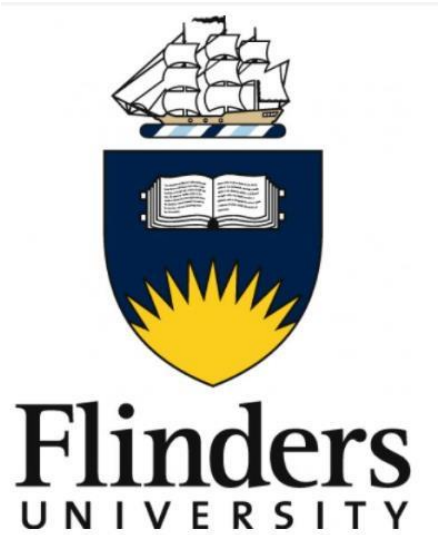

Evaluation of *Limnospira maxima* Biomass and Microwave-Assisted Extracts as Biofertilisers to Support Growth of *Sorghum bicolor*

By

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LIST OF EQUATION

List of Abbreviations

C, N, P, K	Carbon, Nitrogen, Phosphorus, Potassium
Cm	Centimetre
°C	Degree Celsius
G	Gram
ICP-OES	Inductive coupled plasma optical emission spectroscopy
Kg	Kilogram
L	Litre
MAE	Microwave-assisted extract
Mg	Milgram
mL	Millilitre
MQ water	Milli-Q water
Nm	Nanometer
HNO_3	Nitric acid
Ppb	Parts per billion
Ppm	Parts per million
%	Percentage
W	Watt
dS/m	DeciSiemens per meter
meq/100 g	Milliequivalent per 100 g
cmolc/kg	Centimol positive charge per kg

Abstract

The microscopic filamentous cyanobacterium *Spirulina* is used as a complementary supplement. In agriculture, it is a potent source of nitrogen and other elements, enhancing plant growth. As comparative study of different microwave-assisted extracts from *L.maxima*, the pot experiment was carried out using nutrient-deficient soil and fertilised with seasol fertiliser, *L. maxima* biomass, microwave-assisted extracts (MAE) produced from *L. maxima* biomass at 40, 60 and 80^{o}C, whilst compost soil and unfertilised nutrient-poor garden topsoil were used as positive and negative controls, respectively. Inductively-coupled optical emission spectroptroscpy (ICP-OES) and elemental analysis was performed in different pot tretment to estimate the content of P, K and N, C respectively. The N, P, K and C was examined at the start and end of the experiment. Sorghum leaf size, plant height and water holding capacity wre determined durin the experiment and significant growth were found in pots fertilised with *L.maxima* biomass. No significant differences were observed in sorghum biomass for the fresh and dry as well as above and below ground biomass for the different treatments. The present study demonstrated that *L. maxima* biomass can be used as an eco-friendly and cost-effective alternative to chemical fertilisers.

Keywords: Agriculture, *L. maxima* microalgae, Sorghum, Microwave-Assisted Extract (MAE)

DECLARATION

I certify that the thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Sifaben Soheb Ghanchivahoar

2022

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CHAPTER:1

Introduction

1 INTRODUCTION

The global food demand is rapidly increasing as a result of projected population growth (Calicioglu et al., 2019). To meet the desired food requirements, the use of chemical fertilisers is increasing (Atzori et al., 2020). The overuse of chemical fertilisers has severe negative impacts on the Earth's natural resources, principally degradation of soil and water quality (FAO, 2017). Moreover, soils of the northern region of Australia, encompassing Queensland and New South Wales have generally high inherent soil fertility compared to soils of Western Australia which are inherently infertile (Agriculture, 2020). Therefore, improving crop yields without compromising the environment is one of the main challenges (Choudhary et al., 2018).

Sustainable agriculture practices such as the use of organic fertilisers, particularly seaweed and l;microalgae-based fertilisers, is receiving growing attention (Atzori et al., 2020). Among the microalgae, cyanobacteria play a vital role in sustainable agriculture, as they enhance crop growth, soil fertility and are generally considered to be environmentally friendly (Garcia-Gonzalez and Sommerfeld, 2016). *Spirulina* can be used as a rich source of macro-and micronutrients for plants, such as vitamins, amino acids, polypeptides, phytohormones (gibberellins, auxins, cytokinins), anti-oxidants and compounds with antibacterial and antifungal properties (Godlewska et al., 2019).

Globally, *Sorghum bicolour* (L.) Moench is ranked among the top five cereal crops (Ananda et al., 2020). The nutritional composition of sorghum is comparable to wheat whole grain (Ananda et al., 2020). Moreover, it is an important dietary staple for billions of people in arid and semi-arid regions of the world (Mace et al., 2009). Sorghum is a crop adapted to high temperatures and can withstand severe droughts and is therefore appropriate for growth in regions unsuitable for other major grain crops (Ananda et al., 2020). With regards to grain sorghum, Australia ranked ninth globally but is the second largest exporter (Crop Explorer http://www.pecad.fas.usda.gov/cropexplorer/). In north-eastern Australia, grain sorghum is a key component of the dry land cropping systems (Queensland Department of Agriculture and Fisheries 2011).

The positive results, obtained by testing some algal species as bio fertilisers encourage the testing of more algal species for different crops. The current study aimed to determine the fertilizing effects of *Spirulina* biomass and microwave-assisted extracts on the growth and productivity of sorghum.

1.1 Review of literature

1.1.1 Australian soil conditions

Australia has some of the oldest land surfaces on earth and, while rich in biodiversity, its soils and seas are among the most nutrient-poor and unproductive in the world (Agriculture, 2020). Australian soils are being farmed since the British settlement and the soils of Western Australia are inherently infertile, except for the northern region of Australia encompassing Queensland, New South Wales with generally have high inherent soil fertility (Agriculture, 2020)(Fig. 1.1). Several soil-degraded conditions which cause environmental and economic concern have been identified, including acidification, salinity increase, wind and water erosion, loss of organic matter, structural decline, accumulation of pollutants and toxic chemicals (Sonmez et al., 2007) (Table 1.1).

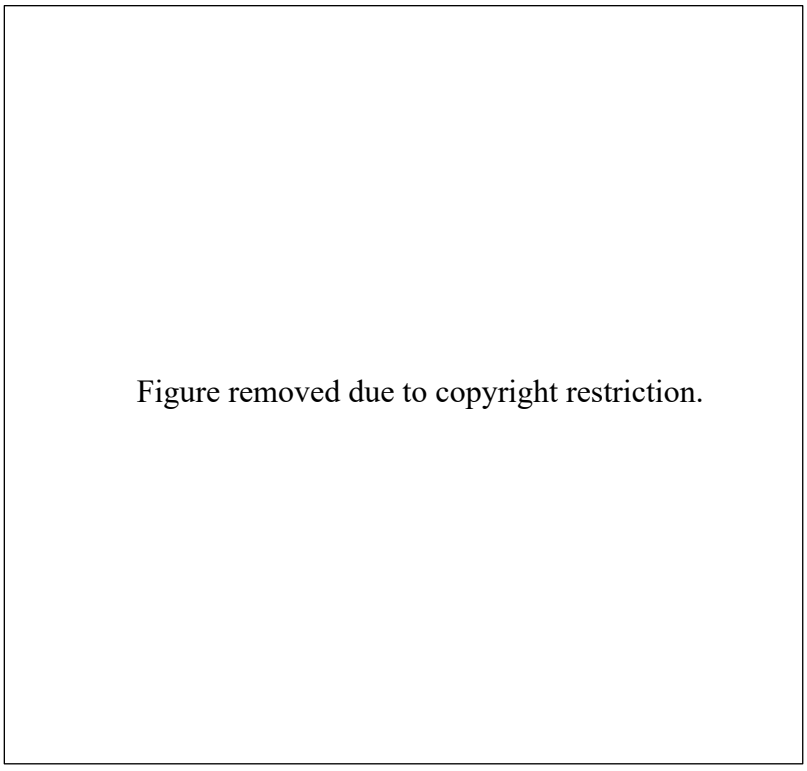

Figure 1-1 Australia's soil fertility map; the portions shown in darker brown has rich volcanic or alluvial soils (http://www.australianpoet.com/boundless.html)

Due to changes in land usage from grazing to cultivation, soil carbon and nitrogen contents are decreasing and as a result, nitrogen is the most limiting nutrient for grain crop production (Dalal and Mayer, 1986). Soil productivity is principally determined by moisture absorption and retention

which greatly depends on soil types and timing of rainfall (Dalal and Mayer, 1986). Insufficient and unreliable rainfall over major parts of Australia negatively affects arable agriculture and pasture production (Johns, 2015). In some areas, principally in Western Australia and New South Wales, the degradation of once-fertile soils is reducing productivity further (Johns, 2015).

In Australia, a major portion of the agricultural land and improved pasture is affected by soil degradation. Population growth and urbanization place additional pressures on available farming land (Nanganoa et al., 2020). Feeding the rapidly growing population is a serious challenge where soil fertility constraints are limiting food production (Stewart et al., 2020, Haileselassie et al., 2011). To meet growing demands, intensive farming practices without adequate soil maintenance further exacerbate land degradation and exacerbate soil fertility problems (Nanganoa et al., 2020). When crops are intensively grown without an adequate supply of nutrients, soils are leached of nutrients, resulting in crops with multi-nutrient deficiencies (Ladha et al., 2003, Seth et al., 2018). Thus, there is an urgent need to implement soil fertility management practices, such as promoting the use of organic compost, green manuring and limited use of chemical fertilisers (Pandey et al., 2018).

Table 1-1 The Main Causes and Impacts of Soil Erosion (Tammana Begum 2021)

Sr. No	Major factors for soil degradation	Their Causes	Impacts of Soil Erosion

1	Deforestation	The increasingly high food demand of the growing population	• Clearing land for agriculture, • Clearing indigenous trees increases the risks of soil erosion especially topsoils
2	Overgrazing	Intensive cattle raising	• Plants do not have the recovery period they need • Top soil sediments are transported elsewhere • Soil can lose its infiltration capacity leading to soils becoming sodden • Can causes floods
3	Agrochemicals	The use of chemicals under the form of pesticides and fertilisers	• The excessive use of phosphoric chemicals causes an imbalance of microorganisms in the soil moisture, stimulating the growth of harmful bacteria. • Accelerate surface runoff and soil erosion
4	Construction and Recreational Activities	Setting up buildings and roads	• Do not allow for the normal circulation of water, instead, it runs off to flood nearby lands, speeding up erosion in these areas.
5	Salination	Salty water is the result of excessive irrigation or extraction of groundwater in coastal the areas	• This can kill many microorganisms and. can make some other bacterial species inactive • Without the soil microbiome, the land would become barren. • It can lead to desertification in the worst-case

1.1.2 Chemical fertilisers - constraints and alternatives

At present, chemical fertilisers are widely used in agriculture, as they immediately provide nutrients and their application is non-labour-intensive (Guo et al., 2010, Howarth et al., 2011). The introduction of chemical fertilisers, known as the green revolution, has resulted in the doubling of food production which increased global per capita food availability, reduced hunger and improved nutrition (Atzori et al., 2020). Over the past century, estimates showed that more than a quarter of

the world population is being fed by food produced with synthetic nitrogen fertilisers only (Ramankutty et al., 2018).

Excessive use of chemical fertilisers contributes to many environmental issues, including increased greenhouse gas emissions, soil degradation, and eutrophication (Guo et al., 2010, Howarth et al., 2011). Even fertiliser applications in ideal conditions only yield 50% of the usage of the applied nitrogen, 2-20% volatilises, 15-25% reacts with organic compounds in the soil and the remaining 2-10% pollute surface and groundwater (Feigin and Halevy, 1989). The latter is due to leaching, drainage, or surface flow, resulting in removal from the root zone (Simpson, 1983). Fertiliser runoff results in increased growth (blooms) of aquatic plants and algae, which can cover the entire surface of a water body, leading to the loss of aquatic animals due to reduced oxygen supply (Neue, 1993). The production of nitrogenous fertilisers also generates greenhouse gases like CO_2, CH_4 and N_2O (Shoji et al., 2001). Some soil bacteria convert nitrogen fertiliser into nitric oxides (NO, N_2O, NO_2) and methane emissions increase when using ammonium-based fertilisers (Shoji et al., 2001). Volatilization of ammonia reaches the atmosphere where it is oxidized to nitric and sulfuric acids, leading to acidic rains (Sharma and Chetani, 2017). All these emissions are responsible for severe air pollution and global climate change (Chen, 2006).

The impact of chemical fertilisers on the soil have been demonstrated to be large and often irreversible e.g., soil acidification and formation of soil crust preventing water penetration (Sonmez et al., 2007). Microbial degradation of nitrogen fertiliser in soils follow slightly different chemical pathways, but acidification due to hydrogen ion production is inherent to all of them (Cooper et al., 2018). The leaching of ammonium-based fertilisers is a major contributor to soil acidification (Sonmez et al., 2007).

Overuse of nitrogen fertilisers has been shown to negatively affect the balance between the three macronutrients, N, P and K and lack of essential micronutrients, Overuse of chemical fertilisers, such as triple phosphate, may also result in a toxic buildup of heavy metals like cadmium, arsenic, and uranium in the soil which bio-accumulate in fruit, vegetable, and grain crops, eventually reaching the human end user via the food web or directly (Sonmez et al., 2007). Hence, improving crop yields without compromising the environment is one of the main challenges nowadays.

