Journal of Natural Sciences
June 2020, Vol. 8, No. 1, pp. 1-15
ISSN 2334-2943 (Print) 2334-2951 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jns.v8n1a1
URL: https://doi.org/10.15640/jns.v8n1a1

Prevalence, Severity and Causative Agents of Dieback in *Calotropis procera* in Semi-Arid Regions of Kenya

Brexidis Mandila[1 2*], Kenneth Odhiambo[2], Alice Muchugi[3], Daniel Nyamai[4], Damaris Musyoka[5]

Abstract

Calotropis procera has a great potential for domestication and commercialization in Kenya for fibre production. However, the shrub experiences dieback condition caused by unidentified fungi. This makes it difficult to prevent dieback during cultivation, a situation that may lead to low productivity and financial losses. This study determined dieback prevalence, severity and causative agents among naturally growing *Calotropis procera* in the semi-arid regions of Kenya. A repeated measure research design was used. Purposive sampling technique was used in selecting Tharaka and Makueni as study sites. Simple and systematic random sampling techniques were used in developing main and sub plots, respectively. Simple random sampling technique was used in selecting 16 cuttings from each block for laboratory analysis. In the laboratory, specimens were obtained from samples, sterilized, rinsed, blotted and incubated at 23°C followed by observation of spores under a Microscope. Mixed analysis of variance (ANOVA) and 2*4*6 factorial ANOVA using SPSS version 25 was used in analysis. There were significant differences in dieback prevalence and severity at different time points with the highest prevalence (78.56%) and severity index (3.54) reported in (September-November) 2019. Fusarium was the dominant dieback causative fungi with dominance ranging from 32.29% to 43.38%. In conclusion, the study established that naturally growing *Calotropis procera* stands in semi-arid regions of Kenya experience dieback throughout the year though at varying levels.

Keywords: Dieback, Severity, Prevalence, Caustaive Agents, Semi-arid

1. Introduction

Calotropis procera, an evergreen shrub in the Asclepiadaceae family grows in the arid and semi-arid regions with less than 1000 mm annual average rainfall, high temperatures and salty soils (Yassin *et al*., 2016 and Coêlho *et al*., 2019). In Kenya, the shrub grows naturally in arid and semi-arid lands (ASALs) of Tharaka, Kajiado, Machakos, Makueni, Turkana, Kitui and Baringo among other ASALs (Mutiso *et al*., 2017).

Traditionally, the shrub is used for medicinal purposes, fuel wood and fodder for goats during dry seasons (Jianchu, 2016). Recently, Mutiso *et al.* (2017) and Jianchu (2016) indicated that *Calotropis procera* can produce high quality calotrope fiber that can be used in the expanding textile industry. This implies that the shrub can be a very important source of raw materials for the textile industry if well managed. However, calotrope fiber supply is very low because farmers in collaboration with World Agroforestry Centre (ICRAF) and other partners are still collecting calotrope fiber from the wild, resulting to very little, unreliable and unsustainable fiber supply (Mutiso *et al*., 2017).

[1] University of Kabianga, School of Natural Resource and Environment, P. O Box 2030-20200, Kericho, Kenya. Email: brexidismandila@yahoo.com
[2] University of Eldoret, School of Natural Resource Management, P. O. Box 1125-30100, Eldoret, Kenya
[3] World Agroforestry Centre (ICRAF), P. O. Box 30677-00100, Nairobi, Kenya
[4] Rongo University, School of Agriculture, Natural Resources and Environmental Studies, P. O. Box 103-40404, Rongo, Kenya
[5] South Eastern Kenya University (SEKU), School of Environment, Water and Natural Resource Management, P.O. Box 170-90200, Kitui, Kenya

Therefore, there is a great opportunity for domesticating *Calotropis procera* for fiber production in ASALs to improve the socio-economic status of communities that mainly rely on limited resources from a fragile ecosystem. Domestication will also ensure sustainable supply of quality calotrope fiber for the expanding textile industry (Muchugi *et al*., 2017).

Although domesticating *Calotropis procera* can be a lucrative venture in ASALs, Mukhtar *et al*. (2013) and Kumar and Khurana (2017) reported over 90 incidences of dieback disease depending on site conditions. Dieback is a condition where plants experience progressive deaths of branches and twigs from their tips towards the trunk due to plant diseases and or unfavorable environmental conditions (Jurskis & Turner, 2002). Dieback conditions may lead to thinning out of crowns of infected trees, limited growth of terminal branches, dying of branches beginning from the top, crown defoliation, crown dieback, discoloration of leaves and shoot wilting, bark and root necrosis, elongated cankerous external and internal lesions on stems that are easily identified with the disease (Sioen *et al*., 2017 & Rolshausen *et al*., 2014). All these may result to death of cultivated stands, low productivity and financial losses to farmers.

According to Mukhtar *et al*. (2014), more research is needed to establish prevalence, severity and causes of dieback conditions between and within agro-ecological zones as a result of variations in physical environments in these regions. This implies that research findings from one region cannot be generalized and applied to other regions. In this regard, research is yet to establish the prevalence, severity and causative agents of dieback in *Calotropis procera* growing naturally in Kenya. Muchugi *et al* (2017) reported that dieback condition in Kenya may be caused by yet to be identified fungi. With unknown fungi, it is difficult to develop proper and correct strategies to prevent the fungi during domestication and cultivation. Therefore, this study established the prevalence, severity and causative agents of dieback condition in *Calotropis procera* in the Kenyan semi-arid regions at different time points from June 2018 to April 2020. This is critical for domestication of *Calotropis procera* regarding calotrope fiber production with respect to developing better strategies for preventing and controlling dieback conditions.

2. Materials And Methods

2.1. The Study Site

The study was conducted in the semi arid regions of Tharaka and Makueni in Kenya. Tharaka is located between Latitudes 00° 07' and 00° 26' South and Longitudes 37° 19' and 37° 46' East. The region's altitude ranges from 600 to 5000 m above sea level (asl). The study was carried out in the lower lands of Kathwana, Kilimangare and Kajiampau that receives unreliable rainfall of about 500 mm annually and higher temperatures (22 – 36) °C. The area is sparsely populated with a population density of 150 persons/km^2 and poverty level of 40%, majority of them depending on agro-pastoralism (Tharaka Nithi County Government, 2018).

Makueni lies on Latitude 1° 35' and 3° 00' South, and Longitudes 37° 10' and 38° 30' East. The region's major physical features include Chyulu, Mbooni, Kilungi and Iuani hills. The study was conducted in the low lands of Makueni (Kyumani and Kyanguli) lying at an altitude of 600 m asl, receiving rainfall ranging from 250 mm to 400 mm annually and experiencing higher temperatures of up-to 35.8 °C. The region has a sparsely distributed human population with population density of 125 persons/km^2 with over 60% poverty level (Government of Makueni County, 2018).

