

**DETECTION AND CHARACTERIZATION OF CASHEW LEAF AND  
NUT BLIGHT-CAUSING FUNGI IN SOUTHERN AND EASTERN  
TANZANIA**

**Dadili Japhet Majune**

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Master's in Life Sciences of the Nelson Mandela African Institution of Science and  
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## ABSTRACT

Cashew (*Anacardium occidentale* L.) is an important crop for export, income and nutrition in Tanzania. However, its productivity is not optimal due to a number of factors including, growing un-improved cashew genotypes, poor soil fertility, insect pests and diseases. Of these factors, Cashew Leaf and Nut Blight Disease (CLNBD) has been cited as among devastating factors for lower nut yields and quality. The present study investigated the CLNBD incidence and severity on farmers' cashew fields in the eastern and southern zones of Tanzania and on artificially inoculated cashew elite hybrid seedlings in the screen house experiment at Tanzania Agricultural Research Institute (TARI)-Naliendele. Samples with disease symptoms collected from surveyed areas were used for detection and characterization of CLNBD-causing pathogens at TARI-Mikocheni. Results showed that there was significant difference ( $P < 0.001$ ) between the CLNBD incidence and severity in the surveyed locations. The highest (71.15%) CLNBD incidence was recorded in Mtwara while the lowest (31.21%) CLNBD incidence was recorded in Bagamoyo Districts. The results for the screen house experiment showed that 38 out of 52 cashew hybrids were tolerant to CLNBD implying their potential in breeding for resistant/tolerant cashew cultivars. The results also showed that CLNBD is caused by a complex of fungal species belonging to genera *Botryosphaeria*, *Pestalotiopsis*, *Colletotrichum*, *Neofusicoccum* and *Lasiodiplodia*. Further studies are recommended to study host range of the new fungal species identified in this study. The identified tolerant cashew genotypes are recommended for further screening in breeding programs for developing resistant varieties against CLNBD in Tanzania.

## DECLARATION

I, Dadili Japhet Majune do here declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concomitantly submitted for degree award in any other institution.

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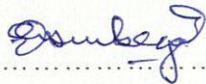
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Dadili Japhet Majune

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Signature



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03.04.2019

Dr. Ernest R. Mbega (Supervisor 1)

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Prof. Peter A. L. Masawe (Supervisor 2)

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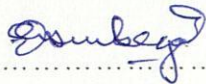
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## TABLE OF CONTENTS

ABSTRACT .....	i
DECLARATION .....	ii
COPYRIGHT .....	iii
CERTIFICATION .....	iv
ACKNOWLEDGEMENTS .....	v
DEDICATION .....	vii
LIST OF FIGURES .....	xi
LIST OF APPENDICES .....	xii
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1 Background Information .....	1
1.2 Problem Statement and Justification .....	3
1.3 Objectives .....	3
1.3.1 General Objective .....	3
1.3.2 Specific Objectives .....	3
1.3.3 Research Questions .....	4
1.3.4 Significance of the Study .....	4
CHAPTER TWO .....	5
LITERATURE REVIEW .....	5
2.1 Production Trend of Cashew Raw Nut in Tanzania .....	5
2.2 Factors Associated with Low Productivity of Cashew Nut in Tanzania .....	5
2.2.1 Cashew Powdery Mildew Disease .....	6
2.2.2 Cashew Dieback Disease .....	6
2.2.3 Cashew Fusarium Wilt Disease .....	7
2.2.4 Cashew Anthracnose Disease .....	7
2.2.5 Cashew Leaf and Nut Blight Disease (CLNBD) .....	7
2.2.6 Control of Cashew Leaf and Nut Blight Disease .....	8
2.3 Molecular Markers Techniques Used in Plant Pathology .....	8
CHAPTER THREE .....	10
MATERIALS AND METHODS .....	10
3.1 Study Area .....	10
3.2 Cashew Materials Used in the Screen House Experiment .....	11

3.3.1 Field Survey .....	12
3.3.2 Disease Incidence and Severity.....	12
3.3.3 Sample Collection and Culturing .....	13
3.3.4 Screen House Experiment.....	14
3.3.5 DNA Extraction .....	15
3.3.6 Polymerase Chain Reaction (PCR).....	15
3.3.7 Construction of Phylogenetic Tree .....	16
3.4 Statistical Analysis.....	16
CHAPTER FOUR .....	17
RESULTS AND DISCUSSION .....	17
4.1 Results .....	17
4.1.1 The Current Status of Cashew Leaf and Nut Blight Disease in Farmers Fields in the Southern and the Eastern zones in Tanzania .....	17
4.1.2 The Status of Cashew Leaf and Nut Blight Disease in Cashew Elite 1996 and 1998 Hybrids .....	18
4.1.3 Cashew Leaf and Nut Blight Disease on Cashew Elite 1996 and 1998 Hybrids Seedlings in the Screen House Experiment .....	22
4.1.4 Molecular-Based Analysis and Phylogenetic Relationship of Fungi Isolates Associated with the Cashew Leaf and Nut Blight Disease.....	25
4.2 Discussion.....	30
CHAPTER FIVE.....	32
CONCLUSION AND RECOMMENDATIONS .....	32
5.1 Conclusion .....	32
5.2 Recommendations .....	32
REFERENCES.....	33
APPENDICES.....	38
RESEARCH OUTPUTS .....	45
Output 1: Published Research Paper and Published Review paper.....	45
Output 2: Poster presentation .....	64

## LIST OF TABLES

Table 1: Cashew hybrid materials used in the screen house experiments .....	11
Table 2: The disease scoring scale of cashew leaf and nut blight disease showing levels, intervals and mid-point in percentages. ....	13
Table 3: CLNBD incidence and severity for “surveyed locations in Mtwara, Nachingwea and Bagamoyo districts” .....	17
Table 4: Incidence and severity of CLNBD on cashew elite 1996 hybrids in Chambezi and Nachingwea experimental sites during the current study. ....	20
Table 5: Incidence and severity of CLNBD on cashew elite 1998 hybrids in Chambezi and Nachingwea experimental sites during the current study. ....	21
Table 6: Cashew leaf and nut blight isolates obtained from lesions of infected cashew leaves and their virulence on cashew variety AC4 .....	26
Table 7: Summary of positive samples with maximum nucleotide identities of 97-99% similarity to CLNBD causing fungi .....	30

## LIST OF FIGURES

Figure 1: Raw cashew nut production trend in Tanzania from year 1945 to year 2017 (Source: CBT, 2017/2018). .....	5
Figure 2: The Cashew growing areas and study locations in Tanzania. ....	10
Figure 3: CLNBD incidence and severity for surveyed villages in Nachingwea, Mtwara and Bagamoyo districts. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ ) .....	18
Figure 4: CLNB Disease severity scored on cashew elite 1996 hybrids in screen house experiment at TARI-Naliendele in March, 2018. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ ). ....	24
Figure 5: CLNB Disease severity scored on cashew elite 1998 hybrids in screen house experiment at TARI-Naliendele in March, 2018. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ ). ....	24
Figure 6: Amplification of a sample of pathogenicity positive isolates by PCR using ITS5 and ITS4 primers. Where; L is DNA ladder, 1 is a control, 2-10 (Lindi1, Lindi2, Lindi3, Lindi4, Lindi5, Lindi6, Lindi7, Lindi8, Lindi9 and Lindi10) samples for detection of CLNBD causing fungi. ....	28
Figure 7: Amplification of a sample of pathogenicity positive isolates by PCR using ITS1 and ITS4 primers. Where L is DNA ladder, 1-10 (1 Mtwara1, 2 control, 3 Mtwara2, 4 Mtwara3, 5 Mtwara4, 6 Mtwara5, 7 Coast1, 8 Coast2, 9 Coast3 and 10 coast4) samples for detection of CLNBD causing fungi. ....	28
Figure 8: Phylogenetic tree reconstructed using the nucleotide sequences of CLNBD isolates. N.B: The phylogenetic tree was constructed with a neighbour-joining method in MEGA7 using bootstrap values over 50%, red blocks show samples sequenced with 97-99% identity to known fungal species. ....	29

## **LIST OF APPENDICES**

Appendix 1: The severity assessment guide for cashew leaf and nut blight disease score....	38
Appendix 2: CLNBD severity on cashew elite 1996 and 1998 hybrids in the screen house experiment at TARI-Naliendele. ....	40
Appendix 3: Ten fungal species associated with cashew leaf and nut blight disease in Tanzania deposited in NCBI gene bank .....	41

## **LIST OF PLATES**

Plate 1: CLNBD symptoms on cashew leaves and young nuts.....	20
Plate 2: Grafting of cashew elite hybrids in screen house during this study.....	23
Plate 3: DNA extraction steps followed during for fungal isolation from the infected cashew leaves. ....	27

## LIST OF ABBREVIATIONS AND SYMBOLS

AZA2	Anacardium Zanzibar 2
AZA17	Anacardium Zanzibar 17
AC4	Anacardium Ceylon 4
<	Less than
CV	Coefficient of variation
%	Percentage
ml	Milligram
°C	Degree centigrade
g	Gram
cm	Centimetre
M	Molarity
S	South
E	East
Masl	Metres above sea level
WG	Water dispersible granule
ANOVA	Analysis of variance
SC	Suspension concentrate
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
NaOCl	Sodium hypo chloride
HCl	Hydrochloric acid
EDTA	Ethylenediamine tetraacetic acid
LSD	Least significant different
NCBI	National Centre for Biotechnological Information
MEGA	Molecular Evaluation Genetic Analysis

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background Information

Cashew (*Anacardium occidentale* Linn.) is a perennial nut crop, native to Brazil that belongs to the Anacardiaceae family (Ohler, 1979; Azam-Ali and Judge, 2000). It is an important crop for nutrition and income generation worldwide (Mange *et al.*, 2014). In Tanzania, cashew is an important export crop in terms of foreign exchange earnings (Masawe and Kapinga, 2010). It is also the leading source of income for over 300 000 households in South-Eastern part of the country (Kasuga, 2013). Cashew nuts contain 21.70% protein and 47.20% fat (Marc *et al.*, 2011). The most important products derived from the cashew trees are cashew nuts which are then processed to get kernel. The cashew apples are important in making juices, jam, alcoholic and soft drinks (Sobhana *et al.*, 2010). Cashew trees can also be used for firewood, charcoal and in carpentry for manufacturing of different furniture (Akinwale, 2000; Opeke, 2005; Menge *et al.*, 2014). In Tanzania cashew provides employment, income and is a leading export crop. Over 80% of the crop production comes from Mtwara, Lindi and Ruvuma (Tunduru) regions (CBT, 2015; CBT, 2018). The area under cashew production in Tanzania has been estimated to be more than 500 000 ha in mono or mixed crop production systems. An average cashew farmer in Tanzania owns about 1-2 ha of cashew trees (Ellias, 1980; Brown *et al.*, 1984; Topper and Boma, 1997). The average yield in farmers' fields ranges from 500 kg/ha to 800 kg/ha (Masawe, 2006).

Despite of its importance, cashew production has been constrained by several factors such as insect pests, diseases and limited use of improved planting materials (Sijaona and Shomari, 1987). Of these factors, diseases such as powdery mildew and others have been very challenging in cashew production (Ellias, 1980; Castellani and Casulli, 1981; Brown *et al.*, 1984; Intini, 1987). For instance, since 1970s, the powdery mildew disease has been associated with crop losses ranging from 70 to 100% (Sijaona and Shomari, 1987). However, management of the disease with Sulphur 99%, Triadimenol, Hexaconazol or Triadimefon has been very successful and cashew production farmers are applying them to date (Sijaona, 1984; Shomari, 1988).

In 2003 a new disease known as "Cashew Leaf and Nut Blight Disease" (CLNBD) was reported for the first time on cashew in Tanzania (Sijaona *et al.*, 2005; Sijaona *et al.*, 2006). In



2012, another new cashew disease namely Fusarium wilt (*Fusarium oxysporum*) was reported attacking cashew in Mkuranga district in Coast region (Tibuha and Shomari, 2016). Currently both diseases are major threats to cashew production in the country. Fungicides for management of these diseases have been recommended, however they are based on a general recommendation with less detail on the causative organism. For instance, of the two diseases, the causative pathogen of CLNBD is very difficult to identify due to its complexity in symptoms yet this species complex is not characterised accurately (CRP, 2006; GPC, 2010). The CLNBD has been reported to be a big problem in wet weather especially during off-season rains, where it causes severe damages on emerging young tender leaves (Sijaona *et al.*, 2005; Sijaona *et al.*, 2006). Visually the disease is characterised by angular lesions, dark tan with a dark reddish brown margin on leaves, and veins contain conidiomata. Lesions subsequently enlarge and coalesce to cause large necrotic lesions and finally defoliation. Older lesions become papery, silver/ grey, and develop shot-holes (Sijaona *et al.*, 2006). During fruit setting, infection of young nuts causes rapid blackening and abscission of nuts, resulting in significant yield losses. Usually infections on older nuts result in sunken, ‘tar spot’-like lesions that frequently extend onto the apples and cause annual crop loss of up to 48.4% if not controlled (Sijaona *et al.*, 2005; Sijaona *et al.*, 2006; Menge *et al.*, 2014).

In recent years, no studies have been undertaken to establish the current status of cashew leaf and nut blight disease on cashew trees in farmers’ fields in the Eastern and the Southern zones of Tanzania. Use of chemical fungicides to control the disease is common among cashew growing farmers. However, the chemicals are associated with different challenges including high costs for farmers to afford, lack of efficacy and untargeted due to accurate identification of the disease causal agent. Given that the symptoms still complicated for identification of the CLNBD causal agents, the powerful approaches such as molecular technologies is required to accurately identify the appropriate fungal strain that is associated with the disease. Use of resistant varieties remains an effective approach for managing diseases including CLNBD as proposed by Masawe (2006) and Zhongrun and Masawe (2014). Thus it is quite important to provide farmers with cashew varieties that are resistant or tolerant to the disease to reduce costs of production and safeguard environment, human health and biodiversity.

TARI-Naliendele has been working tirelessly to minimize or completely eliminate the CLNBD (Dr. Omari Mponda-Centre Director, personal communication, 2018). During the 2016/2017 season, TARI-Naliendele developed more than 52 cashew elite 1996 and 1998 hybrids were ready to be supplied to farmers in cashew growing areas in Tanzania (CRP,

2017). However, these cashew elite hybrids had not been tested for resistance or tolerance against CLNBD but only other traits such as nut size, high yielding and percentage out turn. Thus, based on this background, the present study was undertaken to determine the current status of cashew leaf and nut blight disease - causing fungi in the Southern and the Eastern zones of Tanzania, the accurately detect and characterise causal agent of; and then screening the TARI-Naliendele cashew elite 1996 and 1998 hybrids for their resistance, tolerance and susceptibility to CLNBD.

## **1.2 Problem Statement and Justification**

Cashew leaf and nut blight disease is among the major constraints to cashew production in Tanzania (Sijaona *et al.*, 2006). This disease, if not controlled, can cause crop losses of about 48.4% annually (CRP, 2006; Sijaona *et al.*, 2006). Direct infection by the causal fungi on young nuts leads to blackening and abscission of nuts, resulting in significant yield losses (Sijaona *et al.*, 2006). Affected mature nuts usually possess black spots and thus fetch second lower prices in the markets (Masawe, 2006; Sijaona *et al.*, 2006). Use of the fungicides however has been reported to be expensive, not always accessible to cashew farmers and sometimes are ineffective to control complex symptoms that contradict farmers as no correct identification of disease causing pathogens has been made. Consequently, given the importance of the problem, there was an urgent need to collect information on the current status of the CLNBD and on correct identification of the causal agents using rapid and accurate powerful approaches such as molecular technologies. In addition, it would be imperative to screen cashew elite hybrids for their tolerance/resistance to the CLNBD since use of resistance cultivars is always considered superior to other approaches when targeting disease control.

## **1.3 Objectives**

### **1.3.1 General Objective**

To establish the cashew leaf and nut blight disease status and, to detect and characterize causal agents using molecular techniques as well as screening elite cashew hybrids against the disease in the Southern and the Eastern zones of Tanzania.

### **1.3.2 Specific Objectives**

- (i) To establish the current status of cashew leaf and nut blight disease - causing fungi in the Southern and Eastern zones of Tanzania.

- (ii) To study phylogenetic relationship of fungal organisms associated with the CLNBD in the Southern and Eastern zones of Tanzania.
- (iii) To evaluate selected cashew elite 1996 and 1998 hybrids against virulent fungal isolates obtained from infected cashew materials.

### **1.3.3 Research Questions**

- (i) What is the current status of cashew leaf and nut blight disease in the Southern and the Eastern zones of Tanzania?
- (ii) What is the phylogenetic relationship of CLNBD causing fungi in Tanzania?
- (iii) Are the cashew elite 1996 and 1998 hybrids against virulent fungal isolates isolated from infected cashew materials?

### **1.3.4 Significance of the Study**

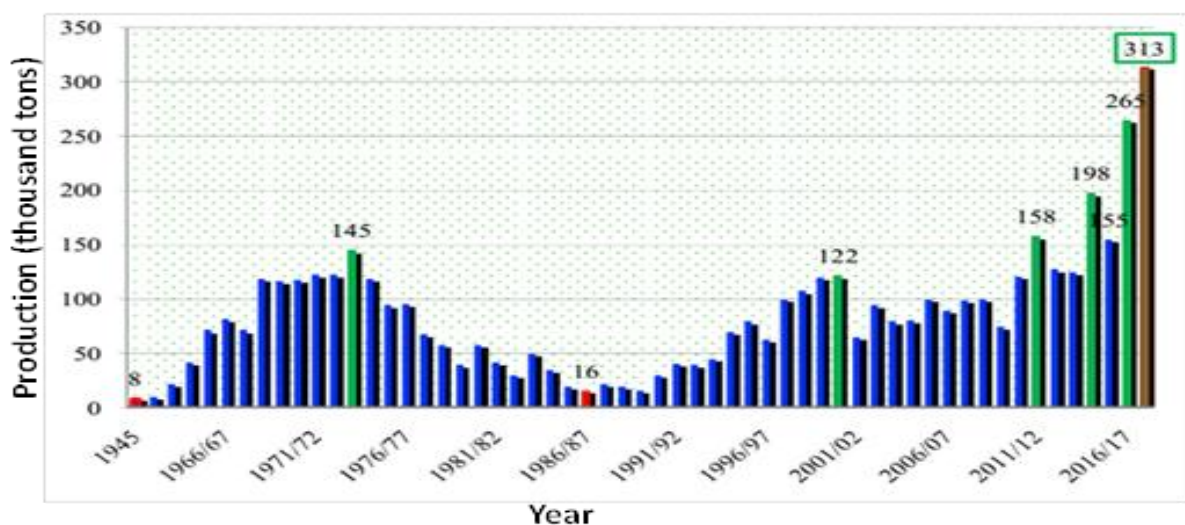
The present study has provided information on the status of the CLNBD in the main cashew production areas of Tanzania (i.e. Nachingwea, Bagamoyo and Mtwara districts). The study has also identified main fungal genera associated with the CLNBD in Tanzania. In addition, this study has identified 38 cashew elite hybrids to have an ability to resist the CLNBD. The information generated here is relevant for breeders, plant pathologists, farmers and policy makers in decision making to commit resources for managing the CLNBD in Tanzania.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Production Trend of Cashew Raw Nut in Tanzania

Tanzania is the world's eighth; and Africa's third largest cashew nut producer after Mozambique and Ivory Coast (CBT, 2011). The cashew raw nut production trend from 1945-2017 is as shown in Fig. 1. Close look of the cashew production from 1945s shows a zigzag production style nevertheless the production is currently increasing possibly due to increased acreage of production. This trend may imply that productivity of cashew is low.



