

**INCIDENCE AND SEVERITY OF SISAL BOLE ROT DISEASE IN SMALL SCALE AND ESTATE FARMERS FIELDS IN MUHEZA DISTRICT**

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**ABSTRACT**

Sisal yields the world's natural strong fiber used to produce several industrial products and boosts economy of many countries. However, sisal production is under threat due to bole rot disease responsible for great loss in production areas. The aim of the study was to determine the incidence (DI) and severity (DS) of bole rot disease in farmer's sisal fields of Muheza district. The assessment was done during dry and wet seasons in three wards, one estate and three small scale farmers per ward obtained through systematic random sampling. Monthly weather data (temperature, relative humidity, rainfall) were from Tanzania Meteorological Agency. Data were transformed using arcsine square root and analysis of variance (ANOVA), means comparison for disease parameters were performed using GenStat® version 16 Software. Means were compared by Duncan Multiple Range Test at 5% probability. ANOVA was used to check the effect of altitude and season on disease parameters. Regression (R<sup>2</sup>) between climatic factors and disease parameters was established and Student's t-test was used to compare mean for (DI), (DS), and disease intensity index (DII) for both seasons. The results revealed that, bole rot disease was present in all surveyed wards with different intensity. DI, DS and DII were significantly different ( $P < 0.05$ ) amongst the surveyed wards in both seasons. For both seasons, Kigombe ward recorded the highest DI, DS and DII while Tanganyika ward recorded the least. Plans are needed to improve farmers' access to improved disease-free planting materials and on the use of integrated disease management practices.

**Keywords:** *Agave sisalana*, *Aspergillus niger*, bole rot, incidence, severity.

**1. INTRODUCTION**

Sisal (*Agave sisalana* L.) contributes nearly 85% of the total fibre production of the World (Sarkar, 2017). Sisal is adapted to warm environments with low rainfall, cultivated in semiarid conditions, characterized by low and irregular precipitations (300–800 mm/ year), long drought periods and annual mean temperatures as high as 32 °C where no other crops can be grown (Sarkar, 2017). It is one of the main sources of hard natural fibre and raw materials for the industry, medicine and handicrafts. Sisal produces a coarse and strong fibre that is used in composite materials for automobiles, furniture, construction and plastic and paper products. Extracts of sisal plants consists of substances with anti-inflammatory, antimicrobial and anthelmintic activities (Cruz-Magalhães et al., 2019). Sisal has several distinguishing characteristics which makes it to be a 'speciality crop' for conservation agriculture since it does not produce pesticide load to the environment because it is not much infested by many disease and insect pest; and therefore the use of chemicals is minimum (Sarkar, 2017). Furthermore, sisal plants reduce soil erosion through its extensive root system and contribute positively to

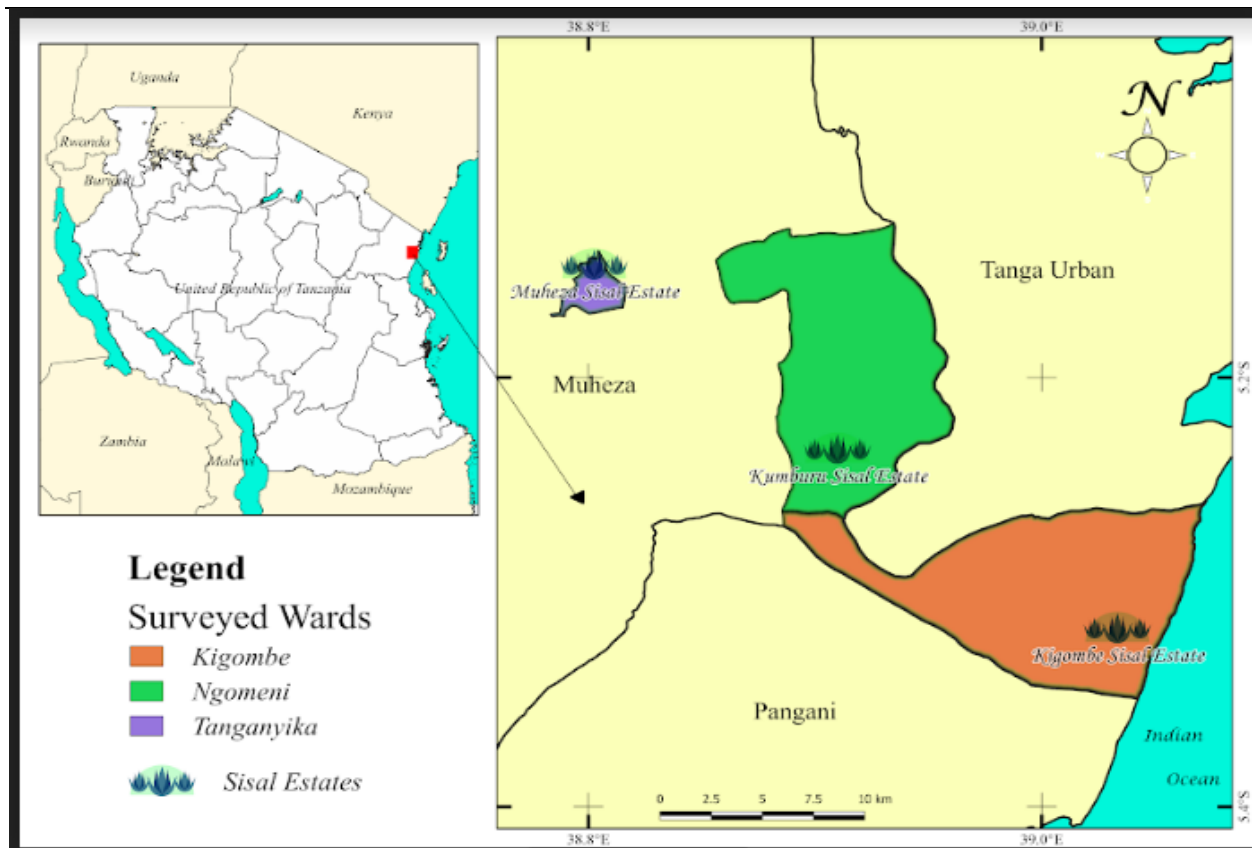
watershed management (Sarkar, 2017). It serves as a source of employment, income for the farmers and provides country foreign currency (Beleko, 2021). Besides all these benefits, sisal bole rot is the main disease of sisal, responsible for substantial losses in producing countries. The disease was first observed in areas of sisal production in Tanzania in the 1930s and reported first in the 1952 (Cruz-Magalhães et al., 2019). It is caused by species of the genus *Aspergillus*, especially the ones belonging in the Nigri complex section. *Aspergillus niger* has been reported to be a major cause of the sisal bole rot disease (Durte et al., 2018), though according to the research done by Santos et al. (2014), there is additional of three species of *Aspergillus* causing bole rot disease; these are *A. tubingensis*, *A. brasiliensis* and *A. welwitschiae*.