2.2. Study Design

The study employed a mixed repeated measure research design, in which according to Kraska (2010) entails multiple measurements of dependent variables on the same subjects or objects or matched subjects or objects under different conditions or over a period of time. In this regard, repeated measures were taken on *Calotropis procera* stems four times over a period of 23 months from June 2018 to April 2020. This was considered appropriate because it enabled assessment of dieback at different climatic seasons over time.

2.3 Sampling Techniques and Sample Sizes

Purposive sampling technique was used in selecting the semi-arid regions of Makueni and Tharaka in Kenya because of availability of prospective ICRAF collaborating partners. Purposive sampling technique was used in selecting farms (blocks) with naturally growing *Calotropis procera* in Tharaka (Kathwana, Kilimangare and Kajiampau) and in Makueni (Kyumani and Kyanguli) noting that the farmers in these sites allowed access during the study.

Simple random sampling technique was used in developing permanent main plots measuring (20 X 20) m in each block. The random points were generated using Google map, QGIS and Geospatial Modeling Environment software. The generated random points were then located on the ground using GPS, and used as center points in developing square permanent main plots in each block. In Kyumani, Kyanguli, Kathwana, Kilimangare and Kajiampau; 14, 4, 5, 8 and 4 permanent main plots were developed respectively. The size of each farm determined the number of plots.

In each plot, 15 permanent sub-plots each measuring (5 x 5) m were established and systematic random sampling technique used in selecting every third sub-plot. The total number of sub-plots included were estimated according to Ralph *et al.* (2002), (Equation 1).

$$\boldsymbol{n = \log\alpha/\log p} \ldots\ldots\ldots\ldots\ldots\ldots \textbf{(1)}$$

Where: n = Sample size; α = permitted error (0.05 correspond with 95% confidence level); p = proportion of sub-plots estimated as having a particular characteristics, in this cases *Calotropis procera*. Since it was not known, it was estimated at 50% (0.5) as recommended by Ralph *et al.* (2002).

As a result, the number of sub-plots in each plot were computed as:

$$\boldsymbol{n = \log 0.05/\log 0.5}$$

= 4.32 plots ≈ 5 subplots

In each sub-plot, all *Calotropis procera* stems were numbered and included as a sample to determine the dieback prevalence and severity. Numbering was important for successive measurements.

In establishing the dieback causative agents, the sample size of infected cuttings from infected stems in each main plot was estimated based on Ralph *et al.* (2002) (Equation 2) expressed as:

$$\boldsymbol{n = \log\alpha/\log p} \ldots\ldots\ldots\ldots\ldots\ldots \textbf{(2)}$$

Where n, α and p remains as defined in equation 1.

Therefore, the sample size was: $\boldsymbol{n = \log 0.05/\log 0.5 =}$ 4.32 ≈ 5 cuttings per main plot.

The cuttings were made on every 4th stem indicating the dieback condition. In case there were less than four stems, then all the stems in the plot indicating dieback conditions were included in the sample. In case there were less than 5 cuttings in the plot, then all cuttings were selected. Samples from all plots in a block were mixed to form a composite sample. From each composite, a sample, whose size was calculated according to Daniel (1999) (equation 3) was selected.

$$\boldsymbol{n = \frac{Z^2 P(1-P)}{d^2}} \ldots\ldots\ldots\ldots\ldots\ldots \textbf{(3)}$$

Where: n = sample size, Z = Z statistic for the level of confidence, in this the Z statistic was 1.96, corresponding to 95% level of confidence, P = expected prevalence of the condition under investigation, in this case dieback. Since it was unknown, Ralph *et al.* (2002) proposes 0.5, d = precision, which according to Naing *et al.* (2006) is P/2, in this case d= 0.5/2 = 0.25.

Therefore, the total number of cuttings that were taken to the lab for analysis from each block's composite sample were:

$$\boldsymbol{n = \frac{1.96^2 * 0.5 * (1-0.5)}{0.25^2} = 15.37 \approx 16 \text{ cuttings from each block}}$$

In case the composite comprised less than 16 cuttings, then all cuttings from that block were taken. In selecting the 16 cuttings, all the cuttings were randomly laid on the ground and every 2nd cutting selected.

2.4. Data Collection

2.4.1. Prevalence

The prevalence of dieback was determined according to Ezeibekwe (2011). All *Calotropis procera* stems in selected sub-plots were counted, and those experiencing dieback (shoots, branches or leaf margins) enumerated. Prevalence was calculated using Ezeibekwe (2011) equation 4.

$$\boldsymbol{P} = \frac{\mathbf{I}}{\mathbf{N}} \times \mathbf{100\%} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{(4)}$$

Where: p = prevalence, I= the total number of infected shrubs of *Calotropis procera* in each sub-plot, and N = total number of shrubs in each sub-plot.

2.4.2. Severity of Dieback

Severity of dieback condition was determined based on 0-5 severity scale as explained by Ezeibekwe (2011) and Wangungu *et al.* (2011). The scale was based on symptoms of the disease as observed, where; 0= healthy shrub and no symptoms of the disease, 1= 5% of the shrub showing dieback of shoots, 2= 25% of the crown showing dieback, 3= 50% of the shrub showing dieback of bigger branches, 4= 65% of the shrub showing severe shoot dieback, 5= >65% shows very severe shoot dieback. The number of shrubs in each scale were counted, and used to calculate sub-plot severity index (Equation 5) expressed as;

$$\boldsymbol{SPsi} = \frac{(\mathbf{0} * \mathbf{a}) + (\mathbf{1} * \mathbf{b}) + (\mathbf{2} * \mathbf{c}) + (\mathbf{3} * \mathbf{d}) + (\mathbf{4} * \mathbf{e}) + (\mathbf{5} * \mathbf{f})}{\mathbf{N}} \ldots\ldots\ldots\ldots\ldots \mathbf{(5)}$$

Where: SPsi = sub-plot severity index; 0, 1, 2, 3, 4 and 5= scales of severity; a, b, c, d, e, and f = number of trees examined in each category of severity; N = total number of *Calotropis procera* assessed in a sub-plot

2.4.3. Identification of Dieback Causative Agent

Growing nutrient media (Malt Extract Agar at 2%) was prepared in six conical flasks. Maltextract weighing 25 g and 5 g of agar were put in each flask. Distilled water was added to 500 Ml in each flask. The flasks were corked using cotton wool and autoclaved at 121 °C for 20 minutes. It was then allowed to cool to 81 ˚C and the autoclave opened to remove the media. In each flask, 25 drops of streptomycin was added to prevent against bacteria. The media was transferred to the sterilized Petri dishes and allowed to cool.

On each cutting from the field, twelve pieces of *Calotropis procera* were chopped from sections of the samples across living and dead tissues and sterilized using hydrogen peroxide for a period of 1 min. Samples were rinsed three times using distilled water to remove excess hydrogen peroxide and then transferred on the filter paper using forceps for the purpose of blotting dry. Samples were then taken to the isolation hood for drying after which plating was done such that each sample had 3 plates with 4 replicates in each plate (Figure 1).

