DECLARATION

I certify that this thesis is my original work and has not been previously submitted for the award of a degree in any other university.

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DEDICATION

To my late father Clifford Omayio Ogero; for inculcating the virtues of discipline and hard work in every honest endeavor of life. Rest in peace dad.

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ABSTRACT

Napier grass (*Pennisetum purpureum* Schum.) is a leading feed for dairy animals in Eastern and Central Africa. In recent years the napier grass production has been threatened by napier head smut (NHS) and napier stunt disease (NSD), caused by *Ustilago kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 respectively. In efforts to manage the two diseases, host plant tolerance has been sought separately for each disease by selection of some tolerant accessions to napier stunt and head smut diseases respectively. However, there is no information available about the selected napier accessions' ability in co-resisting the two diseases amidst reports of their continual spread in Kenya. Further, there is little understanding of the morphological and molecular characteristics of *U. kamerunensis* and its impact on the growth of the napier grass under co-infection treatments in varying nutrients and moisture levels. Thus, it is important to find out the real pathogen variations and their pathogenicity levels from the affected areas to inform the management strategies. The little knowledge available on molecular characteristics of *U. kamerunensis* pathogen has limited the extent of tolerant varieties adoption in different zones due to tolerance breakdown by the hypothesized isolates' varying pathogenicity levels. Therefore, the purpose of this study was to characterize *U. kamerunensis* isolates from the six affected counties in Kenya (Nakuru, Nyandarau, Kiambu, Kirinyaga, Murang'a and Nyeri), using the internal transcribed spacer 1, 2 and 5.8S rRNA genome elements. Further, evaluate their pathogenicity levels against napier grass growth tolerance under co-infection with "*Candidatus* Phytoplasma oryzae" strain Mbita 1 in varying nutrients and moisture levels. The *U. kamerunensis* propagules were systematically collected from the affected counties using geographic positioning system's coordinates. This was done by dusting their ustilospores into pollination bags. Samples were transported to the laboratory for morphological and molecular analysis. Characterization involved *in-vitro* culturing of the isolates, colony colour observation and measurement of colony diameter. Total DNA of *U. kamerunensis* isolates was extracted and sequenced to determine the molecular variations. The two purposively selected *U. kamerunensis* (NAK002 & NYA002) isolates based on their growth *in-vitro* were studied in confined glasshouse conditions under co-infection of napier varieties (KK1, KK2, 16789 & Bana) with "*Candidatus* Phytoplasma oryzae" under four nutrient formulations (complete nutrient solution, nitrogen deficiency, phosphorus deficiency & nitrogen-phosphorus deficiency) and two watering regimes (daily & weekly) in a completely randomized design experiment at ICIPE-Mbita. Growth parameters measured were; tiller height, tiller & leaf number, leaf area, chlorophyll content levels, total fresh, total stem and total leaf weights. Statistical analyses were conducted at confidence level of $P \leq 0.05$. The study revealed differences in *U. kamerunensis* isolates based on morphological and molecular characteristics. Kiambu, Nyandarau and Nakuru isolates clustered together, as well as Murang'a, Nyeri and Kirinyaga. Further, differences were observed on their pathogenic levels. The sole NSD ("*Candidatus* Phytoplasma oryzae") pathogen and NAK002 isolate infections were the most and least virulent respectively in comparison to co-infected treatments. The growth and tolerance levels of the evaluated varieties against the pathogens differed significantly. The varieties under nitrogen formulations and watering daily had high levels of pathogen tolerance. The ability of the varieties to maintain growth, chlorophyll stability and disease tolerance can be used in selecting highly tolerant germplasm. These findings would help farmers, plant pathologists and breeders to integrate information on molecular characterization, pathogenicity levels, varieties growth and tolerance levels under co-infection in streamlining the diseases management towards improving productivity and breeding aspects of Napier grass.

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ACRONYMS AND ABBREVIATIONS

bp	Base pair
CHc	Chlorophyll uninoculated
CHi	Chlorophyll inoculated
CM	Centimeter
CNS	Complete Nutrient Solution
D	Daily watering
DNA	Deoxyribonucleic Acid
HMT	High Magnitude of Tolerance
HSL	High Stress Levels
HV	High Virulence
ICIPE	International Centre of Insect Physiology and Ecology
IPLI	Integrated Parameter Logarithmic Index
IPMLI	Integrated Parameter Mean Logarithmic Index Inoculated / uninoculated but stressed
IPMLIC	Integrated Parameter Mean Logarithmic Index of Control of the four varieties
IPMLIU	Integrated Parameter Mean Logarithmic Index of the Uninoculated / stress free
ITS	Internal Transcribed Spacer
KALRO	Kenya Agricultural and Livestock Research Organization
KK 1	Kakamega 1 Napier grass variety
KK 2	Kakamega 2 Napier grass variety
L.I.I	Logarithmic Index Inoculated
L.I.U	Logarithmic Index Uninoculated
L.I.U.S	Logarithmic Index Uninoculated Stressed
LAc	Leaf Area control
LAi	Leaf Area inoculated
LFNc	Leaf Number control
LFNi	Leaf Number inoculated
LMT	Low Magnitude of Tolerance
LoD	Logarithmic Deviation
LSL	Low Stress Levels
LV	Low Virulence
M.E.I	Magnitude Efficacy Index
M.L.I	Mean Logarithmic Index
MMT	Moderate Magnitude of Tolerance
MSL	Moderate Stress Levels
MV	Moderate virulence
N/P-D	Nitrogen/Phosphorus Deficient Nutrient Solution
NAK-2	Nakuru County-Napier Head Smut Isolate 2
N-D	Nitrogen Deficient Nutrient Solution
NHS	Napier Head Smut
NSD	Napier Stunt Disease
NYA-2	Nyandarua County-Napier Head Smut Isolate 2
OMATEC	Omayio Technologies
OPL	Overall Percentage Levels
PASW	Predictive Analytical Software
PCR	Polymerase Chain Reaction
P-D	Phosphorus Deficient Nutrient Solution
SpD	Specific Percentage Deviation
ssDNA	Single stranded Deoxyribonucleic Acid

TFWc	Total Fresh Weight control
TFWi	Total Fresh Weight inoculated
TLHc	Tiller Height control
TLHi	Tiller Height inoculated
TLNc	Tiller Number control
TLNi	Tiller Number inoculated
TLWc	Total Leaf Weight control
TLWi	Total Leaf Weight inoculated
TSWc	Total Stem Weight control
TSWi	Total Stem Weight inoculated
VHMT	Very High Magnitude of Tolerance
VHSL	Very High Stress Levels
VHV	Very High Virulence
VIRP	Virulence Percentage
W	Weekly watering

DEFINITION OF TERMS

Susceptible **:** A host plant to a particular pathogen that exhibits high degree of damage or symptoms in comparison to another of the same species that is not severely affected (tolerant or resistant).

Ratoon **:** Harvestable napier grass crop obtained within a particular cropping cycle counted from the time of planting basing on the number of harvests. For example; ratoon one is obtained up on harvesting of the first crop from planting. Ratoon two is obtained up on the harvest of the regrowth from ratoon one and so on.

Stay-green trait **:** A trait exhibited by some tolerant plants to a particular stressor (whether biotic or abiotic), where they can be able to limit the degradation of their chlorophyll in a bid to survive pathogen damage and produce some significant yield in comparison to those affected severely (susceptible).

Tiller **:** An individual napier grass plantlet. The many that grow from an individual cutting are known as tillers and they form a stool.

Stool **:** A group of tillers growing from the same seed cane or cutting.

Host plant tolerance **:** Ability demonstrated by some host plants (napier grass varieties or accessions) to a respective pathogen where they are able to express less morpho-pathological symptoms or degree of damage despite being infected by an equal pathogen load, in comparison to those affected severely (susceptible).

Virulence **:**The degree of damage caused by a respective pathogen of concern on a host plant.

Co-infection **:**The infection of a plant system by more than one pathogen simultaneously.

Phylogenetics **:**Study of evolutionary relationships among groups of organisms of the same species, which is determined by construction of genetic tree-like plots that illustrate the extent of evolutionary divergence or convergence using either nucleic acid , amino acid sequences or morphological data.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Napier grass (*Pennisetum purpureum* Schumach), a poaceae is a popular source of fodder in Eastern and central Africa regions (Boonman, 1993; Martha *et al*., 2004; Anitha *et al*., 2006). The fodder crop is highly adopted in both intensive and semi intensive livestock production systems for both dairy and beef (Kabirizi *et al*., 2015). This is because of its edibility by livestock in its leafy stage, enormous biomass, quick regeneration and ability to tolerate frequent cuttings (Van de Wouw *et al*., 1999; Lowe *et al*., 2003; Nyambati *et al*., 2011). This has seen an intensified cultivation of the grass by smallholder dairy farmers in the last decade due the growth of the dairy sector, who then use it mainly through fresh harvest or as silage forms of livestock feed. Further, the fodder crop's production has been heightened due to limited alternative sources of animal feed in the region which has been attributed to the effects of climate change (Woodard *et al*., 1991; Mwangi, 1994; Staal *et al*., 1998; ASARECA, 2010; Omayio *et al.*, 2014a).

In recent years, the vibrant dairy sector in Eastern and Central Africa has been threatened by emergence and continual spread of two economic diseases viz; napier head smut (NHS) and napier grass stunt (NSD) that are constraining the fodder crop production significantly (Lukuyu *et al*., 2012; Kabirizi *et al*., 2015; Mulaa *et al*., 2015). Napier head smut is caused by *Ustilago kamerunensis*; (P. & H. Sydow), and in Kenya it's more prevalent in the Central Kenya region (Omayio *et al*., 2014a). The pathogen is hemibiotrophic causing very significant biomass losses of up to 46% (NAFIS, 2012). The infected napier plant's stems harden and produce smutted premature flower, becoming thin and grassy. The subsequent regrowth is smaller and total dry matter of the affected crop reduces massively (Farrell *et al*., 2002b; Mwendia, 2007). On the other hand, napier grass stunt disease (NSD), is caused by phytoplasma; "*Candidatus* Phytoplasma oryzae" strain Mbita 1, belonging to the 16SrXI group. In Kenya the disease is more prevalent in Western Kenya region causing up to 90% yield losses (Jones *et al*., 2004; Fischer *et al*., 2016). The affected napier crop develops chlorotic leaves, then proliferation of tillers and shortening of internodes to extreme stunting leading to eventual death of the plant within the subsequent third or fourth ratoon of harvests (Ajanga, 2005; Mulaa *et al*., 2015).

Napier head smut and stunt diseases are transmitted by infected planting materials (Jones *et al*., 2004; ASARECA, 2010). In addition, wind transfer of spores aids the spread of head smut (Farrell *et al*., 2000; Omayio *et al*., 2015), while the insect vector transmission by *Maiestas banda* Kramer (Hemiptera: Cicadellidae) has been reported to aid the spread of stunt disease (Obura *et al*., 2009; Omayio *et al*., 2014a). Therefore, to mitigate this threat to the dairy industry in the African regions host plant resistance has been emphasized due to ease of implementation and effectiveness in disease management (Parry, 1990; ASARECA, 2010). However, from recent studies no information is known about the existing napier grass accessions and varieties ability in co-resisting the two diseases amidst reports of continual spread of the diseases to new areas (Lukuyu *et al*., 2012; Kabirizi *et al*., 2015). Furthermore, there is limited understanding on the morphological and molecular characteristics of napier head smut of the Central and Rift-Valley regions of Kenya, largely due to previous studies not addressing this component (Rosete *et al*., 2009). Hence, it is important to expose the real pathogen variations and their pathogenicity levels in the affected Kenyan areas to inform management strategies. In addition, Omayio *et al.(*2015) proposed a possible co-evolution of the head smut pathogen due to adopted germplasm's resistance pressure leading to possible emergence of new variants. This calls for the need of information on the morphological and molecular characteristics of the head smut pathogen in these areas. Moreover, there is no information on the interactions of the napier head smut pathogen with napier stunt pathogen under varying nutrients and moisture levels, coupled with its effect on the general growth and natural tolerance of the napier grass. This is observed in reports by Kabirizi *et al*. (2015) who worked on the effects of moisture and nutrients on head smut but did not assess their effects on the interaction of the two pathogens and how they affect natural resistance dynamics in respective tolerant or resistant accessions. These gaps possess a challenge to successful mitigation of the two diseases in an intergrated management manner, especially if they were to co-occur in the same region simultaneously.

The current study sought to generate information on the morphological and molecular characteristics of napier head smut, virulence levels under abiotic stresses, the interactions of the two pathogens in selected tolerant accessions and identify an accession exhibiting co-tolerance to the two diseases. It was predicted that such a strategy was to provide an effective and sustainable host plant resistance tactic in the management of these diseases. Furthermore, it is not clear as to whether the selected tolerant accessions can grow in different agro-ecological

zones without their resistance breakdown, if diverse isolates of head smut exist (ASARECA, 2010; Kainyu, 2014; Kabirizi *et al*., 2015).

1.2 Statement of the problem

Eastern Africa and specifically Kenya is considered to be the most promising region for dairy production. The region holds over 40% of about 222 million Africa's cattle resource and napier grass forms a major feed source. The produced milk is mainly by smallholder farmers who rely on it as their source of protein and income. The fodder crop is grown by over 70% of these farmers who use it under stall feeding due to population pressure which has resulted to small farms (Kabirizi and Muyekho, 2015). The productivity of the fodder grass is now threatened by two emerging diseases; napier head smut and napier stunt disease. Towards the management of the diseases; host plant tolerance has been sought separately for each disease that led to the selection of five and eleven tolerant accessions to napier stunt and head smut diseases respectively (Omayio, 2013; Wamalwa, 2013). However, from the studies little is known about the existing napier grass accessions' and varieties' ability in co-resisting the two diseases amidst reports of continual spread of the diseases, compounded by some reports of their heightened severity in the newly affected areas. Furthermore, there is limited information on the morphological and molecular characteristics of napier head smut in the affected regions of Kenya. Thus, it is important to expose the real pathogen variations and their virulence levels from the affected areas to inform management strategies. There are also reports suggesting a co-evolutionary scenario of the head smut pathogen that would lead to emergence of new variants. A situation attributed to the pathogen's survival pressure against the adopted resistant varieties in the affected regions. This calls for the need to know the morphology and molecular characteristics of the head smut pathogen in the affected areas. The little knowledge on the molecular characteristics of the pathogen limits how wide tolerant varieties against it can be adopted in different zones without a possible breakdown of the trait. Moreover, there is no information on the co-infection effects of the *Ustilago kamerunensis* pathogen with "*Candidatus* Phytoplasma oryzae" under varying levels of nutrients and moisture. A scenario that is complicated further by lack of knowledge on how the interactions can affect the general growth and natural tolerance of the napier grass against the pathogens. This is especially on their efficacy when subjected to the abiotic stresses under continuous cultivation and harvesting; a

situation that may dilute efforts to mitigate the diseases effectively in an intergrated approach in case of co-occurrence in Kenya using natural resistance and managed soil fertility. Therefore, there is need to identify tolerant accessions to both diseases under co-infection scenario and evaluate them to establish; their efficacy in the management of the diseases under selected abiotic stressors. The study intends to identify the variations at morphological, molecular and virulence levels of the napier head smut pathogen isolates found in affected areas of Kenya.

1.3 Justification

The two diseases have continued to spread to new areas and niches in East and Central Africa and there are indications of imminent overlap of the two diseases (Jorge *et al*., 2014; Kabirizi *et al*., 2015). This has triggered the need to understand the likely reaction of the already selected tolerant accessions to co-infection by the two diseases and possible mitigation strategies if it occurs (Lukuyu *et al.*, 2012, Mulaa *et al*., 2015; Wamalwa *et al*., 2015). Moreover, the advocated cultural practices of managing the diseases like uprooting of diseased plants and subsequent disposal has ended up creating natural reservoirs of the diseases because many farmers dump the plants on roadsides instead of burying or burning them. There is need for a combination of host plant resistance applied together with other integrated pest management practices (Mwendia *et al*., 2007, Omayio *et al*., 2015). Thus, the current study is aimed at generating more knowledge on the natural resistance of napier grass accessions/varieties to napier head smut and stunt diseases. Furthermore, the diseases rapid spread in the region is catalyzed by continuous napier grass cultivation on smallholder farms without rotation, climate change and lack of high yielding alternative fodder grasses for cut and carry zero grazing system. This further emphasizes the need for identifying napier accessions/varieties that are either tolerant or resistant to the two diseases (Farrell *et al*., 2002a; Orodho, 2006; ASARECA, 2010). In addition, the recently selected tolerant accessions to the respective diseases have not been evaluated against the two diseases under co-infection and abiotic stress conditions (Kabirizi *et al*., 2015). This poses a threat of likely reversal of the tolerance and resistance of the selected accessions to the respective diseases. Therefore, the aim of this study is to develop a novel technology that can be able to estimate the apparent resistance levels of the selected accessions to the two diseases and how this result can be optimized to manage the diseases under different abiotic stresses. The findings of this study will be significant to plant pathologists in provision of the tool of estimating the

magnitude of natural resistance, besides providing plant breeders and farmers resistant germplasm to the two diseases that can be deployed in the integrated management of the diseases.

1.4 General objective

To morphologically and molecularly characterize *Ustilago kamerunensis* isolates from affected counties in Central and Rift-Valley regions of Kenya, evaluate their pathogenicity effects on napier grass tolerance under co-infection with '*Candidatus* Phytoplasma oryzae' strain Mbita 1, in varying nutrients and moisture levels.

1.5 Specific objectives

1. To determine the morphological and molecular characteristics of *Ustilago kamerunensis* isolates in affected counties of Kenya.
2. To determine the pathogenicity levels of *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 on selected napier grass varieties under varying nutrients and moisture levels.
3. To determine the co-infection effects of the *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1, on the growth of selected napier grass varieties as influenced by varying nutrients and moisture levels.
4. To determine the levels of tolerance of selected napier grass varieties against the *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 co-infection under varying nutrients and moisture levels.

1.6 Hypotheses

1. There are no differences in the morphological and molecular characteristics of the *Ustilago kamerunensis* isolates in affected counties of Kenya.
2. The pathogenicity levels of the *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1, upon infection of selected napier grass varieties is equal under varying nutrients and moisture levels.

3. There are no differences in the growth of selected napier grass varieties' in response to the co-infection effects of *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1, under varying nutrients and moisture levels.
4. There are no differences in the levels of tolerance of selected napier grass varieties against the *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 co-infection under varying nutrients and moisture levels.

CHAPTER TWO

LITERATURE REVIEW

2.1 Napier grass

Pennisetum purpureum Schumach commonly known as napier grass, is in the division; angiospermae, sub-division; monocotyledonae, class; commelinids, order; poales, and family; poaceae (Bogdan, 1977; 'T mannetje, 1992). Members of the forage crop's family poaceae are distinguished using their flower morphology and growth habit. The species *P. purpureum* which is in the same section *penicillaria* as millets (Farrell, 1998), grows optimally at temperatures range of 25-40^{o}C, though growth at 15^{o}C has been reported. Moreover, maximal growth of the grass is associated with high rainfall of 1000-1500 mm per year. However, tolerance to relatively short periods of drought due to the long root system has been observed. Though, prolonged lack of moisture greatly influences the forage crop's growth significantly (Skerman and Riveros, 1990; Anindo and Potter, 1994).

Napier grass is propagated vegetatively using cuttings from parent plant and exhibits perennial growth habits. The forage crop does not flower easily due to the long vegetative phase it exhibits (Boonman, 1993; Boonman, 1997; Anitha *et al*., 2006). However, when it flowers, it produces an open pollinated inflorescence that is cylindrical, 8-20cm and sometimes 30cm long with 1.5-3cm diameter (Prain, 1934). Therefore, because of the type of pollination it exhibits it usually gives rise to a number of cultivars that are highly heterozygous due to natural crossings from its seeds (Augustin and Teacenco, 1993). The grass' origin is mapped from Sub-Saharan Africa at the Zambezi valley where it is used intensively as a source of fodder (Valk, 1990; Boonman, 1993; Boonman, 1997; Lowe *et al*., 2003).

Kenya among the Eastern Africa countries has adopted immensely the cultivation of the forage crop at both high and medium potential regions of the country to provide dairy feed for its rapidly growing dairy sector (Kariuki, 1998; Muia *et al*., 1999; Bebe *et al*., 2003; Kabirizi *et al*., 2015). In Central Kenya region for example; over 600,000 smallholder farmers in the cut-and-carry system highly rely on napier grass as fodder source, besides the over 90% of the agricultural households growing the grass as a monocrop (Staal *et al*., 1998; Mwangi, 1999).

2.2 Napier grass dependent smallholder livestock systems in Kenya

An estimated 80% of the Kenyan dairy cattle is owned by smallholder dairy farmers (Kariuki *et al*., 1998). Among these farmers the livestock management systems that are napier grass dependent as source of feed vary from intensive zero grazing (stall feeding) to extensive semi-zero (grazing system or free range system) that used to be the dominant system in the 1960s when large tracts of grazing land existed (Staal *et al*., 1998; Orodho, 2006; Farrell *et al*., 2002a; Herrero *et al*., 2008). The zero grazing system is common in highly populated areas like Central Kenya region where 67% practice it compared to 28% who graze in free range (Staal *et al*., 1998). This is attributed to land shortage for grazing due to continuous subdivisions by smallholder dairy farmers (Stotz, 1983; Kariuki *et al*., 1998; Staal *et al*., 1998). The practice has resulted to heightened use of napier grass as a feed source under stall feeding (Stotz, 1983; Anindo and Potter, 1994; Kariuki *et al*., 1999). The farmers are market driven as a result those who do not produce enough napier grass do buy to complement the amount of grass they produce. The free range system has some modified sub-types like; tethered range grazing and grazing supplementation systems where the latter napier grass fed to livestock is supplemented with crop residues in what is referred to by others in the industry as semi-intensive system (Tittonell *et al*., 2009; Lukuyu *et al*., 2012). The system is practiced largely by mixed farmers who are semi-commercial and often keep cross-bred or local zebu cattle (Muyekho *et al*., 2003). The poor subsistence farmers form the lower level of the smallholder livestock systems tree and usually own local cattle tethered in their compound as their main asset (Perry *et al*., 2002). The grazing of livestock by this lower group is usually done on crop residues in fields or on communal patches of pasture. However, even in the system the pasture is supplemented by napier grass (Staal *et al*., 1998; Tittonell *et al*., 2009).

2.3 Napier grass production constraints in Kenya

The constraints to napier grass production are divided into abiotic and biotic stressors based on the causative agent of the stress to the crop's growth and consequently the harvested yield as reviewed:

2.3.1 Effects of abiotic stress on the general growth of napier grass

Several environmental stressors affect the growth of napier grass and its response to disease infection; significant of them being moisture stress (Mwendia *et al*., 2007). The forage's biomass production becomes highly inhibited under prolonged or transient water availability due to its role in the physiological processes of the grass (Yanxian *et al*., 2008; Omayio *et al*., 2015). As a result, the severity of the individual diseases has been observed to increase under limited soil moisture availability (Kabirizi *et al*., 2015). This is a challenge especially with the imminent climate change; the crop might be challenged in coping with the situation. Thus, creating a major problem to its production as a feed source (Barnes *et al*., 2007; Thornton *et al*., 2010). Further, the interaction between the following abiotic factors which are positively correlated namely; temperature, light intensity, water availability, nutrients availability and absorption towards biomass bulking makes the abiotic factors critical in napier grass production (Humphreys, 1991). Especially nitrogen and phosphorus availability highly influence the forage crop's growth and resistance to diseases (Kabirizi *et al*., 2015); like nitrogen for example has been reported to react with phenolic compounds to form pathogen-inhibitive quinine-amino conjugates under the influence of polyphenol oxidase (PPO) (Bittner 2006). Hence, well watered and fertilized napier grass crop has been reported to exhibit some heightened tolerance against napier head smut and napier stunt disease, but of concern is the limited information available on the effect of the nutrients and moisture availability variations on the pathogens virulence, growth and tolerance of the fodder crop under co-infection scenarios (Mwendia, 2007; ASARECA, 2010; McMahon, 2012; NAFIS, 2012; Kabirizi *et al*., 2015).

2.3.2 Biotic stressors on the growth of napier grass

The biotic stressors to napier grass production can be categorized into three groups namely; pests, weeds and diseases (Farrell *et al*., 2002b). The most significant of the three is the diseases. The diseases include: Fungal snow mold disease caused by *Beniowskia sphaeroides* a false mildew, foliar infections eyespot caused by *Helminthosporium ocellum*, napier stunt disease caused by "*Candidatus* phytoplasma oryzae" strain Mbita 1 and napier head smut caused by *Ustilago kamerunensis*. Napier stunt and napier head smut diseases are the leading challenges to its production in Western and Central Kenya regions respectively (Orodho, 2006; Mwendia *et al*., 2007; Kabirizi *et al*., 2015; Fischer *et al*., 2016). Despite most of the napier grass clones and

cultivars being highly susceptible to the snow mold fungi the disease does not affect the growth of the forage and the animals feeding significantly. Hence, the disease is of less economic significance (Boonman, 1997; Nyambati *et al*., 2007). Reports of foliar infections eyespot disease in Kenya do not exist so far however, at the Carribean islands severe cases have been observed (Farrell *et al*., 2002b).

The moles and termites attack the grass with the former being the most destructive (Mwendia *et al*., 2007). Reports of nymphal stages of leaf hoppers (Cicadellidae*)*, aphids (*Macrosiphum euphorbiae*), ants (*Pheidole megacephala*) and mites (Tetranychus sp.) attacking the grass in Kenya have been reported (Farrell *et al*., 2002a). These mites attack correlates with the bronzing of the leaf blades at their underside. Moreover, the longitudinal growth of napier grass is highly affected by these pests upon attack (Farrell *et al*., 2002b). Also, the weeds of the poaceae family affect napier's longitudinal growth especially at the height of 200 cm. Weeds from the Digitaria sp., Eleusine sp., Imperata sp and the sedges are among those leading in limiting the general growth of the forage crop (Farrell *et al*., 2002b).

2.4 Napier grass head smut and napier grass stunt diseases

Among the biotic constraints discussed above two diseases have stood out as the major threats to napier grass production and subsequently the dairy sector sustenance due to reduced feed supply (Kabirizi *et al*., 2015). Napier head smut and stunt diseases caused by a smut fungus and phytoplasma respectively are the two economic diseases of concern currently.

2.4.1 The napier head smut

Napier head smut is caused by *Ustilago kamerunensis* a smut fungus that belongs to the *Ustilago* genus. The fungus belongs to division eumycota and sub-division basidiomycotina which are characterized by their bi-nucleate spores and formation of a dikaryon from vegetative part's fusion (Alexopoulos and Mims, 1979; Holliday, 1989).The smut fungus sub-division is second to rusts' (pucciniales formely uredinales) in the division in terms of species numbers that have a high economic implications (Piepenbring, 2003). The fungus belongs to the class basidiomycetes, sub-class heterobasidiomycetidae which is characterized with numerous identified orders that give rise to over 77 genera under smut and bunts (Zoberi, 1972; Ingold, 1984; Zillinsky, 1987; Hawksworth *et al*., 1995; Piepenbring, 2003).

2.4.1.1 Etiology of the napier head smut disease

Ustilago kamerunensis the causative agent of napier head smut grows within the plant's cells and slowly spreads systemically to the entire plant's tissues. Its hyphae that are branched with internal partitions (septate) produce lobed and curved haustoria that form the feeding structures of this parasite in the host plant or it can feed directly through the cell walls. Its ustilospores are sub-globose with an estimated 7µm diameter. At reproduction the spikelets confine the sori with the ustilospores becoming a black loosely attached mass for easy dissemination (Farrell, 1998; Omayio *et al*., 2015). Because of this the reproductive investment by this systemic pathogen using the host's resources is quite significant that it reduces the plant's biomass extensively (Farrell *et al*., 2002a). This is compounded by the perennial life cycle of the pathogen where it produces ustilospores continuously in huge amounts to the soil (Farrell, 1998). Hence, once a field is infected then for sure one has to ensure the likely management strategy is thorough if the disease problem is to be ameliorated.

2.4.1.2 Epiphytology of napier head smut disease

Epiphytotics of napier head smut can be attributed to certain abiotic conditions like; temperature range of between 5^{o}C and 35^{o}C with an optimum of around 20^{o}C highly favouring the establishment of this pathogen. Moreover, high relative humidity ranging between 65-90% enhances the disease's initiation on susceptible host. This is after successful *Ustilago kamerunensis* spread from a sick crop to health susceptible one that is primarily facilitated by wind transfer of ustilospores from smutted inflorescence to new unaffected areas compounded by ustilospores inoculum on the field soil in natural infections scenario (Farrell, 1998). Secondary transmission of the pathogen is through; animal carrying stuck ustilospores on them, animal's waste fed on the smutted crop, clothes of passersby and planting of diseased canes carrying the pathogen within their tissues (Farrell, 1998; Mwendia *et al*., 2007; ASARECA, 2010; NAFIS, 2012). The most susceptible stage of the crop is during the development stage of the buds into shoots (shoot infection) of a respective cane or when the buds are pushing through the soil a factor explaining why the disease is so severe in the regrowth of a second crop after the first harvest due to the many buds that provide extensive shoots to infect and the damaged stem tissues which also provide entry points of the pathogen (Farrell, 1998).

2.4.1.3 Napier head smut disease symptoms

The disease first manifests itself in susceptible hosts through induced premature flowering covered in a black mass of ustilospores commonly referred to as the smut (Plate 2.1). This occurs even in plants that are below 1.5 m height as opposed to healthy plants that usually flower at heights above 1.5 to 8 metres depending on grass variety, with others even taking so long to do so due to a very long vegetative phase (Farrell, 1998; Boonman, 1993). This visual sign is later compounded by other severe symptoms upon first harvest and regrowth influenced largely by the levels of susceptibility of the grass type including; slow regrowth after cutting, withering and chlorosis setting in with gradual browning towards drying and death of the entire stool of the crop within the subsequent 2-3 cuttings in severe cases (ASARECA, 2010; NAFIS, 2012). Besides the above primary signs other secondary characteristics of the disease like; induced dwarfing (stems are thinner and shorter than normal less than 1.5 m in height) has been observed in serious cases, characterized by short internodes with distorted leaves in shape that are reduced in number and size on stools, with an increased tillering scenario (Farrell, 1998; Mwendia, 2007; NAFIS, 2012).

Plate 2.1: The *Ustilago kamerunensis* induced premature flowers of napier grass covered in a black mass of ustilospores. A morpho-pathological symptom expressed by napier grass challenged by the napier head smut disease (NHS) (Source: own work (surveys)).

2.4.1.4 Research and distribution of napier head smut

Research on ways of mitigating napier head smut is limited and this can be attributed to less attention paid to the crop by virtue of it being a feed source for livestock (Farrell *et al*., 2002b). In Kenya the entry route of the disease is mapped from West Africa, through Uganda (1930), Rwanda (1963), Tanzania (1975) (Farrell *et al*., 2002b), and eventual establishment in the country in the 1990s where it was first reported in press affecting Central's Lari division in Kiambu district by Kung'u and Waller (Farrell, 1998; Farrell *et al*., 2001; Kung'u and Waller, 2001). Since then its distribution within several divisions of the region has been notable and logarithmic (Farrell, 1998; Kung'u and Waller, 2001; Mwendia, 2007). The spread is compounded by reports of its severe occurrence in some new parts of the country like; in Rift-valley (Molo and Londiani) and lower Eastern region (Meru north and south) (Lukuyu *et al*., 2012; Jorge *et al*., 2014). Furthermore, reports of a possible co-evolution of napier head smut pathogen leading to emergence of possible new variants has been reported by Omayio *et al*. (2015). This has necessitated the need for morphological and molecular characterization of the head smut pathogen from affected counties in Kenya which has never been done. As a result, has led to limited understanding of the morphological and molecular characteristics of the pathogen in the affected regions of country, largely due to previous studies not addressing this component (Kabirizi *et al*., 2015).

Rosete *et al*. (2009) molecularly characterized two non-random isolates from Central using ITS primers. However, the sample size of two was limiting to merit a conclusion on the status of variants of head smut disease in Central Kenya. Thus, how wide the selected tolerant accessions can be cultivated and adopted in different agro-ecological zones successfully of the country without resistant breakdown as a result of emerging diverse isolates is not known (ASARECA, 2010; Kainyu, 2014; Kabirizi *et al*., 2015). Further, little is known about the previously selected tolerant napier grass varieties (Kakamega 1, Kakamega 2 and 16789) to the napier head smut pathogen; in co-tolerating both *Ustilago kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 under co-infection. This is coupled with little understanding of the impact of varying moisture and nutrients levels on the pathogenicity of *U. kamerunensis* under co-infection of the napier grass varieties with NSD pathogen which has not been tested (Kabirizi *et al*., 2015). Therefore, the current study aimed to generate information on the status of molecular

characteristics of head smut pathogen in a wide range of regions in Central and Rift Valley areas, besides evaluating the response of the previously selected tolerant napier grass varieties (KK 1, KK 2, 16789) against the two diseases under co-infection.

2.4.2 The napier stunt disease and taxonomic guide to the causative pathogen

Napier grass stunt, abbreviated as NSD (Napier stunt disease) currently causes significant herbage yield losses in napier grass (Mulaa *et al*., 2015). The disease is caused by phytoplasma of the genus "*Candidatus* Phytoplasma" specifically "*Candidatus* Phytoplasma oryzae" (Jung, Sawayanagi, Wongkaew, Kakizawa, Nishigawa, Wei, Oshima, Miyata, Ugaki, Hibi & Namba); strain Mbita 1 abbreviated as "*Ca.* P. oryzae" (Fischer *et al*., 2016). The first sub-species was characterized in Asia in rice plant by Jung *et al*.(2003), and since then in Kenya and India the "*Candidatus* Phytoplasma oryzae" has been reported in different grasses and coconut plants respectively (Manimekalai *et al*., 2014; Adam, 2015; Asudi *et al*., 2016a,b). This pathogen is categorized in pleomorphic group of bacteria of the class Mollicutes. However, it is distinguished from other members of the class by its inability to be cultured or cultivated *in vitro* (IRPCM, 2004). This has created a situation where only the nucleic acids and host characteristics are used to identify the species (De Vos *et al*., 2005; Firrao *et al*., 2005; Duduk and Bertaccini, 2011). According to International Research Programme for Comparative Mycoplasmology; this is not sufficient for a formal taxonomic description of a species under class mollicutes, which requires an organism's culturable characteristics *in vitro* to assume their binomial nomenclature naming format. Hence, the "*Candidatus* status was introduced for such phytoplasma before the genus and species names without italicizing the latter two in quotation marks i.e "*Candidatus* Phytoplasma oryzae" (Murray and Schleifer, 1994; IRPCM, 2004; Firrao *et al*., 2005; Euzeby and Parte, 2013; Fischer *et al*., 2016). The phytoplasmas tryptophan amino acid is encoded by the codon UGG. Further, a spacer region of about 300bp exists between the 16S and 23S ribosomal regions that further differentiates the phytoplasma from other members of the class (McCoy *et al*., 1989; Obura, 2012).

2.4.2.1 Etiology of the napier stunt disease

The species "*Candidatus* Phytoplasma oryzae" (Jung, Sawayanagi, Wongkaew, Kakizawa, Nishigawa, Wei, Oshima, Miyata, Ugaki, Hibi & Namba); strain Mbita 1 belongs to the 16SrXI

group of 16Sr group of phytoplasma that attack napier grass. It is in this group where the Western X-disease belongs; a form of NSD caused by the 16SrIII group of phytoplasma (Arocha and Jones, 2010; Fischer *et al*., 2016). The phytoplasma are quite primitive as they have been observed to lack virulence genes that are exhibited by other pathogenic bacteria in their genomes, besides they lack metabolic pathways (Maust *et al*., 2003; Oshima *et al*., 2004). They also don't exhibit the genes coding for cytoskeleton elements such as flagella or cilia. As a result, their movement is limited through the sieve tube system's pores of the phloem together with the assimilate courtesy of their small pleomorphic sizes (Christensen *et al*., 2005). Despite, these limitations they have developed strategies of slowing phloem functions of transportation of manufactured plant sugars and soluble proteins as they consume them and ended up becoming extremely dependent on the host plant for survival. This leads to maximum exploitation of the plant system thus, affecting the general growth and even altering plant hormone balance (Maust *et al*., 2003; Musetti *et al*., 2005). Phytoplasmas are found in large quantities in mature leaves' tissues with very minimal quantities in roots and stems. However, they are not found in the meristems (Bertaccini and Duduk, 2009; Wanga *et al*., 2017).

2.4.2.2 Epiphytology of napier stunt disease

"*Candidatus* Phytoplasma oryzae" strain Mbita 1(Jung, Sawayanagi, Wongkaew, Kakizawa, Nishigawa, Wei, Oshima, Miyata, Ugaki, Hibi & Namba); the causative agent of NSD is spread by insects in the order hemiptera specifically *Maiestas banda* (Hemiptera: Cicadellidae) (Plate 2.2). These insects attack the plant through their phloem feeding behavior where they parasitize the plant. The insects mainly leaf hoppers, plant hoppers and Psyllids constitute these feeders. Besides direct transmission of the pathogen through feeding, transovarial spread has also been reported in some insects where they do so via their eggs to the off spring which then spread the disease in a persistent manner (Tedeschi *et al*., 2006; Obura, 2012; Fischer *et al*., 2016). Parasitic plants like the dodder (*Cuscuta sp*.) have been reported to aid in the spread, besides propagation of plant propagules hosting the pathogen and grafting of infected scion to uninfected stock (Cordova *et al*., 2003; Razin, 2007).

Plate 2.2: The napier stunt disease (NSD) vector, *Maiestas banda* (Source: Obura, 2012)

2.4.2.3 Napier stunt disease symptoms

The disease manifests itself in susceptible napier grass accessions or varieties firstly through leaf chlorosis (Plate 2.3). With subsequent cutting and regrowth stunting sets in; with characteristic short internodes, bushy growth habit denoted by heightened tillering to eventually death of the stool in high susceptible crop. Plants infected early in the cycle of planting usually exhibit symptoms in the second ratoon and die within that season (Wamalwa *et al*., 2015). Several factors in integration like fertility of the soil, accessions' resistance or tolerance, vectors availability and frequency of cutting in interacting way influence how first the disease progresses (Orodho, 2006; Kabirizi *et al*., 2015).

Plate 2.3: The induced chlorosis and stunting morpho-pathological symptoms expressed by napier grass infected by the napier stunt disease (NSD) caused by '*Candidatus* Phytoplasma oryzae' (Source: own work (surveys)).

2.4.2.4 Napier stunt disease, distribution and research status

The disease was initially reported in eastern Uganda where it was suspected to be viral transmitted (Tiley, 1969). In the 1990s it was reported in Western Kenya specifically the counties of Bungoma and Busia (Jones *et al*., 2004; Mulaa *et al*., 2004). It was first reported by Jones *et al*. (2004) that it's caused by phytoplasma 16SrXI strain, belonging to class mollicutes (ICSB, 1996). The disease was later reported in northern Tanzania as its spread continued unabated (Nielsen *et al*., 2007). The disease now covers Western Kenya, where it causes yield losses of up to 90% in this medium potential dairy region (Mulaa *et al*., 2004). Limited research has been done on selection for tolerant napier grass varieties against the disease except for Obura (2012) and Wamalwa *et al*. (2015) who selected moderately tolerant accessions to the disease among them being 16789, 16807, 16817, 16822 and characterized the pathogen respectively. Recently, Fischer *et al*. (2016) generated a draft sequence of "*Candidatus* Phytoplasma oryzae" strain Mbita 1 which causes the napier grass stunt disease in Kenya. However, research on the possible interaction effects with head smut under co-infection has not been done amidst its continual spread to head smut affected regions (Kabirizi *et al*., 2015). This is vital to enable optimization of its management in areas where the two diseases are likely to co-occur. Furthermore, the impact of varying moisture and nutrients levels on the pathogenicity of the stunt pathogen under co-infection has not been tested. This is coupled with the effect of the abiotic factors on the grass' tolerance magnitude dynamics (ASARECA, 2010; Kabirizi *et al*., 2015). Thus, forming a basis of investigation for this current study. Also, the pathogen has been characterized molecularly but no differences were observed even across related phytoplasma members in 16SrXI group like Hyparrhenia white leaf disease. A factor that was attributed to its primitivity in terms of genome evolution and highly conserved 16S rRNA gene (Nielsen *et al*., 2007; Obura *et al*., 2011). This explains why the current study did not focus on characterizing "*Candidatus* Phytoplasma oryzae" at molecular level and instead did so for *Ustilago kamerunensis*.

2.5 Plant-pathogen molecular level interactions and their influence on a plant's resistance

Plants response to a pathogen challenge is influenced by the presence or absence of resistance genes which are specific or non-specific to the pathogen (Crute and Pink, 1996). Hence, the interactions can either be specific or non-specific (Edreva, 2004; Keane, 2012). The process of

specific interaction begins when the complementary plant's protein and the pathogen's effector protein interact encoded by a major dominant resistance gene and avirulence gene respectively (Staskawicz, 2001; Schirawski *et al*., 2010). At this point the resistance gene can either act in solitude or with a few others in a plant leading to a response; which is a drastic suppression of the pathogen in an incompatible interaction through induced (active) resistance mechanism (Cohen, 2001; Edreva, 2004; Keane, 2012). This is possible due to the pathogen perception by these resistance gene's proteins that leads to the activation of conserved defense signaling systems either directly or indirectly through the pathogen's targeted plant proteins guarded by the R-proteins, a phenomenon common with vertical (race-specific) resistance (Hammond *et al*., 2007; Keane, 2012). This induction of an active resistance against a pathogen has also been reported to be triggered by other biotic and abiotic elicitors in non-specific interactions associated with horizontal (non-specific) resistance (Kuc, 2001; Aziz *et al*., 2003; Edreva, 2004; Keane, 2012). Thus, depending on the plant's interactive levels dictated by its genome, some end up overwhelmed and others not (Pedley and Martin, 2003). The overwhelmed plants usually cannot recognize the proteins encoded by the now virulent gene with their respective resistance gene's proteins or because they lack the resistance genes to encode the matching recognition proteins leading to a disease interaction scenario (Parry, 1990).

Therefore, due to the gene-for-gene relationship described above an eliminative interaction (immunity/complete resistance) can arise through morphological barriers and/or secretion of chemical compounds that eliminates the pathogen totally (Edreva, 2004; Eickhoff *et al*., 2008; Broekgaarden *et al*., 2011; Keane, 2012). This is usually seen on the sharp differential interactions statistically between the plant host and pathogen races in the vertical resistance graphical plot (Parry, 1990; Keane, 2012). Whereas for the horizontal resistance or non-specific resistance which is not based on (R) genes, strong eliminative interactions of the pathogen are not observed. This is because the pathogen is capable of causing some disease or partially establish but does not overwhelm the host. A scenario attribuited to certain plant's biology aspects that slows down the intimate interaction and subsequently the establishment of the pathogen in a compatible interaction way (John, 1998; Keane, 2012).

2.6 The disease management strategies and estimation of their efficacy challenge

The aim of any disease management strategy in practice is to stop build up of a disease in a crop in a cost effective and easy to implement manner by the end user (Parry, 1990). The methods usually influence the disease epidemic progress by affecting the variables like; initial pathogen inocula (X_o), rate of infection (r) and the time (t) of disease establishment, which mathematically is expressed as ($X = X_0 e^{rt}$) where (X) is the amount of disease at any time of disease progress (Van der Plank, 1968; Parry, 1990). Hence, the target in disease management is usually to reduce the amount of disease (X) to as low as possible, a concept which has been used in the attempt to control the napier head smut and stunt diseases currently using the following two key tactics:

2.6.1 Host plant resistance method

This method is a key strategy in disease control in modern plant health management and takes advantage of reducing the rate of infection (r) by either slowing or delaying the progress and initialization of an epidemic respectively (Parry, 1990). Based on this the accession tolerant to napier head smut and napier stunt diseases have been screened by Omayio *et al*. (2015) and Wamalwa *et al*. (2015) respectively to manage the infection rate of these diseases. This has given an opportunity of likely management of these pathogens effectively using varying varietal resistances which according to Van der Plank (1968) is the most effective way of utilizing this tactic of host plant resistance. However, the challenge of estimating the magnitude of this host plant resistance in plants using severity scores introduces errors because its visual based leading to wrong estimations of the intensity of the trait during assessment (Parry, 1990). This is even more challenging with napier diseases which are hemibiotrophic like NSD and head smut (Freeman and Beattie, 2008; Omayio, 2013). These diseases don't show initial visual symptoms for estimation in tolerant or resistant accessions making it very difficult to estimate magnitude of resistance (Farrell, 1998; ASARECA, 2010). Because of this scenario the estimation of the host plant resistance's efficacy and magnitude levels of respective accessions using severity proportions as reported by Parry (1990) is a challenge. Hence, making it an area of focus towards estimating the magnitude of host plant resistance in this study.

Therefore, the key symptoms of the two diseases being reduced herbage yields, induction of dwarfing in tillers and chlorosis. The efforts towards addressing this was based on the premise

that; mean total fresh biomass weight, mean tiller height and the chlorophyll content levels of the varieties being components of cumulative input of growth mechanisms and being affected significantly by the two diseases could be used to estimate the potential of each selected variety's resistance or tolerance (Causton and Venus, 1981; Hunt, 1982). Further, resistance being a relative trait to the highly susceptible end of a plant population system using relativity it can be quantified (Freeman and Beattie, 2008). This could be possible through integration of the combined means of the significantly affected parameters; that is the mean total fresh weight, mean tiller height and chlorophyll content levels' measured of the respective inoculated and uninoculated (control) treatments of all the napier grass varieties ratoons through a modified Van der Plank (1968, 1975) *algorithms 2a,2b,2c* and *2d* of epidemic establishment dynamics (Table 3.6), as explained by Parry (1990) and Andrivon *et al*. (2006).

Translating the same approach of relativity to virulence evaluation in hosts under disease challenge. It can be based on the proposition which is supported by Wolpert (2011), where it is argued that any living organism initiates its processes in growth or in performance from a low potential/value or a unit value. This can be from a single cell, a few undifferentiated countable cells or a simple undefined system for example, a pollen fusing with the ovule towards embryo formation. The former two exhibit a simple structure in terms of the number of cells forming them relative to the embryo they end up forming. Hence, their fusion leads to a multicellular unit whose potential is then triggered when the conditions are right for growth and development into a complex system with enormous potential from it's initial levels in terms of; number of cells, size and performance. Also, an organism can be composed of many cells but with zero potential in growth for example, a seed before germination and development into a plant has zero potential (a state of no growth or cell multiplication) in growth (Wolpert, 2011). As a result, this can be equated to absolute value one (the one seed) before any growth which is equal to zero potential (the logarithm value of one (1) which is zero). Therefore, using this argument the productive/growth potential of a germplasm under pathogen inoculation or a particular stressor versus the germplasm productive/growth potential under uninoculation conditions can be estimated using logarithmic relativity a concept that has been used by Hunt *et al*. (2002) to extensively study plant growth dynamics.

2.6.1.1 Methods of estimating host plant resistance and tolerance

In literature there is limited techniques and strategies to quantitatively estimate tolerance levels of napier grass. The situation is worse with fodder crops especially in their disease management, since in the past they have been grown without serious diseases (Farrel *et al*. 2002b; Mwendia *et al*., 2014; Kabirizi *et al*., 2015; Negawo *et al*., 2017). Despite, the limited research on napier grass, Kawube *et al*.(2014) reported the resistance levels of different napier grass varities which were being screened for resistance against NSD pathogen (‘*Candidatus* Phytoplasma oryzae’). In their method they used descriptive scoring strategy basing on visual symptoms whose means were subjected to Zouzou *et al*.(2008) formula to determine the impact of the disease in percentage, and rate the levels of resistance/tolerance of the various napier grass varieties. This method by Kawube *et al*.(2008) though easy to use, it is prone to errors which arise from differences witnessed among individual raters visual biases. Thus, they end up estimating wrongly since the method is qualitative leading to erroneous evaluation whose output is not consistent and accurate (Reese and Schwenke, 1994; Bock *et al*., 2010; Mutka and Bart, 2015). Moreover, the technique does not integrate different parameters of the napier grass to provide an holistic evaluation. Considering the fodder crop is very unstable in performance under different localities, seasons, stages of growth and management practices applied (Turano *et al*., 2016; Negawo *et al*., 2017).

The same challenges are observed on the method of estimating tolerance of plants using a stress tolerance index (STI) proposed by Fernandez (1992). This method was used by Khayatnezhad and Gholamin (2012) in estimating tolerance levels of maize cultivars against abiotic stressors. The possible use of logarithmic efficacy indices was illustrated by Parry (1990), where the researcher described a logarithmic relativity strategy of determining treatments’ efficacy indices under chemical control strategy. In the description the plants treated with a certain chemical were compared to their untreated controls performance to determine their efficacy levels using logarithmic relativity. However, this strategy did not determine the magnitude in percentage of such indices nor their efficacy indices relative to unit value (1) whose logarithmic value is zero (Thomas, 1998; Umbarger, 2006). The strategy did not also integrate several parameters of the plants under evaluation considering their responses to pathogen attack is very variable and complex to be evaluated by a single parameter, due to the

host plant being influenced by many interacting factors viz; their genotype and environment (John, 1998; Francl, 2001)

Further, Reese and Schwenke (1994) reported a general limitation observed across the methods utilized in tolerance evaluation; where they all utilize a single parameter of plants to estimate tolerance levels without considering it is a product of many factors interacting within and without the plant system (Zhu *et al*., 1996). Another, limitation observed on the methods described by Reese and Schwenke (1994) review, was the inability of the techniques to incorporate the relativeness in performance of a plant to its control using logarithmic functions, instead the techniques utilized absolute values. Thus, logarithmic functions unlike absolute values' functions, are precise and accurate in unearthing the performance of a plant relative to its previous levels, as it was demonstrated by Causton and Venus (1981), Hunt (1982) and Hunt *et al*. (2002). Thus, tolerance index determination methods used in evaluation of plant tolerance to aphids as described by Dixon *et al*.(1990) and Robinson *et al*. (1991), functional plant loss index by Morgan *et al*.(1980), weight loss index by Bramel-cox *et al*.(1986) and Formush *et al*.(1992) exhibit this weakness of utilizing absolute values functions which bias in the estimation of tolerance efficacy indices, besides not integrating many parameters of a plant, cognizant of the fact that host pathogen interactions is a very complex relationship that affects directly or indirectly many aspects of the plant (John, 1998; Francl, 2001; Keane, 2012; Surico, 2013). Thus, the more parameters involved the closer the estimation to the true mean of the levels of host plant resistance. According to Reese and Schwenke (1994), most of the techniques used exhibit a lot of error either by being too descriptive; where they use visual scoring of damage on a plant to estimate tolerance or the measurement does not link the effect of damage on the biomass produced by the plant in estimating tolerance levels. Hence, the current studied technique managed to factor those aspects, which as a result led to the quantitative estimation of the host plant tolerance of the napier grass varieties evaluated.

2.6.2 Cultural practices methods

Several cultural practices like; uprooting of diseased plants then burying or burning them, crop rotation, regular manuring, moisture retaining techniques like tumbukiza and weeding e.t.c., are targeted to reduce the amount of initial pathogen (X_o) inoculum (Zillinsky, 1987; Farrell *et al*., 2002a). These cultural practices are used to complement the host plant resistance tactic in an

integrated pathogen management approach (Kabirizi *et al*., 2015). This is because no single method is totally satisfactory (Parry, 1990). However, most farmers often ignore the burning bit as some bury and others use it as mulch or throw it by the road side (Mwendia *et al*., 2007). Moreover, use of manure from animals not fed to smutted heads has been encouraged to limit ustilospores spread via the manure. Also, regular fertilizer and manure application on the crop to enhance its health which subsequently boosts its resistance is also encouraged (Mwendia, 2007; McMahon, 2012; NAFIS, 2012; Kabirizi *et al*., 2015). However, under co-infection the role of respective nutrients towards co-resistance is gap in knowledge (Kabirizi *et al*., 2015). Finally, warm water treatment of the seed canes at 50^{o}C for 30 minutes before planting, as used in sugarcane smut management is a potential strategy that needs focus (Nike and James, 2006).

Limitations to the use of this tactic entail the refusal by farmers to adopt new cultivation or cultural practices in their farms (Sherwood *et al*., 1998). Thus, reducing the positive impact of the cultural practice in the long run. Also, according to Ragsdale and Sisler (1994), some of these cultural practices have been observed to encourage the development of other problems besides their control of the specific diseases like; development of natural reservoirs of the diseases due to poor dumping of uprooted diseased plants, use of manure with ustilopores in it as a result spreading the disease and soil pH alterations incase of excessive inorganic fertilizer use e.t.c. Hence, in the long run they create a new challenge that needs some attention. Moreover, some practices like fertilization despite having beneficial effects on the crop they do not eliminate the pathogen (Orodho, 2006). Hence, the chances of the plant being overwhelmed by the pathogen still do exist in such a scenario.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The bioassay study was conducted under quarantine conditions at the International Centre for Insect Physiology and Ecology-Mbita screen houses, since the centre provided the appropriate quarantine facilities of the two diseases that do not co-occur in the two Kenyan regions. ICIPE-Mbita is located on latitudes (0^o 25S & 0^o 30S) and longitudes (34^o 10'E & 34^o 15'E). The *Ustilago kamerunensis* isolates were collected from Rift-Valley and Central Kenya hot spot areas basing on the geographic position coordinates and altitude (Table 3.1), through the guidance of KALRO-Muguga south experts.

Table 3.1: The eighteen *Ustilago kamerunensis* isolates from different affected counties' hot spot areas of Central Kenya. Showing their respective co-ordinates and altitude of collection.

				Collection Point Co-ordinates and Altitude		
No.	***Ustilago kamerunensis* Isolates (GenBank codes)**	**Synonyms/ Short forms**	**County of Origin**	**Latitude**	**Longitude**	**Altitude**
1.	MUR001	MUR-1	Murang'a	S 00.81380^o	E 037.03799^o	1931m
2.	MUR002	MUR-2	Murang'a	S 00.72882^o	E 036.87831^o	2102m
3.	MUR003	MUR-3	Murang'a	S 00.68270^o	E 036.90532^o	1935m
4.	KIA001	KIA-1	Kiambu	S 01.18049^o	E 036.64774^o	2157m
5.	KIA002	KIA-2	Kiambu	S 01.17762^o	E 036.74564^o	1928m
6.	KIA003	KIA-3	Kiambu	S 01.08774^o	E 036.78181^o	1871m
7.	NYA001	NYA-1	Nyandarua	S 00.49860^o	E 036.48170^o	2240m
8.	NYA002	NYA-2	Nyandarua	S 00.40371^o	E 036.49390^o	2440m
9.	NYA003	NYA-3	Nyandarua	S 00.87156^o	E 036.57031^o	2698m
10.	NAK001	NAK-1	Nakuru	N 00.00876^o	E 036.25268^o	2063m
11.	NAK002	NAK-2	Nakuru	S 00.02934^o	E 036.20500^o	2268m
12.	NAK003	NAK-3	Nakuru	S 00.17176^o	E 036.12392^o	1929m
13.	KIR001	KIR-1	Kirinyaga	S 00.47394^o	E 037.22716^o	1699m
14.	KIR002	KIR-2	Kirinyaga	S 00.37333^o	E 037.30536^o	1693m
15.	KIR009	KIR-9	Kirinyaga	S 00.54285^o	E 037.30877^o	1344m

16.	NYE001	NYE-1	Nyeri	S 00.58910^{o}	E 036.95040^{o}	1817m
17.	NYE002	NYE-2	Nyeri	S 00.46589^{o}	E 036.94195^{o}	1844m
18.	NYE004	NYE- 4	Nyeri	S 00.37537^{o}	E 036.93737^{o}	1828m

3.2 Objective one; morphological and molecular characterization of *Ustilago kamerunensis* isolates collected from affected counties in Kenya

3.2.1 Collection of *Ustilago kamerunensis* isolates from affected regions in Kenya

A modified purposive sampling strategy as per Kainyu (2014) was carried out across the six napier head smut hot spots counties' viz; Kirinyaga, Nyandarua, Nyeri, Kiambu, Murang'a and Nakuru as reported by Lukuyu *et al*. (2012) and Kabirizi *et al*. (2015). This napier head smut hot spot areas within each county were identified basing on geographic position coordinates (Table 3.1), and sampled through the guidance of KALRO- Muguga south experts. Thus, in a respective field over 22 kilometres apart within each county a smutted napier bush was selected at the mid-point of a x-shaped transect stretching between opposite ends of the field within a range of 0.5 to 5 metres radius depending on the size of the fields. The isolates of napier head smut ustilospores were collected from the individual bush to limit collection of mixed isotypes in case of multiple isotypes infection of an individual field for molecular characterization as per a modified approach of Kainyu (2014). The collected isolates were given names starting with the three initials of the county and a number which signified the percentage of smutted napier grass stools within a particular sampled farm. For instance (001 or 1) mean't the isolate was collected from a field whose smutted napier grass stools incidence was ≥ 90%, (002 or 2) ≥ 80%, (003 or 3) ≥ 70%, (004 or 4) ≥ 60%, (005 or 5) ≥ 50%, (006 or 6) ≥ 40%, (007 or 7) ≥ 30%, (008 or 8) ≥ 20%, (009 or 9) ≥ 10% and (010 or 10) ≥ 0%. Three different isolates were collected from each county in terms of the isolate number assigned. The geographic position coordinates and altitude of the area was recorded using etrex garmin geographic positioning system tool to aid mapping of the isolates using ArcMap application of the ArcGIS. The spores were collected by cutting the smutted heads using a pair of scissors and putting them in pollination bags which were shaken manually to remove the spores from the inflorescence (Omayio *et al*., 2015). These materials were placed in pollination bags, placed in a cool, dry cointainer and taken to the laboratory for storage at a cool dry place at 25 ^{o}C awaiting *in vitro* culture.

3.2.2 *In - vitro* culturing of the *Ustilago kamerunensis* isolates' ustilospores

The respective head smut isolates' ustilospores were cultured on 10 ml petri dishes containing sterilized oxoid malt extract agar at 121^{o}C for 15 minutes as used by Farrell *et al*.(2001) and Sharma and Pandy (2010). This media was treated with 10 ml lactophenol per litre during preparation to inhibit bacterial growth. A 10µl volume pre-standardized pathogen spore inoculum concentration of 5×10^{6} spores ml^{-1} as described by Kinyua (2004), was spot inoculated at the centre of each plate under a lamina air flow chamber (Andrea *et al*., 2005). The inoculations for each isolate was replicated 10 times in a completely randomized design. The inoculated plates were then incubated at 25^{o}C after sealing them using a parafilm in a dark area. After, 4 days of culture which is the minimum recommended culture period for fungal microorganisms at 25^{o}C (Dubey, 2006). The colony growth average diameter was determined at this point before it fully colonized the petri plate, to aid in assessing the vigor in growth *in vitro* of the isolates. The *in vitro* culture of the isolates was repeated twice to validate the outcome. The isolates were sub-cultured to obtain pure cultures of the head smut isolates that exhibited; top white floccose and reverse pale cream colonies. The colonies were used in the extraction of their genomic DNA for sequencing and subsequent phylogenetic analysis. Also, this culture experiment was used to purposively select the *U. kamerunensis* isolates NAK-2 and NYA-2, that had the highest and least vigours in growth respectively. The selected isolates were used as a case study in objective two, three and four, under co-infection with '*Candidatus* Phytoplasma oryzae' strain Mbita 1 under varying nutrients and moisture stress. It was assumed that the isolates with the highest and least vigour in growth will provide general indications on how the pathogenicity potential of the different isolates is likely to differ at the field level due to gene to gene interactions (Staskawicz, 2001; Schirawski *et al*., 2010).

3.2.3 *Ustilago kamerunensis* isolates genomic DNA extraction and amplification

Total DNA was extracted from the respective *Ustilago kamerunensis* isolates' colonies using a modified Bioline® Isolate II Genomic DNA extraction kit as described by Omayio *et al*. (2014a). Towards the extraction lysis buffer G3, wash buffer GW2 and proteinase K was prepared as per the kit manual directions. A 75 mg of the respective fungal colonies was thoroughly ground using a different mortar and pestle for each isolate. Due to the unique fungal structure 0.5 ml extraction buffer was added to enhance extraction. The extract was resuspended

in 180 µl lysis buffer GL and 25 µl proteinase K solution and vortexed vigorously. The mixture was incubated at 56°C for 1 hour 30 minutes. The samples were lysed by vortexing briefly and adding 200 µl lysis buffer G3 then vortexed vigorously and incubated at 70°C for 10 minutes. After the incubation the extracts were briefly vortexed and 200 µl of ethanol (96-100%) was added to the sample followed by a vigorous vortexing. Each sample was placed in ISOLATE II Genomic DNA spin column into a collection tube. The entire sample was added to the column and centrifuged for 1 minute at 11,000 gravity to bind the total DNA. The flow through was discarded and each of the collection tube reused as per the kit instructions. The centrifugation was repeated at a higher gravity force for those whose samples had not completely filtered through the matrix. Then 500 µl wash buffer GW1 was added and centrifuged for 1 minute at 11,000 gravity. The flow through was discarded and the collection tube reused. This was followed by addition of 600 µl wash buffer GW2 to the column and centrifugation for 1 minute at 11,000 gravity. The flow through was discarded and collection tube reused. The resultant product was centrifuged at 11,000 gravity to remove residual ethanol and placed the ISOLATE II Genomic DNA spin column in a 1.5 ml micro-centrifuge tube. Finally, the DNA was eluted by adding 30 µl of preheated elution buffer G at 70°C directly onto silica membrane and incubated at room temperature for 1 minute. The samples were centrifuged at 11,000 gravity for 1 minute then repeated by repassing the 30 µl through the silica membrane; centrifuging again before finally topping up the final volume to 60 µl to ensure the limited *Ustilago kamerunensis* DNA was not diluted but concentrated.

3.2.3.1 Amplification of *Ustilago kamerunensis* isolates genomic DNA

The isolated DNA samples with a negative control (no DNA sample put in this treatment but other PCR reaction reagents are used) was amplified using a modified methodology of Rosete *et al*. (2009). The ITS1-ITS4 primer pair sequences (Table 3.2), were used to amplify the intervening 5.8S rDNA, and the adjacent ITS1 and ITS2 regions. PCR amplification was performed with a volume of 50 µl. Two microliters of each sample was added to the PCR master mixture, which consisted of 5 µl of 10× PCR buffer, 4 µl of a deoxynucleoside triphosphate mixture (0.1 mM each dNTP), 0.8 µl of each primer (40 pmol of each primer), and 0.4 µl (2.0 U) of Ex*Taq* DNA polymerase (Takara Biomedicals, Osaka, Japan), with the remaining volume consisting of distilled water. Amplification consisted of an initial denaturation at 94°C for 4

minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 4 minutes; a GeneAmp PCR system 9600 thermal cycler (Perkin-Elmer Corp., Emeryville, Calif.) was used. Negative control reactions without any template DNA were carried out simultaneously. Amplified products were separated on 1.5% agarose gels in 1× TBE buffer at $10Vcm^{-1}$ for 30 minutes. Amplification products were stained with ethidium bromide and observed with a BioRad UV transilluminator. After the gel was photographed, the bands were located by using UV lamp, cut out and placed in a 2ml eppendorf. The PCR fragments were extracted from the gel using Qiagen Gel extraction kit protocol.

Table 3.2: Primer pairs used to amplify the *Ustilago kamerunensis* isolates genomes

Primer type	Primer sequences
Internal Transcribed Spacer Primers	ITS 1; 5'-TCCGTAGGTGAACCTGCGG-3' ITS 4; 5'-TCCTCCGCTTATTGATATGC-3'

Source: Korabecna (2007)

3.2.4 Sequencing and phylogenetic analysis of the *U. kamerunensis* amplified DNA

Sequencing and phylogenetic analysis was done using the *Ustilago kamerunensis* isolates' polymerase chain reaction products according to modified methodology of Kainyu (2014). The products exhibiting the clear bands under the UV trans-illuminator (Appendix 7) were purified for sequencing using Qiagen kit as per the manufacturer's protocol (QIAGEN Inc., Valencia, CA). Five volumes of binding buffer (BB) was added to one volume of PCR products (100µl to 20µl) and transferred to Qia-quick column in provided 2 ml collection tube. The samples were introduced into the column and spinned for 1 minute. The flow through was discarded and the column returned back in the same tube. A 0.7ml wash buffer (PE) was added to the Qia-quick column and spinned for 1 minute at 13000 rpm. The flow through was discarded and placed back to the column in the collection tubes. A short spin was performed to remove residual wash buffer. The columns were placed in clean 1.5 ml microcentrifuge tube, 30 µl elution buffer (buffer EB) or molecular grade water (pH7) was added to elute DNA and spinned for 1 minute at 13000 rpm. The eluted DNA was used for sequencing at Bioneer laboratory, South Korea. Sequencing reactions were performed using the BigDye Terminator v3.1 sequencing Kit (Applied Biosystems, USA) with the primers ITS1-F, and ITS4-R. A 12 µl of (4µl ss DNA, 2 µg, 4 µl, 0.8 µM primer, 2 µl 10× MOPS buffer and 2 µl 10× Mn[2+] isocitrate buffer) was

added in 1.5 ml microcentrifuge tube, incubated at 65-70^{o}C for 5 minutes to denature DNA and allow primers anneal. The reaction was allowed to cool at room temperature for 15 minutes, and briefly centrifuged to reclaim condensation. To each reaction, 22 µl (7 µl ABI terminator mix (401489), 2 µl diluted Sequenase [TM] (3.25 U/µl), and 1µl 2 mM a-S dNTPs) was added and incubated for 10 minutes at 37 ^{o}C before 20 µl 9.5 M ammonium acetate and 100 µl 95% ethanol was added and vortexed. It was centrifuged again for 15 minutes, and carefully the supernatant decanted. DNA was precipitated in ice-water bath for 10 minutes, centrifuged for 5 minutes at 12000 rpm in a microcentrifuge at 40 ^{o}C and supernatant carefully decanted and rinsed in 300 µl of 70-80% ethanol. The DNA was dried for 5-10 minutes in the Speedy-Vac. Thermal-cycling Conditions included 60 ^{o}C for 30 minutes and holding at 40 ^{o}C. Sequenced products were analyzed in an automatic sequencer, ABI3730XL (Applied Biosystems, USA).

The obtained sequences from the amplified internal transcribed spacer regions were edited in chromas lite to remove the ambiguous bases. They were subjected to BioEdit version 7 to generate consensus sequences from the forward and reverse primer fragments (Hall, 1999). The in-house python script was used on the non-nucleotide characters from the fasta sequences (Appendix 43), before the sequences were submitted to National Center of Biotechnology Information (NCBI) GenBank. The nucleotide alignment was performed by CLUSTAL W that was implemented in BioEdit version 7 upon trimming the ends (Hompson *et al*., 1994; Jeanmougin *et al*., 1998). The gaps in the alignment were deleted by online program Gap Strip/Squeeze version 2.1.0 only allowing 20% gap tolerance. The alignment file was loaded in MEGA 7 where evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and selecting the topology with superior log likelihood value. The analysis involved 17 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd and non-coding. All positions containing gaps and missing data were eliminated. There were a total of 252 positions in the final dataset (Kumar *et al*., 2016). In estimating evolutionary divergence between Sequences, analyses were conducted using the Jukes-Cantor model (Jukes and Cantor, 1969). The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 17 nucleotide sequences and codon positions included were 1st, 2nd, 3rd and non-coding. All

positions containing gaps and missing data were eliminated. There were a total of 252 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Tamura *et al*., 2007; Kumar *et al*., 2016).

3.3 Objective two; pathogenicity levels determination of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 on selected napier grass varieties under varying nutrients and moisture levels

3.3.1 Experimental design of the pathogenicity levels evaluation bioassays

A factorial experiment (6×4×4×2) in completely randomized design was set up in the ICIPE-Mbita glasshouses. The factors studied were; (i.) Inoculation/infection states at 6 levels namely; (NYA002 (*NYA-2) Ustilago kamerunensis isolate + NAK002 (NAK-2) Ustilago kamerunensis isolate + Napier stunt pathogen*)-inoculation, (*NYA002* (*NYA-2) Ustilago kamerunensis isolate + NAK002 (NAK-2 Ustilago kamerunensis isolate*)-inoculation, (*NAK002* (*NAK-2) Ustilago kamerunensis isolate only*)-inoculation, (*NYA002* (*NYA-2) Ustilago kamerunensis isolate only*)-inoculation, (*napier stunt pathogen (NSD) only*)-inoculation and (*uninoculated Control*) treatments. (ii.) Nutrients formulations at 4 levels which were; *N-deficiency(N-D), P-deficiency(P-D), N&P-deficiency(N/P-D)* [as a negative control] and *Complete Nutrient Solution(CNS)* [as a positive control]). (iii.) Napier grass accessions at 4 levels namely; *16789, KK 1* (*Kakamega 1), KK 2* (*Kakamega 2)* and *Bana*. (iv.) Watering regimes at 2 levels which were; daily (D) and weekly (W) watering. This gave a total of 192 treatments which were replicated six times to give a total of 1152 experimental units. The accession 16789 and Kakamega 1 variety were used in this study because they had been selected as tolerant to napier stunt and head smut diseases respectively. Whereas, varieties Kakamega 2 and Bana were used as positive controls to napier stunt and head smut respectively, because from previous studies by Wamalwa *et al*. (2015) and Farrell (1998) they had been selected as being susceptible to the respective diseases. The disease free accessions were acquired from KALRO-Muguga germ plasm bank where they were bulked for experimentation.

3.3.2 Co-inoculation of the varieties with the pathogens

Napier grass varieties were inoculated by the head smut pathogen isolates first using a Mwendia *et al*. (2006) method as modified by Omayio *et al*. (2014b). After two weeks of growth the co-inoculated treatments were subjected to napier stunt infection process. Bana variety was used as a positive check to napier head smut since it had been selected among those which were highly susceptible to the disease by Omayio *et al*. (2015).

3.3.3 Napier grass varieties' preparation for *Ustilago kamerunensis* isolates inoculation

The respective varieties canes were cut at three internode length and sheaths removed to expose two live buds at the nodes in all canes that were head smut inoculated (Farrell, 1998). Two head smut inoculated canes of the respective four accessions were planted per pot of 20 cm diameter to give a total of 768 potted plants inclusive of the replications. This was minus the 192 pots that were napier stunt inoculated only and another 192 uninoculated under the different treatments as described in subsection 3.3.1.

3.3.4 *Ustilago kamerunensis* inoculum preparation and standardization

Inoculum was prepared using two purposively selected isolates (*NAK-2* and *NYA-2*) of *Ustilago kamerunensis* ustilospores which were selected based on their *in vitro* growth as described on subsection 3.2.2. The ustilospores had been collected from affected farmers' fields in Murang'a, Kirinyaga, Nyeri, Nyandarua, Nakuru and Kiambu counties as described in subsection 3.2.1. Fifteen grams of the spores were weighed using an electronic balance and put in a plastic bucket containing 10 litres of distilled water and stirred using a glass rod until the spores were mixed with water. The standardization of the inoculum was done by pipetting and a drop placed on haemocytometer which was mounted on a light microscope and viewed at a lower and high magnifications. The spores were counted on a 12 square grids and the mean of each square count obtained. The mean (19.0) was used to calculate the concentration using the formula; $A/4 \times 10^6$ spores per ml where; A denotes the mean from the grids (Kinyua, 2004). The concentration target was 5×10^6 spores/ml as previously used in the screening of Kakamega 1 (Farrell, 1998).

3.3.5 *Ustilago kamerunensis* isolates inoculation method

The method of inoculation integrated both the dipping and injection methods to ensure no escapes. The napier grass accessions to be inoculated were first injected with 1 ml of distilled water mixed with 1.5mg of ustilospores of respective isolate treatment. The head smut injected canes were dipped in the prepared inoculum in plastic buckets for a period of three hours as described in subsection 3.3.3. After 3 hours the canes were removed and conditioned under high humidity overnight in polythene bags for the buds to assimilate the disease causing spores (Farrell, 1998; Omayio *et al*., 2014a).

3.3.6 Planting of *Ustilago kamerunensis* isolates inoculated napier grass and subsequent inoculation with "*Candidatus* Phytoplasma oryzae" strain Mbita 1

The *Ustilago kamerunensis* inoculated canes of the different napier grass varieties were planted in non-sterile forest soil, which was tested for its nutrients profile before planting was done to confirm the nutrient levels. This was to inform on the conditions of the soil used in planting to enable repetition of the study by another independent researcher as described in subsection 3.3.6.1.