Bole rot disease is responsible for great losses due to its ability to infect sisal plants and exhibit wilting, yellowing of leaves and reddening of the bole and base of the leaves to plant death (Durte et al., 2018). The spread of the disease is faster because the pathogens are found on plant stems and in the soil around the plant rhizosphere and majority of farmers in Muheza district rely on suckers from previous crop for propagation of new sisal fields which increase the chances of spreading the inocula from infected plants and soils to the new fields. The pathogen can also be spread through the use of contaminated tools/machinery, clothes, shoes and human body during harvesting of the crop especially when it is wet. In Tanzania, research on epidemiology of bole rot disease has not been done but such information is available in other sisal growing countries such as Brazil and Mexico where bole rot prevalence has been reported to be as high as 100% whilst the average field incidence is up to 35% in some sisal plantations (Damasceno et al., 2019). Hence, in Tanzania, data is therefore lacking on the incidence and severity of bole rot disease. It is expected that, data from this study will guide the development of intervention strategies against the disease that will safeguard livelihood of sisal farmers in Tanzania.

## **2. MATERIALS AND METHODS**

### **2.1 Study area**

The survey was done in three wards selected randomly at different altitudes of Muheza district. The three wards are: Ngomeni located at the altitude of 107 msl, longitudes of 38.9191 S and latitudes of -5.1866 E; Tanganyika ward located at altitude of 200 msl, longitudes of 38.8055 S and latitudes of -5.1613 E and Kigombe ward located at the altitude of 22 msl, longitudes of 39.0018 S and latitudes of -5.2907 E (Fig. 1).



**Figure 1:** A map of Muheza district shown sisal bole rot disease surveyed wards for disease incidence and severity

**2.2 Anatomy of the infected sisal plants**

The anatomy of the infected sisal plants in farmer’s sisal fields was done by randomly identification of the infected sisal plants in the field and observe the changes in the plant tissue morphology (texture, colour and shapes). The bole of the infected sisal plants was cross-sectionally cut and the changes on the stem tissues were observed.