The use of organic fertilisers can promote sustainable agriculture and is generally considered to be environmentally friendly (Garcia-Gonzalez and Sommerfeld, 2016). Organic farming approaches

aim to recycle organic residues from natural sources, human, and animal activities (Senesi, 1989). Organic fertilisers, including farmyard manure, crop residues, and compost also decrease dependency on synthetic chemical fertilisers (Yang et al., 2008), whilst simultaneously counteracting soil degradation. In addition to different organic sources, macroalgae (seaweeds) and microalgae, in particular, are receiving growing attention recently (Lötze and Hoffman, 2016, Atzori et al., 2020).

The alternatives of chemical fertilizer should be practice for the long term fertility of the soil. The mixture of microalgal extracts along with compost can be used for the comparative study. If there are better results of the soil parameters in terms of N, P and K by the use of microalgal extracts than use of chemical fertilizer should be avoided. Some researchers have used combination of algae and compost to study germination and growth of plants and they found better results compare to chemical fertilizer. It is also found that it will change the physicochemical characteristics of the soil.

1.1.3 Algal biofertiliser use and application

The general term algae refer to a wide group of photosynthetic organisms that include macroscopic, multicellular marine or freshwater algae (seaweed or macroalgae), and the microscopic species (microalgae) (Guo et al., 2020). Microalgae are widespread photosynthetic organisms and include marine and freshwater eukaryotic algae and prokaryotic blue-green algae. These fascinating organisms can also be used in modern agriculture for their ability to enrich the soil with nutrients and enhance the utilisation of macro and micro-nutrients (Tredici, 2010). In addition to improving soil fertility and quality, microalgae also produce plant growth hormones, polysaccharides, antimicrobial compounds and other metabolites to promote plant growth (Guo et al., 2020). Other applications of algae include dietary supplements, healthcare products, cosmetics, as well as being a resource for the biochemical industry (Fradique et al., 2010). Microalgae are an attractive bioresource for the production of high-value co-products, due to high biomass productivity, cultivation on marginal lands in non-potable water, and cultivation does not require the use of pesticides or herbicides (Tredici et al., 2016).

Cyanobacteria (blue-green algae) play a crucial role in sustainable agriculture as they have been shown to improve crop growth and yields and soil fertility, resulting in environmental benefits

(Osman et al., 2016, Singh et al., 2016). Inoculation of soil with dried cyanobacteria to improve soil fertility (termed as “legalization”) and their application in rice fields can increase yields up to 15–20% in compared to uninoculated control plants (Mishra and Pabbi, 2004).

1.1.4 *L. Maxima*

Spirulina was the genus name applied to edible and nontoxic cyanobacteria of the genus *Arthrospira* (Sharoba, 2014). Cyanobacteria are bacteria capable of oxygenic photosynthesis and the plant chloroplast is thought to be acquired through an endosymbiotic event of a host with a cyanobacterium (Sinha and Häder, 2008). *Spirulina* grows well in tropical as well as subtropical regions in alkaline water (pH 9.5) containing high amounts of carbonate and bicarbonate (Ahsan et al., 2008). They grow as helicoid, unbranched trichomes composed of cylindrical cells, but the degree of coiling is variable and not a stable species trait, as it is influenced by growth conditions (Ciferri and Tiboni, 1985). Among a large number of *Spirulina* species, three are produced commercially; *Spirulina platensis* (now *Arthrospira platensis*), *Spirulina maxima* (now *Limnospira maxima)* and *Spirulina fusiformis* (now *Arthrospira fusiformis*), as these species are having high nutritional as well as therapeutic value (Michael et al., 2019). *Limnospira maxima* and other *Spirulina* species are obligate alkaliphiles, thriving in warm, alkaline lakes of the tropical and sub-tropical regions where other organisms struggle to survive (Michael et al., 2019), which limits contamination threat by lesser tolerant microalgae (Touloupakis et al., 2016). For example, *A. fusiformis* dominates microfloral communities in the soda lakes of East Africa, forming almost uni-algal blooms (Michael et al., 2019). *Arthrospira fusiformis* is also dominant in Lake Big Mojela in Tanzania being the major food source for the Lesser Flamingo (Mulokozi, 2016). *Spirulina* is nutritionally comprehensive with a balanced amount of all nutrients. It is high in easily digestible protein (50 and 70% of its dry weight) (Hoseini et al., 2013), essential vitamins, and some dietary minerals such as iron and manganese, and is low in fats (Michael et al., 2019). Moreover, the biomass contains good levels of many antioxidants such as flavonoids, phenolics, vitamin E, and various light-absorbing pigments (e.g., chlorophylls, phycocyanin, phycoerythrin, and carotenoids), which are essential for nutritional protection against free radicals (Michael et al., 2018, Michael et al., 2019). *Spirulina* has shown promise as a biofertiliser. In agriculture, efficient use of *Spirulina* improved food quality, soil physicochemical qualities, prevented soil-borne diseases, added organic matter, and released growth-promoting chemicals (Chittora et al., 2020).

Traditional methods such as maceration, mechanical calcination, and thermal reflux are commonly used to extract bioactive compounds from microalgae. These processes, on the other hand, necessitate long extraction periods, high energy costs and the use of toxic solvents, rendering them both costly and detrimental to human health and the environment (Flores-Gallegos et al., 2020). In this sense, green technologies such as microwave-, ultrasound-, and enzyme-assisted processes have been developed to aid in the extraction of biomolecules (Nanni et al., 2001). Microwave-assisted extraction (MAE) uses electromagnetic waves to disrupt cell structure (Navarrete et al., 2012). High extraction yields of MAE may be the result of a synergistic combination of two transport phenomena: heat and mass gradients acting in the same direction (Veggi et al., 2012), and heat is dissipated volumetrically within the irradiated medium, while heat is transferred from the heating medium to the interior of the sample in traditional extractions (Flores-Gallegos et al., 2020).

1.1.4.1 Application of *Spirulina* in agriculture

Application of nitrogen-fixing cyanobacteria as bio fertilisers (e.g., *Anabaena oryzae, Nostoc, muscorum, Nostoc humifusum*, and *Wollea* sp.) may diminish the requirement of synthetic nitrogen fertilisers (Godlewska et al., 2019). Some cyanobacteria capable of nitrogen fixation can decrease nitrogen mineral fertiliser use by 1/4 or 1/2 of the recommended dose (Hegazi et al., 2010). Species of *Spirulina, Arthrospira* and *Limnospira*, however, are incapable of nitrogen fixation and hence need to be grown in nitrogen-enriched growth media (Hegazi et al., 2010). Commercial cultivation of these species targets the nutrient supplement market, but as industrial production occurs in open ponds, contaminated biomass may be unsuitable for sale. Thus, rather than discard biomass unsuitable for human consumption, utilization as a bio-fertiliser can be considered. Du Jardin (2015) used *Spirulina* formulations as a bio-stimulant for plant growth. *Arthrospira platensis* (*Spirulina platensis*) is a promising source of macro- and micronutrients for plants and also showed some antibacterial and antifungal properties (Godlewska et al., 2019). In most studies, *Spirulina* biomass is applied directly to the soil or added in the form of an algal suspension (Godlewska et al., 2019). Plants biofortified with the macro- and micro-nutrients of cyanobacteria can be used as innovative functional food aiming to prevent malnutrition (Tuhy et al., 2015, Mala et al., 2017). *Arthrospira platensis* was successfully used for agronomic bio-fortification in the red spinach (*Amaranthus dubius*) (Mala et al., 2017). Post-extraction residues after supercritical CO_2

extraction of *A. platensis* enriched with Zn (II), Cu (II) Mn (II) ions using biosorption were used as NPK fertiliser to bio-fortify maize in field trials with these micro-nutrients (Tuhy et al., 2015).

Anitha et al. (2016) tested *A. platensis* bio-fortified with different combinations of bio fertilisers, vermicompost, organic manure, and chemical fertiliser to enhance levels of zinc in cultivars of the vegetable amaranth (*Amaranthus gangeticus*) and tomato. The mutual association with plant roots and *Spirulina* + bio fertilisers combination increased levels of phytosiderophores into the soils. Phytosiderophores chelate zinc ions from the soil, making them bioavailable to plants. Mógor et al. (2018) showed that enzymatic hydrolysates of *A. platensis* contained polyamines (e.g., spermine acquired through decarboxylation of algal L-amino acids), promoting plant growth. Osman et al. (2016) used *A. platensis* as a natural herbicide safener (bioactive organic compounds that increase the tolerance of monocotyledonous cereal plants to herbicides) to withstand harmful effects of the fusilade herbicide (a superior post emergence grass weed herbicide) for *Faba* bean crops, where seeds were primed in the *A. platensis* suspension before cultivation

1.1.5 Sorghum bicolor

Globally, *Sorghum bicolor* (L.) Moench is ranked among the top five cereal crops (Ananda et al., 2020). Sorghum is a multipurpose crop cultivated for grain and forage. Sorghum plays a vital role in global food production and is an important dietary staple of billions of people in 30 countries of arid and semi-arid regions of the world (Mace et al., 2009). The nutritional composition of sorghum whole grain is comparable to wheat whole grain; energy density is 1377 vs 1418 kJ/100 g dry weight, total carbohydrate 74.6 vs 71.1, fat 3.3 vs 2.5 and protein 11.3 vs 13.7 g/100 g dry weight, respectively (Duodu et al., 2003). In western countries, the use of sorghum for human nutrition and as an ingredient in beverages has increased due to its gluten-free nature, but grain, leaves and stalks are still largely used for animal feed (Hariprasanna and Patil, 2015, Venkateswaran et al., 2019). It is also an important resource for the production of alcoholic beverages, bioethanol, and building materials (Ananda et al., 2020). In Australia, sorghum is almost exclusively used as feed for cattle, pigs and poultry (Corporation, 2017). A significant market is also found in the pet food industry, but used as gluten-free breakfast cereals, beer, and baked products for human consumption is still low.

It is one of the cheap alternatives to maize, due to its tolerance of dry growing conditions, requiring less water 450 – 650 mm (compare to 500 – 800 mm for maize) over the total growing period to

produce similar yields (FAO, 2017). According to a study by Farré and Faci (2006), maize yields were higher than for sorghum under well-irrigated conditions, but under moderate or severe water deficits, sorghum out-yielded maize. Sorghum has a superior ability to extract water from the deeper soil layers (Steele et al., 2013).

1.1.5.1 Cultivation of sorghum in Australia

Sorghum is a crop adapted to high temperature and moisture stress (Ananda et al., 2020). It can withstand severe droughts and performs better than maize on low potassium (K) soils, appropriate for growth in regions unsuitable for other major grain crops (Ananda et al., 2020). The average global annual production of sorghum is 50 megatons, with the USA being the world's largest sorghum producer, followed by Nigeria, India, and Mexico (Nations, 2019).

With regards to grain sorghum, Australia ranked ninth globally but is the second largest exporter (Service, 2022). In north-eastern Australia, grain sorghum is a key component for dry land cropping (Queensland Department of Agriculture and Fisheries 2012). Grain sorghum is a summer season crop, with a prolonged season in higher latitudes which includes Central Queensland (approximately 60%) and further north (GRDC grow notes 2015). Sorghum planting areas in northern NSW and Queensland are~160,000 ha and 470,000 ha, respectively (McMullen, 2015). The main sectors for producing grain sorghum include the Liverpool Plains in NSW and east of the Newell Highway and Darling Downs in Queensland. Average farm yields of sorghum are ~one t/ha, reflecting the severity of constraints (GRDC grow notes 2017). Sorghum is primarily exported to China and Japan, and to a lesser extent to Malaysia, the Philippines, Taiwan and Saudi Arabia (GRDC grow notes 2017).

1.1.5.2 Growth requirements of sorghum

In dryland cropping, winter-sown sorghum provides additional sowing opportunities, reducing the chance of heat stress and increasing opportunities for double cropping. In Australia, July-August soil temperatures of $\geq$ 9.4°C do not limit the germination of commercially available sorghum hybrid seeds. A total of 80% of sorghum seeds germinate within about 10 to 12 days at 15ºC (https://www.agrifarming.in). However, as germination is spread over several days, the seedbed must remain moist for at least 9 days for successful germination and emergence (GRDC grow notes 2019). Commercial sorghum hybrid cultivars were evaluated in winter sowing windows on farms in northern New South Wales (NSW) and southern Queensland (Qld). The study showed

that Sorghum sown during the months of July-August was optimal, as the soil temperature is around 12°C. Moreover, winter-sown crops are harvested before mid-December, affording double cropping (Eyre et al., 2019).

