INVESTIGATION OF DETERMINANTS OF *Striga hermonthica* SUPPRESSION AND EVALUATION OF FUNGAL ISOLATES AS POSSIBLE BIOCONTROL AGENTS AGAINST THE WEED

A thesis submitted in partial fulfillment of the requirements for the award of Master of Science in Microbiology and Biotechnology at the University of Nairobi.

By;

OMONDI VICTOR KAGOT

I56/69609/2011

SCHOOL OF BIOLOGICAL SCIENCES

UNIVERSITY OF NAIROBI

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DECLARATION

I hereby declare that this is my original work and has not been presented for a degree in any other University.

Signature: _____ Date: _____

Mr. VICTOR O KAGOT

SUPERVISORS;

This thesis is submitted with our approval as the supervisors;

Prof. SHEILA OKOTH F.S.B

UNIVERSITY OF NAIROBI - SBS

P. O. Box 30197-00100, NAIROBI.

Signature …….. Date……

Dr. FRED KANAMPIU

CIMMYT-KENYA

P.O. Box 1041-00621, NAIROBI.

Signature ….....….. Date……

Dr. PETER OKOTH

CIAT-KENYA

P.O. Box 30677-00100, NAIROBI.

Signature …….. Date……

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DEDICATION

To my beloved mum (Rose Wayona) and dad (John Kagot) who have always encouraged me in my pursuits.

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LIST OF ABBREVIATIONS

CIMMYT – International Maize and Wheat Improvement center.

CIAT – International Center for Tropical Agriculture.

KARI – Kenya Agricultural Research Institute.

IR OPV- Open Pollinated Variety maize seeds coated with imazapyr herbicide.

IR Hybrid – Hybrid maize seeds bred from two parents coated with imazapyr herbicide.

WH 403 – A commercial maize variety suitable for mid altitudes susceptible to *Striga.*

DAP – Diammonium phosphate

CAN – Calcium ammonium nitrate

PPM – Parts per million

ME% - Milliequivalent %

NaOCL – Sodium hypochlorite

ABSTRACT

Striga hermonthica is a parasitic weed which compensates for its lack of an elaborate root system by invading the roots of a host plant sucking away nourishment intended for the host. The weed is a major constraint to maize production in Western Kenya, where it mostly invades small scale farms and depending on the severity, damage caused by the weed can range from 10% to 100%.

Measures recommended for the control of this weed such as the use of chemical fertilizers and herbicides are very expensive and therefore out of reach for farmers. This study aimed at testing different cropping systems and use of different maize varieties as possible options for control of the weed and evaluating fungal isolates pathogenic to the weed for their efficacy as possible biocontrol agents against the weed.

Striga hermonthica seeds were inoculated in plots of maize intercropped with *Desmodium uncinatum,* maize intercropped with groundnuts, pure maize and maize rotated with soybeans in CIMMYT experimental stations in Kibos (Kisumu County) and in Alupe (Busia County). The maize varieties planted were: Open pollinated maize variety coated with imazapyr herbicide (IR OPV), a hybrid variety bred from two parents coated with imazapyr herbicide (IR Hybrid), *Striga* resistant hybrid and a commercial maize variety susceptible to *Striga* (WH 403). Similar experiments were set up in Kibos and Alupe. Emerged *S. hermonthica* were counted in the two experimental blocks. Soil samples collected from the two blocks were analyzed for their physical chemical and biological properties and their means compared using ANOVA tests. Fungi were isolated from diseased *S. hermonthica* and their virulence efficacy against the weed tested in the greenhouse.

