20	38.67^{cde}	1.47^{hij}	197,30^{b-f}	31.80^{cd}
	PGPR 1:5	17.33^{g-l}	3.87^{d-i}	77,00^{i-m}	11.87^{f-k}
	PGPR 1:10	40.00^{b-e}	3.30^{e-j}	189,30^{c-g}	21.70^{def}
	PGPR 1:15	28.00^{d-i}	2.53^{f-j}	136,00^{f-k}	18.23^{e-h}

TOT8420	Control	34.67^{c-f}	4.77^{b-h}	149,00^{d-j}	11.83^{f-k}
	KLP 1:100	34.67^{c-f}	8.00^{b}	124,70^{f-k}	5.33^{jkl}
	KLP 1:40	34.67^{c-f}	6.73^{b-e}	131,00^{f-k}	6.30^{i-l}
	KLP 1:20	26.67^{e-i}	7.00^{b-e}	104,00^{h-l}	8.77^{h-l}
	PGPR 1:5	20.00^{f-k}	7.50^{bcd}	72,30^{j-m}	3.13^{kl}
	PGPR 1:10	44.00^{bcd}	5.30^{b-g}	183,70^{c-g}	11.77^{f-k}
	PGPR 1:15	40.00^{b-e}	6.27^{b-f}	156,70^{d-h}	10.20^{g-l}
Cleome 3	Control	1.33^{lm}	1.33^{hij}	6,00^{m}	0.33^{l}
	KLP 1:100	1.33^{lm}	0.67^{ij}	6,70^{m}	0.67^{kl}
	KLP 1:40	4.00^{klm}	5.50^{b-g}	12,00^{m}	0.63^{kl}
	KLP 1:20	0.00^{m}	0.00^{j}	0,00^{m}	0.00^{l}
	PGPR 1:5	0.00^{m}	0.00^{j}	0,00^{m}	0.00^{l}
	PGPR 1:10	0.00^{m}	0.00^{j}	0,00^{m}	0.00^{l}
	PGPR 1:15	1.33^{lm}	4.00^{c-i}	3,30^{m}	0.10^{l}
Cleome Maseno	Control	8.00^{j-m}	4.00^{c-i}	36,00^{lm}	3.07^{kl}
	KLP 1:100	44.00^{bcd}	2.23^{g-j}	215,00^{b-e}	26.17^{de}
	KLP 1:40	46.67^{abc}	2.43^{g-j}	239,30^{bc}	40.57^{bc}
	KLP 1:20	44.00^{bcd}	2.17^{g-j}	217,70^{bcd}	30.33^{cd}
	PGPR 1:5	16.00^{g-m}	3.97^{d-i}	73,00^{j-m}	8.33^{h-l}
	PGPR 1:10	30.67^{c-h}	2.77^{f-j}	148,70^{d-j}	20.63^{d-g}
	PGPR 1:15	25.33^{e-i}	3.83^{d-i}	119,30^{g-k}	16.00^{e-j}
Cleome Arusha	Control	32.00^{c-g}	4.00^{c-i}	152.00^{d-i}	20.67^{d-g}
	KLP 1:100	14.67^{h-m}	3.77^{d-i}	68.70^{klm}	7.97^{h-l}
	KLP 1:40	29.33^{d-h}	4.00^{c-i}	138.00^{e-k}	16.30^{e-j}
	KLP 1:20	24.00^{e-j}	3.80^{d-i}	104.00^{h-l}	8.07^{h-l}
	PGPR 1:5	12.00^{i-m}	11.90^{a}	30.30^{lm}	1.00^{kl}
	PGPR 1:10	29.33^{d-h}	2.87^{f-j}	132.30^{f-k}	17.03^{e-i}

	PGPR 1:15	18.67$^{f\text{-}k}$	7.73^{bc}	64.00^{klm}	3.50^{kl}
LSD ($p\leq0.5$)		16.244	1.325	77.66	4.051

3.4. Discussion

3.4.1. Effect of genotype on germination parameters of *Abelmoschus esculentus* and *Cleome gynandra*

The plant gene play a major role in the germination process (phase II) where it is responsible for DNA repair and synthesis (Nonogaki et al., 2010).. This has been proven by various studies (Panobianco and Viera, 1996, Babiker et al., 2017, Khayamim et al., 2014). Even though there are not many studies focusing on the effect of genotype on germination parameters (MGT, GI, CVG and TSG) a few exist focusing on standard germination and germination rate.

Similar to the current study **(Table 3.3** and **3.4**), genotypes of the *Glycine max* showed varying germination percentages (Panobianco and Viera, 1996). Genotype MGBR 87-42 had significantly low germination percentage while two genotypes- EMGOPA 309 and MTBR 89-1053 had significantly high germination percentage (Panobianco and Viera, 1996). In a study conducted by Babiker et al. (2017), all genotypes studied (14, 1, 16, 33, 41, 53, 56, 63, 67, 83, 86 and 95) of *Triticum aestevium* had significantly the same seed germination and germination rate. Contrary to the current study, these results suggest that the gene expression of these genotypes is almost similar (especially at the initial stages of growth), all the phases of germination (imbibition, mobilization of food reserves and radicle emergence) were in sync and hence a seedling growth and stand establishment will be synchronised. A study conducted by Khayamim et al. (2014) on *Beta vulgaris* subsp. *vulgaris* convar. *vulgaris* var. altissima discovered that genotypes had varying effects on seed germination. Genotype 7233 p.12 had significantly least germination percentage and genotype 452 had significantly high germination percentage while genotype *B. maritima* had no germination observed (Khayamim et al., 2014). Similar to *A. esculentus* and *C. gynandra* genotypes, *Jatropha curcas* genotypes also showed varying effects on germination (Islam et al., 2009). For instance, genotypes UKM-UJ-016 and UKM-UJ-012 had significantly higher germination percentage and genotypes UKM-UJ-004 and UKM-UJ-005 had significantly low germination percentage (Islam et al., 2009). Furthermore, genotype UKM-UJ-017 had statistically higher germination index while UKM-UJ-004 had least germination index (Islam et al., 2009). The germination ability of genotypes of the species is entirely dependent on their genetic composition.

3.4.2. Effect of biostimulant application and their interaction effect with *Abelmoschus esculentus* and *Cleome gynandra* genotypes on germination parameters.

Seaweed extracts are known to promote seed germination, growth rate, shoot, and root development (Sasikala et al., 2016). In the current study, Kelpak® alone had no positive effect on germination parameters when compared to the control. However, the interaction of Kelpak® with *Cleome* Maseno (all dilutions) and Okra PB1 (1:100) increased the germination of these genotypes. These results are in agreement with the findings by various authors (Carvalho et al., 2013, El-Sheekh et al., 2016, Sasikala et al., 2016), that highlighted the positive effect of brown seaweed extracts on seed germination.

The priming of *Phaseoulus vulgaris* seeds with *Ascophyllum nodosum* significantly increased the speed index of germination when compared to the control (Carvalho et al., 2013). Furthermore, *Sargassum vulgare*, *Colpomenia sinuosa*, and *Padina pavonica*, all at 5% (v/v), positively influenced the germination percentage of *Trigonella foenum-graecum* L. relative to the control (El-Sheekh et al., 2016). These species further increased the mitotic index of *Trigonella foenum-graecum* L., indicating acceleration of radicle emergence and hence improved germination (El-Sheekh et al., 2016). The application of *Sargassum tenerrimum* 0.8% (v/v) to *Solanum lycopersicum* seeds resulted in 100% germination, which was significantly high when compared to control (Sasikala et al., 2016). In constant darkness at 15 ℃, brown seaweed (*Cystoseira barbata*) extracts significantly increased the germination percentage of *Solanum lycopersicum* and *Solanum melongena* seeds when compared to the control (Demir et al., 2006). However, at 25℃, brown seaweed had no positive effect on the germination percentage of *Solanum lycopersicum* while the germination percentage of *Solanum melongena* was significantly improved (Demir et al., 2006). These brown seaweed extracts had no significant effect on the mean germination time relative to the control (Demir et al., 2006). Likewise, in the current study, Kelpak® (all dilutions) did not improve the MGT of both *A. esculentus* and *C. gynandra* seeds. The interaction of Kelpak® treatments with genotypes increased germination in some instance and significantly decreased it in order cases. Kelpak® (1:20) decreased the germination of genotype TOT10212, TOT8420, and *Cleome* 3.

The efficacy of seaweed extracts is affected by their concentration and in most cases, it is enhances plant growth attributes at low concentrations (Michalak et al., 2017). The efficacy of seaweed extracts further vary between species and this is mainly caused by the biochemical contents and location of the species (El-Sheekh et al., 2016). Brown seaweed extracts contain more bioactive compounds when compared to other species of seaweeds (Battacharyya et al., 2015). Seaweed extracts further stimulate and accelerate cell division, elongation, differentiation, and protein synthesis (El-Sheekh et al., 2016). This explains the stimulating effect or acceleration of seed germination by Kelpak®. According to Demir et al. (2006), the application of seaweed extracts under the plant's favorable conditions has limited effect. However, under stress conditions, significant promotion of seed germination is more eminent. The beneficial effects of seaweed extracts on seed germination are attributed to the presence of phytohormones such as gibberellic acid and auxins (Michalak et al., 2017, Hidangmayum and Sharma,

2015, Altindal, 2019). Even though auxins do not directly affect seed germination, they facilitate the biosynthesis of gibberellic acid.

In this study, PGPR treatments did not increase germination. In TOT8420, *Cleome* 3, and Okra PB1, PGPR had no significant effect when compared to the control while it decreased germination in Okra PB2, PB3, PB4, PB5, TOT10212, *Cleome* Maseno and *Cleome* Arusha. These results are in contradiction with the findings by Mangmang et al. (2016), where the application of *A. brasilense* (Sp7, Sp7-S, and Sp245) to tomato seeds, increased germination while in lettuce only Sp7 strain promoted germination value relative to the control (Mangmang et al., 2016). However, they are in agreement with those of Stamenov et al. (2018). In *Allium cepa* seeds, *Bacillus* sp. and *Pseudomonas* sp. reduced germination (%) while *Azotobacter* sp had no significant effect when compared to the control (Stamenov et al., 2018). The authors elucidated that *Bacillus* sp. and *Pseudomonas* sp. produce hydrogen cyanide (HCN) gas, which when available in larger quantities becomes toxic to the seed and hence inhibits germination. Treatment of *Cuscuta campestris* seeds with *Bacillus* sp. had no significant effect on seed germination when measured against the control (Sarić-Krsmanović et al., 2017). *Bacillus subtilis* promoted the germination (%) of sorghum var. CSH-14 and Proagro by 2 and 1% (v/v), respectively with respect to control (Prathibha and Siddalingeshwara, 2013). *B. brevis* significantly increased the germination (%) and rate of *Gossypium hirsutum* (Nehra et al., 2016). Gibberellic acid promotes germination, while auxins promote the biosynthesis of gibberellic acid, which therefore triggers the activities of ∝-amylase (Mangmang et al., 2016). As applicable with seaweed extracts, the efficacy of PGPR is also dependent on the environmental conditions as they can alter activity of the PGPR (Yadav et al., 2010). Furthermore, the efficacy of this type of biostimulant is dependent on the plant species and genotype (cultivar).

3.5. Conclusion

The application of biostimulants influenced the germination parameters of *A. esculentus* and *C. gynandra* and may potentially affect seedling growth and yield. This study showed that the effect of biostimulants varies in different plant species and within genotypes of the same species. Furthermore, the efficacy of biostimulants depended on genotype and biostimulants concentration. Diverse responses, including stimulatory, inhibitory, and neutral effects, were demonstrated for the different treatments. This is because of the observed effect of different Kelpak® concentrations on the two test plant species and their respective genotypes and the toxic impact of PGPR treatments that remained noticeable in both plant species. Overall, this study demonstrated the importance and the contribution of biostimulants-type and concentration on seed germination.

Chapter 4: Effects of biostimulants on the growth and yield responses of *Abelmoschus esculentus* and *Cleome gynandra* genotypes.

4.1. Introduction

Following seed germination, seedling establishment and plant growth are important stages in plant production. During plant growth, several biotic (pest and disease manifestation) and abiotic (e.g. drought, flood and soil nutrient depletion) challenges may arise. Particularly, soil nutrient deficiencies have a negative impact on plant growth. For instance, shortage of nitrogen results in chlorosis, which reduces photosynthetic activity and hence production of soluble sugars for plant growth (Yeh et al., 2000). The need for soil amendments/inputs, such as chemical fertilizers, that improve soil nutritional deficiencies during plant growth remains high which ameliorate soil nutrition deficiencies and improve plant growth. However, an indiscriminate use of chemical fertilizers can have a negative impact on environment (Tahat et al., 2020).

Biostimulants are known for diverse benefits such as increasing crop yield, nutritional content of plant tissue, increase tolerance to abiotic stress, quality traits, and nutrient use and plant metabolism efficiency (Du Jardin, 2015, Calvo et al., 2014). Furthermore, biostimulants improve water efficiency, decrease soil pH and interact with plant signalling processes (Brown and Saa, 2015). Therefore, biostimulants have the potential to increase the growth and yield of multipurpose plants such as *Abelmoschus esculentus* and *Cleome gynandra*. This study evaluated the effect of selected biostimulants (Kelpak® and plant growth promoting rhizobacteria) and genotype on the growth and yield of *A. esculentus* and *C. gynandra*.

4.2. Materials and methods

4.2.1. Source of biostimulants and seeds

Kelpak® was obtained from Kelp Products (Pty) Ltd, Simon's Town, South Africa. Plant growth promoting rhizobacteria (commercial solution) (a mixture of organic acids, *Bacillus* sp., amino/fulvic acid, and soil bacteria) was purchased from Agriman (Pty) Ltd, South Africa. Seeds of *A. esculentus* and *C. gynandra* were obtained from Agricultural Research Council-Vegetables, Industrial and Medicinal Plants, Pretoria, South Africa. . These experiments were conducted in the glasshouse at the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants, Pretoria, South Africa. Seeds were sterilized and soaked as described in section 3.2.2 (following a completely randomized

design). However, *C. gynandra* genotype *Cleome* 3 was excluded due to low germination activity observed in section 3.3).