Sorghum is adapted to resist higher average temperatures than most other cereal crops but the high temperature may affect yields, particularly throughout the sowing season (Hall, 2000). Temperatures of 26-34°C are ideal for growth, up to 45°C are tolerated by Sorghum, while temperatures below 8°C can mutilate the flowering and pollination (Peacock, 1982). The lowest temperatures for germination of sorghum are 7 to 10°C (Rao, 2005), while the optimal range is 21 to 35°C and 25 to 28°C for reproductive growths (Prasad et al., 2008). Sorghum grows well over a pH range of 6.0-8.5 as it tolerates alkaline to saline soils (Carter et al., 1989, Mundia et al., 2019). Being tolerant of wet soils, sorghum can withstand flooding events but the ideal soil moisture for germination ranges between 25% and 50% (Mundia et al., 2019). Sorghum is frequently grown in shallow to medium-deep soils and light to medium-textured soils with a high water-holding capacity (Carter et al., 1989).

1.1.5.3 Nutrient and fertiliser requirements of sorghum

Sufficient soil nutrition is essential to achieve adequate growth and yields (Wylie, 2008). Sufficient nitrogen (N) and phosphorus (P) fertilisation is mandatory for a successful production; other essential nutrients include zinc (Zn), potassium (K) and sulphur (S) (Wylie, 2008). Compared to other cereal crops like wheat and barley, sorghum can withstand lower levels of soil P but soil deficiency is expected after periods of long fallow, with low abundances of soil microbes being the assumed reason (QDAF 2011). In Australia, sorghum is generally grown in rotation with legumes, cereals, pastures, oilseeds and cover crops (WA DA 2001)

In north-eastern Australia, requirements of N fertilisers are increasing, as cropping seasons are short and adequate yields require increased N availability (Lester et al., 2016). Numerous trials have demonstrated that supply of 80 kg N/ha before or at sowing can increase yields from 1.8 to 2.9 t/ha after a short fallow period (GRDC grow notes 2017). Sufficient plant nutrition is necessary at flowering, as maximum nutrient uptake and rapid plant growth occur at this time (GRDC grow notes 2017). To increase yields, application of N is necessary before floral initiation takes place and a nutrient deficiency during this particular period cannot be corrected later on (Vanderlip and Reeves, 1972). Nitrogen fertilisers are typically applied before or at the time of crop sowing, but

a higher concentration of soluble salts negatively affect the germination of seeds and the establishment of young plants (QDAF 2011). Soil nitrogen levels are vulnerable to environmental loss between applications, possibly due to gaseous loss in the form of nitrogen oxides and methane or leaching as a result of intense rainfall events, in addition to high crop demands for growth (Lester et al., 2016).

1.1.6 Soil quality parameters to evaluate soil health

Maintaining soil quality is necessary for the long term prosperity of a cropping system. Soil quality is typically defined as the ability of soil to function while maintaining or improving water and air quality and supporting biota and is assessed using a set of chemical, physical and biological tests (Wienhold et al., 2004). Mainly there are three categories of soil quality indicators: physical, chemical and biological. Any categories do not precisely align with the different soil functions, so integration is required. Although, typical soil testing is based on chemical indicators only; which includes extractable N-P-K, electrical conductivity, cation-exchange capacity (CEC), soil pH, soil moisture, water holding capacity, reactive carbon (RC) etc (Doran and Parkin, 1997).

1.1.6.1 Water holding capacity

The ability of soil to retain water is strongly associated with particle size; e.g. water molecules hold more tightly to the fine particles of clay soil than to the coarser particles of sandy soil, thus clays usually retain more water (Charman and Murphy, 2007). On the other hand, sand provides easier transmission of water through the profile. Clay type, soil structure and organic content also influence soil water retention (Charman and Murphy, 2007).

The maximum amount of water that a given soil can retain is called field capacity**,** whereas a soil so dry that plants cannot liberate the remaining moisture from the soil particles is said to be at wilting point. Available water is that which the plants can utilize from the soil within the range between field capacity and wilting point (Duncan et al., 2013). Water holding capacity can be determined by measuring soil moisture at field capacity and permanent wilting point. The difference between those two soil moisture values is the water holding capacity (Brischke and Wegener, 2019).

1.1.6.2 Soil reaction (pH)

Soil reaction (pH) usually refers to the degree of soil acidity or alkalinity and is chemically defined as the $\log_{10}$ hydrogen ions (H^+) in the interstitial water of the soil. Where, H^+ represents the activity of hydrogen ions in solution, not the concentration of hydrogen ions (Smith and Doran, 1997). A change of just a few pH units can induce significant changes in the chemical environment and sensitive biological processes. The main source of H^+ ions is carbonic acid generated when carbon dioxide from root respiration and decomposing organic matter in the soil atmosphere is dissolved in the interstitial water. Other sources of H^+ ions include a release by roots, nitrification of ammonium from fertilisers, reaction of aluminium ions with water, organic matter mineralization, rainwater, and acid rain. The majority of crops grow well in soils with a pH between 6 and 7.5 (USDA 2015).

1.1.7 Determination of plant growth and yield

A comprehensive evaluation of soil properties along with plant growth is necessary to evaluate the effect of any fertiliser (Hasnain et al., 2020). Several classes of quantitative or qualitative parameters have been used to describe plant development and growth, physiological status, and other aspects of plant development and growth during or after an experimental growth phase (Füzy et al., 2019). The majority of them can be measured non-destructively, such as through optical imaging techniques, e.g., plant height, the number of nodes, shoot diameter, the colour of leaves, leaf number, the state of flowering, podding or grain filling, as well as observations of growth morphological dynamics. Plant responses can also be characterized by the nutritional status of plant shoots, roots or yield. Fresh and dry weight, root and shoot biomass yield, root to shoot ratio, leaf area, grain size, reproductive index and yield are the most basic and obvious parameters (Füzy et al., 2019).

1.2 Biotechnology significance

Significant declines in soil and water quality are currently global environmental and agroeconomic issues. The increased use of chemical fertilisers is one of the leading factors responsible for the deterioration of soils. Bio fertilisers are important pillars in sustainable agriculture to improve production, protect the environment, and generate pollutant-free crops. A cyanobacterium like *Spirulina* (*Arthrospira* or *Limnospira*) offers a comprehensive nutritional product for increasing yields in agriculture, but growth-enhancing effects on sorghum have to date not been investigated.

It is hypothesized that the use of extracts of *L. maxima* as bio fertilisers can improve plant yields and soil quality without compromising the environment. Hence, such an approach is advantageous especially for countries like Australia where soil infertility is a major concern.

1.3 Aim and Objectives:

Evaluate the efficacy of *L. maxima* extracts to improve plant growth overe commercially available gardening Seasol fertiliser, and carbon-rich compost soil.

OBJECTIVES

1. Determination of soil mineral content (C, N, P, K) using inductively coupled plasma optical emission spectrometry (ICP-OES) and elemental analysis.

2. Assessment of the effects of different fertiliser types on plant growth based on

a) Above-ground biomass (fresh and dry)

b) Below-ground biomass (fresh and dry)

c) Number of plant leaves and dimensions

d) Plant height

CHAPTER:2

Material and Methods

2 MATERIALS AND METHODS:

2.1 MATERIALS

2.1.1 Source material:

Twenty kilograms of *Limnospira maxim* organic powder was obtained from Honest to Goodness and seasol fertiliser (Seasol plus nutrients all-purpose including natives and vegies) was purchased from Bunnings (10758). 380 seeds of Sorghum (Broom Corn Ornamental), *Sorghum bicolour*, was purchased from Eden. A soil moisture meter was purchased online from https://bunnings.com.au/gardman-soil-moisture-meter_p2961033; soil moisture meter model number 64737.24 kg of garden soil (compost soil) and 144 kg of screened topsoil (poor soil) was purchased from SA Composters Pty. Ltd.

2.1.2 Chemicals:

HNO_3, Na_2HPO_4, KCl were obtained from the chemical storage room on level 5 of the Health Sciences Building at Flinders University at Bedford Park, SA5042.

2.1.3 Equipment:

27 pots with a diameter of 25 cm and a soil moisture meter (Gardman-soil-moisture-meter_p2961033, model number 64737) were purchased from Bunnings. Microwave extractor (Milestone Innovations), freeze dryer (Virtis, NSW), rotary evaporator (Buchi Flawil, Switzerland), hot air oven (Scientific Equipment Manufacturer SEM), centrifuge (Sartorius microbalance), -80°C freezers, Perkin Elmer ICP-OES Optima 8000, DigiPREP block digestion system, ICP tubes, Elemental Vario, Isotope cube were available in the College of Medicine and Public Health and Flinders Analytical at Flinders University at Bedford Park, SA 5042, respectively.

2.2 METHODS:

2.2.1 Extraction

2.2.1.1 Microwave-assisted water extraction (MAE):

The process was carried out in a Start SYNTH-microwave synthesis lab station, equipped with an industrial magnetron that can deliver up to 1200 Watt. A microwave diffuser which is located

above the microwave chamber evenly distributes the microwaves throughout the cavity, preventing localized hot and cold spots. It is operated using a compact terminal, with a bright, high resolution, touch screen display in which the parameters such as irradiation power, temperature and time are entered. Exactly 10 g of *L.maxima*(*Spirulina* organic powder) was weighed and placed in a 250 mL round bottom flask with a quick-fit wide neck fitting into the condenser tube inside the chamber. All microwave extractions were performed under a set microwave irradiation energy detailed in Table 2.1 for 30 min in 90 mL water at 40, 60 and 80°C. Following the extraction, the extracts were centrifuged at 2,040 rcf for 5 min to obtain the supernatant (extract).

Table 2-1 Extraction conditions for microwave-assisted extraction of *L. maxima* biomass

Extraction	Biomass/Algal powder	Solvent (H_2O)	Temperature	Energy
2	10 g	90 mL	40°C	500 W
3	20 g	180 mL	60°C	800W
4	30 g	270 mL	80°C	1000 W

2.2.2 Determination of soil mineral content (C, N, P, K) using inductively coupled plasma optical emission spectrometry (ICP-OES) and elemental analysis.

Analysis of P and K was carried out by ICP-OES while an elemental analyser was used for determining C and N contents of compost and top soils before planting (day 0) and at the point of harvest (day 55), *L. maxima* powder, and MAE extracts of *L. maxima* at the three different temperatures (Table 2.1). These analyses were conducted by Flinders Analytical.

2.2.3 ICP-OES method for P and K analysis of *L. maxima* extracts:

For P and K analysis via ICP-OES (Table 2.2) samples were diluted 1,000 times in 0.5% HNO_3 in two steps. First 0.1 mL of the sample was diluted with 9.9 mL MQ water, and 1 mL of that solution was diluted with 9 mL 0.5% HNO_3, giving a total dilution of 1,000. The calibration was made in 0.5% HNO_3 for 50 ppb to 10 ppm for K and from 50 ppb to 2 ppm for P. Two wavelengths were used for each element P 177.434 (nm), P 178.221 (nm) and K 766.490 (nm), K 76.896 (nm). The 40°C extracts were analysed using only water and water plus 0.5% HNO_3 to see if the results were

similar, which they were, so the MQ plus 0.5% HNO_3 was used. Samples and standards were analysed on a Perkin Elmer ICP-OES Optima 8000.

2.2.4 ICP-OES method for P and K analysis of *L. maxima* biomass and the freeze-dried extracts:

For P and K analysis via ICP-OES (Table 2.2) samples were acid-digested in a DigiPREP block digestion system in the following way: Between 50 and 100 mg of sample was weighed into 50 mL digestion tubes and 5 mL concentrated highly purified HNO_3 was carefully added to each tube, making sure that no violent reaction occurred. These samples were pre-digested at ambient temperature overnight before placing them into the block digestor. The following digestion method was used: ramping up to 80°C for ~20 min, holding at 80°C for 30 min, following by ramping to from 80 to 120°C over 15 min and holding at 120°C for 2 h. Samples were cooled to room temperature. After cooling, samples were diluted with MQ water to 50 mL, resulting in an HNO_3 concentration of 10%. A 5 mL aliquot of each sample was transferred to 15 mL ICP tubes and diluted to 10 mL with MQ water giving an HNO_3 concentration of 5%. A calibration ranging from 50 ppb to 10 ppm was made in 5% HNO_3 to match the acid matrix of the samples. Two wavelengths were used for each element P 177.434 (nm), P 178.221 (nm), and K 766.490 (nm), K 76.896 (nm). Samples and standards were analysed on a Perkin Elmer ICP-OES Optima 8000

2.2.5 Elemental analysis of C and N in freeze-dried *L. maxima* extracts:

Samples were run using the Elemental Vario Isotope cube with the combustion tube set at 950°C and the reduction tube at 600°C. 15 mL of *L. maxima* MAE extracts obtained at 40, 60, and 80°C were freeze-dried for 4 days at -80˚C. 1.587g, 2.882g and 3.940 g of *L. maxima* MAE extract obtained at 40, 60, and 80°C were used for the analysis respectively. Similarly, 20 mg of compost soil, top soil, and 2 mg of *L. maxima* biomass samples were subjected to elemental analysis of C and N.

