OPTIMIZATION OF MACRO-PROPAGATION PROTOCOLS FOR THE PRODUCTION OF QUALITY PINEAPPLE PLANTING MATERIALS IN UGNADA

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DECLARATION

I **Borniface Maena**, hereby declare that this is my original work and has never been submitted to any University or Institution of higher learning for an award.

Signed…… Date……

APRROVAL

This is to certify that this work was conducted under our supervision as university supervisors and is now ready for submission for examinations.

Signed……. Date……

Prof. Bosco Bua

Signed……. Date……

Dr. Juliet Akello

DEDICATION

This thesis is dedicated to all people who did not give up on me to finish my master's degree. Especially my academic supervisors, family, and classmates who supported my efforts during the course of my studies. Above all, to the almighty God for the knowledge, wisdom and good health.

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ACRONYMS

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BSFF	Black Soldier Fly Frass
CRD	Completely Randomized Design
DAT	Days After Transplanting
DRW	Dry Root Weight
DSW	Dry Shoot Weight
EC	Electrical Conductivity
FoA	Faculty of Agriculture
g	Grams
IAA	Indole-3-Acetic Acid
K	Potassium
KCCA	Kampala Capital City Authority
kg	Kilograms
L	Litres
N	Nitrogen
NAADS	National Agricultural Advisory Services
NDP	National Planning Authority
NGOs	Non-Governmental Organizations
OM	Organic Matter
P	Phosphorus
PHRD	Pineapple Heart Rot Disease
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
UBOS	Uganda Bureau of Statistics
UCU	Uganda Christian University

ABSTRACT

Pineapple production in Uganda remains constrained by inadequate access to quality planting materials, a key limitation to productivity and sustainability. This study was conducted from December 23rd, 2023, to December 23rd, 2024, at Kyambogo University's Faculty of Agriculture, with the overall objective of optimizing macro-propagation protocols for the production of quality pineapple planting materials. Standard laboratory procedures were employed to assess the suitability of various growth substrates (Black soldier fly frass, composite [sand: soil: vermiculite, mixed in a ratio of 1:1:1], lake sand, black forest soil, and vermiculite) for generating pineapple plantlets. A randomized complete block design (RCBD) was used to determine the effect different substrates and plant parts (crowns, slips, and stems) on plantlet production over a 90-day experimental period under field conditions. Plant parts were longitudinally sectioned into quarters and treated with different chemicals including water (control), IAA-hormone, sodium hypochlorite, and a combination of IAA-hormone and sodium hypochlorite prior to planting in the substrates. Accordingly, a completely randomized design (CRD) was subsequently used to evaluate the growth performance of the three-month-old plantlets generated from different substrates and plant parts over a 120-day experimental period in a screen house. Statistical analyses revealed significant ($p<0.05$) differences among substrates, plant parts, and chemical treatments on plantlet yield and growth parameters. Three-way ANOVA indicated significant interaction effects ($p<0.05$) among substrate type, plant part, and sampling time. Black forest soil exhibited the most favorable physiochemical properties including pH and texture for pineapple production. The highest plantlet yield (3.17) was obtained from stem cuttings raised on soil and composite substrates at 90 days after planting. Crowns raised on black soldier fly frass yielded the least number of plantlets. Plantlets generated from slips grown on soil exhibited superior growth performance across majority of the growth parameters measured throughout the 120-day period. Furthermore, plant parts and treated with sodium hypochlorite, and the resultant plantlets outperformed those treated with other chemicals. Considering overall results, it can be concluded that the use of soil substrate with stems or slips provides an effective and reliable protocol for optimizing the production of quality pineapple planting materials in Uganda. The results also demonstrate that treating plant parts with sodium hypochlorite enhances plantlet initiation during the production phase and improves subsequent growth performance of the generated plantlets.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Pineapple (*Ananus comosus* L. Merill) is a perennial monocotyledonous herbaceous fruit widely cultivated in tropical and subtropical regions in more than eighty (80) countries. Pineapple is one of the most economically significant fruit crops in the *Bromeliaceae* family, ranking third in global production after bananas and citrus fruits (Reinhardt *et al.,* 2019; Li *et al.,* 2022). Pineapple contributes approximately 20% of the total global fresh fruit exports (Ocwa *et al.*, 2016, 2018; Firatoiu *et al.*, 2021; Hikal *et al.,* 2021).

Globally, pineapple production is estimated at 28.65 million metric tons (MT), with Costa Rica (3, 328, 100 MT), the Philippines (2, 747, 856 MT), and Brazil (2, 426, 526 MT) being the top three producers, respectively (Hikal *et al.,* 2021). Over the years, pineapple cultivation has expanded significantly, with production increasing from 12.95 million metric tons, on 633, 942 hectares in 1994 to 28.18 million metric tons on 1, 125, 307 hectares by 2019 (Firatoiu *et al.*, 2021).

In the East African region, the top five pineapple producing countries include Tanzania (454, 008 MT), Kenya (278, 179 MT), the Democratic Republic of Congo (193, 367 MT), Rwanda (36, 272 MT) and South Sudan (6, 500 MT), respectively. Accordingly, Uganda (4, 072 MT) is ranked as the lowest pineapple producing country in the region (Hikal *et al.,* 2021).

1.2 Importance of pineapple

Pineapple is an important fruit consumed globally and is associated with a wide range of benefits. Pineapple is consumed as fresh, preserved (dried/canned) and in cooked form supplying multiple nutrients such as calcium, potassium, carbohydrates, crude fiber, and vitamin C (Ascorbic acid), water and minerals which are necessary in digestion and general body health (Chaudhary *et al.,* 2019; Hikal *et al.,* 2021). The crop is also produced mainly for

fresh fruit markets and processing industries where it is processed into several products including pineapple jam, juice, wine, powder, and vinegar while the resulting wastes are used to produce methane, animal feeds and manure respectively (Chaudhary *et al.,* 2019). Besides, pineapple fruits are a source of bromelain used as a meat tenderizing enzyme (Hossain, 2016; Chaudhary *et al.,* 2019). Pineapple also provides raw materials for making leather used for craft works such as bagging and some varieties can be used as ornamentals in compounds (Chaudhary *et al.,* 2019). Additionally, pineapple is a source of employment to a number of people involved along the pineapple value chain including providing income to farmers as well as revenue to the government when sold both locally and in external markets (Baseke, 2009; Robin *et al.,* 2011; Yekoyada *et al.,* 2021).

1.3 Pineapple production in Uganda

In Uganda, pineapple production is majorly by small holder farmers where most of the production is concentrated around the Lake Victoria basin crescent and south of Lake Kyoga covering the districts of Kayunga, Luwero, Mukono and Masaka as well as western Uganda in the districts of Ntungamo and Kabale (Bonabana-Wabbi et al., 2013; Bua *et al.,* 2013; Tenywa *et al.,* 2018). The major pineapple varieties commonly grown in Uganda include Smooth Cayenne, Queens, Red Spanish, Giant Kew and Abacaxi. However, Smooth Cayenne is by far the most dominant variety grown (Oculi *et al.,* 2019). Farmers typically rely on slips and suckers obtained from the mother plants in the old pineapple gardens to establish new fields (Bua *et al.,* 2013; Reinhardt *et al.,* 2018; Chotangui *et al.,* 2019; Oculi *et al.,* 2019).

1.4 Constraints to pineapple production in Uganda

Despite, its importance, pineapple growing is constrained by various abiotic, biotic, and socio-economic challenges (Bua *et al.,* 2018). Key constraints include limited availability of quality planting materials, high fruit perishability, low market prices, restricted access to credit, land shortage, and inadequate transport infrastructure (Ocwa *et al.,* 2016, 2018; Monthe *et al.,*

2024). These socio-economic factors significantly hinder pineapple production (Bua *et al.,* 2013; Baruwa, 2013). Furthermore, poor market access, compounded by the high perishability of pineapples, forces farmers to sell their fruits at very low prices, sometimes as low as 500 Ugandan Shillings during the peak seasons, leading to reduced profit margins (Ocwa *et al.,* 2018).

In addition to the economic constrains, pineapple production is severely affected by pests and diseases, particularly pineapple heart rot disease (PHRD) and mealybug wilt disease (Bua *et al.,* 2013, 2018; Oculi *et al.,* 2019). In cases of severe infestation, PHRD can result in total crop loss, making it a major limiting factor in regions such as the Lake Victoria Crescent in Uganda (Ocwa *et al.,* 2016; Oculi *et al.,* 2019). Abiotic factors such as drought and soil infertility further exacerbate production challenges, contributing to low yields (Bua *et al.,* 2013; Hotegni *et al.,* 2014; Ocwa *et al.,* 2016).

Moreover, the scarcity of quality planting materials has hindered the commercialization of pineapple farming, particularly among smallholder farmers in sub-Saharan Africa, including Uganda (Baruwa, 2013; Bua *et al.,* 2013; Reinhardt *et al.,* 2017). Addressing these constrains through improved access to quality planting materials, disease management, better market access is crucial for enhancing pineapple production and profitability.

1.5 Statement of the problem

Pineapple production in Uganda plays a vital role in improving household incomes and livelihoods, particularly among small holder farmers (Baseke, 2009; Bonabana-Wabbi *et al.,* 2013; Yekoyada *et al.,* 2021). However, the sector continues to face serious constraints, the most critical being the limited availability of quality planting materials (Bua *et al.,* 2013, 2018; Reinhardt *et al.,* 2017, 2018; Monthe *et al.,* 2024). Pineapple production in Uganda predominantly relies on slips and suckers, whose supply is often inadequate and seasonally

restricted due to the plant's growth cycle (Bua *et al.,* 2013, 2018; Reinhardt *et al.,* 2017, 2018). This scarcity results in high costs of planting materials, thereby limiting farmer's ability to expand production.

Besides, the conventional propagation materials are frequently infested with pests and diseases, including pineapple mealybug wilt and heart rot, which compromise field establishment and yields (Bua *et al.,* 2013, 2018; Oculi *et al.,* 2019; Reinhardt *et al.,* 2018). Although tissue culture technology offers potential for rapid and large-scale multiplication of disease and pest free plantlets, its adoption in Uganda still remains low due to the high costs, specialized skills, and infrastructural requirements involved (Bua *et al.,* 2013; Reinhardt *et al.,* 2018; Kumar *et al.,* 2023). Furthermore, the acclimatization of tissue-cultured plantlets to field conditions has been reported to be a challenge in pineapple production (Reinhardt *et al.,* 2018).

Consequently, the lack of cost-effective, scalable, and farmer-accessible propagation methods has created a persistent bottleneck in the pineapple value chain. Addressing this gap is crucial to enhance the availability of quality planting materials, reduce production costs, and sustain productivity among small holder farmers. This study, therefore, sought to develop and optimize macro-propagation approaches that can improve the availability of affordable and quality pineapple planting materials for Uganda's smallholder farmers and beyond.

1.6 Objectives of the study

1.6.1 General objective

The overall objective of the study was to optimize macro-propagation protocols for the production of quality pineapple planting materials using different substrates and pineapple plant parts.

1.6.2 Specific objectives

The specific objectives of the study were to;

1. Establish the suitability of the different substrates for generating pineapple plantlets.
2. Assess the effect of different substrates and plant parts on the production of pineapple plantlets.
3. Evaluate the performance of pineapple plantlets generated using different substrates and plant parts.

1.7 Hypotheses

1. There is a significant difference in the suitability of the different substrates used for generating pineapple plantlets.
2. Substrate type and plant part used have a significant effect on the number of pineapple plantlets produced.
3. There is variability in the performance of plantlets generated using different substrates and pineapple plant parts.

1.8 Justification of the study

Pineapple farming plays a key role in the livelihoods of many stakeholders along the pineapple value chain, including farmers, processors and traders in Uganda. In fact, pineapple contributes significantly to employment creation and the generation of foreign exchange earnings for the country (Baseke, 2009; Tenywa *et al.,* 2018; Yekoyada *et al.,* 2021).

However, one major challenge in pineapple production is inadequate supply of quality planting materials (Bua *et al.,* 2013, 2018; Hotegni *et al.,* 2014; Olah & Okon, 2022). Yet, the currently available propagation techniques such as tissue culture require high capital investment and specialized expertise, making them costly and inaccessible to many small holder farmers (Reinhardt *et al.,* 2018). As a result, the high-cost of plantlets limits the farmers' ability to

expand production, ultimately affecting household incomes and foreign earnings from pineapple exports.

Invariably, if these challenges are left unaddressed, the livelihoods of many households will continue to be compromised. Therefore, the aim of this study was to investigate alternative, low-cost methods for producing quality pineapple plantlets, ensuring a sustainable pineapple subsector in Uganda.

1.9 Significance of the study

This study will provide useful insights into low-cost techniques for producing quality pineapple planting materials. These findings will serve as a guide for various stakeholders, including government extension officers, policy makers, researchers, academia and non-governmental organizations (NGOs), towards supporting farmers in commercializing pineapple production.

By adopting these techniques, farmers are expected to expand the area under pineapple cultivation, leading to increased production over time. This growth will in turn boost both local and international trade, creating more employment opportunities along the pineapple value chain. Ultimately, there will be an improvement in farmer livelihoods and higher foreign exchange earnings for the country through increased pineapple fruit exports.

CHAPTER TWO: LITERATURE REVIEW

2.1 Pineapple propagation techniques

Pineapple is propagated through a number of ways using both micro and macro techniques across the world. Micropropagation is by tissue culture while macro propagation techniques majorly include the use of slips, suckers, crowns, and stems (Agogbua & Osuji, 2011; Reinhardt *et al.,* 2018). However, like in many other parts of the world, pineapple in most producing districts of Uganda is primarily propagated using slips and suckers. Farmers typically rely on slips and suckers obtained from the mother plants in the old pineapple gardens to establish new fields (Bua *et al.,* 2013, 2018; Reinhardt *et al.,* 2018; Chotangui *et al.,* 2019; Oculi *et al.,* 2019).

The macro-propagation techniques are however, characterized by low proliferation/multiplication rate which limits commercialization of pineapple production(Agogbua & Osuji, 2011; Agogbua & Eremrena, 2017). Besides, these methods are also potential avenues for spreading diseases and pests since infected materials may not show symptoms of disease during planting (Reinhardt *et al.,* 2018). In Uganda, farmers rely on slips and suckers for pineapple propagation, whose availability is limited to the plant's lifecycle (Bua *et al.,* 2013, 2018; Reinhardt *et al.,* 2018; Oculi *et al.,* 2019). This creates seasonal scarcity which results into high cost of planting materials (Reinhardt *et al.,* 2018).

The propagation of pineapple plantlets involves the use of different substrates including soil, sawdust, compost, sand, coir dust, burnt puddy husk, perlite, and vermiculite among others to generate planting materials stems and crowns (Ranawana & Eeswara, 2008; Reinhardt *et al.,* 2018; Chotangui *et al.,* 2019). However, the number of pineapple plantlets generated depends on a number of factors including varieties of the pineapple, size, weight of plant parts, and substrates used among others (Agogbua & Osuji, 2011; Shiyam, 2016; Chotangui *et al.,* 2019).

2.2 Substrates and growth requirements for pineapple propagation

Pineapple plant prefers well-draining substrates for their growth and production (Hossain, 2016). According to Magdoff & Van Es (2021), substrates that are sandy-loam in texture possess good aeration qualities which is required for plant root development but also reduces the risks to plant loss due to fungal diseases. In fact, soils with a texture of 15% - 35% clay and more than 15% sand, and those with a texture of 15% clay and more than 70% sand, drain well and with a good content of organic matter to improve their water holding capacity are recommended for pineapple production (Chotangui *et al.,* 2019; Jalil *et al.,* 2021; Magdoff & Van Es, 2021). However, silt soils with less than 35% clay and 15% sand are generally not good for pineapple growth due to their poor structural characteristics that affect aeration and drainage. A soil depth of 60 cm to 100 cm is appropriate for pineapple growth since the roots are concentrated within 15–20 cm of soil depth. According to Reinhardt *et al.* (2018), pineapple thrives well in acidic soils with pH ranging between 4.5 – 5.5 which is considered convenient for nutrient uptake necessary for growth and development.

Pineapple requires a number of nutrients including macro (nitrogen [N], Phosphorus [P], Potassium [K], Calcium [Ca], Magnesium [Mg]) and Sulfur [S]), and micro (Boron [B], Copper [Cu], Iron [Fe], Manganese [Mn], Molybdenum [Mo] and Zinc [Zn]) for their growth and development (Omotoso & Akinrinde, 2012; Mawiyah *et al.,* 2018). Nutrients play specific functions in pineapple growth and development and especially nitrogen, potassium and phosphorus need to be supplied in quantities that are sufficient enough for pineapple growth and development. According to Angeles *et al.* (1990), the critical or lower limits of sufficiency for pineapple in relation NPK range between 1.0% – 1.7%, 0.08% – 0.23% and 1.8% – 4.2% respectively.

However, potassium and nitrogen are respectively are required in high amounts for proper pineapple growth and productivity. Indeed, potassium is considered the number one nutrient required in high amounts for pineapple growth and productivity (Prajapati *et al.,* 2012). Potassium plays vital roles in the plant's growth including transportation of water and minerals in the plant, stomatal activity, enzyme activation and regulation of photosynthesis since it is involved in Adenosine Triphosphate (ATP) which is the primary energy carrier in cellular processes, playing a crucial role in plant metabolism. According to Leghari *et al.,* (2016), nitrogen improves the photosynthetic processes which enhance plant growth and development. Accordingly, the resulting high plant biomass supported by nitrogen availability contributes to improved productivity of the crop. Although demanded less by pineapple compared to other macro nutrients, phosphorus deficiency is expected to compromise growth and development due to poor plant root quality, consequently affecting plant establishment and growth (Duane *et al.,* 2012; Jalil *et al.,* 2021).

