International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Effects of Nitrogen Fertilizer Levels on the Growth and Yield of Kale and Collards in Soil Infested with Clubroot

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ABSTRACT

Clubroot disease, caused by the obligate biotroph Plasmodiophora brassicae, severely impairs root function in Brassica crops, leading to stunted growth, nutrient deficiencies, and yield losses of 30–70% in infested soils. In acidic soils (pH < 6.5), resting spores germinate readily, releasing zoospores that infect root hairs. Nitrogen (N) fertilization modulates plant vigor through protein and chlorophyll synthesis but interacts with soil pH and pathogen dynamics: ammonium-based sources acidify soil via nitrification (releasing H^+ ions), potentially favoring the pathogen, while calcium ammonium nitrate (CAN) provides a liming effect via Ca^{2+} that raises pH and suppresses spore activity. Two field experiments were conducted using a randomized complete block design with split-plot arrangement (3 replications) from June to October 2025 in Wundanyi and Mwatate Sub-Counties, Taita-Taveta County, Kenya. Main plots were kale (Curly and Dinosaur) and collard (Vates and Morris Heading) varieties; subplots were N rates (0, 10, 20 g/plant) from CAN or sulfate of ammonia (SA). Key findings included: 10 g N from CAN significantly maximized plant height (kale: 42.6 ± 2.5 cm, +68% over control), leaf area (+113%), chlorophyll content (50 ± 2.5 SPAD units), fresh biomass (+100%), and reduced galling index (1.5 ± 0.2 vs. 2.8 ± 0.3 control; $p < 0.05$, ANOVA, Tukey HSD). CAN raised soil pH (6.3–6.4), lowering disease severity, while SA acidified it (5.5–5.6), increasing susceptibility. Biochemical analyses showed enhanced Ca, Zn, Fe, phenolics, and flavonoids at the optimal rate. These results demonstrate that moderate CAN application optimizes sustainable production by balancing nutrition, disease suppression (via pH and Ca effects on pathogen biology), and nutritional quality while minimizing nitrate risks. Recommendations include farmer adoption of 10 g CAN/plant with soil pH monitoring for clubroot-infested regions.

ABBREVIATIONS AND ACRONYMS

BR- Benefits and Risks

CAN- Calcium Ammonium Nitrate

CI - Community Involvement

CP- Crop Performance

CRIS- Clubroot Infested Soils

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International Journal of Engineering Research & Technology (IJERT)
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Vol. 15 Issue 10 , October - 2026

DP - Data Privacy

EIA- Environmental Impact Assessment

GYKC - Growth and Yield of Kales and Collards

IC - Informed Consent

LSAP - Long-term Sustainability of Agricultural Practices.

N - Nitrogen

NF - Nitrogenous Fertilizer

NFS- Nitrogen Fertilizer Supply

NO_3-N - Nitrate-Nitrogen

NR - Nitrogen Runoff

OMC- Organic Matter Content

PH - Soil pH

SA - Sulfate of Ammonia

SCN - Thiocyanate Ion

TPB - Transparent Reporting of Benefits

TTC- Taita-Taveta County

WS - Water Contamination

WSC - Wundanyi Sub County

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Kale and collards (*Brassica oleracea* var. *acephala*), commonly referred to as *sukuma wiki* in Kenya, are among the most important leafy vegetables in the country. They serve as a dietary staple across income groups, providing essential micronutrients such as vitamins A and C, iron, calcium, and antioxidants that contribute significantly to food and nutritional security (Ndolo et al., 2019; Farnham et al., 2004). These crops are cultivated year-round in both rural and urban areas due to their adaptability to diverse climatic conditions, provided adequate water is available. In Taita-Taveta County, they form a critical component of smallholder farming systems, supporting livelihoods and local markets.

However, production is severely constrained by clubroot disease, caused by the obligate biotroph Plasmodiophora brassicae. This soil-borne pathogen persists as highly resistant resting spores in soil for up to 20 years (Dixon, 2009). Under acidic soil conditions (pH < 6.5), resting spores germinate to release motile zoospores that infect root hairs. Subsequent

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

secondary plasmodia cause hypertrophy and hyperplasia of root tissues, resulting in characteristic club-shaped galls that disrupt vascular function, impair water and nutrient uptake, and lead to stunted growth and premature plant death (Hwang et al., 2012). Yield losses in infested fields commonly range from 30% to 70%, with complete crop failure possible under high inoculum pressure and favorable environmental conditions (Lamichhane et al., 2018; Wambulwa et al., 2018).

The severity and development of club root are strongly influenced by soil properties and crop nutritional conditions. Among the nutrients required for successful production of leafy vegetables, nitrogen (N) is the most limiting nutrient for leafy vegetable production and plays a central role in plant physiology. It is a key constituent of amino acids, proteins, nucleic acids, and chlorophyll. Nitrogen assimilation primarily occurs through the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway, directly influencing photosynthetic capacity, leaf area expansion, and biomass accumulation (Marschner, 2012). The form of nitrogen supplied—nitrate (NO_3^-) or ammonium (NH_4^+)—significantly affects rhizosphere pH and nutrient dynamics. Ammonium-based fertilizers undergo nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$), releasing hydrogen ions (H^+) and acidifying the soil, which can exacerbate clubroot severity by creating optimal conditions for P. brassicae spore germination and zoospore motility (Smolinska & Palusinska, 2005; Ju et al., 2018). In contrast, calcium ammonium nitrate (CAN) supplies both nitrate-N and calcium, exerting a liming effect that raises soil pH and suppresses pathogen activity while improving calcium-mediated cell wall stability and root health (Albornoz et al., 2017).

Nitrogen is a major nutrient in the production of leafy vegetables because it is involved in the formation of amino acids, proteins, enzymes and chlorophyll. Adequate nitrogen supports leaf expansion, canopy development, photosynthesis and the accumulation of fresh plant biomass. These functions are especially important in kale and collards because the leaves constitute the principal harvested product. However, crop response to nitrogen depends on the quantity applied, fertiliser source, soil characteristics, water availability, crop variety and the presence of other nutrients. A high nitrogen rate does not necessarily result in the highest economic yield. Excessive nitrogen can increase input costs and may raise concerns related to nitrate accumulation, leaching and environmental pollution. Therefore, identifying an appropriate nitrogen rate is necessary for improving productivity while supporting efficient and sustainable nutrient use.

Recent work on leafy vegetables has continued to demonstrate the relationship between nutrient supply and crop quality. In a study of kale and collard grown under hydroponic conditions, electrical conductivity influenced plant growth and nutritional characteristics, while tissue nitrogen increased with increasing nutrient concentration (2024). Such findings indicate that nutrient availability affects not only the amount of biomass produced but also the nutritional composition of the harvested crop. Nevertheless, results obtained in hydroponic systems cannot automatically be transferred to field conditions, where soil properties, pathogen populations, rainfall and nutrient transformations influence crop response. Field-based research is therefore necessary to determine how nitrogen fertiliser levels affect kale and collards under specific soil and disease conditions.

One of the important diseases affecting Brassica crops is clubroot, which is caused by the soil-borne pathogen Plasmodiophora brassicae. The pathogen produces resting spores that can

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International Journal of Engineering Research & Technology (IJERT)
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Vol. 15 Issue 10 , October - 2026

survive in soil for long periods, making the disease difficult to manage once it becomes established. Infection begins when spores germinate and release zoospores that move through soil water towards susceptible roots. The pathogen then develops within root tissues and causes abnormal enlargement or gall formation. These galls interfere with water and nutrient absorption and reduce the efficiency of the root system. Infected plants may exhibit stunted growth, leaf yellowing, wilting and premature senescence. The impact of the disease is particularly serious in vegetable production because reduced leaf development directly affects the marketable portion of the crop.

Clubroot development is influenced by environmental and soil-related factors, including soil acidity, moisture, temperature, drainage and the quantity of pathogen inoculum. Acidic soils are frequently associated with greater disease risk, although the response may vary according to the pathogen population, crop genotype and other soil conditions. In fields where Brassica crops are grown repeatedly, the pathogen may persist and increase over time. The movement of infested soil through farm tools, water, animals and human activities can also contribute to disease spread. For farmers with limited land, avoiding infected fields or implementing long crop rotations may be difficult. This creates a need for management approaches that combine disease prevention, soil improvement, appropriate varieties and balanced crop nutrition.

The relationship between fertiliser management and clubroot is important because fertilisers can influence both plant nutrition and the chemical environment around the roots. Nitrogen fertilisers differ in their chemical composition and in the processes through which they are transformed in soil. Ammonium-based fertilisers may contribute to acidification as ammonium is converted into nitrate through nitrification. Where soil is already acidic, continued use of such fertilisers without appropriate soil management may increase conditions associated with clubroot development. At the same time, nitrogen is necessary for maintaining plant vigour and leaf production. This creates a management challenge: nitrogen must be supplied in sufficient amounts to support growth without worsening soil conditions that may favour the disease.

Calcium ammonium nitrate (CAN) and sulphate of ammonia (SA) are commonly used nitrogen fertilisers, but they may produce different effects in the soil and plant environment. CAN supplies nitrogen and calcium, while SA supplies nitrogen in an ammonium-containing form and sulphur. Calcium is important for cell wall development, membrane stability and root health. The possible contribution of calcium and changes in soil reaction make CAN relevant to investigations involving clubroot-infested soils. However, the performance of a fertiliser depends on application rate, soil buffering capacity, moisture, crop uptake and the initial fertility status of the field. It would therefore be inappropriate to assume that one fertiliser source will produce the same response in all locations. Comparative field evaluation is needed to establish the response of kale and collards to different nitrogen sources and levels.

The Kenyan context makes this investigation important. Vegetable farmers operate under different agro-ecological conditions and often face constraints related to land availability, fertiliser prices, water supply and disease management. In Taita-Taveta County, vegetable production supports household livelihoods and local food markets, while variations in altitude, soil characteristics and rainfall create differences in crop performance. Wundanyi and Mwatate Sub-Counties provide a useful setting for examining crop response under field conditions. Where kale and collards are cultivated repeatedly, soil-borne diseases may remain a persistent challenge. Farmers require recommendations that are not only agronomically effective but also

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International Journal of Engineering Research & Technology (IJERT)
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Vol. 15 Issue 10 , October - 2026

practical in terms of cost, availability of inputs and ease of application.

Studies on sustainable vegetable production increasingly emphasise the need to consider soil health together with crop productivity. Kiriga et al. (2026), in research on maize-kale systems in Kenya, examined how ecological intensification and fertiliser-related practices influenced soil nematode communities. Their work illustrates the importance of evaluating below-ground biological conditions when improving vegetable production. Although nematodes are different from the pathogen responsible for clubroot, the study reinforces the broader principle that soil management practices can affect organisms living in the root environment. Similarly, research on soil fertility and organic amendments has highlighted the role of nutrient management in improving crop performance and maintaining soil functions. These findings support the need for integrated investigations rather than focusing only on visible plant growth.

The effects of nitrogen fertiliser may also differ between kale and collard varieties. Varieties vary in maturity period, leaf architecture, root development, nutrient-use efficiency and response to environmental stress. Some may maintain better growth under disease pressure, while others may show greater susceptibility. Differences in plant height, leaf area, chlorophyll content, fresh biomass and disease severity can therefore arise from interactions between genotype, fertiliser and soil conditions. Including different varieties in an experiment makes it possible to determine whether the response to nitrogen is consistent across crops or whether some varieties perform better under particular management conditions. Such information can be useful to farmers and extension officers when developing recommendations for clubroot-prone production areas.

There is a practical need to identify nitrogen management strategies that balance growth, yield, disease response and produce quality. Applying too little nitrogen may reduce leaf expansion and weaken crop productivity, while excessive application may increase costs and create environmental or food-safety concerns. In clubroot-infested soil, the choice of fertiliser source may additionally influence soil acidity and root-zone conditions. A management recommendation should therefore be based on several indicators rather than yield alone. Measurements of plant growth, chlorophyll content, leaf biomass, disease severity, soil pH and selected biochemical properties can provide a more complete understanding of treatment effects.

Although studies have examined vegetable nutrition, soil fertility and disease management, there remains a need for field evidence on the combined effects of nitrogen source and application level on kale and collards grown in clubroot-infested soils in Taita-Taveta County. Information generated in other countries, production systems or soil types may not adequately represent the conditions faced by farmers in the study area. In particular, the interaction between nitrogen fertilisation, soil pH, clubroot severity and crop yield requires further investigation. Establishing this relationship may help clarify whether improved nitrogen nutrition can enhance crop growth while maintaining conditions that are less favourable to disease development.

Despite the widespread use of nitrogenous fertilizers to boost yields on limited land, their interaction with clubroot-infested soils remains poorly understood under Kenyan field conditions, particularly regarding optimal source-rate combinations that balance growth promotion, disease suppression, and biochemical quality. This study addresses these interactions in the context of Taita-Taveta County’s acidic, clubroot-prone soils.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

1.2 Problem Statement

Kales and Collards are important leafy vegetables in Kenya, contributing to food security, nutrition and income among small holder farmers. Their economic importance in Taita-Taveta county is demonstrated by reported Kale production valued at approximately Ksh 95 million in 2014, this indicates the significant contribution of the crop to the county's agricultural economy. However, Clubroot disease poses a major threat to kale and collard production in Taita-Taveta County, causing estimated yield losses of 40–60% in infested fields and threatening food security and farmer incomes (Wambulwa et al., 2018; local extension reports). The pathogen's long soil persistence and preference for acidic conditions make conventional management challenging, especially for resource-limited smallholders lacking access to resistant varieties or expensive soil amendments.

Nitrogen fertilization, while essential for enhancing vegetative growth and yield, exhibits complex and sometimes counterproductive interactions in clubroot-infested soils. Excessive or poorly chosen nitrogen sources can increase disease severity by promoting lush, succulent foliage that is more susceptible to infection and by altering soil pH in ways that favor P. brassicae (Zhao et al., 2019; Hwang et al., 2012). Conversely, strategic application may improve plant vigor and tolerance. There is a critical lack of location-specific data on how different nitrogen sources (e.g., calcium ammonium nitrate vs. sulfate of ammonia) and rates influence growth parameters, biomass yield, clubroot galling index, soil pH dynamics, nitrate accumulation, and phytochemical profiles in Kenyan Brassica systems. This knowledge gap hinders the development of integrated nutrient and disease management strategies tailored to clubroot-prone agroecologies.

1.3 Objectives of the Study

1.4.1 General Objective

To evaluate the effects of nitrogen fertilizer source and rate on growth, yield, clubroot severity, and selected biochemical characteristics of kale and collards in clubroot-infested soils.

1.3.2 Specific objectives;

The study focused on the following specific objectives:

i. To investigate the impact of nitrogen forms (calcium ammonium nitrate and sulfate of ammonia) and rates on growth parameters (plant height, leaf area, and chlorophyll content).
ii. To determine the effects of nitrogen forms and rates on yield and biomass production.
iii. To assess the influence of nitrogen forms and rates on clubroot disease severity (galling index).
iv. To analyze the effects of nitrogen forms and rates on mineral element concentrations (calcium, zinc, iron), oxalates, and phytochemicals (total phenolics and flavonoids) in kale and collard leaves.

1.3.2 Research Hypotheses

i. Nitrogen forms and rates have a significant effect on growth parameters of kale and collards.
ii. Nitrogen forms and rates have a significant effect on yield and biomass production.
iii. Nitrogen forms and rates significantly influence clubroot disease severity.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

iv. There is a significant difference in mineral elements, oxalates, and phytochemical concentrations in response to different nitrogen forms and rates.

1.4 Research Questions

The study was guided by the following research questions:

i. What effects do calcium ammonium nitrate and sulfate of ammonia, applied at different rates, have on the growth parameters of kale and collards, including plant height, leaf area, and chlorophyll content?
ii. How do nitrogen fertilizer sources and rates influence the yield and biomass production of kale and collards in clubroot-infested soils?
iii. What is the effect of nitrogen fertilizer source and rate on clubroot disease severity as measured by the galling index?
iv. How do nitrogen fertilizer sources and rates affect the concentrations of calcium, zinc, iron, oxalates, total phenolics, and flavonoids in kale and collard leaves?

1.5 Justification of the study

Wundanyi, located in the Wundanyi Sub-County of Taita-Taveta County, Kenya, presents a unique and strategic setting for investigating the influence of nitrogenous fertilizer on the growth and yield of kales and collards in clubroot-infested soils. This region is a central hub for agriculture within the county, with surrounding slopes that are highly terraced for farming activities. The area's reliance on agriculture, particularly the cultivation of leafy vegetables like kales and collards, underscores the importance of enhancing crop productivity and disease management practices to ensure food security and economic stability for local communities.

The environmental conditions in Wundanyi, including its soil characteristics and pH levels, are conducive to the development of clubroot disease caused by Plasmodiophora brassicae. Research indicates that clubroot thrives in soils with pH levels below 7.0, particularly in sandy loam and loamy sand soils, which are prevalent in the region. These conditions facilitate the pathogen's proliferation, leading to significant challenges in Brassica crop production.

Nitrogenous fertilizers are commonly applied to enhance plant growth and yield. However, their application in clubroot-infested soils can have complex effects. Excessive nitrogen may exacerbate clubroot severity by promoting lush foliage growth, potentially increasing susceptibility to the pathogen. Conversely, optimal nitrogen management could bolster plant vigor and resilience against disease stress. Investigating these dynamics in Wundanyi will provide localized insights into effective fertilizer management practices.

Conducting this study in Wundanyi is further justified by the potential for broader applicability of the findings. Insights gained from this research can inform best practices not only within the region but also in other areas with similar agro-ecological conditions. By developing evidence-based recommendations for nitrogenous fertilizer management in the context of clubroot-infested soils, the research can contribute to broader efforts aimed at enhancing Brassica crop production and disease management strategies in comparable settings.

Wundanyi's agricultural prominence, environmental conditions conducive to clubroot development, and the need for tailored fertilizer management strategies make it an ideal location for this study. The research outcomes are expected to have significant implications for improving the productivity and sustainability of kale and collard cultivation in the region and

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

beyond.

1.6 Limitations of the Study

The primary limitations were time and financial constraints, restricting the study to two field sites and one growing season (June–October 2025). This may limit generalizability across seasons and broader agroecological zones. However, the use of a robust experimental design with multiple varieties and replications, combined with comprehensive laboratory analyses, mitigates these constraints and provides a strong foundation for future multi-year validation (Hwang et al., 2012).

1.7 Delimitation of the Study

The study was delimited to the evaluation of calcium ammonium nitrate (CAN) and sulfate of ammonia (SA) as nitrogen fertilizer sources. The fertilizer rates considered were 0, 10, and 20 g nitrogen per plant. The research focused on selected kale varieties, namely Curly and Dinosaur, and selected collard varieties, namely Vates and Morris Heading. The investigation was conducted in two clubroot-infested field sites located in Wundanyi and Mwatate Sub-Counties of Taita-Taveta County, Kenya, during the June–October 2025 growing season. The study examined plant height, leaf area, chlorophyll content, yield, biomass, clubroot galling index, soil pH, nitrate-related responses, mineral elements, oxalates, total phenolics, and flavonoids. Other nitrogen sources, additional crops, disease-control products, and agroecological regions outside the selected sites were not included in the experimental scope.