Table 2-2 Samples provided for ICP-OES and elemental analysis

No	Sample name	Sample volume [mL]	Sample Dry weight [g]
1	Compost soil	-	10
2	Top soil	-	10
3	*L. maxima* MAE extract 40°C	15	1.587
4	*L. maxima* MAE extract 60°C	15	2.882
5	*L. maxima* MAE extract 80°C	15	3.940
6	*L .maxima* biomass	-	10

2.3 Plant growth experiment:

2.3.1 Fertilisation experimental design

A total of 21 plastic pots (25 cm diameter) were used to set up fertilisation regimes in triplicate for six fertilisation treatments – fertilisation with seasol fertiliser, *Limnospira maxima* biomass, MAE-extracts of *L. maxima* biomass obtained at 40, 60, and 80°C, whilst compost and unfertilised sandy topsoils served as positive and negative controls, respectively (Fig. 2.1). Nine Sorghum seeds (*Sorghum bicolor*), were sown in each pot with a uniform distribution. For standardisation of light effects, a randomized design with daily rotation was applied. To maintain even soil moisture in all pots, soil moisture levels were determined and water supply was adjusted in such a way that all pots were returned to the starting soil moisture daily. Plants from each pot were harvested after 55 days of sowing.

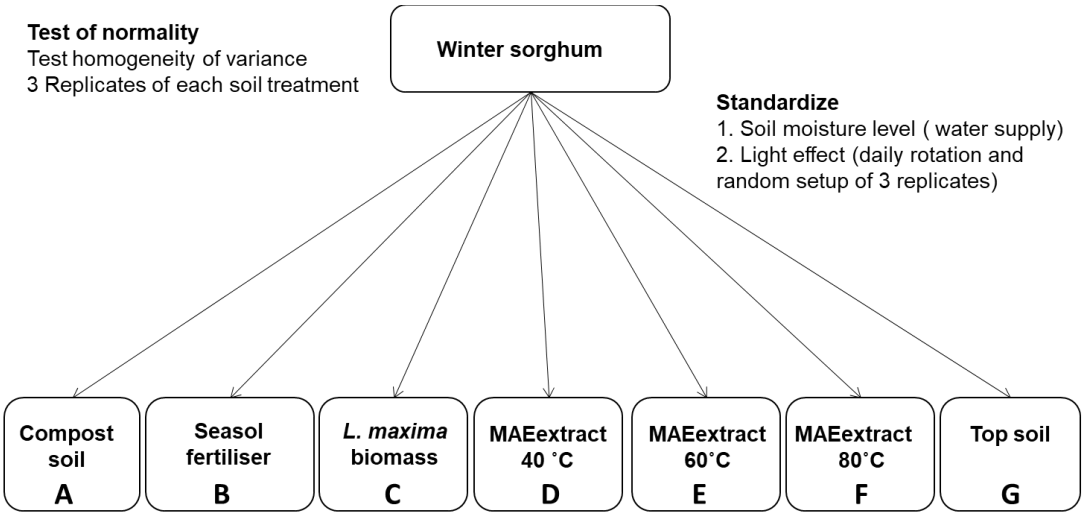

Figure 2-1 The experimental design set-up of the fertilisation treatments

2.3.2 Determination of plant growth and soil N, P, C, and K levels

2.3.2.1 Plant growth characterization

Thirty days after germination, plant heights and leaf sizes were measured for all plants. Plant height was determined by carefully measuring from the ground level to the apex of the growing point using a measuring tape. The leaf size was determined by keeping the leaves flat and a ruler was used to measure the length of each leaf from the pointy part at one end of the leaf to the point where the leaf joins the stalk at the other end. Plant growth was determined by measuring above and below-ground biomass after harvesting. Fresh weight of leaves, stems, and roots was taken for each plant. To determine above and below-ground biomass dry weight, plant materials were washed thoroughly with running tap water and padded dry gently with a paper towel then kept for drying for 2 days in a 37°C hot room.

2.3.2.2 Post fertilisation determination of C, N, P, and K soil contents

Determination of C, N, P, and K contents were carried out by Flinders Analytical for the soil samples taken from each pot, as described for pre-fertilisation section 2.2.2.

2.4 Water Holding Capacity

The water holding capacity of each pot was measured after 10 days for the three replicates for each of the treatments, and the positive and negative controls, respectively. The dry and wet weight of a pot along with soil was measured using a balance. Then, 1000 mL water was added to the pot and the weight of the wet pot was measured again. The total volume of the soil was measure by

adding volume of solids and water. The water holding capacity was calculated using the following formula.

$$\text{Water Holding capacity(VWC\%)} = \frac{\text{Total mass of container with wet soil} - \text{Total Mass of container with dry soil}}{\text{The total volume of the soil}} \times 100$$

……..Equation No 2.1

Water content in root and leaves was calculated in % where fresh and dry weight of leaves were taken

$$\text{water content} = \frac{Wf - Wd}{Wf} \times 100$$

……..Equation No 2.2

2.5 Statistical Analysis

The data were analyzed using the Statistical Software for Social Sciences (SPSS) version 26. Descriptive statistics such as mean and standard deviation were used to describe the data. In addition, a one-way analysis of variance (one-way ANOVA) was used, as the inferential statistics to determine whether there were significant mean differences in the dependent variables between the treatments. Before performing the one-way ANOVA, normality assumptions and test of homogeneity of variance were done for the N, C, P, and K, plant height, leaf size, water holding capacity, as well as, the leaf and root fresh and dry weight data. The test for normality was conducted using the Kolmogorov Smirnov test when the sample size for the N, C, P, and K, plant height, leaf size, water holding capacity, as well as, the leaf and root fresh and dry weight data was greater than 30 and the Shapiro Wilk test if the sample was less than 30. The decision was to conclude that the normality assumption was met if the p-value was greater than the 5% level of significance. On the other hand, the homogeneity of variance assumption was evaluated using the Levene's test based on mean and the decision was to conclude that the assumption was met if the p-value was greater than the 5% significance level. If the results were statistically significant, a

multiple comparison test (post hoc analysis) using Tukey HSD was used to determine which treatments were significantly different. However, when the homogeneity of variance assumption was not met, Welch's F-statistic was used instead of the F value from the ANOVA table. The post hoc analysis assuming unequal variances was conducted using the Gamel-Howell test. In this study, treatment effects were deemed to be significant at a set alpha of <0.05 ($p < 0.05$).

CHAPTER:3

Results

3 RESULTS

3.1 N, C, P, and K concentrations of starting materials

The N, C, P, and K concentrations of the top soil, compost, *L. maxima* biomass, MAE 40˚C, MAE 60˚C, and MAE 80˚C, were calculated to adjust to the levels of N, P, and K found in compost soil that treatments only differed in the amount of carbon, excluding the negative (nutrient-poor top soil) and positive (compost soil) controls, respectively (Table 3-1). P and K concentrations were measured with ICP-OES immediately after extraction (Raw Extract). Then the samples were freeze-dried and reconstituted, and P and K concentrations were quantified again (Reconstituted Freeze-Dried Extracts). For the fertilisation treatments N, P, and K concentrations of the *L. maxima* biomass and MAE extracts were adjusted to meet the levels present in compost soil before sowing the seeds. C contents were highest in biomass and MAE extracts, as were N concentrations (Table 3-1). In addition, P and K contents in the reconstituted freeze-dried extracts were highest in *L. maxima* biomass and MAE extracts (Table 3-1).

Table 3-1 Day-0 concentration of N, P, K, and C of top, compost, and topsoil fertilised with seasol fertiliser, *L. maxima* MAE obtained at 40, 60 and 80˚C

Name	N [g/kg]	C [g/kg]	P in Raw Extract (177.434) [g/kg]	K in Raw Extract (766.490) [g/kg]	P in Reconstitutd Freeze-dried Extracts (P177.434) [g/kg]	K in Reconstituted Freeze-dried Extracts (766.490) [g/kg]
MAE 40˚C	106.6	403.4	1.24987	1.860839	7.515194	8.804349
MAE 60˚C	94.2	357.4	1.534149	1.96564	6.665845	7.388668
MAE 80˚C	82.6	331.9	1.599687	2.031947	6.86167	7.514752
L. maxima	102.3	446			13.22265	14.67547
Top soil	0.13	2.17	0.432339	2.768421		
Compost soil	0.33	4.15	1.187676	6.209598		
Seasol fertiliser	2.14	2.80			0.12	1.92

Seeds of *Sorghum bicolor* did not germinate in the unfertilised top soil (negative control). After harvest of the *Sorghum bicolor* on day 55, the soil concentration of P and K, and N and C was determined by ICP-OES and elemental analysis, respectively (Table 3-2).

Table 3-2: Soil Concentration of Day 55

Statistical analysis on day 55 determined whether there were significant differences in nutrient utilisation (N, C, P and K) of *Sorghum bicolor* in the treatments. For the N concentration, Seasol fertiliser, *L. maxima* biomass, and MAE 40 and 60°C data met the normality assumption test Shapiro Wilk test $p > 0.05$ but the top soil, compost soil, and MAE 80˚C were not normally distributed.

In comparison to the N concentration on day 0, the top and compost soil N concentrations increased by 1.57g/kg and 3.27g/kg, respectively. The N concentrations for the seasol fertiliser, *L. maxima* biomass, MAE 40˚C, MAE 60˚C, and MAE 80˚C treatments on day 55 had decreased by 0.44g/kg, 100.80g/kg, 105.03g/kg, 92.57g/kg, and 80.97g/kg, respectively. N concentrations across all the treatments was statistically similar, $F (5, 5.50) = 4.18$, $p = 0.063$ (Appendix Table 3-1A). This implies that the nitrogen content contributed equally to the plant's growth across treatments.Based on a bar graph, compost soil seemed to have the highest C concentration of all the other treatments (Fig. 3-1B). However, the Welch's F-test showed that the C concentration was statistically similar across the treatments, $F (6, 6.07) = 2.04$, $p = 0.202$ (Appendix Table 3-1B).

The C concentrations of the top soil, compost soils and seasol fertiliser increased by 23.16g/kg, 47.72g/kg, and 27.03g/kg respectively. On the other hand, the C concentration for the *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C treatments decreased by 421.73g/kg, 379.03g/kg, 331.77g/kg and 306.23g/kg, respectively. A bar graph showed that compost soil and MAE 60˚C had the highest P concentration of all the other treatments (Fig. 3-1C). However, Welch's F statistic revealed that P concentration was statistically similar across all treatments, $F (6, 5.72) = 2.89$, $p = 0.12$ (Appendix Table 3-1C). The P concentration of the top soil and the seasol fertiliser increased by 0.17g/kg and 0.37g/kg, respectively. The P concentration for the compost soil, *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C treatments decreased by 0.03g/kg, 12.62g./kg, 7.03g/kg, 5.40g/kg and 6.45g/kg, respectively.

The K concentration for the compost soil and soil treated with seasol fertiliser increased by 0.08g/kg and 1.62g/kg, respectively. The small increase could be attributed to the measurement variations used in this study. The K concentration for the top soil, *L. maxima* biomass, MAE 40˚C,

MAE 60˚C , and MAE 80˚C treatments decreased by 0.15g/kg, 11.62g/kg, 5.54g/kg, 4.37g/kg and 4.50g/kg, respectively. K concentration was statistically similar across all the treatments, F (6, 6.09) = 3.65, p = 0.07 (Table 3-1D).

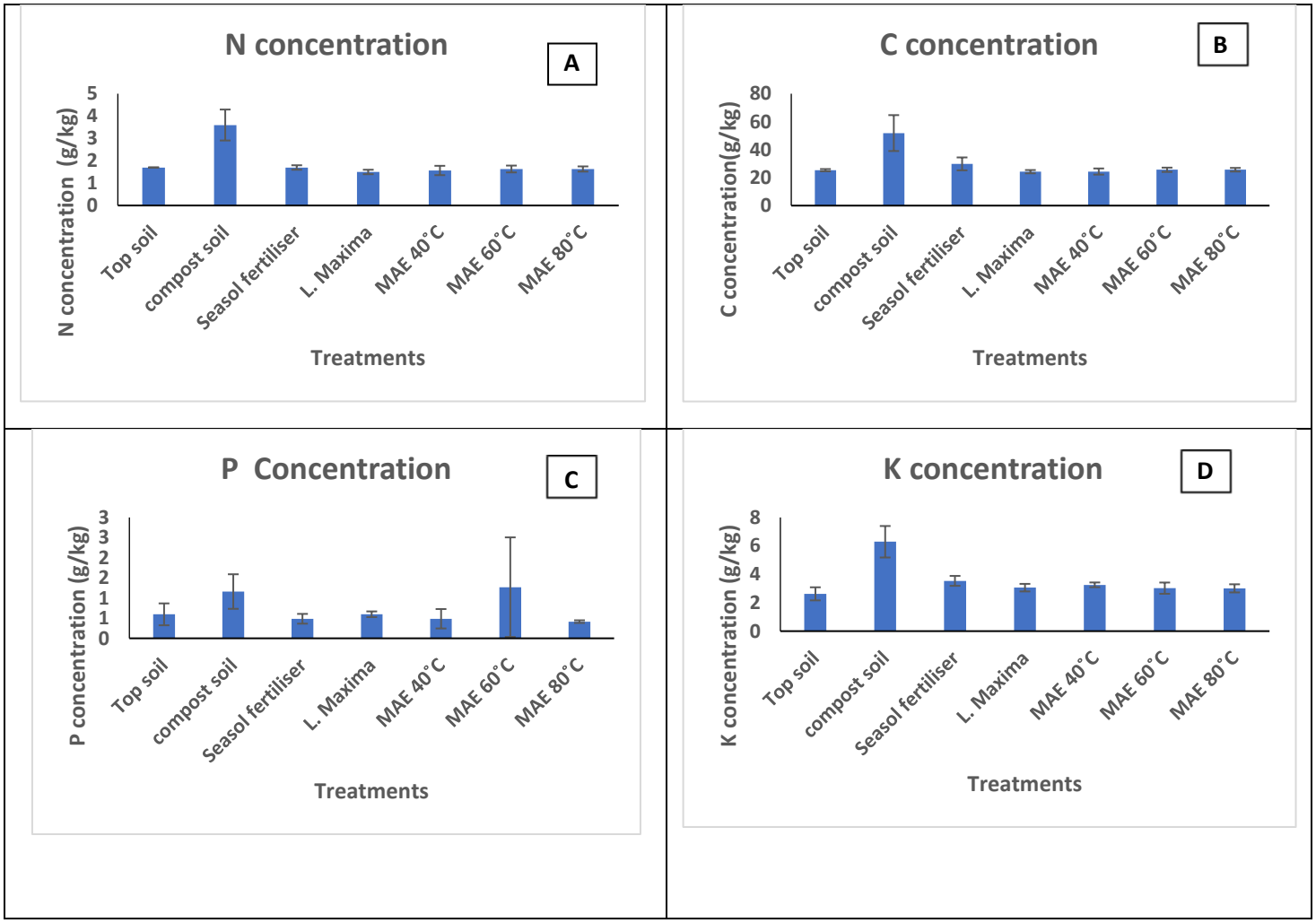

Figure 3-2 Post-harvest Day 55 soil concentrations of N (A), C (B), P (C), and K (D) in untreated top soil (negative control), compost (positive control), and topsoil fertilised with seasol fertiliser *L. maxima*, and MAEs obtained at 40, 60 and 80˚C

