WEED DIVERSITY AND THEIR RESPONSE TO GLYPHOSATE APPLICATIONS IN COFFEE FARMS IN KIAMBU COUNTY, KENYA

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DECLARATION

I declare that this is my original work and has not been presented before in this University or any other institution for a degree. In all cases, where it is relevant, material from the work of others has been acknowledged.

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DEDICATION

To my beloved wife Maryanne Wangui Gakuru, my adorable children Minainah Wambui, Myleen Muthoni and Genay Migwi and my wonderful parents; Joyce Wambui, Erastus Migwi, Irene Muthoni and Joseph Githinji, am extremely humbled by their priceless support and encouragement throughout the study period. MAY THE ALMIGHTY GOD GRACE THEIR WAYS ALWAYS.

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MAY THE ALMIGHTY GOD GRANT YOU ALL HIS SUFFICIENT GRACE

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ACRONYMS

Ae	Acid equivalent
Ave.	Average
ED_{50}	Effective dose 50
LD_{50}	Lethal dose 50
em	Effective mortality
Ksh	Kenya shillings
EPSPS	Enolpyruvyl shikimate phosphate synthase
Exp	Experiment
GPS	Global positioning satellite
Ha	Hectare
IPA	Isoproppylamine
Lts	Litres
Lt/Ha	Litres per hectare
m^2	Square meter
MASL	Meters above sea level
Mls	Millilitres
pH	Negative logarithm of the hydrogen ions concentration
REP	Replication
ROA	Rate of application
TK	Total kill
TOA	Time of application
TOO	Time of observation (Wilting to death period)
UoN	University of Nairobi
UM1	Upper midland 1
UM2	Upper midland 2
UM3	Upper midland 3
F*cal*	Calculated frequency
F*tab*	Tabulated frequency

ABSTRACT

The problem of weed control in the east and west of rift in Kenya differs quite remarkably between the coffee growing areas. This has been verified by past studies conducted with the aim of improving coffee production by use of various means among them being the use of glyphosate in weed management. Various weed species have been identified to be associated with coffee. These programs have been applied routinely in the past decade in the coffee farms that are more than ten hectares. Production costs are a major factor in estimating coffee profitability. The annual average cost of chemical control per hectare is 10,000 Kenya shillings representing about 7% of the direct cost of weeding. Common broadleaved weeds such as black jack and gallant soldier and common soft grasses compete with the coffee crop. Their presence makes spray operations, fertilizer application and harvesting difficult and costly.

Various herbicides rates have been used as influenced by factors such as weed population, resources available to the farmers, farmer practice models such as the tank mixing of two or more different herbicides among others. Particular suspect weed species have developed dominance in such farms and tend to be found in the highlands.

Research studies were thus carried out to investigate the weed species diversity and their responses upon exposure to varied doses of the glyphosate herbicide (Glyphogan 480SL) in coffee farms in Kiambu County, Kenya.

The weeds diversity survey was carried out quantitatively, data analyzed and presented in summary tables. These were their frequency, uniformity, relative equivalents, density and relative abundance. A total of 47 different weed species (39 broadleaved weeds 7 grasses and 1 sedge were identified. *Bidens pilosa* L. was the most frequent weed species at 89%.

Thirteen treatments were applied in a completely randomized design investigating effective mortality, necrosis and chlorosis on treated plants. The plant mortality means against the test doses were established and separated in the data analysis. The efficient lethal concentrations established were 5.0Lt/Ha and 4.5 Lt/Ha for the 2-4 & 6-8 leaves growth stages respectively.

A field evaluation was carried out pursuant to the dose tests. A completely randomized block design with eight treatments was used to establish the efficient doses. This was replicated three times and tested at the same growth stages whose efficient doses established were 5.5 and 4L/Ha respectively. It was established that such high rates are likely not to be economical if used as the only available *Bidens pilosa* L. weed management tool in coffee.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Coffee is classified as one of the most important agricultural commodity as reported by DaMatta in 2004 in the international agricultural trade markets. It is considered to represent a significant source of income to several African, Asian and Latin American countries. Coffee in Kenya is one of the most valued export crop and is rated the fifth leading foreign exchange earner product after remittances from diaspora, horticulture, tea and tourism in such leading order and the country leads as a producer of Arabica coffee in Africa**.** It's mainly used as a principle beverage both locally and internationally.

It was introduced by the Roman Catholic fathers at Saint Austin's in Kiambu in 1889 and commercially planted by the settlers in1893. It is grown in all the main agricultural areas of Kenya where deep volcanic soils are found at altitudes ranging from 1000 – 2000 M above sea level, a pH of 5.3 – 6 and optimal mean annual temperature of between 18-21°C for arabica coffee as was reported by Descroix and Snoeck in 2004. About 160,000 hectares in Kenya are under coffee. 75.5 % is under the co-operatives or societies sub-sector management and 24.5 % in the estates (large growers). 50,000 Ha to date is under active production. The cropped area has been reducing over the years due to production and economic challenges, demographic factors and related influences as well as the change in land use. UM1, UM2 and UM3 are the main zones of growing coffee and Kiambu transverses across these agro ecological zones.

Kiambu County under coffee covers a land area of 12,814 Ha classified as large farms 10,830 Ha being under small farms. The common varieties found are the SL 28, SL 34, K7 varieties & Ruiru 11. Recent varieties being Batian and the grafted types are currently under increased establishment in central and eastern highlands of Kenya.

1.2 Constraints to coffee production in Kiambu County, Kenya

Coffee production is affected by various key constraints such as high costs of inputs, weed management, periodic insect pests and diseases, diminishing soil capacity, adverse weather conditions and highly fluctuating market value leading to low returns on investments.

Weeds competitively and antagonistically interact with coffee trees for moisture and nutrients resulting in a decline in yield and quality. This means that they are therefore more rapid in absorbing space, light, moisture and nutrients throughout their growing season as observed by Ramzan, in 2003; Hayat in 2004 and Hussain *et al*. in 2008). The competitive relationship between the crop and the weeds is dependent on many factors including cultural practices used, characteristics of the crop and weeds as observed by Knezevic *et al*., 2002 as well as the availability and supply of nutrients (Di Tomaso, 1995; Evans *et al*., 2003). The direct and indirect impacts of weeds result in significant increase on the cost of production. Weeds in coffee have been reported to reduce yields by over 50% (Nyabundi *et al.*, 1998).

1.3 Weed management in coffee

Increased cost of weed protection has been a principle item in the economic analysis of coffee production. Such is because of the weed species that are established as dominant and prevalent in areas where they favorably and quickly re-establish.

Various methods of controlling weeds have been used ranging from the ancient primitive methods such as hoeing. Recently, modern technologies with integrated weed control mechanisms that include cultural, mechanical and chemical application are common with growers.

Effective use of herbicides significantly reduces the resources needed to manage weeds in coffee plantations. Proper timing is critical in mitigating the economic threshold level when the weed density that can cause a crop yield loss by exceeding the cost of the control measures (Zadoks, 1985). Aune *et al.*, 2000 observed that zero tillage using herbicides gave higher yields than conventional tillage systems. The most commonly used herbicides include Paraquat, linuron and glyphosate.

1.4 Effect of using herbicides to manage weeds in Coffee.

The use of non-selective herbicides is a common practice. These chemicals eliminate virtually all available vegetation when applied as recommended. Occasionally, this scenario creates a principle avenue for soil erosion as well as a landing ground for new weeds species. As such, they establish and may re-establish to become common weeds. This occurrence antagonizes the flora and fauna balances in the environment as often as it occurs.

Challenges of difficult to control weeds has caused growers to devise supplementary interventions of managing weeds such as by adding 500g of urea (46%) for every

200lts of spray volume water. Others are caustic soda (1kg/200lts of water) and recycled motor oil/diesel (1Lt/200lts of water) as a tank mix with glyphosate herbicide. Such practices pose great dangers in destroying the environmental equilibriums, antagonisms in flora/fauna complexes and change in PH and thus lead to undesirable outcomes such as a direct impact of the quality and yield of coffee and influenced habitats for opportunistic pests. Such practices account for additional costs of production.

Price *et al*. in 2011 and 2012 as well as Culpepper *et al*., (2008); Johnson *et al*., (2009) and Norsworthy *et al*.in 2008 reported that the most significant predicament for commercial crop growers is the management of glyphosate resistant weeds. Subsequently, other weed management strategies have been recommended by growers and research institutions in mitigating such challenges. These include the use of tillage and residual pre-emergent herbicides, tank mixing of glyphosate with other modes of action herbicides, herbicide rotations and use of herbicides with different modes of action (Beckie, 2006, Wilson *et al*., 2007, Norsworthy *et al*., 2012 and Aulakh *et al*., 2011, 2012).

In the last two decades, intensive use of systemic non-selective herbicides such as glyphosate and other residual herbicides in Kiambu coffee plantations has been a routine. Such a scenario is suspect to lead to changes in weed flora referred to as weed. Soft weeds such as annual grasses which are easily controlled have subsequently been replaced by more aggressive noxious weeds resulting in reduced crop yield and increased productivity concerns. Such over reliance on an active ingredient with a particular mode of action can lead to heavy selection pressure on a weed population and may eventually select for resistant individuals.

1.5 Problem statement and justification of the research

Coffee farming profitability is a significant portion on the production cycle's balance sheet for large scale growers. The average production per tree has been declining from an average of 15 Kgs to below 2 Kgs in the last two decades. The average returns per hectare is Ksh. 136,000 (at 400kgs/Ha at 4$/kg (exchange rate of Ksh 85/dollar).

The cost of weed management is estimated to range between 3-12% of the annual growing costs on the assumption that weeds are managed well and where extensive hand weeding is inefficient (Kerkhoven, 2000). This applies where hand weeding is considered to be costly and primitive (Akobundu, 1978). These have a direct impact

on poverty levels management as reiterated by Tamet *et al.* in 1996 and Peacock in 1991.

Variation in rates of herbicides used can lead to varied inefficacy on prevalent weeds. Partially exposed weed species may evolve biotypes resistant or tolerant to a particular or a number of herbicides. This way, some plants escape exposure to fatal dosages of the herbicide or poor foliar delivery thus limiting the amount of herbicide translocated. With successive generations, they build up tolerance and or resistance with repeated applications. These weeds become difficult to eradicate especially in a zero tillage herbicide program. Caseley *et al*., (1991) observed that many reasons may lead to the failure of a herbicide and genetic resistance can be distinguished with comparative studies conducted under controlled conditions.

1.6 Objectives

1.6.1 Broad objective

To increase coffee productivity by improving efficiency of weed control by use of glyphosate.

1.6.2 Specific Objectives

i. To determine the most prevalent weeds in coffee farms with a long history of glyphosate use in Kiambu County.
ii. To determine the glyphosate dose response of the most prevalent weed species in coffee under screen house conditions.
iii. To test the most effective glyphosate rate established by the dose response tests on the most prevalent weed species selected in the field.

1.7 Hypothesis Tested

i. Repeated use of glyphosate herbicide results in prevalence of tolerant/resistant weeds in coffee plantations which can be established by conducting a survey.
ii. Variations in glyphosate rates of application can be used to generate a dose response curve to determine the most effective rate on a given weed.
iii. Glyphosate's herbicidal activity responses under a controlled environment relate to that of the field responses on a given weed.

CHAPTER TWO: LITERATURE REVIEW

2.1 Weed diversity and distribution

Generally, the weed vegetation of a particular area is highly determined by the environment. Other factors contributing to this are edaphic and biological factors such as the soil structure, pH, nutrients and moisture status, associated crops, weed control measures and the field history especially in local geographical variation (Hakim *et al.*, 2010). The natural resource qualities alongside correct estimates of biodiversity of an area are dependent on the sampling design of weed surveys as reported by Knollová *et al*., in 2005.

Burnham & Overton 1978, 1979; Heltshe & Forrester 1983, 1985; Smith & Van Belle 1984; Chao 1984, 1987 observed that weed species richness and diversity was determined with the then developed non-parametric estimators due to their complex nature of diversity. Survey and mapping programs enable the prediction of the potential distribution of a target weed species through modeling where a specific weed's potential range of spread, associated economic and environmental costs are established. Weed species surveys programs are used in defining weed infestation locations, sizes and densities. The accurate pictures generated are used as the guides for weed distribution in estimating the resources required to manage an established problem. *Bidens pilosa* L. is a common weed found in all the agro ecological zones of Kenya and a very common weed species in east Africa's arable land occurring as one of the most important annual weed species (Ivens, 1989).

2.2 Weed Survey

2.2.1 Importance of weed survey for determining diversity and distribution

Surveys are types of field searches used to determine the location and relative abundance of weeds on a landscape scale where they represent samples of an overall weed population. Such searches determine the occurrence in terms of location and abundance of one or more weed species within a delineated management area. This process is achieved by sampling a representative portion of a greater weed population. Weed surveys generate weed maps that are vital to land managers. Regular and planned weed species surveys can enable small infestations to be detected and managed prior to their establishment and expansion. It is reported that Thomas in 1985, McCully *et al*., in 1991 and Frick & Thomas in 1992 observed that weed

surveys are useful for determining the occurrence and relative importance of weed species in crop production systems. Such weed surveys are compared to indicate the effects of new weed control and intervention technologies on farming practices, documentation of herbicide resistant weeds as well as weed species shifts in response to new weed control technologies.