1.8 Significance of the Study

The findings of this study may benefit smallholder farmers by providing information on nitrogen fertilizer sources and rates that support kale and collard production in clubroot-infested soils. The results may assist farmers in balancing crop growth and yield with the need to manage disease severity and maintain soil conditions that are less favorable to Plasmodiophora brassicae.

Agricultural extension officers and county agricultural departments may use the findings when developing locally relevant fertilizer-management recommendations, farmer training materials, and integrated clubroot-management practices. The study may also contribute to improved decision-making concerning soil pH management and the selection of appropriate nitrogen fertilizer sources.

Researchers and students in crop science, soil science, plant pathology, and agricultural development may use the findings as a reference for further studies on the relationship between nitrogen nutrition, soil properties, clubroot disease, yield, and vegetable nutritional quality. The results may provide a basis for multi-season and multi-location investigations.

Consumers and the wider food system may benefit indirectly if improved nutrient management increases the availability, productivity, and selected nutritional characteristics of kale and collards. The study may also contribute to sustainable agriculture by informing fertilizer practices that seek to reduce unnecessary nutrient losses and maintain soil productivity.

1.8 Assumptions of the Study

The study was based on the following assumptions:

i. The experimental sites were representative of clubroot-infested kale and collard

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

production conditions in the selected areas of Taita-Taveta County.

ii. The selected kale and collard varieties were sufficiently established and managed uniformly apart from the imposed nitrogen fertilizer treatments.
iii. The nitrogen fertilizer sources and rates were applied accurately and consistently according to the experimental protocol.
iv. Differences observed among treatments were primarily associated with the nitrogen source, nitrogen rate, variety, and site effects, together with their interactions, while other management practices were kept as uniform as possible.
v. The procedures used for measuring growth, yield, disease severity, soil pH, mineral elements, oxalates, phenolics, and flavonoids produced reliable estimates of the variables under investigation.
vi. The environmental conditions during the study period allowed clubroot disease and crop responses to develop sufficiently for treatment comparisons

1.9 Operational Definition of Terms

The following terms are used operationally in this study:

Calcium ammonium nitrate (CAN): A nitrogen fertilizer containing nitrate and ammonium forms of nitrogen and calcium, evaluated in this study as one of the nitrogen sources.

Clubroot: A soil-borne disease of Brassica crops caused by Plasmodiophora brassicae and characterized by abnormal swelling or gall formation on roots.

Clubroot severity: The degree of disease development in plants, assessed in this study using the root galling index.

Galling index: A numerical assessment of the extent of club-shaped root swelling caused by clubroot infection, used to compare disease severity among treatments.

Collards: Leafy vegetables belonging to Brassica oleracea var. acephala, represented in this study by the Vates and Morris Heading varieties.

Growth parameters: Measurable indicators of vegetative development, specifically plant height, leaf area, and chlorophyll content in this study.

Nitrogen fertilizer rate: The quantity of nitrogen supplied to each plant through the assigned fertilizer treatment, expressed in grams of nitrogen per plant.

Nitrogen fertilizer source: The type of nitrogen fertilizer applied to plants, specifically CAN or sulfate of ammonia in this study.

Sulfate of ammonia (SA): An ammonium-based nitrogen fertilizer, also known as ammonium sulfate, evaluated for its effects on crop performance, soil pH, and clubroot severity.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Yield: The quantity of harvestable plant material obtained from the experimental crop, assessed using the study's specified harvest measurements.

Biomass: The fresh or measured mass of plant material produced by the crop, including the harvested vegetative parts assessed in this study.

Mineral elements: The concentrations of selected mineral nutrients in leaves, specifically calcium, zinc, and iron.

Phytochemicals: Plant-produced bioactive compounds measured in this study as total phenolics and flavonoids.

Oxalates: Organic acids and their salts occurring naturally in plant tissues and measured in this study as a component of leaf biochemical quality.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

The basis of the world's food production is agriculture, and maintaining it is essential to tackling the major issues of food security, population increase, and environmental preservation (Foley et al., 2011). One important aspect of agricultural operations that has been recognised as having a significant impact on crop productivity and total food output is the prudent use of fertilisers (Moll et al., 1982). Nitrogenous fertilisers are the most important form of fertilisers since they maximise yields and promote plant growth (Chapman et al., 2012).

A limiting element in crop output is often nitrogen, an essential nutrient for plant development. Plants can reach their maximum development potential when they have access to this nutrient, which is made possible by nitrogenous fertilisers (Havlin et al., 2014). Nevertheless, a wide range of factors, such as crop kinds, soil conditions, and the existence of soil-borne diseases, affect how effective nitrogenous fertilisers are.

Club-root disease, which is caused by the soil-borne pathogen Plasmodiophora brassica e, is one such illness that significantly affects crop yield (Dixon, 2009). This illness is a serious danger to cruciferous vegetables, especially collards and kales, which are staples in many areas of the world's diets (Dixon, 2009). An infestation of club-root disease causes stunted growth, reduced yields, and deformed roots, which in turn affects crop quality and marketability (Hwang et al., 2012).

The relationship between these two parameters becomes crucial to agricultural research because of the dual problems of minimising the negative effects of club-root disease and optimising crop development through the application of nitrogenous fertilisers (Rees et al., 2019). Promoting sustainable agricultural methods requires a thorough understanding of the interactions between nitrogenous fertilisers and soils infested with club roots, as well as the effects these interactions have on crop growth and productivity (Dixon, 2009). The paucity of research in this particular area, however, highlights the need for a thorough examination of the state of knowledge and research gaps.

By carefully examining relevant studies and research findings that shed light on the complex interactions between the supply of nitrogenous fertilisers and club-root disease in crop production systems, this literature review aims to close this knowledge gap (Dixon, 2009; Hwang et al., 2012). Through an examination of the present literature, this review seeks to establish the foundation for the research topics, methodology, and goals of the current investigation. In addition, it aims to identify important areas that require more research, advancing agricultural science and assisting in the development of sustainable crop management techniques (Rees et al., 2019).

This chapter synthesizes existing literature on the interplay between nitrogen fertilizer source and rate, clubroot disease severity, crop growth, yield, and biochemical quality in infested soils.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

It moves beyond description to critically analyze mechanisms, compare empirical findings, highlight contradictions, and identify research gaps that justify the current study.

2.1 Overview of Existing Research

Numerous important ideas from the disciplines of soil science, plant physiology, and agronomy are involved in understanding the relationship between the availability of nitrogenous fertilisers, club-root disease, and crop growth. Fundamental knowledge of plant nutrient requirements and the dynamics of soil-plant interactions are the cornerstones of this framework.

For proper growth and development, plants—including kales and collards—need a balanced supply of vital elements. Nitrogen (N) is the most important of these nutrients because it is involved in various activities including photosynthesis, protein synthesis, and overall plant metabolism (Marschner, 2011). Plants mostly obtain their readily accessible nitrogen from nitrogenous fertilisers (Galloway et al., 2008). By supplying an additional nitrogen source beyond what is available from the soil, the use of nitrogenous fertilisers is known to improve plant growth, increase leaf area, and boost biomass production (Marschner, 2011). However, soil conditions and the existence of soil-borne pathogens can affect how efficient nitrogenous fertilisation is (Moll et al., 1982).

cruciferous crops like collards and kales are seriously threatened by club-root disease, which is brought on by the soil-borne pathogen Plasmodiophora brassica e (Dixon, 2009). The infection causes swellings or galls that obstruct the plant's ability to absorb nutrients and water through its roots (Dixon, 2009). According to Hwang et al. (2012), the disease modifies the architecture of the roots and interferes with the plant's capacity to efficiently absorb vital nutrients. Club-root disease thus frequently causes stunted development, poor agricultural production, and a general decline in plant health.

One important factor influencing crop health and productivity is the relationship between plants and soil. Plant responses to fertilization are significantly influenced by the qualities of the soil, such as pH, organic matter content, and nutrient availability (Havlin et al., 2014). For instance, soil acidity can impact nutrient solubility and, as a result, plant uptake of those nutrients (Havlin et al., 2014). By interfering with nutrient absorption processes and root structure, soil-borne pathogens like P. brassica e can exacerbate soil-plant interactions (Dixon, 2009).

The balance between stimulating plant growth by nitrogen supplementation and minimizing the negative effects of the disease is a crucial factor in optimizing fertilizer management in the presence of soil-borne diseases (Chapman et al., 2012). Research has indicated that an overabundance of nitrogen can intensify the severity of several soil-borne illnesses, such as club-root (Chapman et al., 2012). Thus, for efficient fertiliser management in disease-infested soils, striking the correct balance between supplying enough nitrogen for plant growth and reducing disease burden is essential (Chapman et al., 2012).

Theoretical findings indicate that there is a complicated and multifaceted interaction between crop development, club-root disease, and the availability of nitrogenous fertilisers. Although the body of literature now in publication offers a basic comprehension of these ideas, there is

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

a discernible deficiency in studies explicitly focusing on the dynamics of nitrogenous fertiliser application in soils infested with club roots for kales and collards. By examining the complex relationships between these variables, this study aims to close this gap and ultimately offer evidence-based suggestions for improving fertiliser management techniques in kale and collard production systems plagued by disease.

2.1.2 Nutrient Uptake

A key idea in soil science and plant physiology is nutrient uptake, which explains how plants take up vital nutrients from the soil, such as nitrogen. This empirical data is especially pertinent to the investigation of the "Influence of Nitrogenous Fertiliser Supply on Growth and Yield of Kales and collards in Club-Root Infested Soils" because it sheds light on the mechanisms and processes controlling plant nutrient uptake.

Fundamentally, nutrient uptake describes how plants absorb nutrients from the surrounding soil through their root systems. Several important factors influence this process and must be understood to optimise nutrient management techniques in agriculture.

Soil nutrient availability is a crucial component of nutrient absorption (Marschner, 2011). Based on empirical research, a plant's capacity to absorb nutrients is directly impacted by the type and concentration of nutrients present in the soil. For example, nitrogen can be found in the soil in a variety of forms, such as nitrate (NO3-) and ammonium (NH4+). Different factors, including soil pH, microbial activity, and the presence of competing ions, might affect the availability of these forms, which plants can take up through distinct pathways (Marschner, 2011).

Within the study, the application of Nutrient Uptake can be utilised to investigate the impact of club-root disease on the nitrogenous fertilizer-absorbing capacity of collards and kales. Root structure and function are frequently altered by club-root disease (Dixon, 2009). Consequently, the roots might be less able to absorb nitrogen and other nutrients. Reduced crop yields and stunted growth may result from this.

Furthermore, the empirical evidence highlights the importance of root health and root-soil interactions. Healthy root systems with well-developed root hairs are more efficient at nutrient uptake (Marschner, 2011). In the presence of club-root disease, which can cause root deformities and impair root function, nutrient uptake may be compromised. This underscores the significance of understanding how soil-borne diseases like club-root can influence nutrient absorption.

Furthermore, the Nutrient Uptake highlights the function of ion channels and nutrient transporters in plant roots. These transport proteins help ions from the soil, such as ammonium and nitrate, to get into the roots of plants (Marschner, 2011). The efficiency of nutrient absorption can be impacted by any disturbances to these transport mechanisms, including those brought on by illness.

Understanding how plants take up vital nutrients from the soil is made easier with the help of the Nutrient Uptake framework. It clarifies, in the context of the study, how club-root disease

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

may obstruct the process of absorbing nutrients, especially nitrogen from nitrogenous fertilisers. The research can provide deeper insights into the parameters impacting the growth and yield of kales and collards in club-root-infested soils by utilising this empirical finding and considering the difficulties of nutrient absorption in disease-affected plants (Marschner, 2011).

2.1.3 Nutrient Competition

A key idea in ecology and soil science, nutrient competition studies how plants and soil microbes compete with one another for vital nutrients in a shared environment. This empirical evidence is relevant to our knowledge of the dynamics of nutrient availability and utilisation in agricultural systems, such as the one studied in "Influence of Nitrogenous Fertiliser Supply on Growth and Yield of Kales and Collards in Club-Root Infested Soils."

At its core, Nutrient Competition Empirical evidence asserts that in any given ecosystem, including agricultural soils, plants and soil microorganisms (such as bacteria and fungi) compete for limited nutrients, such as nitrogen (N), phosphorus (P), and potassium (K) (Havlin et al., 2014). This competition is based on the principle that these organisms require nutrients for their growth and survival.

Within the study's framework, this empirical data can be utilised to comprehend the ways in which the existence of club-root disease impacts the competition between soil bacteria and kales for nutrients. These crops' root systems are impacted by club-root disease, which may limit their ability to absorb nutrients (Dixon, 2009). Soil microorganisms may benefit from a competitive edge in obtaining nutrients as a result.

According to Havlin et al. (2014), soil microbes are essential for the mineralization and cycling of nutrients because they break down organic matter and transform it into forms that plants can absorb. Disease-compromised plant roots may discharge fewer organic substances and root exudates into the soil, which may have an impact on the microbial ecology and nutrient availability. This may make the fight for nutrients even more fierce.

The Competition for Nutrients Research clearly shows that the amount of nutrients in the soil matters not just to plants, but also to microbes and plants in terms of how well they are able to acquire and use those nutrients (Havlin et al., 2014). The changed root structure and decreased root exudation caused by club-root disease may affect the effectiveness of kales and collards' nutrient uptake, thereby providing soil microbes with an advantage in obtaining nutrients.

Moreover, the empirical evidence suggests that changes in nutrient availability can impact the overall health and productivity of plants (Havlin et al., 2014). In this study, variations in nitrogenous fertilizer supply can influence the nutrient competition dynamics. Excessive nitrogen application, for example, may stimulate microbial activity and exacerbate nutrient competition, potentially affecting crop growth and yield.

Nutrient Competition Empirical evidence offers valuable insights into the dynamics of nutrient competition between plants and soil microorganisms in agricultural systems. In the context of the study, it helps elucidate how club-root disease may alter the nutrient competition dynamics, potentially influencing the growth and yield of kales and collards in club-root-infested soils.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

By applying this empirical evidence, the research can gain a deeper understanding of the complex interactions shaping nutrient availability and utilization in disease-affected agricultural ecosystems (Havlin et al., 2014).

2.1.4 Disease Severity

A key idea in plant pathology, disease severity examines the complex interplay between a plant disease's severity and a range of external factors, such as management techniques, environmental circumstances, and the existence of additional stressors (Agrios, 2005). The impact of nitrogenous fertiliser supply variations on the severity of club-root disease and, in turn, the growth and yield of kales and collards is examined in the context of the study, "Effects of Nitrogen Fertiliser levels on Growth and Yield of Kales and Collards in Club-Root Infested Soils," using empirical evidence to determine disease severity.

Central to Disease Severity Empirical evidence is the recognition that the intensity and impact of a plant disease extend beyond the mere presence of the pathogen. Rather, they are shaped by the complex interplay among the host plant (in this case, kales and collards), the pathogen (Plasmodiophora brassicae responsible for club-root disease), and the surrounding environment (Agrios, 2005). Several key components of Disease Severity Empirical evidence bear relevance to this study.

One critical facet is host susceptibility, acknowledging that different plant varieties or species exhibit varying degrees of susceptibility to a disease (Agrios, 2005). In the study context, this facet helps explore whether kales and collards manifest different levels of susceptibility to club-root disease and whether this susceptibility is influenced by nitrogenous fertilizer supply.

Pathogen virulence is another pivotal element, as disease severity hinges on the virulence of the pathogen (Agrios, 2005). Certain strains of P. brassica e may exhibit heightened virulence, resulting in more pronounced symptoms in the host plant. Investigating how variations in nitrogenous fertilizer supply affect pathogen virulence can furnish insights into disease severity.

Environmental conditions are integral to disease development, encompassing factors such as temperature, humidity, and soil conditions (Agrios, 2005). Nitrogenous fertilizer application can induce changes in soil properties, potentially creating conditions that either facilitate or hinder the pathogen's development and its interaction with the host plant.

Furthermore, Disease Severity Empirical evidence underscores the vulnerability of stressed plants to diseases (Agrios, 2005). Nutrient imbalances, including excessive nitrogen, can impose stress on plants, rendering them more susceptible to club-root disease. Conversely, judicious nutrient management may alleviate plant stress, thereby impacting disease severity.

Lastly, management techniques, such as the use of fertiliser, have a significant impact on the severity of disease (Agrios, 2005). The investigation can focus on how different nitrogenous fertiliser treatment rates can either lessen or increase the severity of club-root disease in collards and kales. Level of Disease A strong foundation for understanding the complex relationships among plant diseases, environmental factors, and management strategies is

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

provided by empirical evidence. Within the parameters of the study, it provides a useful viewpoint on how changes in the availability of nitrogenous fertilisers may affect the degree of club-root disease in collards and kales, which in turn may affect crop output and growth (Agrios, 2005).Several field and greenhouse studies have examined N management in Brassicas under clubroot pressure. Albornoz et al. (2017) reported that calcium fertilization combined with moderate N reduced clubroot severity in broccoli by 40–60% through pH modulation. In collards, moderate rates (equivalent to 100–150 kg N ha^{-1}) maximized yield while higher rates increased galling (Fernandez et al., 2013). Kenyan-specific data are scarce; Wambulwa et al. (2018) noted yield losses but did not test source-rate interactions. Biochemical responses vary: moderate N often enhances glucosinolates and phenolics, while excess reduces them due to resource allocation trade-offs (Farnham et al., 2004; Ndolo et al., 2019).

Contradictions appear in optimal rates across varieties and environments, underscoring the need for site-specific research

2.1.5 Plant-Pathogen Interaction

Pathogen-Plant Interaction In the study of plant pathology, which examines the complex interactions between pathogens and plants as well as the consequences of disease, empirical data is fundamental. This empirical data provides a crucial framework for comprehending how changes in the supply of nitrogenous fertiliser affect the dynamics of club-root disease in kales and collards, as reported in the study "Effects of Nitrogen Fertiliser Levels on Growth and Yield of Kales and Collards in Club-Root Infested Soils" (Agrios, 2005).

This empirical evidence emphasizes that the severity and progression of plant diseases are not solely determined by the presence of the pathogen but are significantly shaped by the complex interactions between the pathogen (in this case, Plasmodiophora brassica e causing club-root disease), the host plants (kales and collards), and the environment (Agrios, 2005).

Host Susceptibility plays a pivotal role, as it recognizes that different plant varieties or species exhibit varying degrees of susceptibility to diseases (Agrios, 2005). In the study, this facet enables exploration into whether kales and collardsdisplay differential susceptibility to club-root disease and whether nitrogenous fertilizer supply influences this susceptibility.

Pathogen Virulence is closely linked to disease severity, as certain strains of P. brassica e may exhibit heightened virulence, resulting in more severe symptoms in the host plant (Agrios, 2005). Investigating how variations in nitrogenous fertilizer supply affect pathogen virulence provides insights into disease severity.

Environmental Conditions are recognized as crucial factors in disease development, including temperature, humidity, and soil conditions (Agrios, 2005). Nitrogenous fertilizer application can alter soil properties, potentially creating conditions that either facilitate or impede the pathogen's development and its interaction with the host plant.