**2.3 Assessment of disease incidence and severity in each ward**

For the assessment of disease incidence and severity in each ward, three farms for small scale farmers and one sisal estate were randomly selected and a one hectare sisal farm was measured and surveyed in two seasons (January for dry season and May for wet season). Disease incidence and severity were assessed along the odd sisal rows, systematically. Disease incidence was observed by counting and recording the total number of healthy and infected plants present in all inspected sisal rows. The percentage of disease incidence was calculated as per Yeh et al. (2019), where

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected sisal plants}}{\text{Total number of assessed sisal plants}} \times 100 \dots \dots \dots i$$

For disease severity, the infected sisal plant stems were cross-sectionally cut and the severity was recorded according to the scale proposed by Damasceno et al. (2019), from 0 to 3 where (0) no =

symptom, (1) = initial symptoms, (2) = advanced symptoms, and (3) = plant death (Appendix 1). Disease severity (%) was calculated as per Damasceno *et al.* (2019), where

$$\text{Disease Severity (\%)} = \frac{n \times v}{3N} \times 100 \dots \dots \dots \text{ii}$$

Where, (n) = Number of plants in each category, (v) = Numerical values of symptoms category, (N) = Total number of plants assessed, (3) = Maximum numerical value of symptom category (Fig.2)



**Figure 2:** Disease severity scale; A= (0) no symptoms, B= (1) initial symptoms, C= (2) advanced symptoms but not dead and D= (3) plant death.

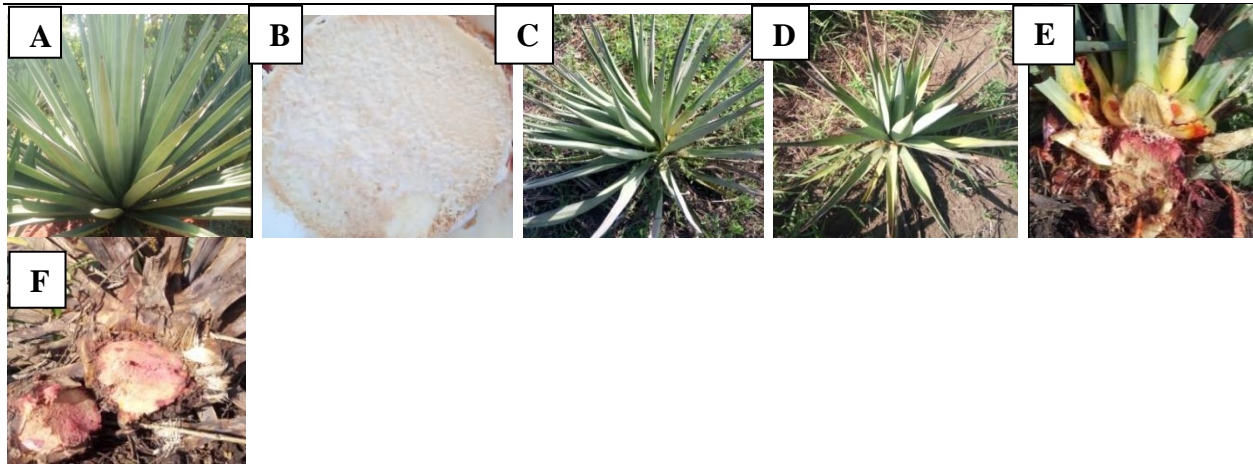
**2.4 Statistical data analysis**

Before analysis, data were transformed using the arcsine square root transformation formula as described by Mousanejad *et al.* (2010) as shown:  $Y = \text{Arcsin } \sqrt{P}$ , Where, Y is transformed data, and p is the observed proportion. Analysis of variance and comparison of means for disease incidence, severity and disease intensity index were performed using GenStat® Executable release 16 Statistical Analysis Software. Means were compared by Duncan Multiple Range Test (DMRT) at 5% probability. Analysis of variance (ANOVA) was used to check the effect of altitude and season on the disease incidence, severity and disease intensity index. Regression ( $R^2$ ) between weather factors and disease parameters was established and a Student’s t-test was used to compare mean for disease incidence, severity, and disease intensity index for the dry and wet seasons

**3. RESULTS**

**3.1 Identified anatomy of the infected sisal plants in farmer’s sisal field**

The survey observed that, plants with bole rot disease exhibit external symptoms such as wilting and yellowing of leaves (Fig 3C-D), brownish to reddening of the stem (bole) and base of the leaves (Figure 3E-F). Healthy plants did not show these symptoms (Figure 3A-B). The internal symptoms were observed as necrosis in the bole and leaf base and all exhibited brownish to reddish rot tissue (Figure 3E-F). The progression of the disease caused plant death (Figure 3F). Transversal sections of the bole indicated that the disease was disseminated along the ground parenchyma from the cortex to the vascular bundle (Figure 3E-F).