The number of weeds was higher in Kibos than Alupe. After twelve weeks of growth 4410weeds had germinated in Kibos while 1771 weeds had germinated in Alupe. This can be attributed to differences in the soil parameters assessed in the two benchmark sites. Increase in soil nitrogen content is known to discourage germination of the weed since nitrogen can limit the production of a *Striga* germination stimulant called strigolactone and this was the case in Alupe which had 0.15% nitrogen content in the soil compared to Kibos which had 0.08% nitrogen content in the soil. The levels of organic carbon was significantly higher in Alupe compared to Kibos with Alupe having 1.36% organic carbon content in the soil when Kibos had 0.75% organic carbon content in the soil. On the other hand, the levels of phosphorus, manganese, magnesium, calcium, and zinc were significantly higher in Kibos compared Alupe. Sandy soils are known to encourage the growth of the weed while clay soils are known to discourage the growth of the weed by lowering the temperature and diluting the germination stimulant and this was the case in Alupe which had clay soils while Kibos had sandy soils.

The different cropping systems and maize varieties planted influenced the emergence of the weed. Maize intercropped with *Desmodium uncinatum* had the least number of the weed while maize rotated with soybeans had the highest number of the weed. WH 403 was the most susceptible maize variety to the weed having the highest number of the weed while IR OPV and IR Hybrid had the least numbers of the weed. All the fungal isolates tested against the weed caused infection and consequently death of the weed. *Fusarium incarnatum* had the highest infection rate of 92% while *F. oxysporum* had the least infection rate of 76%. To the contrary, *F. oxysporum* caused the highest mortality of 60% with *Gibberella intricans* causing the least mortality of only 36%.

Maize intercropped with *Desmodium uncinatum* was the best cropping system in managing the weed while IR hybrid and IR OPV were the best maize varieties in managing the weed. Nitrogen was the most important nutrient with direct effects on the weed. *Fusarium oxysporum* was the most aggressive fungus against the weed portraying its potential for exploitation as a biocontrol agent against the weed.

Key words: *Striga hermonthica*, cropping systems, maize varieties and biocontrol.

CHAPTER ONE

1.1 INTRODUCTION

Striga also known as witch weed is a parasitic weed of plants mainly in the grass family, especially cereals and certain food legumes, for example, cowpeas (*Vigna unguiculata*). *Striga* exists as an obligate parasite in its early life obtaining nourishment from the host plant. It first attaches itself to the roots of its host and then penetrates the phloem and xylem from where it drains off water and nutrients, impairs photosynthesis and causes a phytotoxic effect in a matter of days. This stage of *Striga* growth is known as below ground growth and results into damage of the host crop manifested by stunted growth and a reduction in crop yields (Gacheru et al., 2002). Later in life, after emerging from the soil, the weed becomes a facultative parasite, photosynthesizing approximately 20% of its growth requirements and obtaining the rest from its host (Watson et al., 2007).

Striga can infest a variety of crops, however, the most affected crops in the sub Saharan Africa are staple foods planted by small scale or subsistence farmers, and they include: maize, sorghum, pearl millet, upland rice, sugarcane and cowpeas. Large scale farmers on the other hand, are less affected due to the heavy investment they put in their farms in terms of fertilizers and herbicides which discourages the weed. Oswald, (2005) reported that the weed thrives well in environmental conditions with rainfall between 500 and 2000 mm per annum, an altitude range from the sea level to 1600m and in almost all soil types. Therefore the weed can be found in most areas south of the Sahara with these conditions. The *Striga* menace, in Africa, is further fueled by its excellent survival strategy in the semi-arid conditions of the tropics, coupled with its high fecundity and the persistence of its seed reserves in the tropical soils (Ejeta and Gressel,

2007). *Striga* sets itself apart from other weeds in that, it is both a weed and a parasite. As a parasite, it actually lives at the expense of its host causing damage that is more severe than any other non parasitic weed. It basically drains the life out of its host by poisoning and attenuating it and therefore perpetuating its own growth (Jamil et al., 2012).

There are several species of *Striga* worldwide, of which only a few result in the destruction and the reduction of cereal production in the sub Saharan Africa. In Kenya, the prevalent species are *Striga hermonthica* (Delile) Benth (purple flowered) in the Western Kenya region and *Striga asiatica* (L) Kuntze (red flowered) in the Coastal areas. Of the two, *S. hermonthica* is the most noxious and it causes severe damage to the farming systems in Western Kenya (Gacheru et al., 2002). The weed has been recognized by the natives to be a real threat to cereal crop production and the affected communities have their vernacular names for the weed: in the Luo language it is called *kayongo,* in Luhya it is called *oluyongo* and *kichawi* in Swahili.