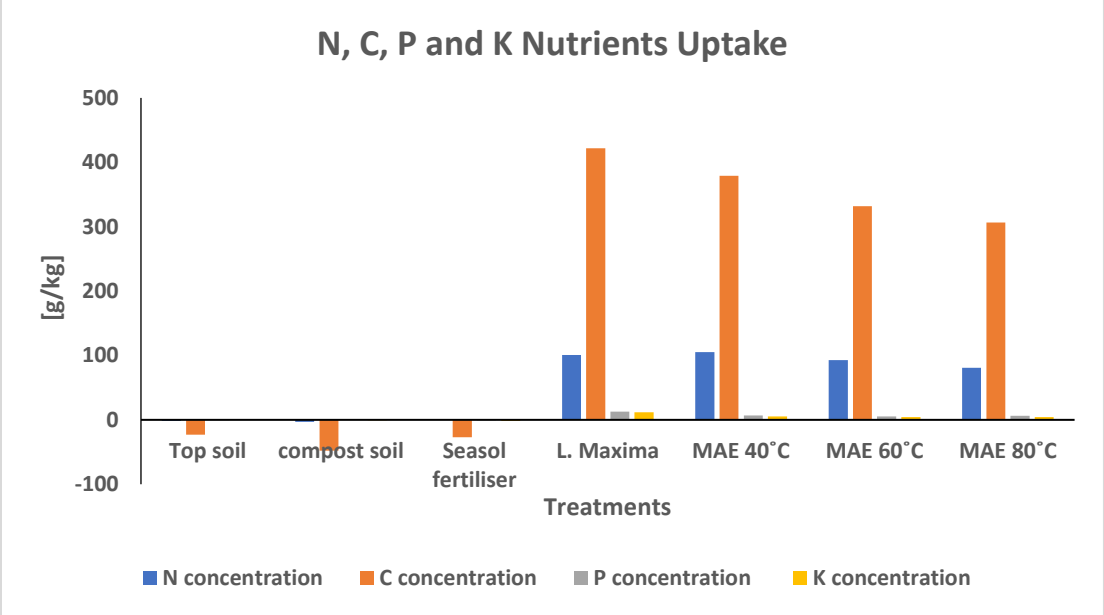

Fig. 3-1A N, C, P and K uptake

3.1 Growth responses of *Sorghum bicolor* to fertilise treatments

Figure 3-1 30-day growth response of *Sorghum bicolor* to no fertilisation (negative control), compost soil (positive control), seasol fertilizer, *L. maxima* biomass, and *L. maxima* MAE extracts at 40, 60, and 80°C (from right to left)

Figure 3-2 55-day growth response of *Sorghum bicolor* to no fertilisation (negative control), compost soil (positive control), seasol fertiliser , *L. maxima* biomass, *and L. maxima* MAE extracts at 40, 60, and 80°C (from right to left)

3.2.1 Leaf sizes at Day 30 and Day 55 after sowing

The normality test assumption was met for leaf size on Day 30 after sowing for all the treatments (Kolmogorov Smirnov, p > 0.05). The homogeneity of variance assumption was also met, F (5, 105) = 2.036, p = 0.079. The one-way ANOVA showed that there was a significant mean difference in leaf size (F (5, 105) = 8.93, p < 0.01). Based on the post hoc analysis (Appendix Table 3-2-1A), *Sorghum bicolor* treated with *L. maxima* biomass had significantly larger leaf sizes than those treated with compost soil, d = 9.43, p < 0.01, seasol fertiliser, d = 5.89, p = 0.006, MAE extract 40°C, d = 7.32, p < 0.01 and MAE extract 60°C, d = 5.52, p = 0.013. In addition, *Sorghum bicolor* raised in top soil fertilised with MAE extract 80°C yielded significantly larger leaf sizes than those treated with compost soil. Thus, concerning leaf sizes on Day 30 of sowing, *L. maxima* biomass was better compared to other fertiliser treatments (Fig. 3-4 A).

Day 55 leaf sizes were normally distributed for all treatments (Kolmogorov Smirnov: > 0.05), except for compost soil with a p-value of 0.014. However, most of the points for compost soil treatment fell along the diagonal line of the q-q plot suggesting that the variable is approximately normally distributed. Thus, the normality assumption was met. Levene's test for homogeneity of variance test showed a violation of this assumption, F (5, 113) = 2.55, p = 0.032. Thus, Welch's F statistics was used to determine whether there was a significant mean difference. The results

showed that there was a significant difference in leaf sizes between the 6 treatments, F (5, 50.89) = 12.88, $p < 0.01$. A post hoc analysis test assuming unequal variance was performed using the Games-Howell test (Appendix Table 3-2-1B). The results revealed that *Sorghum bicolor* raised in top soil fertilised with *L. maxima* biomass had significantly longer leaves than those treated with seasol fertiliser, $d = 8.99$, $p = 0.04$. On the other hand, *Sorghum bicolor* raised in compost soil had significantly shorter leaves than those treated with seasol fertiliser, d = -10.35, p < 0.01, *L. maxima* biomass, d = -19.34 , p < 0.01, MAE extract 40°C, $d = -18.59$, $p < 0.01$, MAE extract 60°C, $d = -13.43$, $p = 0.02$, and MAE extract 80°C, $d = -14.37$, $p = 0.014$ (Fig. 3-4 B).

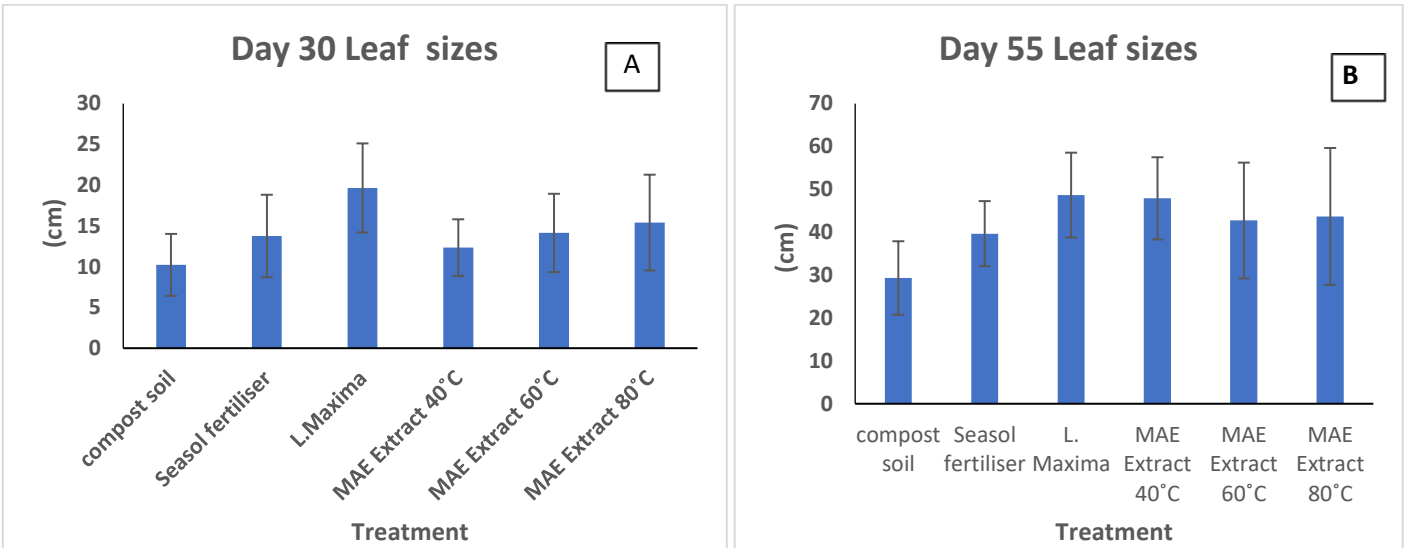

Figure 3-3 Effect of fertiliser treatment on leaf sizes of *Sorghum bicolor* Day 30 (A) and Day 55 (B) of sowing. Standard deviation of n = 3 is shown.

3.2.2 Plant Height at Day 30 and Day 55 after sowing

Plant height for Day 30 was approximately normally distributed for all treatments (Kolmogovor Smirnov test: p > 0.05) However, the homogeneity of variance assumption was violated, F (5, 107) = 3.22, p = 0.010. Thus, Welch's F statistic was used and revealed that there was a significant mean difference in plant heights between the treatments, F (5, 47.54) = 13.70, p < 0.01. Post hoc analysis was conducted using the Gamel-Howell test (Appendix Table 3-2-2A), which revealed that *Sorghum bicolor* grown in top soil fertilised with *L. maxima* biomass produced significantly taller plants compared to plants grown in compost soil, d = 21.33, p < 0.01, seasol fertiliser, d = 13.65, p < 0.01, MAE extract 40°C, $d = 13.72$, $p < 0.01$, and MAE extract 60°C, $d = 13.47$, $p <$

0.01. Moreover, *Sorghum bicolor* raised in MAE extract 40°C, d = 7.61, p = 0.03 and MAE extract 80°C, d = 11.96, p = 0.02, were significantly taller than those raised in compost soil (Fig. 3-5A).

Plant height for Day 55 was normally distributed, p > 0.05 for all treatments, except for compost soil which showed a small deviation from normality, p = 0.006. Thus, it was assumed that the plant height for all the treatments were approximately normally distributed. The homogeneity of variance assumption was also met, F (5, 113) = 0.89, p = 0.49. Therefore, a one-way ANOVA test was conducted, which revealed that there was a significant difference in plant height between the treatments, F (5, 113) = 4.21, p < 0.01. Post hoc analysis was performed using the Tukey HSD test to determine which specific treatments had significantly different plant heights (Appendix Table 3-2-2B). The post hoc results showed that *Sorghum bicolor* raised in compost soil was signficantly shorter than those treated with MAE extract 40°C, d = -16.65, p = 0.013, and *L. maxima* biomass, d = -21.87, p < 0.01. There was no other significant difference in plant height (Fig. 3-5 B).

