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Efficacy of biostimulants on growth, yield and quality of tobacco (*Nicotiana tabacum*) varieties in Zimbabwe



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A dissertation submitted in partial fulfillment of the requirements of the

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APPROVAL FORM

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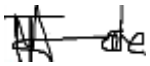
Dr. T Goche  20/06/2026

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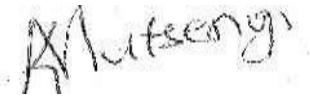
Mr K Mutsengi  19/08/2026

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DECLARATION

I, GOBOZA ARTWELL, registration number B222776B, do hereby declare that this dissertation is my own original work and has not been copied from any source without due acknowledgement of the source.

DEDICATION

I dedicated this dissertation to my family, Mr. and Mrs. Goboza and friends in appreciation of their unwavering love, encouragement and support throughout the whole project period.

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ABSTRACT

Tobacco (*Nicotiana tabacum L.*) is a major cash crop in Zimbabwe yet its productivity and leaf quality are increasingly threatened by declining soil fertility, climate variability and limited access to agronomic inputs particularly among smallholder farmers. Biostimulants have emerged as promising tools for enhancing crop performance under such challenging conditions yet their efficacy across tobacco varieties under Zimbabwean field conditions remain poorly understood. The study assessed the efficacy of biostimulants on the growth, yield and leaf quality of tobacco varieties under field conditions in Zimbabwe. The experiment was conducted as a 2 x 4 factorial arrangement laid out in a Randomized Complete Block Design (RCBD) with factor 1 being biostimulants which had 4 levels and the other factor being tobacco varieties which had 2 levels. The levels consisted of three biostimulants (Calcimax, Fulva wet and Hi-Humic Plus) and a control treatment (without biostimulant) and two varieties (KRK76 and KRK26R). The experiment had 8 treatments in each block which were replicated 3 times to give a total of 24 plots. Analysis of Variance (ANOVA) using GenStat 18th edition software was done at a 5% significance level test on data relating to plant height, stem thickness, leaf area, leaf quality (leaf colour classification) and yield. Biostimulants significantly ($p<0.05$) increased plant height, stem thickness, leaf area, leaf quality and yield mass with Fulva wet outperforming other biostimulants with the largest results on the variety KRK76 in all parameters on different weeks compared to the control treatments without any biostimulant which gave the lowest results. A significant interaction was also observed between the study factors for most of the measured parameters across the different weeks. In this study, the response of tobacco to biostimulant application varied according to the tobacco variety and the stage of crop development. This indicates that biostimulants did not affect all tobacco varieties and growth stages in exactly the same manner. The significant interaction therefore highlights the importance of considering both tobacco genotype and timing of biostimulant application when developing agronomic recommendations. Fulva wet and Hi-Humic Plus, applied to the KRK76 variety, offer the most reliable route to simultaneously improving tobacco growth, cured leaf quality and yield mass under the variable, dry-spell-prone rainfall conditions typical of Zimbabwe's smallholder tobacco-growing areas.

Keywords: biostimulants, fulvic acid, Hi-Humic Plus, quality, smallholder farmers, sustainability, tobacco, yield

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LIST OF ABBREVIATIONS

AMF – Arbuscular mycorrhizal fungi

ANOVA – Analysis of variance

CEC – Cation exchange capacity

CV – Coefficients of Variation

FAO – Food and Agriculture Organisation

IPCC – Intergovernmental Panel on Climate Change

LSD – Least Significant Difference of means

PGPR – Plant growth promoting rhizobacteria

SED – Standard Error of Difference of means

TIMB – Tobacco Industry and Marketing Board

WAT – Weeks after Transplanting

WHC – Water Holding Capacity

ZFU – Zimbabwe Farmers Uni

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Tobacco (*Nicotiana tabacum L.*) ranks among the most important cash crops in Zimbabwe which significantly contribute to foreign currency earnings, national income and rural livelihoods (Shonhe *et al*., 2022). The country has established itself as one of Africa's foremost tobacco producers and also ranks among the leading exporters globally (FAO, 2022; TIMB, 2025). Currently, production of tobacco is expanding, supported largely by smallholder farmers who are operating under contract farming arrangements which provide access to inputs, credits and markets seasonally (Scoones *et al.,* 2018; Moyo & Nyoni 2020)

Current trends in global and Zimbabwe tobacco production reflects a dynamic sector undergoing significant structural and agronomic transformation. At the global level, total tobacco leaf production has been gradually declining in major producing countries such as India, Brazil and China driven by tightening international regulations on tobacco use, reduced smoking prevalence in high income countries and increasing pressure from anti-tobacco health campaigns (FAO, 2023; World Health Organisation, 2023).

Zimbabwe has defied this international downward trend and demonstrated an upward trajectory in its tobacco production. According to the Tobacco Industry and Marketing Board (TIMB), 2025, the country's tobacco production exceeded 354 million kilograms during the 2024/25 season, representing some of the largest quantities produced since records began. As reported in Shonhe *et al*. (2022), the rapid increase in the number of smallholder farmers or A1 farmers (land reform beneficiaries allocated small-scale farming units under Zimbabwe's A1 resettlement model) participating in the national tobacco output process has facilitated these increases. Today, approximately 60 percent of all tobacco produced nationally is grown by smallholders or A1 farmers, up from less than 20 percent ten years prior.

Although there is a marked increase in volume being produced annually, another key issue confronting the Zimbabwean tobacco sector is the quality and value of leaf being produced. Reports from TIMB (2025), along with feedback from other industry stakeholders, suggest that a large portion of the tobacco being sold at auction in Zimbabwe over the past few seasons has received lower-than-average prices because of inconsistencies in leaf quality. Leaf color, texture and chemical composition are typically the most important elements that determine whether tobacco can be classified as high-quality. The reasons cited for these shortcomings include under fertilization and soil management practices as well as increased exposure to

climate variability and its subsequent impact on plant development (Mutetwa *et al*., 2020; Chikowo *et al*., 2022).

Climate variability has become a major concern regarding the continued sustainability of tobacco production in Zimbabwe. The erratic nature of rainfall events combined with increased temperature levels and extended periods without rain have resulted in less favorable growing conditions for crops, reduced yields, decreased leaf quality and ultimately stress on plants (IPCC, 2022; Chikowo *et al*., 2022). Climate-related stresses affect basic physiological functions of plants, including photosynthetic activity, nutrient absorption and biomass accumulation (Liu *et al*., 2024). While the shift to primarily smallholder farming has proven to produce greater volumes of tobacco, it has also exposed previously unaddressed issues concerning producer knowledge and/or lack of access to quality inputs necessary for producing premium-grade tobacco leaves (Scoones *et al*., 2018).

In this regard, there is growing emphasis on sustainable and environmentally responsive tobacco production, driven by both international buyer requirements and domestic policy imperatives. Major international tobacco companies including those operating in Zimbabwe through contract arrangements have increasingly incorporated sustainability criteria into their procurement standards, requiring growers to demonstrate compliance with good agronomic practices, reducing chemical inputs and soil health maintenance (FAO, 2022). The shift in market demands aligns with the global movement towards sustainable intensification which seeks to increase agricultural output while minimizing environmental degradation.

This can be viewed as an area where biostimulants could play a role in improving production systems for smallholders while enhancing sustainability objectives. Therefore, it would seem that evaluating biostimulants on smallholder farms in Zimbabwe an important activity at present and into the future (Rouphael & Colla, 2020; Vanlauwe *et al*., 2022). The term "biostimulants" was defined by du Jardin (2015) and also by Rouphael & Colla (2020), who describe biostimulants as natural substances or microorganisms which stimulate crop physiological processes such as photosynthesis and translocation, but do not supply nutrients.

The authors indicate that products derived from humic substances, fulvic acids, seaweed extracts, protein hydrolysates and beneficial microorganisms have demonstrated capacity to improve nutrient use efficiency, increase stress tolerance, enhance soil health and promote plant development overall (Mukaddas *et al*., 2025). Although global interest in biostimulants has been increasing, adoption of biostimulants among smallholder farmers in sub-Saharan

Africa remains limited due to the lack of locally generated evidence supporting their use and also lack of extension support for their use (Vanlauwe *et al*., 2022).

Advances in tobacco variety improvement have introduced new genotypes of tobacco which possess agronomically superior traits including improved disease resistance, higher yield potential and greater abiotic stress tolerance such as drought and heat (Bandara *et al*., 2025). While both KRK76 and KRK26R are commercial varieties grown in Zimbabwe, they represent different ends of the performance spectrum with KRK76 generally regarded as having higher vigour and being suited to fertile and well managed conditions (Tobacco Research Board, 2020). Thus, understanding how these commercial varieties respond to biostimulant application is fundamental to optimize production systems and ensure that the benefits of both varied improvement and innovation through biostimulants are fully realized under local field conditions.

Although biostimulants have shown positive effects in various crops, their efficacy is known to vary depending on crop variety, prevailing environmental conditions and product formulation (Mangano *et al*., 2020; Khan *et al*., 2023). Nevertheless, in Zimbabwe, there is a limited locally generated information on how biostimulants interact with tobacco varieties under field conditions. This therefore, creates uncertainty and limits their widespread adoption among tobacco farmers. This study therefore evaluates the efficacy of selected biostimulants (Calcimax, Fulva wet and Hi-Humic Plus) on the growth, yield and quality of tobacco varieties (KRK26R and KRK76) under Zimbabwean conditions with the aim of generating context-specific recommendations for sustainable tobacco production.

1.2 Problem statement

Despite the fact that tobacco is one of the major cash crops in Zimbabwe, poor and inconsistent leaf quality is major challenge in Zimbabwe's tobacco industry. Tobacco crops lose grade due to factors like poor color, weight, texture and chemical properties that result in lower price and less income for farmers. As to date, tobacco has settled approximately US$2.49 per kilogram dropping from roughly US$3.30 per kilogram the previous years. One of the major effect of this price fluctuation is based on quality grades. Poor root systems from inadequate nutrition, stressful climate and lack of suitable varieties all contribute to tobacco being graded poorly. Biostimulant products can help improve some of these issues with growing tobacco when used properly; however, there is very little biostimulant-related research in the specific context of Zimbabwe's agricultural conditions.

Droughts, late rains and hot temperatures associated with climate variability are negatively impacting tobacco yields and overall crop production and represent an ongoing risk to tobacco farming. Research has indicated that humic acid-based biostimulants, those based on fulvic acids and those made from seaweed can be effective at improving plant resistance to environmental stresses; however, the performance of these types of biostimulants on tobacco grown in Zimbabwe remain largely unknown. There also remains a need to understand which biostimulant performs best on each type of tobacco variety, in order to promote more sustainable methods of producing tobacco. Therefore, this project will investigate the effects of using biostimulants on tobacco varieties in Zimbabwe.

1.3 Justification of the study

This project was initiated in response to a need for a local evaluation of how various biostimulants impact the plant growth, yield and quality of tobacco plants using genetic types that are grown in Zimbabwe. A number of previous studies have been performed outside of Zimbabwe (Khan *et al*., 2020; Mangano *et al*., 2020) therefore the results from these studies can be used as general guidelines but lack the specific application to Zimbabwe's environment. This study will assist with providing information that has a direct relevance to local growers, researchers and manufacturers of agrochemicals. It will support the expansion of environmentally friendly methods of growing crops. Smallholder producers currently produce the majority of the tobacco produced in Zimbabwe.

However smallholder producers suffer from a significant amount of financial difficulty and many times they do not have an opportunity to obtain commercial fertilizer products. Therefore, using biostimulant based technologies could provide them with a cost-effective method of increasing crop yields. The data collected during this study will allow for better direction of extension services and contract-based farming programs. Also, the findings of this study will aid in furthering the policies set forth by the government of Zimbabwe promoting the use of sustainable and climate smart agricultural practices. Additionally, this study provides a basis for a comparative analysis of biostimulant technology which currently does not exist. The majority of biostimulant studies have focused on one biostimulant at a time. Thus, this study addresses a significant void concerning the differences in performance among genetically diverse tobacco varieties when exposed to multiple biostimulants. Therefore, the research will generate practical and scientific knowledge on the most effective biostimulant products for Zimbabwean tobacco production systems.

1.4 Objectives

1.4.1 General objectives

- To examine the effects of biostimulants (Calcimax, Fulva wet and Hi-Humic Plus) on growth, yield and leaf quality grade of tobacco varieties (KRK76 and KRK26R).