**Figure 1:** Raw cashew nut production trend in Tanzania from year 1945 to year 2017 (Source: CBT, 2017/2018).

#### 2.2 Factors Associated with Low Productivity of Cashew Nut in Tanzania

Cashew production in Tanzania is constrained by several factors that often result into yield fluctuation. Some commonly cited factors include drought, planting unimproved cultivars, decreasing soil fertility, insect pest and diseases (Masawe, 2006). Of these, diseases have been cited to be among important factors that affect cashew yield quantity and quality. The disease of most economic importance include Powdery mildew caused by *Oidium anacardii* Noack (Casulli, 1979; Sijaona, 1984; Shomari, 1988), Anthracnose caused by *Colletotrichum gloeosporoides* Penz (Casulli, 1981), Cashew Leaf and Nut Blight caused by *Cryptosporiopsis* spp (Sijaona *et al.*, 2006), Dieback caused by *Phomopsis anacardii* (Intini

and Sijaona, 1983) and Fusarium wilt disease caused by *Fusarium oxysporum* (Tibuha and Shomari, 2016).

### **2.2.1 Cashew Powdery Mildew Disease**

To date, powdery mildew disease is the main constraint of cashew production in Tanzania. The disease is (as said) caused by *Oidium anacardii* Noack, a fungus of genus *Oidium* of the Deuteromycotina (Fungi Imperfecti) (Shomari, 1996; Sijaona, 1997). Powdery mildew disease infests all tissues of the cashew trees, mainly the tender leaf and inflorescence including the part not well unfolded. The disease seldom attacks old and mature leaves (Sijaona *et al.*, 2006). A white powdery growth is formed on the infested fruit bearing branches and inflorescence (Sijaona and Shomari, 1987). Infected apples turn dull and their skin becomes much coarser. The apples when heavily infected show deep cracks on the surface and gradually shrivel and dry up (Sijaona *et al.*, 2005). The tender nuts when infected are deformed on the shell. Infected nuts deteriorate in quality during storage, decays easily and produce poor quality kernels when processed (Shomari, 1996; Waller *et al.*, 1997; Sijaona, 1997). Powdery mildew is currently controlled mainly using synthetic fungicides including sulphur dusts and wettable powders (Cassulli, 1981; Intini and Sijaona, 1984) and, water based organic fungicides (Topper *et al.*, 1997; Sijaona *et al.*, 2001).

### **2.2.2 Cashew Dieback Disease**

Cashew Dieback disease was reported in Tanzania in 1980 (Intini and Sijaona, 1983). This fungal disease is caused by *Phomopsis anacardii*. It is believed to be facilitated by damage caused by mirid (*Helopeltis* spp) or coconut bug (*Pseudotheraptus wayii*) on cashew plant (Zhongrun and Masawe, 2014). The symptoms of the disease include withering of the panicles, followed by a progressive dieback of small flower stalks. This starts from the tips then advances downward to the main floral shoots (Intini and Sijaona, 1983). The normal greenish colour of the health shoots progressively turns brown resulting in loss of flowers. Infected young nuts and apples become black and fluffy and remains attached to the floral stalks. Heavy infection appears similar to fire damage (Sijaona, 2013). The fungus attacks young and tender shoots and flowers followed by dieback infection starting at tips and spreading downwards. Currently dieback disease is controlled by cultural methods and fungicides containing *Lambda cyhalothrin* and *Alpha cypermethrin*.

### **2.2.3 Cashew Fusarium Wilt Disease**

The cashew Fusarium wilt disease is caused by *Fusarium oxysporum* and it was first reported in Tanzania in 2012 at Magawa village in Mkuranga District in the Coast region (Tibuhwa and Shomari, 2012). There after it was reported in other districts i.e. Masasi (Nanganga), Tandahimba (Lindumbe) and Mtwara (Mnongodi). The cashew fusarium wilt can cause the entire cashew plant to wilt within three to four weeks after appearance of first symptoms. The disease can attack the next nearby cashew trees until trees in the entire field are all wilted (Tibuhwa and Shomari, 2016). Infected cashew plant is characterised by gradual chlorosis (Tibuhwa and Shomari, 2016). Looking from a distance, affected trees appear yellow and green and later wilt within three to four weeks the entire tree(s) (Tibuhwa and Shomari, 2016). Different methods including field sanitation and destruction of infected plant parts have been proposed for controlling Fusarium wilt disease in Tanzania (Tibuhwa and Shomari, 2016).

### **2.2.4 Cashew Anthracnose Disease**

The history of Anthracnose in Tanzania goes back to 1978 (Casulli, 1981). This fungal disease has been reported to be caused by *Colletotrichum gloeosporoides* Penz. The pathogen is not only a problem in cashew but also infects other tropical fruits trees including mango, citrus, avocado and papaya (Sijaona, 2013). The disease attacks all young and tender vegetative organs together with nuts and pseudo fruits/apples. The disease is favoured by relative humidity of 95% - 100% and temperature ranging between 22°C - 28°C during flowering and fruiting period (Sijaona, 2013, Zhongrun and Masawe, 2014). Early symptoms are reddish brown, shinny water-soaked lesions and resin exudation on the affected parts. Infected shoots appear as “hanging nuts”. Hanging nuts may act as a source of disease infection during the next season (Zhongrun and Masawe, 2014). Cashew resistant or partially resistant varieties are used to control anthracnose disease in Tanzania (Masawe, 2006). Other approaches of controlling anthracnose disease are cultural and chemical methods.

### **2.2.5 Cashew Leaf and Nut Blight Disease (CLNBD)**

Cashew leaf and nut blight was first described from Tanzania and elaborated by Global Plant Clinic (GPC). Diseased plant materials were sent to the GPC in October 2003, where the causal fungus was described as *Cryptosporiopsis* spp. (GPC, 2010). Infected cashew leaves develop silver/grey lesions with a dark reddish brown margin that enlarge and coalesce causing defoliation (Sijaona *et al.*, 2006). Infected young nuts blacken while older nuts result

in characteristic dark lesions that under favourable conditions they form white spore masses on nut lesions. The disease has been shown to be most active during wet weather especially during off-season rains (Sijaona *et al.*, 2006). The CLNBD is a major limiting factor that affect cashew nut production in Tanzania, causing crop loss 48.4% annually (CRP, 2006; Sijaona *et al.*, 2006). The cashew leaf and nut blight disease can be transmitted through conidial suspension with the aid of wind and rainy water (Menge *et al.*, 2013; Menge *et al.*, 2014).

### **2.2.6 Control of Cashew Leaf and Nut Blight Disease**

Cultural practices such as clearing off, gathering and burning disease materials and chemical methods including use of Trifloxistrobin 10% SC (2-4g/litre), Difenaconazole WG (water dispersible granule 14 g/litre), and Trifloxistrobin + Tebuconazole (14 ml/litre) have been recommended for controlling the disease (Zhongrun and Masawe, 2014). In addition the CLNBD has been reported to be effectively controlled or minimized by planting resistant cashew cultivars such as AZA 2 and AZA 17 (Masawe, 2006). Effects for managing the disease using resistant varieties are going on at the TARI-Naliendele centre, where 52 genotypes were developed in 1996 and 1998 (Masawe, 2006). The trait for disease tolerance is not affected by weather, soil properties, presence or absence of disease pathogens and plant age or maturity rather than genetic inheritance (Masawe and Kapinga, 2016). Furthermore, even if disease symptoms are not expressed amongst genotypes in a population, choice of resistance or tolerance genotypes can be performed based on markers linked to particular disease tolerance.

### **2.3 Molecular Markers Techniques Used in Plant Pathology**

Internal Transcribed Spacer (ITS) refers to the spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA in the chromosome. The ITS region of 18S-26S nuclear ribosomal DNA (nr DNA) has proved to be a useful source of characters for phylogenetic studies. The ITS region is the most widely sequenced DNA region in molecular ecology of fungi (Peay *et al.*, 2008) and has been recommended as the universal fungal barcode sequence (White *et al.*, 1990). It has been typically most useful for molecular systematics at the species level, and even within species to identify geographic races. This is due to its higher degree of variation than other genic regions of rDNA (for small- and large-subunit rRNA). In addition to the standard ITS1+ITS4 and ITS4+ITS5 primers (Gardes and Bruns 1993; White *et al.*, 1990) they are used by most laboratories, several taxon-specific

primers have been described that allow selective amplification of fungal sequences. In this case, the referred techniques may not be an exception for use in the present study.

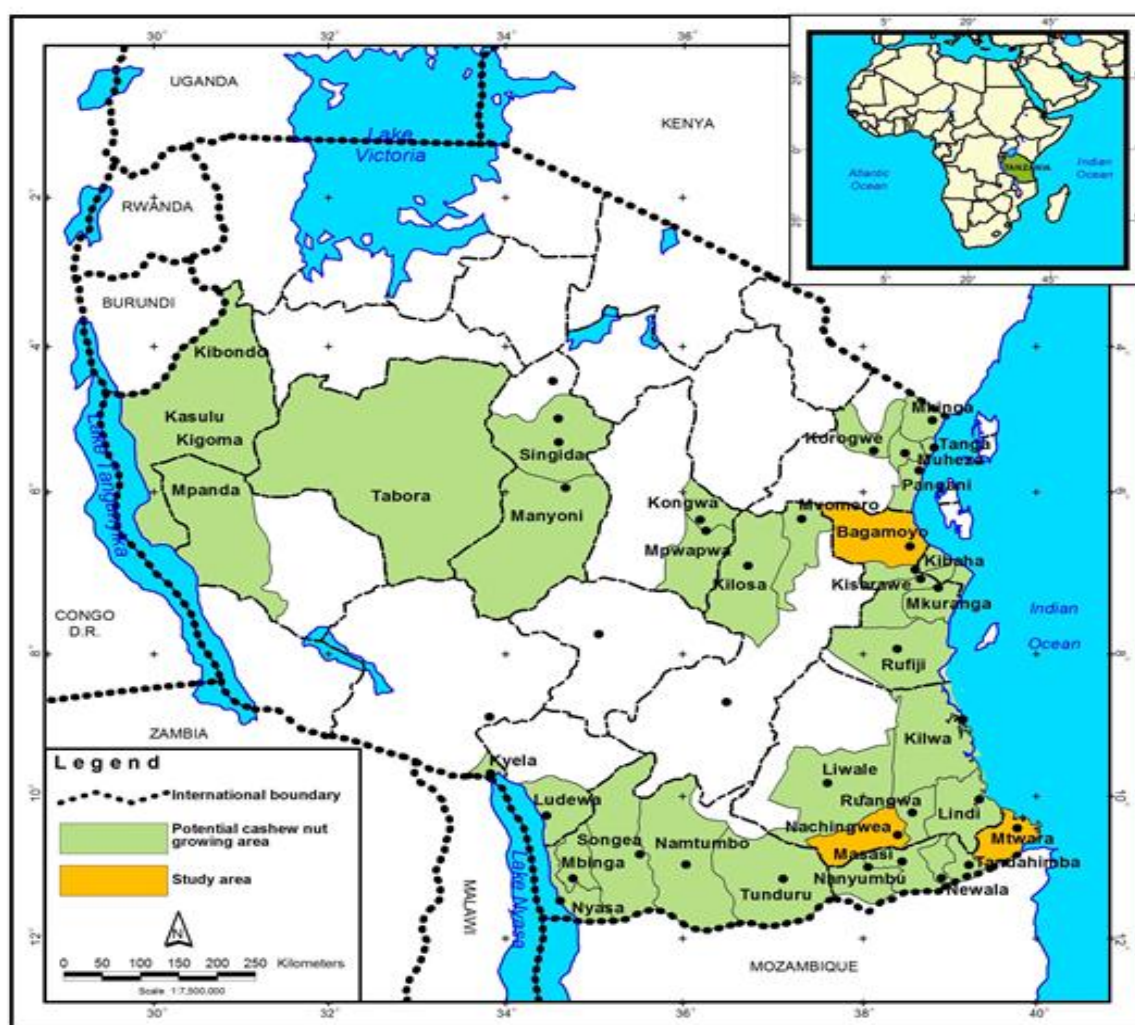


## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study Area

The study was conducted in three districts namely, Nachingwea which is located at 10°19'46"S, 38°46'46"E and 442 metres above sea level (masl) (Lindi region), Bagamoyo located at 6°31'S, 38°55'E and 19 masl (Coast region) and Mtwara at 10°22'22"S, 40°09'35"E and 102 masl (Mtwara region) (Fig. 2). The criteria for selecting the study districts are that they are main cashew growing areas in Tanzania and also, they have been reported to have higher CLNBD (Zhongrum and Masawe, 2014). The areas were also purposely selected due to the fact that they were previously supplied with the cashew elite 1996 and 1998 hybrids which were also used in this study.



**Figure 2:** The Cashew growing areas and study locations in Tanzania.

### 3.2 Cashew Materials Used in the Screen House Experiment

The cashew materials used in the experimentation are as shown in Table 1 below:

**Table 1:** Cashew hybrid materials used in the screen house experiments

S/n	Cashew elite 1996 hybrid	Cashew elite 1998 hybrid
1	C7-1-13	H24.3
2	C9-3	H59.4
3	C1-2	H51.2
4	C4-1	H8.1
5	C10-4-15	H42.4
6	C13-2	H51.4
7	C19-4	H2.1
8	C1-4	H38.4
9	C7-2	H6.1
10	C14-4	H1.3
11	C13-4	H49.4
12	C10-1	H64.4
13	C7-1-6	H34.3
14	C5-2	H68.4
15	C1-3	H26.1
16	C15-2-2	H29.1
17	C13-1	H23.3
18	C15-4	H8.3
19	C3-1	H19.1
20	C10-4-11	H39.4
21	C25-3	H43.4
22	C5-1	H13.1
23	C22-4	H6.3
24	C15-2-8	H37.4
25	C15-2-26	H7.3
26	C15-3	H34.1
27	AZA2 (Resistance)	AZA2 (Resistance)
28	AC4 (Susceptible)	AC4 (Susceptible)

### 3.3.1 Field Survey

In order to determine the current status of the CLNBD, a survey was conducted in the cashew growing locations in the farmers' fields between January 2018 and April 2018; the time when new cashew vegetative shoots were emerging. This was a good timing as it was during the rainy season and also a time considered having conditions favourable for development of CLNBD (Sijaona *et al.*, 2006). The study districts coincided with three agro-ecological zones (Mtwara, Nachingwea and Coast regions) were selected for the study. One district per zone was selected depending of presence of genetic trials planted in 1996 and 1998. From each district, three villages were randomly selected from which three farms per village were purposively selected. The cashew farms selected were those which had cashew trees aged five to twenty years. Ten cashew trees per farm were selected for assessment of CLNBD. Three CLNBD scoring rounds were carried out at an interval of one month to collect disease incidence and severity. In addition, 90 diseased leaf samples were randomly picked during the survey as described in section 3.2.4.

### 3.3.2 Disease Incidence and Severity

The disease incidence was determined by placing a quadrat of 1 m x 1 m on top of the canopy of each cashew tree under assessment on its opposite sides (North and South) as described by TARI Pathologists Protocol (2012). The diseased shoots were counted against total shoots in the quadrat in each selected cashew tree multiplied by 100%. The disease severity was determined by using a scale of 0-6 (Table 2) as per TARI Pathologists Protocol (2012) whereby 0 meant no disease symptoms thus “resistant”, 1 to 2 mild CLNBD symptoms only on leaf tips thus “tolerant” and 3 to 6 plant leaf and or nuts with clear symptoms thus declared “susceptible” (Table 2). From 1 m x 1 m quadrat as described above the total number of shoots in each quadrat was carefully examined by scoring the disease symptoms for each five shoots for five leaves per shoot starting from the top of the shoot downwards, by using colour plates disease severity assessment guide for CLNBD (Appendix 1). The final scores were obtained using the formula below (TARI Pathologists Protocol, 2012).

$$= \sum_0^6 (0*L) + (1*L) + (2*L) + (3*L) + (4*L) + (5*L) + (6*L) / 50(L).$$

Whereby ‘L’ represents the number of leaves scored in two quadrates north and south directions per tree.

**Table 2:** The disease scoring scale of cashew leaf and nut blight disease showing levels, intervals and mid-point in percentages.

Level	0	1	2	3	4	5	6
Interval	0	1-20'	21-40	41-60	61-80	81-99	100
Mid-point	0	10.5	30.5	50.5	70.5	90	100
Grade	Resistance	Tolerant	Susceptible				

Source; TARI Pathologists Protocol, (2012)

### 3.3.3 Sample Collection and Culturing

A total of 90 leaf samples (30 from each zone) which were infested by the disease were randomly collected from different fields during the survey. The collected samples were well labelled and transferred to TARI-Mikocheni for fungal isolations. The fungi were isolated by direct conidial transfer method on potato dextrose agar (PDA) medium. The isolation method used was 39 gram of potato dextrose agar PDA medium prepared in flask dissolved in one litre of water, then autoclaved for 15 minutes in 121°C then poured into sterilized petri dishes for solidification. Every procedure was carried out in aseptic conditions, in a separate culture room free from dusts. The laminar flow was wiped with 70% alcohol, hands were cleaned and tools such as scalpels, forceps and knives were dipped in alcohol and flamed to prevent microbial contaminations.

The sample was cut into small square tissues of 0.5 cm section from the margin of lesions, so that they contain both diseased and healthy tissues. Surface sterilization of the tissue was done using disinfectant solution of 2% sodium hypochlorite (NaOCl) for one minute. Using flamed forceps, leaf pieces were washed in sterilized distilled water through three rinses to remove excessive disinfectant and placed on sterile filter paper to dry before being placed on petri dishes containing a culture medium, for incubation in a growth incubator under constant fluorescent. The leaf bits were placed in petri dishes containing sterilized PDA and incubated for four days at 25±2°C. The sporulated fungal on leaf bits were sub-cultured onto new sterilized PDA medium in petri dishes and incubated for five days at 25±2°C. After obtaining pure cultures, the isolates were transferred to fresh petri dishes containing PDA and cultivated

for five days again until sporulation. Fungal mycelia were scooped using a needle into 1.5 ml Eppendorf tubes for DNA extraction and the remained isolates were maintained on PDA slants for references.

### **3.3.4 Screen House Experiment**

To identify the most virulent fungal isolates, suspensions ( $1 \times 10^6$  conidia per ml) of isolated fungi were each spray inoculated onto four plants of susceptible cashew variety AC4 and placed in the screen house for seven days. Assessment was rated as 0= no disease symptoms after seven days, 1 (or +), disease symptoms occurring after 7 days of inoculation, 2 or ++ disease symptoms occurring 6-7 days after inoculation and 3 or +++ = disease symptoms occurring 5-6 days after inoculation. The most virulent fungal strain was then used for screening 52 cashew elite 1996 and 1998 hybrids (Table 1).

The cashew elite 1996 and 1998 hybrids were screened in the screen house located at TARI-Naliendele. Cashew seeds were raised in polythene tubes (diameter 10 cm and height 20 cm) using forest soil collected under cashew trees as growth media. Cashew seeds started germinating 14 days after sowing, and when the seedlings reached 30 to 45 days after sowing, they were used as rootstalks for grafting. Scions for grafting were collected from selected mother trees of the cashew elite 1996 and 1998 hybrids. Successful grafted seedlings were inoculated with fungal isolates from infected cashew materials at 45 days after grafting.

