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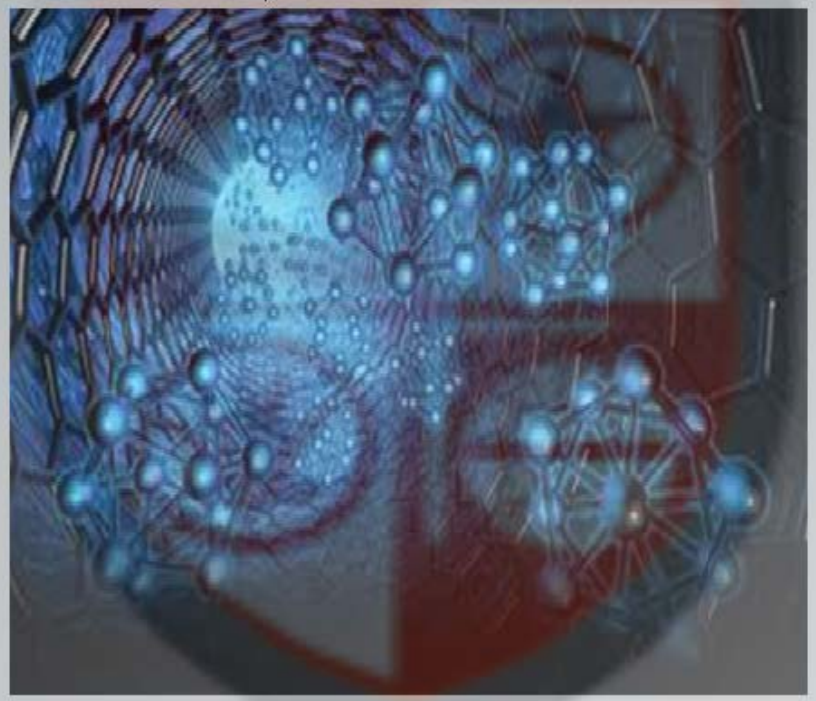

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CONTENTS

Determination of Maximum Residue Levels (Mrls) Of Lambda-Cyhalothrin (Karate 1.75 Ec) and Pre-Harvest Interval (Phi) on Tea in Kenya

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Abstract

Globally, 1031 species of arthropods are associated with the intensively managed tea. All parts of the plant, leaf, stem, root, flower, and seed, are fed upon by at least one pest species, resulting in an 11%–55% loss in yield if left unchecked, hence the need for use of pesticides. With climate change and increasing temperatures the need is greater. Lambda-cyhalothrin is an insecticide currently registered and used in Kenya for control of several insect and mite pests in diverse crops. This study established residue levels for lambda-cyhalothrin in fresh tea leaves, black tea and brewed tea. The study evaluated the effect of tea preparation procedures on pesticide residue levels in tea and monitored the decline of pesticide residues under normal harvest time intervals. The samples were collected at various intervals after application of the pesticide at maximum proposed application rate of 3.0 Lha^{-1} (i.e. worst-case conditions allowable) according to instructions on the label. The study was carried out at Timbilil estate of Tea Research Foundation of Kenya in Kericho. Extraction of lambda-cyhalothrin from Karate treated samples was accomplished using 50% acetone in hexane. Analysis of the samples was done by Gas Chromatography (GC). The pesticide residue concentrations in the tea samples were calculated using the power curve fit; $y = bx^m$. Results show that the levels of the pesticide residues decrease with increase in the pre-harvest interval days. The processing and brewing of tea appear to affect the residues of lambda-cyhalothrin most significantly. The residue levels from the study were lower than the maximum residue levels (MRLs) allowed within the European Union. Therefore, if this pesticide is used according to the established pattern it will pose no risk to the consumers of tea.

Key Words: Residue levels, lambda-cyhalothrin, PHI, MRLs Tea, Kenya

INTRODUCTION

Pesticides have continued to be of interest to toxicologists, biologists, ecologists, agriculturalists and analytical chemists due to their inherent toxicity. Analytical chemists have devoted time to research the specific area of analytical chemistry of pesticides (Moye 1981). With development of chlorinated hydrocarbons and their widespread use in agriculture it became apparent that residues in food were important and thus the study of pesticides in food is a major component in pesticide development. Each crop or food product for which a pesticide is registered must be analyzed for residues and a tolerance established (McEwen and Stephenson, 1979). In the recent years pesticide use has increased tremendously in Kenya, Sudan, Tanzania, Zimbabwe, Cameroon and Ivory Coast. These are countries that engage in high valued cash crop production such as floriculture, coffee, tea, cocoa, and cotton. Although tea in Kenya has been relatively pests and diseases free compared to for example, tea in India or Sri Lanka, serious outbreaks have sporadically been reported. Consequently, measures to control both pests and diseases have been reported. Numerous people commented on the association between increasing temperatures and the prevalence of pests and diseases, an issue of increasing importance to Kenya. Past years' unpredicted and unprecedented pest infestation in Rwanda was seen as a warning of what could happen in Kenya, where the tea mosquito bug (*helopeltis spp*) has recently appeared (Adaptation Workshop, 2011).

Pesticides in the developing countries are mainly available in conventional formulations such as dust, wettable powder, emulsifiable concentrates, solutions, etc. Such conventional formulations

pose problems relating to environmental protection, leaving residues in the ecosystem, food, finished products, etc. (United Nations Industrial Development Organization, 2009).

When pesticides are applied to food crops they degrade through chemical and biological processes at a rate determined by the nature of the chemical and plant surface or soil in which the pesticide is placed. Therefore pesticide residues may not be present as the parent compound. Many pesticides may form metabolites that are as persistent as or more persistent than the initial chemical. This fact is recognized in establishment of tolerances and acceptable daily intake (McEwen and Stephenson, 1979). Lambda- cyhalothrin has short persistence in soil and lacks systemic effect (Pesticide residues in food, 1986).The main concern in the study of pesticide residues in food is to ensure safety of the food supply. It is reported that pesticides can cause allergies and asthma like symptoms and can affect body organs such as the liver, kidneys, and the nervous system.

The problem of residues in food has been addressed at an international level through committees sponsored by United Nations. Acceptable daily intake (ADI) has been established for a number of pesticides and presented with suggested tolerances in a series of annual reports of joint FAO/WHO meetings (McEwen and Stephenson., 1979). It was therefore important to carry out this research so as to establish what maximum residue is likely to be present in fresh tea leaves, processed tea, and brewed tea, when pesticides are used in a manner effective for pest control. In this study, field trials were set up to estimate pesticide residue levels in fresh, black and brewed tea for Karate, a broad-spectrum synthetic pyrethroid for control of foliar insect pests. Reports have shown that normal methods of food preparation significantly reduce pesticide residues (McEwen and Stephenson., 1979). Tea as a product undergoes various preparations from the fresh leaf to the black brewed tea for consumption. The extent of such effects varies with the pesticide residues and the nature of food product. The importance of these procedures in reducing pesticide residues will be established by comparing residue levels in fresh tea leaves, black tea and brewed tea.

Tea is susceptible to a number of insect and mite pests. Therefore application of pesticides such as Karate for control of the pests in tea such as thrips, aphids, and weevils is necessary. Karate is a broad-spectrum synthetic Pyrethroid insecticide used for control of biting and sucking pests in crops. It has a high level activity against a wide range of insects and it also has miticidal activity. The compound has a quick knock down and repellency effect through contact, residual and stomach activity. The chemical is relatively stable to degradation in sunlight; hence it is used as a practical tool in agriculture. Treatments of karate are effective against major pests such as boring caterpillars or leaf miners. Application should be done when insects are noticed and a spray interval of seven days observed depending on the amount of rain and pest infestation. A programme of sprays is usually required particularly during more active growth stages of the plant. Karate is applied at a maximum rate of 3.0l/Ha to mature tea bushes with shoots.

Karate is a pesticide that is foliar sprayed on tea for protection against pests such as boring caterpillars or leaf miners, mites, aphids, whiteflies, thrips and diamond back moth. Karate is commercially available in emulsifiable formulations. It occurs as a 1: 1 mixture of two enantiomeric pair of (S)-α-cyano-3-phenoxybenzyl Z- (1R, 3R)-3-(2-chloro-3, 3, 3-trifluoropropenyl)-2, 2-dimethylcyclopropane carboxylate and (R)-α-cyano-3-phenoxybenzyl Z-(1S, 3S)-3-(2-chloro-3, 3, 3-trifluoropropenyl)-2, 2-dimethylcyclopropane carboxylate. It contains approximately 90% lambda cyhalothrin and small amounts of other cyhalothrin isomers. Lambda-cyhalothrin the active ingredient has the chemical formula $C_{23}H_{19}ClF_3NO_3$ and

molecular weight of 449.9 and the technical material is a viscous, oduorless, liquid. It is insoluble in water but soluble in a range of organic solvents. The structure of the two enantiomeric pairs is given below

(S) (Z)-(IR)-Cis- (S) (Z)-(IS)-Cis-

Figure 1: Structure of Lambda-cyhalothrin (Tomlin, 1997)

Most synthetic pyrethroids have α- Cyano 3- Phenoxybenzyl or a 3-Phenoxybenzyl group as the alcohol moiety and produce 3-phenoxybenzoic acid (PBA) as the ester cleavage metabolite Therefore Lambda-cyhalothrin metabolises to 3-Phenoxybenzoic acid (PBA). A study on thirteen synthetic Pyrethroid insecticides and their ester cleavage metabolite PBA in tea indicate residues of the pyrethroids were found but no 3- Phenoxybenzoic acid (PBA) were detected (Tsumura *et al*, 1994). Therefore the study analyzed the residues of the parent Pyrethroid Lambda-cyhalothrin only.

Residues of this pesticide could reach and affect consumers of tea. Therefore it is necessary to determine the levels of residues likely to appear in drinking tea. In carrying out this endeavor, it was necessary to determine pesticide concentration levels at different stages of tea preparation. This ensured that it was possible to determine the effect of processing on residue levels in tea. This study is also necessary in order to ensure correct use of pesticides on tea in terms of application rates and Pre- Harvest Interval and to permit circulation of tea in the world markets even though they are treated with pesticides as long as the residues comply with harmonized MRLs. More so, it is to ensure that pesticide residues if any remaining in tea are of acceptable levels so that there are no health risks to the consumer. Plant residues are isolated by liquid-liquid extraction (LLE) because they contain water, plant pigments, lipids, proteins, essential oils and waxes. A comparison of the various isolation and cleaning techniques for pesticide residue analysis show that LLE has a good isolation effect and it is universal for food and plant materials. Florisil column chromatography has a good isolation effect and very good cleaning effect for plant materials. Acetone has been used in LLE of synthetic pyrethroids in tea leaves. Classical LLE offers a wide choice of organic solvents for effective analyte isolation from the sample e.g. pure acetone, methanol or their mixtures with medium polar organic solvents are often used for extraction of various pesticide residues from biological matrix (Tekel *et al.*, 2001). Residue data are required for samples of made tea in the world markets. EU and FAO/WHO require producing countries to do field trials to determine the maximum residue levels under their environments. These field experiments must reflect the proposed pesticide use with respect to formulated products, dilutions and rates, modes, number and timings of applications. Enough plots should be set out to permit sampling at intervals after the last application of Karate such as 0, 7 and 14 days so as to establish appropriate pre-harvest interval. Pre-harvesting interval is the period which must be left between application of a pesticide in the farm and the harvesting of a

crop. This is to ensure that pesticide residue on the crop becomes within acceptable and safe limits for human use (Al-Agha *et al.*, 2005).

Methodology

The field experiment conducted in Timbilil estate in Tea Research Foundation of Kenya Kericho. It constituted two replicate Karate 1.75EC treated plots and one untreated control plot. Application of Karate was done at a rate of 3.0ml Karate 1.75 E.C per litre of water. No physical crop maintenance practices such as tilling, hoeing or pruning was carried out during the period of the study. The application was made to mature tea leaves as new growth appeared at the beginning of the dry season in Kericho. The application was made using a knapsack sprayer with a hand held boom as is typical commercial practice. The rate of application was done at the maximum proposed rate, intended to be a worst case treatment pattern to be used in Kenya. Sampling of leaves for residue determination was done 0, 7 and 14 days after application so as to establish the pre-harvest interval. This was to ensure that pesticide residue on the crop was within acceptable and safe limits for human use. Typical local practice were used in the harvest of tea samples including plucking with bare hands, use of rubber aprons, use of net plucking bags and ensuring plucking two leaves and a bud. Leaves were then transferred into plastic lined residue sampling bags on site and transported to TRFK processing facility and laboratory for weighing.

Fresh tea leaf samples, Black Tea Processing and Brewed Tea Preparation

Small fresh tea leaf samples not designated for processing were placed into a freezer at TRFK laboratories until extraction. Black tea processing was performed in a miniature-scale tea processing facility at the Tea Research Foundation of Kenya, Kericho. The process is designed to simulate as closely as possible the commercial black tea processing procedure that is standard in Kenya (Stefan, 1997). The tea processing includes withering which results in moisture loss. Biochemical changes also occur within the leaf matrix. Enzymes begin to gradually ferment the leaf material to add the complexity of Flavor and quality of tea. This is followed by leaf maceration a step in which the cell structure of the leaf matrix is physically destroyed to allow fermentation to occur by a process of crush, tear and curl (CTC). This process allows air to circulate into the tea matrix, where oxygen works with enzymes from the plant cells to ferment the entirerity of the matrix. Fermentation or oxidation is a process by which tea quality is achieved. The macerate leaf is placed in several trays whereby humidified ambient air is blown between the trays in order to supply oxygen to the fermentation reaction and also dissipate heat that is generated by the exothermic reaction. Tea drying terminates the fermentation process. The tea is considered dried at moisture content of approximately 2.5 to 4%. Brewed tea was prepared by weighing 20 g of black tea into a flask then 300 mL of boiling distilled water was added. The contents of the flask were allowed to stand for 5 minutes. The liquid portion was filtered through a filter paper into another flask. Another 300 mL of boiling water was added to the solid black tea remaining in the flask and allowed to stand for 5 minutes. The liquid portion was filtered into the jar containing the first liquid portion. The sample was allowed to cool to room temperature (Samantha and Dan, 1995) and the extraction was then carried out.

Sample Extraction

Hydration of the tea samples was done before extraction to ensure efficient extraction. The Lambda-cyhalothrin extaction was done using hexane: acetone mixture with subsequent drying with anhydrous sodium sulphate (Subbiah and Narayanan, 2009).

Clean-up for lambda-cyhalothrin was conducted using open preparative chromatographic columns packed with 10 g silica gel and 2 g anhydrous sodium sulphate (Na_2SO_4) placed on top of the columns (Lina *et al*., 2010).

Calibration curves

Known concentrations of the pesticides were analyzed to generate a five point calibration curve of the type stated below:

The Power curve fit: $y = bx^m$ (Samantha and Dan, 1995).

Where y = the detector response, peak height
b = y intercept
x = nanograms injected
m = the slope of the line

The standard concentrations were given in terms of nanograms injected for example if 2 µl of 0.1 ppm (0.1ng/ µL) was injected; this is equivalent to 0.2 ng.

The power curve was chosen because it gives all the concentrations as positive values including those of peak heights lower than the y-intercept of the calibration line, which would otherwise be given as negative concentration if a linear curve of the form y=ax + b is used.

Secondly, the rate at which the concentration changes is not constant because the factors responsible for the change are continually changing. These factors include plant growth, sunlight intensity and amount of rainfall. In cases where data do not follow a linear trend, an exponential or power curve fit is used (Frank and Kenneth, 1969).

Calculation of concentrations

The sample peak heights from chromatograms were used in the standard equation obtained to calculate the nanograms found for each sample. The Pyrethrins in all calculations were listed as ppm Pyagro. The ppm of the pesticides was determined from the nanograms found from the calibration equation using the following steps:

g-final weight = g-initial sample wt x mL-aliquot
mL-extraction solvent

$$(ng/mg)\ ppm = \frac{A \times B \times C}{D \times E}$$

A = ng- found
B = final volume, µL
C = dilution factor
D = µL- injected
E = final weight, mg

After obtaining the concentration of each sample, the mean concentration of each triplicate was determined.

Fortification recoveries

Analytical procedures are validated by fortification of control samples, results within the standard range of 70 % to 120% show acceptable accuracy (Stefan, 1997). Percentage recoveries of fortified control samples were calculated using the equation

$$\%\ recovery = \frac{ppm\ found\ in\ fortified\ sample - ppm\ in\ control \times 100}{ppm\ fortification\ level}$$

Treated sample residues were corrected upward using the percentage recoveries for each set of samples.

RESULTS AND DISCUSSION

Time of harvest and pesticide residue levels

Pesticides are generally lost by evaporation, photo degradation, rainfall and growth dilution. The latter is a process where the pesticide present in the plant tissues spreads to new tissues as the plant grows, leading to lower concentrations as the number of tissues increase. The chromatograms of 0, 7 and 14-day samples for Karate and the decay curves reveal that the pesticide residue levels decrease as the number of days after pesticide application increase (Muraleedharan *et, al* 2003).

Residue decay curves

The residue decay curves given in figures 2 and 3 show the decline in the pesticide concentration in the samples collected 0, 7 and 14 days. The figures show that the pesticides decay gradually from the time of application. It shows decay in the fresh leaves, this decay is mainly attributed to growth dilution, photo degradation and rainfall. Figure 3 show residues in black tea samples which significantly lower than the residues of the respective fresh tea samples. The difference is as a result of thermal decomposition during the processing of black tea due to high temperatures.

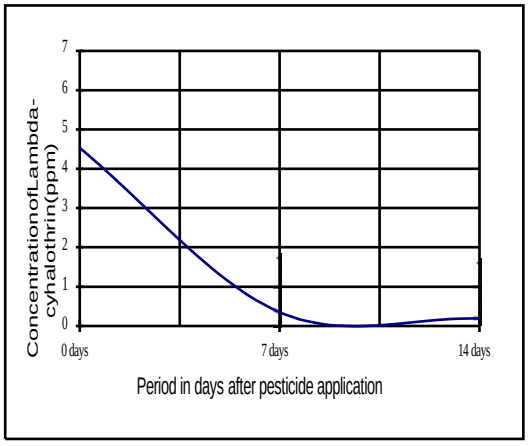

Fig 2: Karate (Lambda-cyhalothrin) Collected on 0.7 and 14 days after Pesticide application

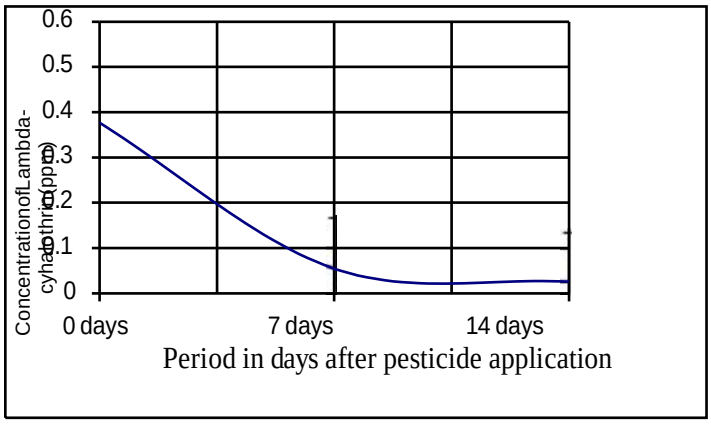

Fig 3: Karate (Lambda-cyhalothrin) concentration in black tea samples collected on 0,7 and 14 days after pesticide application

Effect of processing on pesticide residue levels

Further degradation of pesticide residues takes place due to thermal decomposition during manufacture of black tea, as the tea is exposed to high temperature. More so, during preparation of brewed tea, pesticide residues contained in black tea are further degraded by high temperature. The reduction of the pesticide residues due to processing are shown in figures 4 below. Pesticide residues may leach into the brewed tea or remain in the spent tea depending on their solubility in water.

Figure 4 shows a decline of pesticide residues as black tea is processed from the fresh leaf and as brewed tea is made from black tea. On the day karate was applied, the pesticide residues decline from 4.535 ppm in fresh leaf sample to 0.377 ppm in black tea. This is a percentage decrease of 91.7%. The residues in the samples collected 7 days after application of karate were found to be significantly lower than for the samples collected on the day of pesticide application, for the

fresh leaves the pesticide concentration on the day of pesticide application was found to be 4.535 ppm and 7 days after application the concentration was found to be 0.349 ppm, a 92% decrease. The residue levels decrease further during processing from 0.349 ppm in the fresh leaf samples to 0.059 ppm in black tea (83% decrease).

The bar graph shows a steady decline of the already low lambda-cyhalothrin (Karate) residues in the samples collected 14 days after pesticide application. The decrease from 0.194 ppm in the fresh leaf samples to 0.026 ppm in the black tea, this is equivalent to 86.6% decrease.

The decrease of the residues as the fresh leaf is used in the manufacture of black tea can be attributed to thermal decomposition due to high temperatures of about 120°C during the manufacture of black tea (Stefan, 1997). The decrease of the residues in the same matrix harvested at different pre-harvest interval can be attributed to growth dilution, photo degradation and rainfall (Muraleedharan *et, al* 2003).

No residues were detected in the brewed tea and this may be attributed to the insolubility of lambda-cyhalothrin or decomposition and evaporation during brewing.

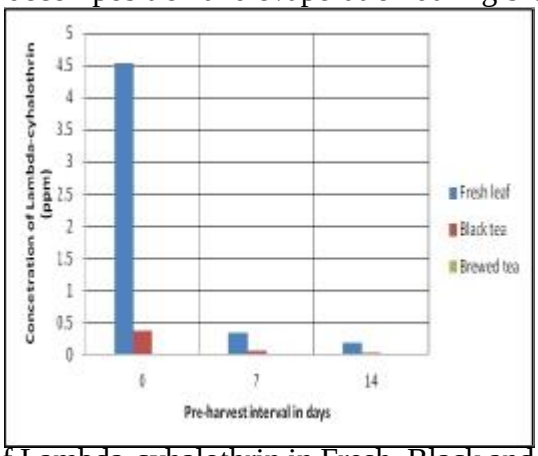

Fig 4: Concentration of Lambda-cyhalothrin in Fresh, Black and Brewed tea after 0, 7 and 14 days after application of Karate

Conclusion

The residue levels shown in the residue decay curves and the bar graph show that application of Karate in fresh leaves result in residues in the tea product and that tea preparation procedures lead to reduction of pesticide residues in the tea product. The residues found in the tea after application of the pesticide at maximum proposed rates indicate that the best time of harvesting (pre-harvest interval) after pesticide application is seven days.

The Kericho trial site was representative of diverse tea growing areas in Kenya. Karate 1.75 E.C is used in Kenya to control pests in tea. The treatment rate was intended as a maximum rate proposed for use on tea in Kenya so as to represent a worst-case treatment. The application parameters considered during the field trial may be proposed for use in Kericho.

Karate residues in the various tea matrices were higher than the method LOQ of 0.01 ppm, except for some samples of black tea taken at 14 days after pesticide application and for all the brewed tea samples that had no detectable residue levels.

Based on the results presented above; treatment of mature tea bushes with Karate 1.75EC according to the intended use pattern tested in Kericho will produce no detectable residue levels of Karate in brewed tea. Generally it will give low levels of Karate residues in fresh and black

tea. The residues found in this study lie below the acceptable Maximum Residue Limits (MRLs) of 0.1ppm in dried (black) tea established within the European Union (E.U). Thus karate if used as recommended will pose no risks to the consumers of tea.

Recommendations

1. Plucking of tea should not be done before a period of seven days after spraying with Karate
2. Spraying should be done after plucking so that a minimum safe period is always maintained, that is before new shoots are available for plucking, which is approximately seven to fourteen days apart.

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Effects of selected plant materials on the whole body compositions and hepatosomatic index of Nile tilapia (*Oreochromis niloticus* L.)

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Abstract

An eight week study was carried out to determine the effect of substituting freshwater shrimps *Caridenea niloticus* with 2 plant materials on the whole body composition and the hepatosomatic index in diets used to feed the Nile tilapia *Oreochromis niloticus* in diets. Cassava leaves (CLM) and Boiled tea leaf residues (BTLR) were used to replace freshwater shrimps from diets used to culture the Nile tilapia (*Oreochromis niloticus* L.). The study was carried out in Sagana Aquaculture Centre both in aquaria set up in a hatchery and in hapas set up earthen ponds where 10 post fingerling fish were used in triplicates. The following were used in the experiments: CLM in aquaria (initial mean weight 10.90±0.64g fish^{-1}; mean length 6.20±0.25cm fish^{-1}) and in hapas set up in fertilized earthen ponds (initial mean weight 10.55g fish^{-1} mean length 8.17±0.23cm fish^{-1}) and BTLR in aquaria (initial mean weight 12.36±1.1g fish^{-1}; mean length 8.43±0.27 cm fish^{-1}) and hapas (initial mean weight 10.98±0.75g fish^{-1} and mean length 8.77±0.3 cm fish^{-1}). Four (300g/kg) practical diets were formulated to contain 0%, 25%, 50% or 100% of the test ingredient. The 0% test ingredient (Sagana diet) was used as the control and the performance of fish fed on the other diets were compared to it. The diets were fed to fish in glass aquaria and in hapas in a pond at 10% of their body weight in triplicates. Carcass chemical composition showed similar effect of diets on body moisture content at 25% CLM and 100% CLM in hapas. At 50% CLM the diets caused a significant ($P<0.05$) increase in the whole body moisture level. Substitution up to 50% CLM showed similar effect on body moisture in fish cultured in aquaria. 100% CLM significantly ($P<0.05$) increased whole body moisture, significantly ($P<0.05$) decreased whole body crude protein and significantly decreased Hepatosomatic Indices (HSI) in fish grown in aquaria. HSI was similar at 0% CLM, 50% CLM and 100% CLM but increased significantly ($P<0.05$) in hapas. The diet had the same effect ($P>0.05$) on the whole body chemistry (moisture, total ash and crude protein) in the fish raised in hapas. In glass aquaria total body ash increased ($P<0.05$) significantly with increase in BTLR inclusion. Crude protein decreased significantly at 100% BTLR. The HSI was significantly ($P>0.05$) high at 100% BTLR in hapas, while in the aquaria there was a significant increase in hepatosomatic index with increase in BTLR inclusion. The study concluded that the two ingredients could be used with limited success in aquaria due to their effect on the *O. niloticus*. However the ingredients can be used to replace freshwater shrimps from diets used to culture *O. niloticus* in fertilized earthen ponds.

Key word: *Oreochromis niloticus*, whole body composition, hepatosomatic index

Introduction

Over the last 30 years, aquaculture has grown faster worldwide than any other animal production sector (FAO, 2007). The average annual growth has been 10% compared with 3% in the cattle industry and 1.6% in capture of aquatic species from natural environments (Garduno-Lugo and Olvera-Novoa 2008). The strong growth in aquaculture has generated a consequent 30% annual growth in the production of aquatic species feeds (Francis *et al*., 2001), and has made raw material supply a continuous challenge in this industry. Tilapia species are used in commercial farming systems in almost 100 countries (Fitzsimmons, 2000). Characteristics that make tilapia a

suitable fish for culture include rapid growth rates, high tolerance to low water quality, efficient feed conversion, ease of spawning, resistance to disease and good consumer acceptance (El-Saidy and Gaber, 2005). Rapid growth of tilapia culture has stimulated the expansion of tilapia feed production and a search for novel protein sources to replace animal proteins like fish meal (Marion & Miguel, 2008). Plant or vegetable sources have given a lot of promise as alternatives and equally their availability is assured in most fish farming areas of Kenya. Aquaculture has been slow in its growth in Kenya since its inception. However there has been intervention by the Government of Kenya's (GOK) to increase aquaculture production through the Economic Stimulus Package (ESP) in the 2009/2010 budget-1.1 billion or 8 million/constituency for the creation of 200 fish farming ponds and covering 140 constituencies countrywide. This move targeted, improvement of nutrition, creation of over 120,000 jobs and income opportunities for citizens (Rothuis *et al*., 2011). As is the trend globally where aquaculture growth has generated a consequent 30% annual growth in the production of aquatic species feeds and has made raw material supply a continuous challenge in this industry, the situation in Kenya is that of poor fish quality and quantity. The use of plant sources to replace animal protein has gained a lot of attention in the recent past with relative degree of success. Despite the abudance of plant ingredients, use in feed formulations can be limited because of their antinutritional components, which can be grouped into three categories: (a) those affecting protein utilization and digestion; (b) those affecting mineral utilization; and (c) anti-vitamins and toxic substances (Marion and Miguel, 2008). Investigations on the effects of test diets on the whole body composition and on the Hepato Somatic Indices (HSI) helps explain the biological availability of the ingredients and also effects on the well being of the test fish. This study looked at the possible effects of the diets on the whole body composition and the Hepatosomatic index which can give an indication of lower digestability and poor food absorption respectively.

Materials and methods

The experiment was performed at the Sagana Aquaculture Center 90 Km northeast of Nairobi, altitude 1230 m, latitude 0°39´S and longitude 37°12´E. The experiments were conducted both in laboratory aquaria (dimension 0.45m x 0.3mx 0.3m; water volume, 60 litres), and upper open end hapas dimension of (1m x 1m x 1m) installed in a single earthen pond. The aquaria were set in a thermo regulated recirculating system, comprising a settling tank for solid removal and an

anaerobic bio filter (tickling filter) to remove ammonia. Filtered and aerated bore hole water was used in filling the tanks. The experimental pond was fertilized weekly at a rate of 20 kg N and 8 kg P ha-1 with Urea and diammonium phosphate (DAP), respectively, and limed once at 2500 kg ha-1 with $CaCO_3$ at the beginning of the experiment. Key water quality parameters: temperature, pH, dissolved oxygen (DO) and chlorophyll *a* were measured three times a week in the aquaria and cage experiments. Dissolved oxygen was measured using model 57 oxygen meter (YSI industries, Yellow springs, OH, USA), while a glass electrode pH meter, Hi-9024 microcomputer (Hanna Instruments Ltd., Chicago, IL., USA), was used to take pH measurements. Chlorophyll *a* was determined as described in American Public Health Association (APHA, 1995).

Experimental diet formulation and feeding practice

Boiled tea leave residues BTLR were sourced from hotels in Sagana town. Freshwater shrimp meal (FSM) was purchased from Kisumu. Cotton seed cake meal (CSM) and wheat bran were bought from animal feed stores in Sagana town. Cassava leaves were sourced from farms surrounding Sagana Aquaculture Centre. Vitamin and mineral premixes were sourced from an agrovet shop in sagana town. The composition of the vitamin and mineral premixes is shown in Table 5. All ingredients were first dried then ground into fine powder before being subjected to proximate analysis. The proximate composition of the ingredients was determined (as shown in Table 1) for the purposes of formulating the diets. An analysis of crude protein, crude fiber, ether extracts, ash and moisture content was done in triplicates, following the procedure by Association of America Chemists (AOAC, 1995). Protein content of the diets was determined using micro-Kjeldhal method, percent fat using ether extraction method, crude fibre by acid-alkali digestion, ash by burning weighed samples at 600^{o}C in a muffle furnace, and moisture by drying samples to constant weight at 100^{o}C (AOAC, 1995). Carbohydrate, estimated as nitrogen-free extracts (NFE), was determined by subtracting the sum of crude protein, crude fat, ash and crude fibre from the dry matter content NFE= 100- (% protein+ % fat +% ash + % fibre).

Substitution with boiled teal leaf residues

The ingredients and proximate composition of diets are presented in Table 1. Boiled tea leave residues were used to substitute the freshwater shrimp meal from the control diet. Four

isonitrogenous diets were formulated to contain 30% crude protein (CP). The substitutions were made at four levels 0 % (control) 25%, 50%, and 100% of the plant ingredient replacing freshwater shrimps.

Table 1: Proximate composition of ingredients used in formulation of the BTLR diet:

Ingredient	DM	CP	EE	CF	ASH	NFE
BTLR	93.61±0.1	23.97±0.3	23.46±0.41	13.71±0.1	4.76±0.03	35.52±13.3
FSM	93.08±0.1	60.11±0.6	10.31±1.04	6.37±3.3	19.08±0.1	2.79±2.92
CSM	93.56±0.7	23.07±1.3	17.55±0.37	7.40±1.75	19.10±0.1	28.44±2.75
WB	94.43±0.3	19.39±0.7	9.28±0.27	12.95±2.92	3.98±0.1	48.83±2.75

The composition of the experimental diets is shown in Table 2 below. After the ingredients were perfectly mixed, cold water was added with continuous turning over until the mixture became suitable for making granules. The wet mixture was passed through a pelletizing machine. The produced pellets were dried at room temperature for 3 days and then packed in plastic bags until used. Fish were hand fed (as is basis) two times a day at 1000hrs and 1600hrs at 10% of live body weight, weighed once every 2 weeks, and the daily ration adjusted accordingly. Sex reversed *O. niloticus* male fingerlings were used for the feeding experiment. Prior to start of the experiment all fish were acclimatized to the experimental conditions for two weeks and were on the control diet during this period. The diets were allocated to the fingerlings held in the aquaria (initial mean weight 12.36±1.1g fish^{-1}; mean length 8.43±0.27 cm fish^{-1}) and in hapas (initial mean weight 10.98±0.75g fish^{-1} and mean length 8.77±0.3 cm fish^{-1}) in triplicate. The feeding experiment lasted 60 days.