1.4.2 Specific objectives

- To determine the effects of Calcimax, Fulva wet and Hi-Humic Plus on growth parameters (plant height, stem thickness and leaf area) of different tobacco varieties.
- To assess the effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf quality grade of different tobacco varieties.
- To determine the effects of Calcimax, Fulva wet and Hi-Humic Plus on yield of different tobacco varieties.
- To examine biostimulant-variety interaction effects on growth, yield and leaf quality of tobacco

1.5 Research hypotheses

$H_{0.}$ There is no significant difference among the different biostimulants on growth parameters (plant height, stem thickness and leaf area) of the different tobacco varieties.

$H_{0.}$ There is no significant difference among the different biostimulants on yield of different tobacco varieties.

$H_{0.}$ There is no significant difference among the different biostimulants on leaf quality (leaf qualification symbols used in Zimbabwe) of the different tobacco varieties.

H_0: There is no significant interaction on growth, yield and leaf quality between biostimulants and tobacco varieties

CHAPTER 2: LITERATURE REVIEW

2.1 Production and uses of tobacco

Tobacco (*Nicotiana tabacum L.*) is cultivated for its leaves, which are processed into various products such as cigarettes, cigars, snuff and value added into other tobacco products that are being used in medicinal uses (Charlton, 2004; Duke *et al*., 2022). The crop remains one of the commercial commodities and cash crops both globally and in Zimbabwe, where it serves as the country's largest agricultural export and a major source of foreign currency (FAO, 2022; TIMB, 2025). Zimbabwe currently is Africa's leading producer of flue-cured tobacco and consistently ranks among the top five tobacco exporters globally (Bandara *et al*., 2025). According to FAO (2023) and World Bank (2023) tobacco contributes approximately 50% of agricultural exports earnings, 30% of total national exports and roughly 10% of the country's gross domestic product (GDP).

The steady increase in tobacco production in Zimbabwe has primarily come from the involvement of smallholder farmers that are supported by numerous buying firms, auction houses, transportation services and contracts. Contract farming systems have provided farmers with direct access to essential input supplies including fertilizers, seeds, agrochemicals, as well as credit for purchasing these items. These factors contribute significantly to continued stability in the amount of tobacco produced in Zimbabwe regardless of fluctuation in the economy or volatile markets (Scoones *et al*., 2018; Moyo and Nyoni, 2020).

2.2 Factors affecting tobacco production

Despite its economic importance and value, tobacco production faces mounting pressure from climate related stressors particularly elevated temperatures and drought which have emerged as critical threats to crop growth, development and productivity (Liu *et al*., 2024). Climate change-induced events such as El Niño related-droughts are known to reduce yield up to 10% during some seasons and impact leaf quality in Southern Africa (Chikowo *et al*., 2022; IPCC, 2022). Stresses from climate change impacts on plant physiology negatively impacting nutrient uptake, chlorophyll biosynthesis and photosynthesis lead to decreased biomass formation and reduced production of high-quality crops (Liu *et al*., 2024; Zhou *et al*., 2022).

Additionally, Liu *et al*. (2020) also stated that heat and drought stress caused by climate changes can alter enzyme and non-enzyme antioxidant systems causing an increase in reactive oxygen species (ROS), which leads to increased lipid peroxidation. These biochemical disorders will negatively affect other important aspects of crop physiology, thus photosynthetic

gas exchange, respiration and nitrogen metabolism, resulting in a decrease in biomass and quality of the crop. Furthermore, declining soil fertility and the rising cost of inorganic fertilizers continue to pose challenges for many smallholder farmers. The continuous reliance on inorganic fertilizers has also raised concerns about soil degradation, reduced microbial activity and increased soil acidity (Vanlauwe *et al*., 2022). These concerns have intensified interest in sustainable soil fertility management practices that integrate organic amendments, conservation agriculture and biostimulants to enhance productivity and resilience (Rouphael and Colla, 2020; Canellas *et al*., 2015).

2.3 Effects of biostimulants in agriculture

Climate change has emerged as the growing concern confronting global agriculture constraining both the quantity and quality of crop production. The agricultural sector faces increasing pressure to achieve sustainable productivity gains within the context of a changing climate. Biostimulants have been identified as a valuable tool for alleviating the negative effects of various environmental stresses on plants including drought, salinity, heavy metal toxicity and extreme temperature fluctuations (Zulfiqar *et al*., 2024).

Biostimulants are defined as substances or microorganisms that enhance plant growth and productivity by improving nutrient use efficiency, stress tolerance and crop quality without functioning as direct nutrient supply (du Jardin, 2015). The use of these products can enhance a plant's normal biological processes including increased photosynthesis, root growth and increased enzymatic antioxidant production (Brito *et al*., 2023). In addition, Mangano *et al*. (2020) identified improved hormone regulation, an increase in nutrient absorption by plants and ultimately an enhancement in total biomass for plants grown under stressful conditions with biostimulant treatment. Biostimulants generally work through many different biochemical pathways affecting gene transcription, metabolic pathways and phytohormones involved in the production of soluble osmotically active molecules and the reduction of oxidative stress through increased levels of antioxidants (Zulfiqar *et al*., 2024).

In many countries where soil fertility is declining and climate variability is increasing, biostimulants offer a promising sustainable strategy for improving crop productivity. Various studies have demonstrated consistence improvements in growth and yield following their application on crops including vegetables, maize and wheat (Zhang *et al*., 2022). The application has been associated with improved nutrient use efficiency, enhanced soil microbial

activity and greater crop resilience to abiotic stresses such as drought and salinity (Vanlauwe *et al*., 2022; Canellas *et al*., 2015).

However, there is still misunderstanding concerning the term biostimulant and many definitions have been proposed in the past (Drobek *et al*., 2019). Many definitions have tried to distinguish biostimulants and fertilizers, since some of the biostimulants can act as organic control agents and may be combined with fertilizers (du Jardin, 2015). Academic interest in biostimulants gained significant momentum following the World Congress on the Integration of Biostimulants in Agriculture held in Strasbourg in November 2012 (Silva *et al*., 2013). After the congress, the term biostimulant became competency material that improves plant growth without being categorized as a fertilizer or chemical crop protectants (du Jardin, 2015). However, it is important to recognise that the biostimulants have inherent limitations and should be used in conjunction with other crop management practices and sustainable resource strategies to ensure optimal yield and quality outcomes under climate change conditions (Zulfiqar *et al*., 2024).

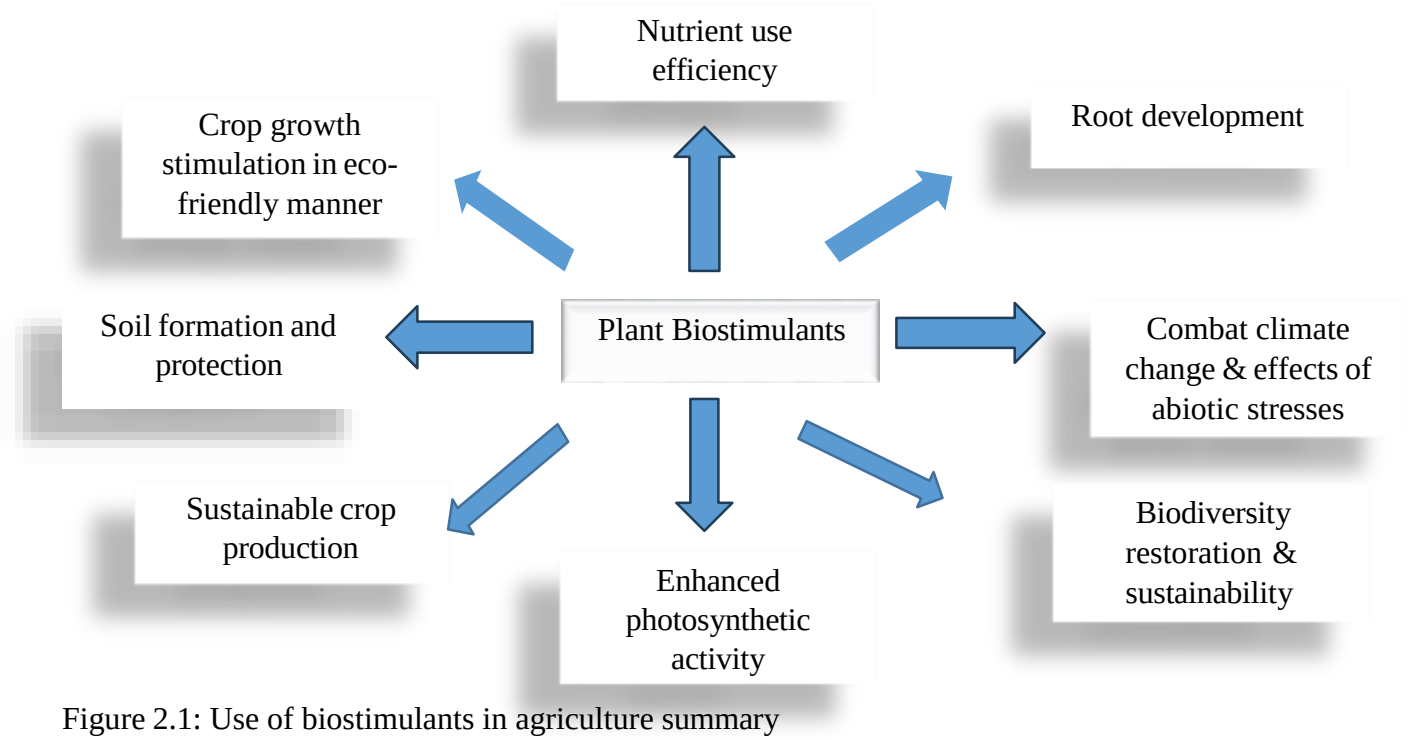

Figure 2.1: Use of biostimulants in agriculture summary

2.4 Application of biostimulants

Foliar sprays are a common delivery method for biostimulants, as is applying to the roots directly, but they also may be delivered via a soil drench or seed treatments. The best delivery

route will depend on the type of biostimulant being applied, what type of crops are being treated and at which point in their developmental cycle the plants are located. Foliar applications deliver nutrients quickly during critical growth periods, while delivering to the soil delivers these same nutrients to support root-microbe relationships and overall nutrient availability (Rouphael & Colla, 2020). The overall efficiency of biostimulant application is influenced by several factors including the crop's growth stage, product formulation, application rate and prevailing environmental conditions at the time of application (du Jardin, 2015). When biostimulants are used in combination with conventional fertilizers, their combined use can substantially improve nutrient use efficiency and overall crop performance (Drobek *et al*., 2019).

2.5 Types of biostimulants

According to the latest European Regulation 2019/1009, "plant biostimulants are defined as products that trigger the nutritional processes of plants independently of the nutrients it contains with the only aim of improving one or more characteristics of the plants or rhizosphere, which include the efficient nutrient use, tolerance to abiotic stress, qualitative features and the availability of nutrients kept in the soil or rhizosphere". Biostimulants are classified as beneficial microorganisms and broad range of natural and synthetic substances. These include humic substances, protein hydrolysates and amino acid-based products, macro and micro algae extracts and seaweed extracts, silicon, Arbuscular Mycorrhizal Fungi (AMF) and plant growth promoting rhizobacteria (PGPR) belonging to the genera such Azotobacter, Azospirilium and Rhizobium spp (European Union, 2019; Ruzzi *et al*., 2024). Both types have shown promise in enhancing crop performance.

2.5.1 Calcium based biostimulant

Calcium is an essential macronutrient that performs a critical function in plants including the maintenance of cell wall integrity, membrane stability, enzyme activation and signal transduction (White & Broadley 2003). Marschner (2012) also added that calcium contributes to the structural rigidity of cell walls by forming calcium pectate compounds which are vital for plant strength and resilience against abiotic stresses. Calcium also participates in signal transduction pathways that regulate plant responses to environmental stresses such as drought and soil salinity (Ahmad *et al*., 2022). Adequate calcium nutrition promotes root and shoots robustness, improves nutrient uptake capacity and enhances plant tolerance to adverse environmental conditions.

Fageria *et al*. (2009) pinpointed that deficiency of calcium often results in weaker root systems, reduced leaf expansion and compromised crop quality. In tobacco, calcium shortage can reduce leaf expansion and compromise curing quality, leading to significant economic losses and market volatility (Zhou *et al*., 2022). Foliar application of calcium-based formulations such as Super Cal 30 has been reported to enhance plant vigor, strengthen disease resistance and improve reproductive development (Fageria *et al*., 2009). Research by Choudhary *et al*. (2020) and Saeed *et al*. (2022) further highlighted that combining calcium based biostimulants with other micronutrients improve chlorophyll content, photosynthetic efficiency and water use efficiency particularly under stress conditions.