Documenting weed management systems facilitates the establishment of priority action plans and systems by agricultural extension professionals and research institutions. Periodic surveys help in the development of flora monitoring areas in cropping regions where weeds are considered to be key concerns in farming systems. This data base has helped greatly in developing a data collection and management software useful while conducting weed surveys. Such software enhances in minimizing errors that are likely to recur thus improving credibility of generated information during surveys. In countries with such developed surveying systems, there is ease of capturing information through data importation thus defining weed species importance under numerous weed management systems.

2.2.2 Weed survey methodologies

Survey type selection depends on the aims, objectives and financial aspects of any given survey project. Field surveys can either be biased or the unbiased type. For several decades now, several diversity indices and species area curves have been used for assessing weed species richness and diversity (Fisher *et al.*, 1943; Sanders 1968). The relevance of these techniques has further been discussed with respect to both on their statistical properties and biological meaning as illustrated by Hurlbert in 1971, Hill in 1973, Peet in 1975, Routledge in 1979, Condit *et al.*, in 1996 and Lande in 1996.

Most weed density estimate techniques are used in field surveys. Field surveys can be carried out to determine if there are any invasive plants present in a given area. They also determine the extent of an invasion by a known invading plant species. A model for sampling weeds for research purposes consists of selecting a given number of quadrats of a certain size, locating it on established grid, and determining the number of weeds of each species within each quadrat. In this case, the mean quadrat density for each weed species is assumed to represent the field. Kuchler and Zonneveld (1988) described the forms of field surveys as exploratory survey, reconnaissance survey, extensive and intensive types of surveys.

Different survey methods (one based on the ground, aerial or remote platform) allow for various sampling techniques such as a swath, a point or a linear. A type of a biased survey method which is achieved by transecting sampling along a chosen area such as roadways and riparian areas is appropriate when looking for specific species that one knows grows in certain areas. It is considered an ideal method for finding most populations of a single species as well as early detection of new invader species. Unbiased survey methods are good for understanding weed distribution across a landscape as most populations of a different species can be detected. Unbiased survey methods are either random (point, swath, stratified) or systematic (grid sampling). A stratified survey involves the use of a perpendicular line to a targeted transect, on a contour transect, a road or on a trail.

2.3 Determination of dose response of herbicides

The inherited ability of that plant to survive and reproduce following exposure to a dose of the herbicide that is normally lethal to the wild type is weed resistance. The criteria used for this status has to fulfill the resistance definition, show practical relevance and provide confirmed heritability through scientific experiments where the weed is identified to its species level. The area of a herbicide resistant weed is established through field observations, passive testing of plant samples collected, related complaints mitigated and active product testing on the target area through appropriate field random sampling techniques.

While screening a large number of putative samples and the response compared with the chosen standard and related non treated checks in pot assays, most researchers use the recommended field doses. The inclusion of more than one dose in the screening test is beneficial because it gives some indication of resistance level among populations where resources allow. Kaloumenos *et al.,* 2011; Maneechote *et al*., 2005; Wise *et al.*, 2009 observed that in their studies of weeds resistance to herbicides, two to four doses had been used in resistance confirmation assays. Single doses are avoided in the initial herbicides resistance tests which preferably require conducting a dose response curve, relative to a susceptible standard. This type of a test shows the magnitude of resistance and the discriminating dose. A single dose can consequently be used in testing other populations of the same plant species.

Shaner *et al.*in 2005 and Kaloumenos *et al*., 2011 observed that a wide range of glyphosate assays doses ranging from 4 -15L/Ha have commonly been evaluated on

perennials such as coach grass. Such doses have been used as standards at 3L/Ha for injury assessments. Researchers quite often repeat their dose response assays whose variations may arise from the control levels initiated and applied to reflect normal growth parameters, but not for other confirmation assays which is associated with the need to refine the dose response curve where the first run is an exploration of the dose range. Post applied herbicides are generally applied at two to four leaves with the recommended surfactants or additives. Lately, glyphosate assays have been conducted with the constant concentration of the plain glyphosate acid formulations with the addition of prescribed surfactants. This practice may not be as frequently administered in most other parts of the world apart from the largely developed countries such as in Europe, America and Asia.

2.4 Herbicide weed control in other countries

Glyphosate and Paraquat are the most widely used post emergence herbicides with glyphosates targeting key perennials like grasses and sedges. 2,4-D amine, Dalapon and Amitrole are also key active ingredients used in coffee weed management. Glyphosate has been reported to be phytotoxic to the vegetative parts of coffee upon contact (Chawdhry 1975). Oxyfluorfen herbicide has been used in selective weed control in Hawaii in young coffee. A combination of post emergent herbicides (Simazine and Ametryl) in Cuba was established to be safe in managing weeds in young coffee plantations. Roe and Whitaker (1985a) observed that there was 22% mulch cover of irrigated coffee estates areas. Roe and Whitaker in 1985 reported that some important cultural practices applied in the East African coffee estates such as sheep grazing, mowing and slashing caused weed shifts to perennial grasses. Friessleben *et al*., in 1991 reported that a regime of clean hoed weeding is not related to weed shifts. Slashing is mainly applied during the rain seasons since it offers considerable soil erosion protection and improved soil structure.

2.5 Glyphosate

2.5.1 Chemistry and behavior of glyphosate

Glyphosate acid (Figure 1) is a principle component in its formulated form integrated weed management systems in coffee production in Kenya. It is normally applied in the form of monoamonium, diamonium, potassium or trimesium isoproppylamine

(IPA) salts (Figure 2 and 3). It is among the most frequently used herbicide in zero tillage and listed among the world's least toxic herbicides (Brent, 2003).

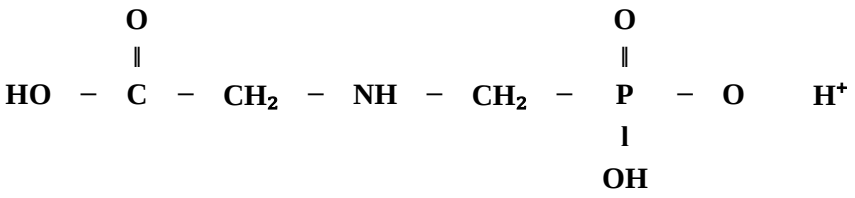

Figure 1: Chemical structure of the parent glyphosate acid.

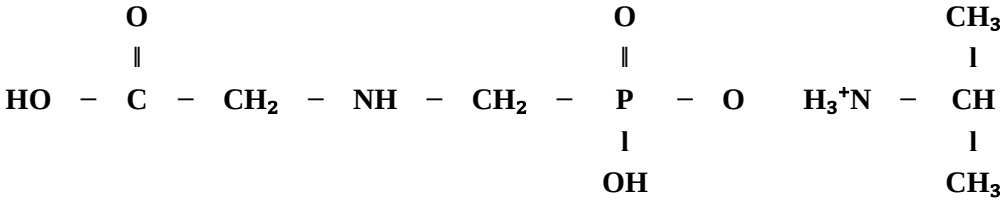

Figure 2: Chemical structure of the Isopropylamine salt of glyphosate.

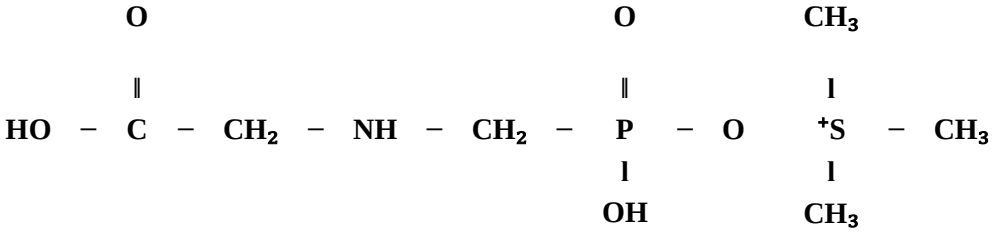

Figure 3: Chemical structure of the Trimesium salt of glyphosate.

The normal formulation of the glyphosate acid was discovered by Monsanto company chemist called John Franz in 1970 and it was brought into the market as a herbicide in the 1970s under the trade name Roundup. Upon expiry of its patent in 2000, hundreds of generics are under production from different parts of the world. It is used in more than 130 countries on agricultural crops, orchards, nurseries, greenhouses, lawns, landscapes and many other target areas. According to the pest control and products board, Kenya has more than 40 registered glyphosate products for weed control with a current annual import level of over one million liters used in agricultural areas. Over 45 million liters of glyphosate are applied annually in the United States farms. The glyphosate acid is formulated to improve handling, its performance & concentration, as a salt component. The herbicidally active part of glyphosate in weight is the acid equivalent (AE). It is a systemic non selective herbicide that is absorbed by leaves and stems and translocated to other living parts. It's therefore effective only as post emergence herbicide. The acid portion of the salt formulation binds at the target site. Thus differences in amount of acid applied between two different formulations may

result in differences in weed control efficiency if the differential amount of acid is significant.

2.5.2 Mode of action

Glyphosate, also commonly referred to as *N* phosphonomethyl glycine is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with agricultural commercial crops in annuals and biennial food and fodder crops. Glyphosate is a non-selective herbicide that severely injures or kills any living plant tissue that it comes in contact with. It belongs to the chemical group 9 (Aromatic Amino Acid Inhibitors) herbicides and controls susceptible plants by inhibiting amino acid synthesis. It's a growth regulator plant chemistry. It functions by blocking the biosynthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) thus inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Once absorbed, it's readily translocated from leaves to growing points of shoots, roots and rhizomes. This is a fundamental characteristic to its ability to control weeds. Wilting and death of plants is achieved in 7-10 days after application. Glyphosate is poorly absorbed into the leaves of resistant plants as indicated by past studies.

2.5.3 Resistance and tolerance of glyphosate by weeds

Abel, 1954 issued warnings that there were possibilities of weeds evolving resistance soon after the phenoxy based herbicides were introduced. In Australia in 1996, the first documented cases were reported in rigid ryegrass in New South Wales on resistance to glyphosate.

In 2006, farmers' associations were reporting 107 biotypes of weeds within 63 weed species with herbicide resistance. The ragweed in 2009 in Canada was identified as a resistant weed. 34 weed species have been confirmed resistant to glyphosate worldwide among them being the common ragweed. Other plants that have been confirmed resistant to glyphosate more recently in Nebraska in the USA as reported by Jhala (2015) are common waterhemp (*Amaranths rudis* (Sauer), the giant ragweed (*Ambrosia trifida* L.), horseweed (*Conyza canadensis* L.), kochia (*Kochia scoparia* L.) and the palmer amaranth (*Amaranthus palmeri* S. Wats.).

However, limitations on research work and technological advancements have hindered establishment of the true status of the glyphosate tolerance especially in the developing countries and areas. Beckie in 2011 and Van Gessel (2001) observed that

the sole reliance on a single herbicide with the same mode of action coupled with a continuous weed management program has resulted in the evolution of herbicide resistant weeds. Weed control issues have been raised on glyphosate resistance where almost exclusive sole reliance on glyphosate for post emergence application has been a practice (Van Gessel 2001, Powles *et al*., 1998 and Culpepper *et al*., 2006).

The plant response to glyphosate may be confounded with the increasing surfactant concentration when an in built surfactant system is used with an increased dose. Fresh weight or dry weight of shoot tissues, with or without visual injury assessments are the response variables evaluated and their responses could be used to estimate resistance levels or amounts of herbicides that would cause a certain level of growth reduction or control. Growers in the south eastern United States who have had issues with glyphosate resistant weeds have adopted alternative practices such as alternative herbicides, hand weeding, and tillage which has resulted to higher production costs causing a decline in the area under no till production systems as well as leading to significant losses of valuable topsoil (Sosnoskie & Culpepper, 2014, Price *et al*., 2011, Aulakh *et al*., 2012, 2013). Ballot *et al.,* in 2009 reported the herbicide resistance effect on ryegrass coleoptile lengths exposed to varied concentrations of glyphosate. Nandula *et al*. in 2007 and Dickson *et al*. in 2011 reported inconsistent burnt down of the Italian ryegrass exposed to glyphosate by recording two to four fold resistance indices. In other resistance studies, Gaines *et al*. in 2010 verified the resistance of the palmer amaranth to glyphosate.

2.5.4 Mechanism of glyphosate resistance and tolerance by weeds

Field sampling is regarded as the most precise method of gathering critical information on the management and biology for varying areas surrounding a herbicide resistant seed source as illustrated by Baumgartner *et al*., 1999; Falk *et al*., 2005; Beckie *et al*., 1999, 2001; Bourgeois and Morrison 1997a, 1997b; Davis *et al*., 2008, Bourgeois *et al*., 1997b; Le´ge`re *et al*., 2000; Llewellyn and Powles 2001; Tucker *et al*., 2006; Beckie *et al*., 2000 and Walsh *et al*., 2001. Resistance evolves after a weed population has been subjected to intense selection pressure in the form of repeated use of a single herbicide. Weeds resistant to such herbicides have been called super weeds such as rye grass, amaranthus and giant ragweed in Georgia against glyphosate.

