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LAIKIPIA UNIVERSITY JOURNAL OF SCIENCE AND APPLIED TECHNOLOGY**Identification of Plant Species along the Riparian Corridor of the Iguameti Stream in Laikipia County Kenya****¹Benson Ojowi Obwanga, ²James Omondi Outa, ¹Veronica Ngure**¹Department of Biological and Biomedical Science Technology, Laikipia University, Kenya² Department of Limnology and Bio-Oceanography, University of Vienna, Austria**Abstract**

This study presents the first characterization of riparian vegetation on Iguameti Stream as well as a description of anthropogenic activities within the riparian corridor. Study points fell within the upper reaches of the stream within Laikipia University and lower reaches with a high density of human settlements and farming activities. Sample plots (30m×10m) in the upper and lower reaches of the stream were selected in the phytocoenoses of the study area in such a way that each sample plot was visually homogenous and that all floristic variation in the area was sampled. Based on physiognomical classification, three types of vegetation formations were identified: afro-montane forest, woodland and swamp vegetation. Human activities include: introduction of exotic species; timber harvesting; damming; stream channelization; livestock grazing; and land tillage. The upper reaches exhibited minimal disturbance, a detailed vertical stratification with ample canopy, understory, shrub, herb layer, and ground cover dominated by indigenous vegetation. *Trichocladus ellipticus*, *Allophylus* sp., *Teclea* sp, *Dombeya goetzinii*, *Ficus natalensis*, *Rhus natalensis*, *Pavonia urens* and *Cyathula cylindrical* were dominant, while in the lower reaches, *Pittosporum viridiflorum*, *Cupressus* sp., *Eucalyptus* sp., *Crotalaria agatiflora*, *Rhus natalensis*, *Hibiscus fuscus*, *Hypoestis verticillaris* and *Rubus* sp. were dominant. Species loss was reported in the lower reaches where the buffer strips <5m on the left and right banks compared to >20m in the upper reaches. Steam damming has caused replacement of riparian vegetation with wetland plants like *Crassula schimperi*, *Hydrocotyl* sp., *Oenanthe palustris*, *Sphaeranthus steetzi*, *Rorippa* sp., *Polygonum pulchrum*, *Typha* sp. and sedges mainly *Cyperus* spp. and *Fimbristylis* sp. Canopy cover decreased from >90% in the upper reaches to < 60% in the lower reaches. Riparian vegetation loss in the upper reaches may cause habitat loss for *Colobus guereza kikuyuensis* and *Aonyx capensis*. Urgent strategic multi-disciplinary management of the riparian zone is needed to limit biodiversity loss.

Keywords: Biodiversity conservation, climate change, Laikipia County, riparian vegetation, species loss

Introduction

The global increase in human population and the resultant effect on aquatic ecosystems (Masese et al., 2009) continues to concern natural resource stakeholders. Anthropogenic impacts on aquatic ecosystems pile pressure specifically on freshwater sources whose composition is only 2.5 percent of the total global water mass with about 68.7 percent of this being locked up in ice caps, 29.9 percent in ground water, and only 0.26 percent occurring in lakes and reservoirs (M'Erimba et al., 2014). In Kenya, the distribution of freshwater resource is limited both spatially and temporally

imposing immense strain on the already scarce resource (Masese et al., 2009; M'Erimba et al., 2014). On the other hand, these are delicate ecosystems which have increasingly lost their integrity and health due to immense anthropogenic utilization pressures (Masese et al., 2009). Rivers and streams integrate effects of land use practices hence suffer impacts of activities in their drainage basins, and they therefore provide a perfect platform to evaluate the environmental health of the drainage basin (Masese et al., 2009). The environmental health of the streams and rivers thus becomes an issue of utmost concern.

The health of a river is measured in terms of its ability of the ecosystem to support and maintain key ecological processes and a community of organisms with a species composition, diversity, and functional organization as comparable as possible to that of undisturbed habitats within the region (M'Erimba 2014). Key land use activities in drainage basins that negatively affect the health of rivers and streams include rapid expansion of farmlands, and degradation of natural forests and wetlands (Masese et al., 2009; M'Erimba et al., 2014). These activities affect the hydrology of the stream and rivers, the quality and quantity of water, and the riparian vegetation through nutrient enrichment, pesticide contamination, sedimentation, destruction of the riparian vegetation among others (Mathooko, 2001; Mathooko and Kariuki 2001; Masese et al., 2009; M'Erimba et al., 2014).

Riparian vegetation is critical to the existence of a river as it provides both physical and biological aspects that determine river health (Oruta et al., 2017). The vegetation is important for the hydraulic, hydrologic, water quality and life support functions in the riparian corridors (Davis et al., 1996; Oruta et al., 2017). The physical and biological functions of the riparian vegetation ensure stabilization of the stream flow, stream morphology and hydrology which in turn attenuates floods. On the other hand, the vegetation plays an important role in providing food, refuge, nesting, areas, habitats and corridors for a great diversity of terrestrial and aquatic fauna (Davis et al., 1996; Oruta et al., 2017). Loss of riparian vegetation leads to destabilization of the stream morphology, alteration of hydrology, poor water quality and loss of related aquatic and terrestrial fauna (Davis et al., 1996; Oruta et al., 2017). Riparian strips are faced with a variety of disturbances, usually natural and human induced perturbations which consequently affect the plant community structure and composition. While the ability of riparian vegetation to recover from spates of natural disturbances like flooding is well reported (Swanson, 1994), the anthropogenic disturbances usually have a long-term negative impact.

There exists a wide diversity of anthropogenic disturbances on the riparian vegetation ranging from damming of river channels, constructing levees, stream channelization, vegetation, clearing and conversion of the riparian vegetation with crops, livestock grazing, development of recreational facilities, sewage discharge, toxic chemical spills among others (Mathooko, 2001). These anthropogenic disturbances are responsible for denying water to the riparian corridor which in turn leads to shrinkage of the riparian corridor, reducing canopy cover, a shift in composition of the riparian vegetation structure and function, soil erosion and compaction, non-native plant invasions, and loss of riparian vegetation related wildlife (Smith et al., 1991; Richardson et al., 2007; M'Erimba et al., 2014).

The interest to study Iguameti stream is premised on the fact that the stream flows through Laikipia County which is described as a water scarce county with human-human and human-wildlife conflicts over water resources being a common occurrence (Butynski & De Jong, 2018). In addition, human activities in the riparian zone like deforestation, cattle grazing, farming among others have led to its destruction and decline compromising its function (Butynski & De Jong 2015; Butynski & De Jong, 2018). This study considered Iguameti Stream a very significant

aquatic ecosystem in Laikipia County and in its provision of ecosystem services in the watershed. We surveyed the riparian vegetation on Iguameti stream with an intent to determine the structure and composition of the riparian vegetation, the width of the riparian buffer strips and to establish the existing anthropogenic induced natural perturbations.

Materials and Methods

The study was conducted on Iguameti Stream located in Nyahururu Sub-County of Laikipia County, Kenya (GoK, 2013). Laikipia County is located in the semi-arid region of the Rift valley with a mixed zone of low-lying drier areas suitable for arid pastoralism and higher, wetter areas that are suitable for high potential farming (Bond, 2014). The County is bordered by Mt. Kenya to the east and southeast and the Aberdare ranges to the southwest as well as the Rift Valley (Butynski & de Jong 2015; Evans & Adams, 2016). Iguameti Stream flows through Laikipia University, through Shamanek Forest that was part of the Marmanet Forest then joins Waseges River which flows towards Lake Bogoria that is found in the neighbouring Baringo County. In the upstream, the stream flows through Laikipia University, where there is minimal anthropogenic impact on the riparian zone. Downstream, the stream flows through privately owned human settlements. There is no data to show whether Iguameti is a seasonal or permanent stream but during the whole duration of the study, the stream flowed consistently. The stream is characterized by silty sediments within its series of pools and ripples, while some artificial impoundments on the stream within and without the University have led to replacement of the riparian vegetation with wetland plants.

The study area and sample points are presented in Fig 1 while the study points and their GPS coordinates are presented in Table 1. A total of 9 study sites were selected to fall within two distinct zones: an upstream with relatively intact riparian buffer strip zones falling within the protected Laikipia University land (T1, T2, T3, CF, DE and WWT) and a downstream section, outside the University boundaries which was characterized by disturbed riparian zones and increased direct human interaction with the stream (W1, W2 and KD). T1 and T2 were points on two ephemeral tributaries that join the Iguameti Stream in the upstream. T3 is the uppermost accessible point on Iguameti Stream. CF was a point at the confluence of the three streams while DE was a study point before the stream joins the first impoundment within the Laikipia University. WWT was a study point located close to a construction site of a waste water treatment plant. W1 and W2 and KD were study sites close to impoundments downstream of the stream. KD was also located close to an unplanned settlement of temporal structures built very close to the stream.

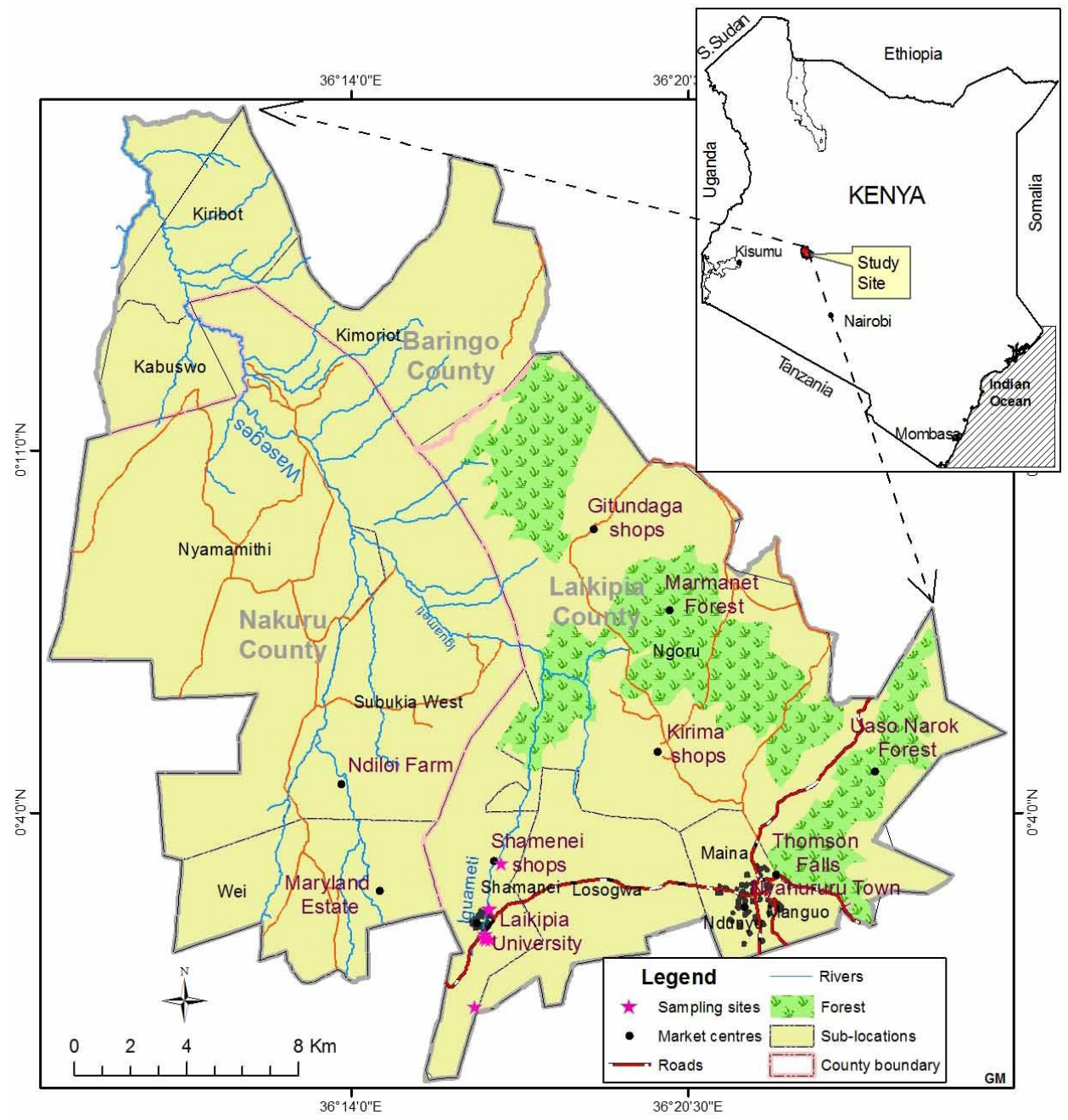


Fig. 1: Study Area and Sampling Sites

Table 1: Study Sites, GPS Coordinates and Altitude

Sites	Co-ordinates	Altitude (m)
Upstream		
T1	N00° 01.568', E036° 16.680'	2372
T2	N00° 01.575', E036° 16.599'	2368
T3	N00° 02.264', E036° 16.378'	2364
CF	N00° 01.604', E036° 16.560'	2361
DE	N00° 01.667', E036° 16.582'	2343
WWT	N00° 02.144', E036° 16.667'	2325
Downstream		
W1	N00° 02.152', E036° 16.659'	2315
W2	N00° 03.039', E036° 16.901'	2304
KD	S 00° 26.122', E 035° 58.361'	2295

Key: T1-Tributary 1; T2-Tributary 2; T3 Tributary 3; CF- Confluence; DE- Dam Entry; WWT- Waste Water Treatment; W1- Wetland 1; W2- Wetland 2; KD-Kamukunji Dam.

The study was carried out between September 2014 and September of 2015. Sample plots (30m×10m) were chosen in the phytocoenoses of the study area in such a way that each sample plot was visually homogenous. All floristic variation in the area was also sampled. Sampling was done at the end of the raining season when most plant species are flowering and producing fruits hence are recognizable. Based on physiognomical classification, the types of vegetation formations were identified. The plants were identified using the handbook by Maundu and Tengnas (2005) and a field guide by Dharani (2002) which combine pictorial images of plants, distribution of plants, plant morphological descriptions, and the plant names in Kenyan dialects and their scientific names.

Although not the original objective of the study, some mammals were identified to associate with the riparian zone. As such, a photographic guide (Stuart & Stuart, 2014) was used to help identify the mammals. Most of the plants were identified on-site, while those that could not be accurately identified were collected for further morphological examination at the Botany Laboratory in Egerton University, Njoro in Kenya. In addition, plant samples were sent to the National Museums of Kenya for confirmation of the species types. Plant press and secateurs were used to collect plant samples. Plant leaves, flowering twigs and fruits where possible were collected, fitted well in the press and labelled.

The cover-abundance values of plant species within each sample plot area were done based on the Braun-Blanquet scale as follows:

- R - Very rare with a negligible cover (usually a single individual)
- + - present but not abundant with a small cover (<1%)
- 1- Numerous but covering 1-5%
- 2 - Covering 5-25%
- 3 - Covering 26-50%
- 4 - Covering 51-75%
- 5- Covering 76-100%

Canopy cover above stream and ground cover by percentage were also estimated in each site. The size of the riparian buffer strips was also measured landward laterally from the river bank. Species richness within each quadrat was determined by counting the total number of different species recorded. The above stream canopy cover, ground cover and height of trees were given as approximations through on-site observation, by averaging the observations of three different people. The Shannon-Wiener diversity index (H') (Brower et al., 1990) was used to assess diversity as shown below:

$$H' = -\sum \left(\frac{n}{N} \times \ln \left(\frac{n}{N} \right) \right)$$

The study team also carried out monthly observations for evidence of anthropogenic impacts on the riparian vegetation and buffer strips; for instance, introduction of exotic species, loggings/clearing of the riparian vegetation, grazing of livestock, and farming within the riparian buffers strip. The importance of the riparian vegetation as refugia for unique organisms was also investigated.