Table 2: Formulation and proximate composition of diets formulated using BTLR as an ingredient .

Diets				
Ingredients	Sagana diet	25%BTLR	50%BTLR	100BTLR
FSM	12	9	6	0
CLM	59	59	59	59
WB	28	28	28	28
BTLR	0	3	6	12
Vitprem	0.5	0.5	0.5	0.5
Minprem	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition				
Dry matter	92.35±0.30	93.54±0.20	93.14±0.93	93.88±1.27
CP	34.40±0.40	33.81±0.82	31.11±0.40	28.93±0.60
EE	14.59±0.60	14.55±0.68	13.09±0.19	14.25±0.50
CF	6.23±0.90	8.76±0.60	11.36±0.10	12.13±0.47
Ash	8.02±0.17	7.33±0.11	6.92±0.29	4.74±0.28
NFE	27.99±0.83	27.76±0.93	29.98±0.30	50.17±7.4

Substitution with Cassava leaf meal

Diets were allocated to the fingerlings held in the aquaria (initial mean weight 10.90±0.64g fish^{-1}; mean length 6.20±0.25cm fish^{-1}) and in hapas (initial mean weight 10.55g fish^{-1} mean length

8.17±0.23cm fish^{-1}) in triplicate. The feeding experiment lasted 60 days. Each aquarium and hapa was stocked with 10 fish. The proximate values of used ingredients and the proximate composition of formulated diets is shown in Tables 3 and 4 respectively

Table 3 Proximate composition of ingredients used to formulate diets with Cassava leaf Meal

Ingredient	DM	CP	EE	CF	ASH	NFE
CLM	92.80±0.10	23.67±0.10	7.7±0.70	17.1±1.80	8.7±0.10	35.40±3.50
FSM	93.08±0.10	60.11±0.60	10.31±1.04	6.37±3.30	19.08±0.10	2.97±2.92
CSM	95.56±0.70	23.07±1.30	17.55±0.37	7.40±1.75	19.01±0.10	28.44±2.75
WB	94.43±0.30	19.39±0.70	9.28±0.27	12.95±2.92	3.98±0.10	48.83±2.75

Table 4: Formulations and proximate compositions of diets formulated using CLM as an ingredient

Diets				
Ingredients	**Control**	**25%CLM**	**50%CLM**	**100%CLM**
FSM	12	9	6	0
CSM	59	59	59	59
WB	28	28	28	28
CLM	0	3	6	12
VitPrem	0.5	0.5	0.5	0.5
MinPrem	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition				
Dry Matter	92.35±0.30	89.11±0.93	90.60±0.10	91.10±0.12
CP	34.40±0.40	34.76±1.34	30.63±1.34	30.26±0.44
EE	14.59±0.60	8.05±0.10	6.58±1.55	9.13±1.08
CF	6.23±0.90	10.09±1.21	13.53±4.9	12.13±0.47
Ash	8.02±0.17	4.49±1.61	5.00±0.11	4.96±0.33
NFE	27.99±0.83	32.89±1.73	29.85±1.06	52.48±5.77

Table 5: Composition of vitamin and mineral premixes used in each formulated diets

Vitamin contents		**Mineral contents**	
Vitamin	**Content**	**Mineral**	**Content(mg)**
A	5,000 000UI	Copper sulphate	1.5
D3	1,000 000	Manganese sulphate	90
E	1,500 IU	Manganese iodide	300
B1	600MG	Zinc oxide	70
B2	2500MG	Nicotinic acid	5500
B6	125MG	Calcium pantothenate	5000
B12	75MG		
K	1250MG		

C	200MG			

Whole body chemical composition analysis and hepatosomatic index analysis

At completion of experiment 3 fish per treatment were sacrificed for whole body chemical analysis. The fish were ground in a blender to get a homogenous sample and stored frozen at 20^{o}C. Fish samples were analyzed for proximate composition according to AOAC (1995). The fish were dried at 60^{o}C overnight and ground to determine the dry matter (DM) content. The crude protein (CP) was determined using the micro-Kjeledahel method (Nx6.25) (AOAC, 1995). Ether extract (EE) was determined in soxhalet apparatus using petroleum ether (60-80^{o}C). Ash content was determined in a muffle furnace at 550^{o}C for 3 hours according to AOAC (1995). Three fish per treatment were also dissected carefully to isolate and weigh the liver. HSI was expressed as the relationship between the liver weight and the whole body weight.

Data analysis

Data on fish performance, effect of the diets on the whole body chemical composition and the effect of the diet on the Hepatosopmatic index (HSI) composition was analyzed by single classification analysis of variance (ANOVA) using the methods described by Sokal & Rolhf, (1981). When significance between means was demonstrated, Duncan's multiple range tests (Duncan, 1955) was used to identify means significantly different from each other. Differences were declared significant at $P \leq 0.05$.

Results

Results of the effects of the diets formulated using BTLR on the HSI and flesh proximate composition are shown in table 6 and 7 respectively. Diets formulated using BTLR had the same effect ($P>0.05$) on the flesh moisture for fish grown both in aquaria and hapas as shown in Table 6 below. The control, 25% BTLR and 50% BTLR treatments had the same effect on the HSI of the fish in hapas. However the HSI was higher ($F= 7.993$ d.f. $=8$ $P< 0.05$) at 100 % BTLR inclusion. In the aquaria, there was a significant difference in the HSI between the 0% BTLR and 25% BTLR and 100% BTLR. However there was no significant difference in the HSI between the 0% BTLR and 50% BTLR for fish grown in the aquaria. In aquaria the diets had the same effect on the flesh crude protein ($F=2.881$, $df=10$, $P>0.05$) at 0%, 25% and 50% BTLR but had a significant decrease at 100% BTLR to record a value of 14.07±0.20%. The diets had the same

effect on the flesh moisture content (F=0.923, df=11, P>0.05) in the fish raised in the aquarium. There was a significant (F=23.30, df=8, P<0.05) decrease in flesh total ash with increase in BTLR in aquaria.

Table 6: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of freshwater shrimps (FSM) with BTLR				
Parameter	Control	25% BTLR	50% BTLR	100% BTLR
Moisture	75.50±2.72[a]	74.45±0.93[a]	74.98±1.24[a]	73.04±1.92[a]
Ash	5.1±0.10[a]	5.0±0.50[a]	5.3±0.10[a]	5.6±0.30[a]
HIS	0.48±026[a]	0.71±0.31[a]	0.44±0.18[a]	1.53±0.28[b]

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Table 7: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in hapas

Levels of substitutions of Freshwater shrimps (FSM) with BTLR				
Parameter	**Control**	**25% BTLR**	**50% BTLR**	**100% BTLR**
Moisture	73.57±1.4[a]	72.94±2.3[a]	75.07±2.0[a]	75.45±0.64[a]
Crude protein	16.16±0.9[a]	17.18±1.8[ab]	14.75±0.5[ab]	14.07±0.20[b]
Ash	5.3±0.05[a]	4.9±0.07[a]	5.8±0.15[cd]	5.80±0.43[d]
HIS	0.48±026[a]	0.71±0.31[a]	0.44±0.18[a]	1.53±0.28[b]

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Effects of the diets on the whole body composition and HSI in aquaria is shown in Table 8 and 9 respectively. In hapas there was a significant difference (F=4.083, df=8, P<0.05) in the effect of the diets on whole body moisture of *O. niloticus*. At 25% CLM and 100% CLM the diets had the same effect on the whole body moisture content. At 50% CLM the diets caused a significant increase in the whole body moisture level. In aquaria there was significant difference (F=2.664, df=8, P<0.05) in the effect of the diets on the whole body moisture. Substitution up to 50% CLM caused the same effect on the whole body moisture compared to the control. 100% CLM substitution caused a significant increase in the whole body moisture. In aquaria increase in CLM inclusion in the diets caused a significant (F=8.155, df=8, P<0.05) decrease in whole body crude protein content. In hapas increase in CLM inclusion caused significant decrease in the body HSI. In aquaria increase in CLM beyond 25% CLM caused significantly high HSI (F=2.207, df=8, P<0.05).

Table 8: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of Freshwater shrimps (FSM) with CLM				
Parameter	**Control**	**25% CLM**	**50% CLM**	**100% CLM**
Moisture	71.23±3.22[a]	72.48±3.05[a]	72.16±0.37[a]	76.53±2.25[a]
Crude protein	27.82±0.39[a]	28.04±0.35[a]	29.88±1.07[a]	24.67±2.33[b]
HIS	1.79±0.29[a]	1.08±0.62[a]	1.58±0.18[ab]	0.94±0.49[b]

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Table 9: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of Freshwater shrimps (FSM) with CLM				
Parameter	**Control**	**25% CLM**	**50% CLM**	**100% CLM**
Moisture	72.97±1.72[a]	75.34±1.7[ab]	76.58±0.76[b]	75.41±0.26[ab]
HIS	0.51±0.34[a]	1.65±0.38[a]	0.85±0.79[ab]	0.81±0.18[ab]

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Discussion

According to Joachim (2006) plant materials are digested to a lesser degree compared to those of animal origin. This may be attributed to high fibre levels responsible for poor protein digestibility, presence of suppressants, low palatability etc. In our study, diets formulated using BTLR had the same effect on whole body moisture levels in hapas. A similar observation was made in previous studies (Afuang *et al*., 2003) in a study carried out in aquaria to test the suitability of raw and methanol-extracted Moringa *Moringa oleifera* (Lam.) leaf meal to replace 10%, 20%and 30% of the total fishmeal-based dietary protein in tilapia feeds. The study did not find significant effect on whole body moisture with increase in Moringa inclusion in the fish diets. The results however are not similar to Garduno-Lugo and Olvera-Novoa (2008) who reported increased whole body moisture while replacing fish meal with peanut *Arachis hypogeal* leaf meal beyond 20%. The experimental environment was similar to the present study where the fish were raised in a pond. The study also reported a decrease in whole body lipids and ash with increased levels of peanut leaf meal in the diets. In the present study carried out in glass aquaria, the diets did not have a significant effect on the whole body moisture, but an increase in inclusion of dietary BTLR at 100% caused a decrease in whole body crude protein. There was

however a decrease in flesh ash for fish in the aquaria with an increase in BTLR inclusion. Moreso analysis done on the ingredients before formulation shows BTLR had less ash content compared to the level in the freshwater shrimps. In aquaria the dietary BTLR caused a decrease in whole body crude protein. A reduced level of whole body crude protein shows a tendency of the fish to convert protein for the purposes of energy production. Low feed uptake in the aquarium may have been a cause for this. There are several reasons which can affect chemical composition of fish flesh. The chemical composition of the flesh can therefore be used to further explain the performance of fish fed on diets formulated using plant materials as ingredients. Despite its high level of crude fibre, Ibrahim and Al-Owafeir and (2004) , incorporated date pits *Phoenix dactylifera* L and their Sprouts in semi-purified diets for Nile Tilapia *O. niloticus* (L.). Ibrahim and Al-Owafeir (2004) concluded that incorporating date pits in semi-purified diets for juvenile tilapia reduced fish growth and negatively influenced the proximate composition of the fish. On the other hand the study found out that substituting corn starch with sprouted date pits at 15% of the diet did not result in a reduction in weight gain or shifts in proximate composition of the fish. In the study, one condition exhibited use of un-sprouted date pits while the other experiment used sprouted date pits. Results showed that inclusion of un-sprouted date pits negatively affected the growth and proximate composition of the fish. This was not experienced in diets with sprouted date pits. The study pointed out the high dietary level of nondigestible carbohydrates in the feed as a likely reason for the poor performance in fish fed on un-sprouted date pits. This was similar to results in other studies where the carp *Cyprinus carpio* L. fed different dietary levels of date pits was used Ibrahim and Al-Owafeir (2004). The authors also showed that total body fat increased and protein decreased as a result of replacement of bran and barley mix (1: 1) with date pits. In a bid to overcome the high levels of high crude fibre content, the study exposed the date pits to sprouting. Ibrahim (2008) suggests that degradedation of date pits using specific enzymes to convert the fibres to simpler forms of carbohydrate molecules may increase utilization of the date pits by animals including fish.

In the present study, increase in CLM replacement of FSM in hapas led to an increase in the whole body moisture content. The results are comparable to those in previous work (Garduno-Lugo and Olvera-Novoa 2008) who reported an increase in the whole body moisture content

with an increase in inclusion of peanut leaf meal to replace fish meal from diets used to culture *O. niloticus*. Fish cultured in glass aquaria and fed on 25% CLM and 50% CLM had similar moisture levels as those fed on the control diet. The findings showed a similar trend with those of a previous study (Al-dosari and Belal, 1999) where there was no significant difference in whole body moisture contents when fish meal was replaced up to 40 % from *O. niloticus* diets with sarliconia meal in glass aquaria experiments. The effect was similar on the whole body crude protein values. Cassava leaves replaced up to 50% freshwater shrimps from diets used to culture *O. niloticus* in earthen ponds. In glass aquaria or recirculating systems like concrete tanks cassava leaves can only replace up to 25% of the freshwater shrimp meal. In the glass aquaria increase in inclusion of cassava leaves led to a decline in fish growth performance. Results of our experiment indicate the potential of the test ingredients are limited by their capacity for biological availability.

Several investigation have been done to find out ways of overcoming biological unavailability of ingredients of plant origin for diets formulated for the *O. niloticus*. Ibrahim (2008) found out that by use of a cellulolytic fungus *Trichoderma reesei* that is efficient in producing large amounts of different cellulase-degrading enzymes was one reasons for the high level of growth rates in *O. niloticus* fed on degraded date pits (DPP). The degradation may have been responsible for the increase of digestible carbohydrates (oligo and monosaccarides), which means that protein was not used for the purpose of providing energy for to the fish but rather for flesh development. In addition degradation may have acted to liberate nutrients blocked within date pits fibres which would have led to more digestible nutrients from the DDP. Cao *et al* (2008) carried out pretreatment of diets with microbial phytase to increase phosphorus availability for *O. niloticus* from a plants ingredient. The study investigated effects of pre-treating plant ingredients including soybean meal and a mixed plant meal of soy bean meal, wheat meal and corn glutten. Results showed that addition of phytase and inorganic phosphorus to the basal diet significantly increased the contents of ash, phosphorus and crude protein in the whole body of Nile tilapia, while dry matter and lipid contents decreased significantly. This shows the importance of phytase in making protein available hence increased levels of crude protein in flesh. A trend is observed in the above results that, use of plant proteins or plant based ingredients without prior treatment prevents uptake of important nutrients into the fish body. In results agreeing to the

ones mentioned above Asraf *et al*., 2007 investigated the growth performance and feed utilization of Nile tilapia *O. niloticus* Linnaeus and tilapia *Galilae sarotherodon galilaeus* L fingerlings fed plant protein-based diets. The work shows that feeding fish with corn glutten meal increased the values of whole body crude proteins and lipids. However in the same study the lowest ash content was observed with diet containing extruded full fat soyabean meal. The study points out the reduction of ash content can be as a result of presence of phytic acid which reduces the availability of several minerals like calcium, magnesium, zinc, iron and phosphorus. Mohsen and Mohammad (2009a) investigated use of live Spirulina (*Arthrospira platensis*) as a growth and immunity promoter for, *Oreochromis niloticus* L., challenged with pathogenic *Aeromonas hydrophila.* Results showed that Spirulina had a growth promoting influence on the *O. niloticus* as was seen in optimum growth and feed utilization. Improved whole body protein and lipid content were also related to the positive effects of the Spirulina.

Management of dietary protein during the growing period on growth performance, feed utilization and whole-body chemical composition of *O. niloticus* L. has also been investigated (Mohsen and Mohammad 2009b). In this study the contents of moisture, crude protein and total lipids in whole-fish body were not significantly affected by protein management except ash content, which was higher in the fish. The study suggested that nutrient digestibility and deposition may not have been affected by protein management but they also added that protein and lipid contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rates. Feeding rate can also have an impact on the proximate body composition (Jamjun and Amararatne, 2005). Investigations show that body lipid increased as well as moisture and ash percentages decreased with increased feeding rate. Studies have also shown that there was a decrease in ash content when the body lipid content increased at higher feeding levels of *O. niloticus.* Equally body protein content of experimental fish increases with increase in feeding rate (Jamjun and Amararatne, 2005).

In addition to the methods used to improve biological availability of the nutrients studies have also been carried out to show the importance of the encouraging natural feed production in earthen ponds. Most farming of *O. niloticus* is done in earthen ponds. Use of formulated feeds incorporating plant ingredients can only be successful under proper earthen pond conditions. A

study by Ali and Mohamed (2002) sheds light on the importance of natural feed resources in earthen ponds. In their study, effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds are investigated. Increased feeding rates had either no effect or irregular effects on the percentages of protein and ash gains in the fish body. Notably there was significant increase in percentages of fat and gross energy gains accompanying linear decreases in percentages of moisture. The study related the increase in percentage of fat gains in the body to increased ingestion. Unfed fish were found to have the lowest percentage of fat and highest percentage of moisture in their flesh. This may have been as a result of low fat and low energy contents of the pond natural food and organisms. Natural food organisms contain low energy but are rich in protein (Ali and Mohamed, 2002), therefore fish consuming only natural food have minimal fat and maximal protein accumulation in their bodies. Supplemental feeding showed high percentages of fat in their bodies and lowest moisture gain. However Azim *et al.*, 2003 found no effects of density or substrate on proximate composition of fish when they investigate the effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. In this study the *O. niloticus* at harvest had their ash content slightly higher while protein content was slightly lower. A study by Mario and Miguel (2008) showed a change in body composition with replacement of fishmeal with peanut leaf meal. The changes are noted with marked increased levels of peanut leaf meal. The study showed an increase in moisture content at 30% Peanut Leaf Meal (PLM) inclusion however the moisture was not higher than the moisture in fish at the beginning of the experiment. The study also found similar results as those of previous study as with protein, fat and lipid levels. This study argues that protein, fat and lipid levels decline with increase in plant protein replacement levels due to lower digestibility and consequent lower nutrient availability in the diets with high plant protein levels. The study by Mario and Miguel (2008) however concluded that the diets formulated using peanut leaf meal had insignificant impact on the growth of the *O. niloticus*, and suggested that the pond environment probably had a positive effect on the growth of fish. The study described different culture conditions as those used in the present study where static ponds were used in comparison to recirculating ponds used by Mario and Miguel (2008). Though their study concluded that up to 20% substitution had no negative effects on growth of *O. niloticus*,

the study recommends further research on the economic evaluation to determine the impact of the substitution on the production costs.

The HSI of fish fed the BTLR-containing diets were noticeably the same but increased significantly at 100%BTLR, as can be observed from the HSIs in hapas. The current study can not explain the increase in HSI at 100% BTLR. However a possible lipid deposition in the liver may have been possible leading to increase in the liver weights as a nutrient storage mechanism. Afuang *et al*., (2003) associated lower nutrient availability and feed intake to low nutrient storage hence linking it to reduced liver sizes.

Conclusion

The present study equally concludes that boiled tea leaf residues (BTLR) that are normally thrown from kitchens as wastes can actually be used to substitute up to 50% of freshwater shrimps from diets used to culture *O. niloticus*. In an intensive indoor production systems replacement is impossible and led to decrease in growth performance with increase in inclusion level. BTLR can therefore replace FSM in *O. niloticus* diets without negative effects on the whole body composition or the HSI. On the other hand cassava leaves can replace up to 50% freshwater shrimps from diets used to culture *O. niloticus* in earthen ponds. In glass aquaria or recirculating systems like concrete tanks, cassava leaves can only replace up to 25% of the freshwater shrimp meal. The study recommends for an economic evaluation study to determine the impact of the substitution on the production costs for effective inclusion in *O. niloticus* production at small scale level. It is also recommended that trials should be done using pretreated BTLR and CLM to find out their potential in diets for *O. niloticus*.

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Effect of Harvest and Postharvest Practices on Seed Quality of Jute Mallow Vegetables

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Abstract

African leafy vegetables (ALVs) are an important source of nutrients, income and traditional medicines in Kenya. However, their production has been constrained by lack of high quality seed due to varied farmers' harvest and post harvest practices. Limited research has been undertaken on the production of quality ALVs seed on farmers' fields. This study was carried out to investigate to identify optimal harvest and post harvest practices as far as seed quality of jute mallow (an ALV) is concerned. Field experiments were established in Kakamega and Siaya districts using Random Complete Block Design (RCBD) with 3 replicates. Agronomic, harvest and post harvest practices identified during a farmers' survey were used in these field experiments. Seed viability (measured by % germination) and vigour (measured by speed of germination index) was determined for the seeds obtained from the field experiments. Data obtained from field experiments was subjected to ANOVA and T-tests using Statistical Analysis Software (SAS). In Kakamega, seeds harvested at black pod stage from non defoliated plants, which were hand shelled and dried in the sun had higher percent germination and speed of germination indices for both seasons. In Siaya, jute mallow seeds harvested from non defoliated plants at black pod stage had significantly higher percent germination and speed of germination indices than other combinations. Season and site significantly affected percent germination and speed of germination index of the seeds harvested during field experiments. It was concluded that seasons, harvest and post harvest practices need to be considered by farmers in their quest to obtain good quality jute mallow seeds.

Key words: Jute mallow; Seed quality, germination indices, vigour

Introduction

Communities in western Kenya have utilized several species of African Leafy Vegetables (ALVs) for food and valued them for their taste, nutritional qualities and medicinal properties (Abukutsa-Onyango, 2004). However, lack of high quality seed remains a major constraint to the production of these vegetables (Adebooye, *et al*., 2005). Seed quality refers to the genetic, physical, physiological and sanitary status of the seed (Hampton, 2000). Of interest to the present study is the physiological status of the seed. Physiological seed quality refers to the germination capacity, viability, characteristics related to dormancy and the vigour of the seed. Seed viability refers to the ability of a seed to give a normal seedling when planted under normal sowing conditions (ISTA, 2004). Seed vigour refers to the sum total of all the attributes that give effective plant stand in the field. Vigour is positively related to the ability of a seed population to establish in optimum and suboptimum soil environments (ISTA, 2004).

Agronomic, harvest and post harvest practices have been shown to influence seed viability and vigour (either positively or negatively) depending on the crop species in question (Fairey and Hampton, 1997). These factors include leaf defoliation, seed harvest time, threshing and drying methods which were investigated in this study. A survey conducted in Kakamega and Siaya districts before the field experiments found that most farmers established their crop by drilling,

dried the pods for 3 days at harvest, and threshed the pods using sticks. There was great variation in weeding and leaf defoliation frequency (Maina et al., 2011). Due to the large number of agronomic, harvest and post harvest practices this study varied stage of leaf harvesting, pod harvest, threshing methods, drying methods and standardized planting methods, weeding frequency and used farm yard manure during planting.

Materials and methods

Field experiments

The experiments were conducted on 2 sites between April to June 2006 for the 1st season and August to October 2006 for the 2nd season. The first site was at Kenya Agricultural Research Institute (KARI) at Kakamega found in the UM_0 zone. The second site was at Agricultural Training Center (ATC) in Siaya which is found in LM_1 zone.

Seed used were obtained from the study area using scientific germplasm collecting techniques with the help of gene bank personnel. Jute mallow seeds exhibited dormancy which was broken by mechanical scarification using sand paper. The seeds were then sown in wooden boxes in a polythene house at Chepkoilel campus – Eldoret. Different morphotypes of jute mallow were characterized and isolated. Jute mallow morphotypes (*Corchorus sp.*) sown in the field experiments were – green stemmed morphotype with large, glossy elliptic leaves (GL) and the red stemmed morphotype with small, non glossy ovate leaves (RL). Seeds for the field experiments were tested in the laboratory the ISTA protocol and found to have on average 95% germination percentage before sowing (ISTA, 2004). Agronomic practices used were those practiced by majority of farmers in the study area. The land was ploughed twice just before the rains at the two sites. The land was then subdivided into plots as shown in the field layout (Figure 1). Randomized complete block design was used with 3 replicates and each plot measured 3m × 2m. Each block had 8 plots.

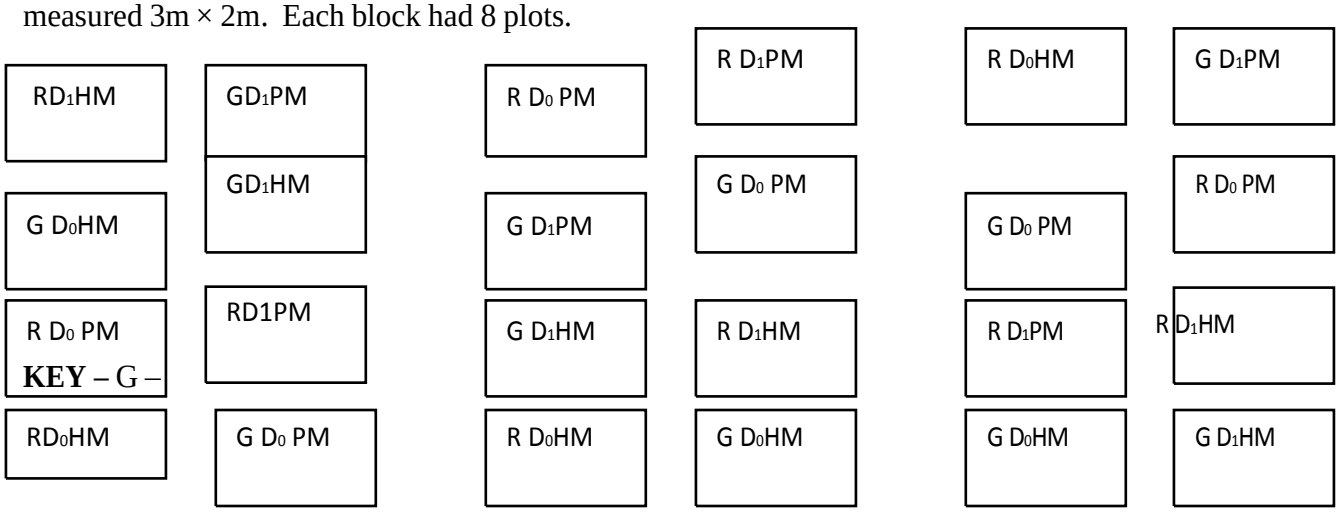


Figure 1 Jute mallow field layout

Glossy leaved morphotype; R – Red leaved morphotype; D_0 – No defoliation; D_1 – defoliated once per week; PM – physiological maturity; HM – Harvest maturity

Small drills were made in the plots using sticks at a spacing of 15 cm from each other. Manure was then applied in the drills at the rate of 2 Kg/m^2. Jute mallow seeds were scarified with sand paper to break dormancy. Seeds of both vegetables were sown in drills and covered lightly with soil. Seed rates used were 2.5 g/m^2 for both vegetables. Weeding was done 3 times during the growing season (at 3 weeks; 6 weeks and 8 weeks).

After 3 weeks, plants in some of the plots were defoliated once a week while others were not defoliated as shown in field layouts (Figure 1). After 90 days, seeds were harvested at brown and black pod stages. Munsell®colour charts for plant tissues were used to determine the pod colour at harvest (Munsell, 1977).

For each pod maturity stage, the pods were divided into two and one half was dried in the sun while other was dried in the shade for 6 hours per day for 3 days. For each drying regime the pods were subdivided into two. One half was threshed using a stick while for the other half seeds were removed from the pods by hand. Seeds obtained from each combination of morphotype/morphotype, harvest and post harvest practices (field experiments) were taken to the laboratory for germination tests.

Laboratory experiments

Jute mallow seeds from the field experiments were subjected to germination tests. Seed germination protocol used was according to International standards for testing seed viability and vigour (ISTA, 2004). Four replicates of 100 seeds of each combination of morphotype, harvest and post harvest practices were placed on individual petri dishes lined with 3 moist filter papers (moist paper substratum). The petri dishes were place in a growth chamber set at 24^0C and 70% relative humidity. Distilled water was added to the petri dishes regularly to ensure the filter paper was kept moist throughout the experimental period. Number of seeds that germinated normally was recorded at 9:00 am for 7 days. The seedlings were removed daily. Percent germination was determined after 7 days. Percent germination was used to determine seed viability. Seed vigour was determined by speed of germination test which was incorporated in the standard germination test described above.

The speed of germination index was determined by the formula below.

$$\textit{Speed of germination index} = \sum N/D$$

N- Number of normal seedlings that germinated per day; D - Day after sowing
The higher the speeds of germination index the higher the seed vigour.

Results

Percent germination and speed of germination index were significantly ($P \leq 0.05$) affected by 3 way interactions between (i) morphotype X seed maturity X defoliation, (ii) morphotype X defoliation X threshing method and (iii) seed maturity X defoliation X threshing methods for both seasons in Kakamega (ANOVA table not shown).
Seeds from glossy and red leafed jute mallow morphotypes that were harvested at black pod stage from non defoliated plants had the highest percent germination and speed of germination index for both the long and short rain seasons (Table 1 and 2). Similarly, seeds from both morphotypes that were harvested at black pod stage and hand shelled had the highest percent

germination and speed of germination index compared to other combinations for both seasons (Table 1 and 2).

Table 1 Percent germination of significant three way interactions between morphotype, harvest and post harvest factors for jute mallow in the long and short rain seasons in Kakamega

PXMXD		LR Mean±SE	SR Mean±SE	PXMXT		LR Mean± SE	SR Mean± SE
GLBR	D0	91.87± 0.81	88.00± 0.79	GLBRHS		89.62±1.38	85.62±1.38
GLBR	D1	87.62± 0.82	83.62± 0.82	GLBR	ST	89.50±1.13	84.62±1.13
GLBL	D0	93.50± 0.67	89.37± 0.71	GLBL	HS	90.00±0.81	86.00±0.81
GLBL	D1	85.62± 0.65	81.62± 0.65	GLBL	ST	89.50±1.02	85.37±1.02
RLBR	D0	91.00± 1.15	87.00± 1.15	RLBR	HS	88.37±0.94	84.37±0.94
RLBR	D1	86.87± 1.00	82.87± 1.00	RLBR	ST	89.50±1.40	85.50±1.40
RLBL	D0	92.75± 0.75	88.75± 0.74	RLBL	HS	91.37±0.78	87.68±0.69
RLBL	D1	88.87± 0.70	85.50± 0.65	RLBL	ST	90.25±0.94	86.56±0.90

MXDXT			LR Mean± SE	SR Mean± SE
BR	D0	HS	93.75±0.83	86.12±1.06
BR	D0	ST	89.12±0.77	88.87±0.78
BR	D1	HS	89.25±0.72	84.25±0.62
BR	D1	ST	85.25±0.80	82.25±1.08
BL	D0	HS	95.31±0.41	90.00±0.58
BL	D0	ST	90.93±0.46	88.12±0.78
BL	D1	HS	89.25±0.62	83.31±0.83
BL	D1	ST	85.25±0.59	83.81±0.81

P – Morphotype; M – Seed maturity; R – Drying; D – Defoliation; T – threshing; GL – glossy leafed morphotype; RL – red leafed morphotype; BR–Brown pod stage; BL–Black pod stage; SHD - Shade drying; SUN - Sun drying ; D0 – No defoliation, D1 – defoliated once a week ; HS – hand shelling , ST – stick threshing

It then follows that, irrespective of the morphotype in question, seeds harvested at black pod stage from non defoliated plants and hand shelled had the highest percent germination and speed of germination index compared to other combinations for both seasons. It is important to note that the percent germination and speed of germination values were high (in the 80s and 90s) for both seasons in Kakamega.

Table 2 Speed of germination index of significant three way interactions between morphotype, harvest and post harvest factors for jute mallow in the long and short rain seasons in Kakamega

PXMXD		LR Mean±SE	SR Mean±SE	PXMXT	LR Mean± SE	SR Mean± SE
GLBR	D0	0.90±0.01	0.87±0.01	GLBRHS	0.87±0.01	0.84±0.01
GLBR	D1	0.86±0.01	0.83±0.01	GLBRST	0.88±0.01	0.83±0.01
GLBL	D0	0.92±0.01	0.88±0.01	GLBLHS	0.89±0.01	0.85±0.01
GLBL	D1	0.83±0.01	0.80±0.01	GLBLST	0.88±0.01	0.84±0.01
RLBR	D0	0.89±0.01	0.86±0.01	RLBRHS	0.87±0.01	0.83±0.01
RLBR	D1	0.84±0.01	0.82±0.01	RLBRST	0.88±0.01	0.84±0.01
RLBL	D0	0.90±0.01	0.87±0.01	RLBLHS	0.89±0.01	0.86±0.01
RLBL	D1	0.86±0.01	0.84±0.01	RLBLST	0.89±0.01	0.85±0.01

MXDXT			LR Mean± SE	SR Mean± SE
BR	D0	HS	0.92±0.01	0.85±0.01
BR	D0	ST	0.88±0.01	0.87±0.01
BR	D1	HS	0.88±0.01	0.83±0.01
BR	D1	ST	0.85±0.01	0.81±0.01
BL	D0	HS	0.94±0.01	0.89±0.01
BL	D0	ST	0.88±0.01	0.86±0.01
BL	D1	HS	0.88±0.01	0.82±0.01
BL	D1	ST	0.84±0.01	0.82±0.01

Key: P - Morphotype; M – Seed maturity; R – Drying; D – Defoliation; T – threshing; GL – glossy leafed morphotype; RL – red leafed morphotype; BR–Brown pod stage; BL–Black pod stage; SUN - Sun drying ; D0 – No defoliation, D1 – defoliated once a week ; HS – hand shelling , ST – stick threshing

In Siaya, percent germination and speed of germination index were significantly ($P \leq 0.05$) affected by 2 way interactions between morphotype and seed maturity, and seed maturity and defoliation for the two seasons (ANOVA tables not shown). The percent germination and speed of germination indices for significant interactions are shown in Figure 2 and 3.