A review done by Holt in 1993 reported that competition fitness between the resistant and the susceptible biotypes of different weeds behave differently. The appearance of

resistance depends on characteristics of different weeds and herbicides, which can be mathematically integrated into models. Theoretical models developed to predict the rate of resistance evolution in weed populations include the relative fitness of resistance and susceptible biotypes. Most studies on resistant weeds that have shown reduced translocation have demonstrated that in these biotypes, glyphosate is rapidly sequestered in cell vacuoles thereby becoming unavailable for translocation while others have indicated that glyphosate is poorly absorbed into the leaves of resistant plants.This selectivity and or resistance can result from a number of factors such as differences in rates of absorption and subsequent translocation, tissue and subcellular localization of the herbicide, metabolism of products, modified phytotoxicity, differences in target site sensitivity and so on thus it is hypothetical to suspect tolerance by common weeds treated with glyphosate.

Hess in 1985 observed the plasma membrane must be crossed by most herbicides before reaching their site of action. The plasma membrane was suggested by Haderlie *et al*. in 1977 that it's possibly a barrier to glyphosate entry into the cell. Less than 1% of the glyphosate in the extracellular medium was reported to have been taken up by suspension cultured carrot cells after a 96 hrs uptake period while the intracellular concentration of glyphosate was estimated to be approximately 25% of the concentration in the uptake medium after 24 hrs. Jachetta *et al.*, in 1986 compared the uptake and translocation of atrazine and glyphosate and observed limited access to the symplast of sunflower stem tissues due to limited permeability more of glyphosate as compared to atrazine. Richard and Slife in 1979 suggested that cellular absorption may offer greater resistance to foliar glyphosate uptake than cuticular penetration due to the negative charges associated with the cell wall and the negative membrane potential of the plasma membrane that repel the anionic glyphosate molecule.

Heavy selection pressure is realized by over reliance on a single active ingredient or mode of action leading to on a weed population with resistant individuals. Over time, the resistant individuals will multiply and become the dominant weeds in a particular field resulting in herbicides that are no longer effective for the control of that specific weed. Akobundu in 1998 observed that information on tillage practices helps in identifying vulnerable stages of a weed's life cycle that can be utilized in weed management systems while Gressel in 1986 observed that effective kill is a measure of the surviving seeds or propagules at the end of the season and not after treatment. Weeds germinate not only throughout the season but also over many seasons.

Susceptible weeds can germinate after a rapidly degraded herbicide has disappeared and produce more seeds before the season is over thus considerably lowering the effective selection pressure. As illustrated by Beckie *et al*., (2000), testing of herbicide resistance is modeled to start with objective field surveys through predetermined sampling methods followed by herbicide screening methods, data analysis and interpretation. The methodologies used should reflect the seasonality differences where may be the case and the results should correlate. Replicating such tests through several seasons is ideal for the results to provide a trend based on conformity through standardization.

2.6 Overview

In conclusion therefore, it was established that there are gaps in commercial coffee farming associated with weed management. These emanate from agronomic experiences raised by growers and other stakeholders in the last two decades by coffee growers in the Kenyan highlands. Such gaps are confirmed by the increasing number of weeds over the years, variation of dosing rates of herbicides over the years and seasons, the continually increasing cost of weed management in such farms, the influence and the trend of documented global trends on herbicides usage, resistance and other findings affecting coffee commercial farming among many other factors. This scenario provides an avenue for research to investigate and document production impacts and outcomes. Modern weed control technologies especially the use of herbicides is an area where cross referencing has been found a challenge in terms of documented practices. Weed diversity in coffee and how weeds respond to herbicide treatments are critical outcomes that once established and documented, they can positively increase the value in coffee production in Kenya.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Weeds survey

3.1.1 Study site

The weed survey was conducted in Kiambu County, Kenya which lies in the coffee-tea zone also referred to as UM1, main coffee zone also referred to as UM2 and the marginal coffee zone (UM3) and whose elevation is between 1411-1926 meters above sea level and lies at latitude 1° S and longitude 37° between 25th November to 5th December 2014.

Table 1: Altitude and global positioning satellite (GPS) coordinate values of the sampled farms

Site (Farm)	Altitude (MASL)	GIS values
Ibonia Estate	1,717	S 01° 10.901' & E 036° 49.170'
Cianda Estate	1,880	S 01° 08.211' & E 036° 46.652'
Gatatha Estate	1,926	S 01° 07.687' & E 036° 45.639'
Nyala Estate	1,640	S 01° 08.069' & E 036° 52.049'
Kays Estate	1,411	S 01° 05.573' & E 036° 54.261'
Karunguru Estate	1,477	S 01° 03.807' & E 036° 57.644'
Benvar Estate	1,536	S 01° 03.306' & E 037° 00.906'
Mutoma Estate	1,585	S 01° 01.433' & E 036° 58.334'
Bendor Estate	1,553	S 00° 58.150' & E 037° 02.550'
Koorali Estate	1,566	S 00° 59.514' & E 037° 01.189'

Key: MASL= Metres above sea level

3.1.1.1 Description of the survey and soil sampling processes applied

Ten farms located within the county (Table 5) were sampled out of 78 farms that were found to be active in production and management by listing alphabetically picking 4th farm. Each sampling site was represented by mature cropped coffee blocks of between 1 and 2 hectares. In the event that the 4th farm was not accessible by foot for the survey and sampling, the subsequent farm was picked to achieve the desired sampling interval.

The starting point of the sampling process was determined by making 50 paces from the corner or convenient points in each field. A normal man walking speed was adopted making twenty (20) steps/paces in a 'W' shape direction to establish independent sampling points (Figure 4) as described by Elzinga *et al.*, in 2001.

A quantitative survey was conducted using simple random sampling technique using a 1m x 1meter quadrat as described by Hakim *et al.*, in 2013. The quadrat was thrown

backwards from a standing position at each of the sampling point and a weed log was established by identifying, counting and recording all the weeds falling within the quadrat. Species that were not immediately identified were tagged with a 16 centimeter PVC plant tags, labelled and taken to botanical and taxonomic laboratories for authentic identification as described by Chancellor and Froud -Williams (1982, 1984).

The coordinates of each surveyed area were established from an upright position in a central part of each sampled block. By switching on the global positioning satellite (GPS) equipment (GARMIN GPS Maps 62S), the point coordinates representing the surveyed area were recorded and later tabulated. This exercise was done in all the ten farms.

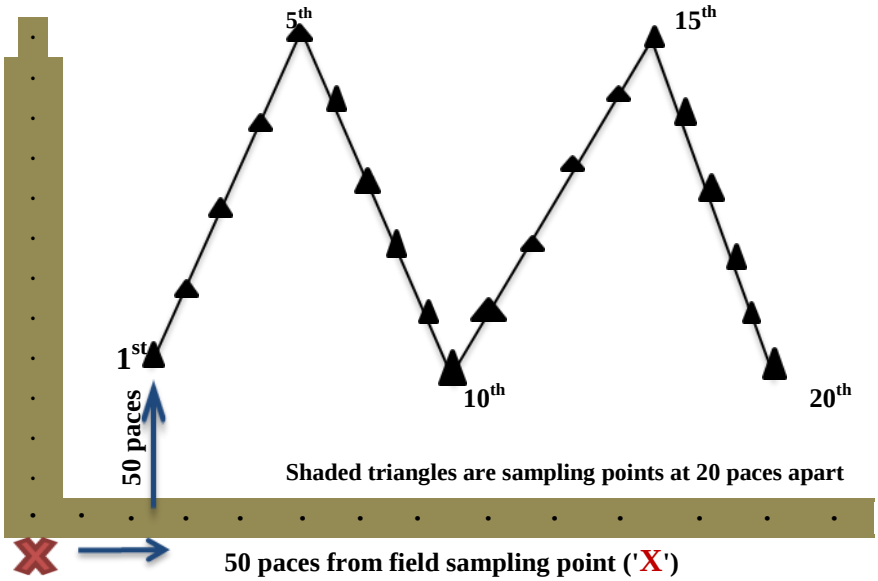

Figure 4: The sampling scheme used in weed species survey and soil sampling in the area of study

3.1.2 Description of the survey and soil sampling processes applied

3.1.2.1 Parameters measured

The parameters established were the mean field density occurrence (MFD), density over all fields (D), weed frequency (F), field uniformity over all fields (FU), and their relative abundance (RA) values.

3.1.2.2 Data analysis

Data was collected, computed and presented in weed species taxonomy tables. Quantitative measures were determined by use of standard formula as described by Thomas in 1985.

Formulae applied in determining the weeds frequency (F*k*) values (1).

$$Fk = \frac{\sum_{i}^{n} Y_i}{n} \times 100$$

Where; Y*i* = presence (1) or absence (0) of species *k* in the field *i*, F*k* = frequency value for species k; and *n* is the number of fields surveyed.

Formulae applied in determining the weeds field uniformity (FU*k*) values (2).

$$\mathbf{FU}k = \frac{\sum_{i}^{n} \sum_{i}^{20} \mathbf{Y}ij}{\mathbf{20} \times \boldsymbol{n}} \times \mathbf{100}$$

Where; Y*ij* = presence (1) or absence (0) of species *k* in quadrat *j* in field *I*, FU*k* = field uniformity value for species *k*, and *n* is the number of fields surveyed.

Formulae applied in determining the weeds density (D*ki*) values (3).

$$\mathbf{D}ki = \frac{\sum_{i}^{\mathbf{20}} \mathbf{Z}_i}{\mathbf{A}i} \times \mathbf{100}$$

Where; D*ki* = density (in numbers m^2) value of species *k* in field *i*, *Zi*= number of plants of a species in quadrat *j* and A*i* being the area in m^2 of 20 quadrats in field *i*.

Formulae applied in determining the mean field density (MFD*k*) values (4).

$$\mathbf{MFD}k = \frac{\sum_{i}^{\boldsymbol{n}} \mathbf{D}Ki}{\boldsymbol{n}}$$

Where MFD*k* = mean field density of species *k*, D*ki* = density (in numbers m^2) of species *k* in field *i* and *n* being the number of fields surveyed.

Formulae applied in determining the relative abundance (RA*k*) of the identified weeds (5).

$$\mathbf{RA}_k = \mathbf{RF}_k + \mathbf{RFU}_k + \mathbf{RMFD}_k$$

The relative frequency (RF*k*), relative field uniformity (RFU*k*), and relative mean field density (RMFD*k*) of *k* species were calculated by dividing the given parameter values by their sums and multiplied by one hundred (100).

Formulae applied in determining the relative frequency (RF*k*) values (6).

$$\mathbf{RF}k = \frac{\text{Frequency (F) value of } k \text{ species}}{\text{Sum of frequency values of all } k \text{ species}} \times \mathbf{100\%}$$

Formulae applied in determining the relative field uniformity (RFU*k*) values (7).

$$\mathbf{RFU}k = \frac{\text{Field uniformity (FU) value for species } k}{\text{Sum of field uniformity values of all } k \text{ species}} \times \mathbf{100\%}$$

Formulae applied in determining the relative mean field density (RMFD*k*) values (8).

$$\mathbf{RMFD}k = \frac{\text{Mean field density (MFD) value for species } k}{\text{Sum of mean field density values of all } k \text{ species}} \times \mathbf{100\%}$$

3.2 Screen house glyphosate dose evaluation

3.2.1 Study area

The dose response studies were set up at the University of Nairobi at the College of Agriculture and Veterinary Sciences field station farm between February and March of 2015 in a clear and well ventilated glass screen house. The site's elevation is at 1400 meters above sea level and lies at latitude 1° 15' S and longitude 36° 44' E. Test parameters of black jack were evaluated at the 2-4 and 6-8 leaves growth stages.

3.2.2 Introduction

The weed diversity survey conducted two months prior to this experiment had established that *Bidens pilosa* L. was the most prevalent weed species. It was therefore selected for the glyphosate dose response experiment.

3.2.3 Treatments applied

Soils from each of the 10 farms were weighed (500gms) and put into round shaped plastic pots of 8cm in diameter and 5cm in depth. The pots were labeled, seeded and watered to field capacity to support germination. A regular watering regime was adopted till target growth stages were attained. Thirteen (13) treatments were applied appropriately (Appendix 3). Thinning and uprooting was done in the 2nd and 3rd weeks after germination to achieve the least number of plants possible per pot after uprooting all other weeds. Each pot had two 2 plants.

A conventional one (1) liter hand sprayer was used to apply the treatments at 2-4 & 6-8 leaf growth stages by exerting adequate pressure on the hand sprayer lever while spraying maintaining very small droplets of the spray solution. Clear tap water was used for each mixture with thorough rinsing before each subsequent application. The herbicide (Glyphogan 480SL) rates were measured using a calibrated 20 milliliter plastic measuring syringe.