Figure 1: Procedure of Culturing Infected *Calotropis procera* in Malt Extract Agar (a- samples laid on the table, b- extraction of specimen from *Calotropis procera cuttings*, c- sterilization with hydrogen peroxide and rinsing in distilled water, d- drying of specimens in isolation hood, e- filling of petri dishes with Malt Extract Agar, f- plating of specimens).

Incubation was done at 23 ˚C and after 3 days, part of the fruiting body developing on the nutrient media were sub-cultured and taken back to the incubator for further growth. After 14 days, spores had formed. The

sporulated areas were scratched with clean inoculating needle and placed on a slide for observation under a dissecting microscope to identify the dieback causative agent.
The dominance of each dieback causative agent per sample collected from the field was calculated using equation 6.

$$\mathbf{Y = \left(\frac{n}{N}\right) * 100} \text{ ..... (6)}$$

Where: Y – the dominance of an identified dieback causative agent, n – Frequency of the agent counted on all plates whose specimen was chopped from a sample, N – Total frequency of agents identified on that sample

2.4.4. Climatic factors

Climatic factors that were assessed include rainfall, temperatures and relative humidity. Data was obtained from National Aeronautics and Space Administration (NASA) satellite (https://power.larc.nasa.gov/data-access-viewer/) using geographical coordinates of study sites.

2.5. Data Presentation and Analysis

Microsoft excel was used to generate graphs and curves for data presentation. Statistical analysis was conducted using IBM SPSS version 25. Data on prevalence and severity of dieback conditions were analyzed using two-way mixed analysis of variance (ANOVA) where research region (Tharaka and Makueni) and research time [(June-August) 2018, (March-May) 2019, (September- November) 2019 and (February – April) 2020] were between-subject factor and within-subject factor respectively. The dominance of identified dieback causative agents were analyzed using a 2*4*6 factorial ANOVA. Post-hoc analsis was conducted base don Bonferroni technique. Linear regression based on generalized estimation equation (GEE) was used to establish associations between climatic factors with dieback prevalence and severity.

3. Results

3.1. Dieback Prevalence and Severity in TharakaNithi and Makueni

Figure 2 indicate the increasing trends of dieback prevalence and severity (Figure 3) from (June-August) 2018 to (September-November) 2019 followed by a slight decrease in (February-April) 2020 in the semi-arid regions of Tharaka and Makueni in Kenya. On average, the overall dieback prevalence for Tharaka and Makueni were 56.54%, 72.58%, 78.56% and 61.82% for the period of (June-August) 2018, (March – May) 2019, (September – November) 2019 and (February-April) 2020 respectively (Table 1). The overall average dieback severity indices were 1.96, 2.71, 3.49 and 2.82 for (June – August) 2018, (March – May) 2019, (September – November) 2019 and (February-April) 2020 respectively (Table 1).

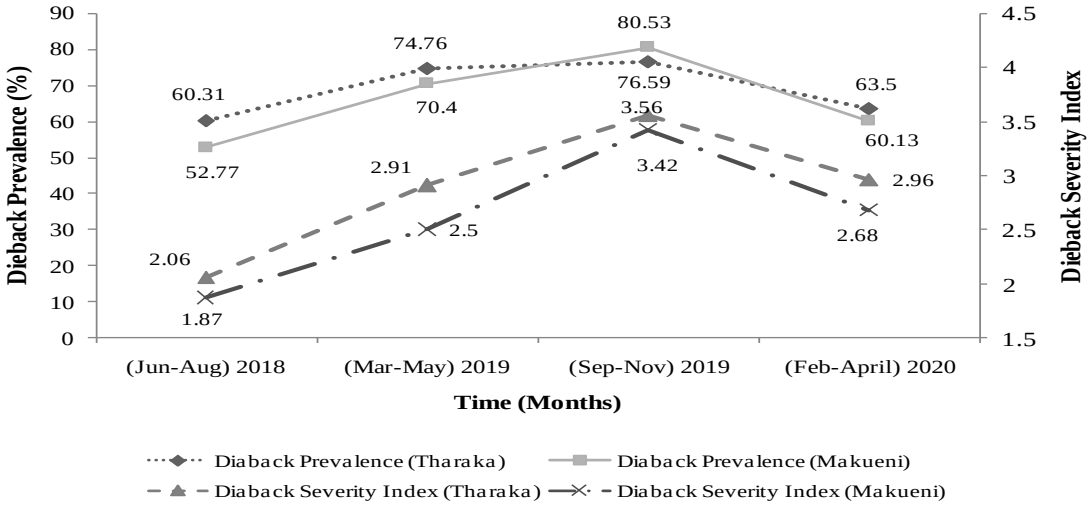

Figure 2: *Calotropis procera*'s Dieback Prevalence and Severity

Table 1: Descriptive Statistics for Dieback Prevalence and Severity

Time	Region	Mean Dieback Prevalence (%)	Mean Dieback Severity Index
(June-Aug) 2018	Tharaka	60.31	2.06
	Makueni	52.77	1.87
	Average	**56.54**	**1.96**
(March-May) 2019	Tharakai	74.76	2.91
	Makueni	70.40	2.50
	Average	**72.58**	**2.71**
(Sept-Nov) 2019	Tharaka	76.59	3.56
	Makueni	80.53	3.42
	Average	**78.56**	**3.49**
(Feb-April)2020	Tharaka	63.5	2.96
	Makueni	60.13	2.68
	Average	**61.82**	**2.82**

Figure 3: Dieback Condition in Tharaka

The assumptions of sphericity and homogeneity of variance were met ($P > 0.05$) by both dieback prevalence and dieback severity data. Table 2 parts a and b indicates that *Calotropis procera* experiences significant differences in mean dieback prevalence and dieback severity at different times points with ($F_{(3, 306)} = 17.201$, $P < 0.001$, $\eta p^2 = 0.144$) and ($F_{(3, 306)} = 49.804$, $P < 0.001$, $\eta p^2 = .320$) respectively. However, there were no significant difference in *Calotropis procera's* dieback prevalence ($F_{(1,102)} = .126$, $P = .723$, $\eta p^2 = 0.001$) and dieback severity ($F_{(1,106)} = .652$, $P = .421$, $\eta p^2 = 0.006$) between the semi-arid regions of Tharaka and Makueni in Kenya (Table 3 parts a and b).

Table 2: Mixed ANOVA Tests Within-Subjects' Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Part a: Tests Within-Subjects' Effects (Time) for Dieback Prevalence							
Time	Sphericity Assumed	26554.250	3	8851.417	17.201	.000	.144
Time * Region	Sphericity Assumed	1795.220	3	598.407	1.163	.324	.011
Error(Time)	Sphericity Assumed	157459.785	306	514.574			
Part b: Tests Within-Subjects' Effects (Time) for Dieback Severity							
Time	Sphericity Assumed	125.304	3	41.768	49.804	.000	.320
Time * Region	Sphericity Assumed	1.094	3	.365	.435	.728	.004
Error(Time)	Sphericity Assumed	266.690	318	.839			

Table 3: Mixed ANOVA Tests Between-Subjects' Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Part a:Tests Between-Subjects' Effects (Regions) for Dieback Prevalence						
Region	198.096	1	198.096	.126	.723	.001
Error	159959.286	102	1568.228			
Part b: Tests Between-Subjects' Effects (Regions) for Dieback Severity						
Region	.987	1	.987	.652	.421	.006
Error	160.499	106	1.514			

Mean dieback prevalence in (June – August) 2018 (56.54%) was significantly lower than in (March – May) 2019 (72.58%) and in (September – November) 2019 (78.56%), but not significantly different from dieback prevalence of (61.82%) in (February-April) 2020 (Table 4 part a). Mean dieback severity index in (June-August) 2018 (1.96) was significantly lower than in (March – May) 2019 (2.71), (September – November) 2019 (3.49) and (February – April) 2020 (2.82) (Table 4 part b).