The design used in the trial was Completely Randomized Design (CRD), plot size was three plants per plot for each cashew hybrid in three replicates. There were two control varieties AZA2 which is resistant to the disease (positive) and AC4 which is susceptible to the disease (negative). The inoculation suspension was prepared by collecting leaves infected by CLNBD with level five and six of the disease scores scale. Using bucket of twenty litres, the infected leaves were soaked in fresh water mixed using stick then left for 24 hours. The suspension was filtered ready for inoculating cashew seedlings. Each plot was uniformly inoculated with a suspension of CLNBD causing pathogen at  $1 \times 10^6$  conidia per ml concentration at an interval of seven days for four weeks (28 days) using a hand sprayer (Menge *et al.*, 2014). Inoculated grafted seedlings were covered with polythene sheet for 24 hours to maintain relative humidity of about 90 - 95% and temperature of about 24-28°C (Menge *et al.*, 2014). The seedlings were examined for disease occurrence on daily basis for 7 days after inoculation and similarly assessed during the second, third and fourth re-inoculation. The disease infection was scored after every seventh day of inoculation in the screen house for 28 days in four rounds. The

varieties with scores of 0 were regarded as resistant, while those with scores of 1-2 and scores of 3-6 were regarded as tolerant and susceptible to CLNBD, respectively.

### **3.3.5 DNA Extraction**

The fungal DNA extraction was performed using a chloroform method (TARI-Mikocheni Protocol's). Fungal mycelia were scooped into 1.5 ml Eppendorf tubes containing 400 µl of extraction buffer, 50 µl of RNase A, and 20 µl of SDS (20%). The mycelia were crushed using plastic pestles before they were vigorously vortexed. The samples were then incubated at 65°C for 30 minutes. Samples were removed from heating blocks and left at room temperature for cooling and then centrifuged at maximum speed for 10 minutes. Equal amount of chloroform (phenol excluded) was added and samples were shaken vigorously and left for seven minutes on ice. The samples were centrifuged at maximum speed for two minutes and the supernatant was placed into a new 1.5 ml Eppendorf tube. Two volumes of cold isopropanol were added and the mixture was gently mixed by inversion. The 1/10 of 3 M NaOAC was added to the mixture inverted two to three times and kept at -20°C for 10 minutes to allow for DNA precipitation. Then it was centrifuged at 1200 rpm for 15 minutes. Supernatant was discarded and DNA pellets were washed with 700 µl of 70% ethanol and centrifuged for two minutes at maximum speed. Ethanol was discarded and the DNA pellets were air dried at room temperature for 40 minutes. The DNA pellets were re-suspended with 50 µl of TE buffer (10 mM Tris HCl and 1 mM EDTA). The DNA obtained was stored at -20°C for PCR analysis.

### **3.3.6 Polymerase Chain Reaction (PCR)**

The primers that target the Internal Transcribed Spacer (ITS) were used. The primers used included ITS1 (CTTGGTCATTTAGAGGAAGTAA) (forward) and ITS4 (GCTGCGTTCTTCATCGATGC) (reverse) and, ITS5 GGAAGTAAAAGTCGTAACAAGG (forward) and ITS4 GCTGCGTTCTTCATCGATGC (reverse) for fungal (White *et al.*, 1990). The reaction consisted of AccuPowerR PCR PreMix (20 µl tubes), 16 µl of water, 1 µl of ITS5 forward, 1 µl of ITS4 reverse and 2 µl of DNA for each. The PCR program was as follows: denaturation at 94°C for two minutes was followed by 35 cycles at 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minute and 72°C for 10 minutes. Then the temperature was held at 4°C for infinity. The agarose gel electrophoresis 50XTAE was prepared 50 mls with 0.5 g of agarose mixed in the beaker and heated at 105°C for 1 minute and gently cooling and, Ethidium Bromide was added in the solution then poured in the plate

for solidification. The PCR product was visualized with the aid of UV light in a Trans illuminator. The PCR products obtained were well parked and labelled sent for sequencing on both strands at Mbeya Referral Hospital Laboratory. Twenty four DNA samples from the three regions were sent for sequencing; however, eight DNA samples from each region were sequenced at Mbeya Referral Hospital Laboratory.

### **3.3.7 Construction of Phylogenetic Tree**

The phylogenetic relationship tree was reconstructed using the nucleotide sequences obtained from each region and from the National Collection of Biotechnological Information (NCBI) using a neighbour-joining by MEGA7. Bootstrap values over 50% are shown in each node. The scale bar indicates the number of expected changes per site. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-894.71) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) 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 then selecting the topology with superior log likelihood value. The analysis involved 24 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 132 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

### **3.4 Statistical Analysis**

The screen house experiment, field and survey data were analysed using Genstat, 16<sup>th</sup> Edition and mean separation was done using Duncan Multiple Range Test at ( $P \leq 0.05$ )

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Results

##### 4.1.1 The Current Status of Cashew Leaf and Nut Blight Disease in Farmers Fields in the Southern and the Eastern zones in Tanzania

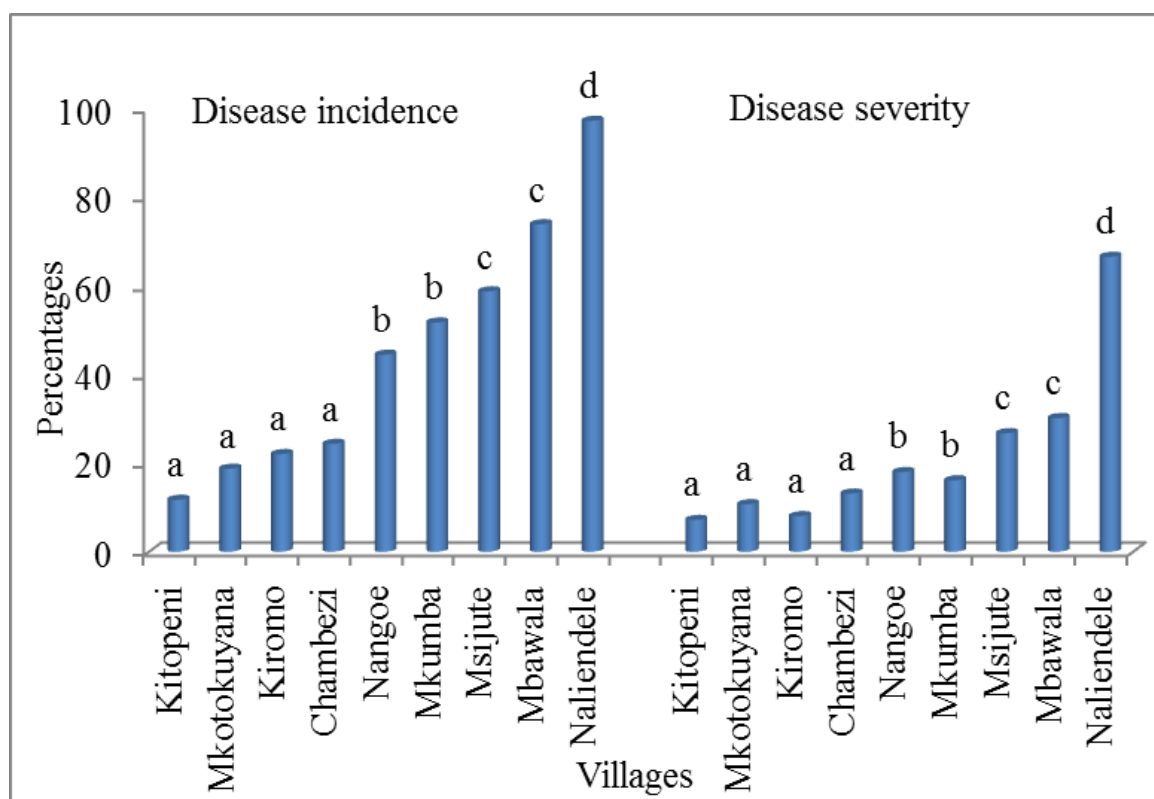
Results from the farmers' fields (on local cultivars) showed that there were highly significant differences ( $P < 0.001$ ) in disease incidence and severity between cashew plants growing in the surveyed locations (Table 3 and Fig. 3). The highest overall disease incidences and severities were recorded in Mtwara (71.15%, 37.90%) and the lowest in Bagamoyo (31.21%, 17.54%) districts. Of the surveyed villages in the study locations, the results show that Naliendele village (Mtwara district) scored the highest disease incidence (97.19%) and severity (66.51%) while Kitopeni village (Bagamoyo district) the lowest scores of disease incidence (11.71%) and severity (7.17%).

**Table 3:** CLNBD incidence and severity for “surveyed locations in Mtwara, Nachingwea and Bagamoyo districts”.

No	District	Disease incidence (%)	Disease severity (%)
1	Bagamoyo	31.21a	17.54a
2	Nachingwea	37.54a	22.56a
3	Mtwara	71.15b	37.90b
	Means	46.63	26.00
	L.S.D <sub>(0.05)</sub>	7.91	6.18
	P <sub>(test)</sub>	***	***

Means with the same letter (s) in the same column are not significantly different following Duncan's Multiple Range Test ( $P \leq 0.05$ ).





**Figure 3:** CLNBD incidence and severity for surveyed villages in Nachingwea, Mtwara and Bagamoyo districts. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ )

#### 4.1.2 The Status of Cashew Leaf and Nut Blight Disease in Cashew Elite 1996 and 1998 Hybrids

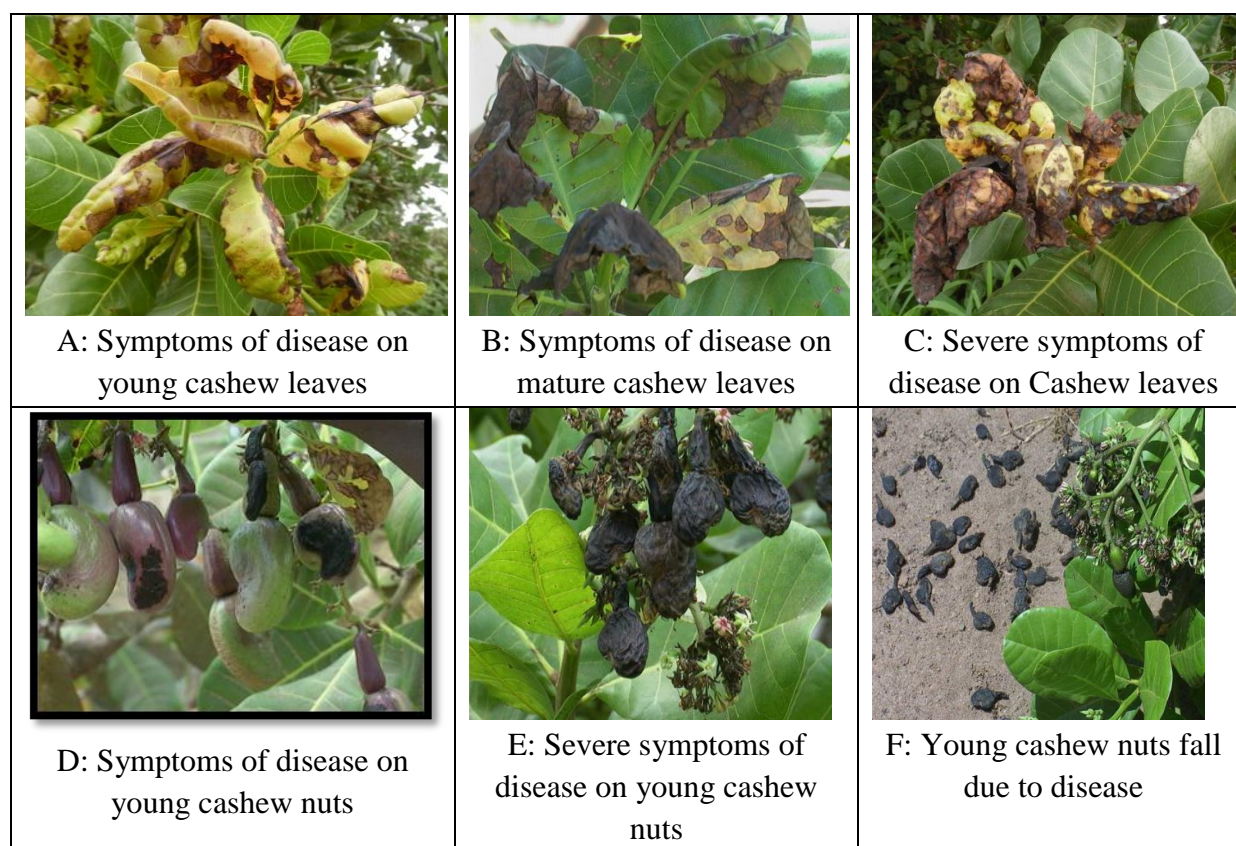
The results for three rounds of monitoring disease symptoms (Plate 1: A to F) in the trials to determine disease incidence and severity are presented in Tables 4 and 5. There were statistically significant differences ( $P < 0.01$ ) between hybrids in disease incidence and severity. When comparing disease incidence for cashew elite 1996 hybrids in Chambezi, hybrid C10-4-11 (2.03%) ranked first and differed significantly ( $P < 0.01$ ) from the last three hybrids C13-1 (24.72%), C10-4-15 (32.48%) and C3-1 (33.15%) (Table 4). Results on disease severity for cashew elite 1996 hybrids at Chambezi indicate that, hybrid C10-4-11 (0.49%) ranked first. However, it differed significantly ( $P < 0.01$ ) with the last four hybrids C9-3 (15.12%), C13-1 (13.95%), C3-1 (18.53%) and C10-4-15 (23.09%) (Table 4).

When comparing disease incidence for cashew elite 1996 hybrids in Nachingwea, hybrid C15-4 (1.28%) ranked first and was statistically significantly different ( $P < 0.01$ ) from the last two hybrids, C10-4-15 (30.38%) and C7-1-6 (34.69%) (Table 4). On the other hand, disease

severity for cashew elite 1996 hybrids in Nachingwea, hybrid C15-4 (0.89%) ranked first. However, the differences were not significant from other hybrids except the last two C10-4-15 (16.40%) and C3-4 (17.27%) (Table 4).

Results for disease incidence on cashew elite 1998 hybrids in Chambezi, hybrids H7.3 (1.21%) ranked first and the differences were not significant from the others, except the last three hybrids H39.4 (28.55%), H29.1 (29.72%) and H6.3 (44.65%) (Table 5). Disease severity for cashew elite 1996 hybrids in Chambezi, hybrid H7.3 (0.48%) ranked first but was not significantly different from the others, except the last five hybrids H34.3 (13.36%), H19.1 (15.80%), H39.4 (16.17%), H29.1 (17.62%) and H6.3 (18.89%) (Table 5).

The disease incidence for cashew elite 1998 hybrids in Nachingwea indicate that, hybrid H8.3 (5.76%) ranked first but it was not significantly different from the others, except the last eight hybrids H19.1 (25.10%), H1.3 (25.10%), H26.1 (27.29%), H39.4 (27.69%), H64.4 (30.29%), H13.1 (30.98%), H29.1 (31.99%) and H34.1 (33.10%) (Table 5). Disease severity for cashew elite 1998 hybrids in Nachingwea, hybrid H8.3 (2.23%) ranked first but was not significantly different from other hybrids, except the last five hybrids H26.1 (17.52%), H64.4 (17.89%), H34.1 (21.38%), H13.1 (21.45%) and H29.1 (24.112%) (Table 5).



**Plate 1:** CLNBD symptoms on cashew leaves and young nuts

**Table 4:** Incidence and severity of CLNBD on cashew elite 1996 hybrids in Chambezi and Nachingwea experimental sites during the current study.

No	Chambezi			Nachingwea		
	Hybrid	Incidence	Severity	Hybrid	Incidence	Severity
1	C10-4-11	2.03a	0.49a	C15-4	1.28a	0.89a
2	C5-1	2.14a	3.82a	C14-4	4.93a	2.80a
3	C19-4	4.04a	2.87a	C15-2-8	9.71a	3.88a
4	C1-4	6.66a	5.04a	C1-2	9.76a	4.81a
5	C15-4	6.68a	5.83a	C19-4	9.84a	8.52a
6	C14-4	8.43a	5.69a	C25-3	10.81a	4.59a
7	C1-3	8.98a	5.92a	C15-2-26	11.00a	10.58a
8	C15-2-2	9.52a	5.57a	C3-1	12.09a	7.33a
9	C15-2-8	10.14a	7.18a	C15-3	13.21a	9.98a
10	C15-3	12.00a	10.58a	C4-1	14.18a	7.91a
11	C1-2	12.41a	4.89a	C7-1-13	14.66a	7.18a
12	C22-4	12.51a	8.14a	C13-2	15.21a	4.93a
13	C4-1	13.01a	6.99a	C7-2	15.23a	9.17a
14	C7-2	14.86a	5.42a	C5-1	15.37a	14.96a
15	C15-2-26	15.44a	11.09a	C10-4-11	15.77a	8.90a
16	C13-4	16.41a	9.01a	C13-1	15.88a	5.88a
17	C10-1	17.73a	11.03a	C10-1	16.26a	9.33a
18	C7-1-6	18.03a	9.31a	C15-2-2	17.86a	9.36a
19	C5-2	18.23a	7.93a	C1-3	18.02a	11.30a
20	C9-3	22.02a	15.12b	C22-4	21.53a	12.01a
21	C13-2	22.22a	9.87a	C1-4	21.55a	14.97a
22	C7-1-13	22.32a	11.24a	C9-3	22.73a	12.47a
23	C25-3	22.74a	11.77a	C5-2	25.84a	13.49a
24	C13-1	24.72b	16.95c	C13-4	26.41a	17.27c
25	C10-4-15	32.48c	23.09e	C10-4-15	30.38b	16.40b
26	C3-1	33.15c	18.53d	C7-1-6	34.69c	14.79a
Means		15	8.98	Means	17.25	9.79
L.S.D <sub>(0.05)</sub>		17.56	10.25	L.S.D <sub>(0.05)</sub>	21.37	9.39
P <sub>(test)</sub>		*	**	P <sub>(test)</sub>	*	***

Means with the same letter (s) in the same column are not significantly different following Duncan's Multiple Range Test ( $P \leq 0.05$ ).

**Table 5:** Incidence and severity of CLNBD on cashew elite 1998 hybrids in Chambezi and Nachingwea experimental sites during the current study.

Chambezi				Nachingwea		
No	Hybrids	Incidence	Severity	Hybrids	Incidence	Severity
1	H7.3	1.21a	0.48a	H8.3	5.76a	2.23a
2	H51.2	4.96a	0.89a	H24.3	7.39a	4.13a
3	H8.3	5.58a	4.26a	H49.4	8.71a	3.82a
4	H51.4	5.76a	2.83a	H59.4	10.79a	3.77a
5	H59.4	6.37a	2.22a	H51.4	13.43a	6.53a
6	H49.4	7.90a	0.90a	H37.4	14.07a	6.57a
7	H24.3	10.55a	5.03a	H8.1	14.11a	10.71a
8	H68.4	10.82a	7.15a	H6.1	14.15a	7.53a
9	H37.4	11.57a	5.18a	H7.3	14.54a	9.83a
10	H43.4	11.8a	4.83a	H38.4	14.72a	9.23a
11	H2.1	12.55a	6.95a	H2.1	15.12a	7.06a
12	H8.1	12.58a	6.40a	H6.3	17.28a	7.16a
13	H6.1	13.37a	10.66a	H42.4	17.62a	10.34a
14	H38.4	14.02a	5.17a	H68.4	18.41a	11.02a
15	H26.1	15.43a	7.54a	H34.3	18.65a	11.18a
16	H19.1	15.83a	15.80c	H23.3	20.68a	16.10a
17	H1.3	16.69a	11.10a	H43.4	23.41a	13.35a
18	H23.3	17.24a	7.38a	H51.2	24.10a	14.60a
19	H64.4	17.72a	11.78a	H19.1	25.10b	15.88a
20	H34.1	17.75a	10.71a	H1.3	25.10b	16.13a
21	H42.4	19.40a	5.48a	H26.1	27.29c	17.52b
22	H34.3	24.31a	13.36b	H39.4	27.69d	15.58a
23	H13.1	25.14a	9.93a	H64.4	30.29e	17.89b
24	H39.4	28.55b	16.17d	H13.1	30.98e	21.45c
25	H29.1	29.72b	17.62e	H29.1	31.99e	24.12d
26	H6.3	44.65c	18.89f	H34.1	33.10f	21.38c
Means		15.44	8.03	Means	19.4	11.74
L.S.D <sub>(0.05)</sub>		21.11	9.29	L.S.D <sub>(0.05)</sub>	15.49	11.85
P <sub>(test)</sub>		*	**	P <sub>(test)</sub>	**	**

Means with the same letter (s) in the same column are not significantly different following Duncan's Multiple Range Test ( $P \leq 0.05$ ).