3.2.4 Experimental design applied

Completely randomized design (CRD) was applied. During the survey, mature black jack seeds were harvested from each farm by hand picking and packing the seeds individually in dry khaki packs. Ten (10) soil samples from the ten farms denoted as S1, S2, S3, S4, S5, S6,S7, S8, S9 and S10 (Appendix 4) of about 70kg each were also collected from each farm as described by Moss (1999). Each plot had 130 pots. The plots were replicated 3 times. These plots were separated by weedfree paths of 0.5 meters (Appendix 6). Two sets of the experiment were conducted at 2-4 & 6-8 leaf growth stages. The 1st set of the experiment was conducted in February 2015 and the 2nd set in March 2015. Data was collected after every three (3) days from the day of treatment, recorded in an excel worksheet and later reorganized for analysis. The timing of treatment was both at the 2-4 leaves stage or at the 6-8 leaves stages and data collected after the 3rd, 6th, 9th, 12th and 15th days after treatment.

3.2.5 Parameters measured

The parameters measured were the number of plants physiologically dead, total days to physiological death, chlorosis and necrosis of each plant. These parameters were coded for ease of analysis. Data in terms of time (TOO) was established at the 3rd, 6th, 9th, 12th and 15th days after treatment. Using a coded scale on visual weed control estimates based on symptoms such as chlorosis, necrosis, and death of the treated

plants compared to the non-treated control plants (Ganie *et al.*, 2015; Sarangi *et al*., 2014) were evaluated. A qualitative measure and score on a scale of one to four (1-4) based on the main shoot observations whose chlorotic or necrotic symptoms signified the effect of the rate applied were scored and recorded (Appendix 5).

3.2.6 Statistical analysis

The analysis of variance (ANOVA) was computed in respect to each parameter using Genstat (Discovery edition 3 by VSN international, 2008) statistical package. The means were separated using Fisher's protected least significance difference where the variate results were established to be significant. The significance level was set at 5%. The 50% lethal concentrations for the herbicide used were determined using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp).

3.3 Glyphosate field efficacy evaluation

3.3.1 Study area

The field evaluation was conducted at Gatatha farmers' cooperative society coffee farm (S3) located in Kiambu county in Kenya between April and July 2015. The farm's elevation is at 1,926 meters above sea level and lies at latitude 1° 07.69' S and longitude 36° 45.64' E.

3.3.2 Introduction

In the preceding dose response studies of glyphogan 480SL on *Bidens pilosa* L., the optimal rates were to be considered in the field efficacy experiments at the similar growth stages. The results indicated glyphogan rates of 5.0 L/Ha and 4.5.0 L/Ha at 2-4 leaves and 6-8 leaves respectively being optimal on effective mortality. These rates were therefore considered to be the optimal and hence included in the field experiment. The study site was prepared by slashing to the ground level all the then present vegetation, followed by broadcasting black jack seeds collected from farms with prolonged use of glyphosate so as to establish a black jack pure stand. Each plot was then thinned in the 2^{nd} to 3^{rd} week after germination so as to retain a plant population of 100. All other weeds were weeded out.

3.3.3 Treatments applied

Eight (8) treatments (Table 2) were applied using a conventional one (1) lt hand sprayer. Glyphogan 480SL doses were measured using a fifty (50) milliliters (mls) calibrated plastic measuring syringe. Adequate pressure was applied on the sprayer lever so as to ensure optimal wetting of the leaf surfaces. The control applied was the recommended glyphogan 480SL rate of application of 2 liters per hectare. Two sets of the experiments were set up. The treatments were applied in May & June 2015 at the 2-4 & 6-8 leaves growth stages respectively.

Counting the number of completely affected (physiologically dead) plants in every plot was done in every 3rd day after treatment upto the 15th day. Data was recorded in an excel worksheet and thereafter harmonized and analyzed.

Table 2: List of treatments applied in the field experiment

T1	Glyphogan 480SL application at 2.0 L/Ha
T2	Glyphogan 480SLcombination with 2,4D application at (2+2) at 2 L/Ha each.
T3	Glyphogan 480SL application at 3.5 L/Ha
T4	Glyphogan 480SL application at 4.0 L/Ha
T5	Glyphogan 480SL application at 4.5 L/Ha
T6	Glyphogan 480SL application at 5.0 L/Ha
T7	Glyphogan 480SL application at 5.5 L/Ha
T8	Glyphogan 480SL application at 6.0 L/Ha

3.3.4 Experimental design applied

Randomized Complete block design was laid out in this experiment with three replicates. The coffee cropped area treated had a spacing of 2.1 meter × 2.1 meter in all directions from one tree to the other. The treatment blocks were separated by a row of non-treated strip here referred to as the separating row (Table 3).

Table 3: Field experiment layout at Gatatha coffee estate farm

6lts/Ha	**4.5lts/Ha**	**5lts/Ha**	**3.5lts/Ha**	**2 + 2;2,4 D Lts/Ha**	**5.5lts/Ha**	**2lts/Ha (Control)**	**4lts/Ha**
Separating row							
5lts/Ha	**2lts/Ha (Control)**	**2 + 2;2,4 –D Lts/Ha**	**6lts/Ha**	**2lts/Ha**	**4lts/Ha**	**3.5lts/Ha**	**4.5lts/Ha**
Separating row							
4lts/Ha	**5lts/Ha**	**2lts/Ha (Control)**	**5.5lts/Ha**	**4.5lts/Ha**	**6lts/Ha**	**3.5lts/Ha**	**2 + 2;2,4 D Lts/Ha**

3.3.5 Parameters measured

The number of plants that succumbed to herbicidal activity also referred to as effective mortality (em) were counted and recorded for statistical analysis.

3.3.6 Statistical analysis

Analysis of variance (ANOVA) was computed in respect to each parameter using Genstat (Discovery edition 3 by VSN international, 2008) statistical package. The means were separated using Fisher's protected least significance difference where the variate results were found to be significant. The significance level was set at 5%. The confidence limits and the 50% lethal concentrations for the herbicide used were determined using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp).

CHAPTER FOUR: RESULTS

4.1 Weed Distribution

The main objective of the coffee weeds survey in the estates with a long history of glyphosate usage was to establish the key weed species based on the five weeds survey quantitative parameters. The data collected during the weeds survey was analyzed and the results summarized in tables. Hierarchical order was used in ranking the weed species in a descending order of their frequencies. The locations of the surveyed sites in elevation ranged between 1411-1926 meters above sea level and with varying coordinates (Table 1). Black jack (*Bidens pilosa* L.) was thus established as the most prevalent weed species in the survey (Table 4).

Table 4: Frequency, Field uniformity, Relative Frequency, Relative field uniformity, Mean field density, Relative mean field density and Relative abundance of the weed species surveyed

	Weed Species	F*k* (%)	FU*k* (%)	RF*k* *(%)*	RFU*k* *(%)*	MFD*k* P/m²	RMFD*k* *(%)*	RA*k* *(%)*
1	Black jack	89.0	79.2	8.7	16.9	462.0	21.1	46.7
2	Double thorn	79.5	63.2	7.8	13.5	259.6	11.9	33.1
3	Wandering jew	65.5	42.9	6.4	9.2	132.0	6.0	21.6
4	Asthma weed	65.0	42.3	6.4	9.0	157.8	7.2	22.6
5	Purslane	58.0	33.6	5.7	7.2	93.0	4.2	17.1
6	Pig weed	52.5	27.6	5.1	5.9	132.2	6.0	17.1
7	Horse weed	50.0	25.0	4.9	5.3	63.2	2.9	13.1
8	Love grass	45.5	20.7	4.4	4.4	78.6	3.6	12.5
9	Star grass	45.0	20.3	4.4	4.3	112.0	5.1	13.8
10	Crab grass	42.5	18.1	4.2	3.9	80.0	3.7	11.7
11	Gallant soldier	41.0	16.8	4.0	3.6	106.8	4.9	12.5
12	Common groundsel	40.0	16.0	3.9	3.4	28.2	1.3	8.6
13	Nut grass	35.0	12.3	3.4	2.6	74.2	3.4	9.4
14	Black nightshade	34.0	11.6	3.3	2.5	38.6	1.8	7.6
15	Mexican marigold	25.0	6.3	2.4	1.3	6.6	0.3	4.1
16	Goat weed	22.0	4.8	2.2	1.0	20.2	0.9	4.1
17	Common wire weed	20.5	4.2	2.0	0.9	21.4	1.0	3.9
18	Garden pink sorrel	19.5	3.8	1.9	0.8	44.8	2.0	4.8
19	Goose grass	18.5	3.4	1.8	0.7	37.4	1.7	4.2
20	Common lambsquarters	18.0	3.2	1.8	0.7	38.0	1.7	4.2
21	Apple of peru	14.5	2.1	1.4	0.5	15.4	0.7	2.6
22	Sow thistle	13.0	1.7	1.3	0.4	8.8	0.4	2.0
23	Thorn apple	11.0	1.2	1.1	0.3	36.0	1.6	3.0
24	Coast morning glory	10.5	1.1	1.0	0.2	11.8	0.5	1.8
25	Parthenium weed	10.5	1.1	1.0	0.2	15.8	0.7	2.0
26	Stink grass	10.5	1.1	1.0	0.2	10.6	0.5	1.7
27	Tropical Mexican clover	10.0	1.0	1.0	0.2	8.2	0.4	1.6
28	Ethiopian kale	9.0	0.8	0.9	0.2	14.4	0.7	1.7

29	Spiny sow thistle	6.5	0.4	0.6	0.1	5.4	0.3	1.0
30	Creeping wood sorrel	6.0	0.4	0.6	0.1	10.6	0.5	1.2
31	Water willow	5.5	0.3	0.5	0.1	1.2	0.1	0.7
32	Hairly ruptureworth	5.5	0.3	0.5	0.1	1.2	0.1	0.7
33	Carolina ponysfoot	5.5	0.3	0.5	0.1	17.2	0.8	1.4
34	Golden wattle	5.0	0.3	0.5	0.1	4.0	0.2	0.7
35	May weed	4.0	0.2	0.4	0.0	12.2	0.6	1.0
36	Puncture vine	4.0	0.2	0.4	0.0	4.8	0.2	0.6
37	Hairy crab grass	4.0	0.2	0.4	0.0	7.2	0.3	0.8
38	Garden cucumber	3.5	0.1	0.3	0.0	3.8	0.2	0.5
39	Dollar weed	2.5	0.1	0.2	0.0	2.2	0.1	0.4
40	Foxtail	2.5	0.1	0.2	0.0	2.0	0.1	0.4
41	Bitter apple	2.5	0.1	0.2	0.0	2.6	0.1	0.4
42	Silver leaf desmodium	2.5	0.1	0.2	0.0	0.8	0.0	0.3
43	Jacobinia	2.5	0.1	0.2	0.0	0.8	0.0	0.3
44	Kenya clover	2.0	0.0	0.2	0.0	4.2	0.2	0.4
45	Egyptian mallow	2.0	0.0	0.2	0.0	1.0	0.1	0.3
46	Climbing asystasia	2.0	0.0	0.2	0.0	2.0	0.1	0.3
47	Wild lettuce	1.0	0.0	0.1	0.0	0.4	0.0	0.1

KEY: F = Frequency, FU=Field uniformity, RF = Relative Frequency, RFU= Relative Field Uniformity, MFD=Mean field density, RMFD = Relative mean field density, RA = Relative abundance.

4.1.1 Weed Taxonomy

A total of fourty seven (47) weed species which included thirty-one (31) annuals and sixteen (16) perennials comprising of thirty-nine (39) broadleaved weeds, seven (7) grasses and one (1) sedge (Representing 83%, 15% and 2% respectively by habitat) were identified. The annuals were greater in number than the perennial weed species. It was observed that overall; the annual broadleaved weed species were more prevalent than perennial broadleaved species and grasses.

Nineteen (19) families were represented; Asteraceae family had the highest number of weed species (12), followed by Poaceae (7), Solanaceae (4), Oxalidaceae (2), Fabaceae (3), Convolvulaceae (3), Acanthaceae (3), Malvaceae (2). The rest of the eleven (11) families were represented by one species each. Asteraceae, Poaceae and Solanaceae families accounted collectively for 50% of the species established (Table 5).