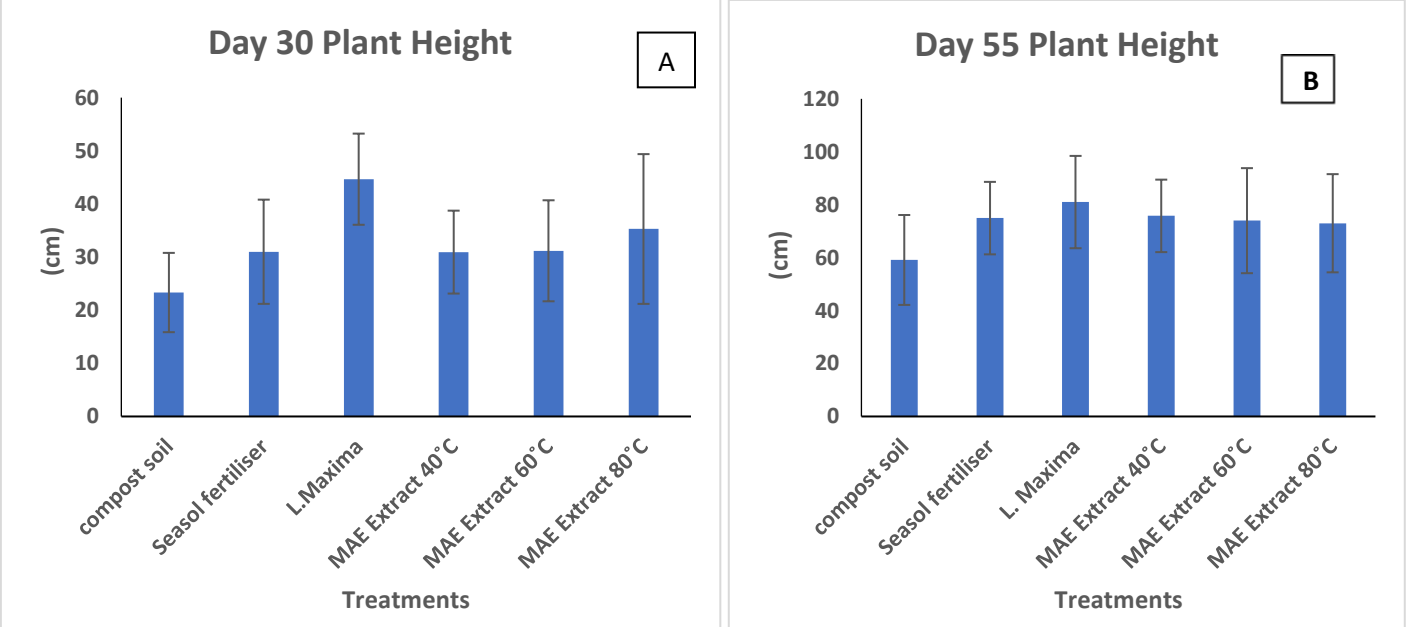

Figure 3-4 Effect of fertiliser treatment on Plant heights of *Sorghum bicolor* after 30 Days and 55 days of sowing. Standard deviation of n = 3 is shown.

3.2.3 Leaf and root fresh and dry weight

The fresh and dry weight of the leaves and roots were also measured for all the treatments in the particular experiment (Fig. 3-7A, B, C and D). One-way ANOVA was performed to determine whether there was a significant difference in fresh and dry leaf weight between the treatments. Fresh and dry leaf weights for all treatments were normally distributed (Shapiro Wilk test: p >

0.05) and the (homogeneity assumption were also met), F (5, 12) = 0.16, p = 0.97 and F (5, 12) = 2.23, p = 0.12, respectively. The one-way ANOVA showed that there were no significant differences in fresh and dry leaf weight between the treatments, F (5, 12) = 0.16, p = 0.97 and F (5, 12) = 0.533, p = 0.75, respectively (Appendix Table 3-2-3A and Appendix Table 3-2-3B). Similar findings were established based on a bar graph (Fig. 3-6A).

The dry leaf and root weight were also analysed to determine whether there was a significant difference between any two treatments. The dry leaf and root weights were normally distributed for all the treatments (Shapiro-Wilks test: p > 0.05) and homogeneity of variance assumtion was satisfied, F (5, 12) = 1.52, p = 0.25 and, F (5, 12) = 1.60, p = 0.23, respectively. The one-way ANOVA results showed that there was no significant mean difference in dry leaf and root weights between the treatments, F (5, 12) = 0.28, p = 0.92, and F (5, 12) = 0.16, p = 0.97, respectively (Appendix Table 3-2-3C). Similar findings were also noted when a bar graph with error bars was plotted (Fig. 3-6 B).

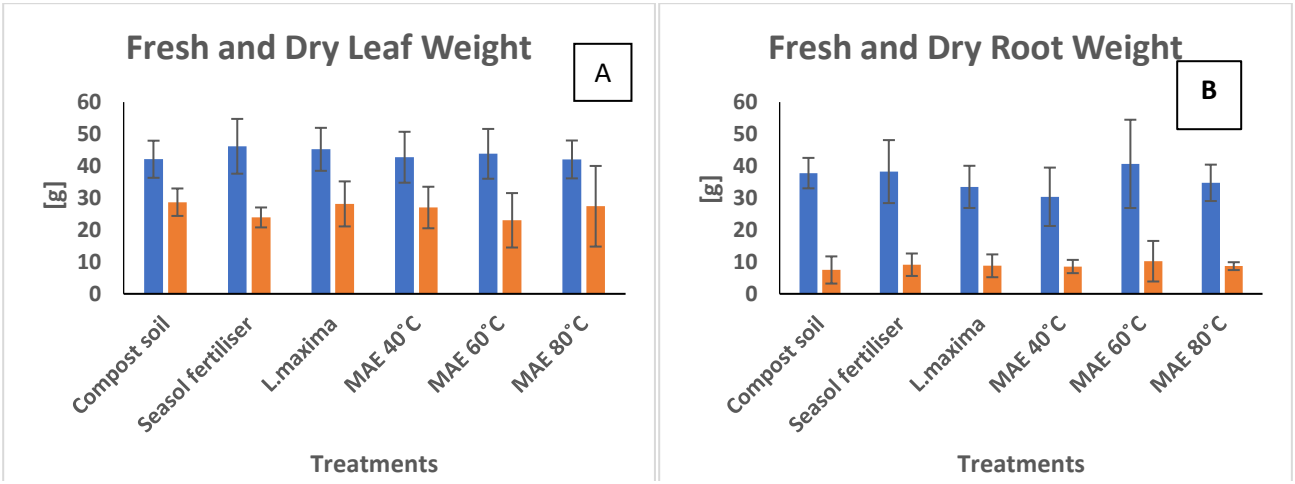

Figure 3-5 Effect of fertiliser treatment on fresh and dry leaf and root weights of *Sorghum bicolor*

Leaves and Roots Water Content

The leaf and roots water contents were also calculated using the formula (water content = (fresh weight-Dry weight)). The data were then analysed using a bar graph. With regard to leaf water content, plant treated with seasol fertiliser (22.26ml) produced plants leaves with the highest water content, followed by MAE extract 60°C (20.80ml). The leaf water content for the plant treated

with L.maxima, MAE extract 40°C, and MAE extract 80°C, were, 17.08ml, 15.73ml and 14.65ml, respectively. However, plants treated with the positive control (compost soil) had the lowest leaf water content, 13.44ml. Regarding the root water content, plants treated with MAE extract 60°C had roots with the highest water content, 30.46ml, followed by those treated with the postive control (compost soil), 30.31ml. The leaf water content for the plants treated with seasol fertiliser, L.maxima and MAE extract 80°C were, 29.14ml, 24.70ml and 26.07ml. However, plants treated with the MAE extract 40°C had the lowest root water content of all the treatment groups, 21/82ml (Fig. 3-7).

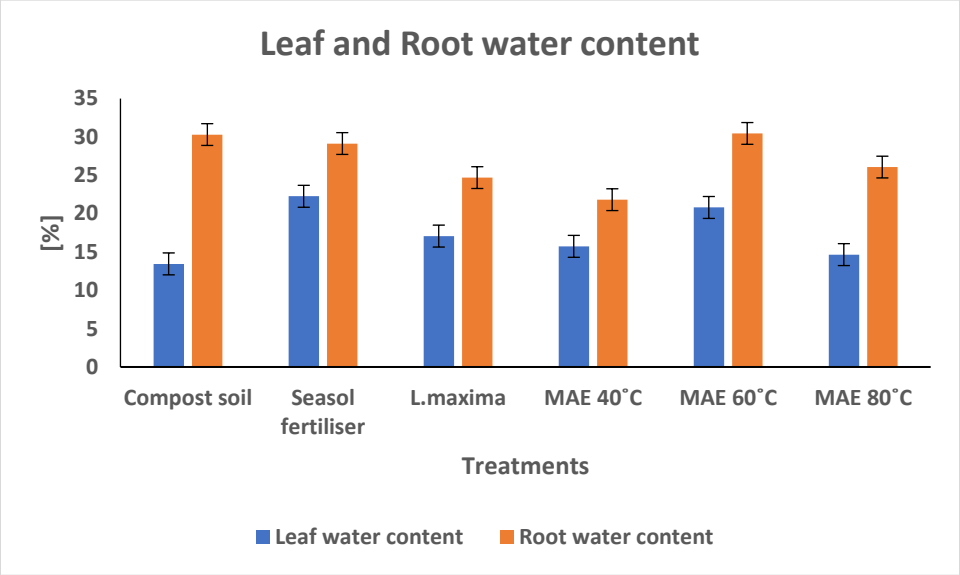

Figure 3-6 Leaf and Root Water content

Figure 3-7 Fresh and dry biomass of *Sorghum bicolor*; were A. Fresh Root, B. Dry Root, C. Fresh Leaf, and D. Dry Leaf

3.2.4 Water Holding Capacity

The water holding capacity of all the pots of different treatments was calculated for 10-55 days after sowing. The mean water holding capacities were, M = 47.56%, SD = 26.26%, M= 43.98%, SD =27.87%, M = 57.35%, SD = 24.31%, M = 42.56%, SD = 17.65%, M = 38.73%, SD =14.84%, M = 41.84%, SD = 16.79%, and M = 46.82%, SD =22.57% for compost soil (positive control), and seasol ferliser, *L. maxima* biomass, and *L. maxima* MAE-biomass extract obtained at 40, 60, and 80°C, and topsoil (negative control), respectively. One-way ANOVA was then performed to determine whether there was a significant mean difference in water holding capacity between the treatments. The normality assumption was met for all the treatments, except for the seasol fertiliser,

(Shapiro-Wilks test: p > 0.05) and verified using Q-Q plots. The homogeneity of variance assumption was also met, F (6, 119) = 0.914, p = 0.488. The one-way ANOVA results showed that there was no significant difference in water holding capacity between the treatments, F (6, 119) = 1.35, p = 0.42 (Appendix Table 3-2-4A). Nonetheless, water holding capacity was highest in treatments fertilised with *Limnospira maxima* biomass (Fig. 3-8).

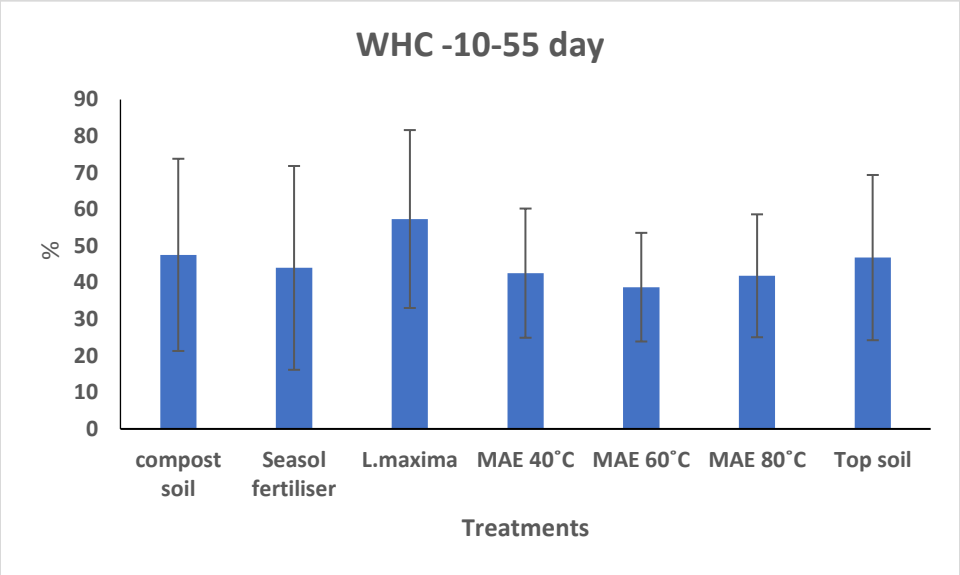

Figure 3-8 Effect of fertiliser treatment on water holding capacity of soil after 55 days of growth. Mean ± standard deviation is shown. n = 3.

CHAPTER:4

Discussion

4 DISCUSSION

Nutrients, macroelements, and microelements are significant for plant metabolisms such as nucleic acid and protein metabolism (Anitha et al., 2016). They affect the chemical composition as well as the growth and development of plants. In fact, Weldegebriel (2020) argued that the application of macronutrients enriched with N, P and K concentrations improves sorghum plants nutritional quality because they increases the amount of proteins of the grains harvested. For plant growth, soil is a vital source of nutrients. The three major nutrients are nitrogen (N), phosphorus (P), and potassium (K). They are known as NPK, a trio. Sulfur, calcium, and magnesium are other essential nutrients (Fernandes et al. 2017). To meet all of their metabolic requirements, plants simply require light, water, and up to 20 elements (Fernandes et al. 2017).

The data were analysed to examine the nutrients uptake by plants. The findings showed a small increase in N concentration for the compost and top soil. The small increase could be attributed to measurement variations. It could also be attributed to the fact that the initial N contents were not consumed since the sorghum plants did not germinate on these soil samples. However, for the seasol fertiliser, *L. maxima* biomass, MAE 40˚C, MAE 60˚C, and MAE 80˚C treatments, the N concentration decreased. This implies that almost all the nitrogen contents were taken up by the plants treated with seasol fertiliser, *L. maxima* biomass, MAE 40˚C, MAE 60˚C, and MAE 80˚C..