**Figure 3:** Anatomy of the healthy and infected sisal plants: healthy plant (A), stem of healthy sisal plant (B), infected sisal plant with wilting and yellowing leaves starting at the stem base (C-D), infected sisal plant stem with brownish and reddish colour (E-F), dead sisal plant (F)

### 3.2 Disease incidence, severity and disease intensity index dispersion

Among the three wards assessed, there was significant difference on bole rot disease in terms of disease incidence, severity and disease intensity index ( $p < 0.05$ ). Kigombe ward was having the highest incidence (36.4%) followed by Ngomeni ward (28.5%) and Tanganyika ward with the least incidence of 19.1% (Table 1, Fig. 4). A significant ( $P < 0.05$ ) variation in severity was observed between the wards surveyed for the bole rot disease. Kigombe and Ngomeni wards had significant high disease severity, 32.54% and 28.94% respectively while Tanganyika ward had the lowest disease severity of 12.82%. The same trend was observed on disease intensity index where Kigombe and Ngomeni wards had significantly high disease intensity index of 425.4 and 311.4 respectively at ( $p < 0.05$ ) while Tanganyika ward had the least disease intensity index of 90.4. Disease severity and disease intensity index of Kigombe ward did not vary significantly with that of Ngomeni ward at ( $P < 0.006$ ) and ( $P < 0.016$ ) respectively with the exception of Tanganyika ward which disease severity and disease intensity index was significantly different with other wards at ( $P < 0.006$ ) and ( $P < 0.016$ ) respectively (Table 1, Fig.4)

### 3.3 Influence of altitude on bole rot disease parameters

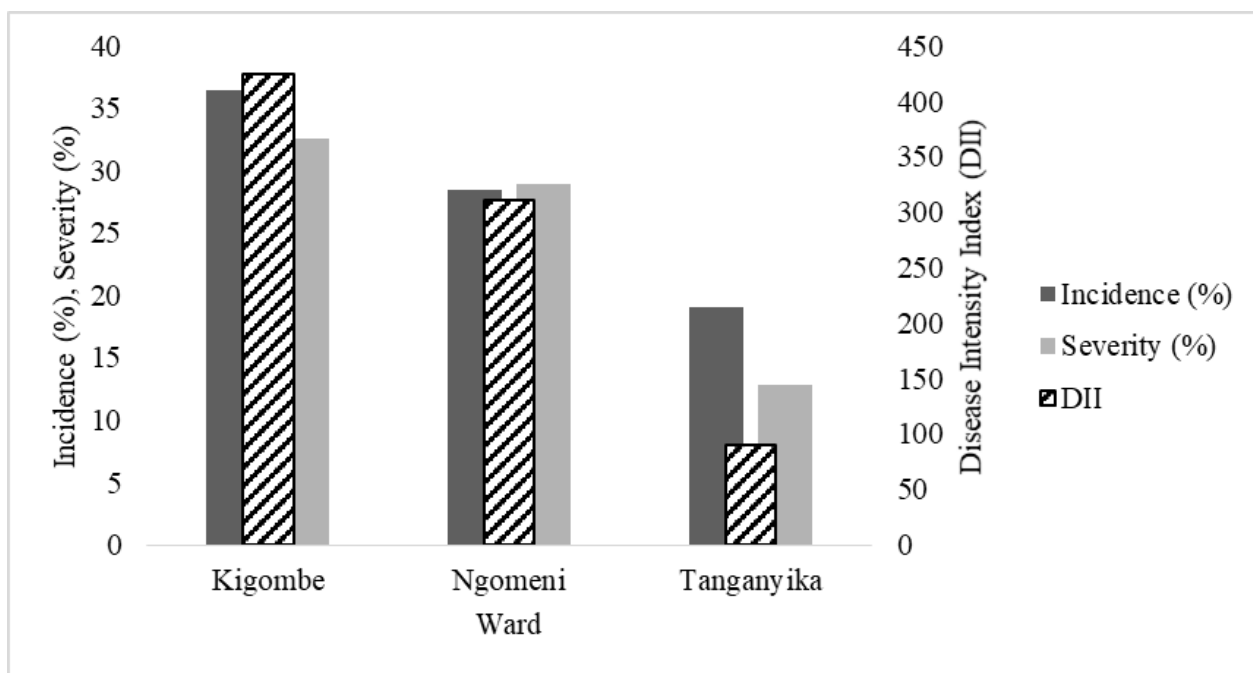
The height above the sea level (altitude) had significant influence on disease incidence, severity and disease intensity index ( $p < 0.05$ ). It was noted that the disease incidence and severity was high in lower altitudes than in higher altitudes in both surveyed wards. Lower altitude (22m) had high disease incidence (36.43%), severity (32.54%) and disease intensity index (425.4). High altitude (200m) had lower disease incidence (19.09%), severity (12.82%) and disease intensity index (90.4) (Table 1, Figure 5).



**Table 1: Bole rot disease parameters on surveyed wards and the influence of altitude on the disease parameters**

Ward	Altitude (m)	Incidence (%)	Severity (%)	Disease Intensity Index
Kigombe	22	36.43 <sup>b</sup>	32.54 <sup>b</sup>	425.4 <sup>b</sup>
Ngomeni	107	28.52 <sup>ab</sup>	28.94 <sup>b</sup>	311.4 <sup>b</sup>
Tanganyika	200	19.09 <sup>a</sup>	12.82 <sup>a</sup>	90.4 <sup>a</sup>
	Grand mean	28.00	24.80	276.0
	SED	5.75	5.67	104.9
	CV%	6.20	18.80	20.7
	p-value	0.025	0.006	0.016

Means carrying the same letter along the column were not significantly different under Duncan Multiple Range Test (DMRT) at  $p < 0.05$ .



