International Journal of Recent Research in Thesis and Dissertation (IJRRTD)
Vol. 1, Issue 1, pp: (7-15), Month: July - December 2020, Available at: www.paperpublications.org

Resistance levels of Improved Sweet Potato Varieties to Alternaria Disease in different Agro-ecological Zones of Western Kenya

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Abstract: **Sweet potato (*Ipomoea batatas*, L) is the third most important food crop in Kenya after maize and Irish potato. Research efforts have been devoted to select, breed, and disseminate improved sweet potato varieties (ISVs) that enhance the productivity and quality to alleviate poverty, nutritional food insecurity and poverty among the rural households. Among the barriers to the introduction of ISVs are the diseases of biological origin. *Alternaria* blight disease has been ranked as the most important fungal disease of sweet potato in East Africa. Several genotypes have been developed by the Kenya Agriculture and Livestock Research Organization (KALRO) that are high yielding, early maturing together with other enhanced nutritional characteristics. However, their performance in the existence of various diseases that prevail in different Agro-ecological zones of Kenya has not been established. This study evaluated ISVs from KALRO for resistance/tolerance to *Alternaria* blightdisease in different AEZs of western Kenya. Nine sweet potato varieties were planted at Kakamega, Kakamega county-high rainfall site and Alupe, Busia county – low rainfall site. A Randomized Complete Block Design (RCBD) with three replications was used. Inoculum of *Alternaria solani* was cultured from infected SPK 004 in KARLO Kakamega laboratory, and applied to all the treatments 6 weeks after planting. All the ISVs were assessed for *Alternaria* disease incidence at different stages using standard scale of 1-5. Yield data was taken at a physiological maturity by harvesting the crops in each plot and weighed. The data was analysed using combined ANOVA and simple correlation using SAS software Version 8. The study revealed that the rate of progression for *Alternaria* disease against improved sweet potato varieties in Kakamega and Alupe was low compared to the unimproved varieties. There was a significant negative effect of *Alternaria* disease on the yield of both the improved and unimproved sweet potato varieties. *Alternaria disease* severity was significantly more vicious at KALRO Alupe than in KALRO Kakamega. None of the varieties was susceptible or resistant to the disease. All varieties were moderately resistant except Kabode and Namunyekera which were moderately susceptible. At Kakamega root yield varied significantly between the varieties where Kenspot1 recorded the highest yield (18.2 t/ha) while SPK013 had the lowest yield (10.1 t/ha). At Alupe yield did not vary significantly between the varieties but Kenspot5 had the highest yield (18.3 t/ha). The study concludes that most improved sweet potato varieties were tolerant to *Alternaria* blight disease in different AEZs of western Kenya. The study recommends that there is need for more baseline surveys to be conducted on adoption and performance of the new disseminated varieties in all high altitude areas of the country, use healthy, clean, fungal free planting materials and proper field sanitation.**

Keywords: **Agro-ecological zones, Alternaria disease, Resistance, Sweet potato.**

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1. INTRODUCTION

Sweet potato (*Ipomoea batatas*, L) is the third most important food crop in Kenya after maize and Irish potato (CIP, 2013). In Kenya, over 75% of sweet potato production is concentrated in western, central and coastal areas of the country. According to the MOA (2011), sweet potato production increased by 89% between 2004 and 2009, a scenario attributed to use of improved cultivars and farming methods which have helped increase yield per unit area (Kenyon *etal*., 2006; MOA, 2010; FAOSTAT 2014). Recently, there have been renewed efforts by the government and other players in the agriculture sector to promote production of traditional high value crops of which sweet potato is among them (Nkirote, 2016). As a result, important research efforts have been devoted to select, breed, and disseminate improved sweet potato varieties (ISVs) that enhance the productivity and quality of food crops, alleviating poverty and food insecurity (Odame *et a*l., 2002).

The yield of sweet potato is constraint by *Alternaria* blight disease that has been ranked as one of the most important fungal disease of sweet potato in East Africa (Rees *et al*., 2003; Agnes, 2009). The fungus causes disease on the foliage stems and fruits (Osiru, 2008) leading to tremendous losses in yield ranging from 2.5 to 10 t/Ha (Wabwile *et al*., 2016) and can be higher in extreme cases. The disease can be considered as the most important infection of sweet potato in East Africa (Anginyah, 2001) and South East Asia (Lopes &Boiteux, 1994). The measure of harm to the sweet potato plant is reliant on the phase of development and prevailing ecological conditions (Skoglund & Smit, 1994).