2.4 Nutrient composition of the substrates and effects on propagation of pineapple plantlets.

Different substrates or growth media possess a range of nutrients required for pineapple propagation. According to Jalil *et al.,* (2021), N, P, K and other nutrient availability from different substrates varies significantly depending on the source and nature of the substrates. The common substrates or growth media used in the propagation of pineapple include compost, sand, soil, vermiculite, cocopeat, peatmoss sawdust, and rice husks, among others. In fact, these substrates are also known to vary in the nutrient composition and levels which significantly affect plant growth parameters such as number of leaves, leaf length and width, plant height, stem girth and finally the yield (Chotangui *et al.,* 2019; Jalil *et al.,* 2021).

2.5 Pineapple plantlet quality and performance

The performance of pineapple plantlets is evaluated using a number of parameters that help to explain the effect of the different treatments including the substrates used in their production (Ranawana & Eeswara, 2008; Chotangui *et al.,* 2019). The widely used parameters for evaluating pineapple plant performance include plant height, the number of leaves, the leaf area, dry shoot and root weights among others. This because they influence the plant's growth performance and subsequently yield attained at the end of the plant's growth cycle (Chotangui *et al.,* 2019; Jalil *et al.,* 2021). For example, the number of leaves is generally associated with an increase in photosynthetic activity which improves plant vigor and yield, whereas leaf area determines the amount of photo-assimilates produced by the plant which significantly affects their growth, development and productivity (Takoutsing *et al.,* 2014; Jalil *et al.,* 2021).

. Also, plant height plays an important role in plant survival and development especially after transplanting and during growth (Takoutsing *et al.,* 2014; Jalil *et al.,* 2021).

CHAPTER THREE

SUITABILITY OF THE DIFFERENT SUBSTRATES FOR GENERATING PINEAPPLE PLANTLETS

3.1. Introduction

Successful propagation of pineapple plantlets is a prerequisite for ensuring good crop establishment and productivity, as well as promoting sustainable production of the crop globally including Uganda (Monthe *et al.,* 2024) . Accordingly, the nutrient composition of the substrates used in pineapple propagation is considered as the key factor influencing pineapple growth and development. In fact, the different substrates serve as the primary medium for root establishment and growth through which plant nutrients and water are channeled for overall plant health (Chotangui *et al.,* 2019).

However, the nutrients needed for crop growth and development are significantly influenced by a number of factors including the physiochemical properties such as pH, organic matter content (OM), electrical conductivity (EC) and texture of the different substrates (Iersel, 2020). These properties affect nutrient availability, substrate aeration, water infiltration and hence directly affecting plant performance (Magdoff & Van Es, 2021). Accordingly, the nutrient composition of the different substrates used for pineapple propagation, especially Nitrogen (N), Phosphorus (P), Potassium (K), among other macro and micronutrients ultimately affects the growth and vigor of pineapple plantlets (Angeles *et al.,* 1990; Chotangui *et al.*, 2019; Magdoff & Van Es, 2021). Hence, understanding the physiochemical properties including pH, N, P, K, OM, EC, and texture of the different substrates is mandatory in order to optimize the conditions for propagating pineapple plantlets. Therefore, the objective of this study, was to establish the suitability of the substrates used for generating pineapple plantlets.

3.2. Materials and Methods

3.2.1. Study area

The study was conducted at Kyambogo University from December, 2023 to December, 2024. Kyambogo University is located eight (8) kilometres from Kampala City Center along the Kampala-Jinja highway. Kyambogo University lies between 00º20’54” N and 32º37’49” E (Oculi *et al.,* 2019). The University is found within Nakawa division, one of the five administrative divisions of Kamapala Capital City Authority (KCCA) in central Uganda at an elevation of 1189 metres above sea level and with predominantly sandy loam soils (Katuromunda *et al.,* 2021). The area experiences a tropical rain forest climate with two annual wet seasons between February to June, and August to December respectively. The average annual rainfall ranges between 63.0 millimetres to 169.3 millimetres with average rainy days ranging between 4.7 days in July and 12.2 days in April. The average monthly relative humidity and mean monthly sunshine hours recorded for the area varies from 66-80.5% and 120-186, respectively. The area records a daily mean temperature of 22.0ºC as the lowest and 23.7ºC as the highest (UBOS, 2016; Khan, 2022) .

3.2.2. Sample collection

The materials used in the study were sourced from different places within Central Uganda. For example, the Black soldier fly frass (BSFF) was sourced from Ento Organic Farm at Makerere University Agricultural Research Institute Kabanyolo in Gayaza, Wakiso district, while lake sand was obtained from local sand traders in Ntinda, Nakawa division, Kampala Capital City. The black forest soil was collected from a tree plantation in Namugongo, Wakiso district, whereas vermiculite was supplied by Ecolite Minerals Uganda Limited, through its agents in Container village, Kampala Capital City. The composite substrate was prepared by mixing sand, soil, and vermiculite in a ratio of 1:1:1 (Rathnayake *et al.,* 2015; Das & Jha, 2018) .

3.2.3 Sample preparation

One sample from each of the five substrates including black soldier fly frass (BSFF), composite, sand, soil, and vermiculite were packed in clean, well labelled polythene bags and delivered to the laboratory to establish their suitability for pineapple propagation. In the laboratory, the samples were ground using a mortar and pestle, and sieved through a 2-milimeter (mm) diameter sieve. The samples were subjected to analysis for pH, NPK, EC, OM, and texture using standard testing procedures/methods (Okalebo *et al.*, 2002).

3.2.4 Determination of pH and Electrical Conductivity of the substrates

The soil water-suspension method (1:1 ratio) was used in determining both the pH and Electrical conductivity (EC) of the substrates. This involved weighing 10 g of well ground, air-dried, and sieved substrate into a clean beaker followed by addition of 10 ml of distilled water (Okalebo *et al.*, 2002) . The mixture (1 part substrate: 1 part water) was thoroughly stirred using a clean spatula for 30 seconds. The suspension was left to settle for 30 minutes with occasional stirring after every 10 minutes. Thereafter, the pH and EC were measured using a multiparameter pocket sensor/reader (serial number: BEOJCIOALOOHN8KBXFL). Prior to taking the readings, the multiparameter pocket sensor was calibrated using standard buffer solutions.

For the sensor preparation and calibration, the electrode of the sensor was rinsed with distilled water and gently dried using a clean laboratory towel and then calibrated for pH and EC. A buffer solution of pH 7.00 was used to calibrate the sensor for pH measurement whereby the electrode was immersed in the buffer solution and left to stabilize to a reading of pH 7.00. To standardize the sensor for EC measurement, an EC standard solution (1413µS/cm) was used to calibrate the sensor by immersing the rinsed electrode inside the standard solution until the reading of 1413µS/cm was stable.

After calibration, the pH electrode sensor was inserted into the substrate-water suspension and stirred gently until the solution was stable, before pH value was recorded. After the pH value was obtained, the pH sensor was rinsed with distilled water followed by inserting the EC sensor in the suspension to obtain the corresponding EC value for the substrate following the same procedure.

3.2.5 Determination of organic matter content

Organic matter of the different substrates was determined using the Weight Loss on Ignition (LOI) method (Okalebo *et al.,* 2002; Robertson, 2011). The procedure involved cleaning and drying crucibles inside a drying oven that was set at 105^{o}C for an hour to ensure complete drying. After drying, the crucibles were cooled in a desiccator and then its weight recorded (W_1). Ten (10) grams of each substrate was added into the crucible and then the weight of the crucible plus the substrate was recorded (W_2). The crucible containing the substrate was placed in the oven set at 105^{o}C to dry the substrate for 24 hours, after which, it was cooled in the desiccator and its weight (Crucible + dry substrate) was recorded (W_3). The crucible containing the dry substrate was then placed into a muffle furnace, heated at 550^{o}C for five (5) hours to burn off the organic matter in the substrate. The crucible with its content (ash) was allowed to cool in the furnace at 200^{o}C, transferred to the desiccator to cool at room temperature and then weight (W_4) determined.

The organic matter of the substrates was calculated following the formular adopted by Robertson, (2011) as stated below;

$$\text{Soil organic matter (\%)} = \frac{\text{pre-ignition weight (g)} - \text{post-ignition weight (g)}}{\text{pre-ignition weight (g)}} \times 100$$

Where; Pre-ignition weight (g) = $W_3 - W_1$ and post-ignition weight (g) = $W_4 - W_1$

W_1 = weight of the crucibles**,** W_2 = Weight of crucible + fresh substrate, W_3 = Weight of crucible + dry substrate, and W_4 = Weight of crucible + ash.

3.2.6 Determination of substrate texture

The texture of the substrates was determined by the Bouyocous (hydrometer method), which measures the percentage of sand, silt and clay based on Stroke's law governing the rate of sedimentation of particles when suspended in water (Okalebo *et al.,* 2002).

Fifty grams (50g) of air-dried substrate sieved through a 2 mm diameter sieve was weighed and transferred to a dispersing cup. Thereafter, 100 ml of 5% dispersing solution (50 g of sodium hexametaphosphate ($Na_6(PO3)_6$/L of deionized water) was added and the dispersing cup was attached to an electric mixer, followed by mixing the sample for 60 seconds. The suspension was transferred from the dispersing cup to 1000 ml graduated cylinder and then filled to the 1000 ml mark with deionized water that was equilibrated to room temperature. The suspension was carefully stirred for 30 seconds until a uniform suspension was obtained. The hydrometer was gently inserted into the suspension and after 40 seconds, the hydrometer reading was recorded indicating the amount of silt plus clay that was suspended in the solution. Another hydrometer reading was recorded after nearly 7 hours (6 hours, and 52 minutes) to quantify the amount of clay in the suspension.

Temperature and density corrections were made by adding 0.2 unit to the readings of the samples every 1^oF above 68^oF, and subtracting 0.2 unit for every 1^oF below 68^oF, and subtracting the density of the blank at each reading from the corresponding density readings for the samples respectively. To determine the texture of the substrates (percentage sand, silt and clay particles), the following calculations were accordingly performed;

$$\text{Percent clay (\% clay)} = \frac{\text{corrected hydrometer reading at 6 hours, 52 minutes} \times 100}{\text{Weight of sample}}$$

Percent silt (% silt) = $\frac{\text{corrected hydrometer reading at 40 seconds} \times 100}{\text{Weight of sample}}$ - % Clay

Percent sand (% sand) = 100% - % silt - % clay

3.2.7 Determination of nutrient composition of the substrates used

3.2.7.1 Preparation of test solution

One gram (1.0g) of air-dried sample was weighed into a conical flask and moistened with 10 drops of distilled water, followed by addition of 4 ml of concentrated sulfuric acid and 10 drops of soil total nitrogen oxidizer (Okalebo *et al.,* 2002). The content of the flask was covered with a small curved funnel, placed on and electric stove with an asbestos wire mesh, and heated on a low fire until a large amount of white fumes swirl around the mouth of the flask for five (5) minutes. The content of the flask was left to cool slightly before transferring to a 100 ml volumetric flask, followed by addition of distilled water to make up the volume. The content of the flask was shaken thoroughly using a rotary shaker and left to stand. Two milliliters (2.0 ml) of the supernatant were pipetted into 100 ml volumetric flask followed by addition of 2.0 ml of soil total nitrogen regulator (1:1 NaOH solution). The content of the flask was shaken thoroughly using a rotary shaker followed by addition of distilled water to make up the volume of the 100 ml volumetric flask. The content was then filtered using a Whatman No. 42 filter paper to obtain the test solution.

3.2.7.2 Total Nitrogen

Total nitrogen content of the extracts from individual substrates was determined following Kjeldahl method using the highly intelligent soil nutrient detector (HM-GT2). Two milliliters (2.0 ml) each of: (i) soil test solution, (ii) distilled water containing 1 drop of soil standard stock solution, and (iii) blank solution were pipetted and placed into three separate test tubes. In each test-tube, 4 drops of soil ammonium nitrogen reagent were added using a pipette. The content of the test tubes was shaken thoroughly using a tube shaker for 10 minutes, before transferring

them to separate cuvettes. The blank, standard, and soil test solutions contained in the cuvettes were placed in the first, second and third channels of the HM-GT2 highly intelligent nutrient detector machine, respectively and total nitrogen content of the substrate samples determined by clicking the N detection button. The total nitrogen content, measured in g/kg was read, recorded and later converted to percentage by multiplying the value by 0.1, as shown below;

N% = N value g/kg × 0.1

3.2.7.3 Total Phosphorus

The total phosphorus content of each substrate was determined by following Bray 1 and 2 procedures using a highly intelligent HM-GT2 nutrient detector. Prior to quantifying the level of Phosphorus in the different samples, three milliliters (3 ml) of distilled water, 2 ml of leaching agent + 1 drop of soil standard stock solution, and 2 ml of soil test solution were pipetted into three small test tubes named 1, 2, and 3, respectively. Four (4) drops of soil available phosphorus reagent were added to test tube one (1) and two (2), and one (1) drop in test tube three (3). The content of the test tubes was shaken thoroughly using a test tube shaker, and left to stand for 10 minutes before transferring it to the cuvettes for determination of phosphorus. The option for "Soil Available Phosphorus" was clicked on the HM-GT2 machine, then the blank, standard and test solutions contained in the cuvettes were respectively placed in channel 1, 2 and 3 of the HM-GT2 machine, followed by clicking of the test button. A value displayed in the corresponding test solution channel was recorded as the soil available phosphorus (g/kg) in the substrate (s). The total phosphorus content (g/kg) was converted to percentage by multiplying the value by 0.1.

3.2.7.4 Total Potassium

For Potassium determination, the Sodium Tetraphenylborate turbidity method was followed using procedures described in the HM-GT2 nutrient detector manual. Three milliliters (3 ml)

of distilled water, 2 ml of potassium extractant + 1 drop of soil standard stock solution, and 2 ml of soil test solution were pipetted into three small test tubes and then 4 drops of soil available potassium reagent were added to test tube 1 and 2. The content of the test tubes were shaken thoroughly using a test tube shaker before transferring to the cuvettes for measurement of available potassium. The "soil available potassium" option was selected on the screen of the highly intelligent HM-GT nutrient detector machine, followed by placing the blank, standard and soil test solutions in slots 1, 2, and 3 respectively. Upon clicking the test button, a value corresponding to the test solution slot was displayed, which indicated the soil available potassium (g/kg). The displayed value was recorded in the data sheet and then multiplied by 0.1 to convert to percentage total potassium of the substrates.

3.3 Data analysis

The results of total nitrogen, phosphorus and potassium per substrate obtained in g/kg were manually converted to percentage (%) by multiplying the results obtained in g/kg with 0.1 using a calculator. The formula for conversion of the results to percentage was: 1 g/kg = 0.1 percent. Thereafter, Analysis of Variance (ANOVA) was conducted for all the parameters. Prior to running ANOVA, the datasets were tested for normality and variance homogeneity (Burt & Burt, 2014). Where the assumptions for ANOVA were not met, the datasets were transformed. Thus, datasets for EC and sand were untransformed. Whereas, datasets for silt and clay were square root transformed while for pH, N, P, K, and OM were log-transformed. Differences in the level of the physiochemical properties of the substrates were compared using Turkey's Studentized range tests at 5% level. All the datasets were analyzed using the SAS statistical software (Version 9.4).

3.5 Results

The physicochemical properties of the various substrates analyzed are summarized in Table 1 and 2. The pH values were statistically different across substrates (p <0.0001). BSFF registered the highest pH followed by vermiculite and these were significantly higher than pH for the other substrates. The lowest pH was recorded in the soil substrate. Overall, pH of the substrates ranged from 5.5 to 8.8 (Table 1). Similarly, the Electrical conductivity (EC) varied significantly across substrates(p<0.0001). The highest and lowest EC of 755 µS/cm and 36.5µS/cm was recorded in BSFF and composite, respectively. The OM content of the substrates was significantly different across all substrates (p<0.0001). The highest and the lowest OM was recorded from BSFF and sand, respectively (Table 1). The texture of the substrates was significantly different (p<0.0001). The highest amount of clay particles was observed in the composite substate while sand exhibited the highest proportion of sand particles. BSFF and vermiculite were respectively siltier compared to other substrates (Table 2). The total nitrogen content of the substrates was statistically different (p<0.0001), and fell between 0.08% and 0.55%. BSFF exhibited the highest nitrogen content compared to the rest of the substrates. Similarly, the total phosphorus content of the substrates varied significantly (p<0.0001), with the highest content of total phosphorus being recorded in the composite substrate. Also, the total potassium (K) content was significantly different across all substrates (Table 1). The highest potassium content was recorded in the BSFF substrate while the lowest was in the composite substrate. Overall, the black forest soil substrate exhibited favorable conditions (pH and texture) for pineapple production.

Table 1: Chemical composition of the different substrates used in generating pineapple plantlets at Kyambogo University, 2023-2024.

