EFFECT OF SOIL AMELIORANT (HUMIC ACID) ON ETHANOL YIELD COMPONENTS OF SWEET SORGHUM (*Sorghum bicolor* [L.] MOENCH) CULTIVARS

By

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ABSTRACT

The sweet sorghum plant is one of the heavy feeders on the soil and its roots can penetrate the soil up to 1 to 2 m deep. This often forces farmers to apply huge amounts of synthetic (inorganic) fertilisers to try and compensate for the nutrient requirements of the crop, whilst achieving high yields. Therefore, increasing performance per hectare is now the solution to increasing agricultural produce or production which is achieved through the proper combination of water, soil, soil nutrition, air and temperature. The main aim of the study was to understand the effects of HA application under dryland field conditions on agronomic performance, juice quality and yield traits of three sweet sorghum cultivars grown for ethanol production in the Free State and Potchefstroom. The specific objectives were (i) to investigate how different levels of HA affects growth, development, juice quality and yield of sweet sorghum genotypes grown for ethanol extraction (ii) to investigate any changes in the physical properties of soil and available organic matter when HA is added to the soil, and (iii) to determine the influence of HA on mineral content, antioxidant activity, phenolic content and total flavonoids in three sweet sorghum cultivars. Two field trials were conducted under dryland conditions in the 2022 and 2023 summer growing seasons at the Glen Agricultural Institute and Agriculture Research Council-Grain Crops (ARC-GC) Experimental farm at Potchefstroom, South Africa. The trial used a factorial design and the layout arrangement was organised in a randomised block design replicated three times. The factors used included locations (Glen and Potchefstroom), three sweet sorghum cultivars (ARC-SS 27, ARC-SS 76, and Hunnigreen), as well as treatment levels of HA (0kg, 5kg, 10kg and 15 kg/ha^{-1}). Soil samples were collected before planting and fertiliser applications were done according to the recommendations from a soil test report. Analysis of variance for the sorghum lines used in the experiment indicated the presence of significant differences in morphological characteristics during the first year. During the first year of planting, there were significant differences at $p<0.005$ on plant height, stalk weight, fresh bagasse, dry bagasse, dry leaf weight, juice weight, juice volume, brix at flowering, brix at harvesting, fresh panicle, dry panicle, and at days to 50% flowering at Potchefstroom. The juice quality and yield parameters of the sweet sorghum lines, humic acid application, and location were significantly different at $p<0.05$ during the first year at Potchefstroom. Brix at flowering differed significantly ($p<0.05$) at Potchefstroom in year two with 18.04% being harvested on Hunnigreen cultivar at 15 kg ha^{-1} of HA while the least was also on Hunnigreen at 0 kg ha^{-1}, HA having been 10.93%. Plant height (PH) was significantly different ($p<0.05$) at Glen in year one, with the tallest plant of 3.520 m being harvested on the Hunnigreen cultivar at 15 kg ha^{-1} of HA while the least was on ARC SS27 at 0 kg ha^{-1} of HA being 2.587 m. There were no differences ($p<0.05$) on juice weight at Glen in year one with 0.3500 g being harvested on the ARC-SS 27 cultivar at 15 kg ha^{-1} of HA, while the least of 0.2000 g was also on Hunnigreen at 0 kg ha^{-1} of HA. At Glen in year two, with the tallest plant being 2.187 m harvested on the ARC-SS 27 cultivar at 15 kg ha^{-1} of HA, while the least was on ARC-SS 76 at 0 kg ha^{-1} of HA being 1.390 m but there were no significant differences. There were significant differences ($p<0.05$) in juice volume in year two at Glen with the highest yield of 17.97 ml at 15 kg ha^{-1} of HA on the ARC SS76 cultivar, while the least was 10.67 ml on ARC-SS 27 at 0 kg ha^{-1} of HA. There were no significant differences on available sucrose, fructose, galactose, trehalose, glucose, maltose after HA application, except only on the total sugars. The significant differences in results of the sweet sorghum varieties were due to differences in their agro-morphology as well as competition for minerals, water, and solar radiation among plants. There were various interactions within the study and these affected various agronomic and juice parameters that were recorded. The season affected plant height, stalk weight, fresh bagasse weight, dry leaf weight, juice volume, brix at flowering, fresh panicle weight, dry panicle weight and the number of days to 50% flowering. The location affected PH, fresh bagasse weight, dry leaf weight, juice volume, brix at flowering, fresh panicle weight, dry panicle weight and days to 50% flowering. Location, cultivar, and HA (LXCXHA) and site, location, cultivar and HA (SXLXCXHA) only affected days to 50% flowering. All three varieties examined (Hunnigreen, ARC SS27, and ARC SS76) differed in agronomic attributes, brix and panicle weight. Hunnigreen proved to be a superior variety, yielding the highest total sugar content. ARC-SS 27 and ARC-SS 76 are important in grain production. This study demonstrates the importance of HA on sweet sorghum and how the various varieties respond with increased levels. Further studies can look at other sweet sorghum varieties and HA in different areas. Results from such research would be necessary useful unravel information and guidelines on the use of HA across diverse agro-ecological zones.

DEDICATION

“This work is dedicated to my daughter Rebaone Botshelo Matsheka and my late mother, Tseleng Conference Mametsi Matsheka.”

DECLARATION

I, **Thato L. B. Matsheka**, do hereby declare that this research project submitted to the Central University of Technology, Free State, for the degree **Master of Agriculture**, is my own independent work, complies with the Code of Academic Integrity as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State, and has not been submitted before to any institution by myself of any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

______________________________	13 November 2025
SIGNATURE	**DATE**

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LIST OF ABBREVIATIONS

GHG	Greenhouse gas
HA	Humic acid
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
S	Sulphur
HS	Humic substances
PH	Plant height
FB	Fresh bagasse
DB	Dry bagasse
DLW	Dry leaf weight
FP	Fresh panicle weight
DP	Dry panicle weight
SW	Stalk weight
DF 50%	Days to 50% flowering

CHAPTER 1: INTRODUCTION

1.1 General introduction

Biofuel feedstock regulations vary significantly from country to country due to differing national legislations. When formulating policies for biofuel crop production, several factors are considered, including the country's economic conditions, food security concerns, water availability, and the extent of arable land. The South African Department of Mineral Resources and Energy, outlined in its Government Gazette, emphasizes that any initiative relying on first-generation biofuels must include measures to protect food security (South African Department of Mineral Resources and Energy, 2020).

The Biofuels Feedstock Protocol, established as part of the 2007 Biofuels Industrial Strategy, aims to ensure food security. It prohibits the use of maize for biofuel production, encourages the cultivation of biofuel feedstocks on fallow land, and promotes the growth of non-irrigated feedstock crops. Additionally, the Biofuels Industrial Strategy supports utilizing former homelands in South Africa—areas primarily occupied by marginalized smallholder farmers—for cultivating crops such as sugarcane, sugar beet, sunflower, canola, and soybeans as biofuel feedstocks. This approach allows the use of existing agricultural land for biofuel production, provided it is integrated into crop rotation practices that enhance farm economic sustainability (South African Department of Minerals and Energy, 2020).

However, the feasibility of using certain authorized crops for sustainable biofuel production is questionable. These crops, classified as first-generation biofuels, may exacerbate the food-fuel conflict. Furthermore, many crops approved by the Biofuels Industrial Strategy require significant agronomic inputs to achieve optimal yields, presenting considerable challenges for impoverished small-scale farmers. South Africa predominantly has semi-arid conditions, making the cultivation of water-intensive crops, such as sugarcane for ethanol, inadvisable. The South African Department of Water and Sanitation discourages irrigation for cultivating biofuel feedstock due to prevailing water scarcity (Malobane *et al.,* 2018). Therefore, classifying these crops as first-generation biofuels raises concerns about their sustainability, as they are likely to intensify the food-fuel conflict.

Moreover, the agronomic requirements of the crops endorsed by the Biofuels Industrial Strategy create significant obstacles for disadvantaged smallholder farmers. Specifically, the growth of water-demanding crops such as sugarcane is ill-suited for South Africa's predominantly semi-arid environment (Malobane *et al.,* 2018). The Department of Water and Sanitation's position on irrigated biofuel feedstock production underscores the urgent problem of water shortages in the country (Mengistu *et al.,* 2016). Effective biofuel production necessitates a consistent supply of biomass that can thrive in poor soils with minimal agronomic inputs, while avoiding the use of arable land (Fernandes *et al.,* 2010). Therefore, it is essential to identify crops that address the food-fuel conflict and can be grown in poor soils to facilitate biofuel production in South Africa (Mengistu *et al.,* 2016).

Lignocellulosic crops, such as sweet sorghum, require few agronomic inputs and can adapt to various environmental conditions, making them particularly suitable for biofuel production in South Africa (Ratnavathi *et al.,* 2011). While there is a wide range of lignocellulosic crops available, it is important to prioritize those that can serve multiple purposes—such as food, feed, and biofuel—over those that are designated solely for biofuel production. Sweet sorghum is an example of a crop with such multifunctionality (Ratnavathi *et al.,* 2011).

1.2 Problem Statement

South Africa is experiencing a shortage of agricultural land suitable for sweet sorghum production, primarily due to land degradation, which leads to a decrease in biological activity, biodiversity loss, and reduced soil fertility (Chimwamurombe *et al.,* 2021). Enhancing performance per hectare could be a solution, requiring a balance of water, soil, nutrients, air, and temperature. The most recent studies focused on organic and animal fertilizers due to environmental concerns associated with inorganic fertilizers. These issues include high energy consumption during production, negative impacts on ecosystems, and significant costs. Sweet sorghum, which is a heavy feeder with roots that can extend 1 to 2 meters deep, often requires large quantities of synthetic fertilizers. This depletes the soil and can hurt future yields due to reduced organic matter. Additionally, high nitrogen fertilizer prices can make them unaffordable for some farmers. To make sweet sorghum profitable in the Free State and other regions, agronomic practices must be improved. Its ability to thrive in poor soils with low inputs offers potential income for small-scale farmers. This study seeks to determine the best application rates of humic acid (HA) for three sweet sorghum cultivars.

1.3 Hypothesis

An application of HA to the soil can improve growth, juice quality and yield of three sweet sorghum cultivars.

1.4 Objectives of the study

The main aim of this study was to assess, under dryland field conditions, the effects of HA addition on the agronomic performance, juice yield, and quality traits of three sweet sorghum cultivars grown for ethanol production in the Free State, South Africa. The specific objectives were:

- to assess the effects of HA levels on growth, development, juice yield, and juice quality of sweet sorghum genotypes; and
- to determine the effects of HA on total sugar, sucrose, fructose, galactose, trehalose, glucose, maltose, and lactose sugar quality.

1.5 References

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CHAPTER 2: LITERATURE REVIEW

2.1 General background on sweet sorghum production

2.1.1 Historical origin

The Poaceae family encompasses the wild grass species known as sorghum, which is indigenous to Africa and exhibits resilience to both heat and drought conditions (Malabadi *et al.,* 2022). The northeastern regions of Africa, particularly Ethiopia and Sudan, have been home to a diverse array of Sorghum species (Naoura *et al.,* 2020). Historically, the Bantu people transported this sorghum crop to the savannahs of western and southern Africa, where it was primarily utilized for beer production, as well as to regions such as Tanzania, Cameroon, and the Congo area during the 1st century B.C. Additionally, sorghum was shipped from East Africa to India as a food source around the first millennium B.C. (Naoura *et al.,* 2020). The varieties cultivated in India share genetic ties with those found in North Eastern Africa and along the coastal stretch from Cape Guardafui to Mozambique (Malabadi *et al.,* 2022). By the beginning of the Christian era, sorghum had further spread along the Southeast Asian coastline and into China. Subsequently, it was introduced to the Western Hemisphere and Australia. In 1853, William Prince, a gardener from New York, imported sorghum seeds from France via China and successfully cultivated sweet sorghum (Malabadi *et al.,* 2022). This was followed by the introduction of grain sorghum varieties in California, significantly boosting sorghum production.