#### **4.1.3 Cashew Leaf and Nut Blight Disease on Cashew Elite 1996 and 1998 Hybrids Seedlings in the Screen House Experiment**

Using 90 diseased leaves samples collected from different locations, about 120 fungal isolates were obtained. Of these only 24 isolates shown in Table 6 induced disease symptoms on cultivar AC4 similar to those observed in the field (Plate 1). Of these, isolate Mtwara 3 was the most virulent after inducing disease symptoms only at about 5-6 days after inoculation (Table 6). This fungal isolate was used for screening cashew elite 1996 and 1998 hybrids and the results with cashew seedlings in the screen house (Plate 2: A to F) shows that there were highly significant differences ( $P < 0.001$ ) between cashew elite hybrids ability to react to infestation by CLNBD-causing pathogens in all trials (Appendix 2). When comparing disease severity for cashew elite 1996 hybrids in the screen house, variety AZA2 (0.88%) ranked first followed by C1-3 (0.93%), C15-2-26 (1.51%), and C9-3 (1.58%) with low severity but was not significantly different from 28 hybrids, except the last three hybrids C13-2 (6.96%), C3-1 (7.01%), C7-1-6 (9.39%) and variety AC4 (14.44%) (Fig. 4). Severity for cashew elite 1998 hybrids in the screen house, variety AZA2 (0.77%) again ranked first followed by H34.3 (1.21%), H13.1 (1.35%), H7.3 (1.71%) and H19.1 (2.64%) in-terms of low disease severity; however, significant differences were observed ( $P < 0.001$ ) for the last four hybrids H26.1 (6.17%), H49.4 (6.51%), H6.3 (6.7%), H68.4 (6.97%) and variety AC4 (18.31%) (Fig. 5).





A: Cashew rootstalk seedlings



B: Cashew seedlings ready for grafting



C: Grafting cashew seedlings



D: Grafted cashew seedlings

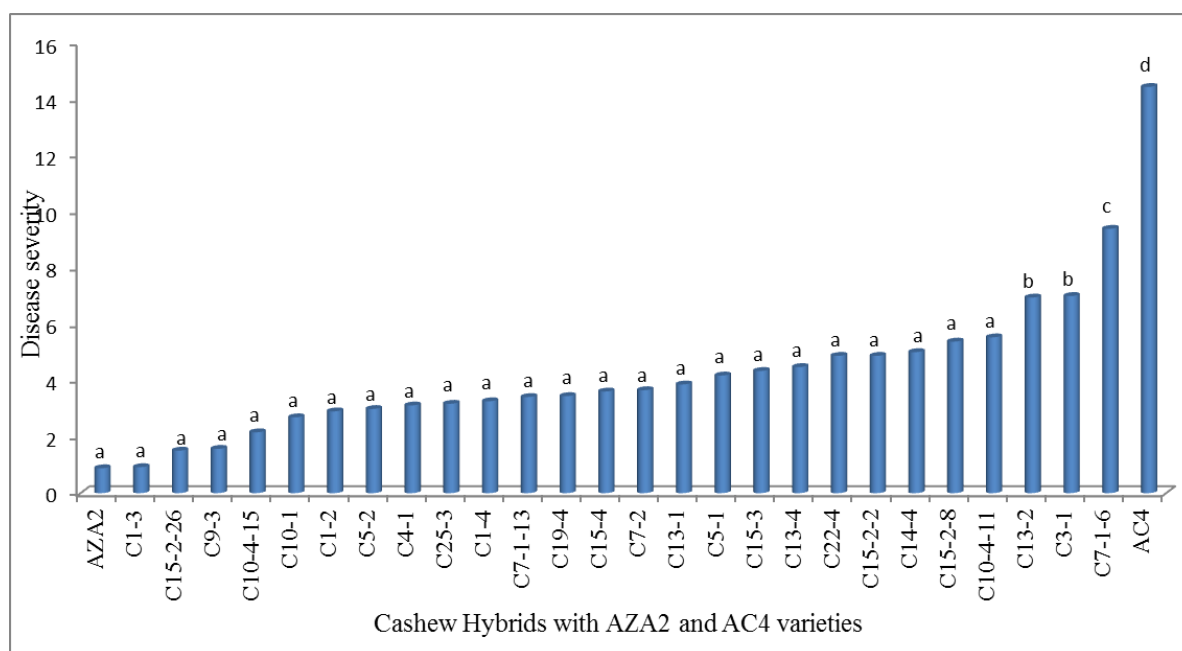


E: Grafted Cashew seedlings ready for  
innoculation with CLNBD-causing fungi

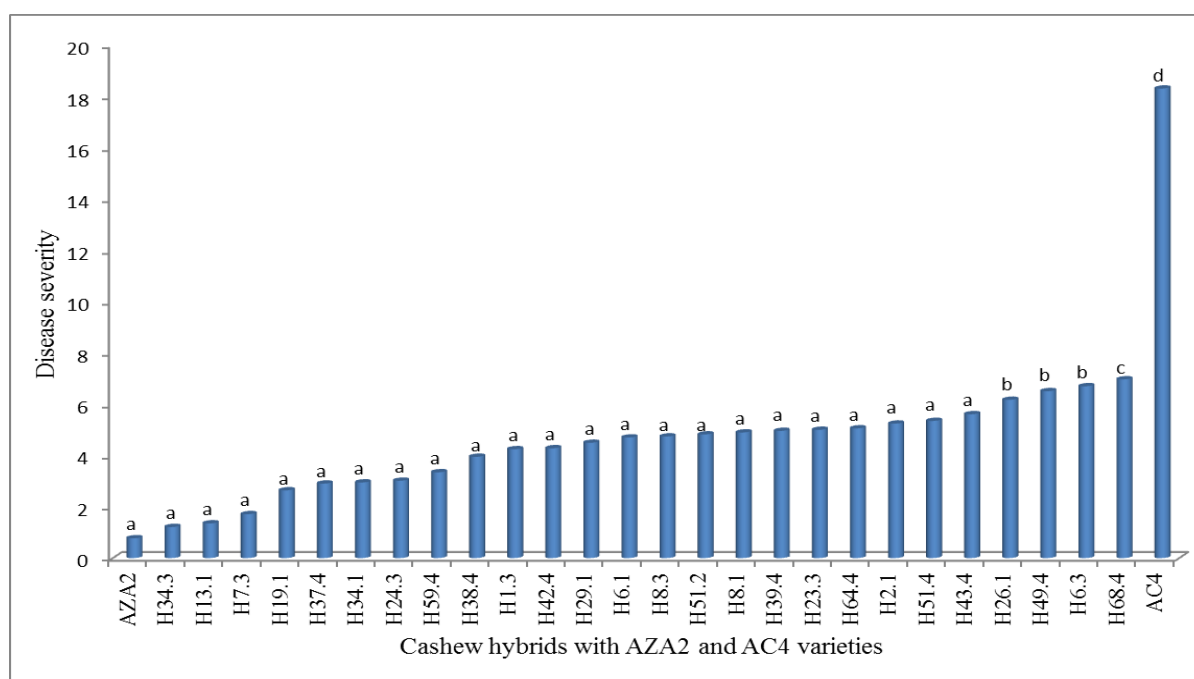


F: Cashew seedlings inoculated with  
CLNBD-causing pathogen

**Plate 2:** Grafting of cashew elite hybrids in screen house during this study



**Figure 4:** CLNB Disease severity scored on cashew elite 1996 hybrids in screen house experiment at TARI-Naliendele in March, 2018. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ ).



**Figure 5:** CLNB Disease severity scored on cashew elite 1998 hybrids in screen house experiment at TARI-Naliendele in March, 2018. Similar letter(s) on bar picks indicated no significantly different based on Duncan's Multiple Range Test ( $P \leq 0.05$ ).

#### **4.1.4 Molecular-Based Analysis and Phylogenetic Relationship of Fungi Isolates Associated with the Cashew Leaf and Nut Blight Disease**


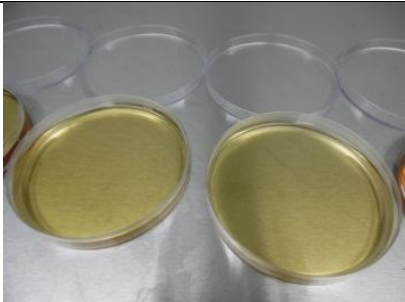
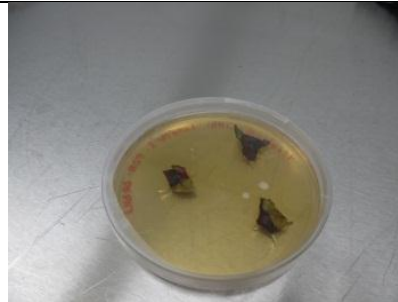



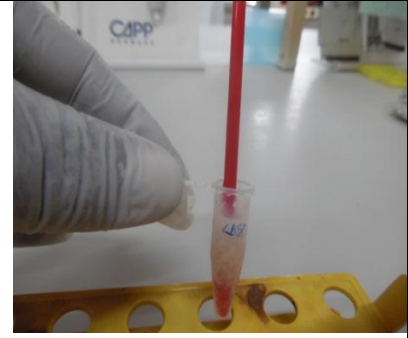


Using fungal isolates presented in Table 6 and procedures highlighted in Plate 3: A to I, the results show that ITS5 (forward) and ITS4 (reverse) amplified DNA from isolates with a product size of about 500 bp (Fig. 6). The results for PCR analysis using primer ITS1 (forward) and ITS4 (reverse) showed an amplification of tested DNA of fungal isolates with a product of about 320 bp (Fig. 7). The fungal isolates had identities of 97-99% similar to fungal species presented in Table 7. The results for BLAST analysis showed that only 10 out of 24 DNA samples sequenced (Appendix 3) were 97-99% identity to known fungal species with those present in the National Center for Biotechnological Information (NCBI) collection (Table 7). Generally five major clusters were created (Fig. 8). Cluster 1 = *Lasiodiplodia* spp, cluster 2 = *Colletotrichum* spp, cluster 3 = *Diaporthe* spp and *Phomopsis*, cluster 4 = *Pestalotiopsis* spp and Cluster 5 = *Curvularia* spp. Representative sequences for each the organisms (highlighted by red colour in Fig. 8) has been deposited and assigned Gene bank accession numbers at NCBI as follows: MH715267, MH715268, MH715269, MH715270, MH715271, MH715272, MH715273, MH715274, MH715275, and MH715276 respectively. Based on this information, it is evident that *Botryosphaeria* spp, *Pestalotiopsis* spp, *Colletotrichum gloeosporioides*, *Colletotrichum fragariae*, *Neofusicoccum* spp, *Colletotrichum* spp, *Lasiodiplodia iranensis* and *Lasiodiplodia theobromae* are organisms associated with CLNBD in Tanzania



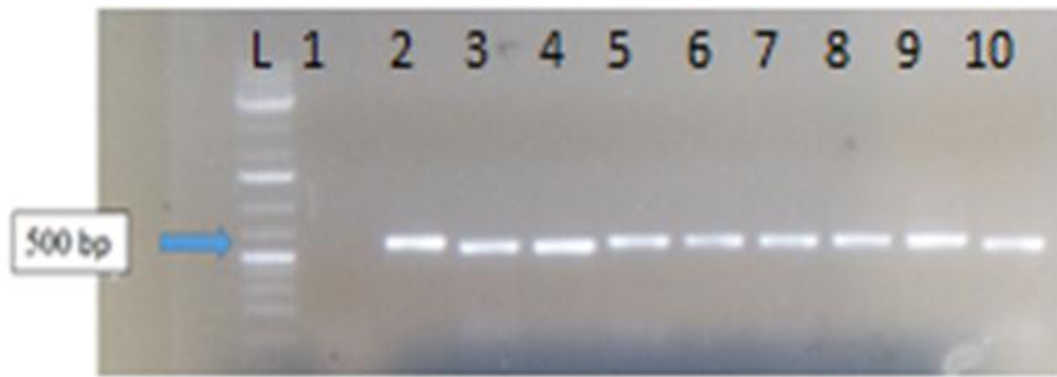
**Table 6:** Cashew leaf and nut blight isolates obtained from lesions of infected cashew leaves and their virulence on cashew variety AC4

No	Collection code	Collection site	Virulence on AC4	Collection per month
1	Lindi1	Nachingwea	++	March 2018
2	Lindi2	Nachingwea	++	March 2018
3	Lindi3	Nachingwea	++	March 2018
4	Lindi4	Nachingwea	++	March 2018
5	Lindi5	Nachingwea	++	March 2018
6	Lindi6	Nachingwea	++	March 2018
7	Lindi7	Nachingwea	++	March 2018
8	Lindi8	Nachingwea	++	March 2018
9	Mtwara1	Naliendele	++	March 2018
10	Mtwara2	Naliendele	++	March 2018
11	Mtwara3	Naliendele	+++	March 2018
12	Mtwara4	Naliendele	++	March 2018
13	Mtwara5	Naliendele	++	March 2018
14	Mtwara6	Naliendele	++	March 2018
15	Mtwara7	Naliendele	++	March 2018
16	Mtwara8	Naliendele	++	March 2018
17	Coastal1	Chambezi	++	March 2018
18	Coastal2	Chambezi	++	March 2018
19	Coastal3	Chambezi	++	March 2018
20	Coastal4	Chambezi	++	March 2018
21	Coastal5	Chambezi	++	March 2018
22	Coastal6	Chambezi	++	March 2018
23	Coastal7	Chambezi	++	March 2018
24	Coastal8	Chambezi	++	March 2018

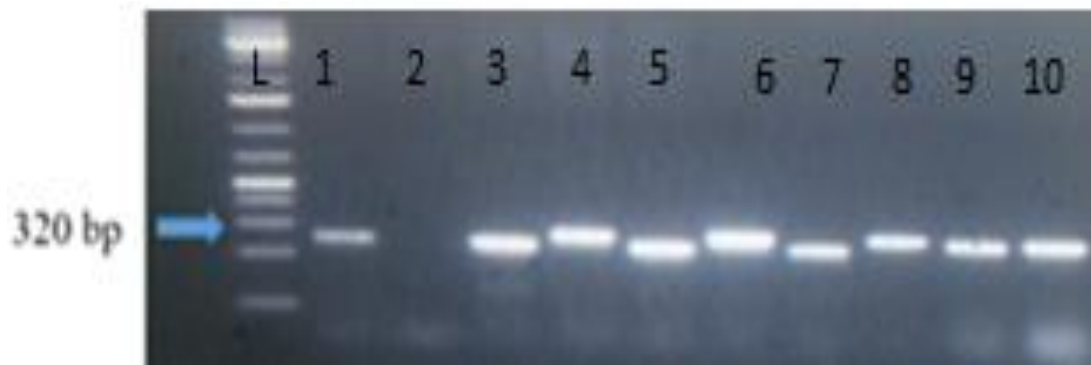
+ = disease symptoms occurring 7days after inoculation, ++ = disease symptoms occurring 6-7 days after inoculation and +++ = disease symptoms occurring 5-6 days after inoculation

		
<p>A: Diseased leaf used for isolation (sample taken from margin of diseased section)</p>	<p>B: Sterilized potato dextrose agar ready for plating samples</p>	<p>C: Diseased leaf bits plated on PDA media</p>
		
<p>D: Appearance of fungal mycelia on PDA on day 5 after plating</p>	<p>E: Appearance of purified fungal isolate on new PDA</p>	<p>F: Full grown fungal mycelia on PDA</p>
		
<p>G: Preparation of fungal material for DNA extraction</p>	<p>H: Preparation of master mix for DNA analysis</p>	<p>I: PCR machine used for PCR reactions</p>

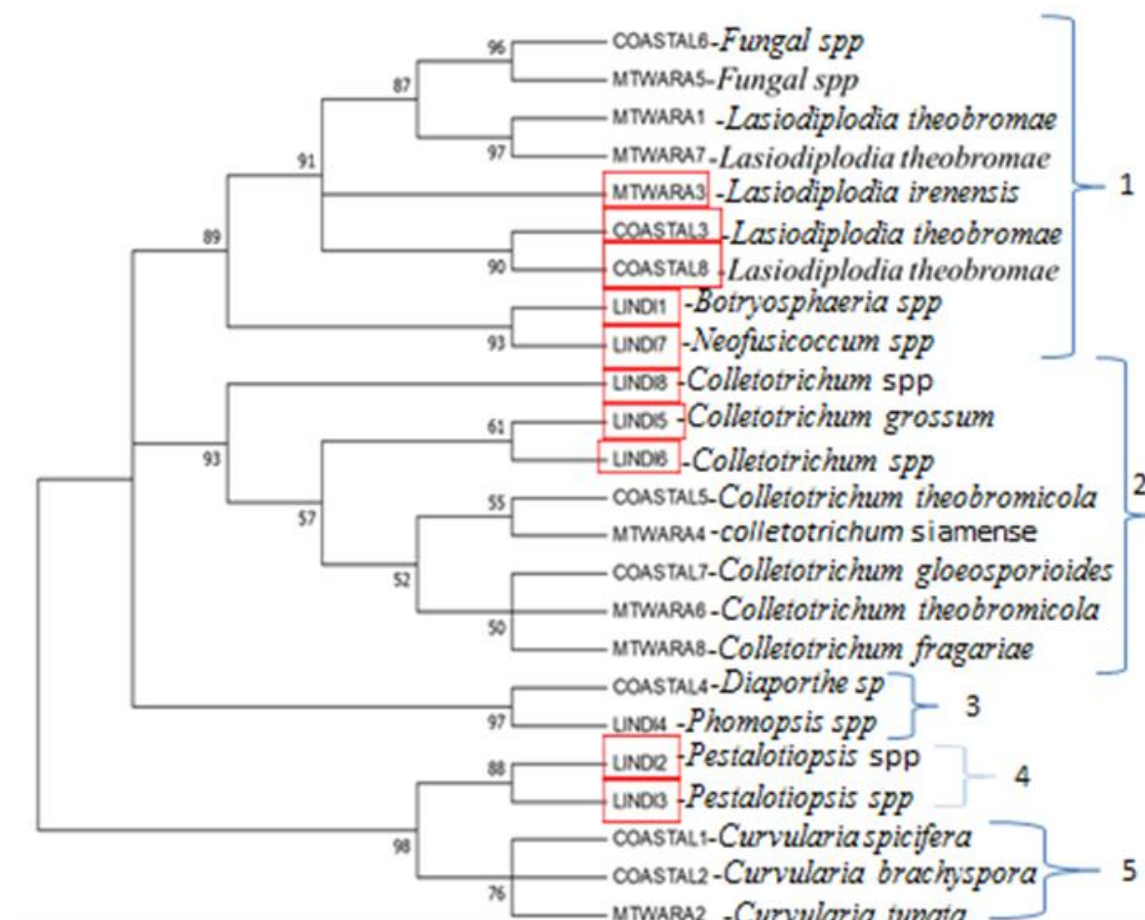
**Plate 3:** DNA extraction steps followed during for fungal isolation from the infected cashew leaves.