3.3.6.1 Testing of the non-sterile forest soil for its nutrient levels before planting the inoculated napier grass varieties canes

The non-sterile forest soil used for planting was collected from the soil surface (0-15 cm from the top soil) of a protected forest field by Kenya Forest Research Institute-Muguga Kiambu county Kenya. Six random replicate samples were obtained from the soil mound for analysis at KALRO-Muguga's soil laboratory, for the organic matter (OM), phosphorus (P) and total nitrogen content as described by Okalebo *et al*. (2002). The soil was sieved through (5 mm aperture sieve) before being thoroughly mixed. To determine the phosphorus levels, 0.3 ± 0.001 grams of oven dried soil at 70^{o}C was weighed for each sample of the soil analyzed into a labelled dry and clean digestion tube. 2.5-ml digestion mixture was added to each tube and the reagent blanks for each batch of the samples. The mixture was digested at 110^{o}C for 1 hour, removed, cooled and three successive 1-ml portions of hydrogen peroxide added. The temperature was raised to 330^{o}C and the heating continued until the solution was colourless. The contents were allowed to cool and 25-ml distilled water added. The contents were mixed well until no more

sediment dissolved. It was allowed to cool and made up to 50 ml with water. The mixture was allowed to settle so that a clear solution could be taken from the top of the tube for analysis. Phosphorus was determined by pipetting 5 ml of the supernatant clear wet-ashed digest solution into a 50-ml volumetric flask for the respective samples along with two blanks as negative control. 20-ml distilled water was added to each flask followed by 10 ml of the ascorbic acid reducing agent, beginning with the standards 0, 1, 2, 3, 4, 5 and 6 ml of the 10 ppm phosphorus working solution into 50 ml volumetric flasks which contained 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm phosphorus respectively. The mixtures were let to stand for 1 hour along with the samples setting to permit full colour development before the absorbances (blue colour) at 880nm wavelength was read in a suitable colorimeter. A graph was plotted of absorbency against standard concentrations. The solution concentrations for each unknown sample were estimated along with the 2 blanks. Finally, the mean blank value was subtracted from the unknowns; this gave a value for the corrected concentration (= c in subsequent calculations). The soil samples phosphorus levels were determined based on the absorbency of a blank sample versus the soil samples', where a phosphorus ppm value was assigned to each sample using the following illustration: For example assuming that the blank = 0.2 ppm and the sample = 4.05 ppm. The corrected phosphorus concentration (c) = 4.05 - 0.20 = 3.85 ppm.

The total nitrogen was determined by a steam distillation apparatus which was set up and NH_3-free distilled water was used where applicable. An aliquot of 10 ml for the respective soil sample solution digest was transferred to the reaction chamber of the still and 10 ml of 1% NaOH added. The mixture was steam-distilled immediately into 5 ml of 1% boric acid containing 4 drops of the mixed indicator. The distillation was continued for 2 minutes from the time the indicator turned green. The distillate was removed and titrated with N/140 HCl, the end point being reached when the indicator changed from green through grey to a definite pink. The amount in ml of the standard HCl used was recorded. Steam was passed through the apparatus for 30 minutes as the steam blank was checked by collecting 50-ml distillate and titrating with N/140 HCl. The nitrogen percentage in the soil was calculated using the following formula as per Okalebo *et al*. (2002):

$$\%\ N\ in\ soil\ sample = \frac{(a-b) \times 0.1 \times v \times 100}{1000 \times w \times al}$$

Where a = volume of the titre HCl for the blank, b = volume of the titre HCl for the sample, v = final volume of the digestion, w = weight of the sample taken and al = aliquot of the solution taken for analysis.

The soil organic matter was determined by taking 10 ± 0.1 grams of soil from the samples that was well mixed in a dry porcelain crucible. The soil was heated slowly in a furnace (raising the temperature setting in steps (100, 200 and 550^{o}C). The final temperature setting of 550 ^{o}C was maintained for 8 hours. The crucible was removed containing a greyish white ash, which was cooled in a desiccator and weighed. The percentage ash and organic matter are calculated by the differences in weight of the crucibles before and after combustion as per Okalebo *et al*.(2002):

$$Ash\ (\%) = \left(\frac{(W3 - W1)}{(W2 - W1)}\right) \times 100$$

Then;

$$Organic\ matter\ (\%)\ = 100 - ash\%$$

Where W1 = the weight of the empty, dry crucible; W2 = the weight of the dry crucible containing soil; and W3 = the weight of the dry crucible containing soil following ignition. Note that the weight of the ash = W3 - W1.

Finally, the soil pH was analyzed using deionized water with a soil to water ratio of 1: 2.5 using a pH meter; to determine the forest soils' contents. The soil mound was thoroughly mixed to evenly distribute the soil components before it was used in the bioassays in ICIPE-Mbita glasshouses.

3.3.6.2 Planting of napier grass varieties canes and co-infection with "*Candidatus* Phytoplasma oryzae" strain Mbita 1

The napier head smut inoculated canes as described in subsection 3.3.5, were planted in plastic pots of 20 cm diameter filled with potting mixture. The potting mixture was modified to comprise the analysed non-sterile forest soil above and gravel at a ratio of 4: 1 respectively without manure. Two canes per pot were planted at an angle with one third of the cane above the soil (Boonman, 1993). The watering was once a day in the evening at 6 p.m under daily watering regime using 100 ml of rain water as used by Ochieno (2010). Whereas under the weekly watering regime it was after seven days using the same amount of water. After two weeks of growth the treatments to be co-inoculated and those to be inoculated with napier stunt only were

set. Bana variety napier plants confirmed to be infected with "*Candidatus* Phytoplasma oryzae" strain Mbita 1, were used as the source of the pathogen inoculum.

The Napier stunt disease vector *M. banda* was obtained from a colony maintained on pearl millet in insect cages (45cm x 45cm x 60cm) at the vector rearing screen house of ICIPE at Mbita point (Plate 3.1). The Napier plants were inoculated using a protocol described by Obura *et al.* (2009) and Wamalwa *et al*. (2015). A diseased plant was placed at the centre of an insect cage (45cm x 45cm x 60cm), surrounded by 6 phytoplasma-free potted plants, hence a total of sixty four cages for the four accessions and their respective treatments. Fifty starved gravid *M. banda* were introduced into each inoculation cage and allowed to feed for 30 days to acquire the phytoplasma from the diseased plant, and transmit it to the stunt-free experimental plants for co-infection to occur. Gravid insects were used to increase the probability of them feeding on the treatments and transmiting the disease. Largely because of their high level feeding behavior towards egg production (Obura *et al*., 2009). Occasionally, the insects were disturbed in the inoculation cages to redistribute the population. After 30 days, the inoculation set up was terminated and the exposed plants left for incubation. Six weeks from this stage the napier grass were harvested as ratoon 1 and the parameters described in subsection 3.3.6.2.3 measured. The harvesting continued to be done on regrowth at a six week interval up to four consecutive ratoons when severities of the diseases were considered to be at their peak (Mulaa *et al*., 2015). The uninoculated pots were used as the control treatments. During the 30 days co-infection period the treatments were not treated with their respective nutrient formulations to allow them exhaust the fertility in the soil used. However, after the period the treatments were applied with respective nutrient formulation prepared as described in subsection 3.3.6.2.1.

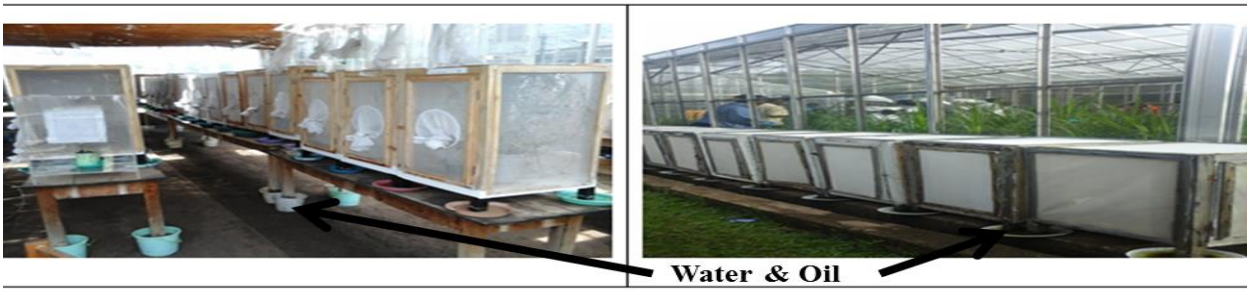


Plate 3.1: The cages used for artificial inoculation of napier grass varieties by napier stunt disease pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) using vector *Maiesta banda.* The water/oil containing plates shown by black arrows on the cages' stands prevents invasion of predators and ants that feed on the vectors and its secretions respectively. (Source: Own work)

3.3.6.2.1 Nutrient formulations applied on the various potted napier grass varieties' under varying pathogen and watering treatments

The factorial experiment described in subsection 3.3.1 tested the effects of 4 levels of nutrient formulations (complete nutrient solution, nitrogen deficiency, phosphorus deficiency and nitrogen-phosphorus deficiency) on the potted napier grass varieties. The four different nutrient formulations simulated plants that were either well nourished or poorly nourished based on the deficiency of one or two nutrients as demonstrated in table 3.3. The chemical compositions of the three nutrient solutions (Table 3.3), that were used in the study were as modified by Ochieno (2010) from Murashige and Skoog (1962).

Table 3.3: Chemical composition of nutrient solutions in milligrams per litre of water

	Nutrient Solution			
Chemical	**Complete Nutrient Solution (CNS*)**	**Nitrogen (N-D) deficient**	**Phosphorus (P-D) deficient**	**Nitrogen & Phosphorus (N/P-D) deficient**
NH_4NO_3	1650	-	1650	-
KNO_3	1900	-	1900	-
KCl	-	1402	93	1495
$CaCl_2.2H_2O$	440	440	440	440
$MgSO_4.7H_2O$	370	370	370	370
KH_2PO_4	170	170	-	-
$NaH_2PO_4.H_2O$	-	-	-	-
KI	0.83	0.83	0.83	0.83
H_3BO_3	6.2	6.2	6.2	6.2
$MnSO_4.4H_2O$	22	22	22	22
$ZnSO_4.7H_2O$	8.6	8.6	8.6	8.6
$Na_2MoO_4.2H_2O$	0.25	0.25	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025	0.025	0.025
$Na_2EDTA.2H_2O$	37.3	37.3	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8

*Complete Nutrient Solution (CNS) as formulated by Murashige and Skoog (1962). The other three solutions were made by omission of N, P or N&P from CNS respectively.

3.3.6.2.2 Potted napier grass varieties growth conditions and application of the nutrient formulations

The potted napier grass varieties were grown under the following screen house conditions (25 ± 3ºC, 70-75 % relative humidity, 12L:12D photoperiod) as used by Ochieno (2010). The containers were perforated at their bases with 6 holes. Plants in respective treatments were well

labeled and completely randomized in the screen house. The potted napier grass varieties' respective treatments were supplied daily with rain water (100 mL) under daily watering regime and after a week under weekly watering regime as described in subsection 3.3.6.2. The respective nutrient solutions were supplied weekly at a volume of 100 mL after the 30 days co-infection with napier stunt disease whose composition is illustrated in table 3.3. This was to allow the napier accessions' treatments exhaust the little phosphorus and nitrogen that was present (Appendix 45) on the planting soil before supplementation with the formulations.

3.3.6.2.3 Napier grass varieties' growth parameters measurement towards quantitative estimation of the pathogens virulence

The following eight parameters idenfified to be affected by the disease were measured viz; leaf number, leaf area (cm^2), tiller number, tiller height (cm), chlorophyl content levels (SPAD units), total stem weight (grams), total leaf weight (grams) and total fresh weight (grams) at intervals of 6 weeks; which constituted a ratoon up to the fourth one (24 weeks) (Kabirizi *et al*., 2015). The leaf and tiller numbers were determined by counting the number of leaves and tillers per pot and the value recorded in data sheets. Leaf area was estimated by multiplying the measured leaf length by width of ten randomly selected mature lower tiller leaves (first leaves from the soil upwards the tiller) and their average was recorded as the leaf area per pot. The chlorophyll content levels in SPAD units was determined using the Chlorophyll meter; where three leaves of a tiller were randomly selected within a potted treatment and their average chlorophyll content levels at ten different points each of the leaves measured. The tiller height of a napier stool in a pot was measured using a tape measure from the base of the soil to the tallest terminal end of the stool. Total fresh weight, total stem weight and total leaf weight parameters were determined using a weighing balance on a 6 week interval by firstly harvesting the crop in each pot by cutting at 1 cm from their base. The total fresh weight in grams per pot was recorded, then leaves removed from the stems for determination of their respective weights. The total of the respective replicates for each treatment combination was recorded.

3.3.7 Determination of pathogenicity levels of the pathogens on the selected napier grass varieties under varying nutrients and moisture levels using the IPLI concept

The determination of pathogenicity levels was preceded by confirmation of the pathogens presence in the inoculated napier grass varieties under the different treatments (Table 3.4), a

dictate of Koch's postulates as an indicator of successful inoculation and pathogen establishment as described (John, 1998).

3.3.7.1 Extraction of total DNA from inoculated napier grass varieties treatments for *Ustilago kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 detection

Six samples per pathogen treatment were obtained randomly from the screening glasshouse at approximately 24 weeks after inoculation (third ratoon harvest) to have a total of thirty six samples under test (Table 3.4). Total DNA was extracted from the napier grass accessions using a modified isolate II genomic DNA extraction kit for Polymerase Chain reaction (PCR) analysis as described by Omayio *et al*. (2014a). Plant leaves were ground in liquid nitrogen using a mortar-pestle and homogenized in extraction buffer (20 ml/g leaf tissue). On the slurry, 100 µl of 20 mg/ml proteinase K was added and incubated for 15 minutes on ice while stirring. The mixture was transferred to a new tube, adjusted to 2% sodium dodecylsulphate and incubated at 65°C for 35 minutes. The homogenate was centrifuged for 10 minutes at 10,000 rpm at room temperature to pellet the cell debris. The supernatant was adjusted to 1.4 M NaCl before adding 0.1 volume of buffer and adjusted to 1% RNase A (20 mg/ml). This was incubated at 65°C for 10 minutes and allowed to cool on ice for 15 minutes. The supernatants were washed twice with an equal volume of chloroform and centrifuged at 10,000 rpm for 10 minutes to further denature plant cell constituents and release nucleic acid. Total nucleic acids were precipitated by addition of 0.8 volume isopropanol followed by incubation on ice for 30 minutes and centrifugation at 10,000 rpm for 20 minutes at 4°C. The final pellets were washed with 70% ethanol, dried at 37°C for 5 minutes and resuspended in appropriate amount of sterile double-distilled water. The DNA samples were amplified using ITS primers (Appendix 44) as described on subsection 3.2.2.1.

Table 3.4: The randomly selected varieties under different treatments for confirmation of the presence or absence of napier head smut and napier stunt pathogens.

The six randomly selected varieties infected by, (NAK-2) isolate from the experiment.
1- Bana + CNS + NAK-2 + W **2-** Bana + N/P-D+ NAK-2 + W **3-** KK1 + P-D + NAK-2 + W **4-**16789 + N-D + NAK-2 + D **5-** KK2 + P-D + NAK-2 + D **6-** KK1 + N-D + NAK-2 + D
The six randomly selected varieties infected by, (NYA-2) isolate from the experiment.
1- 16789 + CNS + NYA-2 + W **2-** KK1 + N/P-D+ NYA-2 + W **3-** Bana + P-D + NYA-2 + W **4-** KK1 + N-D + NYA-2 + D **5-** KK2 + P-D + NYA-2 + D **6-** Bana + N/P-D + NYA-2 + D
The six randomly selected varieties co-infected by, (NAK-2 + NYA-2) isolates from the experiment.
1- KK2 + CNS + NAK-2 + NYA-2 + W **2-** 16789 + N/P-D + NAK-2 + NYA-2 + W **3-** Bana + P-D + NAK-2 + NYA-2 + W **4-** 16789 + P-D + NAK-2 + NYA-2 + D **5-** Bana + N-D + NAK-2 + NYA-2 + D **6-** 16789 + N-D + NAK-2 + NYA-2 + D
The six randomly selected uninoculated varieties from the experiment.
1- KK2 + CNS + UNINOCULATED + W **2-** KK2 + N/P-D + UNINOCULATED + W **3-** KK1 + P-D + UNINOCULATED + D **4-** KK2 + N-D + UNINOCULATED + D **5-** KK1 + N/P-D + UNINOCULATED + D **6-** Bana + N/P-D + UNINOCULATED + W
The six randomly selected varieties infected by, (NSD) from the experiment.
1- KK1 + N/P-D + NSD + W **2-** 16789 + CNS + NSD + W **3-** Bana + P-D + NSD + W **4-** KK1 + N-D + NSD + D **5-** 16789 + N-D + NSD + D **6-** 16789 + P-D + NSD + D
The six randomly selected varieties co-infected by, (NAK-2 + NYA-2 + NSD) from the experiment.
1-KK1 + P-D + NAK-2 + NYA-2 + NSD + D **2-**Bana + N/P-D + NAK-2 + NYA-2 + NSD + D **3-** KK2 + N-D + NAK-2 + NYA-2 + NSD + D **4-** 16789 + N/P-D + NAK-2 + NYA-2 + NSD + W

5- KK2 + P-D + NAK-2 + NYA-2 + NSD + W
6 - Bana + CNS + NAK-2 + NYA-2 + NSD + W

The varieties involved entailed KK1 (Kakamega 1), KK2(Kakamega 2), (Bana) and (16789). The (D) and (W) denote the daily and weekly watering regimes respectively. Whereas, the (CNS),(P-D),(N-D) and (N/P-D) denote the different nutrient formulations described on section 3.3.6.2.1.

3.3.7.1.1 Detection of *Ustilago kamerunensis* in napier grass tissues using ordinary PCR

The presence or absence of head smut pathogen (*Ustilago kamerunensis*) in the inoculated napier varieties, was confirmed using ITS primers through ordinary PCR targeting the fungal pathogen (Omayio *et al*., 2014a). This was done by electrophoresis of polymerase chain reaction product on agarose gel. The method utilized ultraviolet-induced fluorescence emitted by ethidium bromide molecules that are intercalated into the DNA. The electrophoresis buffer (TAE) was prepared by mixing; 0.04 M Tris acetate at pH 8.0 with 1 nM EDTA. The sample buffer entailed 25% Ficoll and 25% Bromophenol blue in 5× TAE, for the analysis of total DNA preparations from plants, standard 1% agarose gels prepared in TAE electrophoresis buffer were used. Agarose powder was added to a TAE buffer (1% w/v) and microwaved for 2 minutes to dissolve the powder. To the cooling solution, 0.005% Ethidium bromide was added to the solution subsequently poured into a tray in which a comb was inserted to form sample slots. The agarose gel was allowed to solidify for approximately 30 minutes before the comb was removed and the gel immersed in the electrophoresis tank containing the TAE buffer. Finally, to 3-10µl of DNA sample, 3 µl of sample buffer was added and the total volume (6-13 µl) loaded into a slot in the gel. A 1Kb DNA ladder (GenScript) was used to aid in identification of the expected band size of about 720 base pairs. The gel was run at 120 volts and maximum current for 45 minutes before being viewed under UV light and photographed. The expected banding of the fungus presence was at 720 base pairs mark to signify its presence.

3.3.7.1.2 Detection of '*Candidatus* Phytoplasma oryzae' strain Mbita 1 in napier grass tissues using the nested PCR

The presence or absence of Napier grass stunt pathogen (NSD) '*Candidatus* Phytoplasma oryzae' strain Mbita 1 in the inoculated napier varieties, was confirmed using nested PCR technique targeting the Ns-phytoplasma as described by Obura (2012). The respective total DNAs extracted from the thirty six napier grass tissues from the different pathogen treatments as described in subsection 3.3.5.2, were subjected to molecular analysis to diagnose for the

phytoplasma using P1/P6 primers, followed by re-amplification with the NapF/NapR primers (Appendix 8), in nested PCR to yield an expected 778 base pair band on the agarose gel as described by Obura (2012). The 16S rRNA sequence target by the primers is shown in appendix 9. The nested PCR reaction mix and PCR conditions used primer pair P1/P6 (Appendix 8), as described by Deng and Hiruki (1991), for the initial amplification cycle. Followed by a nested PCR with NapF/NapR primers developed from the 16S rRNA gene sequence of Ns-phytoplasma. The reaction mixture contained 50 ng of each primer, 125 Mm of each dNTPs, 1U of Taq DNA polymerase (GenScript, USA), 1x PCR buffer with 1.5 mM $MgCl_2$ (GenScript, USA). 0.7 µl of P1/P6 PCR product was used as template in nested PCR. The reaction mixture was adjusted to 25 µl by sterile distilled water. PCR was performed for 35 cycles in a PTC-100 Thermal cycler (MJ Research, Inc.). Reaction conditions were as follows: 2 min at 94 °C, 1 cycle; 1 minute at 94°C, 2 minutes at 52°C (55°C for NapF/NapR), 1 minute at 72°C, 35 cycles; and 10 minutes at 72°C, 1 cycle. The Ready-to-Use™ PCR DNA ladder (GeneScript) was used to aid identification of the expected band size. A confirmed '*Candidatus* Phytoplasma oryzae' Mbita 1 strain infected Bana variety's extracted total DNA was used as a positive control, whereas PCR water was used as negative control. The PCR products were resolved on ethidium bromide stained 1% agarose gel using 1x TAE (40 mM Tris acetate, 1 mM EDTA pH8.0) as running buffer, with the final electropherogram being captured under a UV transilluminator machine BTS-20-M. The DNA template of corresponding uninoculated napier grass varieties under experimentation were also used as control, due to the ability of primers to react probably with sequences of plant genome or dimers leading to potential false positives observation.

3.3.7.2 Pathogenicity levels determination of the pathogens on the selected napier grass varieties under varying nutrients and moisture levels using the IPLI concept

The napier grass accessions reaction to the pathogens virulence under the different treatments was established using a modified approach described by Obura (2012). The modification in this study entailed introduction of a virulence evaluation strategy called IPLI (Integrated Parameter Logarithmic Indexing). The integrated parameter logarithmic indices of the four ratoons combined means were subjected to the modified logarithmic virulence algorithm (Table 3.5). The respective ratoons parameters' combined means of the different treatments which were integrated using natural logarithmic indexing are captured from appendices 11 - 42.

The approach entailed the establishment of the mean logarithmic indices of all the growth parameters' combined means using the natural logarithms across the four cropping cycles/ratoons. This was possible because the natural logarithms aided in disregarding the different parameters' measurement units used, which also aided in harmonization and normalization of the values towards generation of mean logarithmic indices of all the parameters involved (IPMLI). The generated indices were used to determine the deviation (LoD) of each treatment from the mean logarithmic index (IPMLIC) of the four napier grass varieties controls which were uninoculated but under complete nutrient solution and daily watering regime treatment. The use of (IPMLIC) was to standardize the measure of disease virulence estimation instead of relying on individual responses for comparativity purposes, since the effect being estimated is from the pathogen directed towards all the napier grass varieties and not just an individual variety response. Hence , the use of (IPMLIC) which incorporated the mean of uninoculated under CNS and daily watering of the four varieties. This comparative scenario of using the IPMLIC value in *algorithm 1b*, *1c* and *1e* as illustrated on table 3.5, was performed under the assumption that the napier grass under the treatment conditions (uninoculated- disease free, applied with complete nutrient solution and daily watered), were the least stressed from the three stressors which were involved in these experiments namely; disease challenge (*U. kamerunensis* and "*Candidatus* Phytoplasma oryzae"), nutrients deficiency on some nutrient solutions and watering stress especially under weekly watering. The reaction of each accession under the pathogens infection was measured by two parameters: a) disease expression/impact, and b) time taken to express disease. Disease expression was determined using a modified 0-3 integrated rating scale as per Obura (2012) where:

0;High susceptibility = Symptomatic / ≥ 75% Virulence percentage (VIRP)

1;Moderate Susceptibility = Symptomatic / ≥ 50% Virulence percentage (VIRP)

2;Low Susceptibility = No symptoms/symptomatic / ≥ 25% Virulence percentage (VIRP)

3;Very Low Susceptibility = No symptoms/symptomatic/< 25%Virulence percentage(VIRP)

Time taken to express disease was determined in a 1-4 integrated rating scale basing on the number of cuts/harvests witnessed during the experiment as per Obura (2012), where after;

1 cutting / ≥75% VIRP = Very High Virulence/Very High Stress Levels

2 cutting / ≥ 50% VIRP = High Virulence/High Stress Levels

3 cutting / ≥ 25% VIRP = Moderate Virulence/Moderate Stress Levels

4 cutting /no disease symptoms/< 25% VIRP = Low Virulence/Low Stress Levels

The virulence percentage (VIRP) was determined using *algorithm 1d,* which entailed subtracting the overall percentage level (OPL) from 100%. Also, the (VIRP) virulence percentage could be established by multiplying (-1) to (SpD (%)). This is because the specific percentage deviation described the magnitude of deviation from the mean of the controls which is attributed to pathogen measurable degree of damage known as virulence. The virulence describes the level of damage directed towards a host that prevents it from achieving the maximum growth potential relative to unit value, witnessed when the same host is not infected by the pathogen (Surico, 2013). The values (LoD), (IPMLI) and (IPMLIC) were relative logarithmically, where the former (LoD) was a value obtained by comparing the value of (IPMLI) relative to the value of (IPMLIC) using logarithmic indexing with modification but as described by Causton and Venus (1981) and Parry (1990). The latter two (IPMLI) and (IPMLIC) are all relative to absolute unit value (1) whose logarithmic index/value is zero. This is because any logarithmic value of a quotient of any absolute number (X) relative to unit value (1), where (X) is the numerator and one is the denominator. The logarithmic value of the resulting quotient is equal to the logarithmic value of (X) basing on the logarithmic quotient rule (Thomas, 1998; Umbarger, 2006). The point of modification was the integration of parameters to generate a mean logarithmic indices (IPMLI) and (IPMLIC) as highlighted on table 3.5. The virulence levels of the isolates was validated by combining with classical approaches of scoring.

Table 3.5: The modified logarithmic virulence algorithm that was used to determine the levels of virulence of the respective pathogens under the different treatments.Source: Parry (1990).

Algorithm 1a:

(IPMLI); Integrated Parameter Mean Logarithmic Index of the inoculated / uninoculated but stressed

$$\text{i. IPMLI} = \frac{\left(\text{LN}(\text{TFWi} \times \text{TLHi} \times \text{CHi} \times \text{TLNi} \times \text{LFNi} \times \text{TSWi} \times \text{TLWi} \times \text{LAi})\right)}{8}$$

(IPMLIU); Integrated Parameter Mean Logarithmic Index of the Uninoculated / stress free

$$\text{ii. IPMLIU} = \frac{\left(\text{LN}(\text{TFWc} \times \text{TLHc} \times \text{CHc} \times \text{TLNc} \times \text{LFNc} \times \text{TSWc} \times \text{TLWc} \times \text{LAc})\right)}{8}$$

(IPMLIC); Integrated Parameter Mean Logarithmic Index of the Controls of the four varieties

$$\text{iii. IPMLIC} = \frac{\left((\text{KK1}\ \ IPMLIU) + (\text{KK2}\ \ IPMLIU)\ + (\text{Bana}\ \ IPMLIU) + (16789\ IPMLIU)\right)}{4}$$

Algorithm 1b:

(OPL (%)) Overall Percentage Levels;

$$\text{OPL}\ (\%) = \left(\frac{\text{IPMLI}}{\text{IPMLIC}}\right) \times 100\%$$

Algorithm 1c:

(LoD) Logarithmic Deviation;

$$\text{LoD} = \text{IPMLI} - \text{IPMLIC}$$

Algorithm 1d:

(VIRP) Virulence percentage;

$$\text{VIRP}(\%) = 100\% - \text{OPL}$$

Algorithm 1e:

$(SpD)\ Specific\ Percentage\ Deviation;$

$$\text{SpD}\ (\%) = \left(\frac{\text{LoD}}{\text{IPMLIC}}\right) \times 100\%$$

Where: (IPMLI) was the integrated parameters mean logarithmic index of the inoculated/ uninoculated but stressed treatments; (IPMLIU) was the integrated parameters mean logarithmic index of the uninoculated control/ stress free, of each individual napier grass variety under complete nutrient solution and daily watering treatment. (IPMLIC) was the integrated parameters mean logarithmic index of the controls of the four varieties combined (that is the mean of the IPMLIU values of the four napier grass varieties used. Hence, the division by (4) since they were four IPMLIU values). The (OPL) overall percentage levels described the percentage performance of a treatment based on its integrated parameter mean logarithmic index of all the parameters measured out of the integrated parameter mean logarithmic index of the controls used in the experiment for standardization purposes. The (LoD) determined the magnitude of deviation from the mean of the controls. The value (SpD(%)) if multiplied by (-1) could also generate the

magnitude of virulence in percentage (VIRP). The abbreviations for the measured parameters entailed; (TFWc), (TLHc), (CHc), (TLNc), (LFNc), (TSWc), (TLWc) and (LAc). They denoted the means of all the four ratoons combined of the total fresh weight, tiller height, chlorophyll, tiller number, leaf number, total stem weight, total leaf weight and leaf area respectively of the controls (which were uninoculated under daily watering regime and complete nutrient solution). Whereas; (TFWi), (TLHi), (CHi), (TLNi), (LFNi), (TSWi), (TLWi) and (LAi) denoted the means of all the four ratoons combined of total fresh weight, tiller height, chlorophyll, tiller number, leaf number, total stem weight, total leaf weight and leaf area respectively of the treatments which were inoculated with pathogen or uninoculated but under some form of stressor like; limited water (weekly watering regime) or lack key nutrients by being provided with a nutrient solution that lacks one or two nutrients. The division by factor 8 on *algorithm 1a* was to obtain a mean logarithmic index of the eight parameters involved. The control used involved the means of the four varieties indices under uninoculation, daily watering and complete nutrient solution treatment combinations to standardize the evaluation of virulence.

3.4 Objective three; the co-infection effects of the *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 on the growth of napier grass varieties as influenced by varying nutrients and moisture levels

The evaluation of the co-infection effects on the growth of napier grass varieties (KK 1, KK 2, 16789 & Bana) in different treatment combinations by selected *U. kamerunensis* isolates (NAK002 & NYA002) and '*Candidatus* Phytoplasma oryzae' strain Mbita 1, was achieved by analysis of the parameters that were significantly affected by the diseases viz; tiller height, chlorophyl content levels and total fresh weight as measured in subsection 3.3.6.2.3. The analysis provided insights on how the co-infection affected the general growth of the varieties.

3.5 Objective four; determination of the levels of tolerance of selected napier grass varieties against *Ustilago kamerunensis* isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 under varying nutrients and moisture levels

The levels of host plant tolerance of the infected napier grass varieties against Ustilago kamerunensis isolates and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 under varying nutrients and moisture levels, was determined using a modified model as per Parry (1990) and Hunt *et al*.(2002). The significantly affected parameters of napier grass varieties by the diseases were used in the model (Table 3.6). The parameters entailed; the mean total fresh weight, mean tiller height and mean chlorophyll content levels across all the ratoons measured as explained in subsection 3.3.6.2.3. The measured means of these significantly affected parameters (Appendices 11-42), were introduced into the modified model (Table 3.6). This estimated each variety's mean efficacy levels (M.E.I), which was the index that estimated the performance of the varieties'

infected treatments relative to their uninoculated controls efficacy levels (M.E.I) and unit value (1) which was identified as mean logarithmic index (M.L.I) as illustrated in table 3.6. All the ratoons studied were factored because the diseases severity is observed to heighten through subsequent cuttings and the highest levels of damage is observed at ratoon 4 as reported by Mulaa *et al*. (2015).

3.5.1 Rationale behind the indices M.E. I (magnitude efficacy index) and M.L.I (mean logarithmic index)

The first index M.E.I; (magnitude efficacy index) generated by *algorithm 2a, 2b,* and *2c* (Table 3.6); described the napier grass varieties' magnitudes of change from their respective normal/control. The normal or control was their respective uninoculated control under daily watering and complete nutrient solution (CNS) treatment, upon infection by the pathogens. The second index M.L.I; (mean logarithmic index) was generated by computing the mean of the respective natural logarithm of selected parameters affected by the diseases significantly. The (M.L.I) indices' of the different treatments were determined relative to unit value (1) whose logarithmic index is zero which was equated to a zero potential/level. This was based on the publication by Wolpert (2011). The report stated that an organism before initiation of growth and development exhibits zero potential in that aspect. The potential is unlocked upon exposure to suitable environmental conditions that favour growth. However, basing on the interaction of the varying plant genotypes and the environmental conditions which in this case comprised; pathogens, nutrient application and varying water availability, a living organism ends up exhibiting different levels or potentials in growth vigour. Thus, if this vigour could be estimated using relativity to unit value (1) whose logarithmic value is zero, then the impact of the environmental exposure can be estimated and the plant's tolerance levels against the exposure determined. As a result, this led to the M.L.I index that estimated the magnitude of change of the varieties performance from zero potential to maximum potential of growth based on the logarithmic quotient rule where the denominator is unit value (1). The rule states that any logarithmic value of a quotient of any absolute number (A) relative to unit value (1), where (A) is the numerator and one is the denominator. The logarithmic value of the resulting quotient is equal to the logarithmic value of (A) (Thomas, 1998; Umbarger, 2006).

Therefore, using this argument the growth potential of a germplasm under pathogen inoculation or a particular stressor versus the germplasm productive/growth potential under uninoculation

conditions was estimated using logarithmic relativity a concept that was used by Hunt *et al*., (2002) to extensively study plant growth dynamics. The logarithm value zero was equated to a zero potential of an organism before any active response to an input. This was possible because efficacy indices generated by *algorithm 2a,2b,2c* and *2d* were a product of relativity where the standard performance; that is the outputs of napier grass varieties when not diseased was compared with the performance of its diseased treatment's using natural logarithms. Thus, the mean percentages (mean %) of the respective corresponding logarithmic percentages of the M.E.I and M.L.I indices of each treatment were determined from a developed *Omatec natural logarithmic indices' and their corresponding percentages table* as described in subsection 3.5.2, were used to estimate the mean corresponding logarithmic percentage levels of tolerance but unstandardized at this level as illustrated in table 3.6.

Table 3.6: The modified models from Parry (1990) and Hunt *et al*.(2002) used to estimate the mean logarithmic indices and eventually their corresponding logarithmic percentages (mean %) from 'Omatec natural logarithmic indices' and corresponding percentages table' towards quantitative estimation and customization of host plant tolerance magnitude levels for napier grass varieties.

Algorithm 2a:

(L. I. I or L. I. U. S); Logarithmic Index Inoculated or Logarithmic Index Uninoculated Stressed

$$= \frac{(\mathrm{LN}(\mathrm{TFWi1} \times \mathrm{TFWi2} \times \mathrm{TFWi3} \times \mathrm{TFWi4} \times \mathrm{TLHi1} \times \mathrm{TLHi2} \times \mathrm{TLHi3} \times \mathrm{TLHi4} \times \mathrm{CHi1} \times \mathrm{CHi2} \times \mathrm{CHi3} \times \mathrm{CHi4}))}{12}$$

Algorithm 2b:

(L. I. U); Logarithmic Index Uninoculated (uninoculated + daily watering + CNS treatment)

$$= \frac{(\mathrm{LN}(\mathrm{TFWc1} \times \mathrm{TFWc2} \times \mathrm{TFWc3} \times \mathrm{TFWc4} \times \mathrm{TLHc1} \times \mathrm{TLHc2} \times \mathrm{TLHc3} \times \mathrm{TLHc4} \times \mathrm{CHc1} \times \mathrm{CHc2} \times \mathrm{CHc3} \times \mathrm{CHc4}))}{12}$$

Algorithm 2c:

(M.E.I); Magnitude Efficacy Index

$$\mathrm{M.E.I} = \text{Logarithmic Index Inoculated/stressed (L. I. I or L. I. U. S)} - \text{Logarithmic Index uninoculated (L. I. U)}$$

Algorithm 2d:

Mean Corresponding Logarithmic Percentage (Mean %)

$$(\text{Mean \%}) = \frac{(\text{M. E. I corresponding logarithmic \%}) + (\text{M. L. I corresponding logarithmic \%})}{2}$$

Where: (LN) was the natural logarithm of a respective varieties' mean total fresh weight in grams denoted as (TFWi1), (TFWi2), (TFWi3), and (TFWi4) of a respective inoculated/stressed treatment performance as at ratoon 1, 2 3 and 4 respectively. The resulting output was added to the other parameters outputs and the average determined by dividing by twelve (12), since they were twelve parameter values.The components (TFWc1), (TFWc2), (TFWc3), and (TFWc4) described the uninoculated treatment mean performance of total fresh weight as at ratoon 1, 2, 3 and 4. The resulting output was added to the other parameters outputs and the average also determined by dividing by twelve (12). The components (TLHi1), (TLHi2), (TLHi3), and (TLHi4) versus (TLHc1), (TLHc2), (TLHc3), and (TLHc4) described the mean tiller heights in (cm) performance of the inoculated/stressed and uninoculated treatments of ratoons 1,2,3 and 4 as labeled respectively. The values (CHc1), (CHc2), (CHc3), and (CHc4) to (CHi1), (CHi2), (CHi3), and (CHi4) described the chlorophyll levels performance of the uninoculated and inoculated/stressed treatments of ratoons 1, 2, 3 and 4 as labeled respectively. The modified logarithmic infection rate algorithm estimated the magnitude efficacy index (M.E.I) for the accessions regardless whether there was a biomass decrease or increase without introducing a math error scenario due to the defined function's range to domain limitation. A problem which could have been encountered if it was to be estimated as it had been described by Parry (1990) in such a case scenario. Its important to note that the uninoculated treatment used for respective napier variety was the one which was subjected to daily watering and complete nutrient solution treatment as it was assumed it experienced the least stress interactions. It is important to note that

the (L.I.I), (L.I.U.S) and (L.I.U) are sub-types of M.L.I (mean logarithmic index). The sub-types arise because of the different treatment conditions the napier grass varieties are subjected to in the experiment. The M.L.I and M.E.I corresponding percentages were determined from the 'Omatec natural logarithmic indices' and their corresponding percentages table shown in appendix 2.

3.5.2 Generation of a natural logarithmic indices' and their corresponding percentages table towards estimation of the relative host plant tolerance levels in percentage

The estimation of relative host plant tolerance levels of the respective varieties viz; Kakamega 1, Kakamega 2, 16789 and Bana, against *U. kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 under co-infection in this study, was made possible by exploiting the logarithmic scales which exist between absolute numbers (Thomas, 1998; Umbarger, 2006). This enabled assigning of corresponding logarithmic percentages as illustrated in appendix 1. This led to the generation of 'Omatec natural logarithmic indices' and their corresponding percentages table shown in appendix 2 that integrated three logarithmic percentage scales. The mean corresponding logarithmic percentages of M.E.I and M.L.I indices (estimated as illustrated in table 3.6) of respective napier grass variety's treatments was determined from the table in appendix 2. The corresponding logarithmic percentages were then used in customization/standardization of host plant tolerance levels in percentage using two more scales in what holistically was termed as *quintuple scaling strategy* (five scales integration approach). The table in appendix 2 enabled the determination of corresponding logarithmic percentages of the two types of indices (M.E.I and M.L.I) illustrated in table 3.6, that estimated the efficacy in performance of the napier grass varieties under different treatments described in subsection 3.3.1.

The generation of the 'Omatec natural logarithmic indices' and their corresponding percentages table' was made possible by taking advantage of the integer value one which has logarithmic value of zero (since, each living system begins life from a unit value or single cell) and the relative indices it generates when it doubles, triples etc. This is because regardless of an organism's size or units used to measure it; a relative change that doubles or triples and so on is assigned the same magnitude index by logarithms as demonstrated in appendix 1 (Causton and Venus, 1981; Hunt, 1982; Parry, 1990; Thomas, 1998; Umbarger, 2006). Therefore, using excel sheet the test plant's ability numbers were generated using one (1) as a reference point so that the resulting corresponding efficacy indices' percentages were consecutive in a continuum manner

as shown in column C of appendix 3. The logarithmic indices responsible for the corresponding percentages were generated using the integer 1 as the initial performance value using the index generation function demonstrated on the excel screenshot shown in appendix 4. Using relativity the natural logarithmic value of one (which is zero) was substracted from all the natural logarithms of the test plant's ability numbers generated to obtain an efficacy index for each test plant's number using the logarithmic quotient rule (Thomas, 1998; Umbarger, 2006).

Finally, the indices were assigned corresponding percentages using the function shown in the excel screenshot in appendix 5 leading to the first scale of the five (quintuple) scales. The deviation percentage (specific tolerance power) was obtained by a function shown on the excel screenshots in appendix 6 leading to the second logarithmic scale of the five (quintuple) scales. This deviation described the magnitude by which a plant adjusted its processes when infected by a pathogen towards managing the attack. Graph of the percentages corresponding to the efficacy indices was generated to test accuracy of the values in predicting magnitude of a system by observing the level of spacing on the generated trend (the less the spacing the high the accuracy of the table values likelihood in predicting any index's percentage) as shown in appendices 50 and 51. Third scale of the five (quintuple) scales was determined relative to the highest natural logarithmic index (14.51) to estimate each of the logarithmic indices power relative to it in a linear trend as shown in appendix 2. The corresponding percentage levels were determined by dividing each natural logarithmic index as numerator by 14.51 as the denominator. The quotient/answer was then multiplied by 100%. The mean percentage of these three scales in the 'Omatec natural logarithms indices' and their corresponding percentages table' shown in appendix 2, described the mean corresponding logarithmic percentages. These mean logarithmic percentage values were corrected by subtracting 16.67% and multiplying the answer by 1.200048, ((mean corresponding percentage – 16.67%) × 1.200048). This function corrected the percentages to give the absolute value 1 its zero percent magnitude leading to the fulfillment of the rationale based on Wolpert (2011) described in subsection 3.5.1. These corrected mean corresponding percentages were synonymous to host plant tolerance/resistance before standardization and classification.

3.5.2.1 The scales generated towards the development of the natural logarithmic indices' and their corresponding percentages table

The first three scales utilized a continuum of test plant's ability absolute numbers shown in column (B) of appendix 2. The highest test plant's number was 2,000,000 and the lowest was 0.000,000,001 as shown in column (B) of appendix 2. The test plant's numbers represented the ability of a plant in performing a process for example row number 163; Column (B); the test plant's number (2) represented the ability of a plant to double its normal/initial levels or effort in response to a factor. Where the initial levels in this case was unit value (1) shown in row number 130 of column (B) of appendix 2. This was possible because the natural logarithmic constant of any system to double is a constant regardless of its initial value as demonstrated in appendix 1. These constants were also observed to exist in cases of tripling, quadripling and quintupling etc. (Appendix 1).

The first scale of the five determined the overall output of a plant in percentage upon a treatment (in this case the pathogens, nutrient solutions/formulations and watering regimes) relative to the absolute value one whose logarithmic potential was zero (Appendix 50). This scale produced a continuum of values whose maximum percentage value was 100% (Appendix 50), that corresponded with natural logarithmic index 14.51 shown in column (C) of the '*Omatec Logarithmic Indices' and their Corresponding Percentages Table'* shown in appendix 2. Whereas the lowest percentage value was 0% (Appendix 50), that corresponded also with natural logarithmic index -20.72 as shown in column (C) of '*Omatec Logarithmic Indices' and their Corresponding Percentages Table'* (Appendix 2*).* That meant that the highest and lowest potentials a biological system could manage logarithmically relative to unit value one (1) was 100% and 0% respectively. These values are captured in column (D) (Appendix 2*).* This first scale exhibited the overall output potential of a plant in performance without taking into consideration the specific increase or decrease in potential levels of a plant relative to unit value (1) whose logarithmic potential is zero. Therefore, the problem of specific deviation was evaluated using the second scale.

The second scale of the five estimated the specific output of a plant in percentage upon a treatment (in this case the pathogens, nutrient solutions/formulations and watering regimes) relative to the absolute value one whose logarithmic potential was zero (Appendix 51). Also, this

scale produced a continuum of values whose maximum percentage value was 100% (Appendix 51), that corresponded with natural logarithmic index 14.51 shown in column (C) of the *'Omatec Logarithmic Indices' and their Corresponding Percentages Table'* shown in appendix 2. Whereas the lowest percentage value for this scale was -100% (Appendix 51), that corresponded also with natural logarithmic index -20.72 shown in column (C) of *'Omatec Logarithmic Indices' and their Corresponding Percentages Table'* (appendix 2*)*. That meant that the highest and lowest specific input potentials a plant required in response to a treatment led to either an increase or a decrease logarithmically relative to unit value one (1). The negative in the lowest value (-100%) indicate a decline or decrease in the specific input potential. These values are captured in column (E) of the *'Omatec Logarithmic Indices' and their Corresponding Percentages Table'* (*A*ppendix 2).

The third scale of the five estimated the power of each logarithmic index in percentage relative to the highest index 14.51 of the test plant's ability number 2,000,000 that exhibited a perfect 100% for the first, second and third scales as shown in row number 267 of appendix 2. Also, this scale produced a continuum of values whose maximum percentage value was 100% whereas the lowest percentage value was -142.80% (Appendix 52). The highest percentage value corresponded with natural logarithmic index 14.51 shown in column (C) of the 'Omatec Logarithmic Indices' and their Corresponding Percentages Table' (Appendix 2*)*. Whereas the lowest percentage value for this scale was -142.80% that corresponded with natural logarithmic index -20.72 shown in column (C) (Appendix 2*)*. That meant that the power of each index relative to the highest index was largely influenced by the type of the natural logarithm index of a particular test plant's absolute number relative to unit value (1). That meant if it was a negative then the power of the index was also to be a negative and vice-versa. These values are captured in column (F) of the 'Omatec Logarithmic Indices' and their Corresponding Percentages Table' (appendix 2). Their linear trend is captured in appendix 52.

Finally, to develop a table that could predict the corresponding logarithmic levels in percentage of a plant. There was harmonization of the trends of the three scales that ranged from logarithmic to linear trends. Therefore, the mean (average percentage level) of the three scales was established and the various treatment combination's indices were subjected to the table to determine their corresponding mean logarithmic percentages as captured on column (G) of the

'Omatec Logarithmic Indices' and their Corresponding Percentages Table' (Appendix 2). These means highest value was 100% that corresponded with the natural logarithmic index 14.51 and the lowest value was -80.93% that corresponded with the natural logarithmic index -20.72 as shown in column (C) (Appendix 2). However, since the corresponding percentage of absolute value 1 was 16.67% as shown in Column G (Appendix 2). A correction function ((X% – 16.67) × 1. 200048), was applied across the percentage values in Column G which corrected the trend to the one shown in Column H, where the corresponding percentage of absolute value (1) was reduced to 0%. The details on how the function was arrived at are shown in the description below the table in appendix 2. The specific treatment combinations' corresponding mean logarithmic percentages were determined from the table. The fourth and fifth scales of the five were used in customization and standardization of the host plant tolerance levels as shown in the subsection 3.5.3.

3.5.3 Customization and standardization of the host plant tolerance percentages that are equivalent to the mean corresponding logarithmic percentages of the respective napier grass varieties treatments

Towards assigning the respective napier grass treatments' described on subsection 3.3.1, their host plant tolerance levels against *U. kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1, in percentage equivalent to their respective mean corresponding logarithmic percentage values. The host plant tolerance equivalent percentages were determined through the development of a *Customized/standardized napier grass varieties' tolerance magnitudes and classification table* (Appendix 10). Here a linear fourth scale of the five (quintuple) scales, was generated using an ideal minimum's corresponding logarithmic percentage; where the ideal minimum was an hypothesized zero performance of a possible highly susceptible genotype of the napier grass species as demonstrated in appendix 10. Therefore, using Microsoft excel 2007, the corresponding logarithmic percentages in whole numbers between that of the ideal minimum to the experimental maximum were determined all the way to the ideal maximum which was 100%. Where the experimental maximum percentage was the mean corresponding logarithmic percentages which was obtained from the mean logarithmic indices of the four napier grass varieties relative to unit value (1) of their uninoculated controls under complete nutrient solution and daily watering regimes. These treatments of napier grass varieties were assumed to be the least stressed during experimentation. Hence, the use of their mean logarithmic means as the

experimental maximum. This enabled customization/standardization of a scale suitable to the performance of the forage crops' varieties. A continuum of corresponding logarithmic percentages was obtained whose counts were determined. Finally, by dividing 100% by the total counts a logarithmic factor of increase was determined which was multiplied to each corresponding logarithmic percentage in scale four to generate a fifth scale of the five (quintuple) scales which estimated the host plant tolerance levels in percentage as they corresponded to their respective logarithmic percentages as shown in appendix 10. The host plant tolerance magnitudes (fifth scale) were used to rate the accessions performance using the following classifications modified from Obura (2012) and Kawube *et al*.(2014) where;

VHMT (Very High Magnitude of Tolerance/resistance) ≥ 75% magnitude of tolerance

HMT (High Magnitude of Tolerance) ≥ 50% magnitude of tolerance

MMT (Moderate Magnitude of Tolerance) ≥ 25% magnitude of tolerance

LMT (Low Magnitude of Tolerance) < 25% magnitude of tolerance

The table in appendix 10 was used to assign the magnitudes of host plant tolerance to all the other treatments. The ouput was compared with the ouputs in section 3.3.5.1 on disease expression to test the reliability of the approach. Therefore, to check the reliability of the respective bioassays' outputs and tools used; the experiment was designed to check itself to ensure the findings were credible.

3.5.3.1 Customized and standardized table outputs for assigning of the host plant tolerance levels

The customized table utilized the fourth scale of the five scales shown in column (B) of appendix 10 that had a maximum corresponding mean logarithmic percentage of 100% and minimum corresponding mean logarithmic percentage of –36%. The minimum corresponding mean logarithmic percentage (–36%) was determined from the ideal minimum's mean logarithmic index of 0.0000 using the *'Omatec natural logarithmic indices' and their corresponding percantages table*' shown in appendix 2. The experimental maximum mean logarithmic index was 4.7445, which was the mean logarithmic indices of the uninoculated treatments of the four varieties under complete nutrient solution (CNS) and daily watering. The mean logarithmic indices of the four varieties that were used to arrive at 4.7445 are captured in table 4.15. The magnitude efficacy index (M.E.I) of the ideal minimum was – 4.7445 whereas, that of the experimental maximum was 0 (zero) as demonstrated in appendix 10. The magnitude

efficacy index (M.E.I) corresponding percentages from 'Omatec natural logarithmic indices' and their corresponding percentages (Appendix 2), was – 72.23% and 0.00% for the ideal minimum and experimental maximum indices respectively (that is their percentages relative to their control which is presumed to be the experimental maximum). The mean logarithmic index (M.L.I) corresponding percentage for the ideal minimum was 0.00%, whereas the (M.L.I) corresponding percentage for the experimental maximum was 75.23% (that is their percentages relative to unit value (1)) as shown in appendix 10. The mean/average corresponding logarithmic levels in percentage of the ideal minimum was –36.12% which was equivalent (≈) to –36% rounded off to the nearest whole number. Whereas, that of the experimental maximum was 37.62% which was equivalent (≈) to 38% rounded off to the nearest whole number (Appendix 10).

The final fifth scale of the five was the host plant tolerance levels in percentage that is captured on column (D) of appendix 10. This percentage scale was developed by multiplying the individual counts in column (A) with factor 0.729927 to divide the levels into four quarters as used in classical strategies of scoring and evaluation of pathogens. The factor (0.729927) was obtained by dividing 100% by total number of counts in column (A) that is (137 counts) as demonstrated on column (A) of appendix 10. This was the logarithmic factor of increase in percentage of values in column D. The four classes of host plant tolerance levels in percentage arrived at are captured on column (C) of appendix 10. Where the class < 25% implied (LMT) low magnitude of tolerance, the class ≥ 25% implied (MMT) moderate magnitude of tolerance, the class ≥ 50% implied (HMT) high magnitude of tolerance and the class ≥ 75% implied (VHMT) very high magnitude of tolerance (Appendix 10). The highest corresponding logarithmic percentage magnitude of the ideal maximum (100%) shown in column (B) of appendix 10, had corresponding host plant tolerance levels of 100% as shown in column (D) with a classification of very high magnitude of tolerance (VHMT). Whereas, the lowest corresponding logarithmic percentage magnitude (-36%) of the ideal minimum had an equivalent host plant tolerance levels of 0.88% with a classification of low magnitude of tolerance (LMT). The experimental maximum which had a corresponding logarithmic percentage magnitude of 48% had a host plant tolerance levels of 54.39% with a classification of (HMT) high magnitude of tolerance (Appendix 10).

3.6 Data analysis

The normality of the parametric data was tested before analysis using the Shapiro-Wilk tests, with the abnormal data being transformed using logarithmic transformation. A multiple factor analysis of variance was conducted on the various growth parameters viz; leaf number, leaf area, tiller height, tiller number, total fresh weight, total stem weight, total leaf weight and chlorophyll content levels, using PASW GLM procedure. The Tukeys post hoc test at 5% significance level was used to separate the significant means and assessment of any comparisons of interest. Finally, coefficient of variation values were used to test for the stability of natural tolerance trait across the different treatments of the same accession based on the magnitudes that were generated (Zar, 2010).

CHAPTER FOUR

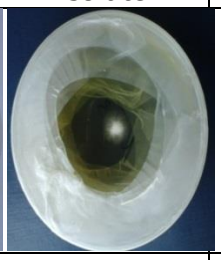
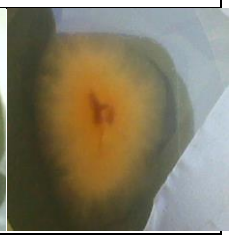
RESULTS

4.1 Morphological and molecular characteristics of *Ustilago kamerunensis* isolates

4.1.1 Morphological characteristics

Based on the *in vitro* cultures of the *Ustilago kamerunensis* isolates on malt extract agar in the laboratory, some easily distinguishable morphological differences in colony size were observed at day four. Based on the colony colour, all of them exhibited predominantly white floccose colonies on top side and pale cream at the reverse (Table 4.1). Significant differences ($df = 17$; $F = 52.22$; $P \leq 0.0001$) and ($df = 17$; $F = 321.88$; $P \leq 0.0001$) were observed in colony growth in both trials one and two respectively (Table 4.2*)*. In trial one NAK002 (NAK-2) isolate from Nakuru county had the highest growth vigour with the largest mean colony diameter of 3.64 ± 0.34 cm, followed by NYA003 (NYA-3) and KIA001 (KIA-1) whose mean colony diameters of 2.34 ± 0.27 cm and 2.19 ± 0.22 cm respectively were not statistically different. The NYA002 (NYA-2) isolate exhibited the smallest mean colony diameter of 1.14 ± 0.13cm, followed by NAK003 (NAK-3) isolate diameter of 1.34 ± 0.22 cm (Table 4.2). In trial two NAK002 (NAK-2) isolate still retained the growth vigour with the largest mean colony mean diameter of 3.56 ± 0.20cm, followed by NAK001 (NAK-1) and NAK003 (NAK-3) with mean colony diameters of 2.55 ± 0.15cm and 2.55 ± 0.19cm respectively that did not exhibit statistical differences. The NYA002 (NYA-2) and KIA003 (KIA-3) isolates had the lowest mean colony diameters of 1.38 ± 0.08 cm and 1.39 ± 0.07cm respectively, which did not exhibit statistical differences with many of the other isolates' mean colony diameters (Table 4.2).

Table 4.1: Morphological characteristics of *Ustilago kamerunensis* isolates on malt extract agar *in vitro* (day four).

***Ustilago kamerunensis* Isolates**	**NAK003 (NAK-3) Isolate**	**NAK002 (NAK-2) Isolate**	**NYA003 (NYA-3) Isolate**	**KIA003 (KIA-3) Isolate**
Top-side Colony Picture				
Reverse-side Colony Picture				

The colonies had been cultured at 25^{o}C and colony diameter measured on day four which is the recommended culture period for fungal cultures for clear observations. It is important to note the pictures on this table are not presented to scale.

Table 4.2: Growth diameter *in vitro* of the *Ustilago kamerunensis* isolates' on malt extract agar.

***Ustilago kamerunensis* Isolates**	**Mean Growth Diameter (cm) (Trial one)**	**Mean Growth Diameter (cm) (Trial two)**
NAK002 (NAK-2)	3.64 ± 0.34 a	3.56 ± 0.20 a
NYA003 (NYA-3)	2.34 ± 0.27 b	1.42 ± 0.10 c
KIA001 (KIA-1)	2.19 ± 0.22 b	1.45 ± 0.15 c
KIA002 (KIA-2)	2.01 ± 0.51 bc	1.53 ± 0.14 c
KIA003 (KIA-3)	1.99 ± 0.41 bc	1.39 ± 0.07 c
KIR002 (KIR-2)	1.88 ± 0.09 cd	1.48 ± 0.09 c
NAK001 (NAK-1)	1.87 ± 0.29 cd	2.55 ± 0.15 c
NYA001 (NYA-1)	1.85 ± 0.12 cd	1.41 ± 0.16 c
NYE002 (NYE-2)	1.72 ± 0.21 def	1.42 ± 0.10 c
NYE004 (NYE- 4)	1.69 ± 0.10 def	1.44 ± 0.12 c
MUR003 (MUR-3)	1.66 ± 0.05 def	2.49 ± 0.07 b
MUR001 (MUR-1)	1.65 ± 0.23 def	2.44 ± 0.12 b
KIR009 (KIR-9)	1.64 ± 0.04 def	1.44 ± 0.08 c
KIR001 (KIR-1)	1.64 ± 0.08 def	1.47 ± 0.14 c
NYE001 (NYE-1)	1.63 ± 0.07 def	1.43 ± 0.08 c
MUR002 (MUR-2)	1.55 ± 0.27 ef	2.41 ± 0.07 b
NAK003 (NAK-3)	1.34 ± 0.22 fg	2.55 ± 0.19 b

NYA002 (NYA-2)	1.14 ± 0.13 g	1.38 ± 0.08 c

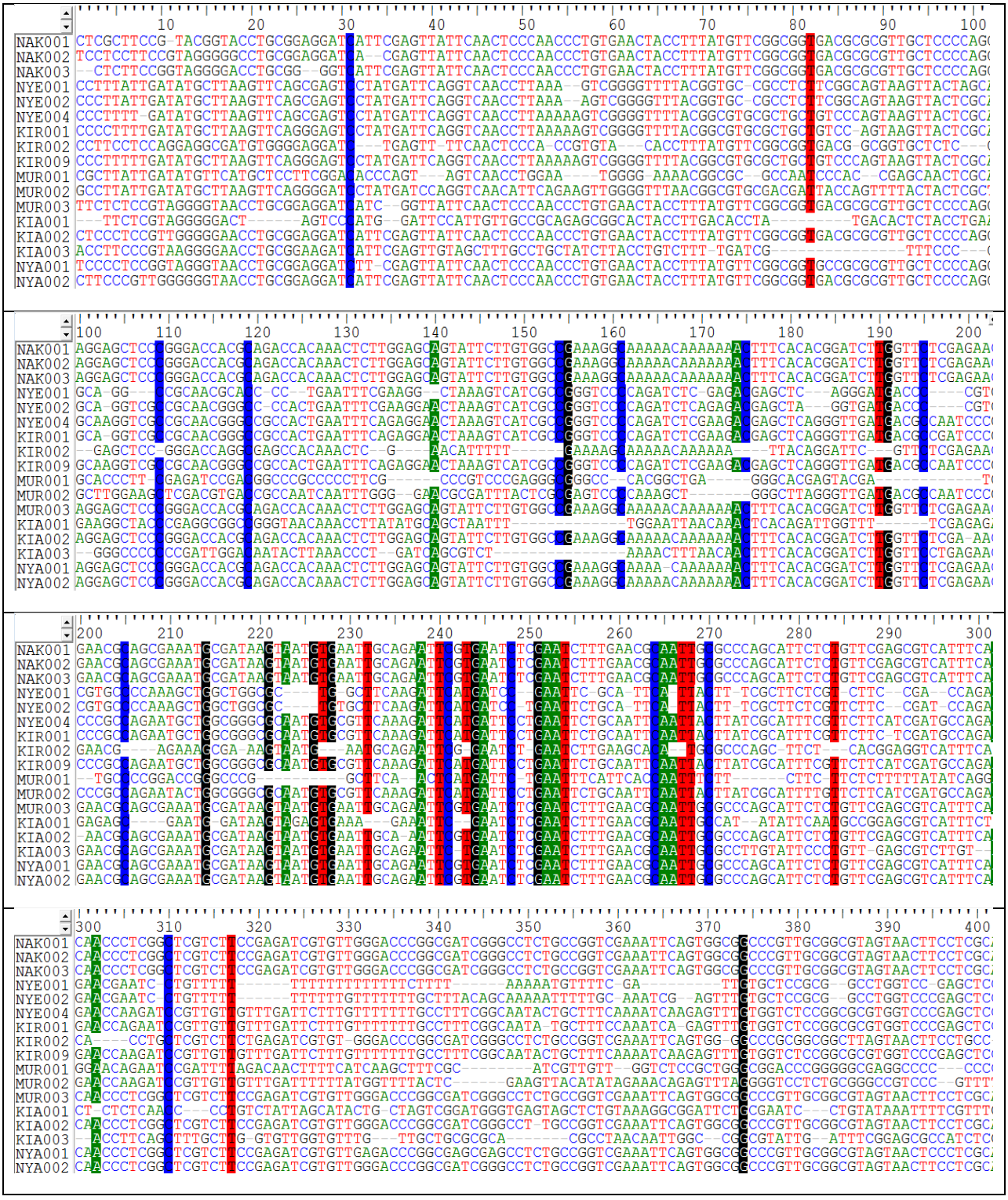
Growth diameter (cm) of the *Ustilago kamerunensis* isolates ± standard deviation. Means having the same letters in the same column do not differ significantly from each other at $p \leq 0.05$. Those with more than one letter within a column are intermediates.

4.1.2 Molecular characteristics

In the analysis of the sampled nine sequences PCR product of the eighteen *Ustilago kamerunensis* isolates' for fragment size quality check, it revealed an estimated 600 base pairs PCR fragment as shown in appendix 46. This confirmed that the required region was successfully amplified. The sequencing of their DNA results (Table 4.3) shows the alignment file, where conserved loci were observed at different positions within the ITS region (approximately at 30, 80, 110, 120, 140, 154, 155, 161 base pairs and many more as shown by the uniform colouring of some regions). Nucleotide substitution and deletions were observed at certain positions of some of the *Ustilago kamerunensis* isolates' nucleic acid sequences comparatively (Table 4.3).

The sequences of the seventeen (17) *Ustilago kamerunensis* shown in the far left of table 4.3 were submitted at the National Center for Biotechnology Information GenBank (Appendix 43). The DNA quality and quantity of isolate NYA003 (NYA-3) was not good and extremely low to support sequencing procedure, hence was left out. Further, of the seventeen that were sequenced; one isolate's sequence, MUR001 did not merit to pass the NCBI filters due to sequence fragmentation and therefore was removed from the submission list. This therefore meant that out of 17 sequences, 16 were assigned accession numbers by which they can be accessed from the Genbank database (Table 4.4). The sequences of the seventeen isolates are illustrated in appendix 43.

Table 4.3: Sequence alignments as viewed in Bioedit version 7. The blocks with the same colour indicate conserved loci.



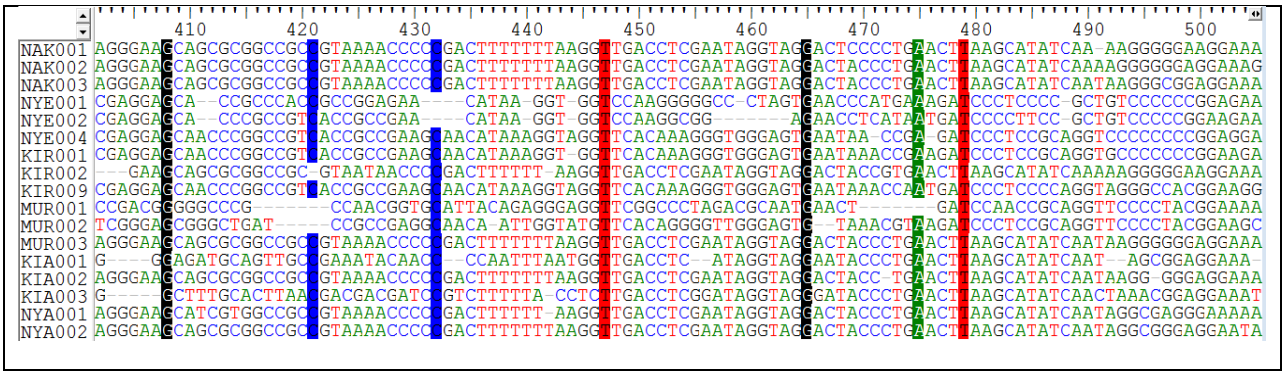


Table 4.4: National Center for Biotechnology Information's (NCBI) GenBank accession numbers of the sample isolates up on submission. Searching the accession number at genbank's domain the details of each isolate's molecular structure is availed.

Sample	Organism name	strain	Accession number
SUB3393968 NAK001	*Ustilago kamerunensis*	NAK001	MG722754
SUB3393968 NAK002	*Ustilago kamerunensis*	NAK002	MG722755
SUB3393968 NAK003	*Ustilago kamerunensis*	NAK003	MG722756
SUB3393968 NYE001	*Ustilago kamerunensis*	NYE001	MG722757
SUB3393968 NYE002	*Ustilago kamerunensis*	NYE002	MG722758
SUB3393968 NYE004	*Ustilago kamerunensis*	NYE004	MG722759
SUB3393968 KIR001	*Ustilago kamerunensis*	KIR001	MG722760
SUB3393968 KIR002	*Ustilago kamerunensis*	KIR002	MG722761
SUB3393968 KIR009	*Ustilago kamerunensis*	KIR009	MG722762
SUB3393968 MUR001	*Ustilago kamerunensis*	MUR001	-
SUB3393968 MUR002	*Ustilago kamerunensis*	MUR002	MG722763
SUB3393968 MUR003	*Ustilago kamerunensis*	MUR003	MG722764
SUB3393968 KIA001	*Ustilago kamerunensis*	KIA001	MG722765
SUB3393968 KIA002	*Ustilago kamerunensis*	KIA002	MG722766
SUB3393968 KIA003	*Ustilago kamerunensis*	KIA003	MG722767
SUB3393968 NYA001	*Ustilago kamerunensis*	NYA001	MG722768
SUB3393968 NYA002	*Ustilago kamerunensis*	NYA002	MG722769

The phylogeny analysis of the isolates' sequences revealed some degree of divergence (variation) from the ancenstral stock (Figure 4.1). The tree with the highest log likelihood (-2319.75) is shown in figure 4.1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All sequences from Nakuru, Nyandarua and Kiambu counties were put in cluster A. Cluster B contained sequences from Nyeri, Kirinyaga and

Murang'a. One of the sequences from Murang'a (MUR003) and Kirinyaga (KIR002) were classified in Cluster A (Figure 4.1). Samples from Nakuru, Nyandarua as well as those from Nyeri appeared very phylogenetically similar as they formed respective sub-clusters.

The number of base substitutions per site from between sequences are shown in table 4.5. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates). The pairwise evolutionary divergence (Table 4.5) showed that the sequences had very high genetic diversity since no sequences had genetic distance zero. The overall genetic distance of all the samples was 2.249 ± 0.670, an indication of high genetic diversity among the *Ustilago kamerunensis* isolates. The strains with the lowest genetic distance was between NYA001 and NYA002 (0.042 ± 0.014) while the highest genetic distance of 6.000 ± 4.865 was observed between strains KIR002 versus NYE001. Followed by 5.693 ± 3.516 and 5.693 ± 4.085 which were observed between the strains KIA001 versus MUR001 and KIR002 versus NYE002 respectively. Generally most of the samples demonstrated a high genetic diversity (Table 4.5). Also, the statistical confidence levels of placing the different clades on their nodes by the bootstrap procedure was very high (Figure 4.1).

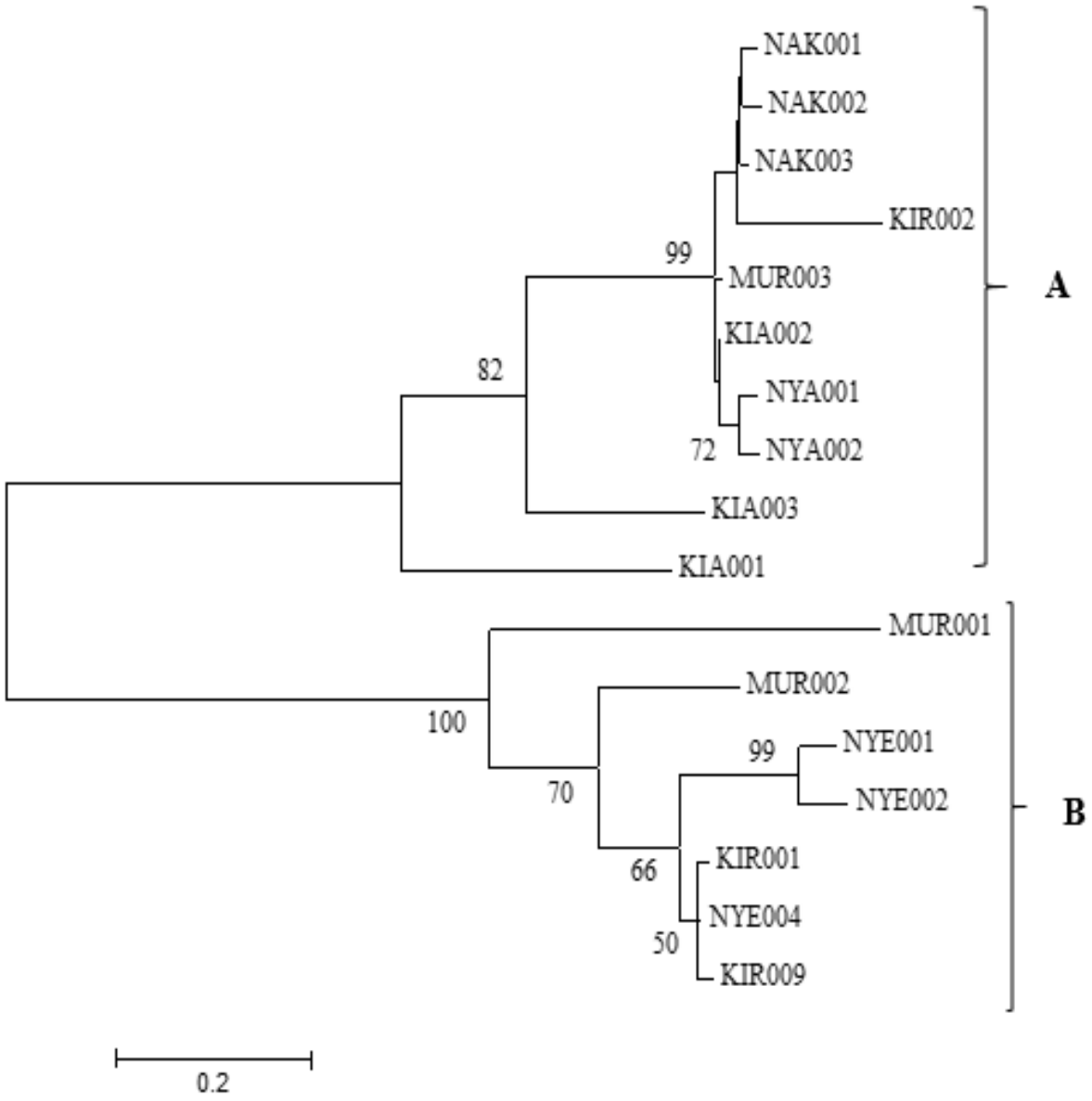


Figure 4.1: A phylogenetic tree based on the molecular phylogenetic analysis by Maximum Likelihood method. The ITS sequences of the different *Ustilago kamerunensis* isolates from affected counties of Kenya were used in the assessment.

Table 4.5: The genetic distances matrix table showing the estimates of molecular divergence between sequences of the different *Ustilago kamerunensis* isolates from affected counties of Kenya.