Substrate	pH	N %	P %	K %	OM %	EC (µS/cm)
BSFF	8.8 [a]	0.55 [a]	0.48 [b]	1.32 [a]	34.25 [a]	750.0 [a]
Soil	5.5 [e]	0.24 [b]	0.43 [d]	0.20 [d]	4.77 [b]	220.0 [b]
Vermiculite	7.0 [b]	0.14 [d]	0.38 [e]	0.26 [c]	1.57 [d]	90.0 [c]
Sand	6.7 [d]	0.09 [e]	0.45 [c]	0.73 [b]	0.49 [e]	51.0 [d]
Composite	6.7 [c]	0.15 [c]	0.62 [a]	0.06 [e]	1.85 [c]	36.5 [e]

BSFF represents Black Soldier Fly Frass.
Composite represents substrate containing sand, soil, and vermiculite in a ratio of 1:1:1.
For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

Table 2: Textual properties of the different substrates used in generating pineapple plantlets at Kyambogo University, 2023-2024.

Substrate	Clay	Sand	Silt	Classification
BSFF	3.84 [d]	0.0 [e]	96.1 [a]	Silty
Soil	14.9 [b]	56.7 [c]	28.4 [c]	Sandy loam
Vermiculite	0 [e]	10.0 [d]	90.0 [b]	Silty
Sand	4.0 [c]	94.8 [a]	1.2 [e]	Sandy
Composite	18.16 [a]	59.3 [b]	22.6 [d]	Sandy loam

BSFF represents black soldier fly frass.
Composite represents substrate containing sand, soil, and vermiculite in a ratio of 1:1:1.
For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

3.6 Discussion

The findings of this study indicate that the physiochemical properties of the substrates varied significantly, influencing the potential for successful pineapple establishment and growth. Notably, the pH levels of the substrates varied from moderate acidity to slightly alkaline conditions. Yet, pineapple typically thrives in slightly acidic environment where the pH values range of 4.5 to 6.5, respectively (Reinhardt *et al.*, 2018; Ajema & Tafa, 2020). The alkaline pH

observed in the BSFF may therefore present unfavorable conditions for the optimal pineapple growth and development. Electrical conductivity (EC) also varied significantly among substrates, with the highest and lowest levels recorded in BSFF and composite, respectively. EC reflects the concentration of ions in a solution, thereby influencing key soil properties such as nutrient availability, osmotic pressure balance, and plant performance (Zhang & Wienhold, 2002). Elevated EC can hinder nutrient uptake due to osmotic stress, while low EC may indicate nutrient deficiency, both of which can negatively impact plant health (Iersel, 2020). Thus, the variation in EC may have a substantial effect on the number of pineapple plantlets produced and their overall growth performance. The organic matter (OM) content across substrates varied widely from 0.49% in sand to 34.25% in BSFF, respectively. Organic matter is essential for improving soil structure, water retention, microbial activity, and nutrient cycling, factors that are crucial during plant establishment (Moyin-Jesu, 2018; Chotangui *et al.,* 2019). The comparatively low OM levels in composite (1.85%), vermiculite (1.57%), and lake sand (0.49%), suggest that these substrates may not adequately support pineapple growth relative to BSFF and black forest soil (Magdoff & Van Es, 2021). Additionally, the texture of the substrates may further influence suitability. Sandy loam texture favors pineapple growth due to its inherent favorable aeration and water infiltration properties, which support root development (Chotangui *et al.,* 2019; Jalil *et al.,* 2021). Conversely, structurally poor sandy and silty substrates may hinder root establishment and overall plant performance. In terms of nutrient composition, the measured concentrations ranged from 0.09 – 0.55% for nitrogen (N), 0.28 – 0.62% for phosphorus (P), and 0.06 – 1.32% for potassium (K). These values were compared against normal thresholds of 1.0 – 1.7% for N, 0.08 – 0.23% for P, and 1.8 – 4.2% for K (Angeles *et al.,* 1990). Phosphorus supports root and shoots development, consequently enhancing overall plant vigor (Azman *et al.,* 2023). While phosphorus levels exceeded the normal range, both nitrogen and potassium were below the required thresholds. These

deficiencies are likely to result in suboptimal conditions for pineapple growth due to their significant roles in photosynthesis, enzyme activity, protein synthesis and water transport (Prajapati *et al.*, 2012; Leghari *et al.*, 2016).

CHAPTER FOUR

EFFECT OF DIFFERENT SUBSTRATES AND PLANT PARTS ON THE GENERATION OF PINEAPPLE PLANTLETS

4.1 Introduction

Pineapple is commonly propagated through vegetative means using various plant parts including crowns, slips, suckers, and stems among others (Reinhardt *et al.*, 2018). However, the success and efficiency of plantlet generation is dependent on a number of factors such as the type of substrates, propagating conditions, phenology and the specific plant parts used (Agogbua & Osuji, 2011; Shiyam, 2016; Chotangui *et al.*, 2019). Therefore, optimizing such factors for pineapple plantlet propagation is critical in enhancing success rates, minimizing production costs and generating uniform plant growth. In fact, these substrates provide adequate support, water and nutrients for plantlet development and survival. Moreover, these substrates affect the regeneration rate of different pineapple plant parts, hence the ability and overall plantlet production efficiency (Agogbua & Osuji, 2011; Omotoso & Akinrinde, 2012; Omotoso, 2014; Shiyam, 2016; Chotangui *et al.*, 2019). Therefore, the objective of this study was to assess the effect of the different substrates on the regenerative capacity of various pineapple plant parts for mass production of quality plantlets.

4.2 Materials and methods

4.2.1 Study area

The study was conducted twice from 23rd December, 2023 to 23rd March, 2024 and from 23rd April, 2024 to 23rd July, 2024 at the Faculty of Agriculture (FoA) experimental field, at Kyambogo University main campus. Details of the location and climatic conditions of Kyambogo University are presented in Chapter three (Section 3.2.1).

4.2.2 Experimental design

The experimental design was a randomized complete block design (RCBD) with three replicates as blocks (Plate 1). Five different substrates (BSFF, composite, sand, soil, and vermiculite), three pineapple plant parts (stem, slip, and crown), and four chemical treatments (sodium hypochlorite [Jik], a combination of sodium hypochlorite [Jik] and rooting hormone [Indole-3-Acetic Acid - IAA], IAA, and control[water]) were used, giving a total of sixty (60) treatments for this study. The different substrates served as the whole plots while plant parts were the sub-plots. Wooden planters/propagation boxes measuring (3.6 × 1.2) metres were constructed each consisting of twelve compartments measuring (0.6 × 0.6 × 0.45) metres. The bottom of the propagation boxes was fitted with black polythene to prevent the substrates from being contaminated with soils and other foreign materials from the ground. The propagation boxes were arranged at a spacing of 0.5m in between each other with temporary shade structure made out of wooden poles and the roof covered with transparent iron sheets constructed to a height of 2 metres above the experiment to prevent rain droplets from hitting the experimental setup. The response variable was the number of plantlets obtained from each experimental unit.

Plate 1: Experimental layout during assessment of the regeneration capacity of the plant parts using different substrates at Kyambogo University, 2023-2024.

4.2.3 Collection of pineapple planting materials

The different pineapple plant parts used in the experiment namely stems, slips, and crowns of Smooth Cayenne, the most commonly cultivated variety were obtained from farmer fields in Kangulumira sub county, one of the major pineapples growing community in Kayunga district. Kayunga district is found in central Uganda, located between 00º42'09N and 32º53'20" E about 58 kilometres from Kampala by road (Jumba & Freyer, 2016). The pineapples were seventeen months old pineapple garden (Plate 2). The seventeen (17) month-old pineapple growth stage was preferred because of the balanced food reserves and phytohormones necessary for plantlet initiation and development (Chotangui *et al.,* 2019). Slips and stems were obtained from healthy, pest and disease-free, and actively growing pineapple plants for use in generating pineapple plantlets.

Plate 2: Collection of pineapple plant parts from Kayunga, 2023-2024.

4.2.4 Preparation and treatment of plant parts

Stems were prepared by removing all the dry and fresh leaves, and roots using a knife. The defoliated stems were then gently washed in running tap water to remove the dirt before

longitudinally splitting them into four sections of 8 centimetres in length (Ranawana & Eeswara, 2008). Slips and crowns were partially defoliated to reduce on the fresh as well as dry leaves at the base before longitudinally splitting them into four sections/quarters. The sections were washed in running tap water as a phytosanitary measure to prevent any possible contamination that would compromise the quality of plantlets generated. These methods were adopted with minimal modifications of the procedures used in previous studies (Ranawana & Eeswara, 2009; Agogbua & Osuji, 2011; Chotangui *et al.,* 2019).

All the prepared plant part sections were subjected to different chemical treatments namely; 3% sodium hypochlorite (JIK), rooting powder (HOMORIL 6 [IAA]), a combination of 3% sodium hypochlorite and IAA, and water (control), respectively (Acheampong *et al.,* 2015). Those treated with 3% sodium hypochlorite were soaked for 5 minutes, while those treated with IAA were gently dipped in the powder until they were coated evenly. Excess powder was dusted off the plant parts by slight finger taping. Those treated with water were soaked for five (5) minutes before planting in the substrates, while those treated with a combination of 3% sodium hypochlorite and IAA-hormone were first soaked in sodium hypochlorite for five (5) followed by gently dipping them in IAA-hormone as above.

4.2.5 Substrates

Five substrates indicated in section 4.2.2 above were used in this study for both trial one and two to determine their effect on the production of treated pineapple plantlets. These substrates were selected due to the ease of accessibility to farmers, affordability and usability as media in growing or production of seedlings of other crops elsewhere in the world (Ranawana & Eeswara, 2008; Chotangui *et al.,* 2019; Lopes *et al.,* 2022).

4.2.6 Preparation of substrates

The substrates were sterilized by steaming using a drum for 30 minutes before the setting up of each trial (Plate 3). Steaming substrates before use eliminates disease pathogens, pests, and weed seeds, hence reducing disease risk and contamination that would compromise the quality of generated plantlets. This creates a clean environment that promotes healthy root development and uniform growth of planting materials (Venturieri *et al.,* 2013; Oculi *et al.*, 2019). The sterilization process involved loading small portions of each substrate (25 kg) in porous sacks and placing them above boiling water to allow the steam go through the substrates and eventually sterilize them. The sterilized substrates were then allowed to cool before placing them in the wooden propagation boxes to provide medium for generating pineapple plantlets.

Plate 3: Sterilizing substrates by steaming at Kyambogo University, 2023-2024.

4.2.7 Planting and management

The different compartments of the propagators were filled with each of the substrate up to three-quarters of their depth. Each of the substrate was then moistened and allowed to stabilize for thirty (30) minutes (Chotangui *et al.,* 2019). Each compartment was planted with four sections of plant parts depending on the different treatment described in section 4.2.4 above. Thus, based on the treatments and number of replicates, a total of 720 treated plant parts were planted across the entire experiment. Stem sections were planted horizontally with the cut side facing down into the substrate, while slips and crown sections were planted upright (vertically), with the surrounding substrate firmly pressed around them to secure the plant parts. Finally, the wooden propagation boxes were covered with a 2-millimeter-thick ultra-violate (UV) polythene to create a conducive microclimate for initiation of plantlet emergence (Chotangui *et al.,* 2019). The UV-polythene covering was opened every 4-5 days for 3 hours to release the accumulated heat and humidity from the propagation boxes (Chotangui *et al.,* 2019) (Plate 4). The experiment was maintained through regular watering as and when necessary, manual weeding by hand-pulling, and pest control particularly targeting termites and mites. During the final two weeks of the three months trial, the UV-polythene was removed to facilitate the hardening off of the developed plantlets prior to transplanting (Plate 4). The methods described in this section were adopted and slightly modified following the procedures used by (Chotangui *et al.,* 2019).

Plate 4: Pineapple plantlets generated from different plant parts using various substrates at Kyambogo University, 2023-2024.

4.3 Data collection and analysis

Data collection was undertaken at 30, 60, and 90 days after planting (Chotangui *et al.*, 2019). The number of plantlets that had emerged were counted from each compartment per replicate and recorded on data sheets using a pencil. The recorded data were then entered into Microsoft excel, cleaned, and subsequently imported into the Statistical Analysis System (SAS – Version 9.4) software for analysis. Levene's statistical test was performed to assess whether the variances of the groups were equal while Shapiro-Wilk and Kolmogorov-Smirnov (KS) tests were used to determine if the data set was normally distributed (Burt & Burt, 2014). A non-significant *p*-value resulting from Levene's test suggests homogenous variance. Similarly, for a normally distributed data set, a higher *p*-value above the 5% significance level resulting from

both tests signified normal distribution (Rao & Richard, 2012). Data were subjected to ANOVA to test for the significance of substrates and plant parts. Where significant differences were detected, means were separated using Tukeys Studentized Range test at 5% level of significance.

4.4 Results

Overall, a total of 1075 plantlets were generated from the two experiments over the 90-day assessment period. The average number of plantlets generated per substrate varied from 0.22 to 3.17 plantlets depending on the trial. Overall, significantly higher number of plantlets were generated in trial one (3.17) than in trial two (2.42) ($p<0.05$). In both trials 1 and 2, results of three-way ANOVA revealed that the number of plantlets generated was significantly affected by the substrate, plant part used and sampling time ($p<0.0001$), but not by the chemical treatment ($p>0.05$). Similarly, results of a three-way ANOVA revealed that there was a significant effect ($p<0.05$) of interaction of substrate and plant parts, substrate and chemical treatment, substrate and sampling time, and plant part and sampling on number of plantlets generated in trial one. For trial two, significant ($p<0.05$) interaction effects of substrate and plant parts, substrate and sampling time, plant part and sampling time, and substrate, plant part and sampling time were observed. In contrast, interactions of chemical treatment and time, substrate, plant part, and chemical treatment, and plant part, chemical treatment and sampling time did not significantly affect ($p>0.05$) plantlet yield in experimental trial one. Relatedly, no significant effect ($p>0.05$) was observed on interactions of substrate and chemical treatment, plant part and chemical treatment, chemical treatment and time, and plant part, chemical treatment and sampling time during experimental trial two. Thus, under each trial, the data sets were analyzed under the different substrates.

4.4.1 Interaction effects of different substrates on the production of pineapple plantlets

4.4.1.1 Black soldier fly frass

In trial one, the number of plantlets generated was significantly affected by plant part (F=24.88, df=2, p<0.0001), chemical treatment (F=8.08, df = 3, p<0.0001), and sampling time (F=6.24, df = 2, p=0.003). Slips produced a higher number of plantlets than other plant parts while treating plant parts with sodium hypochlorite or IAA-hormone produced a highly significant number of plantlets than the combined or control chemical treatments (Table 3). On the other hand, there was no statistical difference in the number of plantlets obtained at 30 and 60 days after planting, but these were significantly lower than the number of plantlets obtained at 90 days after planting. Treating pineapple crowns with sodium hypochlorite produced a significantly higher number of plantlets than the other treatments, while stems treated with IAA-hormone yielded more plantlets than all other treatments. Additionally, significant effects were observed on the number of plantlets derived due to interactions of plant part used and chemical treatment (F=2.73, df = 6, p=0.0181). In contrast, there were no significant effects between interactions of plant part and sampling time (p=0.0833), and chemical treatment and sampling time (p=0.6257). In trial two, the number of plantlets was significantly affected by plant part used (F=9.17, df =2, p=0.0003), and sampling time (F=3.88, df=2, p=0.0244). Slips produced a higher number of plantlets than other plant parts with sodium hypochlorite or IAA-hormone (Table 3). Similarly, there was no statistical difference in the number of plantlets generated at 30 and 60 days after planting, though they were significantly lower than those obtained at 90 days after planting. Pineapple crowns and stems treated with control (water) yielded more plantlets compared to other chemical treatments. Conversely, chemical treatment alone did not significantly affect plantlet yield during trial two (p=0.1435). Also, significant effects were observed between interactions of plant part used and chemical treatments (F=4.26,

df=6, p=0.0009). However, no significant effects were observed between interactions of plant part and sampling time (p=0.5937), and chemical treatment and sampling time (p=0.3966).

Table 3: Effect of plant part and chemical treatment on number of pineapple plantlets generated under black soldier fly frass substrate at Kyambogo University, 2023-2024.

Trail	Chemical treatment	Plant parts		
		Crowns	Slips	Stems
One	Combination	0.00±0.00 [c]	1.22±0.15 [b]	0.00±0.00 [d]
	IAA-hormone	0.22±0.15 [b]	1.33±0.17 [a]	1.22±0.40 [a]
	Sodium hypochlorite	0.78±0.22 [a]	1.22±0.22 [b]	0.67±0.33 [b]
	Control	0.00±0.00 [c]	0.89±0.20 [c]	0.11±0.11 [c]
	Grand mean	0.25	1.17	0.50
Two	Combination	0.44±0.18 [b]	0.33±0.17 [c]	0.22±0.22 [b]
	IAA-Hormone	0.22±0.15 [d]	1.00±0.00 [a]	0.00±0.00 [c]
	Sodium Hypochlorite	0.33±0.17 [c]	1.00±0.00 [a]	0.00±0.00 [c]
	Control	0.56±0.18 [a]	0.56±0.18 [b]	0.78±0.32 [a]
	Grand mean	0.39	0.72	0.42

For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

4.4.1.2 Composite

For the composite substrate, the number of plantlets was dependent on plant part (F=142.86, df = 2, p<0.0001) and sampling time (F=2.32, df = 2, p=0.0409) in trial one. Stems produced the highest number of plantlets compared to slips and crowns (Table 4). The number of plantlets produced at 60 days after planting did not significantly differ from those produced at 90 days after planting, but were significantly higher than those produced at 30 days after planting. Similarly, an interaction occurred between plant part and sampling time (F=9.94, df = 4, p<0.0001) but not between plant part and chemical treatment nor chemical treatment and sampling time (p>0.05). Generally, the number of plantlets obtained at 90 days after planting was significantly higher than those obtained at 30 days after planting.