The performance and resistance/tolerance levels of ISPV to *Alternaria* diseases that prevail in different Agro-ecological zones has not been established. This limits information on the actual yield potential of these ISVs which is important in informing the breeders on the performance of their genotypes.Therefore, there was need to evaluate the ISVs for resistance against *Alternaria* blight disease in different AEZs of western Kenya.

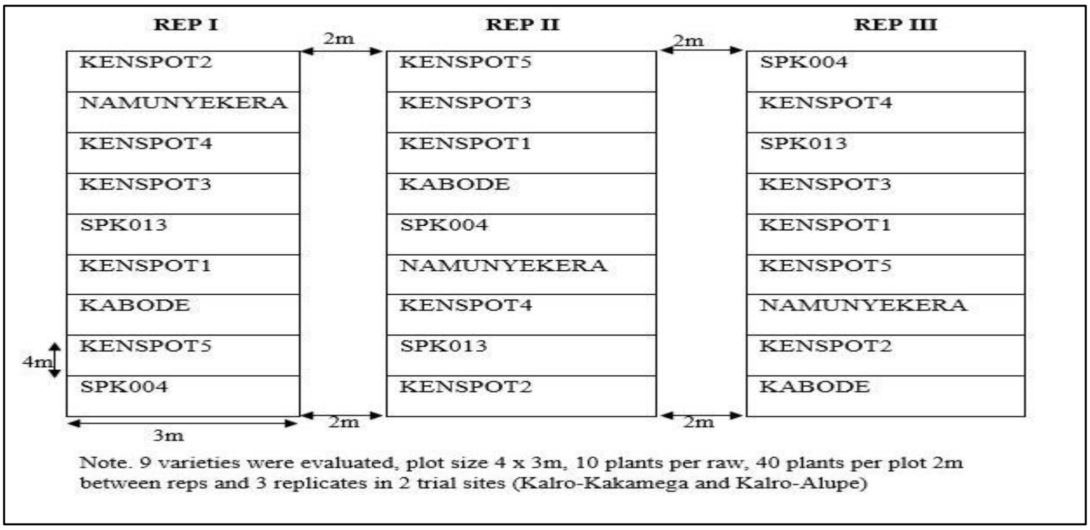
2. MATERIALS AND METHODS

Study sites and field layout

A multi-locational study was conducted to evaluate Improved Sweet Potato Varieties for resistance to *Alternaria* blightdisease in different Agro-Ecological Zones of western Kenya. The two agro-ecological zones were KARLO Kakamega research Centre, Kakamega county – high rainfall agro-ecological zone and KARLO Alupe, Busia county – low rainfall agro-ecological zone.KARLO Kakamega Research Centre lies in the Upper Medium (UM) agro-ecological zone wilt an altitude of 1548m above sea level.The annual rainfall in this zone ranges from 1280.1mm to 2214.1mm with an average of 20.4^{0}C.KARLO Alupe Busia Centre is in the agro-ecological zone LM3 with an altitude of 1222m above sea level.Alupe receives an annual rainfall of between 760mm and 2000mm withaverage temperature is 22.0^{0}C.

The treatment evaluated in the study included 9 ISPVs namely: Kenspot1, Kenspot2, Kenspot3, Kenspot4, Kenspot5, Namunyekera, Kabode and SPK004 with SPK 013 which is susceptible to Alternaria disease acting as a negative control. A Randomized Complete Block Design (RCBD) was used with the treatments replicated three times (Fig. 1).

The planting materials (vines) were sourced from clean plants obtained either from the field or nurseries that were well maintained and inspected and found free from disease infection.