**Figure 4:** Bole rot disease parameters based on ward distributions

### 3.4 Effect of climatic conditions on disease parameters

Climatic conditions; temperature, relative humidity and rainfall per ward were analyzed in combination to evaluate their influence on disease incidence, severity and disease intensity index. The analysis revealed that the disease incidence, severity and disease intensity index varied from one ward to another due to different climatic conditions prevailing in each ward. Disease incidence, severity and disease intensity index among the recorded climatic conditions was significantly different at ( $p < 0.05$ ). A significant high incidence (46.46%), severity (40.24%) and disease intensity index (619.3) was observed at a temperature of 28.1°C, relative humidity of 78.8% and total rainfall of 118.2 mm. Low disease incidence, severity and disease intensity

index was observed at a temperature of 27.9°C, relative humidity of 80.2% and rainfall of 122.8 mm (Table 2, Figure 5).

**Table 2: Bole rot disease parameters based on climatic conditions in three wards**

Ward	Temp.	R.H	Rainfall	DI	DS	DII
Tanganyika (dry)	27.9	80.2	122.8	14.38 <sup>a</sup>	11.55 <sup>a</sup>	59.3 <sup>a</sup>
Tanganyika (wet)	25.8	70.5	60.6	23.80 <sup>a</sup>	14.09 <sup>ab</sup>	121.6 <sup>a</sup>
Kigombe (dry)	30.0	82.6	97.0	26.40 <sup>a</sup>	24.83 <sup>abc</sup>	231.5 <sup>a</sup>
Kigombe (wet)	28.1	78.8	118.2	46.46 <sup>b</sup>	40.24 <sup>c</sup>	619.3 <sup>b</sup>
Ngomeni (dry)	28.8	81.1	54.4	27.99 <sup>a</sup>	27.36 <sup>abc</sup>	291.7 <sup>a</sup>
Ngomeni (wet)	26.3	74.0	76.4	29.05 <sup>a</sup>	30.52 <sup>bc</sup>	331.1 <sup>a</sup>
Grand mean				28.00	24.80	276.0
SED				6.83	7.76	126.6
CV%				6.20	18.80	20.7
p-value				0.009	0.021	0.008

Means carrying the same letter along the column were not significant different under Duncan Multiple Range Test (DMRT)  $p < 0.05$ , SED= Standard error of difference, R.H = Relative Humidity and CV= Coefficient of Variance

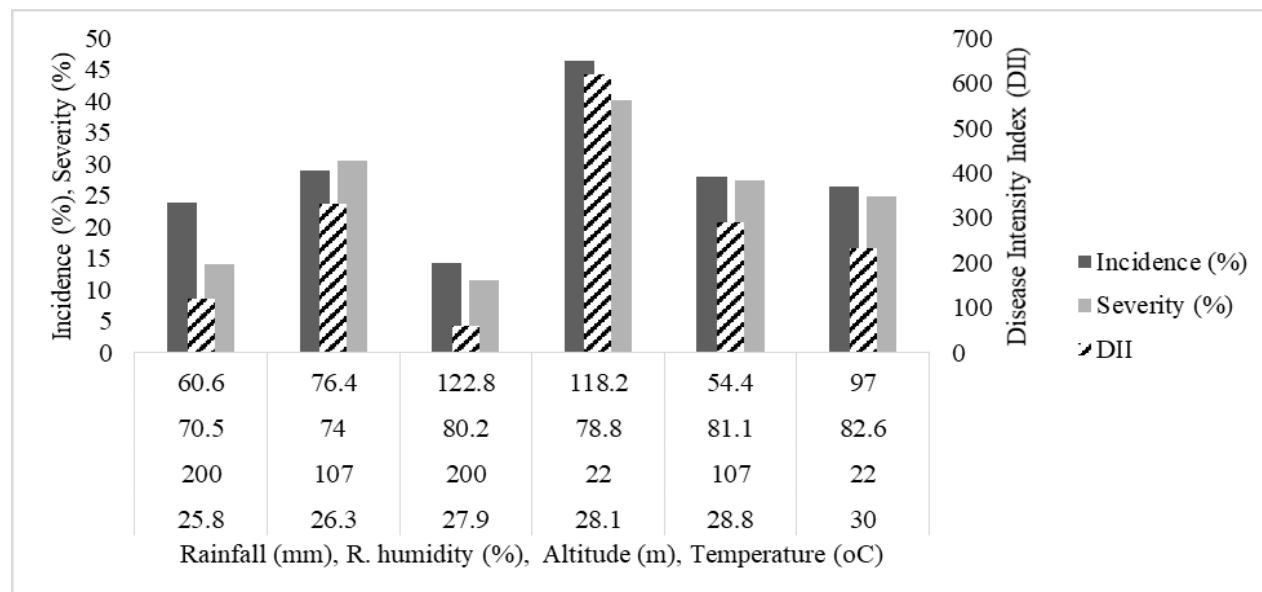


Figure 5: Bole rot disease parameters based on climatic conditions and altitude in three wards of Muheza district