Results

Results on the above stream canopy cover, tree canopy height and buffer strip width at all the study sites is presented in Table 2. The dominant vegetation growth forms are presented in Table 3. In the upstream, indigenous vegetation dominated the study sites and a dense afro-montane forest was exhibited where it was composed of *Allophylus africanus*-*Trichocladus ellipticus* and *Teclea* spp.

Table 2: Above Stream Canopy Cover, Tree Height and Buffer Strip Width in the Study Sites

Sites	Canopy cover (%)	Tree height (m)	Buffer Strip (m)
Upstream			
T1	95	15-40	Right & Left > 20
T2	60	30-50	Right & Left > 10
T3	80	10 - 25	Right 2; Left >5
CF	85	30-40	Right & Left > 10
DE	60	20-30	Right & Left > 5
WWT	80	20-40	Right 3; Left 2
Downstream			
W1	10	5-15	Right 2; Left 2
W2	0	10-30	Right 3; Left 4
KD	60	10-20	Right 2; Left 3

The composition of vegetation forms at the study sites is presented in Table 3. The sites showed a detailed vertical stratification where from top to bottom, the site exhibited a defined canopy, a canopy, an understory, and shrubs.

Table 3: The Dominant Vegetation Growth Forms and Representative Plant Species in the Study Sites

Site	Trees	Shrubs	Herbs	Grass	Climbers	Epiphytes	Sedges/Cattails
Upstream							
T1	<i>Trichocladus elipticus</i> ; <i>Teclea</i> spp., <i>Allophylus</i> sp.	<i>Maytenus senegalensis</i> , <i>Phytolacca dodecandria</i>	<i>Cyathula cylindrical</i> , <i>Pavonia urens</i> , Ferns	<i>Leersia hexandra</i> , <i>Pennisetum clandestinum</i>	<i>Cissus</i> sp. <i>Periploca linearifolia</i>	<i>Loranthus</i> sp.	
T2	<i>Cupressus</i> spp., <i>Rhus natalensis</i> ,	<i>Phytolacca dodecandria</i>	<i>Polygonum pulchrum</i> , <i>Crotalaria agatiflora</i> , <i>Cyathula cylindrica</i>	<i>Pennisetum clandestinum</i> , <i>Agrostis stolonifera</i>	<i>Periploca linearifolia</i> , <i>Momodica foetida</i> , <i>Zehneria</i> sp.(<i>Cyperus rotundus</i>
T3	<i>Allophylus</i> sp, <i>Ficus natalensis</i> , <i>Eucalyptus globulus</i>	<i>Vernonia lasiopus</i>	<i>Circium</i> sp., <i>Crassula schimperii</i> , <i>Hydrocotyl</i> sp.	<i>Cynodon dactylon</i>	<i>Rhynchosia</i> sp		<i>Cyperus</i> sp. <i>Cyperus rotundus</i> , <i>Fimbristylis</i> sp.
CF	<i>Allophylus</i> sp., <i>Cupressus lusitnica</i> <i>Dombeya</i> sp.	<i>Phyllanthus forcesi</i> , <i>Phytolacca dodecandria</i> , <i>Rubus</i> sp.	<i>Oenanthe palustris</i> , <i>Pavonia urens</i> , <i>Triumfetta romentosa</i>	<i>Panicum</i> sp.			<i>Cyperus rotundus</i> , <i>Fimbristylis</i> sp.
DE	<i>Dombeya goetzinii</i> , <i>Rhus natalensis</i> ,	<i>Maytenus senegalensis</i>	<i>Oenanthe palustris</i> , <i>Rorripa sinuate</i> , <i>Polygonum pulchrum</i>	<i>Pennisetum clandestinum</i> , <i>Typha domingensis</i> , <i>Cynodon dactylon</i>	<i>Basella alba</i> , <i>Cissus</i> sp.		<i>Cyperus papyrus</i> , <i>Typha domingensis</i>
W WT	<i>Pittosporum</i> sp., <i>Rhus natalensis</i> , <i>Trichocladus elipticus</i>	<i>Erythrococca bogensis</i> , <i>Rhamnus staddo</i>	<i>Crotalaria agatiflora</i> , <i>Oenanthe palustris</i> , <i>Hypoestes verticillaris</i>	<i>Leersia hexandra</i> , <i>Panicum</i> sp.	<i>Momodica foetida</i> , <i>Periploca linearifolia</i> , <i>Zehneria</i> sp.	<i>Orchids</i>	<i>Cyperus</i> sp. <i>Typha domingensis</i>
Downstream							
W1	<i>Cupressus lusitanica</i> , <i>Pittosporum</i> sp., <i>Rhus natalensis</i>	<i>Hibiscus diversifolius</i> , <i>Maytenus senegalensis</i> , <i>Rhamnus staddo</i>	<i>Polygonum</i> sp., <i>Oenanthe palustris</i> , Ferns	<i>Pennisetum clandestinum</i>	<i>Momodica foetida</i>		<i>Cyperus rotundus</i> , <i>Cyperus</i> sp., <i>Typha domingensis</i>
W2	<i>Cupressus lusitanica</i> , <i>Eucalyptus globulus</i> , <i>Rhus natalensis</i>	<i>Helichrysum</i> sp., <i>Rhamnus staddo</i> , <i>Maytenus senegalensis</i>	<i>Sphaeranthus</i> sp., <i>Crassula schimperii</i> , <i>Comelina</i> sp.	<i>Pennisetum clandestinum</i>	<i>Cissus</i> sp. <i>Rhynchosia</i> sp.		<i>Fimbristylis</i> sp., <i>Cyperus rotundus</i> , <i>Cyperus</i> sp.

KD	<i>Cupressus lusitanica</i> , <i>Dombeya sp.</i>	<i>Rubus sp.</i> , <i>Plectranthus barbatus.</i> , <i>Hibiscus sp.</i>	<i>Oenanthe palustris</i> , <i>Crotalaria agatiflora</i> , <i>Senecio montuosum</i>	<i>Pennisetum clandestinum</i>	<i>Periploca linearifolia</i> , <i>Cissus sp.</i>	<i>Cyperus rotundus</i> , <i>Fimbristylis sp.</i>
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Human activities in the riparian zone and the observed and potential impacts were observed. The potential impacts of the human impacts are described in table 4.

Table 4: Description of Human Activities in the Riparian Zone of Iguameti Stream

Site	Human activities and observed/potential impacts on the riparian zone
Upstream	
T1	¹ Livestock grazing; ² picnicking,
T2	Livestock grazing; Picnicking, ³ Land tilling/Crop farming
T3	Livestock grazing; Picnicking; Land tilling/Crop farming
CF	Livestock grazing
DE	Livestock grazing; ⁴ Stream crossing
WWT	⁵ Construction of waste water plant, Land tilling/Crop farming
Downstream	
W1	⁶ washing laundry; ⁷ Fish farming ⁸ Livestock grazing and watering; Land tilling/Crop farming
W2	⁹ Channelizing of stream; ¹⁰ Charcoal burning; ¹¹ Water harvesting for domestic and irrigation; Land tilling/Crop farming
KD	¹³ Unplanned human settlement (houses); Water abstraction, laundry washing

Key: Description of the observed and potential impacts of activities in the riparian zone:

¹loss of plant species, destruction of plant structure, defecation by livestock;

²Waste disposal (organic and inorganic), trampling on plants

³Species loss, disturbance of vegetation

⁴Interference with riparian vegetation growth; waste disposal;

⁵Loss of riparian vegetation, disposal of sediments in the riparian zone; interference with soil structure in the riparian zone;

⁶Introduction of inorganic wastes from detergents; interference with riparian vegetation growth; ⁷species loss, interference with soil structure, interference with plant growth;

⁸ trampling, species loss, interference with soil structure and root structure

⁹Species loss, interference with soil structure;

¹⁰Species loss

¹¹Species loss & disturbance, inorganic contamination with oils & greases, interference with soil structure

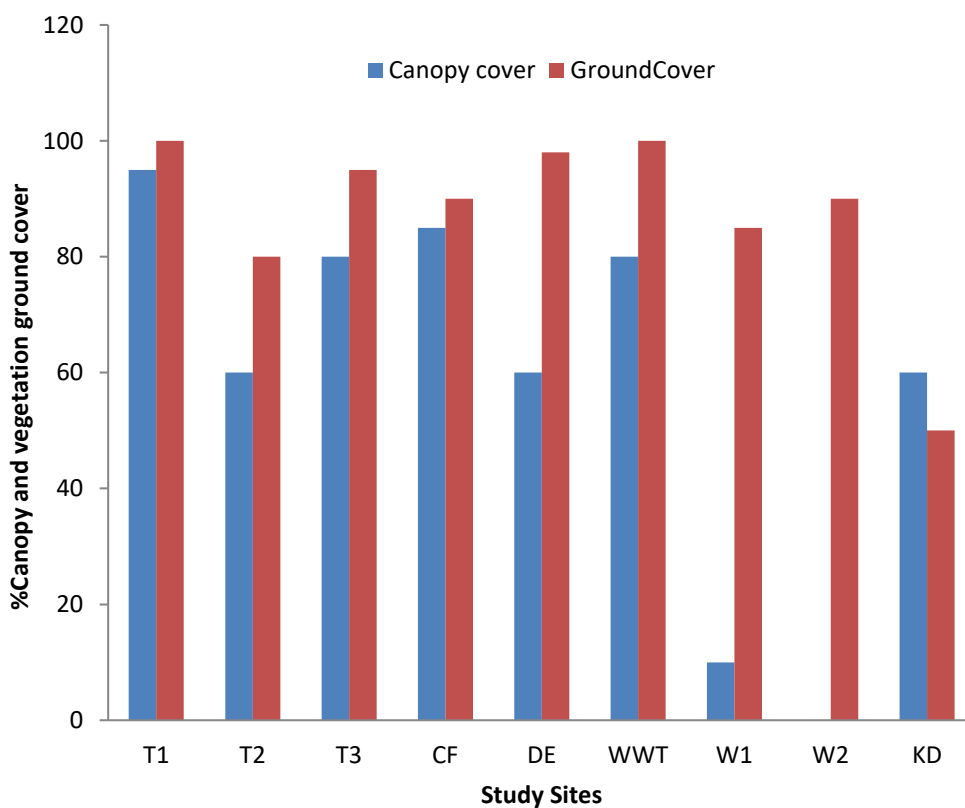
¹²Reduced riparian zone, species loss, interference with soil structure, introduction of alien species ¹³ Species loss, interference with soil structure, defecation

The study reported human activities in the riparian zone or close to the riparian zone both in the upstream and downstream as already shown in Table 4. Livestock grazing, human crossing the stream and picnicking were noted to be the activities that directly impacted the riparian zone in the upstream. A comparison of the human activities in the riparian zone during the wet and dry season is presented in Table 5.

Table 5: Dominant Human Activities Influenced by the Dry and Wet Seasons of the Year

Activity	Wet season	Dry Season	Sampling site
Livestock grazing	√	√	T1, T2, T3,
Picnicking		√	T1,T2
Laundry washing		√	W1,W2,KD
Channelization		√	W1,W2
Stream crossing	√	√	DE, W1, W2, KD
Fish farming	√	√	W1
Water abstraction		√	W1,W2, KD
Tilling/farming	√	√	T1,T2, W1,W2,

Results on canopy cover and vegetation types are presented in Fig 2 and Fig 3 respectively. Except for W1 and W2, the rest of the study sites had more than 50 percent canopy cover. The reduced canopy cover at W1 and W2 can be attributed to the site being a wetland.

**Fig. 2: Percentage Above Stream Canopy Cover and Percentage Ground Cover**

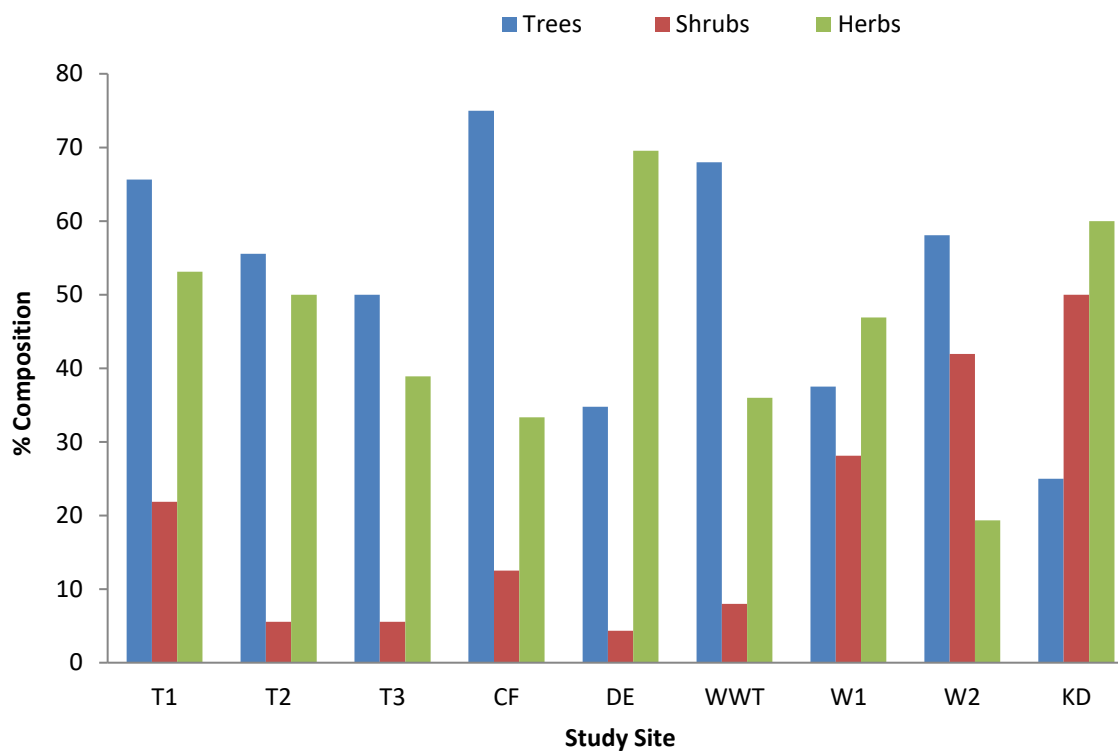


Fig. 3: Percentage Vegetation Type Composition at the Study Sites on Iguameti Stream

The comparison in species diversity Index (H') is shown in Fig 4. The highest diversity indices are recorded on the upper reaches at T1 where there is minimal alteration of the riparian vegetation.