2.5.2 Seaweed extracts

The use of seaweed based biostimulants have gained momentum in crop production systems due to their unique bioactive components and effects. Bioactive components include phytohormones, polysaccharides, amino acids, vitamins and macro and micro nutrients that promote plant growth and development (Battacharyya *et al*., 2015; Shukla *et al*., 2019). They possess phytostimulatory properties that triggers plant growth and yield metrics in several important crops. Treatment by seaweed extracts and products influence a significant change in the microbiome components of soil and plant in support of sustainable plant growth (Ali *et al*., 2021).

In addition, Ali *et al*. (2021) highlighted that seaweed extracts are highly organic and they are ideal suited for organic farming and environmentally sensitive crop production. They also emphasized they have compatibility mode with other inputs thus paving way for an integrated management approach geared towards sustainability. They also evoke phytoelicitor (Shukla *et al*., 2019), triggering plant defence mechanisms that enhance tolerance to pests, diseases, drought, salinity and cold (Mukherjee *et al*., 2020). In semi-arid regions such as Zimbabwe, their hydrogel-like properties help retain moisture in the soil, improve root soil contact and boosting water use efficiency (Zulu *et al*., 2024). Studies conducted on crops such as tomato, maize, sugarcane and wheat have documented significant improvements in nutrient uptake, chlorophyll content and root biomass following seaweed extract application (Jacobassi *et al*., 2022; Khan *et al*., 2009).

2.5.3 Humic acid and Fulvic acid

Humic substances constituent a major fraction of soil organic matter and exert considerable influence on soil structure and fertility regimes. Humic and fulvic acids can be extracted from

soil and other organic sources and are applied to promote plant growth and increase nutrient availability and increase nutrient uptake efficiency (Martins *et al*., 2024; Aditi & Rohit, 2024). Humic acids have the capacity to trigger and stimulate plant growth and yield while suppressing certain diseases and enhances resistance to abiotic stresses.

Fulvic acid in particular has been shown to positively influence root development and to promote germination, growth rate and final crop yield (Shahrajabian *et al*., 2024). Similarly, Alsudays *et al*. (2024) reported that enriching soils with humic acids can increase the availability of nutrients, improve the physicochemical properties of the soil and promote both plant growth and yield. Canellas *et al.* (2015) further note that these substances can stimulate root elongation and increase the availability of key nutrients including phosphorus and various micronutrients.

Humic substances provide two ways to alter the way roots and shoots grow, which allow plants to better absorb and process nutrients. Humic substances may cause changes to a plant's primary and secondary metabolism pathways related to abiotic stress tolerance and ultimately affect a plant's overall fitness or "fitness" (Canellas *et al*., 2015) and therefore influence its ability to grow sustainably.

The inclusion of humic substances into cropping systems will allow for the implementation of sustainable intensive growing practices through an increased ability to create cation exchange sites, stimulate root growth and improve microbial populations within the rhizosphere (Kabra, 2024; Rouphael & Colla, 2020). Fulvic acids were used on tobacco crops and had the opportunity to increase crop yields when grown under low levels of nutrient availability and stressful conditions.

Fulvic acids contribute significantly to the improvement of soil structure, aeration and moisture retention and enhance soil oxygen levels available to plant roots and thus promote plant metabolic functions while reducing water run-off and conserving both water and nutrients. In addition, fulvic acids stimulate microbial action within the soil allowing for faster degradation of organic materials and releasing nutrients that are being held in reserve resulting in long-term soil fertility (Opperman & Specialties, 2023). As a biostimulant, fulvic acids activate plant hormones that promote root elongation, increase crop vigor and enhance biomass production leading to greater yields.

Moreover, fulvic acids facilitate photosynthesis by promoting the absorption of necessary nutrients such as magnesium and iron required for chlorophyll synthesis. By retaining water,

fulvic acids also decrease irrigation needs and waste in order to further support sustainable agriculture. Ultimately, through enhancements of soil quality and improvements to plant performance, fulvic acids assist in developing more environmentally responsible agricultural practices and sustainable agriculture (Pavadharini *et al*., 2025).

2.5.4 Protein hydrolysates

Through enzymatic or chemical hydrolysis of agro industrial proteins derived crop residues, legumes or animal by-products (Choi *et al*., 2015; Martin *et al*., 2022), these compounds have demonstrated capacity to alleviate environmental stress effects, improve vegetative growth and enhance overall crop productivity. Amino acids increase yield and yield components and quality characteristics while phenolic biostimulants improve and regulate oxidation-reduction processes, photosynthesis and various physiological activities (Sun *et al*., 2024). In leaf crops such as tobacco, they may improve leaf thickness, size and vigour by stimulating metabolic processes associated with plant growth and development. A study by Bozhinova (2022) indicated that application of protein hydrolysate trainer (PH Trainer) resulted in a 14.3% increase in nitrogen concentration within treated plants.

2.5.5 Chitosan oligosaccharide

Chitosan based biostimulants have attracted significant attention as potential solutions for improving soil health and optimizing nutrient uptake in agricultural systems (Riseh *et al*., 2026). Chitosan is a natural compound extracted from the polysaccharide chitin. This compound can be used to develop a plant's defense mechanisms for developing tolerance to both biotic and abiotic stress (El Hadrami *et al*., 2010). Chitosan helps plants adapt to stressful conditions through increased access to water and nutrients, an increase in the strength of the plant's antioxidant defense mechanism and the modulation of gene expression related to stress response.

The research of Hadwiger (2013) indicates that chitosan increases the activities of antioxidant enzymes which help strengthen the immune systems of plants against cold, salinity and drought stress. Additionally, chitosan has positive effects on the microbiology of the soil, it stimulates the activity of the beneficial microbes in the soil and creates symbiotic relationships that are beneficial to plant health. It was found that when chitosan was applied to tobacco plants there were significant improvements in root growth, nutrient absorption, and disease resistance. Specifically, Badawy and Rabea (2011) indicated that when tobacco plants received a chitosan treatment, they experienced improved root vitality, increased nutrient uptake, and significantly

reduced susceptibility to diseases such as mosaic virus and *Phytophthora nicotiana*, which causes black shank disease.

2.5.6 Microbial inoculants

Beneficial microorganisms including arbuscular mycorrhizal fungi (AMF) (*Glomus spp*.), *Bacillus* and *Pseudomonas* species have been widely documented to promote nutrient availability and improve stress tolerance in crop plants (du Jardin, 2015). Several studies have reported that microbial biostimulants can contribute to crop yield increase ranging from 10 to 40% (Tarigan *et al*., 2022; Nosheen *et al*., 2021). Mycorrhizal fungi extend root networks, improving water and phosphorus uptake (Ahmed *et al*., 2023). Trichoderma species release metabolites that stimulate root branching and nutrient absorption (Lopez-Bucio *et al*., 2015). Masunaka *et al*. (2011) reported that mineral solubilization in the rhizosphere constitutes one of the primary growth promoting mechanisms of the fungus *Trichoderma koningi*.

Microbial inoculants comprising primarily growth promoting bacteria and fungi exert their beneficial effects through several mechanisms including nutrient supply, hormonal production and pest suppression (Elnahal *et al*., 2022). These microbial inoculants can significantly improve tobacco growth by enhancing nitrogen fixation, phosphorus solubilisation and hormone production (Bal, 2025). These beneficial microorganisms improve soil biological activity and contribute to improved crop productivity by enhancing nutrient cycling and plant stress tolerance (Lu *et al*., 2024). A study specifically focused on tobacco reported that microbial inoculant application increased iron concentration in leaves by 25.5% relative to untreated control plants (Bozhinova *et al*., 2024).

2.6 Effects of biostimulants on tobacco plant height

Research shows that biostimulants, particularly microbial inoculants significantly increase tobacco plant height through improved nutrient uptake and hormonal (du Jardin, 2015). For instance, a pot and field trial application of *Penicillium sp*. PQxj3 (a plant growth promoting fungus) increased tobacco height by 50.19% in early growth stages and 24.05% at maturity relative to untreated controls (Zhang *et al*., 2019) The fungal inoculant promoted root development and nutrient uptake contributing to improved vertical growth. Similarly, *Bacillus* species improved seedling height under saline conditions by up to 91.94% (Musa *et al*., 2023).

These benefits in yield were due to a secondary effect on hormone activity (auxins and gibberellins) as well as through the mitigation of abiotic stresses. For example, drought and

salt stress (Rouphael & Colla, 2020), biostimulants also support an adequate root structure that allows for continued vertical growth during important developmental stages. Biostimulant incorporation into local tobacco production systems in Zimbabwe has increased average plant heights by greater than 45%, most notably in sandy loam soil environments where significant nutrient leaching occurs (Ndlovu *et al*., 2025).

2.7 Effects of biostimulants on tobacco stem thickness

The use of biostimulants especially in combination with biochar and microbial fertilizers, enhances stem diameter and structural integrity. According to Moyo *et al*. (2023), stem girth increased up to 11.48% at 56 days after planting and 9.92% at maturity. Similar findings by Zhou & Chitengu (2024) reviewed a 21.15% improvement when bio-organic fertilizers were combined with conventional fertilizers. These results are closely linked to improved calcium and potassium uptake and enhanced vascular development (Rouphael & Colla, 2020). Improved stem development enhances plant stability and improves the transport of water and nutrients within plant tissues thereby supporting overall plant growth and productivity.

2.8 Effects of biostimulants on tobacco leaf area

Tobacco leaf area has been identified as a key factor influencing photosynthesis and therefore was a focus of several studies. Moyo *et al*. (2023) indicated that the use of biochar treatments produced an up to 54% increase in leaf area when compared to untreated controls. However, biochar combined with peat moss did produce improved leaf expansion under conditions of water stress according to results from research conducted by Tariro and Zhou (2024). Research conducted within a controlled field environment using various applications of seaweed extracts for the purposes of studying the influence of these products on tobacco growth indicated that optimal application rates were capable of producing significant increases in tobacco plant biomass production, growth rate, photosynthesis and other photosynthetic parameters (Tian *et al*., 2025).

The observed effects of biostimulants were attributed to increased cell expansion, nutrient retention and chlorophyll synthesis due to stimulation of metabolic processes (Rouphael & Colla, 2020). In addition, microbial-based biostimulants including *Bacillus subtilis* and *Trichoderma harzanium* were found to positively affect leaf biomass and size (Mangano *et al*., 2020). Studies conducted indicated that biostimulants enhanced the growth and leaf opening of flue cured tobacco (Bozhinova, 2023).

2.9 Effects of biostimulants on tobacco leaf quality

Biostimulants also influence the chemical composition and processing quality of tobacco leaves. Humic substances have been shown to improve leaf quality by enhancing nutrient uptake and increasing the concentration of desirable chemical components such potassium (He *et al*., 2009; Pu *et al*., 2023). Improved nutrient balance contributes to better leaf texture, colour and aroma which are key quality parameters in tobacco production. The application of biostimulants therefore holds considerable potential for simultaneously enhancing both the yield and commercial value of tobacco crops. Studies performed reviewed that the application of fulvic acid exhibited superior performance comprehensive efficacy in enhancing soil quality, facilitating the growth of flue cured tobacco and improving tobacco characteristics. Therefore, it served as an appropriate biostimulant for flue cured tobacco cultivation (Zheng, 2024).

2.10 Effects of biostimulants on tobacco yield

Amino acids have shown great results when used as foliar applications with regards to enhancing leaf yields from tobacco (Muresan *et al*., 2019). They can also help increase leaf grade uniformity and promote better nutrient absorption. Seaweed extracts are another form of biostimulants that can be very beneficial to tobacco producers. These extracts are known to significantly increase leaf biomass and net profit for growers of broad-leafed crops and may provide similar benefits for tobacco (Singh *et al*., 2018). Additionally, research is being conducted into how PGPRs contained within various forms of biostimulants can positively impact leaf yields through improved nutrient utilization efficiencies and reductions in the amount of low-grade leaf produced (Khan *et al*., 2020).

Biostimulant application through microbial-based products has also provided evidence supporting that these products promote crop performance by enhancing root-soil interactions and nutrient availability (Rouphael and Colla, 2020). There is substantial research interest in humic and fulvic substances and their effects on leaf crops (El-Sayed and Abd El-Mageed, 2021). Research demonstrates that humic substances play a regulatory role in plant development, promoting root branching and improving nutrient uptake (Canellas & Olivares, 2014). Studies using tobacco and other closely related crops have illustrated that these developments result in greater amounts of usable leaf mass and an overall increase in yield (Abdo *et al*., 2022). Microbial inoculants were used to treat tobacco plants in a study, resulting in a 5.5 % increase in yield with Europlus and a 6.7% increase with PH Trainer over the untreated plants. This supports that biostimulants containing microbial inoculants or protein

hydrolysate can function as valuable tools for increasing both yield quantity and leaf quality in sustainable tobacco cultivation systems (Bozhinova, 2023).