### 3.5 Comparison between dry and wet seasons on disease parameters

A t-test was carried to compare dry and wet seasons on disease parameters. A null hypothesis “Mean of disease parameters during wet season is equal to the mean of disease parameters during dry season” tested at 95% confidence interval. For disease incidence, null hypothesis was rejected ( $p < 0.05$ ,  $t = -2.13$ ) with dry season having mean disease incidence of 22.92% and wet season having mean disease incidence of 33.10%. The null hypothesis was accepted on disease

severity ( $p > 0.05$ ,  $t = -1.25$ ). This signifies that, mean disease severity during wet season (28.28%) was not significantly different from mean disease severity in dry season (21.25%). For disease intensity index, null hypothesis was accepted ( $p > 0.05$ ,  $t = -1.74$ ) with dry season having 194.2 while wet season had 357.3 (Table 3).

**Table 3: Student’s t-test to compare dry and wet seasons with disease parameters**

Parameter	Season	Mean	Variance	SD	p-value	t-test
Incidence	Dry	22.92±2.88	99.4	9.97	0.045	-2.13
	Wet	33.10±3.82	175.3	13.24		
Severity	Dry	21.25±3.27	128.6	11.34	0.224	-1.25
	Wet	28.28±4.57	250.2	15.82		
DII	Dry	194.20±50.80	30969.0	176.00	0.095	-1.74
	Wet	357.30±78.56	74059.0	272.10		

Means (Mean ± SE); SE=Standard error of means, SD=Standard deviation

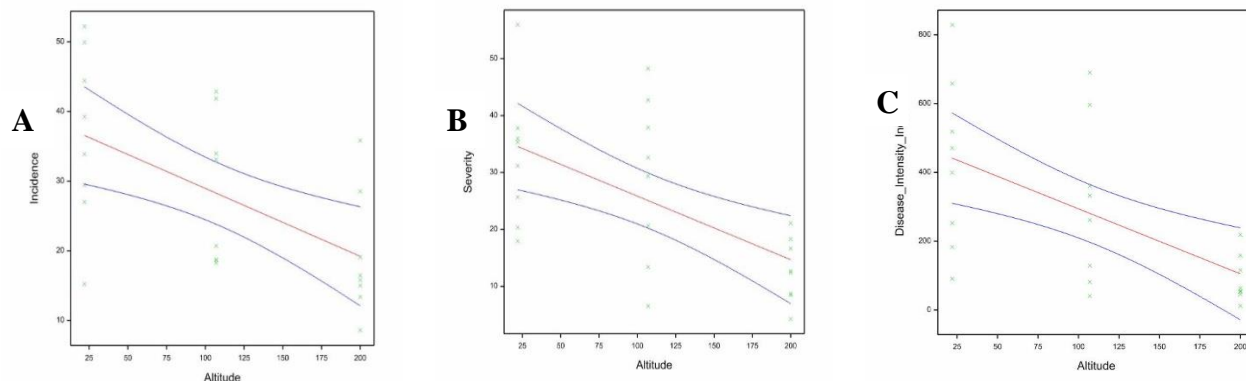
**3.6 Regression relationships between climatic factors, altitude and disease parameters**

Climatic factors and height above the sea level (altitude) were regressed against disease parameters. All climatic factors had no significant ( $p > 0.05$ ) relationship with bole rot disease parameters (Table 4). Significant relationship was observed between the height above sea level (altitude) and quantified disease parameters at ( $p < 0.05$ ). All disease parameters had moderate relationship with altitude where disease incidence had ( $R^2=0.331$ ,  $p = 0.003$ ), severity had ( $R^2=0.355$ ,  $P=0.002$ ), and disease intensity index had ( $R^2=0.344$ ,  $P=0.003$ ) (Table 4, Fig. 6)

**Table 4: Regression relationship between climatic parameters, altitude and disease parameters**

Factor	Disease Incidence	Disease Severity	Disease Intensity Index
Temperature	$P=0.742$ , $R^2=0.005$	$P=0.452$ , $R^2=0.026$	$P=0.601$ , $R^2=0.013$
Altitude	$P=0.003$ , $R^2=0.331$	$P=0.002$ , $R^2=0.355$	$P=0.003$ , $R^2=0.344$
R. humidity	$P=0.994$ , $R^2=0.0002$	$P=0.514$ , $R^2=0.020$	$P=0.652$ , $R^2=0.009$
Rainfall	$P=0.683$ , $R^2=0.008$	$P=0.752$ , $R^2=0.005$	$P=0.492$ , $R^2=0.022$