Diversity Index comparisons

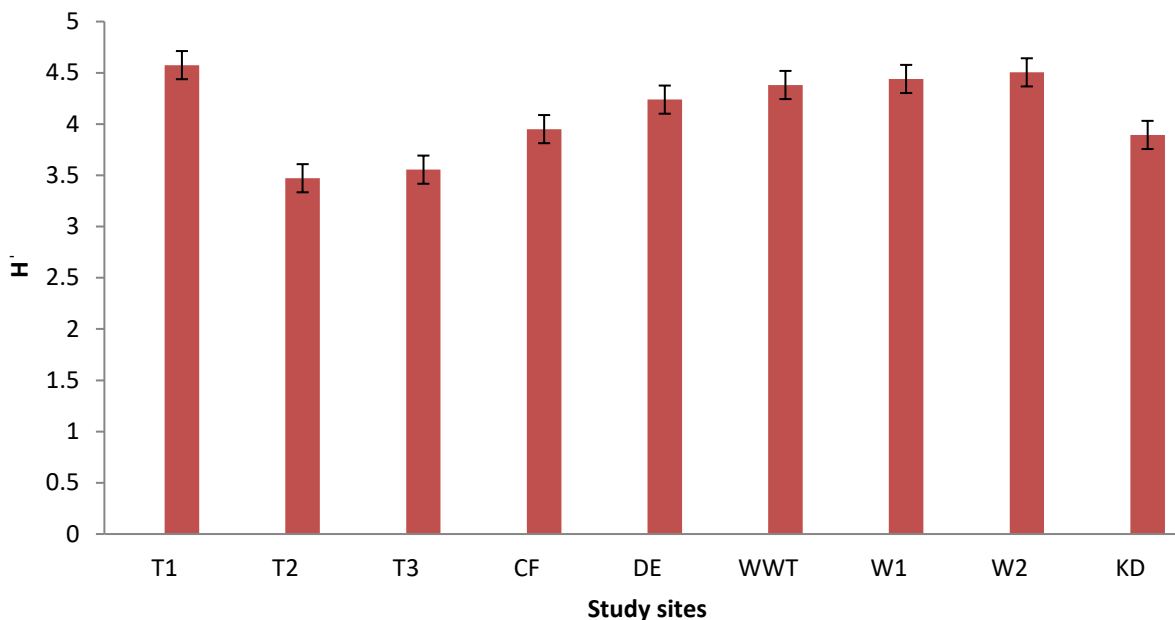


Fig. 4: Diversity index (H') comparison between sites

Discussion

Laikipia County is described to be in a transition zone for three major vegetation types: Somalia-Masai Semi-desert Grassland and Shrubland; Somalia-Masai Acacia-Commiphora Bushland and Thicket; and Afromontane Undifferentiated Montane Vegetation (Butynski & de Jong, 2015). Our study confirms the presence of the Afromontane vegetation as seen in the upstream study sites. Riparian vegetation on Iguameti Stream has an impact on the scarce but biologically important vegetation type in Laikipia County thereby contributing to the closed evergreen forest that is limited to the vicinity of rivers, deeper valleys and higher ground and forms about < 6% or <600km² of the county (Butynski & de Jong, 2014). The rich diversity of intact indigenous vegetation in the upstream and the biodiversity it supports is enough evidence of the critical importance of the riparian vegetation on this stream. The biodiversity includes both flora and fauna whose existence is threatened by habitat loss. However, in the midst of this, the riparian vegetation and the related biodiversity it supports is under threat. Except in the upstream where the riparian vegetation is near intact, there is great vegetation loss on the downstream. This is a scenario replicated in the rest of Laikipia County where change in land use has led to vegetation loss (Bond, 2014; Butynski & de Jong, 2014; M'mboroki et al., 2018).

Over time, much of Laikipia County's natural habitat has been degraded, fragmented, or lost as a result of over-grazing and over browsing by livestock, cutting trees, conversion to farmland and overharvesting of water (Butynski & de Jong, 2018). Studies have also shown that due to the rapid growth of population and the subsequent demand for common resources like water, this pressure may worsen with effects of climate change (Mmboroki et al., 2018). Specifically, Iguameti Stream flows partly through the Shamanek Forest Reserve located to the east of the Laikipia Escarpment/Eastern Rift Valley which has undergone extensive destruction in the recent past (Butynski & de Jong, 2014). The Shamanek Forest Reserve is part of the Marmanet Forest

which overtime has witnessed encroachment and turning of land from the original forest land to agricultural farming with the major crop here being farming of Irish potatoes. In general, the Iguameti Stream watershed has undergone a lot of land degradation just like most parts of Laikipia County. There is scarce documentation of the anthropogenic impacts on riparian vegetation on Iguameti Stream. This knowledge gap creates challenges in the conservation and management efforts.

This study specifically noted that there continues to be adverse anthropogenic effects in the riparian vegetation on the Iguameti Stream attributed to increased human settlement and consequent change in land use activities. The extent of human disturbance is reflected in the composition of riparian vegetation, the forms, the width of the buffer strips, the species richness, the percentage of canopy cover and ground cover. The riparian vegetation on Iguameti Stream exhibits a unique composition specifically among the trees when compared to general description of riparian vegetation elsewhere in Laikipia County, perhaps due to the high altitude. Butynski and de Jong (2014) in their description of riparian vegetation in Laikipia County mentioned that these zones are dominated by tree species: *Acacia xanthophloea*, *Acacia gerrardii*, *Acacia gracilior*, *Syzgium guineense*, *Syzgium cordatum*, *Colodendrum capense*, *Warbugia ugandensis* and *Ficus spp* especially *Ficus sycomorus*.

The altitude in Laikipia County ranges from 1,260m asl to 2400m asl (Butynski & de Jong 2015) while the study points ranged between 2372m asl and 2295m asl which is within the higher points of the County. This explains the species composition specifically among the trees. The upper reaches of Iguameti Stream which have minimal disturbance show a composition that includes a domination of indigenous trees that include *Allophylus africanus*, *Tricholadus*, *Teclea spp.*, *Polyscias kikuyuensis*, *Ficus natalensis* and *Dombeya goetzenii*. The tree layer decreases downstream and are replaced by exotic species like *Eucalyptus globules*, *Gravillea robusta* and *Cupressus lusitanica* that are mostly associated with human settlements hence confirming increased human encroachment, disturbance and depletion of the riparian zone.

The dominance of indigenous trees in the upper reaches depicts minimal disturbance in these reaches and therefore enhances a vertical stratification typical of forest ecosystems. In addition, the presence of abundant ferns at this site indicates the absence of or low levels of disturbance. Beukema and Noordwijk (2004) noted that species richness of terrestrial ferns and fern allies (Pteridophyta) may indicate forest habitat quality. Furthermore, the study also noted the presence of epiphytic wild orchids on trees in the riparian buffer strip. Kawaka et al. (2014) describe tropical wild orchids to be found exclusively in primary forests that are largely undisturbed. The authors further state that orchids are keystone species that can be used to monitor the general health of a wide range of habitats and furthermore, most of the orchids are classified in the International Union for Conservation of Nature (IUCN) red listing as Critically Endangered, Vulnerable, or Threatened. Although the team did not manage to identify the specific orchids as this was beyond the scope of study, it is worth noting that such species may be affected by loss of the host species. Threats to orchid existence have been attributed to increased population growth that has led to deforestation hence occasioning habitat destruction and fragmentation (Kawaka et al., 2014).

Despite the dominance of indigenous vegetation on the upper reaches of the stream, there is still however the presence of exotic tree species like *Cupressus lusitanica*, *Gravillea robusta* and *Eucalyptus globules* which have been introduced during tree planting initiatives in the upstream. Despite the well intended aim of such a strategy, it interferes with the original community structure and function. Exotic tree species like *Cupressus spp* and *Eucalyptus spp* have been found to limit growth of other vegetation through allelopathic activity. Richardson et al. (2007) noted that non-

native plant invasions can cause changes to structure of riparian strips. Kawaka et al. (2014) in their study done on orchids found that most epiphytic orchids occurred on indigenous trees.

While the upstream of Iguameti Stream manifests what would have been of the riparian strip, the lower reaches depict the scenario exhibited in most parts of Laikipia County. Changes in land use have put pressure on water resources in Laikipia County due to competition from pastoralists, small and large scale farmers as well as wildlife and as a result, the riparian zone has not been spared (Bond, 2014). Compared to the upper reaches which fall in a protected area within Laikipia University, the lower reaches exhibit minimal or no management contributing to the destruction and declining of the riparian vegetation. In this zone, there is wanton encroachment and destruction of the riparian zone as shown by results where there is reduced buffer strip, river channel modification, reduced canopy cover among others.

Specifically, at W1 and W2 where there is encroachment onto the riparian zone to pave way for farming of maize, potatoes, vegetables and fruits, there is total destruction of the riparian vegetation. There is also the replacement of the indigenous trees with exotic ones, specifically *Eucalyptus globules* and *Gravillea robusta*. Human disturbance in the riparian zone is manifested at T2, DE, W1 and W2 where the herb layer hosts farm weeds like *Tagates minuta*, *Achyranthes aspera* and *Conyza sp* are present. These species are attributed to human disturbance specifically by land tillage as noted by Mathooko and Kariuki (2001) who attributed human disturbance to an abundance of *A. aspera* in the herb layer of the submontane *Acacia abyssinica* forest belt of the Njoro River. The dominance of the herb layer at KD can also be attributed to the disturbance and proximity to the unplanned human settlement known as Kamukunji. The unplanned settlement did not have access to piped water and sufficient lavatory facilities and hence the dwellers rely on the riparian zone for water abstraction, laundry as well as a site for open defecation.

The anthropogenic disturbance and human interference in the lower reaches also contributed to reduction in the buffer strip. The National Environment Management Authority of Kenya (NEMA) in its National Land Use guidelines recommends buffer strips of between 2m-30m width for rivers or streams depending on the width, water volume, and whether permanent or seasonal and the use of the water (NEMA, 2011). While the guidelines leave the identification and management of the riparian zones to the Water Resource Users Associations (WRUAs), the status of the riparian vegetation in downstream points to lapses or weaknesses in implementation of the NEMA guidelines. The wider buffer strips in the upstream can be attributed to proper management of the riparian zone which should be implemented in the downstream areas.

The Iguameti Stream riparian corridor serves great value given the diverse species of vegetation of great ecological and economic significance. The riparian corridor is rich in indigenous trees with diverse uses including fuel, fodder, flowers for bee foraging and traditional medicine (Dharani, 2002). On the other hand, the importance of Iguameti Stream Riparian as a refugia for different organisms is perhaps highlighted by the presence of two mammalian species. The African clawless otter (*Aonyx capensis*) and the Mount Kenya guereza *Colobus guereza kikuyuensis* were noted to be residents of the riparian zone on the Iguameti Stream.

The *Colobus guereza kikuyuensis* is one of the three primate species in Laikipia restricted to the closed evergreen forest above 1800m asl and endemic to the highlands of central Kenya (that includes the Aberdare Range, Mount Kenya, Ngong Hills and Nairobi) (Butynski & de Jong, 2014). On the other hand, otters are considered some of the top predators of aquatic ecosystems and keystone species of wetland environments. While the *Colobus guereza kikuyuensis* is considered Least Concern on the IUCN Red List (Butynski and de Jong, 2015), the *Aonyx capensis* is considered a near threatened species (NT) on the IUCN Red List for habitat loss (Jacques et al.,

2015; Andarge & Balakrishnan, 2017). Maintenance of the riparian vegetation is critical to the survival of these two mammalian species.

Conclusion and Recommendations

Three types of vegetation formations; afro-montane forest, submontane woodland as well as aquatic and swamp vegetation were identified. The composition of the riparian zone vegetation differed from that described along other rivers in Laikipia County. Various plant species and growth forms (trees, shrubs, herbs, climbers, epiphytes, grasses and sedges) were identified in the study sites. The riparian vegetation at the protected upstream of the stream has had minimal disturbance. Anthropogenic disturbances include: introduction of exotic species, water abstraction, watering of livestock, clearing of riparian vegetation to pave way for agriculture and aquaculture, modification of the stream channel, and conversion of flooding zone into aquaculture ponds. Anthropogenic disturbances were more pronounced during the dry season of the year. Quantification of riparian plant species richness and identification of the existing anthropogenic influence are important for future management of the vegetation and, consequently, the stream. The upper reaches of the riparian zone have played the role of a refugia for two mammalian species; the Mount Kenya Guereza *Colobus guereza kikuyuensis* and the clawless African otter *Aonyx capensis* which necessitates the protection of this unique area and opens up potential for studies into biodiversity supported by the riparian strip.

There are efforts in place to restore and conserve the riparian zone on Iguameti stream. For instance, Laikipia University which is an important stakeholder hosts the annual Laikipia University Marathon whose main theme has been to restore the Shamanek Forest through creation of awareness and tree planting. Such efforts should be supported by relevant stakeholder to ensure increased and guided tree planting, implantation of legal guidelines on riparian area use and management as well as identification mechanisms for sustainable use of the Iguameti Stream riparian corridor. On the other hand, there is need of an integrated approach in the management of the riparian vegetation by making community participation a key component of the management strategy. There is need for the WRUAs to implement the NEMA guidelines for riparian zone identification and management. Key areas to focus on would be restoration of the riparian vegetation in the lower reaches, identification and protection of related biodiversity and mitigation of climate change related effects on use of the Iguameti Stream. Given that this was the first study on the riparian vegetation on the stream, there is need for more research to understand the full ecological and economic value of the buffer strip. The study also recommends more research on the economic importance of the riparian vegetation on the Iguameti buffer strip as well as other biodiversity supported by the riparian zone. This will create opportunities for conservation of the vegetation.

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LAIKIPIA UNIVERSITY JOURNAL OF SCIENCE AND APPLIED TECHNOLOGY**The Cotton Industry in Kenya: Problems, Prospects, and Revival Strategies**

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Abstract

Cotton is presently grown in twenty-two counties in Kenya spread across Central, Coast, Eastern, Nyanza, Rift Valley, and Western regions. Cotton production has been well below potential in recent years due to various constraints, including the non-availability of quality seeds and inappropriate production technologies, especially for smallholder farming, lack of access to credit, high cost of farm inputs, among other constraints. For the cotton industry to be revived, various strategies have to be put in place, including establishing efficient rural finance and credit supply system for smallholders and rural primary agro-processors; ensuring policies, institutional and legal frameworks are investor-friendly; facilitating long term investments in farm improvement technologies; and improving governance of co-operative sector by empowering farmers and farmer groups. This study is based on the principle that policy, institutional and economic content is the base upon which some of the problems in the cotton industry could be addressed in order to arrive at prospects essential in de-limiting the challenges. This will impact cotton industry revival and translate into increased incomes, thereby enhancing food security in cotton-growing areas. Primary data was obtained through field survey using questionnaires and interviews by stratified random sampling method in cotton-growing zones. Secondary data from various sources (KALRO, CODA, & MOA reports) was looked at, compared, and analysed using descriptive and qualitative methods to come up with prospects and strategies for solving production and marketing problems. The qualitative data analysed showed that the cotton industry face problems such as: lack of access to quality seeds; high cost of inputs; competition with other farm enterprises over scarce resources; and collapse of irrigation schemes and ginneries. The prospects suggested include acquisition of new seed varieties, increased employment in cotton industry, and improved economy as well as reduced poverty. The revival strategies included: maximization of African Growth Opportunity Act initiative by United States government that was extended from 2015 to 2025 to make local farmers benefit more; implementing the cotton bill and enforce cotton regulations; expanding cotton acreage/yield; and empowering cotton associations such as KCGA/HAWESA. It was concluded that there was need to address outlaid problems; avail resources for acquisition of quality seed, purchase of farm inputs and rehabilitation of collapsed irrigation schemes and ginneries; and invest in the cotton industry by the government, private sector, and the international community for cotton industry revival.

Keywords: Cotton, problems, prospects, revival strategies

Introduction

In Kenya, the cotton industry is among existing agricultural opportunities that offers best alternative for increased employment, poverty reduction, rural development and generation of

increased incomes in agricultural areas and in arid and semi-arid areas (GOK, 2002:2013). The cotton sub-sector has been identified as one that helps bring about rapid economic development in Kenya and is classified as a core industry. The sub-sector has significant linkages with textile processing and manufacturing industry and manufacturers of soap and detergents, animal feeds, chemicals, fats, and oils. These linkages are particularly important for the exploitation of market opportunities presented by the African Growth and Opportunity Act (AGOA), European and other markets (AFA, 2017).