Striga infestation has been in Western Kenya region since 1936, and the subsistence farmers in the region have bore the brunt of the weed, their poor farming practices have perpetuated the spread of the weed (Atera, 2012). For example, the weed will thrive well in farms with declining fertility, when there are limited agricultural inputs and where mono-cropping is practiced. Once such areas are infested, the weed accelerates the plight of the destitute farmers by declining the yields. Based on the degree of infestation, susceptibility of the host plant and the prevailing environmental conditions, damage caused by the weed can range from 10% to total crop failure in severe situations (Jamil, *et.al*, 2012).

According to Jamil *et.al,* (2012), *Striga* causes an annual cereal loss in the sub-Saharan Africa worth US$ 7 billion. In the Western Kenya Region, losses in maize yield of up to 81% have been

documented and it is also estimated that 1000 *Striga* weeds per hectare can result into 2-3 kg loss in sorghum yield and probably even more in maize (Esilaba, 2006). A typical example is the case of a farmer from the Sega area in Siaya County-Western Kenya, who was quoted saying, "Previously, I harvested up to fourteen 90-kilogramme bags of maize per half a hectare. But due to the infestation of the weed, which I was not able to control, I harvested a total of two and a half bags of maize from my field." (Esipisu, 2012). Farmers are sometimes forced to abandon their farms under severe infestation. This last resort action jeopardizes the struggles to attain food security and economic development in the region (Ejeta and Gressel, 2007).

Several strategies have been tried in *Striga* management, for example, the use of herbicides, manures and chemical fertilizers. However, these approaches are too expensive and are not readily available for subsistence farmers in the developing world and in particular Kenya (Atera, *et.al*, 2013). Hence techniques which would be readily available and affordable to the resource limited famers are urgently needed to alleviate the *Striga* menace so as to improve the lively-hoods of these farmers.

This project aimed at testing alternative, suitable, sustainable and affordable control measures against the weed. The influence of soil physical, chemical and biological properties on the emergence of the weed, the effects of different cropping systems and maize varieties on emergence of the weed and the possibility of an augmentative approach of naturally occurring fungal pathogens as biocontrol agents against the weed were investigated.

1.2 JUSTIFICATION OF THE STUDY

Agriculture is the mainstay economic activity in Western Kenya region. However, *Striga* infestation has become a real threat to agriculture in that region. In the Lake Victoria Basin alone, 0.24 million ha of arable land which is 15% of the total arable land in the region is infested with this weed. This causes yield losses between 10% to total crop failure or monetary losses of US$ 41 million (Jamil et al., 2012). In many cases the damage caused by the weed is so severe that a farmer can only harvest a paltry 500kg of maize instead of 5,000kg in a one-hectare farm or zero yields under heavy infestation (Olwenya, 2012).

This situation is getting worse and the factors aggravating the *Striga* menace are; continuous mono-cropping of maize and sorghum, little effort to control the weed and the increasing population which is pilling pressure on the already limited arable land. These in return, lead to a decline in the soil fertility, thus creating favorable conditions for the weed to flourish, and consequently increasing the weed infestation (Esilaba, 2006). These factors are predisposing crop production in the region to an impending crisis as the deadly weed is rapidly spreading to even highland areas previously thought to be immune to the weed (Gacheru et al., 2002).

The recommended control measures, for example; the use of herbicides, chemical fertilizers and manures have not been effectively integrated and adopted by the farmers as control strategies against the weed due to their high prices. Most farmers practice hand weeding as a control measure against the weed, however, it is a labor intensive exercise with no immediate yield benefits and it can increase the soil seed bank with inappropriate disposal of the uprooted weeds (Atera et al., 2013). Hence it is not preferable and therefore ineffective in the long run. Therefore there is an urgent need to develop effective control strategies which will be affordable and easy to use by the farmers.