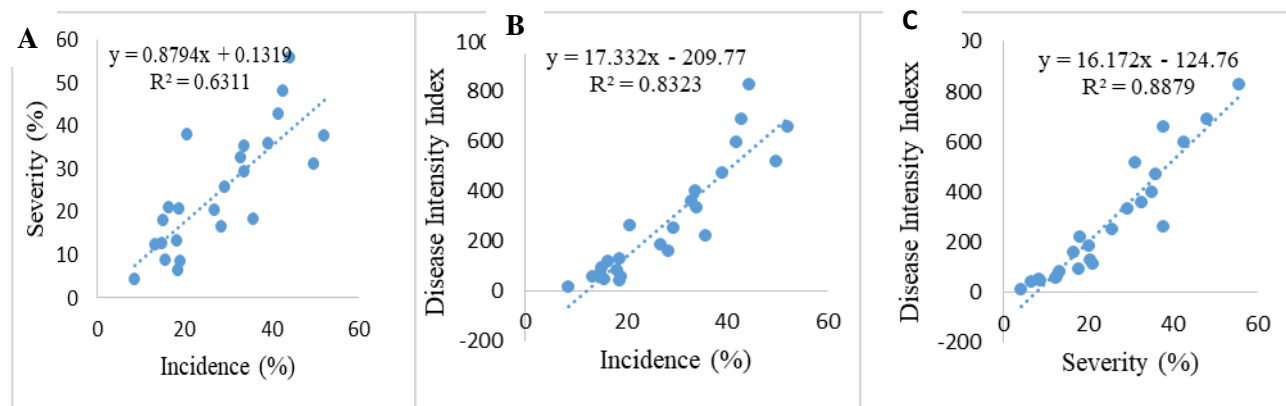




**Figure 6:** Regression relationships between altitude with (A) disease incidence, (B) disease severity and (C) disease intensity index.

### 3.7 Relationship between disease incidence, disease severity and disease intensity index

Disease incidence, severity and disease intensity index was regressed against each other. All parameters were highly significant correlated to each other at ( $p < 0.001$ ). The relationship between disease incidence and severity was 63.11% which is a strong and positive relationship. Disease incidence and disease intensity index had very strong positive relationship of 83.23% while disease severity and disease intensity index had very strong positive relationship of 88.79% (Fig. 7).



**Figure 7:** Correlation relationships between (A) incidence and severity, (B) incidence and disease intensity index and (C) severity and disease intensity index

## 4. DISCUSSION

### 4.1 Anatomy of the infected sisal plants in farmer's field

Although bole rot disease was recognized in the country since 1950s, to date there is no information on its quantification in sisal growing areas. Disease quantification is essential for epidemiological studies, surveys, and management decisions. A result pertaining to anatomy of the infected sisal plants in farmer's sisal fields show that sisal plants with bole rot disease exhibit external symptoms such as wilting and yellowing of leaves, brownish to reddening of the bole

and base of the leaves. The internal symptoms were observed as necrosis in the stems and leaf bases and all of which exhibited brownish to reddish rot tissue and the transversal sections of the bole shows that the disease is disseminated along the ground parenchyma from the cortex to the vascular bundle. Healthy sisal plants do not show these symptoms. The progression of the disease caused plant death. This result coincides with those obtained by Cruz-Magalhães et al. (2019) who pointed out that, sisal plants infected by bole rot disease at advanced stages are identified by the external symptoms which are wilting and yellowing of the aerial parts and the internal symptoms include brownish, rotting and reddening of the stem tissues. Durte et al. (2018) commented that, once sisal plants are infected, bole starts to rot and the internal tissues become brown, surrounded by a reddish border making leaves yellow and collapsing and when the bole is completely rotten, cause plant death. De Souza et al. (2021) observed that, infected sisal plants exhibit wilting and yellowing of leaves and reddening of the bole and base of the leaves followed by plant death.

#### **4.2 Disease incidence and severity dispersion among the surveyed wards**

The survey for disease incidence and severity revealed that, sisal bole rot disease was prevalent in all the surveyed areas but with varying intensity. High disease incidence and severity were along the coastal area, in Kigombe ward followed by Ngomeni ward and Tanganyika ward the least. It was also noted that, the distribution of disease in the surveyed fields was randomly scattered. This finding is in agreement with those of Damasceno et al. (2019) who found that the disease was present in all the studied farms with disease prevalence of 100% and on average, 35% of the plants were infected by the pathogen. Additionally, Duarte et al. (2018) observed that, the incidence of sisal bole rot disease can vary from 5% to 40% in the production areas. Hong et al. (2013) indicated that, species of *Aspergillus* can cause devastating disease in sisal, with incidence in the field varying from 5% to 65%. Similarly, Santos et al. (2014) indicated that the incidence of the bole rot disease was 100% for *A.niger* in production areas.