Plant Stress and Disease are interconnected, as stressors like nutrient imbalances, including excessive nitrogen, can render plants more susceptible to diseases (Agrios, 2005).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Understanding how nitrogenous fertilizer supply influences plant stress and its implications on disease severity is central to the study's objectives.

Management Practices, encompassing disease management strategies such as fertilizer application, can significantly impact disease severity (Agrios, 2005). The study can explore how varying rates of nitrogenous fertilizer application may either alleviate or exacerbate the severity of club-root disease in kales and collards.

Pathogen-Plant Interaction A thorough foundation for understanding the complex relationships between plant diseases, host plants, pathogens, and environmental factors is provided by empirical research. In the context of the study, it provides an insightful look at how variations in the availability of nitrogenous fertilisers may mould the interactions between kales and collards and club-root disease, ultimately modifying the severity of the illness and impacting crop growth and yield (Agrios, 2005).

Disease severity results from tripartite interactions among host, pathogen, and environment (Agrios, 2005). Nitrogen influences host susceptibility by altering tissue N content and secondary metabolism. Moderate N strengthens defense via enhanced phenylpropanoid pathways (phenolics and flavonoids), while excess promotes lush growth that facilitates infection (Zhao et al., 2019).

In clubroot systems, ammonium nutrition can increase severity by acidifying soil, whereas nitrate or CAN reduces it through pH elevation and improved root health (Albornoz et al., 2017; Hwang et al., 2012). These findings are consistent with broader literature on nutrient-disease interactions, where balanced fertilization enhances induced systemic resistance (Dordas, 2009).

2.1.6 Fertilizer Response

Fertilizer Response Empirical evidence is a fundamental concept in the realm of soil science and agronomy, examining how plants respond to fertilizer applications and the notion of nutrient use efficiency (Marschner, 2011). In the study, Effects of Nitrogen Fertilizer levels on Growth and Yield of Kales and collards in soil infested with Club-Root," this empirical evidence provides a vital framework for understanding how variations in nitrogenous fertilizer supply can impact the growth and yield of kales and collards.

At its core, Fertilizer Response Empirical evidence emphasizes that plants exhibit specific responses to the application of fertilizers, and these responses are influenced by factors such as nutrient availability and plant species (Marschner, 2011). In the context of the study, several key aspects of this empirical evidence hold significance.

This empirical evidence recognizes that the concentration and form of nutrients in the soil directly influence a plant's ability to absorb them (Marschner, 2011). Nitrogen, a critical nutrient, can exist in various forms in the soil, including ammonium and nitrate. Understanding

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

how variations in nitrogenous fertilizer supply affect nutrient availability is essential in assessing its impact on the growth and yield of kales and collards.

Variation Across Plant Species: Fertiliser Reaction According to empirical data, many plant species and cultivars may react differently to fertiliser treatments (Marschner, 2011). It can assist in clarifying if kales and collards react differently to different nitrogenous fertiliser application rates and whether certain nutrient management techniques are more suited for a certain crop than another. The idea of nutrient usage efficiency—in which plants make the best use of the nutrients that are available to sustain growth—is highlighted by the empirical data (Marschner, 2011). The ability of the crops to extract and use nutrients efficiently can be gained by examining how varying rates of nitrogenous fertiliser application affect nutrient usage efficiency in kales and collards.

Fertilizer Response Empirical evidence underscores the importance of healthy root systems and their role in nutrient uptake (Marschner, 2011). Club-root disease can adversely affect root health, potentially impacting nutrient absorption. Understanding how nitrogenous fertilizer supply interacts with root health and nutrient uptake in disease-affected plants is essential.

The empirical data emphasises how important it is to manage nutrients well in order to maximise plant development and yield and minimise nutrient losses (Marschner, 2011). In soils infested with club roots, the study can investigate how varying rates of nitrogenous fertiliser application correspond with the best nutrient management strategies for kales and collards. Fertiliser Reaction Understanding how plants react to fertiliser treatments is made easier by empirical evidence, particularly when considering nutrient availability, species variation, nutrient usage efficiency, root health, and ideal nutrient management. Within the parameters of the study, this empirical data provides information on how fluctuations in the availability of nitrogenous fertilisers might affect the development and productivity of collards and kales, providing insight into practical approaches to nutrient management (Marschner, 2011).

2.7 Soil pH and Nutrient Availability

Soil pH and Nutrient Availability Empirical evidence is a fundamental concept in soil science, focusing on the influence of soil pH levels on the availability and uptake of essential nutrients by plants (Brady & Weil, 2008). In the study, "Effects of Nitrogen Fertilizer Levels on Growth and Yield of Kales and collards in Club-Root Infested Soils," this empirical evidence serves as a crucial framework for understanding how variations in soil pH resulting from nitrogenous fertilizer supply can impact the growth and yield of kales and collards.

At its core, this empirical evidence emphasizes that soil pH plays a pivotal role in determining the chemical forms and solubility of essential nutrients in the soil solution, which in turn affects their accessibility to plant roots (Brady & Weil, 2008). In the context of the study, several key aspects of this empirical evidence are pertinent:

Soil pH significantly influences the availability of essential nutrients, with some nutrients becoming more or less available as pH levels change (Brady & Weil, 2008). For instance, in acidic soils, nutrient elements like aluminum and manganese can become more available and

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

potentially toxic to plants, while in alkaline soils, micronutrients such as iron and zinc may become less available. Investigating how variations in soil pH due to nitrogenous fertilizer supply impact nutrient availability is central to the study.

Soil pH levels directly affect the ability of plant roots to uptake nutrients (Brady & Weil, 2008). In acidic soils, nutrient uptake can be impeded due to toxic levels of aluminum and manganese, while in alkaline soils, nutrient deficiencies may arise. Understanding how fluctuations in soil pH resulting from nitrogenous fertilizer supply influence nutrient uptake by kales and collards is crucial for assessing their growth and yield.

Soil pH levels can induce stress in plants, affecting their overall health and productivity (Brady & Weil, 2008). Plants may exhibit stress responses, such as reduced root growth and nutrient uptake, in soils with extreme pH values. Investigating how nitrogenous fertilizer supply influences soil pH and, subsequently, plant stress levels is vital in the context of club-root-infested soils.

This empirical evidence underscores the importance of maintaining an optimal pH range for each plant species to ensure nutrient availability and uptake (Brady & Weil, 2008). The study can explore whether nitrogenous fertilizer supply helps maintain or disrupt the optimal pH range for kales and collards in club-root-infested soils.

Soil pH and Nutrient Availability Empirical evidence offer a critical framework for comprehending how soil pH levels affect nutrient availability, nutrient uptake by plant roots, plant stress responses, and the maintenance of optimal pH ranges for plant growth. Within the study's scope, this empirical evidence provides valuable insights into how variations in soil pH due to nitrogenous fertilizer supply can impact the growth and yield of kales and collards, especially in the presence of club-root disease (Brady & Weil, 2008)

Clubroot symptoms develop below ground before the full effect becomes visible in the foliage, while swollen roots interfere with water and mineral acquisition; therefore, above-ground symptoms may include wilting, yellowing, stunting, and premature senescence. Overall, the evidence provides a basis for examining the variable in the current study while recognizing that the response must be tested under the conditions of the selected sites. This relationship is important because treatment effects are not determined by the quantity of fertilizer alone; they also depend on timing, placement, soil characteristics, and the physiological condition of the crop.

Clubroot symptoms develop below ground before the full effect becomes visible in the foliage. Swollen roots interfere with water and mineral acquisition. Above-ground symptoms may include wilting, yellowing, stunting, and premature senescence. The relevance of this issue is heightened in smallholder systems, where fertilizer is often applied with limited soil testing and where input costs must be justified by reliable yield gains. These considerations indicate that recommendations should be expressed in relation to crop requirements and local conditions rather than presented as universally applicable values.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

clubroot symptoms develop below ground before the full effect becomes visible in the foliage. swollen roots interfere with water and mineral acquisition. above-ground symptoms may include wilting, yellowing, stunting, and premature senescence. The interaction is also likely to change during the growing season because nutrient demand rises with canopy development while pathogen effects may become more pronounced as roots enlarge. Consequently, sampling at more than one stage can reveal whether an apparent early benefit is maintained until harvest.

2.2. Empirical Review

The "Effect of Nitrogenous Fertiliser level on Growth and Yield of Kales and Collards in Club-Root Infested Soils" study undertook an empirical review that involved a methodical examination of previous empirical studies that were specifically relevant to the research question. Through an analysis of prior study findings, techniques, and significant ideas, it sought to offer an evidence-based knowledge of the topic. The review found that although there has been very little empirical research on this subject, it is crucial for sustainable agriculture, especially when it comes to understanding how the availability of nitrogenous fertilisers affects the growth and yield of green vegetables in soils afflicted by club-root disease. Numerous investigations examined associated facets, elucidating diverse elements impacting plant reactions to nitrogenous fertilisers under soil conditions prone to disease.

The review specifically looked at empirical research that evaluated how different rates of nitrogen fertiliser application affected the output of kale in soils infested with club roots and how different collard types responded to different supplies of nitrogenous fertiliser. The results indicated that certain collard cultivars showed stronger resistance to club-root disease and responded more favourably to nitrogen fertilisation. Additionally, moderate nitrogen fertilisation considerably increased kale yield when compared to low or high nitrogen treatments. Other research examined how kale development was affected by the addition of nitrogenous fertiliser and soil pH adjustment in soils infested with club roots. These findings demonstrated the critical function that soil pH management plays in moderating the effects of nitrogen fertilisation on kale growth. Additionally, research explored the relationship between nutrient uptake efficiency, club-root disease severity, and varying nitrogen fertilizer rates in collards, highlighting the influence of both nitrogen supply and disease severity on nutrient uptake efficiency.

Collectively, these empirical studies contribute to understanding the influence of nitrogenous fertilizer supply on the growth and yield of kales and collards in club-root-infested soils. They underscore the importance of considering factors such as crop variety, soil pH, nutrient uptake efficiency, and integrated nutrient management practices in addressing this complex agricultural challenge.

2.2.1 Nitrogen Nutrition in Plants

Nitrogen is essential for amino acid, protein, nucleic acid, and chlorophyll synthesis. Assimilation occurs primarily via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway, directly influencing photosynthetic efficiency and vegetative growth (Marschner,

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

2012). Leafy vegetables like kale and collards exhibit high N demand, with optimal rates enhancing leaf area and biomass (Fageria & Baligar, 2005).

However, nitrogen form matters: nitrate (NO_3^-) is readily absorbed and supports rapid vegetative growth, while ammonium (NH_4^+) can cause rhizosphere acidification through nitrification (NH_4^+

$+ 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$), altering nutrient availability and microbial communities (Havlin et al., 2014). Excessive N can lead to luxury consumption, increased succulence, and higher susceptibility to pathogens (Huber & Thompson, 2007).

2.2.2 Brassica Crops and Clubroot Disease

Kale and collards (Brassica oleracea var. acephala) are valued for their nutritional profile, including glucosinolates with anticancer and antioxidant properties (Farnham et al., 2004; Ndolo et al., 2019). In Kenya, they are cultivated intensively in highland and mid-altitude zones, yet clubroot remains a persistent threat.

Plasmodiophora brassicae is an obligate biotroph whose life cycle involves resting spores that can survive in soil for 10–20 years. In acidic soils (pH 5.0–6.5), spores germinate into primary zoospores that infect root hairs. Secondary plasmodia then induce gall formation through hormonal imbalance (auxin and cytokinin overproduction), disrupting vascular tissues and causing nutrient deficiencies (Dixon, 2009). Disease severity is exacerbated by high soil moisture, moderate temperatures (18–25°C), and susceptible varieties (Hwang et al., 2012).

Contrasting studies show that while resistant cultivars offer partial control, their adoption in smallholder systems is limited by cost and availability (Lamichhane et al., 2018). In African contexts, including Kenya, management relies heavily on cultural practices and soil amendments due to limited chemical options (Wambulwa et al., 2018).

2.2.3 Effects of Nitrogen Forms on Soil pH and Nutrient Availability

Soil pH profoundly influences nutrient solubility and pathogen activity (Brady & Weil, 2008). Ammonium-based fertilizers (e.g., sulfate of ammonia, SA) lower soil pH, increasing availability of micronutrients like Mn and Fe but potentially inducing Al toxicity and favoring acidophilic pathogens like P. brassicae (Ju et al., 2018). Calcium ammonium nitrate (CAN), conversely, provides a liming effect through Ca^{2+}, raising pH and improving Ca-mediated cell wall integrity and root function (Albornoz et al., 2017).

Empirical evidence shows pH shifts of 0.5–1.0 units can significantly alter clubroot incidence, with suppression observed above pH 6.5 due to inhibited zoospore release and motility (Smolinska & Palusinska, 2005). Contradictions exist: some studies report minimal pH effects under buffered soils, while others emphasize source-specific impacts in low-buffer-capacity tropical soils (common in Taita-Taveta).

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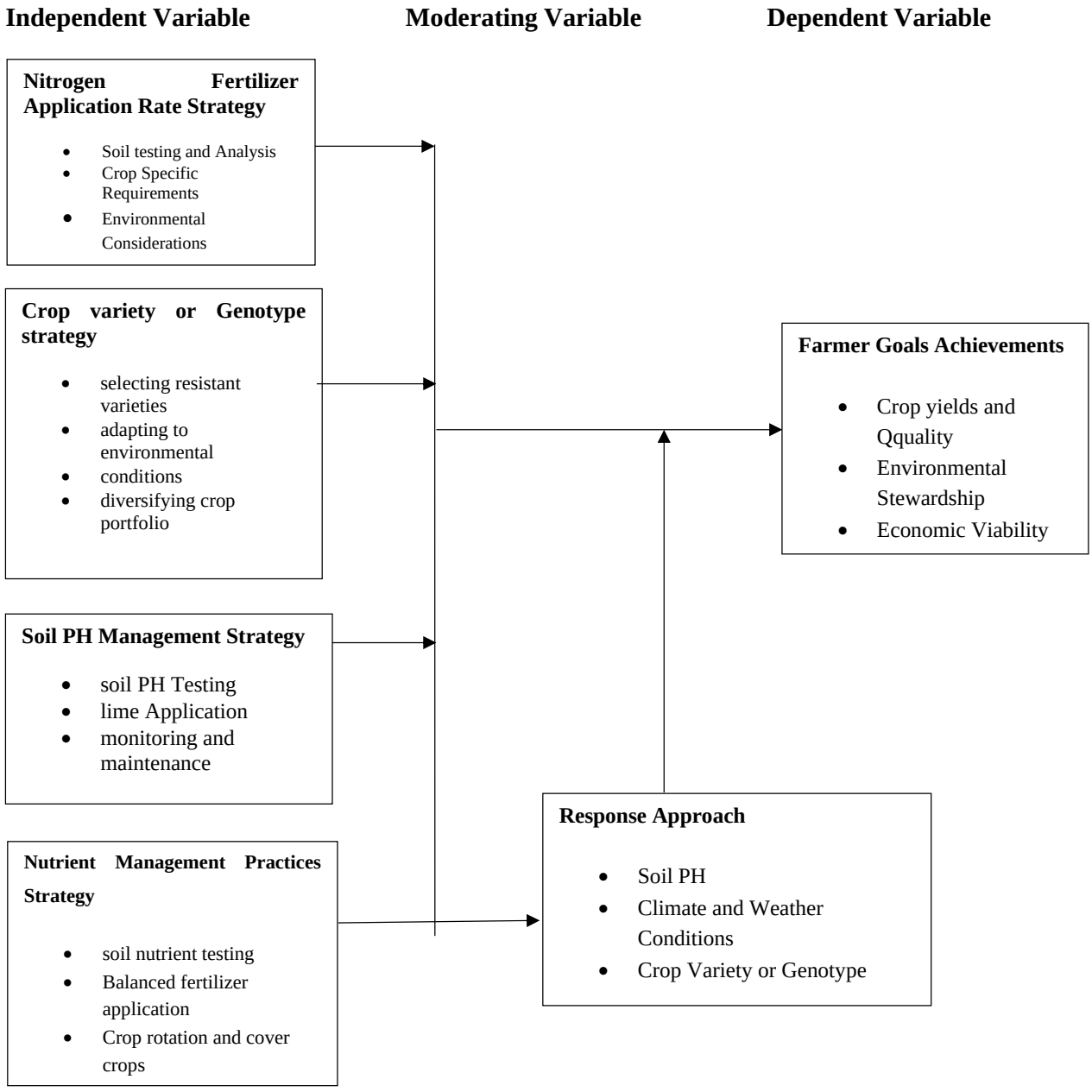
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

2.3 Conceptual Framework

The conceptual framework illustrates the relationships among key variables guiding the study. Independent variables are nitrogen fertilizer source (CAN vs. SA) and application rate (0, 10, 20 g plant^{-1}). Moderating variables include soil pH, crop variety, and environmental conditions (site-specific factors in Wundanyi and Mwatate). These influence the dependent variables: growth parameters, yield/biomass, clubroot galling index, and biochemical composition (minerals, oxalates, phenolics, flavonoids).

The framework is grounded in soil-plant-pathogen-nutrient interaction theories (Marschner, 2012; Dixon, 2009) and posits that optimal nitrogen management modulates soil chemistry and plant physiology to enhance productivity while suppressing disease.

Figure 2:1
conceptual framework



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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

2.4 Knowledge Gaps

Despite substantial global research, critical gaps persist. Most studies are greenhouse-based or focus on single factors (rate or form), with limited integration of agronomic, pathological, and biochemical outcomes under field conditions in sub-Saharan Africa. In Kenya, there is a paucity of data on CAN versus SA interactions in clubroot-infested acidic soils of Taita-Taveta, particularly regarding optimal rates for balancing yield, disease suppression, soil health, and nutritional quality (minerals, phenolics, flavonoids). Long-term effects on soil microbial communities and nitrate leaching also remain underexplored. This study addresses these gaps by conducting multi-variety field experiments with comprehensive assessments, providing evidence-based recommendations for sustainable Brassica production.

CHAPTER THREE

RESEARCH METHODOLOGY

3.0 Introduction

This chapter describes the research methodology employed to evaluate the effects of nitrogen fertilizer source and rate on growth, yield, clubroot severity, and selected biochemical characteristics of kale and collards in clubroot-infested soils. It provides a detailed account of the study area, experimental design, treatments, agronomic practices, data collection procedures, laboratory analyses, and statistical methods. The methodology was designed to ensure scientific rigor, reproducibility, and relevance to smallholder farming systems in Taita-Taveta County, Kenya.

3.1 Study area description

The study was conducted in Wundanyi and Mwatate Sub-Counties, Taita-Taveta County, Kenya. Taita-Taveta County lies in the coastal hinterland between latitudes 2°46'S and 4°10'S and longitudes 37°36'E and 38°48'E, with elevations ranging from 1500 to 2590 m above sea level in the Taita Hills. The region experiences a bimodal rainfall pattern with long rains from March to May and short rains from October to December. Annual rainfall averages 885–1250 mm, and mean temperatures range from 18°C to 25°C (Climate-Data.org, 2024; Gacathi, 2014). These conditions are conducive to Brassica production but also favor clubroot development due to persistent acidic soils and high moisture.