Table 4: Bonferroni's Pairwise Comparisons of Dieback Prevalence and Severity

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Part a: Pairwise Comparison of Dieback Prevalence						
(Jun-Aug) 2018	(Mar-May) 2019	-16.038*	3.639	.000	-25.829	-6.247
	(Sep-Nov) 2019	-22.021*	3.736	.000	-32.074	-11.968
	(Feb-April) 2020	-6.280	3.249	.336	-15.022	2.461
(Mar-May) 2019	(Sep-Nov) 2019	-5.983	3.190	.381	-14.565	2.599
	(Feb-April) 2019	9.757*	3.071	.012	1.495	18.020
(Sep-Nov) 2019	(Feb-April) 2019	15.741*	3.178	.000	7.190	24.291
Part b: Pairwise Comparison of Dieback Severity						
(Jun-Aug) 2018	(Mar-May) 2019	-.840*	.129	.000	-1.185	-.494
	(Sep-Nov) 2019	-1.576*	.125	.000	-1.912	-1.240
	(Feb-April) 2020	-.813*	.112	.000	-1.115	-.511
(Mar-May) 2019	(Sep-Nov) 2019	-.736*	.150	.000	-1.139	-.334
	(Feb-April) 2019	.027	.125	1.000	-.308	.362
(Sep-Nov) 2019	(Feb-April) 2019	.763*	.131	.000	.410	1.116

3.2. Dieback Causing Agents

Table 5 indicates that dieback in naturally growing *Calotropis procera* in the semi-arid regions of Tharaka and Makueni in Kenya was caused by Botryosphaeria, Fusarium, Phomopsis, Altenaria, Cladosporium, and other unidentified agents. Fusarium and Botryosphaeria species (Figure 4) were the most dominant dieback causing agents throughout the time points.

Table 5: Dominance of Dieback Causing Agents on *Calotropisprocera*

Semi-arid Region	Dominance of causative agent	(Jun-Aug) 2018	(Mar-May) 2019	(Sept-Nov) 2019	(Feb-April) 2020
	Botryosphaeria (%)	36.19	34.07	43.81	40.06
	Fusarium (%)	41.89	43.38	38.57	39.42
	Phomopsis (%)	10.08	9.80	8.81	8.65
	Altenaria (%)	7.89	8.33	7.14	8.01
	Cladosporium (%)	1.09	0.49	0.24	.64
Tharaka	Unidentified Agents (%)	2.63	5.39	2.38	4.17
	Botryosphaeria (%)	35.00	37.70	32.64	46.87
	Fusarium (%)	43.00	42.06	39.93	32.29
	Phomopsis (%)	11.00	9.52	10.76	9.72
	Altenaria (%)	8.00	6.77	10.07	9.03
	Cladosporium (%)	0.33	0.0	1.04	.69
Makueni	Unidentified Agents (%)	2.67	4.54	3.82	2.78

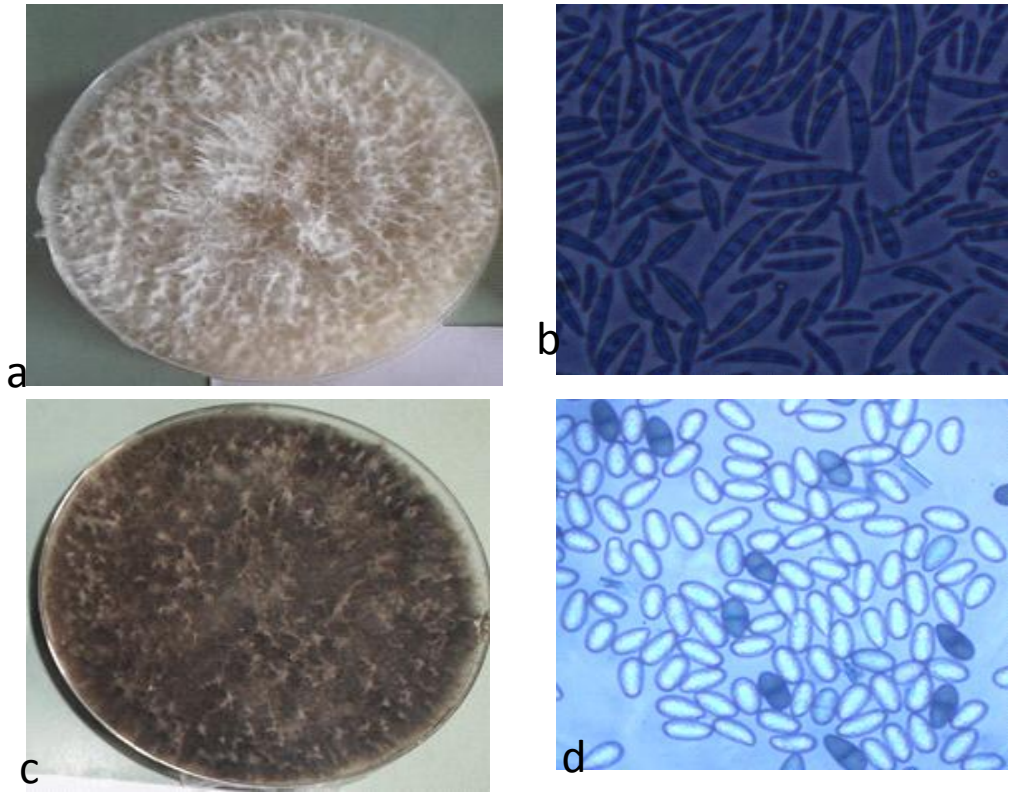

Figure 4: Common Causative Agents of Dieback Condition [a) Fusarium fungi growing on maltextract agar media, b) Fusarium fungi spores observed under dissecting microscope, c) Botryosphaeria fungi growing on maltextract agar media, d) Botryosphaeria fungi spores observed under dissecting microscope].

A 2*4*6 factorial ANOVA (Table 5) indicates that the dominance of dieback causative agents among *Calotropis procera* stands differ significantly among the six agents ($F_{(5, 1314)} = 319.308$, $P < .001$, $\eta p^2 = .549$). However, there were no significant interactions between time points, dieback causative agents and región (Table 5).