REP I	REP II	REP III
KENSPOT2	KENSPOT5	SPK004
NAMUNYEKERA	KENSPOT3	KENSPOT4
KENSPOT4	KENSPOT1	SPK013
KENSPOT3	KABODE	KENSPOT3
SPK013	SPK004	KENSPOT1
KENSPOT1	NAMUNYEKERA	KENSPOT5
KABODE	KENSPOT4	NAMUNYEKERA
KENSPOT5	SPK013	KENSPOT2
SPK004	KENSPOT2	KABODE

Note. 9 varieties were evaluated, plot size 4 x 3m, 10 plants per raw, 40 plants per plot 2m between reps and 3 replicates in 2 trial sites (Kalro-Kakamega and Kalro-Alupe)

Figure 1: Field layout

Inoculum preparation and inoculation

A hundred (100) milliliters spore suspension for the inoculum were prepared in the laboratory by adding 10 drops of tween 20 using Pasteur into 100 ml of distilled H_2O in the beaker and stirred with magnetic stir plate. Ten ml of water solution was put on fungal plate and the pores scraped using the spatula followed by repeated stirring of the mixture in the beaker for 10 mins. The mixture was filtered using cheese cloth and the funnel (Aberkane *et al*., 2002). Quantification of the spores was done using haematocytometer and then visualized in a light microscope. In order to make sure that the spores to be used were be of good quality, concentration of spore suspension were standardized at 1.2 x 10^6 spores per ml. The spore suspensions were prepared with 2 % malt solution and adjusted to a spore density of 1.78 x10^4 spores per millitre. For each suspension 1.4 liters were prepared, placed on ice and taken into the field. The plants were inoculated by spraying the spore suspension starting from the abaxial to the adaxial of the leaves. Suspensions were poured into a backpack sprayer and sprayed with 2 bar onto the plots. After each spore suspension the backpack sprayer is cleaned twice with water in order to prevent contamination. To achieve better conditions for infection, the field was irrigated before and after inoculation.The inoculation was done 6 weeks after planting at both sites. Inoculation was done very early in the morning sites when there was enough moisture favorable for Alternaria fungi to infect and multiply.

Disease progression, severity scoring and yield evaluation

Two criteria were used: a) Number of leaf spot lesions per plant and b) percentage of area of the leaf surface covered by leaf spot lesions were used to assess the disease infection following the procedure of Iiondu & Ayondele, (2003). According to the procedure disease incidences is greatest on older leaves therefore this study was limited to oldest leaves on each plant. The leaves were numbered according to the position of the petiole. The oldest (lowest) leaf were labeled number one (1) where the plants braches, the most prominent branch was selected for the study. Leaf spot lesions on each leaf were physically counted.

Each variety was visually assessed for *Alternaria* severity at different stages using standard 1-5 scale, one (1) being complete resistant and 5 being the complete susceptible (Payak and Sharma, 1982). Based on this rating scale, the sweet potato varieties were classified into four groups namely, resistant (R) genotypes with a score < 2.0; moderately resistant (MR) 2.1-3.0; moderately susceptible (MS) 3.1-3.5 and susceptible (S) > 3.5-5.

Root yield data was taken at a physiological maturity at four and a half months by harvesting the crops in each plot and weighed. Root harvesting was done after plant stand count in each plot and recorded. Roots were harvested, sorted out to determine the marketable roots and non-marketable roots. The marketable and non-marketable roots was counted, weight and recorded separately.

3. RESULTS

Alternaria disease incidences at different growth stages

Overall, Alternaria disease incidence was higher at Alupe than Kakamega. At Kakamega, Kabode had low incidence (3.3 and less) up to 6 weeks after inoculation (WAI) then it increased rapidly from week 8 (20.67%) reaching 27% at week 16. At Alupe, Kabode had 1.67% incidence at 4 WAI which rose to 13% at week 6 (13.33%) and progressed thereafter to 29.67% at 16 WAI (Fig. 2). At Kakamega, Kenspot 2 recorded zero disease up to 6 WAI, however at 8 WAI, 8% disease incidence was observed reaching 11% at 16 WAI. The same variety at Alupe had hit 10% disease incidence at week 6 which progressed rapidly to 26% at 12 WAI and stabilized to the 16 WAI.

