THE DISTRIBUTION OF CURCULIONID WEEVILS, BIOCONTROL MEASURES AND THEIR EFFECTS ON TEA YIELDS IN EASTERN REGIONS OF KENYA

BY

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DECLARATION

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SC/D.Phil/035/09

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DEDICATION

This thesis is dedicated to Kwandap Cheptoo, Cheptoo, Kipng’etich and Cheruiyot

ABSTRACT

Two curculionid weevils namely: *Entypotrachelus meyeri* [Micans/Kolbe] (Kangaita weevil) and *Sphrigodes mixtus* [Hustache] (Nyambene weevil) defoliate tea bushes and affect tea production. Studies were conducted to determine the pests on prevalence, distribution and relation to cultural practices, yield loss, action threshold i.e the number of weevils per bush before any action is done and two control strategies. In this regard, a modeling exercise was undertaken to determine weevil distribution and determine potentially risky areas using Geographical Information System (GIS) with reference to rainfall, temperature and agro-ecological zones (AEZ) as parameters which influence weevil incidence. In addition, interrelations of weevil distribution to agricultural practices were gathered through a questionnaire. three management options were initiated utilizing two local *Beauveria bassiana* (balsom) Vulliemin isolates at a concentration of 10^{13} conidia /ha, Karate 2.5 WG, Water Dispersible Granules which contains Lambda Cyhalothrin 25g/Kg as the active ingredient, a chemical insecticide and push -pull technology based on two shade trees (Fraxinus spp. Milletia dura), avocado and two tea clones (TRFK 306 -purple tea and TRFK 31/8). The trials were laid out in complete random block designs with three replications were used in the field experiment to determine the efficacy of the fungi isolates. Leaves of shade trees or clones were placed in small pots in mixtures or alone and were arranged in a complete random design in cages and replicated three times to determine preference of weevils to plant leaves in the push –pull technology. The risk assessment made to determine whether the weevil species can enter and establish in new areas indicated that based on agro-ecological zone I and climatic conditions, temperature (13.5-23.5^{o}C), rainfall (1000-2700mm), 266,399 ha of tea were at risk to weevils infestation in Eastern tea growing region of Kenya(East of Rift) . The study found that the weevil species distribution was site specific for *E. meyeri* while *S. mixtus* was not. The most prevalent weevil was the *S. mixtus* found in an estimate of 5,135 ha against *E. meyeri* in 1,231 ha. The study also showed that mixed clones of tea, excessive application (High use rate) of nitrogenous fertilizers, intercropping with indigenous trees i.e avocado and eucalyptus had high incidences of weevils. Two isolates of *B. bassiana* (BbGi7a and BbKe6a) were more efficacious ((P=0.05) compared to other isolates at concentration of 1 x 10^3 conidia/ml. The two isolates caused > 50% weevil mortality on day 9 and 14 after inoculation respectively. The tea weevils lowered productivity of tea significantly ($P \leq 0.05$) with reduction in yield of between 30-33%. The performance of *B. bassiana isolates*, applied in form of a solid substrate or sprayed as conidia on the foliage, was comparable to that of Karate at the rate of 2L/ha. The study found a positive correlations between weevil population around the bush and the destroyed leaves. (y=21.127x -11.019, R^2=0. 83333 for Giciaro farm and y=0.2569x+1.3689 R^2=0.5296 for Mununga). The economic threshold level was found to be 2-3 weevils per bush where the destroyed leaves through defoliation was more than 40% to 54% of leaves. The study identified one tea clone purple tea and avocado to be potential for use as a pull crop each.The weevils significantly ($P<0.05$) prefer the purple tea leaves with resultant defoliated area of 90mm^2 and the avocado leaves (120 mm^2) while the shade trees *Milletia dura* (area consumed 23.1 mm^2) and *Fraxinus spp.* (11.8mm^2/ ten weevils/ two days) were less preferred. Therefore, purple tea and avocado are potential "pull crops".

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CHAPTER ONE

INTRODUCTION

1.1. Background

Tea, *Camellia sinensis(L.)* O Kuntze is a perennial crop that provides sole income to farmers and employment to rural populations in many parts of the world. It is a perennial tree belonging to the *Theacea* family (Bokuchava and Skobelava, 1969; Hara *et al.*, 1995) that is managed as a low bush table in continuous phase of determinate vegetative growth. In East Africa it is grown in Kenya, Tanzania, Uganda, Rwanda and Burundi occupying 224,642 ha with a total production of 441 million kilograms of made tea in 2010. In Kenya, tea is the leading cash crop, it was grown on 157,720 ha producing 369 million Kg of made tea in 2012 and contributing to an equivalent of KShs 94 billion of export earnings (Anon. 2013, ITC 2013). Tea growing contributes to poverty alleviation and wealth creation therefore, contributing to better living standards. The tea industry and its allied activities employ over 500,000 families each on the average supporting 6 members (Ogola and Kibiku, 2004). It is estimated that the tea industry activities support approximately a sixth of the Kenyan population with an estimated 5 million people depending on tea for their livelihood both directly and indirectly. In addition, planting of tea contributes towards environmental conservation through enhanced water infiltration, reduced surface erosion, and mitigation of global warming through carbon sequestration (Kamau *et al.*, 2008).

Besides the favourable environmental conditions prevalent in the tea growing zones of Kenya, successful tea cultivation has largely been attributed to focused research and development through adequate funding of the commodity specific Tea Research Foundation and statutory marketing through auctions. However, owing to global climatic changes and non-adherence to good agricultural practices, the crop has become exposed to biotic and abiotic stresses. The devastating biotic stresses include damage by tea mites (red crevice mites, red spider mites and purple mites), scale insects (Aspidiotus sp and green soft scales), weevils (Systates weevils, tea root weevils, *Entypotrachelus meyeri*), tea thrips (Scirtothrips kenyensis), termites, tea mosquito bug (*Helopeltis* sp), tea Crickets and chafer grubs among other arthropods (Anon, 2004). The two main diseases include one major wood rot, *Hypoxylon serpens*, and a sporadic root rot caused by an *Armillaria* s*p.* of fungi (Anon 2004).

Tea weevils comprise about 27 species (Benjamin, 1968). The weevils reported to occur in Kenya include, the Tea Root Weevil (*Aperitmetus brunneus*) [Hust], Nematocerus Weevil (*Nematocerus sulcalus*), Systates Weevil (*Systates* sp.), Kangaita/Kimari Weevil (*Entypotrachelus meyeri*) [micans/Kolbe] and Nyambene Weevils (*Sphrigodes mixtus* Hust.) (Anon, 2006). Adult weevils damage tea by defoliating nursery, newly established and mature tea orchards (Benjamin and Demba, 1974; Muraleedharan and Chen 1997). Kimari/Kangaita weevils are documented to occur throughout the tea growing areas of Kenya. Occasional isolated outbreaks occur causing variable level of damage. (Sudoi *et al.*, 1999) They are polyphagus and feed on over 14 plant families (Benjamin *et al.*, 1968) crossing commodity groups making the weevil a high risk pest (Mannion, 2003). Presently, the management strategies against tea weevils include cultural practices and spraying with an insecticide such as Karate which contains lambdacyhalothrin as the active ingredient. Hand picking is applied where the weevil population is low (Anon., 1986). Biological control have been found to offer great potential against the tea weevils. Sudoi *et al.*, (1999) isolated an entomopathogenic fungus, *Hirsutella* species which gave a high mortality rate on Kangaita weevil. Preliminary investigation at Tea Research Foundation of Kenya (TRFK) has indicated that locally isolated *Beauveria bassiana* using *Galleria* larva moth as an indicator showed good prospects in the control of the Kangaita tea weevil (*E. meyeri*) and the tobacco cricket (Anon 2001, Anon 2003, Anon 2006)

Enthomopathogenic fungi (EF) have been used successfully elsewhere to control several pests in plantations such as the brown leafhopper on rice (Agunda *et al.,* 1988), *Leucenia psylid* on Leucaenia (Ahmad, 1993) and Banana root weevil (Mesquita, 1988, Muraleedharan, *et al.*, 1997; Sevim *et al*., 2013). *Beauveria. bassiana* is a fungus that is found naturally in soils throughout the world and acts as a parasite on various insect species, causing white muscardine disease. *Beauveria.bassiana* strains have successfully been used to control several pests such as: -Tobacco spider mites (*Tetranychus evensi*) Pritchard (*Acarina; tetranychidae)*infesting tomatoes (Wekesa, 2006). Botanigard, an imported formulation of *B. bassiana* is being used commercially in Kenya as a biological insecticide to control a number of sucking insects including thrips, aphids and whiteflies on French beans and snow peas (PCPB, 2011). This fungus has shown success also in management of the voracious diamond back moth *Plutella xylostella* (Vandenberg, *et al.*, 1998) and potato flea beetle *Leptimotarsa decemlinaeta*, (Wraight *et al.,* 2000).

Trudel, (2007) suggested that *B. bassiana* had a potential to persist in the environment and for horizontal transmission between weevils if used. He proposed more investigations on its ability to control populations of white pine weevil *Pissodes* *strobi*. *Beauveria bassiana* has proved to be competitive with chemical insecticides for protection of forests and farms against pests. However, no proven documented report has been published on the variation and potential use of fungal isolates on the control of the tea weevils in Kenya.

The ‘push-pull’ strategy is another promising alternative for reducing pest population. It uses a combination of behavior-modifying stimuli to manipulate the distribution and abundance of insect pests and/or natural enemies. Using this strategy, the pests are repelled or deterred away from the main crop (push) by using stimuli that mask host apparency or are repellent or deterrent. The pests are simultaneously attracted (pull), using highly apparent and attractive stimuli, to other areas such as traps or trap crops where they are concentrated, facilitating their control efficiently away from the commodity product. The most successful example of the push-pull strategy currently being used by farmers was developed in Africa for controlling stem borers on cereal crops (maize) (Kfir *et al.,* 2002; Khan and Pickett, 2004; Cook et al 2007).

The overall goal of this study therefore, is to increase the productivity and quality of tea through the development and application of sound, environmentally friendly sustainable management practices for the tea weevils. The study was geared towards improvement of tea production through the development of two biological control options for the management of tea weevils viz-a-viz the development of local isolates of *B. bassiana* and a pull–push management options that are feasible through determining repellent and attractant plants/trees that can be grown in combination with tea.

1.2 Statement of the problem

Adult weevils feed on the foliage by chewing the leaf edges and also bore irregular holes through the leaf surface. The larvae are occasionally the most destructive, feeding on the tap root and causing stunting, wilting and eventual death of young plants. Stems of young plants may be ring-barked at ground level by the larvae. The tea weevil occasionally defoliate young newly transplanted tea to the field. Therefore, weevils contribute to the reduced production through reduction of leaf area, hence reduced performance of tea through less area for photosynthesis; it can also cause stoppage of growth through effects of feeding on the tap roots. In addition, the tea growers incur loss of time and finances in the process of carrying out the management of tea weevil menace through palliative measures instituted to control them. Therefore the present study was carried out to determine distribution, prevalence level of major two weevil pest in East of Rift and initiate viable control approaches if any for use in the management of the tea weevils in Kenya while enhancing pluckable tea production through applying the devised strategy

1.3 Justification

The tea weevils mostly defoliate nursery stock, mature and newly established tea including the commercially harvestable shoot (Benjamin and Demba, 1968; Muraleedharan and Chen, 1997). Several insect pests have been recorded on tea affecting production and prominent among which is the tea weevils. Decline in tea production has been attributed to pests such as the mites, the mosquito bug and tea weevils which are non-ubiquitous throughout Kenya (TRFK, 2012). Studies on seasonal distribution and extent in area and risk area, on the tea weevils in Kenya are however few, therefore, studies for understanding the population dynamics of the tea weevils and developing strategies for their management were undertaken in order to minimise losses to tea. Presently, management strategy against tea weevils is based solely on a cultural practice of hand picking when weevils are in low population which is labour intensive and spraying with synthetic pyrethroid, Karate which contains lambdacyhalothrin as the active ingredient. Because of problems associated with pest mite resurgence that often resulted from routine chemical applications (Dutcher and Payne 1985), as well as other environmental and regulatory concerns on beverages, research on developing alternative control strategies is warranted. It is therefore, prudent that studies for the development of alternative effective and safer control practices are developed to avoid dependency on a few practices to minimise side effects. Biological control and push–pull technology integration which this study initiated its development offers promising novel strategies development for the future management of tea weevils by farmers in Kenya. Therefore, efforts were made in this study to isolate; and determine efficacy of *B. bassiana* associated with natural mortality of the tea weevils. The two strategies can be combined feasibly as part of an integrated pest management system; with no residue levels thereby ensuring safe and sustainable environment and enhancing global consumer acceptability and confidence in Kenyan tea which can be grown organically. In addition, other produce/crops susceptible to weevil insects can benefit from these approaches.

1.4 Main Objectives

Overall objective was to determine distribution, prevalence level of major two weevil pest in East of Rift and initiate viable control approaches if any for use in the management of the tea weevils in Kenya while enhancing pluckable tea production through applying the devised strategy

The specific objectives of these studies were:

1. To determine the tea weevils distribution in Eastern tea Growing region of Kenya using geographical information system (GIS)
2. To determine the relationship between cultural practices in Eastern tea Growing region of Kenya and weevil distribution
3. To determine the effect of tea weevils on productivity of tea in Kenya
4. To determine the influence of temperature and rainfall on weevil populationas.
5. To assess the efficacy and mode of application of local *B bassiana* isolates in the control of the tea weevil compared to a conventional insecticide
6. To identify a weevil repellent plant and a weevil attractant as a trap crop/plant if any for use in developing a pull – push management strategy.

1.5 Limitation and Scope of the Study

The study on weevil distribution mapped data sets with computer mapping software, (Esri'sArcInfo ArcGIS software at Version 10.1 in order to demarcate areas where different weevil species can potentially thrive.

From nine factory catchment areas with weevil infestation 5 farmers per catchment giving a total of 45 farmers were purposively sampled for the survey.

In the fields efficacy trial on *B.bassiana* isolates, two trials were carried out the first trial being prelimary and the second trial being confirmatory.

The trials to be carried on determination of volatile flavour compounds were not carried out due to lack of equipment. The trials done were on those involving cages

CHAPTER TWO

LITERATURE REVIEW

2.1 General Requirement of Tea and Global production

Tea, *Camellia sinensis* (L.) O. Kuntze, is an intensively managed perennial monoculture crop cultivated on large- and small-scale plantations situated between latitudes 41◦N and 16◦S. It is grown on over 2.71 million ha in more than 34 countries across Asia, Africa, Latin America, and Oceania to produce 3.22 million metric tons of made tea annually. It is predominantly an agro-based export-oriented evergreen crop in Kenya (Lashmi *et al.*, 2009; ITC 2013). Under natural conditions, a tea plant grows to a small tree but it is configured into a bush by sequential pruning and other silvicultural practices, viz tipping, plucking and by harvesting the optimum vegetative produce It is highly heterogeneous with most of it's morphological, physiological and biochemical descriptors showing continuous variation and high plasticity (Lai *et al., 2002*).. Cultivated tea consists of three main species each with specific plant type viz- *Camellia sinensis var sinensis* (China type), *Camellia sinensis var assamica* (assam type) and C*amellia sinensis var assamica* sub-sp. *Lasiocaly* (cambod type) (Wight, 1962). This classification has been based on leaf characteristics, size and shape. Length of pistil and flower sizes have also been used in classification of tea plants (Takeda and Toyao, 1980). Based on these features, a bush with small leaves are characterized as China type while the Assam type is tall with large leaves, and Cambod is thought to be intermediate between China and Assam type (King-dom-ward, 1950, Robert and Wight, 1958).. Tea is either propagated using seeds or cutting. (Anon, 2002). Nevertheless seed grown plants of propagation using cuttings has been developed (Anon, 2002). Grafting can also been used as an alternative propagation technique,although rare and cumbersome (Prakash *et al.,* 1999). Vegetative multiplication from a single original parent forms a stock group of plants known as clones which are genetically identical tea cultivars. Clones have been developed in the attempt to improve tea crop yields and quality has led to development of high yielding clones (TRFK, 2012). Tea grows best in tropical and subtropical areas with adequate and well distributed rainfall of approximately 100mm per month. Rainfall ranges between 1200 to 2700mm annually within the tea growing regions. The minimum annual rainfall considered adequate for the successful cultivation of tea is about 1200mm per annum without irrigation Regardless of whether or not other climatic factors are favourable tea, like other plants does not grow when temperatures are either too low or too high. Air temperature below 13^{o}C are likely to bring damage to foliage and mean maximum temperatures greater than 30^{o}C are likely to be accompanied by low humidity that cessation of active growth is inevitable Free drainage acidic soil (pH 4.5-5.5) with medium texture, good water retaining capacity is essential for tea growing. (Anon, 2002; lakhsmi *et al*., 2009).

2.2 Contraints to Tea Production

Plants are often exposed to plethora of abiotic and biotic stress factors and the tea plants is of no exception. Abiotic stress can cause crop failure and dipping average yields of most crops by more than 50% (Bray *et al.,* 2000).Such abiotic stress factors include; low temperature, salinity and drought. These stress factors are a menace and prevent the plant from reaching its full genetic potential. Globally, 1031 species of arthropods are associated with the intensively managed tea Camellia sinensis (L.) O. Kuntze monoculture. 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 (Lakhsmi *et al.,* 2009). Surveys carried out within the Kenyan tea industry have revealed that the most common pest problems include tea mites (red crevice mites (Brevipalpus phoenics), red spider mites (Olingonychus coffeae) and purple mites (Calacarud carinatus), scale insects (Aspidiotus sp, green soft scales and brown scales), tea weevils (Systates sp. weevils, Apritmethus brunneous, Sphrigodes spp.), tea thrips (Scirtothrips kenyensis), termites, tea mosquito bugs (Helopeltis sp) and chafer grubs. (Sudoi et al., 2011, TRFK 2012)

Diseases affect tea production primarily by lowering field productivity. In Sri Lanka, diseases lower tea production between 27% - 36% (Lakhsmi *et al.,* 2009). The tea diseases documented so far as serious in Kenya are; Armillaria root rot, Hypoxylon wood rot, and stem canker diseases that cause most serious damage to tea in production. Others such as the leaf spots (grey leaf spot & brown leaf spot) and damping off are of concern only in nurseries as a result of too much shading and high humidity. Crown gall -Agrobacterium tumefaciens, Cylindrocarpon root rot, and root knot nematodes are rare. (Otieno 2002; Otieno 2005; Mutai R., 2008)

These problems have been exclusively remedied through research efforts by pest and disease monitoring, surveillance to ensure that old or new pest problems do not get out of control. The pest and disease surveillance is done through frequent visits to scout for the pest problems. Conservation for the purposes of utilization of indigenous natural enemies is promoted as part of the Integrated Pest Management package. The Kenya tea growing environments is rich in biodiversity and is ecologically stable owing to their bordering natural forests and conserved river lines and thus the law of natural control applies in these areas. Pest and disease problems in Kenya have been remedied mainly by cultural practices such as pruning or skiffing; use of adequate/ balanced nutrition that reduce damage from crop pests attack, uprooting and destroying of the affected plant materials is also carried out. Futhermore, new strategies continue to be reviewed and developed for sound sustainable pest management. The strategies include development and availing to tea growers' of resistant or tolerant tea cultivars. Research efforts continue to ensure that the improved clones released for tea growers have substantial resistance to pests and diseases. In geographical locations where pests may pose a threat to tea growing, tea growing is restricted (Brown 1968). In weed management, the most prevalent weeds are perennials and annuals such as grass weeds, couch grass (Penisetum sp,)) wondering jew (Comelina sp.) gallant soldier (Galinsoga sp.), red shark (Poligonum sp.) and Khaki (Borreria sp.). Weed management occupies a special place in tea production for which integrated weed management is used in Kenya, with manual weeding being carried out in young tea with scanty weed population particularly when it is not very wet. Preference is given to manual weed management especially in the smallholder farms. Therefore overally, the use of synthetic pesticides in Kenyan teas is not practiced for production. Instead, the operating philosophy in the Kenyan tea industry is one that promotes use of an integrated pest and disease management (IPM) system with use of pesticides as a last resort (Wachira and Rono 2004; TRFK 2012).

2.3 Economic importance of tea

Economic importance of tea is primarily due to its use as a beverage of international repute. It has received much attention for its aroma, pleasant taste and numerous medicinal benefits and has been socially and habitually consumed by people since 3000B.C. (Lin *et al.,* 2003). Tea is a very important source of revenue for the tea producing countries; in Kenya it contributes about 26% of export earnings and 4% of the Gross Domestic Product (GDP) (Wachira and Ronno, 2004). At the house-hold level tea plant is so-called the crop of the poor, especially in the tropical mountainous areas, because even with the minimal investment required, tea can be planted and harvested weekly or each ten-day period on hard and sloping soils where the other food crops or cash crops could not grow effectively (Vo Thai Dan, 2006). In addition, planting tea plant on the remote mountainous areas is considered as an effective method to cover the spare sloping lands, thus provide a means of soil conservation (Ute, 204). Cultivation of tea in the remote areas also provides many jobs to rural communities and certainly contributes to the development of local infrastructure (Wealth of India, 1950: Lakshmi *et al.*, 2009). Tea is served as a daily drink for two third of the world population (Muktar and Ahmad, 2000). Drinking tea became a special culture ceremony in many countries (such as Japan, China and Vietnam). Tea also cannot be absent in many cultural events such as traditional New Year and wedding ceremony. Other than their use as a beverage, green leaves are also used as vegetables such as 'leppet tea' in Burma and 'Meing tea' in Thailand (Vo Thai Dan 2006). Tea seed can be a potential source of edible and industrial oil as well as a source of oil cakes and husks rich in minerals, polyphenols and traces of residues of essential fatty acids for use in livestock feeds (Njuguna, 2012), they can be used in the manufacture of nematicides and as a lubricant (Wealth of India, 1950).

Pharmacological value of tea

Biological studies have shown that the quality determinants of tea, especially catechins and phenolic acids are associated with medicinal properties such as antidiabetic, antimicrobial, anticancer, antioxidants due to presence of catechins and antiaging activities (Khan and Mukhtar, 2007, Wachira and Kamunya 2005). Tea drinking is associated with cell mediated immune responses of human body and improves growth of beneficial microflora in the intestine. It also imparts immunity against intestinal disorders, protects cell membranes from oxidative damages, prevents dental caries due to presence of fluorine, normalizes blood pressure, prevents coronary heart disease due to lipid depressing activity, reduces blood glucose activity and normalizes diabetes.Tea posses germicidal and germistatic activities against various gram positive and gram negative bacteria that affects human beings (Chen 1999). Several epidemiological studies as well as studies in animal model have shown that green tea can confer protection against various cancers such as those of the skin, breast, prostrate and lung. It has also been shown to be hypocholesterolemiant (Yang *et al.*, 2002) to prevent the development of atherosclerotic plaques (Couturon, 1982), antibacterial, anti-inflammatory and anti-HIV activities (Yang *et al.*, 2002, Khan and Mukhtar 2007, Dona *et al.*, 2003). Among age associated pathologies and neurodegenerative diseases, green tea has been shown to confer significant protection against Parkinson's disease, Alzheimer'sdisease and Ischemic damage (Mangenya *et al.*, 2014). Tea plants thus prove to be a future potential as an important raw material for the pharmaceutical industry.

2.4 History and Tea Growing Regions in Kenya

Tea was discovered more than 2000 years ago in China, but is naturally distributed in the whole of Asian Monsoon regions (Barnejee, 1992) and was first introduced into Kenya by a settler farmer in the early 1904. Seeds were obtained randomly through open pollinated natural hybrids between the Assam and China varieties from Assam region of North East India. The introduced Manipuri hybrid seed was planted in Limuru in 1904. The raised seedlings became the source of seed for future planting. The earlier industry was dominated by the colonial settler community who were the only ones with access to seed. In 1960, special crops development Authority (SCDA) was established to promote the cultivation of the crop within the small-holder agricultural sub-sector. This evolved later to become the Kenya Tea Development Authority (KTDA) whose main function was to facilitate the expansion of Tea cultivation into the native lands (Penwil, 1961).

2.4.1 Eastern Tea Growing Regions of Kenya

The Great Rift Valley divides the country into two tea growing regions almost asymmetrically and defines the two growing regions. To the East of Rift (Eastern Tea Growing Regions) are tea areas within and around the slopes Aberdare Highlands that's in Kiambu, Limuru, Thika, Nyeri, Maragwa and Othaya, areas at the foot of Mt. Kenya mainly Kirinyaga/Kerugoya Embu and Meru (Nkubu, Chukka, Chogoria among other areas) tea growing region and around/within the Nyambene hills, areas between Mikinduri and Maua (TBK, 2003).

2.4.2 Western Tea Growing Regions of Kenya

The West of the Rift is defined by the Mau Escarpment and the areas are the Nandi, Kericho highlands, Mt. Elgon Region (Kapsakwony and Kapsara near Kitale) and Kisii highlands. Tea grow on the slopes of the highlands within the altitude of between 1500 and 2700 metres above sea level (Figure 1) (TBK, 2003).

Tea is mainly grown in the lower Highland sub-zone one (LH1) and a small tea growing area is on the Coffee-tea zone, upper midland one (UM1) which are normally related to be marginal area for lucrative tea growing, they includes the area of upper Meru that is the Chukka, Nkubu Chogoria area, the Tombe Ogembo, Kiamokama area in Kisii and Chavakali/Vihiga-Mudete KTDA catchment of Kakamega

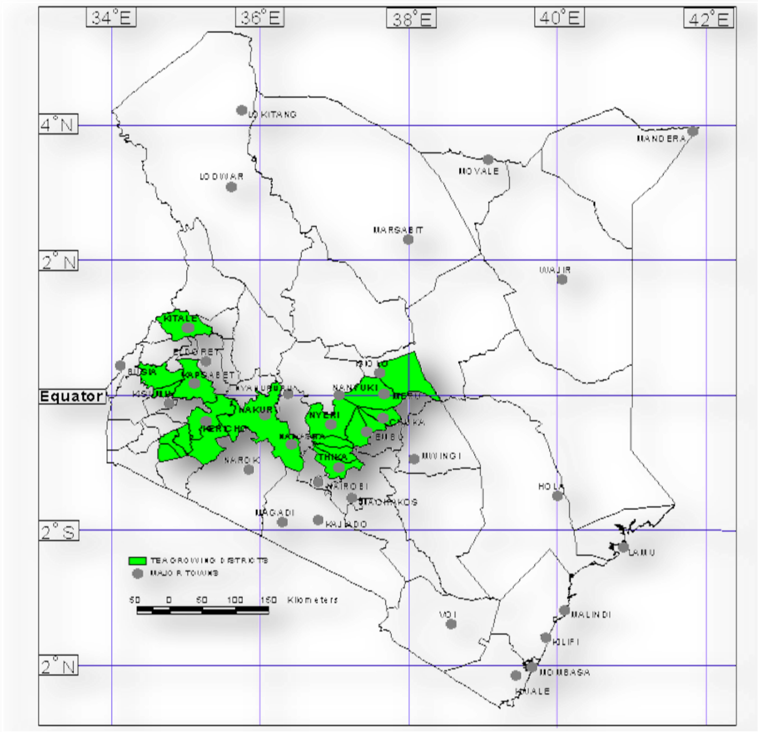


Figure 1: Map of the Tea Growing Regions (green) of Kenya. **(Source: TBK 2003)**

2.5 The tea weevils

The tea weevil species (Coleoptera: Curculionidae) are classified under the Coleoptera class, the largest order of living organism with an estimated 350,000 described species in approximately 23,000 genera (Zimmerman 1968: Endrody-Younga 1985), the super family *Curculionidae*, regarded to represent the most highly evolved of all beetles and within the diverse *Curculionidae* (Zimmerman 1968b,c), The largest family of animals in the world with more than 45,000 described species (Oberprieler and Louw 1985; Picker *et al.*, 2002). The family has a cosmopolitan distribution with members characterised by a globular head produced into a rostrum (Oberprieler and Louw 1985). The classification of *curculionidae* into subfamilies and tribes is probably the largest outstanding problem in the higher classification of coleopteran (Oberprieler and Louw 1985). Traditional Curculionidae are divided into the “short nosed” Adelognaytha and “long nosed Phanerognatha based on the ventral visibility of rostrums (Oberprieler and Louw 1985)

The first’s report of tea weevil menace in Kenya was in 1982 (Kilavuka, 1982). Several species have since been reported to affect tea bushes (Anon, 2002). The *E. meyeri* Kolbe, S. *mixtus* and *Systates* sp. are the most common species. *E. meyeri* Kolbe is commonly referred as “Kimari” or “Kangaita” weevils whereas *S. mixtus* is referred as “Nyambene” weevil (Benjamin *et al.*, 1968). The two weevils are quite similar but not identical and only differ in colour and size. Kangaita weevils are mostly bigger in size and are dark shiny grey in colour while Nyambene weevil are smaller and black in colour. Nyambene weevils, adults are approximately 7-8 mm long while Kangaita weevil can be as long as 12mm, the thorax is covered with raised spots (tubercles).. Both species, as adult weevils defoliate tea by chewing the foliage. They eat the leaf margin causing recognizable notches ford-like (notch-like) indentations in the leaf margin/edges a phenomena that are seen and familiar with the black vine weevils (*Otiorhychus sulcatus* Fabricus) found in the USA (Trundel *et al*., 2006) and the strawberry root weevils, *Otiorhynchus ovatus* (Linnaeus) (Col. Curculionidae) (Sabahi *et al.*, 2008).

In severe attacks there is almost complete defoliation especially with Kangaita weevils. Kimari/Kangaita weevils occur throughout the tea growing areas of Kenya. Occasional isolated outbreaks occurs causing variable level of damage. The weevils are prevalent in the areas around the foot of Mt. Kenya, on the east and southern slopes of Mount Kenya (Kirinyaga and Meru (Nyambene Hills) counties. In the Western Rift Valley side, they are prevalent in Kericho, Sotik and certain parts of

Kisii (Benjamin, *et al.,* 1968). Unimodal seasonal variations of the weevils occur throughout the year and these variations have been reported to be fairly consistent each year but slight shift may occur after some successive years, (Benjamin, *et al.,* 1968) probably due to weather changes and other unknown factors. The tea root weevil (*Aperitmetus brunneus*) [Hust]*)* pest girdles the stem just above ground level. In Sotik the pest has been observed to feed on the bark of twigs and branches of tea. The plant wilts and dies. The larvae feed on taproots causing wilting, stunting and finally death of the plant. In Brazil, The economic importance of these insects is related mainly to the presence of insect fragments in dried tea leaves, hindering the use of machine harvest (Suguino *et al.,* 2013).