Table 5: Taxa of the 47 weed species established in the large scale coffee farms survey

Family	Common Name	Scientific Name	Life Cycle
Acanthaceae	Climbing asystasia	*Asystasia schimperi* L.	A
	Water willow	*Justicia Calyculata* (Deflers) T. Anders	P
	Jacobinia	*Justicia elliotii* S. Moore	P
Amaranthaceae	Pig weed	*Amaranthus graecizans* L.	A
Asteraceae	Black jack	*Bidens pilosa* L.	A
	Gallant soldier	*Galinsoga parviflora* Cav.	A
	Horse weed	*Conyza floribunda* H.B.& K.	A
	Common groundsel	*Senecio vulgaris* L.	A
	Goat weed	*Ageratum conyzoides* L.	A
	Parthenium weed	*Parthenium hysterophorus* L.	A
	May weed	*Matricaria spp*	A
	Sow thistle	*Sonchus oleraceus* L.	A
	Mexican marigold	*Tagetes minuta* L	A
	Spiny sow thistle	*Sonchus asper* (L.) Hill	A
	Hairly rupturewort	*Acanthospernum hispidum* D.C.	A
	Wild lettuce	*Lactuca capensis* Thunb	A
Brassicaceae	Ethiopian kale	*Erucastrum arabicum* Fisch. & Mey	A
Chenopodaceae	Common lambsquarters	*Chenopodium album* L.	A
Commelinaceae	Wandering jew	*Commelina benghalensis* L.	P
Convolvulaceae	Carolina ponysfoot	*Dichondra carolinensis* L.	P
	Coast morning glory	*Ipomea mombassana* Vatke	P
	Kidney weed	*Dichondria rapens* (J.R. & G. Forst)	P
Cucurbitaceae	Garden cucumber	*Cucumis hirsutus* Sond	A
Cyperaceae	Nut grass	*Cyperus rotundus* L.	P
Euphorbiaceae	Asthma weed	*Euphorbia hirta L.* L.	A
Fabaceae	Falcon's claw acacia	*Acacia polyacantha* Willd.	P
	Kenya clover	*Trifolium semipilosum* Fres.	P
	Silver leaf desmodium	*Desmodium sp.* A	P
Malvaceae	Common wire weed	*Sida ovata* Forsk	P
	Egyptian Mallow	*Malva verticilata* L.	A
Oxalidaceae	Garden pink sorrel	*Oxalis latifolia* H.B. & K.	P
	Creeping wood sorrel	*Oxalis corniculata* L.	P
Poaceae	Star grass	*Cynodon dactylon* (L.) Pers.	P

	Coach grass	*Digitaria abyssinica* (A. Rich.) Stapf	A
	Love grass	*Setaria verticilata* (L.) Beauv.	P
	Goose grass	*Eleusine indica* (All.) Gaertn	A
	Stink grass	*Eragrostis cilianensis* (All.) Lut	A
	Crab grass	*Digitaria diagonalis* (Nees) Stapf	A
	Foxtail	*Setaria sphacelata* (Schumach)	A
Polygonaceae	Double thorn	*Oxygonum sinuatum* (Meisn.) Dammer	A
Portulacaceae	Purslane	*Portulaca oleracea* L.	A
Rubiaceae	Tropical American clover	*Richardia brasiliensis* Gomes	P
Solanaceae	Black nightshade	*Solanum nigrum* L.	A
	Thorn apple	*Datura stramonium* L	A
	Apple of peru	*Nicandra physaloides* (L.) Gaertn	A
	Bitter apple	*Solanum incanum* L	A
Zygophyllaceae	Puncture vine	*Tribulus terestris* L	A

Key: A - Annual; P- Perennial

4.1.2 Weed species frequency (F)

The qualitative analysis established that all the weed species were different from one another on all the 5 parameters calculated for each. Black jack was found to have the highest relative abundance (RA) value of 46.7%, the highest frequency (F) value of 89.0%, frequency uniformity (79.2%), relative frequency (RF) of 8.7% as well as the highest relative field uniformity (RFU) value of 16.9% (Table 4).

The following 10 weed species were established to have their F values equal to or greater than 42.5%. In descending order, black jack was leading followed by double thorn, wandering jew, asthma weed, purslane, pig weed, horse weed, love grass, star grass and lastly by crab grass. Nine (9) broadleaved weeds and three (3) grass weeds species topped in the cluster of weeds whose frequency (F) value was $\geq 40\%$.

Black jack topped overall with a frequency (F) value of 89% followed by double thorn, asthma weed, wandering jew, purslane, pig weed, horse weed, love grass, star grass, crab grass, gallant soldier and common groundsel. Their values were 79.5%, 65.5%, 65.0%, 58.0%, 52.5%, 50.0%, 45.5%, 45.0%, 42.5%, 41.0% and 40.0% in descending order respectively coming in the top 12 species. The rest of the broadleaved and grass weed species had a frequency value ranging between 1% and 40% (Table 4).

4.1.3 Field uniformity (FU)

In a similar descending order of field uniformity values, the same seven (7) broadleaved and three (3) grass weed species in the top ten weeds species had their frequency uniformity (FU) values being ≥ 18%. Black jack was leading at 79.2% followed by double thorn (63.2%), wandering jew (42.9%), asthma weed (42.2%), purslane (33.6%), pig weed (27.5%), horse weed (25.0%), love grass (20.7%), star grass (20.2%) and crab grass at 18.1%. The other thirty-two (32) broadleaved species had their frequency uniformity values being ≤ 16.8%. The least FU was established to be wild lettuce at (0.01%). Among the grasses, the highest field uniformity was reported in love grass at 20.7%, followed by star grass at 20.2% and crab grass at 18.1%. The lowest field uniformity in grass weed species was found to be in foxtail at 0.1 % (Table 4).

4.1.4 Mean Field density (MFD)

Black jack was at the top of the list with a mean field density (MFD) value of 462 plants per m². It was followed by double thorn (259.60), asthma weed (157.80), pig weed (132.20), wandering jew (132), star grass (112) and gallant soldier (106) in the top seven (7) weed species. All the other 40 weed species had their mean field densities below 100 plants per m² with the lowest being the wild lettuce at 0.4 plants per m² (Table 4).

4.1.5 Relative abundance (RA)

Black jack had a relative abundance (RA) value of 46.69% and was thus significantly outstanding among all the fourty seven weed species identified in the survey. It topped both as a broadleaf weed species as well as in the overall top eleven (11) weeds species that were established to have a relative abundance (RA) value ≥ 11.7% (Figure 5). In descending order, it was followed by double thorn (33.1%), asthma weed (22.6%), wandering jew (21.6%), purslane (17.1%), pig weed (17.1%), star grass (13.8%), horse weed (13.1%), gallant soldier (12.5%), love grass (12.5%) & crab grass at 11.7% all the other 36 weed species had their relative abundance values being > 11.7% where the wild lettuce was found to have the lowest RA of 0.1% (Table 4).

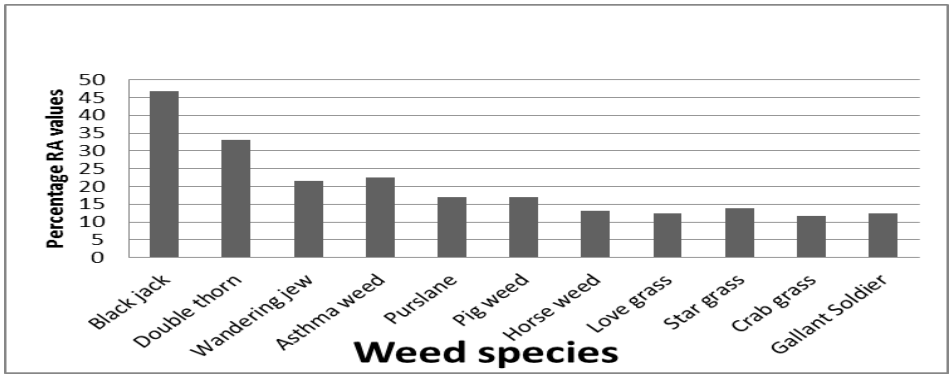

Figure 5: Relative abundance of the 11 leading weeds species established in the survey

4.2 Results of Glyphosate dose tests

4.2.1 Rate of herbicide application

The data that was collected and analyzed indicated varied responses for individual plants against the applied doses. Data was then harmonized, summarized and presented in tables and graphs. The responses at 2-4 leaves growth stage indicated an optimal lethal concentration for Glyphogan 480SL to be about 5.0 L/Ha. The 6-8 leaves growth stage indicated a lower optimal rate of 4.5 L/Ha (Table 6). Optimum mortality means established were 0.123 and 0.083 for the 2-4 and 6-8 leaves growth stages respectively. Their grand means were 0.058 and 0.039 respectively (Appendix 8 and 9). This gave an indictaion that the lethal doses were 5 and 4.5 litres per hectare for the two growth stages. Nevertheless, at the 2-4 leaves stage, the rates of application at 4.5, 5 and 5 litres per hectare were interpreted to be similar. This was likewise similar for the same dose rates at 6-8 growth leaves stage (Table 6).

The farm had a *p* value of 0.8 while the parameter of the farm with rate of application combination had a *p* value of 0.999 (Appendix 8). On the rates of application, the means separated showed 4.5 and 5.5 L/Ha rates at younger growth stage were closely related. This scenario was also reflected by the doses at 5.0 and 5.5 Lts/Ha for the 6-8 leaves growth stage (Table 6). The ED_{50} were established to be 5.27 and 9.32 Lts/Ha and 6.69 and 14.96 Lts/Ha at 95% confidence limits determination for the 2-4 and 6-8 leaves experiments respectively (Appendix 20). At the same growth stages, these rates recorded depressed mortality efficiency that was much lower than the anticipated recommended rate of 2 L/Ha. Consequently, higher rates were required to attain the 50% mortality threshold. These visual assessments indicated that all the test doses performed way below the recommended lethal concentration of 2Lt/Ha.

Table 6: Relationship of the rates of glyphogan 480SL application against the mortality means of *Bidens pilosa* L.

Rate of application	2-4 Leaf stage	6-8 Leaf stage
ROA (Lts/Ha)	Mortality means	Mortality means
5.0	0.123a	0.070ab
5.5	0.097ab	0.070ab
4.5	0.093ab	0.083a
3.5	0.077bc	0.033cde
4.0	0.070bc	0.053abc
6.0	0.070bc	0.047bcd
2.5	0.063bc	0.047bcd
3.0	0.060bc	0.050bc
2.0	0.047cd	0.030cdef
1.5	0.040cde	0.017def
1.0	0.013def	0.003ef
0.5	0.003ef	0.000f
0.0	0.000f	0.000f

Results of lethal concentration where the *Lsd* = 0.469 and p = <0.001 at 2-4 leaves; Lsd =0.418 and p = <0.001 at 6-8 leaves. In the table, means within the same column bearing the same letter are not significantly different at $p = \leq 0.05$

4.2.2 Evaluation on chlorosis

The grand mean on the chlorotic effect was 0.115 while S4 had the highest mean of 0.259. S2 indicated the lowest mean of 0.041 at the 2-4 leaves growth stage. The grand mean on the chlorotic effect at 6-8 leaves growth stage was 0.390 while S2 had the highest mean of 0.492 and S4 a low of 0.285 (Table 7). The variate chlorosis very highly significantly differed at $p \leq 0.05$. At both treatment stages, all the parameters (Farm, rate of application & interaction of the farm with the rate of application) had a *p* value = <0.001 (Appendix 10 and 11).

4.2.3 Evaluation on necrosis

The grand mean of the necrotic effect was 0.647 while S8 had the highest mean of 0.764 and S10 it lowest at 0.538 at 2-4 leaves growth stage (Table 7). The grand mean on the necrotic effect at the 6-8 leaves growth stage was 0.410. S1 had the highest mean of 0.510 and S2 lowest at 0.323 (Table 7). In both the tested growth stages, necrosis did not very highly significantly differ across the farms at $p \leq 0.05$. The rate of application and the interaction between the farm and the rate of application differed very highly significantly (Appendix 12 and 13).

4.2.4 Evaluation on the treatments period

In achieving the optimum effective mortality for the period of treatment, the p value was < 0.001 indicating very highly significant differences (Appendices 14 & 15) for both growth stages. The variate had a grand mean of 0.718. S8 had the highest mean of 1.015 and S10 the lowest mean of 0.554 at 2-4 leaves growth stage. The grand mean on the same period of treatment was 0.519. S4 had the highest mean of 0.646 and S5 the lowest of 0.369 at 6-8 leaves stage (Table 7).

Table 7: Relationship of the variates means at the 2-4 and 6-8 leaves growth stages for all the herbicide rates

	2-4 leaf stage			6-8 leaf stage		
Farm	**Chlorosis**	**Necrosis**	**Treatment period**	**Chlorosis**	**Necrosis**	**Treatment period**
S1 (Ibonia)	0.067	0.633	0.592	0.413	0.510	0.577
S2 (Cianda)	0.041	0.674	0.738	0.492	0.323	0.423
S3 (Gatatha)	0.074	0.633	0.623	0.485	0.426	0.454
S4 (Nyala)	0.259	0.610	0.754	0.285	0.500	0.646
S5 (Kays)	0.115	0.646	0.669	0.423	0.344	0.369
S6 (Karunguru)	0.097	0.654	0.754	0.344	0.431	0.638
S7 (Benvar)	0.144	0.672	0.808	0.369	0.410	0.546
S8 (Mutoma)	0.074	0.764	1.015	0.459	0.359	0.392
S9 (Koorali)	0.174	0.646	0.669	0.331	0.356	0.538
S10 (Bendor)	0.105	0.538	0.554	0.303	0.444	0.608
Grand mean	**0.115±**	**0.647±**	**0.718±**	**0.390±**	**0.410±**	**0.519± Se**

Results of the means of all doses on chlorosis, necrosis and period of treatment where the $Lsd_{0.05}$ (ROA) = <0.001, 0.563 and 0.41 with p = 0.628 at 2-4 leaves and $Lsd_{0.05}$ (ROA) =0.12, 0.14, 0.37 with p = 0.8 at 6-8 leaves respectively.