2.1.2 Sweet sorghum production areas

Sweet sorghum is one of the many varieties of sorghum native to Africa and predominantly grown by small-scale farmers (Malobane *et al.,* 2020). In Africa, Sudan and Nigeria stand out as the foremost producers of sorghum. At the same time, in South Africa, significant production regions include the Free State, Limpopo, Mpumalanga, North West, Gauteng, and KwaZulu-Natal (Dunjana *et al.,* 2022). The annual sweet sorghum yields in South Africa range from 100,000 tonnes (covering 130,000 hectares) to 180,000 tonnes (spanning 150,000 hectares) (Nasidi *et al.,* 2019). The Free State and Mpumalanga provinces are the primary contributors to the area cultivated and overall sorghum production (Motsi *et al.,* 2022).

2.2 Sweet Sorghum Plant

Sweet sorghum is a member of the grass family and is recognized as a C4 graminaceous crop due to its impressive photosynthetic efficiency and high biomass production. This plant features broad, flat leaves and a mature inflorescence that typically appears as a round or elliptical head containing grains (Fretes et al., 2021). Sweet sorghum is particularly noted for its high biomass yield and efficiency as a sugar crop (Yoosukyingsataporn & Detpiratmongol, 2019). It ranks as the fifth-largest cereal crop globally, following wheat, rice, maize, and barley. The grains produced can be used for both human consumption and animal feed, while the sugary juice extracted from its stalks can be processed into syrup or fermented to produce ethanol. Additionally, the byproduct known as bagasse, which remains after juice extraction, can serve as an animal feed source or can be pre-treated, hydrolyzed, and fermented to produce second-generation ethanol (Mutepe, 2012).

Sweet sorghum has a growth cycle of about 4 to 5 months and offers several advantages, including tolerance to waterlogging, resistance to salinity and drought, and the ability to thrive in hot, arid conditions (Yoosukyingsataporn & Detpiratmongol, 2019). It requires minimal inputs such as water and nutrients and can be cultivated on marginal lands, making it an appealing option for farmers. Additionally, sweet sorghum is compatible with crop rotation systems that include maize and soybean (Mathur et al., 2017).

To optimize yield potential, sorghum plants require deep, fertile soils with good drainage, a stable rainfall pattern ranging from medium to good during the growing season, temperate to warm temperatures (between 20 and 30°C), and a frost-free duration ranging from 120 to 140 days (Nasidi *et al.,* 2019). Sorghum is predominantly grown in diverse areas ranging from dry regions, especially on shallow and heavy clay soils (Nasidi *et al.,* 2019). Nutritional elements, particularly N, P, K, Ca, Mg, and S are often the most deficient nutrients required by sorghum and are also the key fertilizers that significantly impact the sustainability of sorghum's role in biofuel production (Nazli *et al.,* 2020). Prior studies have indicated that nitrogen fertilization markedly enhances the yields of sweet sorghum's juice, fresh stem, bioethanol, biomass, and its brix, or fermentable sugar concentration (Mekdad *et al.,* 2016). Nevertheless, nitrogen fertilization constitutes a substantial portion of the overall energy input and production expenses associated with bioenergy production, necessitating cost-effectiveness (Mekdad *et al.,* 2016). Identifying the optimal levels of NPK fertilizers is important in attaining both economically viable and environmentally sustainable biofuel production from sweet sorghum, as inadequate nutrient management can lead to environmental challenges like nitrous oxide (N_2O) emissions and groundwater pollution (Sowiński *et al.,* 2018).

The application of HAs, which are concentrated forms of humic compounds, presents a promising strategy for enhancing bioethanol production and optimizing the cultivation of sweet sorghum in conjunction with NPK mineral fertilizers (Verlinden *et al.,* 2010). These compounds are widely acknowledged as effective soil amendments, constituting a significant portion of soil organic matter and being abundantly available in both aquatic and terrestrial ecosystems (Nazli *et al.,* 2020). HA compounds improve soil fertility, facilitate nutrient absorption, enhance chlorophyll levels, encourage root development and elongation, increase plants' resilience to drought, and stimulate various enzymes and hormones. Collectively, these effects contribute positively to crop growth and yield (Nazli *et al.,* 2020). HA is a crucial component of the organic structure of soil (Lindsey *et al.,* 2021). It is important in boosting crop yields and nutrient absorption when mineral nutrition is sufficient, or in sustaining yield potential even in conditions of nutrient deficiency. Consequently, many farmers have adopted its use to enhance soil quality and promote plant growth (Lindsey *et al.,* 2021).

2.3 Humic acid

Humic acid (HA) has a dark colour, is a black substance formed from the microbial degradation of plant and animal residues, exhibiting resistance to further weathering (Hayes *et al.,* 2020). This complex molecule is naturally found in various environments, including soils, peat, oceans, and freshwater systems. Leonardite serves as a notable source of HA (O'Donnell, 1973). When organic matter breaks down from plants and animals,' this results in the formation of humic compounds that are characterized by their high molecular weight and intricate structures (Ampong *et al.,* 2022). HA consists of functional groups such as carboxyls, alcoholic hydroxyls, ketones, quinoids, and phenolic hydroxyls (Li *et al.,* 2019). Resultantly, the functional properties of these molecules, along with the diverse chemical structures and reaction mechanisms associated with humus, remain inadequately understood (Noroozisharaf *et al.,* 2018). The benefits of HA include enhancing soil aeration, increasing Cation Exchange Capacity (CEC), improving soil fertility, optimizing the availability of mineral nutrients for uptake by plants, protecting water-soluble inorganic fertilizers, and fulfilling the nutritional requirements of plants (Nasidi *et al.,* 2019).

Many soils struggle to retain plant nutrients due to various challenges such as drought, waterlogging, and extreme pH conditions. The application of HA is essential for promoting sustainable agricultural practices to address these issues (Li, 2020). Thus, HA plays a vital role in nutrient absorption and crop yield. As fertilizer costs keep increasing for farmers, the lack of HA in the soil results in high production expenses by restricting nutrient availability to plants. When used as a soil amendment, HA greatly increase the soil's physical, chemical, and biological properties.

2.3.1 Improved fertiliser consumption

The average rate of fertilizers used remains below 50%, indicating a significant gap between the potential yields of fertilizers and their actual efficiency (Noroozisharaf *et al*., 2018). Consequently, fertilizer use rates are crucial for advancing agricultural productivity. Humic acid demonstrated exceptional efficacy in improving these rates. They facilitate the dissolution of phosphorus and the promotion of potassium, thereby optimizing the nutrient balance within the soil (Noroozisharaf *et al*., 2018). Research by Van Tol de Castro *et al.* (2021) highlighted the role of HA's aromatic and aliphatic functional groups in nitrogen uptake, which increases soluble sugars and, ultimately, rice yield.

Urea is the most used nitrogen fertilizer that promotes vigorous stems and leaves growth, resulting in a lush green appearance. In the soil, urea is converted into ammonia by an enzyme called urease, which allows plants to absorb the nutrient. Additionally, humic acid (HA) enhances the effectiveness of urea fertilization by stabilizing urease activity. Phosphate fertilizers, on the other hand, are recognized for increasing the number of spikes and grains produced by plants while also promoting root system development. This helps improve the plants' resilience to cold and drought conditions (Li, 2020). However, the availability of usable phosphorus is often rapidly diminished. HA fertilizers can potentially improve phosphorus uptake by plants while minimizing soil phosphorus fixation. They can activate insoluble phosphorus in the soil, increase the soluble phosphorus levels, or directly interact with phosphate fertilizers. Furthermore, potassium-containing fertilizers can promote rapid plant growth, enhance resilience to environmental stresses, and increase the production of sugars and starches (Li, 2020).

HAs have been shown to enhance the uptake of phosphorus by plants while simultaneously reducing the fixation of available phosphorus within the soil (Laskosky *et al.,* 2020). Furthermore, HAs facilitate the activation of insoluble phosphorus and augment the levels of the soluble phosphorus in the soil, or they may interact directly with phosphate fertilizers (Li, 2020). Potassium fertilizers are known to foster robust plant growth, enhance stress tolerance, and stimulate the synthesis of sugars and starches. However, the rapid release of potassium can lead to significant losses. HAs mitigate the leaching of potassium ions in sandy and highly soluble soils and inhibit potassium fixation in cohesive soils, thereby increasing the pool of exchangeable potassium and enhancing the availability of potassium silicate. An increase in mineral content, such as that from stone, can have a corrosive effect, which in turn elevates the levels of potassium in the soil simultaneously improving the efficiency of potassium fertilizers (Tavares *et al.,* 2019).

2.3.2 Plant agronomic parameters

HAs could stimulate the growth of both roots and shoots through improving the production of hormones (auxin and cytokinin) that promote plant growth and metabolic enzymes (Olaetxea *et al.,* 2020). HAs application improves absorption of both macro and micro-nutrients, leading to an increase in leaf chlorophyll concentration. This, in turn, positively affects shoot growth (El-Bassiouny *et al.,* 2014; Sible *et al.,* 2021). The production of phytohormones and enzymes, along with increases in root and shoot weight, chlorophyll content, and photosynthetic rate after humic acid application, enhances the overall yield (El-Bassiouny *et al.,* 2014).

2.3.3 Soil pH

Soil pH plays a crucial role in determining the availability and supply of nutrients. The ability of humic acid (HA) to modify soil pH depends on the concentration of carboxyl and phenolic functional groups present (Laskosky *et al*., 2020). Changes in soil pH not only affect how accessible nutrients are to plants but also influence the interactions among those nutrients. In acidic conditions, certain elements become less available, while others, such as iron, aluminum, and manganese, can reach toxic levels for plants. Gentili *et al*. (2018) emphasized the importance of soil pH for the uptake of essential nutrients, including nitrogen and magnesium, highlighting its significance in plant development.

Additionally, soil pH is vital for microbial activity, which is essential for maintaining soil health through processes like the decomposition of organic matter, nitrogen fixation, and improving soil structure. Most microorganisms thrive in a pH range of 6.0 to 7.5. The arrangement of soil particles and the spaces between them, known as soil structure, is also influenced by pH (Dewagan *et al*., 2023). Therefore, soils with low pH may exhibit structural issues, leading to increased erosion and reduced water retention (Dewagan *et al.,* 2023). On the other hand, soils with high pH can experience structural degradation, resulting in compaction and impaired water infiltration.

2.3.4 *Soil improvement*

HAs are organic compounds that are crucial in enhancing soil characteristics, promoting the development of plants, and optimizing agronomic outcomes (Chandrakant and Verma, 2023). The various sources of HAs include coal, lignite, soils, and other organic materials. In recent years, HAs have gained widespread use in plant production as a means to promote sustainable agricultural practices. Furthermore, HAs can significantly influence the physical, chemical, and biological properties of soil, such as its texture, structure, water retention capacity, cation exchange capacity (CEC), pH levels, soil carbon content, enzymatic activity, nitrogen cycling, and mineral availability (Ampong *et al.,* 2022). This underscores the vital role of HAs in crop development, plant hormone synthesis, mineral absorption and utilization, enhancement of yield, and protein production. Nevertheless, the effects of HAs on soil properties and crop performance is due to several factors, including the type of HA, application rate, method of application, soil type, solubility, molecular size, and the presence of functional groups.

2.4. Effects of different types of humic acids on soil improvement and plant growth

Rahmi *et al.* (2022) researched on the effects of different humic substances (HS) derived from andisol, spodosol, peat, and lignite on the growth of maize plants (*Zea mays).* The experimental design included two main variables: the different types of HS (Andisol, Spodosol, peat, and lignite) and the application rates (15 and 30 L/ha). The findings indicated that the addition of HA from lignite at a dosage of 30 L/ha was the most effective treatment, significantly enhancing corn plant growth as demonstrated by improvements in leaf width, leaf count, biomass weight, and root dry weight.

Abdellatif *et al.* (2017) assessed the impact of humic acid applied at rates of 4.8, 9.6, and 14.4 kg ha-1 on the growth and yield of two tomato cultivars that is Nema 1400 and Platinum 5043, in hot conditions. The results revealed that humic acid application during the summer season significantly influenced the growth and yields of tomato plants. Specifically, the highest application rate of 14.4 kg ha-1 resulted in increased foliage growth (including plant height and fresh weight), flowering metrics (number of flower clusters and flowers per plant), and yield parameters (fruit count per plant and fruit weight), leading to enhanced early and total yields. Nonetheless, the application of HA had a minimal effect on the number of fruits per plant, including vitamin C and TSS content.