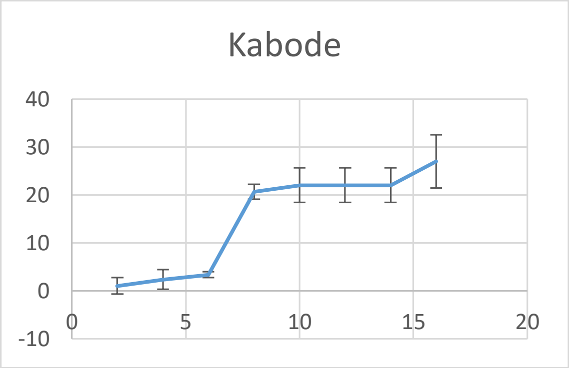
The variety Namunyekera, at Kakamega, had less than 1% incidence up to 6 WAI which then rose to 11% from 8-12 WAI reaching 15% at 16 WAI. At Alupe this variety reached 11.67% at 6 WAI which rose rapidly to 30.33% at 16 WAI. Kenspot5 at Kakamega had lees that 1% incidence at 4 WAI which increased steadily from 6 WAI (4.33%) reaching 22% at 16 WAI. At Alupe, the same variety showed a similar trend in disease progression whereby at 6 WAI 9.33% incidence was recorded which advanced steadily reaching 21% at 16 WAI.

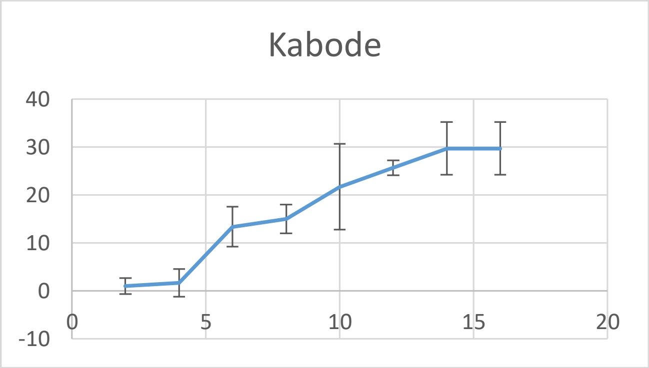
At Kakamega, the variety Kenspot 1 had disease incidence fluctuate between 2-4.33% from week 2 to 6 WAI. This then rose to 16% at 8 WAI and increased to 21.67% at 16 WAI. While at Alupe, the same variety had 4.33% incidence at 2 WAI which increased throughout the weeks reaching 23.33% at 16 WAI. At Kakamega, Kenspot 3 recorded less than 2% disease incidence up to 6 WAI which increased to 10.33% at 8 WAI and stabilized at 14% at 10, 12, 14 WAI and reached 16% at 16 WAI. At Alupe, Kenspot 3 had hit 12.67% disease incidence at 6 WAI which increased to 27% at 12 WAI. The incidence later dropped to 24.33% at 16 and 16 WAI.

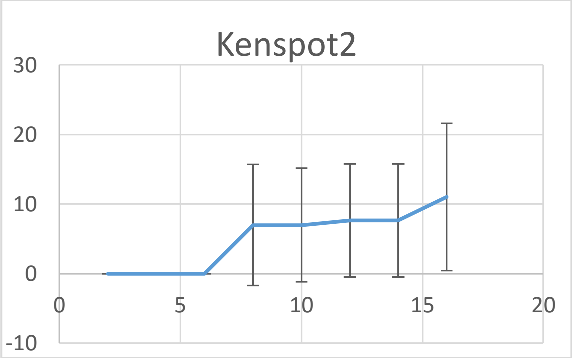
At Kakamega, the variety SPK 013 (susceptible check) fluctuated between 1-4% disease incidences from 2-6 WAI then increased to 10.67% at 8 and 10 WAI and finally reached 18.67% at 16 WAI. At Alupe, the same variety recorded 10.67% diseases incidence at 6 WAI which increased rapidly reaching 25.67% at 16 WAI. Kenspot 4, at Kakamega, had zero incidence at 2 and 4 WAI. It then recorded 1% disease incidence at 6 WAI which increased rapidly reaching 19.67% at 16 WAI. At Alupe, Kenspot 4 had 2-2.33% disease incidence at 2 and 4 WAI, this then rose to 25.67% at 10 WAI which dropped to 18% at 14 and 16 WAI. The variety SPK 004, at Kakamega had no disease incidence up to 4 WAI, but at 6 WAI the variety recorded 1.67% disease incidence which increased rapidly through the weeks reaching 30.33% at 16 WAI. At Alupe, this variety had 1.67% disease incidence at 2 and 4 WAI, which increased sharply at 6 WAI (10.33%) and reached 21% at 16 WAI.