4.2.2. Planting, seedling growth and yield

The experiment was established in potting soil, which consisted of 12% clay (**Table 4.1**). The potting soil consisted a fair amount of soil nutrients (soil properties presented in **Table 4.1**). Seeds were sown directly into 25 cm diameter pots in a glasshouse, with temperatures 25 ℃ for *A. esculentus* and 30/20 ℃, day/night temperatures for *C. gynandra*. The current study involved two factors, where effect of biostimulant application and genotypes were considered. After planting, treatments were arranged in a completely randomised block design, replicated five times. Pots were monitored daily and irrigated every 24 h. Consecutively, 100 ml of biostimulant treatments per plant ([Kelpak® solution (1:100, 1:40 and 1:20) and plant growth promoting Rhizobacteria (PGPR) (1:5, 1:10 and 1:15 v/v)] or distilled water (used as a control) was applied through soil drenching after every two weeks until termination. After successful establishment two months after planting, growth parameters (plant height, number of leaves, stem diameter and chlorophyll content) were measured weekly. *A. esculentus* experiment commenced on 21 September 2019 while *C. gynandra* commenced on 2 April 2020. Harvesting was done after five and three months of planting for *A. esculentus* and *C. gynandra*, respectively. Upon harvesting, fresh and dry weights of the pods for *A. esculentus* while fresh and dry weight of *C. gynandra* of leaves were recorded.

Table 4.1: Chemical and physical properties of potting soil used in the current study.

Properties	Feature	Units	Value
Chemical	Element		
	P	mg/kg	209.7
	K	mg/kg	4210
	Ca	mg/kg	5420
	Mg	mg/kg	1630
	Na	mg/kg	1120
	pH		6.05
	Total Acid	cmol(+)/kg	0
	Total Cations	cmol(+)/kg	56.1005
Physical	Clay Content	%	12

4.2.3. Data analysis

Data were analyzed using analysis of variance (ANOVA) following a completely randomised block designusing Genstat 64-bit Release 18.2 (PC/Windows 8). For statistical significance ($p \leq 0.05$), mean values were separated using Fischer's (least significant difference) LSD test.

4.3. Results

4.3.2. Effect of biostimulant treatments on growth and yield of *Abelmoschus esculentus* and *Cleome gynandra*.

All treatments except Kelpak® (1:100) significantly increased plant height compared to the control (**Figure 4.5**). There was no significant difference in the number of leaves among biostimulant treatments. Application of PGPR (1:5 and 1:10) significantly increased the chlorophyll content while all PGPR treatments significantly improved stem diameter when compared to the control. In terms of yield, Kelpak® (1:20) and all PGPR treatments significantly improved the number of pods, total dry weight of pods and total fresh weight of pods (**Figure 4.6**).

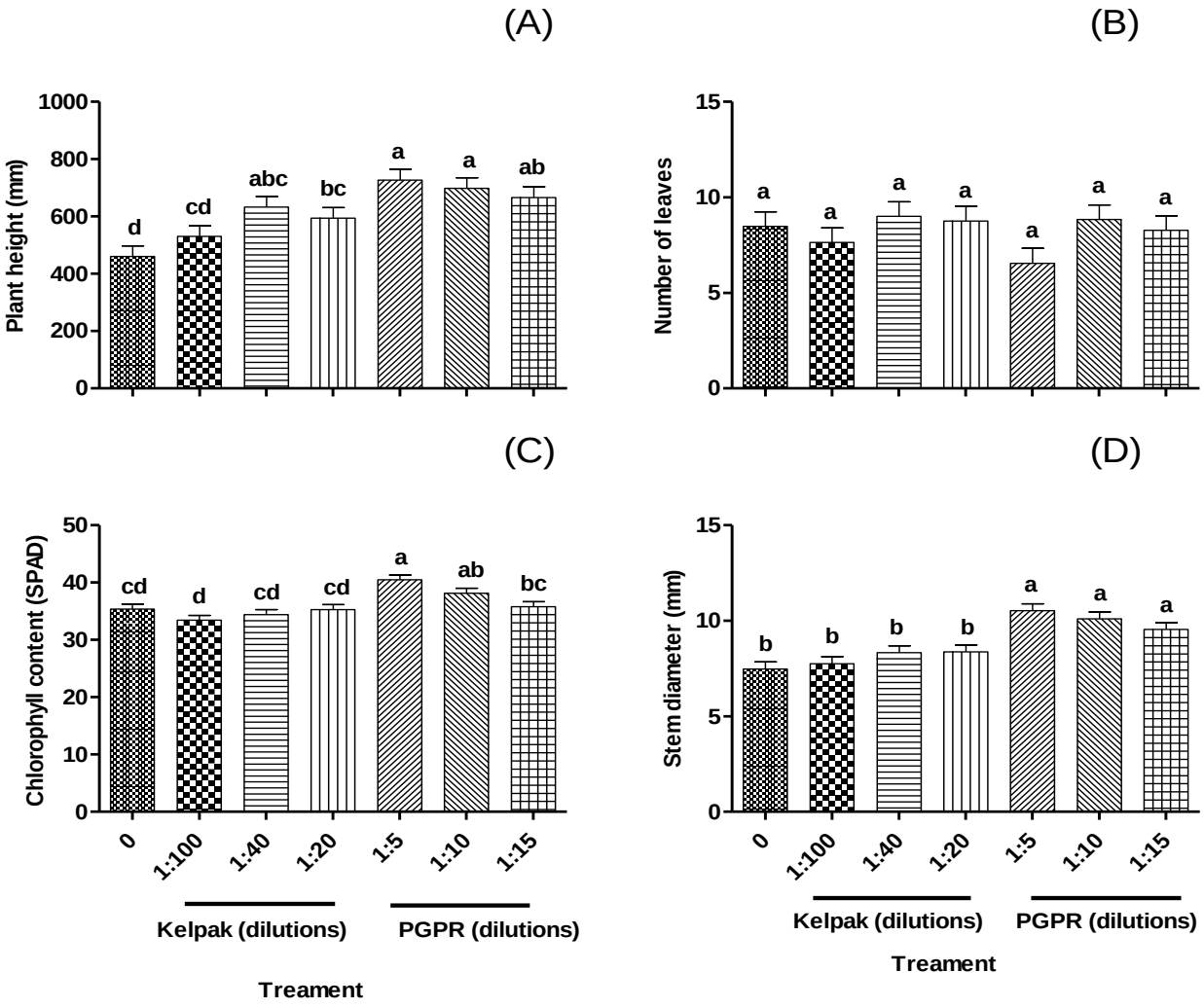

Figure 4.1: Effects of biostimulant treatments on growth parameters of *Abelmoschus esculentus* genotypes (A) plant height (mm), (B) number of leaves, (C) chlorophyll content (SPAD) and (D) stem diameter (mm). Bars with a different letter(s) are significantly different ($p \leq 0.05$).

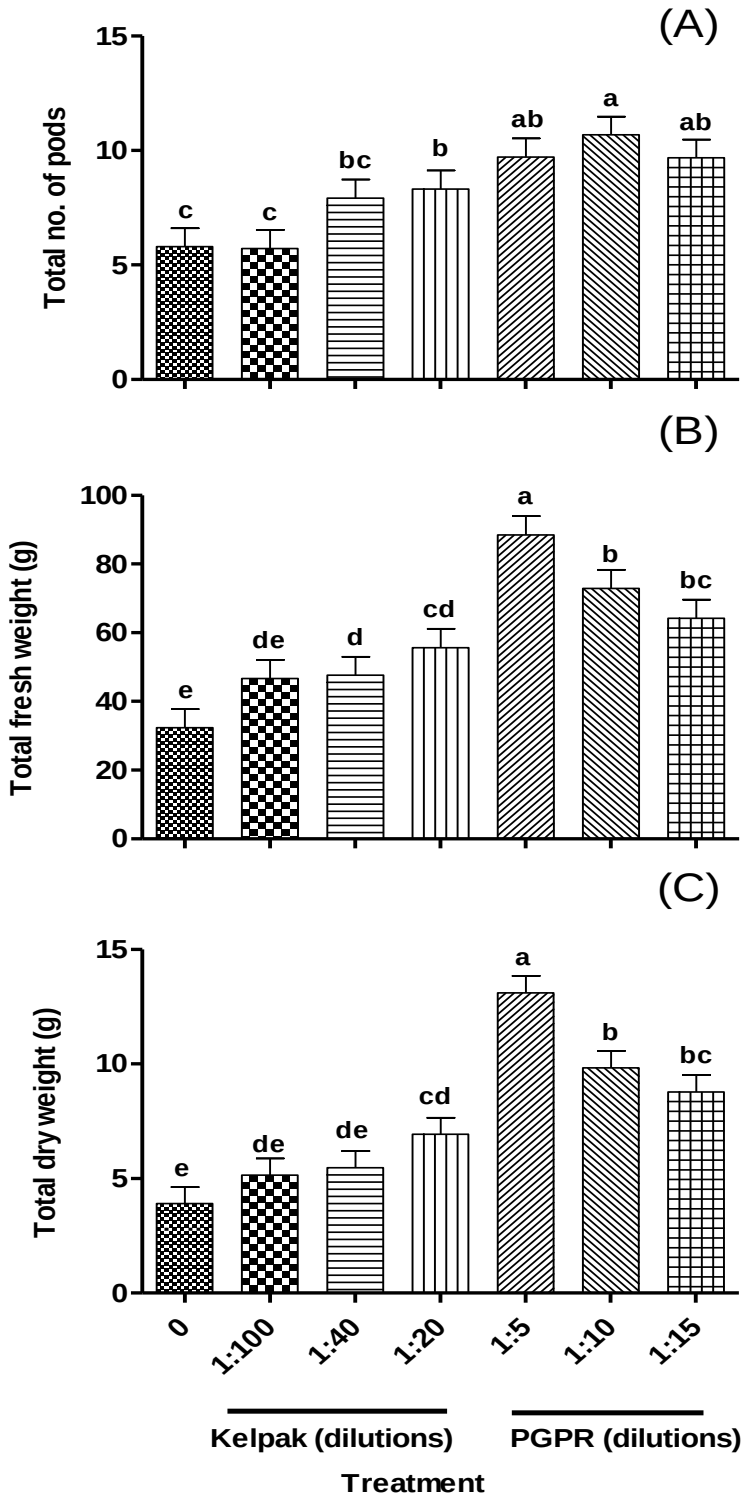

Figure 4. 2: Effects of biostimulant treatments on growth parameters of *Abelmoschus esculentus* genotypes (A) total number of pods, (B) total fresh weight of pods (g) and (C) total dry weight of pods (g). Bars with a different letter(s) are significantly different ($p \leq 0.05$).

Biostimulant application did not affect the plant height and number of leaves of *C. gynandra* genotypes (**Figure 4.7**). However, PGPR (1:5 and 1:10) significantly enhanced stem diameter. In *C. gynandra* yield, Kelpak® (1:100, 1:40 and 1:20) and PGPR (1:15) had no significant effect on both the total leaf fresh and dry weights (**Figure 4.8**). These two parameters (total fresh and dry weight) were significantly improved by PGPR (1:5 and 1:10). According to ANOVA **Table 5** (Appendix), there was no significant treatment effect at $p \leq 0.05$ for chlorophyll content.

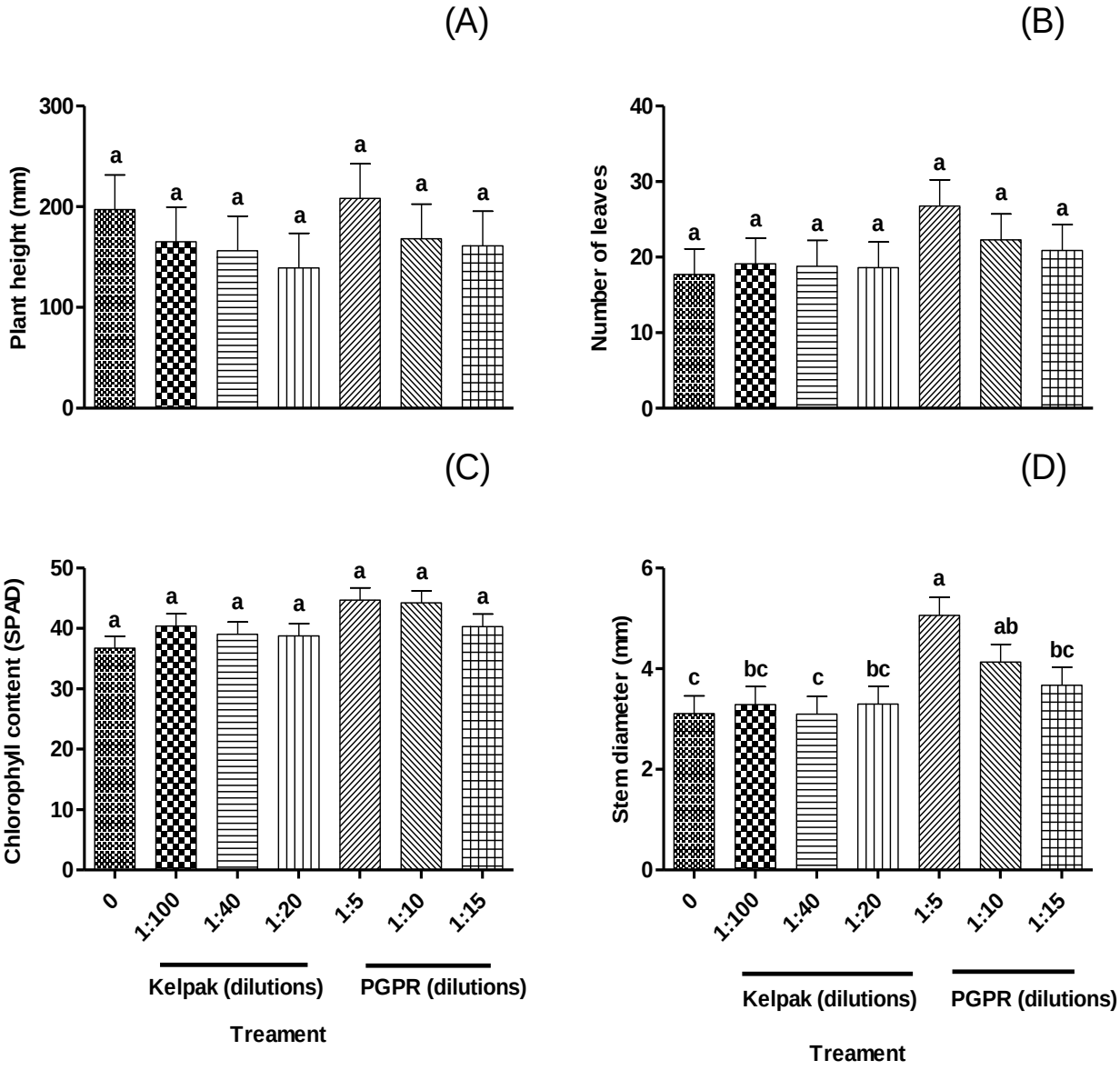

Figure 4.3: Effects of biostimulant treatments on growth parameters of *Cleome gynandra* genotypes (A) plant height (mm), (B) number of leaves, (C) chlorophyll content (SPAD) and (D) stem diameter (mm). Bars with a different letter(s) are significantly different ($p \leq 0.05$).