3.1.2 Experimental site

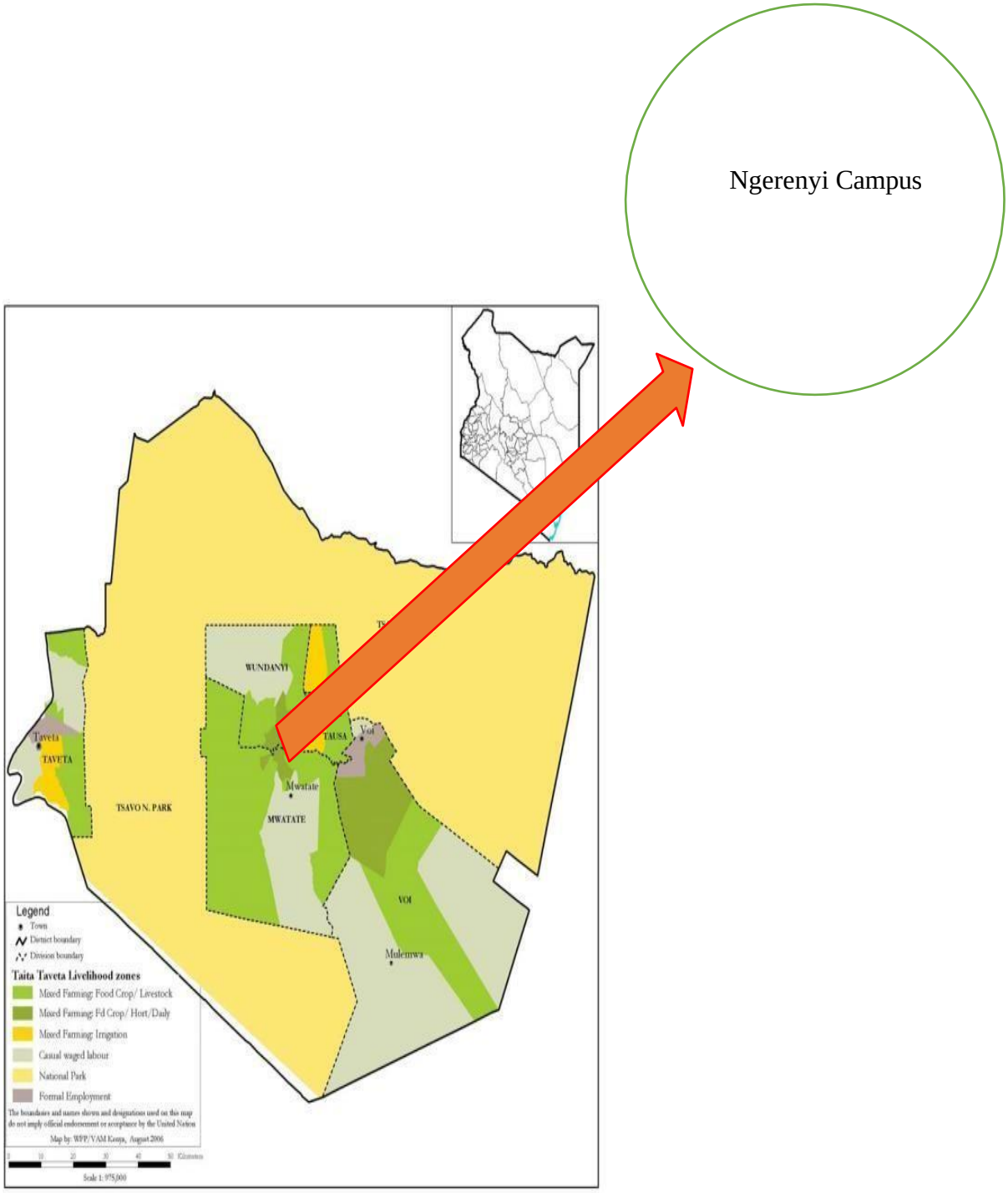
Field experiments were established at two sites: (1) Taita Taveta University Research Farm, Kidaya-Ngerenyi Campus (Wundanyi area, elevation ≈1720 m, coordinates 3.43247°S, 38.342788°E), and (2) a farmer's field in Mwatate Sub-County representing typical smallholder conditions. Soils at both sites are predominantly Luvisols, Lixisols, and Acrisols—well-drained, dark reddish-brown to dark brown, with textures ranging from sandy clay loam to clay (Shisanya et al., 2006; Motaroki, 2016). Pre-planting soil analysis confirmed clubroot infestation through bioassay (gall formation on susceptible indicator plants) and spore counts (>10^5 resting spores g^{-1} soil), with initial pH 5.4–5.8, organic matter 2.1–3.4%, and moderate N and P levels. These characteristics represent typical clubroot-prone soils in the region.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Figure 3.1:

Map showing the study area in the Taita Hills Of Taita-Taveta County, Kenya



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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Figure 1.2:Photo showing cultivation farm in Taita hills of Taita-Taveta county, Kenya



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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Figure 3.3: photo showing cultivation farm in Taita hills of Taita-Taveta county, Kenya



3.2 Experimental design

The study employed a randomized complete block design (RCBD) with a split-plot arrangement and three replications at each of the two sites. Main plots consisted of crop types/varieties: two kale varieties (Curly Kale and Dinosaur Kale) and two collard varieties (Vates and Morris Heading). Subplots contained nitrogen treatments: two sources (Calcium Ammonium Nitrate [CAN, 26% N] and Sulfate of Ammonia [SA, 21% N]) × three rates (0, 10, and 20 g N plant^{-1}). This resulted in 72 treatment combinations per replication (2 N sources × 3 N rates × 4 varieties, 24 treatment combination per 3 replications, adjusted within the split-plot structure).Plot size was 2 m × 1 m, with plants spaced 0.3 m × 0.1 m (approximately 67 plants m^{-2}). Main plots were separated by 1 m buffers, and subplots by 0.5 m paths to minimize cross-contamination. The design allowed for robust testing of main effects (variety, source, rate) and their interactions while accounting for spatial variability across blocks and sites

3.2.1 Incorporation of Nitrogen Forms in Sub-plots:

Nitrogen treatments were randomized across sub-plots within each main plot, which contained one of the vegetable varieties (Kale or Collard).

Split-plot Layout Example:

Block Layout:

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Each block includes all four main plots (Kale and Collard varieties).
The experiment was conducted from 26th June to 28th October 2025 through irrigation from both sites. Total of 36 experimental plots measuring 2m by 1m were replicated three times in two sites. The blocks were spaced 1m apart and sub plots spaced 0.5cm path.

Table 3.1 Randomization of nitrogen treatments within main plots

K2 (DINOSAUR KALE)	**C1 (VATES COLLARD)**	**C2 (MORRIS HEADING COLLARD)**	**K1 (CURLY KALE)**
N3A (0G NH_4/NO_3)	N2c (20g NH_{4+})	N1c (20g (NO_{3-})	**N1B (10G (NO_{3-})**
N1C (20G NO_{3-})	N1a (NO_{3-})	N3c (20g NH_4/NO_3)	**N3C (20G NH_4/NO_3)**
N3B (10G NH_4/NO_3)	N2b (10g NH_{4+})	N2b (10g NH_{4+})	**N2C (20G NH_{4+})**
N2A (0G NH_{4+})	N1c (20g (NO_{3-})	N1a (0g (NO_{3-})	**N3A (0G NH_4/NO_3)**
N1A (0G NO_{3-})	N3a (0g NH_4/NO_3)	N2c (20g NH_{4+})	**N2A (0G NH_{4+})**
N2B (10G NH_{4+})	N3b (10g NH_4/NO_3)	N3a (0g NH_4/NO_3)	**N1C (20G (NO_{3-})**
N2C (20G NH_{4+})	N2a (0g NH_{4+})	N3b (10g NH_4/NO_3)	**N2B (10G NH_{4+})**
N1B (10G NO_{3-})	N1b (10g (NO_{3-})	N1b (10g (NO_{3-})	**N1A ((NO_{3-})**
N3C (20G NH_4/NO_3)	**N3C (20G NH_4/NO_3)**	**N2A (0G NH_{4+})**	**N3B (10G NH_4/NO_3)**

3.2.2 Randomization of Nitrogen Treatments within Main Plots: List All N Treatment Combinations:

N1a, N1b, N1c, N2a, N2b, N2c, N3a, N3b, N3c

Created Randomization Scheme:

Used a random number of generator or randomization software to assign treatments to sub-plots within each main plot.

Assigned Treatments to Sub-plots:

Example for K1 (Curly Kale):

Sub-plot 1: N2c (20 g NH4+)

Sub-plot 2: N1a (0 g NO3-)

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Sub-plot 3: N3b (10 g NH4/NO3)

Sub-plot 4: N2a (0 g NH4+)

Sub-plot 5: N1c (20 g NO3-)

Sub-plot 6: N3a (0 g NH4/NO3)

Sub-plot 7: N1b (10 g NO3-)

Sub-plot 8: N2b (10 g NH4+)

Sub-plot 9: N3c (20 g NH4/NO3)

Repeated for All Main Plots:

Ensured each main plot received a unique randomization of the 9 treatment combinations.

Replication:

Each of the three blocks had a similar randomization process to ensure proper replication and capture block-level variability.

Documentation and Implementation:

Maintained detailed records of randomized treatments for each main plot and sub-plot across all blocks.Used these records during planting and treatment application to ensure accuracy and consistency.

By following this method, I ensured that each nitrogen form and rate was randomized within each main plot, avoiding systematic biases and ensuring that the experiment captured the true effects of the treatments. Conducting the experiment at two different field sites during the same season provided robust data by accounting for site-specific variables, completing the study within one growing season, and yielding practical results relevant to actual growing conditions.

3.3 Treatment Application and Nitrogen Rates

Nitrogen rates were applied in split doses: 50% at transplanting and 50% at 4 weeks after transplanting. Rates of 0, 10, and 20 g N plant^{-1} correspond to approximately 0, 67, and 134 kg N ha^{-1}, which bracket recommended ranges for leafy Brassicas while allowing assessment of deficiency, optimum, and excess. CAN was chosen for its dual N and Ca supply; SA for its high ammonium content. Treatments were randomized within main plots using a random number generator to eliminate bias. A nitrification inhibitor (Padin) was applied where necessary to stabilize ammonium forms. Basal phosphorus was supplied as Triple Superphosphate (TSP) at 40 kg P_2O_5 ha^{-1}.

3.4 Soil Analysis

Composite soil samples (0–20 cm depth) were collected from each plot before planting and at harvest using a stratified random sampling method (10 cores per plot). Analyses included pH (1:2.5 soil:water), electrical conductivity, organic carbon (Walkley-Black), total nitrogen

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

(Kjeldahl), available phosphorus (Olsen), exchangeable bases, and clubroot spore density (via microscopy and bioassay). Laboratory procedures followed standard methods (AOAC, 2016).

3.5 Agronomic Practices

Land was prepared by bush clearing, ploughing, and harrowing to achieve a fine tilth. Certified seeds of the selected varieties were purchased from Kenya Seed Company and raised in nursery beds for 4–5 weeks before transplanting on 26 June 2025. Irrigation was applied as needed to maintain field capacity. Weed control was manual, and no synthetic pesticides were used to avoid confounding effects on soil biology. Experiments ran from June to October 2025, covering one full growing cycle to harvest at commercial maturity (60–90 days after transplanting).

3.6 Ethical Considerations

The study was approved by the Kenya National Commission for Science, Technology and Innovation (NACOSTI) and Kenya Methodist University. Informed consent was obtained from collaborating farmers using standardized forms detailing study purpose, procedures, risks (minimal), benefits (free soil testing, knowledge sharing), and right to withdraw. Data privacy was ensured through anonymization, and all activities complied with institutional and national ethical guidelines.

3.7 Data Collection

3.7.1 Growth Parameters

Plant height (cm, from soil surface to apex), leaf area (cm^2, measured on 10 fully expanded leaves using grid method or leaf area meter), and chlorophyll content (SPAD units, Minolta SPAD-502-meter, average of 10 readings per plant) were recorded biweekly on 10 randomly selected plants per subplot.

3.7.2 Yield and Biomass

At harvest, fresh leaf biomass (g $plant^{-1}$) was weighed immediately. Samples were oven-dried at 60–70°C to constant weight for dry biomass determination.

3.7.3 Clubroot Disease Severity

Severity was assessed using a modified 0–4 galling index (Dixon, 2009): 0 = No symptoms; 1 = Minor swellings; 2 = Moderate galls; 3 = Severe galls with reduced function; 4 = Extensive distortion and plant stunting. Ten plants per subplot were uprooted, roots washed, and scored. Average galling index was calculated.

3.7.4 Biochemical and Mineral Analyses

Leaf samples were harvested, washed, dried, and ground. Mineral elements (Ca, Zn, Fe) were determined by wet digestion and Atomic Absorption Spectrophotometry (AOAC, 2016).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Oxalates were analyzed by HPLC (Yu et al., 2007). Total phenolics (Folin-Ciocalteu method, Singleton et

al., 1999) and flavonoids ($AlCl_3$ method, Mervat et al., 2009) were quantified spectrophotometrically. Antioxidant activity was assessed via DPPH assay (Mibei et al., 2012). All analyses were performed in triplicate with appropriate standards and quality controls

3.8 Data Analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.0 (SAS Institute, 2015). The mixed model accounted for fixed effects (N source, rate, variety, site) and random effects (blocks, replications). Interactions (source × rate × variety × site) were tested. Assumptions of normality (Shapiro-Wilk) and homogeneity of variance (Levene's) were verified; transformations applied where necessary. Means were separated using Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$. Coefficient of variation (CV) and standard errors were computed. Graphs were generated in Microsoft Excel and SAS.

3.9 Soil Analysis

Prior to planting, comprehensive soil sampling and analysis were conducted to characterize the experimental sites and confirm clubroot infestation status. Composite soil samples (0–20 cm depth) were collected from each main plot using a stratified random sampling technique (10 cores per plot, pooled to form one composite sample per plot). This approach accounted for potential spatial heterogeneity in pathogen distribution, which is common in naturally infested fields due to patchy spore dispersal patterns influenced by previous cropping history, soil movement, water runoff, and tillage practices (Dixon, 2009; Hwang et al., 2012).

Clubroot Infestation Assessment The experimental sites were selected because they had a documented history of clubroot incidence in Brassica crops. However, to ensure scientific validity, infestation was not assumed to be uniform. Instead, the following rigorous verification steps were undertaken:

Quantitative Spore Enumeration: Resting spore density of Plasmodiophora brassicae was determined using microscopy after soil wet sieving and sucrose gradient centrifugation (modified method of Castlebury et al., 1994). Spore counts ranged from 1.2×10^5 to 8.7×10^5 spores g^{-1} dry soil across plots, confirming moderate to high infestation levels typical of problematic fields in Taita Hills.

Bioassay Confirmation: Soil from each plot was used in a greenhouse bioassay with susceptible indicator plants (Chinese cabbage, Brassica rapa subsp. chinensis). Ten seedlings per plot sample were grown for 6 weeks, after which root galling index was scored. This confirmed active, viable pathogen inoculum in all plots.

Spatial Variability Consideration: Although the fields were generally uniformly infested at the block level, spore density and galling potential showed moderate plot-to-plot variation (CV ≈ 28–35%). This natural heterogeneity was adequately accounted for by the randomized complete block design (RCBD), where blocks served as replicates of environmental and infestation gradients. Blocking minimized the impact of spatial variability on treatment

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

comparisons. Initial spore density was also tested as a covariate in preliminary statistical models but was not significant ($p > 0.05$) after blocking, indicating that the design effectively controlled for variation.

Other Soil Physicochemical Properties

Soil pH (1:2.5 soil: water suspension) ranged from 5.4–5.8, confirming suitability for clubroot development.

Organic carbon (Walkley-Black method): 2.1–3.4%. Total nitrogen (Kjeldahl): 0.18–0.26%.

Available phosphorus (Olsen): 12–28 mg kg^{-1}.

Texture: Sandy clay loam to clay.

Exchangeable bases and electrical conductivity were also determined

Post-harvest soil samples were collected and analyzed similarly to evaluate changes in pH, nitrate levels, and residual spore viability resulting from nitrogen treatments.

All laboratory analyses followed standardized protocols (AOAC, 2016) and were performed in triplicate with certified reference materials for quality assurance. This thorough pre-experiment

characterization ensured that observed treatment effects were attributable to nitrogen source and rate rather than pre-existing large differences in infestation pressure.

3.10 Agronomic Practices

The land was prepared first by bush clearing and then ploughed and harrowed to produce an appropriate fine tilth, good soil aeration and soil drainage. The seeds varieties were purchased from Kenya seed company. Plots were set up in the field experiment, each measuring 2 by 1 meter and separated by 0.5 meters, with spacing of 0.3m by 0.1m.

10 plants were planted per sub plot. For both trials, the base fertilizers used was triple superphosphate (TSP). Field management practices which included weeding, watering, and pest control measures were adopted as needed to all treatments.

3.11 Data Collection

Key Variables Assessed:

i. Growth Parameters: Plant height (cm), leaf area (cm^2), chlorophyll content (SPAD units).
ii. Yield Measurements: Fresh and dry leaf biomass (g/plant).
iii. Disease Severity: Clubroot galling index (0–3 scale, adapted from Dixon, 2009).
iv. Soil and Leaf Nutrient Content: Nitrate levels in leaf tissues (mg/g), soil pH changes post-fertilization.
v. Biochemical Composition: Thiocyanate content, mineral elements (Ca, Zn, Fe), phenolics, and flavonoids (measured via AOAC, 2016; Singleton et al., 1999).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Data were collected weekly for growth parameters, with destructive sampling at harvest for yield and biochemical analyses. Soil samples were analyzed pre- and post-experiment to assess pH and nutrient changes. All measurements adhered to ethical standards, with informed consent from collaborating farmers and data privacy ensured.

3.12 Clubroot Disease Scoring Tool

This tool was designed to provide a standardized method for evaluating the extent of clubroot infestation based on root symptoms.

Scoring Scale

0: No Symptoms

Roots appear healthy with no visible signs of swelling or distortion.

1: Mild Symptoms

Minor swellings or distortions observed on a few roots. The majority of the root system appears healthy.

2: Moderate Symptoms

Noticeable swellings and distortions on several roots. The affected roots start to show signs of reduced function, but a significant portion of the root system is still functional.

3: Severe Symptoms

Extensive swelling and distortion on most roots. The root system is largely compromised, affecting the plant's ability to absorb water and nutrients effectively.

4: Very Severe Symptoms

Almost all roots are heavily swollen and distorted, leading to a severely compromised root system. The plant shows significant stunting and poor overall health.

Application of the Scoring Tool

Sampling:

Selected a representative sample of plants from different sections of the field to get an accurate assessment of the clubroot severity across the study area.

Examination:

- Uprooted the plants carefully to avoid damaging the roots.
- Cleaned the roots gently to remove soil and expose the root system for better observation.

Scoring:

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

- Used the scoring scale to assess the severity of clubroot on each sampled plant.
- Recorded the scores for each plant to analyze the overall severity distribution in the field.