2.6 Biology and Ecology of the tea weevils

Kangaita/Kimari Weevil (*E. meyeri*) [Kolbe] and N*ematocerus* Weevil (*N. sulcalus* eggs are laid in a fold of leaves and as they hatch the young larvae drop and burrow into the soil (Anon, 2004). The larvae are white, legless and with brown head capsule as that for strawberry weevil mentioned above and similarly pupation takes place in an earthen cell in the soil. The life history details of egg, larva and pupa are not available for Nyambene Weevils (*S. mixtus)*, tea root weevil and others. It is most likely some of the stages development is in the soil, the eggs, larvae and pupae as it is reported that the larvae of most of these weevil species live in the soil and eat the roots, underground stem, and germinating seed (Trundel *et al*., 2006; Suguino *et al.,* 2013). It could be also that it hibernates into the soil as is with most coleopterans when the season is unfavourable (Trudel *et al.,* 2007). The wing covers on adults are fused and subsequently, it does not fly, they are active walkers. Adults feed for several weeks before they begin laying eggs. Several generations can occur each year depending on the climatic conditions. Viewed up close they have long snouts that are a hallmark of the weevils. Their antennae arise from the tip of the snout rather than the base and hind margin of the eye is concave rather than convex (Trundel *et al*., 2006; Sabahi *et al.,* Anon.;2008; TRFK, 2012). The Kangaita and Nyambene weevils are not easy to locate, as soon they feel some movement they drop in to the trash and 'feign dead'. That is the adults weevils exhibit the play dead behaviour, when disturbed, they will tuck their legs in and fall to the ground, this is a phenomenon exhibited by the *otiorhynchid* weevils of apples too (Beers, 2004). Reports from farm managers (personal communication) in area where they occur indicate that they feed mostly in warm sunny afternoon. The *systates* weevils are rarely seen during the daytime. During the daytime they hide under the trash or loose soil and come out at night to feed on edges of leaves producing a characteristic damage (Anon, 2004). *Systate* spp. have been reported to be a pest in coffee plantations too though not common in Kenya (Mugo *et al.*, 2011)

2.7 Current Management Strategies of the Weevils

Laboratory experiments suggested that a neem-based product Godrej Achook formulated as wettable soluble powder, containing 0.03% (300 ppm) azadirachtin has an anti-feedant effect on the tea weevil. *E. meyeri* weevils stop feeding or eat less when the product is applied at concentrations of 10 and 20% (Sudoi, 1998). Use of or broadcast of *B. bassiana* inoculant when attack is in mature tea has shown promising results in the field (TRFK, unpublished) when broadcasted as inoculum in solid state of wheat bran. Hand picking is effective where populations are not high. Although chemical control of adults is difficult due to their ability to hide, spraying trash on the ground with Karate 2.5 WG at 1g per litre of water or any other recommended plant based insecticide (TRFK, 2012) is currently recommended. Therefore, very little in the way of bio-control or cultural control or other control measures are available for management of tea weevils. In essence, the weevils are contained by natural enemies and induced plant defences in Kenya.

In China, *Myllocerinus aurolineatus* (Voss) (Coleoptera, Curculionidae) feeds on the young leaves and tender plants of *C. sinensis.* This species exhibits aggregation behaviour and can severely reduce the yield and quality of tea. Mass trapping with aggregation pheromone, which attracts both females and males, has been found to be the most reliable and cost effective measure for managing this pest (Sun *et al.*, 2010). In India leaf eating weevil, *Myllocerus* sp. **(**Curculionidae: Coleoptera**)** are controlled effective by the application of endosulfan or quinalphos among other insecticides.

2.8 Entomopathogenic Fungus

Entomopathogenic fungus is potentially the most versatile biological control agent, due to their wide host range that often results in natural epizootics (Ferron, 1975). The most important fungal pathogens are *Metarhizium* spp., *Beauveria* spp., (Hypocreales: Clavicipitaceae), *Paecilomyces farinosus*, *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp (Bharathi, 2005). Mycoinsecticides are gaining increased attention as environmentally friendly insect control agents. Although thousands of fungal species infect insects, few have received serious consideration as potential commercial candidates. Use of this group of insect pathogen for practical pest control is limited due to lack of scientific information on the epidemiology of infection, in particular the host parasite system.

B. bassiana appears to have the broadest potential as a viable insect control agent. Adhesion of fungal spores to the host cuticle along with germination are prerequisites for the efficacy of fungal pathogens. Entomopathogenic fungi have been used successfully to control Thrips, aphids and whiteflies on French beans and snow peas in Kenya. (PCPB, 2011), the brown leafhopper on rice in the Philipines (Agunda *et al.,* 1988), *Leucenia psylid* on *Leucaenia* (Ahmad, 1993) and Banana root weevil on Bananas (Ferron, 1981, Mesquita, 1988, Nankinga, 1994, Muraleedharan *et al.,* 1997). *B. bassiana* (Balsamo) Vuillemin have been observed to occur in pecan orchards and contribute to natural control of *Curculio caryae*. (Shapiro *et al.,* 2012). Baruah (1983) showed that many native entomopathogenic fungi occurred in tea ecosystem associated with the mortality of insect pests and concluded that there is a need to study the prospects of utilizing them under the ambit of IPM programme. Locally, in tea for the natural control of tea weevils, *B. bassiana* have been observed contributing to natural control in the field (unpublished observation). Therefore, among the various bio-control agents using entomopathogenic fungi to control tea weevils, *B. bassiana* (Balsamo) is the most promising.

2.8.1 *Beauveria bassiana*

B. bassiana is a fungus that grows naturally in soils throughout the world and acts as a parasite on various insect species, causing white muscardine disease; (Feng *et al.,* 1994; Inglis *et al.,* 1997;). *Beauveria. bassiana* (Balsamo) Vuillemin displays a broad host range and is able to target about 200 species of diverse arthropod species and has been recorded from a number of coleopteran hosts and their larvae. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Bharathi, 2005). These fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. It was formerly also known as *Tritirachium shiotae*. *B. bassiana* is the anamorph (asexually reproducing form) of *Cordyceps bassiana*. The latter teleomorph (the sexually reproducing form) has been collected only in eastern Asia (Lizz *et al.*, 2001). The name *B. bassiana* has long been used to describe a complex of morphologically similar and closely related species. (Rehner and Buckley, 2005) have shown that *B. bassiana* consists of many distinct lineages that could be recognized as distinct phylogenetic species. There are many different strains of the fungus that exhibit considerable variation in virulence, pathogenicity and host range. In culture, *B. bassiana* grows as a white mould. On most common cultural media, it produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short and ovoid, and terminate in a narrow apical extension called rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension. The conidia are single-celled, haploid, and hydrophobic.

The insect disease caused by the fungus is called white muscardine disease. The microscopic fungus have contact mode of action. It contacts the cuticle, forms root like structures known as appresoria, penetrate into the insect system, proliferates and ultimately kills the insect. Afterwards a white mold emerges from the cadaver and produces new spores (Gul *et al.,* 2014). A typical isolate of *B. bassiana* can attack a broad range of insects; various isolates differ in their host range. The factors responsible for host susceptibility are not known. High humidity or free water is essential for conidial germination and infection establishes between 24 and 48 hours under ideal relative humidity (Kumar, 1984; Gul *et al.,* 2014). The infected insect may live for three to five days after hyphal penetration and, after death; the conidiophores bearing conidia are produced on cadaver. The biology of *B. bassiana* with reference to its practical application to the control of insect pests is that mycelial growth and sporulation are two different developmental stages. Environmental conditions most suitable for sporulation differ from those for mycelial growth, but conditions suitable for spore germination are usually similar to the latter. Nonetheless, the high adaptability and high resistance of *B. bassiana* to adverse conditions account for its universal distribution. Wide host range and the practicability of employing it as an agent in the microbial control of insect pests (Teng, 1962, Kumar, 1984; Gul *et al.,* 2014)

Aerial conidia sporulated from infected or mummified cadavers are widely disseminated by wind. Splashing of rain also accounts for spreading but only for short distances. The fungal propagules (conidia spores) have been reported to be disseminated through irrigation water (Bharathi, 2005, Gul *et al.,* 2014).

2.9 Biocontrol by *B.bassiana*

B. bassiana is an important entomopathogenic fungus currently under development as a bio-control agent for a variety of insect pests (Thungrabeab and Tongma, 2007). Besides silkworm, the extensive list of hosts includes such important pests as whiteflies, aphids (PCPB, 2011), grasshoppers (Milat-Bissaad 2011), termites (Avulova and Rosengaus, 2011)., Colorado potato beetle (Posada, 2004), cereal beetle (Golshan *et al.,* 2013).., bark beetles (Mahr 2003), bugs (Vladimir, 2012), European corn borer, stem borer (Maniania 1993; Maniania 1997), codling moths, mites (Ferron, 1985) and chick pea pod borer (Bajya *et al., 2015)*. Natural enemies, such as ladybird beetles, are susceptible too (Mahr, 2003). Although reported to be non-toxic to vertebrates, the potential allergenicity of *Beauveria* species has not been widely studied (Westwood *et al.,* 2006). Strains of *B. bassiana* have been licensed for commercial use against whiteflies, aphids, thrips, and numerous other insect and arthropod pests (PCPB 2011). *Beauveria bassiana* fungal formulations are now being employed in the management of insect pest on a range of vegetables, melons, tree fruits and nuts, as well as organic crops as alternatives to chemical pesticides.These agents are natural occurring and are considered to be non-pathogenic to humans, although a few cases of *B. bassiana* mediated tissue infections have been reported (Westwood *et al*., 2006). *Beauveria bassiana* is available commercially as a microbial insecticide since *B.bassiana* strains can now be mass produced by a fermentation process and formulated to enable the fungus to withstand ultraviolet light, and temperature and humidity extremes commonly encountered in the field.

Fargus and Luz (2000) studied on the effects of both moisture and temperature on the infectivity of *B. bassiana* and reported that the most favourable conditions were 97 per cent relative humidity (RH) and temperature of 20°C. Under less favourable alternating conditions (lower and higher temperature) the amount of inoculums required for killing 50 per cent of first instar nymph was 10 or 20 times higher than the above RH and temperature. Similarly, Akello *et al*., (2007) showed that the fungus viability against Banana weevil *Cosmopolites surdidus* was constrained by abiotic and biotic factors especially temperature, humidity and light. Otherwise, several studies have led to the use or has shown potential use of the fungus as a biological control. Manainia (1997), evaluated formulations of *B.bassiana* on management of Maize stem borer (*Chilo partellus)* and reported 5-100% mortality in stem borers *C. partellus* larvae and 26-86% in *Busseola fusca* , he suggested the fungus potential as a strong microbial agent in control of the pests. Castrillo (2011) showed that the fungi was effective in the control of *Ambrosia* beetle *Xylosandrus germanus* (Coleoptera:Curculioniodae). Wekesa, *et al*., (2006) showed that both *B.bassiana* and *Metarhizium anisopliae* isolates were capable of causing significant mortality in eggs and motile stages of *Tetranychus evansi*. The eggs and the adults being the most susceptible; he proposed *B.bassiana* and *M. anisopliae* as promising control agents. Dembelio *et al*., (2010) noted a high potential of *B.bassiana* in all stages of the life cycle of red palm weevils *Rhynchophorus ferrugineus*. He further found that strains of the fungus could infect eggs, larval and adult stages of *R. ferrugineus* efficiently and transmit the disease to untreated adults. Ngumbi *et al*., (2011) realized high potential with a mortality rate of above 75% when he investigated the pathogenecity of *B.bassiana* and *M. anisophilia* against adult *Phlebotonus duboscqi* (never-lemaire). Limited reported work exists on use of entomophatogenic fungi and *B .bassiana* against weevils of tea (Sudoi *et al.,* 1999, Anon, 2001).

2.10 Push-Pull Strategy for Insect Pest Management

The strategy is diversionary in action. The term 'push-pull' was first conceived as a strategy for insect pest management in Australia by Pyke *et al.,* (1987). They investigated the use of repellent and attractive stimuli, deployed in tandem, to manipulate the distribution of *Heliocoverpa* spp. in cotton to reduce reliance on insecticides, to which the moths were becoming resistant. The concept was later formalized and refined (Miller and Cowles, 1990) who termed the strategy 'stimulo-deterrent diversion' while developing alternatives to insecticides for control of the onion fly, *Delia antiqua*. The most successful example of the push-pull strategy currently being used by farmers was developed in Africa for controlling stem borers on cereal crops (Khan and Picket 2004). The push –pull strategy has been employed by planting grasses around the perimeter of the crop to attract and trap the pests, whereas other plants, like Desmodium planted between the rows of maize repel the pests and has an added advantage of controlling the parasitic plant Striga. The protection employed by desmodium in striga suppression has been established to involve a combination of mechanisms ranging from increased availability of nitrogen, soil shading, and an allelopathic root exudation. Exudates from desmodium roots possess striga seed germination stimulation and radical growth inhibition properties which diminish striga seeds through suicidal germination and a continual reduction of the soil seed bank. (Kfir *et al.*, 2002; Khan *et al.*, 2006; Cook *et al.*, 2007)

The development of a reliable, robust, and sustainable push-pull strategy requires an understanding of the pest's biology and the behavioural/chemical ecology of the interactions with its hosts, conspecifics, and natural enemies. The specific combination of components differs in each strategy according to the pest to be controlled (its specificity, sensory abilities, and mobility) and the resource targeted for protection. Push-pull strategies may involve the behavioural manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive or unsuitable to the pests (push) while luring them toward an attractive source (pull) from where the pests are subsequently removed. The push and pull components are generally nontoxic. Therefore, the strategies are usually integrated with methods for population reduction, preferably biological control. Push-pull strategies maximize efficacy of behaviour-manipulating stimuli through the additive and synergistic effects of integrating their use. By orchestrating a predictable distribution of pests, efficiency of population-reducing components can also be increased. The strategy is a useful tool for integrated pest management programs reducing pesticide input.

Studies related to the use of trap crops in tea are scarce. The use of trap crops has been used based on the principle that more preferred varieties of the cash crop can be grown to reduce damage in less preferred varieties. The more preferred variety not only attracts pests for oviposition and feeding, but serves as a sink for insects or the pathogens they vector (Shelton and Badenes, 2006). A trap crop also manipulates the habitat in an agro-ecosystem, which can be included under the ecological engineering approaches for the purpose of integrated Pest Management (IPM) (Gurr *et al.,* 2004). As such, for example a susceptible tea clones such as Tocklai vegetative clone TV1 to *Helopeltis theivora* have been utilized as the trap crop (Lakhsmi *et al.,* 2009). During pruning, a few tea bushes in the centre is intentionally left unpruned for a day or two to accumulate *H. theivora* adults, which are then sprayed with insecticides to kill the pests (TRA, 1994). This fits well with the concept that trap crop can be used in conjunction with pesticides (Zehnder *et al.,* 2007).

Likewise, the shade tree *Gliricidia sepium* has been found to serves as a diversionary host for *G. dilatatus* (Sivapalan and Seneratne 1977). Limited studies exist on the the preferred crops to various tea pests which can employed for the push-pull technology in Kenya. (Cook *et al.,* 2007; Khan and Pickett 2004) or stimulo-deterrent diversion (Miller and Cowles 1990). A systematic search for these plants is essential, for example, shade trees (Barua, 1994 and Deka *et al.*, 2006) are interplanted with tea plants in Bangladesh, India, Sri Lanka, and Indonesia to provide partial shade for regulation of photosynthesis and leaf temperature. However, this creates a microclimate suitable for *Laspeyresia leucostoma* Mayer (Lepidoptera: Eucosmidae*),* *Emploasca flavescens, Glyptotermes sp*., and *H. theivora* but is unsuitable for *Oligonychus coffeae* (TRA, 1994). These examples are in agreement with the principle that intercropping with distantly related plant species can encourage generalist herbivores (Andow, 1991; Zehnder *et al.,* 2007), but studies on their effect on specialists are lacking. In general, such plants including weeds in field crops can visually or chemically interfere with specialist herbivores by reducing the resource concentration (Root, 1973), presumably creating a less favourable habitat. The trees, however, provide refugia for many generalist predators, especially spiders, but more particularly are ideal habitats for birds and small mammals; each shade tree resembles a beetle bank, which is a refugium for predatory beetles, spiders, birds and small mammals usually in the form of a semi-permanent raised strip in a crop field (Thomas *et al.,* 2001). Citrus interplanted with tea in Georgia encourages build-up of indigenous aphidophagous parasitoids to suppress *Toxoptera auranti* on tea and *Aphis craccivora* Koch (Hemiptera: Aphididae) on citrus (Hazarika *et al.,* 2001).

2.10.1. Attractants and Repellents

The use of attractants in pest management systems can be precise, specific, and ecologically sound. Although the use of attractants against tea pests is not yet popularized, synthetic or natural sex pheromone-based attractants are used for monitoring the population of *H. theivora*, Adoxophyes sp. (Tamaki *et al.*, 1979) *H. coffearia* (Noguchi *et al.*, 1981), and *Ascotis selenaria cretacea* Assc. (Lepidoptera: Geometridae) (Ohtani *et al.,* 2001). Research on repellents against tea pests is still in infancy, as is reflected in the absence of published reports.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Determination of Tea weevil Distribution using GIS in the East of Rift Valley

A digital 1:50,000 topographic map sheet of the tea growing region, which has the study area, Global Positioning system (GPS), Computer hardware and software (Esri'sArcInfo ArcGIS software at Version 10.1) were used for mapping and developing a digital tea weevil distribution map. Before determining the actual weevil distribution, an assessment was made to determine whether the weevil species can enter and establish in new areas, the current distribution of the pest was taken into account and the extent to which the new area contains suitable host plants, climate and the other key factors for successful colonization was also considered (Nelson *et al.,* 1999; Baker, 2015). Therefore potential or risk weevil prone areas in the tea growing regions of Kenya were first determined by modelling using, computer hardware and software and secondary data namely; rainfall, temperature, Agro-ecological zones, tea growing region and guided by initial point sites/areas where there have been reports of weevils at the Tea Research Foundation (TRFK) laboratories(Anon. 2007; Anon 2008) Annual average rainfall, Annual Average mean daily temperature and Agro-ecological zones data (spatial reference data) was derived from a research done by Food and Agriculture Organization of the United Nations (FAO) in 2005 under the AFRICOVER, Kenya(AFRICOVER, Kenya - Spatially Aggregated Multipurpose Landcover database, FAO, Rome, Italy,http://www.africover.org)" (Figures 2-4). The data were placed together (layered) to assit in the analysis (Baker *et al.*, 2015)

3.1.1 Annual rainfall, Mean temperature and Agro-ecological Zones data analysis

Rainfall, Temperature and Agro-Climatic Zone data was analyzed using Esri'sArcInfo ArcGIS software (Version 10.1). Rainfall layer was classified based on the Annual average rainfall for the whole country. The colour coding used assigned red Colour to places with the lowest annual precipitation(< 350mm/year) while those areas with high precipitation (>1000mm/year)-were assigned green colour (Fig 2). Temperature layer was classified based on the Annual average of mean daily temperature for the whole country. The colour coding used assigned colour red to places with the highest annual temperature (> 23.5^{o}C) while areas with lower temperatures (<13.5 ^{o}C) were assigned green colour (Figure 3). Agro Ecological Zones (AEZ) layer was classified based on the Climatic Zones for the whole country. The colour coding used assigned colour red to areas that have low potential to agriculture while areas with high agricultural potential were assigned colour green (Fig 4). Maps for parts of Eastern Kenya Tea growing regions showing rainfall patterns, temperature and Agro-ecological zones were then obtained by extraction from the above whole country data maps. In addition, the data for tea growing zones in parts of Eastern Kenya was obtained from the Tea Boord of Kenya map which was scanned, geo-referenced and digitized using ArcGIS software and inserted on the maps. Factories points provided by KTDA and points where different species of the weevils have been reported were added on the maps for the Eastern Tea Growing Region (Anon 2007; Anon 2008) (Figures 5-7)

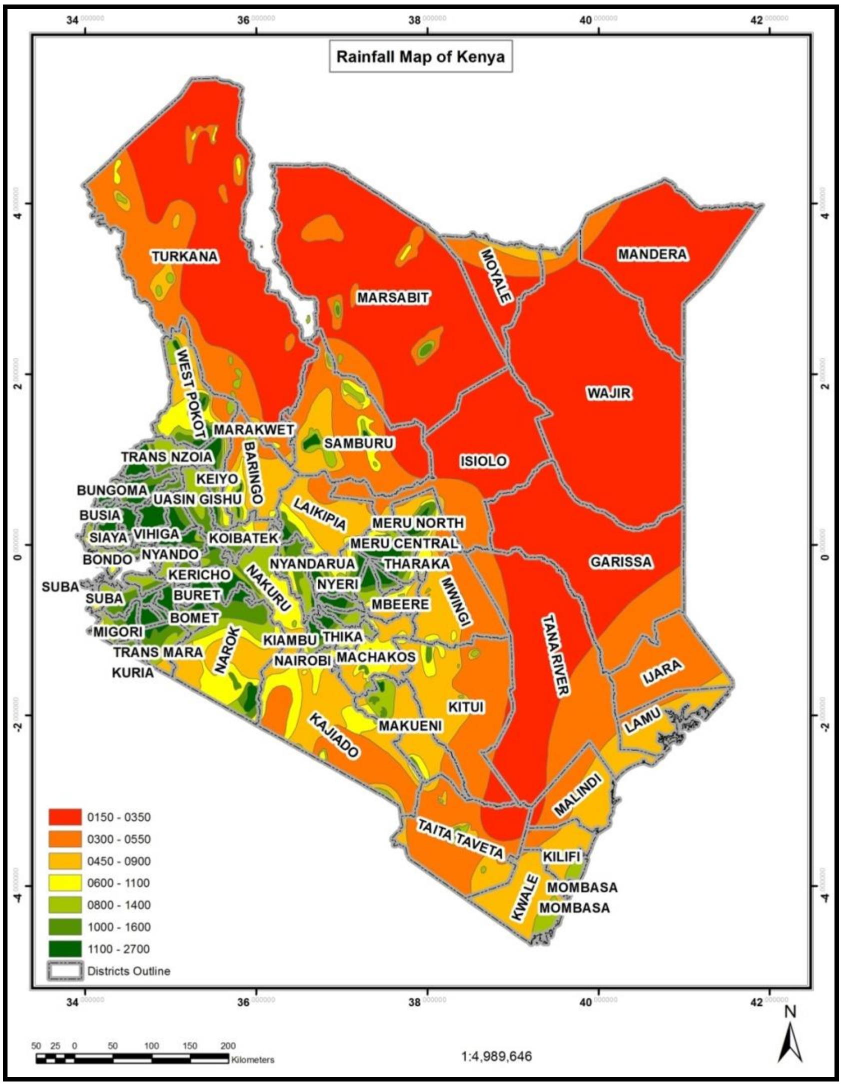


Figure 2 Annual average rainfall of Kenya.**(Source: FAO, Africover, Project, Kenya, 2005)**

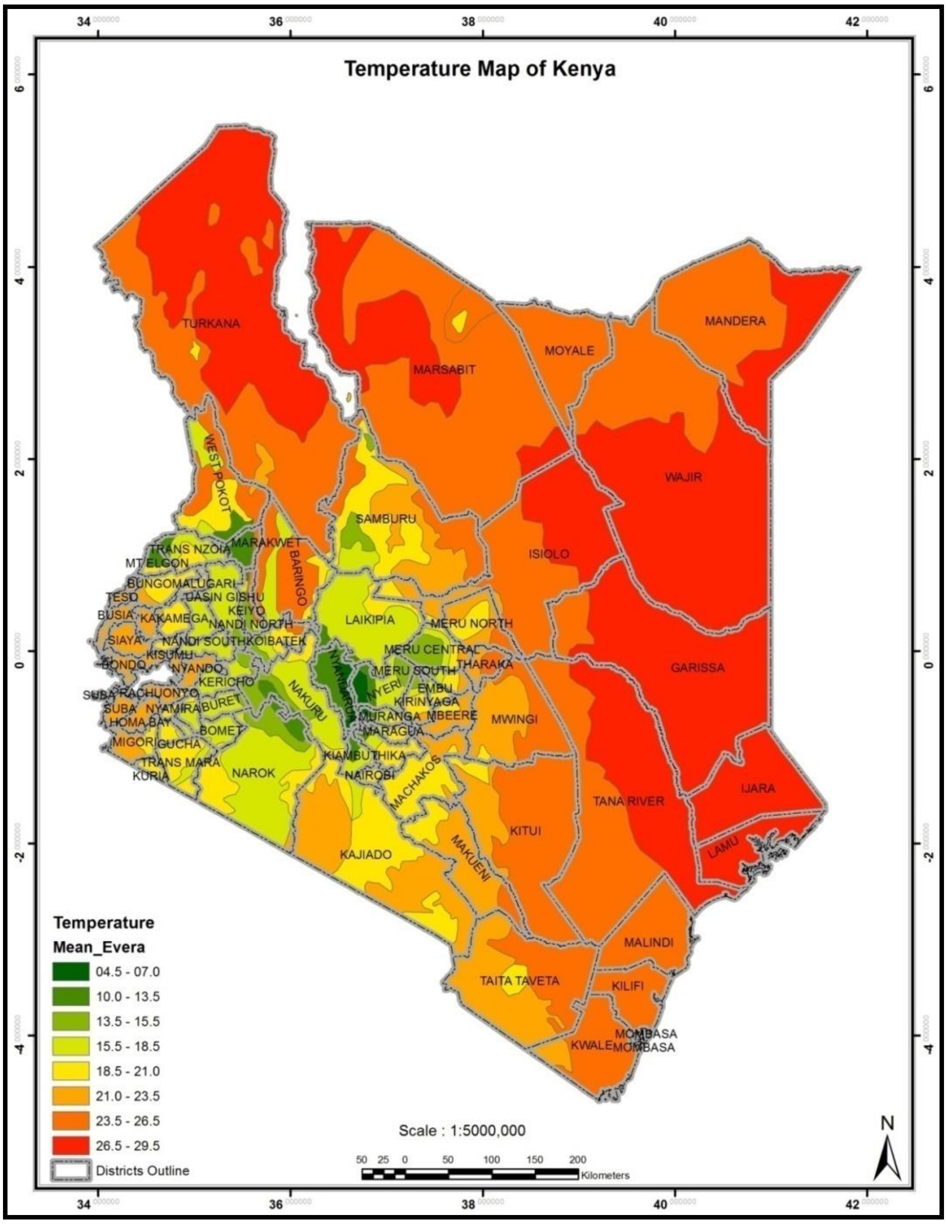


Figure 3: Annual average daily mean temperature of Kenya**. (Source: FAO, Africover Project, Kenya, 2005)**

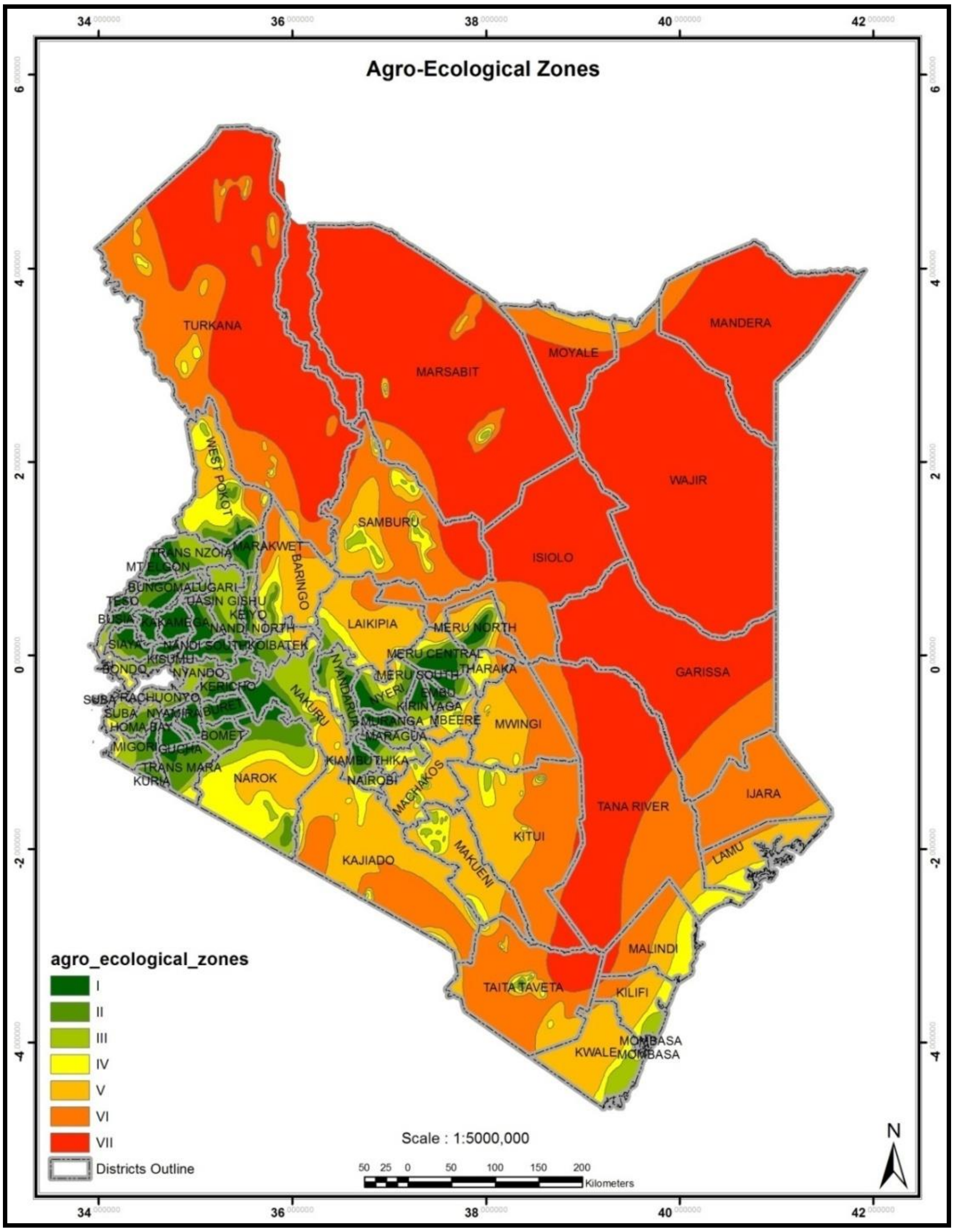


Figure 4: Agro Climatic Zones of Kenya. **(Source: FAO, Africover Project, Kenya, 2005)**

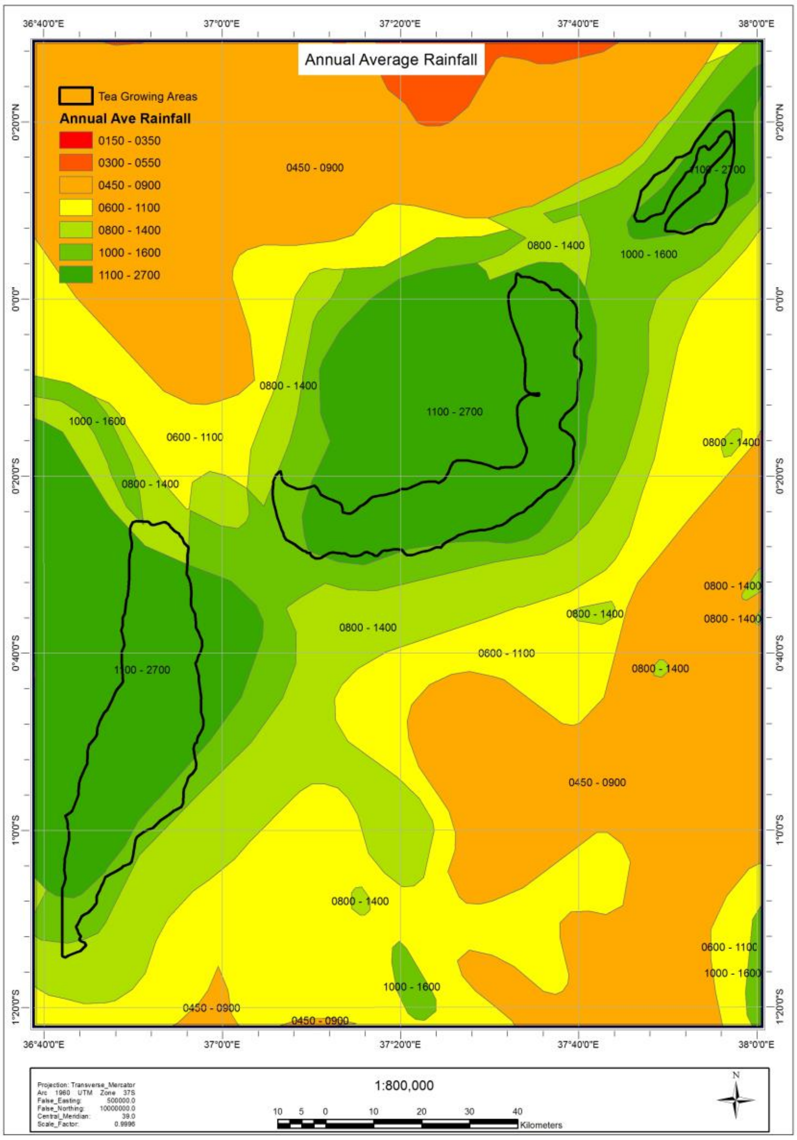


Figure 5: Eastern tea growing areas of Kenyan average rainfall (mm) map: **(Source: FAO, Africover Project, Kenya, 2005 and TBK 2003)**