Kenya is endowed with a well-developed cotton industry that requires a constant supply of cotton lint (World Bank, 2005). However, this industry has been operating below capacity, partly due to the low supply of domestic cotton lint (CODA, 2012). In 2000, a preferential trade agreement under AGOA was signed with U.S. Government, which eliminated all duties and quotas on Kenyan textile exports to the U.S. market. As a result, Kenya's textile exports to U.S. have increased significantly over the past decade, peaking at 300 million USD in 2004 (U.S. DoC & ITC, 2012). Despite this growth in exports, Kenya's local cotton producers have realized very few benefits because the cotton industry and textile sub-industry continue to import most of its factory inputs rather than purchase domestic cotton lint. According to Cotton Development Authority (CODA, 2012), the annual national demand for cotton lint equalled to about 111,000 tonnes of seed cotton, while the average annual production of seed cotton was only about 18,000 tonnes during the period 2005-2010. Therefore, the cotton industry and textile sub-industry largely depend on cotton lint imports to meet its annual demand (FAOSTAT, 2012).

Since market liberalization in 1991, the cotton-to-garment value chain in Kenya has lacked the structure and institutional dynamics required to compete with global players like China, or even with regional competitors and is far from realizing its true potential. According to the World Bank (2005) and MOA (2017), some of the problems responsible for the cotton sector's poor performance in the past and to some extent today include periodic drought, volatile producer prices, delayed payments to farmers, lack of access to quality seeds, high cost of pesticides, competition with other farm enterprises over scarce resources and collapse of co-operative societies and former state-owned textile firms. Others include competition from synthetic fibre substitutes and cheap imports of new and second-hand clothes (MOA, 2018).

In the industry, cottonseed processing stands as a downstream sub-industry for the cotton sector, offering several potential business opportunities with respect to oil, animal feed, and energy production. The Export Processing Zones Authority has identified Kenya's large unmet demand for vegetable oil as an opportunity to further expand and develop its seed cotton processing industry, especially since vegetable oil is the country's second most imported commodity after petroleum and its derivatives (EPZA, 2015). To revitalize the cotton industry in Kenya, the Ministry of Agriculture (MOA) passed the Cotton (Amendment) Bill in 2000, which provided the legal framework to re-organize the sector, allowing stakeholders to regulate the industry through CODA, under the supervision of MOA (Development [CGD Digest], 2005). Although seed cotton production grew after the Bill was adopted, growth was largely due to a small increase in the number of producers, rather than an increase in productivity (Gitonga et al., 2007).

In the early 1970s and 1980s, cotton was grown by 200,000 small-scale farmers distributed in over six out of the eight regions of Kenya. The annual production at that time stood at over 70,000 bales. This production sustained local demand consisting of 24 ginneries, 52 textile firms, 110 large-scale garment manufacturers and spinners and 78,000 small-scale apparel manufacturers hence this provided good employment opportunities and income to a large number of people including the 200,000 farmers and their households (GOK, 2005).

Despite the sector's decline in recent years, cotton is still considered one of the few cash crops with real potential for increasing employment opportunities and food security through income generation in many agricultural lands, including the Arid and Semi-Arid Lands (ASALs) of Kenya (CODA, 2008). Thus, revitalizing the cotton industry is one of the government's key development and industrialization initiatives to be implemented mainly in ASAL and other potential areas as envisaged in Kenya's Vision 2030 Strategic Plan and its' Medium-Term Plan, 2018-2022 (GOK, 2018). Therefore, for the cotton industry to be revived, various strategies have to be put in place.

Objectives of the Study under a Sustainable Livelihood Framework

The general objective of the study was to outline the cotton farming and performance of the cotton industry in Kenya. The specific objectives were; to establish the cotton industry problems in Kenya, find out cotton industry prospects in Kenya, and outline cotton industry revival strategies in Kenya.

The descriptive sustainable livelihood framework was used to come up with what was happening in the cotton industry in Kenya. This included surveys, questionnaires, field visits, interviews, and reports that provided historical problems in the cotton sector. Methods employed in descriptive analytics were observations, case studies, and surveys. The descriptive model was used because it makes it simpler to quantify relationships in data in a way that enabled the user to classify data into problems encountered in the industry and the available prospects, intervention put into groups for the revival of the cotton industry. The research model is presented in figure 1.

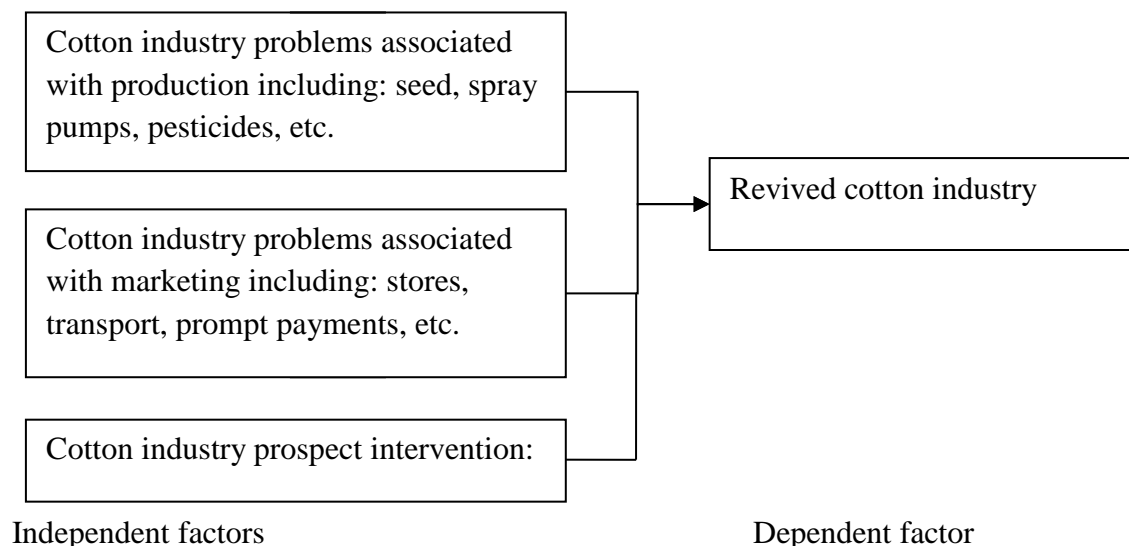


Fig 1: Research Model

From figure 1, the following is noted:

- H1:** There is a positive association between solving cotton production problems and the revival of the cotton industry
- H2:** There is a positive association between solving cotton marketing problems and the revival of the cotton industry
- H3:** There is a positive association between cotton industry prospect intervention and revival of the cotton industry

Research Methodology

Field visits and survey were done to collect data where questionnaires were administered and interviews made on a sampled 100 cotton farmers (20 farmers per cotton-growing zone) and 5 CODA and 5 MOA staff (1 staff each zone) in the five cotton growing catchment zones of Salawa-Baringo, Mwea-Kirinyaga, Mulwanda-Busia, Kibos-Kisumu, and Ndere-Siaya. A stratified random sampling method was used in data collection. The study relied on primary data (survey, questionnaires, and interviews) and secondary data from various sources (Cotton Development Authority-CODA and Ministry of Agriculture-MOA reports), which were looked at, grouped as per research questions (associated with (ploughing, planting, weeding, pest control, harvesting, and transportation of seed cotton to the market by farmers, ginneries and textiles), and analysed based on the present evidence from the cotton-growing areas in Kenya.

Analysis of primary and secondary data was done by descriptive and qualitative methods in the current study (see table 1). It focused on recent and past status of Kenyan cotton sector derived from the questions asked which were grouped based on the industry's problems and prospects intervention. This information was used to come up with revival strategies for the cotton industry in Kenya. Reports and literature were looked at from previous research by KALRO and others researchers. Further, various references of different studies by different people were looked at and conclusion and recommendation made.

Table 1: Data Analysis Output from Cotton Growing Catchment Zones

No.	Problems	Cotton Catchment Zones				
		Salawa-Baringo	Mwea-Kirinyaga	Mulwanda-Busia	Kibos-Kisumu	Ndere-Siaya
1	Untested soil fertility/fertilizer application levels	High	High	High	High	High
2	Variety mix-match	Very high	Very high	Very high	Very high	Very high
3	Collapsed irrigation/schemes/facilities	Low	Medium	Low	Low	Low
4	Collapsed/under operational ginneries	Medium	Low	Very high	medium	Very high
5	Policy issue problems	Low	Medium	Low	Low	Low
6	High cost of inputs	Very high	Very high	Very high	Very high	Very high
7	Poor agronomic practices/untimely planting	High	Medium	High	High	High
8	Inadequate quality seed	Very high	Very high	Very high	Very high	Very high
9	Cotton facing uncompetitive challenges from e.g., food crops and similar low-cost polyester items	Low	Medium	High	Medium	High
10	Inadequate skilled labour supply for textile industry	Low	Low	Low	Low	Low
No.	Prospects					
1	Increased cotton use	Very high	Very high	Very high	Very high	Very high
2	Acquisition of New varieties /selection of superior traits	Medium	Very high	low	medium	Low
3	Increase in employment in cotton industry	High	High	High	High	High

4	Improved economy and reduced poverty	High	High	Medium	Very high	High
5	Increased cloth from EPZ	Medium	Very High	High	Very high	medium
No. Revival Strategies						
1	Implementing AGOA initiative	High	High	High	High	High
2	Implement cotton bill and enforce cotton regulations	High	High	High	High	High
3	Streamline cotton commodity chain	Medium	Very high	Medium	High	Medium
4	Expand cotton acreage/yield	Very high	Very high	Very high	Very high	Very high
5	Integrated crop management/farmer-research link	medium	Very high	High	Very high	medium
6	Enhance CODA through e.g., more finance allocation, staff recruitment to cotton growing counties etc	Very high	Very high	Very high	Very high	Very high
7	Empower cotton associations e.g., KCGA/HAWESA	Medium	High	High	High	Medium

KEY: Number of participants =1-5 (Low); 6-10 (medium); 11-15 (High); 16-20 (very high)

The next section of this paper discusses the findings of this study. The findings are done according to the objectives of the study and are subsequently grouped under three subsections; problems in the cotton industry in Kenya, prospects of the cotton industry in Kenya, and the revival strategies for the cotton industry in Kenya.

Problems in the Cotton Industry in Kenya

Data output from this research (see figure 1) indicate that from independence up to the 1980s, Kenya was a major East African producer of seed cotton for local consumption and export (GOK, 1995; 1999). Other data also show that today the potential of the cotton industry in Kenya remains high. However, poor production methodologies due to diverse problems like lack of appropriate technical skills in agronomic practices and deficient marketing systems for cotton, has resulted in a failure to meet the stakeholders' expectations of cotton demand, quality and pricing (Fibre Crops Directorate, 2018). To mitigate this, the Cotton Development Authority (CODA) under the fibre crops directorate in the Ministry of agriculture, has been setup to coordinate rehabilitation of the cotton industry (MOA, 2017).

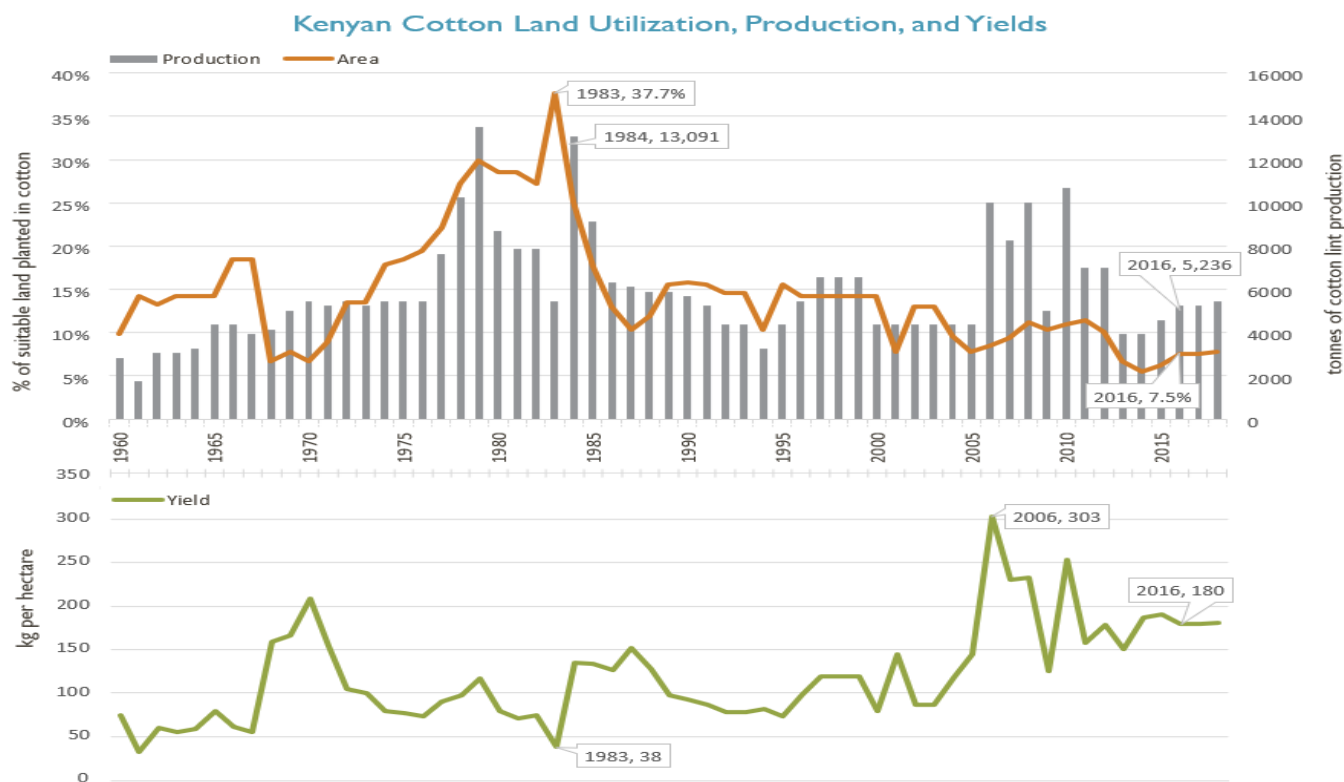


Fig. 1: Trends Oscillating in Land Utilization, Production, and Yields in Kenyan Cotton from 1960-2018

Untested Soil Fertility and Fertilizer Application levels

The research shows problem of untested soil fertility in cotton growing regions that needs soils to be sampled and soil analysis done to ascertain their fertility status. Thereafter, recommended fertilizer and manure application levels for each zone are laid out. Most farmers in cotton growing zones are not aware of their soil fertility status and recommended fertilizer application levels to use in their farms in relation to their farm soil types (MOA, 2018). Research by KALRO (2018) indicates fertilizer and manure use depend on soil type and extent of depletion of nutrients in specific agro-ecological zones. Soil analysis has been carried out extensively in various areas of the country (Fibre Crops Directorate, 2017). To address this problem, proper applications rates of manure and inorganic fertilizer use in specific locations need to be outlaid. Soil analysis is recommended before use (KALRO, 2018; MOA, 2017).