Long rain season

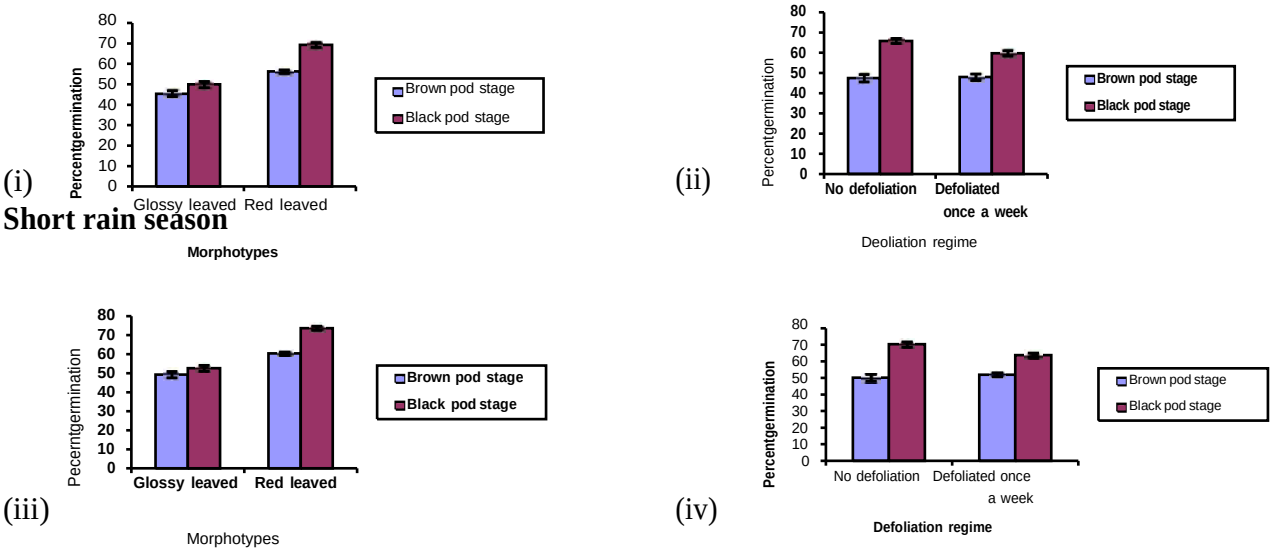
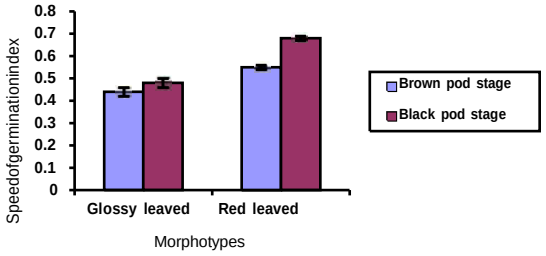


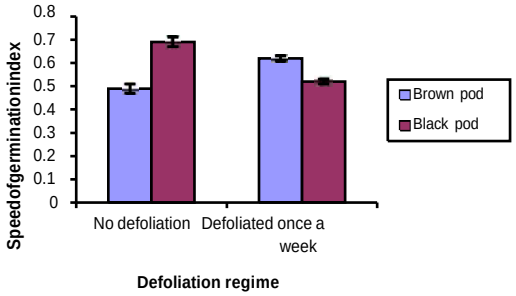
Figure 2 (i) - (iv) Percent germination of significant two way interactions between morphotypes, harvest and post harvest practices for jute mallow seeds in long rain and short rain seasons in Siaya

Seeds from both morphotypes that were harvested at black pod stage had significantly ($P \leq 0.05$) higher percent germination and speed of germination indices compared to those that were harvested at brown pod stage for both the long and short rain seasons (Figure 2 and 3). Seeds from the two morphotypes that were harvested from non defoliated plants at black pod stage had significantly ($P \leq 0.05$) higher percent germination and speed of germination indices than those harvested from plants that were defoliated once a week for both seasons.

Long rain season

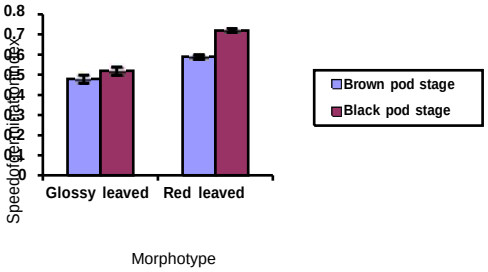


(i)

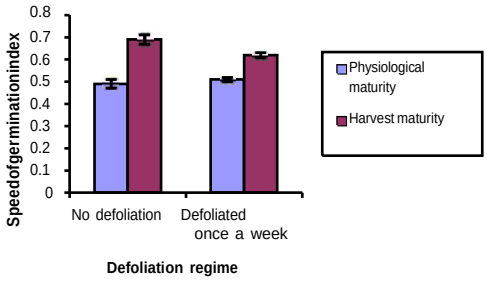


(ii)

Short rain season



(iii)



(iv)

Figure 3 (i) - (iv) Speed of germination index of significant two way interactions between morphotypes, harvest and post harvest practices for jute mallow seeds for the long and short rain seasons in Siaya

Irrespective of the sites and practices seeds produced in the long rain season had significantly ($P \leq 0.05$) higher germination percentages and speed of germination indices than the short rain season (Table 3).

Table 3 Percent germination (PG) and speed of germination indices (SGI) for long and short rain seasons

Season			Sites		
	PG	SGI		PG	SGI
Long rain	74.40±1.11a	0.73±0.01a	Kakamemega	87.82±0.29a	0.86±0.01a
Short rain	70.55±1.09b	0.69±0.01b	Siaya	57.14±0.73b	0.55±0.01b

Means with different letters within each column significantly differed at $P \leq 0.05$

Seeds from Kakamega had significantly ($P \leq 0.05$) higher percent germination and speed of germination indices than those from Siaya. Means of percent germination and speed of germination indices are shown in Table 3.

Discussion

In Kakamega, seeds harvested at black pod stage from non defoliated plants, which were hand shelled and dried in the sun had higher percent germination and speed of germination indices for both seasons. This is similar to results with crops like canola (*Brassica sp.*) where maximum viability and vigour occurred at harvest maturity or black pod stage (Elias and Copeland, 2001). This has been explained by physical changes in hormonal mechanism that occurs after physiological maturity (brown pod stage) which promotes germination. Farmers should therefore harvest jute mallow seeds should be harvested at black pod stage.

Findings that drying seeds in the sun gave significantly ($P \leq 0.05$) better quality seeds differed from those in other studies that indicate that high temperatures associated with sun drying dramatically reduce seed viability and vigour (Walters and Engels 1998). However, a study on spider plant (an ALV) also found that sun drying improved the mean germination time, seedling vigour and overall germination percentage when compared to shade dried seed (K'opondo, et, al., 2005). This may be because sun drying reduces seed moisture levels to 11 - 12% which reduces deterioration of seeds due to moulds and increased respiration in the seed (Kamotho, 2004). Shade drying has been reported to be advantageous only where high temperatures in the sun are capable of damaging seeds with high moisture levels and also where seeds are not fully ripe and therefore require a slow drying process to after ripen and be able to repair mechanical damages (Thomsen and Stubsgaad, 1998). The latter has been observed in tree species and finger millet and does not seem to be the case for the jute mallow seeds.

Jute mallow seeds were not significantly ($P \geq 0.05$) affected by threshing methods as far as percent germination and speed of germination is concerned in Kakamega and Siaya. This implies that the jute mallow seeds have a tough seed coat that was not affected by stick threshing.

In Siaya, jute mallow seeds harvested from non defoliated plants had significantly higher percent germination and speed of germination indices than those harvested from defoliated plants. This is because leaf defoliation reduces photosynthetic area hence less food is manufactured in the leaves for the formation of seeds (Bewley and Black, 1994). In addition to this when leaves are harvested; plants concentrate on recovering lost leaf area essential for photosynthesis rather than on reproduction hence reducing the seed quality. Older leaves left on the plant are also less efficient in photosynthesis hence seed quality is negatively affected (Mnzava and Msikita, 1997). However, some plants are able to recover very fast depending on the time and frequency of defoliation as well as inherent qualities. Some studies have found that most farmers obtained their seeds from the remnants of vegetables after defoliating the plants to obtain vegetables throughout the growing season and rarely cultivated plants deliberately to produce seed (Adebooye, *et al*., 2005). However, few farmers in the Kakamega and Siaya seemed to have realised this and grew plants for seeds separately without defoliation. This needs to be encouraged among the farmers.

Level of interaction between morphotype, harvest and post harvest practices in Kakamega and Siaya, varied imply that to obtain high quality seeds farmers need to take relvant factors into

account to produce quality seeds. In Kakamega, farmers need to harvest their jute mallow seeds at black pod stage from non defoliated plants and remove the seeds from the pods by hand. In Siaya farmers only need to vary two practices that is defoliation and seed maturity at harvest that is harvest the seeds from non defoliated plants at black pod stage.

T-tests were conducted to find out if seasons and sites significantly affected percent germination and speed of germination indices of jute mallow morphotypes irrespective of harvest and post harvest practices. Seeds produced during the long rain season had significantly higher percent germination and speed of vigour than the short rain season. This is because during the long rain season there was sufficient moisture for vigourous vegetative growth which provided essential assimilates for proper seed formation (Bewley and Black, 1994). It has also been noted that jute mallow is susceptible to moisture stress owing to its shallow rooting system (Ogunrinde and Fasinmirin, 2011)

Jute mallow seeds from Kakamega had greater percent germination and speed of germination indices than those from Siaya. This is because Kakamega receives more rainfall than Siaya hence providing sufficient moisture for plant growth and seed formation. There were greater differences in percent germination and speed of germination indices in Siaya than in Kakamega between long and short rain seasons. This was caused by the greater differences in rainfall amounts between the long and short rain seasons in Siaya (GOK, 1998). Percent germination and speed of germination indices were higher in Kakamega than in Siaya indicating that jute mallow is more adapted to Kakamega than Siaya. This is because it has been noted jute mallow requires fairly high levels of moisture to grow well (Abukutsa- Onyango, 2007).

Conclusion

In conclusion significant interactions between seasons, morphotype/morphotype, harvest and post harvest practices for jute mallow as far as seed quality is concerned in Kakamega and Siaya were observed. Farmers in these two regions need to take seasons, harvest and post harvest practices into account in order to obtain high quality seeds.

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Effect of Antiretroviral Drugs on Cd4 Cells and Viral Load in HIV Patients Attending Rift Valley Provincial General Hospital, Nakuru, Kenya

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Abstract

CD4 count and viral load are part of laboratory data, which give guidelines on commencement and subsequent monitoring of chemotherapy. Among the primary goals of Antiretroviral Therapy (ART) are optimal and durable suppression of viral load and the preservation and /or restoration of immunologic function. The objective of the current study was to assess the virological and immunological responses of the human immunodeficiency virus (HIV) -infected individuals with administration of ART and establish the relationship between CD4 count and viral load in the study population, assessing the effect of chemotherapy. The study was conducted on 80 individuals who attended the Voluntary Counseling and Testing (VCT) centre in the Rift Valley Provincial General Hospital, Nakuru, and who consented to the study. Parallel testing for HIV was performed using Determine and Uni-Gold HIV ½ test kits, and discordant results were confirmed by Enzyme Linked Immunosorbent Assay (ELISA). CD3, CD4 and CD8 counts were determined using Beckton Dickson (BD) FACScount while viral loads measured using Exavir load kit prior to commencement of ART regimens. Virologic and immunologic responses were determined by measuring CD4 counts and viral load at two weeks following commencement of chemotherapy and monthly for three months thereafter. Viral loads and CD4 counts for the study population were found to be highly inversely correlated ($r=0.948$, $p<0.001$).

Key words: Human Immunodeficiency Virus, Viral loads, CD4 counts, antiretroviral therapy.

Introduction

The Human Immunodeficiency Virus (HIV) type 1 and type 2 are etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions. HIV-1 is disseminated world wide while HIV-2 is principally found in the West African regions but subsequently found in some European and South American countries. Of the estimated 1.5 million people infected with HIV in Kenya, about 200,000 are in urgent need of antiretroviral (ARV) therapy (NASCOP, 2005). The government of Kenya is committed to increasing access to antiretroviral drugs as part of its wider "Declaration of Total War" on HIV/AIDS, and has therefore developed a plan for the rapid up-scaling of antiretroviral therapy (ART) to government hospitals in every province in Kenya (MOH,2004).

CD4 count is a good indicator of the immune status of the individual as it plays an important role in both humoral and cell mediated immune responses. In HIV infection, CD4 counts are used to determine the progress of disease and to predict the risk of developing HIV related complications (Mary, 2003). When individuals are infected with HIV for a long time, their CD4 count decreases indicating immunesuppression. The lower the CD4 count, the higher the vulnerability to opportunistic infections.

The plasma viral load has been used as a measure of HIV replication (Pallela *et al.,* 2003). During the period of primary infection in adults, viral load initially rises to high levels. Viral load assays are useful for indicating the prognosis of HIV infection, for indicating when asymptomatic patients should be treated, and also as a reference for subsequent monitoring of the virologic response to therapy (Paula *et al.,* 2001). CD4 count and viral load, being part of laboratory data, may give guidelines on initiation and monitoring of chemotherapy. Average normal CD4 counts are between 500-1600 cells/mm^3 of blood (Sheppard *et al.,* 2005) while normal CD4 counts in adults in Kenya ranges from 500-1800 cells/mm^3 (NASCOP, 2001). The primary goals of antiretroviral therapy are maximal and durable suppression of viral load, sustained rises in CD4 counts, preservation and / or restoration of immunologic function, improvement of quality of life and reduction of HIV-related morbidity and mortality (Carmona *et al.,* 2001). Antiretroviral (ARV) drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits. There are three classes of antiretroviral drugs that currently have been licensed: Reverse transcriptase inhibitors (RTIs) target construction of viral DNA by inhibiting activity of reverse transcriptase. There are two subtypes of RTIs with different mechanisms of action: nucleoside-analogue RTIs (NTRIs) are incorporated into the viral DNA leading to chain termination, while non-nucleoside – analogue RTIs (NNRTIs) distort the binding potential of the reverse transcriptase enzyme. Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virions and Fusion inhibitors that block HIV from fusing with a cell's membrane to enter and infect it. In Kenya, the leading regimens to consider are: two nucleoside RTIs and protease inhibitor, two nucleoside RTIs and non-nucleoside RTI and three nucleoside RTIs (NASCOP, 2001). The individuals in the current study were given two nucleoside RTIs (Lamivudine+ Stavudine) and non-nucleaside RTI (Nevirapine).

Materials and Methods

Study Site and Study Population

The study was carried out between May – October, 2006 in the Rift Valley Provincial General Hospital (PGH), located in the Rift Valley Province of Kenya. The hospital is situated on the northern part of Nakuru town, 1.5 kilometres from the town centre. The hospital serves people from the entire Rift Valley Province. As a result of HIV campaigns, patients report to Counseling and Testing (VCT) Centre at the PGH for HIV testing. Other patients are referred for diagnostic HIV testing due to persistent and recurrent opportunistic infections. At the VCT centre, the patients undergo pre-test counseling, which includes being made to understand why it is important to undertake HIV testing, what it entails and what the results may imply. HIV testing is routinely carried out at the VCT centre. Patients who tested HIV positive were referred to the Centre for Comprehensive Care (CCC) for further counseling, and it was at the CCC that patients were advised to have their CD4 counts and viral load determined. Counseling at CCC included talking to patients to accept the results, the importance of living a positive life despite being HIV positive and on how they could improve their immune system by starting antiretroviral therapy (ART). Before the patients started ART, their viral load, CD3, CD4 and CD8 counts were determined after which they commenced ART.

Study Design

A cross sectional study design was used which involved selecting the subjects as they reported in VCT centers and obtaining information. Permission to carry out the study at the Nakuru Provincial General Hospital was approved by the hospital's administration.

Sampling and Sample Size Determination

Stratified sampling was done from the HIV positive individuals attending the VCT centre. They consented to participate in the study by signing a questionnaire. Participants of the study were randomly sampled by use of random numbers. A sample size of 80 patients was used. The patients who were sampled were referred to the CCC for further tests.

Screening for Human Immunodeficiency Virus Patients

A total of eighty individuals participated in the study after being sampled from a population of patients who had been confirmed to be HIV positive using two parallel rapid screening tests, (Determine HIV 1/2, USA and Trinity Biotech Uni-Gold, USA). Twelve males and sixty eight females of various ages participated in the study (Table 1). None of the female patients was pregnant.

Rapid Test Screening for Human Immunodeficiency Virus

Screening for HIV was carried out using two parallel tests simultaneously, the "Determine HIV 1/2" test (Abbot Laboratories, USA) and "Trinity Biotech Uni-Gold" test (Trinity Biotech, USA). Whole blood obtained by finger pricks was used. The determine HIV 1/2 test kits components were: Determine HIV 1/2 Test card, 2 cards (10 tests/card); HIV 1/2 recombinant antigen and synthetic peptide coated; 1 bottle (2.5 ml) Chase Buffer prepared in phosphate buffer. The Trinity Biotech Uni-Gold tests kits comprised of: 20 Test devices containing colloidal gold labelled with recombinant HIV proteins, recombinant HIV proteins as test Zone, and a control line, wash reagent (2ml), 20 disposable pipettes. When using the determine HIV 1/2 test kit, the protocol was carried out as outlined in the manufacture's manual (Piot *et al.*, 1988; Gurtler *et al.*, 1994). Briefly the tests were conducted as follows: to each labelled test card, droplets of whole blood produced by finger prick from an individual patient was applied to the sample pad. After blood was absorbed into the sample pad, one drop of chase buffer was then applied. The result was read after 15 minutes (up to 60 minutes). The test result was positive when two red bars appeared in both the control window and the patient window of the strip in the test card. The test result was negative when one red bar appeared in the control window of the strip and no red bar appeared in the patient window of the strip (Fig. 2).

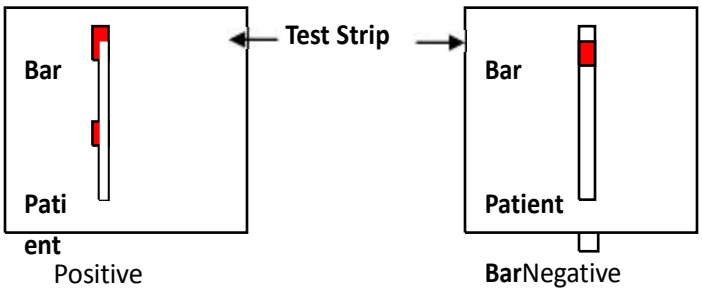

Figure 2:Bar Screening for HIV using Determine HIV1/2 Test

The Trinity Biotech Uni-Gold tests were carried out as outlined by the manufacturer (Feorino *et al.*, 1985; Atler *et al.*, 1987). Briefly, to each labelled test device, droplets of whole blood produced by finger prick from an individual patient were placed onto the device. Two drops of the wash reagent was added to the sample port. After 10-minute incubation time, the result was read. The test results were interpreted as follows: a line of any intensity forming in the test region of the test device, plus a line forming in the control region indicated a positive result while a line in the control region only indicated a negative test result (Fig. 3).

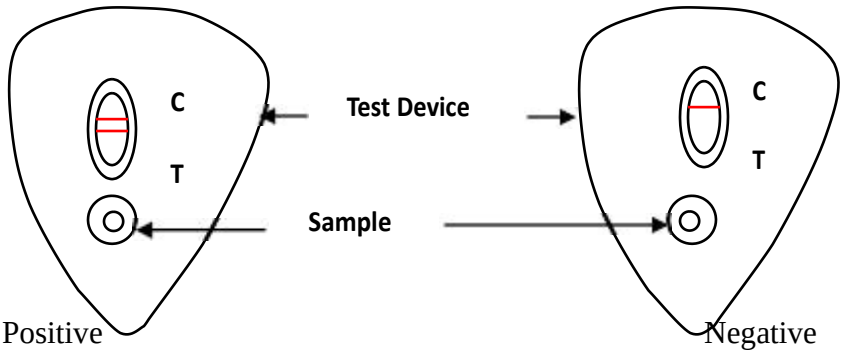

Figure 3: Screening for HIV using Trinity Biotech Uni-Gold test

Enzyme Linked Immunosorbent Assay (ELISA)

Discordant results from the two rapid tests were tested using Murex HIV 1.2.0 kit (Murex Biotech Limited, U.K). The components of the test kit were: Antigen coated wells; 96 microwells coated with HIV antigens; Sample diluent, Conjugate, Anti HIV 1 Positive Control Serum, Anti HIV 2 Positive Control Serum, Negative Control, Substrate diluent, Substrate concentrate and wash fluid. When using the Murex HIV 1.2.0 test kit, the protocol followed was as described by the manufacturer (Gains and Syndons, 1998). Briefly the test was carried out as follows: using test specimens and control sera, to precoated ELISA plates, 50µl of sample diluent was added followed by 50µl of serum sample in each well. After 30 minutes incubation period, unbound antibody was washed away, after which 50µl of HIV antigen conjugated to horseradish peroxidase was added to each well. The plate was incubated for 30 minutes and there after excess conjugate was washed away. Immediately after washing the plate, 100µl of substrate solution containing 3,3, 5,5' tetramethylbenzidine (TMB) and hydrogen peroxide was added to the wells. The plate was incubated for 30 minutes after which a purple colour developed in wells with bound conjugate, which was converted to an orange colour when the reaction was terminated with sulphuric acid and the optical density was read spectrophotometrically at 450nm. The amount of conjugate, and hence colour in the wells directly related to the amount of antibody to HIV in the sample. Guidelines to calculation of results were provided in the Murex HIV 1.2.0 test kit giving the mean absorbance and the cut off value as 0.280;

Results of the assay were considered negative when the samples gave an absorbance less than the cut off values, while the assay was considered positive when the samples gave an absorbance equal to or greater than the cut off value.

Data on Opportunistic Infection

Patients' data on opportunistic infection was recorded from their files as diagnosed before commencement of chemotherapy

CD4 Count Determination

CD4 counts were carried out using Beckton Dickson (BD) FACSCount system (BD Biosciences, USA) according to the manufacturers' protocol (David *et al.*, 2004). Beckton Dickson FACSCount is a complete system incorporating instrument, reagents, controls and software. It utilizes a direct two-colour immunoflourence method for enumerating absolute counts of CD3 lymphocytes, CD4 lymphocytes and CD8 lymphocytes. In addition the system generated a ratio of CD4 and CD8. The BD FACSCount reagent kit consisted of paired reagent sets containing a mixture of monoclonal antibody reagents conjugated to two fluorochromes and a known number of fluorochrome –intergrated polystyrene beads. The first tube in each pair contained CD4 and CD3 antibodies while the second contained CD8 and CD3. The kit also contained formaldehyde fixative. Briefly, the procedure was as follows: whole blood was collected in liquid EDTA; 50µl of whole blood was added to each tube, capped and vortexed. The samples were then acquired and run on the BD FACSCount instrument. The data was processed and reported on a sample print out sheet. CD4 counts were determined for all patients before and after commencement of chemotherapy, first at two weeks of therapy then monthly for three subsequent months.

Viral Load Determination

Viral load was determined using ExaVir Load kit (Cavidi Tech AB, Sweden) according to the protocol provided by the manufacturer (Malmstem e*t al*., 2003; Braun *et al.*, 2003). Briefly the ExaVir Load kit procedure was divided into two main parts: that is the separation and the reverse transcriptase (RT) – assay. In the separation part, the plasma was first treated to inactive cellular enzymes by adding 100µl of plasma treatment additive. 1ml of the sample was pipetted into each of the 32 plasma processing tubes placed in a sample box and incubated for 1 hour in the dark at room temperature. After the 1 hour incubation, 1.5ml of separation gel was added to each plasma processing tube and the sample box was placed on a moving table and incubated at room temperature for 90 minutes. The gel was meant for separating the virus particles from the plasma. After the 90-minute incubation, the gel was sucked dry in all the tubes using a vacuum pump, the gel was then washed four times using 250ml of gel wash buffer. The gel was sucked dry again and washed two times using 250ml of gel reconditioning buffer. 500µl of lysis buffer was added to each tube and the lysates were transferred to lysate collection tube. To obtain the Reverse Transcriptase (RT), the virion was then lysed and the lysate collected for further analysis. During the RT-assay the lysate was analyzed in an ELISA set up. The wells contained the RNA template bound to the bottom. A reaction mixture containing primer and an RT substrate was added to the plate together with the lysates. If the lysate contained any RT enzymes, the enzyme synthesized a DNA-strand. This product was detected with alkaline phosphate conjugate anti bromodeoxyribouridine antibody (α-BradU). The product was quantified by addition of a colorimetric Alkaline Phosphate (AP) substrate.

For comparison of results, in house HIV positive controls and in house negative controls were prepared. In house HIV positive controls: about 100ml of plasma prepared from a pool of EDTA blood from HIV positive patients was prepared by mixing samples with high and low HIV RT activity levels. When no plasma with determined RT amount was available, a pool was prepared that corresponded to 25,000 copies/ml. The material was aliquoted into 1.2ml portion and 1ml of one portion was used as a positive control. In house HIV negative control: about 100ml of a pool of plasma from healthy blood donors was prepared. The material was aliquoted into1.2ml portions and 1ml of one portion used as a negative control. When the AP substrate was added to the product, the plate was incubated in the dark at room temperature. The plate was read at an optical density of 405 (A_{405}) ten minutes after addition of the substrate. The plate was read a second time after two to three hours and a third time after five to six hours or the following day (16 to 24 hours) after addition of AP substrate. Calculation of the viral load values of the plasma samples was performed using the ExaVir Load Analyzer. Viral load determination was carried out in all patients before commencement of chemotherapy and thereafter, first at 2 weeks on therapy and monthly for three subsequent months while on chemotherapy.

Antiretroviral Therapy

Highly active antiretroviral therapy (HAART) was used. Highly active antiretroviral therapy is a combination of three or more antiretroviral drugs in the treatment of HIV infection. The drugs that were used were stavudine (D4T), lamivudine (3TC) and nevirapine (NVP). Doses for patients who were less than 60kg were D4T-30mg twice daily, 3TC-150mg twice daily and NVP-200mg twice daily. Doses for patients who were more than 60kg were D4T – 40mg twice daily, 3TC-150mg twice daily and NVP-200mg twice daily. Patients were advised to take NVP once daily for the first 2 weeks of treatment. They had to return for more drugs after two weeks. Highly active antiretroviral therapy was initiated in all patients with CD4 counts less than 200 cells/mm^3 irrespective of their viral load although 11 patients commenced treatment with counts more than 200 cells/mm^3 due to the severity of opportunistic infections.

Data Management.

CD4 counts and viral loads, as indications of patients' responses were analyzed using Chi-squire test for goodness of fit. The mean CD4 counts and mean viral loads for all the patients during chemotherapy were analyzed using Kruskal-Wallis test. The relationship between the total mean CD4 counts and the total mean viral loads during chemotherapy were analyzed using coefficient of correlation. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS version 18) software; generated data was then presented and interpreted using graphs and tables.

Results:

Twelve males and sixty eight females of various ages participated in the study (Table 1). Six patients (all females) out of eighty (7.5%) had discordant results by parallel testing for HIV. Four patients out of six (5.0%) were HIV positive with Determine HIV 1/2 test but negative when tested with Trinity Biotech Uni-Gold test. Two patients out of six (2.5%) were HIV

negative when tested with Determine HIV 1/2 test but positive when tested with Trinity Biotech Uni-Gold test.

Table 1: Gender and Age of the Study Population

Age in years	Males	Females	Total
Less than 21	0	5	5
21 – 25	2	11	13
26 – 30	3	18	21
31 – 35	2	23	25
36 – 40	3	6	9
More than 40	2	5	7
Total	12	68	80

The serum samples of the six discordant samples were tested for anti HIV antibody by Enzyme Linked Immunosorbent Assay (ELISA) using Murex HIV 1.2.0 Kit (Murex Biotech Limited, UK). All the six samples had absorbance values greater than the cut-off point (0.280) indicating that they were all HIV positive. The absorbance of the six samples were as follows; 0.342, 0.416, 0.402, 0.384, 0.301 and 0.408.

CD4 Levels and Clinical Manifestations

In all the patients included in this study, the highest CD4 count detected at the baseline was 220 cells/mm^3 of blood and the lowest was 8 cells/mm^3 of blood. CD4 counts were grouped into three categories depending on the symptoms and opportunistic infections present. Out of eighty patients, twenty seven (33.75%) had CD4 counts of less than 100 cells/mm^3 of blood at the baseline and a mean CD4 count of 54. The most common opportunistic infections by the patients with CD4 counts less than 100 cells/mm^3 of blood included prolonged weakness, chronic diarrhoea, tuberculosis, Kaposi's sarcoma, candidiasis of the oesophagus, Herpes simplex and pneumonia. Forty two patients (52.5%) had CD4 counts between 100-200 cells/mm^3 of blood at the baseline and a mean CD4 count of 151. They presented with persistent fever, pneumonia, tuberculosis and chronic diarrhoea. Eleven patients (13.75%) had CD4 counts of more than 200 cells/mm^3 of blood at baseline and a mean CD4 count 210. They presented with persistent generalized lymphodenopathy, Herpes zoster, recurrent upper respiratory infections and oral candidiasis.

The overall mean CD4 count before commencement of chemotherapy was 126 and all the patients were put on chemotherapy. After two weeks of chemotherapy the mean CD4 count increased to 148 (17.5% increase), after six weeks of chemotherapy the mean CD4 count increased to 209 (29.2% increase), after ten weeks of chemotherapy the mean CD4 count

increased to 252 (17.1% increase) and after fourteen weeks of chemotherapy the mean CD4 count increased to 278 (9.4% increase; Figure 4).

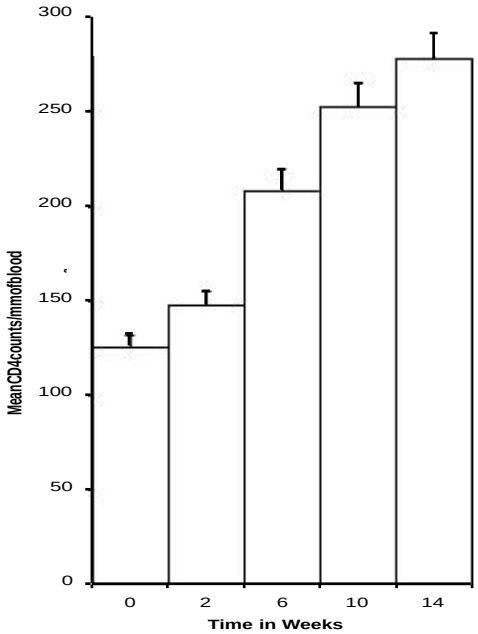

Figure 4: Mean CD4 count during chemotherapy

Viral Load and Clinical Manifestations

In all the eighty patients sampled, the highest viral load detected at the baseline was 1,900,000 copies/ml of plasma and the lowest was 100 copies/ml of plasma. Viral loads were grouped into three categories depending on symptoms and opportunistic infections present. Twenty-six (32.5%) patients had viral load of less than 50,000 copies/ml of plasma at the baseline and a mean viral load of 46,941. They presented with Herpes Zoster, oral candidiasis, recurrent upper respiratory infections and persistent generalized lymphodenopathy. Seven (8.75%) patients had viral load between 50,000 -100,000 copies/ml of plasma at the baseline and a mean viral load of 63,606. These patients presented with persistent fever, pneumonia, tuberculosis, chronic diarrhoea and oral candidiasis. Forty seven (58.75%) patients had viral load more than 100,000 copies/ml of plasma at the baseline and a mean viral load of 308,796. These patients presented with chronic weakness, chronic diarrhoea, Kaposi's sarcoma, candidiasis of the oesophagus, tuberculosis, Herpes simplex and pneumonia.