This project aimed at testing possible alternative methods of managing the weed. The effects of soil type, cropping system and maize variety on *Striga* emergence in the CIMMYT experimental stations in Kibos and in Alupe were investigated. Naturally occurring fungal pathogens were also isolated from diseased *Striga* and tested for their ability to control the weed.

1.3 STATEMENT OF THE PROBLEM

Striga hermonthica infestation is prevalent in small scale farms in Western Kenya reducing yields of the planted crops by more than 50% each season. The currently recommended control strategies against the weed are ineffective due to the big mismatch between the cost of the control strategies and the farmers' socioeconomic status.

1.4 HYPOTHESES

- Type of cropping systems employed influence the emergence of *Striga hermonthica*.
- The emergence of *Stiga hermonthica* is influenced by the variety of maize planted.
- Soil physical, chemical and biological properties influence the emergence of *Striga hermonthica.*
- Fungal strains pathogenic to *Striga hermonthica*, are virulent to the weed.

1.5 OBJECTIVES

1.5.1 Overall objective:

To test the effects of soil parameters, cropping systems and maize varieties on *Striga hermonthica* emergence and the efficacy of fungal isolates as possible biological control agent against the weed.

1.5.2 Specific objectives:

- To test the effects of four cropping systems on *Striga hermonthica* emergence – maize intercropped with ground nuts, maize intercropped with *Desmodium uncinatum*, maize rotated with soybeans and pure maize.
- To evaluate the effects of four maize varieties on *Striga hermonthica* emergence – IR Hybrid, IR OPV, WH403 and *Striga* resistant Hybrid.
- To determine the influence of soil physical, chemical and biological parameters on emergence of *Striga hermonthica* in Alupe and Kibos.
- To isolate and identify fungal strains from *Striga hermonthica* and asses their virulence efficacy on the weed in the greenhouse.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 General characteristics of *Striga hermonthica*

Striga is a parasitic angiosperm which causes severe constrains in cereal crop production in the sub Saharan Africa by parasitizing the roots of the host crop (Atera, 2012). The parasite, attaches itself to the roots of its host from where it withdraws nutrients and water intended for the plant to grow. Several species of *Striga* have been identified worldwide with *Striga hermonthica* being the most notorious and causing damage to the agro economic systems in Kenya and especially in Western Kenya. *Striga hermonthica* is an herbaceous plant with a fibrous four sided stem. Its height does not exceed 1 meter. It has purple/violet flowers though they are sometimes white. The flowers are arranged in spikes of about 15 to 45 cm long. The seeds usually develop in small capsules which upon maturity, burst open to release the seeds for dispersal. One seed capsule can contain between 250 and 500 minute seeds. A single *S. hermonthica* plant therefore, has the capacity to produce over 50,000 seeds (Gacheru et al., 2002). If conditions for germination are not conducive the seeds can remain viable in the soil for up to 20 years (Jamil et al., 2012).

2.1.2 Geographical distribution of *Striga hermonthica*

Striga is mainly found in the tropical-arid and semi-arid zones of Africa, Asia, Australia and America. *S. hermonthica* is believed to have originated from the Nubian hills of Sudan and Semien mountains of Ethiopia. In Africa, the most severely affected countries are Mali, Burkina Faso, Niger, Nigeria, Cameroon, Chad, Sudan and Ethiopia. In other African countries like; Kenya, Gambia, Senegal, Mauritania, Togo, Ghana, Tanzania, Uganda, Botswana, Swaziland and Mozambique only some regions of these countries are affected, where the weed causes yield

losses of more than 50% each season (Faisal, 2011). In Kenya, the Western Kenya region is the most affected (figure 1).

Figure 1: Map of Kenya; districts prone to *Striga hermonthica* infestation in the Western Kenya Region (adopted from Manyong et al., 2007).

KEY:

- The districts in accent red are the districts prone to *Striga hermonthica* in formerly Nyanza province now Kisumu, Migori and Homabay counties.

- The small green squares are *Striga hermonthica* hotspots.