2.5 Effect of humic substances on agricultural productivity

HS are recognized as natural and effective growth promoters, as they trigger important local and systemic physiological responses through hormone-like signal pathways (Vikram *et al.,* 2022). Various factors, such as dosage, origin, molecular size, the extent of hydrophobicity, and aromaticity, including the spatial distribution of hydrophilic and hydrophobic domains, significantly influence plant growth. (Vikram *et al.,* 2022). The low molecular-weight humic substances can penetrate root cells and initiate intracellular signal directly, while high molecular-weight humic substances attach to external cell receptors thereby activating molecular reactions (Vikram *et al.,* 2022). According to Vikram *et al* (2022), HS impact various components, including nutrient transporters, plasma membrane H+-ATPases, hormone pathways, and genes/enzymes that are useful in nitrogen absorption, cell division, and development.

HA and HS can significantly enhance soil quality and plant growth, both in laboratory settings and on the field. They improve soil structure, enhance fertilizer utilization, and promote plant growth by encouraging morphogenesis, formation of lateral roots, and the initiation of root hairs in plants. Additionally, HA stimulates the development of roots and shoots in treated cell calluses (Vikram *et al.,* 2022).

HA also boosts nutrient utilization efficiency and helps to absorb macro and microelements, while inducing carbon, nitrogen, and secondary metabolism. As a result, these improvements support crop growth, ultimately increasing production and cash for farmers (Nadi *et al.,* 2020; Khan et *al.,* 2017; Yanan, 2020).

2.6 Uses of sweet sorghum

Sweet sorghum is an under-utilised grain crop with vast potential, particularly for bio-ethanol production, as it can withstand harsh conditions. Even though it is indigenous to sub–Saharan Africa (SSA), it has largely been neglected and underutilised despite its wide potential in improving the socio-economic status of smallholder farmers in this region (Motsi *et al.*, 2022). Sweet sorghum crop can grow and develop under limited water conditions as it uses water efficiently. It can also be grown under minimal fertile soils compared to its biofuel counterparts such as maize, sugarcane, and sugar beet (Motsi *et al.*, 2022). Furthermore, it can still attain high yields when grown at high plant densities, while early planting dates also result in high yields (Motsi *et al.*, 2022). These attributes of the crop are important in smallholder farming systems as they are prone to socio-economical and agronomic challenges.

2.6.1 Juice extraction

Sweet sorghum production is important in that it provides a direct source of aqueous fermentable sugar. These sugars are available in the stalk juice and are extracted through crushing or squeezing operation. The most common method used in juice extraction is allowing a whole stalk pieces to pass through a set of rollers (Veal *et al.*, 2024). However, using this type of press has shown that, less than 50% of the juice is collected as the press uses manual labour (Veal *et al.*, 2024). Another disadvantage is that the leaves must be removed from the stalks before crushing. Generally,the leaves have few soluble sugars and when very wet and green, they add more water during juice extraction thus diluting the sugar solution. Similarly, when the leaves are very dry and brown, they mimic a sponge, thereby soaking up juice. However, the biggest challenge for industrial utilisation of sweet sorghum juice is the short shelf-life period of fermentable sugars due to the contaminations by other microbes (Hu & Chen, 2022).

2.6.2 Molasses

Molasses is the primary agricultural product that is extracted from sweet sorghum. Molasses is made by crushing and squeezing the sugary juice from the sweet sorghum stalk, then dehydrating the juice so that it is thick, and becomes a viscous sweetener (Veal *et al.*, 2024). If cane press technology is used, it is recommended to strip the leaves off the sweet sorghum before crushing as this increases sugar recovery by more than 30% (Veal *et al.*, 2024). When boiling the juice, constant stirring is required so as to prevent the burning of molasses product. Ultimately, juices change from greenish colour to a deep brown colour because the sugars will concentrate and crystallise.

2.6.3 Ethanol production/Fermentation products

The value of sweet sorghum depends on the high concentration of sugars found in the watery juice extracted from its stalks. Typically, the sugar concentrations in expressed juice from most sweet sorghum varieties range between 12% and 20% (Zhang *et al.,* 2018). These sugars are a mixture of sucrose, glucose, and fructose, with the actual ratios differing among various varieties of sorghum.

Related to the molasses production process, the juice is also extracted from the stalk. When the sugars are present in the juice, fermentation can begin simply by adding yeast to the solution. For successful fermentation, several factors must be taken into account, which include sterilization, the strain of yeast used, the reaction temperature, and the levels of pH. According to research the fermentation process can be done within 24 hours, with up to 90% of the sugars being converted to ethanol simply by the addition of yeast to freshly processed juice (Bridgers *et al.,* 2010).

Apart from ethanol production, the sugars from sweet sorghum are also used to make different fermentation products. Hydrogen and butanol can also be made from the sweet sorghum sugars (Ahmad *et al.,* 2018). Furthermore, during the fermentation processes using sweet sorghum sugars as a feedstock can also make products such as lactic acid, lipids for bio-diesel production, and acetone (Ahmad *et al*., 2018).

2.6.4 Animal feed

Sweet sorghum is used as an animal feed; however proper storage considerations need to be adhered to. The stalk from the field or the bagasse (the solid matter that is left extraction of juice) can be used as feed for animals. Both the bagasse and whole stalk material have high levels of residual sugars that will lead to quick spoilage due to microbial activity (Zhang *et al.*, 2018). As a result, the material must be converted to silage for later use (Xie & Xu, 2019). Ensiling involves tightly compacting the chopped material or wrapping the baled material in plastic so as to limit its exposure to oxygen. As a result, an anaerobic storage condition is created allowing specific bacteria to grow and thus make lactic acid (Alhaag *et al.,* 2019). Consequently, the acid concentration would be high enough to kill off all microorganisms, including the bacteria including the one producing the acid. This would allow the material to be safely preserved.

Sweet sorghum is generally a low-value feed ingredient due to the fact that both starch and protein levels are relatively low. It is because of its ability to high to produce significant tonnage under adverse weather conditions that it is a favoured crop. In other instances when sweet sorghum is mixed with an additional starch source, it provides the same performance as maize silage if fed to dairy cattle (Alhaag *et al.,* 2019).

2.6.5 Other commercial products

There are a lot mentioned below are currently that are being researched and developed to create new markets for sweet sorghum (Whitfield *et al.,* 2012). An interesting possibility is utilizing sorghum's allelopathic properties to produce new, natural herbicides. Allelopathy refers to the ability of a plant to produce chemical compounds that can inhibit the growth of other plants in either a positive or negative manner. Additionally, certain chemical compounds that are found in sorghum have medicinal uses, such as the treatments of diabetes (Hu *et al.,* 2022). The waxes from sorghum can also be used to make edible films and coatings in the food manufacturing industry. Furthermore, the bagasse from the sorghum juice processing has been used as a medium in mushroom production (Veal *et al.,* 2024). Sweet sorghum yields a high tonnage of cellulose, which can be used in developing new second-generation bio-fuels (Minty *et al.,* 2013). From the perspective of cellulosic ethanol, sweet sorghum is a much-favoured feedstock as it has relatively low lignin and hemicellulose contents. The cellulose structure facilitates a more straight forward fuel conversion process compared to other grass feedstock options (Veal *et al.,* 2024).

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CHAPTER 3: MATERIALS & METHODS

3.1 Materials and methods

3.1.1 Site description

Two field experiments were conducted under dryland conditions during the 2022 and 2023 summer growing seasons at the Glen Agricultural Institute near Bloemfontein and the Agriculture Research Council-Grain Crops (ARC-GC) Experimental farm in Potchefstroom, South Africa. The Glen Agricultural Institute is located near Bloemfontein on the R30 road to Brandford in the Free State province (latitude 28º55’47’’S - longitude 26º19’32’’E). The ARC in Potchefstroom is located at 26° 43′ 00″ S and 27° 06′ 00″ E with an average altitude of 1 335 m.

The soil at the Glen Agricultural Institute experimental site is of the Hutton form, based on the South African Soil Classification System (Soil Classification Working Group, 1991). The clay content of the soil is 14% in the A-horizon and 23% in the B-horizon with an effective depth of 180 cm and the ARC Potchefstroom experimental farm has a clay percentage of 34% while the soil is classified as sandy clay loam (Sebetha *et al.*, 2014).

The climatic zone at Glen Agricultural Institute is semi-arid and has an annual rainfall of 547 mm. The lowest minimum temperatures range from -1.1^{0}C to a maximum of 18^{0}C around June, and the highest minimum temperatures range from 15^{0}C to 31^{0}C around January (with current climate change effects leading to maximum summer temperatures of 39^{0}C). The Potchefstroom experimental area has an annual average rainfall of 615 mm and an average temperature of 16.9°C.

3.1.2 Design and Treatments

The factorial experiment design was used and the layout arrangement was in a randomised block design with three replications. Factors that were used include locations (Glen and Potchefstroom), three sweet sorghum cultivars (ARC-SS 27, ARC-SS 76, and Hunnigreen), as well as treatment levels of HA (0.5, 10 & 15 kg ha^{-1}). Soil samples were collected before planting and fertiliser applications were done according to the recommendations from a soil test report (Table 3.1 and Table 3.2 for Glen and Potchefstroom, respectively). Plot dimensions were 3.6 m (breadth) x 5 m (length) with four rows with a spacing of 0.90 m.

Table 3.1: Soil Analysis Report for Glen

Sample	**pH**	**P-mg/kg**	**K-mg/kg**	**Ca-mg/kg**	**Mg-mg/kg**	**Na-mg/kg**	**S-mg/kg**	**Ca/Mg**	**(Ca+Mg)/K**	**CEC-cmol$_c$kg^{-1}**	**Densityg.cm^{-3}**
Glen	5.1	3.0	98.0	855	400.0	128.0	0.33	1.30	30.14	8.36	1.17
Glen Sub Block I	5.5	5.8	133.0	788	335.0	83.0	0.33	1.44	19.66	7.39	1.20

Table 3.2: Soil Analysis Report for Potchefstroom

Sample	**pH**	**P-mg/kg**	**K-mg/kg**	**Ca-mg/kg**	**Mg-mg/kg**	**Na-mg/kg**	**S-mg/kg**	**Ca/Mg**	**(Ca+Mg)/K**	**CEC-cmol$_c$kg^{-1}**	**Density**
Block TB1 (T) Potch - Subsoil	6.0	2.2	198.0	1078	440.0	40.0	24.03	1.50	17.77	9.68	1.00
Block Y22 (L) Potch - Subsoil	6.9	1.3	55.0	1290	573.0	73.0	0.67	1.37	79.24	11.61	1.01

3.2 Data collected and procedures

3.2.1 Growth response parameters (Agronomic)

Days to flowering

Days to flowering were measured as from the date of emergence to when 50% of plants in a plot would have started flowering.

Plant height (cm)

It was measured as the average height from the base of the plant up to the tip of the panicle of the main stem at physiological maturity. A measuring tape was used and this was expressed in cm. A random selection of 10 plants from each plot was taken as the representative, then the average was taken as the final plant height.

Leaf weight (kg)

This was determined by removing all the leaves from the stalk at physiological maturity, placing them in a bucket, and weighing them using a digital electronic scale.

Stalk yield (kg)

This was determined by weighing the harvested stalks without leaves, leaf sheath and panicles before crushing them, then using a digital electronic scale which recorded the weight and expressed in kilograms then converting it to t/ha.

Bagasse fresh and dry weight (kg)

This was done by weighing the bagasse, the fibrous product that was left after the sweet sorghum stalks were crushed to remove the juice.

3.2.2 Juice yield and quality parameters

Brix = Sugar content in juice (BRIX%)

It was measured by a hand-chopping the 10 representative stalks at the fourth internode from the tip and dropping the juice on the hand-held refractometer (a laboratory device that is used for measurements of sugar content in sweet sorghum stalks), three drops were collected on the refractometer and readings were expressed as a percentage (%) at physiological maturity.

Juice volume and juice weight

The juice extraction was done using a hydraulic three-roller press, and juice was measured using a measuring cylinder and expressed in millilitres (ml) then this was converted to litres per hectare (l/ha), using a digital electronic scale.

3.2.3 Panicle and grain yield parameters

Panicle weight in grams or kg

This was done by weighing the dried panicles from each plot before threshing.

Statistical analysis of data

The data was analysed using the analysis of variance (ANOVA) and the means of the results were compared using the least significant difference (LSD) using the Statistical Analyses System (SAS) version 9.2.