In trial two, the number of plantlets produced was significantly affected by plant part (F=36.64, df = 2, p<0.0001) and sampling time (F=83.78, df = 2, p<0.0001). Stems yielded the highest number of plantlets compared to slips and crowns, respectively (Table 4). The number of plantlets produced at 90 days after planting was significantly higher than those obtained at 60 and 30 days after planting. Similarly, significant effects were observed between interactions of plant parts used and sampling time (F=9.55, df = 4, p<0.0001). In contrast, chemical treatment had no significant effect on the number of plantlets generated (p=0.091). Additionally, interactions between plant part used and chemical treatment (p=0.064), and chemical treatment and sampling time (p=0.1463) did not significantly affect number of plantlets produced in trial two of this study.

Table 4: Effect of plant part and sampling time on number of pineapple plantlets under the composite substrate at Kyambogo University, 2023-2024

Trial	Sampling Time (Days)	Plant part		
		Crowns	Slips	Stems
One	30	1.00±0.00 [b]	1.00±0.00 [c]	2.25±0.17 [c]
	60	1.08±0.08 [b]	1.17±0.11 [b]	2.67±0.11 b
	90	1.33±0.14 [a]	1.67±0.14 [a]	3.17±0.18 a
	Grand mean	1.14	1.28	2.69
Two	30	0.17±0.11 [b]	0.83±0.11 [c]	0.75±0.13 [c]
	60	1.00±0.00 [a]	1.17±0.11 [b]	1.17±0.11 [b]
	90	1.00±0.00 [a]	1.67±0.11 [a]	2.33±0.14 [a]
	Grand mean	0.72	1.22	1.42

For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

4.4.1.3 Lake sand

Results from ANOVA revealed that the number of plantlets raised on sand also depended on plant part (F=206.59, df = 2, p<0.0001), and sampling time (F=31.57, df = 2, p<0.0001) during trial one. The highest number of plantlets was produced by stems and was significantly higher

than those produced by slips and crowns (Table 5). The number of plantlets produced by stems at 60 and 90 days after planting was statistically similar, but higher than the number produced at 30 days after planting. Similarly, there was an interaction between plant part and sampling time (F=4.99, df =4, p=0.0012). However, chemical treatment did not significantly affect the number of plantlets produced (p=0.7267). Additionally, interactions between plant part and chemical treatment, and chemical treatment and sampling time did not significantly (p>0.05) affect the number of plantlets produced in trial one. Similarly, the number of plantlets was significantly affected by plant part (F=23.63, df = 2, p<0.0001) and sampling time (F=43.06, df = 2, p<0.0001) in trial two. Stems yielded more plantlets compared to slips and crowns (Table 5). The number of plantlets obtained at 90 days after planting was significantly higher than those produced at 60 and 30 days after planting (Table 5). Relatedly, interactions between plant parts used and sampling time showed significant effects (F=5.5, df = 4, p=0.0006) on the number of plantlets produced. Conversely, chemical treatment alone did not significantly affect number of plantlets produced (p=0.1521). Similarly, interactions between plant part and chemical treatment, and chemical treatment and sampling time had no significant effect on the number of plantlets produced.

Table 5: Effect of plant part and sampling time on number of pineapple plantlets produced under the lake sand substrate at Kyambogo University, 2023-2024.

Trail	Sampling Time (Days)	Plant parts		
		Crowns	Slips	Stems
One	30	0.92±0.08 [b]	1.00±0.00 [b]	2.67±0.14 [b]
	60	1.00±0.00 [b]	1.08±0.08 [b]	2.92±0.15 [a]
	90	1.75±0.13 a	1.92±0.08 [a]	2.92±0.14 [a]
	Grand mean	1.22	1.33	2.84
Two	30	0.5±0.15 [b]	0.58±0.15 [c]	0.92±0.15 [c]
	60	1.00±0.00 [a]	1.00±0.00 [b]	1.42±0.15 [b]
	90	1.08±0.08 [a]	1.25±0.13 [a]	2.42±0.15 [a]
	Grand mean	0.86	0.94	1.59

For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

4.4.1.4 Black forest soil

Except for plant part (F=122.83, df =2, p<0.0001) where the number of plantlets differed significantly, the number of plantlets did not vary across chemical treatments and sampling time (p>0.005) during trial one. The crowns and slips generated a significantly lower number of plantlets than stems (Table 6). However, the number of plantlets produced was affected by the interaction between plant parts and sampling time (F=4.04, df = 4, p=0.0048). No interaction occurred between plant part and chemical treatment nor chemical treatment and sampling time (p>0.05). The number of plantlets obtained at 90 days was significantly higher than those obtained at 30 days after planting across all plant parts. In trial two, the number of plantlets produced was significantly affected by sampling time (F=36.23, df = 2, p<0.0001), but not by plant part nor chemical treatment (p>0.05). Stems consistently produced the highest number of plantlets compared to crowns and slips (Table 6). The number of plantlets produced at 90 days was significantly higher than those obtained at 60 and 30 days after planting. The number of plantlets produced by crowns at 90 and 60 days after planting were statistically similar, but significantly higher than those obtained at 30 days after planting. There was a

significant effect of interactions between plant part and chemical treatment on the number of plantlets produced (F=2.38, df = 6, p=0.0356). In contrast, no significant effects were observed due to interactions between plant part and sampling time nor chemical treatment and sampling time (p>0.05).

Table 6: Effect of plant part and sampling time on number of pineapple plantlets produced under the forest soil substrate at Kyambogo University, 2023-2024.

Trial	Sampling Time (Days)	Plant parts		
		Crowns	Slips	Stems
One	30	0.92±0.08 [b]	1.00±0.00 [c]	2.50±0.15 [c]
	60	1.00±0.00 [b]	1.17±0.11 [b]	2.83±0.27 [b]
	90	1.33±0.14 [a]	1.50±0.15 [a]	3.17±0.17 [a]
	Grand mean	1.08	1.22	2.83
Two	30	0.33±0.14 [b]	0.33±0.14 [c]	0.83±0.08 [c]
	60	1.25±0.13 [a]	1.00±0.12 [b]	0.92±0.19 [b]
	90	1.25±0.13 [a]	1.17±0.17 [a]	1.75±0.33 [a]
	Grand mean	0.94	0.83	1.17

For each parameter, means in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

4.4.1.5 Vermiculite

In trial one, under this substrate, the number of plantlets generated was not affected by chemical treatment (p=0.0608), but depended on plant part (F=93.93, df = 2, p<0.0001), and sampling time (F=14.26, df = 2, p<0.0001). Slips and crowns produced a significantly lower number of plantlets than stems (Table 7). However, the number of plantlets obtained from stems at 30 and 60 days after planting were statistically similar but significantly lower than those obtained at 90 days after planting. Significant differences were observed in the number of plantlets generated due to interactions between plant part and chemical treatment (F=2.52, df = 6, p=0.0274), and plant part and sampling time (F=5.57, df = 4, p=0.0004). There was no interaction effect between chemical treatment and sampling time on number of plantlets

generated (p=0.6382). Except for the stem where no statistical difference existed on the number of plantlets among sampling time (P=0.5005), the number of plantlets produced by both crown (F=20.92, df = 2, p<0.0001), and slip (F=55.50, df = 2, p<0.0001) were significantly higher at 90 days after planting, when compared with those obtained at 30 and 60 days after planting crowns and slips. The number of plantlets produced was significantly affected by plant part (F=45.74, df = 2, p<0.0001), and sampling time (F=60.27, df = 2, p<0.0001) in trial two. Stems yielded more plantlets compared to slips and crowns (Table 7), with the number of plantlets obtained at 90 days being significantly higher than those produced at 30 and 60 days after planting. Chemical treatment did not significantly affect the number of plantlets produced during the three-month trial period. Similarly, significant interactions occurred between plant parts and sampling time (F=6.4, df =4, p=0.0002). Conversely, chemical treatment did not significantly affect the number of plantlets generated. Additionally, there were not significant differences observed in the number of plantlets produced as a result of interactions between plant part and chemical treatment, and chemical treatment and sampling time (p>0.05).

Table 7: Effect of plant part and sampling time on number of pineapple plantlets produced under the vermiculite substrate at Kyambogo University, 2023-2024.

Trail	Sampling Time (Days)	Plant parts		
		Crowns	Slips	Stems
One	30	0.92±0.08 [c]	1.00±0.00 [b]	2.58±0.29 [b]
	60	1.17±0.11 [b]	1.08±0.08 [b]	2.58±0.19 [b]
	90	1.83±0.11 [a]	1.92±0.08 [a]	2.92±0.19 [a]
	Grand mean	1.31	1.33	2.69
Two	30	0.25±0.13 [b]	0.67±0.14 [c]	0.92±0.08 [c]
	60	1.00±0.00 [a]	1.00±0.00 [b]	1.42±0.15 [b]
	90	1.00±0.00 [a]	1.33±0.14 [a]	2.33±0.14 [a]
	Grand mean	0.75	1.00	1.56

For each parameter, means in column followed by similar letters are not significantly different at p<0.05, Tukey's Studentized range test.

4.5 Discussion

The findings of this study revealed significant differences in the number of pineapple plantlets generated across all the substrates used. The number of plantlets produced was highest in composite and soil followed by vermiculite and sand in trial one, respectively. There was however, a significant decrease in the number of plantlets generated by all substrates in trial two. Sand yielded the highest number of plantlets followed by composite and vermiculite, being the best performing substrates in trial two. Black soldier fly frass (BSFF) yielded the least number of plantlets in both trials. The high plantlet yield exhibited by composite and soil substrate could be attributed to their sandy loam texture that favored plantlet growth (Magdoff & Van Es, 2021). Also, the acidic pH exhibited in the soil substrate may have enhanced plantlet production (Reinhardt *et al.*, 2018). Additionally, the excellent porosity and aeration properties of the sand substrate could have influenced plantlet initiation and development during the propagation phase. Accordingly, pH is known to influence nutrient bioavailability, while sandy and sandy loam textures contribute to improved aeration and drainage. Additionally, sandy loam textures improved the water holding capacity of the composite and soil substrates which is necessary for plant growth and development (Magdoff & Van Es, (2021; Jalil *et al.*, 2021). Also, the finding revealed that the high organic matter content of 4.5%, could have favoured plantlets generation in soil (MendonÃ *et al*., 2017; Magdoff & Van Es, 2021). Furthermore, the results revealed that there was a significant substrate and pineapple plant part interaction. Stems yielded the highest number of plantlets in composite and soil, followed by sand and vermiculite, in trial one, while crowns produced the lowest number of plantlets in both trials across all substrates compared to other plant parts used in the study. The high number of plantlets obtained from stems in composite, soil, and sand is significantly higher than the findings obtained by Ranawana & Eeswara, (2008) using semi-mature stems. According to Chotangui *et al.,* (2019), semi-mature stems contain optimum concentration of phytohormones

and food reserves which favoured plantlet generation and growth. The potential of stems as propagation materials for pineapple plantlets is in agreement with the findings of Matthew *et al.,* (2023). Conversely, the number of plantlets obtained from stems and other plant parts was lowest in BSFF during both trials. This could be due to the high pH levels of the substrate that might have presented unfavorable conditions for plantlet generation and growth (Reinhardt *et al.,* 2018b). In fact, the silty texture from BSFF may have presented unsuitable structure associated with poor water drainage and aeration, conditions that are unconducive for plantlet growth and development (Magdoff & Van Es, 2021). These findings are further supported by Lopes *et al.,* (2022) who elaborated on the poor performance of plants grown using BSFF as a fertilizer. This was attributed to a number of aspects which characterizes BSFF such as its varying composition of nutrients, microorganisms, and bioactive compounds, its post-processing requirements for improved biological stabilization, and its unknown action in the plant's metabolism among others. These aspects may have rendered BSFF unsuitable for generating pineapple plantlets, despite its high nitrogen and organic matter content.

In contrast, crowns produced the lowest number of plantlets. Though there was a general decrease in the number of plantlets generated in trial two across all plant parts, the average number of plantlets generated in composite, sand, soil and vermiculite substrates using crowns, slips and stems was greater than or equal to one (1) in trial one and two at ninety (90) days after planting.

The general decrease in the number of plantlets generated across all substrates using the different pineapple plant parts in trial two could be due to the variations in the weather conditions such as temperature, humidity and moisture which could have negatively affected the generation of pineapple plantlets (Matsui & Mochida, 2024). Trial one and two were conducted from 23rd December, 2023 to 23rd March, 2024 and from 23rd April, 2024 to 23rd

July, 2024 respectively, indicating different seasons characterized by varying weather conditions. According to Chotangui *et al*, (2019) sucker initiation and growth are dependent on the food reserves stored in the plant parts. However, the use of the available food reserves relies on enzyme-driven physiological processes of respiration and transpiration which are influenced by microclimatic conditions of temperature and relative humidity. Similarly, light duration plays an important role in breaking bud dormancy and hence contributing to initiation of plantlet growth (Sewhag *et al.,* 2024). Equally, the different substrates might have influenced the microclimate surrounding the plant parts causing the plant parts to respire differently hence affecting bud initiation and development (Chotangui *et al.,* 2019). Therefore, all these variations in weather conditions which may have existed during separate seasons in which trial one and two were conducted might have contributed to the significant differences in the number of pineapple plantlets generated in this study (Sewhag *et al*., 2024). Overall, stems produced the highest number of plantlets across majority (80%) of the substrates used in this study during trial 1 and 2, except in the BSFF substrate. The highest number of plantlets was obtained in soil and composite substrates.

4.5.1 Limitations of the study

Data on weather parameters, such as temperature and humidity, were not recorded during the two trials, which represents a limitation of this study. Environmental differences between trials could have contributed to variations observed in plantlet regeneration and growth. In fact, similar studies have reported that fluctuations in temperature and humidity can significantly affect the production and development of pineapple plantlets (Chotangui *et al*, 2019). Also, light duration plays an important role in breaking bud dormancy and hence contributing to initiation of plantlet development and growth (Sewhag *et al.,* 2024). These findings provide a scientifically supported rationale for the differences observed between the trials and have been used to contextualize the results in this study.

CHAPTER FIVE

PERFORMANCE OF PINEAPPLE PLANTLETS GENERATED FROM DIFFERENT SUBSTRATES AND PLANT PARTS

5.1 Introduction

The successful production of pineapple largely depends on the type of planting material used and the prevalent environmental conditions including temperature, rainfall, and relative humidity, and the edaphic conditions such as including soil texture, structure, fertility, and pH, of where they are planted (Ranawana & Eeswara, 2008; Reinhardt *et al.,* 2018; Chotangui *et al.,* 2019). However, the different substrates used to generate planting materials may influence root system development, plantlet vigor, and acclimatization success which directly enhance survival and growth after transplanting (Hakim *et al.,* 2017). For example, nutrient and water uptake will depend on the quality and level of development of the root system of plantlets transplanted to different growing environments. Therefore, the objective of this study was to assess the performance of pineapple generated from different substrates.

5.2 Materials and methods

5.2.1 Study area

The study was conducted twice from March 23rd, 2024 to July 23rd, 2024 and from July 23rd, 2024 to 23rd November, 2024 at the Faculty of Agriculture, Kyambogo University main campus. Details of the location and climatic conditions of Kyambogo University are presented in Chapter 3 (Section 3.2.1).

5.2.2 Experimental design

Three-month's old pineapple plantlets that were generated in Chapter four from different treatments (i.e. 5 substrates, 3 plant parts, and four chemical treatments) were harvested by detaching from the mother plant and placing inside a tray. After harvesting the plantlets from the respective treatments highlighted in Chapter 4, the plantlets were transplanted into 10-liter

(L) plastic buckets (Plate 5) containing sterile soil. Before use, the soil was sterilized and cooled as described in Chapter 3. In each bucket, approximately 8 kilograms of the soil substrate was added and then watered before planting the plantlets into them. Each bucked contained 1-3 plantlets depending on the number of plantlets that were generated and/or harvested per treatment from the 3-month's experiment highlighted in chapter four. Each plantlet served as a replicate. The buckets containing the plantlets were organized in the screen house using a completely randomized design (CRD) with a 5*3*4 factorial arrangement and evaluated in a screen house environment. The survival and agronomic performance of the transplanted plantlets were evaluated over a period of 120 days. The experiment was maintained using standard agronomic practices, including watering as and when needed, hand weeding to remove unwanted plants, and loosening of soil to prevent compaction and its negative effects on growth. The first trial was conducted from March 23rd, 2024 to July 23rd, 2024 while the second trial was from July 23rd, 2024 to November 23rd, 2024.

Plate5: Evaluation of the performance of pineapple plantlets from substrates and plant parts at Kyambogo University, 2024.

Plate 6: Samples of pineapple plantlets air dried and packed in paper bags for oven drying to collect data on dry shoot and root weights at Kyambogo University, 2024.

5.3 Data collection

Data on plantlet survival, number of leaves, plant height, and leaf area (length × width) were collected at 60, 90, 120 days after transplanting (DAT) (Omotoso & Akinrinde, 2013). At 60 DAT, the plantlets had recovered from transplanting shock and entered into the active growth phase, which is key to ensuring the collection of stable data. Data on number of leaves was collected by counting the leaves on each plantlet and recording it on a data sheet. Plant height was measured using a string placed upright from the surface of the soil in the buckets to the highest tip of plant and then read off in centimeters using a plastic transparent 30 cm ruler following procedures by Karimi *et al*, (2009). Leaf area was obtained as a product of the leaf length and width which were also measured using a string and ruler. Five (5) leaves were sampled per plant to obtain the length and width which were later divided to obtain an average length and width.