4.3 Field evaluation of the lethal concentrations of Glyphogan 480SL

The key objective of the field evaluation was to establish the most effective glyphosate rate that was determined by the dose response tests on the most prevalent weed species from the survey. At the 2-4 leaves growth stage, it was established that the rate of application of the herbicide had p values = <0.001 indicating very high significance differences. This was similar at the 6-8 leaves growth stages (Appendix 16, 17, 18 &19). Comparatively based on the mortality means, the combination dose

was established to have the most outstanding effective lethal dose as graphically illustrated (Appendix 7).

Generally for both growth stages, higher means for the weeds killed were established to be at the 2-4 leaves growth stage as compared to the 6-8 leaves stage. The rate of glyphogan 480SL at 2Lts/Ha and its combination with 2,4 D amine at 2Lts/Ha indicated the highest mortality with means ≥ 47.6. The next closest mortality means was 13.73 in experiment 1 at 4.5 Lts/Ha at 2-4 leaves growth stage (Table 8).

Table 8: Relationship of the rate of glyphogan 480SL and its combination application against mortality means for the field experiments

		Rates of glyphogan 480SL & it's combination							
		2.0	**2+2 (2,4D)**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**
2-4 Leaves	**Experiment 1 (Mortality means)**	4.73	58.33	9.87	11.53	13.73	11.40	9.60	11.13
	Experiment 2 (Mortality means)	6.33	58.73	10.00	11.33	13.07	12.07	9.93	11.53
6-8 Leaves	**Experiment 1 (Mortality means)**	2.67	49.47	4.53	8.73	11.93	10.8	10.87	8.87
	Experiment 2 (Mortality means)	3.13	47.60	5.87	9.13	10.20	9.33	9.73	8.87

Results of the means of varied glyphosate doses applied in the field. The mortality grand mean was 16.29 and 16.62 and *Lsd* = 2.87, 2.93 with p = <0.001 at 2-4 leaves and 13.48 and 12.98 and *Lsd* =2.69, 2.15 with p = <0.001 at 6-8 growth leaves stage respectively.

CHAPTER FIVE: DISCUSSION

5.1 Weeds distribution

In this study, most of the abundant weed species were annual and broadleaved in nature. The methodology used highly borrowed from the description by Kuchler and Zonneveld (1988) on the forms of field surveys being; exploratory survey, reconnaissance survey, extensive and intensive types of surveys which allowed for the linear technique used in this survey. The observations made in this survey reflected the usefulness for determining the occurrence and relative importance of the established weed species in large scale coffee production defined as a cropping system (Thomas in 1985, McCully *et al*., 1991 and Frick & Thomas 1992). The rankings of these weed species differed on the list based on their quantitative parameters.

Cardina *et al.* in 1999 made related weed species compositions findings and their observations were based on an agricultural land where compatible techniques were employed in managing weeds.

In related studies done by Kimemia *et al.* in 1998 on different weed control methods in coffee in Kenya, the observations indicated that *Cynodon dactylon* was the most common grass species while *Bidens pilosa* and *Galinsoga parviflora* were the most abundant broad leaved weed species where *Tagetes minuta* recorded zero dominance. It was also noted that the broadleaved weeds were more in number of species as compared to the rest of the weed species. These findings compare closely with the observations made by Thomas (1985) who observed that in weeds survey, the relative abundance value clearly indicates very few dominated weed species in a given cropping environment. Similarly, Moody and Drost (1983) observed that the dominant weed flora in any crop field is usually about ten (10) species of which the dominant species are rarely more than three (3) to four (4). These observations closely related in the survey findings on the relative abundance values as tabulated in table 4.

5.2 Dose response evaluation

5.2.1 Evaluation on effective mortality

An ideal dose response relationship on a plant species for a given herbicide doses should indicate progressive optimal mortality responses with increasing doses on that sensitive plant species. The various doses used in the screening test were necessary in providing some indications of resistance level among the established plant

populations as reported in confirmation assays by Kaloumenos *et al*., 2011; Maneechote *et al*., 2005 and Wise *et al.*, 2009. The dose response curves established at 2-4 & 6-8 leaves growth stage did not indicate a normal sigmoidal curve, where effective mortality against each concentration applied was expected to be progressive with time and optimizing in about 10 days from the time of treatment as well as obey the principle that the higher the rate of application, the higher the expected phytotoxicity effect. This could probably have occurred due to the impact of resistance or tolerance effect established by black jack upon the application of the herbicide. The high significance levels established in the rates applied indicated a continued effect of the injury upto a given period of herbicidal effect. Generally, increased doses indicated increased injuries. Resistance beyond a given recommended dose of a herbicide is relevant in research because such resistance levels provide clues to resistance mechanisms.

Terry in 1984 observed that such kind of an occurrence signifying resistance to a herbicide may develop from many factors such as growth stage and age of the plant, biophysiological and biochemical processes as well as genetic inheritance and (Georghion, 1986). Resistant genes retention at various frequencies arises from a genetic memory of that given species. In line with such results, this scenario is suspect to have resulted from enhanced tolerance or resistance already developed by *B. pilosa* L. that was investigated. Very varied and less convincing control levels were observed on the treated plants. As observed by Gerwick *et al.,* in 1993 and Shaner *et al.,* (2005), shikimate accumulation assays require the use of young and rapidly expanding plant tissues. Enolpyruvyl shikimate phosphate synthase (EPSPS) enzymes are most active in the meristematic tissues and related enzyme activities decrease rapidly as leaves mature. None of the growth stages as well as the different concentrations recorded an effective mortality level $\geq$ 73%. The younger plant stage appeared to be more susceptible to the herbicide as compared to those tested at the 6-8 leaf stage thus higher mortalities were recorded. Probably due to the glyphosate retention in the active parts, there was more pronounced shoot necrosis as compared to complete chlorosis.

As observed in other studies on protective enzymatic activities in Paraquat resistant *Conyza bonariensis* by Ye and Gressel in 1994, this reduced effective mortality probably was caused by enhanced enzymatic inhibition of the glyphosate molecule during the plant's protein synthesis process. This inhibition seems to have been more

efficient at the 6/8 leaves stage thus lower effective mortality as compared to the 2/4 leaves stage. Consequently, the localized retention of the glyphosate in the leaves by the younger and more active plants probably favored its translocation and this triggered limited systemic injuries but more visual shoot necrosis. The declared mode of action of glyphogan 480SL is through systemic delivery achieved through foliar uptake, a function that is primarily achieved through the active stomata openings. Another probability to these outcomes would have resulted from the leaf absorption areas and stomata absorption capacity being minimal and thus resulted in lower mortalities of the treated *B. pilosa* population.

5.2.2 Evaluation of chlorosis and necrosis

It was observed that chlorosis was more pronounced in the higher rates of application. Though the expectations were that the chlorotic effect progressed to death of affected tissues, there were less and less levels of mortality observed with time. Most plants indicated various levels of chlorotic effect across the rates and with time, most of which recovered. This is probably an indicator of the glyphosate herbicidal characteristic being non injurious or manifesting enhanced reduction of herbicidal activity against expectations. Plants that recovered indicated reduced apical leave sizes as well as their visual chlorophyll content. This could have resulted from the effect of accumulated glyphosate salt in the affected leaf cells. The stems were observed to be normal indicating that probably there was no herbicidal activity that occurred in the stems.

Necrotic effects observed were inconsistently systematic. Some individual leaves indicated partial areas affected while other areas remained none affected. This is an indication that such whole leaves did not receive adequate lethal concentrations. It's also possible that some of the leaves' cells significantly reduced the herbicidal effect upon application.

5.2.3 Evaluation on duration of wilting and death

Wilting and death of treated *B. pilosa* plants was anticipated to be achieved within 7-10 days after application. Efficient time taken for continued injurious effects was noted to be by the 9^{th} upto the 12^{th} days of observation after treatment beyond when no significant whole plant injuries and tissue mortalities were observed. The visual injury assessments based on complete death of tissues, chlorosis and necrosis showed great variations from a normal glyphosate phytotoxicity process. At the higher rates,

there was recovery from the chlorotic effect on all the plants with time through the new leaves that emerged. Continued growth and new tissues were confirmed to be progressive in such circumstances. This characteristic observation could signify a trend that can be investigated to estimate resistance levels of glyphosate on black jack.

5.3 Field evaluation of the lethal concentrations of Glyphogan 480SL

The combined herbicides treatment produced the highest means among all other straight rates. Statistically, there was no difference between the rates of glyphogan 480SL at 4.0, 5.0 and 6 L/Ha applied for both experiments 1 and 2. The glyphogan 480 SL rates of 3.5 and 5.5 L/Ha indicated similarity in both experiments at 2-4 leaves growth stage. It was established that the rates of application on the control means for both experiments at 2-4 leaves growth stage were higher as compared to those at 6-8 leaves growth stage. This is an indication that higher rates were required to provide equally effective outcomes on the younger black jack plants tested.

Such high means indicate the optimum lethal effect that was possibly established. In the means separation for experiment 1, the rates of glyphogan at 2 L/Ha with 2 L/Ha 2,4D combination and glyphogan at 4.5 L/Ha were different from all the other rates applied. However, in experiment 2, the straight rates of glyphogan 480 SL of 3.5, 2 L/Ha and glyphogan 480SL at 2 L/Ha with 2L/Ha of 2,4 D combination were different from all the other rates applied. Statistically, there was no difference between the straight rates of glyphogan at 3.5 and the control in experiment 1 (Table 8). The effective dose (ED_{50}) established at the 2-4 and 6-8 growth stages in the two experiments by probit analysis at 95% confidence levels were 13.04; 9.32 Lts/Ha and 14.74; 11.423 L/Ha respectively (Table 8). These rates are significantly higher than the recommended rate of application for Glyphogan 480SL herbicide of 2 Lts/Ha against black jack.

Weeds resistance to herbicides is one of the primary concerns in modern agriculture. The first weeds resistance report was done by Switzer in 1957 on 2,4-D and wild carrot (*Daucus carota* L.). Heap in 2012 established that since then, resistance to herbicides has been widely established to include over 200 species worldwide involving at least 20 modes of action. Researchers and scientists have been upbeat in modernizing resistance studies for glyphosate. Since there was no previous data base on glyphosate resistance by *B. pilosa* from an existing population, the studies were carried out on its tolerance.

The glyphosate and 2,4 D combination treatment indicated more satisfactory mortalities due to enhanced mode of delivery and action to *B. pilosa* L., an aspect similarly observed by Norsworthy *et al*. in 2012 on herbicides mixtures. As a growth regulator herbicide, 2,4-D is effective under very low concentrations as compared to borax or chlorate herbicides and thus its inclusion contributed principally by causing malformations of sensitive plant tissues (Earl, 1957).

A refined method utilizing transgenic systems that rely on non-plant organisms (such as bacteria or yeast) has been evaluated by Baerson *et al*., in 2002 using *Escherichia coli* mutants that nevertheless were deficient of enolpyruvyl shikimate phosphate synthase (EPSPS) enzyme in order to confirm target site mutations suspected for conferring resistance to glyphosate. As observed by Dickson *et al*., (2011) and Wise *et al*., (2009), the goal of using a high plant population (say 100 plants) in the field assay was achieved in increasing the power of resistance detection as well as through at least three replicates. As Tanaka *et al.,* in 1986 observed in *Erigeron philadelphicus*, the movement of a herbicide can be limited by morphological structures in older plants. Older plants have more aged and non-living structures as compared to younger plants and this likely may hinder the movement and efficacy of a systemic herbicide. The case of localized concentrations and retentions of the glyphosate molecule on the active leaf areas and apices may have resulted in more death in the younger plants as compared to the older plants due to enhanced herbicidal effect.

Devine *et al.,* 1993a, observed that the movement of a herbicide across non-living structures is complex and depends on the nature of the herbicide applied. A common farmer practice of tank mixing glyphosate with 2,4-D that was evaluated as a treatment was necessary to give an indicator of the injury levels expected alongside the straight glyphosate doses. In both experiments, the glyphogan and 2,4 D combinations generated effective mortality above 90% by the 12^{th} day at the 2-4 and 6-8 leaves stages. All the other straight rates did not show much of a difference in terms of the number of plants dead or even improved efficacy within the wilting and death period. This is a reverse expectation with strong indications that the black jack highly tolerated or antagonized the performance on the glyphosate herbicide. The mortality evaluation on the rate of application was very highly significant at $p = <0.001$. A fundamental characteristic for glyphosate to control weeds is in its ability to be translocated from leaves to growing points of shoots, roots and rhizomes.

Studies have indicated that glyphosate is poorly absorbed into the leaves of resistant plants. Probably, enolpyruvyl shikimate phosphate synthase enzyme in this case was less efficiently inhibited in the younger stages as compared to the older growth stage (Attributed to the enzymatic activities involved in the protein making process).

CHAPTER SIX: CONCLUSIONS

6.1 Coffee weeds distribution

The studies indicated that overall, most of the problematic coffee weeds are found across farms with similar and prolonged weed management practices. The dominant weeds were established to be the broad leaf species and a few grass weed species. It is hereby concluded that annual weeds are more prevalent in coffee farms compared to the perennials, grasses and sedges. Broadleaved weeds were more dominant in the higher altitude farms such as Gatatha and Cianda farms as compared to the grasses which were more prevalent in lower altitudes farms such as Benvar and Karunguru. Black jack was concluded as the most prevalent weed species found in the coffee farms. This is an indicator that its presence can be found in all coffee farms.