#### **4.3 Influence of altitude on bole rot disease parameters**

The level of incidence and severity was highly influenced by altitude. Kigombe ward located at lower altitudes, near the coastal areas had high disease incidence and severity compared to Ngomeni and Tanganyika wards located at higher altitudes. Tanganyika ward being at higher altitudes than all wards had the least level of incidence and severity. The sisal lands in Kigombe ward are situated in flat areas where during heavy rainfall the soils get wet easily and water logged favoring bole rot pathogens to grow, proliferate and cause infection to sisal plants at a big rate. Furthermore, the condition of water logging in Kigombe ward allow leaching of major minerals such as Calcium, subject the sisal plants to stresses, easily to be attacked by bole rot pathogens. On the contrary, sisal farms in Tanganyika ward are situated in higher altitudes which do not allow water logged and leaching of Calcium minerals, unfavorable conditions for the bole rot fungi to grow, sisal plants remain strong with no stress and with high resistance to disease infection. The results are in agreement with those of Cruz-Magalhães et al. (2019) who pointed out that the occurrence of sisal bole rot disease was linked to environmental conditions and the nutritional status of the plants. Gashaw et al. (2014) found that, the sampling site of Awi zone in Ethiopia which was characterized by higher altitude, low temperature, and low humidity did not favor the spread of the pathogen, it had low incidence and severity.

#### **4.4 Effects of climatic conditions on disease parameters**

The analysis revealed that the disease incidence and severity varied from one ward to another due to different environmental conditions prevailing in each ward. Sisal is a drought resistant plant that grow well in the arid and semi-arid regions with rainfall range 1000-1250 mm, temperatures ranging from 10°C to 30°C and can also endure temperatures of 40-50°C (Saxena et al., 2011). Excessive rains causing water stagnation and very low temperature causing frost tend to damage the plantation (Sarkar, 2017). It was noted that, in all surveyed wards, disease incidence and severity was high during wet season than during dry season. This was due to decrease in temperature and relative humidity favoring growth, multiplication and infection of bole rot pathogens. The result is in agreement with those of Cruz-Magalhães et al. (2019) pointed out that the occurrence of the bole rot disease was linked to environmental conditions and the nutritional status of the plant. Dennis and Fisher (2018) indicated that, a change in temperature could directly affect the spread of infectious disease and survival between seasons. Additionally, Priyanka et al. (2020) pointed out that, environment do affect plant pathogen survival, vigor, rate of multiplication, sporulation, direction, distance of dispersal of inoculum, rate of spore germination and penetration. Velásquez et al. (2018) indicated that, there are three basic elements required for the development of an infectious disease: a susceptible host, a virulent pathogen and favorable climatic conditions for infection, host colonization and production of propagule. Furthermore, Yáñez-López et al. (2012) observed that, plant diseases develop under a well defined, optimal range of climatic variables such as temperature, rain, and relative humidity and commented that, the occurrence and severity of a disease in an individual plant is defined by the deviation of each climatic variable within the optimal range for disease development. Climatic conditions influence the incidence as well as temporal and spatial distribution of plant diseases.

#### **4.5 Relationships between disease incidence, severity and disease intensity index**

The knowledge of the functional relationships between disease incidence, severity and disease intensity index is important because it improves understanding of the epidemiology of the disease. The relationships between disease incidence, severity and disease intensity index were very strong positive. The results are being justified by Bock and Chiang (2019) who studied incidence– severity relationships for other disease and obtain positive relationship. Furthermore, the result shows that, disease severity and disease intensity index increased with increasing disease incidence. This result is justified by Bock and Chiang (2019) who studied the analysis of incidence–severity relationships for strawberry powdery mildew and obtain a highly significant relationship between powdery mildew incidence and severity. These results suggest that the conditions were favoring auto-infection rather than disease spreading to other areas, which reflected to increased severity for the same level of incidence.

### **5. CONCLUSION**

This study revealed 100% prevalence of the sisal bole rot disease in the surveyed sisal growing wards of Muheza district. Disease incidence and severity varied significantly among the wards under wet and dry seasons, with highest incidence and severity shown during the wet season in all surveyed wards. The disease incidence and severity was highest in Kigombe ward followed by Ngomeni ward and Tanganyika ward being the least. Variation on disease incidence and severity among the wards was contributed by differences in locations (altitude) and environmental conditions prevailing in each ward. It was observed that, in regardless of the

advantages of production of healthy sisal planting materials at Tanzania Agricultural Research Institute (TARI) Mlingano in Muheza District, majority of sisal farmers in Muheza district are still using unhealthy planting materials. Therefore, extension services need to be more focused on increasing the level of awareness among sisal farmers especially on practicing integrated field management practices which will reduce the incidence of bole rot disease. Together with that, extension officers should encourage and direct sisal farmers to buy healthy planting materials from the research institutes and or recognized places.

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