2.11 Interaction effects between biostimulants and tobacco varieties

A major focus for research today is the relationship between different types of biostimulants and the genetic background of tobacco varieties. Biostimulant efficacy varies among genotypes, or varieties, of crops due to variations in their genetic backgrounds (Mangano *et al*., 2020; Khan *et al*., 2023), with some genotypes responding better to certain treatments than others. Differences in root structure, hormone sensitivity, absorption of nutrients, and stress response contribute to these differences. For example, high vigor crops have more developed root structures and will take up and utilize bioactive compounds from biostimulants at a faster rate than low vigor crops (Rouphael & Colla, 2020). Flue-cured tobacco varieties like KRK76 which have larger leaves and higher photosynthesis rates (Tobacco Research Board, 2020) will likely be more responsive to biostimulants that mimic auxins and promote chlorophyll production than other varieties.

Zulfiqar *et al*. (2024) also reported that when biostimulants are applied under stressful conditions the degree of benefit derived from application depends on the ability of the crop to tolerate the level of stress it is experiencing. If a variety has a broader range of genes responsible for stress tolerance, then the application of biostimulants can produce additive effects. Conversely, if a variety is highly sensitive to stress, then its response to biostimulants can vary. Therefore, conducting multiple variety trials under local conditions is essential to developing recommendation specific to individual varieties rather than relying solely on results obtained using one variety. Likewise, Brito *et al*. (2023) found that the increase in antioxidant enzyme activity caused by biostimulants was significantly greater in tolerant varieties than in sensitive varieties. Ultimately, this translates into differences in plant growth characteristics, leaf quality, and yield between varieties receiving the same biostimulant treatment regime.

CHAPTER 3: METHODOLOGY AND EXPERIMENTAL DESIGN

3.1 Study Site

The experimental study was conducted in Mashonaland West Province, Zimbabwe, at Farm 4 Ndiripo, located within Tengwe-Masikati area of Hurungwe District, Karoi, with GPS coordinates -17.104478; 29.655245. The site lies under agroecological region iib of Zimbabwe which is characterised by sandy loam soils ideal for flue cured tobacco production. Annual rainfall at the site ranges between 500-700 mm with pronounced long dry spells, predominantly during the summer months, and approximately 14-16 rain pentads recorded per growing season. Mean annual temperature averages between 21°C and 23°C while average maximum daytime temperatures peak around 26°C to 30°C.

3.2 Experimental Design and field layout

The experiment was conducted as a 2 x 4 factorial treatment structure laid in a Randomized Complete Block Design (RCBD). The experiment involved 2 main factors which were investigated, factor 1 being tobacco (*Nicotiana tabacum L.*) varieties which had 2 levels namely KRK76 and KRK26R, whilst factor 2 consisted of 4 biostimulants treatments which had 4 levels namely Calcimax, Fulva wet, Hi-Humic Plus and an untreated control (no biostimulant). Each treatment combination was replicated 3 times, yielding a total of 24 experimental plots. Land slope was used as the blocking factor to minimize field variability across replications.

Table 3.1: Treatments used to study the effects of different biostimulants on two tobacco varieties

Symbol	Treatment Description
CalK26	Calcimax and KRK26R
HiK26	Hi-Humic Plus and KRK26R
FulK26	Fulva wet and KRK26R
CK26	Control and KRK26R
CalK76	Calcimax and KRK76
FulK76	Fulva wet and KRK76
HiK76	Hi-Humic Plus and KRK76
C76	Control and KRK76

Where: P1, P2 and P3 represent plant biostimulants

K1 and K2 represent tobacco varieties

C represents control treatment (no biostimulant).

Field Layout of the Experiment

Randomised Complete Block Design (RCBD)

2 x 4 Factorial Arrangement (3 replications)

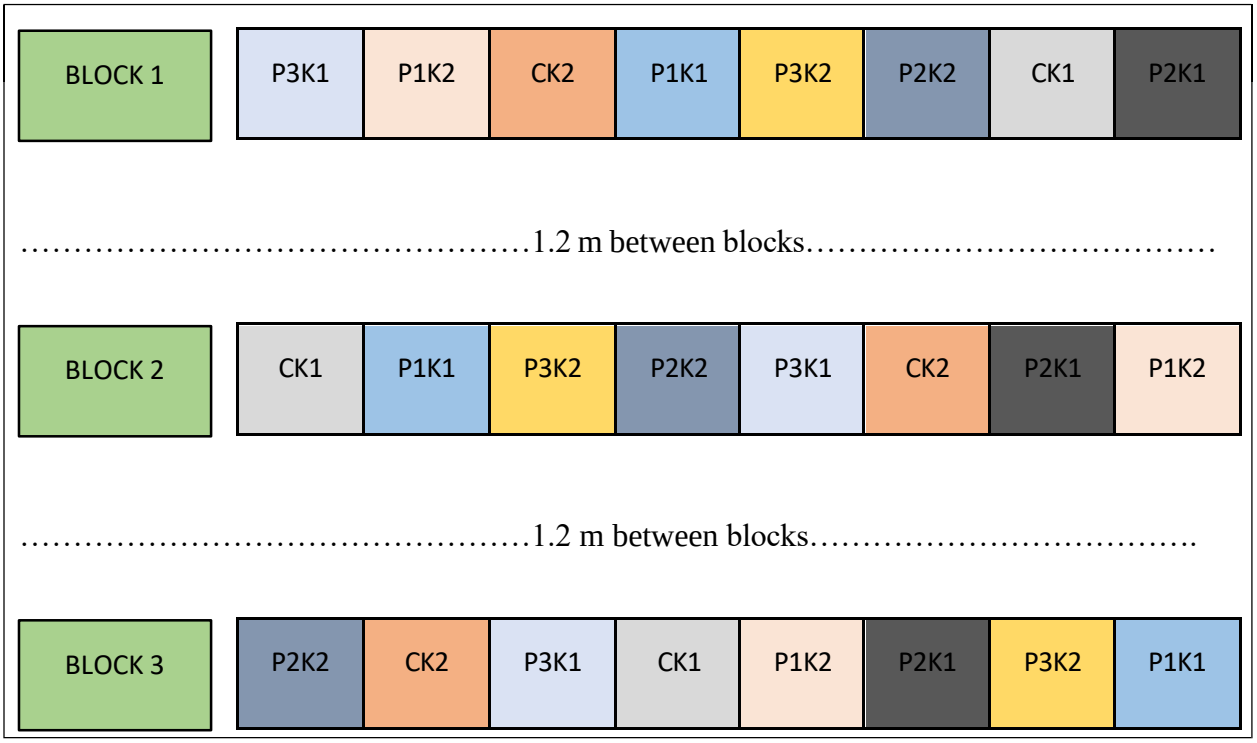

Figure 3.1: Experimental design layout

Layout information

Plots per block 8

Total plots 24

Inrow spacing : 55 cm

Inter-row spacing : 1.2 m

Blocking factor : land slope

3.3 Materials and Methodology

The following materials were used during the field experiment:

3.3.1 Tobacco Varieties

Two tobacco varieties, KRK76 and KRK26R were selected for use in this study. The selection was based on their established yield potential, maturity rate characteristics, ripening rate, adaptability to local growing conditions and resistance to major tobacco diseases.

3.3.2 Biostimulants

Three commercially available plant biostimulants obtained from BOLTAGRO SOUTHERN AFRICA and DAMARA BIO AGRI were used in the study:

1. Calcimax (DAMARA BIO AGRI).

2. Fulva wet (BOLTAGRO SOUTHERN AFRICA)

3. Hi-Humic Plus (BOLTAGRO SOUTHERN AFRICA)

Table 3.2: Indicative chemical composition of the biostimulants used in the study

Product	Category / active basis	Typical composition (indicative)	Physical form
Calcimax	Calcium-based biostimulant	Water-soluble calcium (Ca), typically formulated as calcium chelate/complex with minor N and trace micronutrients (e.g. B, Zn) to aid calcium uptake and translocation	Liquid
Fulva wet	Fulvic acid-based biostimulant	Fulvic acid (extracted from humic substances such as leonardite/peat), low-molecular-weight organic acids acting as natural chelators, trace amounts of K and micronutrients	Liquid, water-soluble
Hi-Humic Plus	Humic acid-based biostimulant	Humic acid derived from humic substances (leonardite/oxidised lignite), with associated fulvic acid fraction, minor K and micronutrient content	Liquid

3.4 Agronomic Practices

3.4.1 Land preparation

Primary tillage was performed using a tractor-drawn mouldbould plough to a working depth of approximately 30 cm, which served to enhance soil aeration, facilitate root penetration and improve water infiltration capacity. Secondary tillage was subsequently carried out using a disk harrow to produce a fine tilth suitable for ridge formation. Ridges were constructed at an inter-row spacing of 120 cm and an in-row spacing of 55 cm. Each ridge constituted an individual treatment plot, with each block containing eight ridges, totaling twenty-four ridges across the three replications.

3.4.2 Planting

Transplanting of tobacco seedlings was conducted in late November, timed to coincide with the onset of seasonal rainfall. Seedlings were placed upright at the centre of each ridge to ensure adequate soil-root contact and to prevent the formation of air pockets around the root zone. Following transplanting and watering, plants were covered with dry soil using a mulch sealing technique (locally referred to as sima-dhinda) to conserve moisture.

3.4.3 Biostimulant application

Each biostimulant was applied strictly in accordance with the manufacturer's recommended guidelines and the specific treatment allocations assigned within the experimental design.

Table 3.3: Biostimulant application rates and details

BIOSTIMULANT	RATE/HA	APPLICATION DETAILS
Calcimax®	20 l/ha	Foliar applied (full cover) at 3 and 6 weeks after transplanting
Hi-Humic Plus®	20 l/ha	Soil applied soon after transplanting
Fulva wet®	500 ml/ha	Incorporated into soil during planting and foliar applied at 4 weeks after transplanting

3.4.4 Fertilizer application

Basal fertilizer (High C) was applied using a number 24 cup at 300 kg/ha, placed approximately 10 cm from each plant ten days after transplanting. The fertilizer was distributed evenly around each plant using the double dolloping method, applying 12 cup per side. Top dressing with Ammonium Nitrate (AN) was subsequently carried out at three weeks after transplanting using a number 5 cup per plant at 60 kg/ha and following the hill placement technique to direct nutrients to the root zone.

3.4.5 Irrigation

Throughout the entire growing period, the crop depended exclusively on natural rainfall with no supplementary irrigation applied. Total rainfall received during the growing season, spanning from November 2025 to March 2026 amounted to approximately 500 mm. Importantly, the total seasonal rainfall received was not, in itself, the limiting factor for crop growth; rather, it was the temporal distribution of that rainfall that constrained the crop. Severe mid-season dry spells, particularly during a critical vegetative-to-early-flowering window, produced intermittent but agronomically significant moisture-stress conditions even though the seasonal total fell within the range considered adequate for a full tobacco growth cycle.

Figure 3.2 illustrates this pattern schematically: the season opened with a slow start, built to good rains in December, then collapsed into a pronounced dry spell in early-to-mid January before rains resumed through February and tailed off in March.

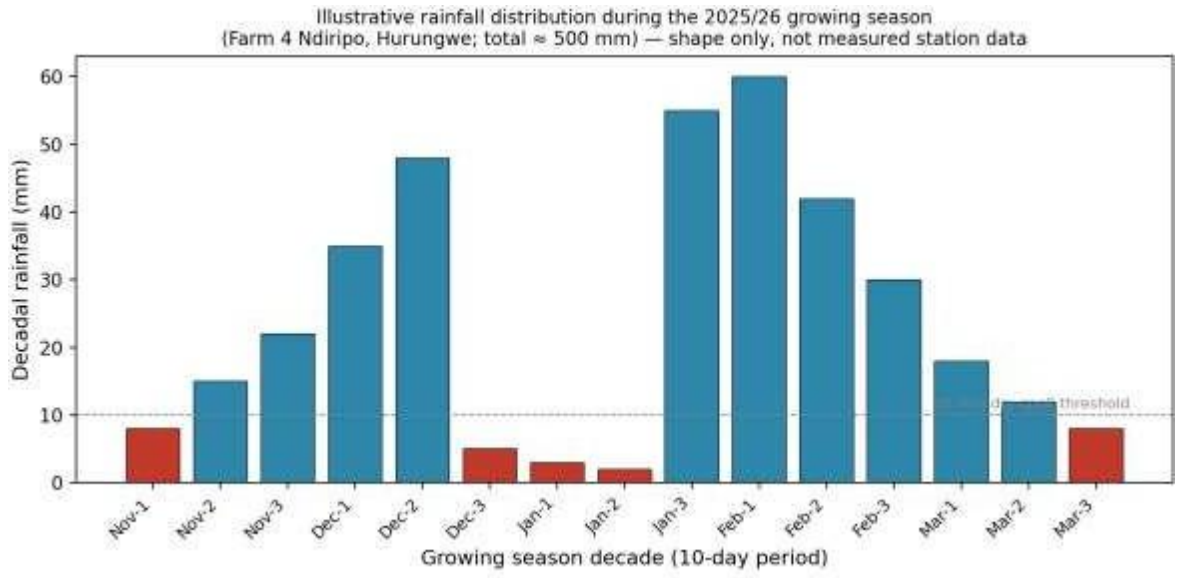

Figure 3.2: Illustrative decadal rainfall distribution during the growing season (Nov 2025-Mar 2026), showing the pronounced mid-season dry spell despite an adequate seasonal total

3.5 Crop Protection

3.5.1 Weed control management

Weed management was achieved primarily through manual hand hoeing which minimised competition between the crop and weeds for nutrients, light and soil moisture. A pre-emergence herbicide, Dual Magnum® 90 EC, was applied at a rate of 1.5 L/ha three days after transplanting using a knapsack sprayer to further reduce weed pressure during the early crop establishment phase and creating a weed free environment.