**Figure 6:** Amplification of a sample of pathogenicity positive isolates by PCR using ITS5 and ITS4 primers. Where; L is DNA ladder, 1 is a control, 2-10 (Lindi1, Lindi2, Lindi3, Lindi4, Lindi5, Lindi6, Lindi7, Lindi8, Lindi9 and Lindi10) samples for detection of CLNBD causing fungi.



**Figure 7:** Amplification of a sample of pathogenicity positive isolates by PCR using ITS1 and ITS4 primers. Where L is DNA ladder, 1-10 (1 Mtwara1, 2 control, 3 Mtwara2, 4 Mtwara3, 5 Mtwara4, 6 Mtwara5, 7 Coast1, 8 Coast2, 9 Coast3 and 10 coast4) samples for detection of CLNBD causing fungi.



**Figure 8:** Phylogenetic tree reconstructed using the nucleotide sequences of CLNBD isolates.

N.B: The phylogenetic tree was constructed with a neighbour-joining method in MEGA7 using bootstrap values over 50%, red blocks show samples sequenced with 97-99% identity to known fungal species.

**Table 7:** Summary of positive samples with maximum nucleotide identities of 97-99% similarity to CLNBD causing fungi

S/N	Description Species	Collection code	Max score	Query cover	Identity	Accession
1.	<i>Botryosphaeria</i> spp.	Lindi1	1011	98%	99%	EU330628.1
2.	<i>Pestalotiopsis</i> spp.	Lindi2	942	97%	99%	HQ607806.1
3.	<i>Pestalotiopsis</i> spp.	Lindi3	958	98%	99%	KF179297.1
4.	<i>Colletotrichum gloeosporiodes</i>	Lindi5	998	97%	99%	JX258732.1
5.	<i>Colletotrichum fragariae</i>	Lindi6	1000	98%	99%	KC209101.1
6.	<i>Neofusicoccum</i> spp.	Lindi7	978	97%	99%	KC706920.1
7.	<i>Colletotrichum</i> spp.	Lindi8	975	98%	99%	KJ493232.1
8.	<i>Lasiodiplodia iranensis</i>	Mtwara3	376	97%	99%	KU997384.1
9.	<i>Lasiodiplodia theobromae</i>	Coastal3	942	99%	99%	KJ596523.1
10.	<i>Lasiodiplodia theobromae</i>	Coastal8	374	97%	99%	FJ904840.1

## 4.2 Discussion

The current status to of cashew leaf and nut blight disease - causing fungi in the Southern and Eastern zones of Tanzania has been established in this study. The results have shown that the CLNBD incidence in the area covered by the present study ranged from 31% in Bagamoyo to 71% in Mtwara districts. Such results implied that CLNBD is wide spread in the study area. These relatively high incidences of CLNBD on cashew could be due to predominant use of susceptible planting materials (TARI Pathologists Protocol, 2012) or favourable environmental conditions for CLNBD development. It has been clearly established that for disease to occur, three factors namely host susceptibility, virulence of the pathogen and favourable environment are necessary (Lopez, 2008; Suffert *et al.*, 2011). In this study, the CLNB disease severity on cashew was also high in Mtwara compared to other Districts. Such observation could also be explained as due to host susceptibility or favorable environment (i.e, temperature, rainfall, high humidity etc) for the pathogens to incite the CLNBD (Sijaona *et al.*, 2005; Sijaona *et al.*, 2006).

Efforts to address the CLNBD through developing cashew hybrids have been going on at the TARI Naliendele (CRP, 2016). For instance, in this study, 52 elite cashew hybrids planted in three different agro-ecological locations were evaluated for CLNBD incidence and severity. The results have indicated that most of the cashew elite 1996 and 1998 hybrids developed are

tolerant to the CLNBD using a protocol by TARI Pathologists Report (2012). The ability of the cashew elite hybrids to tolerate infestation by the CLNBD-causing fungi could be due to their inherent genetic tolerance. The genotypes were consistently tolerant for CLNBD across the surveyed sites (Masawe, 2006; Sijaona, 2013).

To verify and test whether or not the tolerance of the elite cashew hybrid was due to inherent genotypic ability, a screen house experiment was set and the findings clearly concurred with the results from the three different locations. Out of 52 elite cashew hybrids inoculated with the most virulent CLNBD-causing fungi isolated from Mtwara region (Mtwara 3). The results consistently showed that 38 out of 52 cashew elite 1996 and 1998 hybrids developed were tolerant to CLNBD. It seemed interestingly that a CLNBD resistance variety AZA2 (positive control) was not significantly different from other hybrids, while variety AC4 (negative control) ranked last and differences in disease severity were highly significant. Reduced disease infection in variety AZA2 revealed high levels of resistance in disease infection (Menge *et al.*, 2014).

Using the ITS primers and sequence information, it was evident that the CLNBD in Tanzania is caused by different fungal species including those from genera *Lasiodiplodia* spp, *Colletotrichum* spp, *Neofusicoccum* spp and *Pestalotiopsis* spp. These results show a number of studies that indicate pathogenic nature of the identified organisms (Uaciquete, 2003; Sijaona, 2013; Zhongrun and Masawe, 2014). Though the sequence identity for some other isolates were below 95%, it remains possible that two more or three more fungal species such as *Curvularia* spp and others presented in Fig. 8 are associated with the CLNBD in Tanzania. The CLNBD caused by *Cryptosporiopsis* spp reported by Sijaona *et al* (2006) was not detected in the current study. Based on these results the following fungal microorganisms namely *Botryosphaeria* spp, *Pestalotiopsis* spp, *Colletotrichum gloeosporioides*, *Colletotrichum fragariae*, *Neofusicoccum* spp, *Colletotrichum* spp, *Lasiodiplodia iranensis* and *Lasiodiplodia theobromae* are reported for the first time to be associated with CLNBD in Tanzania.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

This study revealed that area surveyed is affected by CLNBD. The highest disease incidence and severity was recorded in Mtwara and the lowest disease incidence and severity was recorded at Bagamoyo districts implying that CLNBD was present in the surveyed locations at different levels. The present study has also identified a total of 38 out of 52 cashew hybrids that are tolerant to CLNBD while 14 were susceptible to CLNBD. These results indicate that there are materials that can be used in breeding programs for developing cashew varieties that are resistant to CLNBD. The present study also have showed that CLNBD is caused by a complex of fungal species such as *Botryosphaeria* spp, *Pestalotiopsis* spp, *Colletotrichum gloeosporioides*, *Colletotrichum fragariae*, *Neofusicoccum* spp, *Colletotrichum* spp, *Lasiodiplodia iranensis* and *Lasiodiplodia theobromae*. These fungal microorganisms are reported for the first time to be associated with CLNBD in Tanzania.

#### 5.2 Recommendations

- (i) Further studies are recommended to study the host range of the new fungal species identified in this study and specific pathogen-host interaction thresholds on cashew in Tanzania.
- (ii) Further studies are also recommended for a wide geographical location to establish the status of each of the identified fungal species so that specific and or integrated disease management approaches may be designed
- (iii) The identified tolerant cashew elite hybrids are recommended for further screening and potential source for resistance to the cashew leaf and nut blight disease in Tanzania.
- (iv) However, further studies are recommended on screening a larger number cashew genotypes collected from different locations and sources in Tanzania against each of the newly identified fungal organisms.

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## APPENDICES

### Appendix 1: The severity assessment guide for cashew leaf and nut blight disease score.

	<p><b>DISEASE LEVEL 0</b></p> <p>Leaf symptoms:</p> <p>No any disease symptom</p> <p>0 % disease severity</p>
	<p><b>DISEASE LEVEL 1</b></p> <p>Leaf symptoms:</p> <p>1 – 3 Darktan angular lessions</p> <p>1 – 2 lessions enlarged not coalesced,</p> <p>% disease; 1 – 20 (Average 10.5%)</p>
	<p><b>DISEASE LEVEL 2</b></p> <p>Leaf symptoms:</p> <p>4 – 8 Darktan angular lessions</p> <p>2 – 3 lessions enlarged not coalesced,</p> <p>% disease; 21 – 40 (Average 30.5 %)</p>



DISEASE LEVEL 3

### DISEASE LEVEL 3

Leaf symptoms:

9 – 12 Darktan angular lesions

4 – 6 lesions enlarged not coalesced,

% disease; 41 – 60 (Average 50.5 %)



DISEASE LEVEL 4

### DISEASE LEVEL 4

Leaf symptoms:

>12 Darktan angular lesions

Lessions enlarged and < 50% coalesced,

% disease; 61 – 80 (Average 80.5 %)



DISEASE LEVEL 5

### DISEASE LEVEL 5

Leaf symptoms:

Lessions enlarged and 75% coalesced causing blighting

% disease; 81 – 100 (Average 90.5 %)

**Appendix 2: CLNBD severity on cashew elite 1996 and 1998 hybrids in the screen house experiment at TARI-Naliendele.**

Source	Mean squares		
	d.f	Severity (1996)	Severity (1998)
Rep	2	15.30	1.44
Hybrids	27	22.49***	29.49***
Error	54	5.46	4.08
Mean		4.28	4.71
L.S.D		3.83	3.28
CV (%)		54.60	42.60

\*P ≤0.05, \*\*P ≤0.01, \*\*\*P ≤0.001

### **Appendix 3: Ten fungal species associated with cashew leaf and nut blight disease in Tanzania deposited in NCBI gene bank**

>Seq1 [Organism=*Botryosphaeria* sp.] Majun1 internal transcribed spacer, partial sequence

CGGACGTACTGATCCGAGGTCACCTTGAGAATAATTCAAAGGTTTCGTCCGGCGGG  
CGACGCCGTGCGCTCCAAAGCGAGGTGTTTTCTACTACGCTTGAGGCAAGACGCC  
ACCGCCGAGGTCTTTAAGGCGCGTCCGCGGAGGACGGAGCCCAATACCAAGCAG  
AGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCTCGGAATACCAAGGG  
GCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACT  
TATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAA  
AGTTTTAGTTTATTAACCTTGTTTTTCAGACTGCGAAGTTCACTGACTGGAGTTTTA  
TGGTCCTCTGGCGGGCGCTGGCCAGCCCCCCCCGAAGGGCGCCGGTGC GGAGGAC  
CGCGGCCCCGCCAAAGCAACAGAGGTAGGTACACATTGGGTGGGAGAGTCGAGCC  
GGAGCTCGAATCAACTCGGTAATGATCCTTCCGCAGGTTCACCTACGAAACCTTG  
TTACACTTTTTACTTC

>Seq2 [Organism=*Pestalotiopsis* sp.] Majun2 internal transcribed spacer, partial sequence

AGGGAATTCTCTGATCGAGGTCAACCACAAAAAATTGGGGGTTTAGCGGCTGGG  
AGTTATAGCACCTAACAAAAGCGAGAAAAAAATTACTACGCTCAGAGGATACTA  
CAAATCCGCCGTTGTATTTTCAGGAACTACAACCTCCTAAGAGAAGTAGATTCCCA  
ACACTAAGCTAGGCTTAAGGGTTGAAATGACGCTCGAACAGGCATGCCCCTAG  
AATACTAATGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCA  
ATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAG  
ATCCGTTGTTGAAAGTTTTGACTTATTAATAAAGACGCTCAGATTACATAAAAT  
AACAAGAGTTTAATGGTCCACCGGCAGCAGCTATAAGGAGACCTATAACTTCTG  
CCGAGGCAACAAAAGGTAAGTTCACATGGGTTGGGAGTTTAGAAAACCTCTATAA  
TGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTTTACTTCCCAA

>Seq3 [Organism=*Pestalotiopsis* sp.] Majun3 internal transcribed spacer, partial sequence

TGCATTCTACTGATCGAGGTCACCACAAAAAATTGGGGGTTTAGCGGCTGGGAGT  
TATAGCACCTAACAAAAGCGAGAAAAAAATTACTACGCTCAGAGGATACTACAA  
ATCCGCCGTTGTATTTTCAGGAACTACAACCTCCTAAGAGAAGTAGATTCCCAACAC  
TAAGCTAGGCTTAAGGGTTGAAATGACGCTCGAACAGGCATGCCCCTAGAAATAC  
TAATGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA



CATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT  
TGTTGAAAGTTTTGACTTATTAATAAGACGCTCAGATTACATAAAATAACAAG  
AGTTTAATGGTCCACCGGCAGCAGCTATAAGAAGACCTATAACTTCTGCCGAGGC  
AACAAAAGGTAAGTTCACATGGGTTGGGAGTTTAGAAAACCTCTATAATGATCCCT  
CCGCTGGTTCACCAACGGAGACCTTGTTACGATTTTACTTCCAAA

>Seq4 [Organism=*Colletotrichum gloeosporioides*] Majun5 internal transcribed spacer,  
partial sequence

AGGGGATTCTGATCCGAGGTCACCTTTGGAAAATTGGGGGGTTTTACGGCAAGAG  
TCCCTCCGGATCCCAGTGCGAGACGTAAAGTTACTACGCAAAGGAGGCTCCGGGA  
GGGTCCGCCACTACCTTTGAGGGCCTACATCAGCTGTAGGGCCCCAACACCAAGC  
AGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCGCCAGAATGCTGGC  
GGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATT  
ACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT  
AAAAGTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGAGT  
TTGGTTATCCTCCGGCGGGCGCCGACCCGCCCCGGGGGGGCGGGAGGCCGGGAGG  
GTCACGGGGACCCTACCCGCCGAAGCAACAGTTGTAGGTATGTTACAAAGGGTT  
GTAGAGCGTAAACTCAGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGT  
TACGACTTTTACTTCC

>Seq5 [Organism=*Colletotrichum fragariae*] Majun6 internal transcribed spacer, partial  
sequence

ATGGCATCACTGATCCGAGGTCACCTTTGGAAAATTGGGGGGTTTTACGGCAAGAG  
TCCCTCCGGATCCCAGTGCGAGACGTAAAGTTACTACGCAAAGGAGGCTCCGGGA  
GGGTCCGCCACTACCTTTGAGGGCCTACATCAGCTGTAGGGCCCCAACACCAAGC  
AGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCGCCAGAATGCTGGC  
GGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATT  
ACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT  
AAAAGTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGAGT  
TTGGTTATCCTCCGGCGGGCGCCGACCCGCCCCGGGGGGGCGGGAGGCCGGGAGG  
GTCACGGGGACCCTACCCGCCGAAGCAACAGTTGTAGGTATGTTACAAAGGGTT  
GTAGAGCGTAAACTCAGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGT  
TACGATTTTTTACTTCCA

>Seq6 [Organism=*Neofusicoccum* sp.] Majun7 internal transcribed spacer, partial sequence

CGGGGTACTGCGGAGCATTACCGAGTTGATTCGAGCTCCGGCTCGACTCTCCCAC  
CCAATGTGTACCTACCTCTGTTGCTTTGGCGGGCCGCGGTCTCCGCACCGGCGCC  
CTTCGGGGGGGCTGGCCAGCGCCCCGCCAGAGGACCATAAAACTCCAGTCAGTGA  
ACTTCGCAGTCTGAAAAACAAGTTAATAAACTAAACTTTCAACAACGGATCTCT  
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA  
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTTGGTATTCCGA  
GGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTATTGGGCT  
CCGTCCTCCGCGGACGCGCCTTAAAGACCTCGGCGGTGGCGTCTTGCCTCAAGCG  
TAGTAGAAAACACCTCGCTTTGGAGCGCACGGCGTCGCCCCGCCGGACGAACCTTT  
GAATTATTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG  
CATATCAATAAGCGAGGAA

>Seq7 [Organism=*Colletotrichum* sp.] Majun8 internal transcribed spacer, partial sequence

CGGGATCGCTGATCGAGGTCACCTTTGGAAATTGGGGGGTTTTACGGCAGAGTCC  
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GTCCGCCACTACCTTTGAGGGCCTACATCAGCTGAGGGCCCCAACACCAAGCAGA  
GCTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATGCTGGCGGGC  
GCAATGTGSGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTA  
TCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAA  
GTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGT  
TATCCTCCGGCGGGCGCCGACCCGCCCCGGGGGGGGCGGGAGGCCGGGAGGGTAC  
GGGGACCCTACCCGCCGAAGCAACAGTTGTAGGTATGTTCAAAAGGGTTGTAGA  
GCGTAAACTCAGTAATGATCCCTCCGCTGGTTCACCAAGGAGACCTTGTTACGAC  
TTTTACTTCC

>Seq8 [Organism=*Lasiodiplodia iranensis*] Majun9 internal transcribed spacer, partial sequence

AGTCGTGGTGAACCTGCGGAAGGATCATTTACCGAGTTTTTCGGGCTTCGGCTCGA  
CTCTCCCACCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTCCGGCCGCCAAAG  
GACCTCCAAACTCCAGTCAGTAAACGCAGACGTCTGATAAACAAGTTAATAAACT  
AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCA

>Seq9 [Organism= *Lasiodiplodia theobromae*] Majun10 internal transcribed spacer, partial sequence

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GGGCGACGCCAACCGCTCCAAAGCGAGGTGTATTCTACTACGCTTGAGGGCTGAA  
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CAAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA  
CATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT  
TGTTGAAAGTTTTAGTTTATTAACCTTGTTTATCAGACGTCTGCGTTTACTGACTGG  
AGTTTGAAGGTCCTTTGGCGGCCGGAGCCGCCAAAGCAACAGAGGTACGTTTACA  
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GTTACCTACGGAAACCTTGTTACGACTTTTACTTC

>Seq10 [Organism= *Lasiodiplodia theobromae*] Majun11 internal transcribed spacer, partial sequence

CAGTGCTGAGTTTTAGTTTATTAACCTTGTTTATCAGACGTCTGCGTTTACTGACTG  
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AAAGGGTGGGAGAGTCGAGCCGGAGCTCGAAAACCTCGGTAATGATCCTTCCGCA  
GGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCTCAAAATGACCAAGA

## RESEARCH OUTPUTS

### Output 1: Published Research Paper and Published Review paper

식물병연구

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# Current Status of Cashew Leaf and Nut Blight Disease (*Cryptosporiopsis* spp.) and Screening of Elite Cashew Hybrids Developed in 1996 and 1998 against the Disease in Eastern and Southern Tanzania

\*Corresponding author

Tel: +255 766 262655

Fax: +255 272 970016

E-mail: majuned@nm-aist.ac.tz

ORCID

<https://orcid.org/0000-0002-2335-5985>

Dadili Japhet Majune<sup>1,2\*</sup>, Peter Albert Masawe<sup>2</sup>, and Ernest Rashid Mbega<sup>1</sup>

<sup>1</sup>School of Life Sciences and Bio-engineering the Nelson Mandela African Institution of Science and Technology (NM-AIST), P. O. Box 447, Arusha, Tanzania

<sup>2</sup>Naliendele Agricultural Research Institute, P. O. Box 509, Mtwara, Tanzania

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Cashew (*Anacardium occidentale* L.) is an export crop and source of income in Tanzania. However, its productivity is challenged by insect pests and diseases. Cashew Leaf and Nut Blight Disease (CLNBD) caused by *Cryptosporiopsis* spp. has been cited as one of the most devastating diseases in Tanzania. Studies were conducted to investigate incidences and severities of CLNBD on cashew in farmers' fields and elite cashew hybrids developed in 1996 and 1998 in eastern and southern zones of Tanzania. Furthermore, a screen house experiment was conducted to screen these hybrids against CLNBD at Naliendele Agricultural Research Institute (NARI), Mtwara, Tanzania. The results indicated significant differences ( $P < 0.001$ ) in CLNBD incidences and severities in cashew in farmers' fields across Bagamoyo, Nachingwea and Mtwara districts. Further, there were significant differences ( $P < 0.001$ ) among hybrids in CLNBD severities in the screen house experiment. In ranking the elite cashew hybrids, 38 were tolerant and 14 were susceptible to CLNBD. This observation suggests that elite cashew hybrids developed in 1996 and 1998 are more tolerant to CLNBD compared to cashew found in farmers' fields. These findings strongly suggest that the elite cashew hybrids can be recommended for commercial farming in Tanzania.