6.2 Glyphogan 480SL dose response evaluation on *B. pilos*a L.

The study established that the effects of the rates of application of Glyphogan 480SL were different both at the younger and older growth stages of *Biden pilosa* L. It is thus possible that different rates of herbicides will give different results on parameters measured. Some of these parameters may not be visually different. It is quite a challenge to achieve acceptable herbicidal results especially while dealing with difficult to kill weeds. It is a prudent strategy to increase herbicides application rate whenever phytotoxicity isn't readily achieved with normal rates. Nevertheless, such strategies would highly negate on the benefits of handling herbicides with undesirable outcomes such as beyond target injuries, safety to the environment and the herbicide handlers and so many others. Weed management costs have gone up steadily occasioned by the frequency and extent of weed establishment in coffee farms. There was consistent in terms of the chlorotic and necrotic symptoms across the impacting dosages upto the last day of observation. This scenario was not expected to get pronounced thereafter since continued vegetative growth produced new shoots that had marginal chlorotic and necrotic symptoms. It therefore indicates that the affected plants entered into the face of tolerance or resistance to the herbicide.

Based on the comparative parameter of the period of observation, it was deduced that there is a time limit when the herbicidal effect on the surviving plants would cease to increase unless a repeat application is done timely. It was also established that for a given herbicide, its varied lethal concentrations can give an indication that its

increment will give efficient desired result upto a given level. This provides the optimal concentration rate beyond which a decline in efficiency results.

6.3 Field evaluation of the lethal concentrations of Glyphogan 480SL

The studies showed that effective mortality in the management of difficult to kill weeds such as *B. pilosa* L. in coffee can be achieved by tank mixing glyphosate with 2,4D amine at the recommended rates of application for each. The inclusion of the 2,4 D herbicide is believed to have enhanced to outcome by working synergistically or alone within the herbicide mixtures. This lethal effect was also noted to be consistent within the period evaluated and any extra time would not further influence the outcomes.

A strong correlation was noted for the optimal rates established as optimal lethal concentrations in the dose response tests with similar doses applied in the field. Nevertheless, the highest straight glyphosate rates applied in the field test did not differ from the dose response doses with any exception. Visually, they would be considered very similar. The studies indicated that it was more beneficial to apply the herbicide at the 2-4 leaves growth stage as compared to the 6-8 leaves stage.

6.4 Recommendations

- It is paramount to establish a coffee weed log for the coffee growing areas. As such would greatly impact on related research work and efficiency in managing weeds in coffee crops commercially.
- A tank mix combination of recommended herbicides can enhance their performance in difficult to kill weeds as compared to individual herbicides. It is also important to note the weed spectrum in a crop and mitigate the weeds management protocol as may be the case where 2 or 3 farms at different altitudes are under the same management in every season.
- Varying herbicides rates with the aim of achieving desired efficiency in weed control is a reasonable consideration. Such doses can be varied upto a given level where it makes economic sense to the user. Given unconfirmed circumstances of particular types of weeds behavior, higher rates of a herbicide applied does not necessarily translate into better control with such rates.

6.5 Recommendations for future research

- It would be prudent to investigate if black jack is a prevalent weed in all coffee growing farms across all altitudes where the crop is established.
- Further studies to establish the exact mode of resistance or tolerance of weeds to herbicides should be carried out in the coffee sector.
- Growers should model coffee weed management tools such as integrated weed control programs that involve various inputs with different recommended or with more than one typical herbicide. This way, they are likely to mitigate on the costs involved.
- It’s also prudent to explore other technologies on weed management such as by use of degradable mulching materials.

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APPENDICES:

Appendix 1: Common names of Bidens pilosa (Alembi 1993)

Language	Common name
Australia	Black fellows
East Africa:	Black jack (E), Eida (Atesa), Ekamogamogia (Gusii), Enyabarashana (Runyankore), Kichoma mguu (Swahili), Labika (Acholi), Muceege (Kikuyu), Nyanyiek-mom (Dholuo), Olukuye (Luyha), Ssere (Luganda).
English	Black jack
	Farmers friend
	Cobblers peg
	Spanish needle
	Hairy beggar ticks
Ethiopia	Abare, Chigogot, Zagogo
French	Sornet
Ghana	Asedura (Asante)
Ivory Coast	Solole (Dan)
Liberia	Niani (Kru-Guere)
Nigeria	Abere (Yoruba)
Philippine Names	Puriket(Bon), Pisau pisau, Nguad
	Pico picho
Portuguese	Picao-preto
	Picao do campo
Polynesian names	Fisiuli
	Kofe Tonge - Niue
South Africa	Gewone knapsekerel
Zimbabwe	Nyamaradzo

Appendix 2: Plant species relative to Bidens pilosa (LeyRoy et al, 1977)

Bidens bipartita L.
Bidens biternata (Lour). Merr.
Bidens coriacea (O. Hoffm) Sherff
Bidens elgonensis (Sherff)
Bidens grantii (Oliv.) Sherff
Bidens incumbens (Sherff)
Bidens kilimandscharica (O. Hoffm) Sherff
Bidens lineata (Sherff)
Bidens quadrangularis DC
Bidens rueppelli (Sch. Bip.) Sherff
Bidens schimperi Sch. Bip.

Bidens steppia Sherff
Bidens subalternans DC
Bidens sundaicus Brume
Bidens superba (Sherff)
Bidens ugandensis Sherff
Bidens tripartita L.
Kerneria dubia Cass
Kerneria tetragona Moench

Appendix 3: List of treatments applied in the dose response experiment

i	T1 stands for Treatment One	(0.5 Lt/Ha)
ii	T2 stands for Treatment Two	(1.0 Lt/Ha)
iii	T3 stands for Treatment Three	(1.5Lt/Ha)
iv	T4 stands for Treatment Four	(2.0 Lt/Ha)
v	T5 stands for Treatment Five	(2.5Lt/Ha)
vi	T6 stands for Treatment Six	(3.0 Lt/Ha)
vii	T7 stands for Treatment Seven	(3.5Lt/Ha)
viii	T8 stands for Treatment Eight	(4.0 Lt/Ha)
ix	T9 stands for Treatment Nine	(4.5Lt/Ha)
x	T10 stands for Treatment Ten	(5.0 Lt/Ha)
xi	T11 stands for Treatment Eleven	(5.5Lt/Ha)
xii	T12 stands for Treatment Twelve	(6.0 Lt/Ha)
xiii	T13 stands for Treatment Thirteen	Control

Appendix 4: List of the coffee farms that were sampled and surveyed

i S1 stands for Ibonia estate
ii S2 stands for Cianda estate
iii S3 stands for Gatatha estate
iv S4 stands for Nyala estate
v S5 stands for Kays estate
vi S6 stands for Karunguru estate
vii S7 stands for Benvar estate
viii S8 stands for Mutoma estate
ix S9 stands for Koorali estate
x S10 stands for Bendor estate

Appendix 5: Visual scoring scale on chlorosis and necrosis

C1 represents a marginally chlorotic apex
C2 represents 1 apical leaf being chlorotic
C3 represents 2 apical leaves being wholly chlorotic
C4 represents more than two apical leaves were chlorotic
N1 represents a marginally necrotic apex
N2 represents 1 apical leaf being necrotic

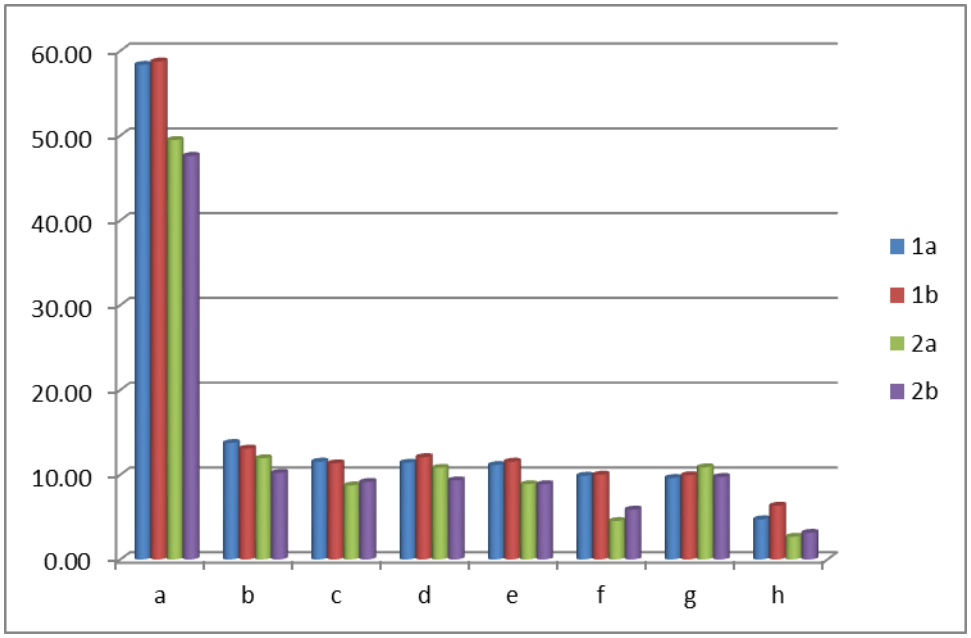
N3 represents 2 apical leaves being wholly necrotic
N4 represents more than two apical leaves were necrotic

Appendix 6: Table on the dose response experiment layout at the University of Nairobi farm

Weedfree path									
S5	**S1**	**S4**	**S6**	**S2**	**S3**	**S7**	**S8**	**S10**	**S9**
3.0 L/ha	**1.0 L/ha**	**0.5 L/ha**	**4.0 L/ha**	**6.0 L/ha**	**0 L/ha**	**1.0 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**5,0 L/ha**
5.0 L/ha	**4.0 L/ha**	**4.5 L/ha**	**2.5 L/ha**	**3.5 L/ha**	**0.5 L/ha**	**4.0 L/ha**	**0.5 L/ha**	**0 L/ha**	**0.5 L/ha**
6.0 L/ha	**3,0 L/ha**	**2.5 L/ha**	**2.0 L/ha**	**0 L/ha**	**1.5 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**4.5 L/ha**	**3,0 L/ha**
3.5 L/ha	**0.5 L/ha**	**5.5 L/ha**	**3.5 L/ha**	**4.0 L/ha**	**3.5 L/ha**	**1.5 L/ha**	**5.5 L/ha**	**5,0 L/ha**	**1.5 L/ha**
1.0 L/ha	**1.5 L/ha**	**1.0 L/ha**	**0 L/ha**	**1.5 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**1.0 L/ha**	**2.5 L/ha**	**6.0 L/ha**
4.0 L/ha	**5,0 L/ha**	**3,0 L/ha**	**6.0 L/ha**	**5.5 L/ha**	**4.5 L/ha**	**5.5 L/ha**	**0 L/ha**	**2.0 L/ha**	**4.0 L/ha**
0.5 L/ha	**2.5 L/ha**	**2.0 L/ha**	**0.5 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**4.5 L/ha**	**2.5 L/ha**	**3.5 L/ha**	**2.5 L/ha**
1.5 L/ha	**3.5 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**4.5 L/ha**	**5.5 L/ha**	**0 L/ha**	**1.5 L/ha**	**1.5 L/ha**	**0 L/ha**
5.5 L/ha	**6.0 L/ha**	**4.0 L/ha**	**1.0 L/ha**	**2.5 L/ha**	**6.0 L/ha**	**3.5 L/ha**	**4.0 L/ha**	**1.0 L/ha**	**4.5 L/ha**
4.5 L/ha	**4.5 L/ha**	**0 L/ha**	**5,0 L/ha**	**3,0 L/ha**	**1.0 L/ha**	**2.5 L/ha**	**4.5 L/ha**	**6.0 L/ha**	**2.0 L/ha**
0.0 L/ha	**2.0 L/ha**	**1.5 L/ha**	**5.5 L/ha**	**2.0 L/ha**	**2.0 L/ha**	**6.0 L/ha**	**3.5 L/ha**	**0.5 L/ha**	**3.5 L/ha**
2.5 L/ha	**5.5 L/ha**	**3.5 L/ha**	**4.5 L/ha**	**0.5 L/ha**	**2.5 L/ha**	**2.0 L/ha**	**2.0 L/ha**	**5.5 L/ha**	**5.5 L/ha**
2.0 L/ha	**0 L/ha**	**6.0 L/ha**	**1.5 L/ha**	**1.0 L/ha**	**4.0 L/ha**	**0.5 L/ha**	**6.0 L/ha**	**4.0 L/ha**	**1.0 L/ha**
Weedfree path									

Table showing the dose response field layout as set up at the University of Nairobi. S1…..S10 represents the sampled farms. Herbicide concentration in Lt/Ha = Liters per hectare

Appendix 7: Graphical relationship of the field experiment mortality means for all the rates tested at two growth stages



Mortality means for the field experiment. (1a and 1b = 1st and 2nd 2-4 leaves stage while 2a and 2b = 6-8 leaves stages; a=Combined doses, b=4.5Lt/Ha, c=4.0 Lt/Ha d=5.0 Lt/Ha e=6.0 Lt/Ha f=3.5 Lt/Ha g=5.5 Lt/Ha and h=2.0 Lt/Ha)

Appendix 8: ANOVA table for B. pilosa mortality at 2-4 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.093	0.046	0.850	
FARM	9	0.293	0.033	0.600	0.800
ROA∗	12	4.944	0.412	7.580	<.001
FARM.ROA	108	3.651	0.034	0.620	0.999
Residual	3,768	204.807	0.054		
Total	3,899	213.787	0.579		

Grand mean = 0.058

Calculated F = 7.58

Tabulated F = 1.76

Conclusion:

The *B. pilosa* mortality effect very highly significantly differed [F (12,3899) = 7.58, $p \leq 0.05$] among the 13 treatments applied at 2-4 leaves growth stage on the dose response evaluation.