At the end of the experiment, the plantlets were harvested gently by pulling out of the moist soil. The soil around the root zones of each plantlet was washed off using tap water. Thereafter, the plants were air dried on raised metallic stands for 30 days inside the screen house (Plate 6)

and then oven-dried at 80^{o}C for 48 hours (Ajema & Shewangizaw, 2021). The dried plant samples were cut using a sharp knife at the root collar to separate the shoot from the root. The dry shoots and roots were then individually weighed using an electric sensitive balance and their respective weights in grams (g) were recorded in the data sheet.

5.4 Data analysis

Data from trials one and two were tested for homogeneity. Since no significant differences were observed between trials for most parameters, those data were pooled for analysis. However, biomass data, which showed significant differences between trials, were analyzed and presented separately. Thereafter, plantlet height, number of leaves, leaf area, and dry root weights were log-transformed while leaf length and dry shoot weight were subjected to square root transformation to obtain homogeneity of variances and normal distribution. (Burt & Burt, 2014; Takoutsing *et al.*, 2014; Lindstromberg, 2020). Data on leaf width were not transformed, as they conformed to normal distribution (Rao & Richard, 2012). Analysis of Variance was conducted to determine the effects of sampling time, substrate, plant part, and chemical treatment on height of plantlets, number of leaves, leaf length and width, and leaf area (Rao & Richard, 2012; Burt & Burt, 2014; Lindstromberg, 2020). Where significant differences were detected, means were separated using Tukey's Studentized test at 5% level of significance. All data sets were analyzed using the SAS statistical software Version 9.4.

5.5 Results

Generally, there was no effect of sampling time and chemical treatment on plant survival and growth except for plantlet leaf width. Irrespective of the growth substrate, plant part, and chemical treatment, all (100%) of the transplanted pineapple plantlets survived over the 120-day assessment period. On average, a height of 13.82±0.15 cm, 17.76±0.22 number of leaves, 14.55±0.22 cm leaf length, and 2.01±0.09 cm leaf width was attained by day 120. The average leaf area was 20.28±0.55cm^{2} across treatments. Results of a three-way ANOVA for pooled

data revealed that plant height, number of leaves, leaf area, leaf width, and leaf area were affected by sampling time (F≥79.04≤1206.66, df = 2, p<0.0001), substrate type (F≥25.31≤107.12, df=4, p<0.0001), plant part (F≥56.78≤145.98, df = 2, p<0.0001), and chemical treatment (F≥3.24≤49.84, df = 3, p<0.0001; or p=0.0211 for width). Significant interaction effects were also observed for all parameters measured including for three-way ANOVA.

5.5.1 Effect of substrate, plant part, and chemical treatment on plant height

Over all, plant height increased over time from 5.59±0.21 to 14.29±0.26 cm but depended on the interaction sampling time × substrate × plant part from the three-way ANOVA (p<0.0001). Thus, the ANOVA that was run under each sampling time showed that plant height was affected by substrate type (F≥30.63≤45.75, df = 4, p<0.0001), plant part (F≥13.27≤49.85, df = 2, p<0.0001), and chemical treatment (F≥14.61≤18.94, df = 3, p<0.0001) as well as the interactions between the three factors at each sampling time. At 60 DAT, plant height depended on the interaction between substrate and plant part (F=6.46, df = 8, p<0.0001) Figure 1a), substrate and treatment (F=2.28, df = 12, p=0.0075), and plant part and chemical treatment (F=2.15, df = 6, p=0.0451) (Figure 1b). Thus, plantlets harvested from crowns had significantly higher height than those harvested from stems or slips for all substrates except for sand where no statistical difference was observed across plant parts (BSFF, F=33.41, df=4, p<0.0001; Composite, F=33.41, df=4, p<0.0001; Soil, F=37.53, df=4, p<0.0001; Vermiculite, F=15.44, df=4, p<0.0001; and Sand, p=9798). Across chemical treatment, no significant differences were noted for plantlets raised on all substrates (p>0.05) except sand and vermiculite treated plantlets where sodium hypochlorite treated plantlets were significantly taller than controls or other chemical treatments (F=11.12, df=3, p<0.0001). Regarding the interaction between plant part and chemical treatment, sodium hypochlorite treated crowns attained a significantly higher height than IAA-treated crowns or those that received combined treatment (F=12.12, df=3,

p<0.0001). However, no significant differences existed across chemical treatments for stems (p=0.1441), while plantlets from IAA-hormone treated slips were significantly taller than the water (control) treatment (F=3.33, df=3, p=0.0197). At 90 DAT, plant height was affected by the interaction between substrate and plant part (F=14.06, df=8, p<0.0001) (Figure 1a), substrate and chemical treatment (F=5.26, df=12, p<0.0001), and plant part and chemical treatment (F=1.94, df=6, p<0.0711) (Figure 1b). Whereas plantlets harvested from crowns had a significantly higher height than those harvested from stems or slips for BSFF (F=6.99, df=2, p=0.0015) and sand (F=14.25, df=2, p<0.0001), no significant differences were noted among plant parts for Composite-raised plantlets (p=0.4959). In soil, plantlets harvested from stems were significantly shorter than those from slips and crowns (F=5.66, df=2, p=0.004), while for Vermiculite, slip-harvested plantlets were taller than stem and crown-derived plantlets (F=31.58, df=2, p<0.0001). Across chemical treatments, no significant differences were noted for plantlets raised on all BSFF and Vermiculite substrates (p>0.05), while sodium hypochlorite treated plantlets were significantly taller than plantlets that received the other chemical treatments for composite-raised plantlets (F=12.68, df=3, p<0.0001). However, for sand, the water (control) and sodium hypochlorite treated plantlets were significantly taller than plantlets that received a combined treatment (F=5.71, df = 3, p=0.0009). On soil, sodium hypochlorite treated plantlets and plantlets that received combined treatment had a significantly higher height than the controls (F=7.46, df = 3, p<0.0001). For Vermiculite substrate, no significant difference was observed across all chemical treatments (p=0.3251). However, IAA-hormone and sodium hypochlorite treated plantlets were taller than those treated with other chemicals. Similarly, at 120 DAT, plant height depended on the interaction between substrate and plant part (F=2.98, df = 8, p=0.0027) (Figure 1a), substrate and chemical treatment (F=3.82, df = 12, p<0.0001), and plant part and chemical treatment (F=4.62, df = 6, p=0.0001) (Figure 1b). Overall, plantlets obtained from crowns were significantly higher than those from

stems for BSFF (F=3.81, df=2, p=0.0259) and sand (F=4.15, df=2, p=0.017). For the composite substrate, slips derived plantlets were significantly taller than crowns (F=7.57, df=2, p=0.0006), while on soil (F=11.7, df=2, p<0.0001) and vermiculite (F=22.12, df=2, p<0.0001), slip derived plantlets were significantly taller than stem-derived plantlets. With the exception of BSFF and Vermiculite raised plantlets where no significant differences were noted across all chemical treatments (p>0.05), the water treated (control) plantlets were significantly shorter than the sodium hypochlorite and the plantlets that received combined treatment and raised on composite (F=5.23, df=3, p=0.0016) and soil (F=3.82, df = 3, p=0.0108), respectively. For sand, the water (control) treated plantlets were significantly taller than plantlets that received combined treatments (F=3.59, df=3, p=0.0144). On the final day of assessment, the different chemical treatments had no effect on plant height for plantlets that were harvested from crowns (p=0.2778) and slips (p=0.3442). However, for the stems, sodium hypochlorite treated plantlets significantly taller than plantlets that received IAA-hormone and water (control).

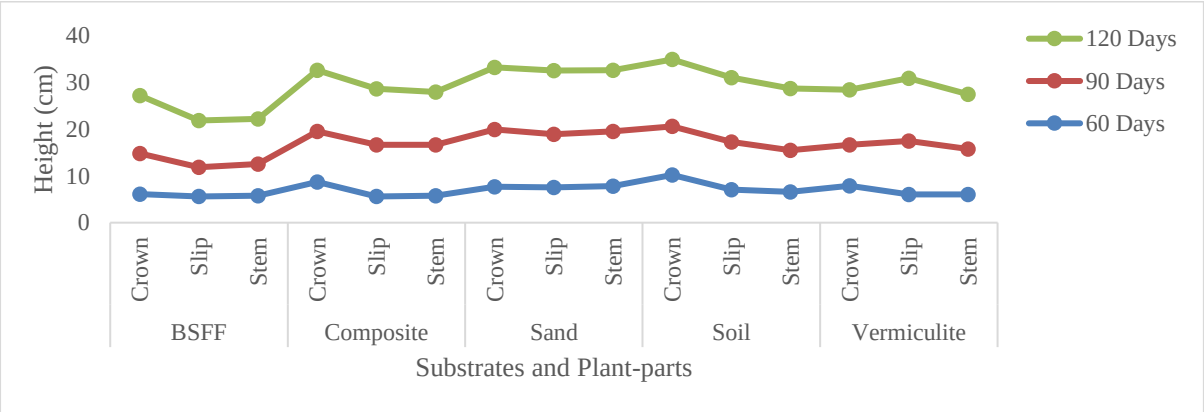

Figure 1a: Effect of substrate and plant part on height of pineapple plantlets under each sampling times.

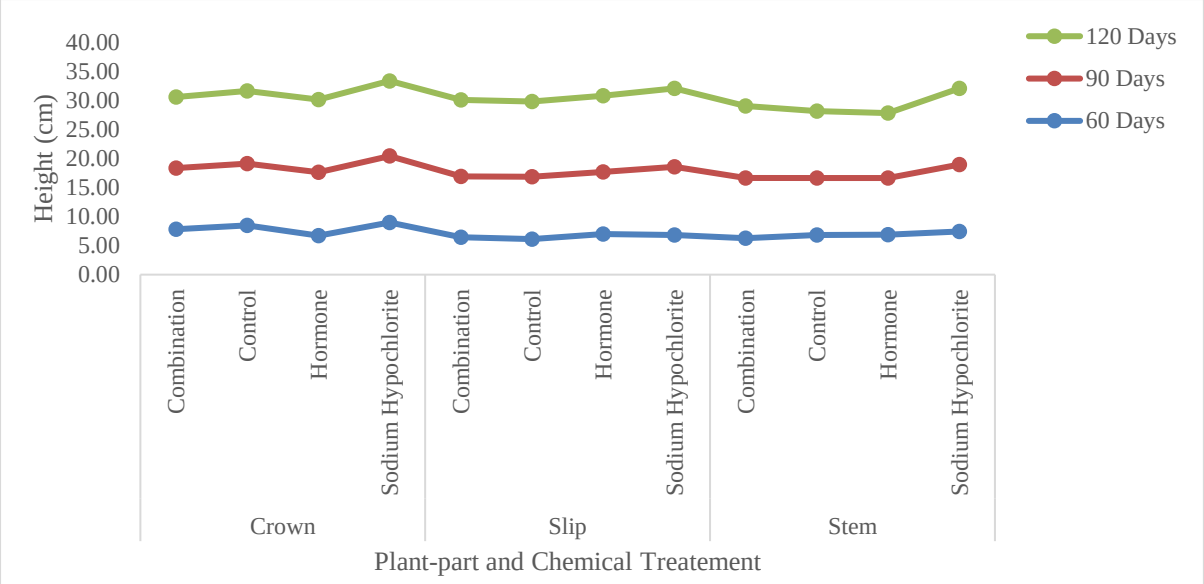

Figure 1b : Effect plant part and chemical treatment on the height of pineapple plantlets under each sampling time.

5.5.2 Effect of substrate, plant part, and chemical treatment on number of leaves

There was a gradual increase in the number of leaves throughout the 120-day assessment period. Like for plant height, the number of leaves depended on interactions of substrate × plant part × chemical treatment from the three-way ANOVA (p<0.0001) (Figure 2a and Figure 2b). For example, under BSFF, the number of leaves was affected by plant part (F=4.16, df=2, p=0.0166), sampling time (F=54.21, df = 2, p<0.0001), but not chemical treatment (p=0.4318). However, the number of leaves depended on interaction between plant part and chemical treatment (F=5.16, df = 6, p<0.0001). While no significant difference existed across treatments for plant parts that were from slips (p=0.0589) and stems (p=0.0812), the water (control) treated crowns produced plantlets with a significantly higher number of leaves than plantlets that received a combined treatment of sodium hypochlorite and IAA-hormone (F=4.02, df =3, p=0.0108). There was no interaction between plant part and sampling time (p=0.8322). For the composite substrate, the number of leaves equally depended on plant part (F=84.07, df=2, p<0.0001), chemical treatment (F=50.04, df=3, p<0.0001), as well as sampling time (F=227.38, df = 2, p<0.0001). However, the number of leaves on the interaction between plant

part and chemical treatment (F=21.74, df=6, p<0.0001), but not between plant part and sampling time (p=0.9604). There was no significant difference across chemical treatments in the number of leaves for plantlets that were harvested slips (p=0.1269). Plantlets harvested from sodium hypochlorite treated crowns (F=31.61, df=3, p<0.0001) or stems (F=18.33, df=3, p<0.0001) had a significantly higher number of leaves than other chemical treatments. Equally, for sand-generated plantlets, plant part (F=46.65, df=2, p<0.0001), chemical treatment (F=42.51, df=3, p<0.0001), and sampling time (F=212.48, df = 4, p=0.2833) had a significant effect on the number of leaves. There was also an interaction between plant part and sampling chemical treatment (F=6.17, df=6, p<0.0001) but not between plant part and sampling time (p=0.2883). Overall, water (control) treated plantlets produced significantly higher number of leaves across all chemical treatments for crowns (F=15.65, df = 3, p<0.0001) while a highly significant number of leaves was noted in sodium hypochlorite treated plantlets harvested from slips (F=9.4, df = 3, p<0.0001) or stems (F=11.37, df=3, p<0.0001) than in other chemical treatments (Figure 4). Similarly, the number of leaves for plantlets that were generated on the soil substrate depended on plant part (F=9.08, df=2, p=0.0001), chemical treatment (F=16.2, df=3, p<0.0001), and sampling time (F=2.06.01, df=2, p<0.0001). The number of leaves were also affected by an interaction between plant part and chemical treatment (F=7.46, df = 6, p<0.0001) (Figure 4) but not between plant part and sampling time (p=0.2978). Whereas plantlets harvested from sodium hypochlorite treated slips (F=7.73, df =3, p<0.0001), and stems (F=5.15, df=3, p=0.0021) had significantly higher number of leaves than controls, and IAA=hormone treated plantlets, respectively, there was no effect of chemical treatments on number of leaves for plantlets that were harvested from crowns (p=0.3748). Under the vermiculite substrate, the number of leaves was affected by plant part (F=63.47, df=2, p<0.0001), chemical treatment (F=10.53, df=3, p<0.0001), and sampling time (F=195.46, df=2, p<0.0001). However, the number of leaves depended on interaction between plant part

and chemical treatment (F=8.91, df=6, p<0.0001) (Figure 4). Plantlets that were harvested from crowns treated with water (control) or sodium hypochlorite had significantly higher number of leaves than plantlets harvested from crowns treated with combined (sodium hypochlorite and IAA-hormone) or sole IAA-hormone (F=13.42, df=2, p<0.0001). Plantlets harvested form slips treated with water (control) registered a significantly lower number of leaves than the plantlets harvested from slips and treated with a combination of sodium hypochlorite and IAA-hormone (F=3.68, df=3, p=0.0126). Plantlets harvested from stems treated with sodium hypochlorite had the highest number of leaves (F=2.99, df =3, p<0.032). There was no interaction between plant part and sampling time (p=0.9981).

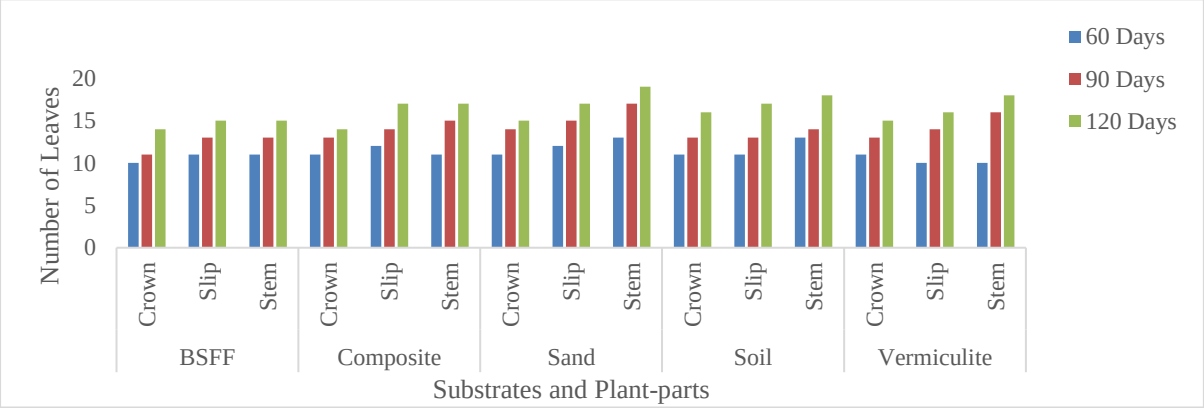

Figure 2a : Effect of substrate and plant part on the number of leaves of pineapple plantlets under each sampling time.