**Keywords:** Cashew, CLNBD, Fungal, Incidence, Severity

## Introduction

Cashew (*Anacardium occidentale* L.) is a perennial nut crop, native to Brazil and belongs to the Anacardiaceae family consisting about 75 genera and 700 species (Menge et al., 2013). It was introduced to East Africa by the Portuguese in the 16<sup>th</sup> century and is now widely cultivated in many tropi-

cal countries including Tanzania and Mozambique (Masawe, 2006). It is an important crop for nutrition and income generation worldwide (Menge et al., 2014). The most important products derived from the plant are the apples and nuts. The cashew nuts are processed into kernels which are consumed mostly as snacks. The apples produce juice, jam, candy and alcoholic beverages like wine, gin and brandy. The cashew nut tree provides food, employment, income and the wood used for carpentry while other products derived from it are firewood and charcoal (Akinwale, 2000). Opeke (2005) reported that major cashew producing countries in the world are India, Tanzania, Mozambique, Nigeria, and Guinea-

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Bissau. However, other countries like Ivory Coast, Brazil, Benin and Ghana are also among big producer.

In Tanzania, cashew is the leading export crop in terms of foreign exchange earnings and the main source of cash income for over 300,000 households in South-Eastern Tanzania (Kasuga, 2013). It is estimated that more than 80% of the national cashew production comes from Mtwara, Lindi and Ruvuma (Tunduru District) regions (CBT, 2015). The area under cashew is estimated to be more than 400,000 hectares in mono or mixed crop production systems. However, the acreage has increased many folds because cashew is now grown in almost all regions in Tanzania (Anonymous, 2017). An average cashew farmer owns 1–2 hectares of cashew trees (Topper et al., 1997). The average yield in farmers' fields ranges from 500 kg/ha to 800 kg/ha (Masawe, 2006). Cashew production increased rapidly in 1960s towards mid-1970s, recording as high as 145,000 Mt. Thereafter, there was drastic decline in production to 16,400 Mt in 1973/1974.

The reasons for the decline in cashew nut production were cited to be powdery mildew disease, insect pests and lack of improved planting materials. The cashew powdery mildew disease was the main reason behind decline in the cashew production (Castellani and Casulli, 1981; Intini, 1987). It can cause crop losses ranging from 70 to 100% (Sijaona and Shomari, 1987). The disease control measures have been developed and adopted by farmers which are cultural methods including undertaking sanitation, removal of water shoots underneath and pruning branches, as well as the application of fungicides, such as sulphur dusts, wettable powders and water-based formulations (Cassulli, 1981; Intini and Sijaona, 1983; Sijaona et al., 2001; Topper et al., 1997).

In 2003, a survey carried out by pathologists at Naliendele Agricultural Research Institute (NARI) in Tanzania, revealed the presence of a new disease known as "cashew leaf and nut blight" (CLNBD). The disease is caused by a fungal pathogen known as *Cryptosporiopsis* spp. and was reported



Fig. 1. Effect of the CLNBD on leaves, nuts and trials conducted in the field and screen house.



to attack cashew for the first time in Tanzania (Sijaona et al, 2005). The disease attacks all tender parts of the plant including young nuts and apples causing annual crop loss up to 48.4% if not controlled (Fig. 1a–1f). The disease is most active during wet weather especially during off-season rains, where severe infections affect emerging young tender leaves. The disease is characterised by angular lesions, dark tan with dark reddish-brown margins formed on leaves. Lesions subsequently enlarge and coalesce causing large necrotic lesions and finally defoliation. If it rains during fruit setting, the infection of young nuts causes rapid blackening and abscission of nuts, resulting in significant yield losses starting on the seventh day onwards. Usually infections on older nuts result in sunken, 'tar spot'-like lesions that frequently extend onto the apples (Menge et al, 2013, 2014).

In recent years no studies have been undertaken to establish the current status of cashew leaf and nut blight disease

on cashew trees growing in farmers' fields in Eastern and Southern zones of Tanzania. However, farmers have been controlling the disease using various fungicides which are expensive. It is the appropriate time to provide farmers with cashew varieties which are resistant or tolerant to the disease in order to reduce cost of production.

NARI is currently working to minimize or completely eliminate the disease. During the 2016/2017 season more than 56 elite cashew hybrids were developed ready to be released for multiplication and distribution to farmers. This study determined the status of these hybrids in terms of resistance, tolerance and susceptibility to CLNBD. The objectives were to determine the current disease status caused by *Cryptosporopsis* spp. in Southern and Eastern zones of Tanzania and to screen selected elite cashew hybrids against the disease. The findings will strengthen information especially for crop resistance in Southern and Eastern zones of Tanzania.



Fig. 2. Map of Tanzania showing potential areas for cashew nut growing and study areas in yellow colour.

## Materials and Methods

**Study area.** The study was conducted in Nachingwea which is located at 10°19'46"S, 38°46'46"E; 442 metres above sea level (masl) (Lindi region), Bagamoyo which is located at 6°31'S and 38°55'E; 19 mals (Coast region) and Mtwara which is located at 10°22'22"S, 40°09'35"E; 102 masl (Mtwara region) (Fig. 2). These districts were selected because they are the main cashew growing areas in the country and the crop in these areas is being affected by the disease. Also, these areas were selected for developing genetic trials of elite cashew hybrids in 1996 and 1998. A survey in the cashew genetic trials and farmers' field was conducted in these areas, whereas screen house experiments were done at NARI nursery due to presence of infrastructure required for this kind of work.

**Field Survey.** In order to determine the current status of the disease, a survey was conducted in the cashew growing fields when the new vegetative shoots were emerging, between January and April 2018, and data were recorded for months during the rainy seasons when the pathogen is most active. Three agro-ecological zones were studied with one district per zone depending on the genetic trials. From each district, three villages each three farms were randomly selected with cashew trees aged five to twenty years. Ten cashew trees per farm were assessed. Three rounds of scoring were carried out at intervals of one month for CLNBD incidence and severity for the cashew hybrids developed in 1996 and 1998 and trees from the farmers' fields.

**Disease incidence and severity.** The disease incidence was determined by placing a quadrat of 1 m×1 m on top of the canopy of each cashew tree on opposite sides (North and South). The diseased shoots were counted against total shoots in the quadrat in a given cashew tree and expressed as a percentage. The disease severity was determined by using a scale of 0–6 (Table 1) where 0 represents resistance, 1

to 2 tolerance and 3 to 6 susceptible. A quadrat of 1 m×1 m was placed on top of the canopy at opposite sides before scoring the total number of shoots and their disease symptoms using a colour plate disease severity assessment guide for CLNBD (Appendix 1). Data was taken for five shoots using five leaves per shoot starting from the top of the shoot downwards. The final score was based on the formula:

$$= \sum_{i=0}^6 (0^*L) + (1^*L) + (2^*L) + (3^*L) + (4^*L) + (5^*L) + (6^*L) / 50(L).$$

Whereby 'L' represents the number of leaves scored in two quadrates north and south directions per tree.

**Screen house experiment.** The elite cashew hybrids developed in 1996 and 1998 were screened in a screen house at NARI. Cashew seeds were raised in polythene tubes (diameter 10 cm and height 20 cm) filled with forest soil collected under cashew trees as growth media. Cashew seeds started germinating at 14 d and between 30 and 45 d rootstalks were ready for grafting. Scions for grafting were collected from selected mother trees of the elite cashew hybrids developed in 1996 and 1998. After 45 d, the grafted cashew seedlings had more than six tender leaves which were suitable for inoculation. The treatments were arranged in a Completely Randomized Design (CRD), with three cashew hybrids per plot and three replicated plots. There were two control varieties, the resistant AZA and the susceptible AC4. The inoculum was prepared by collecting leaves infected by CLNBD with a 5–6 rating of disease and adding them to 20 L of water for 24 h with periodic mixing. The suspension was filtered to remove leaf debris and the seedlings in each plot were uniformly sprayed with  $1 \times 10^6$  conidia per ml, four times each at 7 d intervals for 28 days (Menge et al., 2014) of the same batch of inoculum. Thirty six mls per plot were inoculated on grafted seedlings and covered with a polythene sheet for 24 h to maintain relative humidity of about 90–95% at 24–28°C (Menge et al., 2014). These inoculated grafted

**Table 1.** The scale of cashew leaf and nut blight disease showing levels, intervals and mid-point in percentages

Level	0	1	2	3	4	5	6
Interval	0	1–20	21–40	41–60	61–80	81–99	100
Mid-point (%)	0	10.5	30.5	50.5	70.5	90	100
Grade	Resistance	Tolerant		Susceptible			

Source: NARI Pathologists (2012).

seedlings were examined and scored for disease occurrence daily for 7 d after each round of inoculation. Fig. 1a–1c, show disease symptoms on leaves while those of tender nuts are found in Fig. 1d–1f. The photographs for the trials undertaken in the field and in the screen house are shown in Fig. 1g–1i. Some varieties scoring 0 were regarded as resistant, while those with scores of 1–2 and scores of 3–6 were regarded as tolerant and susceptible to CLNBD, respectively.

**Statistical analysis.** Data were collected from farmers' fields, cashew genetic trials of elite hybrids and screen house experiment. Data from the fields and screen house experiment were analysed using statistical analysis package Genstat, 16<sup>th</sup> Edition, whereas Duncan Multiple Range Test was used to separate the means and Least Significant Difference (LSD) test at ( $P < 0.05$ ).

## Results

**Cashew genetic trials.** Three rounds of monitoring the trials were undertaken and the results for analysis of variance for % incidence and severity for the elite hybrids developed in 1996 and 1998 are presented in Tables 2 and 4. Results showed that there were statistically significant differences between hybrids in incidence ( $P < 0.05$ ) and severity ( $P < 0.01$ ,  $P < 0.001$ ) in the sites studied. Trial means for % incidence for the elite hybrids developed in 1996 and 1998 at Chambezi were 15.00 and 15.44, respectively. On the other hand, trial means for % incidence for the hybrids developed in 1996 and 1998 at Nachingwea were 17.25 and 19.40, respectively. The trial means for severity for the same hybrids at Chambezi developed in 1996 and 1998 were 8.98 and 9.79, respectively; while for those developed at Nachingwea were 8.03 and

11.74. The least significant differences (LSD) for % incidence for the hybrids developed in 1996 and 1998 at Chambezi were 17.56 and 21.37 respectively. On the other hand, the LSD for severity of hybrids developed in 1996 and 1998 at Chambezi were 10.25 and 9.39 respectively. The LSD for % incidence for the same hybrids at Nachingwea developed in 1996 and 1998 were 21.37 and 15.49, respectively; while the LSD for severity of those developed hybrids at Nachingwea were 9.97 and 11.85 respectively. When comparing % incidence for elite cashew hybrids developed in 1996 at Chambezi, hybrid C10-4-11, ranked first but differed significantly from the last three hybrids (C13-1, C10-4-15 and C3-1) only (Table 3). Data on severity for elite hybrids developed in 1996 at Chambezi indicate that, hybrid C10-4-11, ranked first but differences were not significant with 26 hybrids. However, it differed significantly with the last four hybrids C9-3, C13-1, C3-1 and C10-4-15 (Table 3).

When comparing % incidence for elite hybrids developed in 1996 at Nachingwea, hybrid C15-4 ranked first but was statistically significantly different from the last two hybrids C10-4-15 and C7-1-6 (Table 3). On the other hand, when comparing severity for elite hybrids developed in 1996 at Nachingwea, hybrid C15-4 ranked first. However, differences were not significant from other hybrids except the last two (C10-4-15 and C7-1-6) (Table 3).

Results of % incidence on elite hybrids developed in 1998 at Chambezi, hybrids H7.3 ranked first but differences were not significant from the others, except the last three hybrids H39.4, H29.1 and H6.3 (Table 5). When comparing severity for elite hybrids developed in 1996 at Chambezi, hybrid H7.3 ranked first but was not significantly different from the others, except the last five hybrids H34.3, H19.1, H39.4, H29.1 and H6.3 (Table 5).

**Table 2.** Analysis of variance for % incidence and severity for cashew leaf and nut blight disease for "1996 hybrids" at Chambezi and Nachingwea respectively

Source	Degree of freedom	Mean squares			
		% Incidence	Severity	% Incidence	Severity
Rep	2	210.9	134.74	103.2	8.93
Hybrids	25	207.00*	77.74**	272.50*	90.27***
Error	50	114.7	39.05	169.8	32.84
Mean		15	8.98	17.25	9.79
L.S.D		17.56	10.25	21.37	9.39
CV (%)		71.6	69.6	75.5	58.5



**Table 3.** Ranking means for % incidence and severity for elite hybrids developed "1996" at Chambezi and Nachingwea respectively

No	Hybrids	% Incidence	Severity	Hybrids	% Incidence	Severity
1	C10-4-11	2.03a	0.49a	C15-4	1.28a	0.89a
2	C5-1	2.14a	3.82a	C14-4	4.93a	2.80a
3	C19-4	4.04a	2.87a	C15-2-8	9.71a	3.88a
4	C1-4	6.66a	5.04a	C1-2	9.76a	4.81a
5	C15-4	6.68a	5.83a	C19-4	9.84a	8.52a
6	C14-4	8.43a	5.69a	C25-3	10.81a	4.59a
7	C1-3	8.98a	5.92a	C15-2-26	11.00a	10.58a
8	C15-2-2	9.52a	5.57a	C3-1	12.09a	7.33a
9	C15-2-8	10.14a	7.18a	C15-3	13.21a	9.98a
10	C15-3	12.00a	10.58a	C4-1	14.18a	7.91a
11	C1-2	12.41a	4.89a	C7-1-13	14.66a	7.18a
12	C22-4	12.51a	8.14a	C13-2	15.21a	4.93a
13	C4-1	13.01a	6.99a	C7-2	15.23a	9.17a
14	C7-2	14.86a	5.42a	C5-1	15.37a	14.96a
15	C15-2-26	15.44a	11.09a	C10-4-11	15.77a	8.90a
16	C13-4	16.41a	9.01a	C13-1	15.88a	5.88a
17	C10-1	17.73a	11.03a	C10-1	16.26a	9.33a
18	C7-1-6	18.03a	9.31a	C15-2-2	17.86a	9.36a
19	C5-2	18.23a	7.93a	C1-3	18.02a	11.30a
20	C9-3	22.02a	15.12b	C22-4	21.53a	12.01a
21	C13-2	22.22a	9.87a	C1-4	21.55a	14.97a
22	C7-1-13	22.32a	11.24a	C9-3	22.73a	12.47a
23	C25-3	22.74a	11.77a	C5-2	25.84a	13.49a
24	C13-1	24.72b	16.95c	C13-4	26.41a	17.27c
25	C10-4-15	32.48c	23.09e	C10-4-15	30.38b	16.40b
26	C3-1	33.15c	18.527d	C7-1-6	34.69c	14.79a

**Table 4.** Analysis of variance for % incidence and severity for cashew leaf and nut blight disease for "1998 hybrids" at Chambezi and Nachingwea respectively

Source	Degree of freedom	Mean squares			
		% Incidence	Severity	% Incidence	Severity
Rep	2	100.8	15.39	241.69	123.73
Hybrids	25	260.60*	82.48**	185.51**	107.46**
Error	50	165.7	32.06	89.27	52.17
Mean		15.44	8.03	19.4	11.74
L.S.D		21.11	9.29	15.49	11.85
CV (%)		83.4	70.5	48.7	61.5

The % incidence for elite hybrids developed in 1998 at Nachingwea indicate that, hybrid H8.3 ranked first but was not significantly different from the others, except the last eight hybrids H19.1, H1.3, H26.1, H39.4, H64.4, H13.1, H29.1 and H34.1 (Table 5). When comparing severity for elite hybrids developed in 1998 at Nachingwea, hybrid H8.3 ranked

first but was not significantly different from other hybrids, except the last five hybrids H26.1, H64.4, H34.1, H13.1 and H29.1 (Table 5).

**Farmers field survey.** Results from the farmers' fields (local cultivars) showed that there were highly significant

**Table 5.** Ranking means for % incidence and severity for elite hybrids developed "1998" at Chambezi and Nachingwea respectively

No	Hybrids	% Incidence	Severity	Hybrids	% Incidence	Severity
1	H7.3	1.21a	0.483a	H8.3	5.76a	2.23a
2	H51.2	4.96a	0.897a	H24.3	7.39a	4.13a
3	H8.3	5.58a	4.263a	H49.4	8.71a	3.82a
4	H51.4	5.76a	2.830a	H59.4	10.79a	3.77a
5	H59.4	6.37a	2.227a	H51.4	13.43a	6.53a
6	H49.4	7.90a	0.903a	H37.4	14.07a	6.57a
7	H24.3	10.55a	5.037a	H8.1	14.11a	10.71a
8	H68.4	10.82a	7.153a	H6.1	14.15a	7.53a
9	H37.4	11.57a	5.183a	H7.3	14.54a	9.83a
10	H43.4	11.80a	4.833a	H38.4	14.72a	9.23a
11	H2.1	12.55a	6.957a	H2.1	15.12a	7.06a
12	H8.1	12.58a	6.403a	H6.3	17.28a	7.16a
13	H6.1	13.37a	10.667a	H42.4	17.62a	10.34a
14	H38.4	14.02a	5.170a	H68.4	18.41a	11.02a
15	H26.1	15.43a	7.547a	H34.3	18.65a	11.18a
16	H19.1	15.83a	15.807c	H23.3	20.68a	16.10a
17	H1.3	16.69a	11.103a	H43.4	23.41a	13.35a
18	H23.3	17.24a	7.380a	H51.2	24.10a	14.60a
19	H64.4	17.72a	11.783a	H19.1	25.10b	15.88a
20	H34.1	17.75a	10.713a	H1.3	25.10b	16.13a
21	H42.4	19.40a	5.480a	H26.1	27.29c	17.52b
22	H34.3	24.31a	13.363b	H39.4	27.69d	15.58a
23	H13.1	25.14a	9.930a	H64.4	30.29e	17.89b
24	H39.4	28.55b	16.177d	H13.1	30.98e	21.45c
25	H29.1	29.72b	17.627e	H29.1	31.99e	24.12d
26	H6.3	44.65c	18.890f	H34.1	33.10f	21.38cd

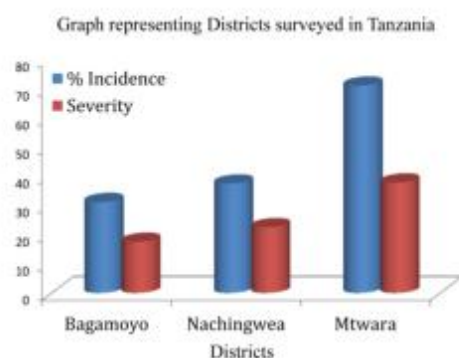
**Table 6.** Analysis of variance for % incidence and severity for cashew leaf and nut blight disease for "Survey done at Mtwara, Nachingwea and Bagamoyo districts"

Source	Degree of freedom	Mean squares	
		% Incidence	Severity
District	2	13831.2***	3375.8***
Error	87	237.80	145.00
Mean		46.63	26.00
L.S.D		7.91	6.18
CV (%)		33.10	46.30

differences ( $P < 0.001$ ) (Table 6) between cashew growing districts in % incidence and severity of the disease. The means for % incidence and severity in farmers' field were 46.63 and 26 respectively; which are very high compared to elite hybrids developed in 1996 and 1998 (Table 2 and Table 4).

When comparing the districts in incidence and severity of the disease in farmers' fields, Bagamoyo district had the lowest incidence and severity followed by Nachingwea district whereas the last was Mtwara district (Fig. 3). The lower the % incidence and severity the lower the CLNBD in the field.