 - The districts in orange are the districts prone to *Striga hermonthica* in formerly Western province now Kakamega, Busia and Bungoma counties.

 - The black lines are district demarcations.

The population dynamics and distribution of *S. hermonthica* can be attributed to the environmental conditions and cropping history of a particular farm. The weed establishes itself preferentially in nutrients deficient fields which have been exhausted by continuous utilization without amendments over time (Atera et al., 2011).

2.1.3 Taxonomy of *Striga hermonthica*

Striga hermonthica is a flowering plant occurring naturally in parts of Africa, Asia and Australia. Table 1 below shows the taxonomic classification of *S. hermonthica* adopted from the United states Department of Agriculture (2012).

Table 1: Taxonomic classification of *Striga hermonthica*

Taxonomic Rank	**Scientific name**	**Common name**
Kingdom	*Plantae*	Plants
Subkingdom	*Tracheobionta*	Vascular plants
Superdivision	*Spermatophyta*	Seedplants
Division	*Magnoloiphyta*	Flowering plants
Class	*Magnoliopsida*	Dicotyledons
Subclass	*Asterdae*	
Order	*Scrophulariales*	
Family	*Scrophlariaceae*	Figwort family
Genus	*Striga*	Lour- witch weed
Species	*Striga hermonthica*	(Delile) Benth – purple witch weed

2.1.4 Life cycle of *Striga hermonthica*

In the soil, the *Striga* seeds require moisture for about two weeks before they are ready to germinate. The seeds will germinate in response to a chemical stimulant (strigolactones) produced by the host plants. A second chemical produced by the host usually aid attachment and penetration of the germinating seedling into the host roots within 3-8 days. This stage of development of *Striga* is known as below ground and the weed entirely depends on the host from which it obtains its nourishment. After 3-6 weeks of below ground growth, the weed emerges above the ground. However, some seedlings may still remain below ground. After 3-4 weeks of over ground growth the plant produces purple flowers followed by seeds 14 days later. The seeds usually develop inside capsules and once they have developed they might mature to form viable seeds even if the plant is uprooted at that stage. The *Striga* seeds are very small and they are usually dispersed by contaminated farm tools, eroded soil, surface run-off, wind, animals and people and once the seeds become established in the soil the cycle continues (Gacheru et al., 2002). It is therefore important that uprooted *Striga* plants should be burned to mitigate the spread of the seeds.

2.1.5 The effects of soil parameters on *Striga hermonthica*

The soil status, in relation to the physiochemical and the biological parameters usually play a crucial role in determining *S. hermonthica* infestation. The infestation of the weed can be considered as an indicator of low fertility and nutrients depletion (Oswald, 2005). The germination of *S. hermonthica* is normally dependent on release of strigolactones by the host plant. Under lower availability of nitrogen and phosphorus rice was found to release more stigolactones and consequently inducing more *Striga* germination. Upon addition of these essential nutrients, less strigolactones were released and hence a reduced *Striga* emergence was

observed (Jamil et al., 2012). Addition of organic matter in the soil is also believed to reduce *Striga* germination by enhancing the decay of the *S. hermonthica* seeds in the soil. However, contradictory results indicate that organic matter may have little influence on *Striga* development in the soil (Ayongwa et al., 2011).

2.1.6 Control strategies of *Striga hermonthica*

According to Oswald, (2005) *S. hermonthica* control methods in Kenya, can be grouped as direct and indirect methods. Direct methods attack the weed directly and even though they immediately reduce the density of the weed in the field they usually don't have much effect on the crop yield in the same season. Examples include: planting resistant varieties, the use of chemicals, the application of biological control agents, the use of catch crops, the use of herbicides and hand weeding. On the other hand the indirect methods are those methods that control the parasite by making its growth conditions less favorable thereby reducing the seed and the weed densities over time. For the indirect methods to be effective several cropping seasons of practicing the method are required. Examples of the indirect *S. hermonthica* control methods include: improved fallow management, adding of organic and inorganic amendments to the soil, intercropping and crop rotation. In Kenya, hand pulling is the widely used control method; however it is not effective because it is time consuming and extremely labor intensive. The control and management of *S.hermonthica* is difficult because it causes most damage to the host during its underground growth stage. If it is not detected before emergence, it is too late to prevent crop loss because the damage is usually already done (Gacheru et al., 2002).