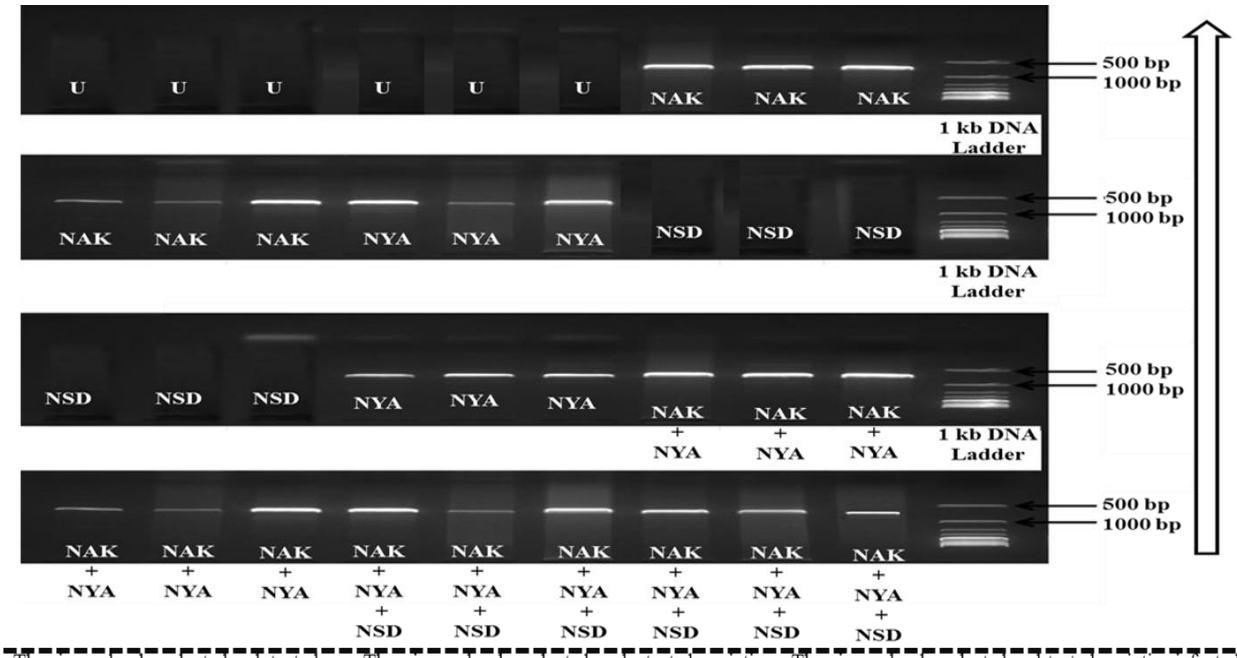
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. NAK001		0.012	0.011	2.148	2.251	1.230	1.148	0.034	0.989	3.988	2.281	0.015	0.162	0.014	0.083	0.019	0.017
2. NAK002	0.038		0.011	2.109	2.049	1.078	1.071	0.030	0.879	2.940	1.810	0.015	0.140	0.015	0.076	0.019	0.015
3. NAK003	0.029	0.033		1.841	1.669	0.961	0.950	0.031	0.795	2.656	2.115	0.013	0.160	0.013	0.086	0.018	0.018
4. NYE001	4.500	4.312	4.138		0.022	0.037	0.040	4.865	0.039	0.159	0.086	2.502	3.421	2.767	2.451	1.862	2.019
5. NYE002	4.500	4.312	3.975	0.094		0.037	0.039	4.085	0.039	0.189	0.087	2.080	2.614	2.247	1.203	1.301	1.996
6. NYE004	3.545	3.300	3.081	0.195	0.208		0.008	2.078	0.009	0.160	0.049	1.072	1.291	1.167	0.918	0.945	0.977
7. KIR001	3.419	3.300	3.081	0.214	0.228	0.016		1.946	0.011	0.150	0.052	1.071	1.275	1.032	0.907	0.853	0.879
8. KIR002	0.189	0.176	0.183	6.000	5.693	4.312	4.312		1.524	4.620	2.498	0.034	0.166	0.034	0.115	0.041	0.035
9. KIR009	3.187	2.980	2.794	0.214	0.228	0.020	0.029	3.823		0.165	0.050	0.885	1.109	0.944	0.870	0.892	0.929
10. MUR001	5.413	4.702	4.702	0.938	1.091	0.938	0.898	5.413	0.958		0.163	2.240	3.516	3.606	3.413	2.558	2.638
11. MUR002	4.920	4.312	4.702	0.516	0.539	0.292	0.308	4.920	0.300	0.938		2.084	3.456	2.277	0.759	1.602	1.700
12. MUR003	0.051	0.055	0.042	4.500	4.312	3.300	3.300	0.195	2.980	4.500	4.500		0.140	0.006	0.075	0.014	0.014
13. KIA001	1.000	0.879	0.979	5.156	4.702	3.545	3.545	1.000	3.300	5.693	5.156	0.879		0.144	0.137	0.167	0.149
14. KIA002	0.042	0.051	0.038	4.702	4.500	3.419	3.187	0.195	3.081	5.156	4.702	0.012	0.898		0.073	0.014	0.014
15. KIA003	0.550	0.493	0.550	4.500	3.300	3.081	3.081	0.742	2.980	5.156	2.707	0.493	0.843	0.483		0.079	0.077
16. NYA001	0.074	0.069	0.065	4.138	3.680	3.081	2.885	0.248	2.980	4.500	3.975	0.046	1.000	0.042	0.516		0.014
17. NYA002	0.060	0.046	0.065	4.312	4.312	3.187	2.980	0.208	3.081	4.702	4.138	0.046	0.918	0.042	0.493	0.042	

The upper blue triangle values are showing the margin of error of the genetic distance between pairwise comparison of isolates. Whereas, the lower black triangle values demonstrate the genetic distance levels between pairwise comparison of *Ustilago kamerunensis* isolates.

4.2 Pathogenicity evaluation of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae" strain Mbita 1 under varying nutrient and moisture levels

The pathogenicity levels evaluation of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 on the napier grass under varying nutrient formulations and moisture levels led to the following observations. The two pathogens presence in the napier grass varieties upon establishment induced some morpho-pathological symptoms of stunting and chlorosis associated with napier stunt disease (NSD) and napier head smut disease (Plate 4.1). However, the smutted inflorescence morpho-pathological symptom associated with *Ustilago kamerunensis* pathogen was not observed in any of the treatments that was infected by *Ustilago kamerunensis* isolates by the end of the fourth cropping cycle. Molecular analysis confirmation of the presence or absence of *Ustilago kamerunensis* isolates in selected napier grass treatments, showed the pathogen's presence in all the treatments under the NAK-2 and NYA-2 *Ustilago kamerunensis* isolates infection (Plate 4.2). The detection of the napier head smut pathogen was made possible by the observation of the ITS region's DNA of about 720 base pairs amplified from the total DNA extracted from the soft tissues of the napier grass varieties (Plate 4.2). The tissues from napier grass varieties treatments under '*Candidatus* Phytoplasma oryzae' strain Mbita 1 (NSD) infection, were positive of stunt disease. This was upon amplification of the 16S rRNA gene segment at approximately 778 base pairs (Plate 4.3). The presence of the pathogens in the artificially inoculated treatments implied that the two pathogens established successfully. The treatments under *Ustilago kamerunensis* infection they failed to show morpho-pathological symptom of smut disease. The uninoculated treatments (controls) tested negative to both pathogens (Plates 4.2 and 4.3).

Plate 4.1: Morpho-pathological symptoms of napier stunt disease (NSD) as observed during the experimentation process showing the sequential development of the symptoms from photo image A is general chlorosis, then followed by photo image B is the general stunting to photo image C is general tillering (grassy characteristics) towards significant biomass reduction of the stool/bush.The (NS) on photo image B shows the non-stunted napier grass whereas (S) on the same image shows the stunted napier grass growing in potted soil that was completely randomized in the screenhouse during experimentation.



The six randomly selected and tested varieties infected by, NAK-2 labeled as 'NAK'.

1- Bana + CNS + NAK-2 + W
2- Bana + N/P-D+ NAK-2 + W
3- KK1 + P-D + NAK-2 + W
4-16789 + N-D + NAK-2 + D
5- KK2 + P-D + NAK-2 + D
6- KK1 + N-D + NAK-2 + D

The six randomly selected and tested; varieties infected by, NYA-2 labeled as 'NYA'.

1- 16789 + CNS + NYA-2 + W
2- KK1 + N/P-D+ NYA-2 + W
3- Bana + P-D + NYA-2 + W
4- KK1 + N-D + NYA-2 + D
5- KK2 + P-D + NYA-2 + D
6- Bana + N/P-D + NYA-2 + D

The six randomly selected and tested varieties infected by, NAK-2 + NYA-2 labeled as 'NAK + NYA'.

1- KK2 + CNS + NAK-2 + NYA-2 + W
2- 16789 + N/P-D + NAK-2 + NYA-2 + W
3- Bana + P-D + NAK-2 + NYA-2 + W
4- 16789 + P-D + NAK-2 + NYA-2 + D
5- Bana + N-D + NAK-2 + NYA-2 + D
6- 16789 + N-D + NAK-2 + NYA-2 + D

The six randomly selected and tested varieties but uninoculated labeled as 'U' as a control.

1- KK2 + CNS + UNINOCULATED + W
2- KK2 + N/P-D + UNINOCULATED + W
3- KK1 + P-D + UNINOCULATED + D
4- KK2 + N-D + UNINOCULATED + D
5- KK1 + N/P-D + UNINOCULATED + D
6- Bana + N/P-D + UNINOCULATED + W

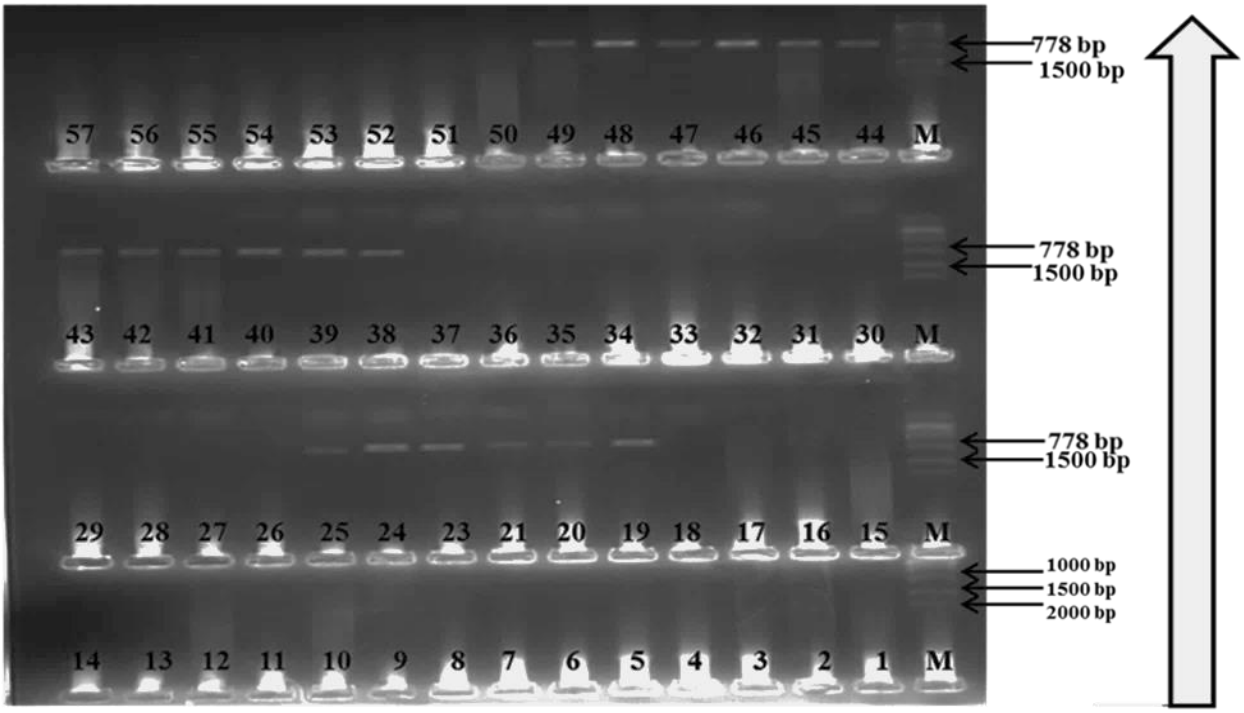
The six randomly selected and tested varieties infected by, NSD labeled as 'NSD'.

1- KK1 + N/P-D + NSD + W
2- 16789 + CNS + NSD + W
3- Bana + P-D + NSD + W
4- KK1 + N-D + NSD + D
5- 16789 + N-D + NSD + D
6- 16789 + P-D + NSD + D

The six randomly selected and tested varieties infected by, NAK-2 + NYA-2 + NSD labeled as 'NAK + NYA + NSD'.

1-KK1 + P-D + NAK-2 + NYA-2 + NSD + D
2-Bana + N/P-D + NAK-2 + NYA-2 + NSD + D
3- KK2 + N-D + NAK-2 + NYA-2 + NSD + D
4- 16789 + N/P-D + NAK-2 + NYA-2 + NSD + W
5- KK2 + P-D + NAK-2 + NYA-2 + NSD + W
6 - Bana + CNS + NAK-2 + NYA-2 + NSD + W

Plate 4.2: Electropherogram showing the DNA bands at approximately 720 bp (base pairs) level where *Ustilago kamerunensis* DNA was visualized upon amplification using ITS primers, of the total DNA that was extracted from the 36 randomly selected napier grass varieties tissues shown below the image under different pathogen combinations infection namely; NAK represents the NAK-2 *Ustilago kamerunensis* isolate from Nakuru County, NYA represents the NYA-2 *Ustilago kamerunensis* isolate from Nyandarua County. The NSD represent the '*Candidatus* Phytoplasma oryzae' strain Mbita 1 and U represent the uninoculated napier varieties used as controls. The (D) and (W) represents the daily and weekly watering regimes wheres the CNS, N-D, P-D and N/P-D the different nutrient formulations. The plus (+) indicate the various pathogen combinations. Six randomly selected napier grass varieties tissues were analyzed for the presence or absence of *Ustilago kamerunensis* after artificial inoculation at the third ratoon; that is week 24 after the experiment's establishment. The arrow on the right indicates direction of electrophoresis flow.



KEY:

M → PCR DNA Ladder
1- 6; Negative control
7- Bana + CNS + NAK-2 + W
8- Bana + N/P-D+ NAK-2 + W
9- KK1 + P-D + NAK-2 + W
10-16789 + N-D + NAK-2 + D
11- KK2 + P-D + NAK-2 + D
12- KK1 + N-D + NAK-2 + D
13- 16789 + CNS + NYA-2 + W
14- KK1 + N/P-D+ NYA-2 + W
15- Bana + P-D + NYA-2 + W
16- KK1 + N-D + NYA-2 + D
17- KK2 + P-D + NYA-2 + D
18- Bana + N/P-D + NYA-2 + D
19 - 25; Positive control
26- KK2 + CNS + NAK-2 + NYA-2 + W
27- 16789 + N/P-D + NAK-2 + NYA-2 + W
28- Bana + P-D + NAK-2 + NYA-2 + W
29- 16789 + P-D + NAK-2 + NYA-2 + D
30- Bana + N-D + NAK-2 + NYA-2 + D
31- 16789 + N-D + NAK-2 + NYA-2 + D
32- KK2 + CNS + UNINOCULATED + W
33- KK2 + N/P-D + UNINOCULATED + W
34- KK1 + P-D + UNINOCULATED + D
35- KK2 + N-D + UNINOCULATED + D
36- KK1 + N/P-D + UNINOCULATED + D
37- Bana + N/P-D + UNINOCULATED + W
38- KK1 + N/P-D + NSD + W
39- 16789 + CNS + NSD + W
40- Bana + P-D + NSD + W
41- KK1 + N-D + NSD + D
42- 16789 + N-D + NSD + D
43- 16789 + P-D + NSD + D
44- KK1 + P-D + NAK-2 + NYA-2 + NSD + D
45- Bana + N/P-D + NAK-2 + NYA-2 + NSD + D
46- KK2 + N-D + NAK-2 + NYA-2 + NSD + D
47- 16789 + N/P-D + NAK-2 + NYA-2 + NSD + W
48- KK2 + P-D + NAK-2 + NYA-2 + NSD + W
49- Bana + CNS + NAK-2 + NYA-2 + NSD + W
50 - 57; Negative control

Plate 4.3: Electropherogram showing the DNA bands at approximately 778 bp(base pairs) level where '*Candidatus* Phytoplasma oryzae' DNA was visualized upon amplification using primers targeting the 16S rRNA gene through the nested PCR technique. This involved the total DNA that was extracted from the 36 randomly selected napier grass varieties tissues shown in the key under different treatments namely; NAK-2 represents the *Ustilago kamerunensis* isolate from Nakuru County, NYA-2 represents the *Ustilago kamerunensis* isolate from Nyandarua County. The NSD represents the '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The (D) and (W) represents the daily and weekly watering regimes. The plus (+) indicate the various factors in combination ranging from the napier grass variety, nutrient formulations,pathogen type/s and watering regime in that order. The positive control was an NSD infected and confirmed Bana variety napier grass at ICIPE-Mbita. Six tissues infected by a respective pathogen/s combination irrespective of the napier grass variety were sampled randomly and analyzed for the presence or absence of the napier grass stunt pathogen after artificial inoculation at the third ratoon that is week 24 after the experiment's establishment. The arrow on the right indicates direction of electrophoresis flow.

4.2.1 General and specific evaluation of virulence levels across selected treatment combinations involving the factors under study

The general means of the various treatments involved in the study revealed varying levels of virulence in the napier grass varieties; as caused by the *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 as follows:

4.2.1.1 Pathogen combinations versus the napier grass varieties effects on virulence trend

The general plot of the respective pathogen combinations' infection of the napier grass varieties exhibited varying levels of virulence. The NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) when used alone produced the highest mean percentage levels of virulence against the napier grass varieties. It was followed by NAK-2 + NYA-2 + NSD pathogens combination; that is NAK-2 and NYA-2 *Ustilago kamerunensis* isolates plus the NSD pathogen co-infected treatments, then NAK-2 + NYA-2 pathogens co-infection, NYA-2 *Ustilago kamerunensis* isolate sole infections and NAK-2 *Ustilago kamerunensis* isolate sole infections in that descending order. The lowest mean percentage virulence levels was demonstrated by the uninoculated controls (Figure 4.2). However, there were some exceptions where NAK-2 *Ustilago kamerunensis* isolate sole infections had the lowest mean percentage virulence levels unlike their uninoculated controls. This was observed in the infection of KK 2 (Kakamega 2 variety) and accession 16789 (Figure 4.2).

In terms of napier varieties; NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) was most virulent on Bana variety followed by accession 16789, while Kakamega 2 (KK 2) and Kakamega 1 (KK 1) variety were the least damaged (Figure 4.2). For NAK-2 + NYA-2 + NSD pathogens co-infection still Bana had the highest virulence, followed by Kakamega 2 (KK 2), Kakamega 1 (KK 1) and 16789 that declining order. The NAK-2 + NYA-2 pathogens co-infection was most virulent on Bana variety followed by Kakamega 1 (KK 1), then Kakamega 2 (KK2) and accession 16789 in a declining order. The infection by NYA-2 *Ustilago kamerunensis* isolate alone caused the highest virulence on Bana variety followed by accession 16789, then Kakamega 2 (KK 2) and Kakamega 1 (KK 1) variety in declining order (Figure 4.2). NAK-2 *Ustilago kamerunensis* isolate alone caused the highest damage on Bana variety followed by accession 16789, then Kakamega 1(KK 1), while Kakamega 2 (KK 2) variety exhibited the lowest levels of damage. The uninoculated but stressed treatments; Bana variety still

demonstrated the highest levels of stress followed by accession 16789, then Kakamega 2 (KK 2) with Kakamega 1 (KK 1) variety producing the lowest mean percentage levels of stress (Figure 4.2). The presence of the smut pathogen especially NAK-2 *Ustilago kamerunensis* pathogen isolate seemed to generally boost slightly the grass' performance.

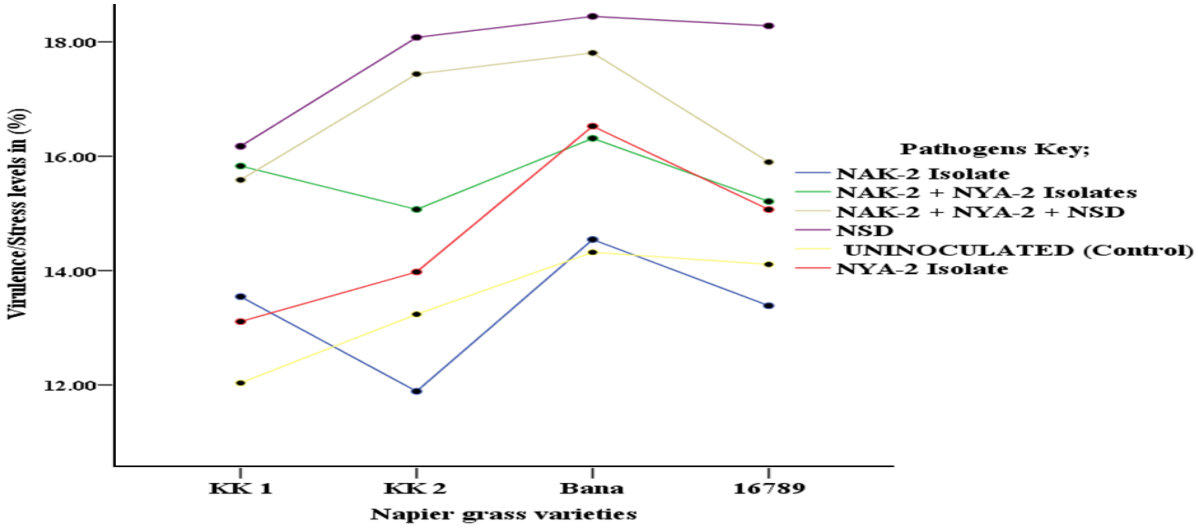


Figure 4.2: Effects of pathogen combinations on virulence caused on the four napier grass varieties. The head smut isolates (*Ustilago kamerunensis*) used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (Napier stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1.

4.2.1.2 Pathogen combinations versus the nutrient formulations effects on virulence trend

Results on the effects of pathogen combinations and nutrients formulations are shown in figure 4.3. The treatments under nitrogen and phosphorus deficient (N/P-D) nutrient solution had the highest virulence levels, followed by those treatments under nitrogen deficient (N-D) nutrient solution, then the phosphorus deficient (P-D) nutrient solution's treatments. The treatments under complete nutrient solution produced the lowest mean percentage virulence levels (Figure 4.3).

The effects of the nutrient formulations on disease virulence under nitrogen and phosphorus deficient (N/P-D) nutrient solution had the NSD pathogen's sole infections show the highest virulence levels, followed by NAK-2 + NYA-2+ NSD co-infected treatments, then NAK-2 + NYA-2 co-infected treatments in that declining order. NAK-2 *Ustilago kamerunensis* isolate when applied alone and uninoculated controls had the lowest virulence levels, while infections

with NYA-2 *Ustilago kamerunensis* isolate alone exhibited the second lowest levels of infection (Figure 4.3). Under the nitrogen deficient (N-D) nutrient solution's treatments, the trends were similar to those under combined nitrogen and phosphorus deficient nutrient solution.

The treatments under phosphorus deficient (P-D) nutrient solution the trend was slightly different. The NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) when applied alone had the highest virulence level, followed by NAK-2 + NYA-2 + NSD co-infected treatments, NYA-2 *Ustilago kamerunensis* isolate sole infected treatments, then the sole infected treatments by NAK-2 *Ustilago kamerunensis* isolate and NAK-2 + NYA-2 co-infected treatments in that declining order. The uninoculated controls had the lowest virulence levels (Figure 4.3). For the treatments applied with complete nutrient solution, the NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) sole infected treatments had the highest levels of virulence, followed by NAK-2 + NYA-2+ NSD co-infected treatments, then NAK-2+ NYA-2 co-infected treatments. NAK-2 *Ustilago kamerunensis* isolate sole infected treatments and uninoculated controls had the lowest virulence levels, while NYA-2 *Ustilago kamerunensis* isolate infected treatments had the second lowest virulence levels (Figure 4.3).

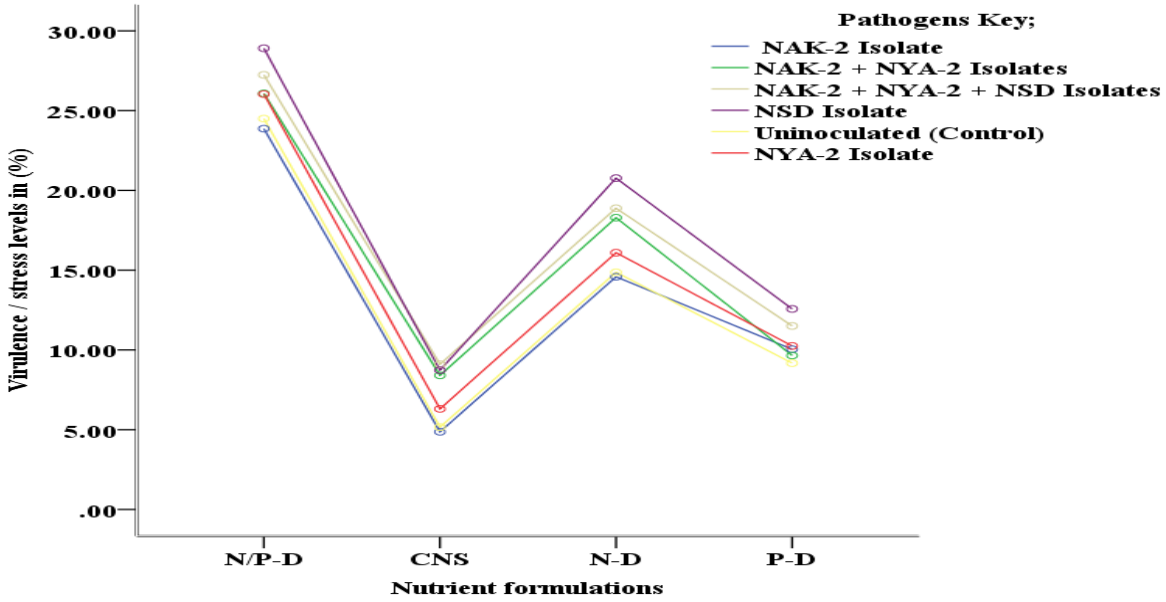


Figure 4.3: Plot of percentage virulence levels of the six pathogen combinations across the different nutrient formulations. The head smut isolates (*Ustilago kamerunensis*) used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (Napier stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The N/P-D (nitrogen and phosphorus

deficient nutrient solution), CNS (complete nutrient solution), N-D (nitrogen solution) and P-D (phosphorus nutrient solution).

4.2.1.3 Evaluation of the general variance of means of the selected treatment combinations' effects on virulence levels

Analysis of variance between the virulence means as influenced by the pathogens combination and nutrient formulations showed significant differences at ($df = 23$; $F = 8.08$; $P \leq 0.0001$) (Appendix 47). The treatment combinations under NSD pathogen + N/P-D (nitrogen and phosphorus deficient nutrient solution) had the highest virulence levels with moderate virulence classification (Appendix 47). This treatment combination was followed by NAK-2 + NYA-2 + NSD-pathogen + N/P-D combination, which also had a moderate virulence classification. Although, the two treatment combinations were not statistically differentits. The treatment combination NAK-2 + CNS (complete nutrient solution) exhibited the lowest virulence which was not statistically different from uninoculated + CNS and NYA-2 + CNS treatment combinations. These treatment combinations had a low virulence classification (Appendix 47). Generally, the treatments under N/P-D (nitrogen and phosphorus deficient nutrient solution) had the highest virulence levels with those under complete nutrient solution (CNS) exhibiting the lowest virulence levels (Appendix 47).

The pathogens combination and watering regimes treatments based on virulence levels, differed significantly ($df = 11$; $F = 11.84$; $P \leq 0.0001$) (Appendix 48). The daily watering treatments irrespective of pathogen combination had significantly lower virulence levels compared to the weekly watering (Appendix 48). The highest severity among weekly watered treatments was with the following treatment combinations; (NSD + Weekly watering), (NAK-2 + NYA-2 + NSD + Weekly watering), (NAK-2 + NYA-2 + Weekly watering) and (NYA-2 + Weekly watering) but their results was not statistically different (Appendix 48). The (NSD + Weekly watering) treatments had a moderate virulence classification levels whereas the other three exhibited low virulence classification levels. The treatments (NAK-2 + NYA-2 + NSD + Daily watering), (NAK-2 + NYA-2 + Daily watering), (NYA-2 + Weekly watering), (Uninoculated + Daily watering) and (NAK-2 + Daily watering) had the lowest virulence levels and low virulence classification (Appendix 48).

The analysis of variance on the combined effects of the pathogens combination, nutrient formulations and watering regimes showed significant differences at (df = 47 ; F= 64.92; $P \leq 0.0001$) (Table 4.6). The treatment combinations (NSD + N/P-D + Weekly watering) and (NAK-2 + NYA-2 + NSD + N/P-D + Weekly watering), had significantly ($P < 0.0001$) the highest virulence with moderate virulence classification level (Table 4.6). The highest two treatment combinations' were however not statistically different. The treatment combination (NAK-2 + CNS + Daily watering) had the lowest virulence, followed by the (uninoculated + CNS + Daily watering) that had a second lowest virulence with low virulence classification levels. The rest of the treatment combinations performed in an intermediate manner as their means overlapped across different classes of performance (Table 4.6).

Table 4.6: Effects of nutrient formulations and watering regimes on the mean pathogen virulence levels in percentage; based on the integrated parameter logarithmic index means cutting across the four cropping cycles.

Pathogen/stressor under different nutrient formulations and watering regimes	Virulence/Stress levels in (%)	Virulence classification
NSD + N/P-D + Weekly watering	36.35 ± 1.22 a	MV
NAK-2 + NYA-2 + NSD + N/P-D + Weekly watering	33.83 ± 1.15 a	MV
NAK-2 + NYA-2 + N/P-D + Weekly watering	33.54 ± 1.29 ab	MV
NYA-2 + N/P-D + Weekly watering	33.19 ± 0.58 ab	MV
Uninoculated + N/P-D + Weekly watering	32.91 ± 0.90 ab	MSL
NAK-2 + N/P-D + Weekly watering	31.32 ± 0.67 ab	MV
NSD + N-D + Weekly watering	30.49 ± 0.54 abc	MV
NAK-2 + NYA-2 + NSD + N-D + Weekly watering	30.05 ± 2.11 abc	MV
NAK-2 + NYA-2 + N-D + Weekly watering	26.51 ± 1.30 bcd	MV
NYA-2 + N-D + Weekly watering	23.71 ± 1.11 cde	LV
NAK-2 + N-D + Weekly watering	23.57 ± 1.12 cdef	LV
Uninoculated + N-D + Weekly watering	22.18 ± 0.72 defg	LSL
NSD + N/P-D + Daily watering	21.47 ± 1.09 defg	LV
NAK-2 + NYA-2 + NSD + N/P-D + Daily watering	20.66 ± 0.28 defgh	LV
NYA-2 + N/P-D + Daily watering	18.88 ± 1.11 efghi	LV
NAK-2 + NYA-2 + N/P-D + Daily watering	18.62 ± 1.82 efghi	LV
NSD + P-D + Weekly watering	18.24 ± 0.70 efghijk	LV
NAK-2 + N/P-D + Daily watering	16.42 ± 1.45 fghijk	LV
Uninoculated + N/P-D + Daily watering	16.09 ± 1.03 ghijk	LSL
NYA-2 + P-D + Weekly watering	15.78 ± 0.47 ghijk	LV
NAK-2 + NYA-2 + NSD + P-D + Weekly watering	15.43 ± 1.56 ghijkl	LV
NAK-2 + NYA-2 + P-D + Weekly watering	15.18 ± 0.95 ghijkl	LV
NAK-2 + NYA-2 + NSD + CNS + Weekly watering	13.82 ± 0.86 hijklm	LV
NAK-2 + P-D + Weekly watering	13.71 ± 1.48 hijklm	LV
NSD + CNS + Weekly watering	13.56 ± 1.16 hijklmn	LV
Uninoculated + P-D + Weekly watering	13.15 ± 1.11 ijklmn	LSL

NAK-2 + NYA-2 + CNS + Weekly watering	13.08 ± 0.83 jklmno	LV
NSD + N-D + Daily watering	11.06 ± 1.23 klmnop	LV
NYA-2 + CNS + Weekly watering	10.78 ± 2.00 klmnop	LV
NAK-2 + CNS + Weekly watering	10.43 ± 1.39 klmnop	LV
Uninoculated + CNS + Weekly watering	10.32 ± 1.55 klmnop	LSL
NAK-2 + NYA-2 + N-D + Daily watering	10.07 ± 1.25 lmnopq	LV
NYA-2 + N-D + Daily watering	8.49 ± 1.86 mnopq	LV
NAK-2 + NYA-2 + NSD + N-D + Daily watering	7.72 ± 1.22 mnopq	LV
NAK-2 + NYA-2 + NSD + P-D + Daily watering	7.57 ± 0.91 mnopq	LV
Uninoculated + N-D + Daily watering	7.55 ± 1.49 mnopqr	LSL
NSD + P-D + Daily watering	6.90 ± 0.63 nopqrs	LV
NAK-2 + P-D + Daily watering	6.38 ± 1.54 nopqrs	LV
NAK-2 + N-D + Daily watering	5.60 ± 0.60 opqrs	LV
Uninoculated + P-D + Daily watering	5.19 ± 1.71 opqrs	LSL
NYA-2 + P-D + Daily watering	4.71 ± 1.23 opqrs	LV
NAK-2 + NYA-2 + NSD + CNS + Daily watering	4.39 ± 1.10 opqrs	LV
NAK-2 + NYA-2 + P-D + Daily watering	4.12 ± 1.67 opqrs	LV
NSD + CNS + Daily watering	3.90 ± 1.52 opqrs	LV
NAK-2 + NYA-2 + CNS + Daily watering	3.73 ± 0.98 pqrs	LV
NYA-2 + CNS + Daily watering	1.83 ± 1.57 qrs	LV
Uninoculated + CNS + Daily watering	0.00 ± 0.38 rs	LSL
NAK-2 + CNS + Daily watering	- 0.70 ± 2.21 s	LV
Test values	**df = 47 ; F= 64.92; P ≤ 0.0001**	

The table shows means ± standard errors in percentage in descending order. The virulence classification MV/MSL; denotes Moderate Virulence/Moderate stress levels which is classified when ≥ 25% whereas LV / LSL denotes (Low virulence / Low stress levels which is classified when < 25%).The head smut isolates (*Ustilago kamerunensis*) used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (Napier grass stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. Nutrient formulations entailed CNS (complete nutrient solution), N/P-D (Nitrogen phosphorus deficient solution), N-D (Nitrogen deficient solution) and P-D (Phosphorus deficient solution). The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

In summary the NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) when applied alone caused more damage, followed by the treatment combinations where NSD was combined with the *Ustilago kamerunensis* isolates (NAK-2 and NYA-2). However, the NAK-2 isolate when applied alone caused the least damage to the napier varieties, followed by NYA-2 isolate sole infections. Under the nutrient solutions the treatments where nitrogen and phosphorus deficient nutrient solution (N/P-D) was applied, they recorded the highest degrees of damage, followed by those where the nitrogen deficient solution was applied. Application of the complete nutrient solution caused the lowest degree of damage, while phosphorus deficient

nutrient solution's treatments caused the second lowest degree of damage on the grass. On the watering regimes the weekly watered treatments caused the highest degree of damage compared to the daily watered treatments These trend of pathogen combination, nutrient formulations and watering regimes' performance seemed consistent in the specific analyses shown in tables 4.7-4.14.

4.2.2 Effects of nutrients formulations and watering on time taken for disease expression of *Ustilago kamerunensis* and '*Candidatus* Phytoplasma oryzae' strain Mbita 1

The results of the effects of nitrogen deficient nutrient solution on the degree of damage are presented in tables 4.8 and 4.9. Under daily watering regime the mean virulence was 8.42 ± 2.91%. The mean disease expression/ time taken for symptom expression score was 3/3 (Table 4.7). The numerator value three (3) of the score meant that the disease expression levels of the varieties in this treatment combination generally were in the category of very low susceptibility class. Whereas, the denominator value three (3) of the score on time taken for symptom expression, meant that the treatments under daily watering and nitrogen deficiency nutrient solution largely expressed their first symptoms after the third cutting/harvest of the crop (Table 4.7). In the weekly watered treatments the mean virulence was 26.08 ± 3.89% with a mean disease expression/ time taken for symptom expression score of 2/3 (Table 4.9). The numerator value two (2) corresponded to the low susceptibility class of the disease expression levels of the varieties. Whereas, on time taken for symptom expression, the denominator value three (3) meant that the treatments under weekly watering regime largely expressed their first symptoms after the third cutting/harvest of the crop (Table 4.9).

The phosphorus deficient nutrient solution treatments had a mean virulence of 5.81 ± 2.63%, with a mean disease expression/ time taken for symptom expression score of 3/4 (Table 4.9). The numerator value three (3) meant that the disease expression levels of the varieties in this treatment combination were in the class of very low susceptibility to the pathogen. Whereas, the denominator value four (4) on time taken for symptom expression meant that the treatments under daily watering and phosphorus deficiency nutrient solution, expressed their first symptoms after the fourth cutting/harvest of the crop (Table 4.9). In the weekly watered treatments the virulence levels had a mean of 15.25 ± 2.53% with a mean disease expression/ time taken for symptom expression score of 3/3 (Table 4.10). The numerator value three (3) meant that the disease expression levels of the varieties was in the category of very low susceptibility.

Whereas, on time taken for symptom expression; the denominator value three (3) meant that the treatments under weekly watering regime expressed their first symptoms after the third cutting/harvest of the crop (Table 4.10).

The mean virulence levels for the nitrogen and phosphorus deficient nutrient solution under daily watered treatments was 18.68 ± 2.90%. The mean disease expression/ time taken for symptom expression score was 3/3 (Table 4.11). The numerator value (3) meant that the disease expression levels of the varieties in this treatment were in the class of very low susceptibility. Whereas, the denominator value three (3) on time taken for symptom expression meant that the treatments under this trial expressed their first symptoms after the third cutting/harvest of the crop (Table 4.11). For the weekly watered treatments the mean virulence was 33.52 ± 2.30% with a mean disease expression/ time taken for symptom expression score of 2/3 (Table 4.12). The numerator value two (2) meant that the disease expression levels of the varieties was generally in the category of low susceptibility. Whereas, on time taken for symptom expression, the denominator value three (3) meant that the treatments under weekly watering regime expressed their first symptoms after the third harvest of the crop (Table 4.12).

The complete nutrient solution's treatments had a mean virulence of 2.19 ± 3.15% under daily watering. The mean disease expression/ time taken for symptom expression score was 3/4 (Table 4.13). The numerator value three (3) meant that the disease expression levels of the varieties in this treatment combination were in the category of very low susceptibility classification. Whereas, the denominator value four (4) on time taken for symptom expression, meant that the treatments expressed their first symptoms after the fourth cutting/harvest of the crop (Table 4.13). In the weekly watered treatments under complete nutrient solution, the virulence mean was 12.00 ± 2.79%. The mean disease expression/ time taken for symptom expression score was 3/3 (Table 4.14). The numerator value three (3) meant that the disease expression levels of the napier grass varieties under this treatment was generally in the category of very low susceptibility classification. Whereas, on time taken for symptom expression; the denominator value three (3) meant that the treatments under weekly watering regime expressed their first symptoms after the third cutting/harvest of the crop (Table 4.14).

Table 4.7: Effects of nitrogen deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under daily watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NSD + N-D + D	3.88	-0.45	-10.40	89.60	10.40	LV	3/2
KK1 + NAK-2+NYA-2 +N-D + D	3.99	-0.34	-7.94	92.06	7.94	LV	3/4
KK1 + NAK-2 + N-D + D	4.01	-0.32	-7.36	92.64	7.36	LV	3/4
KK1 + NAK-2+NYA-2+NSD + N-D + D	4.08	-0.26	-5.96	94.04	5.96	LV	3/2
KK1 + NYA-2 + N-D + D	4.15	-0.18	-4.13	95.87	4.13	LV	3/4
KK1 Uninoculated + N-D + D	4.19	-0.14	-3.30	96.70	3.30	LSL	N/A
KK2 + NAK-2+NYA-2+NSD + N-D + D	3.84	-0.49	-11.31	88.69	11.31	LV	3/2
KK2 + NYA-2 + N-D + D	3.93	-0.41	-9.41	90.59	9.41	LV	3/4
KK2 Uninoculated + N-D + D	3.95	-0.39	-8.89	91.11	8.89	LSL	N/A
KK2 + NSD + N-D + D	3.98	-0.35	-8.16	91.84	8.16	LV	3/2
KK2 + NAK-2+NYA-2 + N-D + D	3.99	-0.34	-7.90	92.10	7.90	LV	3/4
KK2 + NAK-2 + N-D + D	4.11	-0.22	-5.09	94.91	5.09	LV	3/4
Bana + NSD + N-D + D	3.72	-0.61	-14.07	85.93	14.07	LV	3/2
Bana + NYA-2 + N-D + D	3.77	-0.56	-13.03	86.97	13.03	LV	3/4
Bana + NAK-2+NYA-2 + N-D +D	3.79	-0.55	-12.61	87.39	12.61	LV	3/4
Bana Uninoculated + N-D + D	3.99	-0.34	-7.92	92.08	7.92	LSL	N/A
Bana + NAK-2+NYA-2+NSD + N-D + D	4.02	-0.31	-7.20	92.80	7.20	LV	3/3
Bana + NAK-2 + N-D + D	4.11	-0.23	-5.25	94.75	5.25	LV	3/4
16789 + NAK-2+NYA-2 + N-D + D	3.82	-0.51	-11.83	88.17	11.83	LV	3/4
16789 + NSD + N-D +D	3.83	-0.50	-11.60	88.40	11.60	LV	3/4
16789 Uninoculated + N-D +D	3.90	-0.44	-10.11	89.89	10.11	LSL	N/A
16789 + NYA-2 + N-D +D	4.01	-0.32	-7.39	92.61	7.39	LV	3/4
16789 + NAK-2+NYA-2+NSD + N-D + D	4.06	-0.28	-6.41	93.59	6.41	LV	3/3
16789 + NAK-2 + N-D + D	4.13	-0.20	-4.69	95.31	4.69	LV	3/4
			Mean virulence/stress levels in (%)		**8.42 ± 2.91**		**3/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier varieties under (Uninoculated + CNS + D treatments). The letter D denotes daily watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.8: Effects of nitrogen deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under weekly watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NAK-2+NYA-2+NSD + N-D + W	2.96	-1.37	-31.68	68.32	31.68	MV	2/2
KK1 + NSD + N-D +W	3.04	-1.29	-29.73	70.27	29.73	MV	2/2
KK1 + NAK-2+NYA-2 + N-D + W	3.19	-1.15	-26.44	73.56	26.44	MV	2/4
KK1 Uninoculated + N-D + W	3.40	-0.93	-21.48	78.52	21.48	LSL	N/A
KK1 + NYA-2 + N-D + W	3.45	-0.88	-20.38	79.62	20.38	LV	3/4
KK1 + NAK-2 + N-D + W	3.46	-0.88	-20.21	79.79	20.21	LV	3/4
KK2 + NSD + N-D + W	2.97	-1.37	-31.51	68.49	31.51	MV	2/2
KK2 + NAK-2+NYA-2 + N-D + W	3.03	-1.31	-30.16	69.84	30.16	MV	2/4
KK2 + NAK-2+NYA-2+NSD + N-D + W	3.08	-1.25	-28.83	71.17	28.83	MV	2/3
KK2 + NYA-2 + N-D + W	3.25	-1.08	-24.90	75.10	24.90	MV	2/4
KK2 + NAK-2 + N-D + W	3.25	-1.08	-24.89	75.11	24.89	MV	2/4
KK2 Uninoculated + N-D + W	3.42	-0.91	-21.04	78.96	21.04	LSL	N/A
Bana + NAK-2+NYA-2+NSD + N-D + W	2.83	-1.51	-34.79	65.21	34.79	MV	2/3
Bana + NSD + N-D + W	2.98	-1.36	-31.30	68.70	31.30	MV	2/2
Bana + NAK-2+NYA-2 + N-D + W	3.24	-1.09	-25.24	74.76	25.24	MV	2/4
Bana + NYA-2 + N-D +W	3.26	-1.07	-24.72	75.28	24.72	MV	2/4
Bana + NAK-2 + N-D + W	3.27	-1.06	-24.45	75.55	24.45	MV	2/4
Bana Uninoculated + N-D + W	3.28	-1.05	-24.27	75.73	24.27	LSL	N/A
16789 + NSD + N-D +W	3.06	-1.27	-29.40	70.60	29.40	MV	2/3
16789 + NAK-2+NYA-2+NSD + N-D + W	3.25	-1.08	-24.89	75.11	24.89	MV	2/3
16789 + NYA-2 + N-D +W	3.26	-1.08	-24.82	75.18	24.82	MV	2/4
16789 + NAK-2 + N-D + W	3.26	-1.07	-24.74	75.26	24.74	MV	2/4
16789 + NAK-2+NYA-2 + N-D + W	3.28	-1.05	-24.21	75.79	24.21	LV	3/4
16789 Uninoculated + N-D +W	3.38	-0.95	-21.93	78.07	21.93	LSL	N/A
			Mean virulence/stress levels in (%)		**26.08 ± 3.89**		**2/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier varieties under (Uninoculated + CNS + D treatments). The letter W denotes weekly watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.9: Effects of phosphorus deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under daily watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NAK-2 + P-D + D	4.05	-0.29	-6.63	93.37	6.63	LV	3/4
KK1 + NSD + P-D + D	4.10	-0.23	-5.32	94.68	5.32	LV	3/3
KK1 + NAK-2+NYA-2+NSD + P-D + D	4.10	-0.23	-5.28	94.72	5.28	LV	3/3
KK1 + NYA-2 + P-D + D	4.24	-0.09	-2.13	97.87	2.13	LV	3/4
KK1 + NAK-2+NYA-2 + P-D + D	4.28	-0.05	-1.16	98.84	1.16	LV	3/4
KK1 Uninoculated + P-D + D	4.32	-0.01	-0.31	99.69	0.31	LSL	N/A
KK2 + NAK-2+NYA-2+NSD + P-D + D	3.91	-0.42	-9.75	90.25	9.75	LV	3/3
KK2 + NAK-2+NYA-2 + P-D +D	3.95	-0.39	-8.91	91.09	8.91	LV	3/4
KK2 + NSD + P-D + D	4.03	-0.30	-6.95	93.05	6.95	LV	3/3
KK2 Uninoculated + P-D + D	4.07	-0.26	-6.03	93.97	6.03	LSL	N/A
KK2 + NYA-2 + P-D + D	4.13	-0.20	-4.72	95.28	4.72	LV	3/4
KK2 + NAK-2 + P-D + D	4.24	-0.09	-2.16	97.84	2.16	LV	3/4
Bana + NAK-2 + P-D + D	3.92	-0.41	-9.53	90.47	9.53	LV	3/4
Bana + NSD + P-D + D	3.97	-0.36	-8.39	91.61	8.39	LV	3/3
Bana + NAK-2+NYA-2+NSD + P-D + D	4.00	-0.33	-7.70	92.30	7.70	LV	3/3
Bana Uninoculated + P-D + D	4.07	-0.27	-6.16	93.84	6.16	LSL	N/A
Bana + NYA-2 + P-D + D	4.16	-0.17	-3.94	96.06	3.94	LV	3/4
Bana + NAK-2+NYA-2 + P-D +D	4.19	-0.15	-3.41	96.59	3.41	LV	3/4
16789 Uninoculated + P-D + D	3.98	-0.36	-8.27	91.73	8.27	LSL	N/A
16789 + NYA-2 + P-D + D	3.98	-0.35	-8.04	91.96	8.04	LV	3/4
16789 + NAK-2+NYA-2+NSD + P-D +D	4.01	-0.33	-7.56	92.44	7.56	LV	3/3
16789 + NAK-2 + P-D + D	4.02	-0.31	-7.19	92.81	7.19	LV	3/4
16789 + NSD + P-D + D	4.03	-0.30	-6.94	93.06	6.94	LV	3/3
16789 + NAK-2+NYA-2 + P-D + D	4.20	-0.13	-3.01	96.99	3.01	LV	3/4
			Mean virulence/stress levels in (%)		**5.81 ± 2.63**		**3/4**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter D denotes daily watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.10: Effects of phosphorus deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under weekly watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NSD + P-D + W	3.59	-0.75	-17.21	82.79	17.21	LV	3/2
KK1 + NYA-2 + P-D +W	3.60	-0.73	-16.94	83.06	16.94	LV	3/4
KK1 Uninoculated + P-D +W	3.62	-0.71	-16.43	83.57	16.43	LSL	N/A
KK1 + NAK-2+NYA-2 + P-D + W	3.64	-0.70	-16.11	83.89	16.11	LV	3/4
KK1 + NAK-2 + P-D +W	3.66	-0.68	-15.59	84.41	15.59	LV	3/4
KK1 + NAK-2+NYA-2+NSD + P-D + W	3.70	-0.63	-14.53	85.47	14.53	LV	3/3
KK2 + NSD + P-D + W	3.60	-0.73	-16.87	83.13	16.87	LV	3/2
KK2 + NAK-2+NYA-2+NSD + P-D +W	3.69	-0.64	-14.83	85.17	14.83	LV	3/2
KK2 + NYA-2 + P-D + W	3.70	-0.63	-14.65	85.35	14.65	LV	3/4
KK2 + NAK-2+NYA-2 + P-D +W	3.77	-0.56	-12.97	87.03	12.97	LV	3/4
KK2 Uninoculated + P-D + W	3.81	-0.52	-12.07	87.93	12.07	LV	N/A
KK2 + NAK-2 + P-D + W	3.85	-0.48	-11.15	88.85	11.15	LSL	3/4
Bana + NSD + P-D + W	3.50	-0.84	-19.29	80.71	19.29	LV	3/2
Bana + NAK-2+NYA-2 + P-D + W	3.59	-0.75	-17.26	82.74	17.26	LV	3/4
Bana + NAK-2 + P-D + W	3.60	-0.73	-16.87	83.13	16.87	LV	3/4
Bana + NYA-2 + P-D + W	3.64	-0.69	-15.97	84.03	15.97	LV	3/4
Bana + NAK-2+NYA-2+NSD + P-D + W	3.79	-0.54	-12.53	87.47	12.53	LV	3/3
Bana Uninoculated + P-D + W	3.83	-0.50	-11.61	88.39	11.61	LSL	N/A
16789 + NAK-2+NYA-2+NSD + P-D + W	3.47	-0.86	-19.85	80.15	19.85	LV	3/3
16789 + NSD + P-D +W	3.48	-0.85	-19.59	80.41	19.59	LV	3/2
16789 + NYA-2 + P-D +W	3.66	-0.67	-15.57	84.43	15.57	LV	3/4
16789 + NAK-2+NYA-2 + P-D + W	3.71	-0.62	-14.37	85.63	14.37	LV	3/4
16789 + Uninoculated + P-D +W	3.79	-0.54	-12.50	87.50	12.50	LSL	N/A
16789 + NAK-2 + P-D + W	3.85	-0.49	-11.22	88.78	11.22	LV	3/4
				Mean virulence levels in (%)	**15.25 ± 2.53**		**3/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter W denotes weekly watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.11: Effects of nitrogen and phosphorus deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under daily watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NAK-2+NYA-2 + N/P-D + D	3.38	-0.96	-22.08	77.92	22.08	LV	3/4
KK1 + NAK-2+NYA-2+ NSD + N/P-D + D	3.46	-0.87	-20.18	79.82	20.18	LV	3/2
KK1 + NYA-2 + N/P-D + D	3.49	-0.84	-19.47	80.53	19.47	LV	3/4
KK1 + NSD + N/P-D + D	3.52	-0.81	-18.75	81.25	18.75	LV	3/1
KK1 + NAK-2+ N/P-D + D	3.69	-0.64	-14.78	85.22	14.78	LV	3/4
KK1 Uninoculated + N/P-D + D	3.76	-0.58	-13.32	86.68	13.32	LSL	N/A
KK2 + NSD + N/P-D + D	3.37	-0.96	-22.25	77.75	22.25	LV	3/1
KK2 + NAK-2+NYA-2+NSD + N/P-D + D	3.44	-0.90	-20.72	79.28	20.72	LV	3/2
KK2 Uninoculated + N/P-D + D	3.57	-0.76	-17.55	82.45	17.55	LSL	N/A
KK2 + NAK-2 + N/P-D + D	3.58	-0.76	-17.45	82.55	17.45	LV	3/4
KK2 + NYA-2 + N/P-D + D	3.60	-0.74	-16.97	83.03	16.97	LV	3/4
KK2 + NAK-2+NYA-2 + N/P-D + D	3.74	-0.59	-13.62	86.38	13.62	LV	3/4
Bana + NAK-2+NYA-2+NSD + N/P-D +D	3.41	-0.93	-21.42	78.58	21.42	LV	3/2
Bana + NSD + N/P-D + D	3.43	-0.91	-20.95	79.05	20.95	LV	3/1
Bana + NAK-2+NYA-2 + N/P-D + D	3.53	-0.80	-18.45	81.55	18.45	LV	3/4
Bana + NYA-2 + N/P-D +D	3.58	-0.75	-17.32	82.68	17.32	LV	3/4
Bana Uninoculated + N/P-D + D	3.65	-0.68	-15.75	84.25	15.75	LSL	N/A
Bana + NAK-2 + N/P-D + D	3.75	-0.58	-13.46	86.54	13.46	LV	3/4
16789 + NSD + N/P-D + D	3.30	-1.04	-23.93	76.07	23.93	LV	3/2
16789 + NYA-2 + N/P-D + D	3.39	-0.94	-21.77	78.23	21.77	LV	3/4
16789 + NAK-2+NYA-2+NSD + N/P-D + D	3.45	-0.88	-20.31	79.69	20.31	LV	3/2
16789 + NAK-2+NYA-2 + N/P-D + D	3.45	-0.88	-20.30	79.70	20.30	LV	3/4
16789 + NAK-2 + N/P-D + D	3.47	-0.87	-19.99	80.01	19.99	LV	3/4
16789 Uninoculated + N/P-D + D	3.56	-0.77	-17.73	82.27	17.73	LSL	N/A
				Mean virulence levels in (%)	**18.68 ± 2.90**		**3/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter D denotes daily watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.12: Effects of nitrogen and phosphorus deficiency on the virulence levels (VIRP) of the pathogens on napier grass cultivars under weekly watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NSD + N/P-D + W	2.88	-1.45	-33.56	66.44	33.56	MV	2/1
KK1 + NAK-2 + N/P-D + W	2.89	-1.44	-33.24	66.76	33.24	MV	2/4
KK1 + NYA-2 + N/P-D + W	2.92	-1.42	-32.68	67.32	32.68	MV	2/4
KK1 + NAK-2+NYA-2 + N/P-D + W	2.92	-1.41	-32.55	67.45	32.55	MV	2/4
KK1 + NAK-2+NYA-2+NSD + N/P-D + W	2.93	-1.40	-32.29	67.71	32.29	MV	2/4
KK1 Uninoculated + N/P-D + W	3.02	-1.31	-30.30	69.70	30.30	MSL	N/A
KK2 + NSD + N/P-D + W	2.63	-1.71	-39.41	60.59	39.41	MV	2/1
KK2 + NAK-2+NYA-2 + N/P-D + W	2.83	-1.51	-34.73	65.27	34.73	MV	2/4
KK2 Uninoculated + N/P-D + W	2.85	-1.49	-34.33	65.67	34.33	MSL	N/A
KK2 + NYA-2 + N/P-D + W	2.90	-1.43	-33.08	66.92	33.08	MV	2/4
KK2 + NAK-2+NYA-2+NSD + N/P-D + W	2.90	-1.43	-32.99	67.01	32.99	MV	2/2
KK2 + NAK-2 + N/P-D + W	2.99	-1.34	-30.94	69.06	30.94	MV	2/4
Bana + NAK-2+NYA-2+NSD + N/P-D + W	2.72	-1.61	-37.24	62.76	37.24	MV	2/1
Bana + NSD + N/P-D + W	2.74	-1.60	-36.81	63.19	36.81	MV	2/1
Bana + NAK-2+NYA-2 + N/P-D + W	2.76	-1.58	-36.41	63.59	36.41	MV	2/4
Bana + NYA-2 + N/P-D + W	2.82	-1.51	-34.83	65.17	34.83	MV	2/4
Bana Uninoculated + N/P-D + W	2.87	-1.46	-33.71	66.29	33.71	MSL	N/A
Bana + NAK-2 + N/P-D + W	3.03	-1.31	-30.14	69.86	30.14	MV	2/4
16789 + NSD + N/P-D + W	2.79	-1.54	-35.61	64.39	35.61	MV	2/1
16789 Uninoculated + N/P-D + W	2.89	-1.44	-33.30	66.70	33.30	MSL	N/A
16789 + NAK-2+NYA-2+ NSD + N/P-D + W	2.91	-1.42	-32.79	67.21	32.79	MV	2/1
16789 +NYA-2 + N/P-D + W	2.94	-1.39	-32.16	67.84	32.16	MV	2/4
16789 + NAK-2 + N/P-D + W	2.99	-1.34	-30.95	69.05	30.95	MV	2/4
16789 + NAK-2+NYA-2 + N/P-D + W	3.01	-1.32	-30.48	69.52	30.48	MV	2/4
				Mean virulence levels in (%)	**33.52 ± 2.30**		**2/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index(IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter W denotes weekly watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.13: Effects of complete nutrient solution on the virulence levels (VIRP) of the pathogens on napier grass cultivars under daily watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333							
Treatments	**Integrated Parameter Mean logarithmic index (IPMLI)**	**Logarithmic deviation from the mean controls index (LoD)**	**Specific deviation in (%) (SpD)**	**Overall performance levels in (%) (OPL)**	**Virulence levels in (%) (VIRP)**	**Virulence classification**	**Disease expression / Time taken to express symptoms scores (D/T)**
KK1 + NAK-2+NYA-2 + CNS +D	4.06	-0.28	-6.35	93.65	6.35	LV	3/4
KK1 + NSD + CNS + D	4.24	-0.09	-2.13	97.87	2.13	LV	3/3
KK1 + NAK-2+NYA-2+NSD + CNS + D	4.24	-0.09	-2.12	97.88	2.12	LV	3/3
KK1 + NYA-2 + CNS + D	4.32	-0.01	-0.29	99.71	0.29	LV	3/4
KK1 Uninoculated + CNS +D	4.33	0.00	-0.09	99.91	0.09	LSL	N/A
KK1 + NAK-2 + CNS + D	4.47	0.13	3.11	103.11	-3.11	LV	3/4
KK2 + NAK-2+NYA-2+NSD +CNS + D	4.11	-0.22	-5.13	94.87	5.13	LV	3/3
KK2 + NSD + CNS + D	4.17	-0.16	-3.68	96.32	3.68	LV	3/3
KK2 + NAK-2+NYA-2 + CNS + D	4.26	-0.07	-1.67	98.33	1.67	LV	3/4
KK2 Uninoculated + CNS + D	4.37	0.04	0.83	100.83	-0.83	LSL	N/A
KK2 + NYA-2 + CNS + D	4.40	0.07	1.52	101.52	-1.52	LV	3/4
KK2 + NAK-2 + CNS + D	4.56	0.22	5.17	105.17	-5.17	LV	3/4
Bana + NAK-2+NYA-2+NSD + CNS + D	4.03	-0.31	-7.11	92.89	7.11	LV	3/3
Bana + NYA-2 + CNS + D	4.09	-0.25	-5.71	94.29	5.71	LV	3/4
Bana + NAK-2 + CNS + D	4.12	-0.21	-4.89	95.11	4.89	LV	3/4
Bana + NAK-2+NYA-2 + CNS + D	4.20	-0.13	-3.07	96.93	3.07	LV	3/4
Bana + NSD + CNS + D	4.27	-0.07	-1.55	98.45	1.55	LV	3/3
Bana Uninoculated + CNS + D	4.29	-0.04	-0.97	99.03	0.97	LSL	N/A
16789 + NSD + CNS + D	3.98	-0.36	-8.26	91.74	8.26	LV	3/3
16789 + NAK-2+NYA-2 + CNS + D	4.17	-0.17	-3.82	96.18	3.82	LV	3/4
16789 + NAK-2+NYA-2+NSD + CNS + D	4.19	-0.14	-3.20	96.80	3.20	LV	3/3
16789 + NYA-2 + CNS + D	4.21	-0.12	-2.84	97.16	2.84	LV	3/4
16789 + NAK-2 + CNS + D	4.31	-0.03	-0.60	99.40	0.60	LV	3/4
16789 Uninoculated + CNS + D	4.34	0.01	0.23	100.23	-0.23	LSL	N/A
			Mean virulence levels in (%)		**2.19 ± 3.15**		**3/4**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter D denotes daily watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

Table 4.14: Effects of complete nutrient solution on the virulence levels (VIRP) of the pathogens on napier grass cultivars under weekly watering regimes across the four ratoons.

Integrated parameters mean logarithmic index for the controls of the four napier grass varieties (IPMLIC) = 4.3333

Treatments	Integrated Parameter Mean logarithmic index (IPMLI)	Logarithmic deviation from the mean controls index (LoD)	Specific deviation in (%) (SpD)	Overall performance levels in (%) (OPL)	Virulence levels in (%) (VIRP)	Virulence classification	Disease expression / Time taken to express symptoms scores (D/T)
KK1 + NAK-2+NYA-2+NSD + CNS +W	3.73	-0.61	-14.01	85.99	14.01	LV	3/2
KK1 + NAK-2 + CNS +W	3.74	-0.59	-13.66	86.34	13.66	LV	3/4
KK1 + NSD + CNS + W	3.78	-0.55	-12.66	87.34	12.66	LV	3/2
KK1 + NYA-2 + CNS + W	3.80	-0.53	-12.31	87.69	12.31	LV	3/4
KK1+ NAK-2+NYA-2 + CNS +W	3.85	-0.48	-11.06	88.94	11.06	LV	3/4
KK1 Uninoculated + CNS + W	3.95	-0.38	-8.84	91.16	8.84	LSL	N/A
KK2 + NAK-2+NYA-2+NSD + CNS + W	3.64	-0.69	-15.95	84.05	15.95	LV	3/2
KK2 + NSD + CNS + W	3.65	-0.68	-15.79	84.21	15.79	LV	3/2
KK2 + NAK-2+NYA-2 + CNS + W	3.87	-0.46	-10.59	89.41	10.59	LV	3/4
KK2 + NYA-2 + CNS + W	3.92	-0.42	-9.59	90.41	9.59	LV	3/4
KK2 + NAK-2 + CNS + W	3.96	-0.37	-8.59	91.41	8.59	LV	3/4
KK2 Uninoculated + CNS + W	4.04	-0.30	-6.82	93.18	6.82	LSL	N/A
Bana + NYA-2 + CNS + W	3.61	-0.72	-16.68	83.32	16.68	LV	3/4
Bana + NSD + CNS + W	3.67	-0.66	-15.21	84.79	15.21	LV	3/2
Bana + NAK-2+NYA-2+NSD + CNS +W	3.71	-0.63	-14.47	85.53	14.47	LV	3/3
Bana Uninoculated + CNS + W	3.72	-0.61	-14.17	85.83	14.17	LSL	N/A
Bana + NAK-2+NYA-2 + CNS + W	3.72	-0.61	-14.05	85.95	14.05	LV	3/4
Bana + NAK-2 + CNS + W	3.82	-0.51	-11.77	88.23	11.77	LV	3/4
16789 + NAK-2+NYA-2 + CNS + W	3.74	-0.59	-13.67	86.33	13.67	LV	3/4
16789 + NAK-2+NYA-2+NSD + CNS + W	3.81	-0.53	-12.19	87.81	12.19	LV	3/3
16789 + NSD + CNS + W	3.86	-0.47	-10.92	89.08	10.92	LV	3/3
16789 Uninoculated + CNS + W	3.93	-0.40	-9.26	90.74	9.26	LSL	N/A
16789 + NYA-2 + CNS + W	3.99	-0.35	-7.98	92.02	7.98	LV	3/4
16789 + NAK-2 + CNS + W	4.00	-0.33	-7.70	92.30	7.70	LV	3/4
			Mean virulence levels in (%)		**12.00 ± 2.79**		**3/3**

The overall percentage was determined by dividing the integrated parameter mean logarithmic index by the mean logarithmic index (IPMLIC = 4.3333) of the four napier grass varieties under (Uninoculated + CNS + D treatments). The letter W denotes weekly watering whereas the pathogens are denoted as; (NAK-2 isolate, NYA-2 isolate and NSD in different combinations).

4.3 Evaluation of co-infection effects of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 on the growth of napier grass varieties

The results on evaluation of the co-infection effects of the pathogens on the growth of napier grass focuses on the specific individual treatments' performance, then delves into the interactions performances since there were so many treatments that performed intermediately as illustrated in table 4.15.

4.3.1 Co-infection effects of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 on the growth of napier grass varieties across the four ratoons/cropping cycles based on means and logarithmic indices

The total fresh weight, tiller heights and chlorophyll content level parameters of the 192 treatment combinations exhibited significant differences at df = 191; F = 28.217; $P \leq 0.0001$, df = 191; F = 8.05; $P \leq 0.0001$ and df = 191; F = 15.74; $P \leq 0.0001$ respectively (Table 4.15). Majority of the treatments performed in an intermediate manner basing on the post hoc analyses shown in table 4.15. The high variability in parameter performance meant that for proper ranking and evaluation of performance, integration of parameters through logarithmic indexing (where the mean of the natural logarithms of respective growth parameters for each individual treatment is determined as an efficacy index) was critical as demonstrated in table 4.15.

The mean ± standard deviation of the natural logarithmic indices of the 192 treatments was 4.099 ± 0.410. The highest performing specific treatment holistically based on the mean of natural logarithmic indices of total fresh weight, tiller height and chlorophyll content levels was Kakamega 2 variety that was infected by only NAK-2 *Ustilago kamerunensis* isolate on complete nutrient solution and daily watering regime (KK 2 + NAK-2 + CNS + D), that had the highest mean logarithmic index of 4.89 (Table 4.15). This treatment was followed by that of Kakamega 1 variety infected by only NAK-2 *Ustilago kamerunensis* isolate (KK 1 + NAK-2 + CNS + D); and Kakamega 2 variety not infected by any pathogen under complete nutrient solution treatment and daily watering (KK 2 uninoculated + CNS + D), with mean logarithmic indices of 4.88 and 4.81 respectively (Table 4.15). These high logarithmic indices was as a result of the high means witnessed on the general growth of napier varieties under the mentioned treatments in total fresh weight, tiller height and high chlorophyll content levels output (Table 4.15).

In the low performing treatments the specific treatment combination Kakamega 2 variety that was infected by only NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) nourished by nitrogen and phosphorus deficient solution and watered weekly (KK 2 + NSD + N/P-D + W), had the lowest mean logarithmic index of 3.19 (table 4.15). This treatment was followed by accession 16789 that was infected by only NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) nourished by nitrogen and phosphorus deficient solution under weekly watering (16789 + NSD + N/P-D + W) and Bana variety that was infected by only NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) nourished by nitrogen and phosphorus deficient solution under weekly watering (Bana + NSD + N/P-D + W). These two had mean logarithmic indices of 3.26 and 3.30 respectively (Table 4.15). These low logarithmic indices was as a result of the low means witnessed on the general growth of napier varieties under the mentioned treatments in total fresh weight, tiller height and high chlorophyll content levels output (Table 4.15).

In summary from the table 4.15, the *Ustilago kamerunensis* isolates infected only treatments under complete nutrient solution and daily watering seemed to support high mean logarithmic indices as result of high means of the parameters measured viz; total fresh weight, tiller height and chlorophyll content levels, followed by the *Ustilago kamerunensis* isolates infected only treatments under phosphorus deficient nutrient solution and daily watered treatments. Whereas, the NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) infected only treatments under nitrogen and phosphorus deficient nutrient solution on weekly watering seemed to support low mean logarithmic indices resulting from the low means in performance of the total fresh weight, tiller height and chlorophyll content levels (Table 4.15), followed by the NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) infected only treatments under nitrogen deficient nutrient solution and watered weekly as illustrated in table 4.15.

Table 4.15: A table showing the specific performance of the 192 treatment combinations on their respective selected parameters' means that are significantly affected by the diseases under co-infection. Whereas IPLI denote (Integrated Parameter Logarithmic Index of the three parameters). The mean of their respective natural logarithms is the one denoted as Mean IPLI or M.L.I.