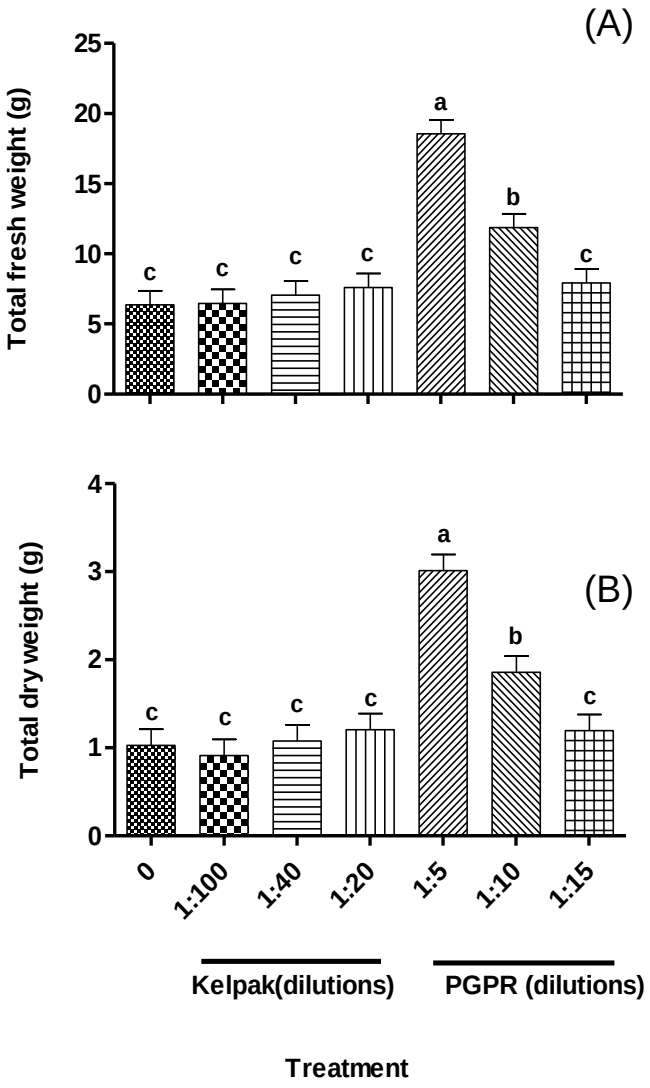

Figure 4.4: Effects of biostimulant treatments on growth parameters of *Cleome gynandra* genotypes (A) total fresh weight of harvested leaves and (B) total dry weight of harvested leaves (g). Bars with different letters are significantly different ($p \leq 0.05$).

4.4. Discussion

4.4.1. Effect of biostimulant application on growth and yield of *Abelmoschus esculentus* and *Cleome gynandra* genotypes.

Biostimulants are well known for their stimulatory effect in many plants (Calvo et al., 2014, Brown and Saa, 2015, Yakhin et al., 2016). In the current study, Kelpak® did not stimulate growth and yield of Okra PB1 and PB5, as well as TOT10212, TOT8420 and *Cleome* Arusha. On the other hand, Kelpak® treatments enhanced the plant height and stem diameter of Okra PB3 and PB4. This is in agreement with the findings by Wang et al. (2017). The authors observed that the plant height of *Malus hupehensis* Rehd. seedlings was significantly enhanced by the application of brown seaweed extracts (*Lessonia nigrescens* and *Lessonia flavicans*) relative to the control (Wang et al., 2017). Furthermore, the biostimulant enhanced the chlorophyll content of *Malus hupehensis*. Similar effect was observed in the current study where *Cleome* Maseno's chlorophyll content was significantly increased following the application of Kelpak®.

Ecklonia maxima extracts had no stimulatory effect on the leaf number of *Brassica rapa* L. subsp. *sylvestris* (Di Stasio et al., 2017). However, a significant increase in SPAD (Soil Plant Analysis Development) index and yield was observed when *Ecklonia maxima* extracts were applied to *Brassica rapa* L. subsp. *sylvestris* (Di Stasio et al., 2017). A brown seaweed *Sargassum vulgare*, significantly increased plant height, number of leaves, root diameter, yield and the chlorophyll content of *Raphanus sativus*, when measured against the control (Mahmoud et al., 2019). Kelpak® is a commercially available brown seaweed extract that is predominantly high in cytokinins, auxins, gibberellins, brassinosteroids, polyamines, phlorotannins, aliginates, amino acids, mannitol but low in abscisic acid, macro-elements and micro-elements (Kocira et al., 2020, Lötze and Hoffman, 2015). A study found that single foliar spraying with Kelpak® (0.4% v/v) significantly increased number of pods of *Phaseolus vulgaris* cultivar Aura while it had no significant effect on Toska (Kocira et al., 2018). Likewise in the current study, Kelpak® (1:40) significantly enhanced the total number of pods, dry and fresh weight of Okra PB1 and dry weight of Okra PB3. In a study conducted by Arthur et al. (2003), soaking of seeds in Kelpak® (0.4% v/v) prior to planting in combination with Kelpak® (0.4% v/v) as foliar spray significantly enhanced the mass of marketable *Capsicum annuum* (var. Indra) when compared to the control, soaking of seeds prior to planting and foliar spraying methods. However, in other *Capsicum annuum* varieties (Orobelle and King Arthur), Kelpak® (0.4% v/v) treatment had no significant effect on the fruit mass (Arthur et al., 2003). Phytohormone content in seaweed extracts may contribute to their stimulating effect. For instance, cytokinins regulate vascular development and promote flower

development while auxins promote cell elongation in the coleoptile and rooting (Farooq et al., 2018). Furthermore, auxins enhance the production of adventitious roots, overall cell division and formation of meristem (Farooq et al., 2018). The synergistic effect of seaweed extracts, genotype and/or environment may contribute to the efficacy of seaweed extracts on plant growth, development and yield (Rathore et al., 2009, Kocira et al., 2020).

Based on increasing evidence (Ahemad and Kibret, 2014, Sansinenea, 2019, Jamal et al., 2018, Ruzzi and Aroca, 2015), PGPR promote plant growth though the synthesis of plant growth regulators, promoting symbiotic N_2 fixation and solubilisation of mineral phosphate and other nutrients. The efficacy of PGPR is dependent on environmental factors such as composition of microbial flora and soil characteristics (Yadav et al., 2010). In the current study, PGPR stimulated growth and yield parameters of *A. esculentus* and *C. gynandra* genotypes, which is in agreement with various studies (Orhan et al., 2006, Samaniego Gámez et al., 2016, Gowtham et al., 2018). In *Rubus idaeus*, *Bacillus* strain M3 significantly enhanced plant height while strain OSU-142 decreased this parameter relative to the control (Orhan et al., 2006). Similarly, plant stem diameter was significantly improved by strain M3 and while being significantly reduced by strain OSU-142 (Orhan et al., 2006). However, both bacterial strains significantly improved number of berries when compared to the control (Orhan et al., 2006). *Bacillus* spp. (strains M9 and K46) had no significant effect on plant height of *Capsicum annuum* while stem diameter and fresh weight were significantly enhanced by strain M9 relative to the control (Samaniego Gámez et al., 2016). Strain M9 further significantly increased chlorophyll content when compared to strain K46 and control (Samaniego Gámez et al., 2016). Inoculation of *Bacillus* strains significantly enhanced stem and leaf dry weight and SPAD value in *Cannabis sativa* 'Finola' when compared to the control (Pagnani et al., 2018). Similarly in the current study, PGPR (1:10) significantly enhanced chlorophyll content of TOT10212 while PGPR (1:5, 1:10 and 1:15) had no stimulatory effect on TOT8420. In *Capsicum annuum*, *B. amyloliquefaciens* and *B. cepacia* application significantly enhanced plant height and number of leaves when measured against the control (Gowtham et al., 2018).

Root exudates further play a role in the efficacy of PGPR. Their interaction with PGPR can either impede or promote plant nutrient cycling and thus reducing the need for chemical fertilizers. Often PGPR is referred to as bio-fertilizers, rhizoremediators and phytostimulators because of their role in plant growth (Jamal et al., 2018). The plant growth promoting effect of *Bacillus* spp. is further dependent on the location it is extracted from. Araújo et al. (2012) discovered that *Bacillus* isolates from locations Taciba, Pirapozinho, Nova Granada, Penapólis and Castilho significantly enhanced plant height of *Zea mays* while isolates from Birigui location had no significant effect when compared to the control. Moreover, *Bacillus* spp. promote the production of lytic enzymes, secondary metabolites and phytohormones (Tsukanova et al., 2017a), which may facilitate the formation of lateral roots, root hairs and primary root elongation. *Bacillus* spp. further plays a role in enhanced nutrient absorption by plants (Sansinenea, 2019, Radhakrishnan and Baek, 2017).

4.5. Conclusion

The application of biostimulants affected the growth and yield of both *A. esculentus* and *C gynandra* genotypes and is most likely to influence the biochemical and mineral elements content. The current study demonstrated that the effect of biostimulant vary with type,concentration, and genotype it is applied to. Kelpak® and PGPR treatments had varying effect on the growth and yield of genotypes of different plants. Even though Kelpak® enhanced growth and yield at a lesser extent when compared to PGPR, it did not inhibit either the growth or yield parameters relative to control. In conclusion, PGPR application had an overall positive impact on the growth and yield of *A. esculentus* and *C. gynandra* and can be used to combat food insecurity, especially in developing regions. This study further demonstrates that biostimulants may have neutral effect on growth and yield of plants, therefore, more studies need to be conducted that will focus on optimizing the promontory effect biostimulants on plant growth and yield.

Chapter 5: Effects of biostimulants on biochemical content and mineral elements of *Cleome gynandra* and *Abelmoschus esculentus* genotypes.

5.1. Introduction

Globally, more than 2 million people suffer from mineral and vitamin deficiencies (Theodore, 2010). Malnutrition results in infections and decreases the immune defence, thus hindering affected individuals from achieving their full mental and physical potentials (Hendricks et al., 2016). Malnutrition is often attributed to inadequate consumption of nutritious foods (Hendricks et al., 2016). The incorporation of nutritious plants in human diets can assist in combatting vitamin and mineral malnutrition. *Abelmoschus esculentus* and *Cleome gynandra* are potential plants for combatting nutritional deficiencies.

Biostimulants have been explored for their potential to reduce the agricultural chemical footprint, their ability to improve phytochemicals and as well as nutritional content (Rafiee et al., 2016). This is because biostimulants have a high range of bioactive compounds that positively interact with the environment to improve plant secondary metabolites (Halpern et al., 2015). In response to the environment, biostimulants may enhance the concentration of bioactive compounds, including phenolic compounds and antioxidants (Yakhin et al., 2016). Biostimulants play a significant role in plant secondary metabolism, amongst other ways, through the provision of co-factors of anti-oxidative enzymes that are provided by mineral elements of seaweed extracts and by that solubilised by microbial inoculants (Van Oosten et al., 2017, Yakhin et al., 2016). Plant biostimulants can either activate signalling processes/metabolic pathways or enhance endophytic molecules and microbial populations that play a role in improving the biochemical content and mineral elements (Brown and Saa, 2015, Yakhin et al., 2016). Furthermore, microbial inoculants enable the absorption of K^+ (responsible for enzymatic activation) and prevent that of Na^+ absorption and accumulation in the rhizosphere and hence contribute to the content of mineral element (Van Oosten et al., 2017). The present chapter aimed to determine the effect of biostimulants on the biochemical and mineral element contents in *A. esculentus* and *C. gynandra* genotypes. The parameters investigated were β-carotene, vitamin C, total phenolic, total flavonoid, condensed tannins, and mineral elements (Ca, Fe, K, Mg, Na, and Zn).

5.2. Materials and methods

5.2.1. Source of plant materials and chemicals

The plant materials were obtained from the harvested *A. esculentus* (five genotypes: Okra PB1, PB2, PB3, PB4, and PB5) pods and *C. gynandra* (four genotypes: TOT10212, TOT8420, *Cleome* Maseno and *Cleome* Arusha) leaves treated with Kelpak® solution (1:100, 1:40 and 1:20) and plant growth promoting Rhizobacteria (PGPR) (1:5, 1:10 and 1:15 v/v). The plant samples were weighed and stored in a -80℃ freezer. Subsequently, the freeze-dried plant materials were ground and used for further analysis. All the chemicals used for the experiments in the current chapter are outlined in Appendix (**Tables 6-9)**.