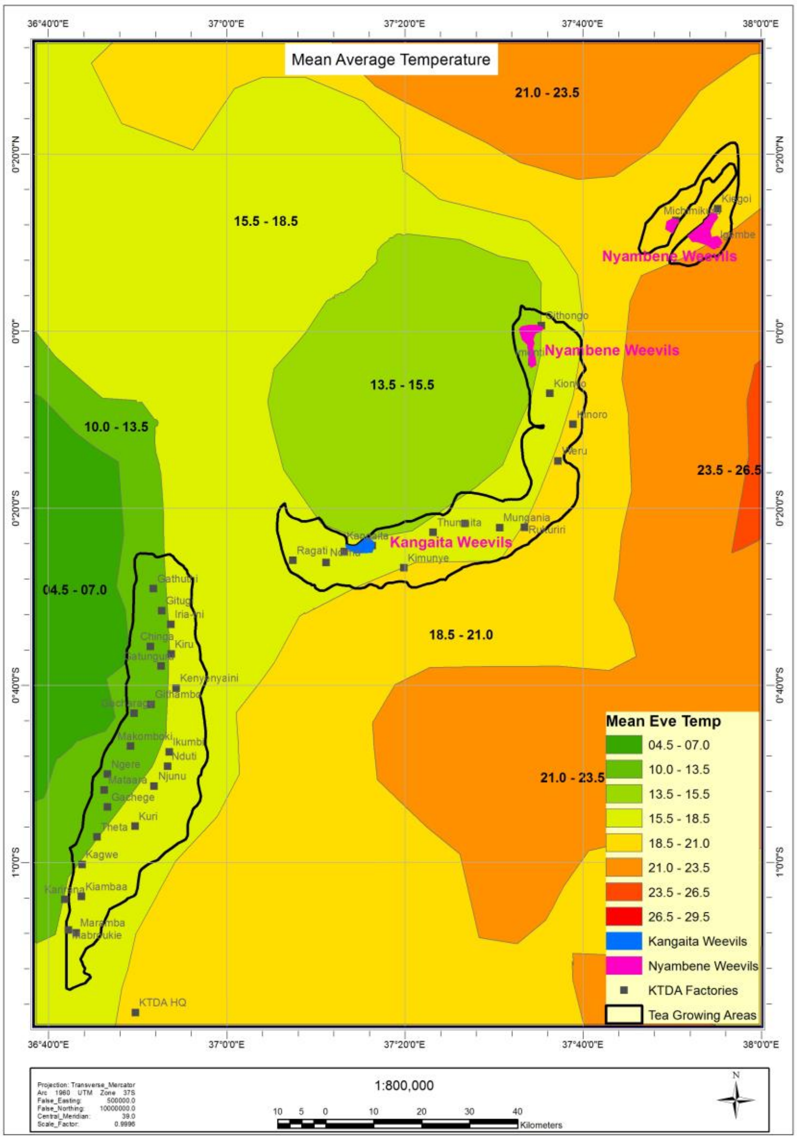


Figure 6: Eastern tea growing areas of Kenyan average Temperatures ^oC map with major weevil prevalence areas. **(Source: FAO, Africover Project, Kenya, 2005 and KTDA/TBK 2003)**

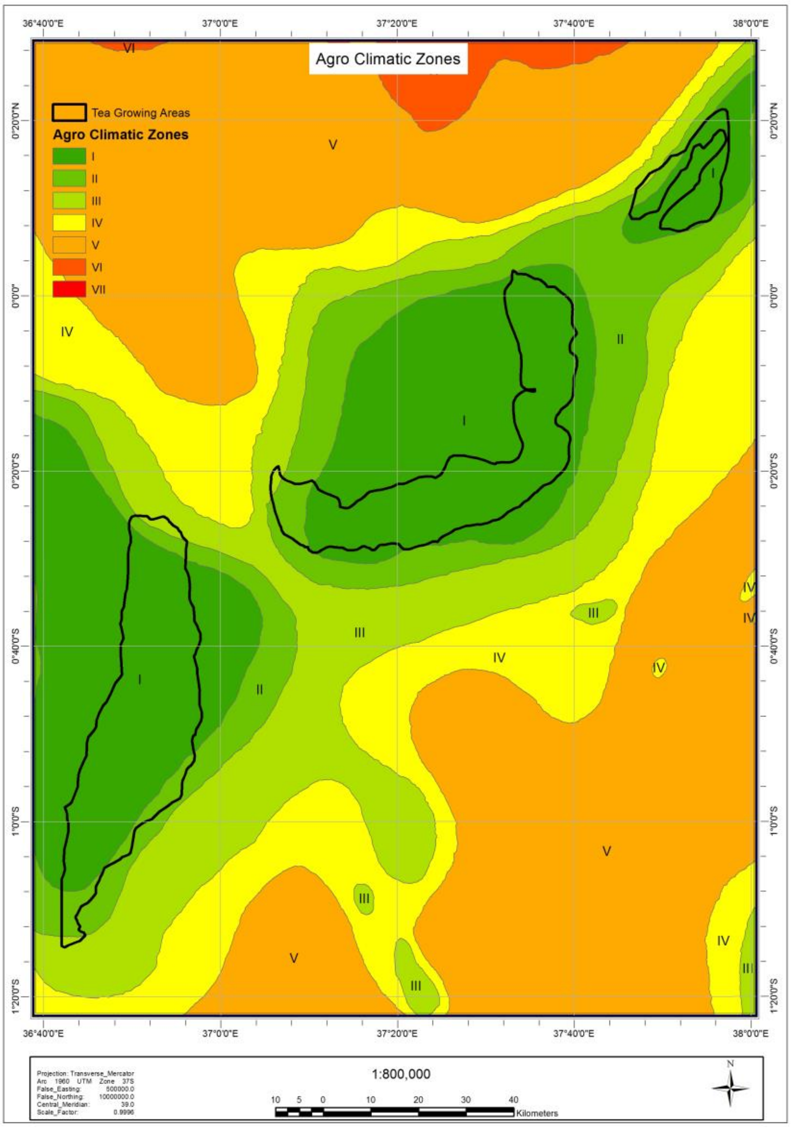


Figure 7: Eastern tea growing areas of Kenyan Agroecological Zone map**. (Source: Farm Management Handbook, 2007)**

3.1.2 Survey to determine actual distribution of weevils on the ground

Ground thruthing followed the above modelling exercise to demarcate the actual areas infested with the tea weevils. The study survey for the actual tea weevil distribution in Eastern Kenya was carried out using purpose sampling. Only areas where reports of the weevils have been noted severally were targeted, to determine the coverage or extent on the ground and the surrounding areas. The survey was conducted in September 2013 in the areas of Michimukuru, Kiegoi, Kimunye, Ragati, Imenti, Kangaita Githongo Mununga, Rukuriri and Mungainia KTDAs and Nyayo tea Zones catchments (Kirinyaga, Embu and Meru Counties-parts of Eastern Tea growing region). The areas chosen were based on areas where weevil infestation was a regular phenomenon (Anon 2007, Anon 2008). Farms were sampled within at least a radius of 1000m from where they have been reports of tea weevil occurrence. The exercise was conducted with the assistance of the KTDA management personnel who are conversant with the area and could easily locate the weevil boundaries. The outcome of the exercise of was confirmed with the same personnel. Coordinates of the boundaries where weevils are found were recorded using a Global Positioning System (GPS) - Mobile Mapper 6 so as to map out weevil infested area. The GPS points were taken based on WGS 84 Datum so as to match the other data sets being used for this exercise.

3.1.3 Survey to determine relationship of weevils and cultural agroecosystem practices

Based on resources available and the time available, a face to face interview using a questionnaire (Appendix 1) with 45 tea growers was also administered as well in the areas surveyed to determine parameters, such as cultural practices, cropping patterns among others, associated with weevil infested tea farms of the Eastern tea growing region. The growers were purposively selected from sub-catchment from around 20-30% of the farmers. Out of the farmers who reported the problems 5 farmers were selected purposively from each catchment Focus group discussion was done in every cacthment area with meeting with farmers and with key informant interviewee, the Technical Extension Service Assistant (TESA) to give more insight. Information on different aspects of tea husbandry and other aspects around tea, viz. tea variety/clone planted, area size of tea farm, age of tea bushes, type of livestock enterprises kept by the tea grower, tea intercropping with trees, fertilizer types, application rates and frequency of application, diseases and pest identification and management were collected. Where possible the damages were visually assessed on tea plants to confirm the presence or absence of the weevil. The data collected were grouped into several categories and sections (where applicable) for statistical analysis, viz. tea farms with weevils verses those without, planting material were grouped into clonal plants and mixed clones, area size planted tea by the grower were grouped into area <1ha 1-10ha, 11-30ha, 31-50ha, 51-100ha 101-300ha and 301-500ha, Composition of livestock were grouped into categories of sections of a mixture of the different species of livestock, Composition of shade trees were grouped into categories of sections, viz. having a mixture of five different species to section having only one species of tree, different rates of NPK fertilizer, manure versus chemical fertilizer, weevils and diseases, weed problems versus weevils .

3.2 Efficacy of *Beauveria bassiana* isolates and effect of tea weevil on productivity of tea in two geographical locations.

3.2.1 Field Experimental Sites,

A study to determine field efficacy of *B. bassiana* and effect of weevil on productivity of tea was conducted from June 2012 to November 2013 in two different major tea weevils occurring geographic regions;(i) Kenya Tea Development Authority (KTDA) Igembe, Giciaro tea Farm at an altitude of 1650 m.a.s.l. and coordinates at Lat. $0^0$08'S, Long.37^0 54'E. Giciaro tea farm is located in Igembe Sub-location, North Meru location of Meru County and about 18km west of Maua Town (ii) Kenya Tea Development Authority (KTDA) Munuga. Mr. Njogu Kiruki's farm, (Reg. No. MU0030185), Kirinyaga at an altitude of 1784 m.a.s.l. and coordinates at Lat. $0^0$27'S, Long.37^0 13'E. The farm is located in Kaguyu Sub location, Mutira location of Kirinyaga Central District about 1 ½ kms from Mununga KTDA Tea Factory. The farm (Mr Njogu's) is planted with about 3,398 tea bushes of mixed clones. The farms were previously having coffee plantation before tea establishment in years 2003 and 1994 for Giciaro and Njogu's farms respectively

The soils in the two areas consist of well-drained, deep reddish brown to dark red friable clay with acidic humic top soil (humic nitosol) and a pH range of 4.4-5.6 (Siderius and Muchema, 1977, Jaetzold *et al.,* 2007). The mean maximum and minimum temperatures are 23^{o}C and 13^{o}C respectively. It receives an average rainfall of above 1000 mm per year. The rainfall is bimodal and long rains are received between October and December while the short rains fall between March and June.

3.2.2 Source of *B. bassiana* Fungus Isolates

Beauveria bassiana was isolated from the soil using the insect bait method. Larvae of the waxy moth (*Galleria mellonella* (Lepidoptera: pyralidae) were used as insect baits to capture entomopathogenic fungi innoculi from soil samples through body surface contamination. Six isolates were collected from different localities in Kenya viz-a viz Nyambene/Igembe (Isolate Bb Gi 7a and Bb Gi 7f), Kericho (isolate Bb Ke 6a) and Chepkoilel campus of the University of Eldoret (Isolate Bb Ch 8c, Bb Ch c2 and Bb Ch c3). The trap insect technique (Bedding and Akhurst, 1975) was originally used for the isolation of entomopathogenic nematodes from soil. A colony of *G.mellonella* which was originally obtained from Kenya Agricultural Research Institute (KARI), National Horticultural Research Centre (NHRC) in Thika, was maintained in the laboratory at room temperature (20 ± 3°C). The larvae were reared on a *Galleria* diet that consisted of 307g maize meal, 225g honey, 45g bees wax and 90g yeast. The diet was prepared by first boiling the bees wax, once melted it was mixed with honey. The mixture of maize meal and yeast was poured into the melted bees wax with honey and stirred while cooking on a Bunsen flame until it became firm and evenly mixed. The mixture was then placed on a bowl with a perforated lid and left to cool overnight.

Moist soil was then placed in a petri dish and approximately 10 medium sized larvae were placed into the soil. The dishes were regularly turned in the beginning of baiting period (first week) to make bait insects penetrate as much soil as possible while they were still vigorous. The larvae were left in the soil until a fungal growth was observed on them growing. Whole infected larvae that already showed hyphal growth on their bodies was first surfaced sterilised with 70% ethyl alcohol prior to incubation on potato dextrose agar (PDA) (fluka Analytical, sigma-Aldrich, Chemie GmbH, Steinheim) to prevent external saprophytic fungi from growing. The larvae were incubated at room temperature until adequate growth of fungus was observed, and then the fungus was transferred to fresh PDA medium and incubated for at least 7 days under the same conditions. PDA culture is known to be among the cultures that has induced the best linear growth for *B. bassiana* according to the findings of Santa *et al.,* (2005). Therefore, after sporulation, microscopic examinations of the fungus were done. Whole living larvae that might have been infected with entomopathogenic fungi were surface-sterilised by dipping consequently in 70% ethyl alcohol and sterile distilled water; each for 3 seconds. Insects were then cultured under the same conditions as decribed above. The *B. bassiana* culture isolates were then stored on media slants placed in bottles and replenished very often mostly after every three months awaiting subsequent efficacy/lab bioassay test on the Nyambene weevil.

3.2.3 Laboratory Bioassay-Susceptibility of weevils to the fungal isolates

Bioassays of B.bassiana against the S. mixtus weevils on six (6) isolates were performed. The weevils were collected from the tea fields in Giciaro Farm. The collection was made 2 days before the experiment was conducted. The weevils were reared in glass cages fitted with mesh screens under room conditions. Fresh tea shoots (tea feeds) were placed in soil in small beakers and replaced every 2 days. Randomly sampled active adults of Nyambene tea weevils of the same ages (approximately 1 month old) as per sizes (Plate 1) were used for the bioassays. The weevils collected from field were assumed to be free of pathogen.   In addition observation were made in cages for one week for any infection before the bioassay. Furthermore, preliminary observations, showed that the weevils' lives for upto 2 months in cages The isolates used in the bioassays were taken from pure cultures in long term storage. PDA cultures of the isolates were flooded with 10ml of sterile water containing 0.1%

Tween 20 solution and the spores were removed by gentle agitation with a sterile loop. The spores were counted with the help of a haemocytometer and the spore concentrations were adjusted to 10^5, $10^4$and 10^3conidia/ml accordingly in distilled water. The bioassays were set up in a completely randomized design with three replicates of 6 month old adult weevils per replicate. The adult weevils were exposed to the fungus by the dipping technique, where they were placed in a large petri-dish (125 mm diameter) containing 30 ml of each fungal suspension for 1 min. Sterile-distilled water containing 0.1% of Tween 20 was used instead of fungal suspension for control treatment. Each batch of adult weevils was transferred to filter paper lined in a Petri dish on the laboratory bench for one hour to dry and then transferred or placed in perforated rearing jars and offered appropriate and equal pieces of tea shoots planted in small beakers to feed on (Plate 2). Weevils' mortalities were recorded up to 30 days post-exposure or up to 100% mortality where duration occurred before 30 days. The eaten tea leaves were replaced with fresh tea leaves every 3 days and the area consumed was measured and calculated by tracing on a graph paper. Dead weevils were counted, removed and incubated on damp filter paper within Petri dishes (20°C) and inspected for the presence of *B.bassiana* mycelium on the cadavers (Plate 3) as in (sabbahi *et al*., 2008). Selection of two isolates that showed promising in the control of the tea weevils was done for use in the successive field experiments.

Plate 1: Active adult Nyambene weevils (*S.mixtus*). **(Source , Author 2015)**

Plate 2: Perforated containers with small beakers planted with pieces of tea shoots for the adult weevils to feed on **(Source Author 2015)**

Plate 3: Cadavers of the Nyambene weevils with *B. bassiana* mycelium (14 days after Innoculation). **(Source: Author, 2015)**

3.2.4. Field Experimental design and Treatments

Field Establishment

Two sets of experiments were set up in each of the two sites. Each set starting June 2012 and September 2012 respectively. The tea fields in Giciaro Farm Igembe that hosted the fields trial comprised of mature tea bushes of clone TRFK 31/8, with a spacing of 4 X2 ft. Mr.Njogu's farm comprised of mature tea bushes of mixed clones of TRFK 6/8, PMC 51 and TRFK 31/8 with a spacing of 5 x21/2. Field efficacy of the isolates against the tea weevil was evaluated using three replicates in a randomized complete block design. Each replicate consisted of 5 (five) plots associated with treatments and one control: Each plot consisted of at least 20 bushes and surrounded by two rows of tea bushes as buffer zone, to safe guard the contamination of the controls and none fungal treated plots.; The treatments were of two efficacious *B.bassiana* isolates at a rate of $1x10^{13}$ conidia/ha (either applied with wheat bran on trash or sprayed on foliage), Karate, a synthetic pyrethriod which contains Lambdacyhalothrin 25% EC as the active ingredient was sprayed on trash and a control which was the formulation that contain no B. bassiana were the other treatment in the experimental layout (Appendix II).

NPK fertilizer of the of (26:5:5-N:P:K) application was done twice in a year at a rate of 100 Kg N /ha /year in both sites. Tea plucking rounds of two leaves and a bud were done between 9-12 days. The fields were maintained weed free for the duration of the study by manual removal (either hand hoeing or hand manual removal). Prunning time was done as per KTDA schedule which is normally in July of the pruning year with either 3 or 4 pruning year cycle. Giciaro tea field for the first set up experiment (starting July 2012) was pruned in July 2011 and later on July 2013 while the second experiment (starting Sept 2012) was pruned in July 2012.
In Mununga field trial area which started in July 2012, pruning was done in July 2010 before setting up the experiment while Mununga second trial tea field was pruned in July 2011 and all the neighbouring, surrounding tea bushes area was pruned in –July 2013. Plucking rounds were between 7-10 days in Munuga and weeding was manually done. Plucked green leaves were weighed using a clockface 5 kg Max, type of “Salter” weighing scale and recorded in Kg of fresh weight.

3.2.5 Formulation and fungi applications for Spraying.

B. bassiana isolates were applied into the demarcated plots, for the different isolates accordingly, using a modified formulation based on modified version as in Burges (1998) review. The *B. bassiana* formulation was made up of 1 % skimmed milk, 2% glycerol, 4% corn oil (modified to corn oil instead of canola oil) and 5 % clay (diatomous earth-known as Kaolin). Oil was used because of it’s excellent adhesive, promoting contact between the active ingredient (the conidia) and the lipophilic insect cuticle while also increasing the conidia’s rain-fastness on the waxy leaf surface of treated host plants (Burges 1998). Clay was added to protect conidia against UV light (Butt 2002). Glycerol was included due to its role as nutrient, as humectant, nutrient and adhesive; whereas skimmed milk acted as additional nutrient and humectant (Burges 1998). For each treatment, water containing the required concentration of conidia was added to the final formulation. Long-term effects of the formulation ingredients on the viability and virulence of the *B. bassiana* are unknown, so the final mixing of the formulation was conducted immediately prior to application, as recommended by Goettel *et al.,* (2002). The concentration was determined prior to application. All treatments were applied at intervals of once every month. The foliar sprays were applied taking care to thoroughly wet both sides of the foliage. A knapsack sprayer was used at a constant pressure of 40 psi and a low volume flow rate at an application rate of 1 X 10^{13} conidia/ha

3.2.6 Formulation and fungi applications with Wheat Bran (Solid substrate)

The fungal isolates was first cultured on potato dextrose agar (PDA) at 25^{o}C for 10 days and stored at 4 ^{o}C until use. In preparation of the seeding (inoculi), aerial conidia taken from a stock culture growing on a PDA agar plate was suspended in sterile water. The number of conidia in the suspension was counted using a haemocytometer, followed by dilution to 1.0×10^{8} conidia/ml. One millilitre of the conidium suspension was inoculated into a 250 ml Erlenmeyer flask containing 100 ml of liquid media, followed by culturing at 25 ± 0.1^{o}C using a rotary shaking incubator operated at 200 rpm. The resulting suspension was then used as the seeding inoculum for solid-state fermentation (SSF) for aerial conidia production.

In preparation of SSF, moistened wheat bran medium was transferred into a container and autoclaved at 121^{o}C for 25 min. The container with moistened solid media was then cooled, after which the seeding culture was added at 10% inoculum size (10^7 conidia/g wet medium) to each container, followed by thorough mixing using a sterilized spoon. The specimens were then incubated at room temperature and relative humidity for a period of 60 days from where it was removed mixed and broadcasted in to the fields at the rate of 1 $x10^{13}$ conidia/hectare(2g of conidia in100kg of wheatbran/ha). All the treatment applications were done once every month for 12 to 15 months

3.2.7 Persistency of the fungi at different Months in the soil

In order to estimate persistence of *B. bassiana* in the soil, approximately 100g of soil samples were obtained randomly around the root rhizosphere of each plot and packed for lab determination of amount of fungus which can be recovered from the soil, before the subsequent monthly application of treatments. *B. bassiana* recovery from the soil was done in the laboratory. Soil samples obtained from the root rhizosphere as indicated above from each plot were weighed. Using a weighing balance, 1g of the sample were weighed and placed in the test tube containing 10mls of sterile distilled water. Serial dilution was done to $10^{-4.}$ A selective media was constituted with 39g Potato Dextrose Agar,10mg Crystal violet, 10mg Cuprocaffaro WP (Copper Oxychloride - 85% equivalent to 50% metallic copper), Copper Oxychloride, 0.76µl Topsin SC (Thiophanate-Methyl 500g/L), 40mg streptomycin.The selective media inhibits the growth of other soil microbes except *B. bassiana* (Modified Shimazu and Sato 1996). Pipetted 0.1mls of the solution was then put onto the selective media on petri-dish then spread evenly and incubated at room temperature for 24-72hrs. Populations of germinating spores were determined from each plate (representing a plot) by counting.

3.3 Determination of a weevil repellant plant and a weevil attractant

Preliminary investigation to determine the repellent and trap plants was conducted by setting up an experiment, in cages in the facility of the Tea Research laboratory, with tea weevils and different types of plants which could be used as trap or intercropped depending on their preference by the weevil. The potential plants tested included pre-selected shade trees such as *Militia dura* and *Fraxinus* spp., fruit trees such as avocado and loquats and six tea clones namely; the purple tea (clone TRFK 306/1), TRFK 6/8, TRFK 31/8, TRFK 303/577, TRFK 301/4, 301/5 (the elite clones) among others. A repeat of the cages experiment to determine preference by the tea weevils was narrowed down to five plants. Selected for further investigation were tea clones TRFK 306 (purple tea) and TRFK 31/8. TRFK 306 is a new clone pre-released in 2011 with very high anthocyanins/antioxidants contents while clone TRFK 31/8 is a universal clone (Wachira *et al.,* 2012) and suitable for tea all tea growing areas in Kenya. The two clones were compared in terms of weevil damage when mixed by growing with potential attractant or repellant plants, avacodo fruit tree, Fraxinus spp. and *Milletia dura* (a shade trees). The two common weevil species, *E. meyeri* and *S. mixtus* collected from Kangaita and Mununga infested tea fields were used. Leaves of these plants were planted in small pots and left in cages for the weevils to eat. They were planced in pure and mixed stands and placed in the cages incomplete randomlyrandom design with 3 replicates. Pure stands which were taken as the standard control, consisted of pots with shoots of clones Purple tea (TRFK 306) and TRFK 31/8. Mixed stands consisted purple tea mixed avocado, purple tea mixed with M. dura, purple tea mixed with fraxinus spp., purple tea mixed with TRFK 31/8, TRFK 31/8 mixed with *M. dura* and TRFK 31/8 mixed with *Fraxinus* spp each plant per pot and 15 weevils was placed in the cages. The leaves were replaced every 2 days and ensured that there are 15 weevils in the cages by replacing dead ones, this was were for 10 days. The area consumed was measured and calculated by tracing on a graph paper

3.4 Data collection and analysis

To determine areas at risk with different weevils' species infestations, maps were developed by combining or layering the three climatic factors affecting the growth and thriving of the weevils species i.e. Rainfall, Temperature and the Agro ecological zones which takes into account soil types and elevation of the area as was done by Baker, (2015).

Esri's ArcGIS software was used to convert the three shape files i.e. Rainfall, Temperature and the Agro Climatic Conditions into Raster files. This was done through the use of Conversion tool in ArcGIS. The Rasterised files obtained were reclassified or overlaid using the Reclass Tool from Spatial Analyst Extension of ArcGIS. Here, only layers that met the conditions desired were considered and three maps of Rainfall, Temperature and the Agro Climatic Conditions were obtained.

Later, the three climatic conditions Raster maps were combined/layered i.e. the areas that are commonly shared by Rainfall, Temperature and the Agro Climatic Conditions were calculated using Esri's ArcGIS Raster Calculator to obtain areas that favour different weevils' species for the entire country. Maps were created that only show the eastern tea growing zones only.

To determine distribution pattern of weevils in the Eastern parts of the tea growing regions in Kenya, Data clubbed under the aforesaid groups were analysed for frequency distribution. The collected data was analysed using Statistical Package for Social Sciences (SPSS). Ver. 10.0. Means were presnted in forms of a pie-chart and Tables

To determine the influence of seasonal variation on the weevils' abundance and distribution, Records of prevailing weather in the field were taken at both Mununga KTDA factory (near Mr Njoki Farm and with similar climatic conditions) and at Igembe KTDA tea Factory (with similar weather conditions as Giciaro Farm) through out the experiment period in order to relate to the weevil incidences. The rainfall was recorded daily using a rain gauge and monthly means determined. The temperature recording was done by use of minimum and maximum thermometer and monthly means calculated. Records of prevailing weather conditions were recorded in order to relate to the records on damage score/percentage damage which will be used to give an indication of weevils incidences throughout the period by correlating with number of weevils collected from around three bushes in the controls plots. Data on damage score, photosynthetically active radiation intercepted by the plant canopy, ground cover and fresh green weight were collected in the field Experiments. In Mununga, Njoki,s farm, because of the mixed clones and to avoid non uniformity, five bushes per plot of clone TRFK 6/8 was tagged and used for data collection. Weevil incidence determination was done by counting the number of weevils in the control plots and relating to the damage scores or percentage damage every month.

Damage Score

The damage score assessment was made every month for 12 mnths. A score scale ranging from of 1-4 was used, where 1 represented damage from 0%-25%, 2 represent damage from 26%-50%, 3 was within the range of 51%-75% damage level and 4 was within the range of 76% to 100% damage (Weigand and Bishara, 1991). Damage score was done vuisually on mature leaves and on fresh pluckable shoots separately and was conducted in a randomly selected area using a grid of 0.40cm X 0.40cm. Data was also collected on the number of damage pluckable leaves versus undamaged and later converted to percent damaged pluckable leaves. Similarly data for mature maintenance leaves was also collected to determine the type of leaves the weevils prefer.

Ground cover (GC)

Ground cover was measured by placing a 1.2 x 0.8m, grid with squares of 0.0036m2 just above each of three randomly selected plants' canopy and the cover in each square assessed from directly above. Following the method used by Burgess (1992), squares were recorded as fully covered (>75%), half covered (25 - 75%) or empty (< 25% cover). The GC was expressed as the product of the total number of squares counted and multiplied by $0.0036m^2$. GC records were taken once every month for 12 months.

Photosynthetic Active radiation intercepted by plant canopy

Photosynthetically active radiation (PAR) intercepted by the plant canopy was determined using tube Solarimeter. Readings was taken between 1200 and 1300 hours once a month for 12 months. By holding the Solarimeter horinzontally, incident PAR reading was taken above plant canopy. Then by holding the solarimeter perpendicular to the tea bushes rows an average of ten readings was taken below the plant canopy of ten randomly sampled plants. The difference between the PAR above plant canopy and that below was taken as the amount of solar radiation intercepted by plant canopy. This was expressed as a percentage fraction (F) of radiation above and calculated using the formula below (Squire 1990). This parameter was essential since defoliation by the weevil ultimately tampered with photosynthetic capacities of tea. Therefore, this aspects of the study was aimed at understanding how any yield effects might occur.

$$\%F = \frac{\text{Above canopy Average PAR-Below canopy Average PAR X 100}}{\text{Above canopy average PAR}}$$

PAR was determined once every month for 12 months in Mununga.

Yield data

The yield was determined in the experimental plots by plucking two leaves and a bud every 9-14 days. Regularly plucking on fresh green leaf was carried out and their weights (Kgs) were recorded in each plot. At end of every month cumulative weight was calculated by addition. Yield of green leaf was converted to kilograms of made tea per hectare (mt/ha) by multiplying by a universal standard factor of 0.225 (Anon 2005). Yield data was collected for the whole trial period. The threshold of number of weevils which can cause significant damage was determined by performing corelations between weevils numbers collected around the tea bushes and damage , the level of damage that caused significant yield loss was used to calculated the number of weevils from the relationship equation.

Data for the Determination of a weevil repellant plant and a weevil attractant

Data collected in the attraction/ repellent experiment involved measuring the area eaten by the weevils by using a tracing paper as earlier described. Later on an analysis of leaves sizes /appearance versus attractives and repellancy was carried out.

Data Analysis

Data on weevil mortalities, damage score, percentage damage, PAR yield and area eaten, was subjected to analysis of variance using SAS Version 9.0. Treatment means were separated using LSD at $P \leq 0.05$. Data with high Coefficient of Variation (CV) (>30%), arising from other variabilities was transformed using $Log_e(x+1)$. Average survival time (AST) was calculated manually using Microsoft excel by adding the multiplied dead weevils by day after inoculation and dividing by the total number of dead weevils at end of experiment(35th day). ANOVA means were tabulated or presented graphically and LSD or error bars was used for their (means) separation repectively. Data on damage percentage was also correlated to weevil population collected in the control plots.