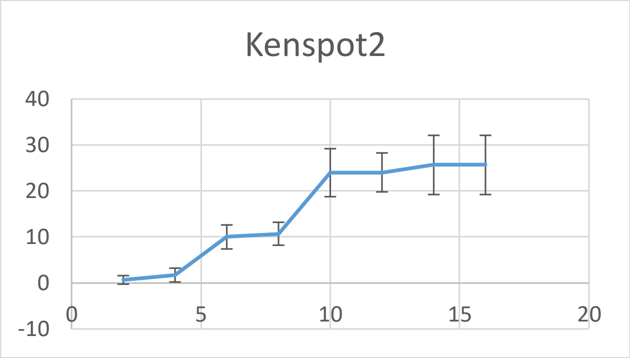
Generally, at Kakamega, Kabode, Kenspot1, SPK013 and Namunyekera had high disease incidences compared to the rest of the varieties (Fig. 2).

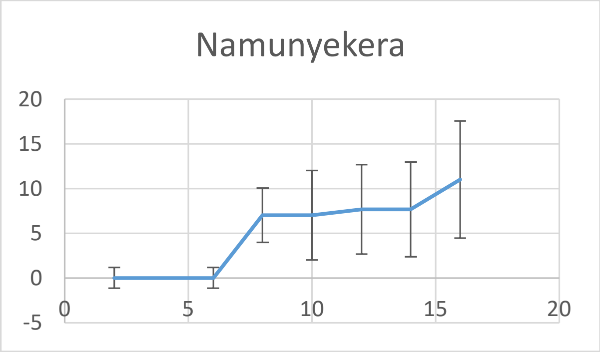
Kakamega **Alupe**

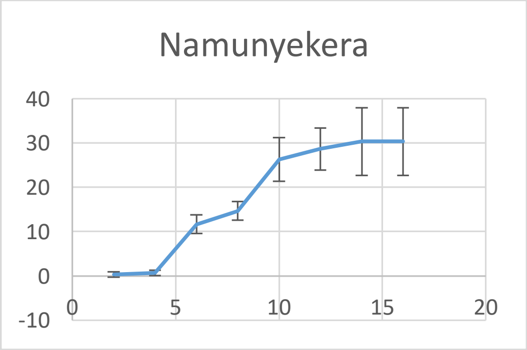


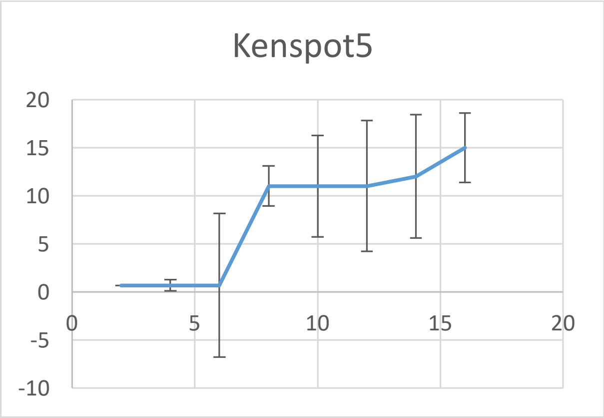


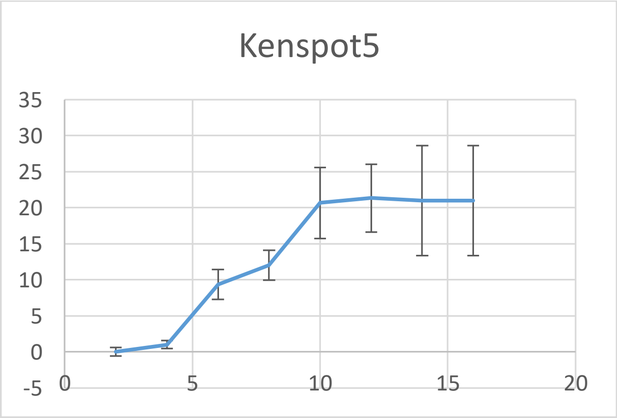












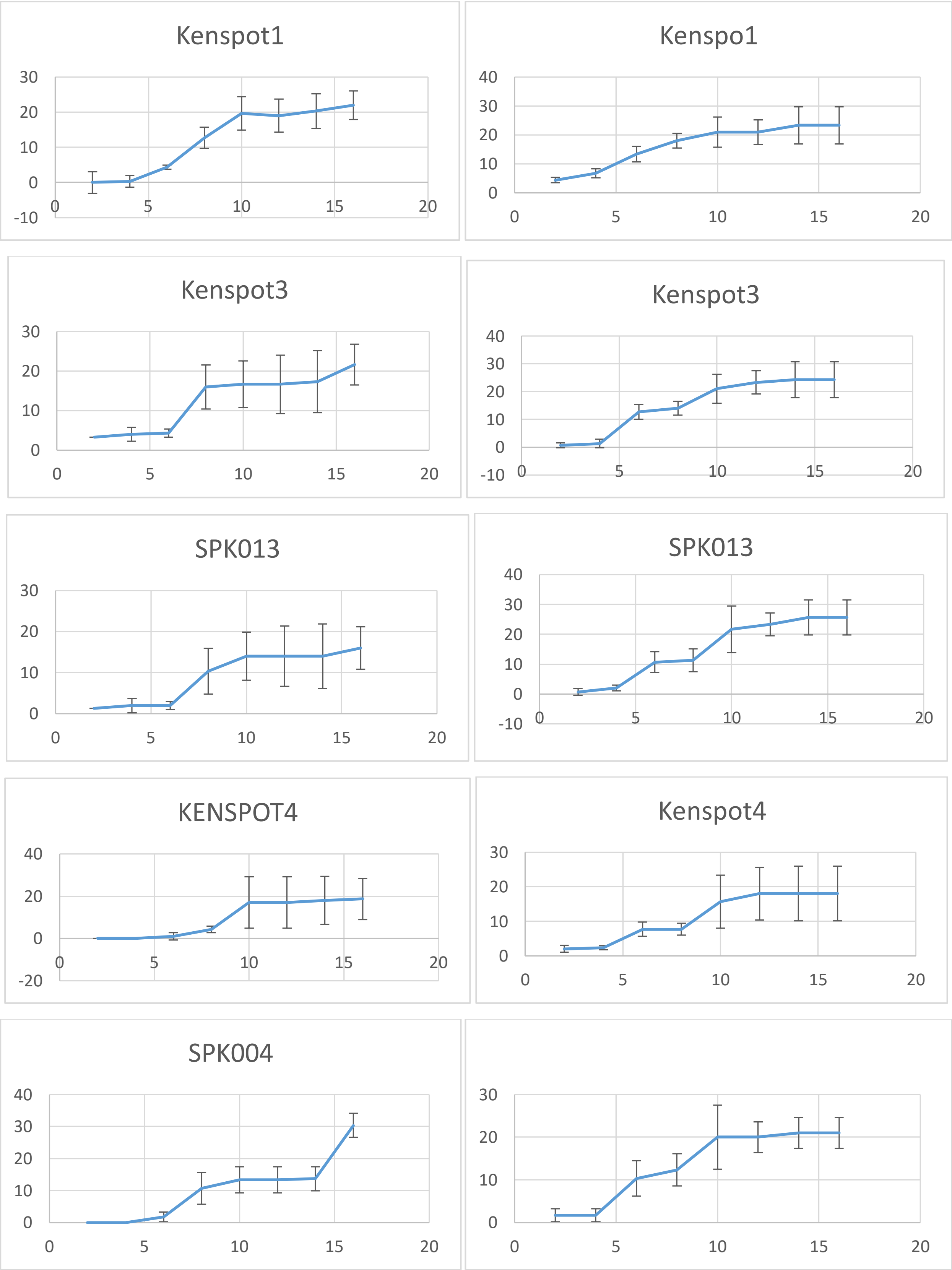


Figure 2: Alternaria disease incidences at different growth stages at Kakamega and Alupe.

Alternaria disease severity at different growth stages

Alternaria disease progression was generally higher at Alupe compared to Kakamega. At 16 WAI overall Altenaria disease severity was significantly higher at Alupe (3.11) than at Kakamega (2.52) across the varieties ($p<0.0001$)

At 2 - 4 WAI all varieties had mean severity below 2 which varied significantly across the varieties with KENSPOT1 having the highest (1.83) although not significantly different from KABODE, KENSPOT3, KENSPOT4 and SPK013. KENSPOT2, KENSPOT5, NAMUNYEKERA and SPK004 had lowest severity which was however not significantly different from KABODE, KENSPOT3, KENSPOT4 and SPK013.