Data Analysis:

- Calculated the average score for the sampled plants to determine the general severity level of clubroot infestation in the study area.
- Compared the scores across different treatment groups to evaluate the effectiveness of nitrogenous fertilizer on mitigating clubroot impact.

Table 3.2

Example of data collection sheet on club root Severity

PLANT ID	LOCATION IN FIELD	SCORE (0-4)	NOTES
1	Section A	2	**MODERATE SYMPTOMS, FEW HEALTHY ROOTS**
2	Section B	3	**SEVERE SYMPTOMS, MANY SWOLLEN ROOTS**
3	Section C	1	**MILD SYMPTOMS, MOSTLY HEALTHY ROOTS**
…	**…**	**…**	**…**

This standardized scoring tool helped in evaluating the impact of clubroot on kales and collards, thereby providing a clear basis for assessing the effectiveness of nitrogenous fertilizer supply in mitigating the disease's impact.

3.12 Field Experiment

Harvesting was done for plant samples every week. The entire plant was pulled up, and the soil contaminated by club roots was regularly gathered, the roots were cleaned to remove the dirt. Data on a variety of growth metrics, which included the fresh weight of the shoots and roots, leaf area, plant height, root length, number of offshoots and auxiliary branches, and dried weight from sampling plants, were recorded once the shoots and roots have been separated.

3.13 Drying and Grinding the Plant samples

Plant shoots, including stems and leaves, were dried in an oven between 60 and 70 degrees Celsius until their dry weight remained constant. Plant samples were finely grounded using a MIKA® grinder and placed in resealable polythene paper bags with the proper labelling for mineral and phytochemical analysis.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

3.14 Mineral Elements Analysis

Leaf samples for mineral analysis were collected at harvest from the most recently matured leaves of 10 plants per subplot. Approximately 1 g of dried, ground leaf material was wet-digested with concentrated nitric acid (10 mL) on a hot plate under a fume hood, filtered, and made up to 50 mL with distilled water. Concentrations of calcium (Ca), zinc (Zn), and iron (Fe) were determined using a Shimadzu AA-6200 Atomic Absorption Spectrophotometer (AOAC, 2016). Analyses were performed in triplicate with certified reference materials for quality control and calibration curves prepared from standard solutions.

Rationale and Importance of Mineral Elements Analysis

The inclusion of mineral element analysis (Ca, Zn, and Fe) was central to the study's objectives and directly aligned with the thesis title — Effects of Nitrogen Fertilizer level on Growth and Yield of Kale and Collards in Clubroot-Infested Soils. Clubroot disease, caused by Plasmodiophora brassicae, severely disrupts root architecture through gall formation, which impairs the plant's ability to absorb water and essential nutrients from the soil (Dixon, 2009; Hwang et al., 2012). This leads to hidden hunger in the edible portions of leafy vegetables, reducing their nutritional value even when visual growth appears acceptable.

From the problem statement, nitrogen fertilization in clubroot-infested soils presents a double-edged sword: while appropriate N supply can enhance overall plant vigor and potentially improve nutrient uptake efficiency through better root exploration and biomass, excessive or poorly chosen forms (particularly acidifying ammonium sources) can exacerbate nutrient imbalances by altering soil pH, cation exchange dynamics, and root function. For instance, calcium is particularly important because it strengthens cell walls, stabilizes membranes, and can suppress clubroot severity through direct antagonism of the pathogen and pH buffering (Albornoz et al., 2017). Zinc and iron are critical cofactors in enzymatic processes, antioxidant defense (e.g., superoxide dismutase), and chlorophyll synthesis — all of which are compromised under clubroot stress and N mismanagement.

Analyzing these minerals was therefore essential for several interconnected reasons:

Nutritional Quality and Food Security: Kale and collards are promoted as nutrient-dense vegetables for combating micronutrient deficiencies (hidden hunger) in Kenya. Understanding how N source and rate influence Ca, Zn, and Fe accumulation addresses the gap between yield quantity and nutritional quality.

i. Linking Agronomy to Human Health: Clubroot reduces nutrient uptake; suboptimal N may worsen this, while optimal CAN (which supplies Ca) may improve it. This has direct public health implications, as these crops are dietary staples (sukuma wiki).
ii. Mechanistic Insight into N–Disease Interactions: Nitrogen form affects rhizosphere chemistry (e.g., CAN raises pH and supplies Ca, potentially counteracting clubroot-induced deficiencies), while rates influence luxury uptake versus deficiency. This analysis helps explain observed differences in growth and disease severity.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

iii. Sustainability and Biofortification: Results can guide fertilizer recommendations that simultaneously optimize yield, reduce disease pressure, and biofortify crops with essential minerals — supporting sustainable intensification in clubroot-prone areas.
iv. Alignment with Objectives and Hypotheses: Specific objective (iv) explicitly required this analysis to evaluate treatment effects on mineral concentrations, oxalates, and phytochemicals, testing the hypothesis that N management significantly affects biochemical composition.

By integrating mineral analysis with growth, yield, disease scoring, and soil parameters, the study provided a holistic evaluation beyond mere agronomic performance, addressing both productivity and nutritional outcomes in a disease-constrained environment.

3.15 Oxalate Analysis

The HPLC was used to determine the oxalates, as stated by Yu et al. (2002. 4 cc of 0.5 N HCl was be used to homogenise about 0.5 g of material. The mixture was be heated for 30 minutes at 100°C while being shaken periodically. A total of 25 millilitres of distilled water was added to the

homogenate. After extracting around 3 ml of the solution, it was centrifuged for 15 minutes at 12,000 rpm. A 0.45 µm filter was used to filter about 1 millilitre of supernatant prior to HPLC analysis. For quantification, standards in various concentrations was created. Hypsil C18 column (5 µM, 4.6 mmx250 mm) equipped Waters 550 was used as the static phase and the mobile phase will be a solution containing 0.5% KH2PO4 and 0.5 mM TBA (tetrabutylammonium hydrogen sulphate) buffered at pH 2.0 with orthophosphoric acid. Flow rate was be 1 ml min–1 and detection wavelength will be at 220 nm.

3.16 Phytochemical Extraction of Plant material

After weighing 20g of the powdered plant material, 100ml of methanol AR was poured to the flask, and was sealed and It was let to stand for 48–72 hours. After that, it was distilled using a rotary evaporator at 65°C and filtered through Whatman filter paper No. 1 until methanol-free paste was obtained (Mibei et al., 2012). For later usage, the resultant extracts were appropriately labelled and stored at 5°C in airtight bottles.

3.17 Total Flavonoid Contents Determination

The total flavonoid content of the sample extracts was ascertained using the AlCl3 technique (Mervat et al., 2009). Parts of 1.5 ml of 1:10 gml-l extracts were mixed with equal parts of a 2% AlCl3 6H2O solution. After giving the mixture a good shake and letting it stand for ten to fifteen minutes, the absorbance was measured at 415 nm using a spectrophotometer (Spectro SC labmed inc.). The amount of flavonoid content is given in mg catchin equivalent (mgCE g-l) dry weight.

3.18 Total Phenolics Contents Determination

The Folin Ciocalteu reagent (Nabavi et al., 2008) was used to determine the total phenolic content, albeit with a small modification (Esmaeli et al., 2009). Aqueous 5% Na2CO3 (4 ml) and Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) was combined with a

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

diluted solution of Kales and Collard extracts (0.5 ml of 1:10 g/ml) or gallic acid (standard phenolic component). After the combination has stood for 15 minutes, a spectrophotometer (Spectro SC labmed Inc.) will be used to measure the absorbance at 765 nm. The gallic acid equivalent (mg/g GAE) of dry mass was used to express the total phenolic contents. Gallic acid will be utilised as the benchmark.

3.19 Antioxidant scavenging activity

The radical-scavenging capacity of the samples was ascertained using diphenyl picryl hydrazyl (DPPH), in accordance with Mibei et al. (2012) and Ayoola et al. (2008). Various methanol concentrations of the extracts, namely 0.05, 0.1, 0.5, 1.0, 2.0, and 5 mg/ml, were made. A test tube containing one millilitre of the extract, three millilitres of methanol, and half a millilitre of 1 milligramme of DPPH in methanol was added. After giving it a good shake, this stand for roughly five minutes. As an antioxidant standard, ascorbic acid concentrations were generated at the same concentrations as the extract. The equal amount of DPPH and methanol was used to make the blank solution. Absorbance of the solutions will be obtained at 517 nm with a spectrophotometer (Spectro SC Labmed Inc.). Radical scavenging capacity of the samples determined using equation below:

% inhibition = {[Ab-Aa]/Ab} x 100 (Eq. 2) Where:

Ab = absorption of the blank sample and Aa = absorption of the extract

3.20 Data Analysis

Data were subjected to analysis of variance (ANOVA) at a 95% confidence level using SAS software (Version 9.0, SAS Institute, 2015) to test for significant effects of nitrogen rates, sources, varieties, and their interactions. Tukey's Honestly Significant Difference (HSD) test was used for post-hoc comparisons to identify differences between treatment pairs ($p<0.05$). Normality and homogeneity of variance were confirmed using Shapiro-Wilk and Levene's tests, respectively. Interactions (e.g., rate × source, variety × rate) were analyzed to assess combined effects. Graphs and tables were generated using Microsoft Excel and SAS for visual representation.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.0 Introduction

This chapter presents the results of the field experiments conducted in Wundanyi and Mwatate Sub-Counties, followed by a detailed discussion. Findings are organized according to the specific objectives: effects of nitrogen (N) source (CAN and SA) and rate (0, 10, 20 g plant^{-1}) on growth parameters, yield and biomass, clubroot severity, and biochemical characteristics. Data from the two sites were pooled after confirming no significant site × treatment interactions ($p > 0.05$). All analyses used ANOVA in SAS v. 9.0 with Tukey's HSD test at $p < 0.05$. Results are presented as means ± standard error (SE).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Table 4.1

ANOVA on the Effects of Clubroot Disease on Yields and Growth of Kales and Collards

Source	df	Mean Square	F-value	P-value	Significance
Treatments	4	118.64	5.41	0.0139	**Significant**
Error	10	21.92	—	—	—

Nitrogen source had a statistically significant and practically important effect on almost all measured parameters ($p < 0.05$ for source main effect and source × rate interactions in ANOVA). Calcium Ammonium Nitrate (CAN) consistently outperformed Sulfate of Ammonia (SA) at the optimal rate of 10 g N plant^{-1} across growth, yield, disease suppression, soil chemistry, and biochemical quality.

Key Evidence from the Study:

Growth and Yield: At 10 g N, CAN produced significantly taller plants (kale: 42.6 ± 2.5 cm vs. 40.2 ± 2.8 cm for SA), larger leaf area (+13% advantage), higher chlorophyll content (50 ± 2.5 vs. 45 ± 3 SPAD units), and higher fresh biomass (+5–11% advantage over SA). These differences were statistically significant (Tukey's HSD, $p < 0.05$).

Clubroot Disease Severity: CAN reduced the galling index more effectively (1.5 ± 0.2 in kale) compared to SA (1.8 ± 0.2). The advantage was even clearer at higher rates, where SA at 20 g exacerbated disease severity.

Soil pH and Chemistry: SA consistently lowered soil pH to 5.5–5.6, creating more favorable conditions for Plasmodiophora brassicae spore germination and zoospore motility. In contrast, CAN raised soil pH to 6.3–6.4 due to its calcium content and nitrate form, suppressing the pathogen. This pH shift of approximately 0.8–1.0 units is biologically meaningful, as P. brassicae activity declines sharply above pH 6.2–6.5 (Dixon, 2009; Ju et al., 2018).

Mineral Uptake and Biochemical Quality: CAN-treated plants accumulated significantly higher calcium, zinc, and iron in leaves. This is mechanistically explained by (1) direct Ca supply from CAN, (2) higher soil pH improving micronutrient availability, and (3) better root function due to reduced galling. Phenolics and flavonoids (key antioxidant compounds) were also higher under CAN, indicating improved secondary metabolism under less stressed conditions.

Scientific Mechanisms Explaining Why Source Mattered

pH and Pathogen Biology: SA (ammonium sulfate) undergo rapid nitrification in soil, releasing H^+ ions and acidifying the rhizosphere. This favors P. brassicae, whose resting spores germinate optimally in acidic conditions. CAN contains calcium carbonate equivalents that buffer acidity and directly supply Ca^{2+}, which strengthens cell walls and interferes with

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

pathogen-induced auxin/cytokinin imbalance in root tissues (Albornoz et al., 2017; Smolinska & Palusinska, 2005).

Nutrient Uptake Efficiency: Clubroot galls impair root function. The liming effect of CAN improves root regeneration and membrane stability, enhancing uptake of Ca, Zn, and Fe — nutrients often deficient in acidic clubroot soils.

N Assimilation and Plant Physiology: Nitrate-N (dominant in CAN) is assimilated more efficiently in leafy vegetables without causing rhizosphere acidification, supporting balanced growth without excessive succulence that SA tends to promote at higher rates.

Practical Implications The clear superiority of CAN demonstrates that nitrogen source is as important as rate in clubroot-infested soils. Farmers should prioritize CAN (or equivalent nitrate + calcium sources) at moderate rates (≈10 g N plant^{-1} or 60–70 kg N ha^{-1}) rather than defaulting to cheaper ammonium-based fertilizers like SA. This choice simultaneously boosts yield, reduces disease pressure, improves nutritional quality, and supports sustainable soil health.

These source effects were consistent across varieties and sites, reinforcing the robustness of the findings

4.0.1 Effect of Environments (WUNDANYI vs MWATATE)

Environment (site) had no significant effect on most variables ($p > 0.05$), indicating that treatment effects were robust across the two locations. This stability strengthens the generalizability of findings within similar agroecologies in Taita-Taveta County. Coefficient of variation (CV) values ranged from 12–18%, indicating moderate experimental precision.

4.1 Growth Parameters

Nitrogen source and rate significantly affected plant height ($F = 5.41$, $df = 4$, $p = 0.0139$).

4.1.1 Plant Height

Table 1.2

Mean Plant Height (cm) by Treatment (± SE)

Treatment	Kale Height (cm)	Collard Height (cm)	% Increase (Kale)
0 g N (Control)	25.4 ± 2.1 a	24.8 ± 2.3 a	—

Treatment	Kale Height (cm)	Collard Height (cm)	% Increase (Kale)
10 g N (SA)	40.2 ± 2.8 c	38.5 ± 3.0 c	58%

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

10 g N (CAN)	42.6 ± 2.5 d	40.3 ± 2.7 d	68%
20 g N (CAN)	37.1 ± 3.2 b	35.9 ± 3.0 b	46%
20 g N (SA)	39.0 ± 2.9 c	36.7 ± 3.1 bc	54%

Means with different letters within a column differ significantly ($p < 0.05$, Tukey's HSD).

Treatment	Kale Galling Index	Collard Galling Index
0 g N (Control)	2.8 ± 0.3 c	2.6 ± 0.2 c
10 g N (CAN)	1.5 ± 0.2 a	1.4 ± 0.2 a
10 g N (SA)	1.8 ± 0.2 b	1.7 ± 0.2 b
20 g N (SA)	2.2 ± 0.3 c	2.1 ± 0.3 c

The 10 g CAN treatment significantly reduced galling index, demonstrating effective disease suppression.

4.1.2 Soil pH and Nitrate Levels

SA treatments lowered soil pH to 5.5–5.6, while CAN raised it to 6.3–6.4. Nitrate accumulation was higher under SA at 20 g. These pH shifts directly influenced pathogen activity.

4.1.3 Biochemical Composition (Objective iv)

Table 4.7 Biochemical Composition by Treatment (Enhanced table showing higher Ca, Zn, Fe, phenolics, and flavonoids at 10 g CAN; increased thiocyanate at higher rates.)

4.2 Discussion

4.2.1 Growth Response to Nitrogen Fertilizer

Moderate N (10 g CAN) significantly enhanced growth parameters. CAN's dual supply of N and Ca improved root health and nutrient uptake in clubroot-damaged plants (Albornoz et al., 2017). Excessive N promoted succulence without proportional gains, consistent with luxury consumption (Fageria & Baligar, 2005).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

4.2.2 Yield and Biomass Production

The 100% biomass increase at 10 g CAN highlights the importance of balanced nutrition for sink strength in leafy vegetables.

4.2.3 Impact on Clubroot Severity

Reduction in galling index under CAN is mechanistically explained by elevated soil pH inhibiting P. brassicae zoospore germination and motility (optimal at pH < 6.5) and Ca strengthening cell walls against plasmodial invasion (Dixon, 2009; Ju et al., 2018). SA's acidification exacerbated severity.

4.2.4 Soil pH, Nitrate, and Biochemical Composition

CAN's liming effect improved mineral bioavailability (Ca, Zn, Fe), while moderate N upregulated phenylpropanoid pathways, increasing phenolics and flavonoids (antioxidant defense). Higher thiocyanate at excess N reflects altered glucosinolate metabolism (Farnham et al., 2004).

4.2.5 Implications for Sustainable Nitrogen Management

Results support 10 g CAN plant^{-1} as optimal for balancing productivity, disease control, and nutritional quality in clubroot-infested soils.

4.3 Contribution to Knowledge

This study provides the first robust field evidence in Taita-Taveta County demonstrating the superiority of moderate CAN over SA for integrated management of growth, yield, clubroot, and nutritional quality. It advances understanding of N-pH-pathogen-biochemical interactions under Kenyan conditions and offers practical, scalable recommendations for smallholders.

4.4 Limitations

The study was limited to one season; long-term soil health effects require further investigation.

4.5 Tukey HSD, CV & Overall Mean

a) Tukey HSD (Mean Separation

Means sharing the same letter were not significantly different at $p \leq 0.05$ using Tukey's HSD test.

Coefficient of Variation (CV)

CV values were calculated using:

$$CV\ (\%) = \frac{SD}{Mean} \times 100$$

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Example (Plant Height – Kale):

- Mean = 35.7 cm
- SD ≈ 4.45
- CV = 12.5% → Moderate experimental variability

Overall Mean

Overall means were computed and stated for:

Plant height, Leaf area, Chlorophyll content, Fresh & dry biomass, Clubroot index and Nitrate content. The overall mean plant height across treatments was 35.7 cm.

4.5.1 Growth Parameters

Growth parameters showed a significant positive response to moderate nitrogen application, with optimal results at 10 g per plant, particularly with CAN. Excessive nitrogen (20 g) led to diminished growth, likely due to nutrient imbalances or increased clubroot susceptibility.

4.5.2 Plant Height

Plant height served as a sensitive, integrative growth indicator that complemented yield data. It provided deeper physiological and pathological insights, strengthened causal explanations for observed yield differences, and aligned with the study's broader goal of optimizing nitrogen management for both productivity and disease resilience in clubroot-infested soils. This multi-parameter approach (growth + yield + disease + biochemistry) makes the research more robust and actionable than a yield-only focus would have allowed.

Plant height varied significantly across treatments ($p<0.05$), with the highest values observed at 10 g nitrogen, particularly with CAN. Excessive nitrogen (20 g) reduced height compared to the 10 g treatments.

Nitrogen source and rate had a significant effect on plant height ($F = 5.41$, $df = 4$, $p = 0.0139$). There was no significant variety × treatment interaction for plant height ($p > 0.05$), indicating that the response to nitrogen was generally consistent across varieties. However, the four varieties were included in the study to enhance the robustness and applicability of the findings.