The overall mean viral load at the baseline for all the patients before commencement of chemotherapy was 419,343 and the patients were put on chemotherapy. After two weeks of chemotherapy the mean viral load decreased from 419,343 to 386,513 (7.83% decrease), after six weeks of chemotherapy the mean viral load decreased to 321,863 (16.73% decrease), after ten weeks of chemotherapy the mean viral load decreased to 289,077 (10.19% decrease) and after fourteen weeks of chemotherapy the mean viral load decreased to 265,537 (8.14% decrease; Figure 5).

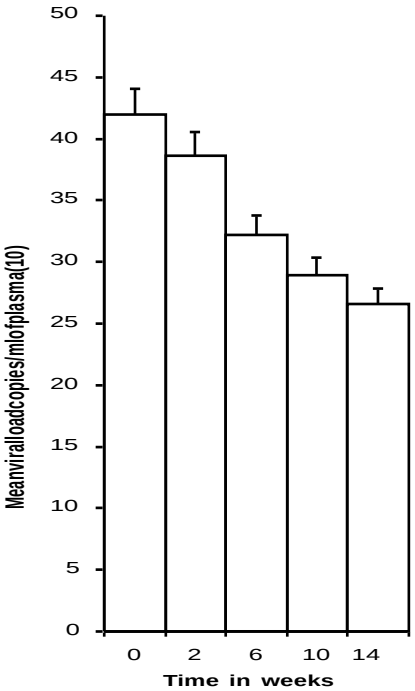

Figure 5: Mean viral load (RNA copies) during chemotherapy

Response to Chemotherapy in terms of CD4 Counts

Response to chemotherapy was monitored every two weeks for a period of fourteen weeks. Patients at different stages of infection were presented separately. After two weeks of chemotherapy, CD4 counts had increased in sixty-four (80%; Table 2), thirteen patients had decreased CD4 counts (16.3%) and there was no change in CD4 counts among three patients (3.7%; Table 2). Among the patients with CD4 counts less than 100 cells/mm3 of blood at the baseline, twenty one patients (77.8%) had increased CD4 counts two weeks after chemotherapy, five patients (18.5%) had decreased CD4 counts and there was no change in one patient (3.7%). Among the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline, thirty five patients (83.3%) had increased CD4 counts, six patients (14.3%) had decreased CD4 counts and there was no change in one patient (2.4%). For the patients with more than CD4 counts 200 cells/mm^3 of blood at the baseline, eight patients (72.7%) had increased CD4 counts in response to chemotherapy, two patients (18.2%) had decreased CD4 counts and there was no change in one patient (9.1%; Table 2).

Table 2: Effect of chemotherapy on CD4 count two weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts <100 cells	100 – 200 cells	> 200 cells	Total Patients
Increased	21 (77.8%)	35 (83.3%)	8 (72.7%)	64 (80%)
Decreased	5 (18.5%)	6 (14.3%)	2 (18.2%)	13 (16.3%)
No change	1 (3.7%)	1 (2.4%)	1 (9.1%)	3 (3.7%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After six weeks of chemotherapy, seventy four patients (92.5%) had increased CD4 counts while six patients (7.5%) had decreased CD4 counts (Table 3). Among the patients with CD4 counts of less than 100 cells/mm^3 of blood at the baseline, twenty five patients (92.6%) increased CD4

counts in response to chemotherapy while two patients (7.4%) decreased CD4 counts (Table 3). Among the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline, forty one patients (97.6%) had increased CD4 counts while one patient (2.4%) had decreased CD4 counts (Table 3). For the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline, eight patients (72.7%) had increased CD4 counts while three patients (27.3%) had decreased CD4 counts (Table 3).

Table 3: Effect of chemotherapy on CD4 count six weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts			Total
	<100 cells	100 – 200 cells	> 200 cells	Patients
Increased	25 (92.6%)	41 (97.6%)	8 (72.7%)	74 (92.5%)
Decreased	2 (7.4%)	1 (2.4%)	3 (27.3%)	6 (7.5%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After ten weeks of chemotherapy, seventy four patients (92.5%) had increased CD4 counts in response to chemotherapy, four patients (5%) had decreased CD4 counts and there was no change in two patients (2.5%; Table 4). Among the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline, twenty six patients (96.3%) had increased CD4 counts and there was no change in one patient (3.7%; Table 4). For those with CD4 counts between 100 – 200 cells/mm^3 of blood at the baseline, 38 patients (90.5%) had increased CD4 counts, three patients (7.1%) had decreased CD4 counts and there was no change in one patient (2.4%). For the patients with more than 200 cells/mm ^{3} of blood at the baseline, ten patients (90.9%) had increased CD4 counts and one patient (9.1%) had decreased CD4 count (Table 4).

Table 4: Effect of chemotherapy on CD4 count ten weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts			Total
	<100 cells	100 – 200 cells	> 200 cells	Patients
Increased	26 (96.3%)	38 (90.5%)	10 (90.9%)	74 (92.5%)
Decreased	- (%)	3 (7.1%)	1 (9.1%)	4 (5%)
No change	1 (3.7%)	1 (2.4%)	- (%)	2 (2.5%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

After fourteen weeks of chemotherapy, seventy three patients (91.2%) had increased CD4 counts, four patients (5%) had decreased CD4 counts and there was no change in three patients (3.8%) from the previous count (Table 5). Among the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline, twenty six patients (96.3%) had increased CD4 counts in response to chemotherapy and there was no change in one patient (3.7%; Table 5). Among the patients with CD4 counts between 100-200 cell/mm^3 of blood at the baseline, thirty seven patients (88.1%) had increased CD4 counts while three patients (7.1%) had decreased CD4 counts and there was no change in two patients (4.8%; Tab.5). For the patients with CD4 counts

more than 200 cells/mm^3 of blood at baseline ten patients (90.9%) had increased CD4 counts in response to chemotherapy and one patient (9.1%) had decreased in CD4 counts (Tab.5).

Table 5: Effect of chemotherapy on CD4 count fourteen weeks post chemotherapy

Effect of Chemotherapy on CD4 counts	Baseline CD4 Counts			Total
	<100 cells	100 – 200 cells	> 200 cells	Patients
Increased	26 (96.3%)	37 (88.1%)	10 (90.9%)	73 (91.2%)
Decreased	-	3 (7.1%)	1 (9.1%)	4 (5%)
No change	1 (3.7%)	2 (4.8%)	-	3 (3.8%)
Total patients	27 (33.7%)	42 (52.5%)	11 (13.8%)	80

CD4 Profile during Chemotherapy

Response to chemotherapy by patients at different levels of HIV infection was compared fortnightly for a period of fourteen weeks. The mean CD4 count among patients with CD4 counts less than 100 cells/mm^3 of blood increased from 54 to 242 during the fourteen weeks of chemotherapy. The mean CD4 count among patients with CD4 counts 100-200 cells/mm^3 of blood increased from 151 to 335 while the mean CD4 count of patients with CD4 counts more than 200 cells/mm^3 of blood increased from 210 to 352 during the same period of chemotherapy.

When the response was compared during the first two weeks of treatment, patients with 100-200 cells/mm^3 were found to have a better response ($p<0.001$; $t=12.5032$) compared to patients with less than 100 cells/mm^3 and more than 200 cells/mm^3. After six weeks of treatment, patients with 100-200 cells/mm^3 were found to have a better response ($p<0.01$; $t=6.4687$) compared to patients with less than 100 cells/mm^3 and more than 200 cells/mm^3. After ten weeks of treatment, patients with less than 100 cells/mm^3 were found to have a better response ($p<0.01$; $t=4.889$) compared to patients with 100-200 cells/mm^3 and more than 200 cells/mm^3 and after fourteen weeks of treatment, patients with less than 100 cells/mm^3 were found to have a better response ($p<0.01$; $t=5.0053$) compared to patients with 100-200 cells/mm^3 and more than 200 cells/mm^3.

Response to chemotherapy between the categories over the entire fourteen weeks were compared by regression analyses. Patients with 100-200 cells/mm^3 were found to have significantly better response ($P<0.01$; $t = 19.7332$) than the patients with less than 100 cells/mm^3 and patients with more than 200cells/mm^3 of blood.

Response to Chemotherapy by Patients with Different Levels of Viral Load

After two weeks of chemotherapy, thirty three patients (41.3%) had decreased viral load, forty six patients (57.5%) had increased viral load and there was no change in one patient (1.2%; Table 8). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, thirteen patients (50%) had decreased viral load in response to chemotherapy, twelve patients (46.1%) had increased viral load and there was no change in one patient (3.9%). Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, four patients

(57.1%) had decreased viral load in response to chemotherapy and three patients (42.9%) had increased viral load. For the patients with viral load more than 100,000 copies/ml of plasma at the baseline category, sixteen patients (34%) had decreased viral load in response to chemotherapy and thirty one patients (66%) had increased viral load (Table 6).

Table 6: Effect of chemotherapy on viral load two weeks post chemotherapy

Effect of Chemotherapy on viral load	Baseline Viral Load			
	<50,000 copies/ml	50,000 – 100,000 copies/ml	>100,000 copies/ml	Total Patients
Decreased	13 (50%)	4 (57.1%)	16 (34%)	33 (41.3%)
Increased	12 (46.1%)	3 (42.9%)	31 (66%)	46 (57.5%)
No change	1 (3.9%)	-	-	1 (1.2%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After six weeks of chemotherapy, thirty four patients (42.5%) had decreased viral load while fourty six patients (57.5%) had increased viral load (Table 7). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, twelve patients (46%) had decreased viral load in response to chemotherapy and fourteen patients (54%) had increased viral load. Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, five patients (71%) had decreased viral load in response to chemotherapy and two patients (29%) had increased viral load. For the patients with more than 100,000 copies/ml of plasma at the baseline seventeen patients (36%) had decreased viral load in response to chemotherapy and thirty patients (64%) had increased viral load (Table 7).

Table 7: Effect of chemotherapy on viral load six weeks post chemotherapy

Effect of Chemotherapy on viral load	Baseline Viral Load			
	<50,000 copies/ml	50,000 – 100,000 copies/ml	>100,000 copies/ml	Total Patients
Decreased	12 (46%)	5 (71%)	17 (36%)	34 (42.5%)
Increased	14 (54%)	2 (29%)	30 (64%)	46 (57.5%)
Percentage of decrease (%)	46.15	71.43	36.17	-
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After ten weeks of chemotherapy, thirty eight patients (47.5%) had decreased viral load while fourty two patients (52.5%) had increased viral load (Table 10). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, thirteen patients (50%) had decreased viral load in response to chemotherapy and thirteen patients (50%) had increased viral load (Table 8). Among the patients with viral load 50,000-100,000 copies/ml of plasma at the baseline, three patients (42.9%) had decreased viral load in response to chemotherapy and four patients (57.1%) had increased viral load (Table 8). For the patients with viral load more than

100,000 copies/ml of plasma at the baseline, twenty two patients (46.9%) had decreased viral load in response to chemotherapy and twenty five patients (53.1%) had increased viral load (Table 8).

Table 8: Effect of chemotherapy on viral load ten weeks post chemotherapy

	Baseline Viral Load			
Effect of Chemotherapy on viral load	**<50,000 copies/ml**	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	13 (50%)	3 (42.9%)	22 (46.9%)	38 (47.5%)
Increased	13 (50%)	4 (57.1%)	25 (53.1%)	42 (52.5%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

After fourteen weeks of chemotherapy, fourty seven patients (58.8%) had decreased viral load while thirty three patients (41.2%) had increased viral load (Table 9). Among the patients with viral load less than 50,000 copies/ml of plasma at the baseline, sixteen patients (61.5%) had decreased viral load in response to chemotherapy and ten patients (38.5%) had increased viral load (Table 9). Among the patients with viral load 50,000-100,000 copies/ml at the baseline, four patients (57.1%) had decreased viral load in response to chemotherapy and three patients (42.9%) had increased viral load (Table 9). For the patients with viral load more than 100,000 copies/ml of plasma at the baseline, twenty seven patients (57.5%) had decreased viral load in response to chemotherapy and twenty patients (42.5%) had increased viral load (Table 9).

Table 9: Effect of chemotherapy on viral load fourteen weeks post chemotherapy

	Baseline Viral Load			
Effect of Chemotherapy on viral load	**<50,000 copies/ml**	**50,000 – 100,000 copies/ml**	**>100,000 copies/ml**	**Total Patients**
Decreased	16 (61.5%)	4 (57.1%)	27 (57.5%)	47 (58.8%)
Increased	10 (38.5%)	3 (42.9%)	20 (42.5%)	33 (41.2%)
Total patients	26 (32.5%)	7 (8.8%)	47 (58.7%)	80

Viral Load Profiles during Chemotherapy

Response to chemotherapy by patients at different levels of viral loads was compared fortnightly for a period of fourteen weeks. The mean viral load of patients with plasma viral load less than

50,000 copies/ml decreased from 46,940 to 26,985 during the fourteen weeks of chemotherapy. The mean viral load of patients with plasma viral load 50,000 -100,000 copies/ml decreased from 63,606 to 42,825 while the mean viral load of patients with plasma viral load more than 100,000 copies/ml category decreased from 308,796 to 195,728 during the same period of chemotherapy.

When the responses were compared during the first two weeks of treatment, patients with viral load 50,000-100,000 copies/ml were found to have a better response ($p<0.001$; $t = 48.4562$) compared to patients with less than 50,000 copies/ml and more than 100,000 copies/ml. After six weeks of treatment, patients with viral load 50,000 -100,000 copies/ml were found to have a better response ($p<0.001$; $t = 16.0503$) compared to patients with less than 50,000 copies/ml and more than 100,000 copies. After ten weeks of treatment, patients with less than 50,000 copies/ml category were found to have a better response ($p<0.001$; $t = 18.9713$) compared to patients with viral load 50,000-100,000 copies/ml and more than 100,000 copies/ml and after fourteen weeks of treatment, patients with less than 50,000 copies/ml were found to have a better response ($p<0.001$; $t = 23.0911$) compared to patients with 50,000 – 100,000 copies/ml and viral load more than 100,000 copies/ml.

Response to chemotherapy between the categories over the entire fourteen weeks were compared by regression analyses. Patients with more than 100,000 copies/ml category were found to have significantly better response (Figure 6; $P<0.001$; $t = 460.7554$) than the patients with 50,000-100,000 copies/ml and less than 50,000 copies/ml categories (Figure 6).

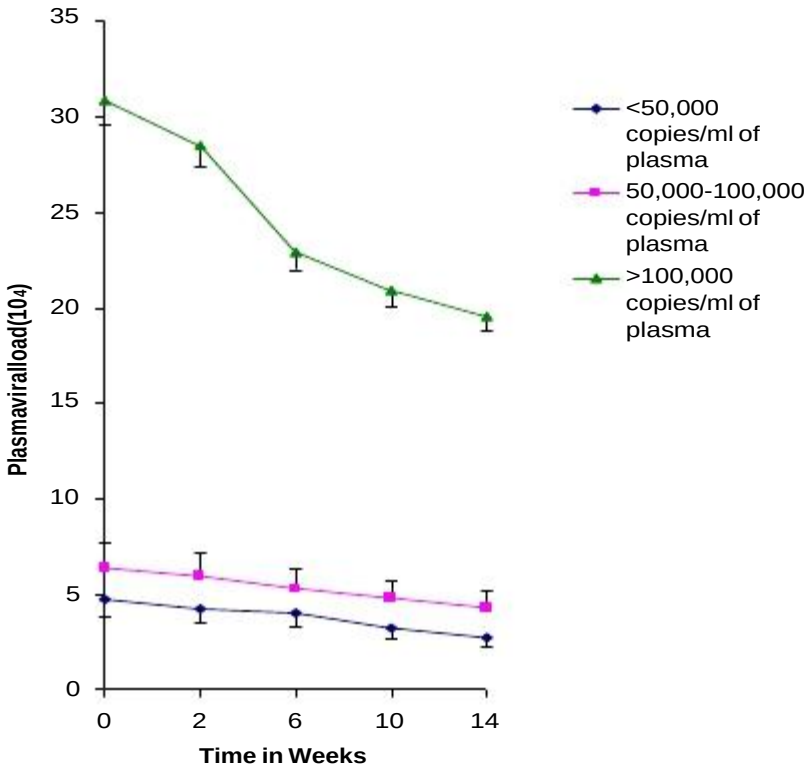

Figure 6: Viral load profile during chemotherapy. Patients categorized according to the level of viral load.

Comparison of Response in CD4 Counts and Viral Load during Chemotherapy

The mean CD4 counts and viral load of all the patients during chemotherapy were compared. Viral load and CD4 counts were found to be strongly inversely correlated (Figure 7; $P<0.001$; $r = -0.992$), that is, as CD4 counts increased, viral load decreased.

Further the relationship between response to CD4 counts and viral loads were compared for each of the categories of CD4 counts and viral loads. The mean CD4 count in the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline and the mean viral load in the patients with plasma viral load more than 100,000 copies/ml at the baseline were compared. The parameters were found to be strongly inversely correlated ($P<0.001$; $r = -0.983$).

Secondly the relationship between mean CD4 count in the patients with CD4 counts between 100-200 cells/mm3 of blood at the baseline and viral load in the patients with plasma viral load of 50,000-100,000 copies/ml at baseline were compared. The two parameters were found to have a very strong inverse correlation ($P < 0.001$; $r = -0.990$).

Finally the relationship between the mean CD4 count in the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline and viral load in the patients with plasma viral load less than 50,000 copies/ml at the baseline category were compared. The two parameters were observed to be strongly inversely correlated ($P<0.001$; $r = -0.969$).

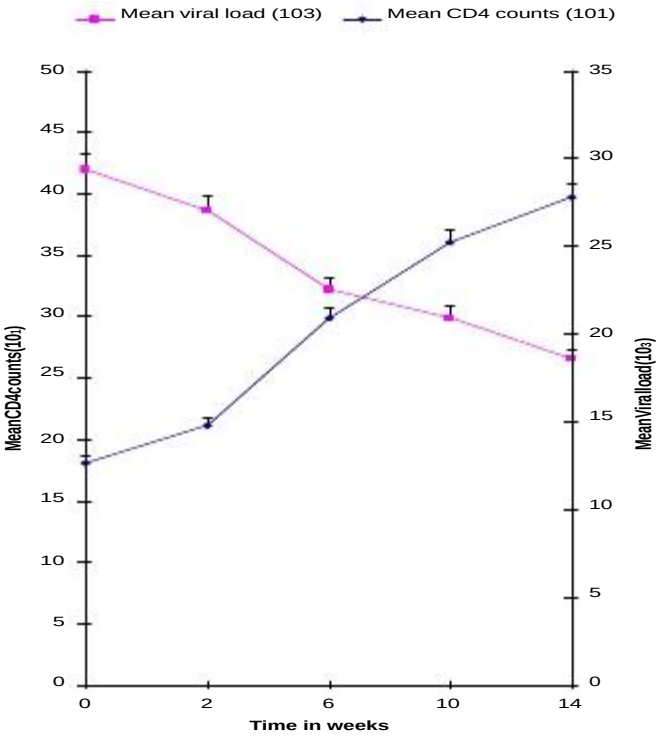

Figure 7: Mean CD4 counts and mean viral loads for all the patients during chemotherapy.

Effect of Chemotherapy on CD3 and CD8 counts

The mean CD3 count increased from 133 to 2078 and the mean CD8 count decreased from 1786 to 835 during the fourteen weeks of chemotherapy.

Effect of Chemotherapy on CD4 and CD8 ratio

The mean CD4:CD8 ratio for all the patients rose from 0.12 to 0.23 during fourteen weeks of chemotherapy (Figure 8). Changes in the CD4:CD8 ratio at different levels of CD4 counts were further examined. The mean CD4:CD8 ratio for the patients with CD4 counts less than 100 cells/mm^3 of blood at the baseline rose from 0.04 to 0.10 during the fourteen weeks of chemotherapy (Figure 8) while CD4:CD8 ratio for the patients with CD4 counts between 100-200 cells/mm^3 of blood at the baseline rose from 0.10 to 0.19 during the fourteen weeks of chemotherapy (Figure 8). The CD4:CD8 ratio for the patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline increased from 0.18 to 0.40 during the fourteen weeks of chemotherapy (Figure 8).

The change in ratio of CD4:CD8 among patients with different levels of CD4 counts during chemotherapy was compared. Patients with CD4 counts more than 200 cells/mm^3 of blood at the baseline were found to have a significantly higher change (Figure 8; $P < 0.001$; $t = 39.91063$) in CD4:CD8 ratio than patients with less than 100 cells/mm^3 and patients with 100-200 cells/mm^3 of blood at the baseline categories.

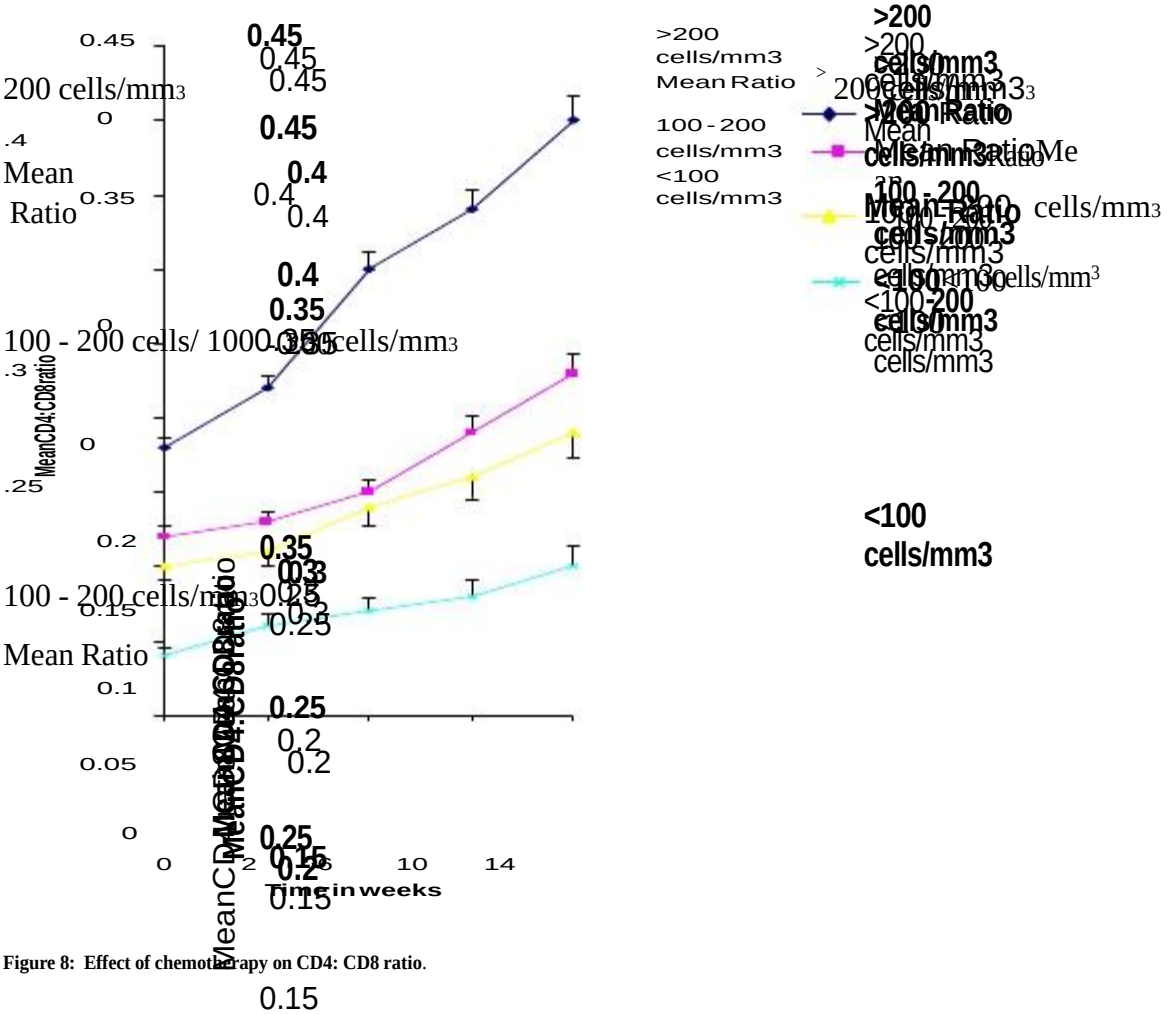

Figure 8: Effect of chemotherapy on CD4: CD8 ratio.

0.1

0.15 0.1

Discussion

In this study, six patients out of eighty had discordant results by pararell testing for HIV antibodies and a third test had to be performed for confirmation. In an earlier study, HIV screening using parallel testing for HIV antibodies recorded discordant results (Hellen, 2002) and a third test had to be used for confirmation. The serum samples of the discordant results in this study were tested for HIV antibody by enzyme linked immunosorbent assay (ELISA) and indicated that they were all HIV positive. This testing agrees with earlier tests carried out on discordant rapid tests which turned HIV positive using ELISA (Healthlink Worldwide, 1999). These results suggest that discordant results following rapid testing should not be concluded as outright negative.

In this study, all the patients had no prior treatment for HIV. They were given fixed dose combinations of stavudine and lamivudine to be taken twice daily and navirapine as an individual drug to be taken once daily. The antiretroviral drugs they received are among the recommended drug regimen to HIV patients by the Government of Kenya as the first line treatment for adults (MOH, 2004).

Overall during the entire period, the patients who started treatment with high viral loads (more than 100,000 copies/ml) had a significantly better response to treatment compared to the patients who started treatment with low viral loads (less than 100,000 copies/ml). This is in agreement with a study by Antony *et al*., (2002) who found better responses in patients who started treatment with high viral loads. As treatment progressed, there was improved health in all the patients. Reduced viral load was linked to improved health. It was observed that the patients who started treatment with viral loads over 100,000 copies/ml of plasma showed better health improvement over the entire period of the study compared to those who started treatment with viral loads below 100,000 copies/ml of plasma. This is in support of an earlier study where improved health was most noticeable in people who started treatment with high viral loads (http://www.atdn.org/simple/viral.html). This means that treatment with ARVs reduces viral load and improves the health of patients.

The mean CD4 count increased while the mean viral load decreased with chemotherapy, an indication of an improvement in immunologic function. Earlier studies have shown increases in mean CD4 counts and reduced viral loads with treatment. For example, one study by O'Brien (1996; http://www.aodsmuc.org/natap) showed that as the CD4 count increased, the plasma viral loads decreased during treatment. In previous studies it was reported that higher pre-treatment viral load and lower pre-treatment CD4 count were associated with greater increase in CD4 counts during the first three months of chemotherapy (Smith, 2004; Alatrakchi, 2005) resulting in the recovery of the immune function. Clinical benefits had been observed between eighth and fourteenth weeks of this study, and the clinicians agreed that the responses to antiretroviral therapy were evident. Generally, all the patients responded well to antiretroviral drugs although few had some delay in initial benefits, but prolonged treatment showed remarkable progress.

Conclusion

Progressive increases in CD4 count and reduction in viral load resulted in reconstitution of the immune system in most individuals in the study population, even in those with advanced disease

who started antiretroviral therapy at very low CD4 counts and very high viral loads. This substantially reduced the risk of clinical disease progression and death. Either CD4 counts or viral load could be used as an accurate measure of response to antiretroviral therapy.

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Phase Transition in High Temperature Superconductivity

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Abstract

The onset of superconductivity is accompanied by abrupt changes in various thermodynamic properties, which is the hallmark of a phase transition. At the superconducting transition, it suffers a discontinuous jump and, therefore, ceases to be linear resulting in change of volume, specific heat and entropy at the critical temperature T_C. Phase transitions are of first order when the latent heat $L = 0$, and are of the second-order phase transition when there is a specific heat jump at the transition temperature T_C. In this study the variation of specific heat capacity with temperature for high temperature superconductors changes discontinuously but does not become infinite at T_C showing a finite specific heat jump at the transition temperature and hence the phase transition is of second order.

Keywords: Phase Transition, Superconductivity, Critical Temperature

Introduction

The vanishing of electrical resistance of a conductor at very low temperature is known as the phenomena of superconductivity. It was discovered by H. K, Onnes in 1911 at Laiden, Holland [1]. He found that when the temperature of pure frozen mercury was reduced below 4.2K, its electrical resistance disappeared resulting in the flow of large electrical currents. A great number of pure metals, alloys and doped semiconductors were found to have this property. It is also found that the metals cooled in the superconducting state in a moderate magnetic field expel the magnetic field from its interior resulting in negative susceptibility of the material and this is the property of a diamagnetic material. Thus a superconductor is a diamagnetic and this is called Meissner effect that was discovered in 1933 by Meissner *et al* [2, 3].

The discovery of high T_C Cuprates and their peculiar properties has stimulated enormous theoretical interest to search for the origin of pairing mechanism in these systems and also to decide whether the superconducting transition is a first-order or second-order phase transition. Superconductors and superfluids exhibit phase transitions [4]. Superconductivity results from correlations between motions of electrons in a metal, induced by electron-phonon interactions.

Its study has been important for our understanding of these interactions and has produced great insight into the physics of electrons in metals at low temperatures.

Since superconducting current is carried by electrons, there will be Coulomb repulsion within them. The Coulomb repulsion could be sometimes very large and or sometimes less than some critical value (V_C); it is found that in the first case, transition to super-conducting state is of first-order and in the second case it is of second-order. In this study the problem focus is to study the order of phase transition in high T_C superconductors [5]. The order of phase transition is determined by knowing whether the latent heat is finite (first-order phase transition) or there is a jump in the specific heat (second-order phase transition)

Theory

In thermodynamics, phase transition is the transformation of a thermodynamic system from one phase to another. The distinguishing characteristic of a phase transition is an abrupt sudden change in one or more physical properties, in particular the heat capacity, with a small change in thermodynamic variables such as the temperature.

The first-order phase transition involves a latent heat. During such transition a system either absorbs or releases a fixed amount of heat [6]. Because energy cannot be instantaneously transferred between the system and its environment, first-order transitions are associated with "mixed-phase regimes" in which some parts of the system have completed the transition and others have not.

The second class of phase transition is the "continuous phase transition", also called second-order phase transitions and not associated with latent heat. Examples of second-order phase transition are the ferromagnetic transition and the Superfluid transition [7, 8].

In superconducting materials, the characteristic of superconductivity appear when the temperature T is lowered below a critical temperature T_C. The value of this critical temperature varies from one material to the other. Conventional superconductors usually have critical temperatures ranging from 1K to 20K. Cuprate superconductors can have much higher critical temperatures. $YBa_2Cu_3O_7$, one of the first Cuprate superconductors to be discovered, has a

critical temperature of 92K, and mercury Cuprates have been found with critical temperatures in excess of 130K[8]. The explanation for these high critical temperatures remains unknown. Electron pairing due to phonon exchanges explains superconductivity in conventional superconductors, but it does not explain superconductivity in the newer superconductors that have a very high T_C

Methodology

Condensation into a superconducting state lowers the free energy F_n of the normal state to F_s of the superconducting state. The critical field H is given by the relation [9].

$$\frac{H_C^2}{2\mu_0} = F_n - F_s \quad \text{..... (1)}$$

The specific heat constant γ is given by

$$\gamma = \frac{1}{3}\pi^2 D(\varepsilon_F) K_B^2 \quad \text{..... (2)}$$

From the BCS theory [3, 6] based on equations (1) and (2) we have the relation,

$$H_C(0) = \left(\frac{3\mu_0}{2}\right)^{\frac{1}{2}} \cdot \frac{3.5}{2\pi}\gamma^{\frac{1}{2}} T_C = 7.65\times 10^{-4}\gamma^{\frac{1}{2}} T_C \quad \text{..... (3)}$$

Thermodynamic description of a superconductor

Although the Meissner effect is described quite accurately by linear relation between the intensity of the magnetization M and the magnetic field H, this linearity does not persist with the arbitrarily high magnetic fields. The most striking non-linear effect is the existence of a critical magnetic field for superconductivity. For instance, for a small value of magnetic field, H_0 a superconductor may exhibit Meissner effect but at some critical field strength H_C, the situation may change abruptly; the specimen losses the property of superconductivity, there is a thermodynamic transition back to the normal state, with a latent heat of transition.

According to Ehrenfest such a transition is called first order phase transition, at constant temperature and constant applied magnetic field, heat must be supplied to the specimen to enable it to make the transition from the superconducting to the normal state. However, when there is no acting external magnetic field $H_C(T_C) = 0$ and the temperature $T \rightarrow T_C$ (the critical temperature for superconducting transition), the latent heat is zero. Such a transition is called the second order phase transition. The electronic specific heat jumps discontinuous to about three times the normal value γT_C as we cool through the transition (γ is a constant value) temperature T_C.

We now have to calculate the jump in the specific heat as the material undergoes transition from the normal to the superconducting state. The change in the Gibb's Free energy density of a system, when the external magnetic field is changed has to be written.

Thus we write;

$$dG = -SdT - \frac{1}{4\pi} B.dH \quad \text{..... (4)}$$

where S is the entropy per unit volume.