Between 8 – 12 WAI all the varieties displayed a moderately resistance severity score (2.1-3.0). At 14 WAI KABODE and NAMUNYEKERA recorded a higher severity (3.17) which was however not significantly different from KENSPOT1, KENSPOT3, KENSPOT5, SPK004 and SPK013. KENSPOT4 and KENSPOT2 had the lowest severity score which was not significantly different from KENSPOT1, KENSPOT3, KENSPOT5, SPK004 and SPK013. At 16 WAI KABODE had the highest severity (3.33) which was however not significantly different from that of NAMUNYEKERA, KENSPOT5, KENSPOT3 and KENSPOT1. KENSPOT4 had the lowest severity (2.33) which was not significantly different from that of KENSPOT1, KENSPOT2, KENSPOT5, SPK004 and SPK013 (Table 1).

Table 1: Alternaria disease severity per variety at different growth stages

Variety	***Mean severity (WAI)**							
	WK 2	**WK 4**	**WK 6**	**WK 8**	**WK 10**	**WK 12**	**WK 14**	**WK16**
KABODE	1.33bcd	1.50ab	2.17a	2.67a	2.67a	2.50ab	3.17a	3.33a
KENSPOT1	1.83a	1.83a	2.33a	2.33a	2.67a	2.67a	2.67ab	2.83abcd
KENSPOT2	1.17cd	1.33b	1.50c	2.17a	2.33a	2.00b	2.50b	2.67bcd
KENSPOT3	1.33bcd	1.50ab	2.33a	2.33a	2.50a	2.33ab	2.67ab	3.00abc
KENSPOT4	1.50abc	1.50ab	1.83bc	2.17a	2.17a	2.17ab	2.33b	2.33d
KENSPOT5	1.00d	1.33b	1.50c	2.33a	2.50a	2.33ab	2.83ab	2.83abcd
NAMUNY	1.33bcd	1.33b	1.83bc	2.33a	2.50a	2.33ab	3.17a	3.17ab
SPK004	1.33bcd	1.33b	1.83bc	2.17a	2.50a	2.17ab	2.67ab	2.67bcd
SPK013	1.67ab	1.67ab	2.00ab	2.17a	2.50a	2.33ab	2.67ab	2.50cd
LSD	0.47	0.48	0.47	0.53	0.55	0.58	0.53	0.56

*Means with the same letter within a column are not significantly different (p=0.05).

At 16 WAI therefore, all the varieties except KABODE and NAMUNYEKERA were moderately resistant to Alternaria disease. KABODE and NAMUNYEKERA were moderately susceptible while none was resistant or susceptible to the disease (Table 2).

Table 2: Classification of resistance levels of the varieties to *Alternaria* disease

Resistant (R)	**Moderate Resistant (MR)**	**Moderately susceptible (MS)**	**Susceptible (S)**
None	KENSPOT4	KABODE	None
	SPK013	NAMUNYEKERA	
	SPK004		
	KENSPOT2		
	KENSPOT1		
	KENSPOT5		
	KENSPOT3		

The effect of Alternaria disease on root yield of improved sweet potato varieties

Table 3 show the root yield of various varieties of sweet potato in the two agro ecological zones. The results indicates that the yields were significantly different between the two sites Alupe and Kakamega

At Kakamega, there was a significant difference in the root yields among the varieties. Kenspot1 had the highest root yield (18.2 t/ha) however this was not significantly different from that of Kenspot2, Kenspot5, Kenspot4, Namunyekera, Kabode and SPK004. SPK13 recorded the lowest yield (10.1 t/ha) which was however not significantly different from the yield of Kenspot4, Namunyekera, Kabode, SPK004 and Kenspot3. At Alupe, Kenspot5 had the highest yield (18.3 t/ha) which was however not significantly different from the rest of the varieties (Table 5).