5.2.2. Determination of total phenolic compounds (TPC)

Total phenolic compounds (TPC) were determined using the Folin-Ciocalteu method as described by Makkar (2000). Ground plant sample (0.2 g) was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, 50 μL of plant extract was transferred into reaction tubes followed by 450 µL of distilled water, 250 µL of 1 N Folin-Ciocalteu reagent, and 1250 μL of Na_2CO_3 (2%) solution. The reaction mixture was sonicated and incubated under dark condition for 40 min at room temperature. Absorbance was measured at 725 nm and a blank was prepared in a similar manner, except the plant extract was replaced with a solvent (50% methanol). TPC was calibrated by using a standard curve of gallic acid.

5.2.3. Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was determined according to the method described by Marinova et al. (2005). A ground sample of 0.2 g was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, an aliquot of 250 µL plant extract was added into a reaction tube. Thereafter, 1 mL of distilled water and 75 µL of 5% $NaNO_2$ was added. After 5 min, 75 µL of 10% $AlCl_3$, 0.5 mL of 1 M NaOH, and 0.6 mL of distilled H_2O was added. The reaction mixture was further sonicated to mix thoroughly and measured for absorbance at 520 nm. A blank was prepared in a similar manner except that plant extract was replaced with a solvent, 50% methanol. TFC was calibrated by using a standard curve of catechin.

5.2.3. Determination of condensed tannins

Condensed tannins were determined by the HCl-butanol method as described by Makkar (2000) with slight modifications. A ground sample of 0.2 g was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, plant extract (500 µL) was added into reaction tubes followed by

3000 µL of butanol-HCl added into the tube, and 100 µL of ferric reagent. The mixture was vortexed to mix thoroughly. The heated and unheated blanks were then prepared. The unheated blank was prepared by adding 0.5 mL of the extracted sample with 3 mL of butanol-HCl reagent and 0.1 mL of ferric reagent, while the heated blank was prepared by adding 0.5 mL of the extracted sample with 3 mL of butanol (only) and 0.1 mL of ferric reagent. The heated blanks and samples were incubated at 100 ℃ for 60 min and were then cooled at room temperature. The absorbance of both blanks was measured at 550 nm. The absorbance of the unheated blank was subtracted from the heated blank. Condensed tannins were calibrated by using a standard curve of cyanidin chloride.

5.2.4. Determination of β-carotene.

The β-carotene content was determined using a method described by Biehler et al. (2010) with modifications. Ground samples (0.2 g) was extracted using 10 mL of ice-cold hexane: acetone (1:1). A total of 15 mL of saturated NaCl was added to the reaction mixture. The mixture was vortexed and centrifuged (HERMLE Z513, Germany) (at 2000 rpm) for about two min each, to achieve phase separation to form a distinct aqueous polar layer and a non-polar layer. Aliquots of 20 µL extracts from the top layer of the nonpolar phase were withdrawn and filtered through a syringe filter (0.45 μm) and were injected into the High-Performance Liquid Chromatography (HPLC) system (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan). The β-carotene content of samples was calculated from peak area generated from β-carotene standard calibration curve.

- HPLC conditions- C18 Luna 150 × 4.5 mm
    - 5 μm column
    - Temperature: 35 ℃
    - Mobile phase: Acetonitrile: Dichloromethane: Methanol (7:2:1)
    - Flow rate:1.0 mL/ min
    - Detection wavelength: 450 nm.

5.2.5. Determination of vitamin C

Vitamin C content was determined using a method described by Odriozola-Serrano et al. (2007) with modifications. Extraction was done by adding 10 mL of 4.5% metaphosphoric acid into 0.2 g of sample in reaction tubes. The tubes were vortexed (Velp Scientifica Vortex Mixer, Europe), ice-cold sonicated for 30 min, and centrifuged at 2000 rpm for about 2 min. The mixture was then filtered into a 10 mL volumetric flask and further transferred into the vial (20 µL) for further analysis using HPLC. The standard curve was calibrated using ascorbic acid.

- HPLC conditions- C18 Luna 150 × 4.5 mm

- 5 μm column
- Temperature: 35 ℃
- Mobile phase: H_2O: Acetonitrile: Formic acid (99:0.9:0.1)
- Flow rate:1.0 mL/min
- Detection wavelength: 245 nm.

5.2.6. Determination of mineral element content

The mineral elements were quantified using an inductively coupled plasma - optical emission spectrometry (ICP-OES) (ICPE-9820, Shimadzu Corporation, Kyoto, Japan) as described by Ang and Lee (2005). Approximately 0.5 g of finely ground dried samples was wet digested using a mixture of nitric acid (65%) and hydrochloric acid (37%) (1:3 v/v). Digestion was further conducted on a 95 ℃ hot plate, after which an amount of 100 mL of distilled water was added to the reaction mixture. Each sample was digested in triplicates. Mineral elements in the digested plant materials were determined using the ICP-OES as according to Ang and Lee (2005).

5.2.7. Data analysis

Data were analyzed using analysis of variance (ANOVA) using Genstat 64-bit Release 18.2 (PC/Windows 8). For statistical significance ($p \leq 0.05$), mean values were separated using Fischer's (least significant difference) LSD test.

5.3. Results

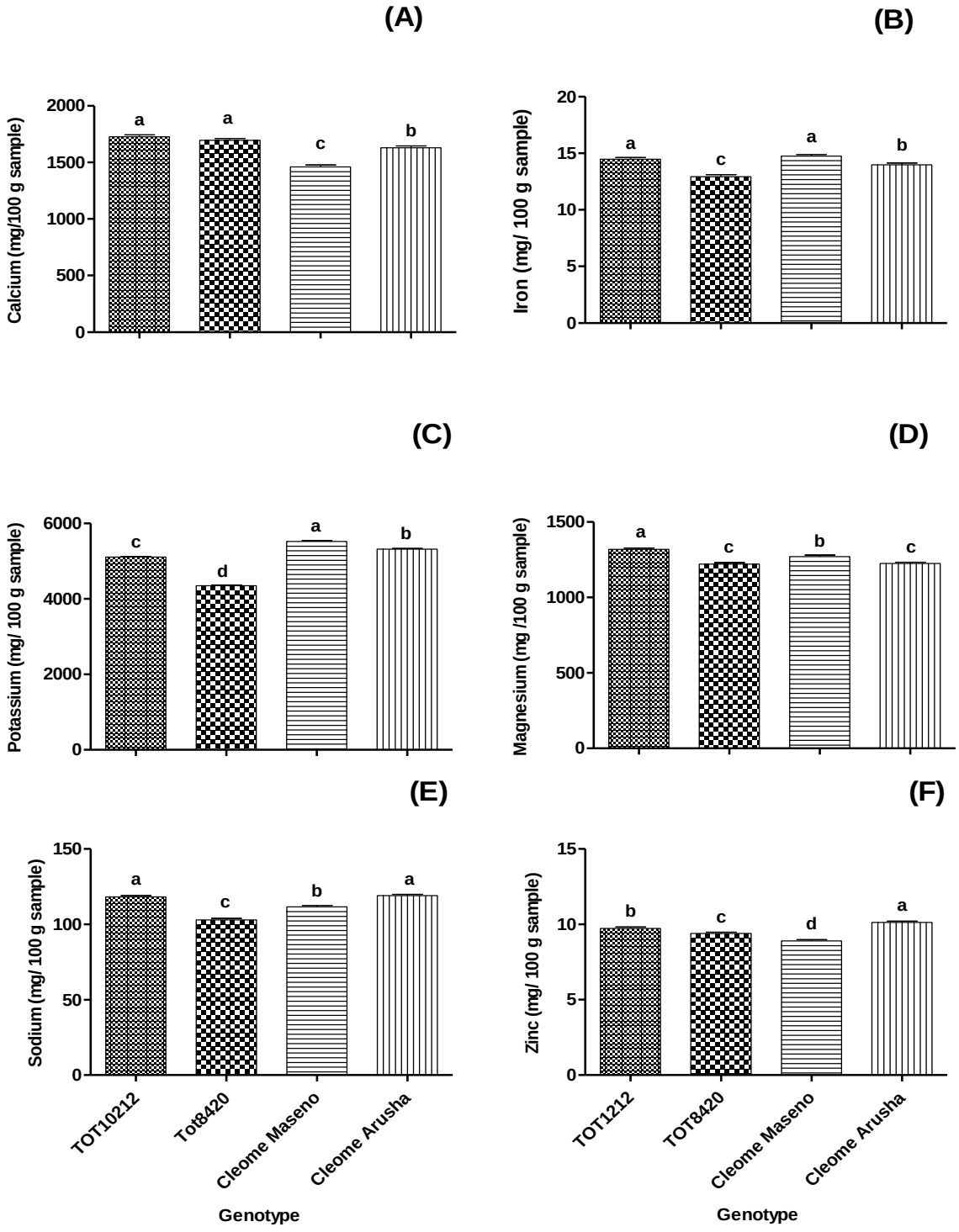

Figure 5.1: Effect of *Cleome gynandra* genotype on (A) Calcium, (B) Iron, (C) Potassium, (D) Magnesium, (E) Sodium and (F) Zinc. In each graph, bars with different letter(s) are significantly different ($p \leq 0.05$).

5.3.2. Effect of biostimulant treatments on biochemical content and mineral elements of *Abelmoschus esculentus* and *Cleome gynandra*

Biostimulant application had caused a significant decrease in the content of β-carotene and vitamin C. PGPR (1:5), Kelpak® (1:40) and Kelpak® (1:20) was characterized by significantly enhancing the total phenolic, total flavonoid and condensed tannins, respectively (**Figure 5.5**). Kelpak® (1:100 and 1:40) significantly enhanced the Ca content. However, PGPR (1:10 and 1:15) significantly enhanced Fe content. Kelpak® (1:20) significantly enhanced Na and Mg content (**Figure 5.6**).

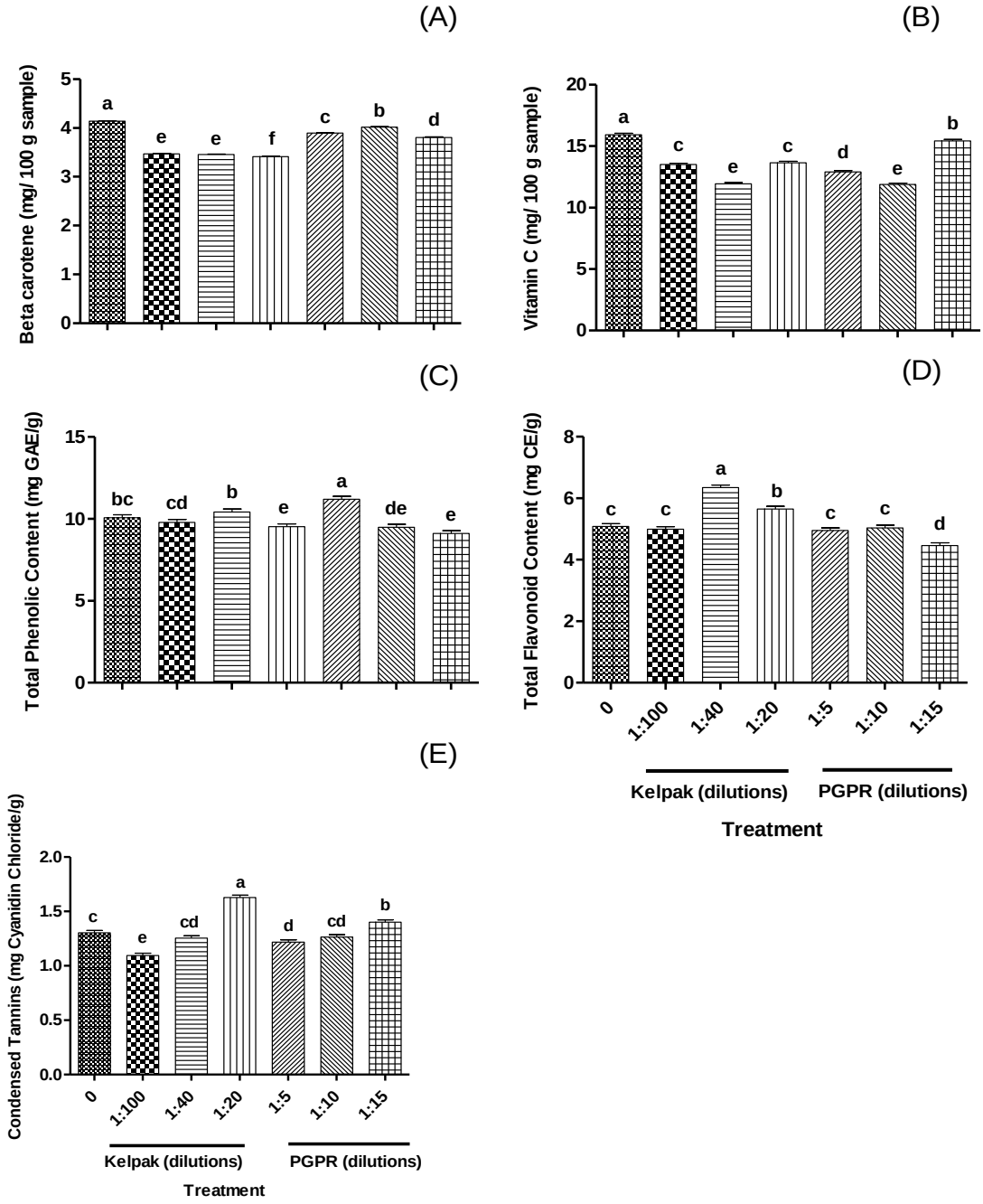

Figure 5.2: Effect of biostimulant ~~treatments~~ application on the concentration of different biochemical parameters in *Abelmoschus esculentus* genotypes. (A) β-carotene, (B) Vitamin C, (C) Total phenolic content, (D) Total flavonoid content and (E) Condensed tannins. In each graph, bars with different letter(s) are significantly different ($p \leq 0.05$).