Equation (6) gives

$$S_s = -\left(\frac{\partial G}{\partial T}\right)_H \quad \text{and} \quad B = -4\pi\left(\frac{\partial G}{\partial H}\right)_T \quad \text{.....} \quad (5)$$

Specific heat C_H is now given as

$$C_{HS} = -T\left(\frac{\partial S}{\partial T}\right)_H \quad \text{..... (6)}$$

Considering a long superconducting cylinder in a magnetic field that is parallel to the axis of the cylinder, that is $H = H_Z$, and then increase the value of H from zero to some value H we have at constant temperature T,

$$G_s(T,H) - G_n(T,H) = \frac{1}{8\pi}\left[\{Hc(T)\}^2 - H^2\right] \text{.........} (7)$$

The entropy S is given by

$$S_s(T,H) - S_n(T,H) = -\frac{1}{4\pi} H_C(T) \frac{dH_C(T)}{dT} \text{.........} (8)$$

And the latent heat Q_L is,

$$Q_L = T(S_s - S_n) = -\frac{1}{4\pi} TH_C(T) \frac{dH_C(T)}{dT} \text{.........} (9)$$

The empirical relation for the variation of $H_C(T)$ with the temperature T is

$$H_C(T) = H_C(0)\left[1 - \left(\frac{T}{T_C}\right)^2\right] \text{.........} (10)$$

The Specific heat based on equation (6) is given as,

$$C_{Hs} - C_{Hn} = \frac{T}{4\pi}\left[\left(\frac{dH_C}{dT}\right)^2 \pm H_C \frac{d^2 H_C}{dT^2}\right] \text{.........} (11)$$

The jump in the specific heat at $T = T_C$ is given by,

$$\left(C_{HS} - C_{Hn}\right) = (\Delta C)_{T=T_C} = \frac{T_C}{4\pi}\left[\left(\frac{dH}{dT}\right)_{T=T_C}\right]^2 \text{.........} (12)$$

Equation (12) is the Rutgers Formula.

First-Order Phase Transition.

In a phase change the change involves a major re-arrangement of structure of the substance, resulting in change of volume, specific heat, entropy viscosity etc at the critical temperature T_C .

Since such changes involve energy, energy change, input or output produced at a finite amount of heat, the latent heat L, the transition takes place at a constant temperature T of ΔT or $dT = 0$. When a substance undergoes a change of phase from phase 1 to phase 2, the accompanied latent heat is

$L_{12} = T_c(S_2-S_1)$ …… (13)

where S_1 is the entropy in phase 1 and S_2 is the entropy in phase 2. Then this equation shows that there is a discontinuity in entropy since the heat capacity C_P is,

$$C_P = T\left(\frac{\partial S}{\partial T}\right)_P \qquad \text{…… …… (14)}$$

Second-Order Phase Transition.

There are other changes of phase involving the initiation of a different kind of ordering in a crystal lattice or the appearance of Superfluid in Helium II, or super-fluidity of a nuclear system and the appearance of the solid and a super-solid ^{4}He, and also superconductivity of such phase changes may involve a change of slope of S against T at the transition point, not a change of the value. In this case the heat capacity changes discontinuously but does not become infinite at T_C. Such changes are called phase change of the order. For such changes, there is no discontinuity in volume ($V_1 = V_2$) and or entropy ($S_1 = S_2$) during the transition.

Phase Transition in Superconductors

Phase transitions are of first-order when the latent heat $L = 0$, and are of the second-order phase transition when there is a specific heat jump at the transition temperature T_C (2.17K in ^{4}He liquid)

A recent study has opened up the possibility of investigating the crossover from a Bose-Einstein Condensate (BEC) to a Bardeen-Cooper-Schrieffer (BCS) Superfluid [9]. In these systems the strength of the interaction can be varied over a very wide range by magnetically tuning the two-body scattering amplitude. For positive values of the s-wave scattering length" a" atoms with different spins are observed to pair into bound molecules which, at low temperatures form a Bose

Condensate [10, 11]. The molecular BEC state is adiabatically converted into a ultra cold Fermi gas with a < 0 and $K_F |a| << 1$, [12, 113], where standard BCS theory is expected to apply. In the cross-over region the value of $|a|$ can be orders of magnitude larger than the inverse Fermi wave vector K_F and one enters a new strongly correlated regime known as unitary limit. In dilute systems, for which the effective range of the interaction R is much smaller than the mean inter particle distance, $K_F R << 1$, the energy of the non-interacting Fermi gas is ε_{FG},

$$\varepsilon_{FG} = \frac{3}{10}\frac{\hbar^2 K_F^2}{m} \quad \ldots\ldots (15)$$

For the ultra cold degenerate Fermi gases, recent work [14] gives that the energy per particle of a dilute Fermi gas is $\frac{E}{N} = \hbar\, \varepsilon_{FG}$ and the energy of a weakly attractive Fermi gas is given by,

$$\frac{E}{N\varepsilon_{FG}} = 1 + \frac{10}{9\pi} K_F a + \frac{4(11 - 2\log 2)}{21\pi}(K_F a) + \ldots \quad \ldots\ldots (16)$$

In order to investigate what kind of phase transition such an assembly can undergo, we multiply the right hand side of equation (16) by the thermal activation factor $e^{-\frac{\varepsilon_{FG}}{KT}}$ and write the total energy as,

$$E = N\varepsilon_{FG}\left[1 + \frac{10}{9\pi} K_F a + \frac{4(11 - 2\log 2)}{21\pi^2}(K_F a)^2\right] e^{-\frac{\varepsilon_{FG}}{KT}} \quad \ldots\ldots (17)$$

Now the specific heat can be obtained from equation (17) as,

$$C = \frac{\partial E}{\partial T} = \frac{\partial}{\partial T}\left\{ N\varepsilon_{FG}\left[1 + \frac{10}{9\pi} K_F a + \frac{4(11 - 2\log 2)}{21\pi^2}(K_F a)^2\right] e^{-\frac{\varepsilon_{FG}}{KT}} \right\} \quad \ldots\ldots (18)$$

$$C = N\varepsilon_{FG} A\left(-\frac{\varepsilon_{FG}}{k}\right)\left(-\frac{1}{T^2}\right)e^{-\frac{\varepsilon_{FG}}{kT}} = N\varepsilon_{FG}\left(\frac{A}{kT^2}\right)e^{-\frac{\varepsilon_{FG}}{kT}} \quad \text{.....} \quad (19)$$

Since $K_F a << 1$, the quantity A can be approximated as,

$$A \approx \left[1 + \frac{10}{9\pi} K_F a + \frac{4(11 - 2\log 2)}{21\pi} (K_F a)^2\right] \quad \text{.....} \quad (20)$$

Using the relation, $e^{-\frac{\varepsilon_{FG}}{kT}} = 1 - e^{-\frac{\varepsilon_{FG}}{kT}} + \text{.....} \approx 1 - e^{-\frac{\varepsilon_{FG}}{kT}}$ and neglecting higher terms in equation (19) gives,

$$C = \eta T^2 e^{-\frac{\varepsilon_{FG}}{kT}} \quad \text{.....} \quad (21)$$

The quantity $\eta = N\varepsilon_{FG}^2 kA$ is a constant. The following parameters were used to calculate the specific heat capacity, C and to plot the variation of C against T for temperature range $T = 0K$ to $T = 120K$

$K_F = 0.25$, $a = 10^{-6}$ and 10^{-8}, mass of electron, $m = 9.1 \times 10^{-24}$ gm, ε_{FG} is calculated from equation (15). The quantity $k = 1.38 \times 10^{-23} J/K$ is the Boltzmann constant

Results and Discussions

Figure 4.1 shows the specific heat dependence with temperature ranging from $0K$ to $120K$. The specific heat value approaches infinity at temperature $T = 120K$ pointing to a possible specific heat jump at this temperature

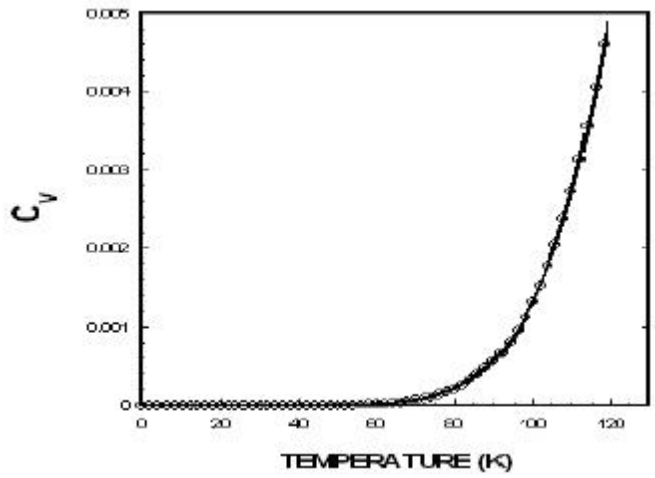

Figure 4.1: A graph of variation of the specific heat capacity, C_V *with the temperature for low temperature superconductors*

Figure 4.2 below shows the temperature dependence of specific heat of high temperature superconductors ranging from $90K$ to $180K$

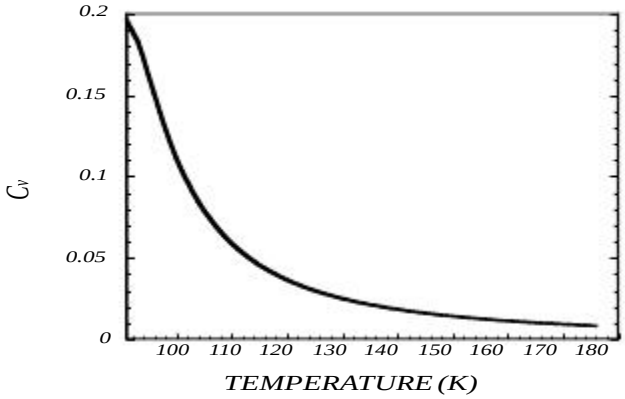

Figure 4.2: Graph of variation of specific heat capacity, C_V *with the temperature, T for high temperature superconductors.*

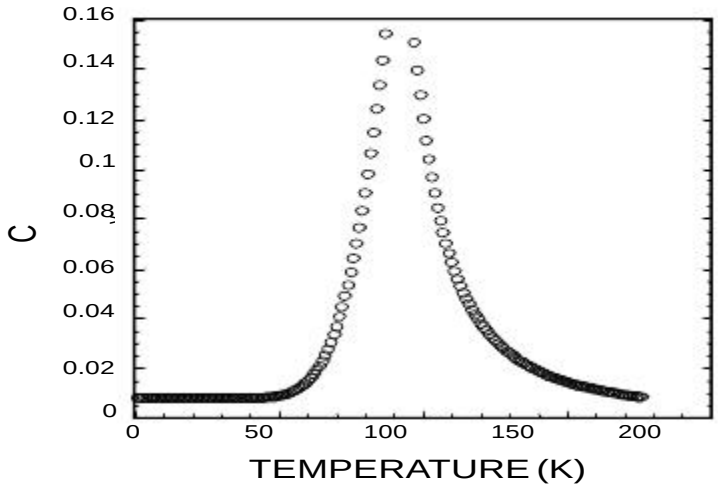

Figure 4.3: Graph of variation of specific heat capacity, C_V *with the temperature, T showing the heat jump.*

The specific heat jump due to the superconducting transition at about $90K$ is observed.

In this work we have investigated the specific heat capacity dependence with temperature for high temperature superconductors showing specific heat jump at the transition temperature $T_C = 90K$. Similar results were obtained in work done by Liu [15] on a possible second-order phase transition at which a gap opens in superconductors showing that there is a second-order phase transition at the temperature T_C.

The superconducting phase transition in heavy fermions $CeCoIn_5$ (T_C=2.3 K in zero field) becomes first order when the magnetic field $H_C(001)$ is greater than 4.7 T, and the transition temperature is below $T_0 \approx 0.31T_C$ [16]. The change from second order at lower fields is reflected in strong sharpening of both specific heat and thermal expansion anomalies associated with the phase transition.

Experimental data obtained from thermodynamic measurements in under doped high temperature superconductors [17] show unusual anomalies in the temperature dependence of the electronic specific heat both in the normal state and at the critical point associated to the superconducting

phase transition. Based on a phenomenological description of the pseudo gap phase, analytical and numerical calculations were performed for the temperature dependence of the specific heat for both the superconducting and normal state. The reduced specific heat jump at the transition point was explained by a modified electronic single particle contribution to the specific heat in the presence of the normal state pseudo gap.

The specific heat $C_v(T)$ of iron-based high temperature superconductor $SmO_{1-x}F_xFeAs$ ($0 \leq x \leq 0.2$) was systematically studied [18]. For the undoped $x=0$ sample, a specific heat jump was observed at $T_C = 130K$. A simple consideration demonstrates that if the temperature of a second-order phase transition is suppressed by fluctuations, the dominating effect at the transition is a maximum, but not a jump of specific heat, C_v [19]

Conclusion

In conventional superconductors or BCS type superconductors, the superconducting transition is a second-order phase transition in the absence of the external magnetic field whereas the transition is of the first-order in a finite magnetic field. Whereas in high T_C superconductors [20], the situation is still not clear. In some materials, the superconducting transition seems to be of the first-order. In those materials in which the nearest neighbours, Coulomb repulsion V between the electrons in the copper oxide planes is sufficiently large, the transition is of first-order, whereas if V is less than some critical value V_C, the transition is of second-order. In recent study [6], the role of attractive interlayer and intralayer interactions in layered high T_C Cuprate superconductors have been investigated using a one- band two layer tight binding Hamiltonian. In another calculation [21] it has been shown that there is a finite specific heat jump at the transition temperature T_C, and hence the phase transition is of second-order

Phase transitions are of first order when the latent heat L = 0, and are of the second-order phase transition when there is a specific heat jump at the transition temperature T_C. In this study the variation of specific heat capacity with temperature for high temperature superconductors changes discontinuously but does not become infinite at T_C showing a finite specific heat jump at the transition temperature and hence the phase transition is of second order.

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Use of Microbiotest Assay and Membrane Filtration Plate Culture Methods in Screening of Microbiological Well Water Quality, Uasin Gishu County- Kenya

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Abstract

Residents of many urban centers including Eldoret are increasingly dependent on groundwater for drinking purposes. This study examined the microbiological suitability of well water available for drinking purposes among residents within Eldoret town. Fifteen sampling stations were established in three residential estates and sampling was done during the wet and dry seasons of the year. The samples were analyzed for the presence of adenosine triphosphate (ATP), using the ATP Microbiotest ® method and confirmed with membrane filtration plate culture method. Results showed that there was a significant difference in the number of bacterial colonies in the sampled sites using the two analytical methods ($\chi 2=0.867$, $p=<0.001$, $\chi 2=3.200$, $p<0.001$) in both the dry and wet seasons respectively. This paper reports high levels of bacterial count during the wet season than in dry season and no significant correlation between relative light units(RLU), *Escherichia coliform (E. coli)* and distance of the pit latrine from the wells in all three sampled residential estates ($p>0.05$). The ATP Microbiotest ® method produced relative light unit (RLU) values which correlated positively with colony forming units from plate culture method ($r=0.64$, $p=<0.001$). The study also showed that wells in the high density residential areas had the greatest number of bacterial contaminants expressed as ATP than the wells in the low density residential areas. Thus the ATP Microbiotest ® method should adequately be applied in the household rapid screening of microbial well water contaminants.

Key words: Microbiotest, RLU, ATP, *E. coli*, culture

Introduction

Natural waters contain living organisms, which like all living creatures on earth will at one moment of time die and be decomposed by bacteria. The amount of microbes in water is therefore a signal for the quantitative importance of bacterial decomposition of biological residues (Nuzzo, 2006) proximity to pit latrines and this may be associated with high rates of waterborne infections (Bianci and Lopardo, 2003). The increasing population of cities and towns by natural growth and by migration from rural areas has made the urban population to be split according to their economic and social well being, and this has eventually lead to high, medium and low residential areas within the towns and cities. However in search of the very basic human needs various anthropogenic activities have been initiated within this residential areas leading to the differences in the groundwater quality (WHO/UNICEF, 2004). All people, whatever their stage of development and their social and economic conditions, have the right to have access to drinking water in quantities and of a quality equal to their basic needs. However in the densely populated and low income areas people have always encountered a lot of challenges in pursuit of the fulfillment of this right. The microbial evaluation of the drinking water has been a major challenge in these highly populated areas. Microbiotest offers a convenient option for measurement of bacteriological quality of water (Chen *et al.*, 2006). The energy source of all living organisms, including bacterial is ATP and the total ATP content of a water sample is composed of both "intracellular" ATP from living biota and "extra cellular" ATP which is the ATP released from dead organisms (Masuda *et al.*, 2000). ATP Microbiotest is a rapid assay that

has become a widely accepted method to monitor drinking water sources and supplies. In the presence of firefly enzyme system (luciferin and luciferase system) from *Vibrio fischeri* bacteria, ATP facilitates the reaction to generate light. The light can be measured by a luminometer and used to estimate the biomass of microbial cells contaminating a water sample (Chen and Sandria, 2006). However an effective surveillance programs on the bio indicators of water quality depends on the existence of national regulatory standards of water quality and codes of practice. This, in turn depends on appropriate national legislation and the establishment of a component surveillance unit or agency within government like National Environmental Management Authority (NEMA) in Kenyan government (WHO/UNICEF, 2004).

Bioluminescence in bacteria can be used to quantify microbial contaminants in groundwater through regulation phenomenon known as auto induction. Auto induction or quorum sensing was first discovered in *Vibrio fischeri,* which is cell-to-cell communication that ties gene expression to bacterial cell density. Quorum sensing involves the self-production of a diffusible pheromone called an auto inducer (AI), which serves as an extra cellular signal molecule that accumulates in the medium and evokes a characteristic response from cells. Using bioluminescence, once the concentration of the AI reaches a specific threshold (above 10^7 cells mL^{-1}), it triggers the energetically costly synthesis of luciferase and other enzymes involved in luminescence. Thus, by sensing the level of AI, the cells are able to estimate their density and ensure that the luminescent product will be sufficiently high to cause an impact in the environment, making the process cost-effective. The AI for *V. fischeri*, N-acylhomoss serine lactone (AHL), was once thought to be species-specific; however recent studies have established that AHL can serve as a signaling molecule for more than 16 genera of gram-negative bacteria. This suggests that the AI protein can facilitate interspecies communication, allowing quorum-sensing bacteria to monitor the population of other species as well as their own. Quorum sensing is now a widespread regulatory mechanism in bacteria, particularly among a number of pathogens, influencing their ecology and associations with eukaryotic organisms (Masuda *et al*., 2000)

In Eldoret municipality there are increased human activities particularly the indiscriminate location of septic tanks, soak away pits and pit- latrines, disposal of refuse and waste, and other materials that can leach into the groundwater posing a major health concern. The municipality population increase of 3.35 % annually has over- stretched the supply of the essential services such as sewerage system and consistent water supply. As a result many households have resulted to the sinking of shallow wells, pit latrines and septic tanks as a source of water and domestic waste management respectively.This practice is rampant in the peri- urban densely populated areas of Langas, Kimumu, Huruma, Kahoya; Munyaka and Kapyemit residential areas (Eldoret Municipal Council, 2010). The study focused on Langas, Kimumu, and Elgon View estates in Eldoret Municipality with the main objective of determining the effectiveness of rapid Microbiotest assay as a method of assessing and monitoring well water quality in high, medium and low density residential areas . The geology of Eldoret town and its environs is mainly made up of metamorphosed basement system formation, overlain by a sequence of tertiary volcanic strata. The basement system rocks weather to a pink or brown sandy soil which is lighter in texture and less fertile than the phonolite soils (Otieno, 2001). The phonolite soil weathers initially to a red brown murram and eventually to friable silt clay, which is very fertile and drains freely hence suitable for agriculture (Hillel, 2004). Poor permeability, porosity and the storage capacity of the rocks in the Eldoret area has set close limits on groundwater exploitation of

shallow wells (such as those found in Langas and Huruma) and produce groundwater contained in the joints of the weathered phonolite.

Methodology

Sampling design and procedure

Purposive sampling method was employed to select residential areas from the municipality to be included in the study. The criterion used in selection was population density and the Municipal service provision like sewer lines and piped water. Thus the Municipality was divided into three strata mainly high population, medium population and low population density residential areas. The selected study areas thus included Elgon View (low density), Kimumu (medium density) and Langas (high density). Stratified random sampling was used to select households based on their water sources and consumption points. All the functional wells were identified from each selected residential area and serial numbers assigned. The wells were then randomly selected based on the serial numbers already given. Locations of selected wells were geo-referenced using Geographical Positioning System (GPS).

Sample Collection

Water samples for bacteriological analysis were collected in 200 ml sterile water sampling bottles which were kept unopened until the time of filling. The cork was removed and bottle held by the other hand around the base of the bottle and it was ensured that the sample bottles were not rinsed with sample during collection and the bottles were not completely filled to allow for shaking prior to analysis. The sampled wells were considered to be representative of the entire study area. For wells equipped with a pump, it was operated for a bout ten minutes to clear any standing water in the water column. The outlet pipe was then sterilized using a flame from a burning cotton swab soaked in Methylated spirit. The pump was operated and allowed to run for 2 minutes and sample collected from the flowing stream of water. Where a bucket was used to draw water a sample from the water collector's bucket was taken, as this was more representative of what was actually being consumed by the household. The sample was poured into the sample bottle directly from the bucket (Yolanda *et al*., 2007). Seven replicate samples were collected both from Langas and from Kimumu and one from Elgon View. The physicochemical analysis of the water samples like pH, temperature and conductivity were all done at the site. The samples were stored in cool boxes containing ice which kept the temperatures between 4^{o}C and 10^{o}C and then taken to the laboratory for analysis. Bacteriological analysis of the samples was done in Eldoret Water and Sanitation Company (ELDOWAS) laboratories in Eldoret and the Moi University Biotechnology Center (MUBC).(Yolanda *et al*., 2007)

Microbiotest technique

A sterile syringe was used to draw 10 ml of the water samples from the sample bottles. A Filtravette™ which is a combination of a filter and a cuvette with a pore size of 0.45µm was placed into a 13-mm Swinex® filter holder. The filter holder was screwed onto the syringe and the water sample was pushed through the filter (Dostalek and Branyik, 2003).The Filtravette was removed from the filter holder after filtration of the water sample and was placed onto a sterile blotting paper. A somatic cell releasing agent (NHD) was used to lyse all non bacterial cells and to release the ATP. With a specially converted 10 ml syringe, air pressure was applied to remove

the nonbacterial ATP through the filter. At this stage, the Filtravette retained bacteria on top of the membrane filter, and the bacterial ATP remained within the bacterial cell membranes throughout this step of the procedure. The Filtravette was inserted into the microluminometer and the bacterial cell releasing agent was then added to lyse the bacterial cells retained on the surface of the filter. The released bacterial ATP was mixed with 50 µl of luciferin–luciferase obtained from *Vibrio fischeri.* The light emission was recorded after a 10-sec integration of the light impulses; the unit was called a relative light unit (RLU). The results were expressed as RLU/ml by dividing the RLU values by the filtered water volume. The detection limit and sensitivity of the luminometer was tested with a serially diluted standard ATP solution, diluted five times, which gave a directly proportional linear relationship between RLU and ATP. Distilled water was used for the dilution. The activity of the luciferin–luciferase was checked by using an ATP standard. The assay was based on the reaction between the luciferase, luciferine and ATP. Light emitted during the reaction was measured quantitatively using the luminometer indicated in Fig 3.1 and correlated with the ATP quantity extracted from bacteria in the water sample. The RLUs are proportional to the amount of ATP, and the amount of ATP is proportional to the number of bacteria within the water sample analyzed (Dostalek and Branyik, 2003).

Membrane Filtration plate culture Technique

The membrane filtration procedure involved the use of membrane filters of 0.45 microns pore size and sterile Petri dishes for holding the sterilized selective media.

The Erlenmeyer flask was connected to the vacuum source and the porous support placed in position with a second flask placed in between the vacuum pump and the Erlenmeyer flask to act as a water trap and protect the electric pump. The filtration unit was assembled by placing a sterile membrane filter on the porous support, using forceps sterilized by flaming and then the upper container was placed in position and secured with special clamps. One hundred ml well water sample was poured into the upper container of the filtration unit then when all the sample had passed through the filter the vacuum pump was disconnected. The filtration unit were taken apart and using the forceps the membrane filter was removed and placed in the Petri dish containing the Endo agar medium with the grid side up without introducing air bubbles. The Petri dishes were inverted and incubated at 37^{o}C for 24 hr (Rivera *et al.,* 2007).

Colonies of Coliform bacteria were medium red or dark red in colour, with a greenish gold or metallic surface sheen. In most cases the sheen covered the entire colony while in others it appeared at the center of the colony. The colonies were counted with the aid of a glass lens. The number of total Coliform per 100 ml was calculated as a percentage number of Coliform colonies counted over 100 ml of the well water sample filtered (Rivera *et al.,* 2007).

Data analysis

The tabulated data was coded in data forms and entered into a computer database. Data was analyzed using SPSS16 computer package and presented in frequency tables and graphics. Median rank test was used to compare differences in the median bacterial count between wells in high, low and medium density areas. Statistical significance for each variable was also calculated

in order to draw appropriate conclusions and test the stated hypotheses. All tests were considered significant at 5% alpha level.

Results

Determination of microbial count within wells in high, medium and low residential

Results from the three studied sites were analyzed both in the dry and wet seasons and presented in table 1 showing the degree of contamination.

Table 1. Median IQR of Microbial count using the ATP Microbiotest ® method

	Dry season		Wet season	
Location	Median microbial count		Median microbial count	
	Direct	Filtration	Direct	Filtration
Langas	1437(1368,1674) (+++)	47900 (45600-55800) (++++)	1790 (1650-192) (+++)	50000 (36000-61500) (++++)
Kimumu	683(570,1020) (++)	22750 (19000-34000) (++++)	900 (788-1200) (++)	30000 (26250-40000) (++++)
Elgonview	444 (++)	14800 (++++)	825 (++)	27500 ++++

Key: Very high ++++ High +++ Significant ++ Relatively low + Very low _

Direct ATP Microbiotest® and the filtration ATP Microbiotest® methods were used concurrently to determine the level of bacterial count in the wells studied. The filtration ATP Microbiotest ® method reported a high number of bacteria with median of 47900(45600,55800) in Langas, 22750 (19000, 34000) in Kimumu and 14800 in Elgon View for the dry season and 50000 (36000,615000) counts in Langas, 30000(26250, 40000) in Kimumu and 27500 in Elgon View for the wet season as compared to the direct ATP Microbiotest® with median of 683(570, 1020) and 900 (788, 1200) in the dry and wet seasons respectively as shown in table 1.

(A) Direct test screening values

RLU VALUE	DEGREE OF CONTAMINATION	NOTATION
< 50	Very low	-
< 200	Low	+
200-1000	Significant	++
> 1000	Very high	+++

(B) Membrane filter test screening values

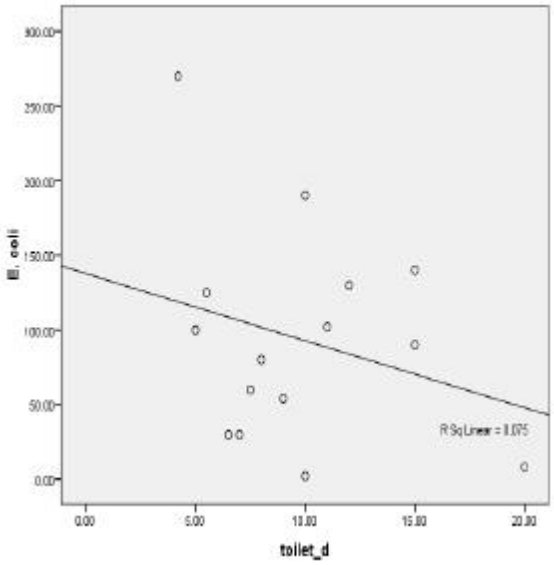
RLU VALUE	DEGREE OF CONTAMINATION	NOTATION
< 200	Very low	-
< 1000	Relatively low	+
> 1000	Significant	++
> 5000	High	+++
> 10000	Very high	++++

Tables A and B above show the standard ATP measurements in form of relative light units (RLU) with the equivalent notation howing the degree of contamination.

Source: Microbiotest® www.microbiotests.be

ATP detection rate and sensitivity

The filtration ATP Microbiotest® method was able to concentrate the bacterial ATP thus increasing the detection and sensitivity rate by an average of 31.5 times than that of Direct ATP Microbiotest ® method. The filtration method was found to be more sensitive in both dry and wet seasons with minimum detection limit (MDL) being <50 and <200 RLU for direct ATP microbiotest and filtration ATP microbiotest methods respectively. The analytical sensitivity of the assay is usually between 0.2 to 20,000pg as established by Chen *et al.*, 2006 using serial dilutions of ATP calibration standards.



Toilet_d= Distance of the pit latrine from the well

r=-0.273, p=0.325

There was a negative correlation between the number of *E .coli* and the distance of the well from the pit latrine (r=-0.273, p=0.325) as indicated in figure 1, though it was not statistically significant (p> 0.001) as in the ATP Microbiotest ® method. The outliers recorded were due to the short distance of the well and the gradient of the well from the pit latrine.

Fig .1: Relationship between *E .coli* and toilet (pit latrine) distance

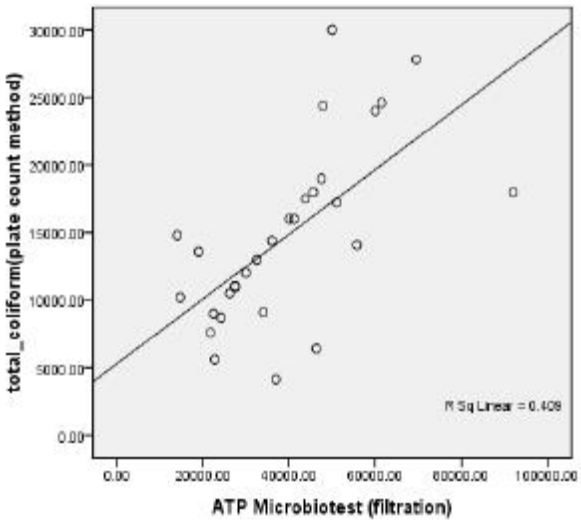
ATP Microbiotest ® method versus conventional membrane filtration plate culture technique

Using the ATP Microbiotest ® method, the median bacterial count for direct ATP microbiotest® and filtration ATP microbiotest® were 1110(653, 1437) and 37000(21750, 47900) per 100mls of water for dry season, and 1300(825, 1790) and 36000(27500, 50000) per 100mls of water for wet season. Using the conventional membrane filtration plate culture method the median bacterial count was 13600(7600, 17200) Cfu and 14400(11000, 24000) Cfu for dry and wet seasons, respectively. Further there was significant difference in the number of total bacterial count per 100ml using the conventional membrane filtration plate culture method and ATP Microbiotest ® method (χ^2=0.867,p=<0.001, χ2=3.200,p<0.001) for dry and wet seasons respectively.

Table 2: Relationship between ATP Microbiotest® rapid assay method and the conventional membrane filtration plate culture methods

Sampling site	MFT (Conventional Method)	Microbiotest	
	Median Total coliform count	Median Direct Count	Median Indirect count (Filtration)
Langas	13,600 (7600-17200) (++++)	1,790 (1650-1920) (++++)	50,000 (36000-61500) (++++)
Kimumu	14,400(11000-24000) (++++)	900 (788-1200) (++)	30,000 (26250-40000) (++++)
Elgon View	12,350 (++++)	825 (++)	27,500 (++++)

The Microbiotest® method showed high values of bacterial count as compared to the membrane filtration technique as shown in table 2. This in turn increased the sensitivity of microbial detection using the Microbiotest filtration technique (indirect) by an average value of 2.7 times in comparison to conventional method. The median direct Microbiotest® count method sensitivity was found to increase by 31.50 times to that of the indirect Microbiotest technique (filtration).



r=0.64, p=0.002

As indicated by the scatter plot in fig 2, there was a significant positive correlation between Cfu and RLU(r=0.640, p=0.002) which showed that, as the ATP numbers (RLU) increases, the amount of bacterial total coliform units (Cfu) also increased. There was a significant difference in the number of bacterial counts between wells with top cover and those without using the ATP Microbiotest ® method which measured the amount of bacteria in RLU (χ^2=0.067, p= 0.037 and χ^2=3.200, p= 0.021and) respectively. Those without top cover had the highest bacterial count as compared to those with top cover.

Fig 2: Relationship between ATP Microbiotest ® method and Convectional membrane filtration plate count method

DISCUSSION

From the study it was found that most of the wells were poorly constructed in almost all the sampled locations, and the ones which were properly constructed with a top then a pit latrine was found located just a few meters from the well, therefore increasing the amount of microbial contaminants. In Elgon View a low population density area of the Eldoret Municipality there was an extensive use of the sewerage system and the low contamination reported was thought to be from the leaking sewer pipes since the existence of a public sewer system does not assure that other sources of wastewater don't contaminate the aquifers (Hirata *et al.*, 2002).