3.5.2 Insect pest control

At the time of planting, insecticides Imidacloprid and Optimator were applied as soil drenches at a rate of 30 cup per plant to provide early season protection against soil dwelling pests. Cutworm infestations were managed through the targeted application of Bullet insecticide. Budworms and leafminers which commonly affect tobacco crops during vegetative phase, were controlled through foliar spray applications of Bullet, Belt and Voltstar at the rates specified in table 3.3.

Table 3.4: Pesticides and application rates

PESTICIDE	RATE/HA	APPLICATION DETAILS
Optimator®	250 ml/ha	Soil drench at planting (30 cup per plant) for cutworm control
BELT®	100 ml/ha	Applied at first budworm appearances using knapsack sprayer
BULLET®	50 ml/ha	Applied as foliar spray for leafminers and budworms
IMIDACHLOPRID	440 ml/ha	Soil drench at planting (30 cup per plant) for aphids, termites and false wireworms

3.5.3 Disease management

Disease control was achieved through an integrated approach combining cultural practices with scheduled fungicide applications. Cultural control measures included the removal of previous

crop residues and maintenance of weed-free field conditions to prevent pathogen buildup and spread.

Table 3.5: Fungicides and application rates

FUNGICIDE	RATE/HA	APPLICATION DETAILS
AZOXY DUO	400 ml/ha	Full foliar spray at 4, 6, 8 and 10 WAT for control of Alternaria and Frogeye

3.5.4 Pruning

Pruning involved the selective removal of the four lowest leaves on each plant, commonly referred to as seedbed leaves. These leaves were removed because they were highly susceptible to diseases carried over from the seedbed nursery, they use up nutrients and fertilizers that would otherwise support productive leaf development and they could not be harvested for commercial use.

3.5.5 Topping and suckering

Topping was performed during the extended bud growth stage. In order for that to be determined, 10 percent of the plants' flower buds had to be observable above their uppermost set of leaves. Tobacco is typically ready to be topped at about 9 weeks post-transplant. The process of topping entailed the removal of an apex (apical) bud on each tobacco plant when there were 18 "harvestable" leaves. The topping process occurred over a total of three stages; with the third and last stage being finished exactly seven days after the beginning of the first stage.

After topping, the axial shoots or suckers started to grow at leaf axil. Suckering occurred immediately upon topping and all suckers greater than two centimeters in size were removed by hand. Suckercide was then applied directly onto the suckers to prevent additional suckering using squeeze bottle method. For this experiment, Flumetrin (a combination of a systemic and contact suckercide) was used to kill and stifle sucker development. At a ratio of 20 liters of water to 0.30 litres of Flumetrin, Flumetrin was applied directly onto each plant from the top so that the Flumetrin flowed downward along the main stem and came into contact with every leaf axil. Care was exercised while spraying to avoid damaging the topmost leaves.

3.5.6 Reaping and curing

Leaves are ready for harvest when visual signs of leaf senescence occur (change in leaf colour from dark green to light green, mottling on older leaves, downward bend of the leaves and the "snap" produced when an old, mature leaf is pulled off). Reaping commenced from the bottom of the plants working up through lugs and cutters until all leaves are removed. Plant leaves were harvested at a weekly basis following a standard 3 leaves per plant removal. No baboon reaping was exercised during the experiment. This was done to avoid mixture of reaps for correct leaf quality and yield assessment. Selected leaf samples were tied together in bundles (T2 strings) for subsequent curing in a conventional rocket barn that used wood as the fuel source.

The curing process involved 4 distinct temperature-controlled phases (colouring phase, colour fixing, leaf drying and Mid-rib drying). The first 72-hour colouring phase occurred at 30-38 degrees celsius. Following this was the 24-hour colour fixing phase, occurred at 38-43 degrees celsius. Then the leaves were dried at 50-55 degrees celcius for 24 hours. Finally, there was the mid rib drying phase lasting 48 hours at 60-70 degrees celcius and within the same 48 hours an overall drying at 70 degrees celcius was achieved and caution was taken to avoid production of red 'moto' tobacco leaves. This sequential curing process allowed the producer to ensure proper leaf colouration, moisture removal and chemical reactions required for the production of marketable quality flue-cured tobacco.

3.6 Data Collection

3.6.1 Plant height (cm)

Plant height measurements were recorded at 2-week intervals from the 4th week up to the 8th week after transplanting using a measuring tape. Five plants per treatment were randomly selected and permanently tagged for repeated measurements throughout the monitoring period. Each height measurement was taken from the point where the main stem emerged from the soil surface to the apical bud and measurements were recorded in centimetres. A total of 3 measurement occasions were conducted across the monitoring period.

3.6.2 Leaf area (cm^2)

Leaf area was assessed at 2 weeks intervals from the 4th week upto the 8th week. Five randomly selected plants per treatment were permanently tagged for continuous measurement of leaf area in centimetres. A 1 m ruler and a string were used to determine the length and maximum width

of the largest fully expanded leaf on each selected plant. Leaf area was subsequently calculated using the following formula:

LA=L x W x K,

Where;

- LA = leaf area (cm^2)
- L = length of the leaf (cm)
- W = width of the leaf at its widest point (cm)
- K = correction factor (K = 0.75) (Elsahookie *et al*., 1993)

3.6.3 Stem thickness (cm)

Stem thickness was measured at 2 weeks intervals from the 4th week upto the 8th week after transplanting. A string was wrapped around the stem circumference on each tagged plant and the measured length of the string was then read against a ruler to determine stem thickness. Five plants per treatment were randomly selected and permanently tagged for repeated measurements throughout the monitoring period. Stem thickness was recorded in centimetres across 3 measurement occasions.

3.6.4 Leaf quality (grade index)

Leaf quality assessment was conducted through visual inspection of cured leaf colour and tactile evaluation of leaf texture. The same 100 leaves used for yield mass measurements were assessed for quality classification purposes. Leaves were assigned to quality lots numbered from 1 to 5, with lot 1 representing the highest quality grade (Choice) and lot 5 representing the lowest quality grade (low). Leaves exhibiting high texture and golden leaf colour were assigned to lots 1 and 2. All quality classifications were conducted in accordance with the major classification symbols officially recognized and used in Zimbabwe as presented in table 3.5.

Table 3.5: Major leaf quality classification symbols used in Zimbabwe

Lot	Grade
1	Choice
2	Very good
3	Good
4	Fair
5	Low
NG	Poor

3.6.5 Yield Mass (kgs)

Yield mass was determined soon after completion of curing. 50 sample of cured leaves per treatment was collected and weighed using a digital balance to determine the yield mass expressed in kilograms per plot. This was done based on the reaping number.

3.7 Data Analysis

All collected data during the study were subjected to Analysis of Variance (ANOVA) using GenStat 18th Edition statistical Software. Where statistically significant treatment differences were detected, mean separation was performed using the Least Significance Difference (LSD) test at 5 % significance level ($p \leq 0.05$). This approach was applied consistently across all variables where significant effects were identified.

3.8 Experimental model

The statistical model employed was as follows:

$$\mathbf{Y_{ijk} = \mu + b_i + p_i + v_k + (pv)_{jk} + e_{ijk}}$$

Where:

$\mathbf{Y_{ijk}}$ = Observation of the i- block, j- biostimulant and k- variety

$\boldsymbol{\mu}$ = Overall experimental mean

b_i = Block effect

p_i = Biostimulant effect

v_k = Variety effect

$(pv)_{jk}$ = Interaction effect between biostimulant and variety

e_{ijk} = Experimental error

CHAPTER 4: RESULTS

4.1 Effects of Calcimax, Fulva wet and Hi-Humic plus on growth parameters of different tobacco varieties

4.1.1 Effects of Calcimax, Fulva wet and Hi-Humic Plus on plant height of different tobacco varieties

According to results from table 4.1, there was a highly significant interaction between biostimulant and variety on plant height across all measurement periods (P=0.001). Biostimulant treatment independently exerted a highly significant effect on plant height (P=0.001). Variety similarly demonstrated a highly significant independent effect on plant height (P=0.001).

Table 4.1: Effects of Calcimax, Fulva wet and Hi-Humic Plus on plant height of different tobacco varieties

	Weeks after planting		
Treatment	**4**	**6**	**8**
Hi-Humic + KRK26R	18.633^{b}	65.17^{b}	96.33^{d}
Calcimax + KRK76	18.9^{b}	60.5^{e}	105^{b}
Control + KRK76	15.567^{d}	52.5^{f}	99^{c}
Calcimax + KRK26R	18.233^{c}	65.83^{b}	97.83^{cd}
Hi-Humic + KRK76	19.467^{a}	63.83^{c}	112.83^{a}
Fulva wet + KRK76	19.7^{a}	61.83^{d}	114.5^{a}
Control + KRK26R	16.333^{c}	63.67^{c}	93.5^{e}
Fulva wet + KRK26R	18.6^{b}	67.17^{a}	101^{c}
Grand Mean	18.179	62.56	102.5
P-value	0.001	0.001	0.001
SED	0.2073	0.543	1.097
LSD	0.4446	1.164	2.352
CV%	1.4	1.1	1.3

Based on the range test at $p \leq 0.05$, means sharing the same letter within a column are not significantly different from each other.

4.1.2 Effects of Calcimax, Fulva wet and Hi-Humic Plus on stem thickness of different tobacco varieties

Table 4.2 showed that at 4 WAT, no significant interaction was observed between biostimulant treatment and tobacco variety on stem thickness (P=0.303). However, highly significant interactions between biostimulant treatment and variety on stem thickness were recorded at both 6 WAT and 8 WAT (P=0.001). Biostimulant treatment exerted a highly significant independent effect on stem thickness (P=0.001). Variety also demonstrated a highly significant independent effect on stem thickness (P=0.001) at 4 and 8 WAT and 6 WAT (P=0.002). At 8 WAT, Fulva wet + KRK76 and Calcimax + KRK26R both measuring 11.833 cm recorded the highest stem girth, whilst control + KRK26R recorded the lowest stem girth at 9.433 cm.

Table 4.2: Effects of Calcimax, Fulva wet and Hi-Humic Plus on stem thickness of different tobacco varieties

	Weeks after planting		
Treatment	**4**	**6**	**8**
Hi-Humic + KRK26R	5^{a}	9.3^{c}	10.5^{b}
Calcimax + KRK76	5.533^{a}	9.9^{a}	11.833^{a}
Control + KRK76	4.833^{a}	8.267^{e}	9.9^{c}
Calcimax + KRK26R	5.067^{a}	9.367^{c}	9.433^{d}
Hi-Humic + KRK76	5.467^{a}	9.667^{b}	11.7^{a}
Fulva wet + KRK76	5.6^{a}	9.967^{a}	11.833^{a}
Control + KRK26R	4.633^{a}	8.733^{d}	9.433^{d}
Fulva wet + KRK26R	5.1^{a}	9.667^{b}	10.5^{b}
Grand Mean	5.154	9.358	10.838
P-value	0.303	0.001	0.001
SED	0.121	0.0949	0.1006
LSD	0.2595	0.2036	0.2158
CV%	2.9	1.2	1.1

Based on the range test at $p \leq 0.05$, means sharing the same letter within a column are not significantly different from each other.