Collapsed Irrigation Schemes or Lack of Irrigation Facilities

The other problem is that of collapsed irrigation schemes that were once vibrant in seed cotton supply up to the 1980s-90s. This includes Hola and Bura irrigation schemes in the coastal region, and Pekerra and Barwessa irrigation schemes in Baringo in Rift valley region. These schemes accounted for over 30 percent of seed cotton production in Kenya (Fibre Crops Directorate, 2018). Their collapse is nearly zero cotton supply and cotton is now mostly grown under rainfall conditions. Data from this research indicate that Kenya has potentially irrigable land areas of 1.3 million ha with the potential to produce over 350,000 bales of cotton lint annually, yet only 105,

800 ha so far have been developed, producing 20,000 bales of lint annually (MOA, 2018). This is way below the requirement for local consumption which is estimated at over 200,000 bales annually. The remedy is increased cotton production done through combination of certified seed production and use of irrigation or availing irrigation facilities that leads to a substantial increase in yields (KALRO, 2017; MOA, 2017).

Collapsed or Under-operational Cotton Ginneries

The other problem is that of collapsed ginneries or the ginneries operating below capacity, partly due to various problems like the low supply of domestic cotton lint and irregular electricity supply which leads to reduced output and damage to equipment and products. In the cotton industry, electricity constitutes up to 60 percent of total costs in some ginneries, spinners, and textile operations (KIA, 2012). Currently, there are 24 ginneries in Kenya with an installed annual ginning capacity of about 140,000 bales that cannot be sustained by the meagre cotton supply of only 20,000 bales annually (GOK, 2018); this has forced many cotton industry stakeholders to collapse.

High Cost of Input

The research outlaid the problem of high cost of inputs, especially chemicals. The cost of purchasing cotton pesticides is exorbitantly high (up to 52%) in Kenya compared to the cost of such chemicals in Uganda and Tanzania. For instance, a litre of pesticides in Kenya goes for about Ksh.1,500 while the same goes for Ksh.700 and Ksh.1000 in Tanzania and Uganda respectively. Farmers cite high input costs, poor quality seed, weather, and low farm gate prices as challenges that prevent re-investment in the sector (FAOSTAT, 2018). Additionally, farmers lack access to finance for high-quality inputs and other investments for farm improvements. Pesticides account for almost a third of farmers' input costs, yet the majority of cotton farmers fail to spray even half of the recommended number of times per season (Ingram, 2005).

Inadequate Quality Seed

Research output indicates there is always inadequate quality seed available to farmers for planting (Fibre Crops Directorate, 2015; KALRO, 2016). A seed bulking and certified support program was started in 2007 by KALRO to try and address this problem. However, this is currently inadequate and requires strengthening in terms of trained manpower and financing. The results have been devastating to the small-scale cotton farmers as yields have declined from expected 1,500kg/ha to 500kg/ha leading to low earnings and reduced interests in cotton production.

In 1979, KARI released a new variety called KSA 81M with average yields of 2,000kg/ha and in 1989 released HART 89M with a production potential of 2,500 kg/ha under rain fed and over 4,000 kg/ha under irrigated conditions (KARI, 2012; CODA, 2012). The two varieties are recommended for commercial production. Their potential is however far from being achieved with current result average yields being 572 kg/ha. Due to lack of seed regulatory systems, the open buying system by ginners and lack of proper seed supply mechanisms has resulted in mixing of seed in the field thereby lowering seed quality due to frequent re-use of cotton seed. To reduce this problem, the government has been buying cotton seed ginned at ginneries and distributing free to farmers for planting as one of the campaign tools for revitalizing the cotton industry in Kenya.

Prospects of the Cotton Industry in Kenya

The national cotton production reached a peak of 38,000 metric tons of seed cotton in 1984/85 and thereafter declined to 14,000 MT by 1995 (as already seen in figure 1) following liberalization of the sector and withdrawal of Government from provision of credit and inputs concurring with Waturu (2001). The Cotton Development Authority in the Fibre Crops Directorate (2017) reports estimates that there are 350,000 ha in Kenya suitable for cotton production and is not cropped, with a potential cotton production of 50,000 tons annually. Before government initiatives to encourage cotton growing began to take effect in 2005, national production stood at only 5,000 tons from 30,000 ha.

In 2008/09 fiscal year, production was estimated at 10,000 tons from 46,000 ha and was reported also by CODA (2012) periodicals. This means that there are high hopes that with the current revitalization strategy of putting in place CODA and cotton Act 2005, the industry could see increased production concurring with MOA (2018) reports. The revitalization of cotton industry will enable all 24 ginneries, 52 textile firms, 110 large-scale garment manufacturers and spinners and 78,000 small-scale apparel manufacturers to work and operate at their installed capacities. These revived industries would create demand for more cotton lint of 60,000 bales per annum on-top-of the current annual demand of 120,000 bales thereby increasing demand for cotton products. Locally, textile manufacturing supplies only 45 percent of Kenyan textiles market while imported new and used clothes account for 37 percent of the market. Demand for textile products in Kenya is estimated to be growing at 3.78 percent annually. This supports reports by EPZA (2010) that more cotton up scales local demand and also concurs with earlier research by Ikiara and Ndirangu (2002) that Kenya has high prospect of cotton industry revival in its dormant fertile agricultural areas.

Acquisition of New Varieties and Selection of Superior Traits

With the revitalization of the cotton industry, there are prospects for provisions of quality and high-yielding cotton varieties as put forward by KALRO (2018) with recently approved Bt cotton variety. Acquiring new varieties, characterizing, evaluating and multiplying seed give breeders a vast Germplasm for the selection of superior varieties. This is done either through direct acquisition or selection from those already maintained at the National Gene Bank of Kenya (NGBK). For example, data output show that at KALRO, 34 new Deltapine lines/varieties (table 1) were obtained from Monsanto in 2008 and are currently being evaluated for performance. It is expected that drought-tolerant and Bt cotton from other countries like India and China will be acquired within the next few years to boost cotton industry revitalization in Kenya. This prospect will enhance cotton yields thereby enhancing volume for the cotton industry.

Table 1: Evaluation Characteristics of Promising Cotton Varieties/Lines at KALRO-Mwea

Variety	Bolls per plant	Days to 1 st flowering	Ginning outturn %	Plant height (cms)	Mean weight (g) of 1 boll sample	Yield in Kg/ha
KSA 81M	139	70.00	39.94	114.43	4.9	2,900
A540	141	73.00	39.39	121.23	4.6	3,700
F962	137	73.50	43.45	110.3	5.1	3,110
Vered	137	75.00	41.67	119.83	5.3	2,420
HART	141	74.25	40.30	122.33	4.6	3,650
89M						
Pb (70) 1	135	71.75	39.56	125.3	4.9	2,760
Sicala v- 1	130	71.00	43.73	108.15	4.6	2,600
K3400-7	130	72.25	38.68	356.50	4.5	3,450
E790	136	74.25	42.49	112.33	4.9	3,140
L433.15	134	73.75	41.88	129.00	5.3	2,810
Cs189+	134	70.25	41.86	112.7	4.5	2,930
Ny (72) 26	141	70.50	41.54	108.08	4.9	2,930
L142.9	136	72.00	44.17	19.10	4.8	3,180

Source: KALRO, 2018

Increased Level of Employment in the Cotton Industry

There is a prospect of an increase in jobs in the cotton industry and its related sectors with the revival of the cotton industry. For instance, reports by MOA (2018) show that the cotton industry used to employ many people in its value chains right from the cotton farms, to the apparels and non-apparel making factories thus creating many job opportunities to many. The same report also indicates that thousands of people were laid off their jobs in Kenya with the closure of many garment making factories like Kisumu Cotton Mills (KICOMI), Raymonds, and Kenya Textile Mills-Thika among others. In the year 2000, there were 10,000 jobs in the textile sector alone. These rose with the enactment of AGOA to 15,000 jobs in 2001, and 30,000 in 2002, and 36,000 in 2003 (ICAC, 2002, 2003). This indicates that cotton sector employability was improving upon regulation and streamlining of Kenya's cotton industry. These are only direct jobs that have a multiplier effect in areas like cotton fields, ginneries, suppliers of cotton seed and farm inputs, and other textile raw materials suppliers thereby indicating prospect of jobs creation with revival of cotton industry in Kenya.

Improved Economy and Poverty Reduction

There are prospects of an improved economy by the cotton industry in Kenya. For example, a vibrant and competitive cotton industry in Kenya would mean to some extent, a reduction of imports, thus improving the balance of trade. Cotton can be exported in linty form or final cotton products themselves. This would earn Kenya foreign currency to boost the economy and even stabilize the falling shilling. Kenya could also work with regional partners to fill in supply gaps based on comparative advantages. Sub-Saharan countries, including Kenya, can take advantage of regional production sharing to boost their economies, which is permissible under AGOA. Moreover, more research needs to be done to determine how much and what kind of fabric is used

and needed by textile companies in the region to target specific investors or take full advantage of regional resources and boost our economy.

Coupled with streamlined cotton production and management technologies, marketing, and research policies in place, this would mean that farmers become empowered financially by proceeds from the sale of cotton, thus reducing poverty levels.

Clothing the Nation from Growth in Kenya's Textile/Apparel Products in EPZ

With the revival of the cotton industry, Kenya can cloth its population and even have enough for export to both far markets and neighbouring markets. This is possible with growth in the Kenya textile/apparel sector currently witnessed in the Export Processing Zone (EPZ), which has a well-established Export Processing Zone (EPZ) and Manufacturing under Bond (MUB) framework which can absorb the extra seed cotton produced supporting report by MOA (2018). Research points out that exports from apparel industry contributed 75 percent of Ksh.20.6 billion which Kenya earned from total export into the USA in 2008 (U.S. DoC & ITC, 2012). Over 80 percent of apparel produced in Kenya for US market is made by companies under the EPZs which are a very good target for the revitalized cotton industry (EPZA, 2010). The same source indicates textile and apparel manufacturing as well as textile accessories that constitute 31 percent of all EPZ activities and employ approximately 20,000 Kenyans. These employment figures are however twice as much compared to five years ago although they have since been declining gradually. This decline is associated with cotton industry problems thus concurring with reports from EPZA (2015) on the need to revamp the cotton industry.

Revival Strategies for the Cotton Industry in Kenya

The revival of the cotton industry leads to the opening of closed mills, rehabilitation of ginneries, and spurring growth in related industries. Its revival also catalyses import substitution and increases exports, thus generating higher foreign exchange earnings for economic growth. Kenya has the potential to produce over 350,000 bales of cotton lint, but it is currently producing less than 45,000 bales of lint. This is way below the requirement for local consumption, which is estimated at over 200,000 bales annually (MOA, 2018). Various strategies can be used to increase output including increased cotton crop production through provision of certified seed, use of irrigation, addressing research issues on low yields and lint quality among others. This will assist boost stakeholder confidence in the cotton industry to join in its revival since enough cotton will be available for use in apparel products making. To revive the cotton industry, some of the strategies to be put in place to encourage players in the sector to participate in bringing the cotton industry to its feet again are discussed.

Implementing African Growth and Opportunity Act Initiative (AGOA)

The cotton industry can be revived through the African Growth and Opportunity Act (AGOA) which is a trade preference programme initiated in year 2000 by US government and currently involving 39 designated Sub-Saharan African (SSA) countries. The act provides preferential market access to more than 6,500 product tariff lines, including apparel/textile, a variety of agricultural products, chemicals, and so on. AGOA has expanded duty-free benefits, which were previously available under the Generalized System of Preferences (GSP), with a special provision for textile/apparels as reported in AGOA website (www.agoa.gov). This provision is to expire in 2025. Since Kenya's accreditation under AGOA in January 2001, there has been a re-opening of three garment factories previously closed, and establishing six new ones. Eighteen companies are

in the process of re-opening. This research found out that most of the closed ginneries have been privatized and some revived, for example, Makueni Ginnery is already ginning and selling to the Export Processing Zone (EPZ) in Athi River.

Implementation of Cotton Amendment Bill and Enforcement of Regulations

In trying to revive the cotton industry, the Kenya government introduced the Cotton amendment Bill of 2006 (that became Act) that provided the legal framework for Government supported re-organization of the cotton sector by establishing Cotton Development Authority (CODA). To enforce the regulations, CODA has recruited inspectors, whose obligations under the cotton Act and the cotton (Amendment) Act 2006 are to; monitor cotton growing and cotton ginning, inspect cotton plants for disease and pests, and carry out quality control of planting seed and raw among others. As a result of this, there has already been some impact for instance with national production rising to 9,800 tons in 2006 from 5,090 tons in 2005 as reported by MOA (2007). However, this increase was mainly due to an increase in the number of producers (hectares under cultivation) rather than any substantial increase in productivity as seed cotton yields remain at 400-600 kg/ ha as reported by MOA (2017).

Expanding Cotton Growing Areas and Productivity

Another way of reviving the cotton industry is through expanding cotton-growing areas by targeting potential areas. Vigorous campaigns can be directed for farmers to increase acreage on cotton. Since cotton in the country is mainly grown in arid and semi-arid areas, irrigation can be done to increase cotton production and productivity. In Kenya, for instance, cotton yields in 2017 averaged 572 kg/ha of seed cotton or 191 kg/ha of lint, which is estimated as 23 percent of the potential yield of 2,500kg/ha as per KALRO, (2018). Poor yields from smallholder cotton farmers in Kenya have been a long-standing problem that has not been significantly altered by the release of new varieties or by other recommendations made based on research findings.

Empowerment of Cotton Related Associations like Kenya Cotton Growers Associations (KCGA) and Handloom Weavers Association (HAWESA)

KCGA is a non-political, not-for-profit, and the only national cotton farmer's representative body formed in the year 2000 out of the need to address the plight of exploited and disempowered cotton farmers. The objectives of KCGA are: providing an advocacy voice for cotton farmers in furtherance of their interests by advising, lobbying and making representations to other stakeholders on matters of policy, legislation, financing and matters pertaining to cotton production; supporting capacity development to the national and county associations; and strengthening and coordinating farmers' roles in acquisition of resources, inputs, grants and other support to cotton production and marketing (CODA, 2006). These will assist in revitalizing the cotton industry.

The other strategy is empowering HAWESA, which comprises forty (40) enterprises drawn from all regions in Kenya, including individual cottage industries, workshops, and groups. The objectives of the association are to create a linkage between the people who trade in handloom woven cotton products and markets members' products under category 9 of AGOA pact through exhibitions and trade shows. They also collaborate with Kenya Industrial Research and Development Institute (KIRDI) to manufacture handlooms and spinner machines for use in the cotton industry, hence spread the market for cotton products, thereby enhancing the industry revitalization process (CODA, 2006).

Conclusion and Recommendation

It is concluded that the cotton industry faces problems of untested soil fertility and fertilizer application levels, lack of access to quality seeds, high cost of inputs/pesticides, collapse of irrigation schemes and former state-owned textile firms and ginneries, non-execution of cotton policies, poor agronomic practices, competition from synthetic fibre substitutes, and inadequate skilled labour supply for the textile industry. The prospects of the cotton industry include; increased cotton use, acquisition of new seed varieties/selection of superior traits, increased employment in cotton industry, improved economy as well as reduced poverty and increased products such as cloth from EPZ. The revival strategies included: implementation of the cotton bill and enforcing cotton regulations, expanding cotton acreage/yield, streamlining cotton commodity chain, integrating crop management and farmer-research link, enhancing Cotton Development Authority, empowering cotton associations such as KCGA/HAWESA, and putting in place policy and regulatory framework that leads to the maximization of the African Growth Opportunity Act initiative by the United States government that was extended from 2015 to 2025 to make local farmers benefit more. Lastly, it is concluded that there is need to address outlaid problems, avail resources for acquisition of quality seed, purchase of farm inputs and rehabilitation of collapsed irrigation schemes and ginneries and the government, the private sector and the international community to invest in the cotton industry for seamless entrenchment of revival strategies in the cotton industry.