CHAPTER FOUR

RESULTS

4.1 Climatic conditions favourable for the major weevils species in Eastern Parts of Tea Growing Regions of Kenya

Maps indicating favourable conditions in terms of temperature, rainfall and Agro-ecological condition suitable for the different weevil species establishment indicated that based on rainfall and cropping patterns (Agroecological zones) both weevils species could thrive well in the tea growing parts of Eastern Kenya. Where temperatures were between15.5^{o}C and 23.5^{o}C, rainfall in the range 1000mm to 2700mm, notably in the agro-ecological conditions zone I. *S. mixtus* prevailed under 15.5^{o}C to 23.5^{o}C, 1000-2700mm, and Zone 1 temperatures rainfall and agroecological regimes respectively, while *E.meyeri* infested tea mostly when the conditions were for 15.5^{o}C-18.5^{o}C, 1000-2700mm and zone 1 temperatures, rainfall and agro-ecological zones (AEZ) respectively. Such favourable ranges are as shown in Appendix IV (a-f)

4.2 Climatic conditions favourable for Kangaita weevils

Figure 8 is the extrapolated maps of the whole country relative to the eastern region maps showing areas with favourable conditions for Kangaita weevils. The map indicates that most of the tea growing regions with temperatures 15.5^{o}C-18.5 and 1000-2700mm rainfall are favourable for the thriving of Kangaita weevil except the Meru North area, near Mt. Kenya in Kirinyaga & Embu and Meru south areas where conditions exceed these threshholds (Figure 8)

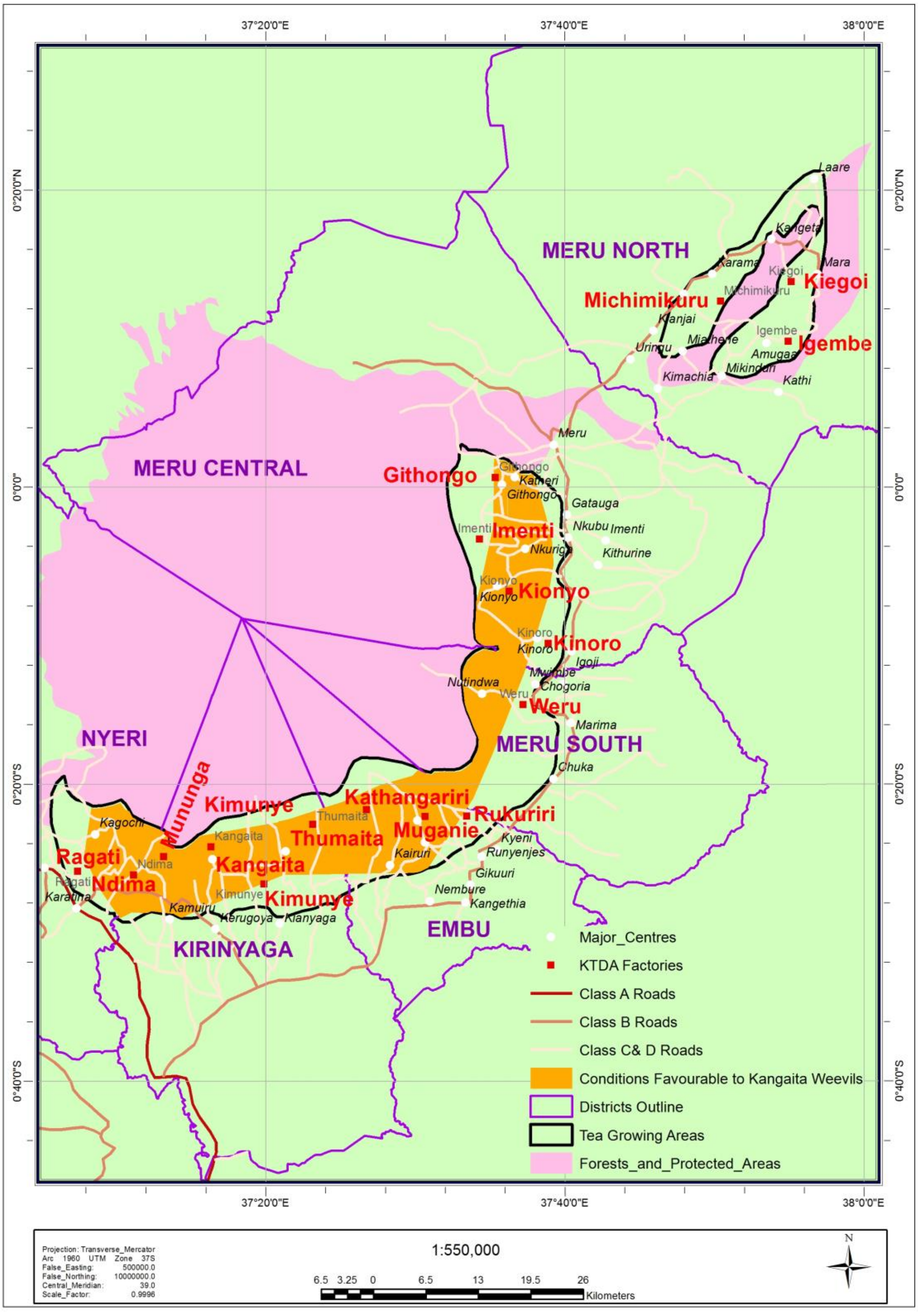


Figure 8: Areas favourable for *E. meyeri* in the Eastern parts of the tea growing regions of Kenya. **(Source Author 2015)**

4.3 Climatic conditions favourable for Nyambene weevils

A similar analysis of the whole country and an extrapolation of eastern region maps showing areas with favourable conditions for Nyambene weevils are as shown in Figure 9. The map indicates that area in the East of Rift, Meru North, around Mt Kenya in Kirinyaga, Embu and Meru south areas are favourable for the thriving of Nyambene weevil where temperatures are between 15.5-23.5^{o}C and rainfall 1000-2700mm.

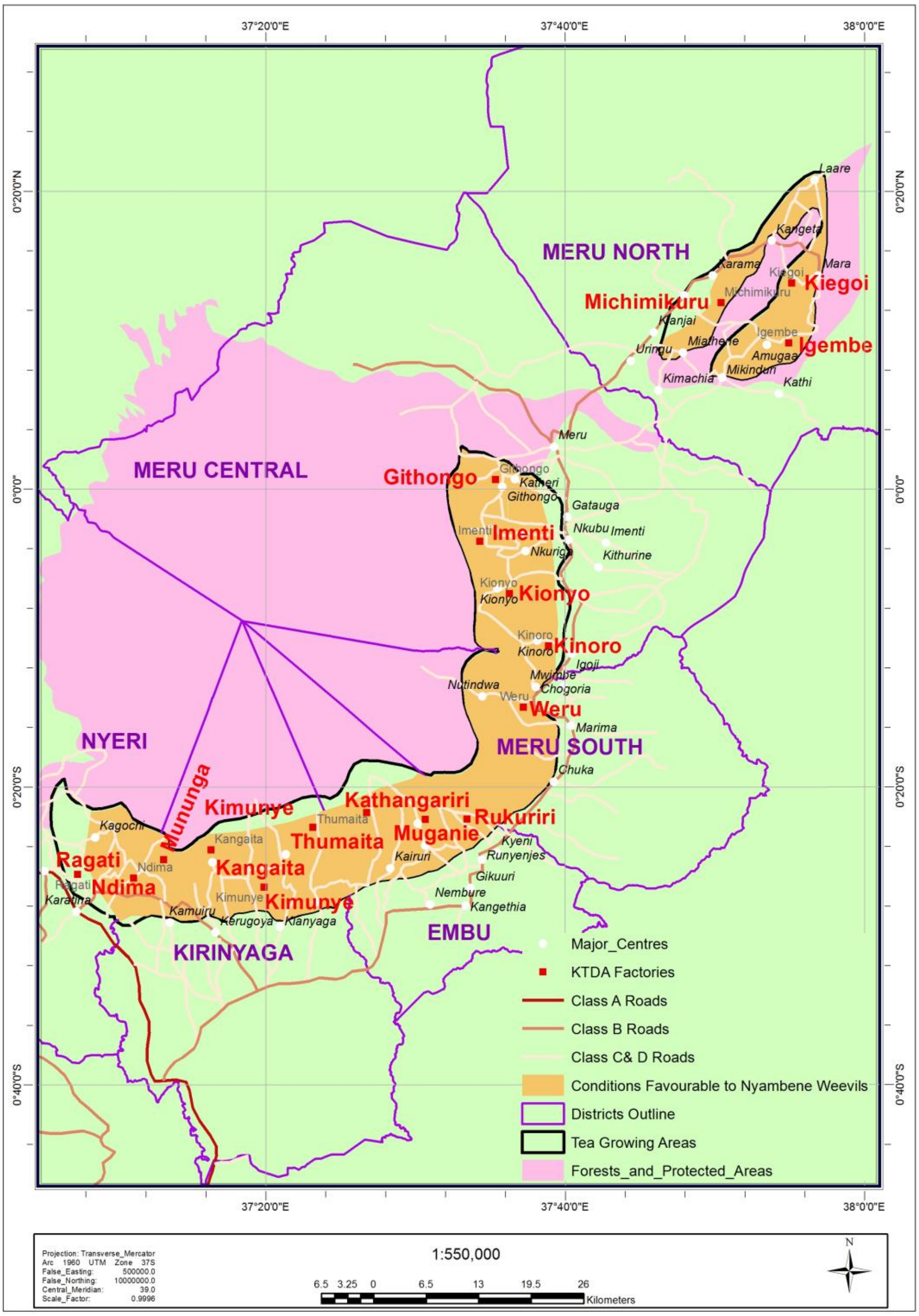


Figure 9: Areas that favour *S.mixtus* to thrive in the Eastern tea growing region (Source Author 2015)

4.4 Area under siege from the two major weevil species in the Eastern tea Growing region of Kenya

Figure 10 shows the outcome of GIS modelling exercise based on temperature, rainfall and Agroecological, the areas that favour the survival of the two major weevil species. Kangaita weevils were specific to an area in parts of Kirinyaga and Embu counties while Nyambene weevil can thrive in the whole of Eastern tea growing areas.

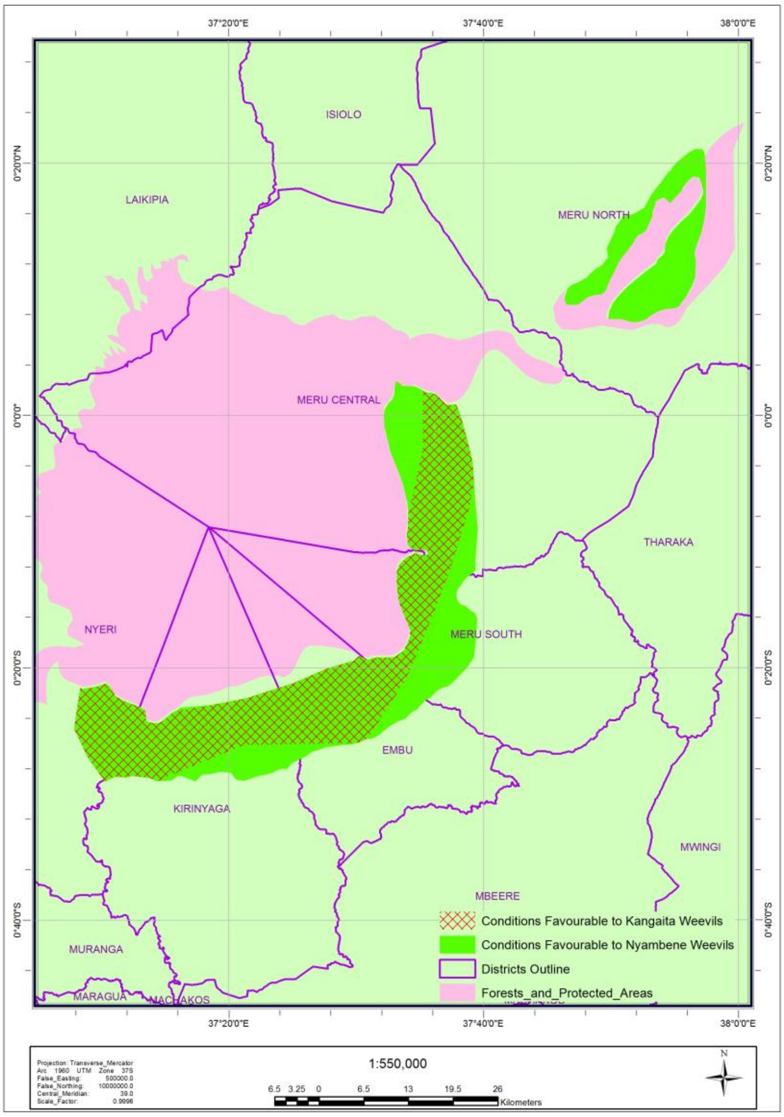


Figure 10: Areas that favour Nyambene and Kangaita weevils to thrive in the Eastern parts of Tea growing Regions of Kenya. (Source Author 2015)

4.5 Weevils distribution relationships to other factors

Actual weevil distribution

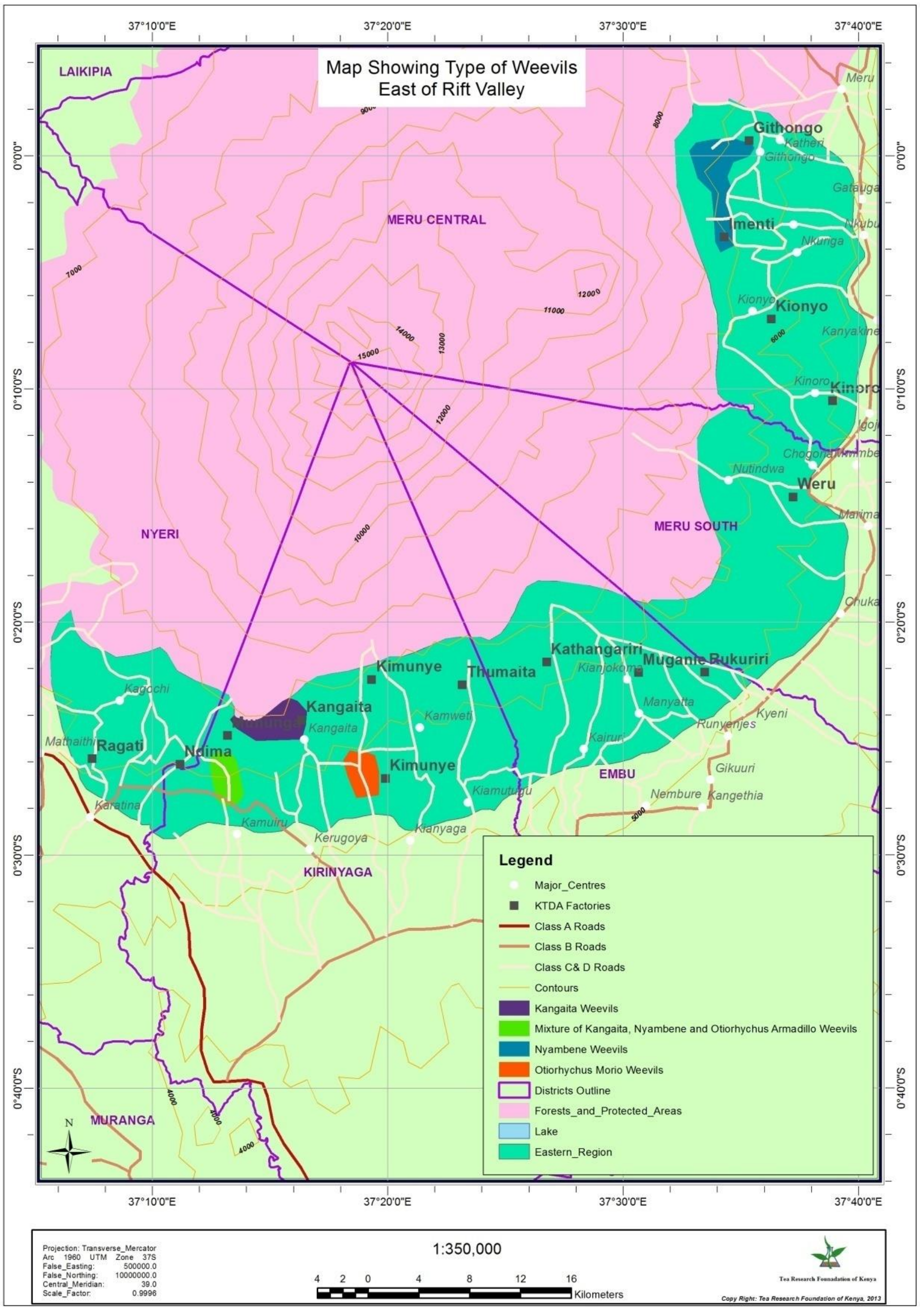
Weevil affected area varied from County to County and the species are specific to an area (Table 1, Figure 11 and 12). The largest area infested by weevils are those areas infested by Nyambene weevils 5,135 ha (blue outline) followed by Kangaita weevils area 1,231 (purple outline) whereas infested area was lowest in Kimunye 822 ha (orange outline) followed by Mununga area 859 ha infested by a mixture of weevils (light green polygon). Nyambene weevil is the predominant species. Thus temperature cover an area of 5135/202673 ha (2.5%), 1231/ 158,818 ha (0.7 %), 822/202671ha (0.4%) and 814/159,818 ha (0.5%) for Nyambene, Kangaita, *Otiorhychus morio* Fabricus, and mixtures of weevil species respectively, while rainfall expand on 5135/266399 ha (1.9%), 1231/266399 ha (0.4%), 822/266,399 ha (0.3 %) and 859/266399 ha (0.3%) of tea variance relatively. Similarly, AEZ expand on 5135 / 219553 ha (2.3%). 1231/219553 ha (0.5%), 822/219553 ha (0.3%) and 859/219533 ha (0.3 %) (Table 1, Figure 11and 12).

Table 1: Estimated Actual area (ha) with different weevil species in the Eastern tea growing region and area under Siege based on temperature, rainfall and cropping systems.

	Weevil species			
Area under siege based on;-	Kangaita weevil(ha)	Nyambene (ha)	*Otiorhychus morio* (ha)	Mixtures* (ha)
Temperature 18.5^{o}C ± 5.0 (*E.meyeri)* 17.5 ^{o}C ±6.0 (*S. mixtus*)	159,818	202,671		
Rainfall 1850±850 (*S. mixtus*) 1850± 850 (*E. meyeri)*	266,399	266,399		
AEZ Zone LH1 and UM1	219,553	219,553		
On Ground trothing (actual area)	1,231	5,135	822 (Kimunye)	859 (Mununga)

**Mixtures of 4 weevil species; Otiorhychus morio,E. meyeri, S. mixtus and Armadilo types of weevil species*

Figure 11 Map showing types of weevils in the Tea Growing Areas, East of Rift Valley



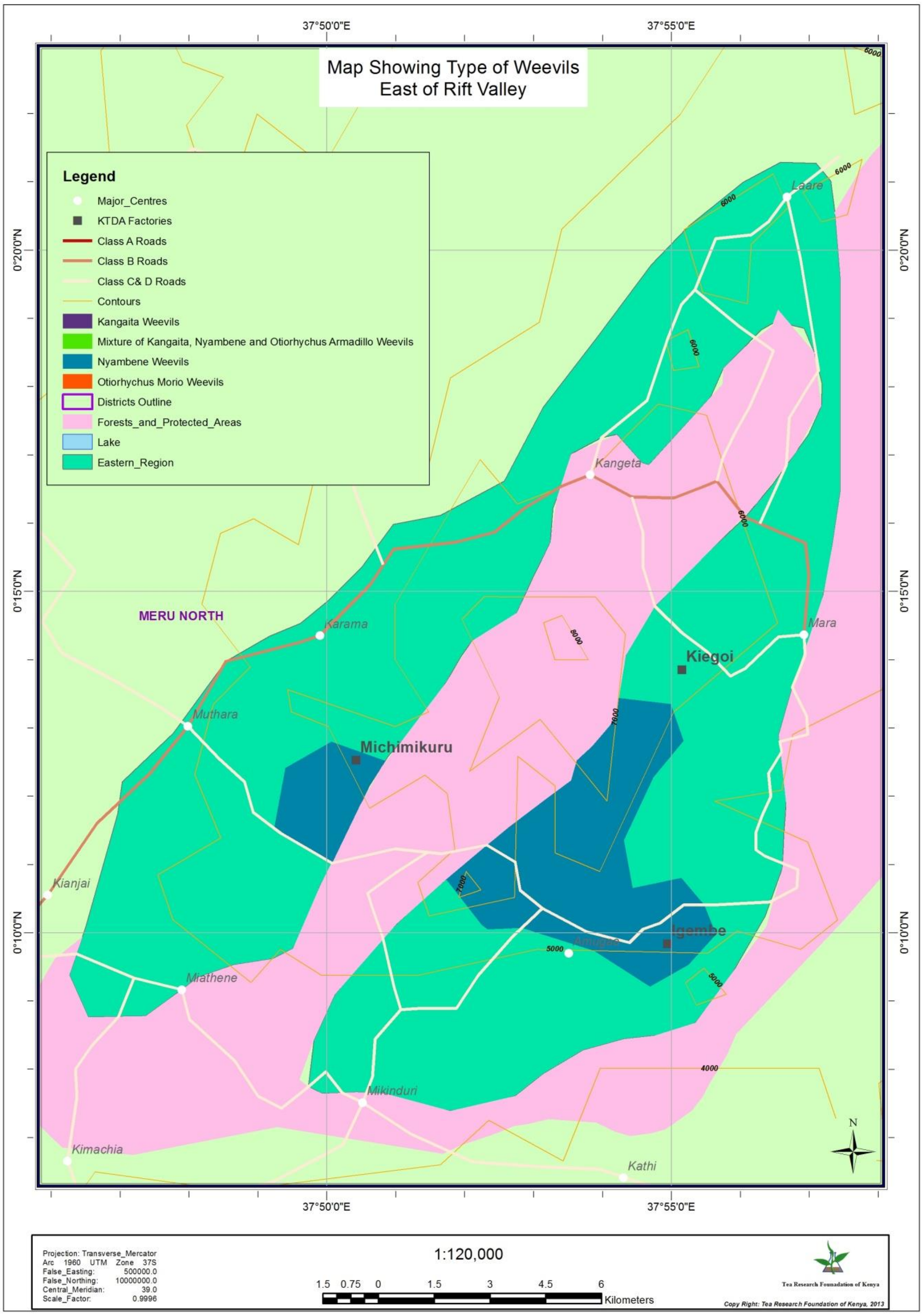


Figure 12: Map showing Actual types of weevils in the Eastern parts of Tea Growing regions (Source Author 2015)

Proportion of tea farmers experiencing pest problem

Most of the tea farmers visited, 90% had experienced general pest problems while 10% said their tea farms had not been infested by pests (Figure 13). About 56.25% of the farmers who had experienced pest problems associated the damages to weevils only while the other 43.75% indicated that other than weevils, they had also observed other pests namely mites, thrips, mole rats, scale insects, aphids and cockchafer

Experience pest problem

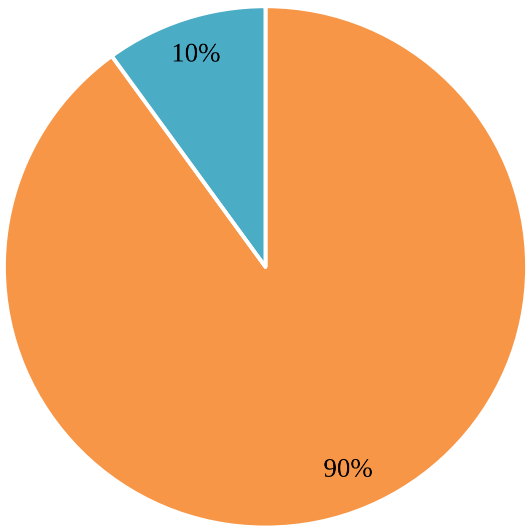


Figure 13 Percentage of farmers that experience pest problem

Relationships of agroecosystems and curculionids

Most of the farms under tea were between 0.41-4.1ha (40%), < 0.41ha (36%), 4-12 ha (4%), 121-202 ha (4%). 21-41 ha (7%), 40-121 ha (7%) and 12-22 (2%). Table 2A shows that the tea farms that had between 4.4 -12 ha, 12-20 ha, 21- 41, 44-121 and 121-202 ha experienced >93% weevil infestation while those between <0.41 and 0.41- 4.1 ha experienced around 73.3% weevil infestation(Table 2A).

Table 2B shows percentage of various clones planted by the tea farmers. Most farmers, about 60% had mixed clones while 12% had planted clone TRFK 6/8. 11.9% of the farmers had clone TRFK 31/8 in their tea farms while about 5.6% had planted TRFK 306, These added up to 89.5%. The other cumulative 10.5% of farmers had planted other clones including TRFK 303/577, TRFK 301/4, TRFK 301/5, D99, BB35, C12 and TRFK 11/4. The table also shows that 54% farms with mixed clones were mostly infested with weevils.

Most of the farms (30%) had tea bushes that were 11-20 years old while 13% had 21-30 year-old bushes. The rest (16%) were 1-10 years(19%), 31-40 years (22%) 41-50 years old. Table 2c shows that the tea farms that (90%) had 11-20 year-old bushes infested with weevils while 57% of 1-10 year-old and 41-50 year-old bushes were less infested.

When it came to livestock enterprise combination in the tea farms (Table 2C), the results of the survey indicated that about 29% of the tea farms incorporated both poultry and dairy/beef farming, 24% reared exclusively dairy/beef cattle, about 10% had poultry, dairy/beef cattle and goats, 20% had poultry, dairy/beef cattle, goats & sheep. A small percentage of 2% had poultry, dairy/beef cattle and sheep in their farms. About 15% of the farmers had no livestock in their farms. The survey indicates that tea growers with dairy/beef cattle and poultry have high weevil infestation while those with additional livestock species of goats had the lowest weevil infestation.

As shown in Table 2E, farmers intercropped their tea (either within or around the tea) mostly with Grevillea (40%), followed by eucalyptus (11%), a combination of macadamia, pine, cyprus (9%), miraa (6%), avocado (4%) and tree tomato (4%). There was 100% weevil infestation in farms where tea was intercropped with indigenous trees, eucalyptus, avocado and tomato tree while farms that had pure tea crop experienced the least weevil attack (55%).

Table 2A,B,C & D:Relationships of agroecosystems and curcullionids

2A	**Area under tea (ha)**	**weevil infestation (%)**	
	4.4-12	100	
	12-20	100	
	40-121	100	
	121-202	100	
	.41-4.1	73.3	
	<.41	73.3	
	21-41	67	
2B	**Type of clone**	**Percentage planted (%)**	**Percentage of weevil infestation(%)**
	Mixed(6/8,31/8,TN14-3,S15/10)	60	54
	TRFK 6/8	12	16
	TRFK 31/8	11.9	11.5
	Others	10.5	15.8
	TRFK 306/1	5.6	5.5
2C	**Livestock Enterprise**		**% weevil infestation**
	Dairy/beef cattle	24	100
	Poultry & dairy/beef cattle	29	89
	Poultry, dairy/beef cattle & goats	10	25
	Poultry, dairy/beef cattle & sheep	20	33
	Poultry, dairy/beef cattle, goats & sheep	20	50
	None	15	38%
2D	**Age of the tea bushes (years)**	**% weevil infestation**	
	11-20	90	
	31-40	71	
	21-30	60	
	1-10	57	
	41-50	57	
2E	**Tree species**	**% of farmers**	**% weevil infestation**
	Eucalyptus	11	100
	Indigenous	4	100
	Avocado	4	100
	Tomato tree	4	100
	Macadamia, Pine, Cyprus	9	75
	Grevillea	40	72
	Miraa	6	67
	Pure tea crop	22	55

Relationship of fertilizer and pesticide application, weeds and weevil incidence

The results presented in Table 3A show that most of the tea farmers, about 82% used NPK fertilizer only (an average of 12bags/ha) while 16% used both NPK (average of 19 bags/ha) and manure (average of 91bags/ha). A small percentage of about 3% did not indicate whether they used any fertilizer or manure in their tea farms. Out of all the tea farms where only NPK was applied, about 65% were infested by weevils while the other 35% did not experience weevil attack. Only 5% of the tea farms where both NPK and manure applied were infested by weevils while 95% were not. The tea farms where farmers did not indicate whether they applied any fertilizer/manure were all infested with weevils.

Table 3B & C further shows that the farms where 7-12 bags/ha of NPK were applied were mostly infested with weevils (32%) while the farms where 0-6 bags/ha and 19-24 bags/ha were applied, were least infested (4%). In the farms where both high amounts of NPK (25-30 bags) & Manure (between 14-220 bags) were applied, there was 100% weevil infestation.

Most of the tea farms, about 72% had observed diseases in the tea bushes while about 28% of the tea farms had not been affected by disease. Table 3D shows that out of the tea farms that experienced diseases, 47% were affected by armillaria root rot only while 19% observed hypoxylon wood rot only,. 22% of them observed armillaria root rot and hypoxylon wood rot, 9% observed armillaria root rot, hypoxylon wood rot &stem canker while 3% of them observed stem canker only. The table further shows that the farms that were affected by armillaria root rot only were mostly infested by weevils at 87% while those that were affected by stem canker only, were not weevil infested.

The farmers that had observed Armillaria root rot uprooted the tea bushes while others dug trenches and planted nappier grass. Those that observed hypoxylon wood rot pruned the affected bushes.

The survey showed that most of the farmers, about 80% don't use any pesticides while about 20% apply pesticides to control pests in their tea farms (Table 3E).

Table 3E shows that out of 32 % of the farmers that applied insecticides to control pests in their tea farms, about 21% of them used karate to control aphids, scale insects, thrips and mites while about 14% use Dithane M45 to prevent stem canker. Another 14% used Ridomil to control damping off in nurseries. About 14% used Gladiator and 7% used Dimethoate and Duduthrin to control termites and other insects. 7% of the farmers applied Fastac (Alpha-cypermethrin 100g/L) and Spectator (Chlorpyrifos 480g/L) to control weevils while another 7% used Fukokill to control mole rats.

Most of the tea farmers, (91%) had experienced weed problem, mostly couch grass, star grass, wandering jew among others, while 9% did not experienced any weed problems in their tea farms. Out of the 91% that had weed problems, 95% experienced damages caused by weevil insects as well. The 9 % that did not have weed problems were also not affected by weevils.

Table 3F shows that a larger percentage, about 48% of farmers did not use any herbicide for the control of weeds in their tea farm, manual weeding was done using jembe and panga instead. About 40% applied herbicides in their farms to control weeds. The herbicides used included Roundup, Weedal, Gramoxone, Twigasate and Kalach. 40% of farms where herbicides were not applied were more infested with weevils compared to those where herbicides were applied (20%).