4.1.3 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf area of different tobacco varieties

According to table 4.3, a highly significant interaction between biostimulant treatment and tobacco variety on leaf area was observed across three measurement periods (P=0.001). Biostimulant treatment exerted a highly significant independent effect on leaf area across all weeks (P=0.001). Variety similarly demonstrated a highly significant independent effect on leaf area across all measurement periods (P=0.001). Fulva wet + KRK76 consistently recorded the highest leaf area values at both 6 and 8 WAT, whilst untreated control treatments consistently recorded the lowest leaf area values across all measurement periods.

Table 4.3: Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf area of different tobacco varieties

	Weeks after planting		
Treatment	**4**	**6**	**8**
Hi-Humic + KRK26R	263.3^{d}	1160^{b}	1493.3^{d}
Calcimax + KRK76	350^{b}	906.7^{d}	1700^{c}
Control + KRK76	305^{c}	916.7^{d}	1250^{e}
Calcimax + KRK26R	243.3^{e}	1083.3^{c}	1450^{d}
Hi-Humic + KRK76	416.7^{a}	1156.7^{b}	2116.7^{b}
Fulva wet + KRK76	410^{a}	1323.3^{a}	2350^{a}
Control + KRK26R	228.3^{f}	1083.3^{c}	1216.7^{e}
Fulva wet + KRK26R	303.3^{c}	1160^{b}	1216.7^{e}
Grand Mean	315	1098.8	1636.7
P-value	0.001	0.001	0.001
SED	5.49	21.71	33.06
LSD	11.78	46.57	70.92
CV%	2.1	2.4	2.5

Based on the range test at $p \leq 0.05$, means sharing the same letter within a column are not significantly different from each other.

4.2 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf quality of different tobacco varieties soon after curing

From table 4.4, there is a highly significant interaction between biostimulant treatment and tobacco variety on leaf quality (P=0.001). Biostimulant treatment exerted a highly significant

independent effect on leaf quality (P=0.001). Variety similarly demonstrated a highly significant independent effect on leaf quality (P=0.001). Referencing the quality classification scale presented in table 3.5, a grade score of 1 represents the highest quality classification (Choice), a score of 2 denotes very good quality and a score of 3 indicates good quality. The highest quality outcomes were achieved by Fulva wet and Hi-Humic Plus treatments applied to KRK76, while untreated control treatments on both varieties recorded the lowest quality outcomes.

Table 4.4: Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf quality of different tobacco varieties soon after curing

Treatment	**Grade/Quality/Scores**
Hi-Humic + KRK26R	2.2^{b}
Calcimax + KRK76	2.033^{b}
Control + KRK 76	3.067^{c}
Calcimax + KRK26R	2.1^{b}
Hi-Humic + KRK76	1.233^{a}
Fulva wet + KRK76	1.133^{a}
Control + KRK26R	3.067^{c}
Fulva wet + KRK26R	2.133^{b}
Grand Mean	2.121
P-value	0.001
SED	0.0652
LSD	0.1398
CV%	3.8

Based on the range test at $p \leq 0.05$, means sharing the same letter within a column are not significantly different from each other.

4.3 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf yield of different tobacco varieties soon after curing

A highly significant interaction between biostimulant treatment and tobacco variety on leaf yield was observed (P=0.001) as shown in figure 4.1. Biostimulant treatments independently exerted a highly significant effect on yield of cured leaves (P=0.001). Variety similarly demonstrated a highly significant independent effect on leaf yield (P=0.001). Treatment combinations involving biostimulants applied to KRK76 consistently recorded the highest leaf

yield values whilst untreated control treatments on both varieties recorded the lowest leaf yield outcomes as illustrated in figure 4.1.

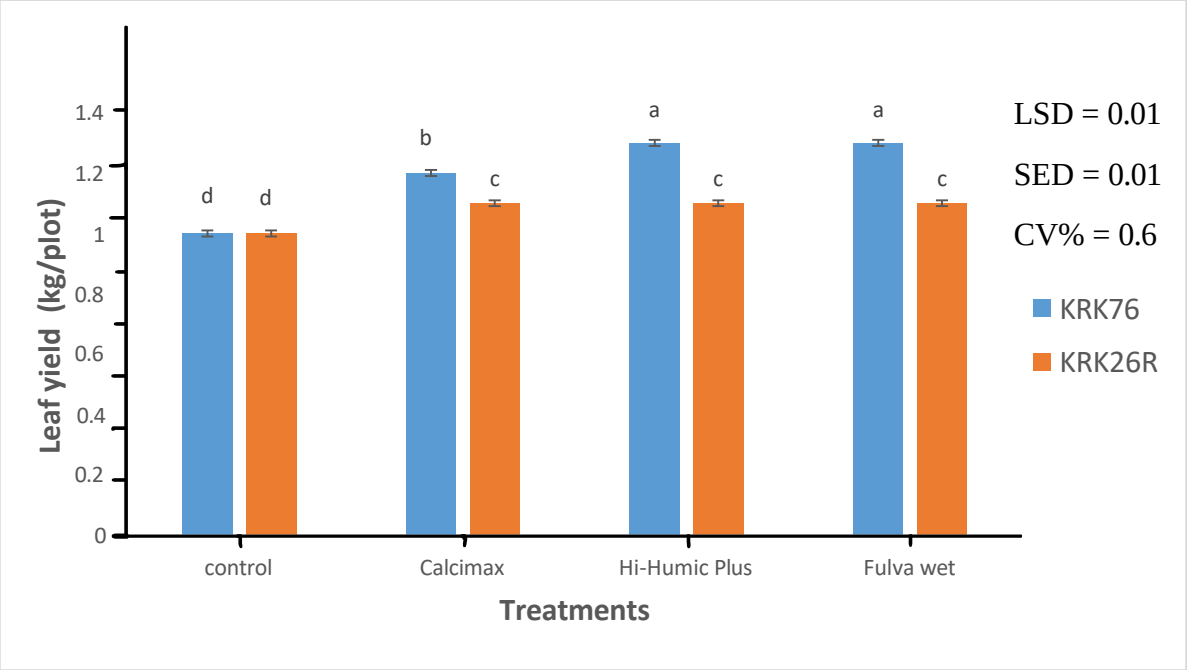

Figure 4.1: Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf yield mass of different tobacco varieties. Based on the range test at $p \leq 0.05$, means sharing the same letter within bars are not significantly different from each other. Bars represent means ±standard error (SE).

CHAPTER 5: DISCUSSION

5.1 Effects of Calcimax, Fulva wet and Hi-Humic plus on growth parameters of different tobacco varieties

5.1.1 Effects of Calcimax, Fulva wet and Hi-Humic Plus on plant height of different tobacco varieties

The study investigated the effects of Calcimax, Fulva wet and Hi-Humic Plus on the growth, yield and quality of tobacco varieties: KRK76 and KRK26R. The results from table 4.1 of this study clearly demonstrated considerable variability among tobacco varieties in their growth responses to biostimulant application. Table 4.1 show that among the biostimulants, the use of Fulva wet and Hi-Humic Plus significantly increased plant height from week 4 upto week 8. Particularly, Fulva wet + KRK76 as well as Hi-Humic plus on KRK76 produced the tallest plants while untreated control treatments on both varieties recorded the lowest height values. This pattern was consistent across all varieties, suggesting a robust and reliable effect of these biostimulants on vegetative growth promotion (Ndlovu *et al*., 2025).

This is a comparison of the effects on tobacco plant height of three different biostimulant treatments: Fulva Wet, Hi-Humic Plus and Calcimax. The two best treatments were Fulva Wet and Hi-Humic Plus, and it's believed that these treatments stimulated tobacco plant growth because they contained some form of humic or fulvic acid (Ndlovu *et al*., 2025). Humic acids contain compounds that promote cell division and elongation, which would help explain why plants treated with these biostimulants grew taller than those treated with Calcimax. Humic acids may increase membrane permeability allowing for greater absorption of certain nutrients by plants (Calvo *et al*., 2014). In addition, humic acids may improve the solubility of certain nutrients so that they can be absorbed by plants. Nitrogen and phosphorous are two important nutrients that are needed for the synthesis of proteins and the generation of energy required for plant cells to divide (Canellas *et al*., 2015).

It is well established that humic acids are beneficial for plant growth. They are used worldwide to improve crop yields. Fulva Wet contains fulvic acid, a compound that acts like an organic chelating agent to make other nutrients available to plants (Pratama *et al*., 2025). This makes the nutrients in the fertilizer more readily available to plants for growth. A study published recently found similar results when researchers studied the effects of using humic and fulvic acids on soybean crops. In another recent study, researchers found that the application of humic

and fulvic acids promoted higher nitrogen assimilation in rice plants. Another recent study, conducted in China, demonstrated that humic and fulvic acids applied to tobacco plants improved photosynthesis rates. As a result of increased nutrient availability resulting from humic and fulvic acid applications, the researchers concluded that the humic and fulvic acids improved the ability of the tobacco plants to grow taller (Wang *et al*., 2016).

A number of studies have provided evidence that show how humic acids influence the physiology of roots and shoots of plants including changes associated with nutrient uptake, assimilation and transport within the plant (Canellas *et al*., 2015). While there is considerable evidence demonstrating that humic acids can provide abiotic stress protection, a consensus exists among scientists regarding their potential benefits for increasing plant growth and fitness. Additionally, based on past research it appears that incorporating humic acids into agricultural production systems may provide a method for achieving sustainable agriculture practices (Canellas *et al*., 2015).

Both Hi-Humic Plus and Fulva Wet produced significant improvements in tobacco plant height. One possible reason for this improvement is that both products contain humic acids and fulvic acids. Like other types of organic matter, humic acids contain carboxyl groups that contribute to anions' adsorption onto positively charged clay surfaces in soils (Nardi *et al*., 2016). However, unlike many forms of organic matter, the carboxyl groups in humic acids are relatively weakly acidic. Therefore, when present in soil at sufficiently high concentrations, humic acids will cause cation exchange capacity (CEC) to increase. An increase in CEC means that more cations will be held tightly onto clay surfaces in the soil. More importantly, this increase in CEC is accompanied by an increase in water holding capacity (WHC) of soils. Increased WHC enables plant roots to absorb more water during times when water becomes limiting (Nardi *et al*., 2016).

In turn, this limits transpiration and reduces drought damage. The increase in CEC also promotes aeration within the soil profile. Soil aeration helps prevent anaerobic conditions within the soil matrix. Anaerobic conditions lead to oxygen deficiency in root tissue which leads to reduced root growth. Reduced root growth contributes directly to reduced nutrient and water absorption capabilities. When combined, these factors all contribute to increased plant height. Moreover, an increase in WHC provides more moisture to support microbial populations in the rhizosphere (Nardi *et al*., 2016). Microorganisms play key roles in decomposing organic materials and converting them into forms that can be utilized by plants

as sources of nutrition. Improved WHC allows microorganisms to remain active longer after irrigation events have ceased.

Therefore, both Hi-Humic Plus and Fulva Wet improve the physical environment in which plant roots function, enabling them to absorb nutrients more efficiently over time. Finally, it is noted here that the superiority of KRK76 over other tested genotypes can be explained by previous experiences in Zimbabwean tobacco cultivation practices. For example, KRK76 is one variety out of several flue-cured varieties grown commercially in Zimbabwe. Flue-cured varieties generally exhibit vigor (Tobacco Research Board, 2020). Vigor indicates responsiveness to improved growing conditions (adequate soil fertility). Tobacco growers in Zimbabwe have long since realized the importance of providing optimal growing conditions through various methods such as fertilizer application. Fertilizer application has contributed greatly to tobacco yield enhancement. Research conducted in Zimbabwe has demonstrated that tobacco production systems benefit from fertilizer application and biostimulants. Biostimulants work synergistically with fertilizers to optimize crop productivity thus increased biomass production (Mashingaidze *et al*., 2021; Maboko *et al*., 2011).

Untreated control treatments predictably recorded the shortest plant height ditto (Table 4.1). This underlines the essential role that biostimulants play in supporting plant vigour under nutrient limited conditions and water stress conditions (Rouphael & Colla, 2020; Vanlauwe *et al*., 2022). Interestingly, the responses of biostimulants across varieties indicated that the products are not limited or restricted to a single genotype (Brito *et al*., 2023).

It is also important to interpret the height differences between treated and control plants against the quality of the rainy season under which the trial was conducted. As described in Section 3.4.5, the 2025/26 season delivered a seasonal total (≈500 mm) that was, in principle, adequate for a full tobacco growth cycle; however, the season's *distribution*, not its total, was the constraining factor, with a pronounced dry spell interrupting rainfall during a critical vegetative growth window (Figure 3.2). Under such a distribution pattern, control plants without biostimulant support would have been more exposed to transient moisture deficits, whereas Fulva wet- and Hi-Humic Plus-treated plants likely buffered these dry-spell effects through the humic/fulvic-acid-mediated increase in water holding capacity and root activity described above. This suggests that part of the height advantage recorded for biostimulant-treated plants (Table 4.1) reflects not only a direct growth-promotion effect but also an improved capacity to withstand the intermittent moisture stress imposed by an erratically distributed rainy season,

reinforcing the practical relevance of biostimulant use in seasons characterised by long dry spells rather than by low seasonal totals.