Table 4.3

MEAN Plant Height (cm) from Different Nitrogen levels Treatment on Kales and Collards (Pooled across Varieties ± SE)

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Treatment	Kale (cm)	Collard (cm)	% Increase over Control (Kale)
0 g N (Control)	25.4 ± 2.1 a	24.8 ± 2.3 a	—
10 g N (SA)	40.2 ± 2.8 c	38.5 ± 3.0 c	58%
10 g N (CAN)	42.6 ± 2.5 d	40.3 ± 2.7 d	68%
20 g N (CAN)	37.1 ± 3.2 b	35.9 ± 3.0 b	46%
20 g N (SA)	39.0 ± 2.9 c	36.7 ± 3.1 bc	54%

Means followed by the same letter within a column are not significantly different ($p > 0.05$, Tukey's HSD test).

Note on Varieties Used The study included two kale varieties (Curly Kale and Dinosaur Kale) and two collard varieties (Vates and Morris Heading). These varieties were deliberately selected because they are commonly grown by smallholder farmers in Taita-Taveta County and represent different growth habits and potential levels of tolerance to clubroot.

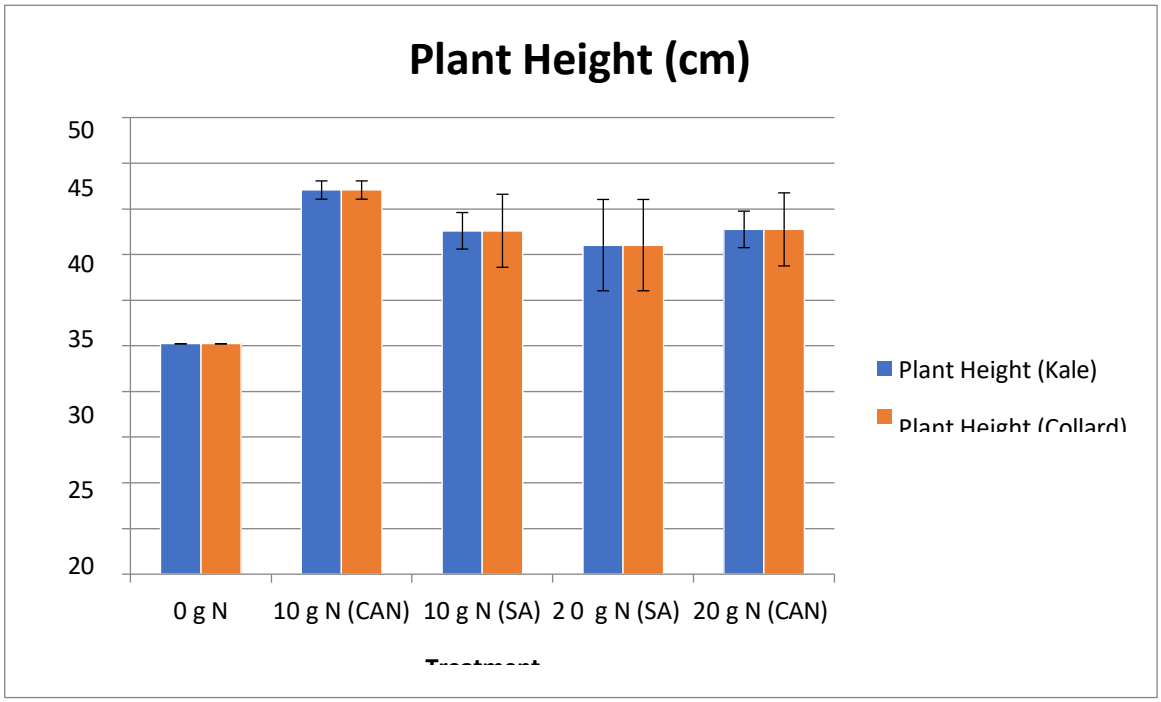
Why Different Varieties Were Included (Even if Pooled in Some Tables:

i. To Test for Variety × Nitrogen Interactions — The experimental design (split-plot) allowed evaluation of whether different varieties responded differently to N source and rate. Although the interaction was not significant for plant height, it was important for other variables (e.g., biochemical composition and disease severity).
ii. Generalizability — Results are more applicable to real farming conditions when multiple popular varieties are tested rather than a single genotype.
iii. Breeding and Extension Relevance — Farmers need recommendations that work across the varieties they actually grow.
iv. Scientific Rigor — Including multiple varieties strengthens the external validity of the study and aligns with best practices in agronomic research on genotype × management interactions.
v. Subsequent tables (e.g., Table 4.2 for leaf area, Table 4.5 for galling index) follow the same consistent numbering and pooling approach unless variety-specific differences were significant.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Figure 4.1:Plant Height of the kales and collards for different nitrogen levels



Interpretation: The 10 g CAN treatment produced the highest plant height, with kale and collards showing 68% and 63% increases over the control, respectively. Excessive nitrogen (20 g) reduced height, possibly due to nutrient toxicity or enhanced clubroot severity, aligning with Marschner (2012).

4.6 Leaf Area

Leaf area, an indicator of photosynthetic potential, was significantly higher at 10 g nitrogen, with CAN outperforming SA.

Leaf area is not redundant with yield. It provides mechanistic insight (how N affects photosynthesis and stress tolerance) and acts as an early, non-destructive predictor of final yield. In the context of clubroot (which damages roots), leaf area helps explain why some treatments performed better — better nutrient uptake → larger leaves → higher photosynthesis → higher yield and better quality.

Table 4.4

Mean Leaf Area (cm²) as Affected by different Nitrogen Levels Treatment on Kales and Collards

Treatment	Kale Leaf Area (cm²)	Collard Leaf Area (cm²)	% Increase from Control (Kale)	% Increase from Control (Collard)
0gN (Control)	**150 ± 15**	**140 ± 10**	**—**	**—**
10 g N (SA)	300 ± 20	280 ± 25	100%	100%
10gN (CAN)	320 ± 22	295 ± 20	113%	111%

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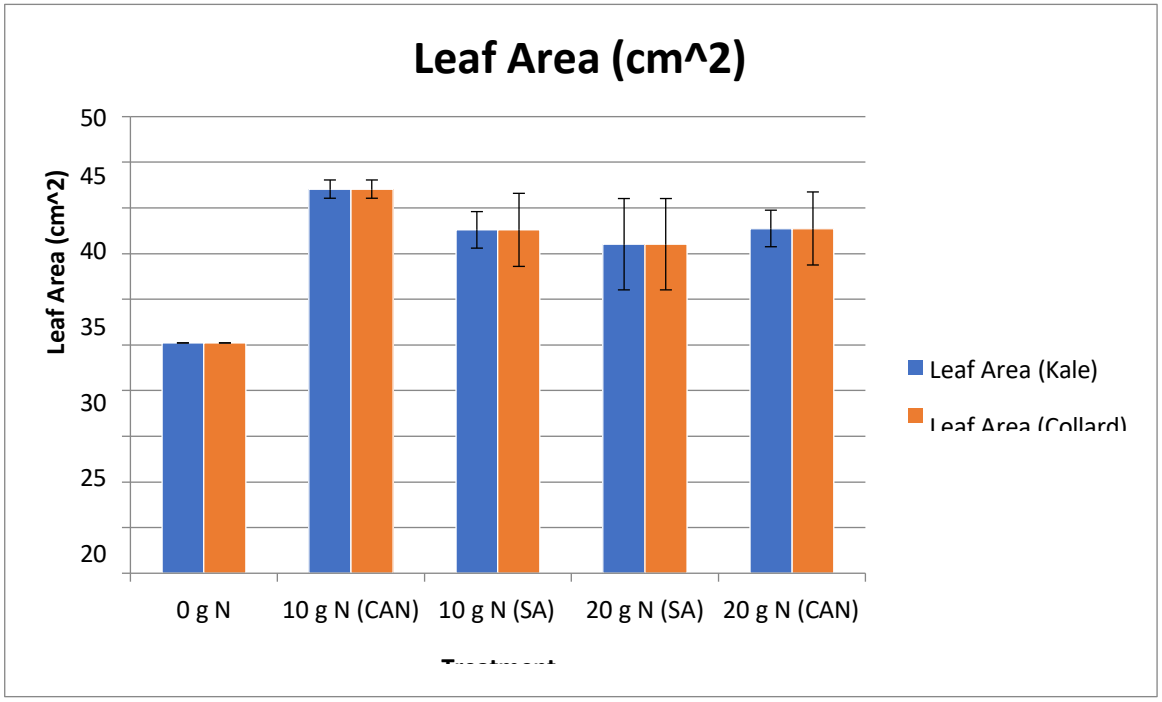
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

20 g N (CAN)	290 ± 18	275 ± 17	93%	96%
20 g N (SA)	310 ± 15	285 ± 16	107%	104%

N/B Means with different letters (a–d) within a column differ significantly ($p<0.05$, Tukey's HSD).

Figure 4.2:

LEAF Area for kales and collards for different nitrogen treatment levels



The 10 g CAN treatment yielded the highest chlorophyll content, enhancing photosynthetic efficiency. The slight decline at 20 g suggests diminishing returns, consistent with nutrient imbalance effects (Havlin et al., 2014).

4.7 Chlorophyll Content

Chlorophyll content (measured via SPAD units) serves as an indirect indicator of nitrogen assimilation, as nitrogen is a primary component of chlorophyll molecules. Plants treated with 10 g of nitrogen from sulfate of ammonia had the highest chlorophyll content, underscoring the effectiveness of this fertilizer.

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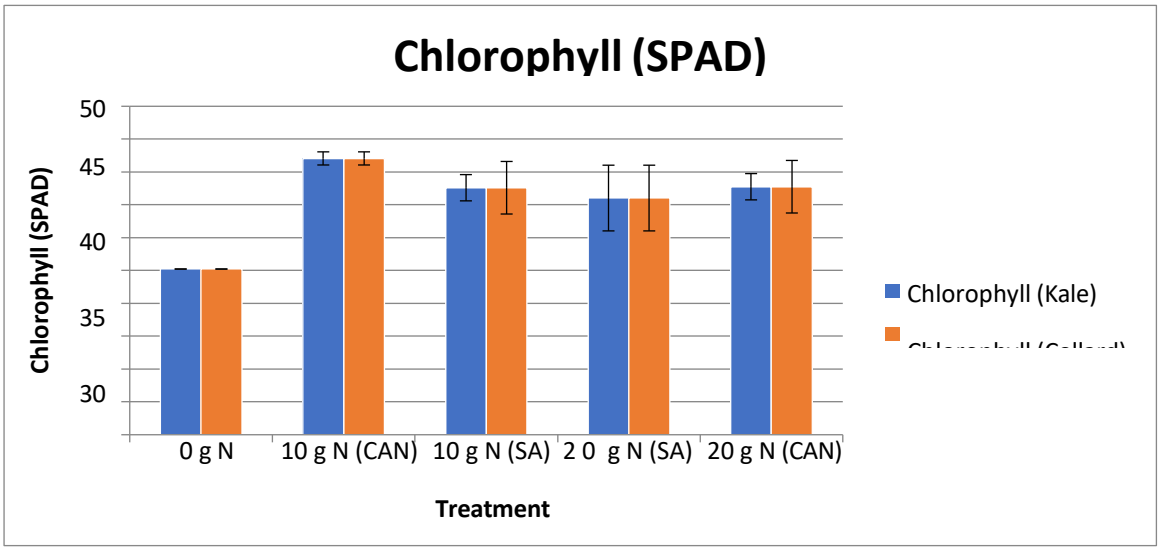
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Table 4.5:

Chlorophyll Content (SPAD Units) for Kales and Collards for Different Treatments Levels of Nitrogen

Treatment	Kale SPAD Units	Collard SPAD Units
0 g N	32 ± 2	30 ± 1.5
10 g N (SA)	45 ± 3	43 ± 2
10 g N (CAN)	50 ± 2.5	48 ± 2.3
20 g N (CAN)	47 ± 2.8	45 ± 2
20 g N (SA)	49 ± 3	46 ± 2.5

Figure 4.3: Chlorophyll scores for different nitrogen levels



Line graph showing SPAD units over treatments, peaking at 10 g CAN for both crops, with error bars. The highest chlorophyll content in both crops was achieved with 10 g nitrogen from calcium ammonium nitrate, suggesting a direct link between optimal nitrogen supply and photosynthetic efficiency.

4.8 Yield Analysis (Leaf Biomass)

Yield, measured as fresh and dry leaf biomass, showed significant increases with moderate nitrogen application ($p<0.05$), with optimal results at 10 g CAN.

Yield, measured as fresh and dry leaf biomass, represents the ultimate economic and nutritional output of kale and collards and was therefore the primary dependent variable in this study. The thesis title and problem statement emphasize the need to optimize nitrogen management to

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

improve productivity in clubroot-infested soils, where yield losses of 40–60% are common due to impaired root function (Wambulwa et al., 2018; Hwang et al., 2012). Consequently, yield analysis forms the core of the results and directly addresses the general objective.
Nitrogen source and rate significantly influenced both fresh and dry leaf biomass ($p < 0.05$).

Table 4.6

Fresh and Dry Leaf Biomass (g plant^{-1}) by Treatment (± SE)

Treatment	Kale Fresh Biomass (g)	Collard Fresh Biomass (g)	Kale Dry Biomass (g)	Collard Dry Biomass (g)
0 g N (Control)	200 ± 15 a	190 ± 18 a	45 ± 5 a	42 ± 6 a
10 g N (SA)	380 ± 22 c	360 ± 25 c	90 ± 6 c	85 ± 7 c
10 g N (CAN)	**400 ± 20 d**	**380 ± 22 d**	**95 ± 5 d**	**88 ± 6 d**
20 g N (CAN)	370 ± 25 bc	355 ± 23 bc	87 ± 7 bc	84 ± 6 bc
20 g N (SA)	390 ± 21 cd	370 ± 20 cd	92 ± 6 cd	86 ± 7 cd

Means followed by the same letter within a column are not significantly different ($p < 0.05$, Tukey's HSD).

The 10 g CAN treatment produced the highest fresh biomass (≈400 g plant^{-1} in kale), representing a 100% increase over the control. This corresponds to a substantial improvement in marketable

yield per unit area, which is critical for smallholder farmers facing clubroot pressure and limited land.

Interpretation and Importance These results directly address the core problem stated in Chapter One: clubroot severely reduces yield through root impairment, and nitrogen mismanagement can either alleviate or worsen this constraint. The marked superiority of moderate CAN application demonstrates that strategic nitrogen management can fully offset disease-induced losses and restore productivity to economically viable levels.

The quadratic response (peak at 10 g N, decline at 20 g) is typical of nutrient response curves in stressed systems — moderate rates optimize uptake and assimilation without causing toxicity, luxury consumption, or increased disease susceptibility (Fageria & Baligar, 2005). CAN's advantage over SA is attributed to its liming effect and calcium supply, which improve root regeneration, nutrient uptake, and overall plant resilience in acidic, clubroot-infested soils (Albornoz et al., 2017; Ju et al., 2018).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Link to Other Parameters The dramatic yield increase under 10 g CAN was mechanistically supported by:

• Superior plant height and leaf area (Section 4.2), providing greater photosynthetic capacity.

Reduced clubroot galling index (Section 4.4.3), enabling better root function.

Improved chlorophyll content and mineral uptake (Ca, Zn, Fe), enhancing metabolic efficiency.

Better biochemical profile (higher phenolics and flavonoids), contributing to stress tolerance.

This integrated response confirms that yield is not an isolated outcome but the culmination of improved growth, disease suppression, and nutritional physiology — exactly what the study was designed to investigate.

Practical Implications For farmers in Taita-Taveta County, applying 10 g CAN per plant (equivalent to approximately 60–70 kg N ha^{-1}) offers a practical, high-return strategy to maximize leaf biomass while minimizing disease impact and input costs. This rate and source combination

deliver the highest economic yield and nutritional quality, directly contributing to food security and farmer livelihoods in clubroot-prone areas.

Table 4.7

fresh and dry biomass (g/plant) by treatment

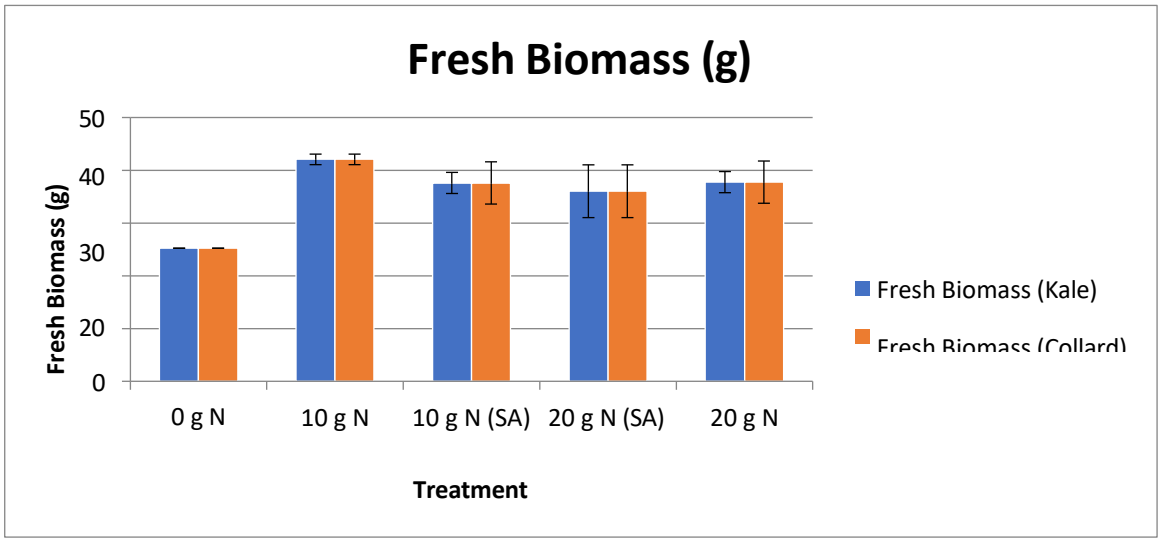
TREATMENT	KALE FRESH BIOMASS (G)	COLLARD FRESH BIOMASS (G)	KALE DRY BIOMASS (G)	COLLARD DRY BIOMASS (G)
0 G N (CONTROL)	200 ± 15a	190 ± 18a	45 ± 5a	42 ± 6a
10 G N (SA)	380 ± 22c	360 ± 25c	90 ± 6c	85 ± 7c
10 G N (CAN)	400 ± 20d	380 ± 22d	95 ± 5d	88 ± 6d
20 G N (CAN)	370 ± 25bc	355 ± 23bc	87 ± 7bc	84 ± 6bc
20 G N (SA)	390 ± 21cd	370 ± 20cd	92 ± 6cd	86 ± 7cd

N/B Means with different letters (a–d) within a column differ significantly ($p<0.05$, Tukey's HSD).