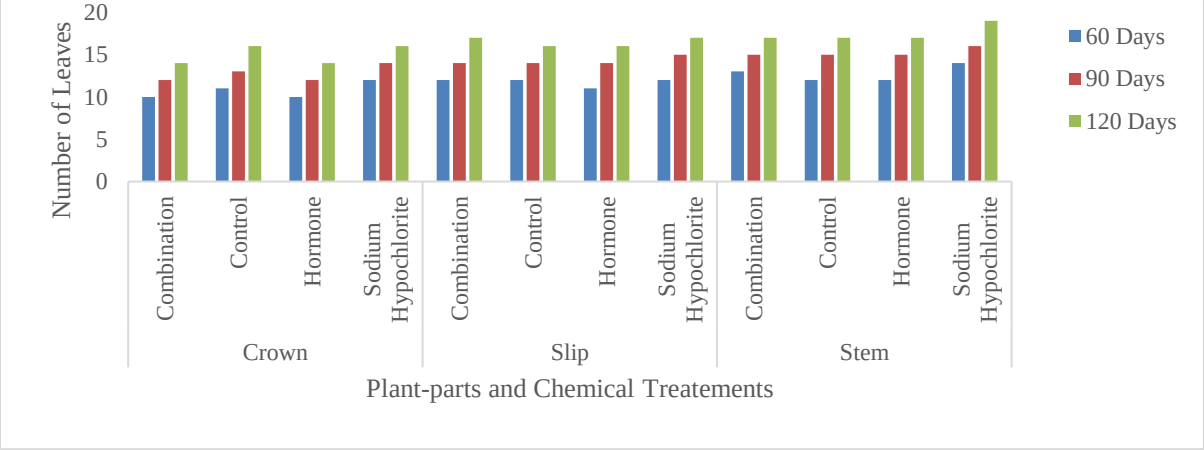

Figure 2b : Effect of plant part and chemical treatment on the number of leaves of under each sampling time.

5.5.3 Effect of substrate, plant part, and chemical treatment on leaf length

The length of the plantlets increased gradually over the 120-day assessment period. Similarly, results of the three-way ANOVA revealed that the plantlet leaf length depended on the interaction between substrate × plant part × chemical treatment (p<0.0001) (Table 8a). In the BSFF substrate, the leaf length of plantlets was affected by plant part (F=11.71, df=2, p<0.0001), chemical treatment (F=8.67, df=3, p<0.0001), and sampling time (F=8.91, df=2, p=0.0002). Also, the leaf length depended on the interaction between plant part and chemical treatment (F=3.93, df=2, p=0.0002). Plantlets harvested from crowns (F=11.63, df=3, p<0.0001) and stems (F=4.37, df=3, p=0.0078) treated with sodium hypochlorite exhibited the highest leaf length across all chemical treatments while plantlets from water treated slips had a significantly higher leaf length than those treated with IAA-hormone and a combination of sodium hypochlorite and IAA-hormone (F=6.35, df=3, p=0.0005). There was no significant effect between plant part and sampling time (p=0.5344). Under the composite substrate, leaf length of plantlets depended on the plant part (F=17.78, df=2, p<0.0001), chemical treatment (F=51.84, df=3, p<0.0001), and sampling time (F=42.47, df=2, p<0.0001). Similarly, there was an interaction between plant part and chemical treatment (F=18.62, df=6, p<0.0001) (Table 8b). As in BSFF substrate, plantlets harvested from crowns (F=30.59, df=3, p<0.0001) and stems (F=31.94, df=3, p<0.0001) treated with sodium hypochlorite had significantly longer leaves than in other chemical treatments. In slips, plantlets treated with IAA-hormone had significantly longer leaves than in a combination of sodium hypochlorite and IAA=hormone (F=3.16, df = 3, p=0.025). In contrast, no significant interaction effect was noted between plant part and sampling time (p=0.9351). For the sand substrate, plantlet leaf length was affected by plant part (F=21.82. df=2, p<0.0001), chemical treatment (F=36.02, df=3, p<0.0001), and sampling time (F=28.21, df=2, p<0.0001). There were interaction effects between plant part and chemical treatment (F=3.62, df=6, p=0.0015) (Table 8b). Plantlets harvested from slips

(F=14.31, df=3, p<0.0001) and stems (F=13.31, df=3, p<0.0001) treated with sodium hypochlorite exhibited a significantly higher leaf length than those treated with IAA-hormone and a combination of sodium hypochlorite and IAA-hormone. Plantlets derived from water (control) treated crowns had significantly higher leaf length than in those treated with IAA-hormone. However, no interaction effects were noted for interactions between plant part and sampling time (p=0.775). The leaf length of plantlets raised on soil were significantly affected by plant part (F=33.03, df=2, p<0.0001), chemical treatment (F=5.83, df=3, p=0.0006), and sampling time (F=45.97, df=2, p<0.0001). Similarly, there was a significant interaction effect between plant part and sampling time (F=5.83, df=6, p<0.0001). A higher leaf length was observed in plantlets harvested from sodium hypochlorite treated slips than in those treated with water (control) and IAA-hormone (F=4.21, df=3, p=0.0063). Plantlets derived from stems treated with a combination of sodium hypochlorite and IAA-hormone had a significantly higher leaf length than those treated with IAA-hormone alone (F=6.75, df=3, p=0.0003). However, no significant effect was noted in plantlets harvested from crowns across all chemical treatments (p=0.1246). In contrast, there was no significant interaction effect between plant part and sampling time on leaf length of plantlets. However, plantlets raised on vermiculite were significantly affected by plant part (F=73.43, df=2, p=0.0001), chemical treatment (F=22.65, df=3, p<0.0001), and sampling time (F=57, df=2, p<0.0001). Similarly, there were significant interaction effects between plant part and chemical treatment (F=10.33, df=6, p<0.0001) (Table 8b). Plantlets harvested from sodium hypochlorite treated crowns had a significantly higher leaf length than those treated with IAA-hormone alone or a combination of sodium hypochlorite and IAA-hormone (F=27.17, df=3, p<0.0001). On the other hand, plantlets harvested from stems treated with a combination of sodium hypochlorite and IAA-hormone had the longest leaves across all chemical treatments (F=7.55, df=3, p<0.0001). There was no significant difference in the leaf length of plantlets harvested from slips across all

chemical treatments (p=0.1464). Similarly, as observed across all substrates, there was no interaction effect between plant part and sampling time on the leaf length of plantlets.

Table 8a: Effect of substrate and plant part on leaf length under each sampling time at Kyambogo University, 2024.

Substrate	Plant part	Sampling time (DAT)		
		60 days	90 days	120 days
BSFF	Crowns	9.93±0.32 [a]	11.27±0.70 [a]	12.78±0.62 [a]
	Slips	9.23±0.52 [b]	10.56±0.59 [b]	11.25±0.24 [b]
	Stems	8.19±0.37 [c]	8.27±0.82 [c]	9.52±0.97 [c]
	Grand mean	9.12	10.03	11.18
Composite	Crowns	11.75±0.70 [a]	11.75±0.39 [b]	12.63±0.78 [b]
	Slips	9.23±0.52 [b]	12.29±0.57 [a]	13.19±0.28 [a]
	Stems	8.27±0.82 [c]	10.99±1.02 [c]	12.33±0.46 [c]
	Grand mean	9.75	11.68	12.72
Sand	Crowns	10.34±0.42 [c]	12.59±0.37 [b]	13.62±0.32 [b]
	Slips	11.84±0.28 [a]	13.97±0.33 [a]	14.26±0.28 [a]
	Stems	10.73±0.46 [b]	12.67±0.53 [b]	13.35±0.44 [c]
	Grand mean	10.97	13.07	13.74
Soil	Crowns	9.92±0.31 [b]	12.21±0.34 [b]	14.77±0.31 [b]
	Slips	11.33±0.43 [a]	13.04±0.34 [a]	14.98±0.32 [a]
	Stems	9.15±0.48 [c]	11.12±0.54 [c]	13.52±0.56 [c]
	Grand mean	10.13	12.12	14.42
Vermiculite	Crowns	8.70±0.31 [b]	11.43±0.34 [b]	12.33±0.34 [b]
	Slips	9.32±0.38 [a]	12.89±0.24 [a]	13.93±0.24 [a]
	Stems	6.88±0.44 [c]	9.94±0.43 [c]	11.19±0.42 [c]
	Grand mean	8.30	11.42	12.48

For each parameter, means + standard errors in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

Table 8b : Effect of plant part and chemical treatment on leaf length at each sampling time at Kyambogo University, 2024.

Plant part	Chemical treatment	Sampling time (DAT)		
		60 days	90 days	120 days
Crowns	Combination	9.90±0.29 [c]	11.02±0.29 [c]	11.90±0.29 [c]
	IAA-Hormone	9.26±0.35 [d]	10.88±0.32 [c]	11.54±0.32 [c]
	Sodium hypochlorite	12.03±0.35 [a]	13.31±0.36 [a]	14.25±0.35 [a]
	Control	10.21±0.37 [b]	11.76±0.38 [b]	12.79±0.36 [b]
	Grand mean	10.35	11.74	12.62
Slips	Combination	11.00±0.30 [b]	12.22±0.30 [b]	13.51±0.29 [b]
	IAA-Hormone	10.82±0.34 [d]	12.41±0.29 [b]	13.47±0.33 [b]
	Sodium hypochlorite	12.08±0.26 [a]	13.55±0.35 [a]	14.57±0.35 [a]
	Control	10.89±0.29 [c]	12.37±0.29 [b]	13.47±0.29 [b]
	Grand mean	11.19	12.64	13.76
Stems	Combination	9.22±0.40 [b]	11.63±0.38 [b]	12.61±0.37 [b]
	IAA-Hormone	8.09±0.61 [d]	9.67±0.61 [c]	10.85±0.59 [c]
	Sodium hypochlorite	11.22±0.45 [a]	12.54±0.51 [a]	13.09±0.38 [a]
	Control	8.55±0.39 c	10.04±0.40 [c]	11.24±0.42 [c]
	Grand mean	9.27	10.97	11.95

For each parameter, means + standard errors in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

5.5.4 Effect of substrate, plant part, and chemical treatment on leaf width

Generally, plantlet leaf width increased gradually across the 120-day assessment period but was affected by interactions between sampling time × substrate × plant part from the three-way ANOVA ($p<0.0001$). Thus, the ANOVA that was run under each sampling time showed that plantlet leaf width depended on substrate ($F \geq 6.03 \leq 22.12$, df=4, $p<0.0001$), plant part ($F \geq 11.17 \leq 57.27$, df=2, $p<0.0001$), and chemical treatment ($F \geq 2.71 \leq 10.77$, df=3, $p<0.0001$ at 90 DAT; $p=0.0166$ at 60 DAT; and $p=0.044$ at 120 DAT), including their interactions at each of sampling time. At 60 DAT, the leaf width of plantlets depended on interactions between substrate and chemical treatment ($F=2.07$, df=12, $p=0.0166$). Thus, plantlets treated with

sodium hypochlorite and raised on composite (F=4.23, df = 3, p=0.0061) and vermiculite (F=2.93, df=3, p=0.0341) had wider leaves compared to other chemical treatments while those treated with water (control) and raised on sand had significantly wider leaves than IAA-hormone treated plantlets. There was no significant effect observed in plantlets raised on BSFF and soil across all chemical treatments at 60 DAT. However, no significant interaction effects were observed between substrate and plant part (p=0.1295), and plant part and chemical treatment (p=0.4081). The widest leaves were recorded in plantlets harvested from slips across all substrates (p<0.05) at 60 DAT. At 90 DAT, the leaf width of plantlets was affected by interactions between substrate and plant part (F=6.17, df=8, p<0.0001) (Table 9a), substrate and chemical treatment (F=3.56, df=12, p<0.0001), and between plant part and chemical treatment (F=4.5, df=6, p=0.0002). Plantlets raised on composite substrates and treated with sodium hypochlorite exhibited the highest leaf width than those treated with water and a combination of sodium hypochlorite and IAA-hormone (F=5.25, df=3, p=0.0016) while those raised on sand and treated with water (control) had the widest leaves those treated with IAA-hormone alone or a combination of sodium hypochlorite and IAA-hormone (F=6.78, df=3, p=0.0002). Sodium hypochlorite treated substrates derived from soil had higher leaf width than those treated with a combination of sodium hypochlorite and IAA-hormone (F=3.86, df=3, p=0.0102). however, there was no significant effect noted in plantlets raised on BSFF (p=0.1949) and vermiculite (p=0.1273) across all chemical treatments. On the other hand, plantlets harvested from slips raised on composite had significantly wider leaves than those from stems and crowns (F=15.58, df=2, p<0.0001) while harvested from crowns raised on sand produced wider leaves than those from stems (F=23.33, df=2, p<0.0001). For soil (F=40.9, df=2, p<0.0001) and vermiculite (F=22.7, df=2, p<0.0001), plantlets harvested from slips had significantly wider leaves than those from stems. In contrast, there was no significant difference observed in plantlets raised on BSFF across all plant parts used (p=0.38). For interactions

between plant part and chemical treatment at 90 DAT (Table 9b), plantlets harvested form crowns treated with sodium hypochlorite had the widest leaves (F=10.4, df=3, p<0.0001) than those from crowns treated with a combination of sodium hypochlorite and IAA-hormone, while those stems treated with IAA-hormone alone produced wider leaves than in a combination of sodium hypochlorite and IAA-hormone. There was no significant effect in the leaf width of plantlets derived from slips across all chemical treatments. At 120 DAT, leaf width of plantlets was significantly affected by interactions between plant part and chemical treatment (F=2.14, df=6, p=0.0469) but not between substrate and plant part (p=0.2497) nor substrate and chemical treatment (p=0.2021). For chemical treatment, plantlets treated with IAA-hormone had significantly wider leaves than those treated with a combination of sodium hypochlorite and IAA-hormone (F=3.35, df=3, p=0.0185). Plantlets harvested from slips had significantly wider leaves than those from stems and crowns (F=14.92, df=2, p<0.0001). In regards to substrates, the widest leaves were observed in plantlets raised on sand than those raised on soil, composite and vermiculite, respectively (F=7.19, df=4, p<0.0001). For interactions between plant part and chemical treatment (Table 9b), plantlets harvested from sodium hypochlorite treated crowns produced the widest leaves than those treated with IAA-hormone alone or in combination with sodium hypochlorite (F=13.9, df=3, p<0.0001) while plantlets derived from IAA-hormone treated stems possessed the widest leaves across all chemical treatments (F=3.23, df=3, p=0.0228). In contrast, there was no significant difference observed in the leaf width of plantlets harvested from slips across all chemical treatments (p=0.06860).

Table 9a : Effect of substrate and plant part on leaf width at each sampling time at Kyambogo University, 2024.

Substrate	Plant part	Sampling time (DAT)		
		60 days	90 days	120 days
BSFF	Crowns	1.37±0.02 [b]	1.61±0.04 [b]	1.75±0.04 [a]
	Slips	1.56±0.02 [a]	1.66±0.05 [a]	1.76±0.27 [a]
	Stems	1.27±0.03 [c]	1.45±0.09 [c]	1.61±0.08 [b]
	Grand mean	1.40	1.57	1.71
Composite	Crowns	1.56±0.03 [b]	1.61±0.04 [b]	1.78±0.06 [c]
	Slips	1.70±0.02 [a]	1.76±0.27 [a]	1.87±0.05 [a]
	Stems	1.45±0.09 [c]	1.50±0.03[c]	1.81±0.08 [b]
	Grand mean	1.57	1.62	1.82
Sand	Crowns	1.40±0.04 [b]	1.72±0.03 [b]	1.81±0.03 [a]
	Slips	1.56±0.02 [a]	1.79±0.02 [a]	1.83±0.02 [a]
	Stems	1.36±0.04 [c]	1.56±0.03 [c]	1.66±0.02 [b]
	Grand mean	1.44	1.69	1.77
Soil	Crowns	1.59±0.02 [b]	1.65±0.03 [a]	1.92±0.02 [b]
	Slips	1.67±0.03 [a]	1.69±0.02 [a]	2.28±0.24 [a]
	Stems	1.31±0.03 [c]	1.44±0.04 [b]	1.71±0.04 [c]
	Grand mean	1.52	1.59	1.97
Vermiculite	Crowns	1.35±0.02 [b]	1.51±0.02 [b]	1.71±0.02 [b]
	Slips	1.52±0.04 [a]	1.69±0.02 [a]	1.85±0.02 [a]
	Stems	1.03±0.04 [c]	1.48±0.04 [c]	1.66±0.03 [c]
	Grand mean	1.30	1.68	1.74

For each parameter, means + standard errors in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

Table 9b : Effect of plant part and chemical treatment on leaf width at each sampling time at Kyambogo University, 2024.