However no significant relationship in electrical conductivity between wet and dry seasons in Langas, Kimumu and Elgon View areas of the municipality. This implies that while seasonal changes caused variations in electrical conductivity levels, rainfall had no significant influence. The findings suggest that direct ingress of bacterial contaminants through the study soils to the well is prevented due to low permeability of the soils. Temporal linked variations could therefore be attributed to the precipitation of ions to the sediment and evaporation or changes in pollution levels, Spatial linked variation could only be as a result of the differences in the soluble mineral

content of the geological material (Otieno, 2001). The median pH for the well water obtained in Langas, Kimumu and Elgon View were found to be within the recommended range by WHO and NEMA being 6.5-8.5 and 6.0 and 9.0 respectively. The recommended range of pH for drinking water acceptable by World health organization(WHO) standards is 6.5-8.5 and 6.0 and 9.0 respectively (Harp, 2000). The study further showed that the median ATP microbiotest bacterial counts were low during the dry season and high in the wet season.

These results when compared to plate culture method showed high bacterial counts in both dry and wet seasons respectively and that the two methods showed a positive correlation when compared together. The results were therefore comparable with a previous study by Chen *et al.*, 2006 on comparison of a rapid ATP Microbiotest® assay and standard plate count methods for assessing microbial contamination of water. A strong positive correlation between relative light units (RLU) and colony forming units (Cfu) in drinking water samples was established as shown in fig 2. In his findings Chen *et al.*, 2006 found out that the ATP Microbiotest® method detected more bacterial contaminants for the same amount of water sample measured than the convectional plate culture method. Both the direct and filtration ATP Microbiotest® method proved to be more reliable and sensitive in the screening of the microbial contaminants which were detected high above those of the convectional membrane filtration plate culture method. The findings from this study therefore seemed to compare very well with those of Chen *et al.*, 2006 as stated above and those of Hirata *et al.* ,2002 were he found out that Poorly constructed wells were the cause of elevated pathogenic bacterial contamination in the Patino Aquifer in Asuncion, Paraguay, where 70% of drilled wells are contaminated with feacal coliforms. Hirata *et al.*, 2002 also found out that in the medium density and low density residential areas of Sao Paolo, Brazil, 60% of drilled wells without top covers had high counts of pathogenic microbial contamination. In another study done by Mangore and Taigbenu (2004) in Zimbabwe it was found that 27% of wells in Bulawayo were contaminated with coliform bacteria which were thought to be caused by leaking sewers.

Therefore from the above study ATP Microbiotest® method provided a rapid means of enumerating total numbers of viable bacterial cells than the convectional membrane filtration plate culture method, and that all the wells in the sampled locations of Eldoret Munipality Uasin Gishu County were all found to be feacally contaminated.

However the above study experienced a shortage of ATP Microbiotest® reagents thus limiting the sampling locations(residential estates) to three which represented the low, medium and high density residential areas within the Municipality.

CONCLUSION

The results showed that ATP Microbiotest® method provided a rapid means of enumerating total numbers of microorganisms in well water as compared to the other conventional methods like the membrane filtration plate culture method and the most probable number (MPN) methods. The high density residential area namely Langas had the highest number of bacterial contaminants. The study also showed that there was no significant correlation between groundwater qualities in all the three studied locations because the sources of groundwater contaminations were all different from each location. The construction of pit latrines, leaking

sewer pipes, and poor abstraction methods were the main cause of contamination or increased level of bacterial ATP count

RECOMMENDATION

The Municipality should provide an increased coverage of the Sewerage system in all the residential areas especially the high density areas where the most urban poor live. The Municipality must also develop a clear definition of water rights (separate from property rights) by enforcing licencing and fee payment for sustainable groundwater exploitation.

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Evaluation of Climate Change Adaptation Strategies among Smallholder Farmers in Bungoma County, Kenya

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Abstract

Climate change exacerbates the already daunting challenge facing the agricultural sector, and this is particularly the case in developing countries. Innovations in agriculture have always been important and will even be more vital in the context of climate change as it allows farmers to adapt efficiently to the changing climate. There are roughly 800 million food insecure people in the world today, each having this status because food is unavailable, unaffordable or they are too unhealthy to make use of it or some combination of the three. Assessing the potential effect of climate change on food production requires understanding the underlying determinants of climate change adaptation strategies in Bungoma County and how they have affected smallholder farming. The objectives of the study were to identify and evaluate indigenous and emerging climate change strategies currently in use by smallholder farmers in the study area. Quality extension services, credit facilities and access to information are usually vital in facilitating adoption of better and affordable climate change coping strategies which enhances small holder's food production. The study identified various indigenous and emerging adaptation strategies and evaluated socio-economic and institutional factors influencing the choice of these strategies. The theory of utility, stated and revealed preference were used in the study. Purposive, multistage and systematic random sampling methods were used to select a sample of 150 smallholder farmers. Structured questionnaires and Participatory Rural Appraisal approach were the techniques used to collect data. The method of data analysis was both qualitative and quantitative. Mulching and soil fertility management were the most common coping and emerging strategies respectively. Unpredictable rainfall pattern and high temperatures were found to have adversely affected food production and rural livelihoods. Adaptations outside of agriculture were also important for livelihood diversification and increasing resilience to climate variability in study area. Government, research institutions and stakeholder need to provide climate change information to farmers through training. Soil fertility and water management were crucial in ensuring farmers adapted to climate change. Investments in infrastructure such as roads and irrigation systems, extension services, credit schemes, and climate information systems would help create the enabling conditions for adaptation to climate change.

Key words: Climate change, adaptation, adaptation strategies, food security and smallholder farmer

Introduction

Climate change has emerged as one of the defining political and socioeconomic issues of the twenty-first century. Although it has been part of the scientific agenda since the 1970s, it only really began to attract widespread international attention during the 1990s. Climate change is a complex issue that covers the full spectrum of scientific, economic, social, and political disciplines, and few people have the opportunity to attain a comprehensive and in-depth understanding of all facets of climate change. Over the past two decades, enormous progress has been made in the understanding of climate science, the likely repercussions of a changing climate on human and natural systems, and the options that are available to reduce the extent of future climate change (Anita *et al.*, 2010)

People have experienced climate change and adapted to it since human species evolved. The invention of agriculture was almost certainly a major adaptation to climate change. Yet much of what people have developed in response to climate change in such areas as domesticated crops, dry land management and many water harvesting techniques have been lost (Agarwal and

Narain, 1997). In times of disaster and climate change people depend on diversity of crops and livestock and their varieties of wild crops and animals which are more resistant to adverse climatic conditions. For many years, people have been fighting loss of biodiversity and adapting to climate change through migration, irrigation, water conservation techniques and reclamation. There are roughly 800 million food insecure people in the world today (FAO, 2002), each having this status because either food is unavailable to them, is unaffordable, or they are unhealthy to make use of it – or a combination of the three. Assessing the potential effects of climate change on food security requires an understanding of the underlying determinants of these three aspects of food security; availability, accessibility and utilization as well as how climate change affects each. From a public policy perspective, it is vital that adaptation strategies are integrated in the farming process as much as possible and those strategies which maintain or increase the resilience of farming systems are promoted.

There are three ways in which climate affects agriculture (Kurukulasuriya and Rosenthal, 2003). Firstly, changes in temperature and precipitation directly affect crop production and can even alter the distribution of agro-ecological zones. Secondly, runoff or water availability is critical in determining the impact of climate change on crop production, especially in Africa. Lastly, agricultural losses can result from climate variability and the increased frequency of changes in temperatures and precipitation (including droughts and floods).

Adaptation to Climate Change

Adaptation to climate change refers to adjustment in natural or human systems in response to actual or expected climatic stimuli or their effects, which moderates harm or exploits beneficial opportunities (IPCC, 2001). Common adaptation strategies in agriculture include use of new crop varieties and livestock breeds that are better suited to current climatic conditions. Other strategies are irrigation, crop diversification, adoption of mixed crop and livestock farming systems, and changing planting dates (Kurukulasuriya and Mendelsohn, 2008). Climate change adaptation strategies are characterized by adjustment in ecological, social or economic systems in response to observed or expected changes in climatic stimuli and their effects and impacts in order to alleviate adverse impacts of change or take advantage of new opportunities. Adaptation can, therefore, involve building adaptive capacity thereby increasing the ability of individuals, groups, or organizations to adapt to changes and implementing adaptations decisions, that is, transforming that capacity into actions. Hence adaptations strategies are continuous stream of activities, actions, decisions and attitudes that informs decisions about all aspects of life, and that reflects existing social norms and processes. Anita *et al.,* (2010) point out that some adaptations occur without explicit recognition of changing risk, while other adaptations incorporate specific climate information and decisions. Since unintentional adaptation has the capacity to reduce the effectiveness of purposeful adaptation, the integration of adaptation actions and policies across sectors remain a key challenge to achieve effective adaptation in practice.

Major types of adaptations include reducing sensitivity of the affected system, which can be achieved, for example, by investing in flood defense or increased reservoir storage capacity; planting drought resistant crops or keeping drought resistant livestock that can withstand more climate variability; or ensuring that infrastructure in flood prone areas is constructed to allow flooding. Altering the exposure of a system to the effect of climate change can be achieved, for example, by investing in hazard preparedness and early warnings, such as seasonal forecasts and anticipatory actions. Also increasing the resilience of social and ecological systems, which can be achieved through generic actions which aims to conserve resources, but also includes specific

measures to enable specific population recover from loss (Anita *et al.*, 2010). The question then becomes how will adaptation be possible, on the basis of what knowledge, with the aid of what kind of innovations, within what institutional arrangements, generating what kind of conflict within and across societies, and how far and what are the limitations of societal systems to adapt to changing climatic conditions.

Studies indicate that Africa's agriculture is negatively affected by climate change (Pearce *et al*., 1996). Sub-Saharan Africa is currently the most food-insecure region in the world (The World Bank, 2008). Climate change could aggravate the situation further unless adequate measures are put in place. For smallholder farmers in Kenya, environmental and social consequences of climate change especially put their livelihoods at risk. In the recent past in Bungoma County, (PDA, 2010) farmers have tried to use indigenous knowledge to adapt to the changes. However, the adaptation strategies that are in place have not shown meaningful improvement and smallholder farmers continue to get less and less yields each year (FAO, 2002).

Adaptation to Climate Change in Kenya

Kenyan economy largely depends on agriculture and like other parts of the world has been experiencing pronounced climatic patterns since 1990. Because Bungoma County's agriculture is mostly rain fed, the pattern of food production has similarly been fluctuating and rapidly tending towards food insecurity. All these have negatively affected livelihood of smallholder farmers in the area. However, farmers in the County have adapted to strategies to counter the effects of changing climatic patterns (PDA, 2010). The effects of the adapted strategies have not been evaluated. Moreover, there has been little research done on evaluation of climate change adaptation strategies and their effect on food production in Kenya in general and Bungoma County in particular. These issues need to be addressed and documented.

The performance of agricultural sector is determined by efficiency of crop and livestock production which depends on a large number of factors. Most important are edaphic and climatic factors. The declining agricultural productivity in Kenya is worrisome and a real challenge for a government with a population of approximately 40 million to feed. Worse still is the expected adverse impact of global warming on agriculture in future. Bungoma County has been rich in crop and livestock production but the yields have been declining from 1990s (PDA, 2010). Against this background of limited arable land, predicted adverse climate conditions and declining agricultural productivity, the biggest challenge facing Bungoma County is how to intensify food production so that output can keep pace with rapid population growth without a large increase in land devoted to food production. Currently agricultural intensification is based on combination of inputs such as fertilizer and pesticides, plant breeding technologies, irrigation and improved agricultural practices such as multiple cropping. However, productivity continues to be undermined by unpredictable weather conditions and declining soil fertility. A better understanding of indigenous coping strategies and ongoing adaptation measure is important to inform policies aimed at promoting successful climate change adaptation strategies. While there is a growing body of knowledge on the effects of soils in agricultural productivity, there is a dearth of literature on the evaluation of climate change adaptation strategies in Bungoma County. In addition, adaptive mechanisms smallholder farmers use to circumvent the welfare impact of climate change have not been adequately studied in Bungoma. The study addressed these research gaps.

Theoretical Framework

One of the theories that are behind consumer behaviour in economics is the theory of utility. Utility as a concept in economics is seen as an abstract measurement of the degree of goal-attainment or want-satisfaction provided by a product or service. This is what informs the theory behind this study. One cannot measure directly how much utility a person may gain from a product or a service. However, inferences can be made about utility based on the person's behaviour, if it is presumed that people act rationally. In economics as explained by Train (2003), there is an assumption that a rational person acts to increase her utility.

Revealed preference theory is a method by which it is possible to discern the best possible option on the basis of consumer behaviour. Essentially, this means that the preferences of consumers can be revealed by their purchasing habits. Revealed preference theory came about because the theories of consumer demand were based on a diminishing Marginal Rate of Substitution (MRS). This diminishing MRS is based on the assumption that consumers make consumption decisions based on their intent to maximize their utility. While utility maximization was not a controversial assumption, the underlying utility functions could not be measured with great certainty. Revealed preference theory was a means to reconcile demand theory by creating a means to define utility functions by observing behaviour. Revealed preference methods use actual choices made by consumers.

Stated preferences are elicited directly based on hypothetical, rather than actual scenarios. Stated preference methods are criticized because the behaviour they depict is not observed and thus they generally fail to take into account certain type of real constraints (Louvier *et al*., 2000). Swait *et al.* (1994) explains that stated preferences can be used to cover a wide to cover a wide range of proposed quality or quantity changes in the attributes of public good. Hence they can be used to consider an array of choices that are fundamentally different than existing ones, as well as exploit information about attributes trade off. Revealed preference d ata have high "face validity" because the data reflect real choices and take into account various constraints on individual decisions such as market imperfections, budgets and time. Recent research indicates that combining the stated and revealed preferences methods through data fusion, which also known as data enrichment method, builds on the strengths and diminishes the drawbacks of each method. Haab *et al.* (2002) notes that the amount of information increases, and findings can be cross-validated. Use of revealed preference data ensures that estimation is anchored in observed behaviour. At the same time inclusion of stated preference responses to hypothetical changes enables identification of parameters that otherwise would be identified.

Conceptual Framework

Farmers will choose a climate change adaptation strategy which will increase their ability to satisfy their need of maximum food production. The indigenous coping strategies are mostly observed in their farms and we see them as revealed preferences. The stated preferences may be the emerging ways of adapting to climate change which may not be currently observed on their farms. The vulnerability context frames the external environment in which people exists. Peoples' livelihood and the wider availability of wealth are fundamentally affected by critical trends as well as by shocks and seasonality over which they have limited or no control. Shocks can destroy wealth directly in case of floods, drought and storm and also force people to abandon their home area and dispose assets such as land, livestock and produce prematurely as part of the adaptation strategy. Trends may be dangerous, though they are more predictable.

They have a particular important influence on rates of return and economics to chosen livelihood strategies. Seasonal shifts in prices, employment opportunities and food availability are one of the greatest and most enduring sources of hardship for poor people in developing countries.

The interactions between dependent and explanatory variables are illustrated in figure1. Human interference through activities can emit greenhouse gases into the atmosphere leading to climate change. Adaptation strategies through policy responses can result into positive outcomes of increased food production as the smallholder famers need to adapt to these climate changes. Effective adaptation coupled with policy responses lead to outcome of increased food production, livelihood diversification, increased farm income, Soil and water conservation and reduced pest and disease infections.

Research Methodology

Study Area

The study covered Bungoma County which occupies a total of about 2,068.5 square kilometers with a population of roughly 1,630,934 people and a population density of 482 persons per square kilometer (KNBS, 2009). The County is located between longitude 34 º 21.4` and 35 º 04` East and latitude 0 º 25.3 and 0º 53.2` North. There is a bimodal rainfall pattern; the long rains (March–July) and the short rains (August-October). The annual rainfall ranges between 1250 and 1800 mm. The altitude ranges between 1200 and 2000 meters above Sea Level (A.S.L) and temperature ranges from 21-25^{0}C during the year (GoK, 2005). The County is endowed with well-drained, rich and fertile arable soils but poor husbandry methods and a bulging population have resulted in declining yields, deforestation and soil erosion. Small scale crop and livestock production has been an important component of agricultural activity in this area. Crops commonly grown include; maize, sunflower, sugarcane, coffee, tobacco, potatoes, beans, kales, groundnuts and bananas. Livestock production includes; dairy cattle, goats, sheep and chicken. Out of the total labour force of about 565,000 people, 52% are engaged in agricultural production which provides 60% of all household incomes, 19% have wage-employment and 13% are self-employed (GoK, 2005). The number of unemployed is estimated at 200,000 people and 60% of the population lives below the poverty line. The poverty incidence in Bungoma is higher than the national average of 53% (GoK, 2005). Bungoma County was selected because it was one of the County's in Kenya which had high agricultural potential and with different agro-ecological and livelihood differences. The livelihood of smallholder farmers' have been affected by declining productivity and this was made worse by climate change. Secondly, the population growth in the County was high compared to the land resource available and thus there was need to evaluate climate change adaptation strategies so as to come up with sustainable food production system. Thirdly, the population growth rate was high compared to the land resource available hence the need to implement strategies to cope with climate change under intensive farming system.

Sample size and sampling procedure

The population for this study consisted of all smallholder farmers in the study area.

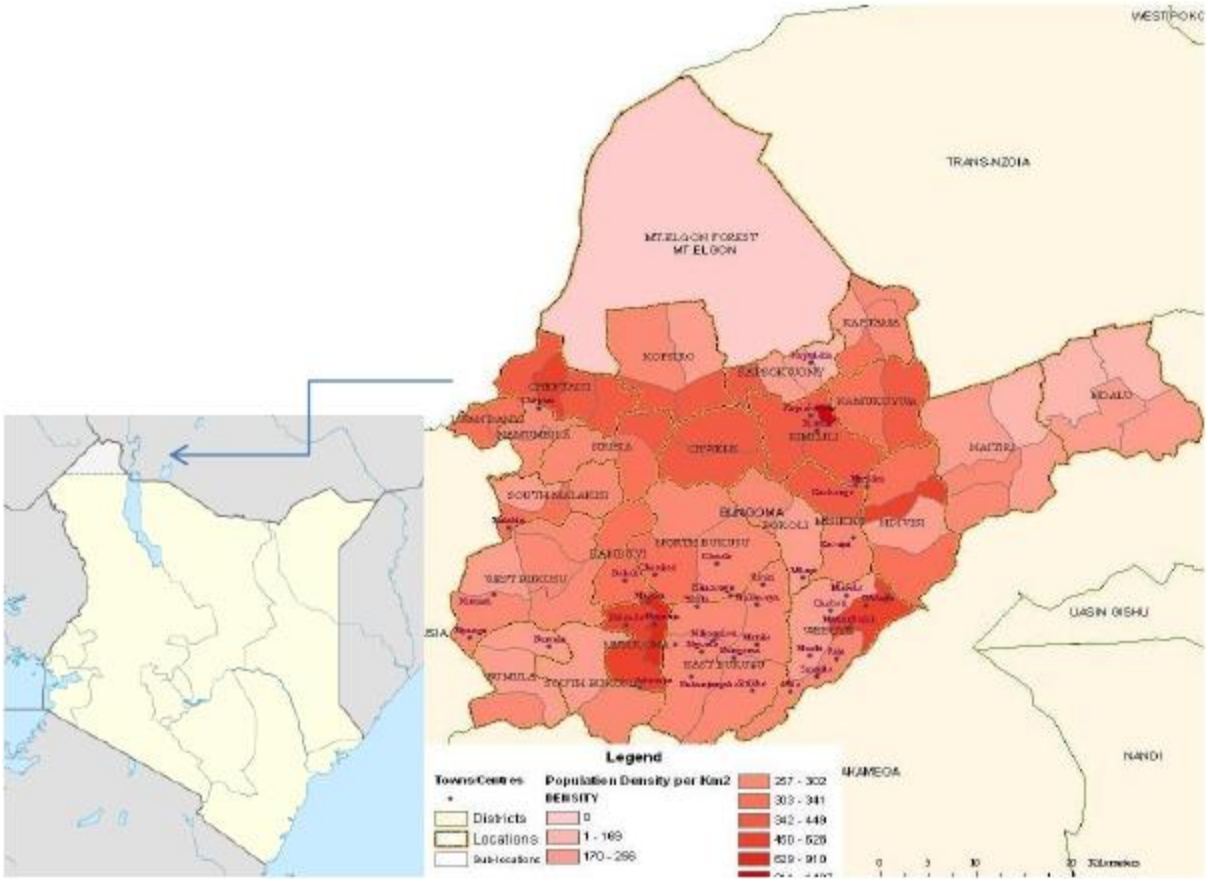

Figure 2: Map of Kenya showing Bungoma County and also map of Bungoma County (Source: Bungoma DDP, 2005-2010

A structured questionnaire was employed to obtain data while multistage sampling procedure was used to select the respondents. Purposive sampling was used to select 3 districts in the county with different livelihoods and agro ecological zones. At the second stage was to employ systematic random sampling was employed to select proportional number of farmers from each of the three districts. A sample of 140 farmers was selected from the population of smallholder farmers in the county. The required sample size was determined by proportionate to size sampling methodology (Anderson *et al.*, 2007).

$$n = \frac{pqZ^2}{E^2}$$

Where n = sample size, p = proportion of the population containing the major interest, q =0.9 = 1-p, z = confidence level ($\alpha = 0.05$) and E = acceptable or allowable error. Since the proportion of the population is not known then, p=0.9, q = 1-0.9 = 0.1, Z = 1.96 and E = 0.0500. This resulted to a sample population of 140 respondents but for easier divisibility of three districts, 150 farmers were selected. Systematic random sampling was used to select respondents from a list alphabetically arranged.

Data Collection and Analysis

The study used both primary and secondary data. Primary data were collected by use of questionnaires and a checklist. Structured questionnaire schedules were used in the individual interviews and administered by trained enumerators. A checklist was facilitated by involvement of the target community in sharing their lived experiences thus enabling generation of practical information on current indigenous (traditional) and adaptation strategies. This was done in two steps. Firstly, smallholder farmers in focal groups were asked to identify and categorize indigenous and emerging climate change adaptation strategies which they were themselves using and those used by other farmers in the study area. Secondly, they were required to evaluate these strategies using a checklist.

Results and Discussion

Household Characteristics of Farmers in Bungoma County

The mean age of the household head in the study area was about 41 years. The mean age for adapters of CCAS was about 40 years while that for non-adapters was about 52 years (Table 1). Age of the household head plays a key role in determining the decision to adapt to CCAS. Result of two-tailed t-test show that age was statistically significant at 1% indicating that non adapters of CCAS were more elderly than adapters. The mean number of children was about 4 for adapters of CCAS and about 7 for non-adapters. Result of two- tailed test show that number of children was statistically significant at 1% indicating that adapters had less children compared to non-adapters. Households with large families are sometimes forced to divert part of labour force to off-farm activities in an attempt to earn income in order to ease consumption pressure imposed by a large family. The mean farm size was 4.06 acres for adapters of CCAS and 5.29 acres for non- adapters (Table 1). The adapters had less land compared to non-adapters. Studies show land size has both positive and negative effect on adaptation. However, result of two-tailed t-test show that land size was statistically insignificant indicating that adapters and non-adapters sizes of land was nearly equal in terms of adaptation to climate change strategies.

Table 1: Description of farm and farmer characteristics in Bungoma County, Kenya.

Characteristic	Mean		Overall	t-ratio	Sig
	Adapters	**Non-adapters**			
Age(Years)	40.28	51.60	41.70	-3.549***	0.001
Number of children	3.72	6.60	4.1	-3.906***	0.000
Land size(Ha)	4.06	5.29	4.22	-1.66	0.245
Experience(Years)	13.40	20.00	14.28	-3.031***	0.003
Income (KES)	22423.32	284717.05	228995.82	-1.053	0.294
Extension(Contacts)	1.21	0.100	1.06	1.946*	0.054
Training(Contacts)	2.44	2.75	2.48	-0.366	0.715
Credit (KES)	32976.92	16500.00	30780.00	1.245	0.215

***** Significant at 1%; and * significant at 10 %**

In terms of farming experience, the mean number of years of farming was about 13 years for adapters of CCAS and about 20 for non-adapters (Table 1). However, result of two-tailed t-test showed that experience was statistically significant at 1% indicating that the more the years of farming experience the less the adaptation of CCAS. This may indicate that some farmers may have had bad experiences of this CCAS and decided to abandon them. This may also imply that those farmers who had less farming experience, mostly young, were more risk takers thus more likely to adapt to climate change strategies.
The mean income was KES. 22,423.32 for adapters of CCAS and KES. 28,471.05 for non-adapters. Result of two-tailed t-test show that off-farm income was statistically insignificant. It also indicated negative relationship between income and adaptation of CCAS. This meant that the more the income the less the adaptation of CCAS.

Adapters of CCAS had a mean of 1.21 contacts per year with extension officers as opposed to a mean of 0.100 contacts for non-adapters. Result of two-tailed t-test show that extension was statistically significant at 10% indicating that adapters of CCAS had more extension services than the non-adapters. The number of contacts with extension officers is a proxy measure for access to information (Adesina *et al*., 2000) and this positively contributes to awareness and subsequent adoption of new technologies. Agricultural extension agents frequently provide different messages throughout the year depending on prevailing activities and this could impact farmers differently.

In terms of training sessions, adapters of CCAS had a mean of 2.44 training contacts per year compared to non-adapters who had a mean of about 2.75 training contact (Table 2). Result of two-tailed t-test show that training was statistically insignificant. Training was negatively related to adaptation of CCAS. This meant that the more farmers were exposed to training the less they adapted CCAS. Training may have been on other aspects of farming other than on climate change. The mean credit for adapters of CCAS was KES 32,976.92 while for non-adapters was KES 16,500.00. The relationship between credit and adaptation of CCAS was positive. However, two-tailed test showed that credit was statistically insignificant indicating that adapters and non-adapters were equally availed with credit facilities.

Education level of the household head between adapters and non-adapters of CCAS was characterized as presented in Table 3. Adapters of CCAS had the highest percentage in Primary (17.7%) and tertiary (16.9%) educational levels while non-adapters had the highest percentage in non-formal (5%) and secondary (75.00%) and university (5%) educational levels. This implies that CCAS is more likely to be understood by farmers who had many years of education. Overall, a low percentage of farmers (5.00%) had attained university education. Result of a chi-square show that education was statistically insignificant showing that education level of the household head between adapters and non-adapters of CCAS was equally distributed.
Among the adapters of CCAS, 25.38% of the household heads were female and 74.62% were male while 20% of the household heads were female and 80% were male among the non-adapters (Table 2). Result of a chi-square show that gender of the household head was statistically insignificant indicating that gender of the household head between adapters and non-adapters of CCAS was equally distributed. Male-headed households, particularly in developing countries, have a higher accessibility to the requisite resources and information that gives them a

higher chance of adopting new innovations (Odendo *et al.,* 2009). In terms of group membership, 62.3% of the adapters of CCAS belong to a farmer group while 37.7% were not in farmer groups (Table 3). Among the non-adapters of CCAS, 45% were in farmer groups while 55.00% do not belong to a farmer group. Result of a chi-square show that group membership was statistically insignificant thus equally distributed.

Table 2: Categorical characteristics of the household head in Bungoma County

Characteristic	Category	Percentage			Chi-square	Sig
		Adapters	**Non-adapters**	**Overall**		
Education	Non formal	3.1	5.00	3.3		
	Primary	17.7	15.00	17.3		
	Secondary	60.8	75.00	62.7		
	Tertiary	16.9	0.00	14.7		
	University	1.5	5.00	2.0		
	Total	86.67	13.33	100	5.248	0.263
Gender	Male	74.62	80.00	75.33		
	Female	25.38	20.00	24.67		
	Total	86.67	13.33	100	0.270	0.603
Group Membership	Yes	62.3	45.00	64.7		
	No	37.7	55.00	40.0		
	Total	86.67	13.33	100	2.163	0.141

***** Significant at 1%; and * Significant at 10%**

Identification of Indigenous Climate Change Coping Strategies carried out by Smallholder Farmers in Bungoma County

Characterization of CCAS was done in order to determine how the practices varied across farmers in the study area. The results are given in figure 2 which shows the proportion of farmers (in percentage) practicing each coping strategy. The indigenous coping strategies were divided into crop and livestock strategies.

Indigenous Crop Coping Strategies

From the results, 24.4 % of small holder farmers used mulching as a strategy to combat climate change. This was because it was easy to get mulching materials as they are locally available and most of them had local knowledge on how to use the strategy. Tree planting strategy was used by 19.2 % of farmers. This was the second common strategy because of accessibility of tree seedlings to be planted and farmers had local knowledge on tree planting thus did not require more training. Planting of cover crops mostly sweet potatoes was at 14.6%. Farmers preferred sweet potatoes because apart from being utilized as food its vines were also used as livestock feed. Intercropping of crops like maize and beans, sugarcane and beans, millet and maize was practiced by 9.1%. Agroforestry, planting drought resistant crops, early planting, crop rotation and growing short seasoned crops strategies were at 8.7%, 7.7%, 6.6%, 5.6% and 4.2% respectively.

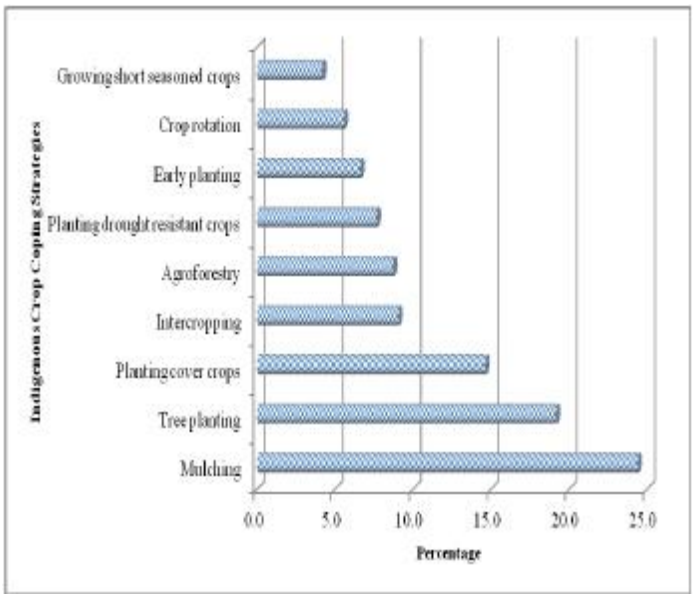

Figure 3: Indigenous crop coping strategies carried out by smallholder farmers in Bungoma County

Indigenous Livestock Coping Strategies

Bungoma County is a predominantly crop growing region. This could be one of the reasons why 32.59% of farmers had no any indigenous livestock coping strategy. Cross breeding was undertaken by 24.11% of farmers as a strategy in improving the local breeds so as to enhance productivity and was also cheaper than rearing pure breeds. 20.54 % of livestock farmers preserved livestock feed which they did use during unfavorable climatic conditions. Rearing local livestock, planting napier grass, Rearing mixed livestock, zero grazing and paddocking strategies were 12.05%, 3.57%, 2.68%, 2.68 and 1.57 % respectively. This can be illustrated below in figure 4

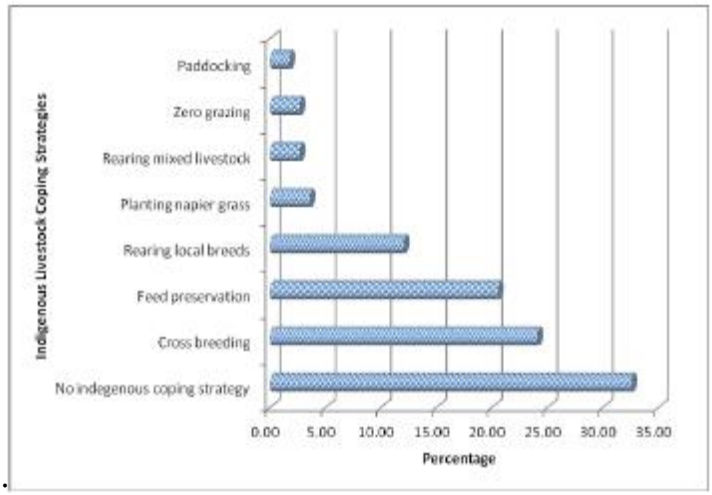

Figure 4: Indigenous livestock coping strategies carried out by smallholder farmers in Bungoma County

Emerging Crop Adaptation Strategies

Tree planting was the most common emerging crop adaptation strategy as it was preferred by 24.9 % of farmers. This has been due to encouragement by the public extension officers to farmers of planting at least 10% of their land acreage. Fast growing tree varieties especially *Eucalyptus species* from South Africa and *Gravilliea spp* have been extensively promoted by the Ministry of Agriculture and Ministry of Environments and Natural Resources for wood fuel and also timber production.