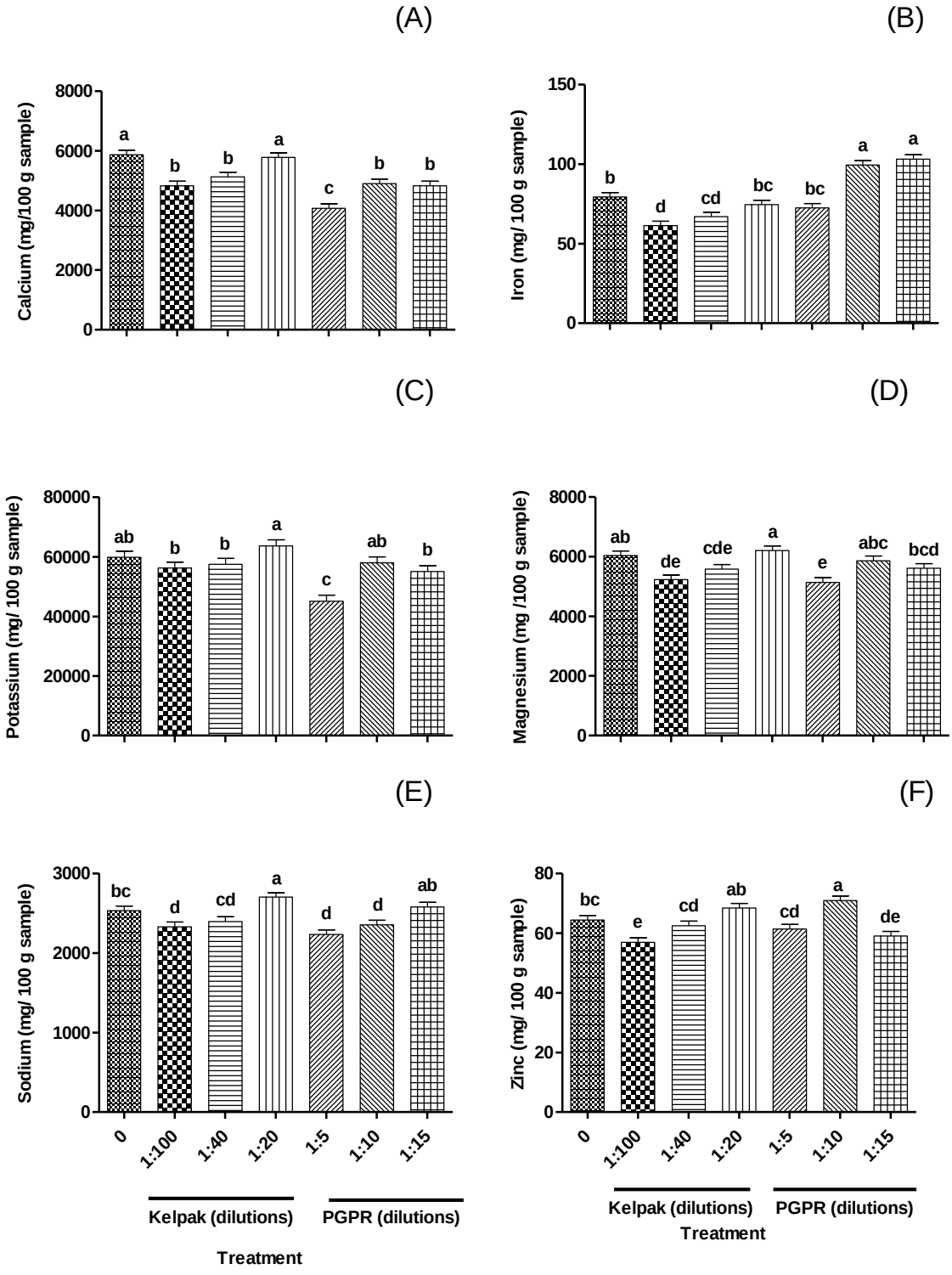

Figure 5.3: Effect of biostimulant application on the concentrations of different mineral elements in *Abelmoschus esculentus* genotypes. (A) Calcium, (B) Iron, (C) Potassium, (D) Magnesium, (E) Sodium and (F) Zinc. In each graph, bars with different letter(s) are significantly different ($p \leq 0.05$).

In *C. gynandra*, biostimulant treatments (Kelpak®- 1:40 and 1:20; PGPR- 1:5, 1:10, and 1:15) significantly enhanced the β-carotene, vitamin C, and total flavonoid content (**Figure 5.7**). Total phenolic content was significantly enhanced by Kelpak® (1:100, 1:40, and 1:20) and PGPR (1:5 and 1:10) while PGPR (1:15) caused a significant reduction on this parameter. Condensed tannins content was significantly reduced by biostimulant application. Treatments had no significant effect on the Ca content of *C. gynandra*, while Mg and Fe content was significantly reduced by biostimulant application (**Figure 5.8**). Relative to the control, Kelpak® (1:100 and 1:40) and PGPR (1:5, 1:10 and 1:15) increased K content. A significant negative lower Na and Zn concentration was observed with PGPR (1:5) treatment.

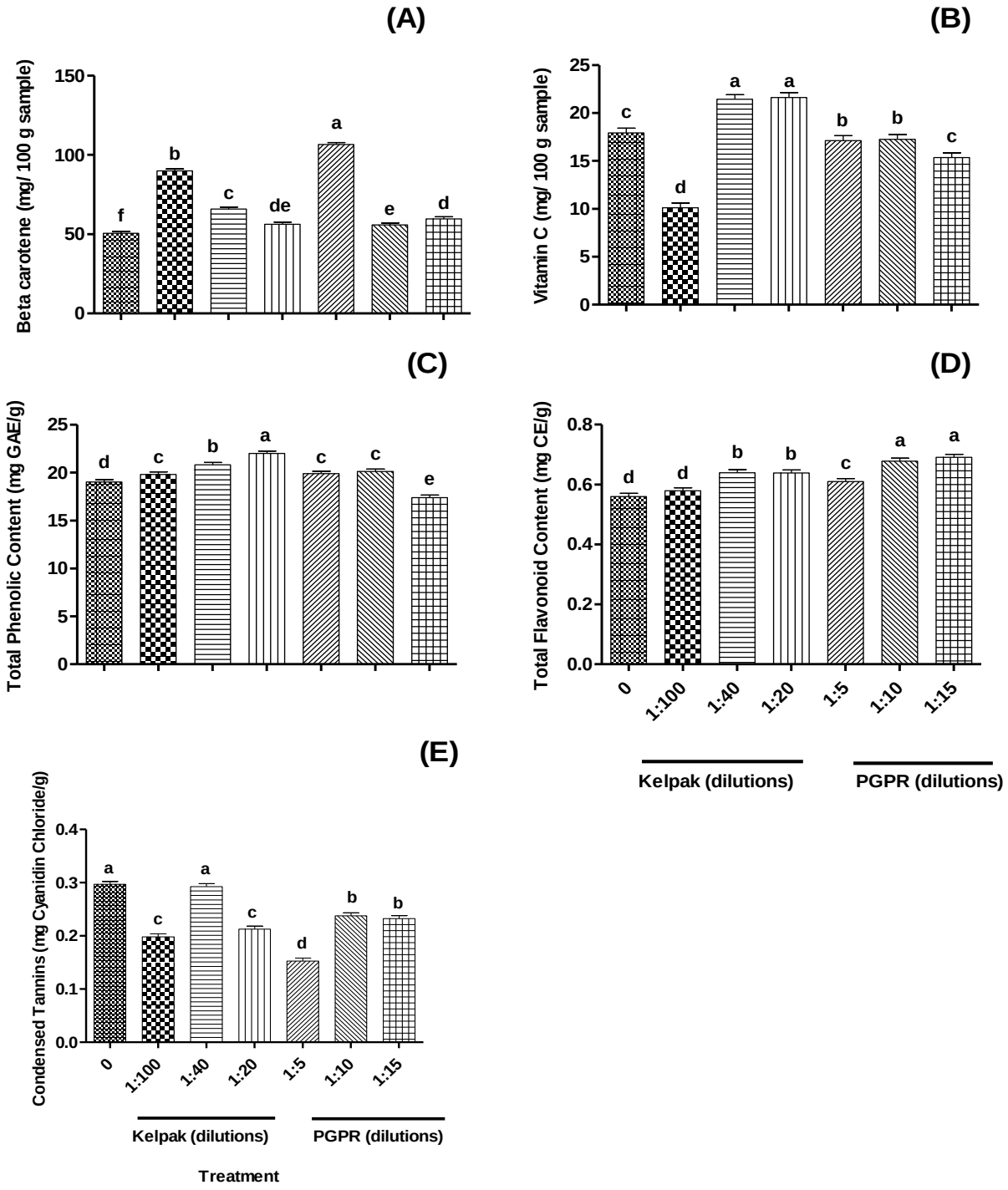

Figure 5.4: Effect of biostimulant application on the concentration of different biochemical parameters in *Cleome gynandra* genotypes. (A) β-carotene, (B) Vitamin C, (C) Total phenolic content, (D) Total flavonoid content and (E) Condensed tannins. In each graph, bars with different letter(s) are significantly different ($p \leq 0.05$).

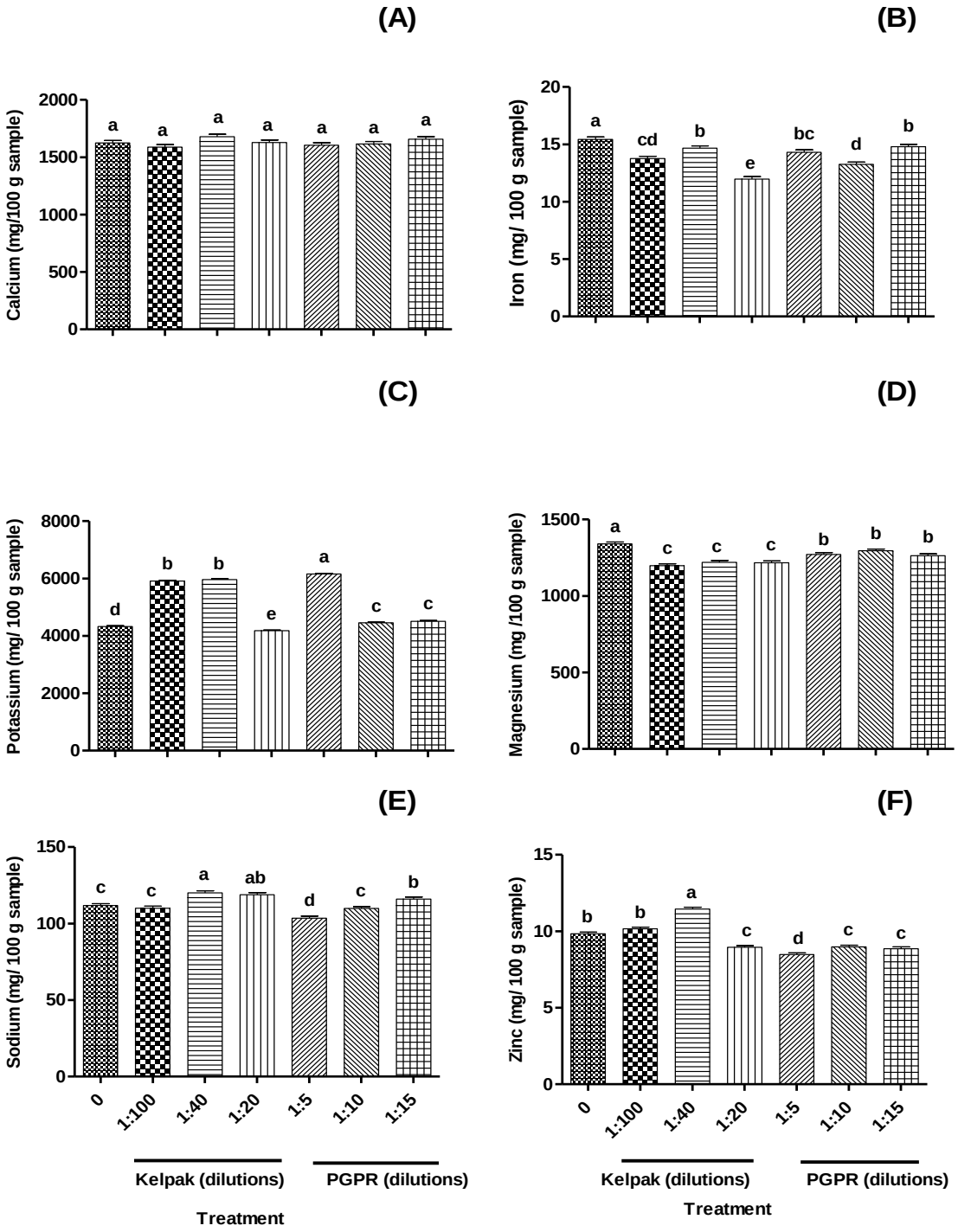

Figure 5.5: Effect of biostimulant application on the concentrations of different mineral elements in *Cleome gynandra* genotypes. (A) Calcium, (B) Iron, (C) Potassium, (D) Magnesium, (E) Sodium and (F) Zinc. In each graph, bars with different letter(s) are significantly different ($p \leq 0.05$).

5.3.3. Interaction effect of genotype and treatment on biochemical content and mineral elements of *Abelmoschus esculentus* and *Cleome gynandra*.