It is recommended that cotton stakeholders including the government address prevailing problems to enhance trade prospects under AGOA thereby benefiting all in the cotton industry right from the farmer, ginner, spinner, weaver and textile industry. This will lead to cotton revitalization and which will in turn offer the best alternative for increased employment, poverty reduction, rural development, and increased incomes with enhanced textile and manufacturing industries.

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LAIKIPIA UNIVERSITY JOURNAL OF SCIENCE AND APPLIED TECHNOLOGY**Genetic Diversity and Geographic Distribution of Maize Streak Virus in Kenya***Daniel Pande*

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Abstract

Maize streak virus (MSV) is one of the most important pathogens responsible for poor maize yields in Africa. For over the past 50 years, the MSV-A₁ genotype has continually been moving back and forth between southern and eastern Africa and from East to West Africa. Despite Kenya being a maize producing country, very little is known of its MSV genetic diversity and geographical distribution of the circulating variants. In this study, a sampling survey was undertaken in the farmers' fields to collect MSV prevalence and symptom severity, where a total of 178 complete MSV genomes were sequenced from both grass and maize. Both phylogenetic and phylogenetic tools were used to illustrate the genetic diversity and geographical distribution. The results showed that the MSVA lineages had a distinct but overlapping geographical distributions in the country and noticeable relationship between the MSV symptom severity and the percentage infectivity.

Keywords: Genetic distribution, geographical distribution, Kenya, Maize streak virus, Mastrevirus

Introduction

Maize streak virus (MSV), one of the most important pathogens responsible for poor maize yields in Sub Saharan Africa is ubiquitously found in Africa; from Egypt in the north (Ammar, 1983) to Sudan in the East, Senegal in the west and South Africa in the south. It is also found on the Atlantic and Indian Ocean islands adjacent to Africa including Madagascar, Mauritius, Réunion, and Sao Tomé (Monjane et al., 2011; Storey, 1936).

Maize streak virus-A, the maize infecting MSV is considered to have originated in southern Africa in mid-1800s and based on full genome data analysed with a range of Bayesian phylogeographic approaches. It is believed to have spread to La Reunion Island between 1888 and 1970 (in the process of diverging into the MSV- A₆ isolate), to East African countries between 1915 and 1969 (in the process of diversifying into the MSV -A₃ genotype), and to West African countries between 1930 and 1968 (in the process of diversifying into the MSV-A₂ genotype) (Monjane et al., 2011). Whereas the MSV-A₄ genotype has apparently remained in southern Africa, over the past 50 years, the MSV-A₁ genotype has continually been moving back and forth between southern and East Africa and from East to West Africa (Monjane et al., 2011).

In the latest study on genetic diversities, geographical distribution and natural host ranges of MSV-A together with other MSV strains, MSV-A apparently moves throughout Africa far more rapidly than both grass-adapted MSV strains (Varsani et al., 2008) and the related African streak virus, PanSV (Varsani et al., 2009). This increased movement rate could be attributable to MSV-A having probably a broader host range or higher probability of being spread by humans through

transport of infected leaf material or viruliferous insects than its grass-adapted relatives (Martin & Shepherd, 2009).

Based on more than 94 percent sequence identity strain demarcation threshold, MSV is classified into eleven strains (named MSV-A to-K) (Muhire et al., 2013). Despite large scale sampling efforts, all indicators are that only MSV-A- causes severe disease in Maize. MSV-B apparently primarily infects grass species in the genus *Digitaria*, MSV -F and -G primarily infect species in the genus *Urochloa*, MSV-J has been established to be infecting a grass in the genus *Pennisetum* and MSV -C, -H, and -K have primarily been found infecting grass species in the genus *Setaria* (Rybicki et al., 1998; Varsani et al., 2008). ‘Grass-adapted’ strains such as MSV – B, -C, -D and -E cannot symptomatically infect any maize genotypes except the most susceptible ones (Martin et al., 2001; Schnippenkoetter et al., 2001; Willment et al, 2001). Although the grass-adapted MSV strains may not be a noticeable threat to crops, they could nevertheless provide invaluable information on the evolution and emergence of MSV-A (Kraberger et al., 2017).

To determine the nucleotide sequences relationships between MSV viruses in different groups, Martin et al. (2001) analysed eight MSV-A isolates, and other grass adapted MSV isolates which were cloned, made agroinfectious and fully sequenced. Besides defining an MSV strain as a group of MSV isolates sharing >93% genome-wide sequence similarity, MSV-A isolates, sharing > 98% sequence identity, were classified into different subtype groupings named MSV-A₁-A₆. Owor et al. (2007a) later further refined the MSV-A classification to account for recombination patterns and characterised MSV -A₁ isolates occurring in Uganda into eight distinct recombinant lineages named MSV- A₁I-MSV- A₁VIII.

Diversity studies in Kenya had been conducted on the East African Cassava Mosaic Virus (EACMV) (Bull et al., 2006). However, not much is known about the MSV diversity in Kenya. Therefore there was need to conduct studies on MSV diversity in the country similar to the diversity study done by Owor et al. (2007a) in Uganda.

This report describes the population structure of MSV isolates sampled from maize genotypes and grass species in Kenyan between the year 2008 and 2011. I used analysis of full-genome sequence data, to identify the major MSV variants circulating in Kenya and then to analyse their population genetic, phylogenetic, and phylogeographic characteristics.

Materials and Methods

Virus Sampling

A range of 122 uncultivated grass specimens and a total of 170 Maize samples displaying symptoms characteristic of MSV infection were collected during the first cropping season (May and June) and second cropping season (November and December) between 2008 and 2011; from the maize growing areas in Kenya, viz: The Kenyan Coast, Central, Rift Valley, Western Kenya and parts of Eastern region. For each sample, the geographical coordinates (determined using a global positioning system; GPS, to increase the precision of sampling location), sampling dates (to determine the inter-seasonal fluctuations in MSV populations and sizes) and host species (to determine the host ranges of various MSV pathotypes (recombinant lineages)) were recorded. There was, however, no criterion used in terms of areas to avoid, so long as the fields had maize or grass displaying MSV symptoms.

Three samples from each field were collected where possible. Every field was divided into two diagonals and the three samples were picked from each field. Two from the first diagonal and the third sample from the second diagonal. Fields with fewer than three diseased plants on the two diagonals, up to any three symptomatic plants were sampled from within the field. Samples were

fresh leaves. The fields were approximately 20 kilometres apart. The first was position determined by the nearest maize field from the county headquarters along the main road and contained maize plants of ages ranging from one month to three and a half months old. Symptomatic grass was of any species and age. The samples were press dried between pages of a ledger book later packaged in small envelopes for long-term preservation before processing.

Field Symptom and Degrees of Virulence Scores

Disease severity was scored using IITA's subjective six-point MSV resistance rating system (where plants rated 0 are immune, where as those rated 5 are highly sensitive) in the field. In addition, a field MSV degree of incidence was determined by the percentage number of maize plants infected against the total number of maize plants along the sampling field diagonal.

Cloning and Sequencing of Full Genomes

Viral genomes were isolated from leaf material, circular viral DNA molecules were amplified from a crude total DNA extract using Phi29 DNA polymerase (TempliPhi™, GE Healthcare, USA) as previously described by Owor et al. (2007b) and Shepherd et al. (2008). Briefly, the amplified concatemers were digested (cut) with *KpnI* or *BamHI* to yield ~2.7-Kb, potentially linearised viral genomes which were gel purified (invisorb spin DNA extraction kit; invitak) and were subsequently ligated to the *KpnI* and *BamHI* sites of pGEMZf+ (Promega Biotech, USA). The clones were fully sequenced by primer walking at Macrogen Inc (Korea).

Phylogenetic and Recombination Analyses of Virus Isolates

A total of 163 MSV-A₁ sequences representing isolates determined in this study, 391 MSV-A₁ sequences from GenBank and one representative of the MSV-A₄ subtype (Pande, 2014) were included in the analyses. A total of 45 genome sequences from grass adapted MSV isolates from Kenya that were determined in this study and 148 MSV B-K genome sequences from GenBank were also included in a separate analysis (Pande, 2014).

All the sequences were aligned by MUSCLE (Edgar, 2004) with manual editing using MEGA 5 (Kumar et al., 2008) and using the CLUSTAL-W (Thompson et al, 1994) based sequence alignment tool which is implemented in MEGA 5. In total, 553 MSV-A₁ sequences and one MSV-A₄ (used to re-root the tree) were firstly analysed for recombination using the computer program RDP4 (with default settings and sequences auto-masked for optimal recombination detection (Martin et al., 2010)).

Maximum likelihood (ML) phylogenetic trees were constructed either using PHYML v1. (Guindon & Gascuel, 2003) with the GTR+I+G₄ nucleotide substitution model (selected as the most appropriate by RDP3 (Martin et al., 2010) or with FastTree2 (with the GTR-CAT nucleotide substitution model and an approximate likelihood ratio test for branch support (Price et al., 2010).

Recombination was analysed using the RDP (Martin & Rybicki, 2000), GENECONV (Padidam et al., 1999), BOOTSCAN (Martin et al, 2005), MAXCHI (Smith, 1992), CHIMAERA (Posada & Crandall, 2001) SISCAN (Gibbs et al., 2000) and 3SEQ (Boni et al, 2007) recombination detection methods implemented in RDP3 (Martin et al., 2010). Default settings in all programmes were used throughout. Only potential recombination events detected by two or more of these methods, together with phylogenetic evidence of recombination were considered as strong evidence of recombination. Phylogeographic analyses of full viral genome datasets were carried out using visual nested clade analyses of geographically labelled sequences in different clades within the phylogenetic tree.

Results and Discussions

Phylogeography of the Maize Adapted MSV-A Strain

Out of the 292 symptomatic maize and grass leaf samples collected, 178 (142 from maize and 36 from grass samples) yielded clones of full MSV genomes which, once sequenced, were all found to belong to the MSV-A₁ subtype. An additional 15 previously sampled MSV full genomes from Kenya were obtained from GenBank (accession numbers: HQ693329, HQ693330, HQ693331, HQ693332, HQ693333, HQ693334, FJ882090, FJ882092, FJ882093, FJ882094, EU152256, EU152257, AF329878, AF329879 and AF329880) along with 375 MSV- A₁ genomes obtained from elsewhere in Africa.

Monjane et al. (2011) classified MSV-A₁ subtypes into 15 recombinant lineages (MSV-A₁I – MSV-A₁XV), retaining both the recombinant lineage naming convention and the recombinant lineage names designated by who had identified. He named just six lineages, and called them haplotypes.

MSV-A₁ subtype has a continent-wide distribution, but its recombinant lineages display a discernible degree of geographical clustering (Monjane et al., 2011). The study focused on the differences in the MSV-A₁ recombinant lineage demographics in different regions of Kenya. This is because the most significant influence on the appearance of virus epidemics is diversity. From the surveyed sequences, seven major Kenyan MSV-A₁ recombinant lineages were identified (MSV-A₁II, MSV-A₁III, MSV-A₁IV, MSV-A₁V, MSV-A₁VI, MSV-A₁VII, and MSV-A₁XIII). As was found in a 2005 survey of Ugandan MSVs (Owor et al., 2007a), the most widely distributed MSV genotypes in Kenya in my 2008-2011 survey were MSV-A₁III and MSV-A₁V which were found in at least half of the regions.

The predominant recombinant lineages per region included: MSV-A₁V in Nairobi (3/3 sequenced MSV-A₁ viruses), MSV-A₁XIII on the coast (10/12 sequenced MSV-A₁ viruses), MSV-A₁V in the eastern region (4/8 sequenced MSV-A₁ viruses), MSV-A₁III in Nyanza (37/76 sequenced MSV-A₁ viruses), MSV-A₁III in the western region (9/12 sequenced MSV-A₁ viruses) and MSV-A₁III and MSV-A₁V in the Rift Valley (17/43 and 10/43 Sequenced MSV-A₁ viruses respectively). These results mirror a phylogeographical finding on the East African Cassava Mosaic Virus (EACMV) strains by Bull et al. (2006) in Kenya where the isolates had a distinct but overlapping geographical distributions.

Based on the subjective field visual symptom assessment scores (see fFig1), the most and least virulent of these recombinant MSV-A₁ lineages were MSV-A₁III and MSV-A₁V. It should be noted, however, that the high degrees of MSV virulence in fields from which MSV-A₁III was isolated may have been influenced by the hot and wet climatic conditions around the Great Lakes region where very high virulence scores were recorded in most surveyed fields irrespective of the recombinant lineage present. Fields from which MSV-A₁III isolates were sampled elsewhere in the country were found to be not as severely affected as those around the lake region. The error bars on figure 1 represent 95 percent confidence intervals of the means.

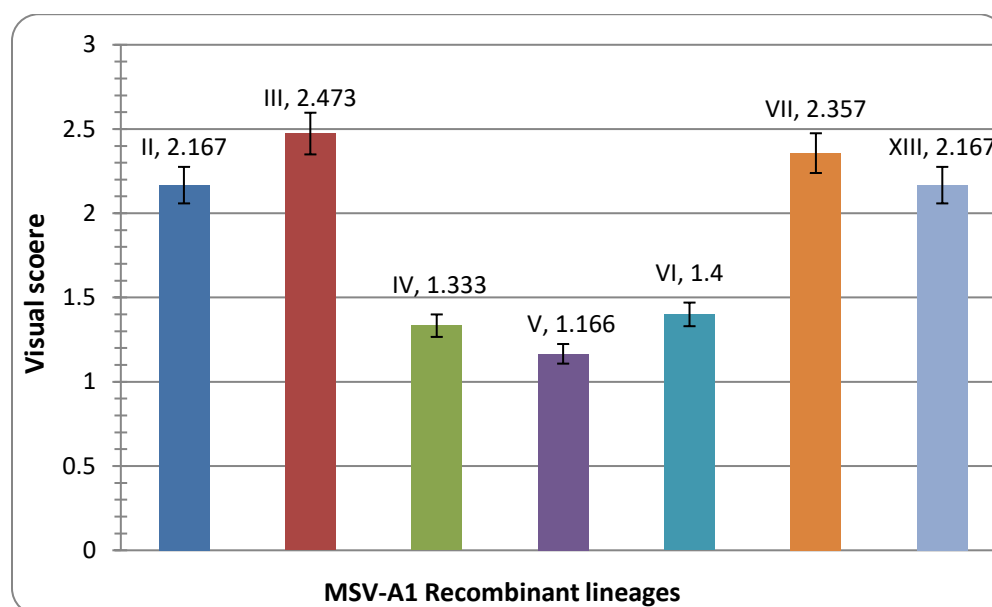


Fig. 1: Subjective MSV-A1 Symptom Severity Ratings (on a scale of 0 to 5)

There was an apparent association between the symptom severity and the incidence (percentage field infected plants, Fig. 2), save for MSV-A₁V and MSV-A₁XIII, which respectively had an elevated and decreased incidence as compared to their relative symptom severities (which were respectively low and high). This may have been due to the fewer numbers of surveyed maize samples showing low incidence: particularly in the coastal region where predominantly MSV-A₁XIII isolates were found. Conversely, in regions where MSV-A₁V was found, infected fields frequently had a 100 percent incidence. The error bars in figure 2 represent 95 percent confidence intervals of the means.