There was also no significant difference in the mean dominance of dieback causative agents between the semi-arid regions of Tharaka and Makueni in Kenya ($F_{(1, 1314)} = .049$, $P = .825$, $\eta p^2 < 0.001$).

Table 5: Factorial ANOVA Tests Within-Subjects' Effects for Dieback Causing Agents

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time	12.327	3	4.109	.019	.996	.000
Region	10.512	1	10.512	.049	.825	.000
Dieback causing agent	342293.508	5	68458.702	319.308	.000	.549
Time * region	18.147	3	6.049	.028	.994	.000
Time * dieback causing agent	3868.036	15	257.869	1.203	.262	.014
Region * dieback causing agent	205.661	5	41.132	.192	.966	.001
Time * region * dieback causing agent	3410.758	15	227.384	1.061	.389	.012
Error	281718.117	1314	214.397			
Total	1028263.285	1362				
Corrected Total	647436.189	1361				

Pairwise comparison (Table 6) indicates that the mean dominance of Botryosphaeria and Fusarium genus were significantly higher than other agents ($P < 0.001$).

Table 6: Pairwise Comparisons of Dieback Causing Agents

(I) Causative agent	(J) Causative agent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Botryosphaeria	Fussarium	-1.9818	1.37440	.701	-5.9042	1.9407
	Phomopsis	28.4878*	1.37440	.000	24.5653	32.4102
	Altenaria	30.1404*	1.37440	.000	26.2180	34.0629
	Cladosporium	37.6658*	1.37440	.000	33.7434	41.5882
	Unidentified Agent	34.8765*	1.37440	.000	30.9541	38.7989
Fussarium	Phomopsis	30.4695*	1.37440	.000	26.5471	34.3919
	Altenaria	32.1222*	1.37440	.000	28.1998	36.0446
	Cladosporium	39.6475*	1.37440	.000	35.7251	43.5700
	Unidentified Agent	36.8582*	1.37440	.000	32.9358	40.7807
Phomopsis	Altenaria	1.6527	1.37440	.836	-2.2697	5.5751
	Cladosporium	9.1780*	1.37440	.000	5.2556	13.1004
	Unidentified Agent	6.3887*	1.37440	.000	2.4663	10.3111
Altenaria	Cladosporium	7.5253*	1.37440	.000	3.6029	11.4478
	Unidentified Agent	4.7360*	1.37440	.008	.8136	8.6585
Cladosporium	Unidentified Agent	-2.7893	1.37440	.326	-6.7117	1.1331

3.3. Climatic Factors Affecting Dieback Prevalence and Severity

Figure 5 indicates that average monthly rainfalls (mm) and monthly relative humidity have negative correlation with dieback prevalence and severity, while monthly average temperature (°C) has positive correlation with dieback prevalence and dieback severity.

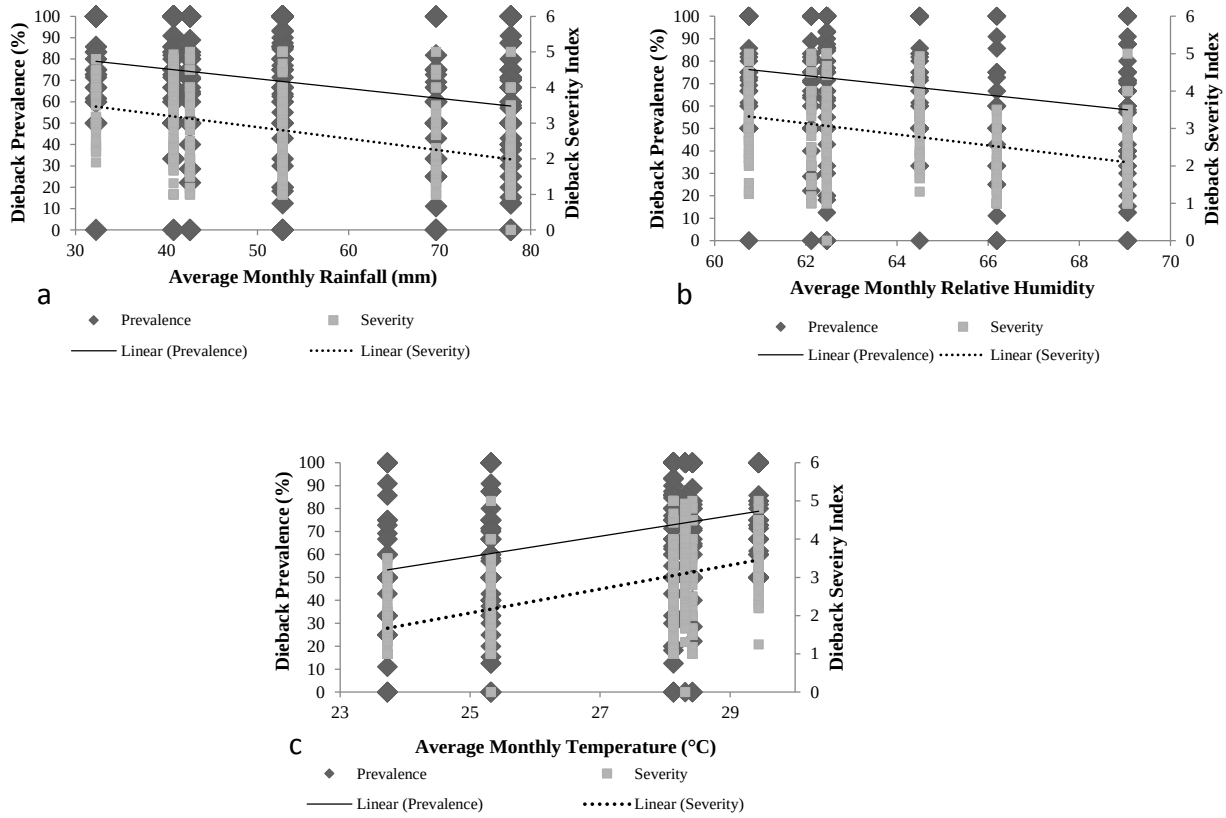

Figure 5: Dieback Prevalence Against Climatic Conditions [a- plot of dieback prevalence (%) and Severity against average monthly rainfall (mm), b- plot of dieback prevalence (%) and Severity against average monthly relative humidity) c- plot of dieback prevalence (%) and Severity against average monthly Temperature (°C)].

Linear regression based on generalized estimation (Table 7) indicates that the association between average monthly relative humidity with dieback prevalence and severity was not significant.

Table 7: Curve Estimation Regression Statistics

	Type III		
Source	**Wald Chi-Square**	**Df**	**Sig.**
Dieback Prevalence			
(Intercept)	9.660	1	.006
Mean monthly rainfall (mm/month)	7.318	1	.012
Mean monthly temperature (°C/month)	4.789	1	.046
Mean monthly relative humidity (%)	1.804	1	.180
Dieback Severity			
(Intercept)	16.020	1	.000
Mean monthly rainfall (mm/month)	20.197	1	.000
Mean monthly temperature (°C/month)	28.418	1	.000
Mean monthly relative humidity (%)	.437	1	.509

Further analysis by eliminating average monthly relative humidity from the model indicated that average monthly rainfall and temperatura were significantly associated with both dieback prevalence and severity (Table 4.8).