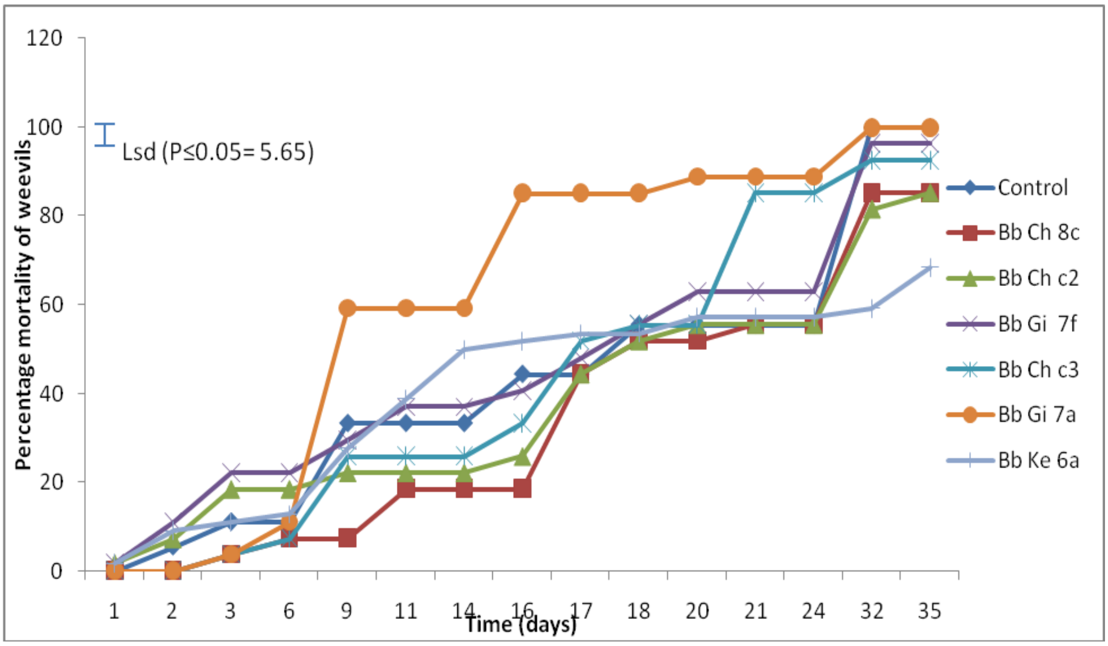
Table 3 A,B,C, D,E & F: Relationship of fertilizer and pesticide application, weeds and weevil incidence

A	**Fertilizer/Manure Applied**	**Percentage of farmers applying**	**Percentage of weevil infested tea farms**
	Unknown	2	100
	NPK only	82	65.4
	NPK & Manure	16	5
	% of farmers applying different types of fertilisers		
B	**Rates (bags/ha)**	**% of farmers applying**	**% weevil of infested farms**
	NPK ONLY (bags)		
	7-12	51	32
	13-18	16	12
	25-30	16	12
	0-6	14	4
	19-24	3	4
C	**NPK & MANURE (bags)**		**% weevil infestation**
	25-30	29	100
	7-12	43	0
	19-24	14	0
	13-18	14	0
	0-6	0	0
D	Disease	Percentage of tea farms affected with the disease	% weevil infestation
	Armillaria Root Rot	47	87
	Hypoxylon Wood Rot	19	67
	Armillaria Root Rot & Hypoxylon Wood Rot	22	57
	Armillaria Root Rot, Hypoxylon Wood Rot & Stem Canker	9	10
	Stem Canker	3	0
E	Pesticide –Trade name(Active Ingredient)		Percentage use
	Fastac 10 EC (Alpha-cypermethrin 100g/L)and Spectator 48 EC(Chlorpyrifos 480g/L)		7.14%
	Dithane M45 wettable powder(Mancozeb 80%m/m)		14.29%
	Karate 2.5 WG(Lambda Cyhalothrin 25g/Kg)		21.43
	Ridomil Gold MZ WG(Metalaxyl-M 40g/Kg + Mancozeb 640g/Kg)		14.29
	Gladiator TC(Chlorpyrifos 48%)		14.29
	Dimeton 40 EC(Dimethoate 400g/L)		7.14
	Diazol 60 EC (Diazinon 600g/L)		7.14
	Fukokil 10 % paste (Zinc Phosphide 10%w/w)		7.14
	Duduthrin 1.7 EC Emulsifiable (Lambda-cyhalothrin 17.5g/L)		7.14
F	**Use of Herbicide**	**Percentage**	Percentage weevil infestation
	No	48	40
	Yes	39.5	20
	Unknown	12.5	9

4.6 Efficacy of *B.bassiana* against tea weevils and effect of Weevils on yield

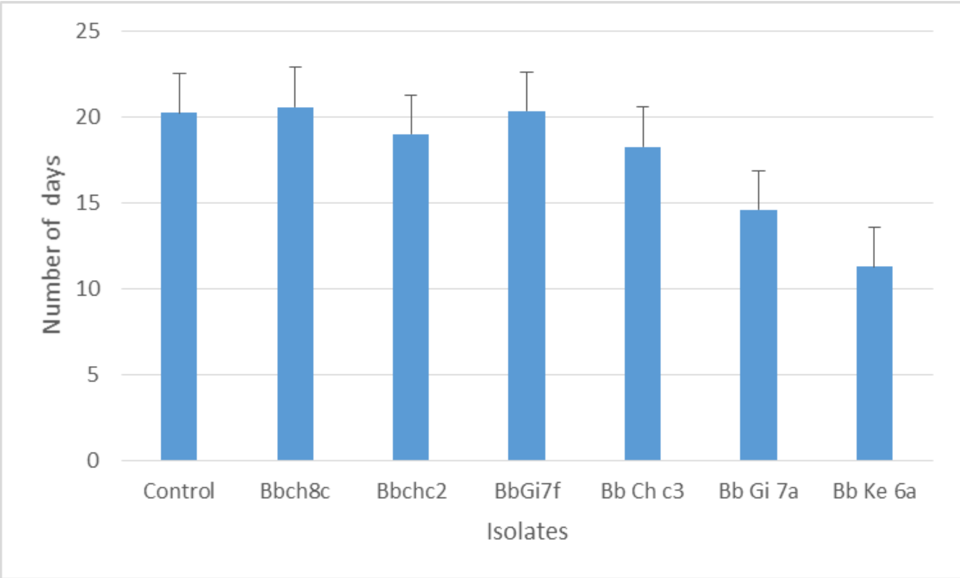
4.7 Effect of *B. bassiana* on population of the tea weevils (*S. mixtus*) in the Laboratory

Mortality caused by the fungus was confirmed based on macro and microscopic characteristics. No mortality attributed to *B. bassiana* infection occurred in the control. Innoculated dead insects showed mycelial growth and this fungal outgrowth was used to validate death by fungal infection. All the isolates varied in their efficacy on *S. mixtus* weevils. Data showing that it took upwards of one week post treatment for *B.bassiana* to kill weevil is as presented in Figure 14 and Appendix V. It needed 2 to 3 weeks to elapse for isolates to be effective when more than 75% mortality was recorded. The data confirms that the isolates Bb Gi 7a and Bb ke 6a achieved > 50% mortality on day 9 and day 14 after inoculation respectively while the next mortality rate compared to the control was isolate Bb ch c3 on day 21 after innoculation at 85% mortality. IsolateBbGe7a (Bb 7a) proved to have higher insecticidal activity against the tea weevils compared to the other isolates (Figure 14). There was no significant difference at ($P \leq 0.05$) in the effects of concentration of conidia of the different isolates (data not shown) meaning that concentration of 10^3 was effective as concentration of 10^5 This implies that concentration of isolate per se had no siginificant ($P \leq 0.05$) influence of effectiveness meaning that epizotic was adequately generated at 10^3 conidia. In addition, weevils treated with Isolates Bb Gi 7a had the lowest average survival time(AST) of 12 days followed by Bb Ke 6a with AST of 14 days while the control and Bb Ch 8c had highest of 20 days (Figure 15) .



120
100
80
60
40
20
0
Lsd (P≤0.05= 5.65)
Percentage mortality of weevils
1
2
3
6
9
11
14
16
17
18
20
21
24
32
35
Time (days)
Control
Bb Ch 8c
Bb Ch c2
Bb Gi 7f
Bb Ch c3
Bb Gi 7a
Bb Ke 6a

Figure 14: Effect of six *Beauveria bassiana* isolates on mortality of *S.mixtus* adults over time



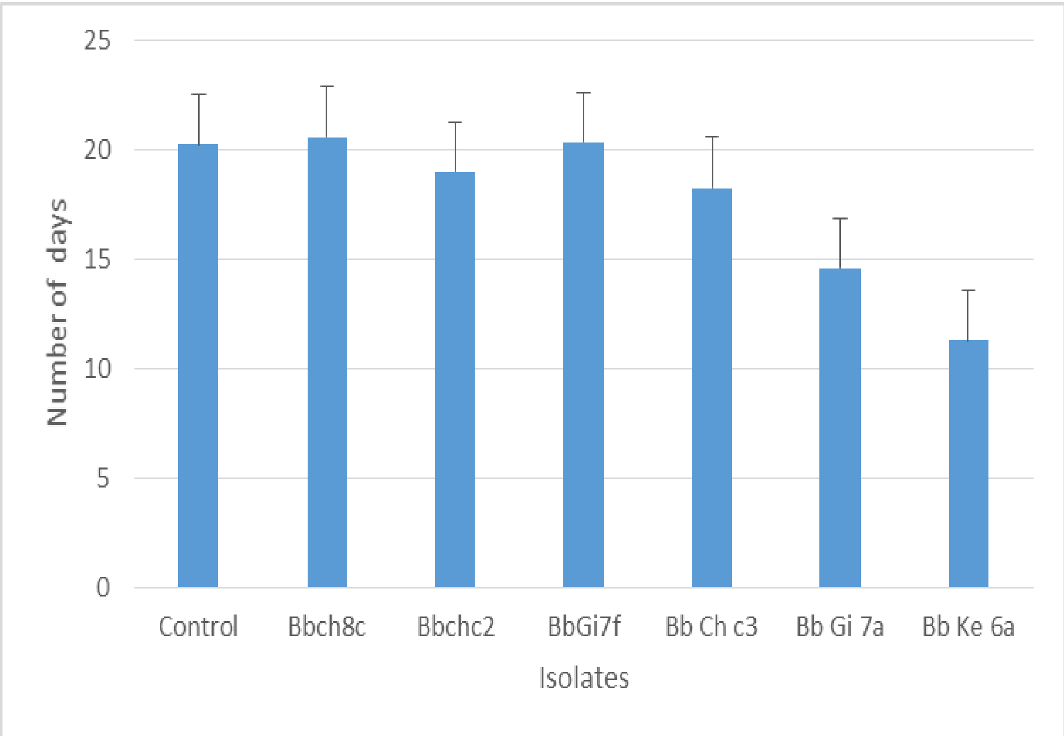


Figure 15: Effect of six *Beauveria bassiana* isolates on Average Survival time (AST) of *S.mixtus* adults

4.8 Effect of *B. bassiana* isolates on feeding of the tea weevil

The results obtained from the experiment shows that there was significant (P=0.05) difference between isolates in area of the leaf consumed (Table 4). Isolate Bb Ch c3 and the control had the largest area eaten (2.02-3.78 cm^2 and 2.80cm^2 respectively). The isolate treatment with least eaten area was Bb Gi 7a (1.11-2.13 cm^2)followed by Bb Ch c2 , Bb Ke 6a, Bb Gi 7f, Bb ch 8c and Bb Gi 7a (Table 4).

Table 4: The mean area consumed on tea leaves (cm^2 per 2 day) by the tea weevils treated *Beauveria bassiana* isolates

Isolates	**Concentration**			**Mean**
	10^5	10^6	10^7	
Bb 7a	2.13a	1.39b	1.11b	1.55a
Bb c2	1.59b	2.19a	1.70b	1.72a
Bb 7f	2.30a	2.00b	2.23a	2.18b
Bb c3	3.78 a	2.02b	3.48a	3.09a
Bb 8c	2.37a	2.72a	2.03b	2.37a
Bb 6a	2.21a	1.69b	2.04b	1.98b
Control	2.80a	-		2.80a
	Isolates treatments			Concentration
CV (%)	29.70			29.70
LSD (P≤0.05)	1.08			NS

Means in a column followed by the same letter are not significantly different at P≤0.05, from each other according to Duncan's Multiple range test.

4.9: Effect of Tea weevils on Productivity of tea and Efficacy of *B.bassiana* Isolates compared to an insecticide

a) Giciaro site

The weevil species found to be occurring in Giciaro area were predominantly the *S. mixtus* (Nyambene weevils). In Giciaro farm (first trial), the culmulative made tea yield data, indicates that the effect of weevils on yield between treatments varied significantly ($P \leq 0.05$). Yield of tea at Giciaro tea field was significantly higher in all the plots treated with B. *Bassiana* isolates (2922.4- 3214 kg mt(made tea)/ha) compared with plots treated with Karate (2518.5 kg) and the control (2469kg), 30 % higher yields in isolate Bb 7a treated plots. Similarly, mean monthly damage scores on pluckable leaves varied significantly ($P \leq 0.0075$) between treatments. Plots treated with, Karate (1.11) Isolates Bb Ke 6a (1.16) and Bb Gi 7a (1.15) had lower damage score compared with the control plots (1.4). Percentage of damaged leaves varied significantly ($P \leq 0.05$) between treatments. Percentage pluckable damaged leaves on plots sprayed with Karate, and the four isolates treatments were similar and had lower number of damaged leaves compared to the control. Similar trends are portrayed by the canopy cover data and mature leaves damage score and damage percentage (Table 5), it was also observed that Infestation of *S. mixtus* in the fields seems to be sporadic i.e. A field may be infested while a neighbouring field with the same clone is not. Despite the above results, it shows that the weevils don’t disperse extensively once they have found a site.

Table 5: The mean yield (kgmt/ha), canopy cover (cm^2), damage score (pluckable and mature) leaves and percentage damage (pluckable and mature leaves) on weevil infested tea and treated with *B. bassiana* in Giciaro Farm (Trial 1 July 2012-June 2013)

Treatments	Cul. yield(kgmt/ha)	Canopy cm^2	Damage score Pluckable	Damage score mature	% damage pluckable	% damage mature
Isolate 7 spray	3215a	7073b	1.15b	1.15ab	16.4b	20.6b
Isolate 7 in wheat Bran	3199a	7135b	1.15b	1.17ab	22.4b	24.4a
Isolate 6 spray	3094a	7699a	1.16b	1.20ab	17.5b	24.3a
Isolate 6 in wheat bran	2922ab	8176a	1.3a	1.18ab	17.7b	22.8
Karate	2519b	7490ab	1.11b	1.09b	16.1b	19.7b
Control	2469b	6224c	1.4a	1.3a	28.3a	29.8a
% CV	12	22.27	23.25	26.12	71.52	51.2
LSD(P≤0.05)	648	789	0.16	0.18	8.1	6.90

Means in a column followed by the same letter are not significantly different at P≤0.05, from each other according to Duncan's Multiple range test.

In the second trial of Sept 2012 –Sept 2013 in a field adjacent to the first one and with the same clone TRFK 31/8. The results indicates that all the other parameters recorded did not show any differences between treatments except for the canopy changes. The canopy cover data portrays that only the plots sprayed with *B. bassiana* isolate Bb Gi 7a had significant higher canopy cover compared to the control and the other treatments (Table 6) meaning that isolate protected tea leaves.

Table 6: The mean yield (kgmt/ha), canopy cover (cm^2), damage score (pluckable and mature) leaves and percentage damage (pluckable and mature leaves) on weevil infested tea and treated with *B. bassiana* in Giciaro Farm 2nd Trial (Sept 2012- Sept 2013)

Treat	Yield *mt/Kg	Canopy cm^2	Damage score Pluckable	Damage score mature	% damage pluckable	% damage mature
Isolate 7 spray	2104	5092a	1	1	9.78	13.6
Isolate 7 in wheat bran	2266	4286b	1.04	1	9.52	13.4
Isolate 6 spray	2221	4566.2b	1	1	11.18	13.9
Isolate 7 in wheat bran	2203	4148b	1	1	13.67	16.6
Karate	20007	4236.6b	1	1	11.67	16.6
Control	2286	3952b	1	1	10.56	18.6
% CV	18.72	22.18	7.8	0	27.51	21.2
LSD($P \leq 0.05$)	NS	718.6	NS	NS	NS	NS

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$, from each other according to Duncan's Multiple range test. *mt/kg= kilogram made tea per annual

b) Mununga site.

Effect of tea weevils sp. on tea production and efficacy of B. bassiana compared to an insecticide in Mununga 1st Trial from July 2012 –Sept 2013

Yield effects varied significantly (P=0.05) among treatments (Table 7). Plots treated with the Isolates (both when sprayed and applied with wheat bran as a carrier) and those sprayed with Karate had significantly (33%) higher yield than the control. Canopy cover varied significant among the treatments (P<0.001) with the control having significantly low canopy cover. The treatments had no significant (P≤0.05) effect on Photosynthetic active radiation (PAR). Damage score on leaves varied significantly with the different treatments (P≤0.0001). Plots treated with sprays of isolates Bb 6a, Bb7a and Karate had lower damage score compared with plots treated with the same isolates in wheatbran (wb) substrate and the control. Percentage damaged leaves varied significantly with the different treatments (P≤0.016). Percentages of both pluckable and mature damaged leaves on plots treated with with Karate, both mode of application of isolates 6 and 7 were significantly (P=0.0016 and P=0.0008 respectively) lower compared with the control (Table 7).

Table 7: The mean yield (kgmt/ha), canopy cover (cm^2), damage score (pluckable and mature) leaves and percentage damage (pluckable and mature leaves) on weevil infested tea and treated with *B. bassiana* in Mununga (July 2012 –Sept 2013 -Trial)

Treatment	Yield in Kg mt/ha	Canopy Cm^2	PAR	Damage rate pluckable	Mature damage rate	% pluckable damage	% mature damage
Iso. 7 spray	2019a	15143b	30.3	1.30d	1..42b	47.4b	52.7b
Iso. 7 in wb	2176a	14549b	30.2	1.60b	1.60b	50.1b	51.5b
Karate	2492ab	16964a	30.5	1.41cd	1.44b	48.2b	51.7b
Iso.e 6 spray	2587ab	14957b	30.1	1.42cd	1.44b	46.7b	53.5b
Isol. 6 in wb	2504bc	16359a	30.34	1.58bc	1.58b	50.1b	54.0b
Control	1937c	12595c	29.25	2.2a	2.13a	53.9a	57.7a
% CV	10.46	15.76	10.28	26.45	29.22	18.30	14.02
LSD ($P<0.05$)	440	956	ns	0.2	0.2	3.6	3.0

Iso =Isolate, wb=wheat bran. Means in a column followed by the same letter are not significantly different at $p<0.05$, from each other according to Duncan's Multiple range test.

Effect of tea weevils' sp. on productivity of tea when treated with *B. bassiana* and Lambdacyhalothrin in Mununga 2st Trial from Sept 2012 –Sept 2013

In the 2nd trial in Mununga, The treatments had no significant ($P>0.05$) effect on yield, however the trial had a similar trend as in the first trial with plots treated with Karate having the highest yield and the control having the lowest. Conopy cover, photosynthetic active radiation (PAR) and damages on mature and pluckable leaves varied significantly ($P<0.001$, $P<0.001$, $P<0.001$,and $P<0.005$ respectively) between treatments. Canopy cover indicates that Karate was more superior although the other treatments performed better than the control. Karate and all isolates treated plots intercept more light compared to the control. Isolate 7 (in both applications) showed the lowest damages on pluckable leaves (Table 8).

Table 8: The mean yield (kgmt/ha), canopy cover (cm^2), damage score (pluckable and mature) leaves and percentage damage (pluckable and mature leaves) on weevil infested tea and treated with *B. bassiana* in Mununga 2nd Trial from Sept 2012-Aug 2013

Treatment	Yield Kg mt/ha	Canopy	PAR	Damage Score on pluckable leaves	Damage Score on mature leaves	% pluckable damage	% mature damage
Karate	2354	16898a	25.2a	1.2cd	1.2bc	47.5a	53.5bc
Isolate 6 in WB	2082	16229ab	25.4a	1.2bc	1.2b	48.7a	56.0ab
Isolate 7 spray	2018	14666ab	25.1a	1.0d	1.2b	50.1a	53.6bc
Isolate 7 in WB	1954	15330ab	25.2a	1.0d	1.3b	48.8a	57.6a
Isolate 6 spray	1602	15616ab	23.7a	1.0d	1.0c	42.8a	51.4c
Control	1545	11893c	25.4b	1.6a	1.6a	49.7b	53.8bc
% CV	27.1	27.0	10.5	27.0	29.6	16.4	13.9
LSD (P≤.05	NS	1753	1.1	0.1	0.16	4.0	3.2

Means in a column followed by the same letter are not significantly different at P≤0.05, from each other according to Duncan's Multiple range test.

Relationship between weevil damage scores/percentage and weevil numbers

Based on the weevils collected in the control plots and the damage scores recorded. There was a positive linear relationship between damage and weevil population which was obtained in the correlation analysis (Figures 16 and 17) in both sites In Giciaro there was a positive correlations between population and the destroyed leaves; y=21.127x -11.019, R^2=0. 83333 which showed that yield losses accrued when an average of 1.4 weevils defoliate more than 40 % of leaves. While in Munuga, the positive correlations between population and the destroyed leaves; y=0.2569x+1.3689

R^2=0.5296 showed that yield loses accrued when an average of 3 weevils defoliate more than 54 % of leaves.

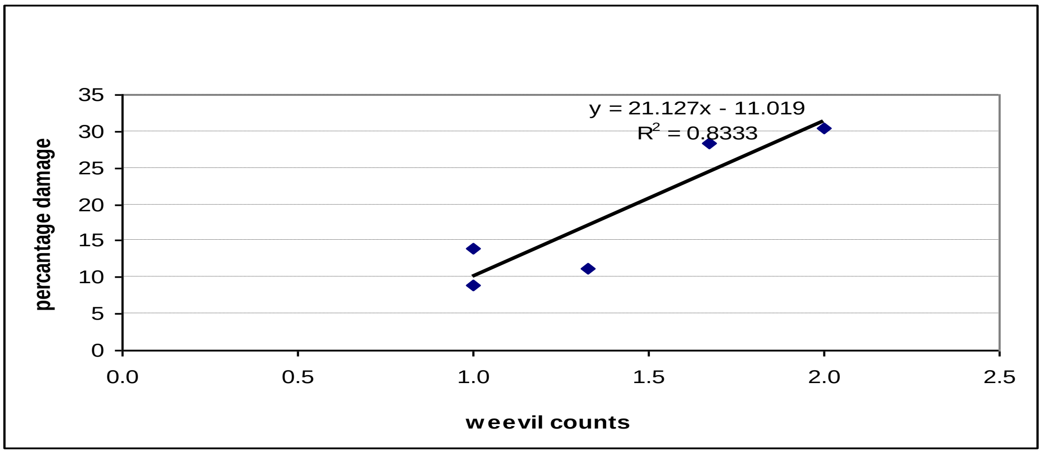


Figure 16 Relationship between weevil damage(percentage) and weevil numbers at Giciaro

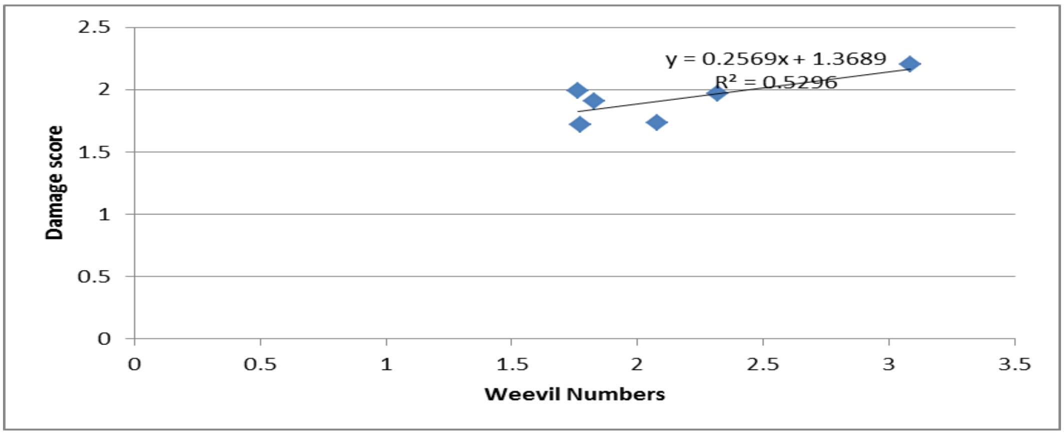


Figure 17: Relationship between weevil damage scores and weevil numbers at Mununga

4.10: Influence of rainfall on weevil damage in Giciaro

Figure 18 shows that the damages that occurred in the field at Giciaro varied and fluctuated with the season of the year. High rainfall >500mm corresponds to low damage of 20-30%. The rainfall was high in the months Oct, Nov, Dec and April and correspond to low or declining pest damage. Low rainfall in July Jan and May correspond to high damage or inclining damage. The response to decreased rainfall is not immediate. In general, the damage was low, below 30% through the experiment period except July. The highest Damage of 40% was in July 2012 at the onset of the field Experiment. Infact, July and March are the only months that contributed to the yield being significantly different between treatments.

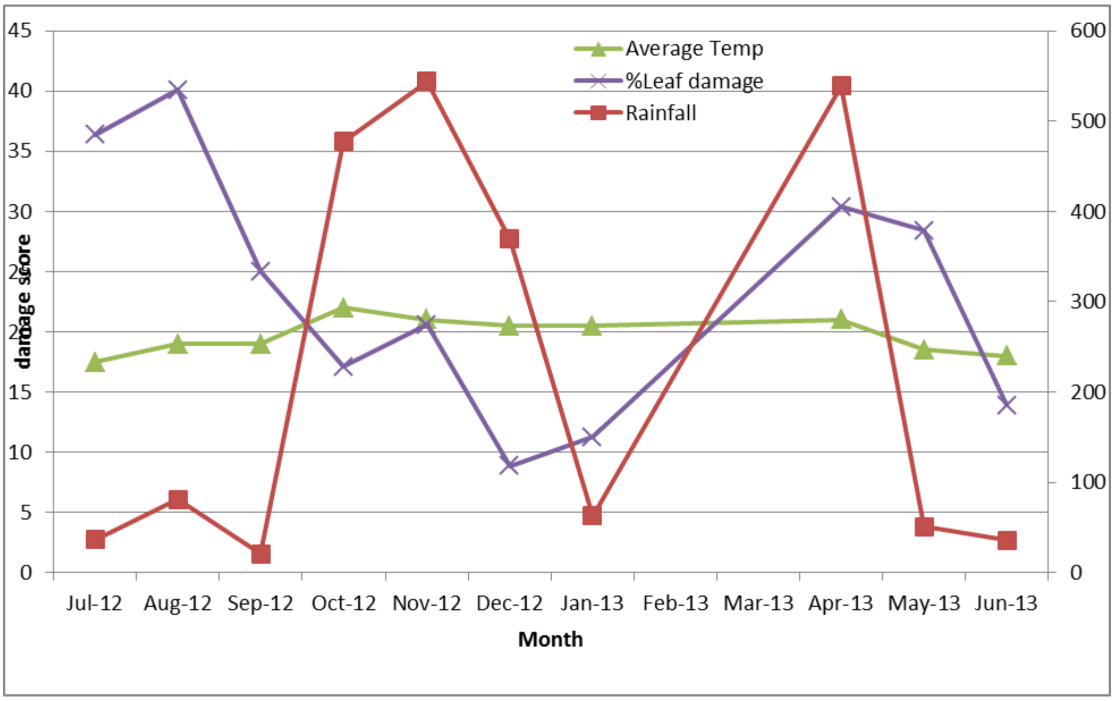


Figure 18: Trends on Nyambene weevil damages on tea Leaves versus rainfall and Average Temperature in Giciaro Farm, Igembe KTDA Cacthment

4.11: Influence of rainfall and temperature on weevil damage in Mununga

Figure 19 shows that the damages that occurred in the field at Mununga varied and fluctuated with the season of the year. Increased rainfall >100m corresponds to a drop in damage and vice versa for temperature of 19^{o}C. The Rainfall was high in the months of October and April >200 mm and correspond with low damage occuring in the field while low rainfall in July/Aug/Sept and Jan/ Feb correspond to high damage, and High rainfall resulted in low damage in the field while high temperatures in some months appear to enhance the weevil damage.

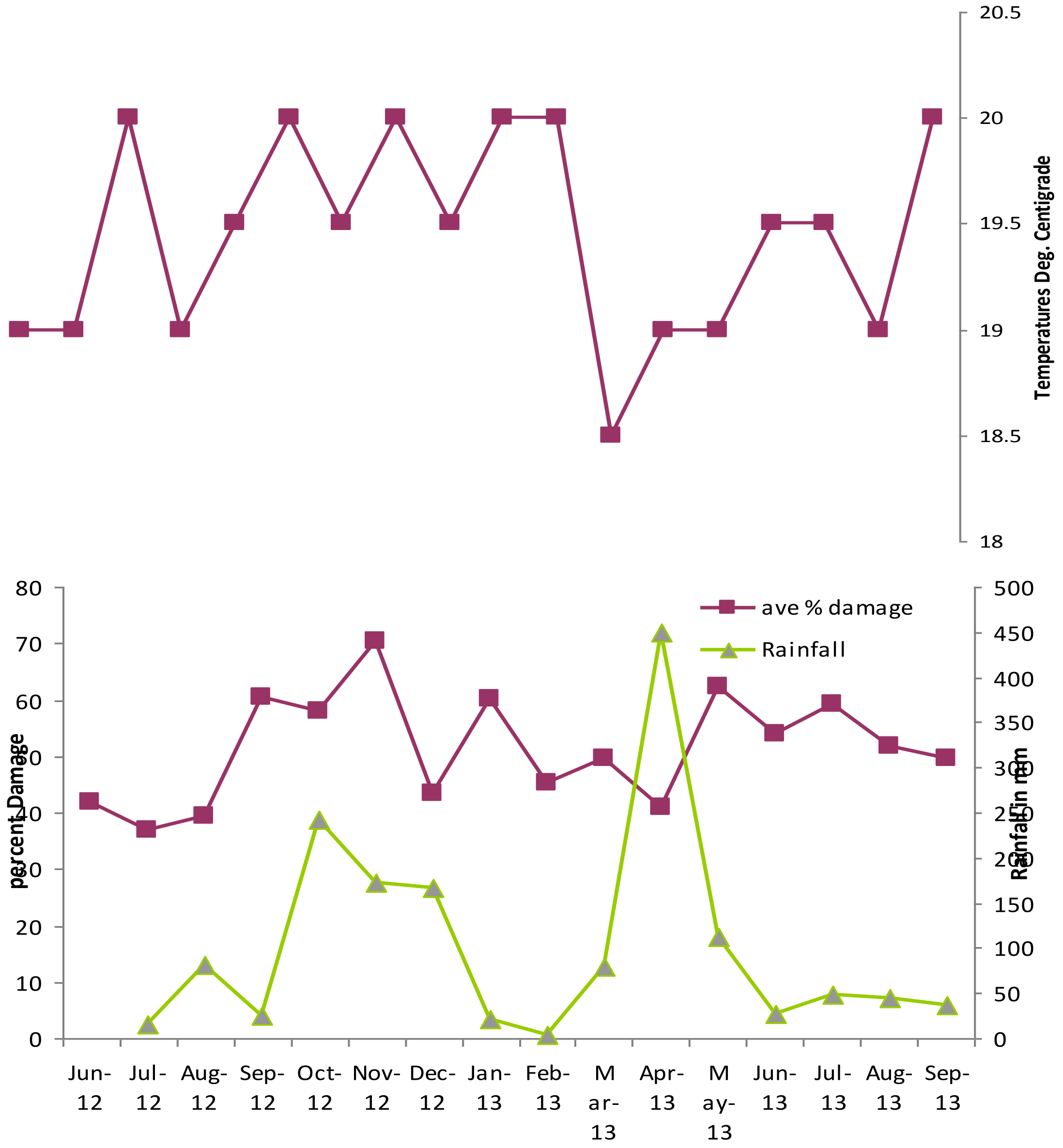


Figure 19: Trends on Nyambene weevil damages on tea Leaves versus rainfall and Temperature in Njogu Farm, Mununga KTDA catchment, Kirinyaga

4.12 Persistency of *B. bassiana* over different months of the year

The results in Mununga showed that *B. bassiana* persist in the cooler and wet months of April, May, June, July and August with Isolate 7a in wheat bran having consistently significantly high number of colony forming units (>1.5 x10^4)(CFU). (Figure 20 and 21) September is unique in that Karate sprayed plots had the highest

CFU of *B. bassiana* meaning the season is favourable for the fungi to thrive in the rich nutrient of decompost beetles earlier knocked down by Karate

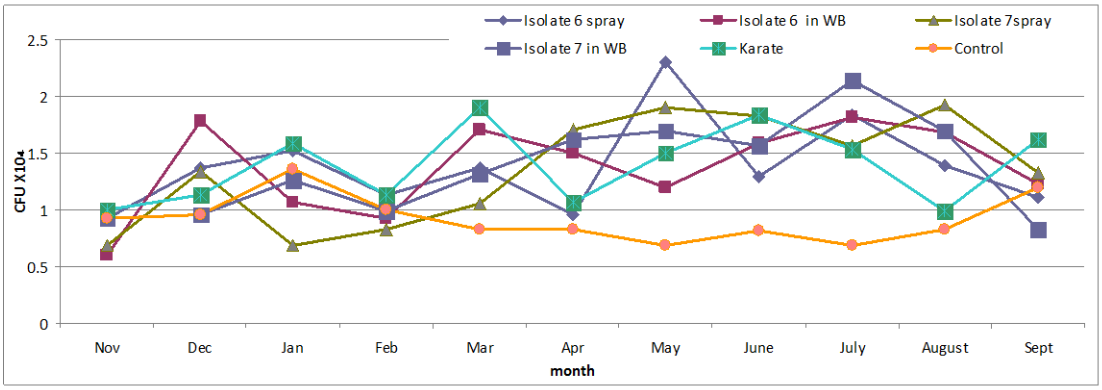


Figure 20: Persistency of B. bassiana inoculum over different months in 1st Mununga trial of July 2012-Nov 2013