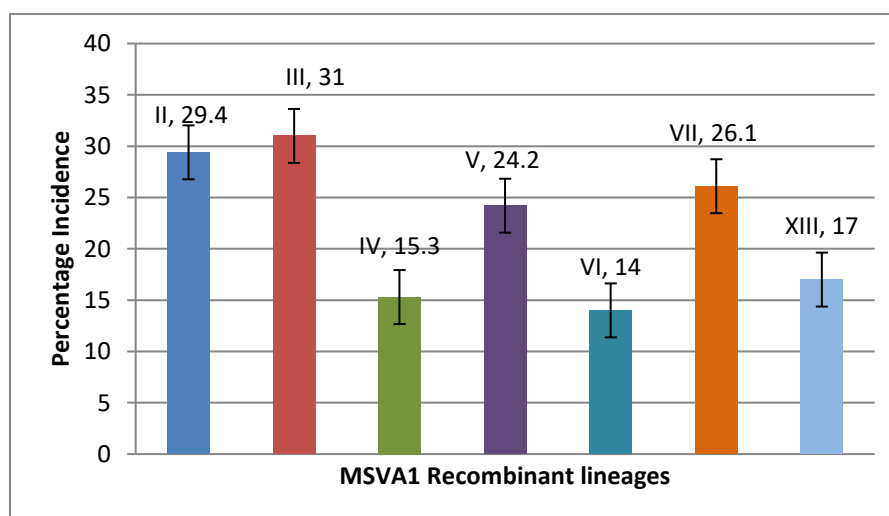


Fig. 2: Average MSV-A1 Percentage Field Incidence

When focusing on the phylogenetic relationships and sampling locations of the 178 MSV-A₁ sequences sampled in Kenya between 2008 and 2011, it is again apparent that MSV-A populations in Kenya might display a slightly greater degree of geographical clustering than those reported in Uganda by Owor et al. (2007a), and was very similar to what was observed in the diversity of cassava mosaic virus in Kenya by Bull et al. (2006). In particular, Nyanza and the Rift valley have very similar MSV population structures and display evidence of extensive mixing between MSV-A₁III and MSV-A₁XIII lineages (Table 1; Figure 3). Similarly, the Eastern and Central regions also have similar MSV population structures and display evidence of frequent movements of MSV-A₁V and MSV-A₁IV lineages between the regions. This implies that there are substantial impediments to the movement of MSV throughout the country, which could be attributed to wind patterns. The distribution of the MSV-A₁XIII lineages in Coastal and Nyanza, Rift Valley regions is intriguing and suggests that there have been some movements of the virus between the three regions but since MSV is only transmitted by leaf hoppers, it remains to be established how this happened because of the vast distance separating the in Coastal and Nyanza, Rift Valley regions.

Table 1: The Regional Distributions of Each of Seven MSV-A₁ Genotypes Found in Kenya

	Western	Nyanza	Rift Valley	Central	Eastern	Coast	Nairobi
MSVA-1II	2	8	6	2	0	0	0
MSVA-1III	9	37	17	0	0	2	0
MSVA-1IV	0	1	1	3	2	0	0
MSVA-1V	0	7	10	19	4	0	3
MSVA-1VI	0	2	2	1	0	0	0
MSVA-1VII	0	10	0	0	1	0	0
MSVA-1XIII	0	11	7	0	1	10	0

The figures shown in table 1 represent the counts of the individual MSV-A₁ genotypes.

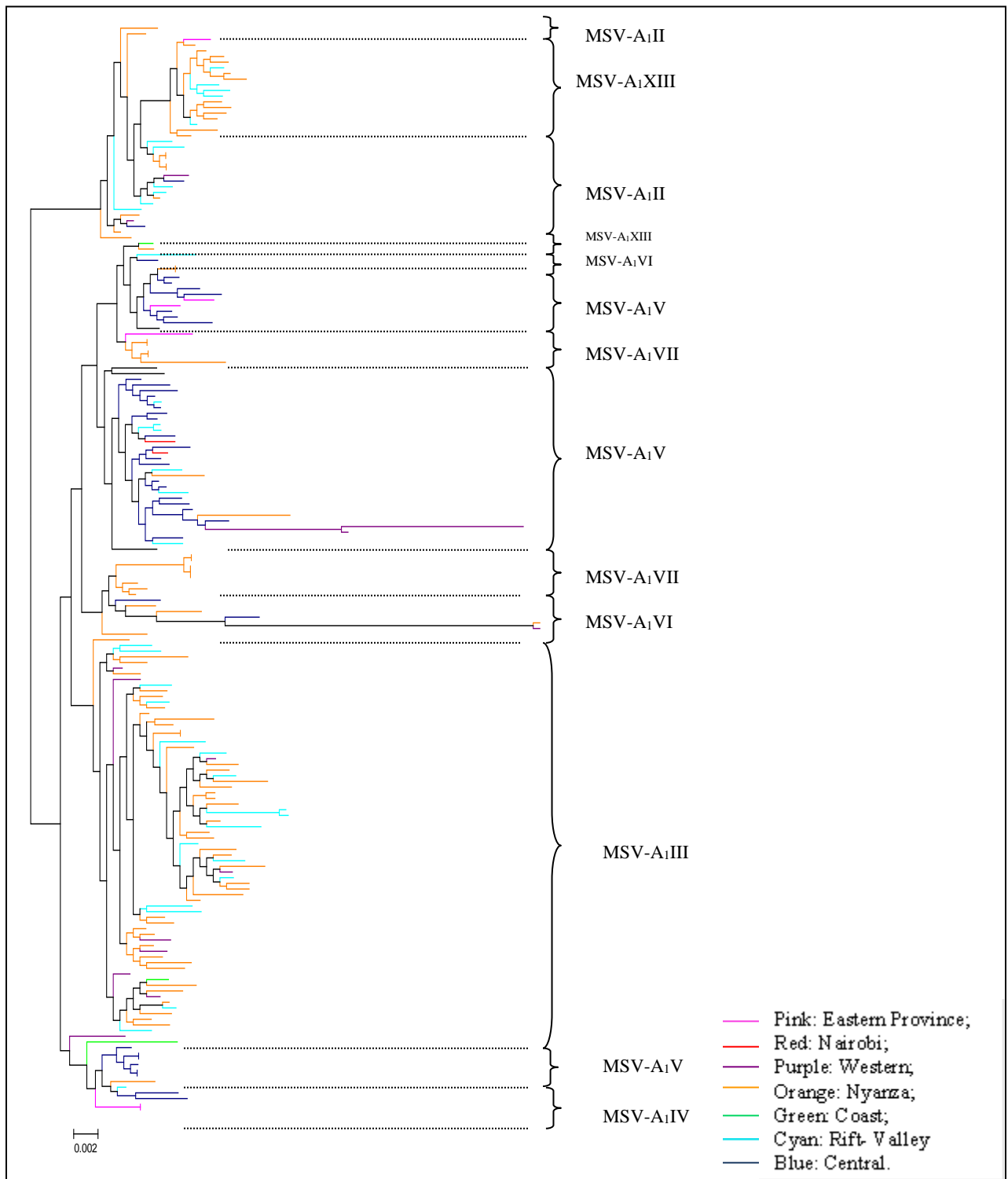


Fig. 3: Maximum Likelihood Phylogenetic Tree of 178 Full Genome Sequences of MSV-A1 Isolates from Kenya

Phylogeography of the Grass Adapted MSV Strains

Out of the 45 grass adapted viruses determined in this study, only the MSV-B, -C, -E, -F, -J and -K strains were identified (see figure 4).

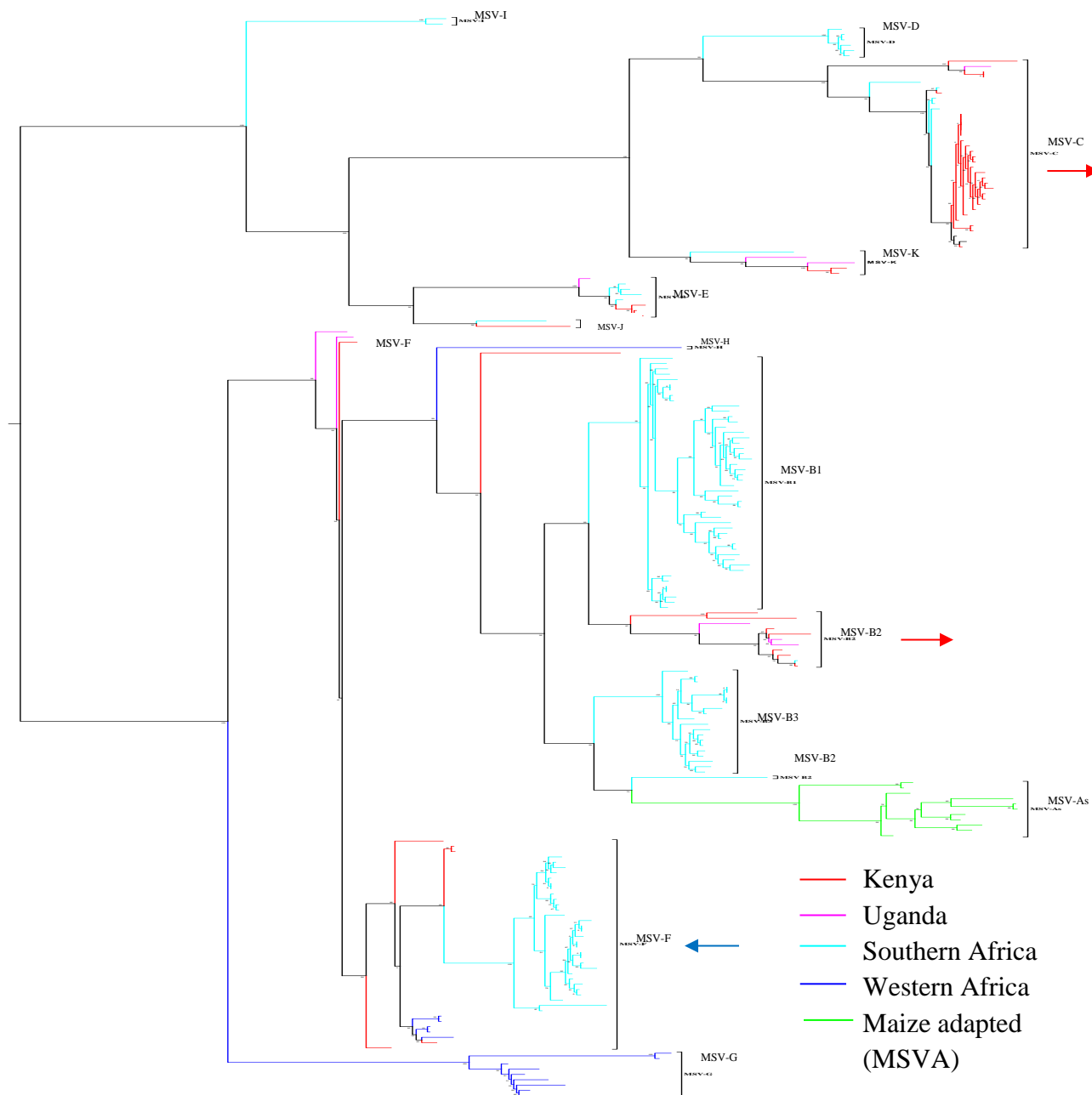


Fig. 4: Maximum Likelihood Phylogeny of 193 Full Genome Sequences of Grass Adapted MSVs and 11 MSV-A Sequences

Figure 4 included six MSV-B isolates, 26 MSV-C isolates, three MSV-E isolates, six MSV-F isolates, one MSV-J isolate, and two MSV-K isolates. Whereas too few MSV-E, -J and K isolates were detected to determine the preferred grass hosts of these MSV-strains, MSV-B isolates were primarily found infecting species in the genus *Digitaria*: a finding similar to that of Varsani et al.

(2008). Also similar to the findings of Varsani et al. (2008), MSV-C isolates were mainly found infecting grasses in the genera *Setaria* and *Digitaria* species.

It is, however, noteworthy that some MSV-A₁ isolates were also detected in and cloned from East African grasses both in this study and in that of Varsani et al. (2008). In this study, they were isolated mainly from *Digitaria sanguinalis*, *D. didactyla*, *D. ciliaris*, *Panicum laetum*, and a *saccharum* hybrid. It is likely that grasses such as these are the over-wintering hosts for MSV-A₁ from which the MSV-A strain re-emerges periodically to infect maize plants during the maize growing season.

Although there are presently too few sampled isolates for most of the grass adapted MSV strains to properly assess their movement dynamics, there is some evidence within the tree presented in Figure 4 of trans-continental movements of MSV-B, -C and -F variants. Based on the nesting of the southern African MSV-C and MSV-B2 sequences within clades of Kenyan sequences in the tree, it is evident that variants of these two lineages have likely moved from East Africa (coloured red and pink in the tree) into southern Africa (coloured cyan in the tree). Conversely the nesting of East African MSV-F variants within the southern African clades indicate that variants of this lineage have likely moved from southern Africa to East Africa. It is curious to note that just like movement of MSV-A from West Africa to East Africa consistently occurs in low frequencies, the grass adapted MSV-G strain which is equally common in West Africa is evidently either very rare or missing in East Africa.

Conclusion

While focusing on the phylogeographic relationship of MSVA₁ sequences sampled in Kenya, it was apparent that MSVA₁ populations showed greater degree of geographical clustering than those reported in Uganda. The study also identified MSV-B, -C, -E, -F, -J and -K strains as grass adapted strains circulating in Kenya. This study therefore recommends a study on topography and the wind patterns in Kenya to understand the clustering of the MSVA₁ haplotypes.

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LAIKIPIA UNIVERSITY JOURNAL OF SCIENCE AND APPLIED TECHNOLOGY**Antimicrobial Activity of Kenyan Laikipia County *Leonotis mollissima* Plant Extract***Esther Wanjiru Kinuthia*

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Abstract

The use of herbal medicine is expanding rapidly across the world due to the high bills involved in the importation of modern medicinal drugs. About 80 percent of the African population use traditional medicine from plants to treat common infectious diseases caused by microorganisms. The main objective of this research was to determine the antimicrobial activity of crude extracts and isolated compounds of Laikipia University *Leonotis mollissima* (Lion's ear) from the *Lamiaceae* family. The plant was studied in this research due to its wide use by local communities of Kenya for medicinal remedies. Its decoction is used by the Marakwets of Kenya to treat microbial diseases. The plant was sampled from Laikipia University Kenya, identified, air dried and voucher specimen kept for reference in the Biological Department of Egerton University in Kenya. Crude extracts from dry powder of the leaves were successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy-two hours. With repeated column chromatography, *Leonotis mollissima* dichloromethane leaves (79.69 g) crude extract yielded compounds **1** (Sederin), **2** (20-hydroxylucidenicacid D2) and **3** (labdane). Identification of pure compounds was achieved by ¹H and ¹³C NMR (500 MHz) spectroscopy. Chemical shifts (δ) were expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling (*J*) in Hz. On screening for antimicrobial activity, all crude extracts showed an MIC (Minimum Inhibition Concentration) of < 0.1 mg/mL to > 0.5 mg/mL on all test microorganism (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Candida albican*). Compounds **1** and **3** had an MIC of > 0.16 mg/mL on all test microorganisms while **2** compound had an MIC of 0.10 mg/mL. The IC₅₀ (Inhibition Concentration that reduces the effect of microorganisms by 50%) for all crude extracts and isolated compounds was less than that of Amoxil[®] and Doxycycline[®] antibiotics on all test micro-organisms on calculation with Graphpad Prism 8 free download computer program at different concentrations. This is a confirmation that *Leonotis mollissima* contains compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

Keywords: Kenya, Laikipia County, *leonotis mollissima*, minimum inhibition concentration (MIC)

Introduction

Many people in the world depend on plants for food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini et al., 2000). Many higher plants have been the source of medical agents since the earliest times and today, they continue to play a dominant role in the primary health care of about 80 percent of the world's population (Tilburt &

Kaptchuk, 2008). In Africa, up to 60 percent of the population consult one of an estimated 200,000 traditional healers especially in rural areas where these healers are more numerous and accessible than allopathic physicians (Van Wyk et al., 2000). The people in Asia, North and South America, as well as Australia and New Zealand have used concoctions prepared from a wide range of medicinal plants for treating the sick. The information on which plant and what part of the plant cures which disease was passed on from generation to generation. This rich heritage of traditional medicinal practices was looked down upon following the slicing of third World countries into fragmented pockets with European spheres of influence. It was branded as primitive although many pharmaceutical drugs and medicinal syrups administered to patients in modern hospitals are of plant origin (De Sa' Ferraira & Ferrao, 1999).