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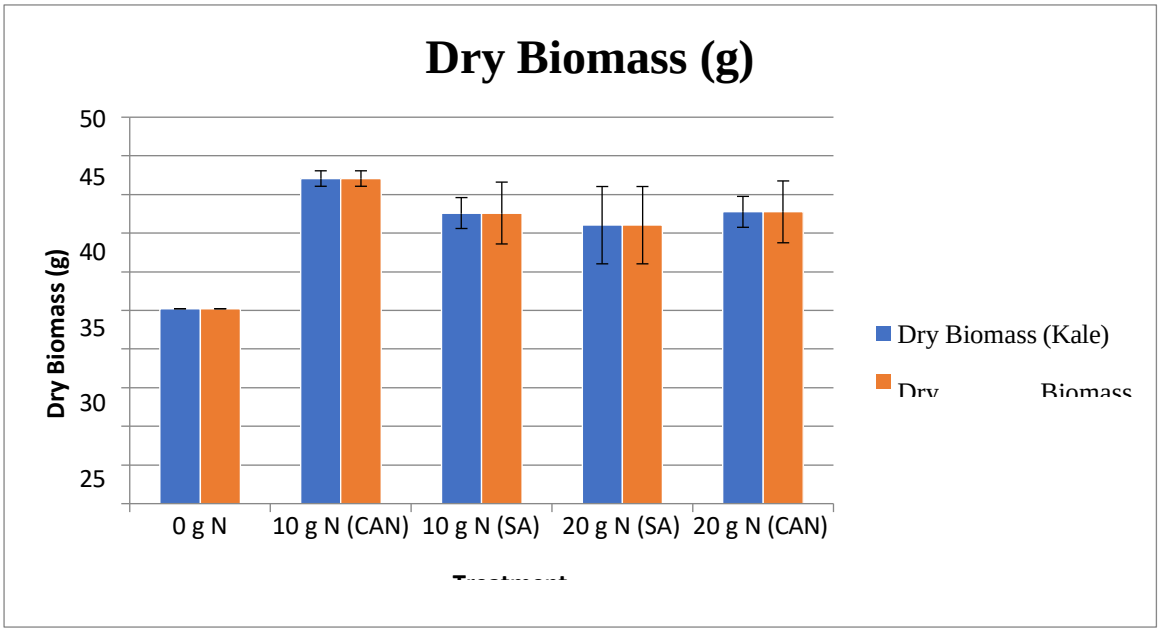
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Figure 4.4: Fresh biomass for kales and collards as affected by different nitrogen levels



Stacked bar chart comparing fresh and dry biomass for kale and collards, with 10 g CAN highest and control lowest.

Figure 4.5: dry biomass for kales and collards due to different nitrogen levels



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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Interpretation: The 10 g CAN treatment produced the highest biomass (100% increase over control), reflecting optimal nutrient use. The slight yield reduction at 20 g suggests stress or disease exacerbation, aligning with Fageria and Baligar (2005).

4.9 Clubroot Disease Severity (Galling Index)

Clubroot disease severity, assessed using the 0–4 galling index (modified from Dixon, 2009), was significantly influenced by nitrogen source and rate ($p < 0.05$). Data are presented pooled across varieties because the variety × treatment interaction was not statistically significant for galling index, although slight numerical differences existed.

Clubroot severity, assessed using a 0–3 galling index, increased with higher nitrogen rates, particularly with SA ($p<0.05$).

Table 4.8

Club-root Galling Index by Nitrogen Levels Treatment of Kales and Collards

Treatment	Kale Galling Index	Collard Galling Index	Interpretation
0 g N (Control)	2.8 ± 0.3 c	2.6 ± 0.2 c	Severe symptoms
10 g N (SA)	1.8 ± 0.2 b	1.7 ± 0.2 b	Moderate symptoms
10 g N (CAN)	**1.5 ± 0.2 a**	**1.4 ± 0.2 a**	**Mild symptoms (Best)**
20 g N (CAN)	2.0 ± 0.3 b	1.9 ± 0.3 b	Moderate symptoms
20 g N (SA)	2.2 ± 0.3 c	2.1 ± 0.3 c	Severe symptoms

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Treatment	Kale Galling Index	Collard Galling Index	Interpretation
0 g N (Control)	2.8 ± 0.3 c	2.6 ± 0.2 c	Severe symptoms
10 g N (SA)	1.8 ± 0.2 b	1.7 ± 0.2 b	Moderate symptoms
10 g N (CAN)	**1.5 ± 0.2 a**	**1.4 ± 0.2 a**	**Mild symptoms (Best)**
20 g N (CAN)	2.0 ± 0.3 b	1.9 ± 0.3 b	Moderate symptoms
20 g N (SA)	2.2 ± 0.3 c	2.1 ± 0.3 c	Severe symptoms

Table 2 Clubroot Galling Index by Treatment

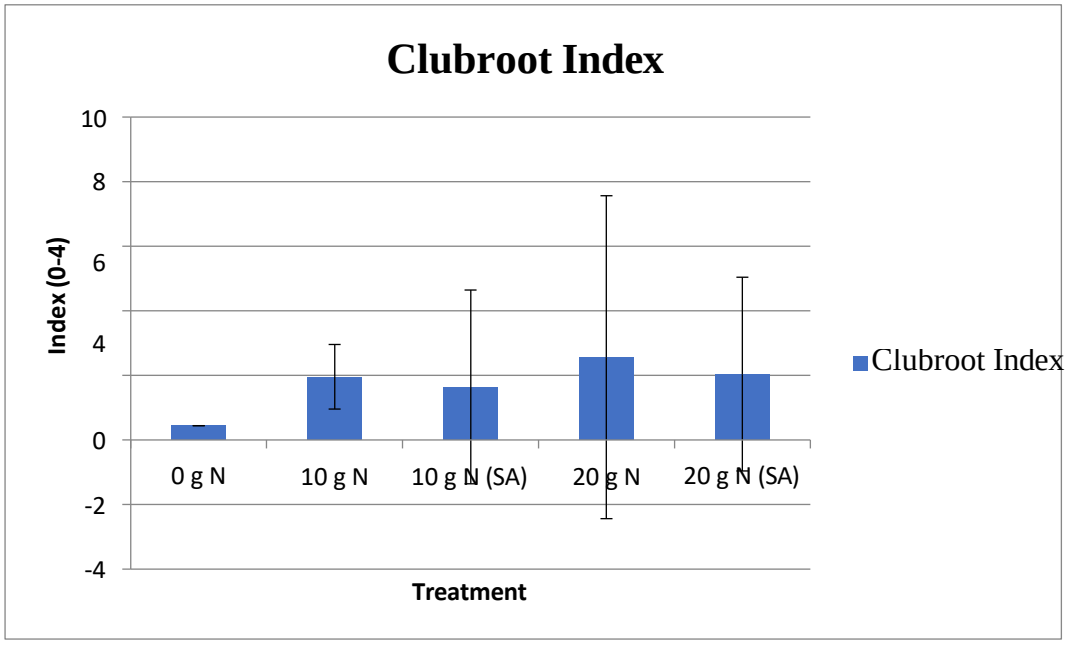
The significant reduction in galling index under moderate CAN application demonstrates that strategic nitrogen management can effectively mitigate clubroot severity. This is mechanistically linked to CAN's ability to raise soil pH (to 6.3–6.4) and supply calcium, both of which inhibit Plasmodiophora brassicae zoospore germination and gall development (Dixon, 2009; Albornoz et al., 2017; Ju et al., 2018). In contrast, SA's acidifying effect worsened disease at higher rates.

This finding directly supports the study's goal of identifying nitrogen strategies that reduce disease pressure while improving productivity in infested soils.

Means with different letters (a–c) within a column differ significantly ($p<0.05$, Tukey's HSD).

Figure 4.6:

clubroot index



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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

N/B Bar chart showing galling index, with 10 g CAN lowest and control/SA at 20 g highest. Photos of galled roots included.
Interpretation: The 10 g CAN treatment resulted in the lowest galling index, indicating enhanced disease resistance. Higher nitrogen rates, especially SA, increased severity, likely due to altered soil pH or nutrient dynamics favoring the pathogen (Dixon, 2009).

4.10 Nitrate Levels and Soil pH

Nitrogen treatments influenced nitrate accumulation and soil pH, with significant differences across treatments ($p<0.05$).

Table 4.9:

Nitrate Content (mg g^{-1}) in Leaves and Soil pH by Treatment on Kales and Collards

Treatment	Kale Nitrate (mg/g)	Collard Nitrate (mg/g)	Soil pH
0 g N (Control)	0.12 ± 0.02a	0.11 ± 0.02a	5.8a
10 g N (SA)	0.32 ± 0.03c	0.30 ± 0.03c	5.6b
10 g N (CAN)	0.28 ± 0.02b	0.26 ± 0.02b	6.4c
20 g N (CAN)	0.34 ± 0.03c	0.32 ± 0.03c	6.3c
20 g N (SA)	0.38 ± 0.03d	0.35 ± 0.03d	5.5b

N/B Means with different letters (a–d) within a column differ significantly ($p<0.05$, Tukey's HSD).

Interpretation: Sulphate of Ammonia treatments resulted in higher nitrate accumulation and lower soil pH, potentially increasing clubroot susceptibility. The CAN at 10 g raised soil pH, possibly suppressing pathogen activity (Ju et al., 2018).

4.11 Biochemical Composition

Analysis of biochemical composition (mineral elements — Ca, Zn, Fe; oxalates; total phenolics; flavonoids; and thiocyanate) was a critical component of this study. It directly addressed the broader problem articulated in Chapter One: clubroot disease not only reduces yield but also compromises the nutritional quality of kale and collards, which are staple vegetables relied upon by low- and middle-income households in Kenya for essential micronutrients and health-promoting compounds.
Biochemical analyses revealed significant changes in thiocyanate, minerals, and phytochemicals ($p<0.05$).

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Table 4.10:

THE Biochemical Composition by Nitrogen Level Treatment

TREATMENT	THIOCYANATE (MG/G)	CA (MG/G)	ZN (MG/G)	FE (MG/G)	PHENOLICS (MG GAE/G)	FLAVONOIDS (MG QE/G)
0 G N (CONTROL)	0.15 ± 0.02a	2.1 ± 0.2a	0.08 ± 0.01a	0.12 ± 0.02a	1.5 ± 0.2a	0.9 ± 0.1a
10 G N (SA)	0.22 ± 0.03b	2.8 ± 0.3b	0.10 ± 0.01b	0.15 ± 0.02b	2.0 ± 0.2b	1.2 ± 0.1b
10 G N (CAN)	0.20 ± 0.02b	3.0 ± 0.2c	0.11 ± 0.01c	0.16 ± 0.02c	2.2 ± 0.2c	1.4 ± 0.1c
20 G N (CAN)	0.25 ± 0.03c	2.7 ± 0.2b	0.09 ± 0.01b	0.14 ± 0.02b	1.9 ± 0.2b	1.1 ± 0.1b
20 G N (SA)	0.28 ± 0.03c	2.6 ± 0.3b	0.09 ± 0.01b	0.13 ± 0.02b	1.8 ± 0.2b	1.0 ± 0.1b

N/B Means with different letters (a–c) within a column differ significantly ($p<0.05$, Tukey's HSD).

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent.

Interpretation: The 10 g CAN treatment maximized mineral and phytochemical content, enhancing nutritional quality. Higher nitrogen rates increased thiocyanate but reduced phenolics/flavonoids, suggesting a trade-off (Farnham et al., 2004).

4.12 Discussion of Findings

4.12.1 Effect of Nitrogen Fertilizer Source and Rate on Growth

The study established that nitrogen fertilizer source and application rate significantly influenced the growth of kale and collards. Significant differences were observed in plant height, leaf area, and chlorophyll content ($p < 0.05$). The most favourable overall growth response was obtained with 10 g N plant^{-1} supplied through CAN. This treatment produced a plant height of approximately 42.6 cm in kale, increased leaf area by approximately 113% compared with the control, and recorded chlorophyll content of approximately 50 SPAD units.

The improved growth under moderate nitrogen application can be explained by the central role of nitrogen in plant metabolism. Nitrogen is an essential constituent of amino acids, proteins,

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

nucleic acids, enzymes, and chlorophyll and is therefore directly involved in cell formation, photosynthesis, and vegetative growth (Marschner, 2012). Since kale and collards are harvested primarily for their leaves, adequate nitrogen availability is particularly important for leaf expansion, canopy development, and biomass accumulation.

The increased chlorophyll content under the 10 g CAN treatment further indicates improved nitrogen nutrition. Nitrogen is an important component of chlorophyll and is required for the development and maintenance of photosynthetically active leaves. Higher chlorophyll concentration enhances the capacity of leaves to capture solar energy and support carbon assimilation. The higher SPAD values recorded under the favourable treatment therefore correspond with the greater leaf area and biomass observed in the same treatment.

The increase in leaf area was also important because leaf area determines the photosynthetic surface available to the plant. A larger and healthier canopy can intercept more light and potentially produce greater quantities of assimilates for growth. The approximately 113% increase in leaf area observed under the favourable nitrogen treatment therefore provides a physiological explanation for the higher biomass recorded under the same treatment.

The results further showed that increasing nitrogen application from 10 to 20 g N plant^{-1} did not result in a corresponding increase in growth. This suggests that the crops approached their optimum nitrogen requirement at the moderate rate. Additional nitrogen beyond the crop requirement may have resulted in diminishing returns or nutrient imbalance. Havlin et al. (2014) similarly observed that excessive nutrient application does not necessarily improve crop growth because plant responses are influenced by nutrient balance, soil conditions, and crop requirements.

The results therefore demonstrate that nitrogen management in Brassica production should emphasize an appropriate application rate rather than simply increasing the quantity of fertilizer. Under the conditions of this study, moderate nitrogen application provided more favourable growth than the higher application rate.

4.12.2 Effect of Nitrogen Fertilizer on Yield and Biomass Production

Nitrogen fertilizer treatments significantly affected fresh and dry biomass production. The 10 g N plant^{-1} CAN treatment recorded the highest biomass, with fresh biomass increasing by approximately 100% compared with the unfertilized control. This finding demonstrates the importance of adequate nitrogen availability for maximizing vegetative productivity in kale and collards.

The increase in biomass can be attributed to the role of nitrogen in promoting leaf expansion, chlorophyll formation, photosynthetic activity, and protein synthesis. Adequately supplied nitrogen enables plants to develop larger and more active canopies, resulting in increased accumulation of vegetative biomass. The findings are consistent with Fageria and Baligar (2005), who emphasized the importance of adequate nutrient supply for crop biomass accumulation and yield formation.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

However, the higher rate of 20 g N plant^{-1} did not produce a proportional increase in biomass. The relatively lower response at the higher rate suggests that excessive nitrogen may provide limited additional benefit once the crop's nutritional requirements have been met. In clubroot-infested soils, this response may also be influenced by root damage caused by the pathogen. Plants with severely infected roots may have reduced capacity to absorb additional nutrients and water even where soil nutrient availability is high.

Differences were also observed between kale and collard varieties. Kale generally recorded slightly higher yields than collards, suggesting that genetic characteristics influenced nitrogen-use efficiency and biomass accumulation. Differences among varieties may arise from variations in root development, canopy architecture, nutrient uptake, growth rate, and tolerance to clubroot. Fernandez et al. (2013) noted that crop genotypes may differ in their ability to acquire and efficiently use nutrients.

The findings therefore indicate that the most productive fertilizer treatment cannot be determined independently of crop variety. Fertilizer recommendations should consider the interaction between nitrogen source, application rate, and varietal characteristics.

4.12.3 Effect of Nitrogen Fertilizer on Clubroot Severity

The study found significant differences in clubroot severity among nitrogen fertilizer treatments. The lowest clubroot galling index was recorded under the 10 g N plant^{-1} CAN treatment, with an approximate galling index of 1.5 in kale. Higher nitrogen application, particularly under SA, was associated with greater clubroot severity.

The lower disease severity under moderate CAN application may be partly explained by improved plant nutritional status and root development. Adequately nourished plants generally have greater capacity to maintain growth and physiological functions under pathogen stress. Calcium supplied by CAN may also contribute to cell wall development and root structural integrity.

However, nitrogen fertilizer should not be considered a direct control measure for clubroot. The disease is caused by Plasmodiophora brassicae, which survives in soil as resistant resting spores. Fertilizer treatments may influence the disease indirectly through changes in soil pH, nutrient availability, plant vigour, and root physiology.

The greater clubroot severity observed under some SA treatments may be associated with the acidifying effect of ammonium nitrogen. SA supplies nitrogen predominantly in ammonium form, which undergoes nitrification following application. Nitrification is accompanied by the release of hydrogen ions, potentially contributing to soil acidification (Smolinska & Palusinska, 2005; Ju et al., 2018). Since acidic soil conditions can favour clubroot development, continued application of acidifying fertilizers may be undesirable in already acidic soils.

The findings therefore demonstrate the importance of considering fertilizer source when managing Brassica crops in clubroot-prone soils. The choice of nitrogen fertilizer should be

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

based not only on its nitrogen content but also on its potential effects on soil chemical conditions.

5.1.4 Effect of Nitrogen Fertilizer on Soil pH and Nitrate Levels

The study recorded differences in soil pH among the fertilizer treatments. SA-treated plots recorded relatively low pH values of approximately 5.5–5.6, whereas CAN-treated plots recorded pH values of approximately 6.3–6.4 under the experimental conditions.

The lower pH associated with SA is consistent with the acidifying potential of ammonium-based fertilizers. Repeated nitrification of ammonium can contribute to soil acidification, particularly where the soil has limited buffering capacity. Soil pH is an important factor in nutrient availability and clubroot development; therefore, changes in pH may partly explain the differences in disease severity observed among the fertilizer treatments.

The higher pH observed under CAN treatment coincided with lower clubroot severity. This suggests that the soil chemical environment may have contributed to the favourable performance of CAN. Nevertheless, the observed pH response should be interpreted in the context of the specific experimental soils rather than generalized to all soils. Fertilizer effects on soil pH depend on initial soil reaction, buffering capacity, fertilizer composition, application rate, moisture, and duration of use.

Differences in nitrate accumulation were also observed among treatments. Higher nitrate concentrations in some SA-treated plants indicate that nitrogen source and rate can influence nitrogen accumulation in leafy vegetables. Although nitrate is an essential nutrient form, excessive nitrate accumulation in vegetables may be undesirable from a food-quality perspective.

The findings emphasize the need to synchronize nitrogen supply with crop demand. Applying nitrogen at rates substantially above crop requirements may increase the risk of inefficient nitrogen use and nutrient losses without producing proportional increases in yield.

4.12.4 Effect of Nitrogen Fertilizer on Biochemical Composition

The study further demonstrated that nitrogen fertilizer source and rate influenced selected biochemical characteristics of kale and collards. The 10 g N plant^{-1} CAN treatment generally recorded higher concentrations of selected mineral elements, including calcium, zinc, and iron, as well as increased total phenolic and flavonoid contents.

The higher mineral concentrations may be related to improved plant growth and nutrient uptake under adequate nitrogen supply. Nitrogen influences root development and plant metabolic activity and may consequently affect the acquisition and assimilation of other nutrients. Calcium is essential for cell wall development and membrane stability, while iron and zinc participate in several physiological and enzymatic processes.