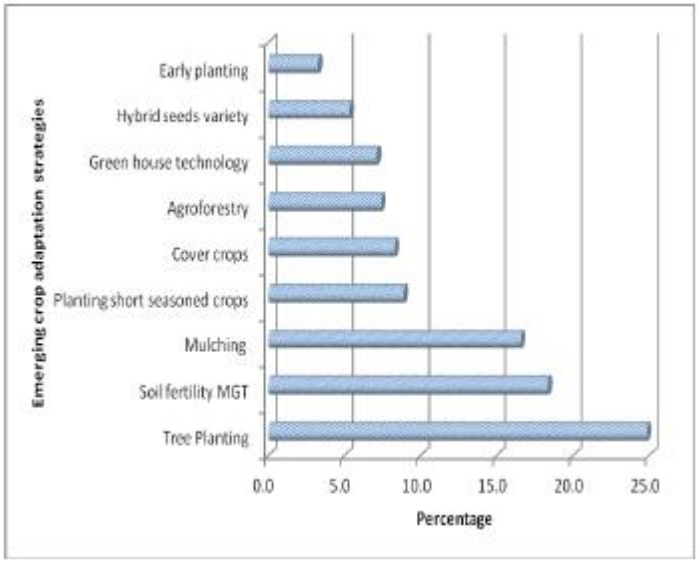

Figure 5: Identified emerging CCAS carried out by smallholder farmers in Bungoma County.

Due to declining fertility of the soils which have negatively affected productivity of farming in Bungoma County 18.3 % of small holder farmers preferred soil fertility management as a strategy in dealing with climate change (Figure 5). Soil fertility management strategies like minimum tillage, organic farming, terracing and building gabions were used by farmers. 16.6 % of farmers practiced mulching as an emerging strategy because mulching materials are cheap, locally available and can also be used as manure and soil conservation measure. As a result of soil cover by vegetation and residues, soil erosion through runoff are eliminated or greatly reduced thus crop production is more reliable. Smallholder farmers who planted short seasoned crops as a means of mitigating against climate change were 8.9 %. Cover crops and Agroforestry strategies were selected by 8.3% and 7.4% respectively. Cover crops like sweet potato were both used as food and livestock feed in form of vines thus having a dual purpose. Agroforestry strategy was being promoted by Kenya Forestry Services and Kenya Forestry Research Institute where farmers are encouraged to grow improved fallow crops. Green house technology was being practiced by 7.1%. Planting hybrid seed variety and early planting strategies were practiced by 5.3 % and 3.3% of farmers in the study area respectively.

Emerging Livestock Adaptation Strategies

Few smallholder farmers in Bungoma County rear livestock as the area is more suitable for growing crops. This was the reason why 30.7 % of farmers did not adopt to any emerging livestock adaptation strategy. Zero grazing was practiced by 18.1 % of smallholder farmers in the study area. This was because of low acreage of farms owned by these farmers. Feed preservation strategy was embraced by 14.7% of farmers. This was mostly through preservation of maize stovers, maize cobs and sugarcane tops. 9.7 % of farmers did paddocking of their land thus ensuring efficient grazing systems on the land. Cross breeds were kept by 9.2 % of farmers and because of increased productivity compared with indigenous cattle. Smallholder farmers who kept pure breeds were 6.7%. This low percentage was due to high costs of purchasing the breeds and also managing them in terms of housing, feeding, breeding and treating pure breeds though they were highly productive. Planting new varieties of napier grass, artificial insemination and rearing different breeds were emerging livestock adaptation strategies by 4.6%, 4.2 % and 2.1 % of farmers respectively (Fig.6).

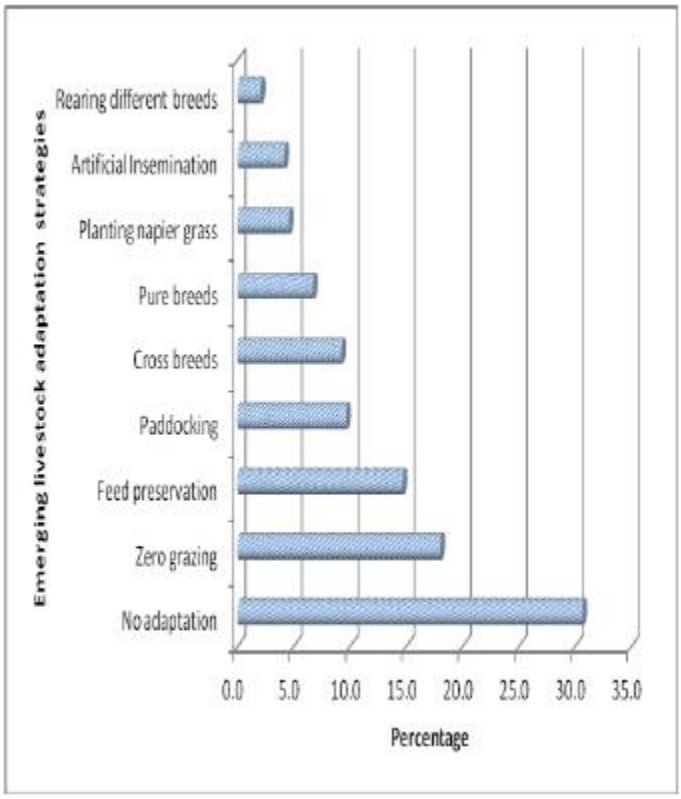

Figure 6: Emerging livestock adaptation strategy carried out by smallholder Farmers.

Conclusion.

Small holder farmers in the study area adapted to climate change by using different methods which were included in this study. Those who did not use any of the methods considered lack of information on climate change adaptation strategies as a constraint to adaptation. Climate change was a new phenomenon in the study area hence most of the trainings organized in the study area were on other issues rather than climate change and that was the reason why training was

insignificant but negatively related to adaptation to climate change. The smallholder farmers in the study area were predominantly crop growers. Climate change had adversely affected production of maize, beans and sugarcane which are their common crops. Most young farmers were willing to adapt to climate change strategies but cited lack of capital as a constraint. From the study, various indigenous coping strategies were identified for both crop and livestock production. Climate change was a new phenomenon in the area though smallholder farmers' perception of climate change was that temperatures were rising while level of precipitation was declining. Mulching was the most common indigenous crop coping strategy because mulching materials are cheap, locally available and most farmers had local knowledge on how to use the strategy. Tree planting, planting cover crops intercropping and planting drought resistant crop varieties were some of the other indigenous crop coping strategies. Cross breeding was the most common indigenous livestock coping strategy. The cross breeds produced more milk compared to local breeds and were also disease resistant and manageable in terms of feeds compared to pure breeds. Feed preservation, rearing mixed livestock and planting napier grass were other livestock coping strategies.

Declining soil fertility has negatively affected productivity of farming in the study area. Soil fertility management strategies and mulching, tree planting, planting short term crops and cover crops are some of the emerging crop adaptation strategies. Zero grazing, paddocking, cross breeds, pure breeds and feed preservation are some of the common livestock adaptation strategies. Farmers in most sites stressed soil and water conservation measures and fertility restoration through the use of manure and compost (but also inorganic fertilizer). Men cited planting trees and cover crops that help improve soil fertility and the need to combat soil erosion. The common farmers' adaptation strategies in the study area were growing a variety of crops, feed preservation, time of planting, rearing different breeds of cattle and soil fertility management. This was done to spread risks involved in farming due to unpredictable weather changes caused by climate change. Participants in the study emphasized community-based organizations and farmers' groups as key to adaptation to climate change. They recognized that such organizations enable farmers to exchange information, establish rotating credit schemes, access training and technologies, and secure better prices and markets. There is, therefore, need to aggressively create more awareness through trainings on climate change in the study area.

Policy Recommendations.

Adaptation of new and appropriate farm practices or technologies requires knowledge and experience. Successful adaptation of these measures will require greater access to information and advice through extension services, and access to inputs, as well as additional financial resources, particularly in the case of more costly investments such as irrigation and agroforestry. There should be more training specifically on climate change adaptation strategies and their impact on food production in the study area. Policymakers can facilitate adaptation of the most promising practices and technologies in several ways like expanding access to credit which can encourage the adaptation of more costly practices and technologies that offer multiple benefits in terms of adaptation, mitigation, and improved productivity. Promoting agricultural intensification to avoid the expansion of cultivated area, through investments in agriculture such as the provision of inputs, capacity development, and additional research and development would further facilitate the adaptation of climate change strategies.

Furthermore, though some carbon markets (such as the Clean Development Mechanism) farmers can be provided with financial incentives to smallholder farmers for soil carbon sequestration. These opportunities should be further explored while international climate negotiators intensify efforts to create additional incentives for agricultural mitigation.

Government investments in infrastructure such as roads and irrigation systems, demand driven extension services, affordable credit schemes, and climate information systems would help create the enabling conditions for adaptation to climate change.

Diversification of income sources is also a key adaptation strategy that should be encouraged further. This may include highly targeted efforts to broaden income-generating opportunities by creating opportunities for off-farm employment. Major changes within the agricultural system may be required in order to protect livelihoods and ensure food security. Responses to climate change need to encompass several levels, including crop and farm-level adaptations; collective action at the community level; and supporting policies and investments at national, regional, and global levels. This will require the involvement of all stakeholders. Potential strategies include infrastructural investment, water-management reform, land-use policy, and food trade. Conducting research on use of new crop varieties and livestock species that are better suited to drier conditions, encouraging informal social networks and investing in irrigation would be better policy interventions.

Further research.

The effect of poverty and household income on the uptake of climate change adaptation technologies should be investigated clinically in order to ensure that farmers are able to afford the technologies. Research on the relationship between farmers' perception on climate change and actual climate data in Bungoma County is important and should be conducted in order to effectively create awareness of climate change impact in the study area.

Research on the impact of climate change on the livelihoods of smallholder farmers in Bungoma County.

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Identification, Characterization and Distribution of *Aspergillus* and *Fusarium* Species Isolated from Maize Kernels from Western Part of Kenya.

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Abstract

Moulds destroy more than 30% of crop yields and produce potentially poisonous mycotoxins. The most prevalent on foods are *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Mucor*. Kenya has experienced dramatic outbreaks of mycotoxin poisoning resulting in loss of lives. The aim of the study was, to isolate and characterize moulds associated with maize from Lake Victoria Basin (LVB). Thirty samples of unaffected maize and mouldy maize were collected from Trans-nzoia, Kakamega and Kuria districts to determine the mould's distribution. These areas are in mid altitude agroecological zones with warm and humid conditions which favors development of moulds and mycotoxins. *Aspergillus* and *Fusarium* were isolated and identified from these areas. Among the genus *Aspergillus*, twelve mycotoxigenic species and two atoxigenic species were identified and among the genus *Fusarium*, fourteen mycotoxigenic species were identified. In all the three districts, the most frequent *Aspergillus* and *Fusarium* species on maize were *A. flavus* at 23.1% and *F. proliferatum* at 20.3% frequency**.** The quantity of the moulds from mouldy and good maize was compared using T- test for each of the district but they were not significantly different.

Key words: Mycotoxins, moulds, maize, *Aspergillus* and *Fusarium*

Introduction

Moulds are opportunistic biological agents of ubiquitous nature (Ryan and Ray, 2004). Because of their powerful arsenal of hydrolytic enzymes, these microorganisms can cause a high degree of deterioration when present in foods and are responsible for considerable economic losses (Souza *et al*., 2005). They are known to destroy 10 to 30% of the total yield of crops and more than 30% of perishable crops in developing countries by reducing their quality and/or quantity (Matasyoh *et al*., 2009). Moulds cause extensive damage on foods, feeds and other agricultural commodities in the field, during transportation, storage and processing, leading to postharvest losses. The ubiquitous nature of moulds, their ability to colonize diverse substrates and lack of effective control measures has contributed to the high incidences of mould and mycotoxin contamination in foods and feeds. Acute mycotoxicoses epidemics occur in Africa leading to death of several hundred people. In 2004, an acute aflatoxicosis outbreak occurred in Kenya resulting in 317 cases and 125 deaths, while cases of esophageal cancer have been linked to high levels of fumonisins in Lake Victoria Basin (Azziz -Baumgartner *et al*., 2005). One of the available methods of controlling moulds is the use of synthetic chemical preservatives like sodium nitrate which have been the cause of appearance of resistant micro-organisms, leading to occurrences of emerging food borne diseases. Furthermore, other methods such as use of solar driers are expensive for the small scale farmers.

Generally when *Fusarium* species invade maize in the field they cause diseases like seedling blights, kernel, root, seed, stalk and ear rots, for example *F. subglutinans* and *F. moniliforme* causes Fusarium ear rot and stalk rots. *Fusarium graminearum* attack maize and cause stalk, cob and root rots. *Fusarium moniliforme* also causes many other diseases like kernel and root rot, seed rot and seedling blight (CIMMYT, 2004). Reports of surveys conducted in some African countries showed *F. moniliforme* as the most prevalent fungus on maize (Marasas *et al.*, 1988; Allah Fadi, 1998; Kedera *et al*., 1999).

Toxigenic fungi can attack maize prior to harvest and further decay the crop during storage. As a result, mycotoxins may form both during crop development and in storage. *Aspergillus, Fusarium, Penicillium* and *Cladosporium* are the predominant fungal genera associated with grain in storage. Mycotoxins are chemicals produced by fungi that are harmful to humans and domestic animals. These chemicals may contaminate staple foods and feeds worldwide, posing a number of significant food safety concerns (Schmale and Munkvold, 2009). They contaminate 25% of agricultural crops worldwide and are a source of morbidity and mortality throughout Africa, Asia and Latin America (Smith *et al*., 1994). They cause diseases referred to as mycotoxicoses in humans and animals (Agrios, 1997). Most mycotoxicoses are caused by the common and widespread moulds namely *Aspergillus, Penicillium* and *Fusarium.* They cause acute liver damage, liver cirrhosis, induction of tumours and attack on the central nervous system, skin disorders and hormonal effects (Erkekoglu *et al*., 2010).

The most important mycotoxins are aflatoxins, deoxynivalenol, fumonisins, ochratoxins and zearalenones. Among these the most famous are the aflatoxins which are produced by *Aspergillus fl*a*vus, A. parasiticus* and several other species of *Aspergillus* in a wide variety of agricultural commodities including grains, legumes and nuts (Turner *et al*., 2003). Acute aflatoxicosis epidemics occur in several parts of Africa and Asia leading to death of several hundred people (Varga *et al*., 2009). Aflatoxins are known to be potent hepatocarcinogens in animals and humans. Ochratoxin A, which is has been experimentally shown to be teratogenic, a potent renal carcinogenic and immunosuppressive is largely produced by *A. ochraceus* and less frequently by *A. niger* (Nielsen *et al*., 2009). As an enzyme inhibitor, ochratoxin A affects lipid peroxidation and has been implicated in Balkan Nephropathy (BENO in humans (Atrosh *et al*., 2000).
Toxin-producing fungi may invade at pre-harvesting period, harvest-time, during post-harvest handling and in storage. Toxigenic fungi can be divided into three groups: (a) field fungi namely, genus *Fusarium,* e. g *F. moniliforme, F. roseus, F. trincinctum* and *F. nivale*; (b) storage fungi which include the genera *Aspergillus* and *Penicilium*, e.g *A. flavus*, and *A. parasiticus*; and (c) advanced deterioration fungi which normally do not infect intact grains but easily attack damaged ones and require high moisture content. Examples of the third group are *A. clavatus, A. fumigatus, Chaetomium, Scopulariopsis, Rhizopus, Mucor,* and *Absidia* (Makun *et al.*, 2009).

Materials and Methods

Sample collection

A total of 30 samples categorized as unaffected maize and mould damaged grain collected randomly from various rural households and markets in Trans-nzoia (Mean Temp: 23^{o}C, RH:37-

81%), Kakamega (Mean Temp: 26^{o}C, RH: 68-83%) and Kuria (Mean Temp: 16.5^{o}C, RH :above 80%). Sampling was done at random, having liaised with the local Agricultural officer to talk to farmers who were growing maize and willing to participate in the study. In Trans-nzoia, samples were collected from Kitale central and Kiminini divisions. In Kakamega, samples were collected from Kakamega municipality division whereas in Kuria district samples were collected from Kehancha and Masaba divisions. Most of these areas are in mid altitude agro ecological zones with warm and humid conditions which favours development of moulds and production of mycotoxins (Kaaya *et al.,* 2006). These areas have unpredictable rainfall patterns making it difficult for small scale farmers to efficiently dry their produce. Ten samples, each weighing half a kilogram were collected from each district in properly labeled khaki paper bags to minimize saprophytic fungal contamination and transported in a cool box to the laboratory for analysis. Each sample was divided into two portions; one was stored at 4^{o}C and the other at -20^{o}C to avoid further accumulation of mycotoxins.

Isolation and Identification of moulds associated with maize

Moulds were isolated from the samples using the direct plating technique. For each sample, 20 seeds were picked randomly and surface sterilized by soaking for 1 minute in 2.5% of sodium hypochlorite, and rinsed in three changes of sterile distilled water. The samples were blotted with sterile filter paper and plated (five seeds per plate) on the surface of malt extract agar (MEA) and potato dextrose agar (PDA) containing 7.5% sodium chloride and 33 mg/l of streptomycin sulphate powder. Addition of streptomycin sulphate is very effective in the inhibition of bacteria. Yet, it does not inhibit the growth of other mould species, including the mycotoxin producers. Thus, identification is not compromised (Diba *et al*., 2007). The plates were incubated at 25^{o}C and monitored daily for fungal growth for up to 14 days. Treatments were replicated four times and the experiment was laid down in a complete randomized design.

Identification and Characterization of the moulds

The resulting cultures of *Aspergillus* species were identified to species level based on cultural and morphological characteristics using taxonomic keys (Kozakiewtez, 1989; Klich, 2002) and *Fusarium* species were identified based on the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006). Morphological features of moulds were studied and the major and remarkable macroscopic features that were looked at are colony diameter, colony colour on agar and reverse and colony texture. Microscopic characteristics that helped in the identification process were conidia heads, stipes, colour and length, vesicles shape and seriation, metula covering, conidia size, shape and roughness (Diba *et al*., 2007). *Aspergillus parasiticus* has green colonies but deeper in shade than *A. flavus*. *Aspergillus ochraceus has* ochre-coloured or buff colonies with submerged mycelium; conidiophores show shades of yellow in the outer layer of the wall which is rough or pitted.

For *Aspergillus niger,* the hyphae are septate and hyaline more or less yellow in colour. The colonies are black coloured and reverse usually colourless *Aspergillus versicolor's* cultures show considerable range of colour. Different strains may be variously pale green, grayish green, buff or, show patches of yellow. *Aspergillus nidulans has c*olonies of a clear green colour, developing

dirty white spots from the centre outwards. Conidiophores smooth walled, or more or less browned. The number of seeds contaminated by *Aspergillus* and *Fusarium* were counted and the infection rate (number of contaminated grain recorded as percentage) was determined and compared for each sample. Target moulds were sub-cultured to obtain pure cultures.

Data analysis

One way analysis of variance (ANOVA) was used to test whether the moulds affecting maize from the three districts are significantly different. Least significant difference (LSD) was used to discriminate which maize and from which district is highly contaminated with moulds. Students't-test was used to test if the two genera of moulds are statistically significant on the maize from the three districts. The statistical level of significance was fixed at $p< 0.05$ (95%).

Results

Moulds isolated from the maize and their incidence

Five different fungal genera; *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Mucor* were isolated from the maize samples collected from Trans-nzoia, Kakamega and Kuria districts. *Aspergillus* was the most frequently isolated genus with a 63.3% frequency.

***Aspergillus* species identified**

The moulds isolated and identified among the genus *Aspergillus* were 12 mycotoxigenic species which included *A. parasiticus*, *A. flavus*, *A. wentii*, *A. ustus*, *A. nidulans*, *A. ochraceus*, *A. tamarii, A. niger, A. versicolor, A. flavipes, A. terreus, A. fumigatus*, and two atoxigenic species, *A. humicola* and *A. sparsus* (Table 1). The resulting cultures were identified based on cultural and morphological characteristics using taxonomic keys (Kozakiewtez, 1989; Klich, 2002). Species of the genus *Aspergillus* are found almost everywhere on every conceivable type of substratum (Venkatesh, 2004).

Table 1 : *Aspergillus* and *Fusarium* species isolated from maize samples in the three districts

Maize sample	District/ Origin	Condition of sample	*Aspergillus* species isolated	*Fusarium* species isolated
KEKIM 01	Trans-nzoia	Good	*A. flavus, A. parasiticus, A. wentii, A. terreus*	*F. proliferatum*
KEKIM 02	Trans-nzoia	Bad	*A. flavus, A. parasiticus*	-
KEKIM 03	Trans-nzoia	Good	*A. flavus, A. ochraceus, A. parasiticus, A. niger*	*F. solani*
KEKIM 04	Trans-nzoia	Good	*A. flavus*	*F. solani*
KEKIM 05	Trans-nzoia	Bad	*A. flavus, A. ochraceus, A. wentii*	-
KEKIM 06	Trans-nzoia	Bad	*A. flavus, A. versicolor*	*F. solani, F. moniliforme*
KEKIM 07	Trans-nzoia	Bad	*A. versioclor*	*F. solani*
KEKIM 08	Trans-nzoia	Good	*A. ustus*	-
KEKIM 09	Trans-nzoia	Good	*A. parasiticus*	-
KEKIM 10	Trans-nzoia	Bad	*A. parasiticus*	*F. solani*

KEKAM 18	Kakamega	Good	*A. versicolor*	*F. scirpi*
KEKAM 19	Kakamega	Good	*A. flavipes*	-
KEKAM 20	Kakamega	Good	*A. flavus, A. niger*	*F. chlamydosporum*
KEKAM 21	Kakamega	Good	*A.flavus, A. niger, A. fumigatus*	*F. graminearum*
KEKAM 22	Kakamega	Good	*A. wentii*	-
KEKAM 23	Kakamega	Good	*A. ustus*	*F. trincinctum, F. chlamydosporum, F. semitectun, F. oxysporum*
KEKAM 24	Kakamega	Bad	*A. nidulans, A. terreus*	*F. nivale, F. proliferatum, F.chlamydosporum*
KEKAM 25	Kakamega	Good	-	*F. graminearum*
KEKAM 26	Kakamega	Good	-	*F. avenaceum, F. subglutinans*
KEKAM 27	Kakamega	Bad	*A. flavus, A. parasiticus*	-
KEKUM 31	Kuria	Bad	*A. flavus, A. versicolor*	*F. merismoides, F. culmorum*
KEKUM 32	Kuria	Good	*A. flavus, A. ochraceus*	*F.crookwellence*
KEKUM 33	Kuria	Good	*A. ustus, A. wentii, A. parasiticus, A. sparsus*	*F. proliferatum, F. nivale*
KEKUM 34	Kuria	Good	*A. flavus, A. niger*	*F. proliferatum*
KEKUM 35	Kuria	Good	*A. humicola*	*F. sporotrichioides*
KEKUM 36	Kuria	Bad	*A. niger, A. nidulans*	*F. merismoides*
KEKUM 37	Kuria	Bad	*A. niger*	*F. merismoides*
KEKUM 38	Kuria	Good	*A. flavus, A. tamari, A. sparsus, A. ochraceus*	*F. chlamydosporum, F. merismoides*
KEKUM39	Kuria	Bad	*A. flavus, A. nidulans*	*F. oxysporum*
KEKUM 40	Kuria	Good	*A. flavus, A. nidulans, A. versicolor, A. fumigatus*	*F. proliferatum, F. solani*

Legend for Maize Sample Codes

KEKIM: KE-Kenya	**KI- Kitale**	**M-Maize**
KEKAM: KE-Kenya	KA- Kakamega	M-Maize
KEKUM: KE-Kenya	KU- Kuria	M-Maize

Occurrence of *Aspergillus* species

The percentage occurrence of *Aspergillus* population was determined by comparing the number of seeds showing each type of mould growth for each sample. When the *Aspergillus* population was separated according to geographical areas of Western Kenya, the predominant *Aspergillus* species in Trans-nzoia was *A. flavus* (23.1%) followed by *A. parasiticus* (15.4%), *A. wentii* (7.5%), *A. ochraceus* (7.5%), *A. versicolor* (7.5%), *A. ustus* and *A. sparsus* at 7.5%. *Aspergillus flavipes, A. nidulans, A. niger* and *A. terreus* were the least isolated species at a frequency of 3.8%, while *A. humicola* and *A. fumigatus* were at 3.6%. In Kakamega district, the most prevalent *Aspergillus* species were *A. flavus, A. niger* and *A. versicolor* at 15%, followed by *A.*

parasiticus, A. flavipes, A. nidulans and *A. fumigatus* at 10%, *A. ochraceus, A. humicola* and *A. sparsus* at 5%. In Kuria district, the most frequent *Aspergillus* species isolated were *A. flavus* (20%), followed by *A. niger* (15%) and *A. parasiticus, A. wentii, A. sparsus,* and *A. humicola* were isolated at 8 %.

***Fusarium* species identified**

Maize was found to be the host of an extremely wide range of *Fusarium* species under natural infection at the sample sites. Sixteen mycotoxigenic species were identified and these included *F. solani, F. proliferatum, F. sporotrichioides, F. moniliforme, F. scirpi, F. chlamydosporum, F. trincinctum, F. oxysporum, F. subglutinans, F. nivale, F. avenaceum, F. graminearum, F. culmorum* and *F. crookwellence.* In Trans-nzoia district, the most frequent *Fusarium* species was *F. solani* at 71.4%, followed by *F. proliferatum, F. avenaceum, F. sporotrichoides*, and *F. moninliforme* at 7.15% as shown in Table 3. In Kakamega district, *F. chlamydosporum* was the highest at 25.1% followed by *F. graminearum* at 16.7%, *F. oxysporum, F. nivale, F. proliferatum, F. subglutinans, F. semitectum*, and *F. trincinctum* at 8.3%, *F. crookwellence* was at 4.3% and *F. scirpi* the least isolated at 4%. The most prevalent *Fusarium* in Kuria district was *F. merismoides* at 36.4% followed by *F. proliferatum* at 27.3%, *F. oxysporum, F. solani* and *F. nivale* at 9.1%, *F. chlamydopsorum* at 5.1% and *F. culmorum* at 4.3% . Generally, the relative level of *Fusarium* distribution within and between the districts ranged from 0% to 71.4%. However, the distribution of the moulds isolated and identified from the three districts were not significantly different at P≤0.05 as shown in Table 4. When the data of the moulds isolated in the three districts was analyzed separately using Tukey HSD, there was no significant (P≤0.05) difference in the number of the moulds in the three districts. The mean difference in the number of the moulds between Trans-nzoia and Kakamega was 0.365, between Trans-nzoia and Kuria was 0.335 and between Kuria and Kakamega was 0.030 (Table 5).

Table 2: Analysis of Variance for Significance of moulds distribution within and between the three districts

Number of moulds					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.014	2	1.007	.846	.434
Within Groups	80.944	68	1.190		
Total	82.958	70			

df=degree of freedom, F= Frequency, Sig=Significance

Table 3: Comparison of the moulds in the three districts

Multiple Comparisons Turkey HSD

(I) Sampling place	(J) Sampling place	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kakamega	Trans-nzoia	-.365	.325	.502	-1.14	.41
	Kuria	-.030	.327	.995	-.81	.75
Trans-nzoia	Kakamega	.365	.325	.502	-.41	1.14
	Kuria	.335	.306	.519	-.40	1.07
Kuria	Kakamega	.030	.327	.995	-.75	.81
	Trans-nzoia	-.335	.306	.519	-1.07	.40

Discussion

The following section discusses the results based on the objectives of the study. This section begins by discussing objective one which aimed at identifying and quantifying the moulds associated with maize from Trans-nzoia, Kakamega and Kuria counties.

The frequency of *Aspergillus* and *Fusarium* species

The high occurrence of moulds in the samples can be attributed to the fact that the three districts are found in warm and humid region which predisposes the maize to the moulds in the field and also after being harvested. These findings were also expected because of the relatively high temperature and relative humidity in Lake Basin region, which was optimum for growth of *Aspergillus* species. In Trans-nzoia, the mean temperature is 23^{o}C annually and relative humidity range is 37-81%. Kakamega has mean temperature of 26^{o}C and relative humidity range of 68-83%. Kuria experience mean tempeature of 16.5^{o}C and relative humidity above 80%. In all the three districts, the most frequently isolated *Aspergillus* species on maize was *A. flavus,* followed by *A. niger, A. parasiticus* and *A. versicolor* (Table 2). Overall, infection of genus *Aspergillus* on the maize seeds in Trans-nzoia district was substantially higher than those collected from Kuria and Kakamega district. The less prevalent *Aspergillus* species throughout the three districts were *A. fumigatus, A. sparsus, A. tamarii, A. ustus, A. humicola*, and *A. terreus.*

The ubiquitous nature of the moulds, ability to colonize diverse substrates and lack of effective control measures could have contributed to the high incidences of the moulds in maize isolated from these regions (Souza *et al*., 2005). *Aspergillus* species are more commonly associated with cereals during drying and storage. *Aspergillus flavus* and *A. parasiticus* have a particular affinity for cereals and can be recognized by yellow-green or grey green colour on corn kernels in the field and in storage (Varga *et al*., 2011). Pre-harvest invasion is partly dependent on insect damage to cobs, but the fungi can also invade down the silks of developing ears and they cause diseases like maize ear and kernel rot. Invasion is primarily due to inadequate drying and improper storage (Pitt, 2000). There is a possibility that since invasion starts in the field and continues in storage, the maize samples collected from markets and various rural households had already accumulated high levels of the moulds especially the *A. flavus* followed by *A. parasiticus* because they commonly attack maize both in the field and in storage.

Damaged maize also favours the growth of *A. flavus* compared to any other *Aspergillus* species and this fact explains why the most frequently isolated *Aspergillus* species in the three districts, was *A. flavus* followed by *A. parasiticus* and other *Aspergillus* species. According to Fandohan *et al*. (2003), postharvest handling favourably and unfavourably affects fungal infection and mycotoxin production in maize. Mechanical damage during and after harvest may also offer entry to the fungal spores either in maize cobs or grains. This possibly explains why very high quantities of the moulds were isolated from the maize samples because some of them had damaged grains that maybe predisposed the grains to the fungus infection.

Fields that vary in cropping history, tillage practices, planting dates, soil types or hybrids can differ greatly in mould and aflatoxin contamination (Munkvold *et al*., 2009). There is likelihood that cropping history, tillage practices, soil types, planting dates and hybrids planted in the three districts are different. These factors may explain the differences in the quantity and type of the moulds isolated from the three districts. That is probably why the overall infection of the

Aspergillus on the grains was substantially higher in Trans-nzoia compared to Kuria and Kakamega districts.

All the *Fusarium* species isolated and identified are considered to be pathogenic to maize. This is in line with observations of Munkvold (2003) and the statement of Leslie and Summerell (2006) that *Fusarium* species is the most common pathogen on maize cobs. *Fusarium* species are destructive pathogens on cereal crops and other commodities, and produce mycotoxins before, or immediately after, harvest (Pitt, 2000). They are commonly considered as field fungi invading more than 50% of maize grains before harvest (Robleda-Robleda, 1991).

Sixteen *Fusarium* species were isolated from the three districts within the Lake Basin region (Table 3). The high number of species isolated could be due to interaction among fungi in maize, which constitutes an important factor influencing fungal infection and subsequent mycotoxins production. Mechanical damage during and after harvest may also offer entry to the fungal spores either in maize cob or grains. Grain damage is a common occurrence in maize especially during postharvest handling of the grains. This possibly predisposes the maize to *Fusarium* attack within the region (Fandohan *et al*., 2003). Another factor that could explain the high level of the *Fusarium* within the three districts is insect invasion. Insects also play an important role in infection of maize by *Fusarium* species. They can act as wounding agents or as vectors spreading the fungus from origin of inocula to plants. Insects are a great havoc to maize in the field as well as in the store and many pests and parasites attack maize during the storage period. According to Gwinner *et al*. (1996), insects are most often considered as the principal cause of grain losses. This therefore means that fungi always invade wherever insects have attacked maize and it justifies the high quantities of *Fusarium* species isolated from the three districts. The occurrence, prevalence and diversity of *Fusarium* species did not vary substantially between the samples.

Relatively high quantities of the moulds were detected from each of the districts sampled. These findings were expected because of the relatively high temperature and relative humidity in Lake Victoria Basin, which predisposes the maize to high infections of the moulds. There was a significant difference in the quantity of moulds between the three districts. In addition, differences were observed in the types of moulds occurring in the three districts probably due to use of different maize varieties by farmers in the respective districts. This is because maize hybrids vary in their resistance to the various types of ear molds (Munkvold *et al*., 2009). Some hybrids are more susceptible to ear mold than others and most probably the maize varieties planted in Trans-nzoia are different from those planted in Kakamega and Kuria. This therefore explains the difference in the type and quantity of moulds in the three districts. Environmental conditions also lead to localized or widespread outbreaks of ear molds that can produce mycotoxins contamination. Factors that affect ear mold development can vary from one portion of a field to another and include previous crop, tillage practices and even small temperature, moisture and humidity differences due to differences in elevation, slope and air movement (Munkvold *et al*., 2009). These three environmental factors determine the direction to which the spores of the ear mold will be blown and their mass. This could have a direct effect on the accumulation of the fungus on the grain. Farmers, therefore, need to be trained on improved pre-harvest and post-harvest practices to decrease the likelihood of mould infection. From the results obtained, there was no difference in the quantity of moulds in the mouldy and good maize indicating that maize which appears good is also contaminated with moulds.