3.3 Results

3.4.1 Growth response parameters

3.4.1.1 Plant height

Analysis of variance for the sorghum lines used in the experiment indicated the presence of significant differences at $p<0.005$ on plant height (Figure 3.1a) at different levels of HA applied. The tallest plant height at year one at Potchefstroom was 3.783 m while the lowest was 2.587 m. Also, there were significant differences ($p<0.005$) that were observed on the overall mean plant height during the first year at Potchefstroom. The tallest plants were 3.687 m from an application rate of 15 kg ha^{-1} of HA while the shortest plants were 2.608 m on 0 kg ha^{-1} of HA (Figure 3.1b). There were no significant differences ($p>0.005$) on plant height of the Hunnigreen cultivar on HA applied at 15 kg ha^{-1} with the tallest plant being 3.153 m and the shortest being 1.877 m on ARC-SS 27 on the 0 kg ha^{-1} of HA at Potchefstroom during the second year of planting. The mean height was 3.100 m for the tallest and the shortest was 1.946 m. Plant height (PH) did not differ ($p<0.05$) at Glen in year one with the tallest plant of 3.520 m being harvested on the Hunnigreen cultivar at 15 kg ha^{-1} HA while the least was also on ARC-SS 27 at an application rate of 0 kg ha^{-1} HA being 2.587 m. The tallest mean plant height harvested was 3.439 m from 15 kg ha^{-1} of HA while the least was 2.633 m at an application rate of 0 kg ha^{-1} of HA. At Glen in year two the tallest plant was 2.187 m being harvested on the ARC-SS 27 cultivar at 15 kg ha^{-1} of HA while the least was on ARC-SS 76 at 0 kg ha^{-1} of HA being 1.390 m. The tallest mean plant height harvested was 2.127 m from 15 kg ha^{-1} of HA and least was 1.522 m at 0 kg ha^{-1} of HA.

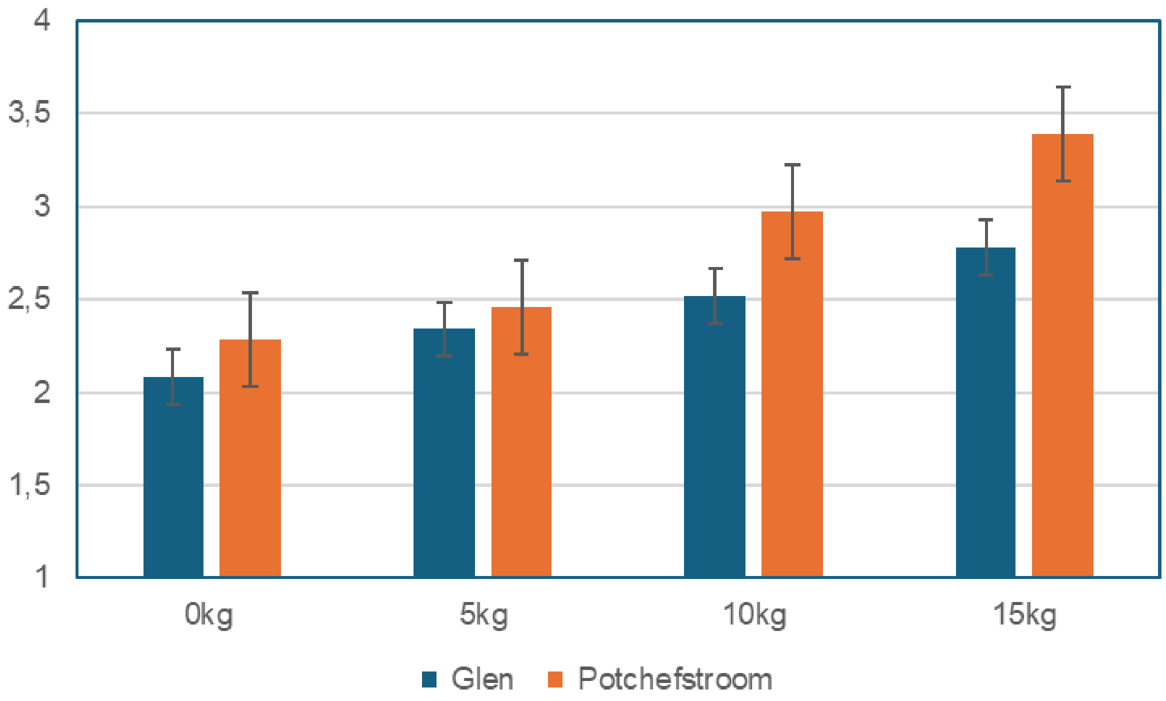

Figure 3.1a: Effects of Humic Acid on plant height of sweet sorghum

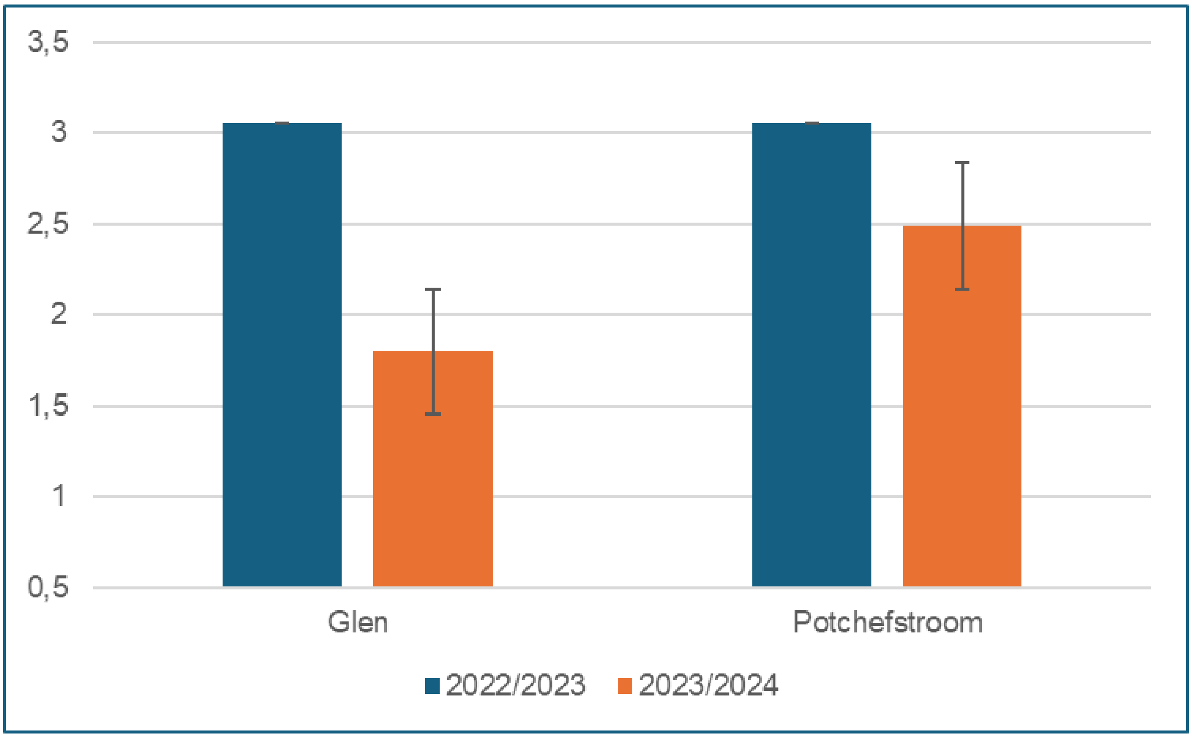

Figure 3.1b: Effects of Humic Acid on plant height of sorghum grown in two locations for two seasons

3.4.1.2 Stalk weight

Stalk weight varied at 15 kg ha^{-1} with 976.7 g having been attained on Hunnigreen, while the least weight of 510.0 kg was on ARC-SS 76 at Potchefstroom in year one (Figure 3.2). The highest mean yield was 141.7 g on Hunnigreen was on 15 kg ha^{-1} and the least on ARC-SS 76 at 0 kg ha^{-1}. There were slight differences in yield among the three cultivars at 15 kg ha^{-1} with the highest yield of 953.3 g by Hunnigreen. The highest mean yield in year one of 813.3 g was attained on 15 kg ha^{-1} while the least was 437.8 g on 0 kg ha^{-1}. Stalk weight also varied significantly (p<0.05) in year two at Potchefstroom with Hunnigreen having 749.3 g at 15 kg ha^{-1} of HA, while the least was on ARC-SS 27 with 162.0 g at 0 kg ha^{-1} of HA. Stalk weight also varied significantly (p<0.05) in year one at Glen with Hunnigreen having 1306.7 g at 15 kg ha^{-1} of humic acid and the least was on ARC-SS 76 with 533.3 g at 0 kg ha^{-1} of HA (Figure 3.2). The largest mean stalk weight was 1141.7 g on Hunnigreen at 15 kg ha^{-1} of HA and the least was 521.7 g on ARC-SS 76 at 0 kg ha-^{1} of HA. Stalk weight also varied significantly (p<0.05) in year two at Glen with Hunnigreen having 499.3 g at 15 kg ha^{-1} of humic acid while the least was on ARC-SS 27 with 188.0 g at 0 kg ha^{-1} of humic acid. The largest mean stalk weight was 466.7 g at 15 kg ha^{-1} of humic acid and the least was 224.7 g on at 0 kg ha^{-1} of HA.

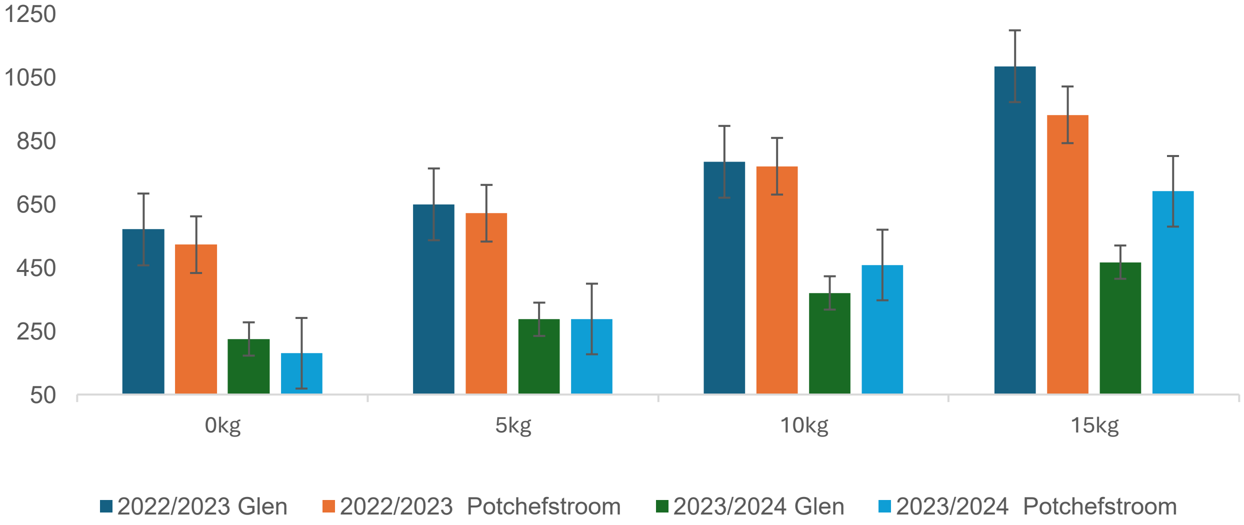

Figure 3.1: Effects of different levels of Humic Acid on stalk weight at different locations

3.4.1.3 Dry bagasse

Dry bagasse (DB) yield at Potchefstroom did not differ on ARC-SS 27 and ARC-SS 76 when applied at a rate of 15 kg ha^{-1} of HA. The highest yield of 496.7 g was on ARC-SS 76 and the least of 230.0 g was attained on Hunnigreen at an application rate of 0 kg ha^{-1} of HA at Potchefstroom (Figure 3.3). The mean yield of dry bagasse varied significantly (p<0.005) with the highest being 518.3 g and the lowest 245.6 g. Also, significant differences (p<0.0 5) were observed on DB weight with 560.0 g on Hunnigreen being harvested on 15 kg ha^{-1} HA and the least 230.0 g on the same cultivar on 0 kg ha^{-1} of humic acid. The largest mean yield of DB of 518.3 g was on 15 kg ha^{-1} of humic acid and the least 245.6 g was attained on 0 kg ha-^{1} of humic acid. At Glen, there were significant differences (p<0.05) on DB weight with 463.7 g on Hunnigreen being harvested on 15 kg ha^{-1} of HA and the least 164.0 g on ARC-SS 27 on 0 kg ha^{-1} of HA (Figure 3.3). The largest mean yield of DB of 437.3 g was on 15kg ha^{-1} of HA and the least 193.7 g was attained on 0 kg ha^{-1} of HA.