Treatments	Total Fresh Weight (Grams)	Tiller height (Cm)	Chlorophyll Content (SPAD Units)	Mean IPLI or (M.L.I)
KK2 + NAK-2 + CNS + D	390.54 ± 1.10 a	124.22 ± 1.08 abcdefghijk	48.40 ± 1.07abcde	4.89
KK1 + NAK-2 + CNS + D	356.18 ± 1.11 ab	139.52 ± 1.05 abcdefgh	45.94 ± 1.06 abcdef	4.88
KK2 Uninoculated + CNS + D	296.83 ± 1.13 abcd	140.93 ± 1.05abcdefg	43.83 ± 1.06 abcdef	4.81
16789 + NAK-2 + CNS + D	235.78 ± 1.18 abcdefghi	142.35 ± 1.05 abcdef	50.17 ± 1.05ab	4.78
KK1 Uninoculated + CNS +D	265.56 ± 1.13 abcdef	133.56 ± 1.05 abcdefghij	46.37 ± 1.07 abcdef	4.77
KK1 + NYA-2 + CNS + D	274.89 ± 1.18 abcde	134.47 ± 1.05 abcdefghi	43.52 ± 1.06 abcdefg	4.76
Bana Uninoculated + CNS + D	262.52 ± 1.17 abcdefg	153.59 ± 1.06 abc	38.55 ± 1.08 abcdefghij	4.75
Bana + NAK-2 + CNS + D	211.76 ± 1.14 abcdefghijk	155.65 ± 1.03 ab	46.30 ± 1.09 abcdef	4.75
KK2 + NYA-2 + CNS + D	302.00 ± 1.12 abc	127.38 ± 1.05 abcdefghijk	38.33 ± 1.10 abcdefghij	4.73
KK2 + NAK-2+ NYA-2 + CNS + D	248.31 ± 1.11 abcdefgh	139.88 ± 1.04 abcdefg	42.16 ± 1.06 abcdefghij	4.73
KK1 + NAK-2+NYA-2 + CNS +D	179.54 ± 1.10 abcdefghijk	134.33 ± 1.07 abcdefghi	51.24 ± 1.17a	4.68
16789 + NYA-2 + CNS + D	198.76 ± 1.09 abcdefghijk	140.59 ± 1.04 abcdefg	43.12 ± 1.05 abcdefghi	4.67
16789 Uninoculated + CNS + D	232.18 ± 1.16 abcdefghij	131.68 ± 1.04 abcdefghij	37.15 ± 1.04 abcdefghij	4.65
16789 + NAK-2+ NYA-2 + CNS + D	206.14 ± 1.15 abcdefghijk	126.68 ± 1.06 abcdefghijk	41.39 ± 1.06 abcdefghij	4.63
Bana + NAK-2+NYA-2 + CNS + D	220.04 ± 1.19 abcdefghijk	109.85 ± 1.05 defghijklm	41.93 ± 1.10 abcdefghij	4.61
KK1 + NAK-2+NYA-2+NSD + CNS + D	215.86 ± 1.14 abcdefghijk	118.63 ± 1.05 bcdefghijkl	40.01 ± 1.05 abcdefghij	4.61
Bana + NYA-2 + CNS + D	172.78 ± 1.10 abcdefghijk	118.59 ± 1.07 bcdefghijkl	49.90 ± 1.08abc	4.61
KK1 Uninoculated + P-D + D	274.37 ± 1.25 abcde	102.44 ± 1.10 efghijklmn	33.90 ± 1.07 bcdefghij	4.59
KK1 + NYA-2 + P-D + D	239.88 ± 1.18 abcdefghi	110.70 ± 1.08cdefghijklm	35.86 ± 1.07 abcdefghij	4.59
KK1 + NSD + CNS + D	199.14 ± 1.14 abcdefghijk	112.51 ± 1.08 cdefghijklm	42.73 ± 1.07abcdefghij	4.59
KK1 + NAK-2 + P-D + D	181.97 ± 1.09 abcdefghijk	125.13 ± 1.05 abcdefghijk	41.86 ± 1.08 abcdefghij	4.59
KK2 + NAK-2+ NYA-2+ NSD + CNS + D	188.37 ± 1.09 abcdefghijk	137.61 ± 1.05 abcdefgh	35.94 ± 1.05 abcdefghij	4.58
Bana + NSD + CNS + D	175.46 ± 1.16 abcdefghijk	122.49 ± 1.07 abcdefghijk	42.60 ± 1.02 abcdefghij	4.58
KK2 + NSD + CNS + D	175.12 ± 1.12 abcdefghijk	114.28 ± 1.04 cdefghijklm	46.41 ± 1.07abcdef	4.58
Bana + NAK-2+ NYA-2+ NSD + CNS + D	155.48 ± 1.09 abcdefghijkl	134.09 ± 1.05 abcdefghi	44.32 ± 1.05 abcdef	4.58
Bana Uninoculated + P-D + D	157.88 ± 1.09 abcdefghijk	143.50 ± 1.04 abcde	40.18 ± 1.05 abcdefghij	4.57
Bana + NAK-2 + P-D + D	39.13 ± 1.17 mnop	125.16 ± 1.05 abcdefghijk	49.53 ± 1.09abcd	4.57
KK1 + NAK-2+NYA-2 + P-D + D	198.38 ± 1.33 abcdefghijk	107.52 ± 1.12 defghijklm	40.90 ± 1.08 abcdefghij	4.56

KK2 + NAK-2 + P-D + D	203.78 ± 1.23 abcdefghijk	117.54 ± 1.08 bcdefghijkl	35.85 ± 1.07 abcdefghij	4.55
Bana + NYA-2 + P-D + D	176.81 ± 1.20 abcdefghijk	117.01 ± 1.08 bcdefghijkl	31.35 ± 1.06 defghijklm	4.55
KK2 Uninoculated + P-D + D	173.11 ± 1.11 abcdefghijk	125.87 ± 1.09 abcdefghijk	37.68 ± 1.05 abcdefghij	4.54
Bana + NAK-2+ NYA-2 + P-D +D	171.46 ± 1.11 abcdefghijk	112.83 ± 1.06 cdefghijklm	41.08 ± 1.05 abcdefghij	4.53
KK2 + NYA-2 + P-D + D	190.55 ± 1.14 abcdefghijk	126.59 ± 1.07 abcdefghijk	32.43 ± 1.08 cdefghijk	4.52
KK1 + NAK-2+NYA-2+NSD + P-D + D	168.53 ± 1.08 abcdefghijk	122.63 ± 1.05 abcdefghijk	37.67 ± 1.06 abcdefghij	4.52
16789 + NAK-2+ NYA-2+ NSD + CNS + D	162.18 ± 1.13 abcdefghijk	111.74 ± 1.08 cdefghijklm	42.35 ± 1.03 abcdefghij	4.52
KK1 + NSD + P-D + D	167.88 ± 1.16 abcdefghijk	115.67 ± 1.07cdefghijklm	38.01 ± 1.05 abcdefghij	4.50
16789 + NSD + CNS + D	136.72 ± 1.06 bcdefghijklmnop	116.86 ± 1.04 cdefghijklm	43.42 ± 1.06 abcdefg	4.48
Bana + NAK-2+ NYA-2+ NSD + P-D + D	132.59 ± 1.16 cdefghijklmnop	107.56 ± 1.03 defghijklm	46.43 ± 1.04 abcdef	4.47
KK1 Uninoculated + N-D + D	190.18 ± 1.11 abcdefghijk	83.23 ± 1.13 hijklmnopq	41.32 ± 1.06 abcdefghij	4.46
KK2 + NAK-2 + NYA-2 + P-D +D	140.98 ± 1.13 abcdefghijklmnop	119.40 ± 1.05 bcdefghijk	38.06 ± 1.05 abcdefghij	4.46
16789 + NAK-2 + P-D + D	160.25 ± 1.08 abcdefghijk	122.77 ± 1.04 abcdefghijk	34.96 ± 1.05 abcdefghij	4.45
16789 + NYA-2 + P-D + D	156.07 ± 1.09 abcdefghijkl	123.97 ± 1.05 abcdefghijk	31.94 ± 1.06 defghijkl	4.44
KK1 + NAK-2 + N-D + D	126.13 ± 1.10 efghijklmnop	117.74 ± 1.05 bcdefghijkl	41.08 ± 1.06 abcdefghij	4.44
16789 + NAK-2 + NYA-2 + P-D + D	132.08 ± 1.40 cdefghijklmnop	152.48 ± 1.17abcd	29.55 ± 1.09 efghijklmnop	4.43
KK2 + NAK-2+ NYA-2+ NSD + P-D + D	120.23 ± 1.13 efghijklmnop	119.94 ± 1.08 bcdefghijk	42.64 ± 1.07 abcdefghij	4.43
KK2 + NAK-2 + N-D + D	158.49 ± 1.12 abcdefghijk	119.07 ± 1.05 bcdefghijk	30.25 ± 1.04 efghijklmnop	4.42
KK2 + NSD + P-D + D	145.10 ± 1.08 abcdefghijklmnop	108.13 ± 1.05 defghijklm	36.68 ± 1.04 abcdefghij	4.42
\16789 Uninoculated + P-D + D	138.30 ± 1.07 bcdefghijklmnop	114.71 ± 1.04cdefghijklm	35.69 ± 1.06 abcdefghij	4.42
KK2 Uninoculated + N-D + D	127.35 ± 1.19 defghijklmnop	115.09 ± 1.06 cdefghijklm	38.97 ± 1.05 abcdefghij	4.42
KK1 + NYA-2 + N-D + D	130.32 ± 1.19 cdefghijklmnop	133.03 ± 1.19 abcdefghij	31.76 ± 1.08 defghijklm	4.41
KK2 + NYA-2 + N-D + D	130.32 ± 1.10 defghijklmnop	115.92 ± 1.10cdefghijklm	36.69 ± 1.05 abcdefghij	4.41
16789 + NAK-2+ NYA-2+ NSD + P-D +D	134.90 ± 1.11 cdefghijklmnop	188.43 ± 1.05 a	34.19 ± 1.06 bcdefghij	4.40
16789 + NAK-2 + N-D + D	161.87 ± 1.10 abcdefghijk	99.87 ± 1.10 fghijklmnop	31.85 ± 1.04 defghijklm	4.38
16789 + NSD + P-D + D	140.80 ± 1.23 bcdefghijklmnop	109.44 ± 1.07 defghijklm	33.28 ± 1.08 bcdefghij	4.38
KK1 + NAK-2+NYA-2 +N-D +D	135.94 ± 1.08 cdefghijklmnop	112.47 ± 1.04 cdefghijklm	33.03 ± 1.06 cdefghijk	4.38
Bana + NSD + P-D + D	130.82 ± 1.32 cdefghijklmnop	119.53 ± 1.12 bcdefghijk	32.10 ± 1.10 defghijkl	4.38
KK2 + NAK-2+NYA-2 + N-D + D	145.10 ± 1.06 abcdefghijklmnop	107.47 ± 1.03 defghijklm	31.24 ± 1.07 defghijklm	4.37
16789 + NYA-2 + N-D +D	141.25 ± 1.07 abcdefghijklmnop	114.09 ± 1.03cdefghijklm	30.93 ± 1.06 defghijklm	4.37
Bana Uninoculated + N-D + D	129.07 ± 1.27 defghijklmnop	87.46 ± 1.15 ghijklmnop	43.37 ± 1.10 abcdefgh	4.37
Bana + NAK-2 + N-D + D	147.06 ± 1.19 abcdefghijkl	86.44 ± 1.06 hijklmnopq	37.21 ± 1.09 abcdefghij	4.36
16789 Uninoculated + N-D +D	113.50 ± 1.13 fghijklmnop	121.03 ± 1.06 bcdefghijk	35.27 ± 1.07 abcdefghij	4.36
16789 + NAK-2 + NYA-2 + NSD + N-D + D	140.71 ± 1.25 bcdefghijklmnop	106.88 ± 1.09 defghijklm	30.74 ± 1.10 defghijklm	4.35

16789 + NAK-2 + NYA-2 + N-D + D	116.82 ± 1.17 efghijklmnop	109.07 ± 1.05 defghijklm	36.63 ± 1.03 abcdefghij	4.35
16789 + NSD + N-D + D	113.07 ± 1.12 ghijklmnop	118.46 ± 1.03 bcdefghijkl	34.22 ± 1.02 bcdefghij	4.35
Bana + NYA-2 + N-D + D	104.31 ± 1.09 hijklmnop	101.27 ± 1.08 fghijklmno	43.42 ± 1.05 abcdefg	4.35
KK1 + NAK-2+NYA-2+NSD + N-D + D	137.25 ± 1.09 bcdefghijklmnop	103.02 ± 1.07 efghijklmn	32.02 ± 1.16 defghijkl	4.34
KK2 + NAK-2+ NYA-2+ NSD + N-D + D	128.09 ± 1.18 defghijklmnop	110.38 ± 1.07 cdefghijklm	31.71 ± 1.09 defghijklm	4.34
Bana + NAK-2+ NYA-2 + N-D + D	104.11 ± 1.10 hijklmnop	100.48 ± 1.07 fghijklmno	43.80 ± 1.05 abcdef	4.34
Bana + NAK-2+ NYA-2+ NSD + N-D + D	137.25 ± 1.10 bcdefghijklmnop	94.42 ± 1.13 ghijklmnop	33.73 ± 1.07 bcdefghij	4.33
KK2 Uninoculated + CNS + W	136.20 ± 1.08 cdefghijklmnop	103.27 ± 1.06 efghijklmn	30.50 ± 1.03 efghijklmnop	4.32
KK2 + NSD + N-D + D	126.86 ± 1.06 efghijklmnop	106.37 ± 1.05 defghijklm	31.93 ± 1.06 defghijkl	4.32
KK1 + NSD + N-D + D	128.70 ± 1.09 defghijklmnop	85.55 ± 1.108 hijklmnopq	37.40 ± 1.04 abcdefghij	4.31
KK1 + NSD + CNS + W	114.82 ± 1.08 efghijklmnop	106.50 ± 1.06 defghijklm	32.68 ± 1.05 cdefghijk	4.30
16789 + NAK-2 + CNS + W	126.38 ± 1.05 efghijklmnop	114.52 ± 1.04 cdefghijklm	26.47 ± 1.10 hijklmnopqrst	4.29
KK2 + NAK-2 + CNS + W	108.39 ± 1.11 hijklmnop	105.35 ± 1.05 efghijklmn	32.92 ± 1.06 cdefghijk	4.29
KK2 + NYA-2 + CNS + W	141.53 ± 1.07 abcdefghijklmnop	80.83 ± 1.10 hijklmnopq	32.23 ± 1.05 defghijkl	4.27
KK1 + NAK-2 + CNS +W	95.68 ± 1.09 hijklmnop	113.21 ± 1.06 cdefghijklm	33.30 ± 1.04 cdefghijk	4.27
KK2 + NAK-2+NYA-2 + CNS + W	106.74 ± 1.31 hijklmnop	101.63 ± 1.09 fghijklmno	32.37 ± 1.07 cdefghijk	4.26
Bana + NSD + N-D + D	93.51 ± 1.11 hijklmnop	103.60 ± 1.10 efghijklmn	36.94 ± 1.05 abcdefghij	4.26
16789 + NYA-2 + CNS + W	120.23 ± 1.26 efghijklmnop	98.53 ± 1.11ghijklmnopq	27.82 ± 1.10 fghijklmnopq	4.24
16789 Uninoculated + CNS + W	97.72 ± 1.09 hijklmnop	101.47 ± 1.06 fghijklmno	33.49 ± 1.04 bcdefghij	4.24
KK1 + NYA-2 + CNS + W	93.68 ± 1.03 hijklmnop	100.08 ± 1.07fghijklmnop	35.54 ± 1.08 abcdefghij	4.24
16789 + NAK-2+ NYA-2 + CNS + W	86.26 ± 1.08 hijklmnop	111.79 ± 1.05 cdefghijklm	32.54 ± 1.03 cdefghijk	4.22
KK1+ NAK-2+NYA-2 + CNS +W	94.59 ± 1.06 hijklmnop	90.24 ± 1.06 ghijklmnop	35.67 ± 1.08 abcdefghij	4.21
Bana Uninoculated + CNS + W	90.68 ± 1.07 hijklmnop	108.85± 1.05 defghijklm	30.60 ± 1.05 defghijklm	4.21
16789 + NAK-2 + NYA-2+ NSD + CNS + W	85.93 ± 1.07 hijklmnop	103.42 ± 1.07 efghijklmn	33.98 ± 1.04 bcdefghij	4.21
Bana + NAK-2 + CNS + W	91.73 ± 1.25 hijklmnop	78.27 ± 1.11 ijklmnopq	40.31 ± 1.11 abcdefghij	4.19
KK1 + NAK-2+NYA-2+NSD + CNS +W	81.44 ± 1.08 hijklmnop	93.27 ± 1.05 ghijklmnop	38.00 ± 1.06 abcdefghij	4.19
Bana + NYA-2 + CNS + W	74.56 ± 1.10 ijklmnop	102.31± 1.08 efghijklmn	37.30 ± 1.05 abcdefghij	4.19
KK2 + NAK-2+ NYA-2+ NSD + CNS + W	77.33 ± 1.08 hijklmnop	104.56 ± 1.05 efghijklmn	34.35 ± 1.03 bcdefghij	4.18
Bana + NAK-2+NYA-2 + CNS + W	66.83 ± 1.24 ijklmnop	137.26 ± 1.21abcdefghi	30.05 ± 1.08 efghijklmnop	4.18
Bana + NSD + CNS + W	84.95 ± 1.33 hijklmnop	95.26 ± 1.13 ghijklmnop	33.79 ± 1.09 bcdefghij	4.17
Bana + NAK-2+ NYA-2+ NSD + CNS +W	74.85 ± 1.36 ijklmnop	96.11 ± 1.10 ghijklmnop	38.02 ± 1.04 abcdefghij	4.17
16789 + NSD + CNS + W	64.44 ± 1.25 ijklmnop	142.32 ± 1.17abcde	29.46 ± 1.08 efghijklmnop	4.17
Bana Uninoculated + P-D + W	97.91 ± 1.11 hijklmnop	76.69 ± 1.08 ijklmnopq	35.49 ± 1.08 abcdefghij	4.16
Bana + NAK-2 + P-D + W	71.07 ± 1.08 ijklmnop	115.51 ± 1.05cdefghijklm	31.85 ± 1.09 defghijklm	4.16

KK2 + NSD + CNS + W	83.34 ± 1.04 hijklmnop	101.73 ± 1.04 fghijklmno	30.35 ± 1.04 efghijklmnop	4.15
Bana + NYA-2 + P-D + W	72.44 ± 1.05 ijklmnop	100.45 ± 1.05 fghijklmnop	35.35 ± 1.08 abcdefghij	4.15
KK2 Uninoculated + P-D + W	90.33 ± 1.07 hijklmnop	101.48 ± 1.05 fghijklmno	27.23 ± 1.04 ghijklmnopqrst	4.14
KK2 + NAK-2 + P-D + W	76.74 ± 1.36 ijklmnop	141.04 ± 1.17abcdef	22.77 ± 1.15 ijklmnopqrst	4.14
KK2 + NYA-2 + P-D + W	83.02 ± 1.20 hijklmnop	100.15 ± 1.07fghijklmnop	29.30 ± 1.07 efghijklmnop	4.13
16789 + NAK-2 + P-D + W	80.51 ± 1.08 hijklmnop	91.74 ± 1.07 ghijklmnop	30.94 ± 1.07 defghijklm	4.11
16789 Uninoculated + P-D +W	82.70 ± 1.06 hijklmnop	89.54 ± 1.03 ghijklmnop	29.42 ± 1.03 efghijklmnop	4.10
KK1 Uninoculated + CNS + W	81.43 ± 1.09 hijklmnop	87.76 ± 1.06 ghijklmnop	30.37 ± 1.08 efghijklmnop	4.10
Bana + NAK-2+ NYA-2 + P-D + W	76.44 ± 1.17 ijklmnop	88.83 ± 1.21 ghijklmnop	32.20 ± 1.04 defghijkl	4.10
KK2 + NAK-2+NYA-2 + P-D + W	86.10 ± 1.15 hijklmnop	93.45 ± 1.06 ghijklmnop	26.31 ± 1.07 hijklmnopqrst	4.09
16789 + NYA-2 + P-D +W	76.30 ± 1.06 ijklmnop	98.04 ± 1.04 ghijklmnop	28.11 ± 1.07 fghijklmnopq	4.09
KK2 + NAK-2+ NYA-2+ NSD + P-D +W	72.31 ± 1.06 ijklmnop	101.46 ± 1.06 fghijklmno	28.73 ± 1.06 fghijklmnopq	4.09
Bana + NAK-2+ NYA-2+ NSD + P-D + W	100.96 ± 1.30 hijklmnop	81.19 ± 1.10 hijklmnopq	24.96 ± 1.05 hijklmnopqrst	4.08
KK1 Uninoculated + P-D +W	77.77 ± 1.15 hijklmnop	84.12 ± 1.08 hijklmnopq	31.28 ± 1.08 defghijklm	4.08
Bana + NSD + P-D + W	60.03 ± 1.09 jklmnop	96.39 ± 1.07 ghijklmnop	34.94 ± 1.06 bcdefghij	4.07
Bana + NAK-2 + N/P-D + D	74.70 ± c1.09 ijklmnop	89.72 ± 1.11 ghijklmnop	28.71 ± 1.04 fghijklmnopq	4.06
KK1 + NAK-2 + P-D +W	70.52 ± 1.24 ijklmnop	85.45 ± 1.11 hijklmnopq	32.09 ± 1.09 defghijkl	4.06
Bana Uninoculated + N/P-D + D	59.45 ± 1.35 jklmnop	100.66 ± 1.22 fghijklmno	32.27 ± 1.06 defghijkl	4.06
KK1 + NYA-2 + P-D +W	76.00 ± 1.26 ijklmnop	88.27 ± 1.09 ghijklmnop	27.97 ± 1.07 fghijklmnopq	4.05
16789 + NAK-2 + NYA-2 + P-D + W	72.72 ± 1.08 ijklmnop	89.67 ± 1.05 ghijklmnop	28.76 ± 1.05 efghijklmnop	4.05
KK2 + NSD + P-D + W	69.72 ± 1.07 ijklmnop	96.28 ± 1.04 ghijklmnop	28.21 ± 1.06 fghijklmnopq	4.05
KK2 Uninoculated + N/P-D + D	79.89 ± 1.12 hijklmnop	99.00 ± 1.05 fghijklmnop	22.87 ± 1.04 ijklmnopqrst	4.04
KK1 + NAK-2+NYA-2 + P-D + W	75.13 ± 1.24 ijklmnop	87.28 ± 1.08 hijklmnopq	27.75 ± 1.08 ghijklmnopqrst	4.04
KK1 + NAK-2+NYA-2+NSD + P-D + W	70.93 ± 1.24 ijklmnop	78.62 ± 1.10 ijklmnopq	32.57 ± 1.08 cdefghijk	4.04
KK1 + NSD + P-D + W	67.09 ± 1.25 ijklmnop	86.73 ± 1.08 hijklmnopq	30.64 ± 1.08 defghijklm	4.03
KK1 Uninoculated + N/P-D + D	72.72 ± 1.08 ijklmnop	73.57 ± 1.06 ijklmnopq	31.95 ± 1.09 defghijkl	4.02
Bana + NYA-2 + N/P-D +D	69.45 ± 1.25 ijklmnop	75.87 ± 1.09 ijklmnopq	33.15 ± 1.08 cdefghijk	4.02
16789 + NAK-2 + NYA-2+ NSD + P-D + W	58.10 ± 1.15 jklmnop	105.59 ± 1.03 defghijklm	26.57 ± 1.08 hijklmnopqrst	4.00
16789 + NSD + P-D +W	56.13 ± 1.17 jklmnop	82.33 ± 1.09 hijklmnopq	32.99 ± 1.06 cdefghijk	3.98
KK2 + NAK-2 + N/P-D + D	54.74 ± 1.30 klmnop	120.37 ± 1.19 bcdefghijk	22.79 ± 1.09 jklmnopqrst	3.97
16789 Uninoculated + N/P-D + D	49.83 ± 1.17 klmnop	102.15 ± 1.19 efghijklmn	29.51 ± 1.04 efghijklmnop	3.97
KK2 + NYA-2 + N/P-D + D	52.48 ± 1.13 klmnop	96.24 ± 1.16 ghijklmnop	27.45 ± 1.07 ghijklmnopqrst	3.95
Bana + NAK-2+NYA-2 + N/P-D + D	49.93 ± 1.22 klmnop	97.79 ± 1.18 ghijklmnop	28.72 ± 1.03 fghijklmnopq	3.95
KK1 + NAK-2+ N/P-D + D	61.66 ± 1.08 jklmnop	84.71 ± 1.03 hijklmnopq	25.87 ± 1.11 hijklmnopqrst	3.94

KK2 + NAK-2+NYA-2 + N/P-D + D	59.34 ± 1.14 jklmnop	101.78 ± 1.18 fghijklmno	22.71 ± 1.07 jklmnopqrst	3.94
KK1 + NYA-2 + N/P-D + D	55.70 ± 1.18 jklmnop	85.74 ± 1.08 hijklmnopq	27.37 ± 1.0 ghijklmnopqrst	3.93
KK1 + NAK-2+ NYA-2 + N/P-D + D	49.83 ± 1.14 lmnop	80.01 ± 1.05 ijklmnopq	29.67 ± 1.10 efghijklmnop	3.89
KK2 + NAK-2+ NYA-2+ NSD + N/P-D + D	51.19 ± 1.12 klmnop	78.74 ± 1.05 ijklmnopq	28.53 ± 1.08 fghijklmnopq	3.88
16789 + NAK-2 + N/P-D + D	54.43 ± 1.27 klmnop	75.69 ± 1.07 ijklmnopq	26.84 ± 1.07 hijklmnopqrst	3.87
KK2 + NSD + N/P-D + D	51.39 ± 1.28 klmnop	84.07 ± 1.09 hijklmnopq	24.39 ± 1.09 ijklmnopqrst	3.86
KK1 + NAK-2+ NYA-2+ NSD + N/P-D + D	42.58 ± 1.14 lmnop	102.57 ± 1.17efghijklmno	24.49 ± 1.03 ijklmnopqrst	3.86
KK1 + NSD + N/P-D + D	57.43 ± 1.13 jklmnop	70.25 ± 1.06 ijklmnopq	25.35 ± 1.11 hijklmnopqrst	3.85
KK2 Uninoculated + N-D + W	48.23 ± 1.22 lmnop	84.16 ± 1.08 hijklmnopq	25.21 ± 1.10 hijklmnopqrst	3.85
Bana + NAK-2+ NYA-2+ NSD + N/P-D +D	52.08 ± 1.21 klmnop	64.89 ± 1.08 ijklmnopq	29.16 ± 1.08 efghijklmnop	3.83
KK1 Uninoculated + N-D + W	51.48 ± 1.28 klmnop	84.11 ± 1.08 hijklmnopq	22.69 ± 1.10 jklmnopqrst	3.83
16789 + NYA-2 + N/P-D + D	51.38 ± 1.08 klmnop	84.06 ± 1.04 hijklmnopq	21.75 ± 1.05 klmnopqrst	3.82
KK1 + NAK-2 + N-D + W	54.95 ± 1.15 jklmnop	74.44 ± 1.08 ijklmnopq	22.26 ± 1.09 jklmnopqrst	3.81
Bana + NSD + N/P-D + D	37.66 ± 1.09 mnop	90.18 ± 1.15 ghijklmnop	26.79 ± 1.04 hijklmnopqrst	3.81
16789 + NAK-2+ NYA-2+ NSD + N/P-D + D	56.56 ± 1.14 jklmnop	81.64 ± 1.07 hijklmnopq	19.31 ± 1.06 lmnopqrst	3.80
16789 + NAK-2+ NYA-2 + N/P-D + D	39.81± 1.23 mnop	100.25 ± 1.16 fghijklmnop	22.68 ± 1.04 jklmnopqrst	3.80
Bana Uninoculated + N-D + W	39.13 ± 1.12 mnop	80.81 ± 1.09 hijklmnopq	27.81 ± 1.11 fghijklmnopq	3.79
KK1 + NYA-2 + N-D + W	44.50 ± 1.19 lmnop	103.97 ± 1.20 efghijklmn	18.23 ± 1.11 lmnopqrst	3.78
16789 + NSD + N/P-D + D	37.15 ± 1.27 mnop	80.02 ± 1.06 hijklmnopq	27.09 ± 1.06 hijklmnopqrst	3.77
16789 Uninoculated + N-D +W	48.23 ± 1.10 lmnop	85.76 ± 1.05 hijklmnopq	18.50 ± 1.04 lmnopqrst	3.75
Bana + NAK-2 + N-D + W	42.90 ± 1.13 lmnop	72.93 ± 1.05 ijklmnopq	22.49 ± 1.07 jklmnopqrst	3.72
16789 + NAK-2 + N-D + W	37.80 ± 1.092 mnop	80.88 ± 1.03 hijklmnopq	22.74 ± 1.07 jklmnopqrst	3.72
Bana + NYA-2 + N-D +W	43.07 ± 1.27 lmnop	63.90 ± 1.13 ijklmnopq	24.84 ± 1.09 hijklmnopqrst	3.71
16789 + NYA-2 + N-D +W	40.12 ± 1.16 lmnop	74.67 ± 1.04 ijklmnopq	21.76 ± 1.09 klmnopqrst	3.70
KK2 + NAK-2 + N-D + W	40.04 ± 1.18 lmnop	74.13 ± 1.07 ijklmnopq	21.56 ± 1.08 klmnopqrst	3.69
Bana + NAK-2+ NYA-2 + N-D + W	36.45 ±1.20 mnop	65.42 ± 1.08 ijklmnopq	26.83 ± 1.08 hijklmnopqrst	3.69
16789 + NAK-2+ NYA-2 + N-D + W	27.86 ± 1.37 mnop	95.27 ± 1.14 ghijklmnop	22.79 ± 1.12 ijklmnopqrst	3.67
KK1 + NAK-2+NYA-2 + N-D + W	24.93 ± 1.18 mnop	81.66 ± 1.12 hijklmnopq	26.43 ± 1.03 hijklmnopqrst	3.64
16789 + NAK-2+ NYA-2+ NSD + N-D + W	43.40 ± 1.17 lmnop	56.82 ± 1.13 mnopq	19.98 ± 1.04 lmnopqrst	3.60
KK2 + NYA-2 + N-D + W	38.53 ± 1.12 mnop	63.61 ± 1.07 ijklmnopq	19.86 ± 1.06 lmnopqrst	3.60
KK2 + NAK-2+NYA-2 + N-D + W	30.14 ± 1.17 mnop	69.00 ± 1.05 ijklmnopq	21.99 ± 1.06 klmnopqrst	3.58
Bana + NAK-2+ NYA-2+ NSD + N-D + W	20.26 ± 1.23 nop	77.59 ± 1.11 ijklmnopq	28.91 ± 1.05 efghijklmnop	3.57
16789 + NSD + N-D +W	29.40 ± 1.13 mnop	80.40 ± 1.06 hijklmnopq	17.86 ± 1.12 opqrst	3.55
KK2 + NAK-2+ NYA-2+ NSD + N-D + W	27.38 ± 1.24 mnop	87.35 ± 1.15 hijklmnopq	17.73 ± 1.07 pqrst	3.55

16789 Uninoculated + N/P-D + W	20.34 ± 1.22 nop	82.22 ± 1.10 hijklmnopq	24.61 ± 1.06 ijklmnopqrst	3.54
Bana + NSD + N-D + W	19.95 ± 1.24 nop	83.54 ± 1.21 hijklmnopq	24.37 ± 1.06 ijklmnopqrst	3.54
Bana Uninoculated + N/P-D + W	24.83 ± 1.13 mnop	80.20 ± 1.06 hijklmnopq	19.45 ± 1.08 lmnopqrst	3.52
Bana + NAK-2 + N/P-D + W	25.95 ± 1.10 mnop	61.49 ± 1.07 ijklmnopq	23.69 ± 1.06 ijklmnopqrst	3.51
KK2 + NSD + N-D + W	20.85 ± 1.05 nop	73.74 ± 1.07 ijklmnopq	24.21 ± 1.10 ijklmnopqrst	3.51
KK1 + NAK-2+NYA-2+NSD + N-D + W	18.84 ± 1.08 nop	81.78 ± 1.14 hijklmnopq	22.51 ± 1.05 jklmnopqrst	3.48
KK1 + NSD + N-D +W	25.31 ± 1.10 mnop	55.70 ± 1.08 opq	23.63 ± 1.05 ijklmnopqrst	3.47
KK1 Uninoculated + N/P-D + W	24.55 ± 1.10 mnop	59.02 ± 1.08 jklmnopq	22.65 ± 1.05 jklmnopqrst	3.47
16789 + NAK-2 + N/P-D + W	25.41 ± 1.10 mnop	67.34 ± 1.05 ijklmnopq	19.10 ± 1.08 lmnopqrst	3.46
16789 + NAK-2+ NYA-2 + N/P-D + W	23.40 ± 1.22 mnop	53.47 ± 1.10 pq	23.32 ± 1.07 ijklmnopqrst	3.43
KK2 Uninoculated + N/P-D + W	18.20 ± 1.09 nop	64.49 ± 1.08 ijklmnopq	24.90 ± 1.03 hijklmnopqrst	3.43
16789 + NYA-2 + N/P-D + W	17.58 ± 1.11 nop	85.05 ± 1.16 hijklmnopq	19.54 ± 1.04 lmnopqrst	3.43
KK1 + NAK-2 + N/P-D + W	20.50 ± 1.12 nop	71.65± 1.06 ijklmnopq	19.72 ± 1.10 lmnopqrst	3.42
Bana + NYA-2 + N/P-D + W	14.10 ± 1.13op	70.46 ± 1.19 ijklmnopq	27.76 ± 1.08 fghijklmnopq	3.41
Bana + NAK-2+NYA-2 + N/P-D + W	16.28 ± 1.14 nop	73.10 ± 1.10 ijklmnopq	22.49 ± 1.07 jklmnopqrst	3.40
KK1 + NYA-2 + N/P-D + W	15.73 ± 1.15 nop	79.09 ± 1.19 ijklmnopq	21.71 ± 1.05 klmnopqrst	3.40
KK1 + NAK-2+NYA-2 + N/P-D + W	20.54 ± 1.12 nop	58.34 ± 1.10 klmnopq	20.44 ± 1.07 lmnopqrst	3.37
KK2 + NAK-2 + N/P-D + W	22.95 ± 1.13 mnop	58.60 ± 1.07jklmnopq	17.97 ± 1.09 mnopqrst	3.36
KK2 + NYA-2 + N/P-D + W	21.88 ± 1.12 nop	67.60 ± 1.08 ijklmnopq	16.27 ± 1.07 st	3.36
KK2 + NAK-2+NYA-2 + N/P-D + W	15.76 ± 1.06 nop	84.63 ± 1.14 hijklmnopq	17.68 ± 1.06 qrst	3.36
KK1 + NAK-2+ NYA-2+ NSD + N/P-D + W	21.59 ± 1.11 nop	56.55 ± 1.10 nopq	18.82 ± 1.07 lmnopqrst	3.35
KK2 + NAK-2+ NYA-2+ NSD + N/P-D + W	19.46 ± 1.14 nop	64.17 ± 1.06 ijklmnopq	18.68 ± 1.06 lmnopqrst	3.35
KK1 + NSD + N/P-D + W	17.82 ± 1.10 nop	59.64 ± 1.06 ijklmnopq	20.72 ± 1.07 lmnopqrst	3.33
16789 + NAK-2+ NYA-2+ NSD + N/P-D + W	22.05 ± 1.07 nop	60.19 ± 1.05 ijklmnopq	15.81 ± 1.06 t	3.32
Bana + NAK-2+NYA-2+NSD + N/P-D + W	15.548 ± 1.16 nop	49.16 ± 1.10 q	27.11 ± 1.05 hijklmnopqrst	3.31
Bana + NSD + N/P-D + W	16.50 ± 1.07 nop	57.58 ± 1.09 lmnopq	21.05 ± 1.05 lmnopqrst	3.30
16789 + NSD + N/P-D + W	15.43 ± 1.08 op	59.43 ± 1.05 ijklmnopq	19.06 ± 1.07 lmnopqrst	3.26
KK2 + NSD + N/P-D + W	14.68 ± 1.10 op	59.66 ± 1.06 ijklmnopq	16.39 ± 1.09 rst	3.19
Test values	**df= 191; F= 28.217; P ≤ 0.0001**	**df= 191; F= 8.05; P ≤ 0.0001**	**df= 191; F= 15.74; P ≤ 0.0001**	**4.099 ± 0.410**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

4.3.1.1 Co-infection effects on the growth of napier grass based on the interactions of the varieties, pathogen combinations, nutrient formulations and watering regimes

The pathogens involved in these experiments had significant exponential interactions with some of the factors under experimentation. Significant interaction at df = 15; F = 3.680; P ≤ 0.0001 were observed between the pathogens and the napier grass varieties for total fresh weight and at df = 15; F = 2.576; P = 0.001 for tiller height parameter. However, there was no significant interaction (P > 0.05) between the pathogens and napier grass varieties for chlorophyll content levels. Also, between the pathogens and nutrient formulations, significant interactions at df = 15; F = 14.274; P < 0.0001, df = 15; F = 6.407; P ≤ 0.0001 and df = 15; F = 3.479; P ≤ 0.0001 were observed for total fresh weight, chlorophyll content and tiller height respectively. Similarly significant interactions were observed between the pathogens and watering regimes at df = 5; F = 4.609; P ≤ 0.0001 and df = 5; F = 2.87; P = 0.014 for the total fresh weight and tiller height respectively. There were no significant differences (P > 0.05) between the treatments for chlorophyll content levels.

4.3.1.1.1 Effects of the pathogen and napier grass interactions on total fresh weight, tiller height and chlorophyll content levels

Significant (df = 23; F = 3.317; P ≤ 0.0001**)** interaction between the pathogen and napier grass was observed based on ratoon/crop 1 and 2 for total fresh weight (Table 4.16). The treatment Kakamega 1 variety not infected by any pathogen (KK 1 uninoculated), had the highest total fresh weight. This treatment was followed by the treatment of Kakamega 2 variety infected by only NAK-2 *Ustilago kamerunensis* isolate (KK 2 + NAK-2). These two treatments were however not statistically different from the others, except for treatments KK 2 + NAK-2 + NYA-2 + NSD pathogen, KK 2 + NSD pathogen and 16789 + NSD pathogen which had the lowest total fresh weight (Table 4.16). In comparison to ratoons/crop 3 and 4 significant (df = 23; F= 2.58; P ≤ 0.0001) interaction was also recorded for total fresh weight (Table 4.17). The treatment Kakamega 1 variety not infected by any pathogen (KK 1 uninoculated), had the highest total fresh weight, followed by the treatment of accession 16789 infected by only NAK-2 *Ustilago kamerunensis* isolate (16789 + NAK-2). However, the means of the two treatments were not statistically different from the other treatments, with except for the treatment Bana

variety infected by only the NSD pathogen (Bana + NSD), which had the lowest total fresh weight (Table 4.17).

The pathogen combination and napier varieties at ratoon/crop 1 and 2 had a significant (df = 23; F = 4.387; P ≤ 0.0001) interaction for tiller height. The treatment Kakamega 1 variety that was infected by NYA-2 *Ustilago kamerunensis* isolate alone (KK 1 + NYA-2), had the tallest tiller heights, followed by the treatment of accession 16789 co-infected by NAK-2 and NYA-2 *Ustilago kamerunensis* isolates (16789 + NAK-2 + NYA-2). The two treatments did not have statistical differences from the other treatments except for (KK 1+ NSD), (KK1 Uninoculated), (KK 2 + NSD pathogen), (Bana + NAK-2 + NYA-2 + NSD pathogen), (16789 + NAK-2) and (16789 + NAK-2 + NYA-2 + NSD). These six treatments had the shortest tillers (Table 4.16). In comparison to ratoons/crop 3 and 4 a significant (df = 23; F = 3.04; P ≤ 0.0001) interaction for total fresh weight was observed (Table 4.17). The treatment Kakamega 2 variety that was not infected by any pathogen (KK 2 uninoculated), had the tallest tiller followed by treatment of accession 16789 infected by NAK-2 isolate alone (16789 + NAK-2). These results were however not statistically different from the other treatments except for KK 1 + NSD pathogen, Bana + NYA-2, Bana + NAK-2 + NYA-2 and Bana + NSD pathogen that had the shortest tillers (Table 4.17).

The pathogen combination and napier varieties at ratoon/crop 1 and 2 had significant (df = 23; F = 3.746; P ≤ 0.0001) interaction for chlorophyll content. The treatment Bana variety infected only by NYA-2 isolate (Bana + NYA-2), had the highest chlorophyll content levels, followed by Bana variety not infected by any pathogen (Bana uninoculated). The means of these two treatments were not statistically different from all the other treatments, except for the treatment of (KK 2+ NSD pathogen), (KK 2 + NAK-2 + NYA-2) co-infected treatments and all treatments of accession 16789 that had the lowest chrolophyll (Table 4.9). In ratoons/crop 3 and 4 significant (df = 23; F = 4.66; P ≤ 0.0001) interactions were observed (Table 4.17). The treatment Bana variety infected by only NYA-2 isolate (Bana + NYA-2), had the highest chlorophyll content levels, followed by Bana variety co-infected by NAK-2 and NYA-2 isolates and NSD pathogen (Bana + NAK-2 + NYA-2 + NSD). The means of the two were however not statistically different from the other treatments, except for the treatments (KK 1+ NYA-2), (KK 2+ NYA-2), (KK-2 + NAK-2), (KK 2 + NAK-2 + NYA-2 + NSD pathogen), (16789 + NYA-2) and (16789 + NAK-2 + NYA-2 + NSD pathogen) which had the lowest chlorophyll (Table 4.17).

Table 4.16: Effects of the respective varieties versus pathogen combinations as at ratoon/crop 1&2 cycles; showing the key selected growth parameters and their means ± standard errors.

Ratoon/crop 1&2; Interactive performance of the respective varieties versus pathogen using selected key parameters affected most by the pathogens.			
Napier grass varieties & pathogen interactions	**Total fresh weight (Yield) in grams**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**
KK 1 UNINOCULATED	140.78 ± 1.11 a	106.83 ± 1.04 bcd	33.74 ± 1.05 abcde
KK 1 + NYA-2	119.88 ± 1.13 abc	142.20 ± 1.07 a	33.13 ± 1.04 abcde
KK 1 + NAK-2	118.40 ± 1.10 abc	117.67 ± 1.03 abcd	32.50 ± 1.06 abcde
KK 1 + NSD	96.54 ± 1.12 abcd	94.82 ± 1.05 d	31.28 ± 1.05 abcde
KK 1+ NAK-2 + NYA-2	90.90 ± 1.13 abcd	113.64 ± 1.04 abcd	34.29 ± 1.04 abcde
KK 1+ NAK-2 + NYA-2 + NSD	83.46 ± 1.11 abcd	112.79 ± 1.05 abcd	31.93 ± 1.04 abcde
KK 2 + NAK-2	138.44 ± 1.13 a	133.31 ± 1.06 ab	31.24 ± 1.05 abcde
KK 2 UNINOCULATED	125.95 ± 1.10 abc	118.67 ± 1.04 abcd	31.49 ± 1.03 abcde
KK 2 + NYA-2	123.03 ± 1.10 abc	111.58 ± 1.06 abcd	31.45 ± 1.05 abcde
KK 2+ NAK-2 + NYA-2	105.57 ± 1.11 abcd	125.55 ± 1.05 abc	30.89 ± 1.04 bcde
KK 2 + NAK-2 + NYA-2 + NSD	76.44 ± 1.11 bcd	118.49 ± 1.04 abcd	32.00 ± 1.05 abcde
KK 2 + NSD	73.28 ± 1.11 cd	102.89 ± 1.03 cd	28.40 ± 1.05 e
BANA UNINOCULATED	128.76 ± 1.09 ab	131.72 ± 1.05 ab	37.95 ± 1.05 ab
BANA + NAK-2	111.61 ± 1.09 abcd	115.33 ± 1.04 abcd	36.17 ± 1.06 abc
BANA + NAK-2 + NYA-2	105.12 ± 1.12 abcd	131.99 ± 1.06 ab	35.97 ± 1.04 abcd
BANA + NYA-2	105.01 ± 1.12 abcd	119.53 ± 1.05 abcd	39.03 ± 1.04 a
BANA + NSD	96.00 ± 1.13 abcd	126.28 ± 1.06 abc	32.89 ± 1.04 abcde
BANA + NAK-2 + NYA-2 + NSD	95.73 ± 1.14 abcd	100.41 ± 1.06 cd	34.20 ± 1.04 abcde
16789 + NAK-2	103.56 ± 1.10 abcd	106.24 ± 1.03 bcd	29.90 ± 1.05 cde
16789 UNINOCULATED	99.43 ± 1.10 abcd	124.44 ± 1.04 abc	30.50 ± 1.03 bcde
16789 + NYA-2	96.33 ± 1.10 abcd	118.07 ± 1.04 abcd	29.30 ± 1.04 cde
16789 + NAK-2 + NYA-2 + NSD	96.05 ± 1.10 abcd	110.18 ± 1.04 bcd	28.89 ± 1.05 cde
16789 + NAK-2 + NYA-2	89.64 ± 1.13 abcd	135.16 ± 1.06 ab	30.57 ± 1.04 bcde
16789 + NSD	67.25 ± 1.12 d	113.77 ± 1.05 abcd	28.61 ± 1.05 de
Test Values	**df = 23; F = 3.317; P ≤ 0.0001**	**df = 23; F = 4.387; P ≤ 0.0001**	**df = 23; F = 3.746; P ≤ 0.0001**

The varieties infected entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD pathogen used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

Table 4.17: Effects of respective varieties and pathogen combinations interactions as at ratoon/crop 3&4 cycles; showing the key selected growth parameters and their means ± standard errors.

Ratoon / crop 3&4; Interactive performance of the respective varieties versus pathogen using selected key parameters affected most by the pathogens.

Napier grass varieties & pathogen interactions	Total fresh weight (Yield) in grams	Tiller height (cm)	Chlorophyll content levels (SPAD UNITS)
KK 1 UNINOCULATED	73.35 ± 1.11 a	73.13 ± 1.04 abc	30.29 ± 1.04 abcd
KK 1 + NAK-2	66.45 ± 1.10 a	82.73 ± 1.04 abc	30.45 ± 1.04 abc
KK 1+ NAK-2 + NYA-2	59.88 ± 1.10 a	73.98 ± 1.04 abc	29.85 ± 1.06 abcde
KK 1 + NYA-2	58.29 ± 1.11 ab	74.11 ± 1.04 abc	25.80 ± 1.04 bcde
KK 1+ NAK-2 + NYA-2 + NSD	56.64 ± 1.11 ab	75.33 ± 1.04 abc	27.78 ± 1.05 abcde
KK 1 + NSD	51.46 ± 1.09 ab	70.34 ± 1.04 bc	29.26 ± 1.04 abcde
KK 2 UNINOCULATED	68.82 ± 1.10 a	87.26 ± 1.04 a	29.73 ± 1.04 abcde
KK 2 + NYA-2	64.23 ± 1.10 a	79.73 ± 1.04 abc	25.00 ± 1.03 cde
KK 2 + NAK-2	61.57 ± 1.12 a	80.87 ± 1.04 abc	24.76 ± 1.05 de
KK 2 + NAK-2 + NYA-2 + NSD	60.00 ± 1.09 a	79.74 ± 1.04 abc	25.64 ± 1.04 bcde
KK 2+ NAK-2 + NYA-2	57.05 ± 1.12 ab	79.91 ± 1.04 abc	25.39 ± 1.04 cde
KK 2 + NSD	56.53 ± 1.11 ab	80.73 ± 1.03 abc	28.84 ± 1.04 abcde
BANA + NAK-2	63.22 ± 1.10 a	76.71 ± 1.04 abc	31.46 ± 1.04 ab
BANA UNINOCULATED	56.21 ± 1.12 ab	76.85 ± 1.05 abc	28.00 ± 1.04 abcde
BANA + NYA-2	49.43 ± 1.10 ab	70.12 ± 1.05 bc	32.84 ± 1.04 a
BANA + NAK-2 + NYA-2	46.98 ± 1.10 ab	69.55 ± 1.05 c	29.57 ± 1.04 abcde
BANA + NAK-2 + NYA-2 + NSD	45.21 ± 1.12 ab	71.32 ± 1.04 abc	32.39 ± 1.03 a
BANA + NSD	35.55 ± 1.10 b	69.82 ± 1.05 c	28.92 ± 1.04 abcde
16789 + NAK-2	73.04 ± 1.10 a	85.92 ± 1.04 ab	28.58 ± 1.03 abcde
16789 + NYA-2	64.13 ± 1.10 a	85.23 ± 1.03 abc	25.42 ± 1.04 cde
16789 UNINOCULATED	61.96 ± 1.09 a	84.05 ± 1.03 abc	29.08 ± 1.03 abcde
16789 + NAK-2 + NYA-2 + NSD	55.91 ± 1.08 ab	73.55 ± 1.04 abc	24.36 ± 1.04 e
16789 + NAK-2 + NYA-2	53.22 ± 1.14 ab	75.40 ± 1.04 abc	27.58 ± 1.04 abcde
16789 + NSD	50.55 ± 1.11 ab	79.79 ± 1.04 abc	28.52 ± 1.04 abcde
Test values	**df = 23; F = 2.58; P < 0.0001**	**df = 23; F = 3.04; P < 0.0001**	**df = 23; F = 4.66; P < 0.0001**

The varieties infected entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD pathogen used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

4.4 Levels of host plant tolerance of the selected napier grass varieties

The levels of tolerance of the napier grass varieties used in this study in respective treatments described in subsection 3.3.1, against *Ustilago kamerunensis* and "*Candidatus* Phytoplasma oryzae" strain Mbita 1 were determined. First, the results addressed their mean logarithmic percentage levels, then their corresponding specific tolerance levels which they were assigned from the customized table (Appendix 10).

4.4.1 Effects of nutrient formulations and watering regimes on the mean logarithmic percentages of the napier grass varieties against *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae" strain Mbita 1

Based on mean logarithmic percentages (mean %), accession 16789 had the highest mean of 30.52% ± 0.30%. This suggested that it was the most tolerant to smut and stunt pathogens under nitrogen deficient nutrient solution and daily watering (Table 4.18). It was followed by Kakamega 1 variety (KK 1) which had mean logarithmic percentage of 29.21% ± 0.80%. The Bana variety treatments had the lowest at 28.36% ± 0.50%, while Kakamega 2 (KK 2) variety's treatments were the second lowest at 28.44% ± 0.60% (Table 4.18). In comparison with the weekly watered treatments the 16789 accession's treatments had the highest mean logarithmic percentage of 18.59% ± 1.00%, followed by Bana variety treatments at 17.33% ± 1.20% (Table 4.19). The Kakamega 2 (KK 2) variety's treatments had the lowest mean logarithmic percentage of 15.86% ± 1.40, while Kakamega 1 (KK 1) treatments were the second lowest at 17.05% ± 1.90% (Table 4.19).

The effects of phosphorus deficiency under daily watering on the mean logarithmic percentages (mean %) of the respective treatments produced the following results. Kakamega 1 (KK 1) variety's treatments had the highest mean logarithmic percentage of 32.22% ± 0.27%, followed by accession 16789's treatments which had 31.84% ± 0.20% (Table 4.20). The Kakamega 2 (KK 2) variety's treatments had the lowest mean logarithmic percentage (mean %) of 30.34% ± 0.41%, while Bana variety's treatments were the second lowest at 31.66% ± 0.55% (Table 4.20). In comparison under the weekly watered treatments the accession 16789 treatments had the highest mean logarithmic percentage (mean %) of 25.10% ± 0.39%, followed by Bana variety

treatments which had 24.68% ± 0.29% (Table 4.21). The Kakamega 1 (KK 1) variety's treatments had the lowest mean logarithmic percentage of 23.07% ± 0.09, while Kakamega 2 (KK 2) variety's treatments were the second lowest at 23.61% ± 0.27% (Table 4.21).

Nitrogen and phosphorus deficiency effects on the mean logarithmic percentages (mean %) had Bana variety's treatments with the highest mean logarithmic percentage of 21.74% ± 0.66%, followed by accession 16789's treatments which had 21.40% ± 0.46% (Table 4.22). The Kakamega 2 (KK 2) variety's treatments had the lowest mean logarithmic percentage of 20.88% ± 0.47%, while Kakamega 1 (KK 1) variety's treatments had the second lowest at 20.89% ± 0.44% (Table 4.22). In comparison to the weekly watered treatments accession 16789 treatments had the highest mean logarithmic percentage of 14.48% ± 0.53%, followed by Bana variety treatments which had 13.37% ± 0.48% (Table 4.23). The Kakamega 2 (KK 2) variety's treatments had the lowest at 11.75% ± 0.31%, while Kakamega 1 (KK 1) variety's treatments had the second lowest at 12.87% ± 0.31% (Table 4.23).

The treatments applied with complete nutrient solution under daily watering, accession 16789 treatments had the highest mean logarithmic percentage of 36.48% ± 1.02%, followed by Kakamega 1 (KK 1) variety's treatments which had 36.03% ± 0.97% (Table 4.24). The Bana variety's treatments had the lowest at 34.59% ± 0.85%, followed by Kakamega 2 (KK 2) variety's treatments that had the second lowest mean logarithmic percentage of 35.47% ± 1.10% (Table 4.24). Comparing the treatments with those under weekly watering regime, the accession 16789 treatments had the highest mean logarithmic percentage (mean %) of 28.08% ± 0.28%, followed by Kakamega 1 (KK 1) treatments which had 26.06% ± 0.46% (Table 4.25). The Bana variety's treatments had the lowest mean logarithmic percentage of 25.74% ± 0.05%, while Kakamega 2 (KK 2) had the second lowest at 26.05% ± 0.44% (Table 4.25).

In summary, the same trend observed under nitrogen deficiency, phosphorus deficiency and nitrogen/phosphorus deficiency was witnessed on treatments that were supplied with complete nutrient solution; where the daily watered treaments exhibited higher mean logarithmic percentages (mean %) in comparison to the weekly watered ones. In addition, the head smut isolates infected treatments demonstrated higher mean logarithmic percentages in comparison to the NSD pathogen infected treatments that had largely lower mean logarithmic percentages. The average of the corresponding percentages of M.E.I and that of the M.L.I determined from the '*Omatec natural logarithmic indices' and corresponding percentages table'* (Appendix 2), was

the one considered as the corresponding logarithmic percentage (mean %) as shown in tables 4.18 - 4.25. Details on the treatments indices types are captured on the same tables. Where L.I.I, meant a logarithmic index of the inoculated treatment by a certain pathogen. Whereas, L.I.U.S, meant a logarithmic index of the uninoculated but stressed due to the limited water availability. The abbreviations L.I.U, meant a logarithmic index of the uninoculated but not stressed due to all nutrients availability and the plants on the treatment being under regular water supply (daily watering regime).

Table 4.18: Effects of nitrogen deficiency on napier grass varieties' corresponding logarithmic percentage (mean %) under daily watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK 1 Uninoculated	4.464	L.I.U.S	-0.30	-9.80	70.84	30.52
KK 1 + NAK-2	4.440	L.I.I	-0.33	-10.37	70.84	30.24
KK 1 + NYA-2	4.407	L.I.I	-0.36	-11.58	70.84	29.63
KK 1 + NAK- 2+ NYA-2	4.377	L.I.I	-0.39	-12.55	70.60	29.03
KK 1 + NAK- 2+ NYA-2 + NSD	4.341	L.I.I	-0.43	-14.01	70.31	28.15
KK 1 + NSD	4.309	L.I.I	-0.46	-14.89	70.31	27.71
					Kakamega 1 (KK 1) Napier grass variety general mean	**29.21 ± 0.80**
KK 2 + NAK-2	4.418	L.I.I	-0.39	-12.55	70.84	29.15
KK 2 Uninoculated	4.418	L.I.U.S	-0.39	-12.55	70.84	29.15
KK 2 + NYA-2	4.408	L.I.I	-0.40	-12.96	70.84	28.94
KK 2 + NAK- 2+ NYA-2	4.366	L.I.I	-0.44	-14.01	70.31	28.42
KK 2 + NAK- 2+ NYA-2 + NSD	4.338	L.I.I	-0.47	-15.14	70.31	27.59
KK 2 + NSD	4.325	L.I.I	-0.48	-15.52	70.31	27.40
					Kakamega 2 (KK 2) Napier grass variety general mean	**28.44 ± 0.60**
Bana Uninoculated	4.367	L.I.U.S	-0.39	-12.55	70.31	28.88
Bana + NAK-2	4.356	L.I.I	-0.40	-12.96	70.31	28.68
Bana + NAK- 2+ NYA-2	4.345	L.I.I	-0.41	-13.29	70.31	28.51
Bana + NYA-2	4.345	L.I.I	-0.41	-13.29	70.31	28.51
Bana + NAK- 2+ NYA-2 + NSD	4.329	L.I.I	-0.42	-13.61	70.31	28.35
Bana + NSD	4.263	L.I.I	-0.49	-15.89	70.31	27.21
					Bana Napier grass variety general mean	**28.36 ± 0.50**
16789 + NAK-2	4.384	L.I.I	-0.26	-8.50	70.60	31.05
16789 + NYA-2	4.373	L.I.I	-0.27	-8.93	70.31	30.69
16789 Uninoculated	4.364	L.I.U.S	-0.28	-9.15	70.31	30.58
16789 + NAK- 2+ NYA-2	4.351	L.I.I	-0.30	-9.80	70.31	30.26
16789 + NAK- 2+ NYA-2 + NSD	4.348	L.I.I	-0.30	-9.80	70.31	30.26
16789 + NSD	4.345	L.I.I	-0.30	-9.80	70.31	30.26
					16789 Napier grass accession general mean	**30.52 ± 0.30**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.19: Effects of nitrogen deficiency on napier grass varieties' corresponding logarithmic percentage (mean %) under weekly watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1 Uninoculated	3.832	L.I.U.S	-0.94	-28.93	67.84	19.46
KK1+NAK-2	3.807	L.I.I	-0.96	-29.63	67.84	19.11
KK1+NYA-2	3.781	L.I.I	-0.99	-30.20	67.84	18.82
KK1+NAK-2+NYA-2	3.639	L.I.I	-1.13	-33.78	66.85	16.54
KK1+NAK-2+NYA-2+NSD	3.485	L.I.I	-1.29	-37.64	65.98	14.17
KK1+NSD	3.471	L.I.I	-1.30	-37.64	65.98	14.17
					Kakamega 1 (KK 1) Napier grass variety general mean	**17.05 ± 1.90**
KK2 Uninoculated	3.845	L.I.U.S	-0.96	-29.63	67.84	19.11
KK2+NAK-2	3.689	L.I.I	-1.12	-33.78	67.84	16.73
KK2+NYA-2	3.598	L.I.I	-1.21	-35.87	66.85	15.49
KK2+NAK-2+NYA-2	3.577	L.I.I	-1.23	-36.47	66.85	15.19
KK2+NAK-2+NYA-2+NSD	3.552	L.I.I	-1.26	-37.07	65.98	14.46
KK2+NSD	3.508	L.I.I	-1.30	-37.64	65.98	14.17'
					Kakamega 2 (KK 2) Napier grass variety general mean	**15.86 ± 1.40**
Bana Uninoculated	3.795	L.I.U.S	-0.96	-29.63	67.84	19.11
Bana+NAK-2	3.720	L.I.I	-1.03	-31.68	67.84	18.08
Bana+NYA-2	3.711	L.I.I	-1.04	-31.68	67.84	18.08
Bana+NAK-2+NYA-2	3.689	L.I.I	-1.06	-31.68	67.24	17.78
Bana+NAK-2+NYA-2+NSD	3.575	L.I.I	-1.18	-35.13	66.85	15.86
Bana+NSD	3.537	L.I.I	-1.21	-35.87	65.98	15.06
					Bana Napier grass variety general mean	**17.33 ± 1.20**
16789 Uninoculated	3.749	L.I.U.S	-0.90	-27.82	67.84	20.01
16789+NAK-2	3.716	L.I.I	-0.93	-28.59	67.84	19.63
16789+NYA-2	3.695	L.I.I	-0.95	-29.27	67.84	19.29
16789+NAK-2+NYA-2	3.670	L.I.I	-0.98	-29.98	66.85	18.44
16789+NAK-2+NYA-2+NSD	3.602	L.I.I	-1.05	-31.68	66.85	17.59
16789+NSD	3.550	L.I.I	-1.10	-32.98	65.98	16.50
					16789 Napier grass accession general mean	**18.58 ± 1.00**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.20: Effects of phosphorus deficiency on napier grass varieties' corresponding logarithmic percentage (mean %) under daily watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1+NAK-2	4.589	L.I.I	-0.18	-5.75	71.30	32.78
KK1 Uninoculated	4.589	L.I.U.S	-0.18	-5.75	71.30	32.78
KK1+NYA-2	4.589	L.I.I	-0.18	-5.75	71.30	32.78
KK1+NAK-2+NYA-2	4.560	L.I.I	-0.21	-6.75	71.30	32.28
KK1+NAK-2+NYA-2+NSD	4.522	L.I.I	-0.25	-8.06	71.30	31.62
KK1+NSD	4.504	L.I.I	-0.27	-8.93	71.08	31.08
					Kakamega 1 (KK 1) Napier grass variety general mean	**32.22 ± 0.27**
KK2+NAK-2	4.554	L.I.I	-0.25	-8.06	71.30	31.62
KK2 Uninoculated	4.539	L.I.U.S	-0.27	-8.93	71.30	31.19
KK2+NYA-2	4.523	L.I.I	-0.28	-9.15	71.30	31.08
KK2+NAK-2+NYA-2	4.457	L.I.I	-0.35	-11.16	70.84	29.84
KK2+NAK-2+NYA-2+NSD	4.429	L.I.I	-0.38	-12.55	70.84	29.15
KK2+NSD	4.421	L.I.I	-0.39	-12.55	70.84	29.15
					Kakamega 2 (KK 2) Napier grass variety general mean	**30.34 ± 0.41**
Bana+NAK-2	4.574	L.I.I	-0.18	-5.75	71.30	32.78
Bana Uninoculated	4.574	L.I.U.S	-0.18	-5.75	71.30	32.78
Bana+NYA-2	4.553	L.I.I	-0.20	-6.35	71.30	32.48
Bana+NAK-2+NYA-2	4.529	L.I.I	-0.22	-7.28	71.30	32.01
Bana+NAK-2+NYA-2+NSD	4.468	L.I.I	-0.28	-9.15	70.84	30.85
Bana+NSD	4.375	L.I.I	-0.38	-12.55	70.60	29.03
					Bana Napier grass variety general mean	**31.66 ± 0.55**
16789+NAK-2	4.454	L.I.I	-0.19	-5.56	70.84	32.64
16789+NYA-2	4.445	L.I.I	-0.20	-6.35	70.84	32.25
16789+NAK-2+NYA-2	4.432	L.I.I	-0.22	-7.28	70.84	31.78
16789 Uninoculated	4.416	L.I.U.S	-0.23	-7.67	70.84	31.59
16789+NAK-2+NYA-2+NSD	4.404	L.I.I	-0.24	-7.67	70.84	31.59
16789+NSD	4.385	L.I.I	-0.26	-8.50	70.84	31.17
					16789 Napier grass accession general mean	**31.84 ± 0.2**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.21: Effects of phosphorus deficiency on napier grass varieties' corresponding logarithmic percentage (mean %) under weekly watering regime across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1 Uninoculated	4.076	L.I.U.S	-0.69	-21.91	68.87	23.48
KK1+ NAK-2	4.057	L.I.I	-0.71	-22.38	68.87	23.25
KK1+NYA-2	4.047	L.I.I	-0.72	-22.84	68.87	23.02
KK1+NAK-2+NYA-2	4.037	L.I.I	-0.73	-23.04	68.87	22.92
KK1+NAK-2+NYA-2+NSD	4.037	L.I.I	-0.73	-23.04	68.87	22.92
KK1+NSD	4.030	L.I.I	-0.74	-23.23	68.87	22.82
					Kakamega 1 (KK 1) Napier grass variety general mean	**23.07 ± 0.09**
KK2 Uninoculated	4.143	L.I.U.S	-0.66	-21.04	69.66	24.31
KK2+ NAK-2	4.138	L.I.I	-0.67	-21.32	69.66	24.17
KK2+NYA-2	4.134	L.I.I	-0.67	-21.32	69.66	24.17
KK2+NAK-2+NYA-2	4.088	L.I.I	-0.72	-22.84	69.30	23.23
KK2+NAK-2+NYA-2+NSD	4.086	L.I.I	-0.72	-22.84	69.30	23.23
KK2+NSD	4.050	L.I.I	-0.76	-23.81	68.87	22.53
					Kakamega 2 (KK 2) Napier grass variety general mean	**23.61 ± 0.27**
Bana Uninoculated	4.164	L.I.U.S	-0.59	-18.83	69.66	25.42
Bana+ NAK-2	4.158	L.I.I	-0.59	-18.83	69.66	25.42
Bana+NYA-2	4.153	L.I.I	-0.60	-19.24	69.66	25.21
Bana+NAK-2+NYA-2	4.098	L.I.I	-0.65	-20.75	69.66	24.46
Bana+NAK-2+NYA-2+NSD	4.076	L.I.I	-0.68	-21.32	68.87	23.78
Bana+NSD	4.072	L.I.I	-0.68	-21.32	68.87	23.78
					Bana Napier grass variety general mean	**24.68 ± 0.29**
16789 + NAK-2	4.113	L.I.I	-0.53	-17.09	69.66	26.29
16789 Uninoculated	4.097	L.I.U.S	-0.55	-17.71	69.66	25.98
16789+NYA-2	4.085	L.I.I	-0.56	-17.98	69.30	25.66
16789+NAK-2+NYA-2	4.047	L.I.I	-0.60	-19.24	68.87	24.82
16789+NAK-2+NYA-2+NSD	4.000	L.I.I	-0.65	-20.75	68.87	24.06
16789+NSD	3.978	L.I.I	-0.67	-21.32	68.87	23.78
					16789 Napier grass accession general mean	**25.10 ± 0.39**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.22: Effects of nitrogen & phosphorus deficiency on napier grass varieties' corresponding logarithmic percentage (mean%) under the daily watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1 Uninoculated	4.016	L.I.U.S	-0.75	-23.52	68.87	22.68
KK1+ NAK-2	3.938	L.I.I	-0.83	-25.78	68.87	21.55
KK1 + NYA-2	3.927	L.I.I	-0.84	-26.28	68.87	21.30
KK1 + NAK-2 + NYA-2	3.894	L.I.I	-0.88	-27.17	67.84	20.34
KK1 + NAK-2 + NYA-2+ NSD	3.860	L.I.I	-0.91	-28.21	67.84	19.82
KK1 + NSD	3.845	L.I.I	-0.93	-28.59	67.84	19.63
					Kakamega 1 (KK 1) Napier grass variety general mean	**20.89 ± 0.44**
KK2 Uninoculated	4.035	L.I.U.S	-0.77	-23.52	68.87	22.68
KK2+ NAK-2	3.973	L.I.I	-0.83	-25.78	68.87	21.55
KK2 + NAK-2 + NYA-2	3.943	L.I.I	-0.86	-26.77	68.87	21.05
KK2 + NYA-2	3.947	L.I.I	-0.86	-26.77	68.87	21.05
KK2 + NAK-2 + NYA-2+ NSD	3.876	L.I.I	-0.93	-28.59	67.84	19.63
KK2 + NSD	3.855	L.I.I	-0.95	-29.27	67.84	19.29
					Kakamega 2 (KK 2) Napier grass variety general mean	**20.88 ± 0.47**
Bana + NAK-2	4.056	L.I.I	-0.70	-22.38	68.97	23.25
Bana Uninoculated	4.057	L.I.U.S	-0.70	--22.38	68.97	23.25
Bana + NYA-2	4.024	L.I.I	-0.73	-23.04	68.97	22.92
Bana + NAK-2 + NYA-2	3.950	L.I.I	-0.80	-25.11	68.97	21.88
Bana + NAK-2 + NYA-2+ NSD	3.833	L.I.I	-0.92	-28.21	67.84	19.82
Bana + NSD	3.806	L.I.I	-0.95	-29.27	67.84	19.29
					Bana Napier grass variety general mean	**21.74 ± 0.66**
16789 Uninoculated	3.973	L.I.U.S	-0.67	-21.32	68.87	23.78
16789 + NAK-2	3.871	L.I.I	-0.78	-24.49	67.84	21.68
16789 + NYA-2	3.817	L.I.I	-0.83	-25.78	67.84	21.03
16789 + NAK-2+ NYA-2	3.804	L.I.I	-0.84	-26.28	67.84	20.78
16789 + NAK-2+ NYA-2+ NSD	3.799	L.I.I	-0.85	-26.28	67.84	20.78
16789 + NSD	3.766	L.I.I	-0.88	-27.17	67.84	20.34
					16789 Napier grass accession general mean	**21.40 ± 0.46**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.23: Effects of nitrogen & phosphorus deficiency on napier grass varieties' corresponding logarithmic percentage (mean %) under weekly watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1 Uninoculated	3.466	L.I.U.S	-1.30	-37.64	65.98	14.17
KK1+ NAK-2	3.425	L.I.I	-1.35	-39.11	65.98	13.44
KK1 + NYA-2	3.401	L.I.I	-1.37	-39.40	65.50	13.05
KK1 + NAK-2 + NYA-2	3.369	L.I.I	-1.40	-40.19	64.88	12.35
KK1+NAK-2 + NYA-2 + NSD	3.347	L.I.I	-1.42	-40.53	64.88	12.18
KK1 + NSD	3.333	L.I.I	-1.44	-40.88	64.88	12.00
					Kakamega 1 (KK 1) Napier grass variety general mean	**12.87 ± 0.31**
KK2 Uninoculated	3.428	L.I.U.S	-1.38	-39.62	65.98	14.17
KK2+ NAK-2	3.364	L.I.I	-1.44	-40.88	64.88	13.44
KK2 + NYA-2	3.363	L.I.I	-1.44	-40.88	64.88	13.05
KK2 + NAK-2 + NYA-2	3.356	L.I.I	-1.45	-41.22	64.88	12.35
KK2+NAK-2 + NYA-2 + NSD	3.352	L.I.I	-1.45	-41.22	64.88	12.18
KK2 + NSD	3.191	L.I.I	-1.62	-44.48	63.78	12.00
					Kakamega 2 (KK 2) Napier grass variety general mean	**11.75 ± 0.31**
Bana Uninoculated	3.522	L.I.U.S	-1.23	-36.47	65.98	14.76
Bana+ NAK-2	3.513	L.I.I	-1.24	-36.47	65.98	14.76
Bana + NYA-2	3.408	L.I.I	-1.34	-38.61	65.98	13.69
Bana + NAK-2 + NYA-2	3.398	L.I.I	-1.35	-39.11	65.50	13.20
Bana+NAK-2 + NYA-2 + NSD	3.313	L.I.I	-1.44	-40.88	64.88	12.00
Bana + NSD	3.301	L.I.I	-1.45	-41.22	64.88	11.83
					Bana Napier grass variety general mean	**13.37± 0.48**
16789 Uninoculated	3.542	L.I.U.S	-1.11	-33.42	65.98	16.28
16789+ NAK-2	3.465	L.I.I	-1.18	-35.13	65.98	15.43
16789 + NAK-2 + NYA-2	3.427	L.I.I	-1.22	-36.47	65.98	14.76
16789 + NYA-2	3.427	L.I.I	-1.22	-36.47	65.98	14.76
16789+NAK-2 + NYA-2 + NSD	3.317	L.I.I	-1.33	-38.61	64.88	13.14
16789 + NSD	3.256	L.I.I	-1.39	-39.84	64.88	12.52
					16789 Napier grass accession general mean	**14.48 ± 0.53**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.24: Effects of complete nutrient solution on napier grass varieties' corresponding logarithmic percentage (mean %) under daily watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1+ NAK-2	4.880	L.I.I	0.11	3.34	75.23	39.29
KK1 Uninoculated	4.771	L.I.U	0.00	0.00	75.23	37.62
KK1+NYA-2	4.764	L.I.I	-0.01	-0.52	75.23	37.36
KK1+ NAK-2+NYA-2	4.676	L.I.I	-0.10	-3.23	75.23	36.00
KK1+ NAK-2+NYA-2+NSD	4.613	L.I.I	-0.16	-5.25	71.52	33.14
KK1+NSD	4.591	L.I.I	-0.18	-5.75	71.30	32.78
					Kakamega 1 (KK 1) Napier grass variety general mean	**36.03 ± 0.97**
KK2+ NAK-2	4.890	L.I.I	0.08	2.41	75.23	38.82
KK2 Uninoculated	4.807	L.I.U	0.00	0.00	75.23	37.62
KK2+ NAK-2+NYA-2	4.732	L.I.I	-0.07	-2.67	75.23	36.38
KK2+NYA-2	4.735	L.I.I	-0.07	-2.67	75.23	36.38
KK2+ NAK-2+NYA-2+NSD	4.582	L.I.I	-0.23	-7.67	71.30	31.82
KK2+NSD	4.581	L.I.I	-0.23	-7.67	71.30	31.82
					Kakamega 2 (KK 2) Napier grass variety general mean	**35.47 ± 1.1**
Bana Uninoculated	4.752	L.I.U	0.00	0.00	75.23	37.62
Bana + NAK-2	4.746	L.I.I	-0.01	-0.52	75.23	37.36
Bana+ NAK-2+NYA-2	4.610	L.I.I	-0.14	-4.65	71.52	33.44
Bana+NYA-2	4.613	L.I.I	-0.14	-4.65	71.52	33.44
Bana+ NAK-2+NYA-2+NSD	4.579	L.I.I	-0.17	-5.56	71.30	32.87
Bana+NSD	4.576	L.I.I	-0.18	-5.75	71.30	32.78
					Bana Napier grass variety general mean	**34.59 ± 0.85**
16789+NAK-2	4.779	L.I.I	0.13	4.28	75.23	39.76
16789+NYA-2	4.667	L.I.I	0.02	0.49	75.23	37.86
16789 Uninoculated	4.648	L.I.U	0.00	0.00	75.23	37.62
16789+NAK-2+NYA-2	4.631	L.I.I	-0.02	-0.52	75.23	37.36
16789+NAK-2+NYA-2+NSD	4.517	L.I.I	-0.13	-4.37	71.30	33.47
16789+NSD	4.483	L.I.I	-0.16	-5.25	70.84	32.80
					16789 Napier grass accession general mean	**36.48 ± 1.02**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance levels.

Table 4.25: Effects of complete nutrient solution on napier grass varieties' corresponding logarithmic percentage (mean %) under weekly watering regimes across the four ratoons.

Treatments	Mean Logarithmic Index (M.L.I)	Index Type	(M.E.I)	(M.E.I) Corresponding (%)	(M.L.I) Levels in (%) relative to unit value (1)	Mean (%)
KK1 Uninoculated	4.299	L.I.U.S	-0.47	-15.14	70.31	27.59
KK1+ NAK-2	4.265	L.I.I	-0.51	-16.31	70.31	27.00
KK1+NYA-2	4.239	L.I.I	-0.53	-17.09	69.66	26.29
KK1+NAK-2+NYA-2	4.209	L.I.I	-0.56	-17.98	69.66	25.84
KK1+NAK-2+NYA-2+NSD	4.191	L.I.I	-0.58	-18.41	69.66	25.63
KK1+NSD	4.096	L.I.I	-0.68	-21.62	69.66	24.02
					Kakamega 1 (KK 1) Napier grass variety general mean	**26.06 ± 0.46**
KK2 Uninoculated	4.323	L.I.U.S	-0.48	-15.52	70.31	27.40
KK2+ NAK-2	4.289	L.I.I	-0.52	-16.73	70.31	26.79
KK2+NYA-2	4.273	L.I.I	-0.53	-17.09	70.31	26.61
KK2+NAK-2+NYA-2	4.256	L.I.I	-0.55	-17.71	70.31	26.30
KK2+NAK-2+NYA-2+NSD	4.178	L.I.I	-0.63	-20.11	69.66	24.78
KK2+NSD	4.153	L.I.I	-0.65	-20.75	69.66	24.46
					Kakamega 2 (KK 2) Napier grass variety general mean	**26.05 ± 0.44**
Bana Uninoculated	4.206	L.I.U.S	-0.55	-17.71	69.66	25.98
Bana+ NAK-2	4.192	L.I.I	-0.56	-17.98	69.66	25.84
Bana+NYA-2	4.186	L.I.I	-0.57	-18.20	69.66	25.73
Bana+NAK-2+NYA-2	4.176	L.I.I	-0.58	-18.41	69.66	25.63
Bana+NAK-2+NYA-2+NSD	4.173	L.I.I	-0.58	-18.41	69.66	25.63
Bana+NSD	4.173	L.I.I	-0.58	-18.41	69.66	25.63
					Bana Napier grass variety general mean	**25.74 ± 0.05**
16789+NAK-2	4.285	L.I.I	-0.36	-11.58	70.31	29.37
16789 Uninoculated	4.238	L.I.U.S	-0.41	-1.329	69.66	28.19
16789+NYA-2	4.235	L.I.I	-0.41	-1.329	69.66	28.19
16789+NAK-2+NYA-2	4.219	L.I.I	-0.43	-14.01	69.66	27.83
16789+NAK-2+NYA-2+NSD	4.206	L.I.I	-0.44	-14.01	69.66	27.83
16789+NSD	4.169	L.I.I	-0.48	-15.52	69.66	27.07
					16789 Napier grass accession general mean	**28.08 ± 0.28**

The mean (%); which is the average between (M.E.I) corresponding (%) and (M.L.I) corresponding (%) relative to unit value (1) were used in standardization/customization of napier grass' host plant tolerance level.

4.4.2 Determination of the tolerance levels' of the napier grass varieties in the respective treatment combination

The results of the respective host plant tolerance levels of the napier grass varieties in different treatments are captured starting with the very specific performances and followed by the various treatment combinations as described.

4.4.2.1 Determination of the specific treatment combinations' tolerance levels in percentage and categorization basing on a customized napier grass' table

The respective treatment combinations were assigned their host plant tolerance levels in percentage basing on their specific mean logarithmic percentages as shown in table 4.26, using the 'Customized/standardized napier grass varieties' tolerance magnitudes and classification table' (Appendix 10). In the specific treatments performance differences were observed across the various combinations involved in the experiment with host plant tolerance percentage levels that ranged between 34.31% - 56.20% as shown in column D of table 4.26. The treatment of accession 16789 infected with NAK-2 *Ustilago kamerunensis* isolate under complete nutrient solution (CNS) and daily (D) watering (16789 + NAK-2+ CNS + D), had the highest levels of tolerance 56.20% with a mean logarithmic percentage of 39.76% and high magnitude of tolerance (HMT) classification (Table 4.26). This treatment was followed by Kakamega 1 variety infected with NAK-2 *Ustilago kamerunensis* isolate only under complete nutrient solution and daily watering (KK 1+ NAK-2 + CNS + D), and Kakamega 2 variety infected with NAK-2 *Ustilago kamerunensis* isolate under complete nutrient solution and daily watering (KK 2 + NAK-2 + CNS + D), which both had a host tolerance level of 55.47%; with a corresponding logarithmic percentage of 39.29%. These two treatments had a high magnitude of tolerance classification (HMT) as shown in table 4.26.

The treatment of Kakamega 2 variety, infected with NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) under nitrogen and phosphorus deficiency (N/P-D) on weekly (W) watering (KK 2 + NSD + N/P-D + W), had the lowest levels of host plant tolerance of 34.31% with moderate magnitude of tolerance classification (MMT) (Table 4.26). Its corresponding logarithmic percentage was 9.65%. This treatment was followed by; (Bana + NSD + N/P-D + W), (KK 2 + NAK-2 + NYA-2 + NSD + N/P-D + W) and (KK 2 + NAK-2 + NYA-2 + N/P-D + W) treatment combinations which all had a host plant tolerance level and corresponding

logarithmic percentage of 35.77% and 11.83% respectively (Table 4.26). Their classification was moderate magnitude of tolerance (MMT) (Table 4.26).

In summary the nutrient formulations played a significant role in the dynamics of host plant tolerance levels. The complete nutrient solution largely performed superiorly, followed by those under phosphorus deficiency solution, then nitrogen deficiency solution treated units with those ones under nitrogen and phosphorus deficiency exhibiting generally low tolerance levels. However, it's worth noting that the effect of watering heighteined the tolerance levels across the treatments, where it disregarded the nutrient formulations to produce better tolerance levels under daily watering as compared to the weekly watering. An example is the daily watered treatments under phosphorus deficient solution performed slightly better than the treatments under complete nutrient solution on weekly watering, despite overally the complete nutrient solution treatments performed better than the phosphorus deficient solution treated ones. A trend which was observed across the different nutrient formulations (Table 4.26).