Appendix 9: ANOVA table for B. pilosa mortality at 6-8 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	0.047	0.023	0.64	
FARM	9	0.187	0.021	0.57	0.826
ROA∗	12	2.777	0.231	6.30	<0.001
FARM.ROA	108	3.756	0.035	0.95	0.636
Residual	3,768	138.387	0.037		
Total	3,899	145.154			

Grand mean = 0.0387

Calculated F = 6.30; Tabulated F = 1.76

Conclusion: The *B. pilosa* mortality effect very highly significantly differed [F (12, 3899) = 6.30, $p \leq 0.05$] among the 13 treatments applied at 6-8 leaves growth stage on the dose response evaluation.

Appendix 10: ANOVA table for B. pilosa chlorosis at 2-4 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.801	0.400	1.620	
FARM∗	9	14.269	1.585	6.400	<.001
ROA∗	12	50.697	4.225	17.060	<.001
FARM.ROA∗	108	156.308	1.447	5.840	<.001
Residual	3,768	933.233	0.248		
Total	3,899	1,155.307			

Grand mean = 0.115

Calculated F = 17.06

Tabulated F = 1.76

Conclusion:

The *B. pilosa* chlorotic effect very highly significantly differed [F (12,3899) = 17.06, p≤0.05] among the 13 treatments applied at 2-4 leaves growth stage on the dose response evaluation.

Appendix 11: ANOVA table for B. pilosa chlorosis variate at 6-8 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	5.78	2.89	4.10	
FARM∗	9	19.75	2.19	3.11	<0.001
ROA∗	12	414.60	34.55	49.03	<0.001
FARM.ROA∗	108	226.95	2.10	2.98	<0.001
Residual	3,768	2,654.96	0.70		
Total	3,899	3,322.03			

Grand mean = 0.390
Calculated F = 49.03
Tabulated F = 1.76

Conclusion:

The *B. pilosa* chlorotic effect very highly significantly differed [F (12,3899) = 49.03, p≤0.05] among the 13 treatments applied at 6-8 leaves growth stage on the dose response evaluation.

Appendix 12: ANOVA table for necrosis variate at 2-4 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	3.671	1.836	1.270	
FARM	9	11.165	1.241	0.860	0.563
ROA∗	12	610.972	50.914	35.200	<.001
FARM.ROA∗	108	392.515	3.634	2.510	<.001
Residual	3,768	5,450.195	1.446		
Total	3,899	6,468.519			

Grand mean 0.647
Calculated F = 35.20; Tabulated F = 1.76

Conclusion:

The *B. pilosa* necrotic effect very highly significantly differed [F (12,3899) = 35.20, p≤0.05] among the 13 treatments applied at 2-4 leaves growth stage on the dose response evaluation.

Appendix 13: ANOVA table for necrosis variate at 6-8 leaves stage on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2.00	3.64	1.82	1.87	
FARM	9.00	14.58	1.62	1.66	0.09
ROA∗	12.00	259.87	21.66	22.25	<0.001
FARM.ROA∗	108.00	377.80	3.50	3.59	<0.001
Residual	3,768.00	3,667.69	0.97		
Total	3,899.00	4,323.59			

Grand mean = 0.410
Calculated F = 22.25

Tabulated F = 1.76

Conclusion:

The *B. pilosa* necrotic effect very highly significantly differed [F (12,3899) = 22.25, p≤0.05] among the 13 treatments applied at 6-8 leaves growth stage on the dose response evaluation.

Appendix 14: ANOVA table for the period of treatment at 2-4 leaves on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	8.349	4.175	0.49	
FARM	9	60.833	6.759	0.79	0.628
ROA∗	12	664.689	55.391	6.45	<.001
FARM.ROA	108	613.357	5.679	0.66	0.997
Residual	3,768	32,346.951	8.585		
Total	3,899	33,694.179	80.589		

Grand mean 0.718

Calculated F = 6.45

Tabulated F = 1.76

Conclusion:

The period of observation very highly significantly differed [F(12,3899) = 6.45, p≤0.05] between the 1st and last day after treatment at 2-4 leaves growth stage.

Appendix 15: ANOVA table for the period of treatment at 6-8 leaves on the dose response evaluation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	8.16	4.08	0.60	
FARM	9	36.94	4.10	0.60	0.796
ROA∗	12	456.93	38.08	5.59	<0.001
FARM.ROA	108	702.00	6.50	0.95	0.614
Residual	3,833	25,653.55	6.81		
Total	3,964	26,857.56			

Grand mean = 0.519; Calculated F = 5.59; Tabulated F = 1.76

Conclusion:

The period of observation very highly significantly differed [F (12,3899) = 5.59, p≤0.05] between the 1st and last day after treatment at 2-4 leaves growth stage.

Appendix 16: ANOVA table for B. pilosa mortality at 2-4 leaves growth stage for field experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	179.62	89.81	6.54	
TOO∗	4	9,867.50	2,466.88	179.76	<.001
ROA∗	7	31,003.19	4,429.03	322.75	<.001
TOO.ROA∗	28	6,904.10	246.57	17.97	<.001
Residual	78	1,070.38	13.72		
Total	119	49,024.79			

Grand mean 16.29; Calculated F = 322.75; Tabulated F = 2.13

Conclusion:

The rate of application very highly significantly differed [F (7,119) = 322.75, p≤0.05] among the 8 treatments applied on *B. pilosa* at 2-4 leaves growth stage for field experiment 1.

Appendix 17: ANOVA table for B. pilosa mortality at 2-4 leaves growth stage for field experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	207.15	103.58	7.41	
TOO∗	4	12,701.83	3,175.46	227.20	<0.001
ROA∗	7	30,825.99	4,403.71	315.08	<0.001
TOO.ROA∗	28	6,116.97	218.46	15.63	<0.001
Residual	78	1,090.18	13.98		
Total	119	50,942.12			

Grand mean 16.62; Calculated F = 315.08; Tabulated F = 2.13

Conclusion:

The rate of application very highly significantly differed [F (7,119) = 315.08, p≤0.05] among the 8 treatments applied on *B. pilosa* at 2-4 leaves growth stage for field experiment 2.

Appendix 18: ANOVA table for B. pilosa mortality at 6-8 leaves growth stage for field experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	151.52	75.76	6.28	
TOO∗	4	9,042.38	2260.6	187.48	<.001
ROA∗	7	23,283.43	3326.2	275.86	<.001
TOO.ROA∗	28	4,620.15	165.01	13.68	<.001
Residual	78	940.48	12.06		
Total	119	38,037.97			

Grand mean 13.48

Calculated F = 275.86

Tabulated F = 2.13

Conclusion:

The rate of application very highly significantly differed [F (7,119) = 275.86, p≤0.05] among the 8 treatments applied on *B. pilosa* at the 6-8 leaves growth stage for field experiment 1.

Appendix 19: ANOVA table for B. pilosa mortality at 6-8 leaves growth stage for field experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	36.867	18.433	2.18	
TOO*	4	9720.05	2,430.013	286.98	<.001
ROA*	7	21,140.77	3,020.11	356.67	<.001
TOO.ROA*	28	4,813.817	171.922	20.3	<.001
Residual	78	660.467	8.468		
Total	119	36,371.97			

Grand mean 12.98

Calculated F = 356.67

Tabulated F = 2.13

Conclusion:

The rate of application very highly significantly differed [F (7,119) = 356.67, p≤0.05] among the 8 treatments applied on *B. pilosa* at 6-8 leaves stage for field experiment 2.

Appendix 20: Table on the relationship of the dose response experiment probit analysis for the ED_{50} determination

Confidence Limits		95% Confidence Limits for ROA		95% Confidence Limits for ROA	
		2-4 Leaves stage		6-8 Leaves stage	
LOGIT	Probability	Estimate	Estimate	Estimate	Estimate
	0.01	-4.78	-4.31	-3.72	-17.95
	0.02	-3.24	-2.22	-2.13	-12.95
	0.03	-2.33	-0.99	-1.19	-9.99
	0.04	-1.68	-0.11	-0.51	-7.87
	0.05	-1.17	0.59	0.02	-6.21
	0.06	-0.75	1.16	0.45	-4.84
	0.07	-0.39	1.65	0.83	-3.66
	0.08	-0.07	2.08	1.15	-2.64
	0.09	0.21	2.46	1.44	-1.72
	0.10	0.46	2.80	1.71	-0.89
	0.15	1.48	4.17	2.76	2.40
	0.20	2.24	5.21	3.55	4.88
	0.25	2.87	6.06	4.20	6.92
	0.30	3.42	6.81	4.77	8.71
	0.35	3.91	7.48	5.28	10.34
	0.40	4.38	8.12	5.77	11.86
	0.45	4.83	8.72	6.23	13.31
	0.50	**5.27**	**9.32**	**6.69**	**14.96**
	0.55	5.71	9.92	7.14	16.17
	0.60	6.15	10.52	7.60	17.62
	0.65	6.62	11.16	8.09	19.14
	0.70	7.12	11.83	8.60	20.77
	0.75	7.67	12.58	9.17	22.56
	0.80	8.30	13.43	9.83	24.60

0.85	9.06	14.47	10.61	27.08
0.90	10.07	15.84	11.66	30.37
0.91	10.33	16.18	11.93	31.20
0.92	10.61	16.57	12.22	32.12
0.93	10.92	16.99	12.54	33.14
0.94	11.28	17.48	12.92	34.31
0.95	11.70	18.05	13.36	35.69
0.96	12.22	18.75	13.88	37.35
0.97	12.87	19.63	14.56	39.47
0.98	13.78	20.86	15.50	42.43
0.99	15.31	22.95	17.09	47.43

Results of the probit analysis showing the effective concentration at 50% (ED_{50}) for the dose response treatments (ED_{50} = 5.27 and 9.32 Lt/Ha at 2-4 leaves and 6.69 and 14.96 Lt/Ha at 6-8 leaves stage
(ROA = Rate of concentration)

Appendix 21: Table on the relationship of the field experiment probit analysis for the ED_{50} *determination*

Confidence Limits		95% Confidence Limits for ROA		95% Confidence Limits for ROA	
		2-4 Leaves stage		6-8 Leaves stage	
LOGIT	Probability	Estimate	Estimate	Estimate	Estimate
	0.01	-12.339	-4.311	-17.951	-17.951
	0.02	-8.455	-2.225	-12.947	-12.947
	0.03	-6.159	-.991	-9.990	-9.990
	0.04	-4.513	-.107	-7.869	-7.869
	0.05	-3.223	.586	-6.207	-6.207
	0.06	-2.158	1.158	-4.835	-4.835
	0.07	-1.247	1.647	-3.662	-3.662
	0.08	-.450	2.075	-2.635	-2.635
	0.09	.261	2.457	-1.720	-1.720
	0.10	.904	2.802	-.892	-.892
	0.15	3.459	4.175	2.400	2.400
	0.20	5.382	5.208	4.878	4.878
	0.25	6.971	6.061	6.924	6.924
	0.30	8.359	6.807	8.712	8.712
	0.35	9.619	7.484	10.336	10.336
	0.40	10.799	8.117	11.855	11.855
	0.45	11.930	8.725	13.312	13.312
	0.50	**13.038**	**9.320**	**14.964**	**11.423**
	0.55	14.146	9.915	16.167	16.167
	0.60	15.277	10.523	17.624	17.624
	0.65	16.457	11.156	19.144	19.144
	0.70	17.717	11.834	20.768	20.768
	0.75	19.105	12.579	22.556	22.556
	0.80	20.694	13.432	24.602	24.602
	0.85	22.617	14.466	27.080	27.080
	0.90	25.172	15.838	30.371	30.371
	0.91	25.815	16.183	31.199	31.199
	0.92	26.526	16.565	32.115	32.115
	0.93	27.323	16.993	33.142	33.142

0.94	28.233	17.482	34.315	34.315
0.95	29.299	18.055	35.687	35.687
0.96	30.589	18.748	37.349	37.349
0.97	32.235	19.632	39.469	39.469
0.98	34.531	20.865	42.427	42.427
0.99	38.415	22.951	47.430	47.430

Results of the probit analysis showing the effective concentration at 50% (ED_{50}) for the field treatments (ED_{50} = 13.04 and 9.32 Lt/Ha at 2-4 leaves and 14.96 and 11.42 Lt/Ha at 6-8 leaves stage.
(ROA = Rate of concentration)