Biostimulants inhibited the β-carotene content of Okra PB1. Kelpak®- 1:20 and 1:40 significantly enhanced vitamin C and phenolic compounds of Okra PB1, respectively (**Table 5.1**). Kelpak® further significantly stimulated condensed tannins content. In Okra PB2, vitamin C content increased with increasing PGPR dilution. Interaction of Okra PB3 and Kelpak® (1:20) significantly enhanced the flavonoid content. Total phenolics content significantly decreased with decreasing PGPR dilution in Okra PB4. Application of PGPR (1:0 and 1:15) significantly enhanced Fe content of Okra PB2 (**Table 5.2**). Even though not significantly higher, K content increased with increasing PGPR dilution in Okra PB3.

Biostimulants (Kelpak®- all dilutions and PGPR- all dilutions) significantly enhanced the β- carotene content of TOT10212 (**Table 5.3**). PGPR treatments caused a significant increase in phenolics of *Cleome* Maseno. However, with the increasing PGPR dilutions, TPC decreased. In *Cleome* Arusha, Kelpak® (1:20) significantly enhanced vitamin C and condensed tannins of *Cleome* Arusha. Furthermore, PGPR (1:10) significantly increased the total flavonoid content in *Cleome* Maseno.

Table 5.1: Interaction effect of *Abelmoschus esculentus* genotypes and biostimulant (KLP =Kelpak® dilution, PGPR = plant growth promoting rhizobacteria, dilution) treatments on biochemical parameters. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Treatment	β-carotene (mg/ 100g sample)	Vitamin C (mg/ 100g sample)	Phenolics (mg GAE/ g)	Flavonoids (mg CE/ g)	Tannins (mg Cyanidin Chloride/ g)
Okra PB1	Control	4.575^{d}	13.80^{j}	10.127^{d-k}	4.825^{h-l}	1.744^{d}
	KLP 1:100	3.154^{t}	11.98^{m-p}	9.409^{i-l}	4.295^{l-o}	1.409^{fg}
	KLP 1:40	3.564^{lm}	8.91^{x}	11.965^{b}	5.865^{def}	1.842^{cd}
	KLP 1:20	3.007^{u}	17.85^{de}	9.481^{i-l}	5.193^{g-h}	2.517^{a}
	PGPR 1:5	3.157^{st}	14.02^{ij}	10.702^{c-h}	5.884^{de}	1.606^{e}
	PGPR 1:10	3.453^{o}	14.15^{hij}	11.226^{bcd}	6.394^{c-d}	1.894^{c}
	PGPR 1:15	4.073^{gh}	10.60^{s-v}	10.009^{e-k}	4.663^{j-n}	2.337^{b}
Okra PB2	Control	3.692^{k}	9.83^{w}	11.102^{b-e}	4.177^{no}	1.229^{i}
	KLP 1:100	3.534^{mn}	12.25^{l-o}	10.363^{c-j}	4.442^{k-o}	0.483^{r}
	KLP 1:40	3.483^{no}	10.38^{uvw}	10.199^{c-k}	11.947^{a}	1.090^{j-n}
	KLP 1:20	4.091^{g}	14.50^{hi}	7.792^{n}	4.687^{j-n}	0.797^{q}
	PGPR 1:5	2.963^{uv}	10.90^{r-u}	8.100^{mn}	4.707^{j-n}	0.950^{op}

	PGPR 1:10	3.529^{mn}	11.41^{pqr}	5.344^{o}	2.632^{r}	1.378^{fgh}
	PGPR 1:15	3.233^{r}	20.03^{b}	9.275^{jkl}	4.021^{op}	1.069^{k-o}
Okra PB3	Control	6.146^{a}	14.76^{h}	7.884^{n}	5.325^{e-h}	0.496^{r}
	KLP 1:100	3.212^{rs}	17.39^{ef}	8.813^{lmn}	4.520^{k-o}	1.003^{nop}
	KLP 1:40	3.558^{lm}	11.23^{qrs}	10.928^{b-f}	4.854^{h-l}	1.241i
	KLP 1:20	3.395^{p}	11.70^{n-q}	9.470^{i-l}	6.753^{c}	1.175^{i-m}
	PGPR 1:5	5.043^{c}	12.36^{lmn}	9.090^{klm}	3.971^{op}	1.047^{l-p}
	PGPR 1:10	5.255^{b}	11.06^{q-t}	9.594^{h-l}	5.149^{g-j}	0.957^{nop}
	PGPR 1:15	4.344^{e}	17.09^{f}	7.910^{n}	4.015^{op}	1.041^{m-p}
Okra PB4	Control	3.346^{pq}	23.00^{a}	10.415^{c-i}	4.530^{k-o}	1.210^{ij}
	KLP 1:100	4.129^{fg}	12.78^{kl}	11.133^{bcd}	5.207^{g-j}	1.398^{fgh}
	KLP 1:40	2.854^{w}	11.22^{qrs}	8.126^{mn}	3.530^{pq}	0.931^{pq}
	KLP 1:20	3.602^{l}	11.64^{opq}	9.778^{g-l}	4.751^{i-m}	1.294^{ghi}
	PGPR 1:5	4.153^{f}	15.72^{g}	18.801^{a}	5.310^{f-i}	0.983^{nop}
	PGPR 1:10	3.833^{j}	12.64^{klm}	9.983^{f-k}	3.183^{qr}	0.612^{r}
	PGPR 1:15	3.482^{no}	18.92^{c}	9.203^{klm}	4.255^{mno}	1.301^{ghi}
Okra PB5	Control	2.938^{v}	18.28^{cd}	10.784^{c-g}	6.546^{c}	1.848^{cd}

	KLP 1:100	3.308^{q}	13.10^{k}	9.173^{klm}	6.491^{c}	1.184^{ijk}
	KLP 1:40	3.823^{j}	17.98^{de}	10.887^{b-g}	5.516^{efg}	1.177^{i-l}
	KLP 1:20	2.976^{uv}	12.58^{klm}	11.087^{b-f}	6.865^{c}	2.365^{b}
	PGPR 1:5	4.156^{f}	11.56^{pqr}	9.286^{jkl}	4.884^{h-k}	1.504^{ef}
	PGPR 1:10	4.017^{h}	10.18^{vw}	11.287^{bc}	7.827^{b}	1.480^{ef}
	PGPR 1:15	3.908^{i}	10.50^{tuv}	9.101^{klm}	5.355^{e-h}	1.265^{hi}
LSD		0.05643	0.6630	1.1113	0.5597	0.13435

Table 5.2: Interaction effect of *Abelmoschus esculentus* genotypes and biostimulant (KLP =Kelpak® dilution, PGPR = plant growth promoting rhizobacteria, dilution) treatments on the concentration (mg/100 g sample) of mineral elements. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Treatment	Calcium	Iron	Potassium	Magnesium	Sodium	Zinc
Okra PB1	Control	6287^{def}	88.40^{d-h}	51933^{e}	6020^{i-l}	2327^{h}	60.53^{jk}
	KLP 1:100	3240^{nop}	45.13^{m-p}	27667^{gh}	3144^{opq}	1336^{lm}	35.45^{n-q}
	KLP 1:40	5493^{fgh}	68.60^{ijk}	54733^{e}	5513^{klm}	2567^{gh}	57.20^{kl}
	KLP 1:20	5393^{fgh}	75.47^{hij}	51600^{e}	4987^{m}	2443^{h}	51.60^{kl}
	PGPR 1:5	3933^{k-n}	81.62^{f-i}	42860^{ef}	4917^{mn}	2514^{h}	73.93^{ghi}

	PGPR 1:10	3080^{nop}	48.67^{mn}	32540^{fg}	3668^{op}	1498^{j-m}	39.13^{mno}
	PGPR 1:15	2517^{opq}	40.93^{nop}	26380^{gh}	2904^{pq}	1363^{lm}	35.40^{n-q}
Okra PB2	Control	7433^{bc}	105.20^{cd}	68467^{d}	7147^{fgh}	3240^{def}	90.27^{cde}
	KLP 1:100	6107^{efg}	74.53^{hij}	72000^{cd}	5767^{j-m}	3350de	67.53^{ij}
	KLP 1:40	5267^{ghi}	78.93^{f-j}	55133^{e}	6707^{g-j}	2540^{gh}	80.67^{e-h}
	KLP 1:20	7673^{bc}	86.87^{e-h}	79267^{bcd}	8040^{def}	3020^{ef}	89.20^{cde}
	PGPR 1:5	3720^{k-n}	62.13^{j-m}	34400^{fg}	3933no	1959^{i}	40.93^{mn}
	PGPR 1:10	7953^{b}	262.67^{a}	100667^{a}	9207^{abc}	3973^{b}	100.40^{ab}
	PGPR 1:15	7127^{bcd}	276.87^{a}	86400^{b}	8233^{cde}	3567^{cd}	91.67^{bcd}
Okra PB3	Control	9107^{a}	120.13^{bc}	100333^{a}	10200^{a}	4460^{a}	103.80^{a}
	KLP 1:100	6893^{cde}	83.20^{f-i}	82067^{bc}	7853^{ef}	3247^{def}	71.67^{hi}
	KLP 1:40	5927^{fg}	83.60^{f-i}	71400^{cd}	7100^{fgh}	3000^{ef}	76.87^{f-i}
	KLP 1:20	7500^{bc}	92.87d^{-g}	82800^{bc}	8173de	3727^{bc}	97.73^{abc}
	PGPR 1:5	4660^{h-k}	74.60^{hij}	52133^{e}	6353^{h-k}	3019^{ef}	74.40^{ghi}
	PGPR 1:10	5467^{fgh}	95.40^{def}	70867^{cd}	7353^{efg}	2953^{f}	90.20^{cde}
	PGPR 1:15	7860^{b}	103.60^{cde}	90267^{ab}	9527^{ab}	4433^{a}	97.60^{abc}
Okra PB4	Control	3015^{nop}	46.00^{mno}	33867^{fg}	3060^{opq}	1161^{m}	30.47^{o-r}

	KLP 1:100	4373^{i-l}	75.93^{g-j}	70467^{cd}	6820^{ghi}	2387^{h}	83.40^{d-g}
	KLP 1:40	1639^{qr}	30.47^{opq}	17327^{hi}	1786^{rs}	765^{n}	29.03^{pqr}
	KLP 1:20	3123^{nop}	50.21^{lmn}	32360^{fg}	3032^{opq}	1445^{lm}	35.51^{n-q}
	PGPR 1:5	1058^{r}	14.78^{q}	13507^{i}	1453^{s}	639^{n}	16.09^{s}
	PGPR 1:10	3407^{mno}	37.25^{nop}	35800^{fg}	3780^{op}	1527^{j-m}	40.93^{mn}
	PGPR 1:15	2367^{pq}	43.53^{nop}	27200^{gh}	2500^{qr}	1677^{i-l}	22.67^{rs}
Okra PB5	Control	3513^{lmn}	37.60^{nop}	44467^{ef}	3773^{op}	1479^{klm}	37.00^{nop}
	KLP 1:100	3545^{lmn}	28.27^{pq}	28933^{gh}	2573^{pr}	1340^{lm}	26.87^{qr}
	KLP 1:40	7333^{bc}	73.67^{hij}	88467^{ab}	6793^{ghi}	3113^{ef}	68.93^{ij}
	KLP 1:20	5213^{g-j}	67.53^{i-l}	72133^{cd}	6807^{ghi}	2887^{fg}	68.33^{ij}
	PGPR 1:5	7007^{b-e}	129.93^{b}	82533^{bc}	9033^{bcd}	3047^{ef}	101.67^{a}
	PGPR 1:10	4613^{h-k}	53.20^{k-n}	49800^{e}	5300^{lm}	1824^{ijk}	84.19^{def}
	PGPR 1:15	4280^{j-m}	51.07^{lmn}	44800^{ef}	4907^{mn}	1862^{ij}	48.07^{lm}
LSD		950.7	17.32	12845.1	997.8	366.8	9.724

Table 5.3: Interaction effect of *Cleome gynandra* genotypes and biostimulant (KLP =Kelpak® dilution, PGPR = plant growth promoting rhizobacteria, dilution) treatments on biochemical parameters. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Treatment	β-carotene (mg/ 100 g sample)	Vitamin C (mg/ 100 g sample)	Phenolics (mg GAE/ g)	Flavonoids (mg CE/ g)	Tannins (mg Cyanidin Chloride/g)
TOT10212	Control	25.91^{n}	16.54^{e-h}	14.54^{n}	0.4900^{kl}	0.4067^{b}
	KLP 1:100	176.09^{b}	14.14^{hi}	15.42^{mn}	0.5733^{hij}	0.1033^{k}
	KLP 1:40	140.75^{c}	45.00^{a}	20.35^{efg}	0.6700^{c-f}	0.3533^{c}
	KLP 1:20	48.84^{h-l}	9.37^{j}	21.31^{de}	0.7233^{abc}	0.0633^{l}
	PGPR 1:5	284.49^{a}	15.55^{f-i}	17.38^{jk}	0.7033^{bcd}	0.1100^{jk}
	PGPR 1:10	33.39^{m}	8.72^{j}	16.15^{klm}	0.6733^{b-f}	0.2800^{e}
	PGPR 1:15	44.84^{l}	9.04^{j}	16.45^{klm}	0.7300^{ab}	0.2333^{fg}
TOT8420	Control	45.87^{kl}	14.70^{ghi}	20.96^{def}	0.6500^{d-g}	0.2000^{hi}
	KLP 1:100	34.65^{m}	8.04^{j}	15.94^{lmn}	0.6233^{fgh}	0.3533^{c}
	KLP 1:40	47.02^{jkl}	8.90^{j}	21.83^{cd}	0.6867^{b-e}	0.2633^{ef}
	KLP 1:20	28.17^{mn}	22.93^{d}	17.20^{jkl}	0.6000^{ghi}	0.0667^{l}