Table 3: Root yield of the test varieties per site

Variety	**Mean root yields (t/ha) per site**			
	Kakamega	S.E (+/-)	Alupe	S.E (+/-)
Kenspot5	17.5ab	3.0	18.3a	9.2
SPK013	10.1c	2.2	17.7a	7.5
Kenspot4	12.8abc	2.0	17.4a	1.0
Namunyekera	14.9abc	1.7	16.3a	0.9
Kenspot2	17.9a	3.5	16.1a	8.4
Kenspot1	18.2a	3.5	15.3a	1.5
Kabode	13.6abc	2.5	14.8a	4.3
SPK004	14.7abc	2.3	10.4a	5.6
Kenspot3	12.1bc	1.7	9.7a	2.8

*Means with the same letter within a column are not significantly different (p=0.05)

4. DISCUSSION

This study has established the resistance levels of ISPV in two agro-ecological zones of western Kenya. After inoculation with Alternaria solani, disease spread was higher at Alupe than Kakamega and disease incidence begun to rise at 6 WAI at Alupe and 8 WAI at Kakamega. Infection by Alternaria has been shown to occur under favorable weather conditions when spores germinate and enter healthy tissues (Skoglund and Smit, 1994). High temperature and humidity enhance the growth of fungi enabling it to quickly express symptoms on the infected plants (Pleysier et al., 2006). Warm, humid (24-29°C) environmental conditions are conducive to infection. In the presence of free moisture and at an optimum of 28-30°C, conidia will germinate in approximately 40 min (Kemmitt, 2002). Alupe is generally warmer than Kakamega due to its low altitude, however the two areas receive almost the same amounts of rainfall. Therefore, the warm weather enhanced the quick establishment of Alternaria solani expressing its symptoms earlier at Alupe than Kakamega hence the high disease incidence observed. Alternaria disease severity was also higher at Alupe than Kakamega. This can still be attributed to warm weather which enabled the pathogen to infect the plants earlier resulting to severe infections. Infections that occur at early stages of plant growth usually increase the severity of the disease in infected plants (Waliyar *et al.*, 2007).

Disease incidence and severity in all the varieties stabilized from 10 WAI. Time from initial infection to appearance of foliar symptoms is dependent on environmental conditions, leaf age, and cultivar susceptibility. Alternaria disease is principally a disease of aging plant tissue (Kemmitt, 2002). At 10 WAI the infected plants started to age and probably had reached maximum infection by the pathogen. Therefore, the infected leaves shade off with no more disease progression as the plant continued to get older.

None of the test varieties were resistant or susceptible to Alternaria disease. All, but two were Moderately Resistant (MR), while the rest were Moderately Susceptible (MS). This implies that most of the ISPVs are tolerant to the disease. This further suggests that the potato varieties vary in their level of resistance/ susceptibility to the disease. This coccurs with the findings by Shtienberg *et al*., (1990) that some varieties are susceptible while others are more resilient towards the scourge. The resistance is either based on the crop age or the variety. The varieties that were significantly affected were NAMUNYEKERA, and Kabode. These results partially are in agreement with the findings of Ochieng, (2018) who separately established that NAMUNYEKERA, SPK 013 and KENSPOT 5 are highly vulnerable to fungal diseases among the ISPVs. However, this study found that none of these varieties was highly susceptible to the disease. The differences in the findings could due to the varying virulence in the strains of *A. solani* used. A study by Kokaeva *et al.*, (2016) showed that there exists various strains of *A. solani* which vary in their virulence and aggressiveness on tomato and potato.

In both zones the findings demonstrated that the yield of improved sweet potato varieties was significantly affected by the disease. The yield in the infected test varieties was much lower than the expected. This was validated by the significant variance between the experimental yield and the expected yields on the two (Alupe and Kakamega) agro ecological zones. Early blight can cause stem lesions and fruit rot on tomato and tuber blight on potato (Aghighi *et al.*, 2004). If uncontrolled, the disease can cause important yield reductions (Adhikari *et al.*, 2017).In potato, major damage by *A. solani* is accredited to premature defoliation of potato plants, which results in tuber yield reduction (Van der Waals *et al.*, 2001).

5. CONCLUSION

Alternaria disease progression in terms of incidences was more rapid in the warm and humid weather of Alupe (22^{0}C) than in the less warm Kakamega (20.4^{0}C).*Alternaria disease* severity was also high at Alupe than Kakamega.Most of the ISPVs (KENSPOT 1-5, SPK004 and SPK013) were found to be moderately resistant to *Alternaria* disease while none was either resistant or susceptible.The study recommends that there is need for more baseline surveys to be conducted on adoption and performance of the new disseminated varieties in all high altitude areas of the country, use healthy, clean, fungal free planting materials and proper field sanitation.

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