Table 4.26: Tolerance levels of the 192 treatments that were evaluated. The levels in Column D were determined by comparing their rounded off values to a whole number logarithmic corresponding percentages in Column C with their host plant tolerance magnitudes and classification on the customized 'Customized napier grass varieties' tolerance magnitudes table' shown in appendix 10. The abbreviations (MMT) and (HMT) stand for Moderate Magnitude of Tolerance and High Magnitude of Tolerance respectively.

COLUMN A	COLUMN B	COLUMN C	COLUMN D	COLUMN E	COLUMN F
Nutrients & Watering regimes applied	**Napier variety & Pathogen/Stressor involved**	**Logarithmic corresponding percentage (%)**	**Host Plant Tolerance Levels in (%)**	**Classification**	**Rank -basing on host plant tolerance magnitudes**
CNS + D	KK1 + NAK-2	39.29	55.47	HMT	1
CNS + D	KK1 Uninoculated	37.63	54.74	HMT	2
CNS + D	KK1+NYA-2	37.36	54.01	HMT	3
CNS + D	KK1+ NAK-2+NYA-2	36.00	53.28	HMT	4
CNS + D	KK1+ NAK-2+NYA-2+NSD	33.14	51.09	HMT	5
CNS + D	KK1+NSD	32.78	51.09	HMT	5
CNS + D	KK2+ NAK-2	38.82	55.47	HMT	1
CNS + D	KK2 Uninoculated	37.62	54.74	HMT	2
CNS + D	KK2+ NAK-2+NYA-2	36.38	53.28	HMT	3
CNS + D	KK2+NYA-2	36.38	53.28	HMT	3
CNS + D	KK2+ NAK-2+NYA-2+NSD	31.82	50.37	HMT	4
CNS + D	KK2+NSD	31.82	50.37	HMT	4
CNS + D	Bana Uninoculated	37.62	54.74	HMT	1
CNS + D	Bana + NAK-2	37.36	54.01	HMT	2

CNS + D	Bana+ NAK-2+NYA-2	33.44	51.09	HMT	3
CNS + D	Bana+NYA-2	33.44	51.09	HMT	3
CNS + D	Bana+ NAK-2+NYA-2+NSD	32.87	51.09	HMT	3
CNS + D	Bana+NSD	32.78	51.09	HMT	3
CNS + D	16789+ NAK-2	39.76	56.20	HMT	1
CNS + D	16789+ NYA-2	37.86	54.74	HMT	2
CNS + D	16789 Uninoculated	37.62	54.74	HMT	2
CNS + D	16789+ NAK-2+NYA-2	37.36	54.74	HMT	2
CNS + D	16789+ NAK-2+NYA-2+NSD	33.47	51.09	HMT	3
CNS + D	16789+NSD	32.80	51.09	HMT	3
CNS + W	KK1 Uninoculated	27.59	47.45	MMT	1
CNS + W	KK1+ NAK-2	27.00	46.72	MMT	2
CNS + W	KK1+NYA-2	26.29	45.99	MMT	3
CNS + W	KK1+NAK-2+NYA-2	25.84	45.99	MMT	3
CNS + W	KK1+NAK-2+NYA-2+NSD	25.63	45.99	MMT	3
CNS + W	KK1+NSD	24.02	44.53	MMT	4
CNS + W	KK2 Uninoculated	27.40	46.72	MMT	1
CNS + W	KK2+ NAK-2	26.79	46.72	MMT	1
CNS + W	KK2+NYA-2	26.61	46.72	MMT	1

CNS + W	KK2+NAK-2+NYA-2	26.30	45.98	MMT	2
CNS + W	KK2+NAK-2+NYA-2+NSD	24.78	45.26	MMT	3
CNS + W	KK2+NSD	24.46	44.53	MMT	4
CNS + W	Bana Uninoculated	25.98	45.99	MMT	1
CNS + W	Bana+ NAK-2	25.84	45.99	MMT	1
CNS + W	Bana+NYA-2	25.73	45.99	MMT	1
CNS + W	Bana+NAK-2+NYA-2	25.63	45.99	MMT	1
CNS + W	Bana+NAK-2+NYA-2+NSD	25.63	45.99	MMT	1
CNS + W	Bana+NSD	25.63	45.99	MMT	1
CNS + W	16789+NAK-2	29.37	48.18	MMT	1
CNS + W	16789 Uninoculated	28.19	47.45	MMT	2
CNS + W	16789+NYA-2	28.19	47.45	MMT	2
CNS + W	16789+NAK-2+NYA-2	27.83	47.45	MMT	2
CNS + W	16789+NAK-2+NYA-2+NSD	27.83	47.45	MMT	2
CNS + W	16789+NSD	27.07	46.72	MMT	3
P-D + D	KK1+NAK-2	32.78	51.09	HMT	1
P-D + D	KK1 Uninoculated	32.78	51.09	HMT	1
P-D + D	KK1+NYA-2	32.78	51.09	HMT	1
P-D + D	KK1+NAK-2+NYA-2	32.28	50.37	HMT	2

P-D + D	KK1+NAK-2+NYA-2+NSD	31.62	50.37	HMT	2
P-D + D	KK1+NSD	31.08	49.64	HMT	3
P-D + D	KK2+NAK-2	31.62	50.37	HMT	1
P-D + D	KK2 Uninoculated	31.19	49.64	HMT	2
P-D + D	KK2+NYA-2	31.08	49.64	HMT	2
P-D + D	KK2+NAK-2+NYA-2	29.84	48.91	MMT	3
P-D + D	KK2+NAK-2+NYA-2+NSD	29.15	48.18	MMT	4
P-D + D	KK2+NSD	29.15	48.18	MMT	4
P-D + D	Bana+NAK-2	32.78	51.09	HMT	1
P-D + D	Bana Uninoculated	32.78	51.09	HMT	1
P-D + D	Bana+NYA-2	32.48	50.37	HMT	2
P-D + D	Bana+NAK-2+NYA-2	32.01	50.37	HMT	2
P-D + D	Bana+NAK-2+NYA-2+NSD	30.85	49.64	HMT	3
P-D + D	Bana+NSD	29.03	48.18	MMT	4
P-D + D	16789+NAK-2	32.64	51.09	HMT	1
P-D + D	16789+NYA-2	32.25	50.37	HMT	2
P-D + D	16789+NAK-2+NYA-2	31.78	50.37	HMT	2
P-D + D	16789 Uninoculated	31.59	50.37	HMT	2
P-D + D	16789+NAK-2+NYA-2+NSD	31.59	50.37	HMT	2

P-D + D	16789+NSD	31.17	49.64	HMT	3
P-D + W	KK1 Uninoculated	23.48	43.80	MMT	1
P-D + W	KK1+ NAK-2	23.25	43.80	MMT	1
P-D + W	KK1+NYA-2	23.02	43.80	MMT	1
P-D + W	KK1+NAK-2+NYA-2	22.92	43.80	MMT	1
P-D + W	KK1+NAK-2+NYA-2+NSD	22.92	43.80	MMT	1
P-D + W	KK1+NSD	22.82	43.80	MMT	1
P-D + W	KK2 Uninoculated	24.31	44.53	MMT	1
P-D + W	KK2+ NAK-2	24.17	44.53	MMT	1
P-D + W	KK2+NYA-2	24.17	44.53	MMT	1
P-D + W	KK2+NAK-2+NYA-2	23.23	43.80	MMT	2
P-D + W	KK2+NAK-2+NYA-2+NSD	23.23	43.80	MMT	2
P-D + W	KK2+NSD	22.53	43.80	MMT	2
P-D + W	Bana Uninoculated	25.42	45.26	MMT	1
P-D + W	Bana+ NAK-2	25.42	45.26	MMT	1
P-D + W	Bana+NYA-2	25.42	45.26	MMT	1
P-D + W	Bana+NAK-2+NYA-2	24.46	44.53	MMT	2
P-D + W	Bana+NAK-2+NYA-2+NSD	23.78	44.53	MMT	2
P-D + W	Bana+NSD	23.78	44.53	MMT	2

P-D + W	16789 + NAK-2	26.29	45.99	MMT	1
P-D + W	16789 Uninoculated	25.98	45.99	MMT	1
P-D + W	16789+NYA-2	25.66	45.99	MMT	1
P-D + W	16789+NAK-2+NYA-2	24.82	45.26	MMT	2
P-D + W	16789+NAK-2+NYA-2+NSD	24.06	44.53	MMT	3
P-D + W	16789+NSD	23.78	44.53	MMT	3
N-D + D	KK 1 Uninoculated	30.52	49.64	HMT	1
N-D + D	KK 1 + NAK-2	30.24	48.91	MMT	2
N-D + D	KK 1 + NYA-2	29.63	48.91	MMT	2
N-D + D	KK 1 + NAK- 2+ NYA-2	29.03	48.18	MMT	3
N-D + D	KK 1 + NAK- 2+ NYA-2 + NSD	28.15	47.45	MMT	4
N-D + D	KK 1 + NSD	27.71	47.45	MMT	4
N-D + D	KK 2 + NAK-2	29.15	48.18	MMT	1
N-D + D	KK 2 Uninoculated	29.15	48.18	MMT	1
N-D + D	KK 2 + NYA-2	28.94	48.18	MMT	1
N-D + D	KK 2 + NAK- 2+ NYA-2	28.42	47.45	MMT	2
N-D + D	KK 2 + NAK- 2+ NYA-2 + NSD	27.59	47.45	MMT	2
N-D + D	KK 2 + NSD	27.40	46.72	MMT	3
N-D + D	Bana Uninoculated	28.88	48.18	MMT	1

N-D + D	Bana + NAK-2	28.68	48.18	MMT	1
N-D + D	Bana + NAK- 2+ NYA-2	28.51	48.18	MMT	1
N-D + D	Bana + NYA-2	28.51	48.18	MMT	1
N-D + D	Bana + NAK- 2+ NYA-2 + NSD	28.35	47.45	MMT	2
N-D + D	Bana + NSD	27.21	46.72	MMT	3
N-D + D	16789 + NAK-2	31.05	49.64	HMT	1
N-D + D	16789 + NYA-2	30.69	49.64	HMT	1
N-D + D	16789 Uninoculated	30.58	49.64	HMT	1
N-D + D	16789 + NAK- 2+ NYA-2	30.26	48.91	MMT	2
N-D + D	16789 + NAK- 2+ NYA-2 + NSD	30.26	48.91	MMT	2
N-D + D	16789 + NSD	30.26	48.91	MMT	2
N-D + W	KK1 Uninoculated	19.46	40.88	MMT	1
N-D + W	KK1+NAK-2	19.11	40.88	MMT	1
N-D + W	KK1+NYA-2	18.82	40.88	MMT	1
N-D + W	KK1+NAK-2+NYA-2	16.54	39.42	MMT	2
N-D + W	KK1+NAK-2+NYA-2+NSD	14.17	37.23	MMT	3
N-D + W	KK1+NSD	14.17	37.23	MMT	3
N-D + W	KK2 Uninoculated	19.11	40.88	MMT	1
N-D + W	KK2+NAK-2	16.73	39.42	MMT	2

N-D + W	KK2+NYA-2	15.49	37.96	MMT	3
N-D + W	KK2+NAK-2+NYA-2	15.19	37.96	MMT	3
N-D + W	KK2+NAK-2+NYA-2+NSD	14.46	37.23	MMT	4
N-D + W	KK2+NSD	14.17	37.23	MMT	4
N-D + W	Bana Uninoculated	19.11	40.88	MMT	1
N-D + W	Bana+NAK-2	18.08	40.15	MMT	2
N-D + W	Bana+NYA-2	18.08	40.15	MMT	2
N-D + W	Bana+NAK-2+NYA-2	17.78	40.15	MMT	2
N-D + W	Bana+NAK-2+NYA-2+NSD	15.86	38.69	MMT	3
N-D + W	Bana+NSD	15.06	37.96	MMT	4
N-D + W	16789 Uninoculated	20.01	41.61	MMT	1
N-D + W	16789+NAK-2	19.63	41.61	MMT	1
N-D + W	16789+NYA-2	19.29	40.88	MMT	2
N-D + W	16789+NAK-2+NYA-2	18.44	40.15	MMT	3
N-D + W	16789+NAK-2+NYA-2+NSD	17.59	40.15	MMT	3
N-D + W	16789+NSD	16.50	39.42	MMT	4
N/P-D + D	KK1 Uninoculated	22.68	43.80	MMT	1
N/P-D + D	KK1+ NAK-2	21.55	43.07	MMT	2
N/P-D + D	KK1 + NYA-2	21.30	42.34	MMT	3

N/P-D + D	KK1 + NAK-2 + NYA-2	20.34	41.61	MMT	4
N/P-D + D	KK1 + NAK-2 + NYA-2+ NSD	19.82	41.61	MMT	4
N/P-D + D	KK1 + NSD	19.63	41.61	MMT	4
N/P-D + D	KK2 Uninoculated	22.68	43.80	MMT	1
N/P-D + D	KK2+ NAK-2	21.55	43.07	MMT	2
N/P-D + D	KK2 + NAK-2 + NYA-2	21.05	42.34	MMT	3
N/P-D + D	KK2 + NYA-2	21.05	42.34	MMT	3
N/P-D + D	KK2 + NAK-2 + NYA-2+ NSD	19.63	41.61	MMT	4
N/P-D + D	KK2 + NSD	19.29	40.88	MMT	5
N/P-D + D	Bana + NAK-2	23.25	43.80	MMT	1
N/P-D + D	Bana Uninoculated	23.25	43.80	MMT	1
N/P-D + D	Bana + NYA-2	22.92	43.80	MMT	1
N/P-D + D	Bana + NAK-2 + NYA-2	21.88	43.07	MMT	2
N/P-D + D	Bana + NAK-2 + NYA-2+ NSD	19.82	41.61	MMT	3
N/P-D + D	Bana + NSD	19.29	40.88	MMT	4
N/P-D + D	16789 Uninoculated	23.78	44.53	MMT	1
N/P-D + D	16789 + NAK-2	21.68	43.07	MMT	2
N/P-D + D	16789 + NYA-2	21.03	42.34	MMT	3
N/P-D + D	16789 + NAK-2+ NYA-2	20.78	42.34	MMT	3

N/P-D + D	16789 + NAK-2+ NYA-2+ NSD	20.78	42.34	MMT	3
N/P-D + D	16789 + NSD	20.34	41.61	MMT	4
N/P-D + W	KK1 Uninoculated	17.17	39.42	MMT	1
N/P-D + W	KK1+ NAK-2	13.44	36.50	MMT	2
N/P-D + W	KK1 + NYA-2	13.05	36.50	MMT	2
N/P-D + W	KK1 + NAK-2 + NYA-2	12.35	35.77	MMT	3
N/P-D + W	KK1+NAK-2 + NYA-2 + NSD	12.18	35.77	MMT	3
N/P-D + W	KK1 + NSD	12.00	35.77	MMT	3
N/P-D + W	KK2 Uninoculated	13.18	36.50	MMT	1
N/P-D + W	KK2+ NAK-2	12.00	35.77	MMT	2
N/P-D + W	KK2 + NYA-2	12.00	35.77	MMT	2
N/P-D + W	KK2 + NAK-2 + NYA-2	11.83	35.77	MMT	2
N/P-D + W	KK2+NAK-2 + NYA-2 + NSD	11.83	35.77	MMT	2
N/P-D + W	KK2 + NSD	9.65	34.31	MMT	3
N/P-D + W	Bana Uninoculated	14.76	37.96	MMT	1
N/P-D + W	Bana+ NAK-2	14.76	37.96	MMT	1
N/P-D + W	Bana + NYA-2	13.69	37.23	MMT	2
N/P-D + W	Bana + NAK-2 + NYA-2	13.20	36.50	MMT	3
N/P-D + W	Bana+NAK-2 + NYA-2 + NSD	12.00	35.77	MMT	4

N/P-D + W	Bana + NSD	11.83	35.77	MMT	4
N/P-D + W	16789 Uninoculated	16.28	38.69	MMT	1
N/P-D + W	16789+ NAK-2	15.43	37.96	MMT	2
N/P-D + W	16789 + NAK-2 + NYA-2	14.76	37.96	MMT	2
N/P-D + W	16789 + NYA-2	14.76	37.96	MMT	2
N/P-D + W	16789+NAK-2 + NYA-2 + NSD	13.14	36.50	MMT	3
N/P-D + W	16789 + NSD	12.52	36.50	MMT	3

Column A exhibits the nutrient formulation used where CNS; -was the complete nutrient solution, N-D; - was the nitrogen deficient nutrient solution, P-D;- was the phosphorus deficient nutrient solution and N/P-D;- was the nitrogen & phosphorus deficient nutrient solution. The D and W in the same column represent daily and weekly watering regimes. The ranking done on Column F is within that particular group per row starting from the largest to smallest in percentage magnitude.

4.4.2.2 Evaluation of the selected treatment combinations' means using tolerance levels as the variable

The host plant tolerance levels of the respective determined treatment combinations in table 4.26 had significant differences at df =31 ; F=134.80; P ≤ 0.0001 (Table 4.26). The means of the treatment of accession 16789 accession applied with complete nutrient solution (CNS) under daily watering (16789 + CNS + Daily watering), had the highest levels of tolerance with a high magnitude of tolerance (HMT) classification (Table 4.27). This treatment however did not differ significantly with (KK 1 + CNS + Daily watering) and (KK 2 + CNS + Daily watering) treatments (Table 4.26). The treatment of Kakamega 2 variety applied with nitrogen and phosphorus deficient nutrient solution application on a weekly watering regime (KK 2 + N/P-D + Weekly watering), had the lowest tolerance levels with the moderate magnitude of tolerance (MMT) classification (Table 4.27). This result was however not significantly different from those of the following treatments, (KK 1 + N/P-D + Weekly watering), (KK 2 + N-D + Weekly watering), (16789 + N/P-D + Weekly watering), (Bana + N/P-D + Weekly watering) and (KK 1 + N/P-D + Weekly watering) as shown in table 4.27.

In summary accession 16789 performed better across the different nutrient formulations and watering regimes where it had the highest tolerance levels in 6 out of 8 nutrient formulations and watering regimes (Table 4.27). Further, this trend can be illustrated from the general means across the different nutrient formulations the watering regimes notwithstanding. For example under complete nutrient solution accession 16789 had the highest tolerance mean of 50.61% ± 2.23% and the same trend was observed under phosphorus, nitrogen and nitrogen-phosphorus deficient nutrient solutions. This accession 16789 was followed by KK 1, KK 2 and Bana variety in that declining order. In the classification of the varieties' tolerance levels, under the complete nutrient solution the accession 16789 and KK 1 variety tolerance means were in the class of high magnitude of tolerance (HMT), whereas, KK 2 and Bana varieties means were in the class of moderate magnitude of tolerance (MMT). Under phosphorus deficient, nitrogen deficient and nitrogen/phosphorus deficient solutions the watering regime notwithstanding the tolerances observed were in the class of moderate magnitude of tolerance (MMT) of all the varieties.

Table 4.27: Mean tolerance levels in percentage of inidvidual napier grass varieties treatments across all the pathogen treatment combinations they were subjected to in this study.

Napier grass, nutrient formulations & watering regimes	Host plant tolerance (%) Means ± S.E	Tolerance Classification	Rank
16789 + CNS + Daily watering	53.77 ± 0.88 a	HMT	1
KK 1 + CNS + Daily watering	53.28 ± 0.75 ab	HMT	2
KK 2 + CNS + Daily watering	52.92 ± 0.88 abc	HMT	3
Bana + CNS + Daily watering	52.19 ± 0.70 bc	HMT	4
KK 1 + P-D + Daily watering	50.61 ± 0.24 bcd	HMT	5
16789 + P-D + Daily watering	50.37 ± 0.19 cde	HMT	6
Bana + P-D + Daily watering	50.12 ± 0.45 cde	HMT	7
KK 2 + P-D + Daily watering	49.28 ± 0.16 cdef	MMT	8
16789 + N-D + Daily watering	49.15 ± 0.36 defg	MMT	9
KK 1 + N-D + Daily watering	48.42 ± 0.36 defg	MMT	10
KK 2 + N-D + Daily watering	47.82 ± 0.25 efg	MMT	11
Bana + N-D + Daily watering	47.69 ± 0.24 fgh	MMT	12
16789 + CNS + Weekly watering	47.45 ± 0.19 fgh	MMT	13
KK 2 + CNS + Weekly watering	46.11 ± 0.40 fgh	MMT	14
KK 1 + CNS + Weekly watering	45.99 ± 0.00 gh	MMT	15
Bana + CNS + Weekly watering	45.99 ± 0.38 gh	MMT	15
16789 + P-D + Weekly watering	45.38 ± 0.29 hi	MMT	16
Bana + P-D + Weekly watering	44.90 ± 0.16 ijk	MMT	17
KK 2 + P-D + Weekly watering	44.17 ± 0.16 ijk	MMT	18
KK 1 + P-D + Weekly watering	43.80 ± 0.00 jk	MMT	19
Bana + N/P-D + Daily watering	42.83 ± 0.52 jk	MMT	20
16789 + N/P-D + Daily watering	42.71 ± 0.41 kl	MMT	21
KK 1 + N/P-D + Daily watering	42.34 ± 0.42 lm	MMT	22
KK 2 + N/P-D + Daily watering	42.34 ± 0.38 lm	MMT	22
16789 + N-D + Weekly watering	40.64 ± 0.36 lm	MMT	23
Bana + N-D + Weekly watering	39.66 ± 0.45 lmn	MMT	24
KK 1 + N-D + Weekly watering	39.42 ± 0.73 mno	MMT	25
KK 2 + N-D + Weekly watering	38.45 ± 0.59 mno	MMT	26
16789 + N/P-D + Weekly watering	37.60 ± 0.37 mno	MMT	27
Bana + N/P-D + Weekly watering	36.87 ± 0.41 no	MMT	28
KK 1 + N/P-D + Weekly watering	36.62 ± 0.58 no	MMT	29
KK 2 + N/P-D + Weekly watering	35.65 ± 0.29 o	MMT	30
Test values	**df =31; F=134.80; P ≤ 0.0001**		

The table shows the mean magnitude of tolerance of the different varieties irrespective of the pathogen used to challenge their growth. Tolerance classification are also indicated where HMT and MMT stands for;-High Magnitude of Tolerance and Moderate Magnitude of Tolerance respectively.The CNS (Complete nutrient solution, N/P-D (Nitrogen/phosphorus deficient solution), P-D (Phosphorus deficient solution) and N-D (Nitrogen deficient solution) were used. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

4.4.3 Step three: Uniformity evaluation of the tolerance levels of the respective napier grass varieties from stage two

The analysis of the uniformity of the tolerance trait across the napier grass varieties was based on coefficient of variation, across the different treatment combinations involving the different pathogen combinations, nutrient formulations and watering regimes in this study as highlighted in table 4.26. The variety Kakamega 2 (KK 2) had the highest coefficient of variation across the different treatments followed by Kakamega 1 (KK 1). The Bana variety had the lowest coefficient of variation followed by the 16789 accession as demonstrated on table 4.28. Thus, 16789 accession was the most stable in performance followed by Bana variety. Kakamega 2 (KK 2) was the least stable in performance, followed by Kakamega 1 (KK 1).

Table 4.28: Analysis of the stability of tolerance levels in four napier grass varieties' across the different treatments of nutrient solutions, watering regime and pathogen combinations.

Napier grass varieties	Mean host plant tolerance level in (%) across the different treatments	Standard deviation (%)	Coefficient of variation (CV)	N
Kakamega 1 (KK1)	45.0756	5.44668	12.08%	48
Kakamega 2 (KK2)	44.5442	5.45812	12.25%	48
Bana	45.0454	4.96694	11.03%	48
16789	45.8973	5.17426	11.46%	48
Total	**45.1406**	**5.24656**	**11.62%**	**192**

However, the variability in performance is low for each variety basing on the low co-efficient of variation values observed which are not so significant.

CHAPTER FIVE

DISCUSSION

5.1 *Ustilago kamerunensis* isolates morphological and molecular characteristics

The *Ustilago kamerunensis* isolates growth *in vitro* displayed a colony colour of white floccose top with a pale cream reverse (Table 4.1), which was similar to the results by Farrell *et al*.(2001) and Omayio *et al*.(2014c). However, the colony diameter growth in the different isolates exhibited differences typical of physiologic isolates which has been observed in many microbial species under culture in relation to their growth kinetics (Table 4.1 and 4.2). This growth is attributed to microbes varying adaptability to their ecological niches brought about by agents of co-evolution in their areas of occurrence (Rausher, 2001; Chase and Leibold, 2003; Fodor, 2011). These variations in growth *in vitro* are largely influenced by the way each isolate has adapted its physiology to survive varying water activity levels and other growth *in vitro* conditions associated with media during culture that leads to different physiologic responses (Andrea *et al*., 2005). In other similar studies such physiologic differences have enabled biological characterization and selection of biological microbes; a case example being the studies by Yahyaoui *et al*. (2002) and Andrea *et al*. (2005).

The molecular characterization of *Ustilago kamerunensis* isolates between the ITS 1 and ITS 2 region where the 5.8S ribosomal RNA gene spans, revealed variations between the *Ustilago kamerunensis* isolates. The isolates from Nyeri and Kirinyaga counties shared similarities in terms of their allelic properties. Hence, formed a common clade except for MUR003 and KIR002 which was classified into another clade. This result can be attributed to susceptible napier grass germplasm transfer by farmers, where they unknowingly exchange cuttings containing the different isolates within the region leading to their introduction in other areas (Jones *et al*., 2004; ASARECA, 2010; Kabirizi *et al*., 2015). All sequences from Nakuru, Nyahururu and Kiambu counties formed a common clade, whereas, the isolates from Nyeri, Kirinyaga and Murang'a formed the other (Figure 4.1). This suggests wind transmission of the smut spores northwards in a regular pattern now that the counties mentioned are next to each other in a northerly manner. Wind has been identified as another mode of transmission of the napier head smut disease as reported by Farrell *et al*. (2000) and Omayio *et al*. (2015).

The molecular differences observed in the isolates of *Ustilago kamerunensis* could be due to environmental restrictions of the pathogen, especially by altitude (Farrell *et al*., 2002a). Environmental selection pressure triggers rapid selection and changes in a pathogen in an effort to adopt to the prevailing threats (Burdon and Thrall, 2009). In other similar studies the smut pathogens have been reported to display rapid changes in their genes through co-evolution especially those involved in the coding for effector proteins towards improving their plant-pathogen interaction levels (Schirawski *et al*., 2010). Similar trend has been reported in some fungal pathogens of wheat which is a poaceae as napier grass (Plissonneau *et al*., 2016). The observed differences in *Ustilago kamerunensis* isolates from Central Kenya is a potential problem. This is because the sequenced regions might not be involved directly in its pathogenicity, but the presence of molecular variations is an indication that it may be extending to their virulence genes. Hence, over time due to environmental pressure we might have highly virulent napier head smut pathogen which might not be limited by altitude differences, and this could lead to massive losses in napier grass production. Future management should focus on slowing down the co-evolution rate especially through integrated pathogen management approaches.

5.2 Pathogenicity evaluation of the *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1

Pathogenicity levels of a pathogen are independent qualities from the disease causing agent which depend on the host plant response patterns and possible quantifiable factors (Casadevall and Pirofski, 2001). This study evaluated pathogenicity levels of *Ustilago kamerunensis* isolates NAK002 (NAK-2), NYA002 (NYA-2) and NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1). The pathogenicity levels and validation of the pathogens presence or absence was evaluated simultaneously using both molecular and classical approaches to ensure that the symptoms being observed were as a result of the pathogens and not any other factors as outlined in Koch's postulates (John, 1998). Morpho-pathological symptoms of stunting and chlorosis were observed on those treatments that were inoculated with the napier stunt disease (NSD) pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) as shown in plate 4.1. These morpho-pathological characteristics were similar to those identified by Wamalwa et al. (2017), during the screening of napier grass cultivars for tolerance against napier stunt disease. The

presence or absence of the napier stunt disease pathogen was confirmed using nested PCR molecular technique (Plate 4.3). The pathogens' DNA bands were observed at 778 base pairs which agrees with the findings of Obura (2012) and Asudi *et al*.(2016a). These findings confirmed the reliability and accuracy of the artificial inoculation procedures used in the current study.

On the contrary, the treatments inoculated by the napier head smut isolates (*Ustilago kamerunensis*), failed to show the morpho-pathological symptom of smutting in all the varieties evaluated in the four ratoons studied. However, the molecular detection confirmed the presence of the *Ustilago kamerunensis* pathogen in the infected treatments (Plate 4.2). The scenario of napier head smut disease being intracellularly present in the napier grass varieties but not expressing morphological symptoms could be a basis for the selection of Kakamega 1, Kakamega 2 and accession 16789 as being tolerant to head smut pathogen by Jorge (2013), Omayio *et al*. (2015) and NAFIS (2017). The non-smutting of the Bana variety which was the positive control to napier head smut could be due to the highly unstable nature of the variety in performance, that has been reported in various studies where depending on the growth conditions it ends up exhibiting some tolerance or susceptibility levels, despite being largely classified as susceptible (Farrel, 1998; Mwendia *et al*., 2013; Omayio *et al*., 2015). This high levels of instability have been reported across a wide range of napier grass varieties, where the genotype, environmental conditions and season significantly influences the chemical composition like heightened production of phenols that enable plants resist a pathogen establishment (Bittner, 2006; Turano *et al*., 2016). Thus, leading to a possible increase or decrease in susceptibility to the napier head smut disease, in cases where it's a minor gene encoded trait which is highly influenced by the environmental dynamics (Keane, 2012). Moreover, Farrell *et al*. (2001) reported the likelihood of the napier head smut pathogen being restricted to high altitude areas above 1660 metres above sea level, which was not the case with ICIPE-Mbita with an altitude of 1200m, where this experiment was conducted. Therefore, the non-smutting of Bana grass might be due to the high temperatures and low dew availability associated with low altitudes that does not favour the disease cycle of the smuts. Similar results have been reported within the *Ustilago* genus for example in, *Ustilago tritici* and *Ustilago avenae* which infect barley optimally under low temperatures and high dew point (RPD, 1990).

Pathogenicity evaluation strategy in this study used a quantitative modified virulence logarithmic indexing algorithm approach that integrated all the napier grass varieties parameters measured. This is because there is a general paradigm shift to integration of variables in the analysis of biological systems to make them accurate and reliable as reported by De Vos *et al*.(2017). This approach is a refinement of the existing approaches in disease virulence estimation which have been using qualitative assessment techniques. For instance visual scoring is used to estimate the level of plant tissue infected and then categorizes the severity magnitude using a scale based on estimations (Bock *et al*., 2010). Despite the ease of using such techniques their reliability, precision and accuracy is low due to many errors that may arise from significant variations accruing between individuals' rating levels, perceptions and bias. As a result, disease monitoring, forecasting and plant resistance screening processes end up being compromised (Mutka and Bart, 2015).

In napier grass research and general assessment not many strategies of virulence assessment have been developed due to neglect of the crop being a fodder crop unlike the food crops (Farrell *et al*., 2002b; Mwendia *et al*., 2014; Negawo *et al*., 2017). However, qualitive approaches like Obura (2012) developed a descriptive scoring scale between (1-4) that estimates the virulence levels' basing on the time it took for morphological symptoms to be expressed after an harvest; where 1= denoted symptoms expression after the first cutting, 2= denoted symptom expression after second cutting, 3= denoted symptom expression after third cutting and 4= denoted symptom expression after fourth cutting. Farrell *et al*.(2000), developed a technique that assesses the percentage of the diseased napier grass stool by assigning a damage class' score based on napier grass' height and proportion of smutted tillers. Other methods involving smut pathogens have been observed in the evaluation of sorghum's loose smut (*Sphacelotheca cruenta*) and covered smut (*Sphacelotheca sorghi*), where effect of the disease on productivity of the crop was based on incidence estimation (Sutherland *et al*., 1996). Also, in sugarcane evaluation of *Ustilago scitaminea* has been based on the scoring of disease incidence and comparison of yields between the undiseased versus the diseased sugarcane stools (Glaz *et al*., 1989; Sutherland *et al*., 1996). All these methods lacked the aspect of quantitative estimation of virulence, that the current study has developed.

Based on the current studies' virulence method; the treatments infected by the NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) only had the highest virulence levels on the

napier grass varieties. This was followed by the co-infected treatments of napier grass varieties by NAK-2 and NYA-2 *Ustilago kamerunensis* isolates alongside the NSD pathogen that had the second highest virulence levels (Figure 4.2). The NAK-2 *Ustilago kamerunensis* isolate only infected treatments had the lowest virulence levels. This high virulence levels by NSD pathogen as compared to napier head smut pathogen is in agreement with previous studies where the former caused up to 100% yield losses in some napier grass varieties as compared to 46% respectively (Farrell *et al*., 2002a; NAFIS, 2012; Kabirizi *et al*., 2015; Fischer *et al*., 2016). Furthermore, the virulence levels were lower under the two pathogens co-infected treatments and this could be attributed to the enhanced plant protective responses which were activated by the initial infection by *Ustilago kamerunensis* isolates, before the varieties were co-infected by the NSD pathogen as per the current study's methodology. Similar results have been observed in some plants by Zhu *et al*.(1996); where when plants are infected by an initial pathogen, the expression of specific protective measures against the microbe, leads to initiation of other non-specific complementary measures that make it more tolerant to other diseases that infect the plant later, especially under incompatible host-pathogen interactions. In addition, the low virulence levels of napier head smut pathogens (*Ustilago kamerunensis*) could be associated with environmental influences of infections and the relatively high tolerance levels which have been observed in Kakamega 1, Kakamega 2 and 16789 accession in previous studies by Omayio *et al*.(2015) and Negawo *et al*.(2017). These intervining factors might have played a significant role in the virulence levels of the napier varieties where Bana variety exhibited high levels across the different pathogen combination while other varieties performed intermediately (Figure 4.2).

Based on the pathogen combinations and nutrient formulations, the treatments that were applied with nitrogen and phosphorus deficient nutrient solution had high levels of virulence from both pathogens, followed by those that were applied with nitrogen deficient solution, then phosphorus deficient solution. Those applied with complete nutrient solution had low virulence levels (Figure 4.3). The same trends were observed on napier grass varieties versus nutrient formulations and napier grass versus watering regimes as shown in appendices 47 and 48. These results confirms the importance of mineral nutrients application in disease management especially nitrogen element. This nutrient has been reported to suppress the virulence of facultative pathogens but not obligate pathogens (Dordas, 2008; Singh, 2015). The nitrogen

effects are supported by the fact that the *Ustilago kamerunensis* is a facultative pathogen (Farrell *et al*., 2001). On the contrary the napier stunt pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1) is an obligate pathogen as reported by Obura *et al*. (2009). The difference between these two types of pathogens is based on the variations witnessed on their nutritional requirements, where obligate pathogens being biotrophic obtain assimilates directly from living cells. Whereas for facultative pathogens being hemibiotrophic they can survive on some senesced cells or tissues within the host. Therefore, any factor that enhances metabolic activity and vigour of the host plant like the nitrogen element leads to enhanced tolerance against facultative pathogens by delaying senescence (Dordas, 2008).

The presence of phosphorus minus nitrogen did not play a big role in suppressing the virulence levels when compared to nitrogen alone minus phosphorus. However, the role of phosphorus cannot be ignored based on the fact that treatments under complete nutrient solution performed better, than those that lacked phosphorus but had the nitrogen. This result can be attributed to the inconsistencies of phosphorus element when it comes to suppressing of virulence of some diseases (Dordas, 2008). Despite, the inconsistencies of phosphorus; positive observations on its involvement in hypersensitive responses and suppression of powdery mildew pathogen in plants exist though its specific role not clear (Huber *et al*., 2012). Therefore, application of nitrogen appears to be more important in the management of the two diseases than phosphorus. This could be attributed to its ability to react with phenolics compounds to form pathogen-inhibitive quinine-amino conjugates under the influence of polyphenol oxidase (PPO), proteins that are critical in host plant resistance against pathogens (Bittner, 2006).

Moreover, the use of this virulence estimation strategy in evaluation of severity levels of the diseases was integrated with modified classical approaches of scoring by Obura (2012) and Kawube *et al*. (2014). The nitrogen deficient treatments under daily and weekly watering exhibited a mean disease expression levels to time it took for symptoms to be observed scores of 3/3 and 2/3 respectively (Tables 4.7 and 4.8). This result suggests very low susceptibility to low susceptibility classifications to the infections by *Ustilago kamerunensis* isolates and ‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1. The time taken for disease expression was after the third cutting as shown by the denominator values three (3). The same trends were observed for nitrogen and phosphorus deficient treatments under the daily and weekly watering regimes (Tables 4.11 and Tables 4.12). The complete nutrient solution (CNS) treatments under the daily

and weekly watering exhibited a mean disease expression levels to time taken for symptom observation of 3/4 and 3/3 respectively (Tables 4.13 and 4.14). These results suggests very low susceptibility classification to the infections by *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The time taken for disease expression was after the fourth and third cutting for daily and weekly watering respectively. The same trend was observed for phosphorus deficient treatments under daily and weekly watering regimes (Tables 4.9 and 4.10). The results on the scoring indicate that the nutrient formulations which had nitrogen in their composition delayed the time it took for symptom to be expressed and the disease susceptibility levels of such treatments were very low. These findings are in agreement with findings that nitrogen is more important than phosphorus in lowering the severity levels of pathogens as reported by Dordas (2008), Huber *et al*.(2012) and Singh (2015). However, the use of classical approaches of scoring as observed do not distinguish effectively the performance of all the various treatments' in comparison to the described quantitative approach of virulence evaluation. Therefore, confirming the results by Mutka and Bart (2015) that qualitative approaches of disease severity estimation are limited in their precision levels and accuracy unlike quantitative approaches.

Finally, the effect of trace elements in virulence suppression in the nutrient formulations can not be ignored, a situation perhaps that led to many treatments performing intermediately (Appendix 47). These trace elements like calcium, boron, manganese, potassium, magnesium, copper and chlorine which had a common presence across the four different nutrient formulations have been reported in similar studies to aid plants in limiting severity of pathogens (Dordas, 2008; Huber *et al*., 2012). Hence, the need to validate the specific and interaction effects of these trace mineral elements in suppressing *Ustilago kamerunensis* and '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The water availability factor seemed very crucial in the general suppression of virulence of *Ustilago kamerunensis* and '*Candidatus* Phytoplasam oryzae' strain Mbita 1; where the treatments under water stress (weekly watering) seemed to be hard hit by the diseases unlike the daily watered ones perhaps due to compounded stress and general effect on plant physiology, a situation that verifies the findings by Orodho *et al*.(2005), Mwendia *et al*.(2007) and Omayio *et al*.(2015).

5.3 Co-infection effects evaluation of *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 on growth of the napier grass varieties

The evaluation of the co-infection's interactive effects on the growth of napier grass varieties by *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1, was preceded by the analysis of the general means based on, total fresh weight, tiller height and chlorophyll content levels. The effects of these parameters were sought because napier grass productivity is reported by Negawo et al.(2017) to be highly influenced by its genotype, the environment and the management practices during growth. It is also reported that plant height and chlorophyll content levels impact significantly on the overall productivity of a plant in terms of its biomass output (Yin *et al*., 2012; Gholizadeh *et al*., 2017). The observed heightened productivity of the *Ustilago kamerunensis* inoculated treatments could be explained by a phenomenon that has been observed in some tolerant members of the grass family against fungal pathogens. The fungal pathogen presence has been observed to trigger the adjustment of the host metabolism in tolerant grass hosts, through stimulation of hormonal secretions especially auxins. This hormones as a result enhance the host's fitness in growth towards tolerating the pathogen's challenge as reported by Tanaka *et al*.(2012). This is supported by reports of heightened concentration of sugars in some fungal pathogen infected plants' cells; where the sugar molecules act as signal factors that interact with hormonal signals leading to a network of events that lead to general tolerance of plants against the microorganism (Morkunas and Ratajczak, 2014). The heightened growth in biomass and tiller height had been reported earlier by Omayio *et al*. (2014c); where some tolerant napier grass varieties infected by *Ustilago kamerunensis* pathogen exhibited higher growth levels in comparison to the susceptible ones.

The significantly higher reduction in total fresh weight and tiller height by NSD pathogen than by *Ustilago kamerunensis* is supported by survey studies that have shown biomass losses of upto 90-100% for NSD and 25 - 46% for *Ustilago kamerunensis* (Farrell *et al*., 2002a; Kabirizi *et al*., 2015; Fischer *et al*., 2016). In terms of severity, NSD seems to exhibit high levels of virulence in its attacks of napier grass varieties. This is probably probably due to its obligate nature as a pathogen. This has been associated with direct utilization of assimilates from living cells of the plant leading to significant damage unlike in facultative pathogens like *Ustilago kamerunensis* which can survive on senesced tissues (Dordas, 2008; Obura *et al*., 2009; Farrel *et*

al., 2001). Another explanation could be altitude where the pathogen appears to be more aggressive at higher altitudes (Farrell *et al*., 2002a). This could have played a role in making the pathogen less virulent. Since, the experiment was carried out at a lower altitude of 1200 m.a.s.l. The effect of altitude could be linked to humidity and precipitation which in turn affects pathogen establishment. This has been reported for onion smut (*Urocystis cepulae*) where infection was localized in cold high altitude areas but not in warm and low altitude zones (Mehrota, 2013) The co-infected treatments by NAK-2 and NYA-2 *Ustilago kamerunensis* isolates alongside the NSD pathogen, as observed in this experiment did not severely affect the napier grass varieties' total fresh weight, tiller height and chlorophyll content levels, as the NSD pathogen infected treatments only did. This could be attributed to enhanced general protective measures which were activated in the napier varieties by the initial infection by *Ustilago kamerunensis* isolates, before the varieties were co-infected by the NSD pathogen. Thus, the situation allowed the napier grass varieties to develop specific protective measures against the initial pathogen *Ustilago kamerunensis* isolates, that led to the initiation of other non-specific additive measures that made the fodder crop more tolerant to the NSD pathogen (Zhu *et al*., 1996).

The study showed co-infection interactions in total fresh weight and tiller heights between pathogen versus napier grass varieties, pathogens versus nutrient formulations and pathogens versus watering regimes. A similar trend was observed on pathogens versus nutrient formulations and pathogens versus watering regimes interactions. However, no significant interaction for chlorophyll content levels was observed. The lack of significant interaction for chlorophyll could be attributed to the stay-green trait. This trait maintains chlorophyll stability, despite the stressors a plant is subjected to leading to some significant productivity amidst the challenge (Luche *et al*., 2015). Further, the possible 'stay- green' character expression and its effect on the napier grass varieties in the present study is supported by the varieties' response nature to the diseases; where the Kakamega 1, Kakamega 2 and accession 16789 have been reported in similar studies to exhibit some tolerance to the smut pathogen (*Ustilago kamerunensis*). These varieties do not smut nor exhibit chlorosis symptoms as observed in susceptible cultivars of napier grass (Jorge, 2013; Kabirizi *et al*., 2015; NAFIS, 2017). Also, the presence of more treatments under napier head smut pathogen infections only and the uninoculated controls in this study in comparison to NSD pathogen infected treatments could

have contributed to a largely stable general means of the chlorophyll content levels. In addition, the *Ustilago kamerunensis* pathogens damage levels restriction by altitude, just like it is for onion smut (*Urocystis cepulae*) seemed to have played a role in chlorophyll content levels' stability (Farrel *et al*., 2002a; Mehrota, 2013). Bana variety being a susceptible check to napier head smut was least affected by the *Ustilago kamerunensis*, in the present study despite the pathogen being present in the tissues of the artificially inoculated napier grass varieties (Plate 4.2). This result could be attributed to the high growth vigour and biomass production of Bana grass in relatively low altitude areas with warm temperatures like ICIPE-Mbita where this experiment was carried out (Ramadhan *et al*., 2015). This is in agreement with findings of Huber *et al*.(2012) which noted that conducive growth conditions can enable plants reduce their susceptibility to pathogens through enhanced vigour and modification of their physiology.

This is unlike the total fresh weight and tiller height traits which are quantitative traits (Yin *et al*., 2012; Gholizadeh *et al*., 2017). Such traits are highly influenced by changes in their surroundings due to many genes involvement in their expression and environmental interactions (Keane, 2012). Furthermore, the trace nutrient elements present in all the four nutrient formulations might have played a role in maintaining the stability of chlorophyll. According to Huber *et al*. (2012) trace elements like iron, magnesium, potassium, zinc and boron play a significant role in chlorophyll development and stability. These significant pathogen versus napier varieties interactions could be attributed to the genotype differences between the napier grass varieties, which has been observed to play a significant role in influencing the way the napier grass respond in terms of growth in the presence of either biotic or abiotic stressors (Rahman *et al*., 2016; Turano *et al*., 2016). Genetic differences have been reported among different napier grass accessions and varieties by Lowe *et al*.(2003) and Anitha *et al*.(2006). Thus, depending on the genotype of the grass, the type of interactions with pathogen can lead to either a compatible or non-compatible response that vary in magnitude (Maleck and Lawton, 1998; Piffanelli *et al*., 1999).

The pathogen and nutrient formulations interactions have been reported to occur in many plants by Dordas (2008) and Singh (2015). In the current study nutrient deficiency increased pathogen virulence (Table 4.6). The most positive interactions that favoured high outputs of total fresh weight and tallest tillers were observed on those treatments infected by NAK-2 *Ustilago*

kamerunensis isolate, which was followed by the uninoculated treatments (Table 4.17). Whereas, the most negative interactions that favoured low biomass outputs and shortest tiller heights were those of NSD pathogen infected only treatments. In most obligate pathogens like '*Candidatus* Phytoplasma oryzae' strain Mbita 1; availability of nitrogen element presence has been observed to increase their proliferation and degree of damage due to their mode of interaction with their host's living cells by siphoning directly the plants photosynthates directly from the cells. Whereas, most of the facultative pathogens like *Ustilago kamerunensis* behave in a hemibiotrophic manner. Where, they can survive on dying plant cells giving room for rejuvenation of the plant under such pathogen attacks on the presence of nutrients (Farrell *et al*., 2001; Obura *et al*., 2009). The high and low performance of the treatments under complete nutrient solution and nitrogen - phosphorus deficiency respectively (Appendix 49) confirmed the findings by Ullah *et al*. (2010), Huber *et al*. (2012) and Kuwahara *et al*.(2016), who reported about the enhanced vigour of the grass and productivity under the application of these nutrients. The pathogen versus watering regime interactions demonstrated that under water stress, the virulence in napier grass increased. This result is in agreement with those reported by Orodho *et al*. (2005), Ajanga (2005), Mwendia *et al*. (2007) and Kabirizi *et al*.(2015). Thus, confirming that the virulence level of a pathogen is an independent trait from the microbe and it largely relies on the hosts' response (Casadevall and Pirofski, 2001). The daily watered treatments had low virulence levels whereas the weekly watered treatment means were generally high. This result could be attributed to the compounded stress effects on the general physiology of the napier grass varieties that arise due to poor photosynthates and minerals transport when water is limited (Osakabe *et al*., 2014).

In terms of the pathogens, nutrient formulations and watering regimes overally the treatment combination of Kakamega 2 variety infected by only NAK-2 isolate on complete nutrient solution under daily watering (KK 2 + NAK-2 + CNS + D) had the highest logarithmic index, followed by Kakamega 1 variety infected by only NAK-2 isolate on complete nutrient solution under daily watering KK 1 + NAK-2 + CNS + D). The treatment of Kakamega 2 variety not infected by any pathogen, on complete nutrient solution under daily watering regime (KK 2 Uinoculated + CNS + D); was third in performance (Table 4.15). The lowest performing treatments in growth were that of Kakamega 2 variety infected by only NSD pathogen, on nitrogen and phosphorus deficient nutrient solution under weekly watering regime (KK 2 + NSD

+ N/P-D + W), followed by accession 16789 infected by only NSD pathogen, on nitrogen and phosphorus deficient nutrient solution under weekly watering regime (16789 + NSD + N/P-D + W). The treatment of Bana variety infected by only NSD pathogen, on nitrogen and phosphorus deficient nutrient solution under weekly watering regime (Bana + NSD + N/P-D + W) was third last in logarithmic indexing of growth. The results confirmed the usefulness of logarithmic indexing in enabling selection incase of highly variable treatments. Further, they verified Wamalwa *et al*.(2017) report of Kakamega 2 relative susceptibility to NSD pathogen and generally the tolerance of Kakamega 1 and 2 to *Ustilago kamerunensis* by Jorge (2013) and Kabirizi *et al*. (2015).

5.4 Levels of host plant tolerance evaluation of the selected napier grass varieties

Based on the results of the omatec natural logarithmic indices; nutrient formulations largely played a significant role in the variations of host plant tolerance levels (Table 4.26). This was demonstrated on general means evaluation of selected treatment combinations (Table 4.27). Treatments applied with complete nutrient solution generally exhibited higher mean logarithmic percentages and corresponding tolerance levels (Tables 4.24 – 4.25). These treatments were followed largely by those which were applied with phosphorus and then nitrogen deficient nutrient (Tables 4.18 - 4.21). Those under nitrogen and phosphorus deficient nutrient solution generally exhibited low mean logarithmic percentages and tolerance levels (Tables 4.22 - 4.23). This can largely be attributed to the importance of the nitrogen in enhancing vigour of plants growth against facultative pathogens like it is demonstrated for *Ustilago kamerunensis* in the present study and other similar studies (Farrell, *et al*., 2001; Dordas, 2008; Huber *et al*., 2012; Veresoglou *et al*., 2013).

However, for obligate pathogens like NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1); the impact of nitrogen nutrient element has been reported to be low in enhancing tolerance levels of the host plant in similar studies, due to the biotrophic nature of such pathogens by Dordas (2008) and Singh (2015). This is because they acquire assimilates directly from actively dividing and developing plant cells. Whereas, for facultative pathogens being hemibiotrophic a times they survive on some senesced cells or tissues within the host. Therefore, when the nitrogen is introduced it seemingly enhances metabolic activity and vigour of the napier grass varieties leading to heightened tolerance levels through slowing senescence for such

facultative pathogens like *Ustilago kamerunensis* (Obura *et al*., 2009; Singh, 2015). Also, in related studies nitrogen has been reported to be involved in antibiosis mechanism of resistance where it reacts with phenolic compounds to form pathogen-inhibitive quinine-amino conjugates under the influence of polyphenol oxidase (PPO). Thus, demonstrating its significance in pathogen management through modification of the resistance levels of plants (Bittner 2006). The role of phosphorus seems also significant basing on the performance of the complete nutrient solution as compared to nitrogen or phosphorus deficient nutrient solution. This observation is supported by previous findings by Huber *et al*. (2012) and Veresoglou *et al*.(2013), who found that phosphorus initiated hypersensitive responses against pathogens like powdery mildew.

Also, many of the treatments as shown in table 4.27, performed intermediately, with the high performing treatments recording high levels of tolerance classification (HMT). The lowest performing treatments exhibited a moderate magnitude of tolerance (MMT). A generally, heightened performance from the napier grass varieties was observed with great variability in performance. In addition, the most susceptible (positive checks) like Bana and Kakamega 2 varieties for *Ustilago kamerunensis* and '*Candidatus* Phytoplasma oryzae' strain Mbita 1 respectively, performed relatively well against the two disease. This can be attributed to the role of the minor mineral nutrient elements like manganese, copper, calcium, potassium, boron, zinc, iron and chlorine which where present in all the four different nutrient formulations with the only alterations being on the nitrogen and phosphorus nutrient elements. This trace nutrient elements have been reported to enhance the tolerance levels of plants against pathogens in different ways. For example potassium has been reported to enhance optimal synthesis of some proteins and cellulose; organic compounds that have been observed to limit pathogen penetration (Dordas, 2008; Singh, 2015). Further, manganese involvement in lignin, phenols biosynthesis and inhibition of aminopeptidase; an enzyme that ensures essential amino acids are available for the pathogen ploriferation has been reported to be very significant in disease management via host plant resistance. Boron involvement in cell wall structure, cell membrane and enhancing plant metabolism has been observed to also enhance plant's tolerance to pathogens. Zinc toxicity to pathogens, calciums role in ensuring stable plant membranes and chlorine's influence of manganese availability make these trace elements very significant in the final tolerance levels of plants (Huber *et al*., 2012). A Phenomenon supported further by the variable nature in

performance of napier grass varieties which has been observed to be highly influenced by the environment of growth and the management practices applied on the crop in similar studies (Turano *et al*., 2016; Negawo *et al*., 2017).

Further, the effect of watering exhibited a significant effect on the tolerance levels across the treatments (Table 4.27), where largely the nutrient formulations under daily watering as compared to the weekly watered ones, produced high tolerance levels. A trend which was observed across the different treatments as demonstrated in table 4.27. Intermittent water availability or prolonged water stress has been reported in related studies by Ajanga (2005), Orodho *et al*.(2005), Mwendia *et al*. (2007) and Omayio *et al*.(2015), to reduce the tolerance of the napier grass varieties against the napier head smut and NSD pathogens. As, much as the napier grass varieties seem to be relatively tolerant to irregular water availability (Yanxian *et al*., 2008). The combined stressors from the pathogen attack and water seemed to overwhelm the crop due to the critical role of water in the plant's physiological processes (Huber *et al*., 2012).

The specific napier grass treatments viz; accession 16789 infected by NAK-2 *Ustilago kamerunensis* isolate applied with complete nutrient solution under daily watering regime (16789 + NAK-2 + CNS + D), had the highest tolerance levels (Table 4.26), followed by Kakamega 1 variety infected by NAK-2 *Ustilago kamerunensis* isolate applied by complete nutrient solution under daily watering regime (KK 1 + NAK-2 + CNS + D). Then Kakamega 2 variety infected by NAK-2 *Ustilago kamerunensis* isolate applied with complete nutrient solution under daily watering regime (KK 2 + NAK-2 + CNS + D), in third position (Table 4.26). The three varieties; Kakamega 1, Kakamega 2 and 16789 have been observed in related studies to exhibit high tolerance levels to *Ustilago kamerunensis*. Hence, their observed tolerance against the same pathogen in this study verified the findings by Kabirizi *et al*.(2015) and NAFIS (2017). These results are comparable to those of objective two and three where these treatments were not affected as much by pathogens virulences in their growth (Appendices 47-49). Hence, confirming the reliability of the omatec tolerance estimation technique used in this study.

On the other hand, the treatment Kakamega 2 infected by NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) applied with nitrogen and phosphorus deficient nutrient solution under weekly watering (KK 2 + NSD + N/P-D + W); had the lowest tolerance levels as shown in table 4.26. This treatment was followed by that of Bana variety infected by NSD pathogen applied with nitrogen and phosphorus deficient nutrient solution under weekly watering (Bana + NSD + N/P-D + W). Then the Kakamega 2 co-infected by NAK-2 and NYA-2 *Ustilago kamerunensis* isolates with NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1) applied with nitrogen and phosphorus deficient nutrient solution under weekly watering (KK 2 + NAK-2 + NYA-2 + NSD + N/P-D + W) in a declining order (Table 4.26). These performances by Kakamega 2 under infection by the NSD pathogen confirmed the susceptibility nature of the variety to NSD pathogen (Wamalwa *et al*., 2017). The *Ustilago kamerunensis* pathogen was tolerated by Bana variety a scenario that could be attributed to the restrictions of the pathogen aggression by altitude. Furthermore, high variability nature of the variety as reported by Ramadhan *et al*.(2015). This analyzed host plant resistance trait seems to be controlled by many genes working together towards the overall estimated trait. This is supported by the significant continuous variation of the trait under different treatment combinations that were involved in this experiment as shown in table 4.26. A trend which is used to identify a possible polygenic resistance also known as tolerance in plants (Freedman and Beattie, 2008; Keane, 2012). The results are comparable to those of objective two and three where the treatments with low tolerance levels were highly affected in terms of growth by the pathogens virulence in this current study (Appendices 47-49). Hence, confirming the reliability of the omatec tolerance estimation technique used in this study.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

Finally based on the experimental findings, the following conclusions are drawn.

1. **Objective 1**; based on the *Ustilago kamerunensis* isolates' ITS 1 and ITS 2 sequences spanned by the 5.8 ribosomal RNA gene, the isolates from Kiambu, Nyandarua and Nakuru counties were found to be sharing more molecular similarities, than those isolated from Murang'a, Nyeri and Kirinyaga counties an indication of evolutionary divergence from a common ancestral stock, and that transmission was vertical across the regions. These differences of the napier head smut isolates extended even to their growth *in vitro* under culture. The study also showed that *Ustilago kamerunensis* isolates were constrained by altitude whereby more pathogen strains were found at higher altitudes than the lower altitudes.

2. **Objective 2**; pathogenicity levels differed between the *Ustilago kamerunensis* isolates and NSD pathogen ('*Candidatus* Phytoplasma oryzae' strain Mbita 1). The NSD pathogen infections only were the most virulent, followed by the co-infection of the napier varieties by NAK-2 isolate + NYA-2 isolate + NSD pathogen, and then the co-infection of the varieties by NAK-2 isolate + NYA-2 isolate. The least virulent isolate's were NYA-2 isolate's infections only and NAK-2 isolate's infections only in a declining order. Mineral nutrients deficiency of phosphorus and nitrogen applied individually or in combination increased the virulence of both *Ustilago kamerunensis* and NSD pathogen. Among the nutrients, nitrogen deficiency caused the highest virulence. A daily supply of water reduced virulence of the pathogens in question, when compared to weekly water availability.

3. **Objective 3**; the co-infected napier grass varieties by *Ustilago kamerunensis* isolates and '*Candidatus* Phytoplasma oryzae' strain Mbita 1, interacted differently with the pathogens. The napier grass varieties co-infected by NSD pathogen and *Ustilago*

kamerunensis isolates were not as affected in their growth than those infected by only the NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1). The *Ustilago kamerunensis* isolates co-infections without NSD pathogen and their individual infections affected least the growth of napier grass varieties. The study also showed that NAK002 (NAK-2) *Ustilago kamerunensis* isolate infections only, on some instances enhanced the growth performance of the infected treatments contrary to what was expected. Also, there was some level of attempt by some of the varieties to maintain the stability of their chlorophyll and productivity amidst co-infection and individual pathogens’ infections. Moreover, there was a high level of napier grass varieties’ interaction with the respective pathogens, nutrient formulations and watering regimes.

4. **Objective 4**; Napier grass varieties possessed varying degrees of tolerance to *Ustilago kamerunensis* isolates and NSD pathogen (‘*Candidatus* Phytoplasma oryzae’ strain Mbita 1). The accession 16789 generally had the highest tolerance levels, followed by Kakamega 1 variety, then Kakamega 2 variety and Bana variety in a declining order. Further, the host plant tolerance levels against the pathogens differed in a continuous manner depending on mineral nutrients and water availability. The presence of phosphorus and nitrogen in nutrient solutions induced high tolerance levels, followed by the presence of nitrogen alone without phosphorus, then phosphorus alone without nitrogen in a declining order. Daily watering enhanced the tolerance levels than weekly watering regime. The presence of all essential nutrients under unlimited water supply enhanced the estimated tolerance levels.

6.2 Recommendations

Basing on the overall review of this study the following are the recommendations per each objective:

1. **Objective 1**; there is need to integrate suitable and compatible management strategies that can effectively slow the observed possible morphological and molecular variation and divergence of the *Ustilago kamerunensis* isolates. Especially encouraging the

planting of mixed varieties/accessions of napier grass with different magnitudes of tolerance to slow the rate of co-evolution of the pathogen due to high plant resistance.

2. **Objective 2**; there is need to manage the varying pathogenicity levels of the pathogens by effectively using a balanced nutrition formulations, with a bias towards nitrogen mineral element supply to successfully lower the virulence levels of these economic pathogens, coupled with regular watering of the fodder crop during its production.

3. **Objective 3**; the ability of the napier grass varieties to maintain the stability of their growth components such as number of tillers and chlorophyll content levels amidst co-infection and sole infections by the two pathogens, needs to be taken advantage of in selecting candidate germplasms for breeding activities, towards development of improved napier grass varieties that can effectively be used in the management of these diseases.

4. **Objective 4**; the napier grass varieties' differences in tolerating the diseases under dynamic conditions needs to be taken advantage of in selecting highly tolerant germplasm whose magnitudes can now be determined through the approaches used in this study. Further, the potential of the germplasms' tolerance can be enhanced by combining with other techniques like 'Tumbukiza'; which is a better water management approach for napier grass production and regular fertilization.

6.3 Suggestions for further research

Basing on the overall review of this study the following are suggestions for further research:

1. Firstly, the seemingly noted altitude restrictions of *Ustilago kamerunensis* isolates needs to be explored further to determine the possible factors behind the phenomenon, and how it can be integrated in the management of the disease.

2. The likely influence of the chemical composition of napier grass varieties due to varying localities of growth, and how they might have influenced their susceptibility to the diseases needs to be explored further.

3. Further investigations should be made into the effect of different nitrogen levels and trace elements effects on the virulence levels of *Ustilago kamerunensis* isolates and NSD pathogen '*Candidatus* Phytoplasma oryzae' strain Mbita 1.

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APPENDICES

APPENDIX 1: Demonstration table of the rationale behind the logarithmic indices derivation and their corresponding percentages

Using the natural logarithms ($\log_{e\ or\ LN}$) to demonstrate how efficacy indices are constant for doubling and tripling scenarios alongside their corresponding percentages.

Plant Samples	Hypothetical initial measure of parameter (Y)	Hypothetical Second measure of parameter (Y)	$\log_{e\ or\ LN}$ Relative Efficacy Index; Generating Function	Resulting Efficacy Index	Percentage Level Corresponding with the Respective Index; Generating Function	Resulting Percentage Level
Incase of doubling scenario; Due to genetic variations if we have two plants (A& B); each having a different initial/normal performances that is a performance exhibited when a plant is not diseased of parameter (Y) for example. Then after being infected they end up doubling their previous performance as shown below. Thus, regardless of their starting initial performance as observed, both end up with an equal magnitude of 66.67%.						
PLANT-A	1	2	LN(2)-LN(1)	0.6931	$\frac{2}{(1+2)} \times 100\%$	66.67%
PLANT-B	6	12	LN(4)-LN(2)	0.6931	$\frac{12}{(6+12)} \times 100\%$	66.67%
Incase of tripling scenario; Due to genetic variations if we have two plants (A& B); each having a different initial/normal performances that is a performance exhibited when a plant is not diseased of parameter (Y) for example. Then after being infected they end up tripling their previous performance as shown below. Thus, regardless of their starting initial performance as observed, both end up with an equal magnitude of 75%.						
PLANT-A	1	3	LN(3)-LN(1)	1.0986	$\frac{3}{(1+3)} \times 100\%$	75%
PLANT-B	6	18	LN(18)-LN(6)	1.0986	$\frac{18}{(6+18)} \times 100\%$	75%

Using the natural logarithms ($\log_{e\ or\ LN}$) to demonstrate how efficacy indices are also constant for quadrupling and quintupling scenarios alongside their corresponding percentages.

Plant Samples	Hypothetical initial	Hypothetical Second	$\log_e$ Relative	Resulting Efficacy	Percentage Level Corresponding with the	Resulting Percentage

	measure of parameter (Y)	measure of parameter (Y)	Efficacy Index generation (Function)	Index	index generated (Function)	Level
Incase of quadripling scenario; Using the natural logarithm, a different logarithmic index for quadripling is generated of 1.3863 but the magnitude remains the same at 66.67% like for natural logarithms, as shown below.						
PLANT-A	1	4	LN(4) – LN(1)	1.3863	$\frac{4}{(1+4)} \times 100\%$	80.00%
PLANT-B	6	24	LN(24)-LN (6)	1.3863	$\frac{24}{(6+24)} \times 100\%$	80.00%
Incase of quintupling scenario; Also in case of quintupling scenario the index generated is 1.6094 and its corresponding magnitude in percentage is 75% like for natural logarithm as shown below.						
PLANT-A	1	5	LN (5)-LN(1)	1.6094	$\frac{5}{(1+5)} \times 100\%$	83.33%
PLANT-B	6	30	LN(30)-LN(6)	1.6094	$\frac{30}{(6+30)} \times 100\%$	83.33%

The relative efficacy index is determined by subtracting the natural logarithm of the previous performance from the natural logarithm of the succeeding performance. If index is a negative it implies the succeeding performance reduced in magnitude to its previous one (Hunt, 1982; Parry, 1990). Since the magnitude/size of the number is not a factor in the generation of the efficacy indices. Integer one was used to derive all the possible indices relative to it. Hence, resulting to the Omatec natural logarithmic indices' and their corresponding percentages table shown in appendix *2*. The indices on this table are constant regardless of the size, magnitude or even units of measurement used. This trend of constants continue to be exhibited for the different levels of change which makes it a unique attribute for natural and standard logarithms.