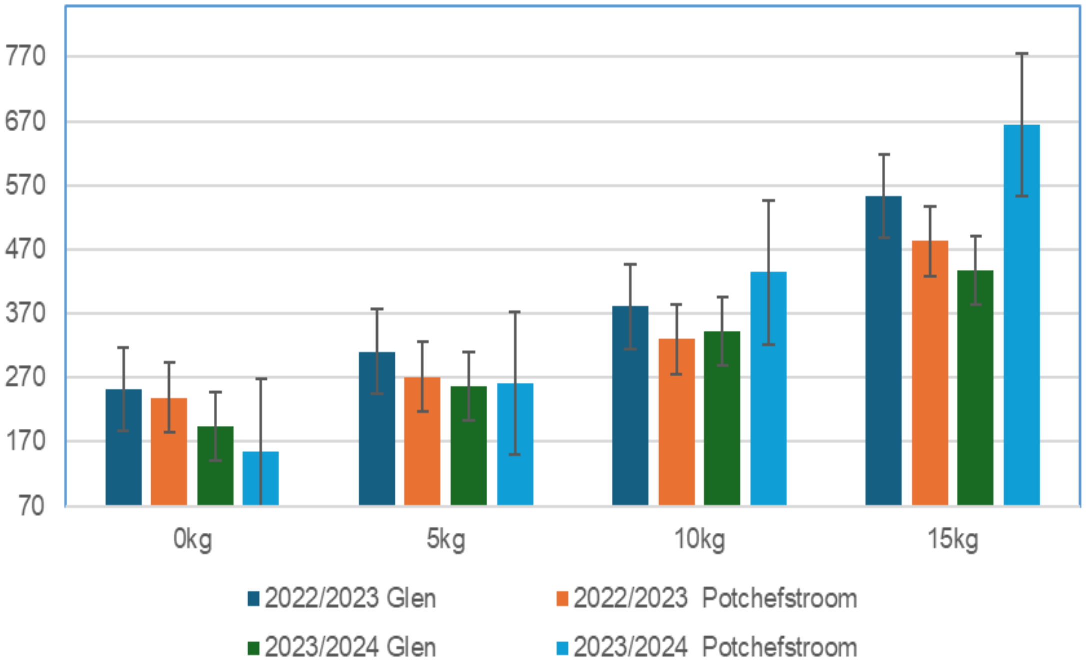

Figure 3.3: Effects of different levels of Humic Acid on dry bagasse at different locations

3.4.1.4 Fresh bagasse

There were significant differences observed on fresh bagasse (FB) during the second year with Hunnigreen having 737.3 g at 15 kg ha^{-1} of HA while the least was ARC-SS 27 at 150.0 g at

0 kg ha^{-1} of HA (Figure 3.4). Yield of FB also varied significantly at Potchefstroom with 678.0 g on 15 kg ha^{-1} of HA being the largest and the least was 168.7 g and 0 kg ha^{-1} of HA. There were significant differences (p<0.05) on fresh panicle weight with 85.93 g on ARC-SS 27 being harvested on 15 kg ha^{-1} of humic acid and the least 36.73 g on ARC-SS 27 on 0 kg ha-^{1} of humic acid. The largest mean yield of fresh panicle weight of 78.84 g was on 15 kg ha^{-1} of humic acid and the least 41.78 g was attained on 0 kg ha^{-1} of HA. During year one at Glen, there were significant differences (p<0.05) (Figure 3.4) on FB, with the largest weight of 953.3 g being on Hunnigreen at 15 kg ha^{-1} of HA while the least was 310.0 g on ARC-SS 27 at 0 kg ha^{-1} of HA. The largest FB mean weight recorded was 813.3 g and the least was 437.8 g. During year two at Glen, there were no significant differences (p>0.05) on FB with the largest weight of 477.3 g on Hunnigreen at 15 kg kg ha^{-1} of humic acid while the least was 176.7 g on ARC-SS 27 at 0 kg ha^{-1} of humic acid. The largest FB mean weight recorded was 451.6 g on 15 kg ha^{-1} of HA and the least was 206.8 g on 0 kg ha^{-1} of HA.

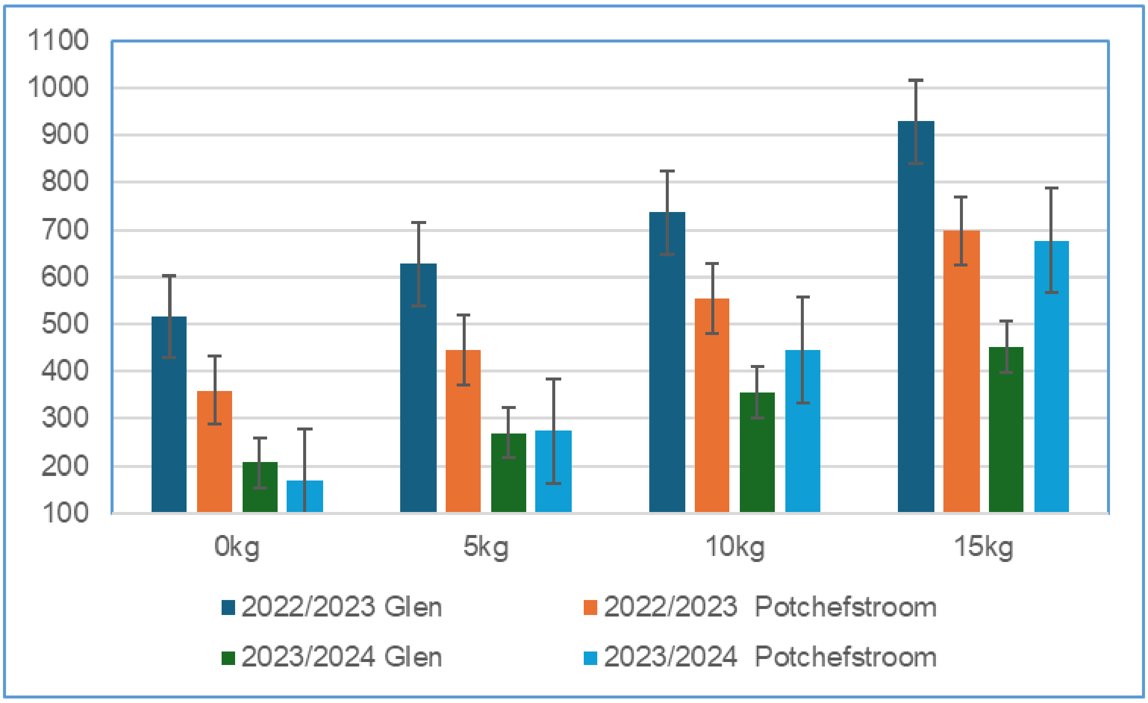

Figure 3.4: Effects of different levels of Humic Acid on fresh bagasse at different locations

3.4.1.5 Dry leaf weight

As such no significant differences (p<0.005) were observed on all cultivars on dry leaf weight (DLW) with Hunnigreen having 90.35 g and the least was on ARC SS76 at 38.78 g in year one at Potchefstroom (Figure 3.5). The total HA yield also varied significantly with the highest being 89.53 g and the least 49.59 g. DLW varied across treatments with the largest being 96.43 g recorded on 15 kg ha^{-1} of HA on Hunnigreen at Glen in year one, while the least DLW was 38.78 g on 0 kg ha^{-1} of HA. The largest DLW mean was 89.53 g and the least was 49.59 g for Glen during year one. Significant differences (p<0.05) were observed on DLW with the largest being 80.45 g recorded on 15 kg ha^{-1} of HA on Hunnigreen at Glen in year two, while the least DLW was 27.56 g on 0 kg ha^{-1} of HA (Figure 3.5). The largest DLW mean was 58.4 g and the least was 33.70 g for Glen in year two.

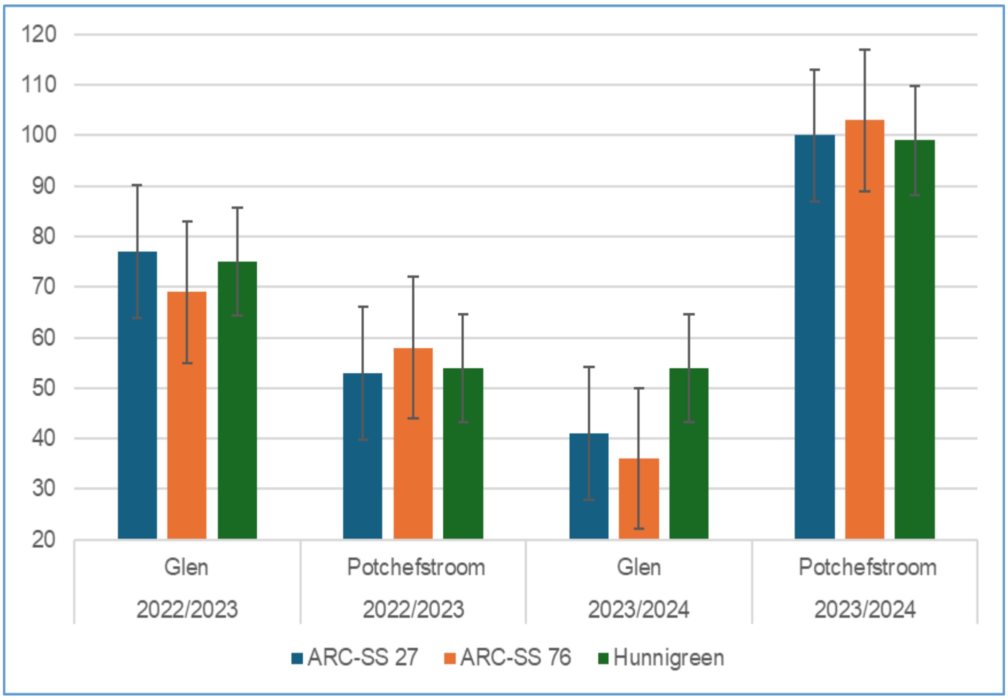

Figure 3.5: Effects of different levels of Humic Acid on leaf weight at different locations and seasons

3.4.1.5 Fresh panicle weight

At Potchefstroom in year one, fresh panicle weight (FP) varied significantly (p<0.005) when HA was applied at 15 kg ha^{-1} with a highest yield of 85.93 g for ARCSS27 and the least weight of 36.73 g for the same cultivar at 0 kg ha^{-1} of HA (Figure 3.6). The highest mean yield of FP was 78.84 g while the least was 41.78 g. At Potchefstroom in year two, significant differences were observed on FP weight with 81.92 g on Hunnigreen being harvested on 15 kg ha^{-1} of humic acid and the least 41.61 g on ARC-SS 27 observed on 0 kg ha^{-1} of humic acid. The largest mean yield of FP weight of 78.84 g was on 15 kg ha^{-1} of humic acid, and the least of

41.78 g was attained on 0 kg ha^{-1} of HA (Figure 3.7). At Glen, there were significant differences ($p<0.05$) on FP weight with 47.50 g on Hunnigreen being harvested on 15 kg ha^{-1} of humic acid and the least of 17.97 g on ARC-SS 76 on 0 kg ha^{-1} of humic acid (Figure 3.6). The largest mean yield of FP weight of 58.14 g was on 15 kg ha^{-1} of humic acid and the least 31.85 g was attained on 0 kg ha^{-1} of HA (Figure 3.7).