(June P<0.03,LSD=0.606;July P<0.005,LSD=0.68)

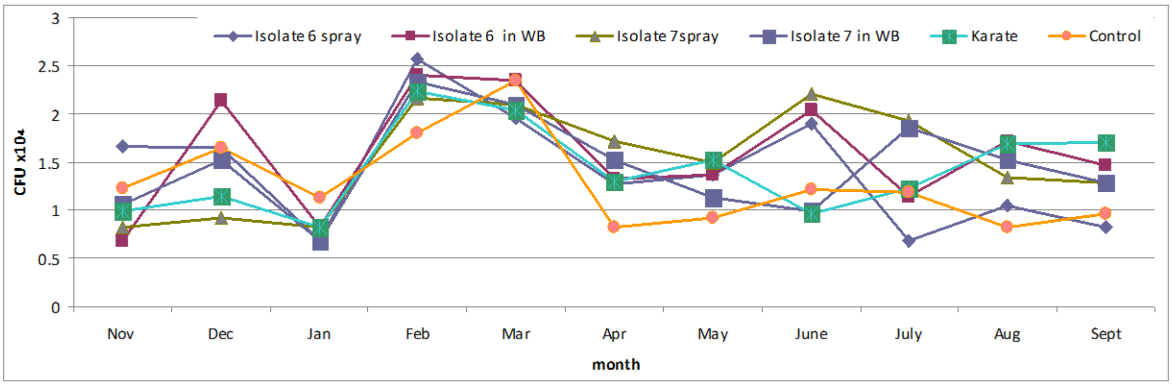


Figure 21: Persistency of *B. bassiana* inoculum over different months in 2nd Mununga trial of September 2012-2013

The results in Giciaro showed that *B.bassiana* persist in the wet months of March April and August with the control having very low CFU compared with the treated plots of Isolate 6 in wheat bran with significantly high number of colony forming units(CFU) (Figure 22). October is unique in that Karate sprayed plots had the highest CFU of *B. bassiana*

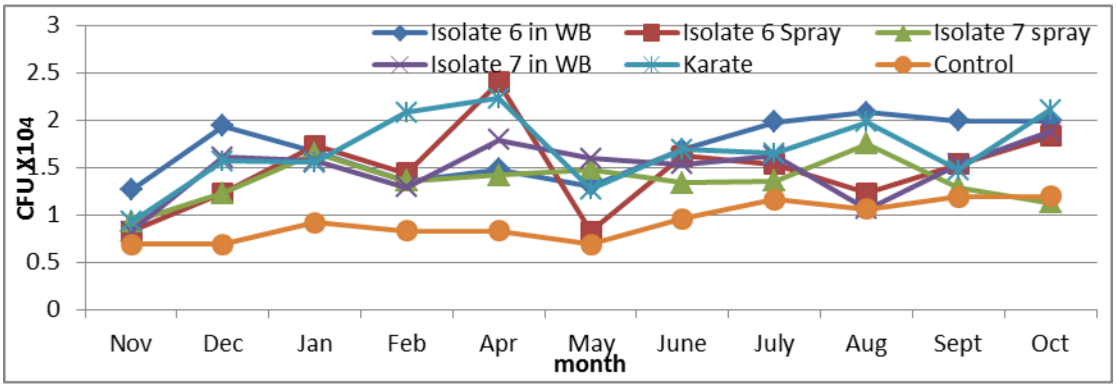


Figure 22: Persistency of *B. bassiana* inoculum over different months in Second Giciaro trial of Sept 2012- Oct. 2013

(May $P<0.03$,LSd 0.947; June $P<0.04$,LSD=0.6013; Sept $P<0.04$,LSD=0.740; Nov.$P<0.04$,LSD=0.704)

4.13. Determination of Potential Repellant and Trap/Attractant Plants.

Nyambene weevils preferred leaves from the Avocado and clone TRFK 306 (Purple Tea) plants. All the other tested clones were less preferred including Loquats and coffee. Area eaten is as presented on Fig 21. Weevil attracted on avocado feed up 125 mm^2 the ones on 306 (purple tea) fed on $90mm^2$, TRFK 301/5 ate $64mm^2$,TRFK 6/8 $25mm^2$, M.dura $23mm^2$, TRFK 303/577 $16mm^2$, 31/8 $12.5mm^2$, SFS 150-$12mm^2$, TRFK 301/4 $12mm^2$, TRFK430/90 $12mm^2$, Fraxinus $11.8mm^2$, coffee $8mm^2$ and loquats nothing (0) (Fig 22)

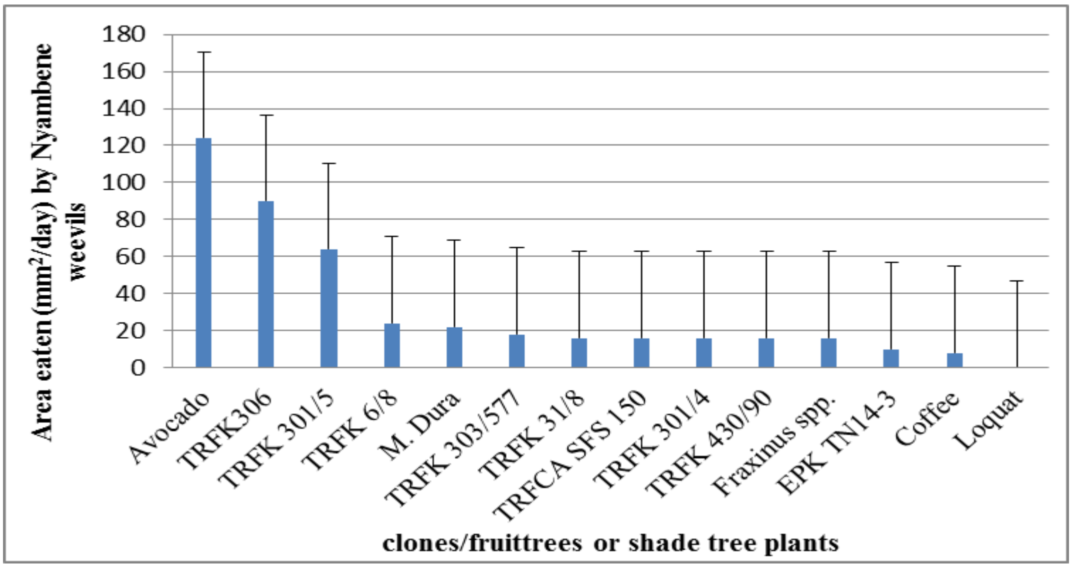


Figure 23: Nyambene Weevils (20) Preference to tea Clone and trees based on area eaten in mm^2 per days

When preference is compared with leaf size and appearance in (Table.9). The most preferred leaves are the big sized leaves of Avocado even in the tea clone TRFK 301/5 with larger leaf area than most of the other green clones is more prefered. Colour may also be contributing to preference as in purple tea versus green yellow clones . Glossiness may be to be part of the criteria for preferring although leaves for coffee are glossy and is not preferred.rred is preferred by the weevils. Semi-erect leaves are not preferred by the weevils.

Table 9: Shade trees, Avocado and Tea clone leaves Characteristics in terms of area size and leaf appearance

No.	Tree plant/clone	Leaf area consumed in mm^2/day/20 weevils	Average Leaf size (mm^2/leaf)	Appearance/Glossyness
1	Avocado	125	76.7	Green & glossy Leaves elliptical/oval in shape
2	TRFK 306 (Purple tea)	90	26.70	Purple , the green colour is masked & is glossy
3	TRFK 301/5	64	32.03	Yellow green (mature-green), straight, obtuse, acute, biserrate, semi-erect
4	TRFK 6/8	25	23.1	Yellow green (mature-green), recurved, acute, attenuate, biserrate, erect
5	*M. dura*	23.1	15.1	Leaflets 15-19, oblong, glabrous, except on the margins and midribs, leaf asymmetric, tip acuminate
6	TRFK 303/577	16	23.7	Yellow green, recurved, rounded, obtuse, serrulate, semi-erect
7	TRFK 31/8	12.4	22.2	Yellow green (mature-green), straight, attenuate, acute, serrulate, horizontal
8	TRFCA SFS 150	12	22.4	Yellow green
9	TRFK 301/4	12	37.1	Yellow green, straight, obtuse, acute, bisserrate, semi-erect
10	TRFK 430/90	12	21.5	Yellow green
11	*Fraxinus spp.*	11.8	20.1	Leaves opposite, mostly pinnately compound
12	TN 14-3	8.78	32.5	Dark Green
13	Coffee	8	47.3	Dark green & glossy. Semi erect
14	Loquat	0	50.0	Mature leaves are dark green and glossy while new leaves are yellow green. Leaves are semi erect

Clones TN 14-3, Purple tea (TRFK 306), TRFK 31/8 and an avocado were preferred by the Kangaita weevil while shade trees *Milletia dura* and *Fraxinus* spp. were least preferred (Table 10). Although EPK TN 14-3 was more preferedd by Kangaita weevil, it is not commonly grown in the tea weevil prone tea region of Kenya

Table 10: Preference of Kangaita weevil to tea clones and shade tree plants based on Area eaten in cm^2 / day

Clone/Tree plant	Leaf area eaten in cm^2 *
M. Dura	1.83 (1.04)b
Fraxinus	3.62 (1.53)b
TRFK 31/8	7.50 (2.14)a
TRFK 6/8	6.10 (1.96)ab
Purple Tea (TRFK 306)	8.30 (2.23)a
TRFK 303/577	6.77 (2.05a)
EPK-TN14-3	8.78 (2.28)a
Avocado	5.89 (1.93)ab
CV (%)	16.77
LSD($P \leq 0.05$)	0.56

** Figures in Parenthesis are log. e $^{(x+1)}$ transformations of leaf area eaten. Means in a column followed by the same letter are not significantly different at $p<0.05$, from each other according to Duncan's Multiple range test.*

The area consumed by *E. meyeri* from plants in pure stand or when mixed were significantly different. Kangaita weevils are highly attracted to the Avocado leaves, followed by the purple tea, then TRFK 31/8. *M. dura* and the *Fraxinus* spp. are less preferred. Kangaita weevils consumed the smallest area on clone TRFK 31/8 when mixed with Avocado compared with when clone TRFK 31/8 alone or when mixed with m.dura or fraxinus spp or purple tea. Preference to purple tea did not vary as a pure stand or when mixed with other plants (Figure 24)

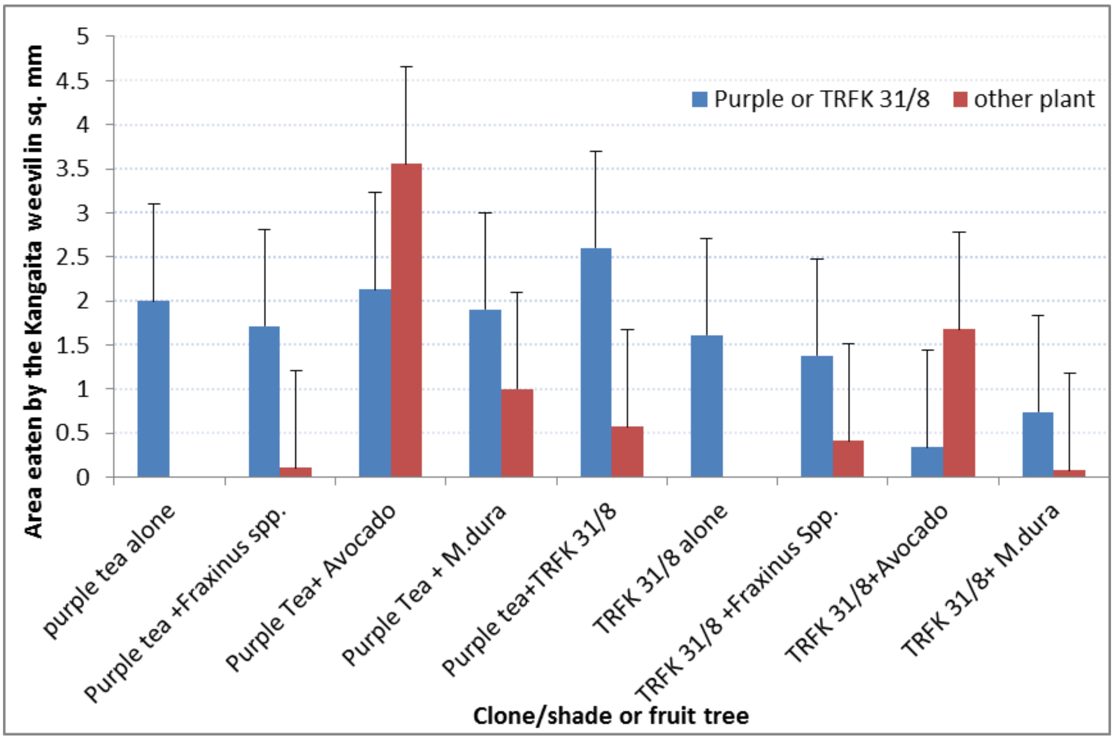


Figure 24: Preference/attraction of Kangaita weevils to tea clones when mixed with shade and fruit trees (Area eaten in square mm)

The area consumed by *S. mixtus* from plants in pure stand or when mixed were significantly (p>0.05) different. *S. mixtus* is more attracted to the purple tea regardless of whether purple tea is alone, mixed with Avocado or *M.dura* or TRFK 31/8 but when TRFK 31/8 is mixed Avocado this weevil prefer the avocado leaves, thus smallest area significantly (p>0.05) comsumed was obtained in clone TRFK 31/8 when in a mixture with Avocado and when in a mixture with purple tea. *M. dura* and the *F*raxinus spp. are not attracted to the *S. mixtus* (Figure 25)

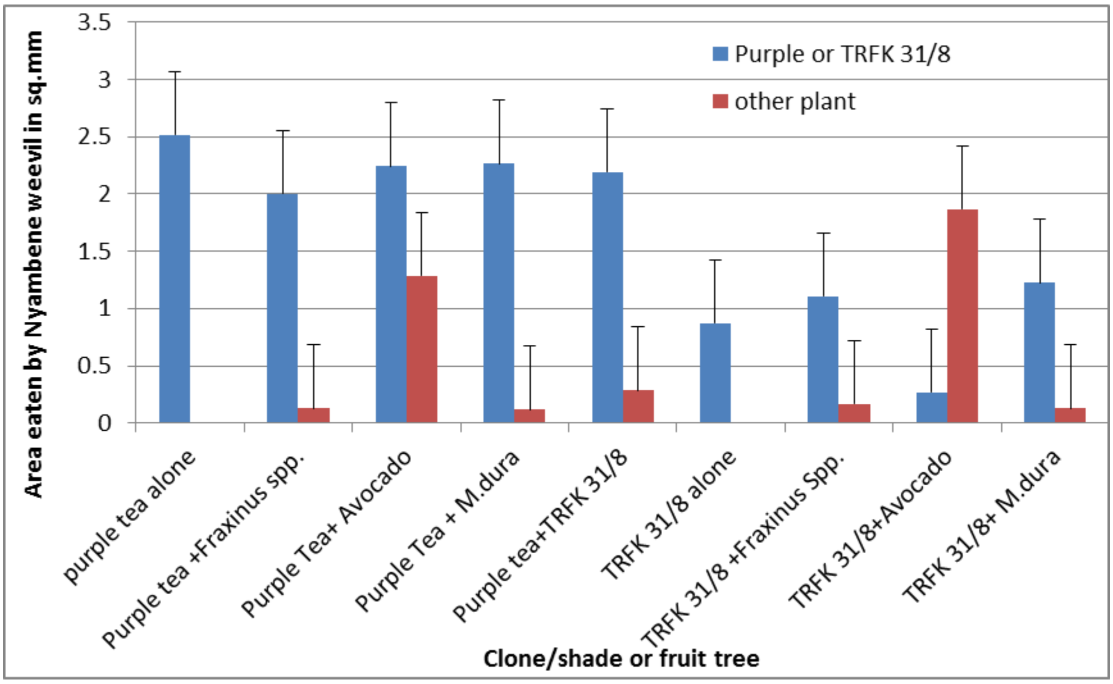


Figure 25: Preference of Nyambene weevils to tea clones when mixed with shade and fruit trees (Area eaten in square mm)

CHAPTER FIVE

DISCUSSION

The GIS modelling exercise using climatic conditions, temperature, rainfall and cropping patterns showed that all the tea growing regions East of Rift were at risk to weevil infestations. Mununga, Kangaita, Imenti, Githongo Michimikuru and Igembe areas had weevil infestation. Temperature, rainfall and land use played a role in containing or localising the weevils. The biological attributes of the weevils could be playing a role in the attributes for the location of the pest i.e the bigger sized Kangaita weevils could be playing a role in their being able to withstand lower temperatures compared to Nyambene weevils. The pest management strategies employed such as environment conservation by restriction to forest encroachment (Ngeno, 2015) and preservation of river lines (Wachira and Ronno, 2004) could also be contributing to the containment of the pests at a higher level. Studies as far back as 1959 by Sen has showed that incidence of pests and diseases and the intensity of infestation may vary widely over districts and even within districts with variation in climate, elevation and even the clone/jat of tea. Similarly Majumder *et al.,* (2011) while conducting a survey on the distribution pattern of looper pests (*Ascotis selenaria cretacea* Assc. (Lepidoptera: Geometridae)) in the plantations of Doars, West Bengal, found that loopers normally are observed during spring and summer meaning that temperature and other climatic conditions may play a role in their occurrence and distribution. He also found that the pest infestation varied with varying planting materials such as clonal plants alone or mixed plants i.e either clone or jats or clone and seedling. Pests have also been found to be area specific as was the case of Kangaita weevil in this study. Sudoi (1997) and Sudoi *et al*., (2011) while screening clones for susceptibility to mite pests found that some mites species are area specific. Red crevic mite (*Brevipalpus phoenics*) was found to occur mainly in the East of rift while the red spide rmite (*Oligonychus coffeae)* occur in all the tea growing areas of Kenya.

Agroecosystem is a well-known phenomenon that interelates cultural practices to pest incidences (Olujide and Adeogun 2006; Lakshmi *et al*., 2009) in the case of tea cultural practices notably routine husbandry measures always result in either enhancing or detering attack (Sudoi *et al.*,1997) at times , adjustment of the practices can augment deliberate control measures that are administered. This regularly occurs in plantations and amounts for the low infestations of pests on tea in Kenya (Wachira and Rono, 2004)

Similar kind of association between various vegetative factors and pest incidence has been studied by various workers in different crops and crop area has been reported to influence the occurrence of insect pest, similar to the findings of this study(TRA, 1994; Thomas *et al.*,2001; Zehnder *et al.*, 2007). Litsinger (1994) observed that in case of rice, larger crop area favoured occurrence of stem borer and brown hoppers. The factors playing the role of having 100% infestation in large farms could be attributed to vast area of monocluture leaving the weevil with minimal host plant and its inability to fly hence limited mobility. Shade is an important component of tea plantations. Though there remains controversy over the use of shade. It is certain that shade trees are important / responsible for modulating the environment of tea ecosystem, enriching soil fertility, reducing temperature and evaporative capacity, conserving soil moisture and helping in the control of certain pests and diseases which are thermotrophic in nature (Barua 1989). In contrast, trees have also been reported to serve as potential source of root diseases, encourage quick germination of fungal spores , invite and habour pests and diseases among different components of tea eco system. The high infestation of weevils relating to certain trees demonstrate finding in other studies. Hasan (1963) and Majumder *et al*., (2011) reported about the tea looper ((*Ascotis selenaria cretacea* Assc. (Lepidoptera: Geometridae)) in west bengul , India, where higher percentage of the looper infestation in sections having *Derris robusta* and *Albizzia odoratissima* as shade trees species could be accounted for the preference of adult moths for egg laying on them and young larvae defoliating their foliage before invading tea (Majumder *et al.,* 2011).

Livestock species in addition to cattle providing milk for brewing tea as is the culture can contribute in enriching soil fertility. On the other hand they can cause harm by being carriers of minute pests such as mites. Poultry can serve as a generalist predator as is the role of birds mentioned by Thomas *et al.,* (2001), although it seems not to be the case in this study, may be the poultry lack preference for the weevils or the confinement of livestock species in the survey area could have contributed to lack of a interpretable trend as regards to livestock species.

Increase in infestation seem to have occurred on 11 to 20 years tea bushes and this could be associated to the period when agricultural expansion was at the peak which involved the clearing of huge areas of forested land to pave way for crops reducing biodiversity of flora hence less host plants for the weevils thus the persistency on the tea monoculture (Mbugua, 2015). Alternatively the high weevil's presences' in the 11-20 years old tea could be related to the effects of long term tea monocropping on the soil acidity, ratios of base nutrients and microbial biomass (Kamau *et al.,* 2009). This is an area for more research.

It is evident from the results of the laboratory bioassay study that two *B. bassiana* isolates are most virulent on the tea Nyambene weevil. Bb Gi 7a isolate, is native to East of the tea growing region. This observation may be attributed to the locality of the isolate which is the common habitat of the Nyambene weevil and therefore this mean's that a local isolate can be more promising as a control agent since it has adapted to the local conditions and minimizes environmental risks related to field application. Many researchers have noted that the highest level of virulence were observed when isolates were used on the same species or closely related species on which they have been collected (Poprawski *et al.,* 1985; Sabbahi *et al.,* 2008). The varied efficacy of *B.bassiana* isolates concurs with reports that the fungs contains diverse assemblage of genetypes and probably comprises of species complexes (Inglis *et al.,* 2001; Sevim *et al.,* 2012). Although this study did not determine, it may also be conceivable to have individual isolates or pathotypes which exhibit a substantially specificity on the host range. Average survival time (AST) for this most virulent isolate was found to be 12 days at a concentration range of 1x 10^3 in this study. Sabbahi *et al.,* (2008) attained. AST of 7.8 days with a virulent *B. bassiana* against *O. ovatus* at a higher contration of 1 x 10^8. The virulent type affects the feeding rate of the weevil pest, this implies that the more the *B. bassiana* penetrates the body of an insect the weaker it becomes hence feed less. The results of most previous feeding studies, involving noctuids, lymantriids (Lepidoptera), acridids (Orthoptera), and chrysomelids (Coleoptera)(Mohamed 1982; Mohamed *et al*. 1982; Thorvilson *et al.,*. 1985; Lord *et al.,*. 1987; Hajek 1989; Sieglaff *et al*. 1997; Noma and Strickler 2000) are in agreement with the result of this study. In such cases, feeding of insects infected with *B. bassiana* or other fungi was reduced (Mohamed 1982, Mohamed *et al*. 1982, Hajek 1989, Sieglaff *et al*. 1997) or not affected (Thorvilson *et al.,*. 1985, Lord *et al.,*. 1987) or contrary to Noma and Strickler,(2000) studies which increased. Mohamed (1982) suggested that reduction in food consumption may be attributed to an effect of the invading fungus on hormonal control of feeding. Bordoloi *et al.,* (2012) while conducting an experiment on entomopathogenic fungi found that *Helopeltis* spp. infected with *Cladosporium* spore suspension caused reduced feeding rate and he attributed it to the effect of fungus on metabolites. In the current study, mode of action of the fungus was not elucidated but definitely sickened the weevils to an extent that their feeding was reduced.

Weevil occurrence in a tea field area was found to be sporadic, this can be due to the aggregative behaviour of the tea weevils and several reports have indicated this behaviour (Sun *et al*., 2010) and have attributed this behaviour to volatiles involved in the attractants of conspecifics to either locate host plant or as a pheromone synergist. The very low damage of between 9 to 18% in the second trial of Giciaro farm can be attributed to this phenomena of conspecies being attractant to originally infested fields. The very low damage could be the reason for none significance difference between treatments. This also explains why diversionary host plants can deter attack.

Compared to controls all the pesticides (chemical pesticide and biopesticides) used (except karate in Giciaro) demonstrated their efficacy on reducing either weevil damage or yield loss as a result of high weevil infestations. The poor performance of Karate insecticides in Giciaro farm (trial 1) in terms of yield can either be attributed to Karate having phytotoxicity effect to the clone in that field or the weevils have developed resistance in to the active ingredient lambdacyhalothrin in those fields or karate plots had low yields right from the start of the experiment. This results corroborated those of Kpindou *et al.,* (2013) where the results of chemicals such as imidacloprid, lambdacyhathrin and flubendiamide and biologicals, *B.bassiana* and *M. anisopilae,* significantly reduced the density of pest compared to the controls and they concluded that there is a posiblilitiy of *B. bassiana* and *M. anisopilae* being used as alternatives to chemical insecticides for cotton pests' control. The study has also shown that weevils can cause damage at certain seasons of the year with yield loss ranging between 30-33% and is more prominent on tea gardens which are due for prunning and that the damage is relative to weevil population. The study showed that the reduced yield effect by the weevils may be attributed to reduced photosynthetic organs caused by defoliation. The low PAR nust have contributed too to low yield. There is documented evidence that the rate of photosynthetic (dry matter production) is largely dependent on incoming solar radiation and is proportional to the amount of solar intercepted and efficiency to which it is converted to dry matter (Squire, 1990).

Maximun mean Temperatures in Giciaro tea farm ranged between 17.5-22^{o}C while Mumungas' had a narrower range of 18.5 to 22 ^{o}C. Temperature plays a role in the pest population as evident in the modelling exercise above. Mununga seems to be slightly warmer than Giciaro site and thus the higher damage and It can be inferred that higher temperatures may have an increasing weevil population leading to increased damage. Increase in pest as a result of increased temperatures is not a new phenomena several studies has shown this (Sudoi *et al.*, 2011). The results explain the weevil outbreaks which normal occur during the dry weather and the low damages during the following rainy season in the areas of study. It appears that monthly rainfall above 100mm is adequate to suppress the weeil numbers.

When it comes to persistency of the fungus in the soil, the months of March, April and May when it is rainy (moist environments) and it's cool, the fungus persist in the soil (Sabahi *et al.*,2008). The persistence study also revealed that there seems to be high inoculum of *B. bassiana* in plots sprayed with Karate in the month of November. This may be attributed to high mortalities of weevils which provides rich nutrient for the natural inoculum in the soil for *B. bassiana* to thrive well and thus the increase.

Studies in cages proved that the two types of commom tea weevils (*E. meyeri* and *S. mixtus*) highly prefered the Avocado and purple tea leaves in cages and there was a reduction in leaf area eaten on clone TRFK 31/8 when planted in a mixture. Therefore, Purple tea and Avocado are potential "pull crop". Quite a number of studies on mixed planting of tea plants and fruit or shade trees compared with monoplantation has been implicated in the role of chemical stimuli. Hongfei *et al.,* (2014) found the number of the tea green leafhopper *(Empoasca vitis)* in mono-tea plantation were significantly higher than in mixed planting plantation. Mixed planting of *Dimocarpus longan* (a friut tree) and tea plantation were the best mixed types because of moderate light, highest production and most predators and less pests. The presence of predators and less pest can be attributed to the many factors among them could be the attractive volatiles in the fruit tree as may be attributed to the results of this study. In another study (Majumder *et al.,* 2011), it was found that well shaded tea had more looper than poorly shaded tea, particularly where *Indigofera teysmanii, Derris robusta* was grown as a shade tree that might have initially attracted more loopers towards it. Plant architectural traits have been reported to impact on pest and disease. Properties/characteristics of a plant may allow it (or not) to escape or survive attacks . The organs in which nature shape and position may influence pest attack and development. These traits can vary between genotypes within species and can be used to draw plant protections (Costes *et al*., 2012) as shown by the weevil preferring broad leafed large flat plain leaves.

CHAPTER SIX

Conclusion and recommendation

Conclusion

The following conclusion can be made from the study:

The tea growing regions in East of Rift are at risks to weevil infestation and weevil species distribution was site specific for *E. meyeri* as opposed to *S. mixtus* and the most prevalent weevil was *S, mixtus.*

Cultural practices notably routine husbandry measures and keeping of livestock can be manipulated to either enhancing or deterring pest attack or improve crop production for examples, mixed clones of tea, excessive use rate of nitrogenous fertilizers, indigenous trees, avocado and eucalyptus intercropping enhanced weevils infestation.

Weevils affects green leaf yield with a loss of between 30-33%, through reduced canopy, reduced PAR intercepted by the plant canopy as a result chewing the leaves, resulting in less photosynthetic organs.

The economic threshold level was found to be 2-3 weevils per bush where the destroyed leaves through defoliation was more than 40% to 54% of leaves.

Weevil presence/damage fluctuated with the season of the year. Increase in rainfall corresponds to low damage and relation to damage and weevil numbers is highly position.. Increased temperatures enhances weevil population and in extension damage.

Beauveria. bassiana isolates has the potential for use in the management of tea weevils as an alternative to chemical insecticides. *B. bassiana* isolates Bb 7a and isolates Bb 6 performance compared with Karate insecticide, either as sprays or by broadcasting in solid substrate like wheat bran

Purple tea and avocado are potential "pull crop" (attractants to weevils or are preffered by the tea weevils.

Recommendation

Patterns of changing climatic conditions may results in variations in weevil distribution over years as a result to climate change and clearing of forest for agricultural purposes, such as deforestation for Agricultural crops, therefore it is prudent to continue to determine weevil distribution over time in order to establish whether there is a shift in distribution overtime while relating weevil population dynamics to extreme weather parameters (climate change conditions – extremes temperatures and rainfall). The distribution trend or tipping points may have an impact on the effectiveness of current management strategies and thus the need for ongoing monitoring and assessment of pest species responses to environmental changes and their management at local and regional scales. It also indicates a need for research aimed at identifying potential tipping points in relation to significant meteorological events.

Using attractants/semiochemicals of tea and its bio-ecology under the different Kenyan climate can provide barriers which can contain the spread of the weevils incase their sporadic outbreaks gets triggered by factors such as the climate change, changing cultural systems among others

B. bassiana isolates Bb Gi 7a and isolates Bb Ke 6 can be recommended for the management of the weevils either as sprays or by broadcasting in solid substrate like wheat bran. There is therefore need to develop further an appropriate formulation of these isolates for use by the tea growers affected by the weevils in times of posterity. In addition, various application strategies, formulation and modes of introducing the fungus into plantations should be consistently studied and integrated into routine practices of tea husbandry

Identification of of a repellant /attractant plants to the determination of volatiles involved in preference and in the development of biological control push-pull technology in the field and its integration to tea research is required to perfect the ‘push’ component for the strategy to work in the field and be used by resource poor tea growers.

REFERENCES

Akello, J., Dubois, T., Coyne, D., Gold, C. J. and Kyamanywa, S. (2007). Colonization and persistence of entomopathogenic fungus *Beauveria bassiana*, in tissue culture of banana. *Africa crop science Conference Proceedings*, Vol. 8 857-861.

Andow, D. A. (1991). Vegetational diversity and arthropod population response. *Annu. Rev. Entomol*. 36:561–86.

Agunda, R.M., Rombach, M.C., Shepard, B.M. (1988). Infection of brown plant hopper (BPH) with insect fungi in the laboratory. International Rice Research Newsletter, 13 1- 34.

Ahmad, S. S. (1993). Prevalence of an entomopathogenic fungus, (*Hirsutella cubana*) in Malaysia. *Dertanmika J. Agrioc. Sc.*, 16(2), 95-99.

Anon. (1986). *Tea Growers Hand book*, Tea Research Foundation of Kenya (4th Edition) 172-173.