APPENDIX 2: Omatec logarithmic indices' and their corresponding percentages table

OMATEC LOGARITHMIC INDICES' AND THEIR CORRESPONDING PERCENTAGES TABLE; ©2018

COLUMN A	**COLUMN B**	**COLUMN C**	**COLUMN D**	**COLUMN E**	**COLUMN F**	**COLUMN G**	**COLUMN H**
ROW NUMBER	TEST-PLANT'S ABILITY NUMBERS IN ABSOLUTE VALUES	NATURAL LOGS (LN/ LOG_e) EFFICACY (INDICES)	**(SCALE 1)** OVERALL MAXIMUM POTENTIAL IN PERCENTAGE UPON AN INPUT	(**SCALE 2**) SPECIFIC INPUT POTENTIAL IN PERCENTAGE	(**SCALE 3)** PERCENTAGE LEVELS OF LOGARITHMIC INDICES RELATIVE TO THE HIGHEST LOGARITHMIC INDEX (14.51)	MEAN PERCENTAGE OF THE THREE SCALES **COLUMNS D, E & F** **X%**	CORRECTED MEAN PERCENTAGE OF THE THREE SCALES GIVING THE CORRESPONDING LOGARITHMIC (%) **(CORRECTION FUNCTION)** **((X% – 16.67) × 1. 200048)**
1.	0	*****	0.00	-100.00	*	*	*
2.	0.000000001	**-20.72**	0.00	-100.00	-142.80	-80.93	**-117.12**
3.	0.000122071	**-9.01**	0.01	-99.98	-62.10	-54.02	**-84.83**
4.	0.000244141	**-8.32**	0.02	-99.95	-57.34	-52.42	**-82.91**
5.	0.000294141	**-8.13**	0.03	-99.94	-56.03	-51.98	**-82.38**
6.	0.000366211	**-7.91**	0.04	-99.93	-54.51	-51.47	**-81.77**
7.	0.000488281	**-7.62**	0.05	-99.90	-52.52	-50.79	**-80.96**
8.	0.000549317	**-7.51**	0.05	-99.90	-51.76	-50.54	**-80.66**
9.	0.000610352	**-7.40**	0.06	-99.88	-51.00	-50.27	**-80.33**
10.	0.000732422	**-7.22**	0.07	-99.85	-49.76	-49.85	**-79.83**

11.	0.000782422	**-7.15**	0.08	-99.84	-49.28	-49.68	**-79.62**
12.	0.000854493	**-7.07**	0.09	-99.83	-48.73	-49.49	**-79.40**
13.	0.000976563	**-6.93**	0.10	-99.80	-47.76	-49.15	**-78.99**
14.	0.001126563	**-6.79**	0.11	-99.77	-46.80	-48.82	**-78.59**
15.	0.001220704	**-6.71**	0.12	-99.76	-46.24	-48.63	**-78.36**
16.	0.001330704	**-6.62**	0.13	-99.73	-45.62	-48.41	**-78.10**
17.	0.001440704	**-6.54**	0.14	-99.71	-45.07	-48.21	**-77.86**
18.	0.001464844	**-6.53**	0.15	-99.71	-45.00	-48.19	**-77.84**
19.	0.001564844	**-6.46**	0.16	-99.69	-44.52	-48.02	**-77.63**
20.	0.001664844	**-6.40**	0.17	-99.67	-44.11	-47.87	**-77.45**
21.	0.001764844	**-6.34**	0.18	-99.65	-43.69	-47.72	**-77.27**
22.	0.001953125	**-6.24**	0.19	-99.61	-43.00	-47.47	**-76.97**
23.	0.002953125	**-5.82**	0.29	-99.41	-40.11	-46.41	**-75.70**
24.	0.00390625	**-5.55**	0.39	-99.22	-38.25	-45.69	**-74.83**
25.	0.00490625	**-5.32**	0.49	-99.02	-36.66	-45.06	**-74.08**
26.	0.005859375	**-5.14**	0.58	-98.83	-35.42	-44.56	**-73.48**
27.	0.0068125	**-4.99**	0.68	-98.65	-34.39	-44.12	**-72.95**

28.	0.0078125	**-4.85**	0.78	-98.45	-33.43	-43.70	**-72.45**
29.	0.0088125	**-4.73**	0.87	-98.25	-32.60	-43.33	**-72.00**
30.	0.0098125	**-4.62**	0.97	-98.06	-31.84	-42.98	**-71.58**
31.	0.01171875	**-4.45**	1.16	-97.68	-30.67	-42.40	**-70.89**
32.	0.013671875	**-4.29**	1.35	-97.30	-29.57	-41.84	**-70.21**
33.	0.015625	**-4.16**	1.54	-96.92	-28.67	-41.35	**-69.63**
34.	0.01953125	**-3.94**	1.92	-96.17	-27.15	-40.47	**-68.57**
35.	0.0234375	**-3.75**	2.29	-95.42	-25.84	-39.66	**-67.60**
36.	0.02734375	**-3.60**	2.66	-94.68	-24.81	-38.94	**-66.73**
37.	0.03125	**-3.47**	3.03	-93.94	-23.91	-38.27	**-65.93**
38.	0.0390625	**-3.24**	3.76	-92.48	-22.33	-37.02	**-64.43**
39.	0.046875	**-3.06**	4.48	-91.04	-21.09	-35.88	**-63.06**
40.	0.05078	**-2.98**	4.83	-90.33	-20.54	-35.35	**-62.43**
41.	0.0546875	**-2.91**	5.19	-89.63	-20.06	-34.83	**-61.80**
42.	0.0625	**-2.77**	5.88	-88.24	-19.09	-33.82	**-60.59**
43.	0.0685	**-2.68**	6.41	-87.18	-18.47	-33.08	**-59.70**
44.	0.07031	**-2.65**	6.57	-86.86	-18.26	-32.85	**-59.43**

45.	0.078125	**-2.55**	7.25	-85.51	-17.57	-31.94	**-58.33**
46.	0.085025	**-2.46**	7.84	-84.33	-16.95	-31.15	**-57.39**
47.	0.088125	**-2.43**	8.10	-83.80	-16.75	-30.82	**-56.99**
48.	0.09375	**-2.37**	8.57	-82.86	-16.33	-30.21	**-56.26**
49.	0.1	**-2.30**	9.09	-81.82	-15.85	-29.53	**-55.44**
50.	0.109375	**-2.21**	9.86	-80.28	-15.23	-28.55	**-54.27**
51.	0.11719	**-2.14**	10.49	-79.02	-14.75	-27.76	**-53.32**
52.	0.12	**-2.12**	10.71	-78.57	-14.61	-27.49	**-52.99**
53.	0.125	**-2.08**	11.11	-77.78	-14.33	-27.00	**-52.41**
54.	0.13	**-2.04**	11.50	-76.99	-14.06	-26.52	**-51.83**
55.	0.140625	**-1.96**	12.33	-75.34	-13.51	-25.51	**-50.62**
56.	0.143	**-1.94**	12.51	-74.98	-13.37	-25.28	**-50.34**
57.	0.15	**-1.90**	13.04	-73.91	-13.09	-24.65	**-49.59**
58.	0.15625	**-1.86**	13.51	-72.97	-12.82	-24.09	**-48.91**
59.	0.165	**-1.80**	14.67	-71.67	-12.41	-23.14	**-47.77**
60.	0.171875	**-1.76**	14.67	-70.67	-12.13	-22.71	**-47.26**
61.	0.18	**-1.71**	15.25	-69.49	-11.78	-22.01	**-46.42**

62.	0.1875	**-1.67**	15.79	-68.42	-11.51	-21.38	**-45.66**
63.	0.196	**-1.63**	16.39	-67.22	-11.23	-20.69	**-44.83**
64.	0.203125	**-1.59**	16.88	-66.23	-10.96	-20.10	**-44.13**
65.	0.208	**-1.57**	17.22	-65.56	-10.82	-19.72	**-43.67**
66.	0.21875	**-1.52**	17.95	-64.10	-10.48	-18.88	**-42.66**
67.	0.22	**-1.51**	18.03	-63.93	-10.41	-18.77	**-42.53**
68.	0.234375	**-1.45**	18.99	-62.03	-9.99	-17.68	**-41.22**
69.	0.2421875	**-1.42**	19.50	-61.01	-9.79	-17.10	**-40.53**
70.	0.25	**-1.39**	20.00	-60.00	-9.58	-16.53	**-39.84**
71.	0.255	**-1.37**	20.32	-59.36	-9.44	-16.16	**-39.40**
72.	0.258	**-1.35**	20.51	-58.98	-9.30	-15.92	**-39.11**
73.	0.27	**-1.31**	21.26	-57.48	-9.03	-15.08	**-38.10**
74.	0.28125	**-1.27**	21.95	-56.10	-8.75	-14.30	**-37.17**
75.	0.28225	**-1.26**	22.01	-55.98	-8.68	-14.22	**-37.07**
76.	0.296875	**-1.21**	22.89	-54.22	-8.34	-13.22	**-35.87**
77.	0.3	**-1.20**	23.08	-53.85	-8.27	-13.01	**-35.62**
78.	0.3125	**-1.16**	23.81	-52.38	-7.99	-12.19	**-34.63**

79.	0.319	**-1.14**	24.18	-51.63	-7.86	-11.77	**-34.13**
80.	0.328125	**-1.11**	24.71	-50.59	-7.65	-11.18	**-33.42**
81.	0.34	**-1.08**	25.37	-49.25	-7.44	-10.44	**-32.53**
82.	0.34375	**-1.07**	25.58	-48.84	-7.37	-10.21	**-32.26**
83.	0.359375	**-1.02**	26.44	-47.13	-7.03	-9.24	**-31.09**
84.	0.369	**-1.00**	26.95	-46.09	-6.89	-8.68	**-30.42**
85.	0.375	**-0.98**	27.27	-45.45	-6.75	-8.31	**-29.98**
86.	0.385	**-0.95**	27.80	-44.40	-6.55	-7.72	**-29.27**
87.	0.395	**-0.93**	28.32	-43.37	-6.41	-7.15	**-28.59**
88.	0.40625	**-0.90**	28.89	-42.22	-6.20	-6.51	**-27.82**
89.	0.416	**-0.88**	29.38	-41.24	-6.06	-5.97	**-27.17**
90.	0.421875	**-0.86**	29.67	-40.66	-5.93	-5.64	**-26.77**
91.	0.4375	**-0.83**	30.43	-39.13	-5.72	-4.81	**-25.78**
92.	0.4475	**-0.80**	30.92	-38.17	-5.51	-4.25	**-25.11**
93.	0.4575	**-0.78**	31.39	-37.22	-5.38	-3.74	**-24.49**
94.	0.46875	**-0.76**	31.91	-36.17	-5.24	-3.17	**-23.81**
95.	0.478	**-0.74**	32.34	-35.32	-5.10	-2.69	**-23.23**

96.	0.484375	**-0.72**	32.63	-34.74	-4.96	-2.36	**-22.84**
97.	0.5	**-0.69**	33.33	-33.33	-4.76	-1.59	**-21.91**
98.	0.51	**-0.67**	33.77	-32.45	-4.62	-1.10	**-21.32**
99.	0.52	**-0.65**	34.21	-31.58	-4.48	-0.62	**-20.75**
100.	0.53125	**-0.63**	34.69	-30.61	-4.34	-0.09	**-20.11**
101.	0.546875	**-0.60**	35.35	-29.29	-4.14	0.64	**-19.24**
102.	0.5625	**-0.58**	36.00	-28.00	-4.00	1.33	**-18.41**
103.	0.57	**-0.56**	36.31	-27.39	-3.86	1.69	**-17.98**
104.	0.58	**-0.54**	36.71	-26.58	-3.72	2.14	**-17.44**
105.	0.59375	**-0.52**	37.25	-25.49	-3.58	2.73	**-16.73**
106.	0.61	**-0.49**	37.89	-24.22	-3.38	3.43	**-15.89**
107.	0.625	**-0.47**	38.46	-23.08	-3.24	4.05	**-15.14**
108.	0.63	**-0.46**	38.65	-22.70	-3.17	4.26	**-14.89**
109.	0.64	**-0.45**	39.02	-21.95	-3.10	4.66	**-14.41**
110.	0.65625	**-0.42**	39.62	-20.75	-2.89	5.33	**-13.61**
111.	0.67	**-0.40**	40.12	-19.76	-2.76	5.87	**-12.96**
112.	0.6875	**-0.37**	40.74	-18.52	-2.55	6.56	**-12.13**

113.	0.7	**-0.36**	41.18	-17.65	-2.48	7.02	**-11.58**
114.	0.71875	**-0.33**	41.82	-16.36	-2.27	7.73	**-10.73**
115.	0.73	**-0.31**	42.20	-15.61	-2.14	8.15	**-10.22**
116.	0.75	**-0.29**	42.86	-14.29	-2.00	8.86	**-9.37**
117.	0.76	**-0.27**	43.18	-13.64	-1.86	9.23	**-8.93**
118.	0.78125	**-0.25**	43.86	-12.28	-1.72	9.95	**-8.06**
119.	0.80	**-0.22**	44.44	-11.11	-1.52	10.60	**-7.28**
120.	0.8125	**-0.21**	44.83	-10.34	-1.45	11.01	**-6.79**
121.	0.83	**-0.19**	45.76	-9.29	-1.31	11.72	**-5.94**
122.	0.84375	**-0.17**	45.76	-8.47	-1.17	12.04	**-5.56**
123.	0.86	**-0.15**	46.24	-7.53	-1.03	12.56	**-4.93**
124.	0.875	**-0.13**	46.67	-6.67	-0.90	13.03	**-4.37**
125.	0.89	**-0.12**	47.09	-5.82	-0.83	13.48	**-3.83**
126.	0.90625	**-0.10**	47.54	-4.92	-0.69	13.98	**-3.23**
127.	0.9375	**-0.06**	48.39	-3.23	-0.41	14.92	**-2.10**
128.	0.96	**-0.04**	48.98	-2.04	-0.28	15.55	**-1.34**
129.	0.96875	**-0.03**	49.21	-1.59	-0.21	15.80	**-1.04**

130.	1	**0.00**	**50.00**	**0.00**	**0.00**	**16.67**	**0.00**
131.	1.03	**0.03**	50.74	1.48	0.21	17.48	**0.97**
132.	1.05	**0.05**	51.22	2.44	0.34	18.00	**1.60**
133.	1.0625	**0.06**	51.52	3.03	0.41	18.32	**1.98**
134.	1.09	**0.09**	52.15	4.31	0.62	19.03	**2.83**
135.	1.125	**0.12**	52.94	5.88	0.83	19.88	**3.85**
136.	1.14	**0.13**	53.27	6.54	0.90	20.24	**4.28**
137.	1.15625	**0.15**	53.62	7.25	1.03	20.63	**4.75**
138.	1.1875	**0.17**	54.29	8.57	1.17	21.34	**5.60**
139.	1.2	**0.18**	54.55	9.09	1.24	21.63	**5.95**
140.	1.23	**0.21**	55.16	10.31	1.45	22.31	**6.77**
141.	1.25	**0.22**	55.56	11.11	1.52	22.73	**7.27**
142.	1.28	**0.25**	56.14	12.28	1.72	23.38	**8.05**
143.	1.3125	**0.27**	56.76	13.51	1.86	24.04	**8.84**
144.	1.34	**0.29**	57.26	14.53	2.00	24.60	**9.52**
145.	1.375	**0.32**	57.89	15.79	2.21	25.30	**10.36**
146.	1.4	**0.34**	58.33	16.67	2.34	25.78	**10.93**

147.	1.4375	**0.36**	58.97	17.95	2.48	26.47	**11.76**
148.	1.46875	**0.38**	59.49	18.99	2.62	27.03	**12.43**
149.	1.48	**0.39**	59.68	19.35	2.69	27.24	**12.68**
150.	1.5	**0.41**	60.00	20.00	2.83	27.61	**13.13**
151.	1.55	**0.44**	60.78	21.57	3.03	28.46	**14.15**
152.	1.58	**0.46**	61.24	22.48	3.17	28.96	**14.75**
153.	1.625	**0.49**	61.90	23.81	3.38	29.70	**15.64**
154.	1.64	**0.49**	62.12	24.24	3.38	29.91	**15.89**
155.	1.6875	**0.52**	62.79	25.58	3.58	30.65	**16.78**
156.	1.72	**0.54**	63.29	26.47	3.72	31.16	**17.39**
157.	1.75	**0.56**	63.64	27.27	3.86	31.59	**17.90**
158.	1.8125	**0.59**	64.44	28.89	4.07	32.47	**18.96**
159.	1.83	**0.60**	64.66	29.33	4.14	32.71	**19.25**
160.	1.875	**0.63**	65.22	30.43	4.34	33.33	**19.99**
161.	1.9	**0.64**	65.52	31.03	4.41	33.65	**20.38**
162.	1.96	**0.67**	66.22	32.43	4.62	34.42	**21.30**
163.	2	**0.69**	66.67	33.33	4.76	34.92	**21.90**

164.	2.0625	**0.72**	67.35	34.69	4.96	35.67	**22.80**
165.	2.08	**0.73**	67.53	35.06	5.03	35.87	**23.04**
166.	2.125	**0.75**	68.00	36.00	5.17	36.39	**23.66**
167.	2.22	**0.80**	68.94	37.89	5.51	37.45	**24.94**
168.	2.25	**0.81**	69.23	38.46	5.58	37.76	**25.31**
169.	2.3	**0.83**	69.70	39.39	5.72	38.27	**25.92**
170.	2.375	**0.86**	70.37	40.74	5.93	39.01	**26.81**
171.	2.395	**0.87**	70.54	41.09	6.00	39.21	**27.05**
172.	2.5	**0.92**	71.43	42.86	6.34	40.21	**28.25**
173.	2.55	**0.94**	71.83	43.66	6.48	40.66	**28.79**
174.	2.625	**0.97**	72.41	44.83	6.69	41.31	**29.57**
175.	2.7	**0.99**	72.97	45.95	6.82	41.91	**30.29**
176.	2.75	**1.01**	73.33	46.67	6.96	42.32	**30.78**
177.	2.8	**1.03**	73.68	47.37	7.10	42.72	**31.26**
178.	2.875	**1.06**	74.19	48.39	7.31	43.30	**31.96**
179.	2.95	**1.08**	74.68	49.37	7.44	43.83	**32.59**
180.	3	**1.10**	75.00	50.00	7.58	44.19	**33.03**

181.	3.125	**1.14**	75.76	51.52	7.86	45.05	**34.06**
182.	3.25	**1.18**	76.47	52.94	8.13	45.85	**35.02**
183.	3.3	**1.19**	76.74	53.49	8.20	46.14	**35.37**
184.	3.375	**1.22**	77.14	54.29	8.41	46.61	**35.93**
185.	3.5	**1.25**	77.78	55.56	8.61	47.32	**36.78**
186.	3.625	**1.29**	78.38	56.76	8.89	48.01	**37.61**
187.	3.75	**1.32**	78.95	57.89	9.10	48.65	**38.38**
188.	3.875	**1.35**	79.49	58.97	9.30	49.25	**39.10**
189.	3.9	**1.36**	79.59	59.18	9.37	49.38	**39.25**
190.	4	**1.39**	80.00	60.00	9.58	49.86	**39.83**
191.	4.125	**1.42**	80.49	60.98	9.79	50.42	**40.50**
192.	4.25	**1.45**	80.95	61.90	9.99	50.95	**41.14**
193.	4.375	**1.48**	81.40	62.79	10.20	51.46	**41.75**
194.	4.5	**1.50**	81.82	63.64	10.34	51.93	**42.31**
195.	4.625	**1.53**	82.22	64.44	10.54	52.40	**42.88**
196.	4.75	**1.56**	82.61	65.22	10.75	52.86	**43.43**
197.	4.875	**1.58**	82.98	65.96	10.89	53.28	**43.93**

198.	5	**1.61**	83.33	66.67	11.10	53.70	**44.44**
199.	5.125	**1.63**	83.67	67.35	11.23	54.08	**44.89**
200.	5.25	**1.66**	84.00	68.00	11.44	54.48	**45.37**
201.	5.375	**1.68**	84.31	68.63	11.58	54.84	**45.81**
202.	5.5	**1.70**	84.62	69.23	11.72	55.19	**46.23**
203.	5.625	**1.73**	84.91	69.81	11.92	55.55	**46.66**
204.	5.75	**1.75**	85.19	70.37	12.06	55.87	**47.04**
205.	5.875	**1.77**	85.45	70.91	12.20	56.19	**47.43**
206.	6	**1.79**	85.71	71.43	12.34	56.49	**47.79**
207.	6.125	**1.81**	85.96	71.93	12.47	56.79	**48.15**
208.	6.25	**1.83**	86.21	72.41	12.61	57.08	**48.49**
209.	6.375	**1.85**	86.44	72.88	12.75	57.36	**48.83**
210.	6.5	**1.87**	86.67	73.33	12.89	57.63	**49.15**
211.	6.625	**1.89**	86.89	73.77	13.03	57.90	**49.48**
212.	6.75	**1.91**	87.10	74.19	13.16	58.15	**49.78**
213.	6.875	**1.93**	87.30	74.60	13.30	58.40	**50.08**
214.	7	**1.95**	87.50	75.00	13.44	58.65	**50.38**

215.	7.125	**1.96**	87.69	75.38	13.51	58.86	**50.63**
216.	7.25	**1.98**	87.88	75.76	13.65	59.10	**50.92**
217.	7.375	**2.00**	88.06	76.12	13.78	59.32	**51.18**
218.	7.5	**2.01**	88.24	76.47	13.85	59.52	**51.42**
219.	7.625	**2.03**	88.41	76.81	13.99	59.74	**51.69**
220.	7.75	**2.05**	88.57	77.14	14.13	59.95	**51.94**
221.	7.875	**2.06**	88.73	77.46	14.20	60.13	**52.15**
222.	8	**2.08**	88.89	77.78	14.33	60.33	**52.39**
223.	8.125	**2.09**	89.04	78.08	14.40	60.51	**52.61**
224.	8.25	**2.11**	89.19	78.38	14.54	60.70	**52.84**
225.	8.375	**2.13**	89.33	78.67	14.68	60.89	**53.07**
226.	8.5	**2.14**	89.47	78.95	14.75	61.06	**53.27**
227.	8.625	**2.15**	89.61	79.22	14.82	61.22	**53.46**
228.	8.75	**2.17**	89.74	79.49	14.96	61.40	**53.68**
229.	8.875	**2.18**	89.87	79.75	15.02	61.55	**53.86**
230.	9	**2.20**	90.00	80.00	15.16	61.72	**54.06**
231.	9.125	**2.21**	90.12	80.25	15.23	61.87	**54.24**

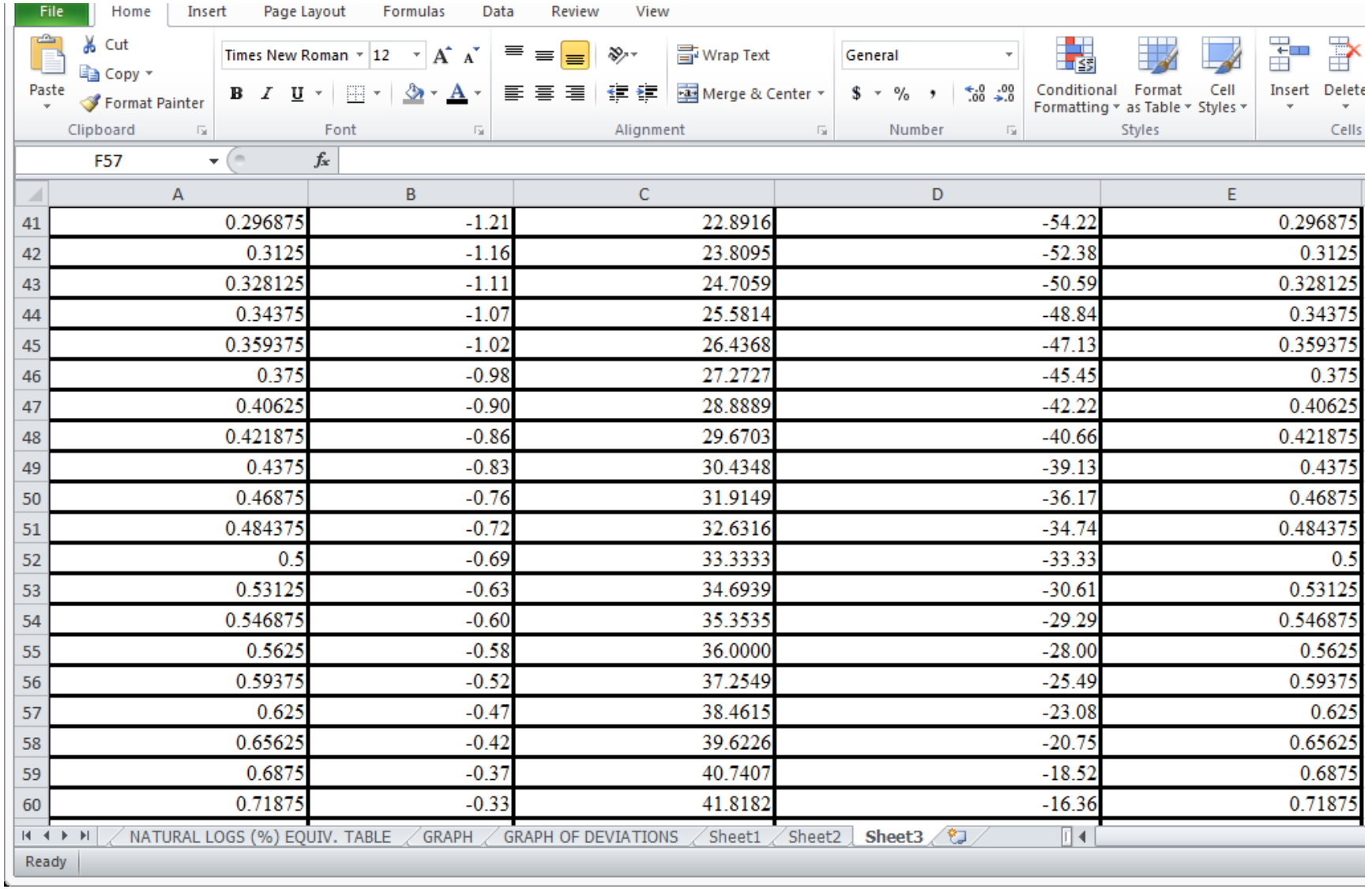
232.	9.25	**2.22**	90.24	80.49	15.30	62.01	**54.41**
233.	9.375	**2.24**	90.36	80.72	15.44	62.17	**54.60**
234.	9.5	**2.25**	90.48	80.95	15.51	62.31	**54.77**
235.	9.625	**2.26**	90.59	81.18	15.58	62.45	**54.94**
236.	9.75	**2.28**	90.70	81.40	15.71	62.60	**55.12**
237.	9.875	**2.29**	90.80	81.61	15.78	62.73	**55.27**
238.	10	**2.30**	90.91	81.82	15.85	62.86	**55.43**
239.	10.125	**2.32**	91.01	82.02	15.99	63.01	**55.61**
240.	10.25	**2.33**	91.11	82.22	16.06	63.13	**55.75**
241.	11	**2.40**	91.67	83.33	16.54	63.85	**56.62**
242.	12	**2.48**	92.31	84.62	17.09	64.67	**57.60**
243.	13	**2.56**	92.86	85.71	17.64	65.40	**58.48**
244.	14	**2.64**	93.33	86.67	18.19	66.06	**59.27**
245.	15	**2.71**	93.75	87.50	18.68	66.64	**59.97**
246.	16	**2.77**	94.12	88.24	19.09	67.15	**60.58**
247.	17	**2.83**	94.44	88.89	19.50	67.61	**61.13**
248.	18	**2.89**	94.74	89.47	19.92	68.04	**61.65**

249.	19	**2.94**	95.00	90.00	20.26	68.42	**62.10**
250.	20	**3.00**	95.24	90.48	20.68	68.80	**62.56**
251.	22	**3.09**	95.65	91.30	21.30	69.42	**63.30**
252.	25	**3.22**	96.15	92.31	22.19	70.22	**64.26**
253.	30	**3.40**	96.77	93.55	23.43	71.25	**65.50**
254.	35	**3.56**	97.22	94.4	24.53	72.05	**66.46**
255.	40	**3.69**	97.56	95.12	25.43	72.70	**67.24**
256.	50	**3.91**	98.04	96.08	26.95	73.69	**68.43**
257.	60	**4.09**	98.36	96.72	28.19	74.42	**69.30**
258.	70	**4.25**	98.59	97.18	29.29	75.02	**70.02**
259.	80	**4.38**	98.77	97.53	30.19	75.50	**70.60**
260.	90	**4.50**	98.90	97.80	31.01	75.90	**71.08**
261.	100	**4.61**	99.01	98.02	31.77	76.27	**71.52**
262.	1000	**6.91**	99.90	99.80	47.62	82.44	**78.93**
263.	10000	**9.21**	99.99	99.98	63.47	87.81	**85.37**
264.	100000	**11.51**	100.00	100.00	79.32	93.11	**91.73**
265.	1000000	**13.82**	100.00	100.00	95.24	98.41	**98.09**

266.	1500000	**14.22**	100.00	100.00	98.00	99.33	**99.20**
267.	2000000	**14.51**	100.00	100.00	100.00	100.00	**100.00**

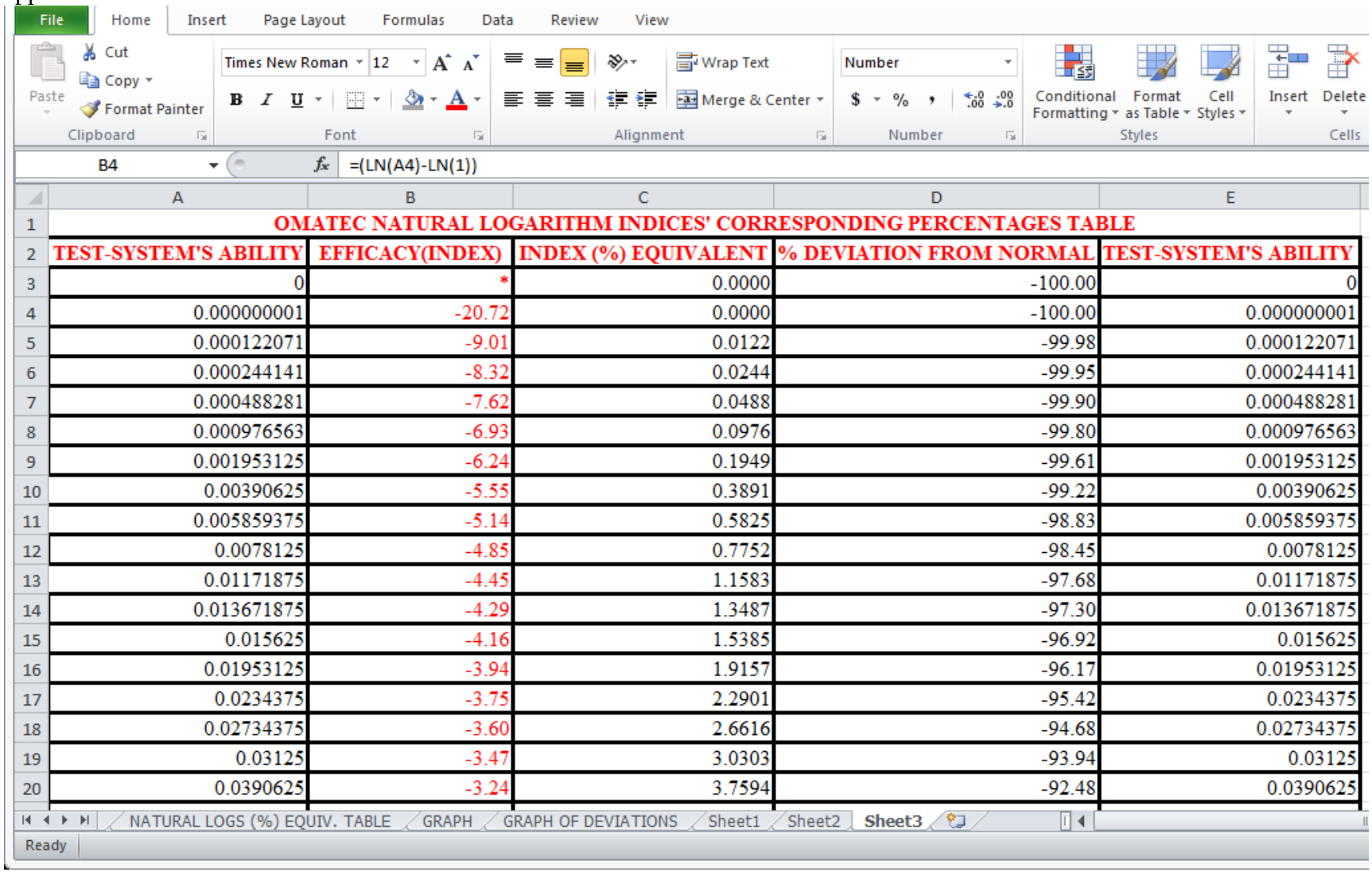
The symbol (*) indicates that the value generated on that cell is negative infinity. The Column B; displays the different abilities that living systems can exhibit in response to different aspects like a disease etc. Example Row Number 163; Column B; the number (2) represents the ability of a system to double its normal/initial levels or effort in response to a factor relative to a control, unit value or a standard. The Columns (C); displays the corresponding derived natural logarithm of the system's absolute values relative the unit value (1) or a control/standard. Example Row Number 163; Column (C); the logarithmic value is 0.69 which is the value one gets when a system doubles using natural logarithms. The Column (D); displays the corresponding overall percentages of the logarithmic values basing on their absolute values. Example Row Number 163; Column (D); equivalent percentage is 66.67%. The Column E; displays the specific percentage/ percentage deviation of a living system from a normal/standard. Example Row Number 163; Column (E); the deviation/specific percentage (power) is 33.33% for a system that doubles its normal/initial levels or effort in response to a factor. The specific percentage(specific power) demonstrates the effort an organism/system produces due to a response to environmental dynamics. The Column F; displays the percentage levels of logarithmic indices relative to the highest logarithmic index value namely 14.51 for natural logs shown in row 267 column (C). The Column (G); demonstrates the average magnitude of a system basing on the three percentages namely; overall percentage (Column D), specific percentage (Column E) and logarithmic indices percentages (Column F). The mean corresponding logarithmic percentage (Column G) was corrected using a function **((X% – 16.67) × 1. 200048),** which gave the corrected mean corresponding logarithmic percentages in (Column H) that agrees with the rationale of the idea which was based on Wolpert (2011) , as described in the materials and methods chapter. The factor 1.200048 on the correction factor was determined by substracting the highest value in Column G, which was 100% minus 16.67% which resulted to 83.33%. Therefore, by dividing 100% by 83.33% the correction factor 1.200048 was arrived at which was then used to correct all the values in Column G. Further, the value 16.67% in the correction model was the mean corresponding percentage of test plant's value (1) in Column B row number 130. Since, the logarithmic potential of (1) is zero which translates to 0% potential. Therefore, the correction function enabled the establishment of such a trend as shown in Column H. The table is read by comparing a natural logarithmic index of choice in Column C with its mean corresponding percentage in Column H. And if the logarithmic index is not directly captured on the table the two logarithmic indices where it falls in between, their mean corresponding logarithmic percentage is captured as an estimate of the corresponding percentage of the index in question from Column H.

APPENDIX 3: Excel screenshot of omatec natural logarithms indices' table showing the test – plant's ability numbers on column A. The numbers were generated to produce a continuum of corresponding consecutive percentages in column C.



	A	B	C	D	E
41	0.296875	-1.21	22.8916	-54.22	0.296875
42	0.3125	-1.16	23.8095	-52.38	0.3125
43	0.328125	-1.11	24.7059	-50.59	0.328125
44	0.34375	-1.07	25.5814	-48.84	0.34375
45	0.359375	-1.02	26.4368	-47.13	0.359375
46	0.375	-0.98	27.2727	-45.45	0.375
47	0.40625	-0.90	28.8889	-42.22	0.40625
48	0.421875	-0.86	29.6703	-40.66	0.421875
49	0.4375	-0.83	30.4348	-39.13	0.4375
50	0.46875	-0.76	31.9149	-36.17	0.46875
51	0.484375	-0.72	32.6316	-34.74	0.484375
52	0.5	-0.69	33.3333	-33.33	0.5
53	0.53125	-0.63	34.6939	-30.61	0.53125
54	0.546875	-0.60	35.3535	-29.29	0.546875
55	0.5625	-0.58	36.0000	-28.00	0.5625
56	0.59375	-0.52	37.2549	-25.49	0.59375
57	0.625	-0.47	38.4615	-23.08	0.625
58	0.65625	-0.42	39.6226	-20.75	0.65625
59	0.6875	-0.37	40.7407	-18.52	0.6875
60	0.71875	-0.33	41.8182	-16.36	0.71875

APPENDIX 4: Excel screenshot of omatec natural logarithms indices' table showing the function = (LN(A4)-LN(1)) for cell (A4) as an example; that was used against the test plant's ability numbers to generate the efficacy indices on column B.The function was applied across all the individual cells on column B.



File Home Insert Page Layout Formulas Data Review View

Cut Copy Format Painter Paste Clipboard | Times New Roman 12 Font | Wrap Text Merge & Center Alignment | Number Number | Conditional Formatting Format as Table Cell Styles Styles | Insert Delete Cells

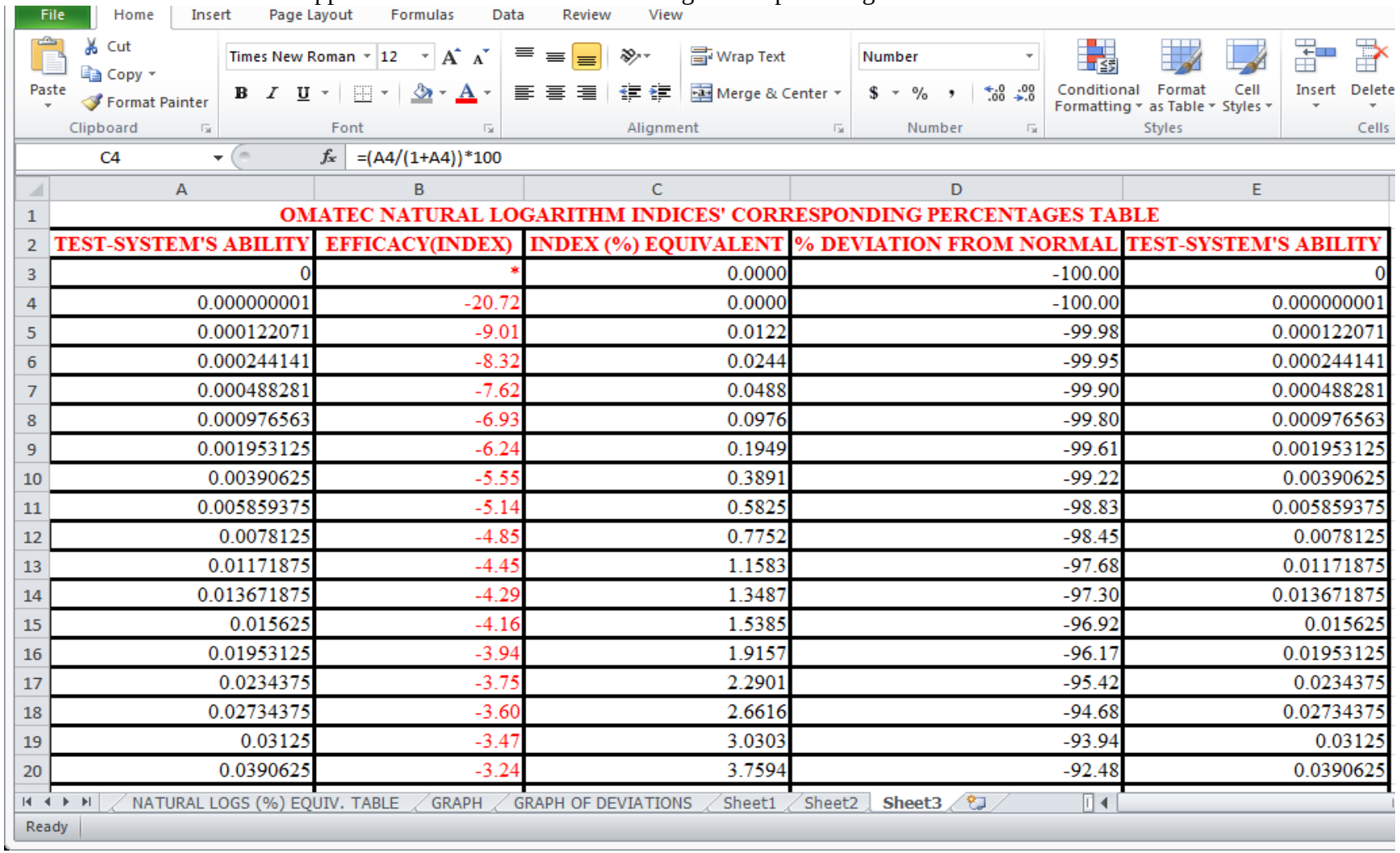
B4 =(LN(A4)-LN(1))

	A	B	C	D	E
1	OMATEC NATURAL LOGARITHM INDICES' CORRESPONDING PERCENTAGES TABLE				
2	TEST-SYSTEM'S ABILITY	EFFICACY(INDEX)	INDEX (%) EQUIVALENT	% DEVIATION FROM NORMAL	TEST-SYSTEM'S ABILITY
3	0	*	0.0000	-100.00	0
4	0.000000001	-20.72	0.0000	-100.00	0.000000001
5	0.000122071	-9.01	0.0122	-99.98	0.000122071
6	0.000244141	-8.32	0.0244	-99.95	0.000244141
7	0.000488281	-7.62	0.0488	-99.90	0.000488281
8	0.000976563	-6.93	0.0976	-99.80	0.000976563
9	0.001953125	-6.24	0.1949	-99.61	0.001953125
10	0.00390625	-5.55	0.3891	-99.22	0.00390625
11	0.005859375	-5.14	0.5825	-98.83	0.005859375
12	0.0078125	-4.85	0.7752	-98.45	0.0078125
13	0.01171875	-4.45	1.1583	-97.68	0.01171875
14	0.013671875	-4.29	1.3487	-97.30	0.013671875
15	0.015625	-4.16	1.5385	-96.92	0.015625
16	0.01953125	-3.94	1.9157	-96.17	0.01953125
17	0.0234375	-3.75	2.2901	-95.42	0.0234375
18	0.02734375	-3.60	2.6616	-94.68	0.02734375
19	0.03125	-3.47	3.0303	-93.94	0.03125
20	0.0390625	-3.24	3.7594	-92.48	0.0390625

NATURAL LOGS (%) EQUIV. TABLE GRAPH GRAPH OF DEVIATIONS Sheet1 Sheet2 Sheet3

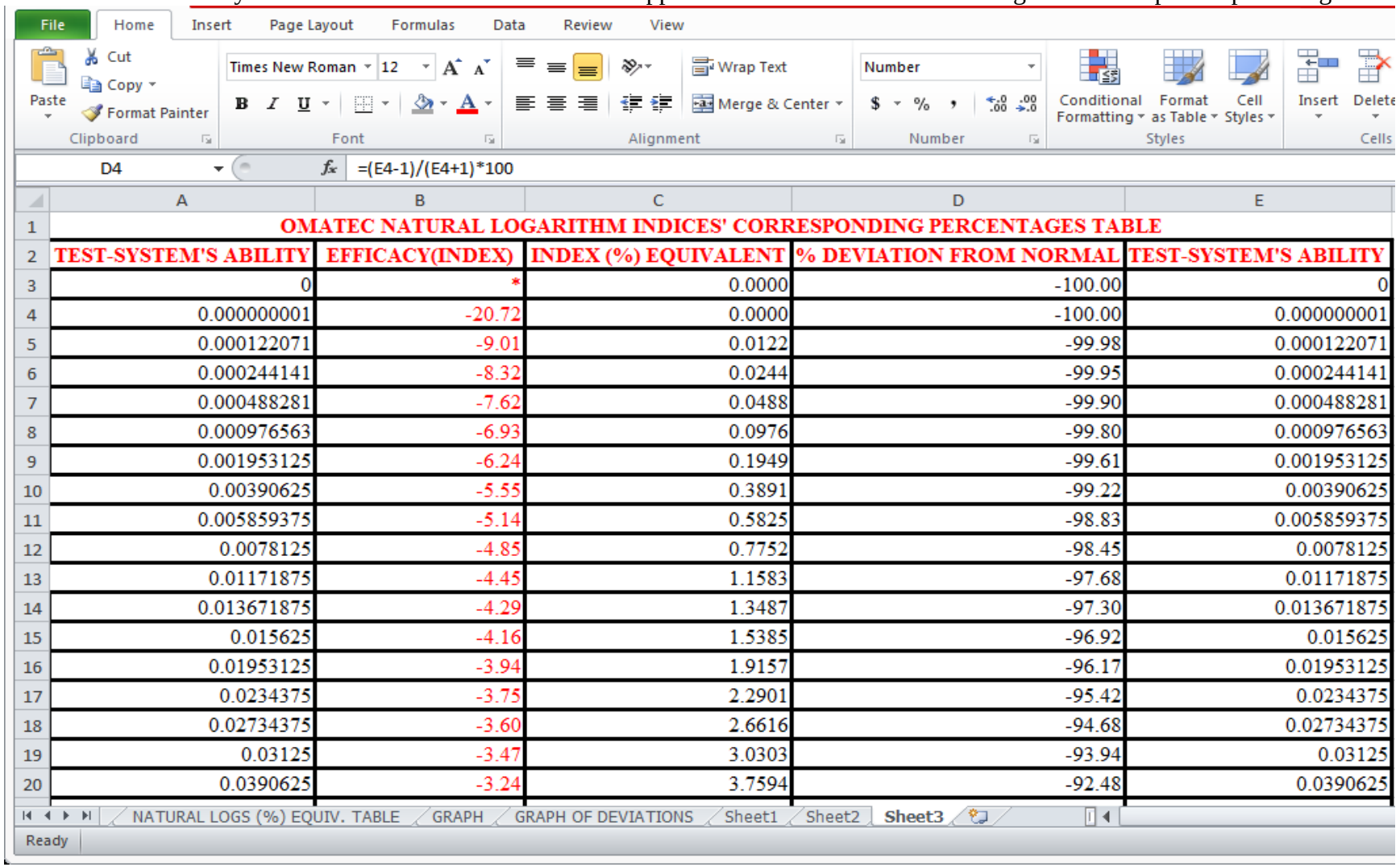
Ready

APPENDIX 5: Excel screenshot of omatec natural logarithms indices' table showing the function =(A4)/(1+A4))*100 for cell (A4) as an example; that was used against the test plant's ability numbers to generate the efficacy indices corresponding percentages on column C. The function was applied on each cell on column C to generate percentages.



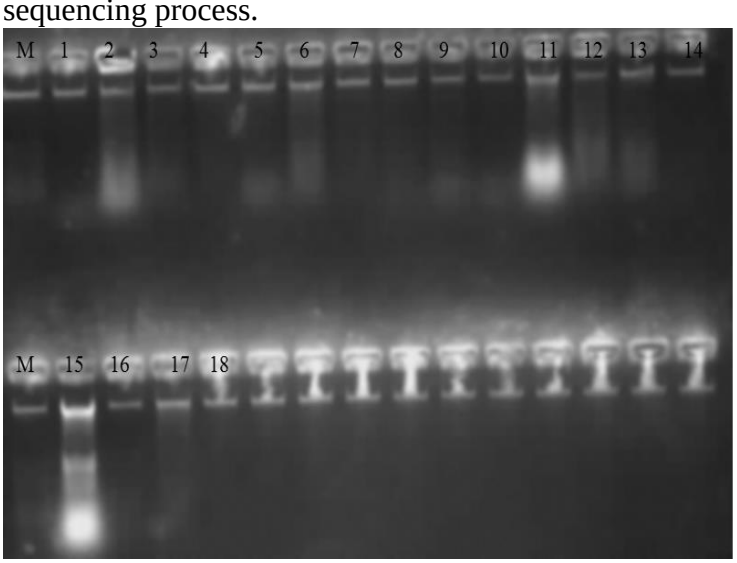
	A	B	C	D	E
1	OMATEC NATURAL LOGARITHM INDICES' CORRESPONDING PERCENTAGES TABLE				
2	TEST-SYSTEM'S ABILITY	EFFICACY(INDEX)	INDEX (%) EQUIVALENT	% DEVIATION FROM NORMAL	TEST-SYSTEM'S ABILITY
3	0	*	0.0000	-100.00	0
4	0.000000001	-20.72	0.0000	-100.00	0.000000001
5	0.000122071	-9.01	0.0122	-99.98	0.000122071
6	0.000244141	-8.32	0.0244	-99.95	0.000244141
7	0.000488281	-7.62	0.0488	-99.90	0.000488281
8	0.000976563	-6.93	0.0976	-99.80	0.000976563
9	0.001953125	-6.24	0.1949	-99.61	0.001953125
10	0.00390625	-5.55	0.3891	-99.22	0.00390625
11	0.005859375	-5.14	0.5825	-98.83	0.005859375
12	0.0078125	-4.85	0.7752	-98.45	0.0078125
13	0.01171875	-4.45	1.1583	-97.68	0.01171875
14	0.013671875	-4.29	1.3487	-97.30	0.013671875
15	0.015625	-4.16	1.5385	-96.92	0.015625
16	0.01953125	-3.94	1.9157	-96.17	0.01953125
17	0.0234375	-3.75	2.2901	-95.42	0.0234375
18	0.02734375	-3.60	2.6616	-94.68	0.02734375
19	0.03125	-3.47	3.0303	-93.94	0.03125
20	0.0390625	-3.24	3.7594	-92.48	0.0390625

APPENDIX 6: Excel screenshot of omatec natural logarithms indices' table showing the function =(E4-1)/(E4+1)*100 for cell (E4) as an example; that was used against the test plant's ability numbers to generate the specific percentage /deviation percentage from the normal/standard of a system on column D. The function was applied for each cell in column D to generate the specific percentages.



	A	B	C	D	E
1	OMATEC NATURAL LOGARITHM INDICES' CORRESPONDING PERCENTAGES TABLE				
2	TEST-SYSTEM'S ABILITY	EFFICACY(INDEX)	INDEX (%) EQUIVALENT	% DEVIATION FROM NORMAL	TEST-SYSTEM'S ABILITY
3	0	*	0.0000	-100.00	0
4	0.000000001	-20.72	0.0000	-100.00	0.000000001
5	0.000122071	-9.01	0.0122	-99.98	0.000122071
6	0.000244141	-8.32	0.0244	-99.95	0.000244141
7	0.000488281	-7.62	0.0488	-99.90	0.000488281
8	0.000976563	-6.93	0.0976	-99.80	0.000976563
9	0.001953125	-6.24	0.1949	-99.61	0.001953125
10	0.00390625	-5.55	0.3891	-99.22	0.00390625
11	0.005859375	-5.14	0.5825	-98.83	0.005859375
12	0.0078125	-4.85	0.7752	-98.45	0.0078125
13	0.01171875	-4.45	1.1583	-97.68	0.01171875
14	0.013671875	-4.29	1.3487	-97.30	0.013671875
15	0.015625	-4.16	1.5385	-96.92	0.015625
16	0.01953125	-3.94	1.9157	-96.17	0.01953125
17	0.0234375	-3.75	2.2901	-95.42	0.0234375
18	0.02734375	-3.60	2.6616	-94.68	0.02734375
19	0.03125	-3.47	3.0303	-93.94	0.03125
20	0.0390625	-3.24	3.7594	-92.48	0.0390625

APPENDIX 7: The electropherogram showing the tested quality of total DNA extracted from the eighteen *Ustilago kamerunensis* isolates before they were subjected to the sequencing process.



KEY

M-DNA Ladder
1-Sample 1
2-Sample 2
3-Sample 3
4-Sample 4
5-Sample 5
6-Sample 6
7-Sample 7
8-Sample 8
9-Sample 9
10-Sample 10
11-Sample 11
12-Sample 12
13-Sample 13
14-Sample 14
15-Sample 15
16-Sample 16
17-Sample 17
18-Sample 18

APPENDIX 8: Sequences of NapF and NapR nested PCR primers and conventional P1/P6 primer pairs used in the detection of '*Candidatus* Phytoplasma oryzae' Mbita 1 strain.

Primer	Sequence (5'-3')	Expected size
P1	AAGAGTTTGATCCTGGCTCAGGATT	1500 bp
P6	CGGTAGGGATACCTTGTTACGACTTA	
NapF	AGGAAACTCTGACCGAGCAAC	778 bp
NapR	ATTTTTCATTGGCAGTCTCGTTA	

Source: (Obura, 2012))

APPENDIX 9: The targeted 16S rRNA gene sequence of *'Candidatus* Phytoplasma oryzae' pathogen showing the underlined primer annealing sites for the respective primers; NapF and NapR shown , (Genbank AY377876).

TCTTTTAATTTTTAAAAGACCTTTTTCGAAAGGTATGCTTATTCAGGGGATTGCGACACATTAGTTAGTT
GGTAGGGTAAAAGCCTACCAAGACTATGATGTGTAGCTGGACTGAGAGGTCGAACAGCCACATTGGGACT
GAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGGCAATGGAGGAAACTCTGACCGA → NapF PRIMER
GCAACGCCGCGTGAACGATGAAGTATTTCGGTATGTAAAGTTCTTTTATTGAAGAAGAAAAAATAGTGGA
AAAACTATATTGACGCTATTCAATGAATAAGCCCCGGCAAACTATGTGCCAGCAGCCGCGGTAATACATA
GGGGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGTGCGTAGGCGGTTTAATAAGTCTGTAGTTTAA
TTTCAGTGCTTAACACTGTCCTGCTATAGAAACTATTAGACTAGAGTGAGATAGAGGTAAGCGGAATTCC
ATGTGTAGCGGTAAAATGCGTAAATATATGGAGGAACNCCAGAGGCGTAGGCGGCTTACTGGGTCTTTAC
TGACGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GAGTACTAAGTGTCGGGGAAACTCGGTACTGAAGTTAACACATTAAGTACTCCGCCTGAGTAGTACGTAC
GCAAGTATGAAACTTAAAGGAATTGACGGGACTCCGCACAAGCGGTGGATCATGTTGTTTAATTCGAAGA
TACACGAAAAACCTTACCAGGTCTTGACATACTCTGCAAAGCTATAGCAATATAGTGGAGGTTATCAGGG
ATACAGGTGGTGCATGGTGGTCGTCAGCTCGTGTCGTGAGATGTTAGGTTAAGTCCTAAAACGAGCGCAA
CCCTTATCGTTAGTTACCAGCATGTTATGATGGGGACTTTAACGAGACTGCCAATGAAAAATTGGAGGAA → NapR PRIMER
GGTGAGGATCACGTCAAATCATCATGCCCCTTATGATCTGGGCTACAAACGTGATACAATGGCTGTTACA
AAGAGTAGCTAAAACGCGAGTTTATAGCCAATCTCAAAAAAACAGTCTCAGTTCGGATTGAAGTCTGCAA
CTCGACTTCATGAAGTTGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCTCGGGGT
TTGTACACACCGCCCGTCAAACCACGAAAATCGGTAATACTCGAAAGCGGTGGCCTAACTTCTTCGGAAG
AGGGAACCGTCTAAGGTAGGATTGATGATTGGGGTTAAGTCGTAACAAGGTATCCCTACCGGAAGGTGGG
GATGGATCACCTCCTTTCTAAGGAAAAGTTTTTAAATTTTCATCTTCAGTTTTGAAAGACTTAGTTCTTA
TAAGTTTTTCCTTTTTT

APPENDIX 10: 'Customized/standardized napier grass varieties' tolerance magnitudes and classification table' showing the host plant tolerance/resistance magnitude levels in Column D and the classification of their magnitudes in Column E where; (LMT) stands for Low Magnitude of Tolerance, (MMT) stands for Moderate Magnitude of Tolerance, (HMT) High Magnitude of Tolerance and (VHMT) Very High Magnitude of Tolerance. The percentages in Column D were obtained by multiplying the factor 0.729927 with the respective counts in Column A to divide the magnitudes in four quarters as used in classical strategies of scoring and evaluation of pathogens. The factor (0.729927) was obtained by dividing 100% by 137 (the total number of counts in Column A) to determine factor of increase of the percentage in Column D.

Logarithmic Index for the ideal minimum & experimental maximum Index types; (L.I.I & L.I.U)	**Mean Logarithmic Index (M.L.I) of the Controls (Index type is L.I.U)**	**M.E.I**	**M.E.I Corresponding (%)**	**(M.L.I) Levels in (%) relative to unit value (1)**	**Mean (%)**
0.0000	**4.7445**	**-4.7445**	**-72.23**	**0.00**	**-36**
4.7445		**0**	**0.00**	**75.23**	**38**
COLUMN A	**COLUMN B**	**COLUMN C**	**COLUMN D**	**COLUMN E**	
Counts of the respective magnitudes in ascending order	**Generated Linear continuum of mean Logarithmic magnitudes of tolerance in (%)**	**Magnitudes in four main classes (Classical strategy)**	**Host Plant Tolerance/Resistance Levels in (%)**	**Classification of magnitudes**	
1	**-36**	**< 25% Class**	**0.7299**	**LMT**	
2	-35	< 25% Class	1.4599	LMT	
3	-34	< 25% Class	2.1898	LMT	
4	-33	< 25% Class	2.9197	LMT	
5	-32	< 25% Class	3.6496	LMT	
6	-31	< 25% Class	4.3796	LMT	
7	-30	< 25% Class	5.1095	LMT	

8	-29	< 25% Class	5.8394	LMT
9	-28	< 25% Class	6.5693	LMT
10	-27	< 25% Class	7.2993	LMT
11	-26	< 25% Class	8.0292	LMT
12	-25	< 25% Class	8.7591	LMT
13	-24	< 25% Class	9.4891	LMT
14	-23	< 25% Class	10.2190	LMT
15	-22	< 25% Class	10.9489	LMT
16	-21	< 25% Class	11.6788	LMT
17	-20	< 25% Class	12.4088	LMT
18	-19	< 25% Class	13.1387	LMT
19	-18	< 25% Class	13.8686	LMT
20	-17	< 25% Class	14.5985	LMT
21	-16	< 25% Class	15.3285	LMT
22	-15	< 25% Class	16.0584	LMT
23	-14	< 25% Class	16.7883	LMT
24	-13	< 25% Class	17.5182	LMT
25	-12	< 25% Class	18.2482	LMT

26	-11	< 25% Class	18.9781	LMT
27	-10	< 25% Class	19.7080	LMT
28	-9	< 25% Class	20.4380	LMT
29	-8	< 25% Class	21.1679	LMT
30	-7	< 25% Class	21.8978	LMT
31	-6	< 25% Class	22.6277	LMT
32	-5	< 25% Class	23.3577	LMT
33	-4	< 25% Class	24.0876	LMT
34	**-3**	**≥ 25% Class**	**24.8175**	**MMT**
35	-2	≥ 25% Class	25.5474	MMT
36	-1	≥ 25% Class	26.2774	MMT
37	0	≥ 25% Class	27.0073	MMT
38	1	≥ 25% Class	27.7372	MMT
39	2	≥ 25% Class	28.4672	MMT
40	3	≥ 25% Class	29.1971	MMT
41	4	≥ 25% Class	29.9270	MMT
42	5	≥ 25% Class	30.6569	MMT
43	6	≥ 25% Class	31.3869	MMT

44	7	≥ 25% Class	32.1168	MMT
45	8	≥ 25% Class	32.8467	MMT
46	9	≥ 25% Class	33.5766	MMT
47	10	≥ 25% Class	34.3066	MMT
48	11	≥ 25% Class	35.0365	MMT
49	12	≥ 25% Class	35.7664	MMT
50	13	≥ 25% Class	36.4964	MMT
51	14	≥ 25% Class	37.2263	MMT
52	15	≥ 25% Class	37.9562	MMT
53	16	≥ 25% Class	38.6861	MMT
54	17	≥ 25% Class	39.4161	MMT
55	18	≥ 25% Class	40.1460	MMT
56	19	≥ 25% Class	40.8759	MMT
57	20	≥ 25% Class	41.6058	MMT
58	21	≥ 25% Class	42.3358	MMT
59	22	≥ 25% Class	43.0657	MMT
60	23	≥ 25% Class	43.7956	MMT
61	24	≥ 25% Class	44.5255	MMT

62	25	≥ 25% Class	45.2555	MMT
63	26	≥ 25% Class	45.9854	MMT
64	27	≥ 25% Class	46.7153	MMT
65	28	≥ 25% Class	47.4453	MMT
66	29	≥ 25% Class	48.1752	MMT
67	30	≥ 25% Class	48.9051	MMT
68	**31**	**≥ 50% Class**	**49.6350**	**HMT**
69	32	≥ 50% Class	50.3650	HMT
70	33	≥ 50% Class	51.0949	HMT
71	34	≥ 50% Class	51.8248	HMT
72	35	≥ 50% Class	52.5547	HMT
73	36	≥ 50% Class	53.2847	HMT
74	37	≥ 50% Class	54.0146	HMT
75	38	≥ 50% Class	54.7445	HMT
76	39	≥ 50% Class	55.4745	HMT
77	40	≥ 50% Class	56.2044	HMT
78	41	≥ 50% Class	56.9343	HMT
79	42	≥ 50% Class	57.6642	HMT

80	43	≥ 50% Class	58.3942	HMT
81	44	≥ 50% Class	59.1241	HMT
82	45	≥ 50% Class	59.8540	HMT
83	46	≥ 50% Class	60.5839	HMT
84	47	≥ 50% Class	61.3139	HMT
85	48	≥ 50% Class	62.0438	HMT
86	49	≥ 50% Class	62.7737	HMT
87	50	≥ 50% Class	63.5036	HMT
88	51	≥ 50% Class	64.2336	HMT
89	52	≥ 50% Class	64.9635	HMT
90	53	≥ 50% Class	65.6934	HMT
91	54	≥ 50% Class	66.4234	HMT
92	55	≥ 50% Class	67.1533	HMT
93	56	≥ 50% Class	67.8832	HMT
94	57	≥ 50% Class	68.6131	HMT
95	58	≥ 50% Class	69.3431	HMT
96	59	≥ 50% Class	70.0730	HMT
97	60	≥ 50% Class	70.8029	HMT

98	61	≥ 50% Class	71.5328	HMT
99	62	≥ 50% Class	72.2628	HMT
100	63	≥ 50% Class	72.9927	HMT
101	64	≥ 50% Class	73.7226	HMT
102	65	≥ 50% Class	74.4526	HMT
103	**66**	**≥ 75% Class**	**75.1825**	**VHMT**
104	67	≥ 75% Class	75.9124	VHMT
105	68	≥ 75% Class	76.6423	VHMT
106	69	≥ 75% Class	77.3723	VHMT
107	70	≥ 75% Class	78.1022	VHMT
108	71	≥ 75% Class	78.8321	VHMT
109	72	≥ 75% Class	79.5620	VHMT
110	73	≥ 75% Class	80.2920	VHMT
111	74	≥ 75% Class	81.0219	VHMT
112	75	≥ 75% Class	81.7518	VHMT
113	76	≥ 75% Class	82.4818	VHMT
114	77	≥ 75% Class	83.2117	VHMT
115	78	≥ 75% Class	83.9416	VHMT

116	79	≥ 75% Class	84.6715	VHMT
117	80	≥ 75% Class	85.4015	VHMT
118	81	≥ 75% Class	86.1314	VHMT
119	82	≥ 75% Class	86.8613	VHMT
120	83	≥ 75% Class	87.5912	VHMT
121	84	≥ 75% Class	88.3212	VHMT
122	85	≥ 75% Class	89.0511	VHMT
123	86	≥ 75% Class	89.7810	VHMT
124	87	≥ 75% Class	90.5109	VHMT
125	88	≥ 75% Class	91.2409	VHMT
126	89	≥ 75% Class	91.9708	VHMT
127	90	≥ 75% Class	92.7007	VHMT
128	91	≥ 75% Class	93.4307	VHMT
129	92	≥ 75% Class	94.1606	VHMT
130	93	≥ 75% Class	94.8905	VHMT
131	94	≥ 75% Class	95.6204	VHMT
132	95	≥ 75% Class	96.3504	VHMT
133	96	≥ 75% Class	97.0803	VHMT

134	97	≥ 75% Class	97.8102	VHMT
135	98	≥ 75% Class	98.5401	VHMT
136	99	≥ 75% Class	99.2701	VHMT
137	**100**	**≥ 75% Class**	**100.0000**	**VHMT**

The 0.0000 is the ideal minimum that is hypothesized basing on the decimal points used of the control (experimental maximum) which was 4.7445 the average of the mean logarithmic indices of the four napier grass varieties performance under complete nutrient solution /daily watering treatments. The ideal minimum (0.0000) mimics the likely expected performance of a highly susceptible napier grass variety. Hence, used to establish the lowest end of the corresponding logarithmic percentages as shown in Column B (-36%). The M.E.I corresponding percentages and M.L.I (%) are obtained from Omatec Logarithmic indices' and corresponding percentages table (appendix 2). The M.E.I index is obtained by applying *algorithm 2c* described in the materials and methods. When determining the host plant tolerance levels in percentage one has to round off the corresponding logarithmic percentage to a whole number of napier grass plant.