Plant part	Chemical treatment	Sampling time (DAT)		
		60 days	90 days	120 days
Crowns	Combined	1.49±0.03 [b]	1.66±0.03 [b]	1.79±0.03 [b]
	IAA-Hormone	1.38±0.03 [d]	1.57±0.02 [c]	1.69±0.02 [c]
	Sodium hypochlorite	1.53±0.03 [a]	1.73±0.03 [a]	1.88±0.03 [a]
	Control	1.41±0.03[c]	1.54±0.02[c]	1.68±0.02[c]
	Grand mean	1.45	1.63	1.76
Slips	Combined	1.59±0.02 [c]	1.72±0.02 [a]	1.88±0.02 [a]
	IAA-Hormone	1.55±0.03 [c]	1.73±0.02 [a]	1.88±0.02 [a]
	Sodium hypochlorite	1.62±0.02 [b]	1.73±0.02 [a]	1.89±0.02 [a]
	Control	1.67±0.12 [a]	1.70±0.02 [a]	1.81±0.02 [b]
	Grand mean	1.61	1.72	1.87
Stems	Combined	1.28±0.03 [b]	1.44±0.03 [c]	1.63±0.02 [b]
	IAA-Hormone	1.29±0.06 [b]	1.59±0.04 [a]	1.71±0.04 [a]
	Sodium hypochlorite	1.38±0.04 [a]	1.50±0.03 [b]	1.65±0.03 [b]
	Control	1.25±0.03 [c]	1.41±0.02 [c]	1.58±0.02 [c]
	Grand mean	1.30	1.49	1.64

For each parameter, means + standard errors in column followed by similar letters are not significantly different at $p \leq 0.05$, Tukey's Studentized range test.

5.5.5 Effect of substrate, plant part, and chemical treatment on leaf area

As noted in the other growth parameters measured over the 120-day assessment period, there was a gradual increase in the leaf area of the plantlets. From the three-way ANOVA, the leaf area was affected by interactions between sampling time × plant part × substrate ($p<0.0001$). Hence the ANOVA run under each sampling time showed that leaf area of plantlets was affected by substrate ($F \geq 29.56 \leq 45.75$, df=4, $p<0.0001$), plant part ($F \geq 42.69 \leq 55.65$, df=4, $p<0.0001$) and chemical treatment ($F \geq 15.57 \leq 18.94$, df=4, $p<0.0001$) as well as their interactions at each sampling time ($p<0.05$). At 60 DAT leaf area depended on substrate ($F=45.75$, df=4, $p<0.0001$), plant part ($F=55.65$, df=2, $p<0.0001$), and chemical treatment

(F=18.94, df=3, p<0.0001). Plantlets treated with sodium hypochlorite had a significantly higher leaf area compared to IAA-hormone treated plantlets (F=10.95, df=3, p<0.0001). For the different plant parts, plantlets generated from slips exhibited a significantly higher leaf area than those from stems and crowns (F=48.57, df=2, p<0.0001). In the substrate factor, plantlets raised on sand produced leaves with the greatest leaf area than all the other substrates used (F=34.32, df=4, p<0.0001). There were significant interaction effects between substrate and plant part (F=6.46, df=8, p<0.0001), substrate and chemical treatment (F=2.28, df=12, p=0.0075), and plant part and chemical treatment (F=2.15, df=6, p=0.0451) on leaf area of plantlets. For interactions between substrate and chemical treatment, plantlets treated with sodium hypochlorite and raised on composite had the highest leaf area than those treated with water (control), combination of sodium hypochlorite and IAA-hormone, and IAA-hormone alone (F=8.09, df=3, p<0.0001), while those raised on sand and treated with sodium hypochlorite exhibited the highest leaf area compared to IAA-hormone or in combination with sodium hypochlorite (F=5.96, df=3, p=0.0006). In vermiculite substrate, plantlets treated with sodium hypochlorite produced leaves with the widest leaf area than those treated with IAA-hormone alone (F=5.2, df=3, p=0.0017). However, there was no significant difference observed in plantlets raised on BSFF (p=0.155) and soil (p=0.3391) across all chemical treatments used. In regards to interactions between substrate and plant part (Figure 3a), plantlets harvested from slips raised on composite had the greatest leaf area than those harvested from crowns and stems (F=8.59, df=2, p=0.0002). In sand, plantlets harvested from slips similarly exhibited a higher leaf area than those from stems and crowns (F=10.44, df=2, p<0.0001), while plantlets from slips raised on soil produced significantly greater leaf are compared to those from stems (F=28.66, df=2, p<0.0001). As for vermiculite substrate, a significantly higher leaf area was noted in plantlets harvested from slips (F=34.78, df=2, p<0.0001). There was no significant difference observed in the leaf area of plantlets raised on

the BSFF substrate at 60 DAT (p=0.0598). In regards to interaction between plant parts and chemical treatments (Figure 3b), plantlets derived from crowns treated with sodium hypochlorite registered the highest leaf area than those from crowns treated with IAA-hormone alone or in combination with sodium hypochlorite (F=7.1, df=3, p=0.0001). Similarly, plantlets harvested from stems treated with sodium hypochlorite yielded leaves with the highest leaf area (F=4.74, df=3, p<0.003). However, there was no effect noted in the leaf area of plantlets raised on BSFF substrate across all plant parts used at 60 DAT. Relatedly, at 90 DAT, leaf area was affected by substrate (F=29.56, df=4, p<0.0001), plant part (F=42.69, df=2, p<0.0001), and chemical treatment (F=16.31, df=3, p<0.0001). Plantlets raised on the sand substrate had the highest leaf area than those from soil, BSFF, vermiculite and composite (F=25.08, df=4, p<0.0001). In regard to plant parts, plantlets harvested from slips exhibited a higher leaf area than those from stems and crowns (F=38.86, df=2, p<0.0001). For chemical treatment, plantlets treated with sodium hypochlorite registered a significantly higher leaf are than those treated with IAA-hormone alone or in combination with sodium hypochlorite (F=10.55, df=3, p<0.0001). There was a significant interaction effect observed in the leaf area of plantlets generated from the different treatment. For example, leaf area of the plantlets depended on interactions between substrate and plant part (F=3.48, df=12, p<0.0001), substrate and chemical treatment F=df=5.14, df=8, p<0.0001), and between plant part and chemical treatment (F=3.3, df=6, p=0.0032). For substrate × plant part interaction (Figure 3a), plantlets from crowns grown on BSFF had the highest leaf area than those harvested from stems (F=3.83, df=2, p=0.0254) while plantlets harvested from slips raised in composite exhibited the highest leaf area than those harvested from stems and crowns (F=9.19, df=2, p=0.0001). In sand, plantlets harvested from slips had a higher leaf area than those from stems and crown (F=9.17, df=2, p=0.0001) whereas those harvested from slips in soil recorded the greatest leaf area compared to stems and crowns (F=17.25, df=2, p<0.0001). Similarly, plantlets harvested

from slips raised on vermiculite substrate produced leaves with the greatest leaf area than those harvested from stems and crowns at 90 DAT (F=26.37, df=2, p<0.0001). Regarding interactions between substrate and chemical treatment, plantlets treated with sodium hypochlorite and raised on composite had the greatest leaf area than those treated with water (control) and a combination of sodium hypochlorite and IAA-hormone (F=11.35, df=3, p<0.0001). In sand, plantlets treated with water (control) produced leaves with the greatest leaf area than those treated with IAA-hormone or in combination with sodium hypochlorite (F=8.42, df=3, p<0.0001). A higher leaf area was noted in plantlets treated with sodium hypochlorite and raised on vermiculite substrate than those treated with IAA-hormone alone (F=3.42, df=3, p=0.0179). In contrast, plantlets raised on BSFF and soil substrates exhibited no significant differences in leaf area at 120 DAT. For interactions between plant part and chemical treatment (Figure 3b), plantlets harvested from crowns treated with sodium hypochlorite had the highest leaf area those treated with a combination of IAA-hormone and sodium hypochlorite, IAA-hormone alone, and control (F=9.51, df=3, p<0.0001). Similarly, plantlets harvested from sodium hypochlorite treated stems had the greatest leaf area than those treated with IAA-hormone alone or water (control) (F=4.22, df=3, p=0.0061). However, there was no significant difference in the leaf area of plantlets harvested from slips across all chemical treatments at 90 DAT (F=2.19, df=3, p=0.0885). At 120 DAT, the leaf area of plantlets depended on substrate (F=2.98, df=8, p=0.0027), plant part (F=49.85, df=2, p<0.0001), chemical treatment (F=15.57, df=3, p<0.0001). Plantlets raised on the sand substrate had a significantly higher leaf area than all other substrates used (F=27.34, df=4, p<0.0001). For plant parts, plantlets harvested from slips exhibited the highest leaf area than those from stems and crowns, respectively (F=46.45, df=2, p<0.0001). In chemical treatments, plantlets obtained from plant parts treated with sodium hypochlorite had the highest leaf area compared to all other chemical treatments used (F=8.27, df=3, p<0.0001). Also, significant

interaction effects were observed between substrate and plant part (F=2.98, df=8, p=0.0027), substrate and chemical treatment (F=3.82, df=12, p<0.0001), and plant part and chemical treatment (F=0.62, df=6, p=0.0001) at 120 DAT. For example, in regards to substrate × plant part (Figure 3a), the highest leaf area was noted in plantlets harvested from slips than stems and crowns across all substrates used (p<0.0001), except for the BSFF substrate (p=0.4189). For substrate × chemical treatment, plantlets harvested from plant parts treated with sodium hypochlorite generally outperformed those from other chemical treatments across most of the substrates used (BSFF, p=0.0066; composite, p<0.0001; vermiculite, p=0.0031). In the sand substrate, plantlets treated with water (control) exhibited the highest leaf area compared to other chemical treatments (F=4.99, df=3, p=0.0023). However, there was no significant effect on leaf area of plantlets raised on soil substrate across all chemical treatments (p=0.6635). In relation to interactions between plant part and chemical treatment (Figure 3b), plantlets derived from crowns treated with sodium hypochlorite exhibited a significantly wider leaf area than plantlets from crowns treated with IAA-hormone or any other chemical treatment (F=13.29, df=3, p<0.0001). For plantlets harvested from slips (p=0.1525) and stems (0.0638), there was no significant difference in their leaf area at 120 DAT. Overall, plantlets derived from slips grown on soil and slips treated with sodium hypochlorite outperformed those derived from stems and crowns for most of the parameters measured across substrates and chemical treatments used.

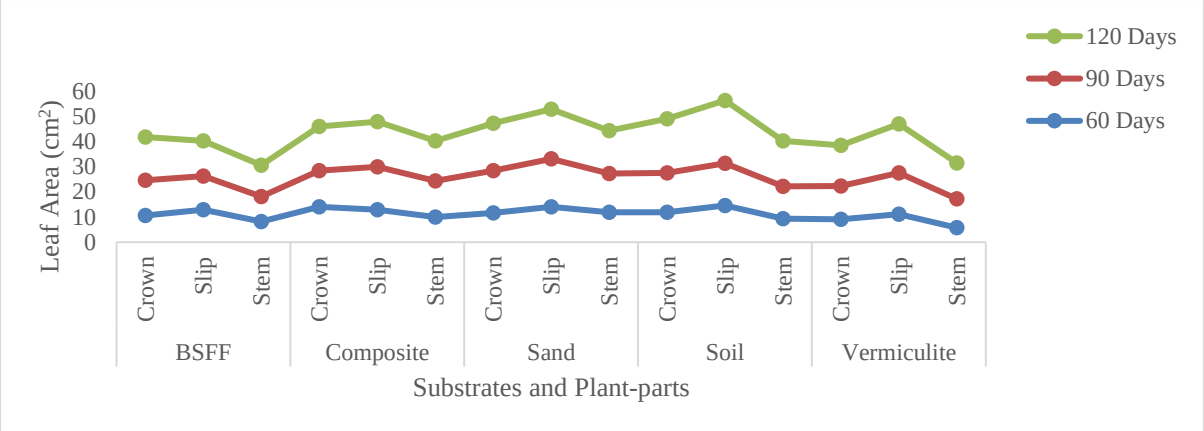

Figure 3a : Effect of substrate and plant part on leaf area of pineapple plantlets under each sampling time at Kyambogo University, 2024.

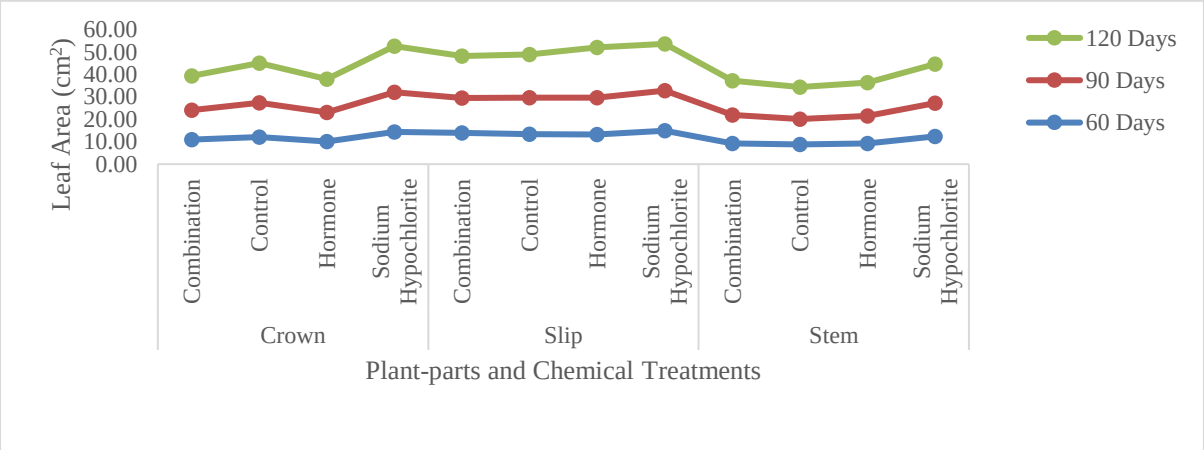

Figure 3b : Effect plant part and chemical treatment on leaf area of pineapple plantlets under each sampling time at Kyambogo University, 2024.

5.5.6 Effect of substrate, plant part, and chemical treatment on biomass

The above-ground and below-ground dry weights depended on the trial with the dry weights of plants recorded in experiment one being significantly higher than those registered in trial two (dry shoot weight [DSW], F=56.31, df=1, p<0.0001; dry root weight [DRW], F=25.77, df=1, p<0.0001). Thus, data analysis was carried out by experiment. In trial one, DSW depended on the substrate (F=4.67, df=4, p<0.0001), plant part (F=31.92, df=3, p<0.0001), and treatment (F=5.81, df=3, p=0.0072). However, DSW depended on the interaction between substrate and plant part (F=5.1, df=8, p<0.0001) (Figure 4a). Irrespective of substrate, plantlets harvested from slips registered a significantly higher DSW than those harvested from crowns

or stems (Composite, F=9.7, df=2, p<0.0001; sand, F=14.62, df=2, p<0.0001; soil, F=23.53, df=2, p<0.0001; vermiculite, F=33.17, df=2, p<0.0001) except for BSF substrate where DSW did not differ across plant parts (p=0.5691). There was also an interaction between substrate and treatment (F=2.9, df=12, p=0.0007) as well as between plant part and treatment (F=2.36, df=9, p=0.0129). On BSF, soil, composite and vermiculite substrates, DSW did not vary across treatments (p>0.05). However, on sand, plantlets treated with a combination of IAA and JIK registered a significantly higher DSW than the water-treated (control) plantlets (F=3.31, df=3, p=0.0224). For the interaction between plant part and treatment, there was no significant effect of treatments for plantlets that were harvested from slips (p=0.5128) and stems (p=0.8493). On the other hand, the DSW of plantlets that were harvested from crown and treated with JIK were significantly higher than for those treated with IAA (F=4.36, df=3, p=0.0054). For dry root weight (DRW), there was an effect of plant part (F=18.49, df=3, p<0.0001) but not substrate (p=0.1218) nor treatment (p=0.2918) on the final weight after harvest at 120 days after transplanting. However, DRW was affected by the interaction between substrate and treatment (F=2.73 df=12, p=0.0013, and between substrate and plant part plant (F=7.04, df=8, p<0.0001) (Figure 4a) but not between part and treatment (p=0.1446). Whereas no statistical difference was observed across plant parts for DSW of BSF-raised plantlets (p=1536), the plantlets harvested from slip and crown contained a significantly higher DRW than plantlets that were harvested from stems and raised on Vermiculite substrate (F=9.23 df=2, p=0.0002). There was no effect of treatment on DRW for plantlets that were raised on BSF, composite, sand and soil substrates (p>0.05); however, for vermiculite raised plantlets, the water-treated (controls) recorded a significantly higher DRW than the other treatments (F=5.14, df=3, p=0.0022). Similarly, no effect of treatment on DRW was observed for plantlets that were harvested from slips (p=0.4536) and stems (p=0.445); however, for crown-harvested plantlets, JIK-treated plantlets registered a significantly higher DRW than IAA-treated plantlets (F=3.01, df=3,

p<0.0314). Overall, plantlets derived from slips recorded the highest DSW and DRW across most of the substrates (80%) used during trial one. The heaviest DSW was recorded in plantlets derived from slips grown on vermiculite, while those from slips raised on soil had the highest DRW. In trial two, DSW depended on the substrate (F=15.28, df=4, p<0.0001), plant part (F=3.33, df=3, p=0.0365), but not on chemical treatment (p=0.0647). Also, DSW depended on the interaction between substrate and plant part (F=2.74, df=8, p=0.0057) (Figure 4b). On BSF (p=0.0771) and soil (p=0.4598) substrates, DSW was not affected by plant part. However, the DSW of plantlets that were harvested from slips and raised on composite (F=6.88, df=2, p=0.0015) and vermiculite (F=4.27, df=2, p=0.0163) substrates were significantly higher than for crown-harvested plantlets that were raised on the same substrates. For plantlets that were raised on sand, the DSW of plantlets that were harvested from stems was significantly higher than those of crown- and slip-harvested plantlets (F=4.52, df=2, p=0.0129). In contrast, DSW was not affected by the interaction between substrate and chemical treatment (p=0.7716) or plant part and chemical treatment (p=0.0601).