Anon. (2001). Preliminary trial on infectivity of *B. bassiana* on Kangaita weevil. In: *Annual Report for the year 2001*, Tea Research Foundation of Kenya, pp. 186.

Anon. (2002). Tea Growers Handbook, Tea Research foundation of Kenya. Fifth 5th Edition 261.

Anon. (2003). Integrated control of tobacco crickets: Use of Entomopathogenic fungi (*Beauveria bassiana* innoculat) and insecticide bait to reduce plant losses to Tobacco in the field. (Kaptel, North Nandi). In: *Annual Technical Report for the year 2003*, Tea Research Foundation of Kenya Tea Board of Kenya. 113.

Anon. (2004). Pests samples received and identified in Plant Protection Laboratory in the year 2004 In: *Annual Technical Report for the year 2003*, Tea Research Foundation of Kenya Tea Board of Kenya 129.

Anon. (2006). Entomopathogenis fungi for control of tea pests: Preliminary trial on infectivity of *Beauveria bassiana* on Kangaita weevil (*Entypotrachelus Meyeri*) In: *Annual Report 2006*. Tea Research Foundation of Kenya. 140.

Anon. (2007). Monitoring of pests through pests and disease surveillance. Plant Protection Investigations. In: *Annual Technical Report for the year 2007*, Tea Research Foundation of Kenya Pg. 119.

Anon. (2008). Monitoring of pests through pests and disease surveillance. Plant Protection Investigations. Im: *Annual Technical Report for the year 2007*. Tea Research Foundation of Kenya Pg. 79.

Anon. (2013). International Tea Committee (ITC) Supplement to Annual Bulletin of Statistics 2013 9 pp.

Avulova, S. and Rosengaus R.B. (2011). Losing the battle against fungal infection: suppression of termite immune defenses during mycosis. *J. Insect Physiol.* 57: 966-971

Bajya, D.R., Ranjith, M. and Raza, S. K. (2015). Evaluation of *Beauveria bassiana* against Chickpea Pod boerer, *Helicoverpa amigera* and it's safety to natural enemies. *Indian Jour. Of Agric. Sc*i. 85(3):378-381

Baker, R. (2015). The role of Geographis Information Systems in Pest Risk Analysis. *Zbornik predavanj in referatov 5. Slovenskega posvetovanja o varstvu rastlin*. 22-26.

Barnejee, B., (1992). Botanical Classification of Tea. In: *Tea cultivation to consumption. (Eds).* Wilson *K.C.* and Clifford, *M. N.*, 25-51. Chapman and Hall London

Bharathi, H. T. (2005)**.** PH.D thesis, on Isolation and characterization of entomopathoegenic fungi and their effectiveness, University of Agricultural Sciences, Dharwad.

Barua, D. N. (1994). Science and Practice in Tea Culture. Calcutta: Tea Res. Assoc. 509 pp.

Barua, D. N. (1989) Introduction to tea culture. In: *Science and practice in tea Culture* .Tea Research Association , Kolkota pub. 8 pp

Baruah, G.C.S. (1983) Fungi in Biological control of Tea Pests and Diseases in N. E. India *Two and a Bud* 30: 5-7

Bray, E. A., Bailey-Serres, J., Weretilnyk, E. (2000). Responses to abiotic stresses. In Gruissem, *W*. Buchannan, B. Jones, *R.* (eds), *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249.

Bedding, R. A. & Akhurst, R. J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nenlatologica*, 21: 109-110.

Beers, E. H. 2004. Curculionids and Pacific Northwest tree fruits: past and present. *Curculio*, (49) 13-18.

Benjamin, D.M. (1968). Insects and mites on tea in Africa and adjacent islands of E. Afric. *E. Africa Agric and Forest J.*., 33, 345-357.

Benjamin, D.M. and Demba, W.M. (1968). Notes on the biologies of three parasites of the eggs of *Entypotracherus meyeri* Micans *E.meyeri* Hust, (Col. Curculionidae) laid on tea and cinchona. *Bull. Ent. Res.*, 59, 473-478.

Benjamin, D.M. and Demba, W.M. (1974). The bionomic of *Entypotrachelus meyeri meyeri* Kolbe and *E.m micans* Hust, (Col, curculionidae) on tea in East Africa. *Bul.Ent. Res.*, 63, 431-451.

Benjamin, D.M.; Freeeman, G.H.; Brown E.S. (1968). The determination of irregularly shaped areas of leaves destroyed by chewing insects. *Ann. Appl. Biol.*, 64, 13-17.

Bharathi, H. T. (2005). PH.D thesis, on Isolation and characterization of entomopathoegenic fungi and their effectiveness, University of Agricultural Sciences, Dharwad

Bordolei, M., Madhab, M., Dulta, P., Borah T, Nair, S. C, Phukan, 1. Debnath, S. and Barthakur, B. K. (2012) Potential of entomopathogenic fungi for the management of *Helopeltis theivora* (watrhouse). *Two and a Bud* 59:21-23

Bokuchava, M. A. and Skobelava, N. I. (1969). The Chemistry and Biochemistry of tea and tea manufacture. *Advances in Food Research*, 17, 215-292

Burges,. D K (1998). Formulation of mycoinsecticides in formulation microbial biopesticides: In: *Beneficial microorganisms, Nematodes and seed treatments (ed).* Burges *H. D.*, The Netherlands: Kluwer Academics publishers, pp 31-185

Butt, T. M.,(2002). Use of entomogenous fungi for the control of insect pests. In: *The Mycota XI Agricultural Applications*.(Ed.) by Kempken *F*, Springer, Berlin, 111–134.

Castrillo, L. A., Griggs, M. H.; Ranger, C. M.; Reding, M. E. and Vandenberg J. D. (2011). Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. *Biol. Control* 58 121–126.

Chen, Z. (1999). Pharmacological functions of tea. In: *Global advances in tea science.* (Ed,) Jain *N. K.* Aravali Books International Ltd., India, pp 333-358.

Cook, S.M., Khan, Z.R. and Pickett, J. A. (2007). The use of 'push-pull' strategies in integrated pest management. *Annual Review of Entomology* 52: 375-400.

Costes, E., Lauri, P.E, Simons, S. and Andrieu B. (2012). Plant Achitecture, its Diversity and Manipulation in agronomic conditions, in relation with pest and pathogen attacks. *European Journal of Plant Pathology* vol. 135 issue No. 3 pp 455-470

Couturon. E., (1982) Obtention de'haploides spontanes de coffea canephora Pierre par I'utilisation du greffagee d' embryons.cafe cacao *The*, 26 (3):155-160

Deka, A., Deka, P.C., Mondal, T. K. (2006) Tea. In *Plantation Crops*, (ed.) Parthasarathy, *V. A.*, Chattopadhyay, *P. K.*, Bose,*T. K.*, 1:1–147. Kolkata: Naya Udyog

Dembilio, O, Enrique Quesada-Moraga, Cándido Santiago-Álvarez and Josep A. Jacas (2010) Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the Red Palm Weevil, *Rhynchophorus ferrugineus*, *Journal of Invertebrate Pathology* Volume 104, Issue 3, Pages 214-221

Dona, M., Dell 'Aica, I., Calabrese, F., Benelli, R., Moroni, M., Albini, A., Garbisa, S., (2003) Neutrophil restraints by green tea: Inhibition of inflammation, associated angiogenesis and pulmonary fibrosis. *J. Immunol*, **170**(8): 4335-4341

Dutcher, J. D. and J. A. Payne. (1985). The impact of pecan weevil control strategies on non-target arthropods, pp.39Ð50. In *Pecan weevil: research perspective*. Neel *W. W.,* (ed.), Quail Ridge Press, Brandon, MS.

Endrödy-Younga, S. (1985). Coleoptera, introduction. In: Scholtz, *C.H.* & Holm, *E.* (Eds.) *Insects of Southern Africa*. 188. Butterworths, Durban.

Fargues, J. and, Luz, C. (2000). Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. *J Invertebr Pathol.* Apr;75(3):202-11

Feng, U.G., Poprawshi, J.J. and Khaohatourians, G.G. (1994). Production, formulation and application of entomopathogenic fungus *Beauveria bassiana* for insec control, *Current Status Biocontrol Science and Technology* 4;3-34

Ferron, P. (1975). Biological control of insects by entomogeneous fungi *Ann. Rev. Ent* 23:409-42

Ferron, P. (1981). Pest control by fungi *Beauveria* sp. and *Metarhizium* .sp. In; *Microbial control of pests and plant diseases* 1970-1980. Burges *H.D.*, ED Academic press .London pp 465-482

Goettel, S. M., Hajek, A,E., Siegel, P. J. and Harry, E. C. (2001). Safety of fungal biocontrol agents.CAB international, Fungi as bio control agents, 347-35

Golshan, H., Saber, M., Majidi-Shilsar, F. and Karimi, F. (2013). Efficacy of Several Isolates of *Beauveria bassiana* (Balsamo) Vuillemin against Adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Acta Entomol. Sinica*, 56: 201-206.

Gul, H. T., Saeed S. and Khan F. Z. (2014). Entomopathogenic Fungi as an Effective Insect Pest Management Tactic: A Review. *Applied Science and Bussiness Economics* Vol.1 No.1 10-18

Gurr, G. M., Wratten, S. D., Altieri, M. A., eds. (2004). Ecological Engineering for Pest Management: *Advances in Habitat Manipulation for Arthropods*.Wallingford, UK: CABI Publ.

Hajek, A. E. (1989). Food consumption by *Lymantria dispar* (Lepidoptera: Lymantridae) larvae infected with *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Environ. Entomol.* 18: 723-727.

Hara, Y.; Liu, S. J.; Wickermasinghe, R. L.; Yamanishi, T. (1995). Special Issue on tea. *Food Review, International*, 11, (3): 371-545

Hasan, K. A. (1963). Shade trees for tea. Part 1: Their functions and behaviour. *Tea J. Bangladesh.*, 1(2), 9-16.

Hazarika, L. K, Puzari, K. C, Wahab, S. (2001). Biological control of tea pests. In Biocontrol Potential and Its Exploitation in Sustainable Agriculture. Vol. 2: Insect Pests, (ed.) *RK* Upadhyay, KGMukerji, *BP* Chamola, pp. 159–80. New York: Kluwer Academic. 421 pp.

Hongfei, S., Liuxia, Y., Wenzhong, Z., Xinghua, W., Xuekum, Y. and Tinghong, C., (2014) The Effects of Mixed Planting in Tea Production, Main Predators and Insects Pests in Tea Plantation. *Journal of Tea Science* 34(2) 122-128

Inglis, G.P., Johnson, D. L. and Goettel, M.S. (1997) Field and Laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsama) Vuillemin against grasshoppers. *The Canadian entomologist* 129, 171-186

Inglis, G.D., Goettel, M. S., Butt, T. M., Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: Butt, *T.M.*,Jackson, *C.*, Magan, *N.* (Eds.), . *Fungi as Biocontrol Agents. Progress, Problems and Potential.* CABI Publishing: 23–69

ITC, (2013). International Tea Committee, Annual Bulletin of statistics, Aitken Spence Printing (PVT) ltd, Colombo, Sri Lanka 156 pp.

Jaetzold, R., Schmidt, H., Hornets, B. and Shisanya, C. (2007). Farm Management Handbook of Kenya vol II *Natural Conditions and Farm Management Information part II/C East Kenya* (2^{nd} Edition) Project supported by Ministry of Agriculture and German Technical Cooperation (GTZ) pp 319

Kingdom-ward, F., (1950). Does wild tea exist? *Nature*. 165; 297-299

Kfir, R., Overholt, W.A., Khan, Z.R. and Polaszek, A. (2002). Biology and management of economically important cereal stem borers in Africa. *Annual Review of Entomology* 47: 701-731.

Kamau, D.M., Spiertz, J.H.J., Oenema, O. (2008). Carbon and nutrient stocks of tea plantations differing in age, genotype and plant population density.*Plant & Soil* 307, 29-39.

Khan, Z.R. and Pickett, J.A. (2004) The 'push-pull' strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. In *G*. Gurr, *S.D.* Waratten, and *M.A.* Altieri (Eds.), *Ecological engineering for pest management: advances in habitat manipulations for arthropods*. CSIRO and CABI Publishing. 155-164

Kamau, D.M., Onenema, O., Spiertz, H., Owuor, P.O. (2009) Changes in soil Properties following conversion of Forests into Intensively Managed *Camellia sinensis* L. Plantations along a Chronosequence. *Tea* 30(1) 42-53.

Khan, N. and Mukhtar, H. (2007) Review on Tea Polyphenols for health promotion. *Life Sci*. **81** (**7**): 519-533

Khan Z. R., Pickett J. A., Wadhams L. J., Hassanali A. and Midega C. A.O. (2006) Combined control of *Striga hermonthica* and stemborers by maize-*Desmodium* spp. intercrops. *Crop Prot.* 25:989–95

Kilavuka, C.I. (1982) Advisory services, Pests, Diseases and Weather Damages. *Tea* Vol 3. Number 2. Dec. 1982 Pp 6-10

Kpindou Douri, O.K., Parasio, A., Djegui, D.A., Glitho, I. A., Maffon, S., Tamo, M., (2013) Comparative Study of the Efficacy of Entomopathogenic Fungi, Chemicals and Botanical Pesticides in the Management of cotton Pests and Their Natural Enemies in Benin. *International Journal of Science and Advanced Technology* (ISSN2221-8386) Vol.3 No I pg. 21-33

Kumar, R. (1984), Insect pest control with special reference to African Agriculture, Camelar press Ltd, Great Britain.

Lakshmi, K., Hazarika, Mantu bhuyan and Budhindra N. Hazarika (2009) Insect Pests of Tea and Their Management *Annu. Rev. Entomology* 54: 267-84

Lai, J., Wei-chen Yang, Hsiao, Y. (2002). An assessment of genetic relationship in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. *Bot. Bull. Acad. Si*n (42), pp 93-100.

Lin, Y.S., Tsai, Y.J., Tsay, J. S. and Lin, J. K. (2003). Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *Journal of Agricultural Food Chemistry*, vol. **51**(7) pp, 1864-1873

Litsinger, J. A. (1994). Cultural, mechanical and physical control of rice insects. In *E.A.* Henrichs (Ed.) *Biology and Management of Rice Insects*. Ed. E. A. Heinrichs International Rice Research Institute, Philippines. 549-584

LiZZ, Li. C.R., Huang, B., Fan, M. Z. (2001). "Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus". *Chinese Science Bulletin* 46: 751–3.

Lord, J. C., Magalhaes, B. P. and Roberts, D.W.. (1987) Effects of the fungus *Beauveria bassiana* (Bal.) Vuill. Behavior, oviposition, and susceptibility to secondary infections of adult *Cerotoma arcuata* (Olivier, 1791) (Coleoptera: Chrysomelidae). *An. Soc. Entomol. Brasil*. 16: 187-197

Mahr, S. (2003) Know your friends. The entomopathogen *Beauveria bassiana* (htpp://www.entomology.wisc.edu/mbcn/kyf410html. Diakses 21 Januari 2003). *J. Entomol. Ind*., April 2006, Vol. 3, No. 1, 30-40

Majumder, A.B., Pathak, S.K., Hath, T. K., Gupta, D. S. and Majumdar, D. (2011) A Survey on the Distribution pattern of looper pests in the tea plantation of dooars, West Bengal. *Two and a Bud* 59: 49-54.

Manainia,. N K, (1993**).** Effectiveness of the entomopathogenic fungus *Beauveria bassiana* (Balsi) Vuill for control of stem borer *Chilo partellus* (Stalk borer) in maize in Kenya. *ICIPE annual reports* 1993

Maniania, N.K. (1997), Evaluation of formulations of *Beauveria bassiana* for control of stem borer*, Chilo partellus. ICIPE annual report*,1997.

Mannion, C. (2003). High risk insect Pests; Monitoring and diagnosis *Proc. Fla. State Hort. Soc.*116:78-79

Mbugua, D. (2015). Forest Outlook Studies in Africa (FOSA) country Report – Kenya. FAO CORPORATE DOCUMENT REPOSITORY pp70 .www.fao.org/docrep/003/ab569e/AB569E03.htm retrived on 2nd July 2015

Mesquita, A.L.M. (1988). Control biolo'gico des brocas bananeira cosmopolotes sorditus (Germar, 1824) a Hermisterus (Line, 1764) com fungus entomogenos. En: Reunion ACROBAT Medullin AUGURA, 311-324

Milat-Bissaad, F.Z., Bounaceur, F., Halouane, F., Behidj, N., Chebouti, N. and DoumandjiMitiche, B. (2011). Effect of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* var. acridum on the haemolymph of the desert locust Schistocerca gregaria. *Tunisian Journal of Plant Protection* 6: 127-132.

Miller, J. R., and Cowles, R.S. (1990). Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology* 16: 3197-3212.

Mohamed, A.K.A. (1982). Pathogenicity of *Nomuraea rileyi* and its effect of food consumption and utilization by *Heliothis virescens* larvae. *J. Ga. Entomol. Soc*. 17: 369- 376

Mohamed, A.K.A., Brewer, F. W., Bell, J. V. and Hamalle, R. J. (1982) Effect of *Nomuraea rileyi* on consumption and utilization of food by *Heliothis zea* larvae. *J. Ga. Entomol. Soc.* 17: 351-356

Mangenya T. M., Kelvin O. Moseti, K. O., Wanyoko, J. K., Kinyua, J. K., Daniel Kariuki, D., Esther N. Magiri, E.N. and Obanda, M. A. (2014) Selected

Inorganic Nutrients in Black Tea from Three Tea Growing Agro-Ecological Areas in Kenya *American Journal of Plant Sciences*, 5, 473-479.

Mugo, H. M., Irungu, L. W. and Ndegwa, P. N (2011) The Insect Pests Of Coffee And Their Distribution In Kenya In *International Journal of Science and Nature* (I.J.S.N). ISSN 2229 – 6441, VOL. 2(3) 2011: 564 -569

Muraleedharan, N. and Chen, Z.M. (1997). Pests and diseases of tea and their management. *J. Planatation Crops*, 25, (1) 15-43.

Muktar, H. and Ahmad, N. (2000). Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nutr.* 1698S-1702S

Mutai, R. (2008). Recent research achievements in tea disease management.*Tea* 29, 46-56

Nankinga, C.M (1994) Potential of indigenous fungal pathogens for the biological control of banana weevil, *Cosmopoliter sordidus* (Germar) in Uganda, Msc thesis, Department of crop Science . Faculty of Agriculture and Forestry, Makerere University Uganda, 95pp

Nelson, M. R., Orum, T. V., Jamie-Garcia, R. and Nadeem, A. (1999). Aplications of Geograpic Information Systems and Geostatistics in Plant disease Epidemiology and Management. The American Phytopathology Soceity. *Plant diseases*, Vol. 83 (4) 308-319.

Ngeno, C. (2015) Government creates tea buffer zones in Mau Forest. The Standard Newspaper 1st July 2015 P. 15

Ngumbi,. P. M., Irungu,. L. W., Ndegwa,. P. N. and Maniania, N. K. (2011), Pathogenecity of *Metarhizium anisophia* (Melch) sorok and *Beauveria bassiana* (Bals) Vuill to adult *Phlebotonus duboscqi* (Never-lemaire) in the laboratory, *Journal of vector borne diseases*, 48(1)**,** 37-40

Njuguna, D. G. (2012). Determination of Essential and Toxic Elements, Polyphenols and Fatty Acids Residues in Tea seed oil cake and Husks of Selected Cultivars in Kenya. Master Thesis Egerton University October 2012 93pp

Noguchi, H., Tamaki, Y., Arai, S., Shimoda, M., Ishikawa, I. (1981). Field evaluation of synthetic sex pheromone of the oriental tea tortrix moth, *Homona magnanima Diakonoff* (Lepidoptera: Tortricidae). Jpn. *J. Appl. Entomol. Zool*. 25:170–75

Noma T. and Strickler K. (2000). Effects of Beauveria bassiana on Lygus hesperus (Hemiptera: Miridae) Feeding andOviposition. Entomological Society of America, *Environmental Entomology*, 29(2):394-402. 2000.

Oberprieler, R. G. and Louw, S. (1985). Curculionoidea. I.: Scholtz, C. H. and Holm, E. (Eds) *Insects of Sounthern Africa*. 270, Butterworths. Durban 276-280

Ohtani, K., Witjaksono, T., Fukumoto, T., Mochizuki, F., Yamamoto, M., Ando, T. (2001) Mating disruption of the Japanese giant looper in tea gardens permeated with synthetic pheromones and related compounds. *Entomol. Exp. Appl.* 100:203–9

Ogola, S. O., Kibiku, P. N. (2004) smallholder tea growing enterprise. Productivity and profitability. Tea Board of Kenya Survey Report, Tea Board of Kenya Nairobi 44 pp

Olujide, M. G. and Adeogun, S. O. (2006) Assessment of Cocoa growers' farm management practices in Ondo state, Nigeria. *Spanish Journal of Agricultural Research* 4(2), 173-179

Otieno, W. (2005). Basis of Good Agricultural Practices in Sustainable Crop Protection: The Case of Disease Management on Tea (Camellia sinensis) in Kenya

Otieno, W. (2002). Armillaria Root rot of tea in Kenya . Characterization of the pathogen and approaches to disease management. Phd thesis.

Penwil, D.J. (1961). Future tea Development among smallholders in the African areas of Kenya. *Tea* 2(2), 15-22.

PCPB . (2011). Pest control Products Registered for use in Kenya 7th Edition pp 184

Picker, M., Griffiths, C. & Weaving, A. (2002) Field Guide to Insects of South Africa. Struik Publishers, Cape Town.

Poprawski, T. J., Marchal, M., Robert, P.H. (1985) Comparative susceptibility of *Otiorynchus sulcutus* and *Sitona lineatus* (coleopteran: Curculionadae) early stages of five entomopathogenic hyphomycetes. *Environ. Entomol.* 14 247-253.

Posada, F., Vega, F. E., Rehner, S. A., Blackwell, M., Weber, D., Suh, S.-O. & Humber, R. A. (2004). *Syspastospora parasitica*, a mycoparasite of the fungus *Beauveria bassiana* attacking the Colorado potato beetle *Leptinotarsa decemlineata*: A tritrophic association. *Journal of Insect Science*, *4*, 24.

Prakash, O., Sood, A., Sharma, M., Ahuja, P., (1999). Grafting micropropagated tea (*camellia sinensis* (L) O. kuntze) shoots on tea seedling-a new approach to tea propagation. *Plant cell Rep.* 18:137-142

Robert, E. A. H and Wight, W. (1958). Paper chromatography as an aid in the taxonomy of *Theo camellian*. *New Phytot*. 57(2):211-215.

Pyke, B., Rice, M., Sabine, B. and Zalucki, M. P. (1987). The push-pull strategy -- behavioural control of Heliothis. Australian Cotton Grower. May-July: 7-9.

Rehner, S.A., Buckley, E (2005). "A *Beauveria bassiana* phylogeny inferred from nuclear ITS and EF1-{alpha} sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs". *Mycologia* 97: 84–98. Retrieved from "http://en.wikipedia.org/wiki/*Beauveria_bassiana*" on 23rd September 2014

Root, R B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassicae oleracea*). *Ecol. Monogr*. 43:95–124

Sabbahi, R., Merzouki, A. and Guertin, C. (2008) Efficacy of *Beauveria bassiana* against straw berry pests. J. *Appl. entomol. Zeitschrift fur angewandte Entomologie* 2008 Mar v.132, No. 2 pp 151-160.

Santa, H. S., Santa, O. R., Brand, D., Vandenberghe, L. P., Soccol C. R. (2005). Spore production of *Beauveria bassiana* from agro-industrial residues. Braz. *Arch. Boil. Technol.,* 48: version prints ISSN, 1:1516-8913.

Sevim, A., Hofte, M., Demirbag, Z. (2012) Genetic variability of *Beauveria bassiana* and *Metarhizium anisopliae* var. *anisopliae* isolates obtained from the Eastern Black Sea Region of Turkey. *Turkisk Journal of Biology* 36: 255–265.

Sen, A.R. (1959). Pest and diseases surveys. *Two and bud*, 4(4):16.

Sevim, A., Demir, I., Sonmez, E., Kocacevik and Demirbag Z. (2013). Evaluation of entomopathogenic fungi against the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae) *Turkish Journal of Agriculture and Forestry* 37: 595-603

Shelton, A.M., Badenes-Perez, F.R.(2006). Concepts and applications of trap cropping in pest management. *Annu. Rev. Entomol*. 51:285–308

Shimazu, M. and Sato, H. (1996). Media for selective isolation of an entomogenous fungus, *Beauveria bassiana* (Deutromycotina:Hyphomycetes). *Appl. Entomol. Zool.* 31 (2): 291–298.

Siderius, W. and Muchema, F.N. (1977). Soil and environmental conditions of Agricultural research stations in Kenya. Ministry of Agriculture. Kenya Soil Survey, Miscellaneous paper No. 17, 1977

Sieglaff, D. H., R. M. Pereira, and J. L. Capinera. (1997) Pathogenicity of *Beauveria bassiana* and *Metarhizium flavoviride* (Deuteromycotina) to *Schistocerca americana* (Orthoptera: Acrididae). *J. Econ. Entomol.* 90: 1539-1545.

Sivapalan P, and Seneratne K. A. D. W. (1977). Some aspects of the biology of the tea termite, Glyptotermes dilatatus. *PANS* 23:9–12

Shapiro-Ilan, D. I., Wayne, A., Gardner , L. W. and Bruce W. (2012) Wood Cumulative Impact of a Clover Cover Crop on the Persistence and Efficacy of *Beauveria bassiana* in Suppressing the Pecan Weevil (Coleoptera: Curculionidae). *Environmental Entomology* 41(2):298-307.doi: http://dx.doi.org/10.1603/EN1122. Retrived on 23rd September 2014

Sudoi, V. (1997) Tea Pests with special Reference to mites: Research Achievements and Futurre Thrusts. *Tea* (2) 156-165

Sudoi, V. (1998). Evaluation of Neem seed oil product for the control of tea pests in Kenya, *Tea* 19(2) 62-65.

Sudoi, V., Langat, J. K. and Bor, S. K. (1999). Evaluation of entomopathogenic fungus for control of tea weevil *Entypontrachelus meyeri* micans in Kenya: Preliminary indications.*Tea* 20, 72-75.

Sudoi, V., Cheramgoi, E., Langat, J. K., Kamunya, S. M. and Wachira, F. K. (2011) Screening of Kenyan Tea Clones for Susceptibility to Mites Attack at Different Ecological Zones.' *International Journal of Current Research,* Vol. 33(6): 328-332.

Sun, X. L., Wang, G. C., Cai, X. M., Jin, S., Gao, Y., and Chen, Z. M. (2010). The tea weevil, *Myllocerinus aurolineatus*, is attracted to volatiles induced by conspecifics. *J. Chem. Ecol.* 36(4): 388-395 DOI 10.1007/s10886-010-9771-9

Suguino, E., Santos-Cividanes, T.M.D., Cividanes F. J., De Faria, A.M. and Martins, A. N. (2013). First Report of the Occurrence of Weevils (Insecta:Coleoptera) in Plantations of Green Tea in Brazil Florida .*Entomologist* 96(3), 1217-1220

Squire, G.R. (1990). The physiology of tropical crop production. C.A.B International for the Overseas Development Administration.

Takeda, Y and Toyao, T. (1980). Identification and classification of green tea varieties by the morphological character of flower organs. *Tea Res. J.* 52:1-6.

Tamaki, Y., Noguchi, H., Sugie, H., Sato, R. (1979). Minor components of the female sex attractant pheromone of the smaller tea tortrix moth (Lepidoptera: Tortricidae) isolation and identification. *Appl. Entomol. Zool*. 14:101–13

Teng C. (1962). Studies on the Biology of *Beauveria Bassiana* (Bals.) Vuill. with Reference to Microbial Control of Insect Pests. *Acta Botanica Sinica* Volume 10 Issue 3, 210-232

Thomas S. R., Goulson, D., Hollard, J. M. (2001). Resource provision for farmland gamebirds: the value of beetle banks. *Ann. Appl. Ecol*. 41:524–31

Thorvilson, H. G., Pedigo, L. P. and Lewis, L. C. (1985). Soybean leaf consumption by *Nomuraea rileyi* (Fungi: Deuteromycotina) -infected *Plathypena scabra* (Lepidoptera: Noctuidae) larvae. J. *Invertebr. Pathol*. 46: 265-271.

Thungrabeab M. and Tongma S. (2007). Effect of entomopathogenic fungi, *Beauveria bassiana* (balsam) and *Metarhizium anisopliea* (Metsch) on non target insect. *KMITL* 7(1): 8-12

TBK, (2003) The Tea map of Kenya, Compiled, revised and drawn by Tourist Maps (K) Ltd for the Tea Board of Kenya.

TRA. (1994). Pests of Tea in North-East India and Their Control. Memo. No. 27. Jorhat, India: Tea Research Association (TRA),Tocklai Exp. Stn. 231 pp.

TRFK. (2012). Tea Pest and Disease Handbook 1st Ed. Inkit , Nairobi 68 pp

Trudel, R., Lavelee , R., Guertin, C. and Kope, H. (2007), Potential of *Beauveria bassiana (Hyphomycetes: Moniliales*) for controlling the white pine weevil, *Pisssides strobe* (Col., Curculionidae), *Applied Entomology* 131(2),90-97

Ute Williges (2004). Status of Organic Agriculture in Sri Lanka with special emphasis on tea Production Systems (*Camellia sinensis* (L) O. Kuntze . Ph. D Thesis pp 127.

Vladimir, G., Svetlana, G., Skinner, M., Hamilton, G., Jae Su K. and Bruce, L. Parke (2012) Virulence of selected entomopathogenic fungi to the brown marmorated stink bug, *Halyomorpha halys* (Stal) (Heteroptera: Pentatomidae) *Pest Management Sci*.68(2) 149–320. DOI: 10.1002/ps.2310 Retrived on 23rd September 2014

Vandenberg, J. D., Shelton, A. M., Wilsey, W. T. and Ramos, M. (1998), 'Assessment of *Beauveria bassiana* Sprays for Control of Diamondback Moth (Lepidoptera: Plutellidae) on Crucifers', *Journal of Economic Entomology*, 91, 624_630.

Vo Thai Dan, (2006). An assessment on genetic diversity in Vietnam tea using morphology, ISSR and microsatellites. *Gottingen*, pp 14

Wachira, F. N. and Ronno, W. (2004). Current research on tea in Kenya. In: *Proceedings of 2004 International Conference on O-Cha (Tea) Science,* pp 59-65 Shizouka, Japan

Wachira, F. N. and Kamunya, S. K. (2005). Kenyan teas are rich in antioxidants. *Tea* 26 81-89.

Wachira, F. N., Kamunya, S. K., Chalo, R., Maritim, T. and Kinyagi, T. (2012) *TRFK Clonal Catalogue,* (1st Edition). Tea Research Foundation of Kenya (TRFK) 28-29 &112-113

Wealth of India. (1950). A directory of Indian raw materials and industrial products. Vol. II S. S. Bhatnagar, chairman Editorial committee,. Council of scientific and industrial Research, New Dheli, India. pg. 26-51

Weigand, S. and Bishara, S. I. (1991) Status of insects pests of Faba bean in the meditteranean region and methods of Control. International Center for Agricultural Research in the Dry Areas (ICARDA) Egypt .*Options Méditerranéennes*, 10: 67-74.

Wekesa, V.W., Knapp, M., Maniania, N. K. and Boga, H. I. (2006) Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality, fecundity and egg fertility of *Tetranychus evansi*. *Journal of Applied Entomology* 130(3),155-159

Westwood, G., Huang, S., Keyhani, N.(2006). Molecular and immunological characterization of allergens from entomopathogenic fungus *Beauveria bassiana, Clinical and Molecular Allergy Journal* vol 4,pp12-21

Wraight, S.P., Carruthers, R.I., Jaronski, S.T., Bradley, C.A., Garza, C.J., and Galaini-Wraight, S. (2000), 'Evaluation of the Entomopathogenic Fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for Microbial Control of the Silverleaf Whitefly, *Bemisia argentifolii'*, *Biological Control*, 17, 203-217.