	PGPR 1:5	48.80^{h-l}	9.56^{j}	15.94^{lmn}	0.5667^{hij}	0.0500^{l}
	PGPR 1:10	47.63^{i-l}	17.58^{ef}	18.99^{ghi}	0.7000^{bcd}	0.1367^{j}
	PGPR 1:15	52.37^{g-k}	16.01^{e-i}	18.61^{hij}	0.6933^{b-e}	0.1300^{jk}
Cleome Maseno	Control	50.29^{g-l}	17.17^{efg}	17.90^{ij}	0.4500^{lm}	0.2267^{gh}
	KLP 1:100	92.56^{d}	8.53^{j}	20.30^{efg}	0.7000^{bcd}	0.1000^{k}
	KLP 1:40	22.46^{n}	8.57^{j}	16.23^{klm}	0.5500^{ij}	0.1933^{i}
	KLP 1:20	55.81^{gh}	18.42^{e}	20.93^{def}	0.5767^{hij}	0.2733^{e}
	PGPR 1:5	27.95^{mn}	13.89^{hi}	20.71^{def}	0.5233^{jk}	0.2767^{e}
	PGPR 1:10	54.58^{ghi}	18.45^{e}	19.80^{fgh}	0.7633^{a}	0.2700^{e}
	PGPR 1:15	52.35^{g-k}	13.71^{i}	18.97^{ghi}	0.7000^{bcd}	0.3167^{d}
Cleome Arusha	Control	80.28^{e}	23.31^{d}	22.74^{c}	0.6500^{d-g}	0.3533^{c}
	KLP 1:100	56.30^{g}	9.78^{j}	27.65^{a}	0.4200^{m}	0.2367^{fg}
	KLP 1:40	53.02^{g-j}	23.30^{d}	24.89^{b}	0.6533^{d-g}	0.3600^{c}
	KLP 1:20	92.28^{d}	35.84^{b}	28.57^{a}	0.6567^{d-g}	0.4467^{a}
	PGPR 1:5	64.87^{f}	29.55^{c}	25.60^{b}	0.6467^{d-g}	0.1733^{i}
	PGPR 1:10	87.51^{d}	24.32^{d}	25.63^{b}	0.5767^{hij}	0.2633^{ef}
	PGPR 1:15	89.16^{d}	22.70^{d}	15.61^{mn}	0.6400^{efg}	0.2500^{efg}

LSD	7.139	2.786	1.4240	0.05674	0.03127	

Table 5.4: Interaction effect of *Cleome gynandra* genotypes and biostimulant (KLP =Kelpak® dilution, PGPR = plant growth promoting rhizobacteria, dilution) treatments on the concentration (mg/100 g sample) of mineral elements. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Treatment	Calcium	Iron	Potassium	Magnesium	Sodium	Zinc
TOT10212	Control	1942^{a}	21.82^{a}	7413^{a}	1432^{ab}	131.3^{b}	12.757^{b}
	KLP 1:100	1425^{l-o}	12.00^{mno}	3300^{i}	1109^{l}	105.7^{g-k}	9.200^{g-j}
	KLP 1:40	1711^{efg}	13.54^{h-k}	7207^{b}	1339^{cde}	127.8^{bc}	8.807^{i-l}
	KLP 1:20	1726^{efg}	13.06^{i-m}	3507^{h}	1439^{ab}	121.8^{cd}	8.753^{i-l}
	PGPR 1:5	1691^{e-h}	13.83^{hij}	6780^{c}	1241^{ghi}	108.0^{g-j}	9.153^{hij}
	PGPR 1:10	1743^{d-g}	12.87^{j-m}	3660^{g}	1277^{d-g}	106.7^{g-k}	9.893^{ef}
	PGPR 1:15	1862^{a-d}	14.18^{f-i}	3887^{f}	1387^{bc}	126.2^{bcd}	9.500^{fgh}
TOT8420	Control	1395^{mno}	10.73^{p}	2680^{j}	1187^{ijk}	99.7^{klm}	7.247^{op}

	KLP 1:100	1925^{ab}	14.07^{f-j}	7153^{b}	1237^{ghi}	111.3^{ghi}	10.207^{de}
	KLP 1:40	1761^{c-g}	12.23^{lmn}	3347^{i}	1109^{l}	99.9^{klm}	10.773^{cd}
	KLP 1:20	1513^{k-n}	10.40^{p}	2747^{j}	1203^{hij}	94.2^{m}	9.297^{f-i}
	PGPR 1:5	1875^{abc}	15.07^{d-g}	6693^{cd}	1387^{bc}	105.7^{h-k}	8.780^{i-l}
	PGPR 1:10	1749^{c-g}	12.58^{klm}	3867^{f}	1345^{cd}	106.6^{g-k}	10.810^{cd}
	PGPR 1:15	1655^{ghi}	15.47^{de}	3947^{f}	1077^{l}	102.7^{jkl}	8.620^{i-m}
Cleome Maseno	Control	1517^{j-m}	18.32^{c}	3940^{f}	1484^{a}	110.5^{ghi}	9.020^{h-k}
	KLP 1:100	1467^{k-n}	12.95^{j-m}	6540^{e}	1186^{ijk}	110.1^{g-j}	11.340^{c}
	KLP 1:40	1661^{f-i}	13.34^{i-l}	6767^{c}	1297^{d-g}	132.3^{b}	10.757^{cd}
	KLP 1:20	1467^{k-n}	13.10^{i-m}	3873^{f}	1095^{l}	112.1^{fgh}	8.530^{j-m}
	PGPR 1:5	1462^{k-n}	15.26^{def}	7253^{b}	$1277d^{-g}$	104.0^{i-l}	7.607^{no}
	PGPR 1:10	1324^{o}	14.70^{e-h}	3573^{gh}	1285^{d-g}	106.9^{g-k}	6.897^{p}
	PGPR 1:15	1328^{o}	15.55^{de}	6713^{cd}	1267^{fgh}	105.3^{h-k}	8.063^{mn}
Cleome Arusha	Control	1643^{g-j}	10.90^{op}	3300^{i}	1263^{fgh}	105.7^{g-k}	10.293^{de}
	KLP 1:100	1541^{i-l}	16.05^{d}	6647^{cde}	1259^{fgh}	113.1^{efg}	9.857^{efg}
	KLP 1:40	1582^{h-k}	19.57^{b}	6540^{e}	1137^{jkl}	120.5^{cde}	15.487^{a}
	KLP 1:20	1813^{b-e}	11.36^{nop}	6593^{de}	1133^{kl}	147.5^{a}	9.223^{f-i}

	PGPR 1:5	1390no	13.09^{i-m}	3900^{f}	1183^{ijk}	96.7^{lm}	8.367^{klm}
	PGPR 1:10	1647^{ghi}	12.87^{j-m}	6720^{cd}	1272^{efg}	119.5^{def}	8.320^{lm}
	PGPR 1:15	1785^{c-f}	14.01^{g-j}	3507^{h}	1321^{c-f}	129.7^{b}	9.247^{f-i}
LSD		125.52	1.2075	146.15	68.04	7.464	0.6768

5.4. Discussion

5.4.1. Effect of genotype on the biochemical and mineral element content in *Abelmoschus esculentus* and *Cleome gynandra*

Biochemical characteristics of plants vary with genotypes and are considered to be amongst the important quality attributes in agricultural production and food security. In the present study, genotypes of *A. esculentus* and *C. gynandra* had varying biochemical composition (**Table 5.4**). Generally, this varying response has been reported for different plants (Irakli et al., 2019, Sokrab et al., 2011). The chemical composition (β-carotene and total phenolic content) in *Cannabis sativa* varied with genotypes (Irakli et al., 2019). For instance, *Cannabis sativa* genotype Futura had significantly higher β-carotene content while Tygra had the least quantity. In addition, Futura had the highest phenolic content followed by Finola and Felina (Irakli et al., 2019). Similar trend was observed in the content of polyphenols in *Zea mays* genotype (Sokrab et al., 2011). *Prunus cerasus* genotypes had varying effects on the total phenolic and total flavan-3-ol content (Ciccoritti et al., 2017). Genotype BO- FD had significantly higher total phenolic and total flavan-3ol content while MM-OD had the least quantity of bioactive compounds (Ciccoritti et al., 2017). Findings by Yasaminshirazi et al. (2020) also strongly support the effect of genotypes on the total phenolic content of *Beta vulgaris* subsp. *vulgaris*. Genotype Monty RZ F1 had significantly higher TPC while Sniezna Kula and Burpees Golden had significantly low levels (Yasaminshirazi et al., 2020). Given that genotypes play a role in the phenolic compound content of various plants (Palmieri et al., 2017), it is important to carefully select an appropriate genotype.

Similar to the secondary metabolite content, mineral element concentration varied with genotypes used in the current study. Sokrab et al. (2011) demonstrated that mineral element content are influenced by genotypes of *Zea mays*. Genotype Mugtama-45 had significantly high total Na content while Hudiba-1 had least quantity of Na (Sokrab et al., 2011). Total K, Mg and Ca content was high in PAN-6480, TL-98B-6225-9×TL617 and S-98TLW-GHA, and least in S-98TLW-GHA, Banglore-9733 and Hudiba-1, respectively (Sokrab et al., 2011). Furthermore, Fe and Zn was high in TL-98B-6225-9×TL617 and PAN-6480, respectively while Mugtama-45 and S-98TLW-GHA had least Fe and Zn contents, respectively (Sokrab et al., 2011). In *Vigna unguiculata*, genotypes played a significant role in the mineral elements content (Gerrano et al., 2019). For instance, genotype IT90K-59 had high Ca content while genotype CH14 had least Ca content (Gerrano et al., 2019). A genotypic variation Fe content was also observed in the *Vignia unguiculata* genotypes, where genotype IT845-2246 had significantly high and Bechuana white had least Fe content (Gerrano et al., 2019). The varying effects of genotypes on the mineral elements in the current study was also collaborated by Moatshe et al. (2020). Moatshe et al. (2020) found that *Carthamus tinctorius* genotype Gila had significantly high Ca content while genotype Sina had least. Furthermore, genotype Kiam had significantly increased levels of Na and genotype Pi527710 had least levels (Moatshe et al., 2020).

5.4.2. Effect of biostimulant treatments and their interaction effect with *Abelmoschus esculentus* and *Cleome gynandra* genotypes biochemical and mineral elements content

The effect of Kelpak on the phytochemical and nutritional content varies across genotypes. In the current study, Kelpak® (1:40) significantly enhanced phenolic content of Okra PB1. Likewise, Kelpak® (at varying levels) affected the biochemical content of two common bean cultivar's (var. Aura and Toska) (Kocira et al., 2018). In *Phaseleous vulgaris* (var. Toska), Kelpak® application [single spraying- 0.2 and 0.4% (v/v), double spraying- 0.2 and 0.4% (v/v)] had no significant effect on the total phenolic content (Kocira et al., 2018). However, single spraying of Kelpak® (0.2% v/v) significantly enhanced total phenolic content of the same cultivar. Kelpak treatments had no significant effect on both total phenolic and flavonoid content in *Phaseleous vulgaris* (var. Aura) (Kocira et al., 2018). Even though Kelpak® increased the phenolic content of Okra PB1, it did not increase that of TOT8420. Similarly, the application of brown seaweed (*Ascophyllum nodousum*) extract had no significant effect on the phenolic content of *Solanum lycopersicum* cultivars (Black Cherry, Brandywine, German Johnson and Roma) but enhanced the β-carotene content of German Johnson (Sokrab et al., 2011). In *Phaseolus vulgaris*, Kelpak® application significantly enhanced average total flavonoid content in respect to control (Kocira et al., 2020).

In the current study, the mineral content of genotypes were affected by Kelpak® application at varying levels. Similar trend have been reported in other plant species (Ngoroyemoto et al., 2020, Rouphael et al., 2018). Based on the findings by Ngoroyemoto et al. (2020), the application of Kelpak® had diverse effects on the mineral composition of *Amaranthus hybridus*. While Kelpak®had no significant effect on Na, Zn and Mg, it significantly reduced the accumulation of Ca, Mg and Fe of *Amaranthus hybridus* (Ngoroyemoto et al., 2020). *Ecklonia maxima* extracts significantly enhanced mineral element composition (K and Mg) of *Spinacia oleracea* but had no stimulatory effect on the Na content (Rouphael et al., 2018).

Seaweed extracts are common in agriculture for their ability to influence absorption, translocation and retention of mineral nutrients (Battacharyya et al., 2015). Compounds (eckol and phloroglucinol) found in *Ecklonia maxima* enhanced the activity of enzymes (such as α-amylase and MDH) and increased secondary metabolites (Rengasamy et al., 2015). This enhanced metabolism enhances the production and activity of enzymes involved in various biological processes including glycolysis and nitrogen assimilation, thereby increasing the production of secondary metabolites (Kocira et al., 2019, Mahmoud et al., 2019). The ability of seaweed extracts to facilitate the production of secondary metabolites is further attributed to their polysaccharides content, which activates defence responses (Sharma et al., 2013). According to Kocira et al. (2020), the efficacy of biostimulants on production of phenolic content is dependent on the applied concentration.