APPENDIX 11: Specific effects of pathogens co-infection under nitrogen deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 UNINOCULATED	11.37 ± 1.11 a	89.64 ± 1.24 bc	42.72 ± 1.08 abcd	60.84 ± 1.12 efgh
KK 1 + NYA-2	8.28 ± 1.19 abc	245.76 ± 1.25 a	42.78 ± 1.09 abcd	298.54 ± 1.31 a
KK 1+ NAK-2 + NYA-2 + NSD	8.27 ± 1.20 abc	113.50 ± 1.10 bc	37.65 ± 1.05 bcd	113.50 ± 1.11 bcdef
KK 1 + NAK-2	7.56 ± 1.14 abc	132.36 ± 1.05 bc	42.87 ± 1.07 abcd	43.99 ± 1.13 h
KK 1 + NSD	6.29 ± 1.14 abc	82.84 ± 1.19 c	36.23 ± 1.07 bcd	57.65 ±1.12 fgh
KK 1+ NAK-2 + NYA-2	5.66 ± 1.24 abc	127.78 ± 1.04 bc	39.61 ± 1.04 abcd	86.10 ± 1.07 bcdefgh
KK 2 + NAK-2	7.31 ± 1.10 abc	138.35 ± 1.05 bc	32.60 ± 1.08 cd	102.33 ± 1.12 bcdefg
KK 2 + NSD	6.33 ± 1.13 abc	113.08 ± 1.05 bc	30.08 ± 1.06 d	123.50 ± 1.04 bcde
KK 2 + NYA-2	5.85 ± 1.23 abc	128.63 ± 1.19 bc	40.97 ± 1.06 abcd	82.22± 1.14 bcdefgh
KK 2+ NAK-2 + NYA-2	5.52 ± 1.10 abc	119.05 ± 1.03 bc	37.33 ± 1.05 bcd	67.87 ± 1.08 cdefgh
KK 2 UNINOCULATED	4.28 ± 1.35 abc	124.45 ± 1.09 bc	40.26 ± 1.07 abcd	132.33 ± 1.06 bcd
KK 2 + NAK-2 + NYA-2 + NSD	3.80 ± 1.23 bc	134.91 ± 1.08 bc	44.75 ± 1.11 abc	87.77 ± 1.21 bcdefgh
BANA UNINOCULATED	8.59 ± 1.10 abc	149.89 ± 1.04 ab	56.38 ± 1.08 a	153.70 ± 1.21 ab
BANA + NAK-2	8.24 ± 1.21 abc	103.79 ± 1.08 bc	42.78 ± 1.14 abcd	143.44 ± 1.20 abc
BANA + NAK-2 + NYA-2 + NSD	5.94 ± 1.16 abc	107.62 ± 1.26 bc	32.95 ± 1.08 cd	135.42 ± 1.15 bcd
BANA + NAK-2 + NYA-2	3.64 ± 1.34 bc	121.71 ± 1.04 bc	43.11 ± 1.10 abcd	53.09 ± 1.08 gh
BANA + NYA-2	3.36 ± 1.36 c	120.96 ± 1.12 bc	51.13 ± 1.07 ab	68.65 ± 1.14 cdefgh
BANA + NSD	3.32 ± 1.26 c	131.42 ± 1.04 bc	36.17 ± 1.09 bcd	44.50 ± 1.17 h
16789 + NAK-2 + NYA-2 + NSD	9.29 ± 1.16 ab	151.35 ± 1.09 ab	44.72 ± 1.05 abc	138.04 ± 1.21 bc
16789 + NAK-2	7.39 ± 1.14 abc	113.04 ± 1.11 bc	36.00 ± 1.05 bcd	90.85 ± 1.18 bcdefgh
16789 UNINOCULATED	7.34 ± 1.19 abc	140.38 ± 1.04 abc	37.28 ± 1.10 bcd	49.17 ± 1.07 gh
16789 + NYA-2	5.77 ± 1.28 abc	126.20 ± 1.04 bc	35.68 ± 1.04 bcd	71.89 ± 1.21 cdefgh
16789 + NSD	4.85 ± 1.15 abc	122.96 ± 1.04 bc	35.24 ± 1.04 cd	64.07 ± 1.14 defgh
16789 + NAK-2 + NYA-2	4.04 ± 1.19 bc	123.48 ± 1.04 bc	39.89 ± 1.01 abcd	54.33 ± 1.11 fgh
Test Values	**df =23 ; F = 3.310; P ≤ 0.0001**	**df = 23 ; F = 3.401; P ≤ 0.0001**	**df = 23 ; F = 3.903; P ≤ 0.0001**	**df = 23 ; F = 10.733; P = 0.009**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 12: Specific effects of pathogens co-infection under nitrogen deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NYA-2	232.63 ± 1.27 ab	139.10 ± 1.30 ab	89.47 ± 1.22 abcd	161.19 ± 1.15 a
KK 1 UNINOCULATED	229.97 ± 1.16 ab	139.64 ± 1.14 ab	85.11 ± 1.23 abcd	164.32 ± 1.05 a
KK 1+ NAK-2 + NYA-2	177.15 ± 1.06 ab	100.77 ± 1.08 abc	75.57 ± 1.05 abcd	43.48 ± 1.16 abc
KK 1+ NAK-2 + NYA-2 + NSD	171.13 ± 1.14 ab	77.92 ± 1.21 abc	85.44 ± 1.12 abcd	48.23 ± 1.09 ab
KK 1 + NAK-2	149.62 ± 1.16 ab	80.04 ± 1.20 abc	57.99 ± 1.29 cd	46.24 ± 1.12 abc
KK 1 + NSD	147.63 ± 1.12 ab	84.30 ± 1.16 abc	61.78 ± 1.09 bcd	35.89 ± 1.07 abcd
KK 2 + NAK-2	229.97 ± 1.17 ab	91.90 ± 1.09 abc	125.89 ± 1.24 abc	52.28 ± 1.07 ab
KK 2 + NAK-2 + NYA-2 + NSD	212.16 ± 1.21 ab	122.56 ± 1.24 abc	86.10 ± 1.20 abcd	34.41 ± 1.18 abcd
KK 2 + NYA-2	171.13 ± 1.09 ab	103.51 ± 1.12 abc	66.58 ± 1.04 bcd	37.44 ± 1.08 abcd
KK 2+ NAK-2 + NYA-2	160.32 ± 1.07 ab	81.91 ± 1.14 abc	72.72 ± 1.09 abcd	35.21 ± 1.05 abcd
KK 2 UNINOCULATED	151.94 ± 1.37 ab	88.78 ± 1.40 abc	57.99 ± 1.37 cd	34.15 ± 1.30 abcd
KK 2 + NSD	128.33 ± 1.10 b	171.61 ± 1.15 abc	54.95 ± 1.06 cd	40.89 ± 1.07 abcd
BANA UNINOCULATED	324.84 ± 1.16 a	157.88 ± 1.21 a	164.06 ± 1.12 a	59.11 ± 1.11 a
BANA + NAK-2	200.29 ± 1.32 ab	105.52 ± 1.34 abc	93.33 ± 1.33 abcd	50.12 ± 1.22 ab
BANA + NAK-2 + NYA-2 + NSD	184.79 ± 1.14 ab	100.00 ± 1.20 abc	79.74 ± 1.10 abcd	42.82 ± 1.11 abc
BANA + NAK-2 + NYA-2	150.20 ± 1.06 ab	75.28 ± 1.06 abc	73.85 ± 1.07 abcd	28.29 ± 1.16 cd
BANA + NYA-2	146.78 ± 1.06 ab	84.14 ± 1.07 abc	62.13 ± 1.04 bcd	24.36 ± 1.16 cd
BANA + NSD	100.38 ± 1.29 b	51.68 ± 1.26 c	46.42 ± 1.22 d	21.22 ± 1.11 d
16789 + NAK-2 + NYA-2 + NSD	310.22 ± 1.30 a	164.06 ± 1.32 a	143.99 ± 1.28 ab	59.11 ± 1.19 a
16789 + NAK-2	191.28 ± 1.11 ab	100.77 ± 1.14 abc	88.44 ± 1.09 abcd	47.71 ± 1.07 abc
16789 + NAK-2 + NYA-2	171.79 ± 1.24 ab	92.26 ± 1.26 abc	79.43 ± 1.22 abcd	23.71 ± 1.15 cd
16789 + NYA-2	157.88 ± 1.12 ab	85.44 ± 1.13 abc	70.79 ± 1.12 abcd	35.89 ± 1.17 abcd
16789 UNINOCULATED	150.20 ± 1.16 ab	75.57 ± 1.18 abc	74.70 ± 1.14 abcd	40.43 ± 1.10 abcd
16789 + NSD	121.15 ± 1.19 b	58.21 ± 1.21 bc	62.37 ± 1.17 bcd	34.54 ± 1.11 abcd
Test Values	**df = 23; F = 2.682; P ≤ 0.0001**	**df = 23 ; F = 2.326; P ≤ 0.0001**	**df = 23 ; F = 3.140; P ≤ 0.0001**	**df = 23 ; = 5.423; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 13: Specific effects of pathogens co-infection under nitrogen deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1+ NAK-2 + NYA-2	6.28 ± 1.19 a	117.02 ± 1.20 ab	28.23 ± 1.05 ab	53.29 ± 1.16 cdefg
KK 1 + NYA-2	5.23 ± 1.15 ab	175.96 ± 1.34 a	23.79 ± 1.11 ab	147.91 ± 1.33 a
KK 1 UNINOCULATED	5.12 ± 1.16 ab	95.22 ± 1.12 ab	23.43 ± 1.13 ab	49.17 ± 1.09 defg
KK 1 + NAK-2	4.98 ± 1.16 abc	100.06 ± 1.08 ab	20.04 ± 1.17 bc	64.81 ± 1.17 bcdefg
KK 1+ NAK-2 + NYA-2 + NSD	4.46 ± 1.12 abc	112.44 ± 1.26 ab	20.09 ± 1.06 bc	71.07 ± 1.14 abcdefg
KK 1 + NSD	3.09 ± 1.07 abc	69.05 ± 1.12 b	20.51 ± 1.06 bc	61.42 ± 1.10 cdefg
KK 2 UNINOCULATED	6.05 ± 1.18 a	100.17 ± 1.05 ab	27.85 ± 1.11 ab	109.65 ± 1.05 abc
KK 2 + NYA-2	5.01 ± 1.17 abc	78.09 ± 1.08 b	19.10 ± 1.11 bc	58.21 ± 1.09 cdefg
KK 2 + NAK-2 + NYA-2 + NSD	4.04 ± 1.18 abc	134.80 ± 1.24 ab	17.27 ± 1.13 bc	89.81 ± 1.21 abcde
KK 2 + NAK-2	3.90 ± 1.27 abc	87.29 ± 1.07 ab	22.17 ± 1.14 abc	38.46 ± 1.19 fg
KK 2 + NSD	3.72 ± 1.16 abc	80.87 ± 1.11 b	25.00 ± 1.06 ab	35.89 ± 1.12 fg
KK 2+ NAK-2 + NYA-2	3.36 ± 1.14 abc	82.47 ± 1.06 b	22.63 ± 1.10 abc	51.68 ± 1.06 cdefg
BANA + NAK-2 + NYA-2	5.42 ± 1.27 ab	78.68 ± 1.14 b	26.75 ± 1.16 ab	52.89 ± 1.09 cdefg
BANA + NYA-2	5.25 ± 1.29 ab	92.19 ± 1.14 ab	27.88 ± 1.15 ab	46.77 ± 1.18 efg
BANA + NAK-2	4.98 ± 1.32 abc	81.13 ± 1.07 b	22.88 ± 1.12 abc	52.78 ± 1.10 cdefg
BANA UNINOCULATED	2.97 ± 1.31 abc	113.65 ± 1.09 ab	38.17 ± 1.09 a	89.13 ± 1.16 abcde
BANA + NSD	2.24 ± 1.21 bc	133.27 ± 1.36 ab	26.43 ± 1.11 ab	108.39 ± 1.33 abcd
BANA + NAK-2 + NYA-2 + NSD	1.96 ± 1.26 c	108.63 ± 1.13 ab	30.11 ± 1.06 ab	61.66 ± 1.20 cdefg
16789 + NAK-2	6.46 ± 1.16 a	87.13 ± 1.05 ab	25.33 ± 1.11 ab	41.37 ± 1.18 efg
16789 + NAK-2 + NYA-2 + NSD	5.94 ± 1.09 a	83.58 ± 1.13 ab	20.09 ± 1.06 bc	61.19 ± 1.16 cdefg
16789 + NAK-2 + NYA-2	5.32 ± 1.20 ab	135.44 ± 1.22 ab	21.00 ± 1.21 bc	138.57 ± 1.24 ab
16789 + NSD	4.97 ± 1.14 abc	88.49 ± 1.09 ab	12.94 ± 1.15 c	32.86 ± 1.13 g
16789 UNINOCULATED	4.53 ± 1.14 abc	95.55 ± 9.07 ab	18.38 ± 1.08 bc	78.52 ± 1.11 abcdef
16789 + NYA-2	3.16 ± 1.30 abc	79.14 ± 1.07 b	24.67 ± 1.07 ab	61.66 ± 1.10 cdefg
Test Values	**df = 23; F = 2.997; P ≤ 0.0001**	**df = 23 ; F = 2.398; P ≤ 0.0001**	**df = 23 ; F = 3.972; P ≤ 0.0001**	**df = 23 ; F = 6.824; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 14: Specific effects of pathogens co-infection under nitrogen deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NAK-2	90.85 ± 1.17 a	49.93 ± 1.26 a	36.45 ± 1.09 ab	29.40 ± 1.13 ab
KK 1 UNINOCULATED	74.13 ± 1.41 ab	43.15 ± 1.44 ab	28.40 ± 1.43 abc	30.32 ± 1.14 a
KK 1 + NYA-2	64.57 ± 1.31 abc	34.15 ± 1.39 abc	27.65 ± 1.44 abc	28.73 ± 1.16 ab
KK 1 + NSD	33.75 ± 1.17 abcde	18.27 ± 1.21 abcdefg	14.96 ± 1.13 abc	17.31 ± 1.05 ab
KK 1+ NAK-2 + NYA-2 + NSD	20.18 ± 1.12 de	7.88 ± 1.19 efg	11.89 ± 1.08 c	22.47 ± 1.10 ab
KK 1+ NAK-2 + NYA-2	18.62 ± 1.35 e	6.71 ± 1.31 g	11.61 ± 1.39 c	28.40 ± 1.24 ab
KK 2 UNINOCULATED	81.60 ± 1.07 ab	38.02 ± 1.09 abc	42.99 ± 1.07 a	30.78 ± 1.14 a
KK 2 + NYA-2	54.95 ± 1.10 abcde	28.73 ± 1.17 abcde	24.64 ± 1.04 abc	26.51 ± 1.13 ab
KK 2+ NAK-2 + NYA-2	51.09 ± 1.22 abcde	26.51 ± 1.29 abcde	23.00 ± 1.18 abc	22.56 ± 1.11 ab
KK 2 + NAK-2	42.99 ± 1.37 abcde	17.71 ± 1.45 abcdefg	23.26 ± 1.32 abc	25.12 ± 1.23 ab
KK 2 + NSD	22.47 ± 1.08 cde	6.97 ± 1.34 fg	12.78 ± 1.10 bc	20.81 ± 1.11 ab
KK 2 + NAK-2 + NYA-2 + NSD	18.84 ± 1.16 e	8.07 ± 1.22 defg	10.43 ± 1.13 c	19.20 ± 1.16 ab
BANA + NYA-2	85.77 ± 1.40 a	43.99 ± 1.53 ab	40.89 ± 1.49 a	34.54 ± 1.19 a
BANA UNINOCULATED	62.61 ± 1.07 abc	33.63 ± 1.13 abc	26.92 ± 1.09 abc	22.05 ± 1.20 ab
BANA + NAK-2	59.80 ± 1.22 abc	30.55 ± 1.20 abcd	28.29 ± 1.24 abc	32.86 ± 1.26 a
BANA + NAK-2 + NYA-2	55.80 ± 1.35 abcde	25.80 ± 1.40 abcdef	28.29 ± 1.33 abc	34.67 ± 1.23 a
BANA + NSD	45.53 ± 1.17 abcde	20.11 ± 1.27 abcdefg	22.73 ± 1.56 abc	17.82 ± 1.15 ab
BANA + NAK-2 + NYA-2 + NSD	37.30 ± 1.20 abcde	11.01 ± 1.46 cdefg	21.38 ± 1.22 abc	13.54 ± 1.15 b
16789 + NAK-2 + NYA-2 + NSD	61.90 ± 1.32 abcd	37.73 ± 1.31 abc	22.13 ± 1.36 abc	29.29 ± 1.11 ab
16789 UNINOCULATED	61.66 ± 1.16 abcd	33.88 ± 1.19 abc	26.92 ± 1.16 abc	27.12 ± 1.10 ab
16789 + NAK-2	49.55 ± 1.11 abcde	22.13 ± 1.11 abcdefg	27.23 ± 1.13 abc	36.45 ± 1.12 a
16789 + NYA-2	45.53 ± 1.25 abcde	22.13 ± 1.24 abcdefg	23.00 ± 1.26 abc	23.62 ± 1.28 ab
16789 + NSD	37.01 ± 1.15 abcde	19.13 ± 1.15 abcdefg	17.71 ± 1.16 abc	27.54 ± 1.16 ab
16789 + NAK-2 + NYA-2	27.86 ± 1.33 bcde	11.79 ± 1.31 bcdefg	15.97 ± 1.32 abc	30.20 ± 1.16 a
Test Values	**df = 23; F = 4.838; P ≤ 0.0001**	**df = 23 ; F = 5.767; P ≤ 0.0001**	**df = 23 ; F = 3.409; P ≤ 0.0001**	**df = 23 ; F = 2.609; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 15: Specific effects of pathogens co-infection under nitrogen deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NAK-2	25.22 ± 1.14 a	104.74 ± 1.07 ab	39.36 ± 1.11 ab	39.66 ± 1.11 bcd
KK 1+ NAK-2 + NYA-2 + NSD	24.27 ± 1.06 a	93.51 ± 1.09 ab	27.23 ± 1.33 abcde	58.88 ± 1.12 abc
KK 1 UNINOCULATED	20.50 ± 1.06 abc	77.29 ± 1.14 abc	39.96 ± 1.09 ab	51.29 ± 1.10 abcd
KK 1 + NYA-2	19.65 ± 1.11 abcd	72.01 ± 1.07 bc	23.62 ± 1.04 cde	58.88 ± 1.04 abc
KK 1+ NAK-2 + NYA-2	16.60 ± 1.09 abcdefg	98.99 ± 1.06 ab	27.54 ± 1.10 abcde	39.05 ± 1.12 bcd
KK 1 + NSD	15.49 ± 1.19 abcdefg	88.36 ± 1.07 ab	38.61 ± 1.05 abc	32.61 ± 1.08 cd
KK 2+ NAK-2 + NYA-2	19.80 ± 1.07 abcd	97.01 ± 1.04 ab	26.30 ± 1.10 bcde	43.32 ± 1.13 abcd
KK 2 + NAK-2	19.13 ± 1.12 abcde	102.48 ± 1.06 ab	28.08 ± 1.03 abcde	51.29 ± 1.09 abcd
KK 2 + NSD	11.79 ± 1.09 cdefgh	100.06 ± 1.08 ab	33.88 ± 1.11 abcde	77.92 ± 1.10 a
KK 2 + NYA-2	11.18 ± 1.10 efgh	104.47 ± 1.06 ab	32.86 ± 1.06 abcde	41.21 ± 1.07 abcd
KK 2 UNINOCULATED	9.77 ± 1.27 gh	106.42 ± 1.07 ab	37.73 ± 1.07 abc	78.22 ± 1.12 a
KK 2 + NAK-2 + NYA-2 + NSD	7.88 ± 1.11 h	90.31 ± 1.08 ab	22.47 ± 1.04 de	48.60 ± 1.31 abcd
BANA + NAK-2	19.20 ± 1.14 abcde	71.98 ± 1.05 bc	32.36 ± 1.09 abcde	52.89 ± 1.12 abcd
BANA + NAK-2 + NYA-2 + NSD	16.22 ± 1.03 abcdefg	82.85 ± 1.07 ab	34.54 ± 1.11 abcde	63.58 ± 1.15 ab
BANA + NSD	15.73 ± 1.08 abcdefg	81.67 ± 1.17 ab	37.73 ± 1.06 abc	39.66 ± 1.14 bcd
BANA UNINOCULATED	12.21 ± 1.11 cdefgh	51.03 ± 1.17 c	33.37 ± 1.15 abcde	29.29 ± 1.15 d
BANA + NYA-2	11.93 ± 1.14 cdefgh	84.79 ± 1.06 ab	36.87 ± 1.04 abcd	39.96 ± 1.05 bcd
BANA + NAK-2 + NYA-2	10.92 ± 1.09 fgh	82.95 ± 1.11 ab	44.50 ± 1.03 a	31.62 ± 1.16 cd
16789 + NAK-2	22.30 ± 1.06 ab	88.24 ± 1.16 ab	28.18 ± 1.06 abcde	57.99 ± 1.07 abc
16789 + NYA-2	17.11 ± 1.06 abcdef	103.14 ± 1.04 ab	26.81 ± 1.09 bcde	64.32 ± 1.11 ab
16789 UNINOCULATED	13.54 ± 1.10 bcdefgh	104.35 ± 1.09 ab	33.37 ± 1.11 abcde	37.44 ± 1.17 bcd
16789 + NAK-2 + NYA-2 + NSD	13.23 ± 1.07 bcdefgh	75.48 ± 1.07 abc	21.13 ± 1.10 e	52.89 ± 1.28 abcd
16789 + NSD	12.16 ± 1.12 cdefgh	114.13 ± 1.05 a	33.24 ± 1.02 abcde	52.89 ± 1.08 abcd
16789 + NAK-2 + NYA-2	11.70 ± 1.10 defgh	96.33 ± 1.08 ab	33.63 ± 1.05 abcde	43.65 ± 1.12 abcd
Test Values	**df =23 ; F = 8.061; P ≤ 0.0001**	**df = 23 ; F = 4.154; P ≤ 0.0001**	**df = 23 ; F = 4.041; P ≤ 0.0001**	**df = 23 ; F = 4.359; P = 0.009**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 16: Specific effects of pathogens co-infection under nitrogen deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 UNINOCULATED	157.28 ± 1.16 a	77.03 ± 1.20 a	77.62 ± 1.14 a	92.26 ± 1.08 abc
KK 1 + NSD	112.20 ± 1.11 abc	49.93 ± 1.18 abcd	60.26 ± 1.07 ab	54.84 ± 1.09 efgh
KK 1+ NAK-2 + NYA-2 + NSD	110.07 ± 1.07 abc	48.23 ± 1.16 abcd	52.68 ± 1.15 abc	92.26 ± 1.13 abc
KK 1 + NAK-2	106.33 ± 1.12 abc	46.42 ± 1.16 abcd	58.88 ± 1.09 ab	108.39 ± 1.11 ab
KK 1+ NAK-2 + NYA-2	104.31 ± 1.09 abc	36.39 ± 1.20 abcd	63.83 ± 1.05 ab	84.14 ± 1.06 abcde
KK 1 + NYA-2	73.00 ± 1.08 bcd	32.36 ± 1.08 bcd	39.81 ± 1.12 abc	86.43 ± 1.07 abcd
KK 2+ NAK-2 + NYA-2	132.32 ± 1.10 ab	59.57 ± 1.09 abc	70.79 ± 1.10 ab	79.43 ± 1.04 abcde
KK 2 + NSD	125.41 ± 1.07 abc	66.32 ± 1.11 ab	50.12 ± 1.21 abc	63.10 ± 1.05 cdefg
KK 2 + NAK-2	109.23 ± 1.09 abc	48.79 ± 1.12 abcd	60.49 ± 1.06 ab	88.78 ± 1.07 abcd
KK 2 UNINOCULATED	106.74 ± 1.15 abc	56.45 ± 1.16 abc	42.99 ± 1.25 abc	45.01 ± 1.21 gh
KK 2 + NYA-2	99.24 ± 1.14 abcd	44.33 ± 1.22 abcd	53.09 ± 1.09 abc	45.88 ± 1.10 gh
KK 2 + NAK-2 + NYA-2 + NSD	77.33 ± 1.21 bcd	35.21 ± 1.25 abcd	40.58 ± 1.19 abc	38.02 ± 1.15 h
BANA + NAK-2	107.98 ± 1.20 abc	42.82 ± 1.21 abcd	64.07 ± 1.20 ab	93.33 ± 1.14 abc
BANA + NAK-2 + NYA-2 + NSD	101.94 ± 1.06 abc	35.89 ± 1.08 abcd	64.07 ± 1.10 ab	70.25 ± 1.06 bcdefg
BANA + NSD	87.10 ± 1.10 abcd	40.89 ± 1.14 abcd	46.42 ± 1.07 abc	65.82 ± 1.02 cdefg
BANA + NYA-2	74.13 ± 1.09 bcd	28.51 ± 1.11 cd	45.88 ± 1.09 abc	39.81 ± 1.07 h
BANA + NAK-2 + NYA-2	72.17 ± 1.12 bcd	30.20 ± 1.18 bcd	41.37 ± 1.09 abc	74.99 ± 1.06 bcdef
BANA UNINOCULATED	51.29 ± 1.28 d	22.82 ± 1.29 d	27.75 ± 1.29 c	48.60 ± 1.10 fgh
16789 + NAK-2	136.98 ± 1.15 ab	56.45 ± 1.19 abc	79.74 ± 1.12 a	117.94 ± 1.08 a
16789 + NYA-2	126.38 ± 1.06 ab	60.72 ± 1.07 abc	65.31 ± 1.05 ab	78.83 ± 1.05 abcde
16789 + NSD	105.52 ± 1.17 abc	47.86 ± 1.22 abcd	43.82 ± 1.28 abc	37.58 ± 1.06 h
16789 UNINOCULATED	85.77 ± 1.17 abcd	39.81 ± 1.18 abcd	45.53 ± 1.16 abc	65.56 ± 1.06 cdefg
16789 + NAK-2 + NYA-2	79.43 ± 1.19 bcd	37.30 ± 1.23 abcd	40.89 ± 1.16 abc	54.74 ± 1.04 efgh
16789 + NAK-2 + NYA-2 + NSD	63.83 ± 1.17 cd	28.51 ± 1.18 cd	34.94 ± 1.17 bc	56.89 ± 1.05 defgh
Test Values	**df = 23 ; F = 4.071; P ≤ 0.0001**	**df = 23 ; F = 3.442; P ≤ 0.0001**	**df = 23 ; F = 3.489; P ≤ 0.0001**	**df = 23 ; = 15.154; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 17: Specific effects of pathogens co-infection under nitrogen deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NSD	16.09 ± 1.26 a	44.94 ± 1.06 bcd	27.23 ± 1.06 a	24.08 ± 1.12 bcdef
KK 1 + NAK-2	14.40 ± 1.12 ab	55.38 ± 1.06 abcd	24.74 ± 1.02 a	34.15 ± 1.07 abcdef
KK 1 UNINOCULATED	13.75 ± 1.15 ab	74.29 ± 1.10 a	21.96 ± 1.18 ab	18.98 ± 1.21 def
KK 1 + NYA-2	12.12 ± 1.11 abc	61.44 ± 1.04 abc	13.96 ± 1.16 b	45.88 ± 1.13 ab
KK 1+ NAK-2 + NYA-2	10.39 ± 1.07 abcd	59.82 ± 1.05 abc	24.74 ± 1.04 a	50.70 ± 1.06 ab
KK 1+ NAK-2 + NYA-2 + NSD	6.29 ± 1.16 de	59.47 ± 1.04 abc	25.22 ± 1.06 a	40.89 ± 1.11 abcd
KK 2 + NAK-2	11.22 ± 1.10 abcd	62.95 ± 1.10 abc	20.97 ± 1.08 ab	35.75 ± 1.13 abcde
KK 2 + NSD	10.47 ± 1.12 abcd	67.24 ± 1.07 ab	23.44 ± 1.08 a	34.41 ± 1.17 abcdef
KK 2 + NYA-2	9.81 ± 1.05 abcd	51.82 ± 1.07 abcd	20.65 ± 1.05 ab	48.23 ± 1.08 ab
KK 2 UNINOCULATED	7.16 ± 1.32 cde	70.72 ± 1.13 a	22.82 ± 1.17 a	38.46 ± 1.17 abcde
KK 2+ NAK-2 + NYA-2	6.14 ± 1.12 de	57.73 ± 1.03 abcd	21.38 ± 1.07 ab	17.65 ± 1.17 ef
KK 2 + NAK-2 + NYA-2 + NSD	6.03 ± 1.10 de	56.61 ± 1.06 abcd	18.20 ± 1.07 ab	45.01 ± 1.10 abc
BANA + NAK-2 + NYA-2	11.13 ± 1.08 abcd	54.40 ± 1.04 abcd	26.92 ± 1.06 a	15.43 ± 1.14 f
BANA UNINOCULATED	8.74 ± 1.02 abcd	57.47 ± 1.06 abcd	20.26 ± 1.16 ab	30.32 ± 1.14 abcdef
BANA + NAK-2	8.16 ± 1.08 bcde	65.56 ± 1.07 abc	22.30 ± 1.09 ab	22.39 ± 1.09 bcdef
BANA + NYA-2	7.94 ± 1.07 bcde	44.30 ± 1.16 cd	22.13 ± 1.09 ab	25.12 ± 1.17 bcdef
BANA + NSD	7.82 ± 1.12 bcde	52.36 ± 1.13 abcd	22.47 ± 1.05 ab	37.58 ± 1.15 abcde
BANA + NAK-2 + NYA-2 + NSD	4.28 ± 1.10 e	55.42 ± 1.09 abcd	27.75 ± 1.07 a	19.72 ± 1.25 cdef
16789 + NAK-2 + NYA-2	10.39 ± 1.13 abcd	67.01 ± 1.12 abc	24.74 ± 1.13 a	59.80 ± 1.32 a
16789 + NYA-2	9.96 ± 1.11 abcd	70.46 ± 1.05 a	19.20 ± 1.17 ab	37.58 ± 1.26 abcde
16789 + NSD	8.78 ± 1.12 abcd	73.04 ± 1.08 a	24.64 ± 1.12 a	18.55 ± 1.35 def
16789 + NAK-2 + NYA-2 + NSD	8.41 ± 1.14 abcd	38.63 ± 1.15 d	19.88 ± 1.04 ab	30.08 ± 1.19 abcdef
16789 UNINOCULATED	8.35 ± 1.15 bcd	76.98 ± 1.06 a	18.62 ± 1.06 ab	50.70 ± 1.16 ab
16789 + NAK-2	8.19 ± 1.13 bcde	75.07 ± 1.03 a	20.42 ± 1.06 ab	30.55 ± 1.18 abcdef
Test Values	**df = 23; F = 5.833; P ≤ 0.0001**	**df = 23 ; F = 4.887; P ≤ 0.0001**	**df = 23 ; F = 2.660; P ≤ 0.0001**	**df = 23 ; F = 5.680; P = 0.01**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 18: Specific effects of pathogens co-infection under nitrogen deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 UNINOCULATED	35.75 ± 1.38 a	14.02 ± 1.45 ab	21.22 ± 1.09 a	62.61 ± 1.06 a
KK 1+ NAK-2 + NYA-2	33.37 ± 1.11 a	15.49 ± 1.16 ab	17.71 ± 1.08 a	43.32 ± 1.10 abcd
KK 1 + NAK-2	33.24 ± 1.12 a	14.07 ± 1.15 ab	18.91 ± 1.10 a	56.45 ± 1.09 ab
KK 1 + NYA-2	30.67 ± 1.17 ab	15.25 ± 1.15 ab	15.14 ± 1.18 ab	49.17 ± 1.09 abc
KK 1 + NSD	18.98 ± 1.04 abc	5.05 ± 1.20 bc	13.08 ± 1.02 ab	56.45 ±1.14 ab
KK 1+ NAK-2 + NYA-2 + NSD	17.58 ± 1.12 abc	5.93 ± 1.21 abc	11.13 ± 1.10 abc	27.44 ± 1.16 def
KK 2 + NAK-2 + NYA-2 + NSD	39.81 ± 1.46 a	17.99 ± 1.50 a	21.63 ± 1.42 a	24.17 ± 1.06 ef
KK 2 + NAK-2	37.30 ± 1.10 a	15.08 ± 1.13 ab	22.22 ± 1.09 a	48.23 ± 1.07 abc
KK 2 UNINOCULATED	28.51 ± 1.39 ab	11.31 ± 1.43 abc	17.11 ± 1.37 ab	31.99 ± 1.29 cdef
KK 2 + NYA-2	27.02 ± 1.07 ab	9.37 ± 1.13 abc	17.38 ± 1.06 a	40.89 ± 1.02 abcde
KK 2 + NSD	19.35 ± 1.04 abc	7.76 ± 1.08 abc	11.39 ± 1.04 abc	38.31 ± 1.08 abcde
KK 2+ NAK-2 + NYA-2	17.78 ± 1.09 abc	6.63 ± 1.10 abc	10.92 ± 1.10 abc	27.33 ± 1.11 def
BANA + NAK-2	30.78 ± 1.09 ab	10.19 ± 1.13 abc	20.50 ± 1.08 a	40.12 ± 1.08 abcde
BANA UNINOCULATED	24.45 ± 1.10 abc	10.80 ± 1.10 abc	13.65 ± 1.10 ab	39.81 ± 1.06 abcde
BANA + NAK-2 + NYA-2	23.80 ± 1.12 abc	7.53 ± 1.11 abc	16.22 ± 1.13 ab	51.88 ± 1.07 abc
BANA + NYA-2	21.63 ± 1.10 abc	8.45 ± 1.12 abc	12.83 ± 1.11 ab	30.20 ± 1.09 cdef
BANA + NAK-2 + NYA-2 + NSD	11.01 ± 1.31 bc	4.92 ± 1.31 bc	6.07 ± 1.32 bc	21.54 ± 1.10 f
BANA + NSD	8.74 ± 1.22 c	4.06 ± 1.30 c	4.07 ± 1.23 c	30.67 ± 1.11 cdef
16789 UNINOCULATED	37.73 ± 1.06 a	16.03 ± 1.11 ab	21.30 ± 1.04 a	43.15 ± 1.13 abcd
16789 + NYA-2	35.35 ± 1.22 a	14.07 ± 1.29 ab	20.42 ± 1.18 a	40.58 ± 1.08 abcde
16789 + NAK-2 + NYA-2 + NSD	30.43 ± 1.08 ab	11.79 ± 1.08 abc	18.41 ± 1.09 a	34.94 ± 1.08 bcdef
16789 + NAK-2	28.84 ± 1.07 ab	10.55 ± 1.08 abc	18.13 ± 1.07 a	33.75 ± 1.14 bcdef
16789 + NAK-2 + NYA-2	27.86 ± 1.80 ab	12.02 ± 1.81 abc	15.61 ± 1.79 ab	37.15 ± 1.20 abcdef
16789 + NSD	23.35 ± 1.21 abc	7.59 ± 1.26 abc	15.08 ± 1.19 ab	35.75 ± 1.09 abcdef
Test Values	**df = 23 ; F = 3.377; P ≤ 0.0001**	**df = 23 ; F = 3.278; P ≤ 0.0001**	**df = 23 ; F = 4.107; P ≤ 0.0001**	**df = 23 ; F = 6.384; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 19: Specific effects of pathogens co-infection under phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 UNINOCULATED	11.28 ± 1.20 a	149.12 ± 1.07 b	40.62 ± 1.12 bc	140.17 ± 1.10 bcd
KK 1+ NAK-2 + NYA-2	8.25 ± 1.22 abc	153.95 ± 1.11 b	43.33 ± 1.05 abc	147.63 ± 1.15 bc
KK 1+ NAK-2 + NYA-2 + NSD	6.48 ± 1.20 abc	144.24 ± 1.02 b	43.36 ± 1.06 abc	52.28 ± 1.34 g
KK 1 + NSD	6.43 ± 1.17 abc	136.04 ± 1.09 b	43.30 ± 1.06 abc	71.61 ± 1.13 defg
KK 1 + NYA-2	6.19 ± 1.12 abc	145.59 ± 1.05 b	45.80 ± 1.06 abc	68.39 ± 1.15 efg
KK 1 + NAK-2	5.98 ± 1.11 abc	147.28 ± 1.05 b	54.79 ± 1.04 ab	61.66 ± 1.11 fg
KK 2 + NSD	7.41 ± 1.12 abc	121.87 ± 1.07 b	40.47 ± 1.04 bc	53.29 ± 1.11 g
KK 2 + NAK-2	7.18 ± 1.13 abc	142.62 ± 1.11 b	41.27 ± 1.10 bc	158.49 ± 1.21 b
KK 2 + NYA-2	6.40 ± 1.14 abc	148.25 ± 1.06 b	43.17 ± 1.06 abc	87.10 ± 1.12 bcdefg
KK 2 + NAK-2 + NYA-2 + NSD	5.92 ± 1.19 abc	150.92 ± 1.05 b	50.46 ± 1.12 abc	60.95 ± 1.14 fg
KK 2+ NAK-2 + NYA-2	4.83 ± 1.19 abc	128.44 ± 1.05 b	35.28 ± 1.09 c	49.93 ± 1.13 g
KK 2 UNINOCULATED	4.52 ± 1.15 bc	171.36 ± 1.04 b	38.36 ± 1.08 bc	71.34 ± 1,16 defg
BANA + NYA-2	7.81 ± 1.15 abc	155.07 ± 1.07 b	50.47 ± 1.07 abc	75.86 ± 1.08 cdefg
BANA + NAK-2 + NYA-2 + NSD	7.75 ± 1.30 abc	120.01 ± 1.04 b	50.54 ± 1.06 abc	50.31 ± 1.12 g
BANA UNINOCULATED	7.25 ± 1.08 abc	154.28 ± 1.03 b	46.92 ± 1.03 abc	60.03 ± 1.08 fg
BANA + NSD	7.19 ± 1.13 abc	168.81 ± 1.15 b	46.91 ± 1.10 abc	114.38 ± 1.14 bcdef
BANA + NAK-2 + NYA-2	6.31 ± 1.25 abc	132.81 ± 1.07 b	46.30 ± 1.05 abc	128.83 ± 1.12 bcde
BANA + NAK-2	3.64 ± 1.19 c	147.34 ± 1.03 b	60.69 ± 1.13 a	70.79 ± 1.12 defg
16789 + NAK-2 + NYA-2	8.58 ± 1.17 ab	273.24 ± 1.19 a	37.97 ± 1.09 bc	499.27 ± 1.22 a
16789 UNINOCULATED	7.63 ± 1.11 abc	121.74 ± 1.05 b	39.36 ± 1.03 bc	60.03 ± 1.08 fg
16789 + NAK-2 + NYA-2 + NSD	6.87 ± 1.17 abc	140.74 ± 1.07 b	39.01 ± 1.07 bc	83.18 ± 1.08 bcdefg
16789 + NSD	6.45 ± 1.22 abc	132.70 ± 1.05 b	34.88 ± 1.09 c	119.31 ± 1.18 bcdef
16789 + NYA-2	6.06 ± 1.18 abc	140.73 ± 1.04 b	35.24 ± 1.08 c	61.90 ± 1.06 fg
16789 + NAK-2	4.74 ± 1.26 bc	131.51 ± 1.03 b	39.69 ± 1.03 bc	67.61 ± 1.21 efg
Test Values	**df = 23 ; = 4.532; P = 0.005**	**df = 23 ; F = 5.019; P ≤ 0.0001**	**df = 23; F = 3.707; P ≤ 0.0001**	**df = 23 ; F = 14.284; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 20: Specific effects of pathogens co-infection under phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 UNINOCULATED	551.65 ± 1.29 a	322.35 ± 1.33 a	220.46 ± 1.24 a	76.15 ± 1.14 a
KK 1 + NYA-2	464.16 ± 1.17 ab	291.74 ± 1.22 ab	164.06 ± 1.12 ab	55.17 ± 1.05 abc
KK 1+ NAK-2 + NYA-2	344.09 ± 1.49 abcd	194.98 ± 1.54 abcd	145.10 ± 1.44 ab	59.11 ± 1.23 abc
KK 1 + NSD	261.02 ± 1.22 abcd	148.48 ± 1.25 abcd	108.39 ± 1.18 abc	41.69 ± 1.07 abc
KK 1 + NAK-2	230.85 ± 1.06 abcd	157.28 ± 1.07 abcd	46.95 ± 1.43 c	32.73 ± 1.06 bc
KK 1+ NAK-2 + NYA-2 + NSD	192.75 ± 1.10 bcd	118.85 ± 1.10 abcd	74.70 ± 1.10 bc	48.05 ± 1.16 abc
KK 2 + NAK-2	354.81 ± 1.29 abcd	168.53 ± 1.32 abcd	157.28 ± 1.31 ab	44.50 ± 1.18 abc
KK 2 + NYA-2	262.02 ± 1.23 abcd	150.78 ± 1.25 abcd	110.92 ± 1.22 abc	39.05 ± 1.11 abc
KK 2 UNINOCULATED	258.03 ± 1.11 abcd	168.53 ± 1.15 abcd	81.91 ± 1.11 abc	41.69 ± 1.03 abc
KK 2 + NAK-2 + NYA-2 + NSD	183.37 ± 1.08 bcd	91.20 ± 1.12 cd	88.44 ± 1.08 abc	39.20 ± 1.11 abc
KK 2 + NSD	172.45 ± 1.13 bcd	90.50 ± 1.20 cd	76.15 ± 1.07 bc	38.61 ± 1.08 abc
KK 2+ NAK-2 + NYA-2	150.20 ± 1.16 cd	75.86 ± 1.20 cd	72.17 ± 1.15 bc	36.87 ± 1.11 bc
BANA + NSD	386.07 ± 1.32 abc	223.01 ± 1.37 abc	154.29 ± 1.26 ab	46.59 ± 1.14 abc
BANA + NYA-2	370.11 ± 1.16 abcd	198.76 ± 1.20 abcd	160.32 ± 1.16 ab	47.13 ± 1.11 abc
BANA + NAK-2 + NYA-2	229.97 ± 1.14 abcd	129.32 ± 1.12 abcd	97.35 ± 1.18 abc	51.09 ± 1.19 abc
BANA + NAK-2	196.49 ± 1.09 bcd	111.34 ± 1.15 abcd	78.83 ± 1.06 abc	30.78 ± 1.11 c
BANA + NAK-2 + NYA-2 + NSD	195.73 ± 1.24 bcd	80.66 ± 1.24 cd	112.20 ± 1.24 abc	51.09 ± 1.28 abc
BANA UNINOCULATED	191.28 ± 1.05 bcd	106.33 ± 1.09 abcd	80.04 ± 1.10 abc	41.85 ± 1.06 abc
16789 + NSD	269.15 ± 1.19 abcd	138.04 ± 1.18 abcd	128.83 ± 1.21 abc	49.17 ± 1.23 abc
16789 + NAK-2 + NYA-2	268.12 ± 1.53 abcd	140.17 ± 1.61 abcd	123.50 ± 1.45 abc	63.34 ± 1.22 ab
16789 + NAK-2	217 10 ± 1.10 abcd	114.38 ± 1.09 abcd	101.16 ± 1.12 abc	30.32 ± 1.15 c
16789 + NYA-2	199.53 ± 1.10 bcd	103.51 ± 1.10 bcd	81.28 ± 1.22 abc	38.02 ± 1.08 bc
16789 + NAK-2 + NYA-2 + NSD	169.82 ± 1.16 cd	86.10 ± 1.19 cd	82.54 ± 1.14 abc	48.05 ± 1.39 abc
16789 UNINOCULATED	141.25 ± 1.11 d	70.79 ± 1.13 d	64.17 ± 1.19 bc	48.71 ± 1.09 abc
Test Values	**df=23;F=3.377; P ≤ 0.0001**	**df=23;F= 3.462; P ≤ 0.0001**	**df= 23; F = 3.339; P ≤ 0.0001**	**df=23 ; F= 2.791; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 21: Specific effects of pathogens co-infection under phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1+ NAK-2 + NYA-2 + NSD	7.95 ± 1.56 a	94.75 ± 1.10 bc	35.25 ± 1.13 ab	64.07 ± 1.19 cdef
KK 1 + NAK-2	6.89 ± 1.18 ab	120.60 ± 1.13 bc	37.68 ± 1.14 ab	116.14 ± 1.34 bcd
KK 1 + NSD	6.58 ± 1.23 ab	108.37 ± 1.11 bc	34.74 ± 1.10 ab	46.06 ± 1.31 ef
KK 1+ NAK-2 + NYA-2	5.55 ± 1.21 ab	106.44 ± 1.10 bc	29.51 ± 1.17 ab	65.31 ± 1.15 cdef
KK 1 UNINOCULATED	5.33 ± 1.08 ab	94.28 ± 1.14 bc	32.15 ± 1.10 ab	38.31 ± 1.22 f
KK 1 + NYA-2	4.93 ± 1.21 ab	107.63 ± 1.13 bc	27.44 ± 1.13 b	44.33 ± 1.15 ef
KK 2 + NYA-2	5.93 ± 1.35 ab	123.78 ± 1.11 bc	33.78 ± 1.08 ab	105.12 ± 1.19 bcd
KK 2 UNINOCULATED	5.92 ± 1.18 ab	111.65 ± 1.05 bc	25.72 ± 1.06 b	123.50 ± 1.07 bc
KK 2+ NAK-2 + NYA-2	5.58 ± 1.15 ab	110.51 ± 1.07 bc	33.38 ± 1.06 ab	86.10 ± 1.10 bcde
KK 2 + NSD	5.34 ± 1.28 ab	107.40 ± 1.02 bc	29.69 ± 1.07 ab	65.82 ± 1.15 cdef
KK 2 + NAK-2	4.89 ± 1.19 ab	233.68 ± 1.24 a	31.25 ± 1.09 ab	310.22 ± 1.31 a
KK 2 + NAK-2 + NYA-2 + NSD	4.86 ± 1.16 ab	113.23 ± 1.04 bc	28.41 ± 1.05 ab	67.60 ± 1.09 cdef
BANA + NAK-2 + NYA-2 + NSD	8.00 ± 1.27 a	113.94 ± 1.11 bc	26.28 ± 1.07 b	84.14 ± 1.09 bcde
BANA UNINOCULATED	6.44 ± 1.18 ab	79.63 ± 1.12 c	43.50 ± 1.04 a	86.43 ± 1.09 bcde
BANA + NAK-2	4.48 ± 1.16 ab	132.94 ± 1.03 b	25.29 ± 1.13 b	61.66 ± 1.09 cdef
BANA + NYA-2	4.00 ± 1.27 ab	117.50 ± 1.04 b	32.62 ± 1.05 ab	84.14 ± 1.18 bcde
BANA + NSD	3.00 ± 1.17 b	109.90 ± 1.08 bc	34.40 ± 1.08 ab	73.56 ± 1.06 bcdef
BANA + NAK-2 + NYA-2	2.63 ± 1.20 b	132.64 ± 1.06 bc	35.67 ± 1.05 ab	152.52 ± 1.08 ab
16789 UNINOCULATED	5.88 ± 1.18 ab	99.31 ± 1.03 bc	29.66 ± 1.07 ab	105.52 ± 1.07 bcd
16789 + NSD	5.84 ± 1.15 ab	105.56 ± 1.07 bc	35.75 ± 1.07 ab	54.74 ± 1.07 def
16789 + NAK-2 + NYA-2	5.52 ± 1.16 ab	104.76 ± 1.04 bc	29.92 ± 1.10 ab	107.56 ± 1.04 bcd
16789 + NYA-2	4.63 ± 1.21 ab	109.74 ± 1.05 bc	29.25 ± 1.12 ab	57.77 ± 1.15 cdef
16789 + NAK-2 + NYA-2 + NSD	4.10 ± 1.28 ab	111.75 ± 1.04 bc	33.15 ± 1.06 ab	58.21 ± 1.14 cdef
16789 + NAK-2	4.00 ± 1.27 ab	108.17 ± 1.10 bc	32.68 ± 1.06 ab	119.31 ± 1.06 bc
Test Values	**df = 23; F = 2.032; P = 0.004**	**df = 23; F = 4.374; P = 0.004**	**df = 23; F = 2.127; P = 0.002**	**df=23 ; F = 9.585; p ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 22: Specific effects of pathogens co-infection under phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NAK-2	142.34 ± 1.37 abc	89.81 ± 1.35 abc	39.05 ± 1.68 abc	40.58 ± 1.09 ab
KK 1+ NAK-2 + NYA-2	116.14 ± 1.38 abc	58.21 ± 1.46 abcd	52.89 ± 1.35 abc	36.73 ± 1.19 ab
KK 1 UNINOCULATED	105.93 ± 1.19 abc	63.10 ± 1.18 abcd	42.33 ± 1.23 abc	34.41 ± 1.13 ab
KK 1+ NAK-2 + NYA-2 + NSD	100.00 ± 1.40 abc	45.53 ± 1.47 abcd	53.50 ± 1.35 abc	44.50 ± 1.19 a
KK 1 + NYA-2	98.86 ± 1.38 abc	57.10 ± 1.45 abcd	39.81 ± 1.32 abc	23.53 ± 1.24 ab
KK 1 + NSD	90.16 ± 1.48 abc	40.58 ± 1.58 abc	45.01 ± 1.43 abc	42.49 ± 1.19 a
KK 2 + NAK-2	201.06 ± 1.25 ab	81.91 ± 1.38 abc	95.50 ± 1.27 ab	36.03 ± 1.15 ab
KK 2 + NYA-2	121.15 ± 1.35 abc	63.34 ± 1.37 abcd	53.70 ± 1.38 abc	26.00 ± 1.22 ab
KK 2+ NAK-2 + NYA-2	115.26 ± 1.26 abc	57.32 ± 1.29 abcd	56.89 ± 1.24 abc	38.76 ± 1.13 ab
KK 2 UNINOCULATED	109.65 ± 1.03 abc	56.02 ± 1.05 abcd	52.08 ± 1.07 abc	33.50 ± 1.13 ab
KK 2 + NAK-2 + NYA-2 + NSD	77.92 ± 1.12 bc	37.87 ± 1.16 abcd	39.05 ± 1.08 abc	28.62 ± 1.10 ab
KK 2 + NSD	60.26 ± 1.07 c	25.51 ± 1.09 cd	34.15 ± 1.06 bc	22.56 ± 1.11 ab
BANA + NAK-2 + NYA-2 + NSD	243.59 ± 1.37 a	128.83 ± 1.41 a	112.63 ± 1.34 a	38.31 ± 1.25 ab
BANA UNINOCULATED	119.31 ± 1.16 abc	55.80 ± 1.10 abcd	62.61 ± 1.17 abc	34.81 ± 1.41 ab
BANA + NAK-2 + NYA-2	114.38 ± 1.07 abc	73.85 ± 1.09 abcd	38.31 ± 1.14 abc	20.34 ± 1.14 ab
BANA + NAK-2	85.11 ± 1.08 abc	50.31 ± 1.10 abcd	34.41 ± 1.06 bc	26.71 ± 1.11 ab
BANA + NYA-2	69.45 ± 1.06 bc	31.26 ± 1.10 bcd	34.67 ± 1.13 bc	18.69 ± 1.13 b
BANA + NSD	65.56 ± 1.14 c	31.50 ± 1.21 bcd	32.73 ± 1.10 bc	24.55 ± 1.10 ab
16789 + NAK-2	105.12 ± 1.04 abc	48.60 ± 1.11 abcd	53.29 ± 1.06 abc	43.82 ± 1.14 a
16789 UNINOCULATED	95.87 ± 1.10 abc	38.76 ± 1.14 abcd	55.59 ± 1.08 abc	36.59 ± 1.12 ab
16789 + NAK-2 + NYA-2	89.47 ± 1.10 abc	42.99 ± 1.11 abcd	45.19 ± 1.11 abc	28.08 ± 1.10 ab
16789 + NYA-2	83.18 ± 1.08 abc	36.03 ± 1.05 bcd	46.77 ± 1.11 abc	23.90 ± 1.13 ab
16789 + NSD	73.00 ± 1.13 bc	31.50 ± 1.15 bcd	40.12 ± 1.14 abc	33.75 ± 1.10 ab
16789 + NAK-2 + NYA-2 + NSD	48.23 ± 1.27 c	22.65 ± 1.24 d	25.22 ± 1.30 c	18.41 ± 1.24 b
Test Values	**df = 23 ; F = 2.818; P ≤ 0.0001**	**df = 23 ; F = 2.978; P ≤ 0.0001**	**df = 23 ; F = 2.169; P = 0.002**	**df =23; F = 2.961; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 23: Specific effects of pathogens co-infection under phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NYA-2	23.99 ± 1.11 a	84.18 ± 1.10 bc	28.08 ± 1.07 bcde	38.61 ± 1.08 bcd
KK 1+ NAK-2 + NYA-2	22.56 ± 1.14 a	75.10 ± 1.15 bc	38.61 ± 1.16 ab	48.42 ± 1.32 abcd
KK 1 + NSD	20.73 ± 1.04 ab	98.35 ± 1.10 abc	33.37 ± 1.06 abcd	44.16 ± 1.07 abcd
KK 1+ NAK-2 + NYA-2 + NSD	20.34 ± 1.05 ab	194.26 ± 1.07 abc	32.73 ± 1.07 abcde	29.97 ± 1.12 cd
KK 1 + NAK-2	16.16 ± 1.10 ab	106.31 ± 1.06 abc	31.99 ± 1.12 abcde	40.58 ± 1.08 abcd
KK 1 UNINOCULATED	15.91 ± 1.12 ab	70.37 ± 1.08 c	28.29 ± 1.02 abcde	26.51 ± 1.31 d
KK 2 + NSD	22.56 ± 1.24 a	95.95 ± 1.06 abc	33.24 ± 1.06 abcde	48.23 ± 1.09 abcd
KK 2 UNINOCULATED	17.58 ± 1.20 ab	92.45 ± 1.10 abc	37.01 ± 1.07 ab	45.53 ± 1.10 abcd
KK 2 + NYA-2	16.66 ± 1.09 ab	108.10 ± 1.10 ab	24.36 ± 1.09 cde	40.43 ± 1.32 abcd
KK 2 + NAK-2	16.34 ± 1.09 ab	96.87 ± 1.09 abc	31.14 ± 1.07 abcde	70.25 ± 1.25 ab
KK 2 + NAK-2 + NYA-2 + NSD	13.39 ± 1.16 abc	87.55 ± 1.11 abc	36.03 ± 1.03 abc	25.41 ± 1.16 d
KK 2+ NAK-2 + NYA-2	12.54 ± 1.27 abc	111.00 ± 1.09 ab	41.05 ± 1.05 ab	39.81 ± 1.10 abcd
BANA + NYA-2	19.65 ± 1.08 ab	88.29 ± 1.09 abc	33.88 ± 1.05 abcd	48.79 ± 1.11 abcd
BANA + NAK-2 + NYA-2	16.85 ± 1.11 ab	95.86 ± 1.08 abc	36.45 ± 1.06 abc	77.33 ± 1.09 ab
BANA + NAK-2 + NYA-2 + NSD	13.70 ± 1.11 ab	96.41 ± 1.03 abc	42.66 ± 1.05 a	36.59 ± 1.14 bcd
BANA + NSD	12.07 ± 1.16 abc	84.64 ± 1.10 bc	21.96 ± 1.07 e	44.33 ± 1.21 abcd
BANA UNINOCULATED	11.98 ± 1.09 abc	133.47 ± 1.07 abca	34.41 ± 1.08 abcd	53.91 ± 1.08 abcd
BANA + NAK-2	6.81 ± 1.21 c	106.31 ± 1.08 abc	40.23 ± 1.11 ab	40.75 ± 1.14 abcd
16789 + NAK-2 + NYA-2 + NSD	18.55 ± 1.07 ab	99.65 ± 1.04 abc	29.97 ± 1.07 abcde	31.02 ± 1.10 cd
16789 + NAK-2 + NYA-2	16.72 ± 1.13 ab	85.09 ± 1.10 bc	23.00 ± 1.10 de	85.44 ± 1.26 a
16789 + NAK-2	16.47 ± 1.12 ab	96.70 ± 1.04 abc	30.78 ± 1.09 abcde	54.33 ± 1.06 abcd
16789 + NYA-2	15.14 ± 1.09 ab	109.20 ± 1.07 ab	28.95 ± 1.10 abcde	47.32 ± 1.06 abcd
16789 UNINOCULATED	12.07 ± 1.07 abc	108.09 ± 1.05 ab	32.36 ± 1.11 abcde	61.19 ± 1.10 abc
16789 + NSD	10.35 ± 1.31 bc	90.26 ± 1.11 abc	31.74 ± 1.14 abcde	72.17 ± 1.18 ab
Test Values	**df = 23 ; = 4.532; P ≤ 0.0001**	**df = 23 ; F = 2.788; P ≤ 0.0001**	**df = 23; F = 4.519; P ≤ 0.0001**	**df = 23 ; F = 4.350; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 24: Specific effects of pathogens co-infection under phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1+ NAK-2 + NYA-2 + NSD	147.34 ± 1.11 a	70.52 ± 1.16 a	74.70 ± 1.07 a	118.40 ± 1.05 a
KK 1 + NAK-2	143.44 ± 1.15 a	66.32 ± 1.19 a	76.44 ± 1.11 a	48.42 ± 1.10 def
KK 1 UNINOCULATED	136.46 ± 1.27 a	69.18 ± 1.27 a	66.58 ± 1.27 a	69.45 ± 1.15 abcdef
KK 1 + NYA-2	123.97 ± 1.10 a	61.19 ± 1.15 ab	54.12 ± 1.20 ab	94.41 ± 1.13 abc
KK 1+ NAK-2 + NYA-2	114.38 ± 1.42 ab	52.68 ± 1.48 ab	59.11 ± 1.37 a	109.65 ± 1.14 ab
KK 1 + NSD	107.98 ± 1.15 ab	51.29 ± 1.22 ab	55.16 ± 1.10 ab	74.13 ± 1.08 abcde
KK 2 + NYA-2	138.57 ± 1.12 a	66.33 ± 1.15 a	71.34 ± 1.10 a	68.65 ± 1.07 abcdef
KK 2+ NAK-2 + NYA-2	132.33 ± 1.21 a	63.58 ± 1.25 ab	66.83 ± 1.18 a	63.10 ± 1.22 bcdef
KK 2 + NSD	122.09 ± 1.09 a	51.29 ± 1.14 ab	69.72 ± 1.08 a	77.03 ± 1.18 abcd
KK 2 + NAK-2	117.04 ± 1.29 ab	54.33 ± 1.27 ab	62.85 ± 1.29 a	74.42 ± 1.08 abcde
KK 2 UNINOCULATED	116.14 ± 1.09 ab	54.53 ± 1.19 ab	51.68 ± 1.20 ab	69.98 ± 1.12 abcdef
KK 2 + NAK-2 + NYA-2 + NSD	78.83 ± 1.18 ab	35.08 ± 1.22 ab	43.15 ± 1.14 ab	73.28 ± 1.16 abcde
BANA UNINOCULATED	130.32 ± 1.16 a	74.99 ± 1.20 a	52.89 ± 1.11 ab	54.95 ± 1.06 cdef
BANA + NAK-2 + NYA-2	127.84 ± 1.13 a	56.67 ± 1.14 ab	69.72 ± 1.13 a	71.34 ± 1.11 abcdef
BANA + NAK-2	109.65 ± 1.33 ab	47.50 ± 1.40 ab	58.88 ± 1.28 a	39.05 ± 1.18 f
BANA + NAK-2 + NYA-2 + NSD	89.81 ± 1.15 ab	37.15 ± 1.15 ab	52.28 ± 1.15 ab	80.35 ± 1.11 abcd
BANA + NYA-2	84.46 ± 1.14 ab	35.48 ± 1.18 ab	48.60 ± 1.12 ab	78.22 ± 1.07 abcd
BANA + NSD	44.33 ± 1.21 b	21.22 ± 1.30 b	22.30 ± 1.15 b	45.53 ± 1.11 def
16789 UNINOCULATED	135.42 ± 1.10 a	57.32 ± 1.15 ab	77.92 ± 1.07 a	38.76 ± 1.07 f
16789 + NYA-2	122.09 ± 1.11 ab	55.80 ± 1.13 ab	65.56 ± 1.08 a	40.89 ± 1.02 ef
16789 + NAK-2	119.77 ± 1.05 a	51.29 ± 1.06 ab	67.87 ± 1.06 a	62.37 ± 1.10 bcdef
16789 + NAK-2 + NYA-2 + NSD	107.15 ± 1.12 ab	51.48 ± 1.14 ab	55.38 ± 1.10 ab	71.61 ± 1.08 abcdef
16789 + NSD	74.70 ± 1.32 ab	30.55 ± 1.31 ab	43.48 ± 1.34 ab	59.11 ± 1.25 cdef
16789 + NAK-2 + NYA-2	65.06 ± 1.58 ab	29.51 ± 1.56 ab	35.48 ± 1.60 ab	64.32 ± 1.14 abcdef
Test Values	**df=23;F=2.212; P ≤ 0.0001**	**df=23;F=2.111; P = 0.003**	**df= 23; F = 2.257; P ≤ 0.0001**	**df=23 ; F= 6.072; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 25: Specific effects of pathogens co-infection under phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1+ NAK-2 + NYA-2 + NSD	18.27 ± 1.09 abc	65.24 ± 1.15 b	30.08 ± 1.08 abc	46.06 ± 1.23 abcde
KK 1 + NYA-2	17.05 ± 1.18 abc	72.39 ± 1.09 ab	28.51 ± 1.04 abc	29.85 ± 1.22 cde
KK 1 + NAK-2	15.43 ± 1.12 abcde	60.54 ± 1.08 ab	27.33 ± 1.08 abcd	23.17 ± 1.14 e
KK 1 UNINOCULATED	14.51 ± 1.13 abcde	75.06 ± 1.09 ab	30.43 ± 1.14 abc	27.44 ± 1.26 de
KK 1 + NSD	13.08 ± 1.16 abcdef	69.41 ± 1.09 b	27.02 ± 1.11 abcd	34.81 ± 1.18 bcde
KK 1+ NAK-2 + NYA-2	10.84 ± 1.14 abcdef	71.57 ± 1.10 a	26.10 ± 1.07 abcd	50.89 ± 1.17 abcde
KK 2 + NAK-2 + NYA-2 + NSD	20.73 ± 1.20 a	90.92 ± 1.10 a	29.06 ± 1.12 abc	40.12 ± 1.13 bcde
KK 2 + NAK-2	16.92 ± 1.10 abc	85.12 ± 1.07 a	16.60 ± 1.28 d	92.26 ± 1.19 a
KK 2+ NAK-2 + NYA-2	16.22 ± 1.03 abcd	79.01 ± 1.08 ab	20.73 ± 1.09 cd	60.26 ± 1.10 abcd
KK 2 UNINOCULATED	14.34 ± 1.13 abcde	92.24 ± 1.07 a	28.84 ± 1.06 abc	61.90 ± 1.17 abcd
KK 2 + NSD	13.39 ± 1.15 abcde	86.30 ± 1.07 a	26.81 ± 1.10 abcd	44.33 ± 1.07 abcde
KK 2 + NYA-2	7.50 ± 1.17 ef	81.04 ± 1.05 a	25.41 ± 1.11 abcd	69.72 ± 1.12 ab
BANA + NAK-2 + NYA-2 + NSD	18.91 ± 1.06 ab	57.85 ± 1.08 c	23.71 ± 1.07 abc	24.27 ± 1.21 e
BANA UNINOCULATED	16.85 ± 1.03 abc	73.85 ± 1.13 ab	28.95 ± 1.12 abc	49.17 ± 1.17 abcde
BANA + NYA-2	15.49 ± 1.22 abcde	85.88 ± 1.07 a	38.31 ± 1.16 a	36.73 ± 1.07 bcde
BANA + NAK-2	9.73 ± 1.26 bcdef	100.37 ± 1.08 a	40.12 ± 1.08 a	39.36 ± 1.06 bcde
BANA + NSD	7.88 ± 1.08 def	84.55 ± 1.12 a	35.48 ± 1.10 ab	38.31 ± 1.09 bcde
BANA + NAK-2 + NYA-2	6.19 ± 1.21 f	59.49 ± 1.41 a	29.06 ± 1.04 abcd	63.10 ± 1.25 abc
16789 + NAK-2	22.91 ± 1.13 a	77.80 ± 1.07 ab	29.29 ± 1.13 abc	62.85 ± 1.11 abc
16789 + NYA-2	21.13 ± 1.13 a	87.59 ± 1.06 a	27.02 ± 1.08 abcd	39.66 ± 1.08 bcde
16789 UNINOCULATED	17.38 ± 1.07 abc	80.74 ± 1.02 a	29.17 ± 1.02 abc	77.92 ± 1.16 ab
16789 + NAK-2 + NYA-2	16.09 ± 1.23 abcd	76.76 ± 1.08 ab	27.65 ± 1.04 abcd	63.10 ± 1.10 abc
16789 + NSD	11.61 ± 1.07 abcdef	64.21 ± 1.14 b	30.43 ± 1.09 abc	26.00 ± 1.34 e
16789 + NAK-2 + NYA-2 + NSD	8.71 ± 1.31 cdef	99.77 ± 1.04 a	21.30 ± 1.11 bcd	40.74 ± 1.20 abcde
Test Values	**df = 23; F = 5.838; P ≤ 0.0001**	**df = 23; F = 2.030; P = 0.004**	**df = 23; F = 3.348; P = 0.003**	**df=23 ; F = 5.767; p ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 26: Specific effects of pathogens co-infection under phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NYA-2	58.43 ± 1.40 ab	26.30 ± 1.47	30.67 ± 1.37 abc	60.72 ± 1.15 abcde
KK 1 UNINOCULATED	57.10 ± 1.18 ab	23.09 ± 1.23	33.11 ± 1.17 abc	60.95 ± 1.11 abcde
KK 1+ NAK-2 + NYA-2 + NSD	50.31 ± 1.27 ab	21.38 ± 1.32	27.86 ± 1.25 abc	59.80 ± 1.05 abcde
KK 1 + NSD	49.93 ± 1.20 ab	22.91 ± 1.30	20.18 ± 1.22 abc	53.91 ± 1.13 abcdef
KK 1+ NAK-2 + NYA-2	48.60 ± 1.27 ab	21.71 ± 1.34	25.61 ± 1.23 abc	43.82 ± 1.12 bcdef
KK 1 + NAK-2	34.94 ± 1.08 ab	15.79 ± 1.09	19.28 ± 1.09 bc	56.23 ± 1.11 abcdef
KK 2 + NSD	80.66 ± 1.11 a	31.50 ± 1.17	47.86 ± 1.08 a	48.82 ± 1.16 abcdef
KK 2 UNINOCULATED	74.42 ± 1.12 ab	33.50 ± 1.16	40.58 ± 1.10 ab	55.80 ± 1.08 abcdef
KK 2 + NAK-2 + NYA-2 + NSD	67.09 ± 1.05 ab	27.86 ± 1.08	38.61 ± 1.04 ab	80.04 ± 1.14 a
KK 2+ NAK-2 + NYA-2	64.32 ± 1.13 ab	25.90 ± 1.18	37.30 ± 1.11 abc	62.37 ± 1.06 abcde
KK 2 + NYA-2	56.89 ± 1.16 ab	25.61 ± 1.14	31.26 ± 1.18 abc	36.59 ± 1.15 ef
KK 2 + NAK-2	29.29 ± 1.54 b	11.35 ± 1.69	15.85 ± 1.51 c	77.92 ± 1.10 ab
BANA UNINOCULATED	80.35 ± 1.13 a	31.50 ± 1.16	48.23 ± 1.12 a	67.87 ± 1.05 abcd
BANA + NYA-2	75.57 ± 1.09 ab	28.08 ± 1.16	46.77 ± 1.07 a	59.34 ± 1.15 abcde
BANA + NAK-2	59.34 ± 1.13 ab	25.02 ± 1.19	33.37 ± 1.08 abc	38.46 ± 1.15 def
BANA + NSD	54.95 ± 1.13 ab	24.64 ± 1.18	28.73 ± 1.12 abc	41.21 ± 1.03 cdef
BANA + NAK-2 + NYA-2	51.09 ± 1.29 ab	22.64 ± 1.39	27.12 ± 1.12 abc	30.90 ± 1.15 f
BANA + NAK-2 + NYA-2 + NSD	41.85 ± 1.22 ab	17.31 ± 1.28	23.71 ± 1.19 abc	73.85 ± 1.08 abc
16789 UNINOCULATED	71.34 ± 1.05 ab	23.00 ± 1.12	46.95 ± 1.03 a	63.31 ± 1.10 abcde
16789 + NAK-2 + NYA-2 + NSD	69.98 ± 1.13 ab	30.67 ± 1.15	38.76 ± 1.12 ab	48.42 ± 1.23 abcdef
16789 + NYA-2	69.98 ± 1.09 ab	26.20 ± 1.14	43.15 ± 1.07 ab	58.43 ± 1.17 abcde
16789 + NAK-2	61.66 ± 1.12 ab	23.26 ± 1.14	38.02 ± 1.12 abc	87.43 ± 1.09 a
16789 + NAK-2 + NYA-2	59.11 ± 1.08 ab	22.73 ± 1.13	35.62 ± 1.06 abc	65.06 ± 1.13 abcde
16789 + NSD	43.15 ± 1.30 ab	16.60 ± 1.36	26.00 ± 1.28 abc	39.36 ± 1.10 def
Test Values	**df = 23 ; F = 1.908; P = 0.009**	**df = 23 ; F = 1.168; P = 0.274**	**df = 23 ; F = 3.232; P ≤ 0.0001**	**df =23; F = 5.277 ; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 27: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2;Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime

Treatment combinations	Tiller Number	Tiller height (cm)	Chlorophyll content levels (SPAD UNITS)	Leaf Area (cm^2)
KK 1 + NAK-2	7.78 ± 1.26 a	89.28 ± 1.04 bcde	24.02 ± 1.18 abc	68.13 ± 1.13 defg
KK 1 UNINOCULATED	7.33 ± 1.25 a	89.31 ± 1.03 bcde	35.53 ± 1.17 ab	84.14 ± 1.09 cdefg
KK 1 + NYA-2	7.27 ± 1.08 a	105.25 ± 1.11 abcde	30.40 ± 1.10 abc	54.33 ± 1.21 fg
KK 1 + NSD	6.07 ± 1.15 a	79.97 ± 1.09 de	28.48 ± 1.16 abc	60.03 ± 1.13 efg
KK 1+ NAK-2 + NYA-2	4.78 ± 1.24 b	83.89 ± 1.05 cde	33.98 ± 1.09 ab	54.74 ± 1.14 fg
KK 1 + NAK-2 + NYA-2 + NSD	4.24 ± 1.29 b	166.75 ± 1.26 abcd	23.96 ± 1.03 abc	167.24 ± 1.20 abcd
KK 2+ NAK-2 + NYA-2	10.52 ± 1.13 a	175.60 ± 1.27 abcd	28.57 ± 1.10 abc	206.54 ± 1.18 abc
KK 2 + NAK-2	6.27 ± 1.38 a	219.69 ± 1.28 a	28.21 ± 1.11 abc	252.15 ± 1.36 a
KK 2 + NYA-2	5.96 ± 1.23 a	148.43 ± 1.25 abcde	33.28 ± 1.08 ab	153.11 ± 1.25 abcde
KK 2 + NAK-2 + NYA-2 + NSD	4.44 ± 1.16 b	90.10 ± 1.07 bcde	38.38 ± 1.08 ab	56.89 ± 1.08 fg
KK 2 UNINOCULATED	4.24 ± 1.15 b	116.74 ± 1.08 abcde	24.66 ± 1.06 abc	69.45 ± 1.21 defg
KK 2 + NSD	3.98 ± 1.28 c	112.63 ± 1.10 abcde	25.18 ± 1.19 abc	68.92 ± 1.15 defg
BANA + NSD	9.36 ± 1.23 a	137.27 ± 1.23 abcde	25.85 ± 1.05 abc	111.77 ± 1.12 abcdef
BANA + NYA-2	5.27 ± 1.34 a	96.94 ± 1.13 abcde	40.52 ± 1.11 a	57.10 ± 1.25 fg
BANA UNINOCULATED	4.96 ± 1.28 ab	196.64 ± 1.26 ab	33.32 ± 1.09 ab	250.23 ± 1.28 abcd
BANA + NAK-2 + NYA-2 + NSD	4.88 ± 1.18 ab	71.71 ± 1.15 e	32.33 ± 1.15 abc	59.80 ± 1.16 efg
BANA + NAK-2 + NYA-2	4.67 ± 1.18 b	155.34 ± 1.30 abcde	31.23 ± 1.04 abc	162.18 ± 1.27 a
BANA + NAK-2	4.21 ± 1.12 b	140.79 ± 1.05 abcde	29.24 ± 1.08 abc	120.69 ± 1.05 abcdef
16789 + NAK-2	7.82 ± 1.15 a	89.53 ± 1.11 bcde	26.05 ± 1.13 abc	46.77 ± 1.20 fg
16789 + NYA-2	6.22 ± 1.33 a	89.59 ± 1.06 bcde	23.34 ± 1.04 bc	33.88 ± 1.23 g
16789 + NAK-2 + NYA-2	5.06 ± 1.36 a	153.65 ± 1.28 abcde	24.16 ± 1.07 abc	103.51 ± 1.42 abcdef
16789 + NSD	4.65 ± 1.18 b	87.83 ± 1.08 bcde	25.55 ± 1.07 abc	66.58 ± 1.12 defg
16789 + NAK-2 + NYA-2 + NSD	4.32 ± 1.20 b	101.05 ± 1.09 abcde	19.42 ± 1.12 c	90.50 ± 1.12 bcdef
16789 UNINOCULATED	4.02 ± 1.27 b	184.62 ± 1.26 abc	28.72 ± 1.08 abc	224.73 ± 1.29 ab
Test Values	**df = 23; F = 1.739; P = 0.021**	**df=23;F= 4.202; P ≤ 0.0001**	**df = 23; F = 3.001; P ≤ 0.0001**	**df=23 ; F =9.502; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$.

APPENDIX 28: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime

Treatment combinations	Total Fresh Weight (Yield in grams)	Total Stem Weight (Grams)	Total Leaf Weight (Grams)	Leaf Number
KK 1 + NYA-2	90.16 ± 1.29 abc	50.31 ± 1.33 ab	39.20 ± 1.23 ab	44.16 ± 1.07 ab
KK 1 UNINOCULATED	85.77 ± 1.10 abc	42.49 ± 1.09 abc	42.99 ± 1.11 ab	43.99 ± 1.19 ab
KK 1 + NSD	78.22 ± 1.19 abc	41.05 ± 1.23 abc	35.75 ± 1.16 ab	32.73 ± 1.15 ab
KK 1 + NAK-2	70.79 ± 1.12 abc	31.50 ± 1.14 abc	37.87 ± 1.13 ab	43.48 ± 1.18 ab
KK 1+ NAK-2 + NYA-2 + NSD	67.87 ± 1.14 abc	37.15 ± 1.20 abc	28.84 ± 1.06 ab	29.29 ± 1.18 ab
KK 1+ NAK-2 + NYA-2	53.09 ± 1.10 abc	25.12 ± 1.15 abc	27.12 ± 1.09 ab	21.05 ± 1.15 b
KK 2 UNINOCULATED	118.40 ± 1.17 ab	74.13 ± 1.19 a	43.65 ± 1.14 ab	26.81 ± 1.08 ab
KK 2 + NAK-2	110.07 ± 1.46 ab	58.31 ± 1.55 a	48.79 ± 1.40 ab	33.24 ± 1.22 ab
KK 2 + NSD	109.23 ± 1.33 ab	63.58 ± 1.37 a	43.99 ± 1.29 ab	29.29 ± 1.18 ab
KK 2+ NAK-2 + NYA-2	91.20 ± 1.13 abc	46.77 ± 1.19 ab	41.05 ± 1.10 ab	50.12 ± 1.10 a
KK 2 + NYA-2	81.28 ± 1.14 abc	34.54 ± 1.15 abc	45.71 ± 1.14 ab	37.01 ± 1.14 ab
KK 2 + NAK-2 + NYA-2 + NSD	69.72 ± 1.21 abc	38.61 ± 1.18 abc	29.63 ± 1.28 ab	25.51 ± 1.13 ab
BANA UNINOCULATED	131.32 ± 1.17 a	61.42 ± 1.23 a	66.32 ± 1.17 a	33.50 ± 1.18 ab
BANA + NYA-2	104.31 ± 1.44 ab	47.68 ± 1.51 ab	53.91 ± 1.41 a	39.05 ± 1.23 ab
BANA + NAK-2	104.31 ± 1.04 ab	59.57 ± 1.09 a	42.17 ± 1.06 ab	25.22 ± 1.08 ab
BANA + NAK-2 + NYA-2	94.77 ± 1.27 ab	44.84 ± 1.35 ab	46.95 ± 1.21 ab	37.30 ± 1.10 ab
BANA + NAK-2 + NYA-2 + NSD	84.79 ± 1.34 abc	41.21 ± 1.32 abc	42.33 ± 1.38 ab	46.95 ± 1.29 ab
BANA + NSD	52.28 ± 1.07 abc	25.12 ± 1.15 abc	25.41 ± 1.02 ab	34.28 ± 1.10 ab
16789 UNINOCULATED	89.13 ± 1.18 abc	43.15 ± 1.26 ab	42.99 ± 1.14 ab	21.79 ± 1.15 ab
16789 + NAK-2 + NYA-2 + NSD	85.44 ± 1.20 abc	43.65 ± 1.22 ab	41.05 ± 1.18 ab	30.67 ± 1.14 ab
16789 + NAK-2	74.70 ± 1.50 abc	36.87 ± 1.53 abc	37.01 ± 1.48 ab	48.23 ± 1.19 ab
16789 + NYA-2	59.80 ± 1.12 abc	30.20 ± 1.13 abc	28.84 ± 1.13 ab	37.58 ± 1.23 ab
16789 + NAK-2 + NYA-2	38.61 ± 1.51 bc	14.07 ± 1.72 bc	22.13 ± 1.41 ab	29.40 ± 1.38 ab
16789 + NSD	28.73 ± 1.33 c	10.96 ± 1.25 c	17.44 ± 1.37 b	22.73 ± 1.15 ab
Test Values	**df = 23; F = 2.389; P ≤ 0.0001**	**df = 23; F = 2.894; P ≤ 0.0001**	**df = 23; F = 1.954; P = 0.007**	**df = 23; F = 2.441; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates

APPENDIX 29: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime

Treatment combinations	Tiller Number	Tiller height (cm)	Chlorophyll content levels (SPAD UNITS)	Leaf Area (Cm^2)
KK 1 + NYA-2	5.04 ± 1.11 ab	130.03 ± 1.31 a	19.80 ± 1.06 abcd	77.62 ± 1.27 ab
KK 1+ NAK-2 + NYA-2 + NSD	4.69 ± 1.12 ab	69.57 ± 1.12 ab	21.17 ± 1.08 abcd	36.17 ± 1.06 bc
KK 1 + NAK-2	4.25 ± 1.15 ab	84.27 ± 1.10 ab	17.17 ± 1.14 abcd	40.74 ± 1.08 bc
KK 1+ NAK-2 + NYA-2	4.12 ± 1.25 ab	76.84 ± 1.09 ab	22.70 ± 1.04 ab	37.87 ± 1.16 bc
KK 1 UNINOCULATED	3.90 ± 1.12 ab	80.45 ± 1.08 ab	21.14 ± 1.08 abcd	61.19 ± 1.06 abc
KK 1 + NSD	3.89 ± 1.12 ab	67.46 ± 1.10 ab	18.24 ± 1.09 abcd	42.49 ± 1.08 bc
KK 2 + NAK-2	3.33 ± 1.24 ab	75.38 ± 1.09 ab	20.38 ± 1.15 abcd	64.57 ± 1.18 abc
KK 2 UNINOCULATED	3.29 ± 1.20 ab	69.50 ± 1.12 ab	26.65 ± 1.03 a	53.50 ± 1.20 abc
KK 2+ NAK-2 + NYA-2	3.02 ± 1.24 ab	122.63 ± 1.24 a	17.51 ± 1.10 abcd	90.85 ± 1.22 ab
KK 2 + NYA-2	2.99 ± 1.28 ab	78.81 ± 1.14 ab	13.74 ± 1.08 d	64.32 ± 1.29 abc
KK 2 + NAK-2 + NYA-2 + NSD	2.79 ± 1.19 ab	74.74 ± 1.08 ab	19.32 ± 1.11 abcd	66.07 ± 1.12 abc
KK 2 + NSD	2.61 ± 1.18 ab	68.12 ± 1.10 ab	13.88 ± 1.09 cd	38.02 ± 1.23 bc
BANA + NAK-2	3.10 ± 1.23 ab	69.88 ± 1.12 ab	22.51 ± 1.08 abc	57.32 ± 1.23 abc
BANA + NYA-2	3.04 ± 1.30 ab	107.22 ± 1.32 ab	26.41 ± 1.08 a	132.32 ± 1.49 a
BANA + NSD	2.97 ± 1.31 ab	65.92 ± 1.14 ab	18.68 ± 1.07 abcd	28.95 ± 1.31 c
BANA + NAK-2 + NYA-2 + NSD	2.90 ± 1.26 ab	58.59 ± 1.11 b	27.10 ± 1.08 a	50.12 ± 1.18 abc
BANA + NAK-2 + NYA-2	2.38 ± 1.33 b	102.99 ± 1.14 ab	24.40 ± 1.11 a	58.43 ± 1.20 abc
BANA UNINOCULATED	2.29 ± 1.06 b	92.59 ± 1.09 ab	18.25 ± 1.16 abcd	54.95 ± 1.15 abc
16789 + NAK-2 + NYA-2 + NSD	6.89 ± 1.19 ab	66.70 ± 1.09 ab	14.22 ± 1.04 bcd	67.87 ± 1.09 abc
16789 + NAK-2 + NYA-2	6.89 ± 1.19 a	78.40 ± 1.06 ab	25.06 ± 1.08 a	46.77 ± 1.24 bc
16789 + NYA-2	4.56 ± 1.14 ab	125.92 ± 1.27 a	18.27 ± 1.05 abcd	68.92 ± 1.23 abc
16789 + NSD	3.80 ± 1.17 ab	67.79 ± 1.08 ab	19.74 ± 1.14 abcd	42.33 ± 1.23 bc
16789 + NAK-2	3.11 ± 1.18 ab	75.97 ± 1.07 ab	17.01 ± 1.15 abcd	62.61 ± 1.06 abc
16789 UNINOCULATED	2.67 ± 1.31 ab	112.10 ± 1.13 ab	26.85 ± 1.10 a	61.66 ± 1.16 abc
Test Values	**df = 23; F = 7.764; P ≤ 0.0001**	**df = 23; F = 2.823; P < 0.15**	**df = 23; F = 4.691; P ≤ 0.0001**	**df = 23; F = 3.100; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 30: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime

Treatment combinations	Total Fresh Weight (Yield in grams)	Total Stem Weight (Grams)	Total Leaf Weight (Grams)	Leaf Number
KK 1 UNINOCULATED	35.62 ± 1.08 ab	19.72 ± 1.09 ab	15.37 ± 1.10 ab	18.13 ± 1.07 ab
KK 1+ NAK-2 + NYA-2 + NSD	28.62 ± 1.14 ab	11.05 ± 1.16 ab	17.18 ± 1.15 ab	22.05 ± 1.11 ab
KK 1 + NAK-2	27.02 ± 1.18 ab	13.96 ± 1.24 ab	12.40 ± 1.11 b	18.98 ± 1.14 ab
KK 1+ NAK-2 + NYA-2	25.22 ± 1.21 ab	11.05 ± 1.16 ab	11.44 ± 1.43 b	21.54 ± 1.22 ab
KK 1 + NSD	23.26 ± 1.16 ab	11.64 ± 1.17 ab	11.24 ± 1.16 b	24.45 ± 1.11 ab
KK 1 + NYA-2	21.96 ± 1.17 ab	8.07 ± 1.38 ab	10.39 ± 1.20 b	15.12 ± 1.10 ab
KK 2 + NYA-2	34.15 ± 1.12 ab	18.41 ± 1.19 ab	14.13 ± 1.11 ab	18.48 ± 1.26 ab
KK 2 + NAK-2	29.97 ± 1.22 ab	13.91 ± 1.25 ab	15.61 ± 1.21 ab	18.13 ± 1.18 ab
KK 2 UNINOCULATED	22.05 ± 1.11 ab	10.12 ± 1.16 ab	11.79 ± 1.09 b	17.25 ± 1.15 ab
KK 2+ NAK-2 + NYA-2	18.69 ± 1.08 b	6.79 ± 1.21 b	10.76 ± 1.12 b	17.38 ± 1.17 ab
KK 2 + NAK-2 + NYA-2 + NSD	17.71 ± 1.29 b	8.51 ± 1.37 ab	8.68 ± 1.16 b	14.62 ± 1.17 b
KK 2 + NSD	15.61 ± 1.16 b	6.92 ± 1.17 b	8.61 ± 1.16 b	15.73 ± 1.17 b
BANA UNINOCULATED	36.31 ± 1.19 ab	22.73 ± 1.26 a	13.30 ± 1.09 b	12.26 ± 1.06 b
BANA + NAK-2	32.73 ± 1.18 ab	15.97 ± 1.25 ab	15.97 ± 1.11 ab	19.65 ± 1.19 ab
BANA + NAK-2 + NYA-2 + NSD	24.45 ± 1.20 ab	12.16 ± 1.26 ab	11.75 ± 1.16 b	16.53 ± 1.14 ab
BANA + NYA-2	20.57 ± 1.18 ab	7.36 ± 1.39 ab	11.53 ± 1.11 b	17.51 ± 1.25 ab
BANA + NSD	20.10 ± 1.09 ab	10.00 ± 1.12 ab	10.12 ± 1.06 b	20.26 ± 1.25 ab
BANA + NAK-2 + NYA-2	15.37 ± 1.23 b	7.33 ± 1.28 ab	7.27 ± 1.25 b	13.44 ± 1.32 b
16789 + NAK-2 + NYA-2	47.32 ± 1.14 a	13.59 ± 1.34 ab	30.67 ± 1.13 a	37.30 ± 1.09 a
16789 + NAK-2	32.48 ± 1.17 ab	15.73 ± 1.16 ab	16.66 ± 1.19 ab	16.85 ± 1.13 ab
16789 + NAK-2 + NYA-2 + NSD	28.40 ± 1.07 ab	12.40 ± 1.11 ab	15.55 ± 1.07 ab	15.91 ± 1.15 b
16789 UNINOCULATED	24.08 ± 1.44 ab	11.89 ± 1.48 ab	10.76 ± 1.49 b	14.96 ± 1.26 b
16789 + NYA-2	21.38 ± 1.08 ab	11.57 ± 1.10 ab	9.62 ± 1.06 b	22.22 ± 1.12 ab
16789 + NSD	17.85 ± 1.13 b	8.19 ± 1.20 ab	9.23 ± 1.07 b	17.78 ± 1.15 ab
Test Values	**df = 23; F = 3.034; P ≤ 0.0001**	**df = 23; F =2.380; P ≤ 0.0001**	**df = 23; F = 3.199; P ≤ 0.0001**	**df = 23; F = 2.058; P = 0.004**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 31: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime

Treatment combinations	Tiller Number	Tiller height (cm)	Chlorophyll content levels (SPAD UNITS)	Leaf Area (cm^2)
KK 1 + NAK-2	30.67 ± 1.20 ab	80.37 ± 1.05 ab	27.86 ± 1.13 abcd	49.74 ± 1.03 ab
KK 1 UNINOCULATED	25.51 ± 1.18 abc	60.60 ± 1.07 abc	28.73 ± 1.05 abc	42.33 ± 1.14 abcd
KK 1 + NSD	16.16 ± 1.09 bcdef	61.72 ± 1.06 abc	22.56 ± 1.16 abcde	41.69 ± 1.11 abcd
KK 1 + NYA-2	15.08 ± 1.08 cdefg	69.85 ± 1.08 ab	24.64 ± 1.05 abcde	90.09 ± 1.06 g
KK 1+ NAK-2 + NYA-2 + NSD	11.53 ± 1.08 defghi	63.09 ± 1.05 abc	25.02 ± 1.04 abcde	50.89 ± 1.09 ab
KK 1+ NAK-2 + NYA-2	8.00 ± 1.21 ghi	76.31 ± 1.07 ab	25.90 ± 1.19 abcde	34.94 ± 1.13 abcde
KK 2+ NAK-2 + NYA-2	22.73 ± 1.08 abcd	59.00 ± 1.05 abc	18.06 ± 1.05 e	43.15 ± 1.09 abcd
KK 2 + NYA-2	11.89 ± 1.18 defgh	62.41 ± 1.08 abc	22.65 ± 1.07 abcde	57.32 ± 1.11 a
KK 2 UNINOCULATED	10.59 ± 1.14 efghi	83.96 ± 1.04 a	21.22 ± 1.06 abcde	23.00 ± 1.17 def
KK 2 + NAK-2 + NYA-2 + NSD	10.59 ± 1.08 efghi	65.35 ± 1.03 abc	21.22 ± 1.05 abcde	48.42 ± 1.06 abc
KK 2 + NAK-2	6.33 ± 1.18 hi	65.95 ± 1.06 abc	18.41 ± 1.12 de	60.95 ± 1.14 a
KK 2 + NSD	5.84 ± 1.15 i	62.75 ± 1.09 abc	23.62 ± 1.06 abcde	50.70 ± 1.17 ab
BANA + NAK-2	32.21 ± 119 a	57.17 ± 1.07 bc	28.18 ± 1.05 abc	35.35 ± 1.08 abcde
BANA + NYA-2	12.98 ± 1.10 cdefg	59.38 ± 1.07 abc	27.12 ± 1.09 abcde	28.08 ± 1.20 bcdef
BANA + NAK-2 + NYA-2 + NSD	11.05 ± 1.14 efghi	58.71 ± 1.07 abc	26.30 ± 1.07 abcde	60.28 ± 1.16 fg
BANA + NSD	10.43 ± 1.07 efghi	59.24 ± 1.08 abc	27.75 ± 1.06 abcd	39.81 ± 1.05 abcd
BANA + NAK-2 + NYA-2	8.29 ± 1.07 fghi	61.56 ± 1.09 abc	26.40 ± 1.03 abcde	56.45 ± 1.10 a
BANA UNINOCULATED	8.25 ± 1.31 fghi	51.53 ± 1.17 c	31.26 ± 1.09 a	60.49 ± 1.30 a
16789 + NAK-2 + NYA-2	18.41 ± 1.10 abcde	65.41 ± 1.03 abc	21.30 ± 1.03 abcde	60.26 ± 1.08 a
16789 UNINOCULATED	13.96 ±1 .08 cdefg	56.51 ± 1.11 bc	30.32 ± 1.05 ab	43.99 ± 1.08 abcd
16789 + NAK-2	12.26 ± 1.14 defgh	63.98 ± 1.07 abc	27.65 ± 1.08 abcd	18.27 ± 1.18 ef
16789 + NSD	9.81 ±1.10 efghi	72.91 ± 1.08 abc	28.73 ± 1.08 abc	39.36 ± 1.20 abcd
16789 + NAK-2 + NYA-2 + NSD	8.84 ± 1.12 efghi	65.95 ± 1.04 abc	19.20 ± 1.04 cde	48.60 ± 1.09 abc
16789 + NYA-2	8.68 ± 1.13 fghi	78.87 ± 1.05 abc	20.26 ± 1.10 bcde	25.41 ± 1.17 cdef
Test Values	**df = 23; F = 8.938; P ≤ 0.0001**	**df=23;F=5.437; P ≤ 0.0001**	**df = 23; F = 5.019; P ≤ 0.0001**	**df=23 ; F = 5.183; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$.