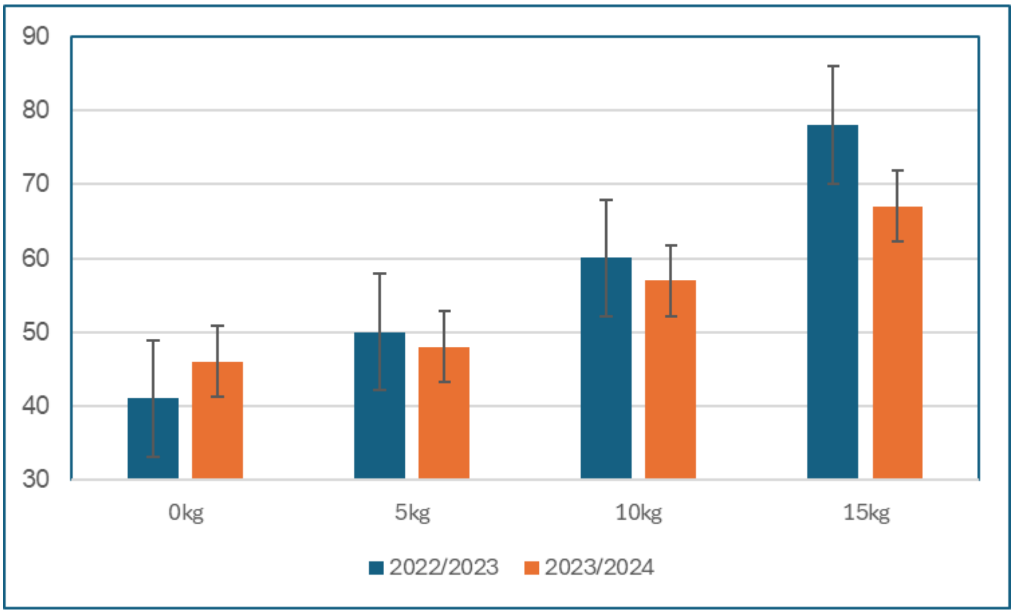

Figure 3.6: Effects of different levels of Humic Acid on fresh panicle weight on sweet sorghum at different locations

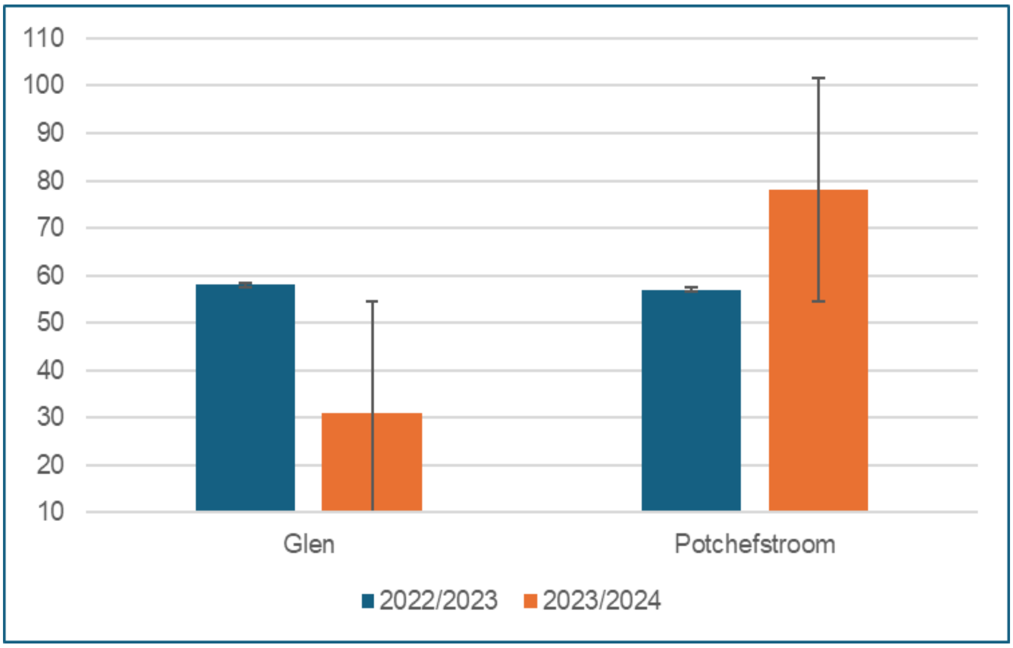

Figure 2.7: Effects of Humic Acid on fresh panicle of sweet sorghum at two locations and different seasons

3.4.1.6 Dry panicle weight

There were significant differences ($p<0.005$) on weight of dry panicle (DP) of Hunnigreen cultivar on HA applied at 15 kg ha^{-1} with a high yield of 50.68 g and the least being 20.70 g on ARC-SS 27 on the 0 kg ha^{-1} of HA at Potchefstroom in year one (Figure 3.9). The highest mean yield was obtained on the 15 kg ha^{-1} of HA at 46.98 g while the least was 27.60 g. Significant differences were observed only in the second year between Glen and Potchefstroom on DP (Figure 3.8). During the second year, Glen had a mean DP of 25.06 g while Potchefstroom had a mean yield of 71.14 g.

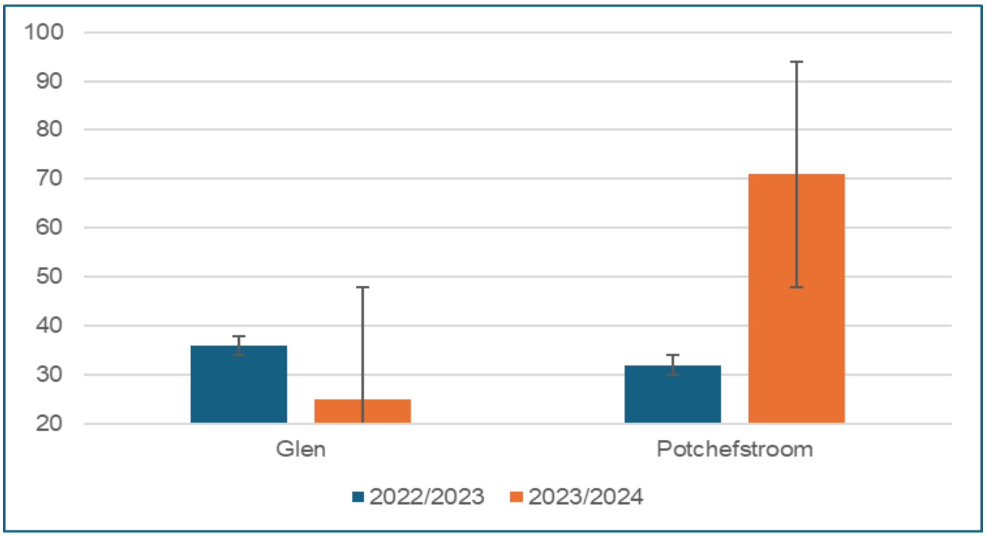

Figure 3.8: Effects of Humic Acid on dried panicle of sorghum genotypes grown in two locations and seasons

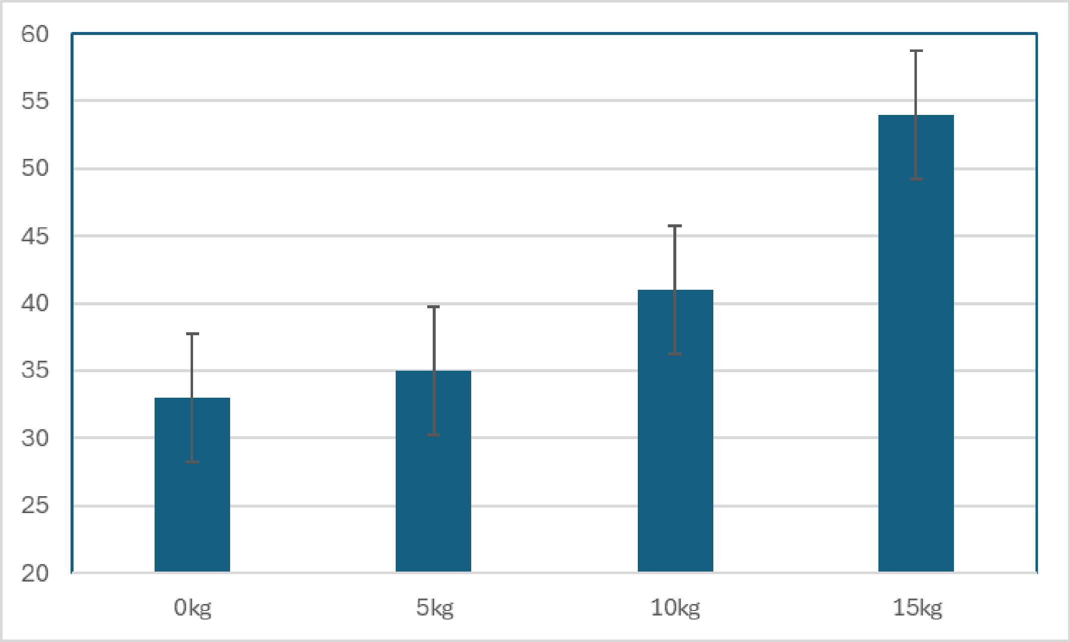

Figure 3.9: Effects of different levels of Humic Acid on dried panicle of sweet sorghum genotypes

3.4.1.6 Days to 50% flowering

The days to 50% flowering significantly differed (*p*<0.005) for ARC-SS 27 with a highest of 80.67 days on 15 kg ha^{-1} of HA while the least was Hunnigreen at 70.00 days at HA on 0 kg ha^{-1} at Potchefstroom in year one (Figure 3.10). There were significant differences in days to flowering between Glen and Potchefstroom in the 2023/2024 season on days to 50% flowering particularly at 5 kg ha^{-1} of HA with all other different HA levels applied. At Glen, ARC-SS 76 had 116.33 days to 50% flowering in the 2023/2024 season. At Potchefstroom, the same cultivar had 79.33 days to 50% flowering even with increased humic acid of up to 15 kg ha^{-1} .

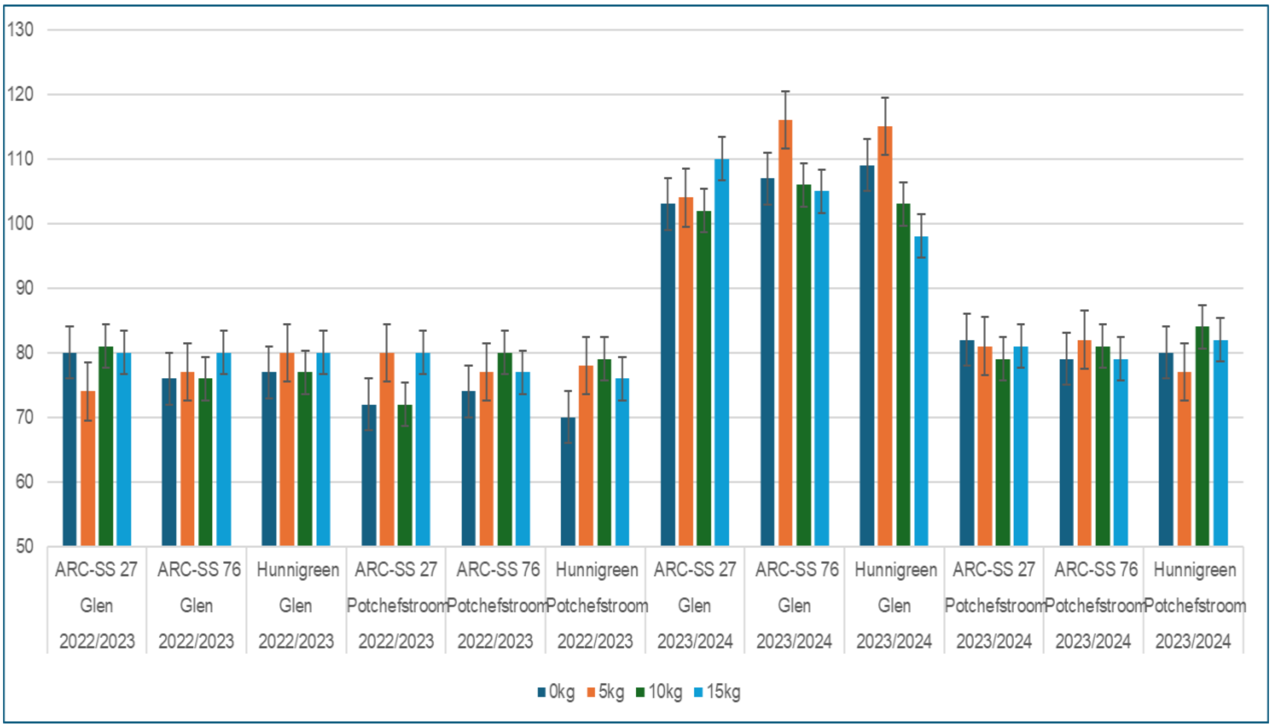

Figure 3.10: Effects of Humic Acid, planting seasons and location interaction on 50% flowering of sweet sorghum genotypes in two different seasons

3.4.2 Juice yield and quality parameters

3.4.2.1 Juice volume

There were no differences ($p<0.05$) on juice volume in year one at Glen, with the highest yield of 356.6 ml at 15 kg ha^{-1} of humic acid on ARC-SS 76 cultivar, while the least of 209.1 ml was on Hunnigreen at 0 kg ha^{-1} of humic acid. The largest mean juice volume collected was 338.3 ml on 15 kg ha^{-1} of humic acid while the least was 222.2 ml on 0 kg ha^{-1} of HA.