Despite the numerous studies on the effects of PGPR application on improving growth and yield in crop plants, there are only few reports on its effect on biochemical and nutritional parameters. In the current study, PGPR significantly enhanced the β-carotene and TPC of Okra PB4 (1:5, 1:10 and 1:15) and *Cleome* Maseno (1:5 and 1:10). Likewise, inoculation of *Cannabis sativa* 'Finola' with PGPR significantly enhanced the TPC when compared to the control (Pagnani et al., 2018). Interestingly, the TPC of PGPR-inoculated plants was similar to that of nitrogen fertiliser-treated plants (Pagnani et al., 2018). In the current study, the application of PGPR had varying effect on the mineral elements of both *A. esculentus* and *C. gynandra* genotypes. *Bacillus megaterium* significantly influenced the content of microelements (Fe, Cu, Zn and Mg) when compared to the un-inoculated control in both pot and field conditions (Kumar et al., 2014). PGPR are well-known for their siderophore-producing and mineral solubilisation ability, which play a role in the uptake of mineral elements (Kumar et al., 2014). Orhan et al. (2006) studied the effect of two *Bacillus* strains OSU-142 and M3 *Rubus ideaeus* nutrient content and the findings indicated that Ca content in the plants inoculated with both strains (OSU-142 and M3) was significantly higher than other nutrients. Furthermore, *Bacillus* stain M3 significantly enhanced the Ca, Fe and Mg content relative to strain OSU-142 and control (Orhan et al., 2006). The interaction of plant and bacteria organic acids in the rhizosphere has the potential to maintain the soil pH and thus improve the availability of mineral elements (Orhan et al., 2006). Based on the study by Esitken et al. (2010) on the nutritional properties of *Fragaria ananassa*, both *Bacillus* strains OSU-142 and M3, had no significant effect on Fe, Mg and Na content while M3 significantly enhanced Zn relative to control. PGPR have the ability to produce volatile organic compounds including antioxidants which can promote plant absorption and endophytic metabolic pathways leading to the production of volatile organic compounds (Aloo et al., 2019). The ability of PGPR to enhance mineral element availability and absorption on the rhizosphere by plant roots contributes to its positive effect on mineral elements (Almaghrabi et al., 2013). PGPR can further enhance the enzymatic actions involved in the antioxidative responses of plants and can facilitate the production of abscisic acid which further plays a role in inducing some biochemical responses involved in the plant stress responses (Calvo et al., 2014). Some plant genotypes support the stimulatory effect of PGPR through production of root exudates that act as substrates to the inoculants (Calvo et al., 2014). The current study suggests that the efficacy of PGPR is strongly dependent on the plant species.

Unfavourable conditions may lead to high production of secondary metabolites (total phenolics, condensed tannins and total flavonoids) regardless of biostimulant application. Secondary metabolites are produced in abundance in stressful conditions (nutrient deprivation) (Masondo et al., 2019), however, this is entirely species-dependent. This is similar to the findings of Rengasamy et al. (2016), where the secondary metabolites were significantly higher in control plants than those treated with eckol.

5.5. Conclusion

In the current study, the effect of biostimulants on the biochemical and mineral elements content varies with different genotypes of the two plants. Biostimulant concentration played a major role in the biochemical and mineral elements content in the investigated plants. Therefore, biostimulants can be used to relieve increase the nutritional content of some *A. esculentus* and *C. gynandra* and hence combat nutritional deficiencies. Genotype, biostimulant application and the interaction of genotype and biostimulant application played a role on the biochemical and mineral elements content. The current study highlighted the significance effects of plant genotype, biostimulants-type and concentration on the biochemical and mineral elements in okra and cleome. This study further illustrated that biostimulants can be used to increase the biochemical content of medicinal plants, so as to increase their efficacy in treating various diseases. However, the varying effects observed in the current study suggest the need for further research to optimise the use of biostimulants for accumulation of important secondary metabolites in plants.

Chapter 6: General conclusion and recommendations

Soil infertility contribution to insufficient and limited agricultural production remains one of the major concern as it ultimately increases food insecurity in developing countries. Literature indicates that biostimulants (Kelpak® and PGPR) can be used in agricultural production to increase plant yields and quality without degrading soil infertility. The current study evaluated the physiological and biochemical role of biostimulants on two important multipurpose plants in Africa, *Abelmoschus esculentus* and *Cleome gynandra* genotypes.

Seed germination remains the most crucial step in crop production as it responsible for controlling seedling emergence and stand establishment. Biostimulants influenced the germination parameters of *A. esculentus* and *C. gynandra* genotypes. Seaweed extracts have been widely used as germination promoting agents because of their hormonal content (). These hormones are known for breaking dormancy and improving seed germination ().On the other hand, PGPR can fix nitrogen and produce phytohormones, including GA which promotes germination (Garcia-Cristobal et al., 2015). Seaweed and PGPR have been widely used to stimulate seed germination in various species (Hernández-Herrera et al., 2013, Mangmang et al., 2016, Prathibha and Siddalingeshwara, 2013, Sasikala et al., 2016, Stamenov et al., 2018). In the current study, the application of Kelpak® had a neutral effect on the germination parameters of *A. esculentus* genotypes while PGPR treatments had an inhibitory effect on germination parameters.Overall, biostimulants had no stimulatory effect on the germination of both *A. esculentus* and *C. gynandra* genotypes seeds. The overall promontory effect of seed germination parameters by Kelpak® treatments was significantly higher than that of PGPR.

Seedling growth and stand establishment are important stages in agricultural production (plant growth and yield). Plant growth is affected by a number of parameters including but not limited to plant height, number of leaves, chlorophyll content and stem diameter. Genotypes of the same species often have different responses to the application of external stimulus, e.g. biostimulants. The function of biostimulants often vary depending on their nature and origin. Biostimulants affect the metabolic processes through various mechanisms including production of phytohormones, improving nutrient uptake, translocation and utilization which ultimately affect the yield, nutritional and biochemical content (Kocira et al., 2020). Seaweed extracts are a rich source of various phyto-stimulators including auxins, cytokinins, polyamines, gibberellins, abscisic acid, and brassinosteroids (Rengasamy et al., 2015). In the current study, the effect of Kelpak® remained limited, this is because of the observed stimulatory effect of Kelpak® (1:20) on the growth and yield of *A. esculentus* genotypes, while no stimulatory effect by Kelpak® treatments was observed on the growth and yield of *C. gynandra* genotypes. Furthermore, Kelpak® improved the β-carotene content of *C. gynandra* genotypes. PGPR

colonizes the rhizosphere, interact with plant roots and promote plant growth through various mechanisms such as the production of plant growth regulators, fixation of nitrogen and production of siderophores (Yadav et al., 2010). Application of PGPR (1:5 and 1:10) increased the total fresh and total dry weight of harvested leaves. Based on the results obtained in the current study, PGPR treatments were efficient in stimulating the growth, yield, biochemical and mineral elements content of the two selected multipurpose plants. PGPR improved the growth and yield of *A. esculentus* and *C. gynandra* when compared to Kelpak® treatments. Therefore, PGPR remains the preferred biostimulant to enhance the growth and yield of plants in crop production.

. .. Biostimulants application had no constant effect on the biochemical and mineral elements content. The effect of biostimulant application varied form one plant to the next and also from genotypes of the same species. This raises the need for more research focusing on enhancing the effect biostimulants on the biochemical and mineral elements.

The current study findings authenticate the potential use of biostimulants (Kelpak® and PGPR) on seed germination, plant growth and yield, and biochemical and mineral elements content. Biostimulant use can therefore be adopted as a tool of improving food security and tackle nutritional deficiencies on the rising world population. Based on the current study's findings, biostimulant application can potentially be used on the cultivation of multipurpose plants which are of great importance, especially on the rural communities. The current study further revealed that the efficacy of biostimulants are plant genotype and concentration-dependent. However, a more in-depth assessment of the efficacy of biostimulants on seed germination, and biochemical and mineral element content is encouraged in order to establish methods that will enhance the effect of biostimulants on these parameters. The current study provided insights on the effect of biostimulants on different plant genotypes and the efficacy of biostimulant (types and concentrations) on the two selected multipurpose plants (*A. esculentus* and *C. gynandra*).

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Appendix

Table 1: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Abelmoschus esculentus* seed germination. FGP = final germination percentage, MGT = mean germination time, GI = germination index, CVG = coefficient of velocity of germination, GRI = germination rate index, TSG = time spread of germination.

Source of variation	df	MS					
		FGP	MGT	GI	CVG	GRI	TSG
Genotype (G)	4	358.75***	0.3290 n.s	6189.8***	0.870 n.s	1019.47***	13.319***
Biostimulant treatment (B)	6	5963.53***	2.2002***	75466.8***	31.687***	6540.91***	5.321*
G ×B	24	333.31***	0.1838 n.s	4142.6***	2.756 n.s	327.56***	1.630 n.s
Residual	70	65.98	0.1497	757.9	1.684	77.26	1.990
Total	104						

* = $p \leq 0.05$, *** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 2: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Cleome gynandra* seed germination. FGP= final germination percentage, MGT= mean germination time, GI= germination index, CVG= coefficient of velocity of germination, GRI= germination rate index, TSG= time spread of germination.

Source of variation	df	M.S					
		FGP	MGT	GI	CVG	GRI	TSG
Genotype	6	1946.13***	0.89 n.s	45802.5***	5.818***	313.065	15.867***
Treatment	6	126.58***	5.873 n.s	2769.3***	0.9518***	18.985	8.016**
G ×B	24	80.36***	3.18 n.s	1625.7***	0.6644***	8.789	4.139
Residual	70	30.32	3.986	626	0.2324	3.057	2.61
Total	104						

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 3: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Abelmoschus esculentus* growth parameters.

Source of variation	df	M.S			
		Plant height (mm)	Number of leaves	Chlorophyll Content (SPAD)	Stem Diameter (mm)
Genotype	4	102823*	38.59*	6.14 n.s	6.662 n.s
Treatment	6	225161***	18.53 n.s	143.32***	35.094***
G ×B	24	28253 n.s	12.58 n.s	21.34 n.s	2.419 n.s
Residual	140	34271	14.72	18.2	3.249
Total	174				

* = $p \leq 0.05$, *** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 4: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Abelmoschus esculentus* yield parameters.

Source of variation	df	MS		
		No. of pods	Total fresh weight of pods	Total dry weight of pods
Genotype	4	33.41 n.s	930.0 n.s	18.07 n.s
Treatment	6	94.28***	8696.3***	255.88***
G ×B	24	24.01 n.s	710.6 n.s	15.64 n.s
Residual	136	16.25	740.3	13.44
Total	174			

*** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 5: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Cleome gynandra* growth parameters.

Source of variation	df	M.S			
		Plant height (mm)	Number of leaves	Chlorophyll Content (SPAD)	Stem Diameter (mm)
Genotype	3	45688 n.s	518.2 n.s	30.44 n.s	1.732 n.s
Treatment	6	11553 n.s	344.6 n.s	169.02 n.s	10.261***
G ×B	18	13222 n.s	137.7 n.s	61.73 n.s	2.616 n.s
Residual	108	23761	233.8	81.73	2.485
Total	139				

*** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 6: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Cleome gynandra* yield parameters.

Source of variation	d.f	M.S	
		Total fresh weight of leaves	Total dry weight of leaves
Genotype	3	15.42 n.s	0.6031 n.s
Treatment	6	395.64***	11.1019***
G ×B	18	25.22 n.s	0.7653 n.s
Residual	107	20.07	0.6707
Total	138		

*** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 7: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Abelmoschus esculentus* biochemical content.

Source of variation	df	MS				
		Beta-carotene	Vitamin C	Total phenolic content	Total flavonoid content	Condensed tannins
Genotype	4	3.07***	17.79***	17.84***	9.18***	3.42***
Treatment	6	1.31***	37.38***	7.34***	5.49***	0.43***
G ×B	24	1.27***	34.95***	12.71***	7.96***	0.31***
Residual	70	0.00	0.17	0.47	0.12	0.01
Total	104					

*** = $p \leq 0.001$.

df = Degrees of freedom, MS = Mean squares.

Table 8: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Abelmoschus esculentus* mineral elements.

Source of variation	df	MS				
		Ca	Fe	K	Mg	Na
Genotype	4	57997755.00***	27175.10***	7.83×10^9***	79595816.00***	15911508.00***
Treatment	6	5700772.00***	3781.20***	4.93×10^8***	2373265.00***	401018.00***
G ×B	24	6410035.00***	7071.20***	1.15×10^9***	10056126.00***	1522153.00***
Residual	70	340822	113.1	6.22×10^7	375455	30.00
Total	104					

*** = $p \leq 0.001$.

df = Degrees of freedom, MS = Mean squares.

Table 9: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Cleome gynandra* biochemical content.

Source of variation	df	MS				
		Beta-carotene	Vitamin C	Total phenolic content	Total flavonoid content	Condensed tannins
Genotype	3	17603.76***	475.78***	152.18***	0.01***	0.06***
Treatment	6	5234.67***	183.364***	24.56***	0.03***	0.03***
G ×B	18	8560.67***	213.41***	33.37***	0.02***	0.3***
Residual	56	19.04	2.902	0.76	0.00	0.00
Total	83					

*** = $p \leq 0.001$.

df = Degrees of freedom, MS = Mean squares.

Table 10: Analysis of variance (ANOVA) for the effect of biostimulant application and genotype difference on *Cleome gynandra* mineral elements.

Source of variation	df	MS				
		Ca	Fe	K	Mg	Na
Genotype	3	298903.00***	13.35***	5518017.00***	43881.00***	1174.07***
Treatment	6	11304.00 n.s	15.91***	9419949.00***	30494.00***	404.84***
G ×B	18	93022.00***	22.3289***	9240245.00***	36363.00***	383.99***
Residual	56	5889.00	0.55	7984.00	1730.00	20.82
Total	83					

*** = $p \leq 0.001$, ns = not significant.

df = Degrees of freedom, MS = Mean squares.

Table 11: List of chemicals used in the study and their manufacturers.

Chemical	Manufacturer
Acetone	Sigma-Aldrich, USA
Aluminium chloride	Fluka Analytical, USA
Ascorbic acid	Rochelle Chemicals, South Africa
Butanol	AnaloR® Merck, Germany
Catechin	Sigma-Aldrich, USA
Cyanidin chloride	Sigma-Aldrich, USA
Ferric reagent	PAL Chemicals, India
Folin-Ciocalteu reagent	Sigma-Aldrich, USA
Gallic acid	Sigma-Aldrich, USA
HCl	Minema Chemicals, South Africa
Hexane	VWR, PROLABO® Chemicals, France

Hydrochloric acid	Mayise lab, South Africa
Metaphosphoric acid	Sigma-Aldrich, USA
Methanol	Merck KGaA, Germany
NaCl	Sigma-Aldrich, USA
$NaNO_2$	Rochelle Chemicals, South Africa
NaOH	Rochelle Chemicals, South Africa
Nitric acid	Mayise lab, South Africa
Sodium Hypochlorite	Sigma-Aldrich, USA
β-carotene	Sigma-Aldrich, USA