Equally, in trial two, DRW was affected by substrate (F=20.22, df=4, p<0.0001) as well as the interaction between substrate and plant part (F=3.18, df=8, p=0.0016) (Figure 4b). Generally, for BSF- and soil- raised pineapple plantlets, there was no effect of plant part on DRW (p>0.05). For composite-raised plantlets, the plantlets that were harvested from the crown recorded a significantly lower DRW when compared with those that were harvested from the slips or crowns and raised on the same substrate (F=7.69, df=2, p=0.0007). On the other hand, for sand-generated (F=3.45, df=2, p=0.0351) and vermiculite-raised (F=4.73, df=2, p=0.0106) substrates, plantlets that were harvested from the stems and slip, respectively, registered a significantly higher DRW compared to those harvested from the crowns. DSW, however, was not dependent on the plant part (p=0.0995) nor on treatment (p=0.1156). Similarly, there was no interactions between substrate and chemical treatment (p=0.3295) or plant part and chemical

treatment (p=0.0833). For trial two, plantlets derived from stems had heavier DSW and DRW across most of the substrates (60%), followed by those generated from slips.

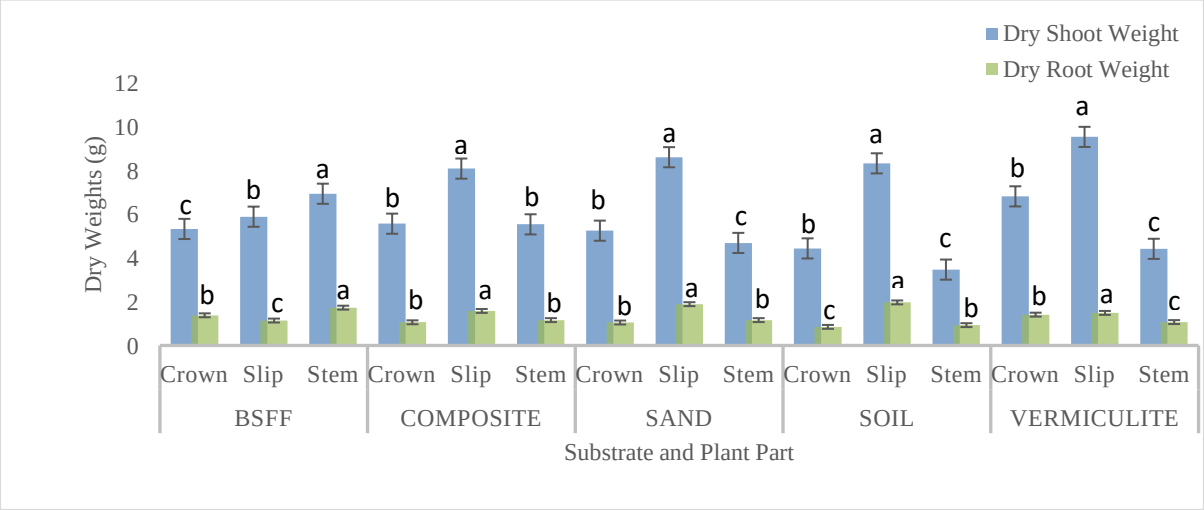

Figure 4a : Effect of substrate type and plant part on DSW and DRW of pineapple plantlets in trial one at Kyambogo University, 2024.

Bars with different letters indicate statistically significant differences at $p \leq 0.05$, Tukey's Studentized range test.

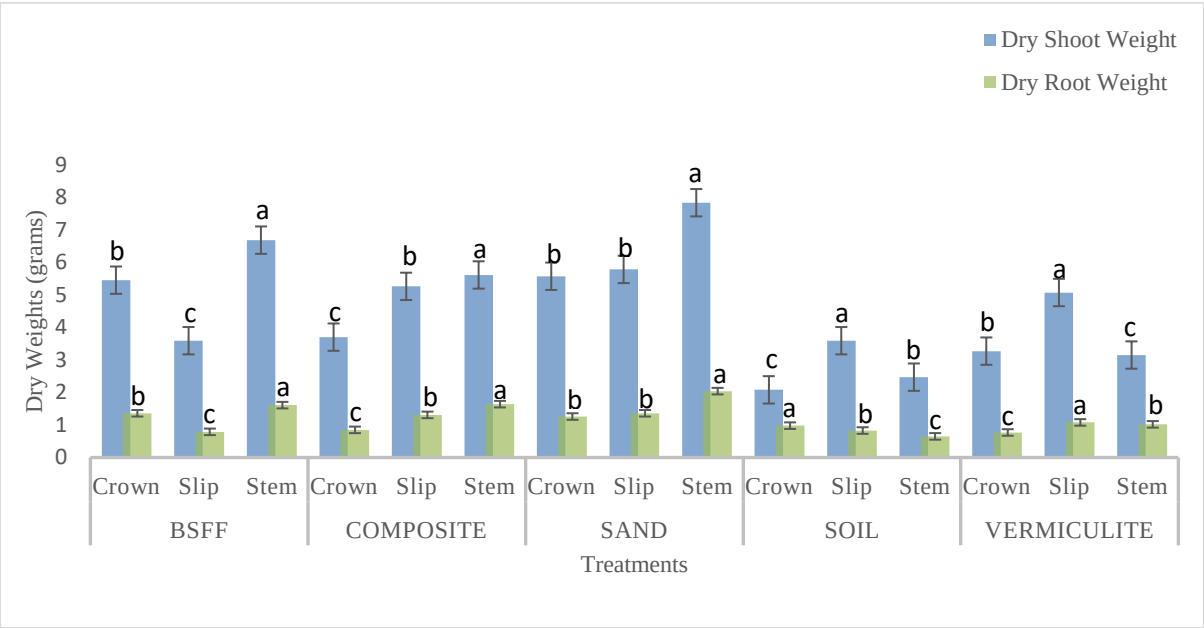

Figure 4b : Effect substrate and plant part on DSW and DRW of pineapple plantlets in trial two at Kyambogo University, 2024.

Bars with different letters indicate statistically significant differences at $p \leq 0.05$, Tukey's Studentized range test.

5.6 Discussion

5.6.1 Effect of substrate on morphological growth parameters

The type of substrate significantly affected the morphological traits of pineapple plantlets. For example, plantlets propagated using sand exhibited superior performance across all growth parameters assessed as opposed to the other substrates used in this study. The physical properties of sand, especially porosity and drainage are known to enhance root aeration and reduce waterlogging stress, facilitating optimal root development during the propagation stage (Kumar, 2015; Magdoff & Van Es, 2021). Besides, the developed root system could have enhanced efficient nutrient uptake of plantlets hence promoting good establishment and growth of the plantlets. Conversely, plantlets propagated in soil demonstrated inferior performance across most morphological parameters assessed yet it possessed favorable physiochemical properties including pH, soil texture, and organic matter content. Accordingly, the relatively high proportion of silt and fine particles may have contributed to the reduction of soil porosity hence, limiting oxygen availability to the developing roots. Moreover, the higher bulk density may have impeded root elongation and nutrient absorption during propagation (Lampurlanés & Cantero-Martínez, 2003; Magdoff & Van Es, 2021). Consequently, the combined effects of these limitations could have resulted in the restriction of the above above-ground growth and development among other.

5.6.2 Effect of plant part on growth performance of pineapple plantlets

Plant part significantly influenced growth performance over the 120-day propagation period. Slip-derived plantlets were more vigorous, outperforming those from stems and crowns in almost all morphological growth parameters, including leaf length, leaf width, and leaf area. These results align with earlier findings that slips possess a greater regenerative potential due to their active meristematic regions and substantial carbohydrate reserves (Reinhardt *et al.*, 2003; Duane *et al.*, 2012). These physiological traits could have enhanced faster development

of roots and shoots in the plantlets during the propagation stage, which consequently enhanced early establishment and growth during the 120-day trial period. In contrast, stems consistently underperformed, possibly due to limited active tissue or increased lignification, which may have restricted rooting and shoot initiation (Maisyarah *et al.,* 2019). Crown-derived plantlets also lagged, particularly in leaf number, possibly due to the persistence of apical dominance and slower vegetative reactivation (Monthe *et al.,* 2024).

5.6.3 Effect of chemical treatment on growth performance of pineapple plantlets

The findings of this study revealed that chemical treatments significantly influenced plantlet growth and development. Sodium hypochlorite-treated plant parts produced the most vigorous plantlets across most growth parameters. For example, plantlets from plant parts treated with sodium hypochlorite recorded the highest height, number of leaves, leaf length, and leaf area, suggesting a dual role of both a disinfectant and growth stimulant. Sodium hypochlorite reduces surface microbial load, enhancing tissue health and potentially stimulating cellular differentiation and consequently vigorous plantlets (Acheampong *et al.,* 2015; Amantayev *et al.,* 2023). On the other hand, IAA-hormone increased leaf width, but had limited positive effects on other growth parameter, and in other cases appeared to suppress shoot elongation. This could be attributed to the possibility of imbalanced exogenous auxin concentration (George *et al.,* 2008). The plantlets derived from plant parts treated with water consistently exhibited the poorest performance, confirming the value of chemical pre-treatment of plant parts in enhancing macro-propagation success.

5.6.4 Effect substrate and plant part on growth performance of pineapple plantlets

The interaction between substrate and plant part significantly influenced morphological outcomes in plantlets. The highest vegetative growth was observed in plantlets generated from slips grown in soil, which produced the longest leaves, widest leaves and largest leaf area. This synergy likely arises from combination of slip's physiological vigor (Reinhardt *et al.,* 2003;

Duane *et al.,* 2012), and soil's favorable physiochemical properties which could have enhanced plantlet development during propagation stage (Grossnickle & MacDonald, 2018; Magdoff & Van Es, 2021). Consequently, this may have contributed to good plantlet establishment and growth during the trial period. In contrast, stems grown in BSFF performed poorest, exhibiting the shortest leaves, narrowest leaves, and smallest leaf area. This may be attributed to BSFF's physiochemical limitations such as high pH, silt texture, and the likely effect of microbial imbalances, making it unfavorable for macro-propagation when used alone (Magdoff & Van Es, 2021; Lopes *et al.,* 2022) . Interestingly, while soil promoted elongation and leaf expansion, sand supported grater leaf proliferation in plantlets generated from stems, likely due to its better aeration facilitating multiple shoot formation. These findings highlight the importance of selecting compatible substrate-plant part combinations to enhance propagation efficiency.

5.6.5 Effect of plant part and chemical treatment on growth performance of pineapple plantlets

The interaction between pineapple plant part and chemical treatment similarly influenced plantlet performance during the trial period. Plantlets from slips treated with sodium hypochlorite exhibited the highest values for height, leaf length, leaf width, indicating a synergistic effect between the inherent regenerative capacity of slips and the antimicrobial and stimulatory properties of sodium hypochlorite. This finding supports previous studies showing that disinfected plant material exhibits improved early growth due to reduced microbial interference and enhanced root initiation (Reinhardt *et al.,* 2003; Duane *et al*., 2012; Acheampong *et al.,* 2015). Despite sodium hypochlorite influencing improved leaf elongation and width in plantlets propagated from slips, the highest number of leaves was observed in plantlets obtained from stems also treated with sodium hypochlorite. This could be a compensatory response, where stress induced by sterilization triggered axillary bud activation (Cox, 2018). On the contrary, IAA-hormone treatment especially in crowns and stems was

associated with poor performance of the resultant plantlets, suggesting inappropriate auxin concentration or timing may have interfered with shoot development (George *et al.*, 2008).

5.6.6 Effect of substrate and plant part on biomass accumulation in pineapple plantlets

Dry shoot and root weight, important indicators of a plant's good growth performance (Omotoso, 2014) varied significantly across substrate-plant part combinations. Plantlets derived from slips grown in vermiculite during trial one yielded the highest DSW. These results are consistent with literature attributing vigorous growth in slips to their metabolic activity and carbohydrate reserves (Reinhardt *et al.,* 2003**).** Vermiculite's favorable water retention and aeration also likely contributed to high biomass accumulation during the propagation stage which enhanced establishment and growth of plantlets (Kumar, 2015; Magdoff & Van Es, 2021). Stem-driven plantlets performed best in sand and BSFF during trial two, highlighting their adaptability to substrate with high organic matter content and pH. Black soldier fly frass seemed to favor stems more than slips or crowns, probably due to its nutrient profile and microbial composition, which may have benefited more lignified tissues in stems (Maisyarah *et al.,* 2019; Lopes *et al.,* 2022). However, performance in BSFF varied widely between trials, suggesting the need for further optimization of its physical and microbial properties to ensure consistency. Soil based propagation exhibited marked variation between trials possibly due to seasonal shifts in bulk density and moisture dynamics (Lampurlanés & Cantero-Martínez, 2003). This reinforces the need to consider substrate quality and stability over time in plantlet production. Crown-derived plantlets consistently produced least biomass, further confirming their low stability for high-efficiency macro-propagation.

5.6.7 Limitations to the study

This study was conducted under screen house conditions for a relatively short period of 120 days to evaluate the performance of pineapple plantlets generated from different substrates and

plant parts. While this controlled environment minimized external variability, it may not fully represent field conditions where plantlets are exposed to a wider range of biotic and abiotic factors such as soil microorganisms, temperature fluctuations, and rainfall variability. Additionally, the short evaluation period may have limited the observation of log-term growth responses and plantlet establishment potential. Therefore, further studies under field conditions and extended evaluation periods are recommended to validate and complement these findings.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The study aimed at optimizing macro-propagation protocols including different substrates, plant parts, and chemical treatments for the production of quality pineapple planting materials. The findings demonstrated that the quality pineapple plantlets and propagation success were significantly influenced by the choice of substrate, plant parts, and chemical treatments, respectively. Among the substrates used, soil and composite influenced the highest plantlet yield, while BSFF was the poorest substrate for propagation of pineapple plantlets. The superiority of composite and soil is attributed to their generally favorable textural conditions that could have promoted root development during plantlet propagation stage, and subsequently enhanced nutrient uptake during the growth phase. Additionally, soil exhibited a conducive pH for pineapple production. Sand and vermiculite, also influenced plantlet production, giving a comparably high plantlet yield respectively. Overall, a superior performance was observed in pineapple plantlets derived from soil across most of the measured growth parameters. Slips emerged as the most effective plant parts for macro-propagation, exhibiting superior performance across majority of plantlet morphological and biomass parameters. Their excellent performance is likely due to their advanced meristematic activity and carbohydrate reserves, making them more vigorous and responsive to favorable conditions during propagation and growth phases. Chemical treatment also exhibited significant effects on the growth performance of pineapple plantlets. Sodium hypochlorite-treated plant parts produced the most vigorous plantlets, likely due to the compound's dual function in microbial sterilization and stimulation of tissue regeneration. In contrast, the use of rooting hormone yielded inconsistent results, emphasizing the need for proper concentration and application of protocols. Furthermore, significant interactions were observed between substrate, plant part,

and chemical treatments. Notably, slips grown in soil and treated with sodium hypochlorite yielded the most robust plantlets morphologically supported by their physiological capacities. Conversely, stems and crowns propagated in BSFF or water (control) consistently underperformed, indicating that these combinations are less suitable for plantlet production.

6.2 Conclusions

This study demonstrated that different pineapple plant parts and substrates vary significantly in their suitability for macro-propagation of quality plantlets. Although stems produced the highest number of plantlets, slips consistently exhibited superior quality across all measured growth parameters. Crowns performed poorest in both yield and growth performance. Among substrates, composite and soil exhibited the highest potential for pineapple propagation, producing high plantlet yields and comparably good growth performance of the resultant plantlets, likely due to its favorable soil texture and pH. In contrast, black soldier fly frass (BSFF) produced the fewest plantlets and generally showed poor growth performance, although pre-treatment of plant parts with sodium hypochlorite enhanced plantlet establishment and growth across most parameters. These findings indicate that soil is a reliable growth medium for pineapple propagation, while BSFF, despite showing limited performance has potential for improvement through refinement and optimization.

6.3 Recommendations

Based on the findings and conclusions of this study, the following recommendations are proposed:

1. Slips and stems grown using soil should be prioritized by farmers and nursery operators for optimum propagation of quality pineapple planting materials, given their superior regenerative capacity and consistent performance.

2. Sand in combination with soil and vermiculite (composite) offers a balanced mix of aeration and nutrient retention, making it especially suitable for resource constrained small holder farmers which needs to be popularized.
3. Pre-treating planting materials with sodium hypochlorite enhances plantlet vigor and sanitation. Its integration into propagation protocols can reduce microbial contamination and improve plantlet establishment, particularly in smallholder or community nursery settings.
4. Future use of BSFF should involve pre-treatment or amendment with other materials to improve its physiochemical properties and microbial balance for pineapple propagation.
5. Additional studies need to be conducted to the determine optimal concentrations and application methods of rooting hormones, especially those in powder form.
6. Extension services and agricultural training programs should equip farmers and nursery operators with knowledge on the use of slips and stems in combination appropriate substrates (soil, sand, composite and vermiculite), and chemical treatments to ensure sustainable production of quality pineapple planting materials.
7. Further studies to evaluate performance under field conditions and extended evaluation periods are recommended to validate and complement these findings.

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