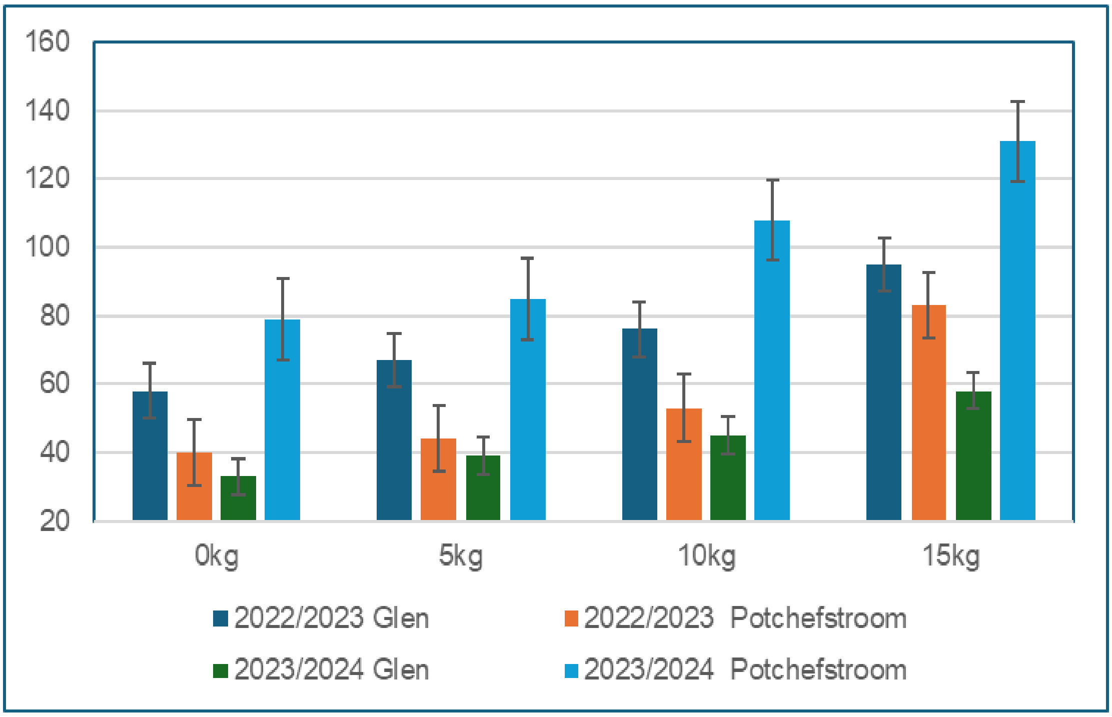

Figure 3.11: Effects of different levels of Humic Acid on juice volume of sweet sorghum at two different locations and seasons

3.4.2.2 Juice and sugar yield

There were significant differences ($p<0.05$) were observed at Potchefstroom in year one among the cultivars with Hunnigreen attaining 0.3800 g at 15 kg ha^{-1} and the lowest being 0.2267 g at 0 kg ha^{-1} of humic acid. Juice yield varied significantly at 0 kg ha^{-1} with a yield of 0.2328 g while at 15 kg ha^{-1} of HA there was 0.3489 g. Brix at flowering differed significantly ($p<0.05$) at Potchefstroom in year one with 18.50% being harvested on the Hunnigreen cultivar at 15 kg ha^{-1} of HA, while the least was also on Hunnigreen at 0 kg ha^{-1} of HA having 12.27%. Again, the mean yield of brix at flowering varied significantly ($p<0.05$) with the highest being 17.47% and the least at 12.78%. At Potchefstroom during year one, brix yield at harvesting significantly varied ($p<0.05$) at 15 kg ha^{-1} of HA attaining a yield of 17.60% for Hunnigreen. The least yield was on ARC-SS 27 at 9.63% at 0 kg ha^{-1} of humic acid at harvesting. The highest mean brix yield at harvesting was 17.58% for Hunnigreen at an application rate of 15 kg ha^{-1} of humic acid, while the least yield was on both Hunnigreen and ARC-SS 76 at 9.32% at 0 kg ha^{-1} of humic acid.

Brix at flowering differed significantly ($p<0.05$) at Potchefstroom during year two with 18.04% being harvested on the Hunnigreen cultivar at 15 kg ha^{-1} of humic acid, while the least was also on Hunnigreen at 0 kg ha^{-1} of humic acid having 10.93%. The highest mean brix harvest at flowering was 15.35% on ARC-SS 27 observed at 15 kg ha^{-1} of humic acid while the least was 14.58% on ARC-SS 76 at 0 kg ha^{-1} of humic acid. Significant differences ($p<0.05$) were observed on brix at harvest with the highest yield of 21.23% at 15 kg ha^{-1} of humic acid on the Hunnigreen cultivar, while the least was 9.43% on ARC-SS 27 at 0 kg ha^{-1} of humic acid. The largest brix yield was 20.54% on 15 kg ha^{-1} of humic acid, and the least was 11.87% on 0 kg ha^{-1} of HA.

Even though there were no significant differences ($p>0.05$) at Glen in year one, a juice weight of 0.3500 g was collected on ARC-SS 27 cultivar at 15 kg ha-1 of humic acid while the least of 0.2000 g was also on Hunnigreen at 0 kg ha^{-1} of humic acid (Figure 3.12). The largest mean juice weight was 0.3489 g at 15 kg ha^{-1} of humic acid, while the least was 0.2328 g at 0 kg ha^{-1} of HA. There were no significant differences ($p<0.05$) observed at Glen in year one on brix at flowering with 18.10% being harvested on the ARC-SS 76 cultivar at 15 kg ha^{-1} of humic acid while the least of 12.33% was on ARC-SS 27 at 5 kg ha^{-1} of humic acid. The largest mean brix at flowering was 17.47% at 15 kg ha^{-1} of humic acid while the least obtained was 12.78% at 5 kg ha^{-1} of HA. On the quantity of brix there were no significant differences at harvesting as it was 17.57% on the Hunnigreen cultivar at 15 kg ha^{-1} of humic acid, while the least was 9.00% on the same cultivar at 0 kg ha^{-1} of humic acid. The highest mean brix in year one at Glen was 17.58% on the Hunnigreen cultivar, and the least was 9.32% of the same cultivar at 0 kg ha^{-1} of humic acid.

Significant differences ($p<0.05$) were observed on brix at flowering in year two at Glen, with the highest yield of 17.97 ml at 15 kg ha^{-1} of HA on the ARC-SS 76 cultivar, while the least at 10.67 ml was on ARC-SS 27 at 0 kg ha^{-1} of humic acid. The largest mean brix at flowering collected was 15.76 ml on 15 kg ha^{-1} of humic acid, while the least was 11.98 ml on 0 kg ha^{-1} of humic acid. At Glen in year two, there were no significant differences on brix at harvesting with 15.67% being harvested on the ARC-SS 76 cultivar at 15 kg ha^{-1} of humic acid while the least of 15.59% was on ARC-SS 27 at 5 kg ha^{-1} of HA. The largest mean brix at harvesting was 15.76% at 15 kg ha^{-1} of HA, while the least obtained was 11.98% at 5 kg ha^{-1} of humic acid. At flowering brix differed significantly($p<0.05$) in year two at Glen, with the highest yield of 17.97 ml at 15 kg ha^{-1} of humic acid on the ARC-SS 76 cultivar, while the least was 10.67 ml on ARC-SS 27 at 0 kg ha^{-1} of humic acid (Figure 3.12). The largest mean brix at flowering collected was 15.76 ml on 15 kg ha^{-1} of HA, while the least was 11.98 ml on 0 kg ha^{-1} of HA. At Glen in year two there were no significant differences observed on brix at harvesting, with

15.67% being harvested on the ARC-SS 76 cultivar at 15 kg ha^{-1} of HA, while the least of 15.59% was on ARC-SS 27 at 5 kg ha^{-1} of HA. The largest mean brix at harvest was 15.76% at 15 kg ha^{-1} of humic acid, while the least obtained was 11.98% at 5 kg ha^{-1} of humic acid.

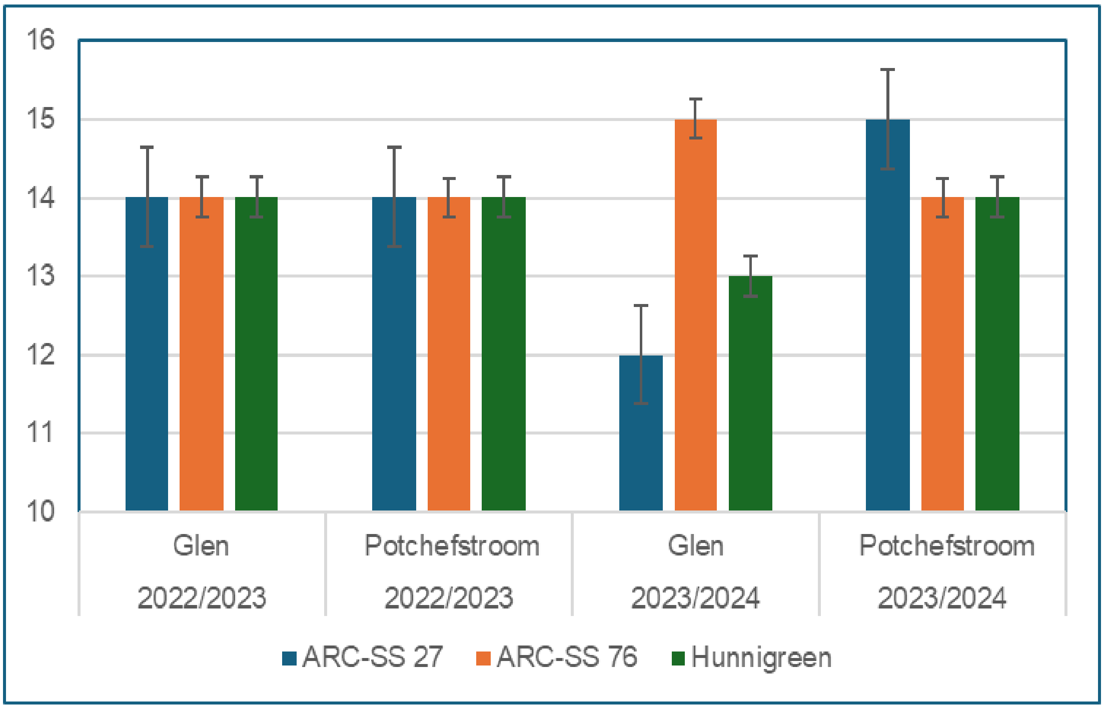

Figure 3.12: Effects of Humic Acid on sweet sorghum brix content at two locations and seasons

There were no significant differences on sucrose, fructose, galactose, trehalose, glucose, maltose and lactose despite the different levels of humic acid that were applied (Figure 3.13). Only the total sugars were significantly affected (Figure 3.14).