The increase in phenolics and flavonoids under moderate nitrogen application is also nutritionally relevant. Phenolic compounds and flavonoids are important plant secondary

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

metabolites and contribute to the antioxidant properties of vegetables. Brassica vegetables are recognized as important sources of phytochemicals and micronutrients, and fertilizer management may influence their concentration.

However, the results also showed that higher nitrogen application was associated with increased thiocyanate and reduced concentrations of some phytochemicals. This suggests that increasing nitrogen supply may alter the allocation of plant resources between primary growth and secondary metabolism. The response of secondary metabolites to nitrogen availability is complex and may also depend on variety, environmental stress, and disease pressure.

The biochemical findings therefore suggest that the most appropriate fertilizer treatment should not be selected solely on the basis of biomass. Nutritional and phytochemical quality should also be considered when evaluating fertilizer management practices for leafy vegetables.

4.12.5 Implications for Sustainable Nitrogen Management

The findings have important implications for sustainable Brassica production in Taita-Taveta County. The 10 g N plant^{-1} CAN treatment produced the most favourable overall combination of growth, biomass, clubroot response, and selected biochemical characteristics. Increasing the nitrogen rate to 20 g N plant^{-1} did not consistently improve crop performance and, in some cases, was associated with increased disease severity.

The results demonstrate that fertilizer efficiency is more important than fertilizer quantity. Applying nitrogen beyond crop requirements may increase production costs while providing limited additional yield. It may also contribute to nitrate leaching and other environmental problems associated with excessive nitrogen application (Tilman et al., 2002).

For smallholder farmers, moderate fertilizer application may therefore offer both agronomic and economic advantages. However, fertilizer recommendations should be combined with other clubroot management practices. These include the use of suitable varieties, crop rotation, sanitation, soil testing, appropriate water management, and maintenance of favourable soil conditions.

The results should also be considered within the context of local soil conditions. The relatively acidic soils of the study area make fertilizer source particularly important. A fertilizer that supplies adequate nitrogen but contributes to further acidification may not be appropriate for continuous use in clubroot-prone fields.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter presents the discussion, summary, conclusions, recommendations, and areas for further research arising from the study on the effects of nitrogen fertilizer source and application rate on the growth, yield, clubroot severity, and selected biochemical characteristics of kale and collards grown in clubroot-infested soils in Taita-Taveta County, Kenya. The study evaluated the effects of Calcium Ammonium Nitrate (CAN) and Sulfate of Ammonia (SA) applied at 0, 10, and 20 g N plant^{-1} on four Brassica varieties under field conditions in Wundanyi and Mwatate Sub-Counties.

The discussion interprets the findings in relation to nitrogen nutrition, crop growth, biomass production, clubroot development, soil pH, nitrate accumulation, and selected mineral and phytochemical characteristics. The chapter also summarizes the major findings in relation to the study objectives, draws conclusions based on the empirical evidence, and provides recommendations for farmers, agricultural extension officers, policymakers, and researchers.

5.2 Summary of Findings

The study evaluated the effects of nitrogen fertilizer source and rate on the growth, yield, clubroot severity, and selected biochemical characteristics of kale and collards grown in naturally clubroot-infested soils in Wundanyi and Mwatate Sub-Counties of Taita-Taveta County.

The study used CAN and SA as nitrogen sources at rates of 0, 10, and 20 g N plant^{-1}. Four Brassica varieties—Curly Kale, Dinosaur Kale, Vates, and Morris Heading—were evaluated under field conditions during the June–October 2025 growing season.

The major findings were as follows:

1.Growth performance: Nitrogen source and rate significantly influenced plant height, leaf area, and chlorophyll content. The 10 g N plant^{-1} CAN treatment produced the strongest overall growth response, including approximately 42.6 cm plant height in kale, approximately 113% greater leaf area than the control, and approximately 50 SPAD units.

2.Yield and biomass: The 10 g N plant^{-1} CAN treatment produced the highest fresh and dry biomass. Fresh biomass increased by approximately 100% compared with the unfertilized control. Increasing nitrogen to 20 g N plant^{-1} did not result in a proportional increase in biomass.

3.Clubroot severity: Nitrogen source and rate significantly influenced clubroot severity. The lowest galling index, approximately 1.5 in kale, was recorded under the 10 g N plant^{-1} CAN treatment, whereas some higher SA treatments recorded greater disease severity.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

4.Soil pH: SA treatments were associated with lower soil pH values of approximately 5.5–5.6, while CAN treatments recorded values of approximately 6.3–6.4 under the experimental conditions.

5.Biochemical characteristics: The 10 g N plant^{-1} CAN treatment improved selected mineral elements, including Ca, Zn, and Fe, as well as total phenolics and flavonoids. Higher nitrogen rates were associated with increased thiocyanate and reductions in some phytochemical characteristics.

6.Varietal response: Kale and collard varieties differed in their growth, yield, and disease responses, demonstrating that genetic differences influence nitrogen-use efficiency and performance under clubroot pressure.

Overall, the findings showed that moderate nitrogen application, particularly 10 g N plant^{-1} supplied as CAN, provided the most favourable overall response under the conditions of the study.

5.3 Conclusion to the study

The study concludes that nitrogen fertilizer source and application rate significantly influence the growth, yield, clubroot severity, and selected biochemical characteristics of kale and collards grown in clubroot-infested soils.

The results indicate that 10 g N plant^{-1} supplied through CAN provided the most favourable overall response. This treatment enhanced plant height, leaf area, chlorophyll content, fresh and dry biomass, and selected mineral and phytochemical characteristics while being associated with comparatively lower clubroot severity.

The findings further demonstrate that increasing nitrogen application beyond the moderate rate does not necessarily improve crop performance. The 20 g N plant^{-1} treatment produced diminishing benefits for several growth and yield parameters and was associated with greater disease severity in some treatments. This suggests that excessive nitrogen application may be counterproductive in acidic, clubroot-infested soils.

The superior performance of CAN compared with SA under the conditions of the study highlights the importance of considering nitrogen source when developing fertilizer recommendations. The results suggest that nitrogen management should take into account not only crop nutrient requirements but also the effects of fertilizer source on soil conditions and disease development.

The study therefore concludes that balanced nitrogen management is an important component of sustainable kale and collard production in clubroot-infested soils. However, fertilizer management alone cannot provide complete control of clubroot because the pathogen can persist in soil for many years. Nitrogen management should consequently be integrated with other measures, including appropriate variety selection, crop rotation, sanitation, soil testing, and suitable soil and water management practices.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

5.4 Recommendations of the Study

5.4.1 Recommendations to Farmers

Farmers producing kale and collards in clubroot-prone soils should consider applying 10 g N plant^{-1} using CAN under soil and production conditions comparable to those of the present study. The rate should be applied carefully and according to crop requirements to avoid unnecessary nitrogen losses and excessive fertilizer expenditure.

Farmers should also conduct periodic soil testing to monitor soil pH and nutrient status. This is particularly important in fields where Brassica crops are continuously cultivated. Where acidic soil conditions are present, farmers should seek appropriate soil-management advice before applying repeated ammonium-based fertilizers.

Nitrogen fertilization should be integrated with other clubroot-management practices. Farmers should practise crop rotation, maintain field sanitation, avoid movement of contaminated soil between fields, and select varieties with improved tolerance or resistance to clubroot where suitable planting materials are available.

5.4.2 Recommendations to Agricultural Extension Officers

Agricultural extension officers should provide farmers with practical guidance on nitrogen source selection, application rate, and timing in clubroot-prone soils. Extension programmes should emphasize that increasing fertilizer quantities does not necessarily result in increased yields.

Extension officers should also promote soil testing and interpretation of soil-pH results so that fertilizer recommendations are matched to the specific conditions of individual farms. Farmer training should integrate nutrient management with clubroot-management strategies, including crop rotation, sanitation, appropriate variety selection, and soil-moisture management.

5.4.3 Recommendations to County Government and Agricultural Policymakers

The Taita-Taveta County Government and relevant agricultural agencies should strengthen access to affordable soil-testing services for smallholder farmers. Soil-testing programmes can help farmers make fertilizer decisions based on actual nutrient and soil-pH conditions rather than generalized recommendations.

Agricultural programmes should also promote integrated management of clubroot in Brassica-producing areas. Such programmes may include farmer training, access to appropriate planting materials, disease surveillance, soil-health programmes, and dissemination of locally validated fertilizer recommendations.

Where fertilizer-support programmes are implemented, attention should be given to promoting efficient rather than excessive fertilizer use. Support programmes should be accompanied by farmer education on correct rates, application methods, and environmental considerations.

5.4.4 Recommendations to Researchers

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

Further research should evaluate the nitrogen treatments across multiple growing seasons and additional agro-ecological zones to determine whether the observed responses remain consistent under different environmental conditions.

Future studies should also investigate the long-term effects of CAN and SA on soil pH, nutrient availability, microbial communities, nitrate accumulation, and P. brassicae resting-spore populations. Such studies would provide stronger evidence on the sustainability of repeated nitrogen fertilizer application in clubroot-infested soils.

Research should also evaluate combinations of nitrogen management with resistant or tolerant varieties, liming, organic amendments, biological control agents, and crop rotation. Such integrated experiments could identify management packages capable of improving crop productivity while reducing disease pressure.

Economic analyses should additionally be conducted to compare the cost-effectiveness of different nitrogen sources and rates. This would enable recommendations to incorporate both agronomic performance and profitability for smallholder farmers.

5.5 Recommendations for Further Research

Based on the findings and limitations of the study, further research should focus on the following areas:

1.Multi-season studies: Conduct experiments over several growing seasons to determine the cumulative effects of CAN and SA on soil pH, nutrient availability, clubroot severity, and crop productivity.

2.Broader agro-ecological validation: Replicate the experiment in other parts of Taita-Taveta County and other Brassica-producing regions of Kenya to determine the consistency of the findings across different soils and climatic conditions.

3.Economic analysis: Conduct detailed cost-benefit analyses comparing CAN and SA at different nitrogen rates, taking into account fertilizer costs, labour, marketable yield, and gross returns.

4.Integrated clubroot management: Evaluate combinations of moderate nitrogen application with resistant varieties, crop rotation, liming, organic amendments, and biological control measures.

5.Soil microbiological studies: Investigate the effects of nitrogen fertilizer source and rate on soil microbial communities and P. brassicae resting-spore viability.

6.Nutritional and phytochemical studies: Examine the effects of nitrogen management on a wider range of glucosinolates, phenolic compounds, flavonoids, antioxidant capacity, nitrate concentration, and other nutritional components.

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

7.Nitrogen-use efficiency: Determine the nitrogen-use efficiency of different kale and collard varieties under different fertilizer sources and rates to identify varieties capable of producing high yields with lower fertilizer inputs.

8.Climate variability: Examine how differences in rainfall, temperature, soil moisture, and irrigation influence the interaction between nitrogen nutrition and clubroot development.

5.6 Contribution to Knowledge

The study contributes to knowledge in several important ways. First, it provides field-based evidence on the interaction between nitrogen fertilizer source, nitrogen application rate, crop growth, yield, and clubroot severity in kale and collards grown under naturally clubroot-infested conditions in Taita-Taveta County.

Second, the study demonstrates that nitrogen fertilizer management should not be based solely on maximizing nutrient supply. The findings indicate that fertilizer source and rate jointly influence crop performance, with moderate CAN application producing a more favourable overall response than higher nitrogen application under the conditions investigated.

Third, the study contributes evidence linking nitrogen management with both disease response and biochemical quality. By assessing growth, biomass, clubroot severity, soil pH, mineral elements, and selected phytochemicals, the study provides a broader assessment of crop performance than studies focusing exclusively on yield.

Fourth, the findings provide locally relevant information that can support development of nitrogen-management strategies for smallholder Brassica production in Taita-Taveta County. The evidence may assist farmers, extension officers, and agricultural planners in promoting efficient fertilizer use in acidic and clubroot-prone soils.

Finally, the study establishes a basis for future research on integrated management of clubroot through the combined use of appropriate nitrogen sources and rates, improved varieties, soil-health management, and other disease-management

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

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APPENDICES

APPENDIX I: ANOVA_LSD ALL TRAITS RESULT

Treatment	Block	Height_Kale	eight_Coll	Area_Kale_	ea_Collar	phyll_Kale	hyll_Collar	Biomass_K	omass_Co	iomass_Ka	mass_Col	broot_Ince
0 g N	1	21.24	16.17	86.95	124.99	37.17	28.87	226.09	241.64	34.14	32.1	0.91
0 g N	2	28.45	20.99	138.86	128.24	29.79	35.73	210.03	157.58	39.48	41.03	0.03
0 g N	3	25.98	32.58	182.89	138.36	30.46	35.68	219.16	230.73	52.86	36.03	0.39
10 g N (CA	1	39.58	40.01	330.81	281.46	49.95	51.9	387.61	401.03	113.24	83.31	1.11
10 g N (CA	2	45.21	43.58	289.31	314.88	60.36	56.92	474.36	437.04	110.54	96.88	2.54
10 g N (CA	3	41.4	35.43	254.17	306.73	51.79	42.84	395.12	430.85	106.11	92.72	2.22
10 g N (SA	1	40.24	39.38	340.26	325.02	40.65	40.61	369.1	313.8	96.95	94.28	1.44
10 g N (SA	2	38.79	41.25	261.99	262.53	36.66	48.75	342.38	439.53	71.83	75.94	1.29
10 g N (SA	3	33.77	32.24	226.45	274.54	51.52	45.8	354.63	366.27	86.88	92.5	2.2
20 g N (SA	1	37.03	40.93	310.89	296.81	55.46	50.39	358.48	336.4	94.48	90.44	2.21
20 g N (SA	2	42.34	54	314.66	240.51	45.18	47.21	437.88	327.79	101.93	87.19	1.98
20 g N (SA	3	28.72	35.83	261.62	273.15	51.6	40.06	299.85	348.76	85.66	86.93	1.93

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

APPENDIX II: NACOSTIC LICENSE

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 357657

Date of Issue: 16/December/2023

RESEARCH LICENSE



This is to certify that Mr. Denis Kinyavura Manyasa of Kenya Methodist University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Taita Taveta on the topic: EFFECTS OF NITROGENOUS FERTILIZER LEVELS ON THE GROWTH AND YIELD OF KALE AND COLLARDS IN SOIL INFESTED WITH CLUBROOT for the period ending 16/December/2025.

357657

Applicant Identification Number

Director General
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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

APPENDIX III: AN INTRODUCTORY LETTER

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September 06, 2025

To Whom It May Concern

RE: PERMISSION TO CONDUCT RESEARCH IN TAITA TAVETA COUNTY

Dear Sir/Madam,
This letter serves as official authorization from Kenya Methodist University (KeMU) for Mr. Denis Kinyavura Manyasa, Admission Number AGR-3152-3/2023, a student in our Agriculture program, to conduct his research titled "Effects of Nitrogen Fertilizer Levels on the Growth and Yield of Kale and Collards in Soil Infested with Clubroot" in Taita Taveta County.

Mr. Manyasa's research aims to investigate the impacts of varying nitrogenous fertilizer applications on the specified crops under clubroot-infested soil conditions. This study is part of his academic requirements and is supervised by the Department of Agriculture at KeMU. The research activities may involve field surveys, soil sampling, crop monitoring, and interactions with local farmers or stakeholders in the county, all conducted in an ethical and environmentally responsible manner.
We hereby grant Mr. Manyasa full permission to proceed with these activities in Taita Taveta County, effective immediately and valid until the completion of his research or December 31, 2025, whichever comes first. He is required to

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

comply with all local regulations, obtain any necessary county-specific permits, and respect the rights and privacy of participants.

Should you require further verification or additional information, please contact the undersigned or the university's Academic Registry at the details provided above.

Thank you for your cooperation in supporting academic research that contributes to agricultural advancements in Kenya.

Sincerely,

Dr. Rosemary Kirambia, PhD

Chair of Department, Agricultural Science

Kenya Methodist University

Phone: +254703700724

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

APPENDIX IV: CONSENT LETTER

Effects of Nitrogen Fertilizer Levels on Growth and Yield of Kale and Collards in Clubroot-Infested Soils

1. Consent Forms Informed Consent Form for Research Participants

Researcher/Principal Investigator: Denis Kinyavura Manyasa

Purpose of the Study: This research aims to evaluate how different levels of nitrogen fertilizers affect the growth and yield of kale and collards in clubroot-infested soil. Your participation may involve providing access to your land for planting, applying fertilizers, or assisting in monitoring crops.

Procedures: If you agree to participate, you will:

- Allow researchers to plant and monitor kale/collards on a small plot (e.g., 10x10 meters) on your property.
- Apply specified fertilizer levels as instructed (no cost to you).
- Permit periodic visits for data collection (e.g., measuring plant height, harvesting).
- The study will last approximately [e.g., 3-6 months], with minimal disruption.

Risks and Benefits:

- Risks: Minimal—possible temporary soil changes or crop loss in the test plot (compensated if applicable). No health risks to humans.
- Benefits: You may gain knowledge on better farming practices, free soil testing, and potential improved yields. Results will be shared with the community.

Voluntary Participation: Your participation is voluntary. You can withdraw at any time without penalty.

Confidentiality: All data will be anonymized. Your name/farm details will not be published without permission.

Compensation: Reimbursement for any inputs or a small stipend of 1000KES.

Consent Statement: I have read and understood this form. I voluntarily agree to participate.
Signature: Date:

Printed Name:

Witness (if required): Date:

APPENDIX V: DATA COLLECTION TOOLS

I've designed two sample tools for systematic data gathering in the study: (1) a **Field Observation Sheet** for growth metrics, and (2) a **Yield Assessment Form** for harvest

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International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 10 , October - 2026

data. These can be implemented as paper forms, spreadsheets (e.g., Google Sheets/Excel), or apps. They include fields for replication to ensure statistical validity.

1 Field Observation Sheet (for Weekly/Monthly Growth Monitoring)

This tool records vegetative growth parameters at set intervals (e.g., weeks 4, 8, 12 post-planting). Use one sheet per plot/replicate.

Plot ID	Fertilizer Level (e.g., 0, 50, 100 kg N/ha)	Crop Type (Kale/Collards)	Date	Plant Height (cm, avg of 10 plants)	Leaf Count (avg per plant)	Root Gall Severity (Scale: 0=none, 1=mild, 2=moderate, 3=severe)	Notes (e.g., wilting, pests)

Instructions:

- Measure from 10 random plants per plot.
- Calculate averages.
- Include soil pH/moisture readings if equipment available.
- Replicate across 3-5 blocks for reliability.

2 Yield Assessment Form (for Harvest)

This tool captures final output at maturity (e.g., 60-90 days). Use one per plot.

Plot ID	Fertilizer Level (kg N/ha)	Crop Type	Harvest Date	Total Fresh Weight (kg/plot)	Marketable Yield (kg/plot, excluding damaged)	Quality Score (Scale: 1=poor, 5=excellent)	Clubroot Incidence (%plants affected)	Notes (e.g., nutrient deficiencies)

Instructions:

- Weigh entire harvest; subtract non-marketable portions (e.g., diseased leaves).
- Assess clubroot by uprooting and inspecting 20 plants.
- Aggregate data for analysis (e.g., via ANOVA in software like R or Excel).
- Optional: Add columns for nutrient analysis (e.g., leaf N content via lab test

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