Table 8: 2nd Fixed Effect Test of Climatic Factors Affecting dieback prevalence and severity

Source	Type III Wald Chi-Square	df	Sig.
Dieback Prevalence			
(Intercept)	19.848	1	.000
Mean monthly rainfall (mm/month)	14.017	1	.001
Mean monthly temperature (°C/month)	13.288	1	.002
Dieback Severity			
(Intercept)	35.857	1	.000
Mean monthly rainfall (mm/month)	20.860	1	.000
Mean monthly temperature (°C/month)	39.942	1	.000

Model estimates (Table 9) indicates that an increase in average monthly rainfall reduces dieback prevalence and severity by an odds ratio of .714(95% CI, 1.001 to 1.909), Wald χ^2 (1) = 14.017, P = .001 and .696(95% CI, .723 to .834), Wald χ^2 (1) = 20.860, P < .001 respectively. On the other hand, an increase in temperature increases dieback prevalence and severity by an odds ratio of 1.427(95% CI, 2.790 to 3.303), Wald χ^2 (1) = 13.288, P = .002, and 1.380(95% CI, 1.231 to 1.3.461), Wald χ^2 (1) = 39.942, P < .001 respectively.

Table 9: Parameter Estimates of Climatic Factors Affecting Dieback Prevalence and Severity

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square	df	Sig.	Exp (B)	95% Wald Confidence Interval for Exp(B) Lower	Upper
Dieback Prevalence										
(Intercept)	30.10	1.991	31.50	41.969	19.848	1	.000	2.100	1.035	1.087
Monthly rainfall (mm/month)	-2.48	2.495	-7.372	2.408	14.017	1	.001	.714	1.001	1.909
Monthly temperature (°C/month)	8.853	3.791	20.679	26.974	13.288	1	.002	1.427	2.790	3.303
Dieback Severity										
(Intercept)	-20.66	4.285	-34.060	-17.262	35.857	1	.000	1.170	.614	.986
Monthly rainfall (mm/month)	-.016	.003	-.022	.009	20.860	1	.000	.696	.723	.834
Monthly temperature (°C/month)	.948	.150	.654	1.242	39.942	1	.000	1.380	1.231	3.461

4.0. Discussions

4.1. Dieback Prevalence and Severity on *Calotropis procera*

Naturraly growing *Calotropis procera* stems in the semi-arid regions of Tharaka and Makueni were experiencing crown dieback, cankerous, leaf scorching and discoloration; which according Bergdahl and Hill (2016) are indicators of dieback disease. It was established that the shrub experienced dieback conditions at all research time points from June 2018 to April 2020. This concurs with Kumar and Khurana (2017) that found serious leaf spot dieback problem on almost every naturally growing *Calotropis procera* stem in India at all times regardless of existing climatic conditions. According to McKinney *et al.* (2014), it is difficult to find a stand without dieback condition at any instance because even young stems may be infected by their parents especially when the cause is fungus pathogens.

Dieback prevalence and severity varied significantly at different time points of the year. These variations concurred with Handiso and Alemu (2017) that seasons and site conditions contribute significantly to the prevalence and severity of dieback conditions. Seasons contribute to dieback variations because different seasons pose varying levels of environmental stresses like drought that affect plants differently (Kozlowski & Pallardy, 1997).

However, the findings of this study contradict Zarafi and Abdulkadir (2013) that found insignificant variations of dieback instances on Jatropha among months under review. The difference may be explained by differences in methodology between the two studies. This is because Zarafi and Abdulkadir (2013) concentrated on dieback caused by one fungal pathogen (*Fusarium* spps) on Jatropha, while this study looked at dieback conditions caused by multiple causative agents on *Calotropis procera*. In addition, the the plant species of these two studies were different. This contradiction implies that dieback prevalence/incidence depends on the plant species and causative agents.

There was an insignificant variation in dieback prevalence and severity on *Calotropis procera* between the semi-arid regions of Tharaka and Makueni in Kenya. These findings contradict Handiso and Alemu (2017) and Mukhtar *et al.* (2014) that reported variations in dieback prevalence and severity between regions. This contradiction is because according to Tharaka Nithi County Government (2018) and Government of Makueni County (2018), the study areas (Tharaka and Makueni) experience almost similar environmental conditions, are located within the same agro-ecological zone IV and have almost similar altitude. Therefore, a difference in dieback prevalence and severity reported by Mukhtar *et al.* (2014) is as a result of a study conducted in different agro-ecological zones. Different agro-ecological zones mean different environmental and site conditions that influences dieback conditions.

4.2. Dieback Causative Agents

In this study, six dieback causative agents were identified, namely: Botryosphaeria, Fusarium, Phomopsis, Altenaria, Cladosporium and other unidentified agents. Amongst the six, Fusarium and Botryosphaeria species were the most dominant at all four time points in the two semi-arid regions. Botryosphaeria species has been reported to be causing stem and branch canker by colonizing and killing phloem and cambium (Mehl *et al.*, 2013). Fusarium species have been identified in Kenya as a dieback causing fungi (Amata *et al.*, 2009). This fungus is normally soil-borne, meaning that they degrade roots to a level that causes vascular wilts through root rot and root necrosis invasion (Zarafi & Abdulkadir, 2013; Davison, 2014). They also proliferate xylems and phloem where they block water, mineral and food transportation within the plant; causing dieback. According to Mukhtar (2007) the dominance of Fusarium is expected to be low in *Calotropis procera* because the plant has high extract contents that inhibit fungal growth. However, it is unclear why the dominance of a vascular wilt (Fusarium species) remained high in Tharaka and Makueni with dominance ranging from 32.29% to 43.38%.

In Kenya, Amata *et al.* (2009) reported that Altenaria species are notable dieback causing fungi among citrus fruits. However, the presence of Altenaria species on *Calotropis procera* has been reported in India and other regions (Kumar & Khurana, 2017). According to Kumar and Khurana, (2017), the fungi grow on leaves as dark brown bloom, which reduces the photosynthetic area of the plant that eventually affects its photosynthetic abilities. Although Kumar and Khurana, (2017) found that the prevalence of Altenaria species on *Calotropis procera* is high in wastelands (desert and uncultivated regions), it is unclear why in this study, Altenaria's dominance was low (6.77% to 10.07%) compared to Botryosphaeria and Fusarium species. However, this may be because all samples were taken from stems and branches, but not leaves where Altenaria was reported to be prominent.

Cladosporium species has also been reported as a known dieback causing agents on *Calotropis procera* (Barreto *et al.*, 1999; Korekar & Chavan, 2015). These species forms black soot on leaves that eventually causes leaf distortion especially during rainy seasons (Barreto *et al.*, 1999; Talgo *et al.*, 2011). Phomopsis species are also known to cause abnormal bunching and discoloration of foliage, thus resulting to dieback (Janis, 2015; Mahadevakumar & Janardhana 2016). In this study, it was found that the dominance of Phomopsis remained low ranging from 8.65% to 11.00% and did not vary significantly at different times of the year. These findings contradict Janis (2015) that found higher dominance of Phomopsis species in spring where new growth is still wet. The reason may be that Tharaka and Makueni were all located in semi-arid regions experiencing very low amount of rainfalls with high temperatures. These harsh conditions may have inhibited the growth of Phomopsis species.