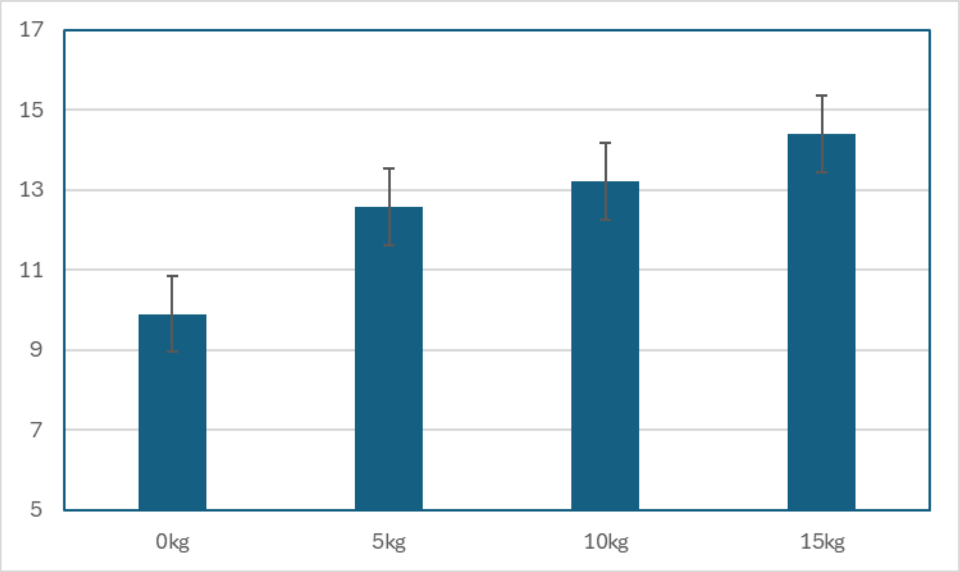

Figure 3.13: Effects of different levels of Humic Acid on sugar content

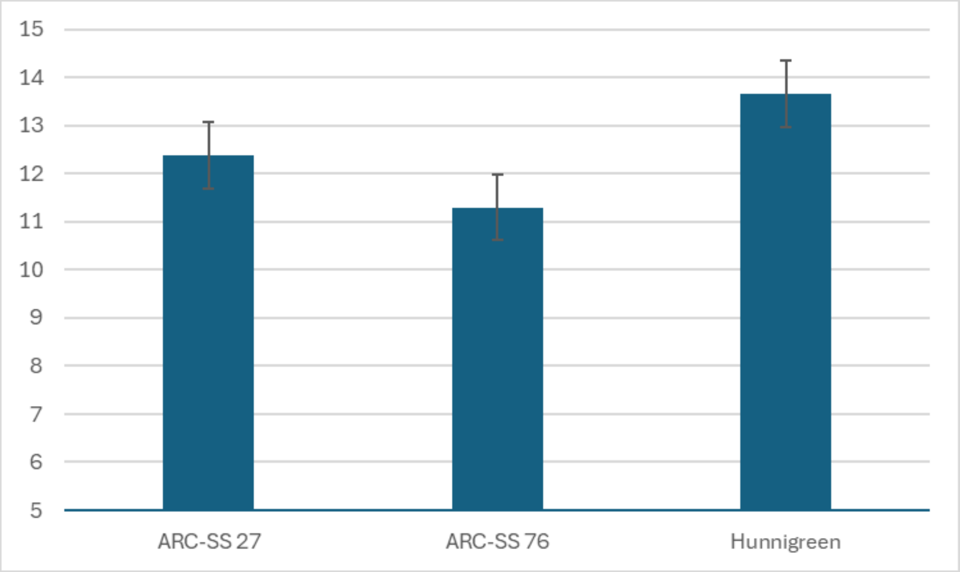

Figure 3.14: Effects of different levels of Humic Acid on the different cultivars at two locations and seasons

3.4.2 Effects of interactions on agronomic, juice yield and quality parameters

There were various interactions within the study, and these affected various agronomic and juice parameters that were recorded. The season affected plant height, stalk weight, fresh bagasse weight, dry leaf weight, juice volume, brix at flowering, fresh panicle weight, dry panicle weight and the number of days to 50% flowering. The location significantly affected plant height, fresh bagasse weight, dry leaf weight, juice volume, brix at flowering, fresh panicle weight, dry panicle weight and days to 50% flowering. However, the cultivar type affected stalk weight, fresh bagasse, dry bagasse weight, juice volume, brix during flowering, and the number of days to 50% flowering. Both HA and a combination of season and location (SXL) affected all agronomic characters, such as plant height, stalk weight, fresh bagasse weight, dry bagasse weight, dry leaf weight, juice volume, brix at flowering, fresh panicle weight, dry panicle weight and days to 50% flowering. However, site and cultivar (SXC) only affected dry bagasse weight and juice volume. Similarly, location and cultivar interaction (LXC) had an effect on two parameters, namely dry leaf weight and brix at flowering. The effect of season and HA (SXHA) was evident on dry bagasse weight, juice volume and fresh panicle weight. Location and HA (LXHA) affected plant height, dry bagasse weight, dry leaf weight, juice volume and brix at flowering. Cultivar and HA (CXHA) effect was on dry leaf weight, brix during flowering, and the number of days to 50% flowering. Season, location and cultivar (SXLXC) affected stalk weight, the dry leaf weight and brix during flowering. The effect of season, location and HA (SXLXHA) was on stalk weight, fresh bagasse, dry bagasse, the dry leaf weight, juice volume and days to 50% flowering. However, season, cultivar and HA (SXCXHA) had no effect on any agronomic and juice parameters measured. Location, cultivar and HA (LXCXHA) and site, location, cultivar and HA (SXLXCXHA) only affected days to 50% flowering.

CHAPTER 4: DISCUSSION

In both locations in both seasons one and two, noticeable differences in plant height were observed among cultivars treated with different levels of humic acid. The interaction between varieties and doses of HA differed in growth variables at both Potchefstroom and Glen. A comparison of plant height at Potchefstroom during the first year of planting showed that the three cultivars only varied at 15 kg/ha of HA. These results align with Zhapayev *et al.* (2023), who observed significant variations in plant height of sixteen genotypes of sweet and grain sorghum due to different soil and climatic conditions. Adinurani *et al.* (2020) also confirmed significant differences among genotypes of sweet sorghum at varying altitudes.

The average bagasse yield of all genotypes increased by 27.6 t/ha during the second year of study. Yucel *et al.* (2023) also noted differences in bagasse and silage yield in sweet sorghum between different years, with bagasse quantity being affected by year and genotype interactions. In another experiment, it was found that the addition of humic substances affected bagasse combustion quality, leading to increased mineral and ash concentrations (Nazli *et al.,* 2020). Harshlata *et al.* (2018) reported that continuous addition of nitrogen up to 120 kg N/ha increased dry biomass in sweet sorghum.

However, all cultivars had more dry leaf weight (DLW) at Potchefstroom. Samanhudi *et al.* (2021) observed significant differences in Numbu and Kawali varieties in dry stover weight after assessing the effects of various organic manures on sweet sorghum growth. Conversely, Lestari *et al.* (2021) did not find any differences among sorghum varieties when evaluating grain production and sugar content at different plant densities. Younis Al-Ghazal *et al.* (2023) recorded significant differences between levels of HA and seaweed extract in assessing the effect of bio-fertilizer, HA, and sea algae extract on the growth and yield of Sudan grass in two different locations, likely due to improved soil structure and increased permeability of cell membranes from the addition of HA.

Continuous addition of HA in other treatments and of the same cultivar had no effect. Aml et al. (2021) found no differences between the two hybrids in days to 50% flowering after analyzing humic and fulvic acids applied as a foliar application in grain sorghum. Significant differences between treatment concentrations were only observed in the second season, with cultivar and site being the main factors contributing to these differences. These findings are consistent with those of Galicia-Juarez *et al.* (2022), who observed that genotype affected the production and quality of sweet sorghum juice, biomass, and sugar. Most of the variation was attributed to genotype and interactions regarding fresh biomass and juice yields.

For both high sugar and grain yield, the Hunnigreen cultivar had the highest brix at harvest across both locations. Lestari *et al.* (2021) noted that sugars are stored in the stem due to metabolic and transport processes, resulting in high sugar contents due to the retention of carbohydrates in the stem. However, Nazli *et al.* (2020) contradicted these results, finding that nitrogen and HA applications did not affect the brix of sweet sorghum when assessing the interactive effects of nitrogen and HA applications on bioethanol production. Significant differences were observed comparing different levels of HA at both locations. Maria *et al.* (2021) also noted that juice yield increased with higher nitrogen levels, and that genotype and site variations can impact juice yield in sweet sorghum varieties. ARC SS27 and Hunnigreen are promising varieties across varying HA levels.

The Hunnigreen cultivar consistently showed dominance in juice yield from the first to the second year at both sites, indicating its potential as a promising cultivar. These variations can be linked to nutrient release and absorption by the cultivars during their initial growth year. Research by Galicia-Juarez *et al.* (2022) demonstrated that HA addition increased juice content due to superior absorption efficiency in three evaluated cultivars. Kanbar et al. (2021) also reported notable differences in brix and sugar-related traits in 14 sorghum genotypes.

Dried panicle weight did not exhibit significant variation throughout the two growing seasons at both locations. Hassan Ali Abd et al. (2023) observed differences in grains per panicle due to higher levels of bio-fertilizers, impacting different sorghum genotypes. Additionally, Shen et al. (2024) used potassium humate to enhance yield in foxtail millet. Plant height is a critical trait positively correlated with stalk yield and juice volume, with lower brix levels and reduced panicle weight indicating increased susceptibility to lodging in grain sorghum. Nundwe (2018) observed wide genetic variations in sugar-related traits in sweet sorghum varieties. Chalachew and Rebuma (2018) noted that variations in ethanol yield due to fertilizer applications were likely due to fresh stalk sugar and juice yield. Nundwe (2018) also found that nitrogen and phosphorous fertilizations can increase sugar-related traits except for brix quantity and other agronomic characteristics, leading to increased ethanol yield in sweet sorghum varieties.

Dawood *et al.* (2019) observed increased carbohydrates and seed yield in soybeans with HA addition, which also led to increased sorghum growth. HA treatments, including those under water stress, showed increased growth according to Dawood *et al.* (2023), enhancing metabolic processes and plant resistance to abiotic stresses. HA can enhance cell membrane permeability, water uptake, and nutrient absorption and utilization. In this study, HAs did not affect most sugar levels but significantly increased total sugar content. Makhlouf *et al.* (2022) reported enhancements in various sugar-related traits with HA application, including root length, fresh weights, leaf area, and overall sugar yields per hectare, except for sodium content. de Moura *et al.* (2023) emphasized the role of humic substances (HS) in plant development, including root and leaf growth, mineral uptake, and enzyme regulation. The efficacy of HAs depends on factors like plant type, treated organ, plant age, HA dosage, HA source, and chemical properties.

4.4 References

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CHAPTER 5: CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

The significant differences in results of the sorghum varieties tested could be attributed to the site, agro-morphology characteristics of the varieties, and competition for resources such as nutrients, water, and especially solar radiation among plants. All three varieties examined (Hunnigreen, ARC SS27, and ARC SS76) differed in terms of agronomic, brix, and panicle weight. Hunnigreen was found to be a superior variety, with the highest potential in sugar and grain production among the varieties examined in this study. ARC SS27 and ARC SS76 show promise and could be developed for grain production purposes. This indicates that when there is little or no fertilizer, ARC SS27 outperforms all other cultivars in both locations. The addition of HA allowed Hunnigreen to grow faster than all other cultivars, especially at 5 kg ha^{-1} and 10 kg ha^{-1} of HA, resulting in more stalk weight in both Glen and Potchefstroom.

The Hunnigreen cultivar had the highest brix at harvest and could potentially be used for both sugar and grain production. Over the two seasons at the two sites, there were no significant differences in dry panicle weight. This suggests that despite the different growth and response characteristics of the three varieties evaluated in this study, all the varieties are equally important in terms of dry panicle weight.

This study demonstrates the value of sorghum as a multi-purpose and resilient crop, as well as the potential to increase yields through the use of HA. ARC SS27 had the highest yield without the use of HA, making it a potential variety for soils with low nutrient levels. The use of HA at a rate of 5 kg ha^{-1} to 10 kg ha^{-1} produced the best results over the two years that the three varieties were planted in both Potchefstroom and Glen. In some cases, an increase of up to 15 kg ha^{-1} of HA did not significantly differ from the 10 kg ha^{-1} of HA.

This study highlights the potential use of HA on sweet sorghum and how different varieties respond to increased levels. While promising, the use of HA needs to be validated across diverse agro-ecological environments and soil conditions before widespread recommendations can be made. It is important to assess various types of HA and how they respond under different soil types and with different sweet sorghum varieties. This study serves as an initial foundation for utilizing HA in crop management to enhance yields and resilience of sorghum and other dryland cereals.

5.2 Recommendations

Future studies should examine additional sweet sorghum varieties and different HAs in different areas. Findings from the study would provide information that is useful in the cultivation of this crop in diverse agro-ecological zones.