5.1.2 Effects of Calcimax, Fulva wet and Hi-Humic Plus on stem thickness of different tobacco varieties

Stem thickness was assessed across all biostimulant treatments and tobacco varieties throughout the monitoring period (Table 4.2). The absence of a significance biostimulant-variety interaction at 4 WAT followed by the emergence of highly significant interactions at 6 and 8 WAT (Table 4.2), suggests the structural benefits of biostimulant application on stem development require a cumulative period of nutrient uptake and assimilate partitioning before becoming measurable at the above grounded level (Canellas *et al*., 2015; Nardi *et al*., 2016). Fulva wet +KRK76 and Calcimax + KRK26R recorded the highest stem girth values at 8 WAP whilst the untreated control + KRK26R recorded the lowest (Table 4.1). As supported by Canellas *et al*. (2015), Fulva wet and Hi-Humic Plus likely boosted vascular development through improving nutrient uptake and hormonal stimulation particularly auxin-like activity associated with fulvic and humic acid substances Furthermore, improved photosynthetic activity contributes to greater biomass accumulation and stem strengthening (Wang et al., 2016) (Wang *et al*., 2016).

According to results from table 4.2, Calcium based Calcimax also attributed significantly to stem thickness, especially in KRK76, can be explained by calcium's crucial role in strengthening cell walls and improving structural rigidity which is essential for supporting the substantial leaf biomass characteristics in tobacco crops (Hawkesford *et al*., 2012). This explains why Calcimax treated plants showed competitive performance at later growth stages. Similar findings by Zhou & Chitengu (2024) indicated an increased stem girth when bio-organic fertilizers were combined with conventional fertilizers. These results from the study are linked to improved calcium and potassium uptake and enhanced vascular development (Rouphael & Colla, 2020). Stronger stem development ultimately enhances plant structural stability and improves the efficiency of water and nutrient transport within plant tissues, supporting overall growth and productivity.

The superior performance of KRK76 again reflects varietal adaptability and efficient nutrient utilization. In Zimbabwe, KRK76 is widely reported to have better growth characteristics and structural robustness under improved agronomic practices compared to other varieties such as KRK26R (Tobacco Research Board, 2020). The lowest records observed in control treatments

(Table 4.2) are consistent with findings by Chitamba *et al*. (2020) who reported that nutrient deficient and poor nutrient in tobacco plants tend to allocate fewer resources to structural development, resulting in thin stems compared to biostimulant treated plants and that can negatively affect leaf support and final yield.

5.1.3 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf area of different tobacco varieties

The efficacy of biostimulant treatments on leaf area development was assessed throughout the monitoring period (Table 4.3). The results of this study revealed that biostimulant application produced a consistently positive effect on leaf area, with Fulva wet and Hi-Humic Plus emerging as the two superior treatments (Table 4.3). A closer reading of Table 4.3, however, shows that this superiority was not uniform across the whole growing period. At 4 WAT, Hi-Humic Plus + KRK76 (416.7 cm^2) and Fulva wet + KRK76 (410.0 cm^2) shared the same mean separation letter (a), indicating that the two treatments were not significantly different from one another at this early stage; Hi-Humic Plus was therefore statistically at par with, rather than inferior to, Fulva wet at 4 WAT. It was only from 6 WAT onward that Fulva wet + KRK76 pulled significantly ahead of Hi-Humic Plus + KRK76 (1323.3 cm^2 versus 1156.7 cm^2 at 6 WAT, and 2350.0 cm^2 versus 2116.7 cm^2 at 8 WAT), establishing Fulva wet as the consistently superior treatment only in the mid-to-late growth stages.

Fulva wet and Hi-Humic Plus performed comparably during early canopy establishment, with Fulva wet's advantage becoming statistically distinct as the season progressed, most plausibly because the auxin-like, cell-elongation effects associated with fulvic acid (Jannin *et al*., 2013) continue to operate once canopy establishment is underway, giving Fulva wet a growing advantage over Hi-Humic Plus as the season progresses. Leaf area remains a critical determinant of photosynthetic capacity and, ultimately, yield in tobacco (Tian *et al*., 2025)

Fulva wet was the most effective biostimulant in increasing leaf area across varieties tested. Fulva wet acted as stimulant interfering with the internal plant physiological functions such as nutrient and water uptake improving photosynthesis through increased leaf area. The increase observed under Fulva wet can be attributed to fulvic acid's role in enhancing chlorophyll synthesis, stomatal conductance and nutrient uptake (Jannin *et al*., 2013). Fulva wet has been found to increase nutrient uptake thus greatly influence leaf area (Iftikhar *et al*., 2025). These findings are consistent with those of Abdul *et al*. (2025) who demonstrated that fulvic acid

application increased leaf area by 63.7% compared to untreated control plants under stress conditions. Larger leaf area increases light interception thereby promoting photosynthetic efficiency and biomass accumulation.

Hi-Humic Plus also enhanced leaf expansion likely through improved soil fertility and root activity. Hi-Humic Plus contain humic acids which are known to stimulate enzymatic activity and metabolic processes leading to accelerated leaf growth (Nardi *et al*., 2016). Pratama *et al*. (2025) also reviewed that improved soil properties influence efficient nutrient and water use efficiency thereby promoting nutrient assimilation to leaf organs thus increased leaf size. These findings are consistent with global studies showing that humic and fulvic acid based biostimulants significantly increase leaf area in solanaceous crops including tobacco (Schiavon *et al*., 2017).

In the Zimbabwean context, leaf size is a critical quality and yield determinant in flue cured tobacco fetching high market price. Similarly, larger leaves are associated with higher cured leaf mass and better grading potential (TIMB, 2025). The superior performance of KRK76 under biostimulant treatments (Table 4.3) suggests that this variety has a higher capacity for nutrient assimilation and canopy development.

Calcimax resulted in the average leaf area though its effects were less compared to Fulva wet and Hi-Humic Plus (Table 4.3). The contribution of Calcimax to leaf area development can be attributed to calcium's critical role in maintaining cell wall stability, membrane integrity, enzyme activation and signal transduction within plant tissues (White & Broadley, 2003). These findings are consistent with those of Choudhary *et al*. (2020) and Saeed *et al*. (2022), who demonstrated that combining calcium based biostimulants with other micronutrients improves chlorophyll content, photosynthetic efficiency and water use efficiency, especially under stress conditions, effects were manifested in this study through improved leaf area development.

The control treatments without any biostimulant resulted in the lowest leaf area (Table 4.3) emphasizing the need of integrating biostimulants in crop productivity especially under stress conditions brought by climate change. This is in agreement by Anjum *et al*. (2011) findings which demonstrated that plants under unoptimized conditions without biostimulant resulted in lower leaf area compared to the treated plants. The results (Table 4.3) reinforce the importance of external inputs in enhancing tobacco growth under field conditions. This is particularly

relevant in Zimbabwe where continuous cultivation and limited organic amendments often lead to declining soil fertility (FAO, 2019) as well as where climate variability is experienced.

5.1.4 Soil characteristics and biostimulant efficacy

The magnitude of the growth responses reported across Sections 5.1.1-5.1.3 is best understood against the background of the site's inherent soil characteristics (Section 3.1). Farm 4 Ndiripo lies on the coarse-grained, granite-derived sandy to sandy loam soils typical of agroecological region IIb, which are characterised by low native organic matter, low cation exchange capacity (CEC) and limited water- and nutrient-holding capacity, and which are inherently deficient in nitrogen, phosphorus and, in places, potassium and micronutrients (Grant, 1981; Nyamapfene, 1991). Because such soils offer little in the way of a residual nutrient or moisture buffer, the crop's performance was comparatively more dependent on external inputs, both fertilizer and biostimulant, than would be the case on heavier, more fertile clay soils.

This soil context helps explain why the humic- and fulvic-acid-based products (Hi-Humic Plus and Fulva wet) produced the largest and most consistent gains in plant height, stem thickness and leaf area (Tables 4.1-4.3). As discussed in Section 5.1.1, humic and fulvic acids raise the effective CEC of the root zone and increase water holding capacity (Nardi *et al*, 2016); on a soil that is inherently low in both properties, this amendment effect is likely to be proportionally larger than it would be on a soil that already has appreciable clay-associated CEC and moisture retention.

In other words, the low baseline fertility and low water-holding capacity of the trial site plausibly amplified the relative advantage of Fulva wet and Hi-Humic Plus over the untreated control, since the control plants had no means of compensating for the site's limited native nutrient and moisture reserves. This is consistent with the wider observation that biostimulant benefits tend to be most pronounced under marginal or input-limited growing conditions (Rouphael & Colla, 2020), and it strengthens the practical case for biostimulant use specifically on the sandy, low-fertility soils that dominate Zimbabwe's smallholder tobacco-growing areas.

5.2 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf quality of different tobacco varieties

The study demonstrated that biostimulants significantly influence leaf quality across tobacco varieties (Table 4.4). Fulva wet and Hi-Humic Plus applied to KRK76 were recorded having the highest quality while control treatments recorded the least quality (Table 4.4). The findings

presented in Table 4.4 indicate that biostimulants do not only increase plant growth, they also positively affect the post-harvest characteristics of plants. A better quality of leaves is an indication of a well-balanced intake of nutrients and enhanced physiological processes during growth. Humic substance-based biostimulants improve the synthesis and storage of carbohydrates which is crucial for determining curing quality in tobacco (Marschner, 2012). In addition, proper carbohydrate content will determine leaf color, texture and combustibility, all being important parameters when grading flue-cured tobacco. Similarly, Hi-Humic Plus probably had positive effects on both physical or chemical structure of leaves and overall chemical composition of leaves due to improving the fertility of the soil and/or metabolic activity of the plants (He *et al*., 2009; Pu *et al*., 2023)

Fulva wet may have enhanced sugar accumulation and uniform leaf maturation due to improved nutrient translocation and metabolic activity. These observations align with research conducted by Zheng (2024), who demonstrated that fulvic acid application exhibited superior and comprehensive efficacy in enhancing soil quality, facilitating the growth of flue cured tobacco and improving leaf characteristics, thereby establishing fulvic acid as a particularly appropriate biostimulant for flue cured tobacco cultivation. Fulva wet is the ideal plant, root and soil stimulant.

Calcimax also showed average contribution to leaf quality although it showed least results compared to Fulva wet and Hi-Humic Plus (Table 4.4). This can be attributed to calcium's role in regulating signal transduction pathways that govern plant responses to abiotic stresses including drought and salinity (Ahmad *et al*., 2022). This response to abiotic stress helped Calcimax to contribute to tobacco leaf quality as adequate calcium nutrition improves nutrient uptake efficiency and enhances plant tolerance to adverse environmental conditions.

The control treatments without any biostimulant resulted in least grade scores (Table 4.4) emphasizing the significance of integrating biostimulants in promoting leaf quality under stress conditions. In Zimbabwe's tobacco marketing environment, leaf grading is highly sensitive to quality attributes including colour, texture and maturity. Studies by the Tobacco Industry and Marketing Board (TIMB, 2022) indicate that improved nutrient management significantly enhances leaf grade and market value.

5.3 Effects of Calcimax, Fulva wet and Hi-Humic Plus on leaf yield of different tobacco varieties

Overall, referring to figure 4.1 it is evident that leaf weight varies across different varieties with all biostimulants treated leaves recording highest yield mass on KRK76, whilst control treatments recorded lowest. KRK26R treatments showed an intermediate mass and this confirms that both variety selection and biostimulant play a role in determining tobacco productivity (Bandara *et al*., 2025). These findings suggest that humic and fulvic based biostimulants enhance biomass accumulation through improved nutrient uptake, root development and physiological efficiency (Shahrajabian *et al*., 2024).

The observed yield increase can be accredited to enhanced nutrient use efficiency, improved photosynthetic activity and increased tolerance to abiotic stress conditions (du Jardin, 2015; Rouphael & Colla, 2020). The results from fig 4.1 are consistent with trials involving tobacco and related crops that demonstrated improvements in usable leaf mass and overall yield (Abdo *et al*., 2022). Furthermore, fulvic acid has been found to increase crop yield under drought and normal conditions due to their efficient nutrient use (Chen *et al*., 2025). Humic substances promote root architectural development and increase the bioavailability of essential nutrients including phosphorus and various micronutrients collectively supporting higher biomass production (Canellas *et al*., 2015).