Unidentified agents included all agents that either did not indicate fungal properties on the growing nutrient media, or the specimen on the plate did not grow any agent. According to Mukhtar *et al* (2014), there are other edaphic, biotic and abiotic factors excluding fungi that cause dieback. Therefore, the category of unidentified agents was other agents that might have been outside the scope of this research, meaning that they were not individually isolated and determined. For instance, high temperaturas, low rainfall, the presence of aphids, spiders and insects may have contributed to dieback condition.

In this study, the dominance of each dieback causing agent did not vary significantly from time to time and from region to region. This contradicts Amata *et al.* (2009) that fungi causing dieback differ from one region to the other depending on the prevailing ecological condition. This contradiction may be because the study areas (Tharaka and Makueni) are located in the same agro-ecological zone, meaning that the prevailing ecological conditions were almost the same.

4.3. Climatic Factors Affecting Dieback Prevalence and Severity

This study established that average monthly rainfall and average monthly relative humidity correlate negatively with dieback prevalence and severity, while temperature correlates positively with the same. This implies that an increase in rainfalls and relative humidity reduce dieback prevalence and severity, while an increase in temperature exacerbates dieback prevalence and severity and the reverse is true. However, linear regression based on GEE indicated that only average monthly rainfall and temperature were significantly associated with dieback prevalence and severity. This concur with Sevanto *et al.* (2014), Brunner *et al.* (2015) and Vose *et al.* (2016) that, high temperaturas and low rainfalls subject plants to hydraulic failure that makes plants lose water through transpiration. This condition creates high xylem water tension that leads to the loss of cavitations and conductivity of xylem which restrict water up-take that eventually leads to wilting and dieback (Brunner *et al.*, 2015; Kennelly *et al.*, 2012).

According to Moustafa and Sarah (2017), although *Calotropis procera* can flourish under dry conditions, excessively high temperatures and low rainfalls reduces its photosynthetic pigments by shading leaves to reduce transpiration rates. Therefore, from Figure 5 and odd ratios below 1.427 for average monthly temperature and above .696 for average monthly rainfall means that the correlation between climatic factors and prevalence in the study areas was weak. This may be because the temperatures were not excessively high while the average monthly and rainfall were not excessively low. This weak association supports Ahmad *et al* (2019) that found weak relationship between dieback prevalence and climatic factors on *Dalbergia sissoo* in Pakistan.

5.0. Conclusions and Recommendations

5.1. Conclusions

The following conclusions were drawn from this study:

1). Naturally growing *Calotropis procera* in the semi-arid regions of Tharaka and Makueni in Kenya experience dieback conditions throughout the year. However, dieback condition is worse during dry seasons with high temperaturas as evidenced by highest dieback prevalence and severity índices of 78.58% and 3.49 in (september – November) a period with low rainfall and high temperatures

2). Dieback conditions on *Calotropis procera* in the semi arid regions of Tharaka and Makueni in Kenya is caused by Botryosphaeria, Fusarium, Phomopsis, Altenaria, Cladosporium, and other unidentified agents. However, Botryosphaeria and Fusarium are the most dominant in the two regions at all times.

3). Prevailing climatic factors, mainly rainfall and temperature were influencing dieback prevalence and severity levels in the two study areas.

5.2. Recommendations

1). To manage dieback condition caused by identified fungi, owners of farms with naturally growing *Calotropis procera* need to be educated on the need to avoid wounding the plant, apply appropriate cultural systems, detecting the condition at an early stage and spray with appropriate fungicides.

2). Understanding the contribution of climatic factors on dieback needs long-term research (over 3 years). This is because the effects of climatic factors are not immediate. For example, after drought, it takes time for the plant to recover from drought effects even when heavy rains are being experienced.

Acknowledgement

Authors thank German Academic Exchange Service (DAAD) for financial support through in-region PhD scholarship under ICRAF-DAAD collaboration (Grant ID: DAAD-1157). We thank Kenya Forestry Research Institute (KEFRI) pathology laboratory staff for their technical support.

References

Ahmad, I., – Atiq, M., Nawaz, M., F. Ahmed, S., Asif, M., Gull, S., Tanvir, M. A., Abdullah, M., Azhar, M., & Rajput, N. A. (2019). Prediction of Dieback Disease of *Dalbergiasissoo* (Shisham) Based upon Environmental Factors and Tree Age. A*pplied Ecology and Environmental Research*, 17(3): 6483-6495.

Amata, R.L., Otipa, M.J., Waiganjo M., Wabule, M., Thuranira, E.G., Erbaugh, M., & Miller, S. (2009). Incidence, Prevalence and Severity of Passion Fruit Fungal Diseases in Major Production Regions of Kenya. *Journal of Applied Biosciences,* 20: 1146 – 1152.

Barreto, B. R., Evans, H. C., & Pomella, A. V. (1999). Fungal pathogens of Calotropisprocera (rubber bush), with Two New Records from Brazil. *Australasian Plant Pathology*, 28: 126-130.

Bergdahl, A. D., & Hill, A. (2016). *Diseases of Trees in the Great Plains. Fort Collins, CO: U.S*. Department of Agriculture, Forest Service: Rocky Mountain Research Station.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., &Sperisen, C. (2015). How Tree Roots Respond to Drought. *Front Plant Sc.* 2015(6): 547.

Coêlho, M.R.V., Rivas, R., Ferreira-Neto, J.R.C., Pandolfi, V., Bezerra-Neto, J.P. &Benko-Iseppon, A.M. (2019) Reference Genes Selection for *Calotropisprocera* under different Salt Stress Conditions. *PLoS ONE,* 14(4): e0215729.

Daniel, W. (Ed). (1999). *Biostatistics: A Foundation for Analysis in the Health Sciences*, (7thed). New York: John Wiley & Sons.

Davison, E. (2014). Resolving Confusions about Jarrah Dieback - Don't Forget The Plants. *Australasian Plant Pathology*, 43(6): 691-701.

Ezeibekwe, I. O. (2011). Study of Citrus Disease Prevalence on Four Citrus Varieties at the National Institute of Horticultural Research (NIHORT) Mbato, Okigwe, Imo State, Nigeria. *African Journal of Plant Science*, 5(6): 360-364.