This study also found a large increase in carbon contents for the top, compost soil, and seasol fertiliser samples. This implies that the C concentraton was not used for the top, compost and seasol fertiliser samples. For the top and compost, the increase could be attributed to the top soil and compost soil samples being obtained from soil that already had high C concentration.. The increase in carbon content could be prevented by using top and compost soil samples without saw dust, leaves, and hay, which are very rich in carbon contents. On the other hand, the C concentration decreased by large amount for the plants treated with *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C. This implies that almost all the C was taken up by the plants for the plants treated with *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C

The findings also showed a small increase in P concentrations in the soil samples treated with seasol fertiliser and top soil. The small increase could be attributed to measurement variations. However, the P concentrations decreased by larger amounts for the soil treated with *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C, indicating that almost all the P contents were consumed by the plants. In other words, P concentration greatly contributed to plants growths,

particlarly when treated with compost soil, *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C. The large amount of phosporous content used by the plants under the various treatments helped in cell division and development of new tissue.

K concentration for the compost soil (positive control) and soil treated with seasol fertiliser. This negligible increase in concentration could be due to measurement errors. However, there was a decrease in K concentrations for the top soil and soil treated with *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C. This implies that almost all the K contents was taken up by the plants treated with *L. maxima* biomass, MAE 40˚C, MAE 60˚C , and MAE 80˚C. This large potassium uptake by sorghum plants was necessary to regulate the water content of the plants by controlling the closing and opening of stomata. More importantly, potassium uptake fostered enzyme activation within the plants, thus enhancing the production of protein, starch and adenosine triphosphate (ATP) (Prajapati and Modi, 2012).

Leaf sizes were also analysed on day 30 of sowing and results showed that *Sorghum bicolor* raised in top soil fertilised with *L. maxima* biomass had significantly larger leaf sizes than those treated with compost soil, seasol fertiliser, MAE extract 40°C, and MAE extract 60°C. In addition, sorghum treated with MAE extract 80°C yielded significantly larger leaf sizes than those treated with compost soil. Although *L.maxima* biomass yielded larger leaf sizes than MAE extract 80°C , the difference was not statistically significant. This implies that *L. maxima* biomass significantlyincreased the plant leaves sizes, relative to other treatments such as seasol fertiliser, MAE extract 40°C, and MAE extract 60°C. Thus, concerning leaf sizes during Day 30 of sowing, *L. maxima* biomass was better compared to other fertiliser treatmentsWhen analysed on day 55 of sowing, the results showed that sorghum treated with *L. maxima* biomass had significantly larger leaf size than those treated with seasol fertiliser. However, there was no significant difference in leaf sizes between The findings also showed that seasol fertiliser, *L. maxima* biomass, MAE extract 40°C, MAE extract 60°C, and MAE extract 80°C were significantly better in increasing sorghum leaf sizes compared to those treated with compost soil. This study findings also revealed that *L. maxima* biomass significantly yielded taller plants than those treated with compost soil, seasol fertiliser, MAE extract 40°C, and MAE extract 60°C, based on analysis of data gathered on Day 30 of sowing. Similarly, soil treated with MAE extract 40°C, and *L. maxima* biomass, yielded significantly taller plants than compost soil during Day 55 of sowing. The taller plants in soil treated with *L.maxima* biomass and with MAE extract 40°C, could be due to the high phophorus

and nitrogen contents uptakes by these plantsThe study also examined the effects of compost, topsoil, seasol fertiliser, *L. maxima* biomass, and *L. maxima* MAE-biomass extracts obtained at 40, 60, and 80°C on the fresh and dry leaf and root weight of the sorghum plants.

In addition, this study examined whether the different seven treatments (compost, topsoil, seasol fertiliser, *L. maxima* biomass, and *L. maxima* MAE-biomass extracts obtained at 40, 60, and 80°C) had significant effects on the water holding capacity. The findings showed that there was no significant difference in water holding capacity between the treatments. The difference in findings could be attributed to the different experimental conditions such as watering regime, plant material used, physical factors as well as other environmental conditions. The present study findings and literature findings were also summarised (Table 4-1)

Table 4-1 Comparison of leaf sizes, plant height, fresh & dry root/leaf weight, and water holding capacity in response to different treatments

Treatment /variable	Compost	Top soil	*L. maxima biomass*	Seasol fertiliser	MAE 40°C	MAE 60°C	MAE 80°C
Leaf size(this study)	Sig. smaller	Sig. smaller	Sig. better	Sig. better	Sig. better	Sig. better	Sig. better
Plant height (this study)	Sig. smaller	Sig. smaller	Sig. better	Sig. smaller	Sig. smaller	Sig. smaller	Sig. smaller
Fresh and dry leaf and root weight (this study)	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. difference
Water holding capacity (this study)	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. difference	No sig. Difference
Leaf size			Sig. better (Michalak et al., 2016)	Not sig. better (Arshad et al., 2014)	Not Sig. better (Michalak et al., 2016)	Not Sig. better (Michalak et al., 2016)	Not Sig. better (Michalak et al., 2016)
Plant height			Sig. better (Wuang et al., 2016, Uddin et al., 2019)	Sig. better (Kwon et al., 2019)	Not Sig. better (Michalak et al., 2016)	Not Sig. better (Michalak et al., 2016)	Not Sig. better (Michalak et al., 2016)
Fresh and dry leaf and root weight			Sig. better (Wuang et al., 2016)	Not. Sig. better (Okechukwu, 2011)			
Water holding capacity	Sig.better (Ramos, 2017)		Sig.better (Malik et al., 2013)	Not Sig. better (Zhou et al., 2017)			

5 CONCLUSION

The main aim of the study was to assess the efficacy of L.maxima biomass in improving plant growth compared to other fertilise treatment. In particular, this study focused on determining the soil mineral content (C, N, P, K) using inductively coupled plasma optical emission spectrometry (ICP-OES) and elemental analysis. It also examined the effects of different fertiliser types on plant growth based on plant height, leaf size, dry leaf and root weight, fresh leaf and root weight, and water holding capacity. The experiment was carried out using *Sorghum bicolor* in pots utilizing various fertiliser types such as compost soil, seasol fertiliser, *L.maxima* biomass, and MAE extracts obtained at 40, 60, and 80˚C. The ICP-OES and elemental analysis studies demonstrated that the pots that were given *L. maxima* biomass had an increased level of C, N, P, and K at the end of the experiment. Concerning soil analysis, this study found that N, C, P and K were necessary for plant growth. In particular, almost all the N, C, P and K contents were used by the plants grown on soil treated with seasol fertiliser, *L.maxima* biomass, and MAE extracts obtained at 40, 60, and 80˚C. Thus, the N, C, P, and K contents should be determined before sowing the seeds and necessary adjustments made to significant enhance the growth and production of *Sorghum bicolor*. However, the current study did not establish whether the N, C, P and K contents significantly improved plant growth.. Concerning the effects of different types of fertilisers on plant height and leaf sizes, it was found that *L.maxima* biomass yielded significantly taller plants and leaf sizes compared to other treatments. This implies that the *L.maxima* biomass should be adopted by farmers as a strategy to improve the growth of *sorghum bicolar*. However, the different types of fertilisers did not have significant effects on fresh and dry leaf/root weight as well as water holding capacity. This research contributes to the understanding of how *L.maxima* biomass or MAEs promote *Sorghum bicolor* growth when compared to widely available gardening fertiliser Seasol and carbon-rich compost soil. Future studies could look into NPK fertilisers, algal extracts (*L. maxima*), and compost soil to see how fertilisation affects crop development (including water holding capacity, above and below ground biomass fresh and dry weight). To determine whether these N, P, K, and C content significantly promote plant growth, researchers must undertake soil analysis utilising pre- and post-fertilized soil tests. Future research should look at the effects of fertilisation treatments

carried out at the same temperature, water level, light intensity, and photo phase of the light/dark cycle as this study.

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7 APPENDIX

3-1 N, C, P, and K concentrations of starting materials

Table 3-1A. Robust Tests of Equality of Means in N concentration

N_concentration				
	Statistic[a]	df1	df2	Sig.
Welch	4.177	5	5.506	.063
a. Asymptotically F distributed.				

Table 3-1B Robust Test of Equality of means in C concentration

C(%)	Statistic		df1	df2	Sig.
Welch	2.043		6	6.066	0.202

Table 3-1C Robust Test of Equality o f means (P178.221 axial [mg/g])

	Statistic	df1	df2	Sig.
Welch	2.89	6	5.716	0.116

Table 3-1D: Robust Tests of Equality of Means (K 766.490 [mg/g])

	Statistic	df1	df2	Sig.
Welch	3.654	6	6.085	0.069

3-2-1: Leaf size Day 30 Outputs

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
compost soil	20	10.2210	3.79860	.84939	8.4432	11.9988

Seasol fertiliser	15	13.7620	5.05972	1.30641	10.9600	16.5640
L.maxima	20	19.6555	5.46825	1.22274	17.0963	22.2147
MAE Extract 40˚C	22	12.3341	3.47725	.74135	10.7924	13.8758
MAE Extract 60˚C	15	14.1360	4.80275	1.24006	11.4763	16.7957
MAE Extract 80˚C	19	15.4132	5.87155	1.34703	12.5832	18.2432
Total	111	14.2360	5.57376	.52904	13.1876	15.2845

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Leaf_length_30days	Based on Mean	2.036	5	105	.079
	Based on Median	1.812	5	105	.117
	Based on Median and with adjusted df	1.812	5	93.846	.118
	Based on trimmed mean	2.076	5	105	.074

ANOVA

Leaf_length_30days

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1019.252	5	203.850	8.926	.000
Within Groups	2398.099	105	22.839		
Total	3417.351	110			

3-2-1A Post Hoc Tests

Multiple Comparisons

Dependent Variable: Leaf_length_30days

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
compost soil	Seasol fertiliser	-3.54100	1.63235	.261	-8.2797	1.1977
	L.maxima	-9.43450*	1.51126	.000	-13.8217	-5.0473
	MAE Extract 40˚C	-2.11309	1.47651	.708	-6.3995	2.1733
	MAE Extract 60˚C	-3.91500	1.63235	.166	-8.6537	.8237
	MAE Extract 80˚C	-5.19216*	1.53101	.012	-9.6367	-.7476
Seasol fertiliser	compost soil	3.54100	1.63235	.261	-1.1977	8.2797
	L.maxima	-5.89350*	1.63235	.006	-10.6322	-1.1548
	MAE Extract 40˚C	1.42791	1.60023	.948	-3.2176	6.0734
	MAE Extract 60˚C	-.37400	1.74505	1.000	-5.4399	4.6919
	MAE Extract 80˚C	-1.65116	1.65065	.917	-6.4431	3.1407
L.maxima	compost soil	9.43450*	1.51126	.000	5.0473	13.8217
	Seasol fertiliser	5.89350*	1.63235	.006	1.1548	10.6322
	MAE Extract 40˚C	7.32141*	1.47651	.000	3.0350	11.6078
	MAE Extract 60˚C	5.51950*	1.63235	.013	.7808	10.2582
	MAE Extract 80˚C	4.24234	1.53101	.070	-.2022	8.6869
MAE Extract 40˚C	compost soil	2.11309	1.47651	.708	-2.1733	6.3995
	Seasol fertiliser	-1.42791	1.60023	.948	-6.0734	3.2176
	L.maxima	-7.32141*	1.47651	.000	-11.6078	-3.0350
	MAE Extract 60˚C	-1.80191	1.60023	.870	-6.4474	2.8436
	MAE Extract 80˚C	-3.07907	1.49673	.318	-7.4241	1.2660
	compost soil	3.91500	1.63235	.166	-.8237	8.6537

MAE Extract 60˚C	Seasol fertiliser	.37400	1.74505	1.000	-4.6919	5.4399
	L. maxima	-5.51950*	1.63235	.013	-10.2582	-.7808
	MAE Extract 40˚C	1.80191	1.60023	.870	-2.8436	6.4474
	MAE Extract 80˚C	-1.27716	1.65065	.971	-6.0691	3.5147
MAE Extract 80˚C	compost soil	5.19216*	1.53101	.012	.7476	9.6367
	Seasol fertiliser	1.65116	1.65065	.917	-3.1407	6.4431
	L. maxima	-4.24234	1.53101	.070	-8.6869	.2022
	MAE Extract 40˚C	3.07907	1.49673	.318	-1.2660	7.4241
	MAE Extract 60˚C	1.27716	1.65065	.971	-3.5147	6.0691

3-3-1, Leaf size Day 55 Outputs

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
compost soil	23	29.3213	8.58067	1.78919	25.6107	33.0319
Seasol fertiliser	16	39.6750	7.59134	1.89783	35.6299	43.7201
L. Maxima	20	48.6625	9.89896	2.21348	44.0296	53.2954
MAE Extract 40˚C	23	47.9083	9.58903	1.99945	43.7617	52.0549
MAE Extract 60˚C	17	42.7476	13.49391	3.27275	35.8097	49.6856
MAE Extract 80˚C	20	43.6955	15.96060	3.56890	36.2257	51.1653
Total	119	41.8903	12.90851	1.18332	39.5470	44.2336

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Leaf_length_55days	Based on Mean	2.548	5	113	.032
	Based on Median	2.408	5	113	.041
	Based on Median and with adjusted df	2.408	5	90.077	.043
	Based on trimmed mean	2.606	5	113	.029