The results (Figure 4.1) align with Shahrajabian *et al*. (2024) who reported that fulvic acid positively influence root development and promotes germination rate, vegetative growth and final crop yield. Similarly, Alsudays *et al*. (2024) demonstrated that enriching soils with humic and fulvic acids increases available nutrient content, improves soil physiochemical properties and promotes both plant growth and final yield. From soil amelioration to a photosynthesis boost, fulva wet is the ideal for improved tobacco yield. The results (Figure 4.1) are further supported by Chang *et al*. (2018) who documented a moderate but consistent positive effect of humic substances on crop yield across multiple crop species.

The results of this study (figure 4.1) also coincide with earlier findings that biostimulants enhanced crop productivity and yield through chemical biological and physiological enhancement mechanisms (Calvo *et al*., 2014; Nardi *et al*., 2016). Similarly, Bozhinova in 2023 reported on an improvement to yields for tobacco after applying biostimulant application. They also stated that they enhanced leaf mass and overall plant performance. In conjunction

with its role in structuring plant development and reducing physiological disorders by enhancing cell wall strength Calcimax appears to have resulted in a moderate yield improvement in tobacco (Fageria *et al*., 2009; White & Broadley, 2003). However, it's apparent from figure 4.1 that these combined hormonal and nutrient effects of biostimulants will be more predictive of maximum yields than single-nutrient products (Canellas *et al*., 2015).

Control treatments resulted in the lowest yields ever documented (figure 4.1), and are thus important to the use of biostimulants in modern systems for producing tobacco especially under stressful climate conditions where soil fertility is declining. As such, global studies have demonstrated the importance of providing biostimulant inputs to prevent lower growth rates, poorer leaf development and ultimately lower yields (Calvo *et al*., 2014). The experiment described here has demonstrated that integrating biostimulants like Fulva wet and Hi-Humic Plus, Calcimax with responsive varieties can significantly enhance tobacco growth, quality and yield under field conditions in Zimbabwe where climate variability remains a major threat to tobacco productivity.

Simultaneously, the type of biostimulant used and the genotype of tobacco used in this study all played a role in the final yield mass. As Mangano *et al*. (2020) found differential responses between cultivar genetic backgrounds to biostimulants, this study supports the need for genotype specific recommendations.

The yield differences reported above must also be read against the rainfall pattern experienced during the 2025/26 season. The season's *total* rainfall (≈500 mm) was not, on its own, the constraint; the constraint was the season's poor *distribution*, in particular the pronounced mid-season dry spell illustrated in Figure 3.2. Because yield mass in tobacco is strongly influenced by moisture availability during leaf expansion and bulking, a dry spell occurring at this stage would be expected to depress yield in proportion to a plot's capacity to buffer against transient water deficits. The consistently lowest yields recorded in the untreated control plots (Figure 4.1) are therefore attributable not only to nutrient limitation but also, in part, to the control plants' reduced capacity to withstand the dry-spell period without the water holding capacity and root development benefits associated with humic- and fulvic-acid-based biostimulants (Nardi *et al*, 2016). This distribution effect reinforces the practical value of biostimulant use as a buffer against the erratic, dry-spell-prone rainfall that increasingly characterises Zimbabwe's tobacco-growing seasons, rather than as a response to low seasonal rainfall totals per season.

5.4 Interaction effects between biostimulants and tobacco varieties

All tested characteristics of the interaction of different biostimulants and varieties of tobacco were statistical important ($p \leq 0.001$) for almost all features from the 6th week onward, and therefore do not indicate an independent effect of both types of factors on the yield of crops (Tables 4.1, 4.2, 4.3, 4.4, Fig. 4.1). Variety KRK76 has shown a higher degree of sensitivity to all used kinds of biostimulants than KRK26R. It can be assumed that KRK76 has a genetic structure that is better adapted to take advantage of the physiological changes caused by the use of biostimulants. The difference in the genetic material could also explain the differences in the intrinsic vigor and in the size of the roots of the plants of both varieties. Both factors affect the ability of the plants to absorb nutrients (Tobacco Research Board, 2020). The fact that KRK26R showed lower results with regard to all applied treatment options compared to KRK76, but generally had better values than the untreated control (Tables 4.1, 4.2, 4.3, 4.4, Figure. 4.1) indicates that the productivity of the crops depends on both the variety of tobacco and on the type of product used.

The findings of this research are supported by numerous studies conducted in other countries. These studies show that there are genetically based differences in how well plants respond to biostimulants (Mangano *et al*., 2020; Brito *et al*., 2023). Mangano *et al*. (2020) found out that different varieties of tobacco differ in terms of their sensitivity to different plant hormones and therefore react differently to various forms of biostimulants. Brito *et al*. (2023) investigated whether differences in how well plants grow under stressful conditions due to drought depend on how well they tolerate stress. They came to the conclusion that plants that are more tolerant of drought also exhibit increased levels of antioxidants as a result of the application of biostimulants. Increased levels of antioxidants are indicative of reduced oxidative stress in plants.

The results obtained in this investigation demonstrate that KRK76 reacts much more sensitively to Fulva wet than KRK26R because Fulva wet contains large amounts of fulvic acids, which are known for their hormonal activity. Thus, it appears likely that KRK76 exhibits a higher level of sensitivity to signaling pathways initiated by fulvic acids that play a role in regulating the processes of elongation and division of cells as well as nutrient allocation within the plant (Janinn *et al*., 2013; Shahrajabian *et al*., 2024).

No significant interaction was detected for stem diameter at 4 WAT; however, highly significant interactions were identified at 6 and 8 WAT (Table 4.2). This finding supports the

understanding that structural plant growth developments stimulated through the application of biostimulants necessitate a delay prior to becoming discernible. As described previously, this time dependent process corresponds to the biochemical-physiological mechanism associated with humic and fulvic acid substances where initial effects are evident at the biochemical or root level and subsequent structural benefits become visible in above-ground biomass over time (Nardi *et al*., 2016; Canellas *et al.*, 2015). Therefore, these data emphasize the need for repeated applications beginning at transplanting to provide cumulative advantages by harvest time. Practically speaking, these findings support early initiation of biostimulant applications and continued regular applications throughout the entire growing season.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The purpose of the study was to assess the effectiveness of commercially available biostimulants (Calcimax, Fulva Wet and Hi-Humic Plus) for promoting growth, enhancing yield and improving leaf quality of two Flue Cured Tobacco Varieties (KRK76 and KRK26R) grown under field conditions within the Mashonaland West Province, Zimbabwe. Data collected during this research demonstrates that applications of biostimulants enhance several aspects of tobacco plant development under local agroecological conditions. In all cases, both Fulva Wet and Hi-Humic Plus performed better than Calcimax when compared with the respective control treatments. Therefore, based upon data obtained from the experimentations, we recommend applying Fulva Wet to KRK76 as the best treatment option for maximizing both growth and yield while also maintaining high quality leaves under Zimbabwean agroecological conditions. Additionally, this study has demonstrated that the variety affects morphological response to growth, yield and leaf quality, therefore genotype contributes substantially to the magnitude of crop responses to applications of biostimulants. Finally, the interaction of biostimulants and variety clearly indicates the necessity of developing targeted agronomic recommendations.

6.2 Recommendations

To Farmers

- Adopt biostimulants as a promising tool as a sustainable and ecofriendly practice to improve productivity.
- Engage into climate smart agriculture and use of responsive varieties such as KRK76
- Integrate biostimulants with existing fertiliser programs.

To Researchers

- Investigate optimal application rates and timing
- Conduct multi-season trials for validation of these findings

To Policy makers

- Consider incorporating proven biostimulant products such as Fulva wet and Hi-Humic Plus into standard seasonal input programmes for smallholder tobacco producers.

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APPENDIX

Appendix 1: Analysis of variance of plant height at 4 WAT

Variate: Plant_height_at_4_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.11083	0.05542	0.86	
Block. *Units* stratum					
Factor_1_Biostimulant	3	40.92125	13.64042	211.60	<.001
Factor_2_Variety	1	1.26042	1.26042	19.55	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	3.14458	1.04819	16.26	<.001
Residual	14	0.90250	0.06446		
Total	23	46.33958			

Appendix 2: Analysis of variance of plant height at 6 WAT

Variate: Plant_height_at_6_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.8125	0.4062	0.92	
Block. *Units* stratum					
Factor_1_Biostimulant	3	167.6146	55.8715	126.42	<.001
Factor_2_Variety	1	201.2604	201.2604	455.38	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	73.7813	24.5938	55.65	<.001
Residual	14	6.1875	0.4420		
Total	23	449.6562			

Appendix 3: Analysis of variance of plant height at 8 WAT

Variate: Plant_height_at_8_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.750	0.875	0.49	
Block. *Units* stratum					
Factor_1_Biostimulant	3	432.833	144.278	80.00	<.001
Factor_2_Variety	1	682.667	682.667	378.51	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	121.500	40.500	22.46	<.001
Residual	14	25.250	1.804		

Total	23	1264.000			

Appendix 4: Analysis of variance of stem thickness at 4 WAT

Variate: Stem_thickness_at_4_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.06583	0.03292	1.50	
Block. *Units* stratum					
Factor_1_Biostimulant	3	1.45792	0.48597	22.13	<.001
Factor_2_Variety	1	1.00042	1.00042	45.55	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	0.08792	0.02931	1.33	0.303
Residual	14	0.30750	0.02196		
Total	23	2.91958			

Appendix 5: Analysis of variance of stem thickness at 6 WAT

Variate: Stem_thickness_at_6_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.09083	0.04542	3.36	
Block. *Units* stratum					
Factor_1_Biostimulant	3	6.22833	2.07611	153.65	<.001
Factor_2_Variety	1	0.20167	0.20167	14.93	0.002
Factor_1_Biostimulant.Factor_2_Variety	3	0.88833	0.29611	21.91	<.001
Residual	14	0.18917	0.01351		
Total	23	7.59833			

Appendix 6: Analysis of variance of stem thickness at 8 WAT

Variate: Stem_thickness_at_8_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.04750	0.02375	1.56	
Block. *Units* stratum					
Factor_1_Biostimulant	3	11.12125	3.70708	244.23	<.001
Factor_2_Variety	1	5.51042	5.51042	363.04	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	0.50458	0.16819	11.08	<.001
Residual	14	0.21250	0.01518		

Total	23	17.39625			

Appendix 7: Analysis of variance of leaf area at 4 WAT

Variate: Leaf_area_at_4_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	100.00	50.00	1.11	
Block. *Units* stratum					
Factor_1_Biostimulant	3	30200.00	10066.67	222.53	<.001
Factor_2_Variety	1	73704.17	73704.17	1629.25	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	4512.50	1504.17	33.25	<.001
Residual	14	633.33	45.24		
Total	23	109150.00			

Appendix 8: Analysis of variance of leaf area at 6 WAT

Variate: Leaf_area_at_6_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	3700.0	1850.0	2.62	
Block. *Units* stratum					
Factor_1_Biostimulant	3	266945.8	88981.9	125.83	<.001
Factor_2_Variety	1	12604.2	12604.2	17.82	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	115912.5	38637.5	54.64	<.001
Residual	14	9900.0	707.1		
Total	23	409062.5			

Appendix 9: Analysis of variance of leaf area 8 WAT

Variate: Leaf_area_at_8_WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	6108.	3054.	1.86	
Block. *Units* stratum					
Factor_1_Biostimulant	3	1696967.	565656.	344.94	<.001
Factor_2_Variety	1	1135350.	1135350.	692.34	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	584550.	194850.	118.82	<.001
Residual	14	22958.	1640.		
Total	23	3445933.			

Appendix 10: Analysis of variance of leaf quality

Variate: Leaf Quality (Reap/plot/barn)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.010833	0.005417	0.85	
Block. *Units* stratum					
Factor_1_Biostimulant	3	7.791250	2.597083	407.77	<.001
Factor_2_Variety	1	1.550417	1.550417	243.43	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	1.357917	0.452639	71.07	<.001
Residual	14	0.089167	0.006369		
Total	23	10.799583			

Appendix 11: Analysis of variance of yield mass

Variate: Yield_(kg/plot)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.00110833	0.00055417	10.23	
Block. *Units* stratum					
Factor_1_Biostimulant	3	0.21571250	0.07190417	1327.46	<.001
Factor_2_Variety	1	0.08283750	0.08283750	1529.31	<.001
Factor_1_Biostimulant.Factor_2_Variety	3	0.03697917	0.01232639	227.56	<.001
Residual	14	0.00075833	0.00005417		
Total	23	0.33739583			