**Screening of seedlings in the screen house.** After inoculation of seedlings with conidia of *Cryptosporiopsis* spp. small brown spots appeared on the young leaves at the first time of observations, 7 d. The spots enlarged and coalesced as the time increased and resembled the symptoms observed in the field. The analysis of variance showed that there were highly significant differences between cashew elite hybrids in all trials ( $P < 0.001$ ). Trial means for severity of elite cashew hybrids developed in 1996 and 1998 in the screen house were 4.28 and 4.71, respectively. Their least significant



**Fig. 3.** Ranking means for % incidence and severity for farmers' field surveyed at Nachingwea, Mtwara and Bagamoyo districts.

**Table 7.** Analysis of variance for severity for cashew leaf and nut blight disease for elite cashew hybrids developed "1996 and 1998" in the screen house at NARI

Source	Degree of freedom	Mean squares	
		Severity (1996)	Severity (1998)
Rep	2	15.30	1.44
Hybrids	27	22.49***	29.49***
Error	54	5.46	4.08
Mean		4.28	4.71
L.S.D		3.83	3.28
CV (%)		54.60	42.60

differences (LSD) for all trials are 3.83 and 3.28 respectively (Table 7). When comparing severity for elite cashew hybrids developed in 1996 in the screen house, variety AZA2 ranked first with low severity but was not significantly different from 28 hybrids, except the last three hybrids and variety AC4 (Fig. 4). When comparing severity for elite cashew hybrids developed in 1998 in the screen house, variety AZA2 again ranked first in terms of low disease severity; however, significant differences were observed it was compared with the last eleven hybrids and variety AC4 (Fig. 5).

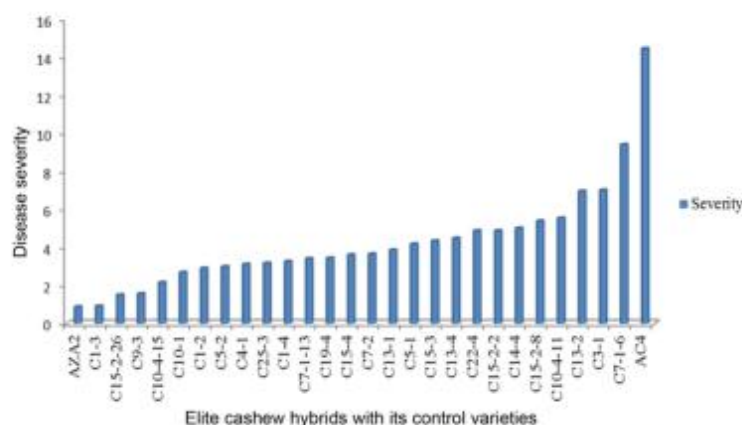
## Discussion

### The elite cashew hybrids developed in 1996 and 1998.

The overall highest score % incidence and severity for elite hybrids developed in 1996 and 1998 are 19.4 and 11.74 respectively. These findings compare to the farmers field results where highest % incidence and severity at Nachingwea, Bagamoyo and Mtwara districts were 46.63 and 26.0 respectively. Indeed, most unimproved cashew varieties have a lower level of resistance or tolerance than the hybrids (Masawe 2006; Sijaona, 2013). The tolerance of the elite hybrids would benefit farmers with reduced fungicide costs and higher yields.

### Farmers field survey.

The farmers field survey results in-



**Fig. 4.** Ranking means for severity for elite cashew hybrids developed "1996" in the screen house at NARI.

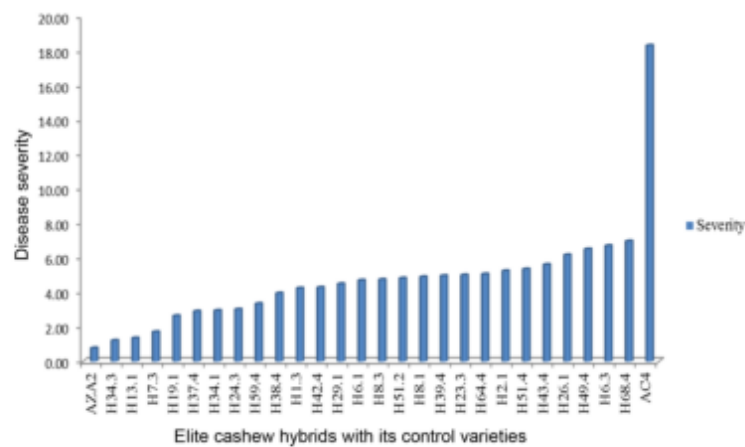


Fig. 5. Ranking means for severity for elite cashew hybrids developed "1998" in the screen house at NARI.

indicated that the highest % incidence and severity at Nachingwea, Bagamoyo and Mtwara districts were 46.63 and 26.00 respectively. The results suggested that the materials used by farmers in the surveyed areas are in group three which is the susceptible group. The susceptible group is the one vulnerable to infection by the CLNBD when compared to other groups. The cashew planting materials used by farmers are highly affected by CLNBD. Through this study farmers are advised to use elite cashew hybrids developed in 1996 and 1998 which are resistant or tolerant to CLNBD and are available at NARI.

**Screening of seedlings in the screen house.** The mean severity of the disease for the elite hybrids developed in 1996 and 1998, 4.28 and 4.71 respectively, was very low in the tolerant group, possibly due to size of the tree canopy. However, analysis of variance showed that the elite hybrids had variable tolerance ( $P < 0.001$ ). The screen house studies showed that variety AZA2 (positive control) rank first with no significant difference between hybrids in all trials. On the other hand, variety AC4 (negative control) ranked last and differences in disease severity were highly significant from all the other genotypes in all trials. This suggests that all the elite cashew hybrids rank in AZA2 group are tolerant to cashew planting materials. However, these materials are to be

adopted by cashew growing farmers for high yielding and tolerant to CLNBD.

### Conclusion and Recommendation

This study revealed that farmers cultivating cashew in Nachingwea, Bagamoyo and Mtwara districts are using cashew planting materials which susceptible to CLNBD. However, a screen house study with inoculation by the pathogen showed CLNBD tolerance in 38 elite cashew hybrids developed in 1996 and 1998. Based on these findings farmers of these three districts and other areas with similar climatic conditions in the country are advised to cultivate these elite cashew hybrids in order to reduce the cost of production, increase household income which will also lead into increased foreign exchange earnings for the country. It is recommended that the study be undertaken all over cashew growing areas in the country so that if similar results are achieved it will justify up-scaling of these hybrids for higher incomes and sustainable revenue for the government.

### Conflicts of Interest

No potential conflict of interest relevant to this article was reported.









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**Appendix 1.** The severity assessment guide for cashew leaf and nut blight disease scores

 <p>DISEASE LEVEL 0</p>	<p>DISEASE LEVEL 0</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• no disease symptoms</li> <li>• 0 % disease severity</li> </ul>
 <p>DISEASE LEVEL 1</p>	<p>DISEASE LEVEL 1</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• 1–3 dark tan angular lesions</li> <li>• 1–2 lesions enlarged not coalesced,</li> <li>• % disease; 1–20 (Average 10.5%)</li> </ul>
 <p>DISEASE LEVEL 2</p>	<p>DISEASE LEVEL 2</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• 4–8 dark tan angular lesions</li> <li>• 2–3 lesions enlarged not coalesced,</li> <li>• % disease; 21–40 (Average 30.5%)</li> </ul>
 <p>DISEASE LEVEL 3</p>	<p>DISEASE LEVEL 3</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• 9–12 dark tan angular lesions</li> <li>• 4–6 lesions enlarged not coalesced,</li> <li>• % disease; 41–60 (Average 50.5%)</li> </ul>
 <p>DISEASE LEVEL 4</p>	<p>DISEASE LEVEL 4</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• &gt; 12 dark tan angular lesions</li> <li>• lesions enlarged and &lt; 50% coalesced,</li> <li>• % disease; 61–80 (Average 80.5%)</li> </ul>
 <p>DISEASE LEVEL 5</p>	<p>DISEASE LEVEL 5</p> <p>Leaf symptom:</p> <ul style="list-style-type: none"> <li>• lesions enlarged and 75% coalesced causing blighting</li> <li>• % disease; 81–99 (Average 90%)</li> </ul>

# Status and Management of Cashew Disease in Tanzania

Dadili Japhet Majune<sup>1,2\*</sup>, Peter Albert Masawe<sup>2</sup> and Ernest Rashid Mbega<sup>1</sup>

<sup>1</sup>School of Life sciences and Bio-engineering the Nelson Mandela African Institution of Science and Technology (NM-AIST), P. O. Box 447, Arusha, Tanzania

<sup>2</sup>Naliendele Agricultural Research institute, P. O. Box 509, Mtwara, Tanzania

\*Correspondence: [majuned@nm-aist.ac.tz](mailto:majuned@nm-aist.ac.tz)

**Abstract**— Cashew (*Anacardium occidentale* L.) is one of the most important export crops and the main source of cash income in the southern part of Tanzania. However it is challenged by a number of factors such as drought, declining soil fertility, un-improved low yielding cashew genotypes, insect pests and diseases. Of these factors, diseases have been cited to result in high production costs, poor nut quality and low market price. The most devastating diseases that attack cashew are powdery mildew, cashew leaf and nut blight, dieback and fusarium wilt. Other minor diseases include anthracnose, damping off and leaf spots. Despite the negative role that these diseases possess to cashew growers, there is limited or no critical updated information on their current infection status and management in Tanzania. Thus, this review article discusses the status of the most important cashew diseases and their management options in the country. Such information will be vital to cashew farmers and other stakeholders in making appropriate improvements in cashew production in Tanzania.

**Keywords**— Cashew, dieback, fusarium wilt, cashew Leaf and nut blight, powdery mildew.

## I. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a perennial nut crop, native to Brazil that belongs to the Anacardiaceae family (Ohler, 1979; Masawe, 2006; Zhongrun and Masawe, 2014). It was introduced to East Africa by the Portuguese in the 16th century and it is now widely cultivated, especially in Tanzania. (Masawe, 1994; Topper and Boma, 1997). The most important product derived from the plant is cashew nuts that are processed into kernels. The crop also produces other products such as juices, jam, alcohol and non-alcoholic beverages; all of which are produced from the cashew apples (Sobhana *et al.*, 2010). In Tanzania cashew is the main cash crop and the leading source of income for over 300,000 households in South-Eastern Tanzania (Kasuga, 2013). It is estimated that more than 80% of the national cashew production comes from Mtwara, Lindi and Ruvuma (Tunduru District) regions (CBT, 2015). The area under cashew is

estimated to be more than 400,000 hectares in mono or mixed crop production systems. An average cashew farmer owns 1-2 hectares of cashew trees (Topper *et al.*, 1997). The average yield in farmers' fields ranges from 500kg/ha to 800kg/ha (Masawe, 2006).

Cashew has been one of the most important export crops since independence in Tanzania. The cashew production increased rapidly in 1960s towards mid-1970s, recording as high as 145,000 MT (metric ton). Thereafter there was drastic decline in production up to 16,400 MT in 1973/74. The reasons for the decline in cashew nut production were drought, declining soil fertility, unimproved low yielding genotypes, insect pests and diseases (Ellias, 1980; Brown *et al.*, 1984). Of the factors, diseases (Table 1) have been a major challenge in cashew since the 1970s. Of the diseases cashew powdery mildew disease (PMD) has been cited to be among important constraints to cashew production causing crop losses if not controlled ranging from 70 to 100% (Castellani and Casulli, 1981; Sijaona, 1984; Sijaona and Shomari, 1987; Intini, 1987; Shomari, 1988). The historical timeline for cashew diseases in Tanzania is shown in Table 1. In 2003, a second deadly disease known as 'cashew leaf and nut blight' caused by *Cryptosporiopsis* spp was reported for the first time, attacking cashew at all growth stages (Sijaona *et al.*, 2005; Sijaona *et al.*, 2006). The disease causes crop losses of up to 48.4% annually if not controlled (ACRR, 2006). The third major disease was reported in 2012 by Tibuhwa and Shomari (2012), as cashew fusarium wilt caused by *Fusarium oxysporum*. This disease affects cashew trees leading to yield losses of up to 100% if not controlled (Tibuhwa and Shomari, 2016).

The current status of these major and minor diseases such as dieback, anthracnose, pestalotia leaf spot and damping off is discussed in this article. Such disease status information and how they are managed is vital for different cashew stakeholders in Tanzania. It can alert policy makers on how to collectively address cashew disease problems and plant breeders and or pathologists on how to develop resistant crop varieties and or integrated pest management options, respectively, against



the diseases. A chronological history of occurrence of 1.  
major cashew diseases in Tanzania is as shown in Table



Fig.1: Distribution of cashew growing areas and major diseases affecting the crop in Tanzania (Source: ARI-Naliendeke). Green coloured regions represents all cashew growing areas in Tanzania but affected by powdery mildew, dieback, and anthracnose; deep blue coloured circles represent cashew producing areas affected by cashew leaf and nut blight disease and the red coloured blocks represent locations affected by cashew fusarium wilt disease.



Table.1: Historical time line of cashew diseases occurrence in Tanzania.

S/n	Disease	Causal agent	Year reported	References
1	Cashew powdery mildew disease	<i>Oidium anacardii</i> Noack	1970	Casulli, (1979) Sijaona, (1984) Shomari, (1988)
2	Anthracnose disease	<i>Colletotrichum gloeosporoides</i> Penz	1978	Casulli, (1981)
3	Dieback disease	<i>Phomopsis anacardii</i>	1980	Intini and Sijaona (1983)
4	Pestalotia Leaf Spot disease	<i>Pestalotia</i> spp.	1980	Intini and Sijaona (1983)
5	Damping off disease	<i>Fusarium</i> spp., <i>Pythium</i> spp., <i>Phytophthora palmivora</i> Butler,	1980	Intini and Sijaona (1983)
6	Cashew leaf and nut blight disease	<i>Cryptosporiopsis</i> spp	2003	Sijaona <i>et al.</i> , (2005) Sijaona <i>et al.</i> , (2006)
7	Cashew fusarium wilt disease	<i>Fusarium oxysporum</i>	2012	Tibuhwa and Shomari, (2016)

## II. CASHEW PRODUCTION TREND AND DISTRIBUTION OF MAJOR DISEASES IN TANZANIA

Tanzania is the world's eighth and Africa's third largest cashew nut producer after Mozambique and Ivory Coast (CBT, 2011). The main producing areas and distribution of major cashew diseases in Tanzania is as shown in Figure 1 and the production trend from 1945s shows a zigzag production style in Figure 2, nevertheless the production is currently increasing possible due to increased acreage of production.

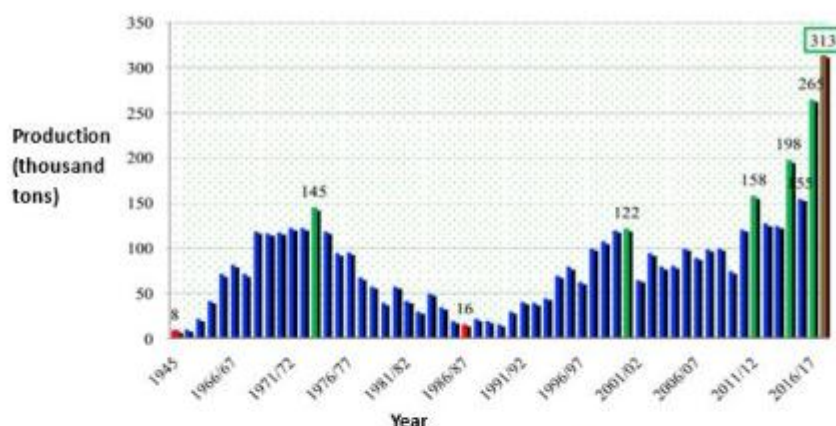


Fig. 2: Raw cashew nut production trend in Tanzania since year 1945 to year 2017 (Source: Cashewnut Board of Tanzania).

## III. HISTORY, EFFECT, CONDITIONS FAVOURING CASHEW DISEASE AND THEIR MANAGEMENT IN TANZANIA

### Cashew Powdery mildew disease

The history of powdery mildew goes back to the 1950s; however it was not economically important in Tanzania until mid-1970s (Casulli, 1979; Castellani and Casulli, 1981; Sijaona, 1984; Sijaona and Shomari, 1987; Shomari, 1988). In 1975 the rate of powdery mildew disease infection on cashew started to increase (Sijaona

and Shomari, 1987) which resulted into decline in cashew production from 145,000 MT in 1973/74 to 16,4000 MT in 1986/87 (CRP Report, 2006). The decline in cashew production was consistent in all cashew-growing areas in the country, which resulted into huge losses of revenue for both growers and the government (Topper *et al.*, 1997). The increased outbreak of the powdery mildew disease was then associated with change of environmental conditions and poor cashew management resulting from villagilization program which took place in 1974/1975

(Elias, 1980; Brown *et al.*, 1984). To date, powdery mildew disease is the main constraint of cashew in Tanzania. The disease is caused by *Oidium anacardii* Noack, a fungus of genus *Oidium* of the Deuteromycotina (Fungi Imperfecti) (Shomari, 1996; Sijaona, 1997). Powdery mildew disease infests all tender tissues of the cashew trees, mainly the tender leaf and inflorescence including the part not well unfolded. The disease seldom attacks old and mature leaves (Sijaona *et al.*, 2006). A white powdery growth is formed on the infested fruit bearing branches and inflorescence. The lesions of the infected parts turn to brown and after 2-3 weeks they shrink gradually and become dry and shed, leading to drying out and drop of numerous flowers and tender fruits (Sijaona and Shomari, 1987). Infected apples turn dull and their skin becomes much coarser. The apples when heavily infected show deep cracks on the surface and gradually shrivel and dry up (Sijaona *et al.*, 2005). The tender nuts when infected are deformed on the shell. The lesions turn grey on infected tender apples and nuts. Infected nuts deteriorate in quality during storage, decays easily and produce poor quality kernels when processed (Shomari 1996; Waller *et al.*, 1997; Sijaona, 1997).

All cashew varieties are susceptible to the powdery mildew disease but at different levels. Most unimproved cashew varieties succumb more to the disease compared to improved varieties, which have a certain level of resistance or tolerance (Masawe 2006; Sijaona, 2013). The conducive environment for PMD are cold nights which are followed by warm daytimes leading to mist and fog conditions in the early mornings. An optimum temperature ranges between 25-28°C with optimum at 26°C. Relative humidity that is conducive to the environment ranges between 80-100% with optimum at 95% (Noak 1898; Ohler 1979; Casulli, 1979; Chacko *et al.* 1990; Sijaona, 2013). The PMD spores are mainly dispersed by wind as rainfall inhibits its development. However, perennation and survival of the pathogen from one season to another takes place in fallen infested leaves, water shoots and off season flowers (Sijaona *et al.*, 2006).

Powdery mildew disease is not dormant and can occur on the tree canopy all the year around by wind dispersal (Shomari, 1996; Sijaona, 1997). Undertaking sanitation, which is basically the removal of water shoots underneath tree canopies, pruning branches to allow aeration, clearing of dropped branches and leaves to remove the source of the inoculum can reduce and delay occurrence of this disease for weeks (Casulli, 1979). Overlapping branches and twigs under the crown without penetration of sunlight and lack of rains are optimum condition for the powdery mildew fungus to survive (Shomari, 1996; Zhongrun and Masawe, 2014). Powdery mildew is currently controlled mainly using fungicides (chemical control). Several

fungicides including sulphur dusts, wettable powders (Casulli, 1981; Intini and Sijaona, 1984) and water based organic fungicides (Topper *et al.*, 1997; Sijaona *et al.*, 2001) have been recommended for control of the PMD in Tanzania but more efforts are still going on to have more fungicides. Cashew varieties partially resistant to PMD have been developed in Tanzania (Masawe, 2006). These varieties have been commercialized and made available to farmers as grafted seedlings through Cashew Development Centres (CDCs) located in the main cashew growing areas in Tanzania. Polyclonal Seed Orchards (PSG) was established using these varieties for production of planting materials in form of seeds. Still there is a need of developing other new resistant materials for controlling powdery mildew disease to increase cashew production.