Medicinal agents derived from plants are also an essential feature in the health care system of the remaining 80 percent of the population residing mainly in developing countries. Of the world's twenty-five best-selling pharmaceutical agents, twelve are derived from natural products, which continue to play an important role in drug discovery programmes of the pharmaceutical industry and other research organizations (Chen et al., 2016). Without plants, most medicines taken would not exist. Over 40 percent of medicines now prescribed in USA contain chemicals derived from plants extracts (Facchini et al., 2000).

Throughout the world, botanists and biochemists search the plant kingdom for new medicines. For example, the native Pacific yew was burned as trash generated by logging operations in the Pacific Northwest. In 1975, a substance in its bark, taxol, was found to reduce the production of cancerous tumours (Facchini et al., 2000). A comprehensive search of known plants for medicinal chemicals is an enormous task. Of the estimated 250,000 plant species on earth, only 2 percent have been thoroughly screened for chemicals with potential medicinal use. Many native plant habitats are destroyed almost daily and therefore many medicinally valuable plants will be gone before scientists can investigate them (Facchini et al., 2000). Although plant extracts have been used in the treatment of diseases, research has shown some secondary metabolites present in these medicinal plants to be potentially toxic and carcinogenic, thus care should be taken before use (De Sa' Ferraira & Ferrao, 1999). Secondary metabolites are molecules that are not necessary for the growth and reproduction of a plant. They may serve some role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. They are often involved in key interactions between plants and their abiotic and biotic environments that influence them (Facchini et al., 2000).

Lion's ear (*Leonotis mollissima*) belongs to the mint family: Lamiaceae, genus *Leonotis* that comprises about 10 species (Nurdan & Aysel, 2007). The plant is known to treat cold, cough, fever, headache and asthma (Fowler, 2006). Among the Marakwets, the root decoction is used to treat wounds, festering sore and intestinal worms. Young leaves and buds are used to treat conjunctivitis and indigestion and are also chewed for cramp in the stomach (Kokwaro, 1976). However, no antimicrobial activity has been reported on the Kenyan Laikipia *Leonotis mollissima* crude extract. This research, sought to extract and isolate chemical compounds from the *Leonotis mollissima* leaves, and test their antimicrobial activity on selected bacteria.

Materials and Methods

Leaf samples of *Leonotis mollissima* were obtained from Laikipia University Laikipia county in Kenya. These were put in sample bags in June 2014, and air dried in shade. The air dried and ground leaves were sequentially extracted using hexane, dichloromethane, ethyl acetate and methanol for 72 hours. Exactly 10 μ L to 50 μ L of 10 mg/mL crude extracts and 5 μ L to 40 μ L of

4mg/mL isolated compounds were tested for antimicrobial activity on *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876 and an isolate of *Candida albicans* in duplicates. Extraction solvent was used as negative control (Oshomoh, 2012). The crude dichloromethane extract showed more compounds and its fraction was purified by step gradient isolation (dichloromethane/methanol) followed by repeated column chromatography (ethyl acetate/hexane).

Determination of **MIC** (Minimum Inhibition Concentration) was carried out for all the crude extracts and isolated compounds in a serial dilution. Methanol was used as negative control (Oshomoh, 2012). The lowest concentration with the smallest inhibition zone was taken as the **MIC**. For the **IC₅₀**, different concentrations of Amoxil® and Doxycycline® antibiotics (10.000 mg/L, 4.000 mg/L, 1.000 mg/L, 0.400 mg/L, 0.100 mg/L, 0.040 mg/L, 0.010 mg/L and 0.004 mg/L in methanol) were prepared using serial dilutions method. The **IC₅₀** for Amoxil® and Doxycycline® antibiotic was determined using probit analysis software (GraphPad Prism was used to plot inhibition zone against log of concentration of Amoxil® and Doxycycline® antibiotics). The **IC₅₀** for the crude extracts and the pure compounds were determined in a similar way. Their **IC₅₀** were then compared with the **IC₅₀** for Amoxil® and Doxycycline® antibiotics (Oshomoh, 2012).

Results

From dichloromethane crude extract of Laikipia leaves sample (79.69 g), three compounds **1** (Siderin, white pow 7.70 mg), **2** (20-hydroxylucidenicacid D2, 7.10 mg) and **3** (13R)-19 α ,13 α -epoxylabda-6 β (19).16(15)-dioldilactone, 21.20 mg) were isolated with repeated CC and Thin Layer Chromatography (TLC) (Kinuthia et al., 2018) (see figure 1).

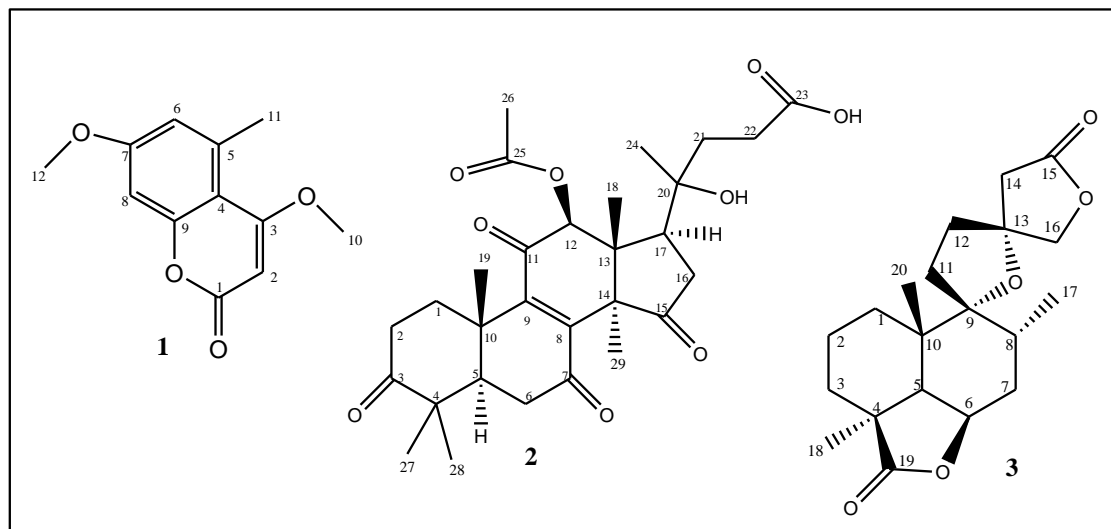


Fig. 1: Structures of Compounds Isolates from Laikipia County, *leonotis mollissima*

All the crude extracts had very significant antimicrobial activity on *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* at a concentration of 1mg/mL as indicated in table 1. All the organic extraction solvents did not show any activity. Of all the compounds isolated, only compound **2** showed significant antimicrobial activity on *Escherichia coli* at a concentration of 0.4 mg/mL as indicated in table 2.

Table 1: Inhibition Zone (mm) of Crude Extracts at a Concentration of 1 mg/mL

Sample	Micro-organism					Control
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Candida albicans</i>	
Hexane extract	16	16	9	17	11	-
Dichloro-methane extract	12	20	12	20	11	-
Ethyl acetate extract	9	14	12	12	10	-
Methanol extract	10	11	11	15	11	-

Table 2: Inhibition Zone (mm) of Compound 2 (20 Hydroxylucidenicacid D2) at Different Concentrations

Micro-organism	Concentrations (mg/mL)							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
<i>Bacillus cereus</i>	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	6	6	7	9
<i>Salmonella typhimurium</i>	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	-	-	-	-	-	-	-	-

The crude extracts had an MIC of < 0.1 mg/mL to > 0.5 mg/mL on all test microorganism as indicated in table 3. Compounds **1** (Siderin) and **3** (Labdane) had an MIC of > 0.16 mg/mL on all microorganisms while compound **2** (20-hydroxylucidenicacid D2) had an MIC of 0.08 mg/mL on *Escherichia coli* as indicated in table 3. Siderin is known to have a variety of bioactivities including anticoagulant, estrogenic, dermal photosensitizing, anti-microbial, vasodilator, molluscicidal, antihelminthic, sedative and hypnotic, analgesic and hypothermic activity (Divakar & Parminder, 2017). Lanostene-tetracyclic triterpenes possess anti-tumor, anti-inflammation, antioxidant, antimicrobial and blood fat reducing effects (Qing et al., 2014).

Additionally, a variety of biological activities have been encountered in labdane diterpenes such as antibacterial, antifungal, antiprotozoal, enzyme inducing, anti-inflammatory activities and modulation of immune cell functions. They also exhibit significant cytotoxic and cytostatic effects against leukemic cell lines of human origin (Costas & Konstantinos, 2001). This is an indication that *Leonotis mollissima* have compounds that can be developed to treat infectious microbial diseases.

Table 3: MIC of Crude Extracts and Pure Compounds on Test Micro-organism

Sample	MIC (Minimum Inhibition Zone) mg/mL				
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Candida albicans</i>
Hexane extract	< 0.1	0.1	0.1	< 0.1	> 0.5
Dichloromethane extract	< 0.1	< 0.1	0.1	0.2	>0.5
Ethyl acetate extract	> 0.5	0.3	< 0.1	0.2	>0.5
Methanol extract	0.4	> 0.5	< 0.1	0.1	>0.5
Siderin (1)	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
20-hydroxylucidenicacid D2 (2)	> 0.16	> 0.16	0.08	> 0.16	> 0.16
Labdane (3)	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16

The IC₅₀ for dichloromethane crude leave extracts (see figure 1 and also table 4) was 4 and 12 times less that of Amoxil[®] antibiotic on *Bacillus cereus* (figure 2). The IC₅₀ for Doxycycline[®] antibiotic on *Escherichia coli* was found to be 4.5 and 4.7 times more on comparison with both dichloromethane and ethyl acetate crude extracts (table 4). These concentrations were too low compared to those of the two antibiotics. Compound 2 (20-hydroxylucidenicacid D2) had an IC₅₀ of 0.141 mg/mL (see figure 3 and also table 4) on *EC* which was 11 times less that of Amoxil[®] antibiotic and 1,600 times that of Doxycycline[®] antibiotic (table 4). This is an indication that compound *Leonotis mollissima* has compounds that can be used to treat diseases caused by *Escherichia coli*.

Table 4: IC50 of Crude Extracts and Pure Compounds on Test Micro-organism

Sample	IC ₅₀ (mg/mL)				
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Candida albicans</i>
DCM extract	0.210	52.602	52.602		
EtOAc extract			49.889		
Siderin (1)					
(20-hydroxylucidenicacid D2 (2)			0.141		
Labdane (3)					
Amoxil [®] antibiotic	0.775	1.178	1.486	3.811	1.776
Doxycycline [®] antibiotic	0.044	1.200	233.884	1.276	0.632

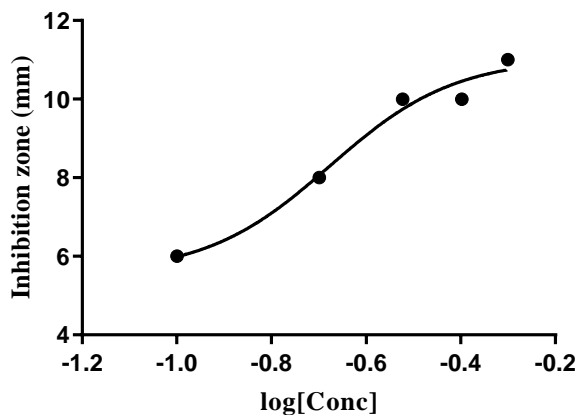


Fig. 2: Dichloromethane Crude Extract IC₅₀ on *Bacillus cereus* = 0.210 mg/mL

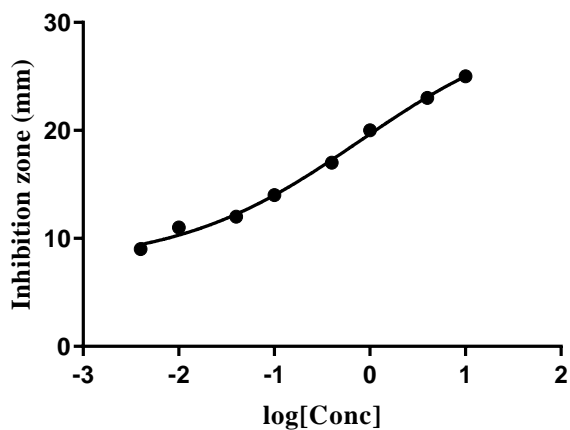


Fig. 3: IC₅₀ of Amoxil® Antibiotic on *Bacillus cereus* = 0.775 mg/mL

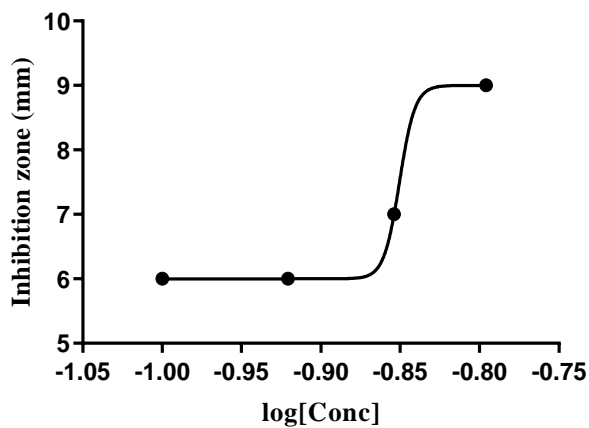


Fig. 4: IC₅₀ of Compound 2 on *Escherichia coli* = 0.141 mg/mL

Conclusion

Leonotis mollissima crude extracts showed significant antimicrobial activity on all the test microorganism (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Candida albicans*) at a concentration of 1 mg/ml. They had lower MIC (Minimum Inhibition Concentration) and IC₅₀ (Inhibition Concentration that reduces the effect of microorganisms by 50%) as compared to the Amoxil[®] and Doxycycline[®] antibiotics that were used as positive control for comparison.

From dichloromethane leaves crude extract, three compounds **1** (Siderin), **2** (20-hydroxylucidenicacid D2) and **3** (13R)-19 α ,13 α -epoxylabda-6 β (19).16(15)-dioldilactone were isolated (Kinuthia et al., 2018) (Figure.1). All had an MIC of > 0.16 mg/ml. Compound **2** had significant IC₅₀ of 0.141 mg/ml on *Escherichia coli*. Their IC₅₀ was lower than for Amoxil[®] and Doxycycline[®] antibiotics. Methanol was used as negative control.

Antimicrobial activity of all the compounds isolated were lower as compared to the crude extracts. This is a confirmation that the plant contains compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

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