ANOVA

Leaf_length_55days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5539.942	5	1107.988	8.866	.000
Within Groups	14122.369	113	124.977		
Total	19662.311	118			

3-2-1B Post Hoc Analysis

Dependent Variable: Leaf_length_55days							
	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Games-Howell	compost soil	Seasol fertiliser	-10.35370*	2.60825	0.0040	-18.2158	-2.4915
		L. Maxima	-19.34120*	2.84617	0.0000	-27.8802	-10.8022
		MAE Extract 40˚C	-18.58696*	2.6831	0.0000	-26.5841	-10.5898
		MAE Extract 60˚C	-13.42634*	3.7299	0.0150	-24.9087	-1.944
		MAE Extract 80˚C	-14.37420*	3.99227	0.0140	-26.5677	-2.1807
	Seasol fertiliser	compost soil	10.35370*	2.60825	0.0040	2.4915	18.2158
		L. Maxima	-8.98750*	2.91569	0.0430	-17.7884	-0.1866

		MAE Extract 40˚C	-8.23326	2.75673	0.0520	-16.5235	0.0569
		MAE Extract 60˚C	-3.07265	3.78321	0.9630	-14.7139	8.5686
		MAE Extract 80˚C	-4.0205	4.04213	0.9160	-16.3608	8.3198
	L. Maxima	compost soil	19.34120*	2.84617	0.0000	10.8022	27.8802
		Seasol fertiliser	8.98750*	2.91569	0.0430	0.1866	17.7884
		MAE Extract 40˚C	0.75424	2.98283	1.0000	-8.1735	9.6819
		MAE Extract 60˚C	5.91485	3.951	0.6690	-6.1327	17.9624
		MAE Extract 80˚C	4.967	4.19958	0.8420	-7.7608	17.6948
	MAE Extract 40˚C	compost soil	18.58696*	2.6831	0.0000	10.5898	26.5841
		Seasol fertiliser	8.23326	2.75673	0.0520	-0.0569	16.5235
		L. Maxima	-0.75424	2.98283	1.0000	-9.6819	8.1735
		MAE Extract 60˚C	5.16061	3.8352	0.7580	-6.5774	16.8986
		MAE Extract 80˚C	4.21276	4.09082	0.9040	-8.2237	16.6493
	MAE Extract 60˚C	compost soil	13.42634*	3.7299	0.0150	1.944	24.9087
		Seasol fertiliser	3.07265	3.78321	0.9630	-8.5686	14.7139
		L. Maxima	-5.91485	3.951	0.6690	-17.9624	6.1327
		MAE Extract 40˚C	-5.16061	3.8352	0.7580	-16.8986	6.5774
		MAE Extract 80˚C	-0.94785	4.84231	1.0000	-15.539	13.6433

	MAE Extract 80˚C	compost soil	14.37420*	3.99227	0.0140	2.1807	26.5677
		Seasol fertiliser	4.0205	4.04213	0.9160	-8.3198	16.3608
		L. Maxima	-4.967	4.19958	0.8420	-17.6948	7.7608
		MAE Extract 40˚C	-4.21276	4.09082	0.9040	-16.6493	8.2237
		MAE Extract 60˚C	0.94785	4.84231	1.0000	-13.6433	15.539

3-2-2 Plant_height_30days

Descriptives								
Plant_height_30days								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound		
compost soil	20	23.3250	7.45914	1.66792	19.8340	26.8160		
Seasol fertiliser	15	31.0000	9.80707	2.53217	25.5690	36.4310		
L.Maxima	20	44.6500	8.57950	1.91844	40.6347	48.6653		
MAE Extract 40˚C	22	30.9318	7.80155	1.66330	27.4728	34.3908		
MAE Extract 60˚C	15	31.1800	9.50340	2.45377	25.9172	36.4428		
MAE Extract 80˚C	21	35.2810	14.09126	3.07497	28.8667	41.6952		
Total	113	32.8637	11.65483	1.09639	30.6914	35.0361		

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Plant_height_30days	Based on Mean	3.215	5	107	.010
	Based on Median	2.503	5	107	.035
	Based on Median and with adjusted df	2.503	5	95.153	.036
	Based on trimmed mean	3.281	5	107	.009

Robust Tests of Equality of Means				
Plant_height_30days				
	Statistic[a]	df1	df2	Sig.
Welch	13.701	5	47.543	.000
a. Asymptotically F distributed.				

3-2-2A Post Hoc Tests

Multiple Comparisons						
Dependent Variable: Plant_height_30days						
Games-Howell						
		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) Treatment	(J) Treatment				Lower Bound	Upper Bound
compost soil	Seasol fertiliser	-7.67500	3.03214	.153	-17.0113	1.6613
	L.Maxima	-21.32500*	2.54211	.000	-28.9588	-13.6912
	MAE Extract 40˚C	-7.60682*	2.35552	.028	-14.6560	-.5576
	MAE Extract 60˚C	-7.85500	2.96697	.121	-16.9747	1.2647
	MAE Extract 80˚C	-11.95595*	3.49819	.020	-22.5801	-1.3318
Seasol fertiliser	compost soil	7.67500	3.03214	.153	-1.6613	17.0113
	L.Maxima	-13.65000*	3.17684	.002	-23.3603	-3.9397

	MAE Extract 40˚C	.06818	3.02960	1.000	-9.2534	9.3898
	MAE Extract 60˚C	-.18000	3.52603	1.000	-10.9559	10.5959
	MAE Extract 80˚C	-4.28095	3.98338	.888	-16.3039	7.7420
L.Maxima	compost soil	21.32500*	2.54211	.000	13.6912	28.9588
	Seasol fertiliser	13.65000*	3.17684	.002	3.9397	23.3603
	MAE Extract 40˚C	13.71818*	2.53908	.000	6.1069	21.3294
	MAE Extract 60˚C	13.47000*	3.11470	.002	3.9636	22.9764
	MAE Extract 80˚C	9.36905	3.62434	.129	-1.5836	20.3216
MAE Extract 40˚C	compost soil	7.60682*	2.35552	.028	.5576	14.6560
	Seasol fertiliser	-.06818	3.02960	1.000	-9.3898	9.2534
	L.Maxima	-13.71818*	2.53908	.000	-21.3294	-6.1069
	MAE Extract 60˚C	-.24818	2.96438	1.000	-9.3523	8.8560
	MAE Extract 80˚C	-4.34913	3.49599	.812	-14.9623	6.2640
MAE Extract 60˚C	compost soil	7.85500	2.96697	.121	-1.2647	16.9747
	Seasol fertiliser	.18000	3.52603	1.000	-10.5959	10.9559
	L.Maxima	-13.47000*	3.11470	.002	-22.9764	-3.9636
	MAE Extract 40˚C	.24818	2.96438	1.000	-8.8560	9.3523
	MAE Extract 80˚C	-4.10095	3.93400	.900	-15.9762	7.7743
MAE Extract 80˚C	compost soil	11.95595*	3.49819	.020	1.3318	22.5801
	Seasol fertiliser	4.28095	3.98338	.888	-7.7420	16.3039
	L.Maxima	-9.36905	3.62434	.129	-20.3216	1.5836
	MAE Extract 40˚C	4.34913	3.49599	.812	-6.2640	14.9623
	MAE Extract 60˚C	4.10095	3.93400	.900	-7.7743	15.9762

7.2.2B.Plant_height_55days

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
compost soil	23	59.1304	16.98744	3.54213	51.7845	66.4764
Seasol fertiliser	16	74.9375	13.68926	3.42231	67.6430	82.2320
L. Maxima	20	81.0000	17.43861	3.89939	72.8385	89.1615
MAE Extract 40˚C	23	75.7826	13.69126	2.85482	69.8621	81.7032
MAE Extract 60˚C	17	73.9706	19.84193	4.81238	63.7688	84.1724
MAE Extract 80˚C	20	73.0000	18.54440	4.14665	64.3210	81.6790
Total	119	72.6008	17.90613	1.64145	69.3503	75.8514

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Plant_height_55days	Based on Mean	.889	5	113	.491
	Based on Median	.762	5	113	.579
	Based on Median and with adjusted df	.762	5	97.752	.579
	Based on trimmed mean	.817	5	113	.540

ANOVA					
Plant_height_55days					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5939.595	5	1187.919	4.209	.002

Within Groups	31894.695	113	282.254		
Total	37834.290	118			

3-2-2B Post Hoc Tests

Multiple Comparisons

Dependent Variable: Plant_height_55days

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Compost soil	Seasol fertiliser	-15.80707	5.46926	.051	-31.6636	.0495
		L. Maxima	-21.86957*	5.13659	.001	-36.7617	-6.9775
		MAE Extract 40˚C	-16.65217*	4.95417	.013	-31.0154	-2.2890
		MAE Extract 60˚C	-14.84015	5.37355	.071	-30.4192	.7389
		MAE Extract 80˚C	-13.86957	5.13659	.083	-28.7617	1.0225
	Seasol fertiliser	compost soil	15.80707	5.46926	.051	-.0495	31.6636
		L. Maxima	-6.06250	5.63503	.890	-22.3997	10.2747
		MAE Extract 40˚C	-.84511	5.46926	1.000	-16.7017	15.0114
		MAE Extract 60˚C	.96691	5.85184	1.000	-15.9988	17.9327
		MAE Extract 80˚C	1.93750	5.63503	.999	-14.3997	18.2747
	L. Maxima	compost soil	21.86957*	5.13659	.001	6.9775	36.7617
		Seasol fertiliser	6.06250	5.63503	.890	-10.2747	22.3997
		MAE Extract 40˚C	5.21739	5.13659	.912	-9.6747	20.1095

		MAE Extract 60˚C	7.02941	5.54219	.802	-9.0386	23.0974
		MAE Extract 80˚C	8.00000	5.31276	.661	-7.4028	23.4028
	MAE Extract 40˚C	compost soil	16.65217*	4.95417	.013	2.2890	31.0154
		Seasol fertiliser	.84511	5.46926	1.000	-15.0114	16.7017
		L. Maxima	-5.21739	5.13659	.912	-20.1095	9.6747
		MAE Extract 60˚C	1.81202	5.37355	.999	-13.7671	17.3911
		MAE Extract 80˚C	2.78261	5.13659	.994	-12.1095	17.6747
	MAE Extract 60˚C	compost soil	14.84015	5.37355	.071	-.7389	30.4192
		Seasol fertiliser	-.96691	5.85184	1.000	-17.9327	15.9988
		L. Maxima	-7.02941	5.54219	.802	-23.0974	9.0386
		MAE Extract 40˚C	-1.81202	5.37355	.999	-17.3911	13.7671
		MAE Extract 80˚C	.97059	5.54219	1.000	-15.0974	17.0386
	MAE Extract 80˚C	compost soil	13.86957	5.13659	.083	-1.0225	28.7617
		Seasol fertiliser	-1.93750	5.63503	.999	-18.2747	14.3997
		L. Maxima	-8.00000	5.31276	.661	-23.4028	7.4028
		MAE Extract 40˚C	-2.78261	5.13659	.994	-17.6747	12.1095
		MAE Extract 60˚C	-.97059	5.54219	1.000	-17.0386	15.0974
		MAE Extract 80˚C	2.78261	5.03436	.993	-12.3978	17.9630
		compost soil	14.84015	5.97542	.160	-3.2837	32.9640

	MAE Extract 60˚C	Seasol fertiliser	-.96691	5.90518	1.000	-18.9902	17.0563
		L. Maxima	-7.02941	6.19389	.863	-25.7841	11.7252
		MAE Extract 40˚C	-1.81202	5.59544	.999	-18.9637	15.3396
		MAE Extract 80˚C	.97059	6.35246	1.000	-18.2301	20.1713
	MAE Extract 80˚C	compost soil	13.86957	5.45357	.137	-2.4705	30.2097
		Seasol fertiliser	-1.93750	5.37652	.999	-18.1701	14.2951
		L. Maxima	-8.00000	5.69210	.724	-25.0794	9.0794
		MAE Extract 40˚C	-2.78261	5.03436	.993	-17.9630	12.3978
		MAE Extract 60˚C	-.97059	6.35246	1.000	-20.1713	18.2301

3-2-3 Leaf and Root fresh and dry weight

Table 3-2-3A ANOVA for Fresh Leaf weight

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	43.605	5	8.721	0.168	0.97
Within Groups	623.56	12	51.963		
Total	667.165	17			

Table 3-2-3B ANOVA for Fresh Root Weight

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	208.949	5	41.79	0.533	0.747

Within Groups	940.126	12	78.344		
Total	1149.075	17			

Table 3-2-3C ANOVA for Leaf and Root Dry Weight

		Sum of Squares	Df	Mean Square	F	Sig.
Leaft_g	Between Groups	81.871	5	16.374	0.279	0.916
	Within Groups	703.443	12	58.62		
	Total	785.314	17			
Root_g	Between Groups	11.858	5	2.372	0.159	0.973
	Within Groups	178.565	12	14.88		
	Total	190.423	17			

3.2.4 Water Holding Capacity

Table 3-2-4A ANOVA for Water Holding Capacity

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3897.358	6	649.56	1.346	0.242
Within Groups	57444.43	119	482.726		
Total	61341.79	125			