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Relationship between Household Size and Access to Improved Water Sources and Basic Sanitation in Bomet Municipality, Kenya

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Abstract

Improved water sources, sanitation facilities and good hygiene are fundamental to health, survival, growth and development. The principal sources of water in Bomet municipality as well as sanitation are unimproved. As a result, diarrhoea, cholera and typhoid cases are still reported in the area. This study was conducted to determine if there is a relationship between household size and household access to improved water sources and basic sanitation in Bomet municipality. Multi-stage random sampling method was used to obtain the sample. 151 households were selected for the study. The questionnaire was the main instrument for data collection. Analysis of data was done using the SPSS. Data on household size and household level of access to basic sanitation and water were summarized using frequencies and percentages. Correlations at 5% level of significance were used to assess the relationship between household size and household access to improved water sources and basic sanitation. Results from the study indicated that there was a negative relationship between household size and household access to improved water sources as indicated by the correlation coefficient of -0.532 and that there was no relationship between household size and household access to basic sanitation as indicated by the correlation coefficient of -0.072. The amount of water used per person per day significantly decreases as the household size increases. Shifting from larger to smaller households can bring a reduction in household water use. There is need for a study to establish what impacts on household access to basic sanitation in Bomet municipality.

Keywords: household size, improved water sources, basic sanitation

Introduction

Safe drinking water, sanitation and good hygiene are fundamental to health, survival, growth and development (WHO/UNICEF, 2006). However, these basic necessities are still a luxury for many of the world's people. Over 1.1 billion people do not use drinking water from improved sources while 2.6 billion lack basic sanitation (UN/WWAP, 2003). The crisis is worst in Sub-Saharan Africa, where 2 in 5 people lack safe water (AMREF, 2010). According to UN Environment Programme (UNEP), 300 million people in Africa still do not have reasonable access to safe drinking water and nearly 230 million people defecate in the open (Vidal, 2012). While Kenya has launched broad ranging water sector reforms and has stepped up investment in water supply, Sanitation and Hygiene (WASH), the country still faces considerable challenges in reaching the water and sanitation Millennium Development Goals (MDGs). Thirteen million Kenyans lack access to improved water supply and 19 million lack access to improved sanitation (USAID, 2011).

WHO/UNICEF (2006) found that large number of people without adequate provision for safe water and sanitation live in urban areas. Insufficient water supply and sanitation is very often associated with an unsustainable exploitation of natural resources (WHO/UNICEF, 2006). According to Allain (1994), demographic factors contribute heavily to shape water requirements.

Population growth has been found to be a direct determinant of increases in water demand for domestic uses (Gleick, 2003). Another key demographic factor is change in the geographic distribution of population, which modifies the spatial pattern of demand for domestic uses. Urbanization, in particular, through increased population density and the concentration of demand, can make the latter a serious constraint on local resources (Allain, 1994). Urban poverty also contributes to the lack of adequate water and sanitation in poor households (Dungumaro, 2007). Lawrence *et al*. (2002) noted that socioeconomic status is a significant determinant of household access to water and basic sanitation in households. Other variables closely connected with the availability of water and adequate sanitation include, among others, household size and gender of the household head (Dungumaro, 2007).

Rapid pace of urbanization is one of the factors that have been attributed to failure to ensure sustainable access to water and adequate sanitation in many African countries Kenya included (Dungumaro, 2007). The expanding urban population growth creates unprecedented challenges among which provision for water and sanitation have been the most pressing and painfully felt when lacking (UN-HABITAT, 2010).This is because many disease vectors tend to thrive where there is an inadequate provision of these services (WHO, 1999). A lack of safe drinking water and sanitation results in faecal-oral diseases such as diarrhoea and outbreaks of malaria and cholera (UN-HABITAT, 2010). Access to and use of improved drinking water sources and sanitation can make an immense contribution to improved health, productivity, and social development. As part of the Millennium Development Goals (MDGs), the international community has set a goal of reducing the proportion of people without sustainable access to safe drinking water by 50 percent by the year 2015 compared to its level in 1990 (UN, 2010). The importance of water and adequate sanitation is also recognized at the International Decade for Action 'Water for Life' (2005-2015); and the 2008 International Year of Sanitation, to mention just a few. In spite of these concerted efforts, water and adequate sanitation remain a challenge for many people.

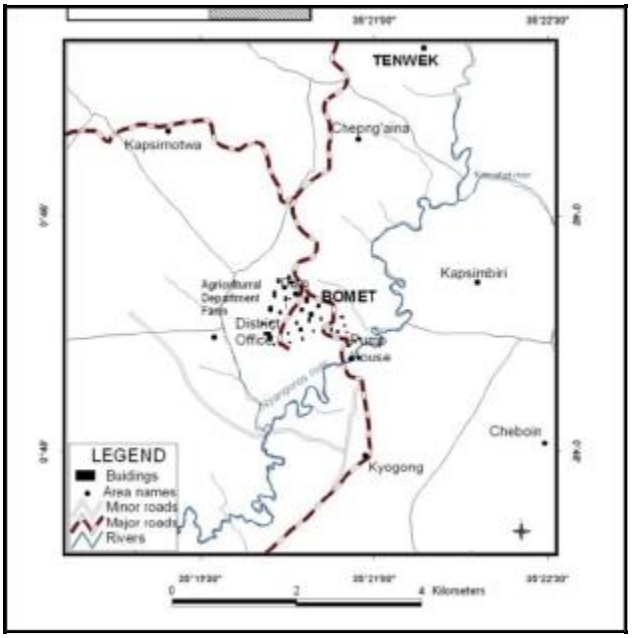
The people most vulnerable to water-borne diseases are those who use an unimproved drinking water source and sanitation (Mintz *et al.*, 2001). Diarrhoea claims the lives of an estimated 1.5 million children under the age of five each year (AMREF, 2010). Lack of access to improved water, sanitation and hygiene affects the health, security, livelihood and quality of life for women and girls. They are much more likely than men and boys to be burdened with caring of family members who are ill and collecting drinking water (WHO, 2002). Waterborne diseases represent a real public health problem in Kenya. Water, sanitation and hygiene (WASH) -related diseases and associated conditions (e.g., anaemia, dehydration and malnutrition) are the number one cause of under-five hospitalization, mortality and over 50% of hospital visits (USAID, 2011). Bomet Municipality is one of the areas in Kenya that are still reporting cases of diarrohea, cholera and typhoid, which are caused by unclean water and poor sanitation. Malaria, which is caused by mosquitoes, is also still reported in the area (Ministry of State for Planning, 2008). This will inevitably decline the attainment of sustainable development since health is regarded as the pillar for sustainable development.

Improved water sources include sources that, by nature of their construction or through active intervention, are protected from outside contamination, particularly faecal matter and are more likely to provide water suitable for domestic use than unimproved technologies (WHO/UNICEF,

2006). These include piped water into dwelling, plot or yard, public tap/ standpipe, tube well/ borehole, protected dug well, protected spring and rainwater collection**.** Unimproved drinking water sources include unprotected dug well, unprotected spring, cart with small tank/ drum, tanker, surface water (river, dam, lake, pond, stream, canal, irrigation and channel) and bottled water. Basic sanitation is the management of human waste at the household level. It is the lowest-cost technology for securing sustainable access to safe, hygienic and convenient facilities and services for excreta and sullage disposal that provide privacy and dignity while ensuring a clean and healthful living environment both at home and in the neighborhood of users (WHO/UNICEF, 2006).

The principal sources of water in Bomet are unimproved: they include wells, dams and rivers. Most households also use unimproved sanitation facilities such as the pit latrines. Baseline survey of the Mara River Basin (2004) reported that on average, households in Bomet get their water at a distance of 4 kilometers. Only about 3% of the households in the district have access to piped water and none is connected to sewer. The environment and natural resources in Bomet have in the recent years been under threat due to increased dependence on natural resources to meet basic needs. The population growth rate has over time become higher than the economic growth rate, hence the increasing pressure on the natural resources (Mara River Basin Survey, 2004). This study therefore investigates the nature of relationship between household size and access to improved water sources and basic sanitation in Bomet municipality.

2. Study Area



Bomet municipality is located in Bomet County in Rift Valley Province, Kenya (figure 1). It lies between 0^0 39' and 1^0 02' south of the Equator and between longitudes 35^0 00' and 35^0 32' east of prime meridian (33^3 East of the Greenwich meridian). It is the capital of Bomet County. It received its township status in 1989 and became a Municipality in 1992. Bomet municipality has six wards: Cheboin, Emkwen, Itembe, Mutarakwa, Township and Tuluapmosonik (Ministry of planning and National Development, 2008).

Bomet Municipality is characterized by gentle topography that gives way to flatter terrain in the south (Ministry of State for Planning, 2008).

Fig. (1). Location of the study area (S*ource:* Survey of Kenya, 2007).

The overall slope of the land is towards the south; consequently, drainage is in that direction and the altitude rises to 2018 M above sea level. The main river in the district, River Nyangores, flows from southwest Mau forest, and proceed southwards through Tenwek in Bomet Municipality (Fig 1). The soils are generally fertile with altitude, temperatures and rainfall as the

main determinants of farming practices in each area. Clay soil which covers 43.6 per cent of the district including the municipality does not allow water to percolate easily and therefore toilets (pit latrines) overflow pouring the sludge on the surface thus causing a threat to human health (Ministry of State for Planning,2008). The area experiences two rainy seasons; the long rains, which occur from March to May, and the short rains, which occur from August to October. Apart from November and December, all the months have mean rainfall of between 1100mm and 1500mm (Ministry of State for Planning, 2008).

Bomet is one of the fastest growing towns in Kenya and is the largest urban centre within the Mara river basin. Rising birth rates and natural growth of the urban population in the region along with rural to urban migration occasioned by rural poverty have contributed to the growth. The population of those currently living in the area is estimated at 76,694 people. The municipality has a population density of 419 persons per square kilometer and the average household size is six (Ministry of State for Planning, 2008). The population of Bomet municipality rose by 134 % in 10 years between 1999 and 2009. Rapid urbanization and increased migration into urban areas within the District have resulted in urban decay, loss of environmental quality and health deterioration, water pollution, loss of biodiversity and encroachment of fragile ecosystems (NEMA, 2011). In both urban and rural areas, access to safe drinking water and basic sanitation is a critical environmental and health concern.

Methodology

Multistage random sampling technique was used to obtain the sample. Stage 1 was the division of the study area to various zones based on the distance from the Central Business District (CBD). Seven zones were created. The second stage involved listing of all households within the different zones out of which simple random sampling was used to select a sample of 22 households. One hundred fifty one households were selected for the study. Random sampling was done following a method described by Franzel and Crawford (1987). This technique is used as follows; a researcher starts from the estimated centre of a study area and proceeds in different directions using the available routes in the study area. The selection of routes is based on probability sampling procedures so as to remove bias and to make it possible get valid conclusions (Arye *et al*., 1972). Three different routes (roads) were used to transect each selected area. The data were obtained from households through personal interviews by use of a semi-structured questionnaire. The study focused mainly on household heads for interviewing to ensure uniformity of data collection process.

The data were obtained from households through observation and by use of semi structured questionnaire. A structured interview-administered questionnaire was designed to carry out a survey about household size and household access to water and sanitation among 151 residents. This involved questions on the number of household members, type of toilet facility used as well as their source of drinking water. Additionally, photographs of the various water sources and sanitation facilities in the study households were taken. The photographs have helped to illustrate the various water sources and sanitation facilities that were used by the households. The data collected was analyzed using correlation. Correlations at 5% level of significance were used to assess the relationship between household size and household access to improved water sources

and basic sanitation in Bomet municipality while data on household size and household level of access to basic sanitation and water were summarized using frequencies and percentages. The survey information was represented using tables.

Results

Household Size

The results (Table 1) show that the greater proportion of households (58 %) in Bomet municipality has up to seven members. About 25 % of households have eight members and above. It would therefore appear that majority (77 %) of households have large families. Households with less than three family members constitute only 17 %. It was also established that the average household size is six.

Table 1: Household sizes

Household size	Frequency	Percentage
Below 3 members	25	17
4 to 7 members	88	58
8 to 10 members	29	19
11 members and above	9	6
Total	151	100

Households' Access to Improved Water Sources.

In this study, an assessment of access to improved drinking water sources was based on WHO/UNICEF Joint Monitoring Programme (JMP) variables. According to WHO/UNICEF Joint Monitoring Programme (JMP) definition of access, distance covered, time spent, quantity of water collected, location of water source, water sources classified as improved and reliability of such sources are all essential for a declaration of access to water (WHO/UNICEF, 2008). The descriptive results presented in figure 2 show that majority of the households (57 %) used unimproved water sources; unprotected dug well, unprotected spring and surface water. Surface water obtained from rivers, dams and streams is the major source of drinking water for about 40 % of the households in the sample. It was established that out of the 40% households that used surface water, majority used the worst drinking water source -a dam. Access to improved water sources remains limited. The proportion of households who get water from tap or private standpipe is only about 21 %. Furthermore, the proportion of households who get water from borehole is only about 2 %.

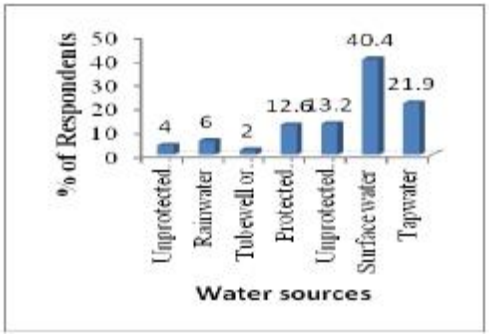


Fig. 2; Principal water sources used

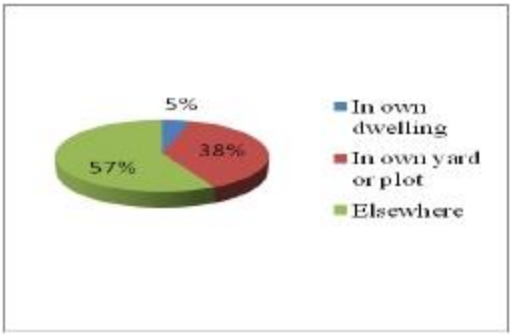


Fig. 3: Location of water sources in Bomet in Bomet municipality

(a) (b) (c)

(d) (e) (f)

Plate 1: Water sources used by households in Bomet Municipality

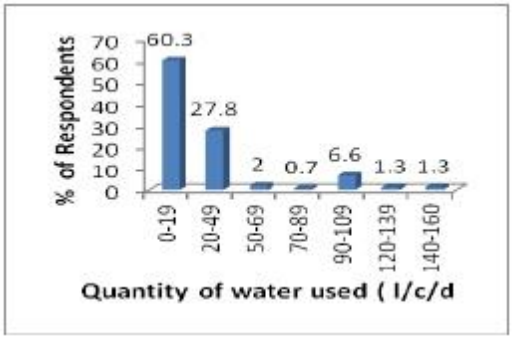
(a) Dam (b) River (c) Rainwater (d) Unprotected dug well (e) Spring (f) Tap water

Results show that only few households (5%) have their water source located in own dwelling (Fig. 3). Most sources (57%) are located elsewhere. The households that fetched water from a source that was not immediately accessible to the household transported using a donkey and human-powered transport. Women and children in Bomet almost exclusively do the considerable labour involved in water collection (see plate 2).

(a) (b)

Plate .2: Transportation of water in Bomet Municipality: (a) Using donkey (b) Human-powered transport

On the quantity of water used per capita per day, most persons (60%) used 19 liters and below per day (Fig.4). Only few individuals (22 %) met the minimum limit of the 20 liters per person per day set by World Health Organization (WHO). This implies that there is water shortage in Bomet municipality. Besides, the average time taken to water sources, fill containers and come back was one hour. Most households (88 %) took 30 minutes or less than half an hour (Fig.5). WHO/UNICEF (2008) recommends 30 minutes in a (normal) round trip to fetch water as adequate for a person to access a minimum of 20 litres of potable water needed per day and still have enough time to do other activities.



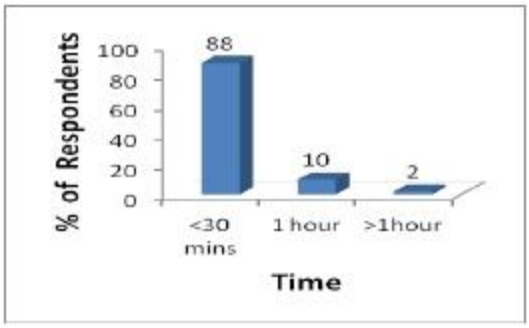
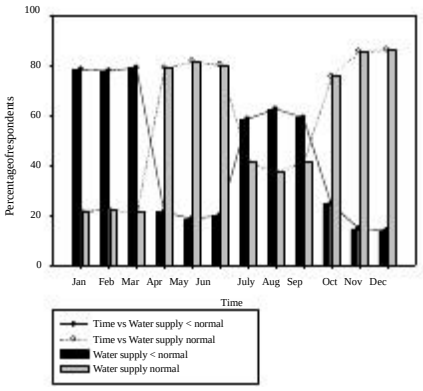


Fig.4: Total domestic water used In liters/person/day

Fig.5: Time spent collecting water

The average distance from households to water source was 0.8-kilometer. Most of the households (52%) traveled less than a kilometer to get to water source from their dwelling (Fig.6). Results from the findings (Fig. 6) indicate that the supply of water in Bomet municipality is less than normal to most of the households on January (78%),February (77.5%) and March (78.8 %) while to some of the households, the water supply is less than normal on July (58%), August (62%) and September (59 %). Only few of the respondents had water supply less than normal on April, May, June, October, November and December.



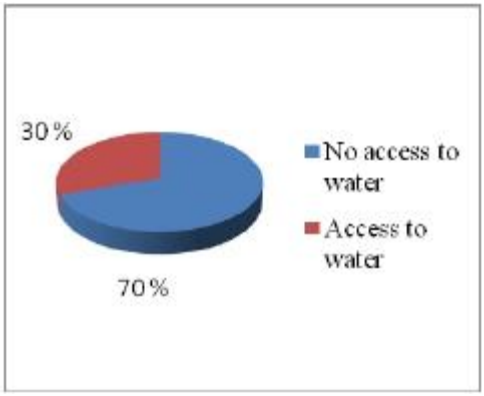


Fig 6. Seasonal variation in household access to water in Bomet municipality

Fig.7: Households access to improved source

The results indicate that only 30% of the households in Bomet municipality had access to improved water sources while 70% had no access (Fig. 7). This implies that there is acute water shortage in the Municipality.

Relationship between Household Size and Household Access to Improved Water Sources

Spearman's rank correlation coefficient was used to measure the level of association between household size and access to improved domestic water sources. The findings show that there was a significant negative relationship between household size and household access to improved water sources as indicated by correlation coefficient of -0.532** (Table 2). This means that an increase in household size would lead to a decrease in access to improved water by the household. These findings are in agreement with those of Arbués *et al. (*2010) and Lawrence *et al*. (2002) who observed a similar relationship between household size and access to water.

Table 2: **Relationship between household size and access to water in Bomet municipality**

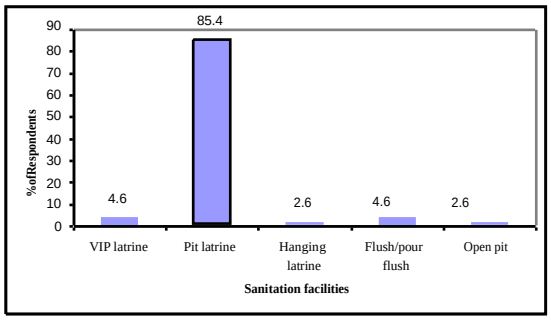
			Household size	Access to improved water
Household size	Correlation Coefficient		1	-0.532**
	Sig. (2-tailed)		.	0.000
Access to domestic water	Correlation Coefficient		-0.532**	1
	Sig. (2-tailed)	0.000	.	
	N		151	151

**Correlation is significant at the 0.05 level (2-tailed).

Households' Access to Basic Sanitation

An assessment of access to basic sanitation was also based on WHO/UNICEF Joint Monitoring Programme (JMP) variables. The WHO/UNICEF Joint Monitoring Programme (JMP) recommend the following indicators to be used in household access to basic sanitation: diarrhea prevalence, hygienic sanitation facilities, sanitation facilities classified as improved, safe and private toilet facilities (WHO/UNICEF, 2008).

The results (Fig.8) show that only few households (9 %) used improved sanitation facilities such as Flush/pour flush toilet and ventilation improved pit (VIP) latrine. Majority of the households (91 %) used unimproved sanitation facilities (pit latrine without a slab, open pit and hanging latrine). Majority of the households (53 %) had no sewerage facilities as (Fig. 9). There was evidence of wastewater flowing out of the compounds uncontrolled, which constituted a threat to health of residents of Bomet (see plate 4).



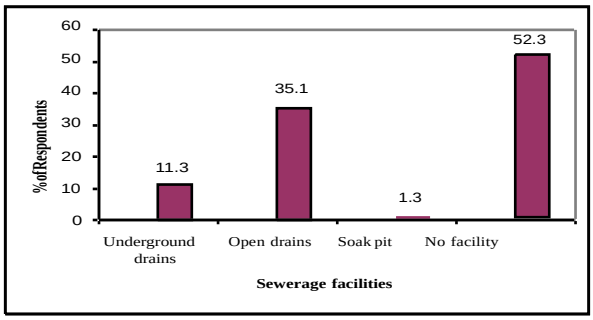


Fig. 8: Sanitation facilities in Bomet

Fig. 9: Sewerage facilities in Bomet municipality

(a) (b) (c)

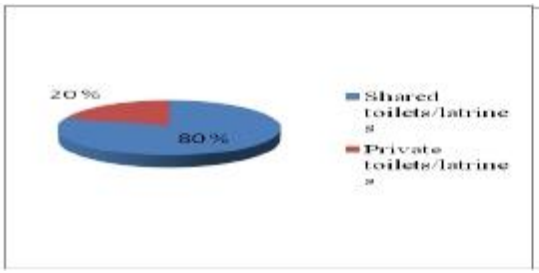
(d) (e) (f)

Plate 3: Sanitation facilities used in Bomet municipality

(a) Flush toilet (b) Pour flush toilet (c) open pit latrine (d) VIP latrine (e) Pit latrine with a slab (f) Pit latrine

Plate 4: Open drains in Bomet municipality

Results (Fig. 10) indicated that only 20 % of the households sampled in Bomet Municipality used private sanitary facilities. Most households (80%) shared a toilet or used public facilities. It was also noted that most households (69%) used unhygienic sanitation facilities as shown in Fig.11.



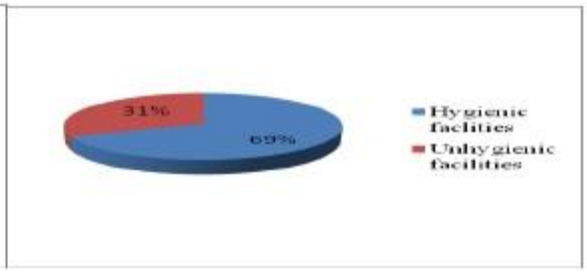
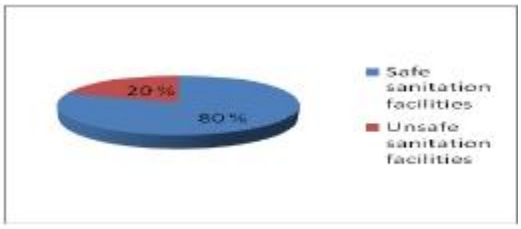


Fig.10: Privacy of sanitation facilities **Fig.11:** Toilets/latrines hygiene

Most of the sanitation facilities (80%) were safe for every member of the household (Fig.12). Unsafe facilities may be attributed to lack of private sanitation facilities in some households. Only 11% of the households sampled reported their children (< 36 months) to have had diarrhea in the two preceding weeks (Fig.13). The diarrhoea cases reported in Bomet may be attributed to pit latrines that overflow when it rains due to high water table.



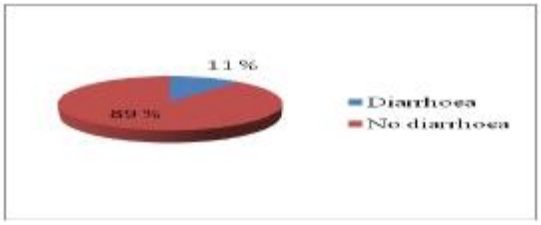
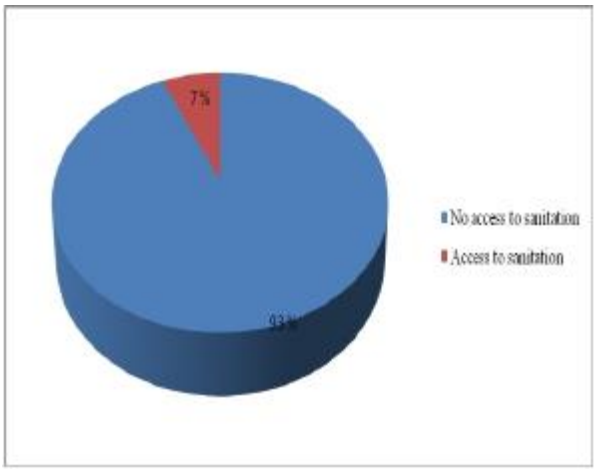


Fig.12: Safety of sanitation facilities **Fig.13**; Instances of diarrhoea reports

The results indicate that only 7 % of the households in Bomet municipality had access to basic sanitation while 93% had no access as shown in figure 13.



Household Size and Household Access to Basic Sanitation

The spearman's rank correlation coefficient was run to test the significance of relationship between household size and access to improved basic sanitation. Results (Table 3) indicate no relationship between household size and household access to basic sanitation as indicated by correlation coefficient of -0.072.

Table 3: Relationship between household size and access to basic sanitation in Bomet municipality

		Household size	Access to basic sanitation
Household size	Correlation Coefficient	1	-0.072
	Sig. (2-tailed)	.	0.379
Access to basic sanitation	Correlation Coefficient	-0.072	1
	Sig. (2-tailed)	0.379	.
	N	151	151

DISCUSSIONS

Majority of households (58% and 19%) in Bomet municipality have large families. Household size is an important consideration in household water availability as it determines the amount of water that is required for use in the household (Demeke, 2009). Majority of the households (57 %) used unimproved water sources; unprotected dug well, unprotected spring and surface water. Surface water (dug wells, dam and river) is the main source of water for domestic use in Bomet Municipality. The main source of river water is Nyangores River. According to KNBS (2010), the source of drinking water is an indicator of whether it is suitable for drinking.

WHO/UNICEF Joint Monitoring Programme (JMP) recommends the source be located within 1 kilometre of the dwelling. Most households (57%) in Bomet fetched water from a source that was not immediately accessible to the household**.** From the results, it was evident that the responsibility of drawing water, according to the Kipsigis customs, lies with women and children. Girls carried containers full of water on their backs (see plate 2). Water sources that are considered safe for use can become contaminated between point - of - collection, storage and household use (KNBS, 2010). When water is obtained from sources outside the home, average consumption drops to roughly one-third of the average consumption at a compound tap and one tenth that of household with water piped into the house (Well, 1998). Most of the households in Bomet municipality relied on water systems in which water was supplied on a community or group basis.

On the quantity of water used per capita per day, most persons (60%) used 19 liters and below per day. This figure is slightly lower than the WHO guidelines, which state that the per capita water consumption should be at least 20 liters per day (Mengesha *et al.*, 2003, Minten *et al.*, 2002 and Collick, 2008). The distance between the nearest water access point and each household is one indicator of the access to improved water sources. The average distance from households to water source in the municipality was 0.8-kilometer. November and December are the drought months, water quantity is lowest at these times of the year and many of the improved sources dry up in some locations of the Municipality such as Kapkesosio, Ithembe and Kyogong forcing women and children to travel longer distances in search of water from unimproved water sources. It was equally noted that lack of sufficient amount of water in other times of the year

with most respondents was not due to climatic factors. The respondents however cited reasons such as power failure and increasing population within the municipality as some of the factors for inadequate water in other times of the year.

Overall, only 30 % of he households in Bomet municipality had access to improved water sources**.** This implies that only 30% of the sampled households had their water source located within one kilometer from their homes, spent 30 minutes or less to fetch water, used water sources classified as improved and were able to reliably obtain at least 20 liters per member of a household per day as recommended by WHO/UNICEF.

Results indicated that there was a significant association between household size and household access to improved water sources in Bomet municipality. An increase in household size would lead to a decrease in access to improved water by the household. The results are in agreement with those of Shonnar (2007) who found that the larger the family size, the more the amount of water consumed or demanded. There are more people in the municipality and increasing consumption of water for domestic use. These have created demands for clean water which, in turn, exacerbate water shortages hence people are most likely to use water from unimproved sources.

Access to basic sanitation in Bomet municipality is also limited. Majority of the households (91 %) used unimproved sanitation facilities; pit latrine without a slab, open pit and hanging latrine. The high percentage of people using pit latrines can be explained by the fact that pit latrines can be build and maintained at low cost**.** Pit latrines are all that most people in the developing world can afford (Pickford, 1995). Given sensitive guidelines and a little technical help, families can build pit latrines for themselves at very low cost. Many households (57 %) drained wastewater from washrooms within their compounds although they knew the consequences of such action. There was also evidence of uncontrolled wastewater flowing out of the compounds that constituted a threat to public health (see plate 4). Lack of sewerage facilities may be attributed to high per capita cost and poor access to water services in the Municipality.

A household is classified as having an improved toilet if the toilet is used only by members of one household (that is, it is not shared) and if the facility used by household separates the waste from human contact (WHO/UNICEF, 2006). Most households (80%) shared a toilet or used public facilities. The use of public facilities by Bomet residents may be attributed to greater numbers of tenants. Most households (69%) used unhygienic sanitation facilities. The dirty latrines were the shared household latrines because of poor management. If a toilet is dirty and smelly, no one will want to use it — and it may spread disease (UNDP, 2005).

The nature of the construction and distance between households is also another indicator of latrine availability. Most of the sanitation facilities (80%) were safe for every member of the household. It was established that the unsafe facilities were the public facilities. Maintenance of shared facilities is often problematic and is not used by all members of the household (UNDP, 2005). In addition, distance may be a factor affecting convenience and therefore use (ibid). Latrines were not used by young children (>5 years of age) and women in some areas of the municipality because they were poorly constructed, located in a bush or far from home. World Bank (2011) found that for sanitation to be effective, facilities must be correctly constructed, properly maintained and in a safe place.

The proportion of children in the households sampled who had diarrhoea at the time the information was collected or who have had it anytime in the two preceding weeks was also considered. Only 11% of the households sampled reported their children (< 36 months) to have had diarrhea in the two preceding weeks. It was established that the households that reported diarrhoea cases used unimproved sanitation facilities. Improvements in sanitation have been shown consistently to result in better health as measured by fewer diarrhoeas, reductions in parasitic infections, increased child growth, and lower morbidity and mortality (Bendahmane *et al.,* 1999).

Overall, only 7 % of the households in Bomet municipality had access to basic sanitation while 93% had no access. This implies that only 7% of the households used improved, hygienic, private and safe sanitation facilities as recommended by WHO/UNICEF.

There was no relationship between household size and household access to basic sanitation in Bomet municipality. Although it appear logical to think that sanitation decreases as the population grows, this is not true as reported by (WRI, 1996). Technological advancements have greatly increased sanitation. There are various sanitation technologies in Bomet ranging from the lowest cost technology (such as pit latrines, simple defecation trenches etc) to highest cost technology (such as private sewer connection, flush toilets etc). Pit latrines are affordable and can be shared between several families (Pickford, 1995).

Conclusions and Recommendations

The study suggests that the type of water source used by household was significantly influenced by the size of the household. Average daily water consumption varies depending on household size. As the household size increases, the amount of water used per person per day significantly decreases (Demeke, 2009). Thus households with more members are likely to use water from unimproved source. Especially, it has emerged from the study that the number of household members is the fundamental factor, which compels households to rely on unimproved sources. Shifting from larger to smaller households can bring a reduction in household water use. The government should take action to slow the growth in demand for freshwater by slowing population growth. Continuing and expanding family planning programs can help assure that population growth eventually slows to sustainable levels in relation to the supply of freshwater. Policymakers and the general public need to be educated about water resources, sanitation and population dynamics, with an emphasis on making human activities sustainable with respect to water availability.

There was no relationship between household size and household access to basic sanitation in Bomet municipality. Despite increase in population, technological advancement has greatly increased sanitation. A wide range of sanitation technologies exist ranging from the lowest cost technology (such as shared household latrines) to highest cost technology (such as individual household latrines). Shared household latrines are cheaper to construct than individual household latrines and can be shared between several families where crowding prevents household solutions. There is need for a study to establish what impacts on household access to basic sanitation in Bomet municipality.

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