Wight, W. (1962). Tea classification revised.*Curr. Sci*, 31: pp 298-299.

Yang, C. S.; Maliaka and Meng, X. P. (2002). Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol Toxicol.* 42, 25-54

Zehnder, G., Gurr, G. M., Kuhne, S., Wade, M. R., Wratten, S. D., Wyss, E. (2007). Arthropod pest management in organic crops. *Annu. Rev. Entomol*. 52:57–80

Zimmerman, E.C. (1968a) *Cosmopolites pruinosus*, a new pest of banana. *Journal of Economic Entomology* 61: 870-871

Zimmerman, E. C. (1968b) Rhynchophorinae of Southeastern Polynesia. *Pacific Insects* 10:.47-77

Zimmerman, E. C. (1968c). The cosmopolites banana weevils (Coleaptera Curculionidae: Rhynchophorinae), *Pacific Insects* 10: 295-299

APPENDIX I: QUESTIONNAIRE

Survey for Tea Weevil Distribution In Kenya

1.0 BACKROUND

Sample site No………..Farmer’s Name……………. Region(Province)………………

District………………..Division…………………..Location…………………………

Sublocation……………..Village………… Agro-ecozone…………………………..

Altitude………………..GPS(lat.&Long.) ……………………………………..

Farm size……………………………

Tea Variety/Clone…..…………….. Time crop planted…………………

Recorder…………..

Date…………………………..

2.0 LAND USE:

2.1 How much is your farm under tea cultivation

Local variety(seedling tea)………………….acres

Improved variety (clones)……………..acres,which clones?..............................

2.2 Which of the livestock enterprise do you have in your farm;

Poultry…………………..

Dairy/Beef cattle..............

Goats……………………

Sheep……………………

Pigs…………………………

Donkey…………………..

Others (Specify)…………

3.0 TEA CROPPING TYPE

3.1 Do you grow tea crop pure or intercropped with trees?

Pure………………….

Grevilla intercrop…….

Mellitia dura intercrop…..

Avacado intercrop…..

Other (Specify)………

4.0 FERTILIZER / MANURE USE

4.1 What type of fertilizer do you apply on your crop?

Type of fertilizer / manure / pulp / foliar	Month	Rates
A)		
B)		
C)		
D)		
E)		
F)		

5.0 DISEASES AND PESTS CONTROL ON TEA

5.1 Diseases Control

5.1.1 Do you experience any disease problems?

( ) YES

( ) NO

If yes, which ones?

(a)……………..

(b)…………..

(C)…………

5.1.2 List down any pesticide (fungicide) you use to control any of the diseases

Pesticide (fungicide) Type Applied	Months	Rates
1)		
2)		
3)		
4)		
5)		

5.2 PEST CONTROL

5.2.1 Do you experience any pest problem on tea crop?

( ) YES

( ) NO

If yes, which ones;

(a)……………….

(b)………………….

(c)………………..

(d)………………..

Have ever experienced the damage below or seen the weevil insects below in your tea bushes

Plate 23: *Adult Nematocerus weevil*

Plate 24: *Adult systates weevils*

Plate 25: *A young tea damaged on leaves by Systates weevils*

Plate 26: *Pinned specimens of Kangaita weevils*

Plate 27: *Leaf damage by Kangaita weevils*

Plate 28: *Leaves damaged by Nyambene weevil, note the weevil on the leaf*

Plate 22: *Tea stems damaged by tea Root weevils*

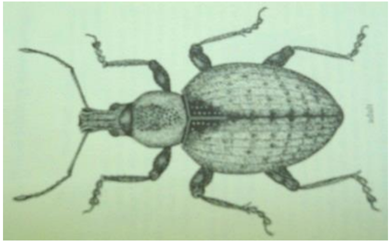


Plate 21: *Tea Root Weevils-adults*

(Source TRFK 2012)

5.2.2 Do you apply insecticides (any pesticides) to control any of the pests?

( ) YES

( ) NO

If yes, which insects do you apply?

Insecticide	Month	Rates
1)		
2)		

5.2.3 Apart from insecticide (pesticide) which control method do you apply?

Method	Insect pest controlled	Comment on results
1)		
2)		

6. 0 WEED CONTROL

6.1 Do you experience any weed problem in your field?

( ) YES

( ) NO

If yes, which ones;

1)……………………………..

2)………………………………

3)……………………………..

4)………………………………

6.2 Do you apply herbicides to control weeds in the field?

( ) YES

( ) NO

If yes, which herbicides do you apply?

Herbicide	Month(s)	Rate
1)		
2)		
3)		

APPENDIX II; FIELD LAYOUT IN MUNUNGA

	EAST			Rep 3
	6	**2**	**1**	
	18	17	16	
Road	**4**	**3**	**5**	
	13	14	15	
	2	**4**	**3**	Rep 2
	12	11	10	
	6	**5**	**1**	
	7	8	9	
	4	**1**	**3**	Rep 1
	6	5	4	
	5	**6**	**2**	
	1	2	3	

Key 1,2,3,4,……..18 =plot number

5,6,2,………… .=Treatment numbers

Where Treatment 1 is *B. bassiana* Isolate Bb Ke 6a

2 is *B. bassiana* Isolate Bb Ke 6a in wheat bran

3 is *B. bassiana* Isolate Bb Gi.7a

4 is *B. bassiana* Isolate Bb Gi.7a in wheat bran

5 is Karate at 1g/L

4 iscontrol

APPENDIX III; GICIARO FARM FIELD LAYOUT

	Offices					
	Plot No.	Rep 1 Treatments	Plot No.	Rep 2 Treatments	Plot No.	Rep 3 Treatments
	6	4	12	3	18	6
	5	1	11	4	17	2
Walk	4	3	10	2	16	1
way	3	2	9	1	15	5
	2	6	8	5	14	3
	1	5	7	6	13	4
Road						

Where Treatment

1- *B. bassiana* IsolateBbKe 6a

2- *B. bassiana* IsolateBb ke 6a in wheat bran

3- *B. bassiana* Isolate Bb Gi 7a

4- *B. bassiana* Isolate Bb Gi 7a in wheat bran

5 – Karate at 1g/L

6- control

APPENDIX IV MAPS SHOWING FAVOURABLE CONDITIONS TO KANGAITA AND NYAMBENE WEEVILS

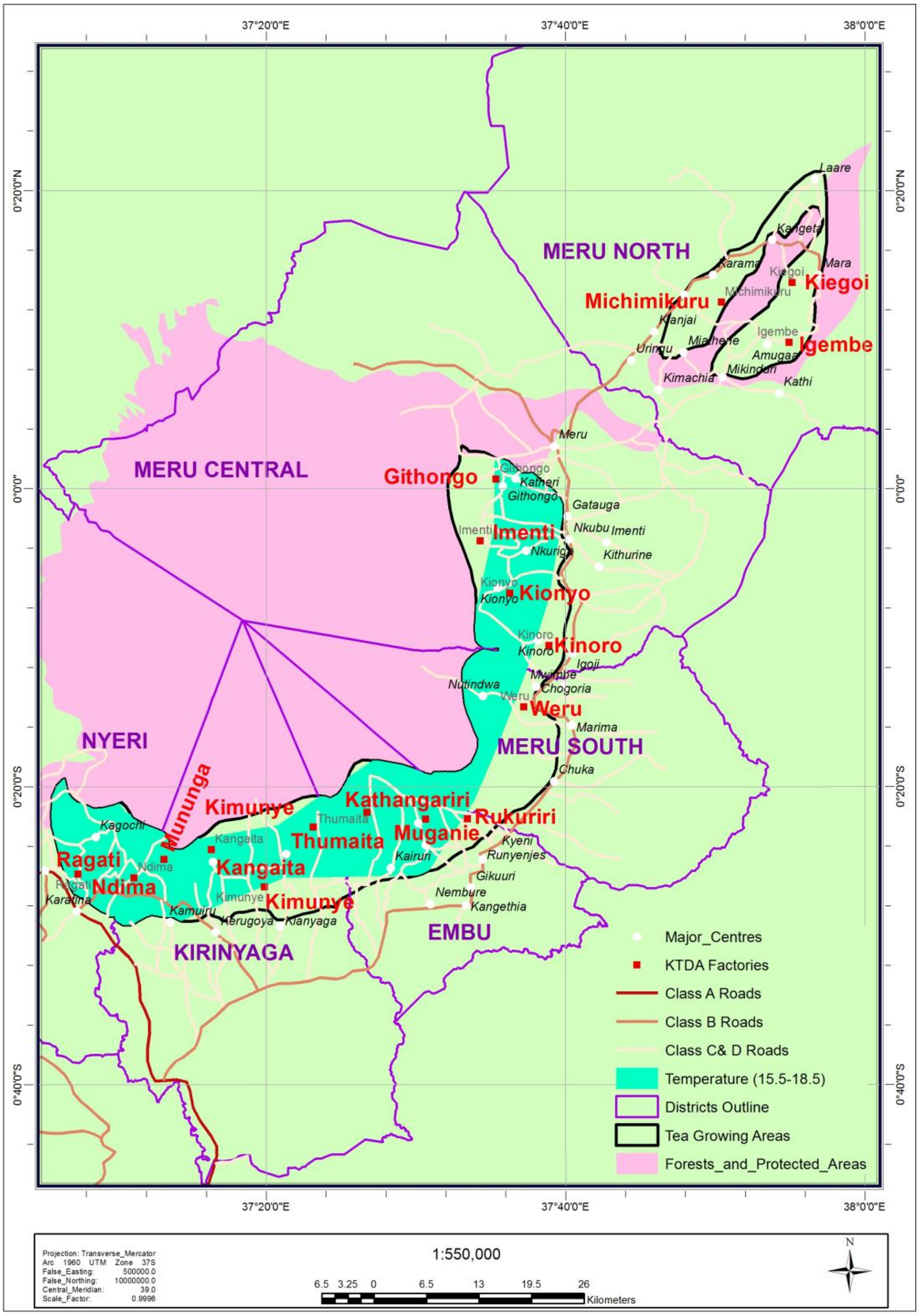


Figure a: Areas Favourable for Kangaita weevil (green) according to Temperature **(Source Author 2015)**

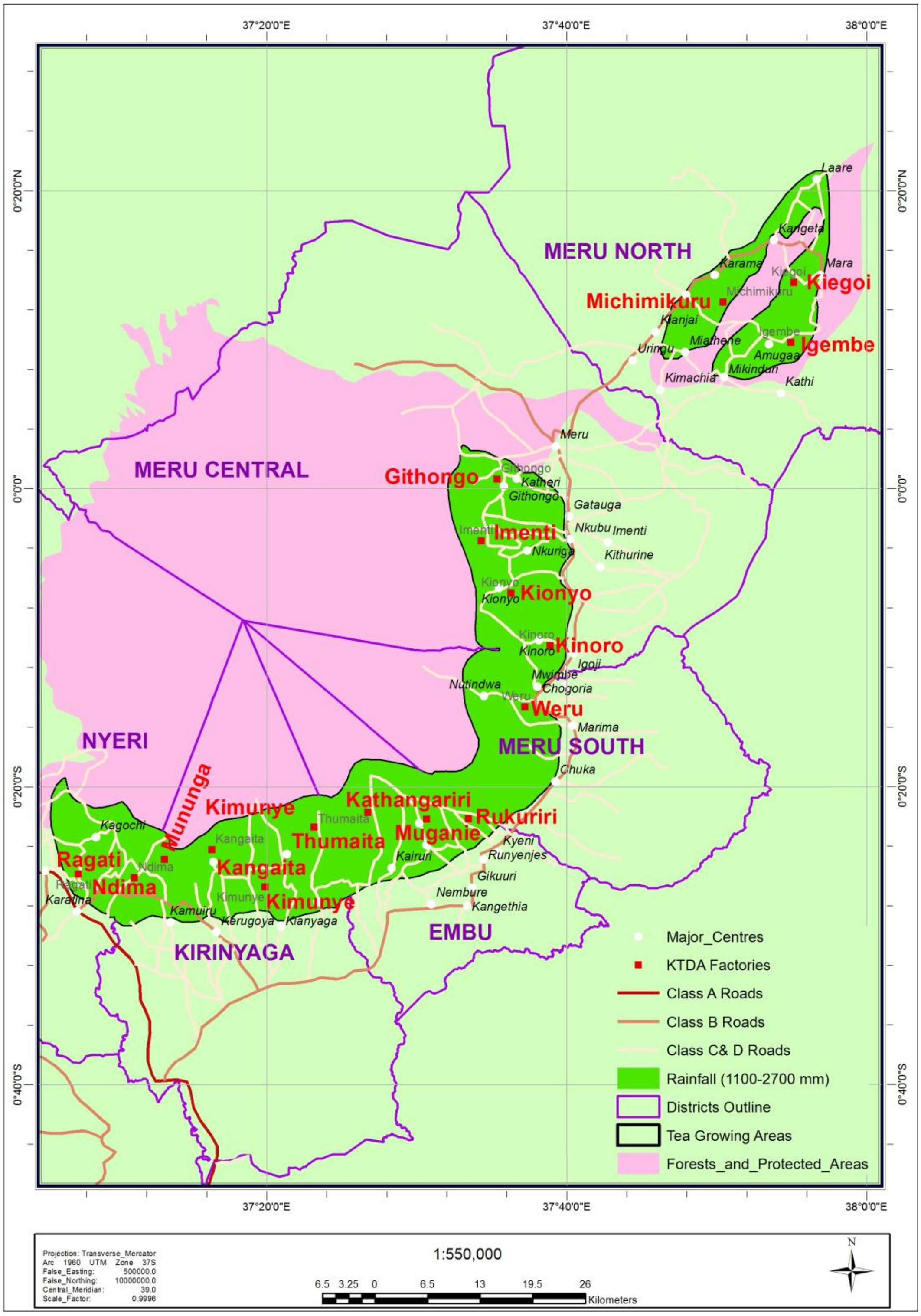


Figure b: Areas Favourable for Kangaita weevil (green) according to Rainfall
(Source Author 2015)

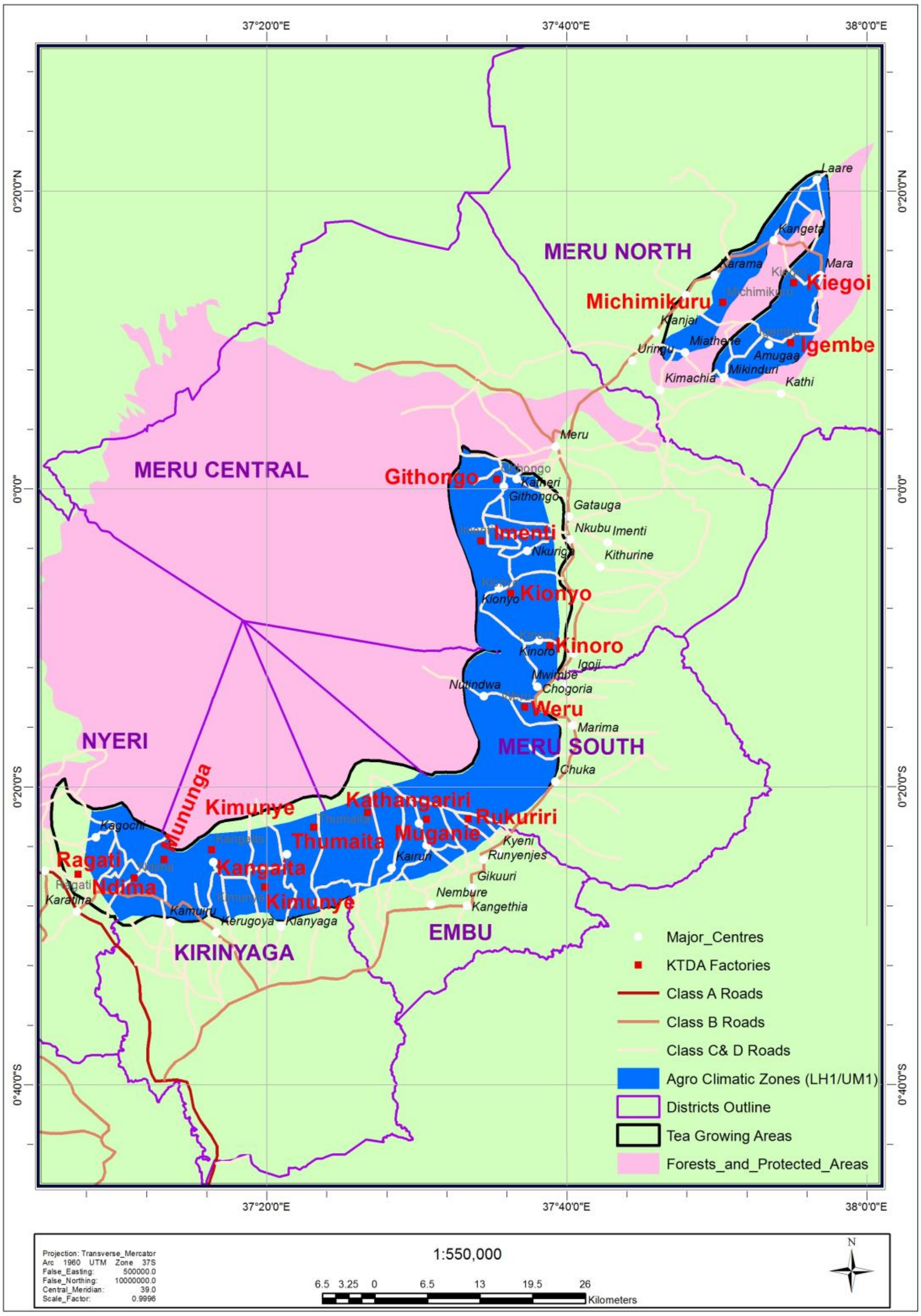


Figure c: Areas Favourable for Kangaita weevil (blue) according to Agro Ecological Zones **(Source Author 2015)**

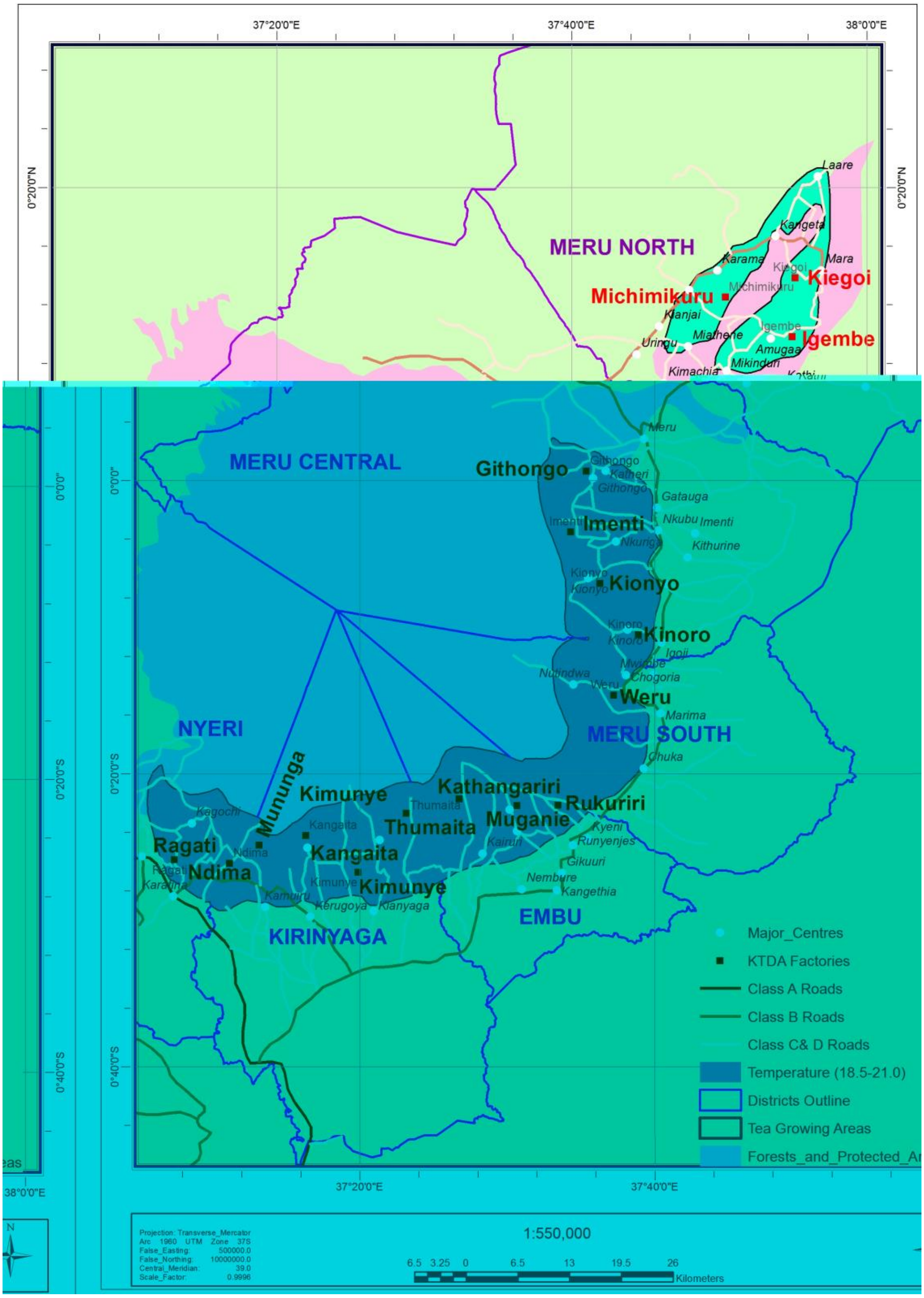


Figure d: Areas Favourable for Nyambene weevil (green) according to Temperature Map **(Source Author 2015)**

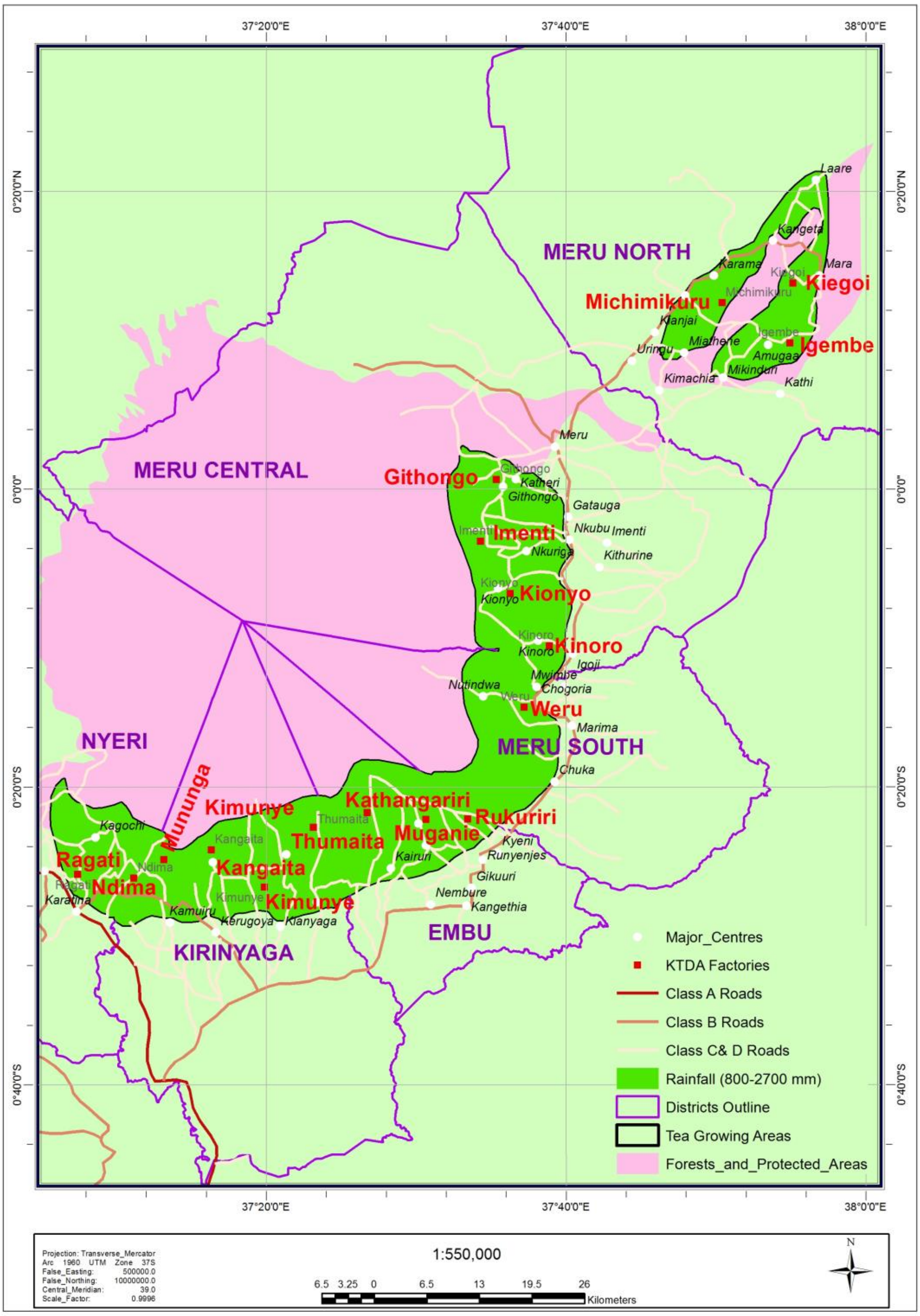


Figure e: Areas Favourable for Nyambene weevil (green), according to Rainfall Map

(Source Author 2015)

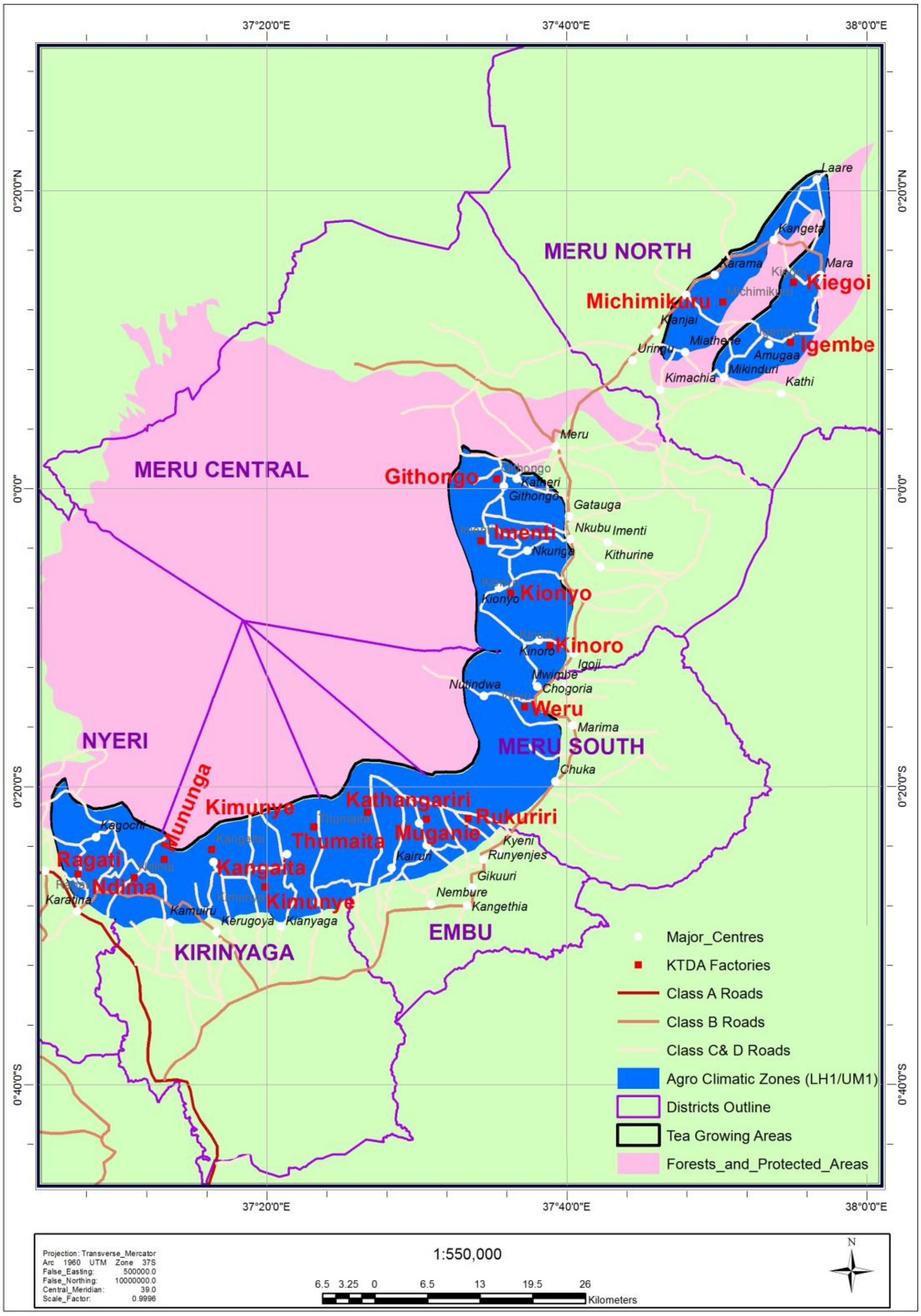


Figure f: Areas Favourable for Nyambene weevil spp. according to Agro-ecological Zones **(Source Author 2015)**

Appendix V: Effect of five *Beauveria bassiana* isolates on mortality of adult tea weevils on Day (D) 1-35

	Number of weevils exposed							Number of weevils dead							Percent mortality (%)						
	Isolates							Isolates							Isolates						
D	C	1	2	3	4	5	6	C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	54	54	53	53	54	54	53	0	0	1	1	0	0	1	0	0	2	2	0	0	2
2	51	54	50	48	54	54	49	3	0	3	5	0	0	4	6	0	6	9	0	0	7
3	48	52	44	42	52	52	48	3	2	6	6	2	2	1	6	4	11	11	4	4	2
6	48	50	44	42	50	48	47	0	2	0	0	2	4	1	0	4	0	0	4	7	2
9	36	50	42	38	40	22	39	12	0	2	4	10	26	8	22	0	4	7	19	48	15
11	36	44	42	34	40	22	33	0	6	0	4	0	0	6	0	11	0	7	0	0	11
14	36	44	42	34	40	22	27	0	0	0	0	0	0	6	0	0	0	0	0	0	11
16	30	44	40	32	36	8	26	6	0	2	2	4	14	1	11	0	4	4	7	26	2
17	30	30	30	28	26	8	25	0	14	10	4	10	0	1	0	26	19	7	19	0	2
18	24	26	26	24	24	8	25	6	4	4	4	2	0	0	11	7	7	7	4	0	0
20	24	26	24	20	24	6	23	0	0	2	4	0	2	2	0	0	4	7	0	4	4
21	24	24	24	20	8	6	23	0	2	0	0	16	0	0	0	4	0	0	30	0	0
24	24	24	24	20	8	6	23	0	0	0	0	0	6	0	0	0	0	0	0	11	0
32	0	8	10	2	4	0	22	24	16	14	18	4	0	1	44	30	26	33	7	0	2
35	0	8	8	2	4	0	17	0	0	2	0	0	0	5	0	0	4	0	0	0	9

Where Isolates;, 1=Bbch 8c, 2=Bb Ch C2, 3=Bb Gi 7f, 4=Bb Ch C3, 5=Bb Gi 7a and 6= Bb Ke 6a and D=Days after treatment, C=Control