Government of Makueni County. (2018). *Makueni County Integrated Development Plan (CIDP) 2018-22*. [Online] Available: https://roggkenya.org/wp_content/uploads/Makueni_CIDP_2018-2022_County-Integrated-Development-Plan-1.pdf

Handiso, S. &Alemu, T. (2017). The Nexus between Incidence and Severity of Chili Anthracnose (*Colletotrichumcapsici* (Syd.) Bisby and Butler) on Chili in SNNPR, Ethiopia. *American Scientific Research Journal for Engineering, Technology, and Science,* 32(1): 298-302

Janis, R. (2015). *Tree and Shrub Diseases: Phomopsis Blight, Anthracnose, and Black Knot. In Lawn Care*. [Online] Available: https://blog.lawneq.com/tree-and-shrub-diseases-phomopsis-blight-anthracnose-and-black-knot/

Jianchu, X. (2016). *CalotropisFibre: The Hope of Africa*. Kunming, China: World Agroforestry Center.

Jurskis, V., & Turner, J. (2002). Eucalypt Dieback in Eastern Australia: A Simple Model. *Australian Forestry*, 65(2): 87-98.

Kennelly, M., O'Mara, J., Rivard, C., Miller, G.L., & Smith, D. (2012). Introduction to Abiotic Disorders in Plants. *The Plant Health Instructor,* 10(1094): 10-20.

Korekar, S. L., &Chavan, S. P. (2015). *Studies on Fungal Diseases of Some Medicinal and Aromatic Plants from Osmanabad District*.Solapur, India: Laxmi book publication.

Kozlowski, T. T., & Pallardy, S. G. (1997). Environmental Regulation of Vegetative Growth. *Physiological Ecology*, 1997:195-322.

Kraska, M. (2010). Repeated Measures Design. In Salkind, N. J. (Ed). *Encyclopedia of Research Design*. Thousand Oaks: Sage Publications, Inc.

Kumar, N., & Khurana, S. P. (2017). Serious Leaf Spot Disease Problem of *Calotropisprocera* (Aiton) W.T.Aiton. By Alternariaalternata in Gurgaon (Haryana), India. *Int. J. Curr. Microbiol. App. Sci.* 6(5): 403-407.

Mahadevakumar, S. & Janardhana, G.R. (2016). Leaf Blight and Fruit Rot Disease of Brinjal Caused by Diaporthevexans (Phomopsisvexans) in Six Agro-Ecological Regions of South West India. *Plant Pathology & Quarantine,* 6(1): 5–12.

Mehl, J., Slippers, B., Roux, J., & Wingfield, J. (2013). Cankers and other Diseases Caused by the Botryosphaeriaceae. In Gonthhier, P. (Ed). *Infectious Forest Diseases* pp. 298-317. Washington: CABI.

McKinney, L., Nielsen, L., Collinge, D., Thomsen, I., Hansen, K., & Kjær, E. (2014). The Ash Dieback Crisis: Genetic Variation in Resistance can Prove a Long-Term Solution. *Plant Pathology*, 2014(63): 485–499

Moustafa, A. R., & Sarah, S. Q. (2017). Population Ecology and Economic Importance of *Calotropisprocera* as an Exotic Medicinal Plant. *Journal of Ecology & Natural Resources*, 1(1): 1-11.

Muchugi, A., Gachuiri, A., Gacheri, N., Mutiso, F., Kimiti, J., Jamnadass, R. &Xu, J. (2017).*Calotropisprocera*: A New Investment for African Drylands. Future Agriculture: Socio-Ecological Transitions and Bio-Cultural Shifts. Tropentag, 20–22 September, Bonn.

Mukhtar, I. (2007). Comparison of Phytochemical and Chemical Control of *Fusariumoxysporium* f. sp. ciceri. *Mycopath,* 5(2)**:** 107-110

Mukhtar, I., Bajwa, R., &Nasim, G. (2014). Trees Survival Exposed to Dieback Disease Implies Evolutionary Modulation Resistance in Shisham (Dalbergiasissooroxb.) In Various Agro Ecological Zones of Punjab (Pakistan). Pakistan Journal of Phytopathology, 26 (2): 289-300

Mukhtar, I., Khokhar, I., & Mushtaq, S. (2013). First Report of Leaf Spot Disease of CalotropisgiganteaCaused by Passaloracalotropidis in Lahore, Pakistan. *The Journal of Animal & Plant Sciences,* 23(2): 670-671.

Mutiso, F. M., Kimiti, J., Muchugi, A., Gachuiri, A., Jamnadass, R., Xu, J., & Kimatu, J. (2017). Introduction from the Wild and Growth Characterization of Three Provenances of Calotropisprocera (Ait) in a Domesticated State in Dry lands of South Eastern Kenya. *Journal of Natural Sciences Research*, 7(24): 102-112.

Naing, L., Winn, T. & Rusli, B. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. Medical Statistics. *Archives of Orofacial Sciences*, 1: 9-14.

Ralph, B., Holleran, D. S. & Ramakrishnan, R. (2002). Sample Size Determination. *ILAR Journal*, 43(4): 207-213.

Rolshausen, P., Baumgartner, K., Travadon, R., Fujiyoshi, P., Pouzoulet, J., & Wilcox, W.F. (2014). Identification of *Eutypa spp*. Causing Eutypa Dieback of Grapevine in Eastern North America. *Plant Disease*, 98(4): 483-491.

Sevanto, S., McDowell, N., Dickman, L. T., Pangle, R., &Pockman, W. (2014). How do Trees Die? A Test of the Hydraulic Failure and Carbon Starvation Hypotheses. *Plant, Cell and Environment,* 37: 153–161.

Sioen, G., Roskams, P., Cuyper, B. & Steenackers, M. (2017). Ash Dieback in Flanders (Belgium): Research on Disease Development, Resistance and Management Options. *Swedish University of Agricultural Sciences,* 3: 61–67.

Talgo, V., Sundheim, L., Gjaerum, H. B., Herrero, M.L., Suthaparan, A., Toppe, B. &Stensvand, A. (2011). Powdery Mildews on Ornamental Trees and Shrubs in Norway. *The European Journal of Plant Science and Biotechnology*, 5(1): 86-92.

Tharaka Nithi County Government. (2018). *Development Plan CIDP 2018-2022*. [Online] Available:https://roggkenya.org/wp-content/uploads/Tharaka-Nithi_CIDP_2018-2022_County-Integrated-Development-Plan.pdf

Vose, J. M., Clark, J. S., & Luce, C. H. (2016). *Effects of Drought on Forests and Rangelands in the United States: A Comprehensive Science Synthesis*. Colorado: United States Department of Agriculture.

Wangungu, C., Maina, M. & Mbaka, J. (2011). Proposed Assessment Scale For Dieback Disease Severity on Passion Fruit. *Journal of Animal & Plant Sciences*, 12(2): 1583-1589

Yassin, M. A., Nawar, S., & Anwar, A. K. (2016). Ecology of Invasive Species in Saudi Arabia, *Calotropisprocera* (Ait) W.T. Ait.: Floristic Composition and Associated Plant Communities. *International Journal of Ecotoxicology and Ecobiology*, 1(3): 127-140.

Zarafi, A.B. &Abdulkadir, I.D. (2013). The Incidence and Severity of Jatropha dieback disease in Zaria, Nigeria. *Archives of Phytopathology and Plant Protection*, 46(8).