#### Cashew Anthracnose disease

The history of Anthracnose in Tanzania goes back to 1978 (Casulli, 1981). This fungal disease caused by *Colletotrichum gloeosporoides* Penzance not only a problem in cashew but also infects other tropical fruits trees including mango, citrus, avocado and papaya (Sijaona, 2013). The disease attacks all young and tender vegetative organs together with nuts and pseudo fruits/apples. The disease is favoured by relative humidity of 95% - 100% and temperature ranging between 22°C - 28°C during flowering and fruiting period (Sijaona, 2013; Zhongrun and Masawe, 2014). Early symptoms are reddish brown shiny water-soaked lesions and resin exudation on the affected parts. Infected shoots appear as "hanging nuts" Hanging nuts may act as a source of disease infection during the next season (Zhongrun and Masawe, 2014). Cashew varieties resistant or partially resistant to anthracnose were developed in Tanzania (Masawe, 2006). These varieties have been commercialized and made available to farmers as grafted seedlings. Other approaches of controlling anthracnose disease have been discussed in table 2.

#### Cashew Dieback disease

Cashew Dieback disease was reported in Tanzania in 1980 according to Intini and Sijaona (1983). This fungal disease caused by *phomopsis anacardii* Early and Punithalingam is believed to be facilitated by damage caused by mirid (*Helopeltis* spp) or coconut bug (*Pseudotheraptus wayii*) on cashew plant (Zhongrun and Masawe, 2014). The symptoms of the disease include withering of the panicles, followed by a progressive dieback of small flower stalks. This starts from the tips then advances downward to the main floral shoots (Intini and Sijaona, 1983). The normal greenish colour of the health shoots progressively turns brown resulting in loss



of flowers. Infected young nuts and apples become black and fluffy and remains attached to the floral stalks. Heavy infection appears similar to fire damage (Sijaona, 2013). Damage caused by insect attack (*Helopeltis* spp or *Pseudotheraptus wayii*) are considered as predisposing factors to dieback infection. The fungus attack young and tender shoots and flowers followed by dieback infection starting at tips and spreading downwards. The dieback disease of cashew is found in every cashew growing areas. The different methods used to control the infections are well explained in Table 2.

#### Cashew Pestalotia leaf spot disease

Pestalotia leaf spot is a disease caused by a fungus known as *Pestalotia heterocornis* Gubaand it was reported in 1980 (Intini and Sijaona, 1983). The fungus attacks mature leaves, forming angular to irregular leaf lesions, reddish brown on upper surface and pale gray to whitish on underside of leaves. Later on lesions become thinner, papery and necrotic. Severe infection may cause defoliation (Intini and Sijaona, 1983). The infected leaves show regular or irregular polyclonal lesions or round lesions. These lesions mostly appear on the leaf tip and they enlarge gradually and coalesce, expanding from the leaf tip downwards to more than half of the leaf within masses of conidia appearing on both lower and upper sides of the leaves (Zhongrun and Masawe, 2014). The pathogen develops at an optimum temperature and relative humidity range between 26°C-28°C and 80-100% respectively (Sijaona, 2013). The dispersal mechanism of the pathogen is mainly wind and free running water. The disease can best controlled by application of copper based fungicides (Zhongrun and Masawe, 2014), such as Kocide at the rate of 3-5 gm per litre of water at two week intervals. Other management methods are described in Table 2.

#### Cashew Damping off disease

Damping off cashew disease is caused by number of fungal organisms including *Fusarium* spp, *Pythium* spp, *Phytophthora palmivora* Butler, *Cylindrocladium scoparium* Morgan, *Sclerotium rolfsii* Sacc and *Pythium ultimum* Trow; most of which occurs mainly at nursery (Intini and Sijaona, 1983). It was reported in Tanzania in 1980's and mainly infects young cashew seedlings in the nursery with poor drainage or container-raised young plants (Zhongrun and Masawe, 2014). The infected plantlets cease to grow and wither gradually and show circular water soaked stripes on the root collar (Sijaona, 2013). The roots may rot, leading to lodging of the plants (Zhongrun and Masawe, 2014). The nursery or the land for container raised young plants should be well drained to stop waterlogging. Seed beds can be leached by

spraying with carbendazim 50% WP, Chlorothalonil 75% WP, carbendazim thiram zineb 80% WP, or cymoxanil manacozeb 72% WP. Further details on disease management are as described in Table 2.

#### Cashew Leaf and nut blight disease

The cashew leaf and nut blight disease was reported for the first in Tanzania in August 2002, (Sijaona *et al.*, 2005). The disease was reported in Nanyanga, Mtopwa sub-station and Chiwindi in Newala District. It was also observed in a neighbouring country at Itoculo farm in Monopo District, Nampula Province, Mozambique in 2005 (Sijaona, 2005 and Sijaona *et al.*, 2006). The disease has been reported to be more active during wet weather especially during off-season rains, where severe infections affect the young flushing material (Sijaona *et al.*, 2006). Infected cashew leaves develop silver/grey lesions with a dark reddish brown margin that enlarge and coalesce causing defoliation (Sijaona *et al.*, 2006). Infected young nuts blacken while older nuts results in characteristic dark lesions that under favourable conditions form white spore masses of the fungus within the nut lesions (Menge, 2013 and Menge, 2014). Cultural methods are done by removing, gathering, burning and burying all diseased fruits and branches and twigs left in the cashew plantation to reduce pathogen source in the field (Zhang and Masawe, 2014). Chemical methods are done by spraying fungicides such as Trifloxystrobin 10% SC, Difenaconazole WG, Picoxystrobin and Trifloxystrobin + tebuconazole (Table 2). Disease control commences when first symptoms occurs particularly during fruiting season. Two varieties which are tolerant or resistant to cashew leaf and nut blight disease have been developed and these are AZA 2 and AZA 17 (Masawe, 2006). Farmers are advised to use resistant varieties to control this disease. These varieties have been commercialized and made available to farmers as grafted seedlings (Masawe, 2006; Zhang and Masawe, 2014).

#### Cashew Fusarium wilt disease

The cashew fusarium wilt disease caused by *Fusarium oxysporum* was first reported in Tanzania in 2012 at Magawa village in Mkuranga District in the Coast region (Tibuhwa and Shomari, 2012). There after it was reported in Masasi District (Nanganga), Tandahimba District (Lindumbe) and Mtwara District (Mnongodi). The cashew fusarium wilt can cause the entire cashew plant to wilt within three to four weeks after first symptoms. The disease can attack the next nearby cashew trees until trees in the entire field are all wilted (Tibuhwa and Shomari, 2016). Infected cashew plant is characterised by gradual loss of natural green colour of leaves of some branches and then turns yellow (chlorosis) (Tibuhwa and Shomari,

2016). Looking from a distance, affected trees appear yellow and green and later within three to four weeks the entire tree(s) wilt (Tibuhwa and Shomari, 2016). Different methods including field sanitation and destruction of infected plant parts have been proposed controlling fusarium wilt disease. No other fusarium wilt managerial

option has been proposed to the moment. Some fungicides have shown positive response in controlling the disease at laboratory level and field trials are in progress (Tibuhwa and Shomari, 2016). The room is open for scientists to work on in order to have positive control for this new devastating cashew disease.

Table.2: Current management options for cashew diseases in Tanzania

Disease	Causal agent	Management and description option
Cashew powdery mildew	<i>Oidium anacardii</i> Noack	<b>Cultural methods:-</b> Include sanitation by removing suckers, clearing of dropped branches and leaves also thinning and pruning unwanted branches (Casulli, 1981; Shomari, 1996) <b>Chemical methods:-</b> Sulphur dusts and Wettable powder at 250gm per tree at 14 days interval. Water based organic fungicides e.g Triadimenol, Hexaconazole and Penconazole at the rate of 10-15ml per litre per tree at an interval of 21 days (Intini and Sijaona, 1983; Sijaona, 1984; Topper <i>et al.</i> , 1997; Sijaona <i>et al.</i> , 2001)
Cashew anthracnose	<i>Colletotrichum gloeosporoides</i> Penz	<b>Cultural methods:-</b> Clearing off and burning of diseased and dead shoots, leaves, fruits on trees and fallen dry twigs, fruits and leaves after harvest (Sijaona, 2006). <b>Chemical methods:-</b> Chlorothalonil 75% WP 1ml per 0.6-0.8 litre, Prochloraz 25% EC 1ml per 0.8-1.5 litres, copper oxychloride, copper hydroxide, captafol, benomyl, Anilazine, Triadimenol and Dithianon (Sijaona, 2013; Zhongrun and Masawe, 2014)
Cashew dieback	<i>Phomopsis anacardii</i>	<b>Cultural methods:-</b> Remove all infected leaves on the entire trees (Intini and Sijaona, 1983). <b>Biological methods:-</b> Use weaver ants to control sucking pests (Sijaona <i>et al.</i> , 2001) <b>Chemical method:-</b> use Lambda cyhalothrin EC 5 mls per litre, Beta-cypermethrin 4.5% EC 1ml per 2.5-3 litres, Dimethoate 40% EC 1ml per 1-1.5 litre and Trichlorfos 90% crystal 1g per 1 litre Zhongrun and Masawe, 2014).
Cashew pestalotia leaf Spot	<i>Pestalotia sp.</i>	<b>Chemical methods:-</b> Metalaxl Mancozeb 58% WP (1g per 0.8-1litre), Chlorothalonil 75% WP (1g per 0.8-1litre) and propamocarb 72.29% (1g per 0.8-1 litre) (Zhongrun and Massawe, 2014).
Damping off disease	<i>Fusarium spp.</i> , <i>Pythium spp.</i> , <i>Phytophthora palmivora</i> Butler,	<b>Cultural methods:-</b> The nursery and the land container raised young plants should be well drained to stop waterlogging (Sijaona, 2013; Zhongrun and Massawe, 2014). <b>Chemical methods:-</b> Carbendazin 50% WP (1g/0.5-1 litre), chlorothalonil 75% WP (1g/0.8/1 litre) and cymoxanil mancozeb 72% WP (1g/0.5/1 litre) (Zhongrun and Massawe, 2014).
Cashew leaf and nut blight	<i>Cryptosporiosis spp</i>	<b>-Cultural methods:-</b> Clear off, gather, burn and bury all the diseased fruits and leaves infected in the field (Sijaona, 2006). <b>-Chemical method:-</b> Fungicides are used such as difenaconazole 14g/litre, Picoxystrobin 10 mls per litre, Chlorothalonil 14 g/l Trifloxistrobin+Tebuconazole 14mls per litre (Sijaona, 2006) <b>-Resistant varieties:-</b> Such as AZA2 and AZA 17 (Masawe, 2006)
Cashew fusarium wilt	<i>Fusarium oxysporum</i>	<b>-Cultural methods:-</b> Clean the equipment after use and plant satisfied materials from authorised institutes (Tibuhwa and Shomari, 2016)

#### IV. CONCLUSION

This document highlights the status of the most important cashew diseases and their management options in the country. The information provided here is vital to cashew

farmers and other stakeholders in making appropriate improvements in cashew production in Tanzania. However, there is need to conduct in-depth research especially on characterizing pathogen strains and



developing appropriate management options that will discourage use of chemical pesticides for environmental safety and improved cashew production in Tanzania.

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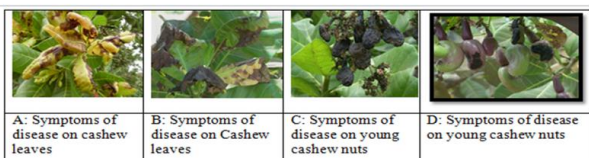
## Output 2: Poster presentation

### DETECTION AND CHARACTERIZATION OF CASHEW LEAF AND NUT BLIGHT-CAUSING FUNGI IN SOUTHERN AND EASTERN TANZANIA

Dadili Japhet Majune, Prof. Peter A. L. Masawe, Dr. Ernest Rashid Mbega  
Department of Sustainable Agriculture and Biodiversity Ecosystem Management  
Nelson Mandela African Institution of Science and Technology, P.O.Box 447, Arusha, Tanzania.

#### Introduction

Cashew (*Anacardium occidentale* L.) is the export crop and the source of income in Tanzania. However, its productivity is not optimal due to number of factors such as un-improved cashew genotypes, insect pests and diseases. Of these factors, Cashew Leaf and Nut Blight Disease (CLNBD) caused by a fungal pathogen including *Cryptosporopsis* spp has been cited as devastating diseases resulted to lower cashew nut yields and poor nut quality. In 2003 a new disease known as "Cashew Leaf and Nut Blight Disease" (CLNBD) was reported for the first time on cashew in Tanzania. The disease cause annual crop loss of 48.4% if not controlled (Sijana *et al.*, 2006; Menge *et al.*, 2014; Majune *et al.*, 2018). Visually the disease is characterised by angular lesions, dark tan with a dark reddish brown margin on leaves, and veins contain conidiomata. During fruit setting, infection of young nuts causes rapid blackening and abscission of nuts. Usually infections on older nuts result in sunken, "tar spot"-like lesions that frequently extend onto the apples. The present study evaluate selected cashew elite 1996 and 1998 hybrids against virulent fungal isolates obtained from infected cashew materials. Also the detection and characterization of CLNBD causing fungi in eastern and southern zones of Tanzania



#### Objectives

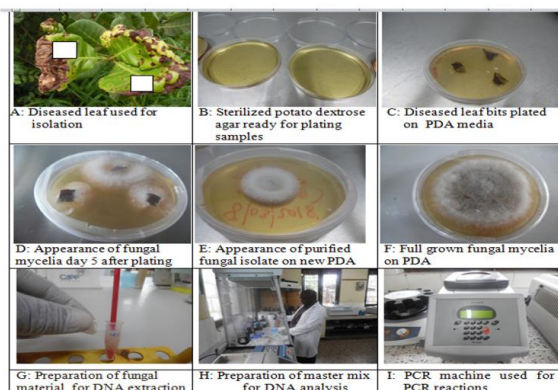
- To evaluate selected cashew elite 1996 and 1998 hybrids against virulent fungal isolates obtained from infected cashew materials
- To study phylogenetic relationship of fungi isolates associated with the CLNBD in the Southern and Eastern zones of Tanzania

#### Materials and Methods

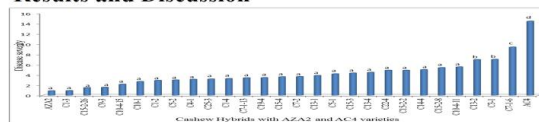
The cashew elite 1996 and 1998 hybrids were screened in the screen house located at TARI-Naliendele. Cashew seeds were raised in polythene tubes (diameter 10 cm and height 20 cm) using forest soil collected under cashew trees as growth media. Cashew seeds started germinating 14 days after sowing, and when the seedlings reached 30 to 45 days after sowing, they were used as rootstocks for grafting. Scions for grafting were collected from selected mother trees of the cashew elite 1996 and 1998 hybrids. Successful grafted seedlings were inoculated with fungal isolates  $1 \times 10^6$  conidia per ml concentration from infected cashew materials at 45 days after grafting. Completely Randomized Design (CRD), plot size was three plants per plot in three replicates. AZA2 positive and AC4 negative. The inoculation suspension was prepared by collecting leaves infected by CLNBD from susceptible AC4.



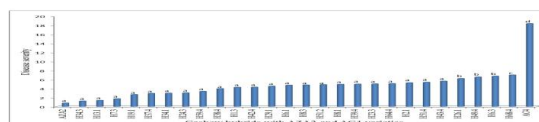
A total of 90 leaf samples (30 from each zone) which were infected by the disease were randomly collected from different fields during the survey. The collected samples were well labelled and transferred to TARI-Mikocheni for fungal isolations. The fungi were isolated by direct conidial transfer method on potato dextrose agar (PDA) medium



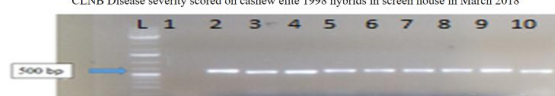
#### Results and Discussion



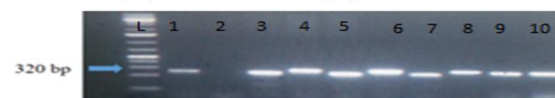
CLNBD Disease severity scored on cashew elite 1996 hybrids in screen house in March 2018



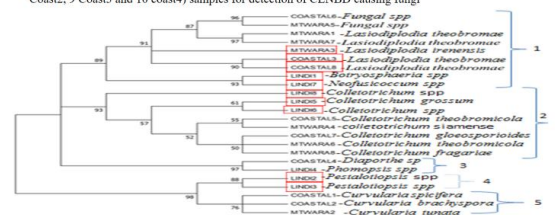
CLNBD Disease severity scored on cashew elite 1998 hybrids in screen house in March 2018



Amplification of a sample of pathogenicity positive isolates by PCR using ITS5 and ITS4 primers. Where: L is DNA ladder, 1 is a control, 2-10 (Lindi1, Lindi2, Lindi3, Lindi4, Lindi5, Lindi6, Lindi7, Lindi8, Lindi9 and Lindi10) samples for detection of CLNBD causing fungi.



Amplification of a sample of pathogenicity positive isolates by PCR using ITS1 and ITS4 primers. Where L is DNA ladder, 1-10 (1 Mtwar1, 2 control, 3 Mtwar2, 4 Mtwar3, 5 Mtwar4, 6 Mtwar5, 7 Mtwar6, 8 Coast1, 9 Coast2, 10 Coast3 and 10 coast4) samples for detection of CLNBD causing fungi



Phylogenetic tree reconstructed using the nucleotide sequences of CLNBD isolates. N.B: The phylogenetic tree was constructed with a neighbour-joining method in MEGA7 using bootstrap values over 50%, red blocks show samples sequenced with 97-99% identity to known fungal species

#### Discussion

The cashew elite 1996 and 1998 hybrids against virulent fungal isolates obtained from infected cashew materials were evaluated. In this study, 52 elite cashew hybrids planted in three different agro-ecological locations were evaluated for CLNBD severity. The results have indicated that most of the cashew elite 1996 and 1998 hybrids are tolerant to CLNBD. The results consistently showed that 38 out of 52 cashew elite 1996 and 1998 hybrids were tolerant to CLNBD. It seemed interestingly that a CLNBD resistance variety AZA2 was not significantly different from other hybrids.

The phylogenetic relationship of fungi isolates associated with the CLNBD in the Southern and Eastern zones of Tanzania were studied. Using the ITS primers and sequence information, it was evident that the CLNBD in Tanzania is caused by different fungal species including those from genera *Lasiodiplodia* spp, *Colletotrichum* spp, *Neofusicoccum* spp and *Pestalotiopsis* spp. These results show a number of studies that indicate pathogenic nature of the identified organisms.

#### Conclusion

The present study has identified a total of 38 out of 52 cashew hybrids that are tolerant to CLNBD while 14 were susceptible to CLNBD. These results are promising indicator for cashew farmers and stakeholders to reduce the cost of production if will be released for commercial activities. The results also have showed that CLNBD is caused by a complex of fungal species such as *Bortyosphaeria* spp, *Pestalotiopsis* spp, *Colletotrichum gloeosporioides*, *Colletotrichum fragariae*, *Neofusicoccum* spp, *Colletotrichum* spp, *Lasiodiplodia iranensis* and *Lasiodiplodia theobromae*. These fungal microorganisms are reported for the first time to be associated with CLNBD in Tanzania. The recommendation is that more study to be done to confirm these results because experiment was set for only one season.

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