APPENDIX 32: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under daily watering regime

Treatment combinations	Total Fresh Weight (Yield in grams)	Total Stem Weight (Grams)	Total Leaf Weight (Grams)	Leaf Number
KK 1 UNINOCULATED	61.66 ± 1.10 a	27 69 ± 1.14 a	33.37 ± 1.08 a	87.43 ± 1.12 ab
KK 1 + NAK-2	53.70 ± 1.09 ab	24.08 ± 1.13 a	28.95 ± 1.06 a	87.77 ± 1.11 ab
KK 1+ NAK-2 + NYA-2	46.77 ± 1.28 b	21.38 ± 1.39 ab	22.91 ± 1.23 b	40.27 ± 1.11 def
KK 1+ NAK-2 + NYA-2 + NSD	46.71 ± 1.13 b	12.16 ± 1.21 c	12.98 ± 1.16 c	45.88 ± 1.06 bcdef
KK 1 + NSD	42.17 ± 1.15 bc	17.25 ± 1.16 b	24.36 ± 1.16 ab	56.45 ± 1.12 bcde
KK 1 + NYA-2	34.41 ± 1.07 c	13.49 ± 1.11 c	20.73 ± 1.05 b	78.83 ± 1.19 abcd
KK 2 UNINOCULATED	53.91 ± 1.05 ab	21.79 ± 1.07 ab	31.74 ± 1.04 a	47.68 ± 1.12 bcdef
KK 2+ NAK-2 + NYA-2	38.61 ± 1.17 bc	12.02 ± 1.18 c	26.20 ± 1.17 ab	83.82 ± 1.13 abc
KK 2 + NAK-2 + NYA-2 + NSD	37.58 ± 1.07 bc	15.43 ± 1.08 bc	22.05 ± 1.07 b	46.24 ± 1.08 bcdef
KK 2 + NYA-2	33.88 ± 1.13 c	12.54 ± 1.16 c	21.05 ± 1.15 b	54.33 ± 1.20 bcde
KK 2 + NAK-2	27.32 ± 1.27 cd	12.74 ± 1.27 c	14.45 ± 1.27 c	29.97 ± 1.15 ef
KK 2 + NSD	24.17 ± 1.29 d	10.80 ± 1.29 c	13.18 ± 1.30 c	24.45 ± 1.17 f
BANA + NAK-2	53.50 ± 1.11 ab	20.57 ± 1.16 ab	32.48 ± 1.10 a	115.26 ± 1.16 a
BANA + NYA-2	46.24 ± 1.25 b	16.53 ± 1.31 b	29.29 ± 1.22 a	48.42 ± 1.19 bcde
BANA + NAK-2 + NYA-2 + NSD	31.99 ± 1.16 c	11.13 ± 1.13 c	20.57 ± 1.14 a	48.04 ± 1.18 bcdef
BANA + NSD	27.12 ± 1.09 cd	11.66 ± 1.09 c	15.31 ± 1.11 bc	45.19 ± 1.11 bcdef
BANA UNINOCULATED	26.92 ± 1.62 cd	12..64 ±1.67 c	12.64 ± 1.65 c	46.24 ± 1.29 bcdef
BANA + NAK-2 + NYA-2	26 30 ± 1.21 cd	12.35 ± 1.20 c	12.69 ± 1.30 c	41.85 ± 1.07 def
16789 + NSD	48.05 ± 1.47 ab	20.81 ± 1.51 ab	26.40 ± 1.45 ab	43.48 ± 1.12 cdef
16789 + NYA-2	44.16 ± 1.09 b	18.27 ± 1.13 b	25.61 ± 1.07 ab	57.32 ± 1.20 bcde
16789 + NAK-2 + NYA-2	41.05 ± 1.09 bc	16.09 ± 1.09 b	24.74 ± 1.09 ab	57.32 ± 1.06 bcde
16789 + NAK-2	39.66 ± 1.28 bc	15.14 ± 1.34 b	23.35 ± 1.27b	53.09 ± 1.06 bcde
16789 + NAK-2 + NYA-2 + NSD	37.44 ± 1.08 bc	15.14 ± 1.12 b	21.79 ± 1.06 b	40.74 ± 1.07 def
16789 UNINOCULATED	27.86 ± 1.11 cd	11.61 ± 1.13 c	15.79 ± 1.12 bc	63.83 ± 1.07 abcd
Test Values	**df = 23; F = 2.050; P ≤ 0.0001**	**df = 23; F =1.557; P ≤ 0.0001**	**df = 23; F = 2.693; P ≤ 0.0001**	**df = 23; F = 7.138; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 33: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime

Treatment combinations	Tiller Number	Tiller height (cm)	Chlorophyll content levels (SPAD UNITS)	Leaf Area (Cm^2)
KK 1+ NAK-2 + NYA-2	14.91 ± 1.18 a	44.29 ± 1.14 bc	18.41 ± 1.12 bcd	17.58 ± 1.28 cdef
KK 1 + NSD	14.79 ± 1.22 a	52.74 ± 1.07 abc	23.58 ± 1.09 abcd	12.40 ± 1.26 f
KK 1+ NAK-2 + NYA-2 + NSD	12.35 ± 1.16 ab	45.97 ± 1.13 bc	16.72 ± 1.12 cd	17.71 ± 1.26 cdef
KK 1 UNINOCULATED	10.39 ± 1.18 abc	43.30 ± 1.06 bc	24.27 ± 1.07 abcd	28.78 ± 1.80 abcdef
KK 1 + NAK-2	9.37 ± 1.12 abcdef	60.92 ± 1.05 ab	22.65 ± 1.13 abcd	12.74 ± 1.46 ef
KK 1 + NYA-2	8.51 ± 1.18 abcdef	48.11 ± 1.07 abc	23.80 ± 1.08 abcd	32.73 ± 1.19 abcde
KK 2 + NAK-2	14.40 ± 1.19 a	45.55 ± 1.03 bc	15.85 ± 1.07 b	30.90 ± 1.12 abcdef
KK 2 + NAK-2 + NYA-2 + NSD	9.73 ± 1.19 abcde	55.09 ± 1.05 ab	18.06 ± 1.05 bcd	35.35 ± 1.14 abcd
KK 2 + NYA-2	9.40 ± 1.14 abcde	57.98 ± 1.03 ab	19.28 ± 1.10 abcd	17.18 ± 1.27 def
KK 2+ NAK-2 + NYA-2	6.61 ± 1.15 bcdefg	58.41 ± 1.04 ab	17.85 ± 1.09 bcd	37.87 ± 1.14 abcd
KK 2 UNINOCULATED	5.47 ± 1.08 cdefg	59.84 ± 1.11 ab	24.17 ± 1.06 abcd	29.29 ± 1.18 abcdef
KK 2 + NSD	3.62 ± 1.15 g	52.26 ± 1.05 abc	19.35 ± 1.15 abcd	36.45 ± 1.13 abcd
BANA + NAK-2	8.91 ± 1.08 abcdef	54.10 ± 1.04 ab	24.93 ± 1.08 abc	31.50 ± 1.11 abcdef
BANA + NAK-2 + NYA-2	6.76 ± 1.09 bcefg	51.88 ± 1.03 abc	20.73 ± 1.11 abcd	15.37 ± 1.18 def
BANA + NSD	6.68 ± 1.09 bcdefg	50.30 ± 1.10 abc	23.71 ± 1.05 abcd	18.84 ± 1.08 cdef
BANA + NYA-2	5.52 ± 1.26 cdefg	46.31 ± 1.14 bc	29.17 ± 1.05 a	33.88 ± 1.17 abcd
BANA + NAK-2 + NYA-2 + NSD	4.79 ± 1.20 defg	41.25 ± 1.15 bc	27.12 ± 1.07 ab	24.36 ± 1.23 abcdef
BANA UNINOCULATED	4.50 ± 1.09 fg	69.46 ± 1.05 a	20.73 ± 1.05 abcd	22.30 ± 1.20 bcdef
16789 + NAK-2 + NYA-2	9.96 ± 1.15 abcd	36.46 ± 1.08 c	21.71 ± 1.11 abcd	21.05 ± 1.35 bcef
16789 + NSD	9.89 ± 1.17 abcde	52.11 ± 1.03 abc	18.41 ± 1.05 bcd	24.55 ± 1.09 abcdef
16789 + NYA-2	6.89 ± 1.11 bcdefg	57.45 ± 1.05 ab	20.89 ± 1.07 abcd	44.50 ± 1.11 abc
16789 + NAK-2 + NYA-2 + NSD	6.51 ± 1.15 bcdefg	54.31 ± 1.07 ab	17.58 ± 1.08 cd	48.79 ± 1.03 ab
16789 + NAK-2	5.75 ± 1.05 cdefg	59.70 ± 1.07 ab	21.46 ± 1.03 abcd	57.77 ± 1.06 a
16789 UNINOCULATED	4.75 ± 1.18 efg	60.31 ± 1.06 ab	22.56 ± 1.06 abcd	29.74 ± 1.24 abcdef
Test Values	**df = 23; F = 7.764; P ≤ 0.0001**	**df = 23; F = 3.823; P ≤ 0.0001**	**df = 23; F = 3.564; P ≤ 0.0001**	**df = 23; F = 5.057; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 34: Specific effects of pathogens co-infection under nitrogen & phosphorus deficiency and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Nitrogen & phosphorus deficiency effects on the performance of key selected growth parameters under weekly watering regime

Treatment combinations	Total Fresh Weight (Yield in grams)	Total Stem Weight (Grams)	Total Leaf Weight (Grams)	Leaf Number
KK 1 UNINOCULATED	16.91 ± 1.07 abcd	6.48 ± 1.12 abc	10.15 ± 1.05 abcd	35.08 ± 1.17 abcd
KK 1+ NAK-2 + NYA-2	16.72 ± 1.09 abcd	6.94 ± 1.13 ab	9.55 ± 1.09 abcd	46.06 ± 1.12 ab
KK 1+ NAK-2 + NYA-2 + NSD	16.28 ± 1.11 abcd	5.15 ± 1.16 abc	10.88 ± 1.09 ab	53.09 ± 1.19 a
KK 1 + NAK-2	15.55 ± 1.10 abcd	5.35 ± 1.16 abc	10.00 ± 1.09 abcd	41.53 ± 1.09 abc
KK 1 + NSD	13.65 ± 1.04 abcd	4.43 ± 1.09 abc	9.19 ± 1.05 abcd	54.33 ± 1.20 a
KK 1 + NYA-2	11.26 ± 1.22 cd	5.45 ± 1.17 abc	5.69 ± 1.29 cde	35.89 ± 1.16 abcd
KK 2 + NAK-2 + NYA-2 + NSD	21.38 ± 1.08 a	8.35 ± 1.12 a	12.83 ± 1.04 a	37.58 ± 1.13 abcd
KK 2 + NAK-2	17.58 ± 1.08 abcd	6.10 ± 1.08 abc	11.09 ± 1.11 ab	42.01 ± 1.14 abc
KK 2 UNINOCULATED	15.02 ± 1.11 abcd	6.53 ± 1.14 abc	8.38 ± 1.08 abcde	21.88 ± 1.06 de
KK 2 + NYA-2	14.02 ± 1.09 abcd	4.32 ± 1.25 abc	9.02 ± 1.08 abcd	41.85 ± 1.12 abc
KK 2 + NSD	13.80 ± 1.12 abcd	4.47 ± 1.18 abc	9.19 ± 1.10 abcd	16.92 ± 1.10 e
KK 2+ NAK-2 + NYA-2	13.28 ± 1.07 abcd	6.73 ± 1.14 abc	5.62 ± 1.17 de	24.45 ± 1.16 cde
BANA + NAK-2	20.57 ± 1.03 ab	6.92 ± 1.06 ab	13.70 ± 1.03 a	34.81 ± 1.06 abcd
BANA + NAK-2 + NYA-2	17.25 ± 1.19 abcd	4.94 ± 1.28 abc	12.02 ± 1.18 a	31.87 ± 1.10 abcd
BANA UNINOCULATED	16 18 ± 1.06 abcd	6.14 ± 1.14 abc	10.55 ± 1.04 abc	21.71 ± 1.09 de
BANA + NSD	13.54 ± 1.08 abcd	4.40 ± 1.19 abc	8.74 ± 1.03 abcde	31.14 ± 1.09 abcd
BANA + NAK-2 + NYA-2 + NSD	9.89 ± 1.14 d	3.30 ± 1.21 bc	6.41 ± 1.13 bcde	21.79 ± 1.10 de
BANA + NYA-2	9.66 ± 1.08 d	3.66 ± 1.27 abc	4.73 ± 1.15 e	28.73 ± 1.16 bcde
16789 + NAK-2	19.88 ± 1.05 abc	7.47 ± 1.07 ab	12.26 ± 1.05 a	22.13 ± 1.03 de
16789 UNINOCULATED	17.18 ± 1.19 abcd	7.67 ± 1.21 ab	9.55 ± 1.18 abcd	22.05 ± 1.05 de
16789 + NAK-2 + NYA-2 + NSD	17.11 ± 1.06 abcd	5.45 ± 1.11 abc	11.48 ± 1.05 ab	28.62 ± 1.12 bcde
16789 + NYA-2	14.45 ± 1.20 abcd	6.41 ± 1.25 abc	7.61 ± 1.20 abcde	27.02 ± 1.08 bcde
16789 + NSD	13.34 ± 1.08 abcd	4.45 ± 1.19 abc	8.38 ± 1.07 abcde	40.74 ± 1.05 abc
16789 + NAK-2 + NYA-2	11.57 ± 1.27 bcd	2.93 ± 1.31 c	8.58 ± 1.26 abcde	38.17 ± 1.08 abcd
Test Values	**df = 23; F = 3.308; P ≤ 0.0001**	**df = 23; F =2.795; P ≤ 0.0001**	**df = 23; F = 5.147; P ≤ 0.0001**	**df = 23; F = 7.498; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 35: Specific effects of pathogens co-infection under complete nutrient solution and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Complete nutrients solution effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1+ NAK-2 + NYA-2 + NSD	10.05 ± 1.23 ab	134.17 ± 1.05 bcd	44.95 ± 1.08 abc	42.17 ± 1.12 f
KK 1 + NYA-2	6.78 ± 1.29 abcd	155.96 ± 1.04 abcd	48.13 ± 1.07 abc	42.82 ± 1.16 f
KK 1 UNINOCULATED	6.66 ± 1.17 abcd	150.36 ± 1.03 abcd	47.41 ± 1.12 abc	59.80 ± 1.18 def
KK 1 + NAK-2	6.34 ± 1.10 abcd	157.70 ± 1.04 abcd	47.07 ± 1.03 abc	68.92 ± 1.12 cdef
KK 1 + NSD	6.23 ± 1.22 abcd	136.35 ± 1.10 bcd	49.14 ± 1.12 abc	139.64 ± 1.05 ab
KK 1+ NAK-2 + NYA-2	4.02 ± 1.21 cd	169.80 ± 1.02 ab	48.70 ± 1.05 abc	82.54 ± 1.06 bcde
KK 2 + NYA-2	9.76 ± 1.18 ab	151.18 ± 1.05 abcd	49.97 ± 1.12 abc	53.50 ± 1.09 ef
KK 2 + NAK-2	9.54 ± 1.15 ab	136.40 ± 1.14 bcd	55.34 ± 1.07 ab	163.43 ± 1.14 a
KK 2 + NSD	7.53 ± 1.13 abc	119.12 ± 1.07 d	46.06 ± 1.13 abc	57.32 ± 1.16 ef
KK 2 UNINOCULATED	7.50 ± 1.17 abc	161.48 ± 1.03 abcd	42.35 ± 1.05 abc	40.89 ± 1.10 f
KK 2 + NAK-2 + NYA-2 + NSD	6.02 ± 1.15 bcd	151.14 ± 1.06 abcd	35.57 ± 1.06 c	54.95 ± 1.13 ef
KK 2+ NAK-2 + NYA-2	5.96 ± 1.14 bcd	152.16 ± 1.05 abcd	45.86 ± 1.10 abc	53.91 ± 1.10 ef
BANA + NSD	9.39 ± 1.19 abc	149.60 ± 1.07 abcd	42.53 ± 1.03 abc	113.50 ± 1.05 abcd
BANA + NAK-2 + NYA-2 + NSD	7.50 ± 1.07 abc	160.88 ± 1.01 abc	42.15 ± 1.03 abc	38.90 ± 1.07 f
BANA UNINOCULATED	6.86 ± 1.18 abcd	183.39 ± 1.04 a	49.41 ± 1.09 abc	60.49 ± 1.15 def
BANA + NYA-2	6.30 ± 1.24 abcd	149.90 ± 1.05 abcd	52.82 ± 1.09 abc	68.13 ± 1.17 def
BANA + NAK-2 + NYA-2	5.25 ± 1.25 bcd	130.64 ± 1.03 bcd	51.88 ± 1.05 abc	62.85 ± 1.22 def
BANA + NAK-2	3.09 ± 1.07 d	169.56 ± 1.03 abc	59.28 ± 1.08 a	44.98 ± 1.13 ef
16789 UNINOCULATED	14.62 ± 1.15 a	151.26 ± 1.03 abcd	38.75 ± 1.05 bc	53.29 ± 1.15 ef
16789 + NAK-2 + NYA-2 + NSD	9.74 ± 1.19 ab	127.17 ± 1.09 cd	43.34 ± 1.05 abc	130.82 ± 1.08 abc
16789 + NAK-2	7.85 ± 1.17 abc	133.65 ± 1.08 bcd	53.19 ± 1.10 ab	55.59 ± 1.17 ef
16789 + NAK-2 + NYA-2	7.08 ± 1.21 abcd	147.74 ± 1.04 abcd	38.05 ± 1.06 bc	43.65 ± 1.12 ef
16789 + NSD	6.11 ± 1.14 bcd	127.04 ± 1.03 bcd	47.73 ± 1.08 abc	53.29 ± 1.15 ef
Test Values	**df = 23 ; F = 3.654; P = 0.004**	**df = 23 ; F=3.564; P ≤ 0.0001**	**df = 23; F = 2.459; P ≤ 0.0001**	**df =23; F = 10.329; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 36: Specific effects of pathogens co-infection under complete nutrient solution and daily watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Complete Nutrient solution effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NAK-2	380.19 ± 1.17 abc	220.46 ± 1.17 ab	156.08 ± 1.18 abc	42.66 ± 1.05 bcd
KK 1 + NYA-2	329.86 ± 1.32 abc	222.16 ± 1.32 ab	96.61 ± 1.42 abc	52.28 ± 1.28 abcd
KK 1 UNINOCULATED	319.89 ± 1.18 abc	201.84 ± 1.17 abc	113.07 ± 1.22 abc	49.55 ± 1.15 abcd
KK 1 + NSD	318.66 ± 1.12 abc	156.08 ± 1.24 abcd	125.89 ± 1.23 abc	47.86 ± 1.15 bcd
KK 1+ NAK-2 + NYA-2 + NSD	254.10 ± 1.25 abc	160.32 ± 1.24 abcd	86.43 ± 1.31 abc	62.85 ± 1.16 d
KK 1+ NAK-2 + NYA-2	228.21 ± 1.07 abc	162.18 ± 1.08 abcd	58.21 ± 1.17 c	27.07 ± 1.11 ab
KK 2 + NAK-2	518.81 ± 1.07 a	288.40 ± 1.10 a	221.31 ± 1.08 a	59.11 ± 1.15 abc
KK 2 + NYA-2	413.68 ± 1.14 ab	223.01 ± 1.14 ab	189.82 ± 1.14 ab	60.49 ± 1.06 abc
KK 2 UNINOCULATED	386.07 ± 1.15 abc	239.88 ± 1.17 ab	136.98 ± 1.18 abc	59.80 ± 1.13 abc
KK 2+ NAK-2 + NYA-2	250.23 ± 1.17 abc	139.64 ± 1.17 abcd	109.65 ± 1.17 abc	38.61 ± 1.09 bcd
KK 2 + NAK-2 + NYA-2 + NSD	187.64 ± 1.11 bc	111.34 ± 1.11 abcd	74.42 ± 1.14 bc	46.95 ± 1.12 bcd
KK 2 + NSD	177.15 ± 1.21 bc	89.81 ± 1.22 bcd	84.14 ± 1.22 abc	51.68 ± 1.13 abcd
BANA + NAK-2 + NYA-2	361.69 ± 1.27 abc	198.00 ± 1.27 abc	159.71 ± 1.28 abc	46.24 ± 1.19 bcd
BANA UNINOCULATED	331.13 ± 1.29 abc	204.96 ± 1.28 abc	120.23 ± 1.31 abc	50.70 ± 1.22 abcd
BANA + NSD	291.74 ± 1.20 abc	179.20 ± 1.24 abcd	106.33 ± 1.17 abc	62.37 ± 1.20 ab
BANA + NYA-2	220.46 ± 1.08 abc	126.86 ± 1.11 abcd	81.44 ± 1.20 abc	40.74 ± 1.14 bcd
BANA + NAK-2 + NYA-2 + NSD	175.79 ± 1.12 bc	114.38 ± 1.10 abcd	56.89 ± 1.22 c	45.10 ± 1.05 bcd
BANA + NAK-2	173.78 ± 1.14 bc	105.52 ± 1.15 bcd	65.56 ± 1.16 c	27.02 ± 1.08 d
16789 UNINOCULATED	305.49 ± 1.30 bc	147.34 ± 1.28 abcd	159.10 ± 1.31 abc	95.50 ± 1.15 a
16789 + NYA-2	236.23 ± 1.13 abc	128.33 ± 1.12 abcd	107.56 ± 1.16 abc	53.09 ± 1.07 abcd
16789 + NAK-2 + NYA-2	194.24 ± 1.25 bc	94.41 ± 1.27 bcd	97.35 ± 1.25 abc	35.08 ± 1.10 bcd
16789 + NAK-2 + NYA-2 + NSD	188.36 ± 1.11 abc	80.79 ± 1.17 cd	101.55 ± 1.11 abc	58.88 ± 1.14 abc
16789 + NAK-2	171.79 ± 1.34 bc	72.72 ± 1.47 d	86.76 ± 1.32 abc	57.32 ± 1.15 abc
16789 + NSD	159.71 ± 1.08 c	78.22 ± 1.16 cd	77.92 ± 1.05 bc	30.90 ± 1.06 cd
Test Values	**df = 23; F = 3.625; P ≤ 0.0001**	**df = 23; F = 4.204; P ≤ 0.0001**	**df = 23; F = 3.176; P ≤ 0.0001**	**df = 23; F = 4.852; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 37: Specific effects of pathogens co-infection under complete nutrient solution and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Complete nutrients solution effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NYA-2	7.33 ± 1.17 ab	115.61 ± 1.04 cd	39.18 ± 1.05 ab	44.67 ± 1.14 h
KK 1 + NSD	6.46 ± 1.24 ab	105.32 ± 1.04 cd	32.10 ± 1.13 abc	55.80 ± 1.17 gh
KK 1+ NAK-2 + NYA-2 + NSD	5.88 ± 1.16 ab	96.50 ± 1.04 cd	41.02 ± 1.09 ab	56.23 ± 1.10 gh
KK 1+ NAK-2 + NYA-2	4.72 ± 1.29 ab	103.71 ± 1.10 cd	35.59 ± 1.10 abc	97.35 ± 1.10 cdefgh
KK 1 UNINOCULATED	4.71 ± 1.31 ab	130.87 ± 1.06 cd	36.04 ± 1.03 abc	136.46 ± 1.06 bcde
KK 1 + NAK-2	3.54 ± 1.23 b	131.72 ± 1.08 c	36.16 ± 1.07 abc	66.58 ± 1.08 defgh
KK 2 UNINOCULATED	7.45 ± 1.20 ab	125.83 ± 1.06 cd	32.62 ± 1.05 abc	112.63 ± 1.05 bcdefg
KK 2 + NAK-2	6.82 ± 1.25 ab	109.73 ± 1.08 cd	30.62 ± 1.06 bc	149.62 ± 1.09 abc
KK 2 + NYA-2	6.15 ± 1.22 ab	73.72 ± 1.19 d	36.70 ± 1.04 ab	58.32 ± 1.08 gh
KK 2+ NAK-2 + NYA-2	5.94 ± 1.09 ab	135.21 ± 1.12 c	36.33 ± 1.13 abc	149.62 ± 1.21 abc
KK 2 + NSD	4.68 ± 1.21 ab	114.84 ± 1.05 cd	29.13 ± 1.08 bc	80.66 ± 1.15 cdefgh
KK 2 + NAK-2 + NYA-2 + NSD	3.95 ± 1.14 b	122.83 ± 1.05 cd	37.60 ± 1.04 ab	59.80 ± 1.15 fgh
BANA + NAK-2	7.56 ± 1.09 ab	113.72 ± 1.12 cd	50.40 ± 1.16 a	130.82 ± 1.18 bcdef
BANA + NAK-2 + NYA-2	5.37 ± 1.07 ab	261.27 ± 1.31 a	36.37 ± 1.06 ab	238.96 ± 1.28 ab
BANA + NAK-2 + NYA-2 + NSD	5.36 ± 1.29 ab	95.64 ± 1.17 cd	38.47 ± 1.08 ab	59.57 ± 1.37 fgh
BANA + NSD	3.92 ± 1.18 b	146.99 ± 1.14 abc	43.23 ± 1.09 ab	113.50 ± 1.25 bcdefg
BANA UNINOCULATED	3.45 ± 1.15 b	129.64 ± 1.03 cd	32.60 ± 1.07 abc	67.61 ± 1.17 defgh
BANA + NYA-2	2.91 ± 1.19 b	131.61 ± 1.07 c	40.60 ± 1.06 ab	64.07 ± 1.08 efgh
16789 + NYA-2	11.87 ± 1.14 a	137.86 ± 1.11 bc	32.38 ± 1.18 abc	115.70 ± 1.14 bcdefg
16789 UNINOCULATED	6.94 ± 1.20 ab	113.25 ± 1.10 cd	31.37 ± 1.07 bc	114.38 ± 1.08 bcdefg
16789 + NSD	6.13 ± 1.19 ab	242.64 ± 1.25 ab	32.75 ± 1.09 abc	312.61 ± 1.29 a
16789 + NAK-2	5.50 ± 1.22 ab	127.49 ± 1.07 cd	22.94 ± 1.19 c	145.10 ± 1.07 abcd
16789 + NAK-2 + NYA-2	5.26 ± 1.29 ab	130.70 ± 1.05 cd	34.79 ± 1.03 abc	74.42 ± 1.14 cdefgh
16789 + NAK-2 + NYA-2 + NSD	4.75 ± 1.26 ab	127.33 ± 1.04 cd	34.88 ± 1.02 abc	93.68 ± 1.12 cdefgh
Test Values	**df = 23; F = 2.684; P ≤ 0.0001**	**df = 23; F = 5.559; P ≤ 0.0001**	**df = 23; F = 2.930; P ≤ 0.0001**	**df = 23 ; F = 10.325; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 38: Specific effects of pathogens co-infection under complete nutrient solution and weekly watering on selected growth parameters as at ratoons/crops 1&2 cycles combined; showing their means ± standard errors.

Ratoons 1&2; Complete nutrients solution effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 UNINOCULATED	158.49 ± 1.07 abc	94.41 ± 1.10 ab	61.42 ± 1.08 abc	35.21 ± 1.22 abcd
KK 1 + NAK-2	118.85 ± 1.12 abc	62.85 ± 1.19 abc	50.50 ± 1.13 abcd	19.50 ± 1.14 d
KK 1+ NAK-2 + NYA-2	115.70 ± 1.07 abc	61.66 ± 1.13 abc	51.09 ± 1.03 abcd	38.31 ± 1.19 abcd
KK 1 + NSD	110.92 ± 1.06 abc	63.10 ± 1.06 abc	46.24 ± 1.11 abcd	35.75 ± 1.15 abcd
KK 1 + NYA-2	94.77 ± 1.05 abc	43.15 ± 1.10 abc	49.74 ± 1.05 abcd	45.36 ± 1.13 ab
KK 1+ NAK-2 + NYA-2 + NSD	71.61 ± 1.02 bc	37.97 ± 1.04 bc	33.37 ± 1.03 bcd	29.51 ± 1.12 abcd
KK 2+ NAK-2 + NYA-2	255.07 ± 1.25 a	138.57 ± 1.27 a	116.14 ± 1.23 a	43.48 ± 1.06 abc
KK 2 UNINOCULATED	179.20 ± 1.09 ab	93.33 ± 1.07 ab	83.82 ± 1.12 ab	44.50 ± 1.13 abc
KK 2 + NYA-2	153.11 ± 1.06 abc	76.74 ± 1.12 abc	74.42 ± 1.04 abc	36.45 ± 1.08 abcd
KK 2 + NAK-2	111.77 ± 1.20 abc	53.29 ± 1.24 abc	57.99 ± 1.17 abcd	45.88 ± 1.20 ab
KK 2 + NSD	91.90 ± 1.06 abc	47.13 ± 1.08 abc	44.16 ± 1.06 abcd	26.71 ± 1.09 bcd
KK 2 + NAK-2 + NYA-2 + NSD	88.10 ± 1.13 abc	48.79 ± 1.18 abc	34.94 ± 1.18 bcd	25.31 ± 1.17 bcd
BANA + NSD	203.39 ± 1.47 ab	134.38 ± 1.54 a	56.67 ± 1.35 abcd	29.97 ± 1.24 abcd
BANA + NAK-2	202.61 ± 1.33 ab	108.81 ± 1.36 ab	83.82 ± 1.35 ab	40.27 ± 1.12 abcd
BANA + NAK-2 + NYA-2	128.33 ± 1.26 abc	85.77 ± 1.30 abc	36.73 ± 1.25 bcd	32.36 ± 1.15 abcd
BANA UNINOCULATED	103.12 ± 1.13 abc	45.19 ± 1.19 abc	55.38 ± 1.11 abcd	26.40 ± 1.12 bcd
BANA + NYA-2	96.61 ± 1.14 abc	50.19 ± 1.15 abc	41.85 ± 1.22 abcd	20.50 ± 1.15 cd
BANA + NAK-2 + NYA-2 + NSD	58.88 ± 1.79 c	37.30 ± 1.83 bc	20.57 ± 1.76 d	37.87 ± 1.39 abcd
16789 + NYA-2	205.75 ± 1.35 ab	95.87 ± 1.40 ab	104.31 ± 1.31 a	60.72 ± 1.14 a
16789 + NAK-2	146.78 ± 1.02 abc	78.83 ± 1.04 abc	67.87 ± 1.02 abc	43.48 ± 1.14 abc
16789 UNINOCULATED	116.14 ± 1.14 abc	55.80 ± 1.20 abc	56.23 ± 1.13 abcd	42.99 ± 1.13 abc
16789 + NAK-2 + NYA-2	102.33 ± 1.04 abc	50.31 ± 1.02 abc	52.08 ± 1.06 abcd	26.61 ± 1.11 bcd
16789 + NAK-2 + NYA-2 + NSD	100.77 ± 1.05 abc	54.33 ± 1.06 abc	46.24 ± 1.05 abcd	33.24 ± 1.18 abcd
16789 + NSD	57.99 ± 1.51 c	26.61 ± 1.66 c	27.75 ± 1.40 cd	42.66 ± 1.12 abcd
Test Values	**df = 23; F =3.320; P ≤ 0.0001**	**df = 23; F= 3.006; P ≤ 0.0001**	**df= 23; F = 3.996; P ≤ 0.0001**	**df= 23; F= 3.927; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 39: Specific effects of pathogens co-infection under complete nutrient solution and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Complete nutrients solution effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NAK-2	26.61 ± 1.13 ab	123.43 ± 1.09 abc	44.84 ± 1.14 ab	56.02 ± 1.09 abcd
KK 1+ NAK-2 + NYA-2 + NSD	21.46 ± 1.09 abcd	104.88 ± 1.08 abc	35.62 ± 1.06 ab	35.21 ± 1.17 d
KK 1 + NYA-2	20.26 ± 1.19 abcd	115.93 ± 1.07 abc	39.36 ± 1.10 ab	39.05 ± 1.10 bcd
KK 1 UNINOCULATED	19.35 ± 1.07 abcd	118.65 ± 1.08 abc	45.36 ± 1.09 ab	39.05 ± 1.10 bcd
KK 1 + NSD	18.27 ± 1.12 abcd	92.85 ± 1.11 c	37.15 ± 1.07 ab	68.92 ± 1.22 ab
KK 1+ NAK-2 + NYA-2	13.44 ± 1.16 cd	106.28 ± 1.09 abc	53.91 ± 1.37 a	40.12 ± 1.06 bcd
KK 2 + NYA-2	24.27 ± 1.23 abc	107.33 ± 1.07 abc	29.40 ± 1.11 b	33.50 ± 1.20 d
KK 2 UNINOCULATED	20.34 ± 1.07 abcd	122.98 ± 1.07 abc	45.36 ± 1.10 ab	41.37 ± 1.12 bcd
KK 2 + NSD	18.20 ± 1.10 abcd	109.64 ± 1.06 abc	46.77 ± 1.06 ab	50.70 ± 1.02 abcd
KK 2+ NAK-2 + NYA-2	16.79 ± 1.11 abcd	128.59 ± 1.06 abc	38.76 ± 1.06 ab	39.66 ± 1.08 bcd
KK 2 + NAK-2	14.85 ± 1.09 bcd	113.13 ± 1.09 abc	42.33 ± 1.11 ab	57.99 ± 1.15 abcd
KK 2 + NAK-2 + NYA-2 + NSD	12.74 ± 1.09 d	125.30 ± 1.06 abc	36.31 ± 1.07 ab	58.66 ± 1.10 abcd
BANA + NSD	29.85 ± 1.03 a	100.30 ± 1.08 bc	42.66 ± 1.03 ab	57.32 ± 1.08 abcd
BANA + NAK-2 + NYA-2	17.92 ± 1.14 abcd	92.37 ± 1.06 c	33.88 ± 1.17 b	49.55 ± 1.09 abcd
BANA + NYA-2	14.57 ± 1.11 bcd	93.81 ± 1.10 c	47.13 ± 1.05 ab	38.31 ± 1.12 bcd
BANA + NAK-2 + NYA-2 + NSD	13.65 ± 1.05 cd	111.76 ± 1.06 abc	46.59 ± 1.06 ab	47.68 ± 1.07 abcd
BANA + NAK-2	12.49 ± 1.25 d	142.89 ± 1.04 ab	36.17 ± 1.11 ab	44.50 ± 1.14 abcd
BANA UNINOCULATED	12.26 ± 1.17 d	128.63 ± 1.07 abc	30.08 ± 1.09 b	49.17 ± 1.11 abcd
16789 UNINOCULATED	18.13 ± 1.07 abcd	114.64 ± 1.06 bc	35.62 ± 1.05 ab	45.71 ± 1.08 abcd
16789 + NAK-2 + NYA-2	17.51 ± 1.11 abcd	108.62 ± 1.09 abc	45.01 ± 1.09 ab	36.87 ± 1.14 cd
16789 + NAK-2	17.44 ± 1.07 abcd	151.63 ± 1.04 a	47.32 ± 1.04 ab	40.58 ± 1.07 bcd
16789 + NAK-2 + NYA-2 + NSD	16.03 ± 1.10 bcd	98.18 ± 1.11 abc	41.37 ± 1.04 ab	77.03 ± 1.18 a
16789 + NSD	15.55 ± 1.16 bcd	107.16 ± 1.06 abc	39.51 ± 1.07 ab	44.16 ± 1.15 abcd
16789 + NYA-2	15.08 ± 1.10 bcd	125.50 ± 1.06 abc	42.82 ± 1.13 ab	65.56 ± 1.12 abc
Test Values	**df = 23 ; F = 3.810; P = 0.004**	**df = 23 ; F=3.137 ; P ≤ 0.0001**	**df = 23; F = 4.744; P ≤ 0.0001**	**df =23; F = 3.694; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 40: Specific effects of pathogens co-infection under complete nutrient solution and daily watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Complete Nutrient solution effects on the performance of key selected growth parameters under daily watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1 + NAK-2	333.68 ± 1.15 a	167.88 ± 1.19 a	163.42 ± 1.12 a	81.60 ± 1.09 abc
KK 1 + NYA-2	229.09 ± 1.20 abcde	115.70 ± 1.21 abc	112.63 ± 1.20 abcde	121.15 ± 1.16 ab
KK 1 UNINOCULATED	220.46 ± 1.20 abcde	115.70 ± 1.22 abc	104.31 ± 1.18 abcde	109.23 ± 1.07 ab
KK 1+ NAK-2 + NYA-2 + NSD	183.37 ± 1.13 abcde	90.85 ± 1.15 abc	92.61 ± 1.12 abcde	120.69 ± 1.06 ab
KK 1+ NAK-2 + NYA-2	141.25 ± 1.16 bcde	71.34 ± 1.19 abc	69.98 ± 1.13 cdef	45.36 ± 1.11 e
KK 1 + NSD	124.45 ± 1.16 cde	57.32 ± 1.21 bc	65.56 ± 1.12 cdef	83.18 ± 1.09 abc
KK 2 + NAK-2	293.99 ± 1.15 ab	156.08 ± 1.17 a	134.90 ± 1.14 abc	78.83 ± 1.11 bcd
KK 2+ NAK-2 + NYA-2	246.41 ± 1.16 abcd	128.83 ± 1.19 abc	115.70 ± 1.15 abcde	100.38 ± 1.07 ab
KK 2 UNINOCULATED	228.21 ± 1.19 abcde	126.86 ± 1.23 abc	95.13 ± 1.19 abcde	111.77 ± 1.05 ab
KK 2 + NYA-2	220.46 ± 1.15 abcde	106.33 ± 1.18 abc	113.94 ± 1.13 abcde	129.82 ± 1.11 a
KK 2 + NAK-2 + NYA-2 + NSD	189.09 ± 1.16 abcde	87.10 ± 1.21 abc	100.38 ± 1.12 abcde	50.31 ± 1.06 de
KK 2 + NSD	173.11 ± 1.16 abcde	77.03 ± 1.20 abc	94.41 ± 1.14 abcde	102.72 ± 1.07 ab
BANA + NAK-2	258.03 ± 1.24 abc	139.10 ± 1.24 ab	116.59 ± 1.25 abcd	80.35 ± 1.22 abcd
BANA UNINOCULATED	208.13 ± 1.19 abcde	112.20 ± 1.18 abc	92.61 ± 1.21 abcde	83.82 ± 1.17 abc
BANA + NAK-2 + NYA-2 + NSD	137.51 ± 1.13 bcde	56.89 ± 1.18 bc	79.43 ± 1.10 abcdef	43.32 ± 1.06 e
BANA + NYA-2	135.42 ± 1.17 bcde	56.89 ± 1.18 bc	77.33 ± 1.13 bcdef	53.09 ± 1.10 cde
BANA + NAK-2 + NYA-2	133.86 ± 1.19 bcde	54.95 ± 1.21 bc	78.52 ± 1.17 abcdef	80.97 ± 1.11 abcd
BANA + NSD	105.52 ± 1.10 e	52.28 ± 1.22 c	43.15 ± 1.11 f	107.56 ± 1.04 ab
16789 + NAK-2	323.59 ± 1.13 a	172.45 ± 1.14 a	147.91 ± 1.10 ab	105.52 ± 1.03 cde
16789 + NAK-2 + NYA-2	218.78 ± 1.18 abcde	112.20 ± 1.20 abc	106.33 ± 1.17 abcde	101.94 ± 1.09 ab
16789 UNINOCULATED	176.47 ± 1.12 abcde	88.78 ± 1.12 abc	87.43 ± 1.12 abcdef	104.31 ± 1.03 ab
16789 + NYA-2	167.24 ± 1.11 abcde	85.77 ± 1.18 abc	70.52 ± 1.17 bcdef	52.28 ± 1.08 ab
16789 + NAK-2 + NYA-2 + NSD	139.64 ± 1.25 bcde	69.18 ± 1.38 abc	54.95 ± 1.26 ef	82.54 ± 1.08 abc
16789 + NSD	117.04 ± 1.06 de	58.43 ± 1.08 bc	58.21 ± 1.04 def	61.19 ± 1.07 cde
Test Values	**df = 23; F = 4.535; P ≤ 0.0001**	**df = 23; F = 4.310; P ≤ 0.0001**	**df = 23; F = 4.896; P ≤ 0.0001**	**df = 23 ; F = 12.600; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 41: Specific effects of pathogens co-infection under complete nutrient solution and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Complete nutrients solution effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Tiller Number**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**	**Leaf Area (Cm^2)**
KK 1 + NSD	37.73 ± 1.41 a	73.12 ± 1.08 abc	28.73 ± 1.10 ab	31.26 ± 1.10 ef
KK 1 UNINOCULATED	19.35 ± 1.12 abc	86.65 ± 1.03 ab	29.63 ± 1.09 ab	55.80 ± 1.13 abcde
KK 1+ NAK-2 + NYA-2	18.98 ± 1.06 abcd	78.52 ± 1.04 abc	35.75 ± 1.04 ab	54.74 ± 1.05 abcde
KK 1+ NAK-2 + NYA-2 + NSD	18.20 ± 1.08 bcd	90.15 ± 1.09 ab	35.21 ± 1.07 ab	38.31 ± 1.09 cdef
KK 1 + NAK-2	15.14 ± 1.10 bcde	97.20 ± 1.06 a	30.67 ± 1.04 ab	37.58 ± 1.07 cdef
KK 1 + NYA-2	15.08 ± 1.06 bcde	86.64 ± 1.12 ab	32.24 ± 1.15 ab	35.21 ± 1.12 def
KK 2 + NYA-2	20.50 ± 1.10 ab	88.63 ± 1.08 ab	28.29 ± 1.08 ab	42.17 ± 1.06 bcdef
KK 2 UNINOCULATED	16.79 ± 1.12 bcd	84.74 ± 1.08 ab	28.51 ± 1.03 ab	69.98 ± 1.14 abc
KK 2+ NAK-2 + NYA-2	11.89 ± 1.11 bcde	76.40 ± 1.08 abc	28.84 ± 1.07 ab	41.53 ± 1.21 cdef
KK 2 + NAK-2	11.44 ± 1.14 bcde	101.14 ± 1.07 a	37.58 ± 1.08 a	85.11 ± 1.14 a
KK 2 + NAK-2 + NYA-2 + NSD	9.44 ± 1.09 de	89.00 ± 1.07 ab	31.38 ± 1.03 ab	55.17 ± 1.13 abcde
KK 2 + NSD	7.61 ± 1.15 e	90.11 ± 1.02 ab	31.62 ± 1.05 ab	48.98 ± 1.07 abcdef
BANA UNINOCULATED	16.60 ± 1.28 bcd	91.39 ± 1.06 ab	28.73 ± 1.07 ab	27.02 ± 1.17 f
BANA + NAK-2	11.70 ± 1.17 bcde	53.87 ± 1.09 c	32.24 ± 1.13 ab	42.33 ± 1.06 bcdef
BANA + NAK-2 + NYA-2	11.39 ± 1.04 bcde	72.11 ± 1.07 abc	24.83 ± 1.12 ab	51.09 ± 1.11 abcde
BANA + NYA-2	10.51 ± 1.08 bcde	79.54 ± 1.11 abc	34.28 ± 1.08 ab	39.51 ± 1.04 cdef
BANA + NAK-2 + NYA-2 + NSD	10.12 ± 1.11 cde	96.59 ± 1.11 a	37.58 ± 1.04 a	60.26 ± 1.14 abcd
BANA + NSD	10.12 ± 1.08 cde	61.73 ± 1.12 bc	26.40 ± 1.13 ab	30.32 ± 1.22 ef
16789 + NAK-2 + NYA-2 + NSD	19.95 ± 1.21 abc	84.00 ± 1.09 ab	33.11 ± 1.09 ab	35.35 ± 1.09 def
16789 + NSD	18.98 ± 1.14 abcd	83.48 ± 1.07 ab	26.51 ± 1.14 ab	84.79 ± 1.18 a
16789 UNINOCULATED	18.76 ± 1.12 abcd	90.92 ± 1.06 ab	35.75 ± 1.05 ab	79.43 ± 1.12 ab
16789 + NAK-2 + NYA-2	16.85 ± 1.09 bcd	95.62 ± 1.04 a	30.43 ± 1.05 ab	33.37 ± 1.04 def
16789 + NYA-2	16.16 ± 1.06 bcd	70.42 ± 1.13 abc	23.90 ± 1.08 b	46.06 ± 1.26 abcdef
16789 + NAK-2	14.51 ± 1.13 bcde	102.87 ± 1.03 a	30.55 ± 1.03 ab	80.97 ± 1.08 a
Test Values	**df = 23; F = 6.434; P ≤ 0.0001**	**df = 23; F = 4.027; P ≤ 0.0001**	**df = 23; F = 2.318; P ≤ 0.0001**	**df = 23; F = 7.840; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

APPENDIX 42: Specific effects of pathogens co-infection under complete nutrient solution and weekly watering on selected growth parameters as at ratoons/crops 3&4 cycles combined; showing their means ± standard errors.

Ratoons 3&4; Complete nutrients solution effects on the performance of key selected growth parameters under weekly watering regime				
Treatment combinations	**Total Fresh Weight (Yield in grams)**	**Total Stem Weight (Grams)**	**Total Leaf Weight (Grams)**	**Leaf Number**
KK 1+ NAK-2 + NYA-2 + NSD	92.61 ± 1.16 abcd	46.06 ± 1.17 ab	44.50 ± 1.18 abcd	53.29 ± 1.10 bcd
KK 1 + NYA-2	92.61 ± 1.04 abcd	41.85 ± 1.06 abc	49.93 ± 1.05 abcd	64.57 ± 1.05 bc
KK 1 UNINOCULATED	83.18 ± 1.04 abcd	36.45 ± 1.07 abcde	46.42 ± 1.03 abcd	73.28 ± 1.06 abc
KK 1+ NAK-2 + NYA-2	77.33 ± 1.05 abcde	33.63 ± 1.07 abcde	42.99 ± 1.05 abcd	78.22 ± 1.07 abc
KK 1 + NAK-2	77.03 ± 1.10 abcde	36.17 ± 1.12 abcde	40.58 ± 1.09 abcde	62.61 ± 1.11 bc
KK 1 + NSD	59.80 ± 1.11 abcde	19.13 ± 1.19 bcde	39.05 ± 1.10 abcdef	127.84 ± 1.35 a
KK 2 + NYA-2	130.82 ± 1.14 a	58.21 ± 1.16 a	72.17 ± 1.12 a	45.36 ± 1.10 cd
KK 2 + NAK-2	105.12 ± 1.13 ab	45.71 ± 1.16 ab	57.99 ± 1.12 ab	59.57 ± 1.09 bcd
KK 2 UNINOCULATED	103.51 ± 1.05 ab	46.95 ± 1.08 ab	56.45 ± 1.04 abc	68.39 ± 1.07 bc
KK 2 + NSD	75.57 ± 1.05 abcde	31.26 ± 1.11 abcde	42.99 ± 1.04 abcd	32.61 ± 1.13 d
KK 2 + NAK-2 + NYA-2 + NSD	67.87 ± 1.10 abcde	32.24 ± 1.10 abcde	35.21 ± 1.10 abcdef	43.82 ± 1.09 cd
KK 2+ NAK-2 + NYA-2	44.67 ± 1.39 cde	17.99 ± 1.46 cde	26.10 ± 1.36 cdef	46.95 ± 1.14 cd
BANA + NAK-2 + NYA-2 + NSD	95.13 ± 1.26 abc	47.13 ± 1.30 ab	47.32 ± 1.24 abcd	60.03 ± 1.12 bcd
BANA UNINOCULATED	79.74 ± 1.02 abcd	34.54 ± 1.03 abcde	44.84 ± 1.01 abcd	71.61 ± 1.13 abc
BANA + NYA-2	57.54 ± 1.12 bcde	24.36 ± 1.14 abcde	32.86 ± 1.12 abcdef	56.67 ± 1.09 bcd
BANA + NAK-2	41.53 ± 1.15 de	16.41 ± 1.18 de	25.02 ± 1.13 def	59.80 ± 1.17 bcd
BANA + NSD	35.48 ± 1.25 e	15.97 ± 1.28 e	19.13 ± 1.24 ef	47.68 ± 1.09 cd
BANA + NAK-2 + NYA-2	34.81 ± 1.27 e	16.47 ± 1.27 de	18.13 ± 1.27 f	43.82 ± 1.10 cd
16789 + NAK-2	108.81 ± 1.08 ab	51.88 ± 1.10 a	56.89 ± 1.07 abc	65.56 ± 1.12 bc
16789 UNINOCULATED	82.22 ± 1.11 abcd	31.62 ± 1.14 abcde	49.93 ± 1.10 abcd	75.86 ± 1.10 abc
16789 + NAK-2 + NYA-2 + NSD	73.28 ± 1.13 abcde	40.58 ± 1.08 abcd	30.43 ± 1.22 bcdef	93.33 ± 1.22 ab
16789 + NAK-2 + NYA-2	72.72 ± 1.14 abcde	28.29 ± 1.20 abcde	43.65 ± 1.11 abcd	52.89 ± 1.09 bcd
16789 + NSD	71.61 ± 1.23 abcde	33.63 ± 1.25 abcde	37.58 ± 1.22 abcdef	74.13 ± 1.07 abc
16789 + NYA-2	70.25 ± 1.35 abcde	33.88 ± 1.35 abcde	36.03 ± 1.36 abcdef	62.13 ± 1.07 bc
Test Values	**df = 23 F =4.906; P ≤ 0.0001**	**df = 23; F = 4.837; P ≤ 0.0001**	**df= 23; F = 4.866; P ≤ 0.0001**	**df= 23; F= 5.661; P ≤ 0.0001**

The varieties entailed; KK 1 (Kakamega 1), KK 2 (Kakamega 2), Bana and 16789. The head smut isolates; (NAK-2, NYA-2) and Napier stunt Disease pathogen (NSD); '*Candidatus* Phytoplasma oryzae' strain Mbita 1 were used respectively. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

Appendix 43: The molecular sequences of the seventeen *Ustilago kamerunensis* isolates sequenced.

>NAK001

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>NAK002

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>NAK003

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>NYE001

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>NYE002

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>NYE004

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>KIR001

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>KIA002

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>KIA003

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>NYA001

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>NYA002

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CGCGGCCACGCCGTAAAACCCCCGACTTTTTTTAAGGTTGACCTCGAATCAGGTAGGACTACCCTGAACTTAAG
CATATCAATAGGCGGGGAGGAATA

Appendix 44: Primer pairs used to amplify the *Ustilago kamerunensis* DNA during pathogen presence in the tissues confirmation.

Primer type	Primer sequences
Internal Transcribed Spacer (ITS 1 / 4) Primers	Forward 1; 5'-TCTGTAGGTGAACCTGCGG-3' Reverse 4; 5'-TCCTCCGCTTATTGATATGC-3'

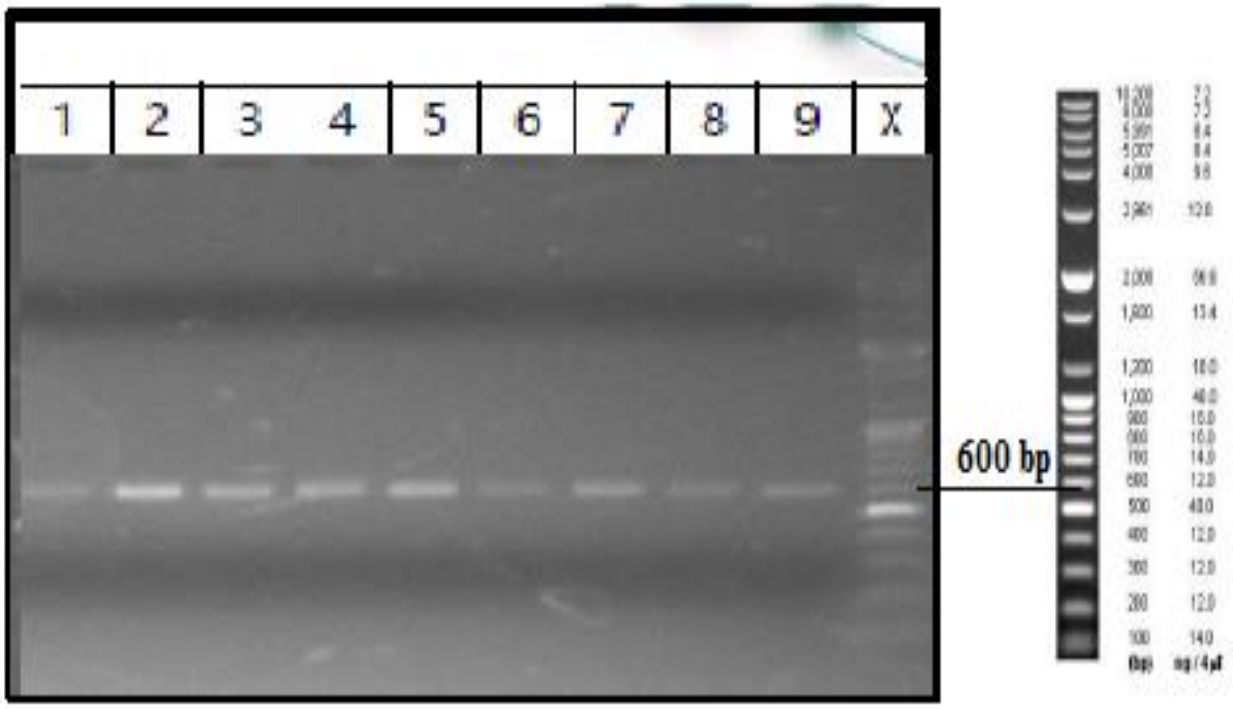
Source: Rosete *et al*.(2009) and Omayio *et al*. (2014a)

Appendix 45: Soil pH and nutrient levels of the soil used in the planting of the napier grass accessions under test against the pathogenicity and virulence of selected *Ustilago kamerunensis* isolates and *Candidatus* Phytoplasma oryzae pathogens.

BIOASSAYS' SOIL PROPERTIES	MEAN ± S.E	df	t-Value	P-Value
Soil pH	7.20 ± 0.1	5	1.387	0.224
Soil (%) nitrogen	0.27 ± 0.1	5	4.475	0.007
Soil (%) organic carbon	2.63 ± 0.1	5	34.6	0.0001
Soil Phosphorus (ppm)	120 ± 11.0	5	10.9	0.0001

The results indicate mean score of four soil parameters of six soil samples collected randomly from the soil used in the bioassays. The individual scores were subjected to one sample T-test using neutral pH (7.0) and (0.0) as means' test value for the soil pH and the other three soil parameters respectively.

Appendix 46: An electropherogram of the nine PCR sample products. X is the ladder, showing the DNA bands at approximately 600 bp (base pairs) level of *Ustilago kamerunensis* visualized upon amplification of the total DNA using ITS primers towards sequencing.



Appendix 47: Nutrient formulations effects on the mean pathogen virulence and involved stressors' levels in percentage; evaluated basing on the integrated parameter logarithmic index means cutting across the four cropping cycles; showing their means ± standard errors in percentage in descending order. The virulence classification MV/MSL; denotes Moderate Virulence/Moderate stress levels which is classified when ≥ 25% whereas LV/LSL denotes (Low virulence/Low stress levels which is classified when < 25%).

Pathogens/stressors and nutrient formulations	Virulence levels in (%)	Virulence/stress levels classification
NSD + N/P-D	28.91 ± 2.91 a	MV
NAK-2 + NYA-2+ NSD + N/P-D	27.24 ± 2.55 ab	MV
NAK-2 + NYA-2 + N/P-D	26.08 ± 3.00 abc	MV
NYA-2 + N/P-D	26.03 ± 2.77 abc	MV
Uninoculated + N/P-D	24.50 ± 3.24 abcd	MSL
NAK-2 + N/P-D	23.87 ± 2.91 abcde	LV
NSD + N-D	20.77 ± 3.72 abcdef	LV
NAK-2 + NYA-2+ NSD + N-D	18.88 ± 4.37 abcdefg	LV
NAK-2 + NYA-2 + N-D	18.29 ± 3.22 abcdefg	LV
NYA-2 + N-D	16.10 ± 3.05 abcdefg	LV
Uninoculated + N-D	14.87 ± 2.87 bcdefg	LSL
NAK-2 + N-D	14.59 ± 3.45 bcdefg	LV
NSD + P-D	12.57 ± 2.19 cdefg	LV
NAK-2 + NYA-2+ NSD + P-D	11.50 ± 1.70 defg	LV
NYA-2 + P-D	10.25 ± 2.18 efg	LV
NAK-2 + P-D	10.04 ± 1.70 efg	LV
NAK-2 + NYA-2 + P-D	9.65 ± 2.27 fg	LV
Uninoculated + P-D	9.17 ± 1.78 fg	LV
NAK-2 + NYA-2+ NSD + CNS	9.10 ± 1.90 fg	LV
NSD + CNS	8.73 ± 2.03 fg	LV
NAK-2 + NYA-2 + CNS	8.40 ± 1.87 fg	LV
NYA-2 + CNS	6.30 ± 2.06 g	LV
Uninoculated + CNS	5.16 ± 2.09 g	LV
NAK-2 + CNS	4.87 ± 2.43 g	LV
Test values	**df =23 ; F = 8.08; P < 0.0001**	

The head smut isolates (*Ustilago kamerunensis*) used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (Napier grass stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. Nutrient formulations entailed CNS (complete nutrient solution), N/P-D (Nitrogen phosphorus deficient solution), N-D (Nitrogen deficient solution) and P-D (Phosphorus deficient solution). The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

Appendix 48: Watering regimes effect on the mean pathogen virulence and involved stressors' levels in percentage; evaluated basing on the integrated parameter logarithmic index means cutting across the four cropping cycles; showing their means ± standard errors in percentage in descending order. The virulence classification MV; denotes Moderate Virulence/Moderate stress levels which is classified when ≥ 25% whereas LV / LSL denotes (Low virulence / Low stress levels which is classified when < 25%).

Pathogens/Stressors involved under the different watering regimes	**Virulence/Stress levels in (%)**	**Virulence classification**
NSD + Weekly watering	24.66 ± 2.40 a	MV
NAK-2 + NYA-2 + NSD + Weekly watering	23.28 ± 2.36 a	LV
NAK-2 + NYA-2 + Weekly watering	22.08 ± 2.22 a	LV
NYA-2 + Weekly watering	20.86 ± 2.25 a	LV
NAK-2 + Weekly watering	19.76 ± 2.20 ab	LV
Uninoculated + Weekly watering	19.64 ± 2.33 ab	LSL
NSD + Daily watering	10.83 ± 1.79 bc	LV
NAK-2 + NYA-2 + NSD + Daily watering	10.08 ± 1.67 c	LV
NAK-2 + NYA-2 + Daily watering	9.13 ± 1.69 c	LV
NYA-2 + Daily watering	8.48 ± 1.79 c	LV
Uninoculated + Daily watering	7.21 ± 1.60 c	LSL
NAK-2 + Daily watering	6.92 ± 1.73 c	LV
Test values	**df = 11 ; F= 11.84 ; P < 0.0001**	

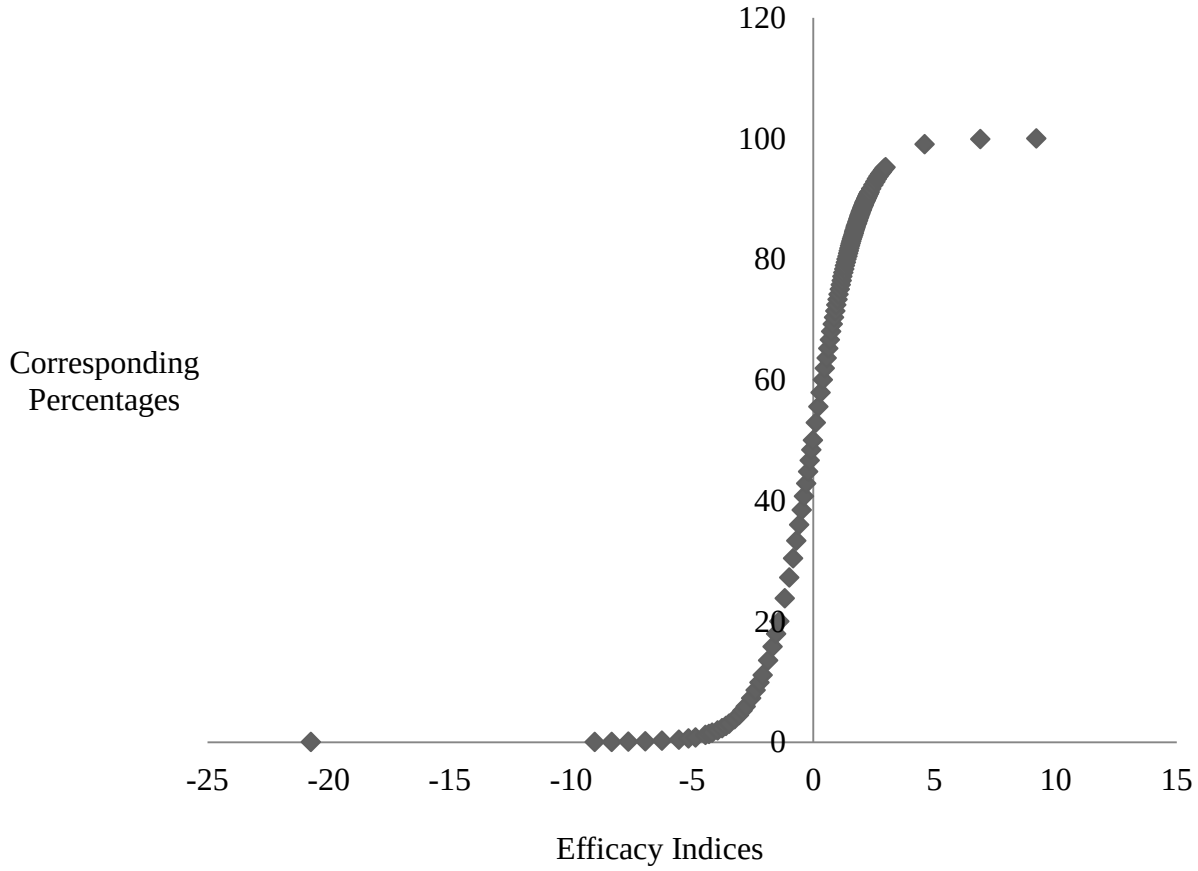
The head smut isolates (*Ustilago kamerunensis*) used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (Napier grass stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

Appendix 49: Interactions analysis of the nutrient formulation versus pathogen combinations as at ratoon/crop 3&4 cycles; showing the key selected growth parameters and their means ± standard errors.

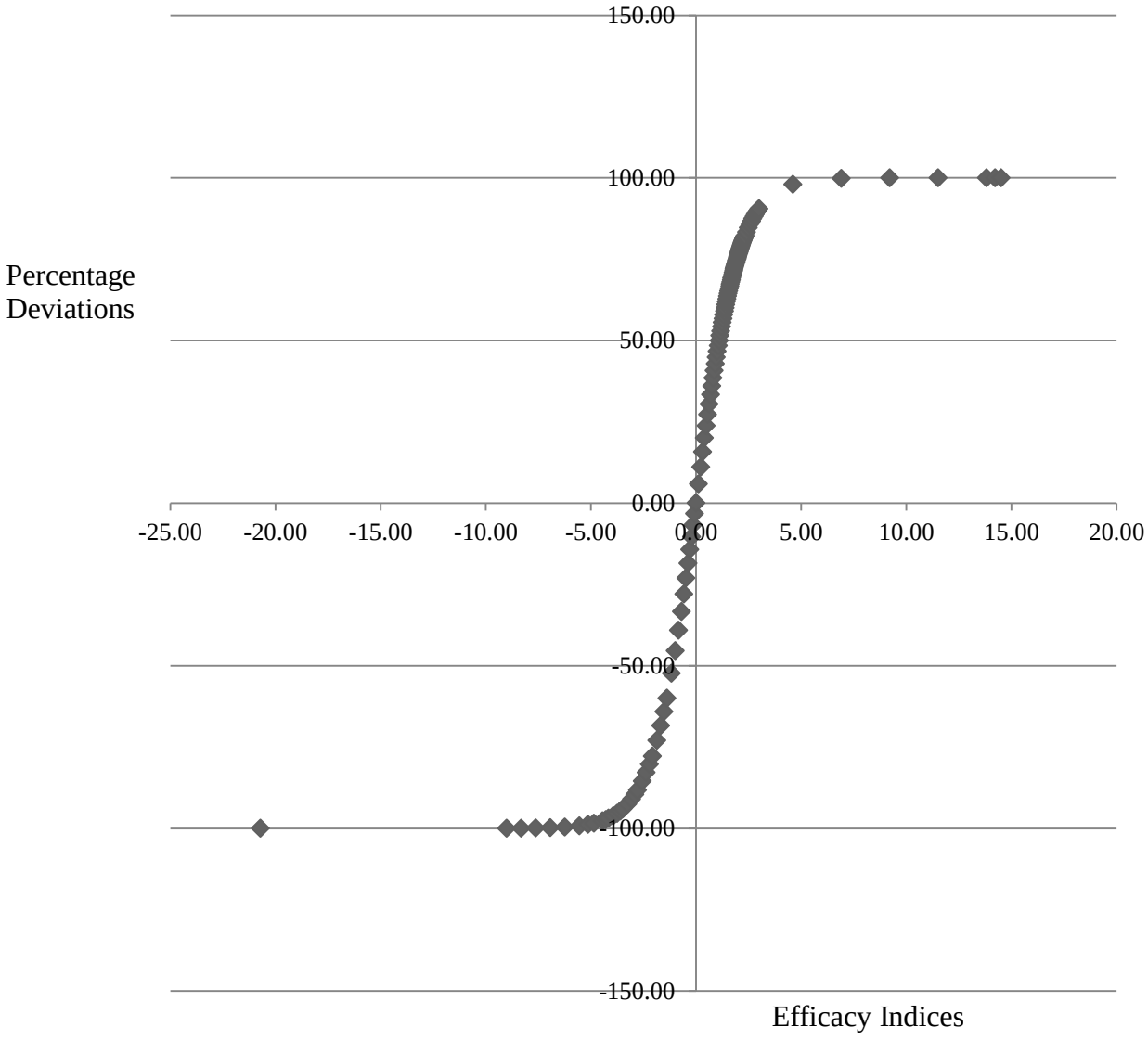
Ratoon 3 & 4; Overall performance of the pathogens using selected key parameters			
Nutrient formulations & pathogen combinations	**Total fresh weight (Yield) in grams**	**Tiller height (cm)**	**Chlorophyll content levels (SPAD UNITS)**
CNS + NAK-2	152.96 ± 1.09 a	106.46 ± 1.04 a	37.22 ± 1.03 a
CNS + UNINOCULATED	134.06 ± 1.06 ab	103.46 ± 1.03 ab	34.29 ± 1.03 abc
CNS + NYA-2	124.03 ± 1.08 ab	94.39 ± 1.04 abcd	33.90 ± 1.04 abc
CNS + NAK-2 + NYA-2 + NSD	114.32 ± 1.07 abc	99.21 ± 1.03 abc	36.89 ± 1.02 a
CNS + NAK-2 + NYA-2	98.57 ± 1.10 abcd	93.15 ± 1.03 abcde	35.43 ± 1.06 ab
CNS + NSD	86.22 ± 1.07 bcde	88.36 ± 1.03 abcdef	34.18 ± 1.04 abc
N/P-D + NAK-2	27.70 ± 1.07 hi	60.26 ± 1.02 ij	22.94 ± 1.04 hi
N/P-D + UNINOCULATED	25.61 ± 1.09 i	59.68 ± 1.04 ij	25.12 ± 1.03 defghi
N/P-D + NAK-2 + NYA-2	23.29 ± 1.08 i	55.40 ± 1.03 j	21.07 ± 1.04 i
N/P-D + NAK-2 + NYA-2 + NSD	22.71 ± 1.06 i	55.54 ± 1.03 j	21.05 ± 1.03 i
N/P-D + NYA-2	21.88 ± 1.08 i	59.23 ± 1.03 ij	23.26 ± 1.03 ghi
N/P-D + NSD	21.47 ± 1.08 i	57.58 ± 1.03 j	23.22 ± 1.04 ghi
P-D - UNINOCULATED	95.32 ± 1.06 abcde	88.82 ± 1.03 abcdef	31.05 ± 1.03 abcd
P-D + NYA-2	86.43 ± 1.07 bcde	88.81 ± 1.03 abcdef	29.02 ± 1.03 bcdef
P-D + NAK-2 + NYA-2	76.63 ± 1.10 cdef	80.45 ± 1.06 defgh	29.53 ± 1.04 bcde
P-D + NAK-2 + NYA-2 + NSD	75.93 ± 1.07 cdef	86.04 ± 1.04 bcdefg	30.06 ± 1.03 abcd
P-D + NAK-2	73.21 ± 1.10 cdef	89.88 ± 1.03 abcdef	30.03 ± 1.05 abcd
P-D + NSD	67.22 ± 1.08 defg	83.42 ± 1.04 cdefg	29.70 ± 1.04 bcde
N-D + NAK-2	60.90 ± 1.08 efg	76.47 ± 1.04 efgh	26.43 ± 1.03 defgh
N-D + NYA-2	50.60 ± 1.08 fg	71.11 ± 1.04 ghi	23.53 ± 1.04 fghi
N-D + NAK-2 + NYA-2	48.56 ± 1.11 fg	74.67 ± 1.03 fgh	28.04 ± 1.03 cdefgh
N-D + NAK-2 + NYA-2 + NSD	43.57 ± 1.11 gh	66.46 ± 1.04 hij	24.08 ± 1.05 efghi
N-D - UNINOCULATED	42.29 ± 1.10 efg	78.09 ± 1.05 defgh	28.60 ± 1.05 bcdefg
N-D + NSD	42.01 ± 1.11 gh	74.53 ± 1.04 fgh	29.54 ± 1.03 bcde
Test Values	**df = 24; F = 56.72; P < 0.0001**	**df = 24; F = 33.16; P < 0.0001**	**df = 24; F = 22.04; P < 0.0001**

The effects of nutrient formulations factor on pathogen combination effects on the performance of three major growth parameters affected by the diseases. The CNS (Complete nutrient solution, N/P-D (Nitrogen/phosphorus deficient solution), P-D (Phosphorus deficient solution) and N-D (Nitrogen deficient solution) were used. The head smut isolates used were NAK-2 from Nakuru County and NYA-2 from Nyandarua County. The NSD (napier grass stunt pathogen) used was '*Candidatus* Phytoplasma oryzae' strain Mbita 1. The means ± standard error with the same letter/s within the same column exhibited no significant differences at $P \leq 0.05$. Those with more than one letter within a column are intermediates.

Appendix 50: A plot validating the closely placed logarithmic plots of corresponding overall maximum potential in percentage levels against natural logarithmic efficacy indices. The linear equation (y = 23.682x + 50.096) describes the exponential phase (linear phase) of the curve whose coordinates' relationship strength stood at (R^2 = 0.9992). Confirming how close consecutive percentages are of different efficacy indices; increasing the reliability of the table in assigning percentage magnitudes of any unknown index on test. This generated indices and respective magnitudes in percentage which were used to assign the host plant resistance magnitudes based on the relative performance of the diseased napier grass accessions (artificially inoculated napier accessions) from their controls (uninoculated napier accessions). Details on different natural resistance levels are shown in appendix 2*: Omatec Logarithmic Indices'and their Corresponding Percentages Table;* Column (D).



Appendix 51: A plot showing the logarithmic plot relationship between natural logarithmic efficacy indices of different levels and their equivalent specific input potential in percentages. These values estimate the levels in percentage of deviation of the diseased napier grass accessions' relative to their non-diseased controls (uninoculated); in terms of their plant processes mounting against the diseases challenge. Details on different deviation levels are shown in appendix 2*: Omatec Logarithmic Indices' and their Corresponding Percentages Table*; Column (E). The concentration of the values consecutive to each other demonstrate the ability of the model to predict any other value with minimal error.



Appendix 52: A plot of corresponding percentage levels against their natural logarithmic efficacy indices obtained relative to the maximum logarithmic index (14.51). This maximum logarithmic index had a corresponding specific input percentage magnitude of 100% (appendix 2). These values estimate the power of respective logarithmic efficacy indices in percentage relative to the maximum index 14.51 value which is natural logarithm value of 2000000. The percentages were determined by dividing them as numerators by 14.51 as the denominator, with the answer being multiplied by 100%. Details on different percentage levels are shown in appendix 2*: Omatec Logarithmic Indices' and their Corresponding Percentages Table* Column (F). The concentration of the values consecutive to each other demonstrate the ability of the model to predict any other value within the continuum of values with high accuracy and reliability.