2.1.7 Beneficial uses of *Striga hermonthica*

In the African traditional medicine, *S hermonthica* has been widely used as a remedy to many ailments. For example it can be used as a remedy for leprosy, ulcers, pneumonia and jaundice. It

also has trypanocidal, antiplasmodial and antibacterial effects. The plant is also believed to be an antioxidant due to its rich content of phenolic compounds (Hammad et al., 2011). However, in agriculture, the damage it causes as a weed overrides its purported health and medicinal benefits.

2.2 Biological control of weeds

Biological control is the use of living natural enemies (parasites, predators, pathogens) – to control pests, weeds and diseases. Of interest in this study is to find a suitable fungal isolate that can be used to augmentatively control *S. hermonthica*. As biological control agents the antagonistic nature of fungi is exploited to reduce the effects of the undesirable pests and weeds and favor the desired crops. As an antagonistic organism fungi employs mycoparasitism, antibiosis and cell wall degrading enzymes to invade its host, acquire the nutrition it requires and consequently kill the host. These features make fungi excellent candidates for use as biological control agents against *S. hermonthica*. Three approaches can be employed in biological control and they include: cultural practices that favor existing antagonistic organisms, the classical approach and the augmetive approach where hypovirulent organisms are inoculated to immediately reduce the pest and weed population.

Herbicides have been used to control weeds but they are becoming less attractive due to their negative environmental impacts. According to Elzein et al., (2008) the use of mycoherbicides is more advantageous in that they are more host-specific, less expensive and they are more environmentally friendly. Two fungal strains of *Fusarium oxysporum*, the Foxy 2 and the PSM197 have been found to be aggressive against *S.hermonthica*. However, their potential has not been fully harnessed due to the unique storage facilities required by this fungus (Elzein et al., 2008). *Cubitermes* termites mound amendments to the soil also have the ability to reduce damage done by *S. hermonthica*, however, it is very difficult to collect enough mound for use in

large scale (Andrianjaka et al., 2007). Examples of fungi which have been successfully developed into mycoherbicides, include: *Cercospora rodmanii* a mycoherbicide against water hyacinth (*Eichhornia crassipes*) which is available in the USA as ABG5003. *Phytophthora palmivora* a mycoherbicide against milk weed vine (*Morrenia odorata),* which is available in the trade name De VINE. *Alternaria cassia* a mycoherbicides against sickle weed (*Cassis obtusifolia),* which is available in the trade name CASST. Apart from fungi, insects can also be exploited as biological control agents against weeds for example Lantana bug (*Ortheza insignis*) and Lantana caterpillar (*Lannophaga pusillidactyla*) can suppress the growth of Lantana (Bhatnagar, 2011).

2.3.0 The effects of intercropping on weeds

Intercropping is the practice of growing more than one crop in the same field simultaneously. The crops are usually grown in alternating rows with the main objective of maximizing the output from that field. In Kenya, the most practiced intercrop system is cereal intercropped with legume for example maize intercropped with beans. This cropping system is widely accepted and practiced because it is purported to improve food security, soil fertility and maximize use of limited farm land (Odhiambo et al., 2011). Intercropping generates beneficial biological interactions between crops thus improving the plant health and increasing the yield. Intercropping can also play a vital role in weed control, this is because; some intercrop plants can release allelopathic compounds which can hinder the growth of the weeds. Intercropping also provides an efficient utilization of environmental resources thus depending on the availability of the environmental resources, the growth of weeds can be decreased (Eskandari and Kazemi, 2011). This project will investigate various intercrop systems so as to find out the best intercrop system in *S. hermonthica* management in western Kenya.

CHAPTER THREE

3.0 METHODOLOGY

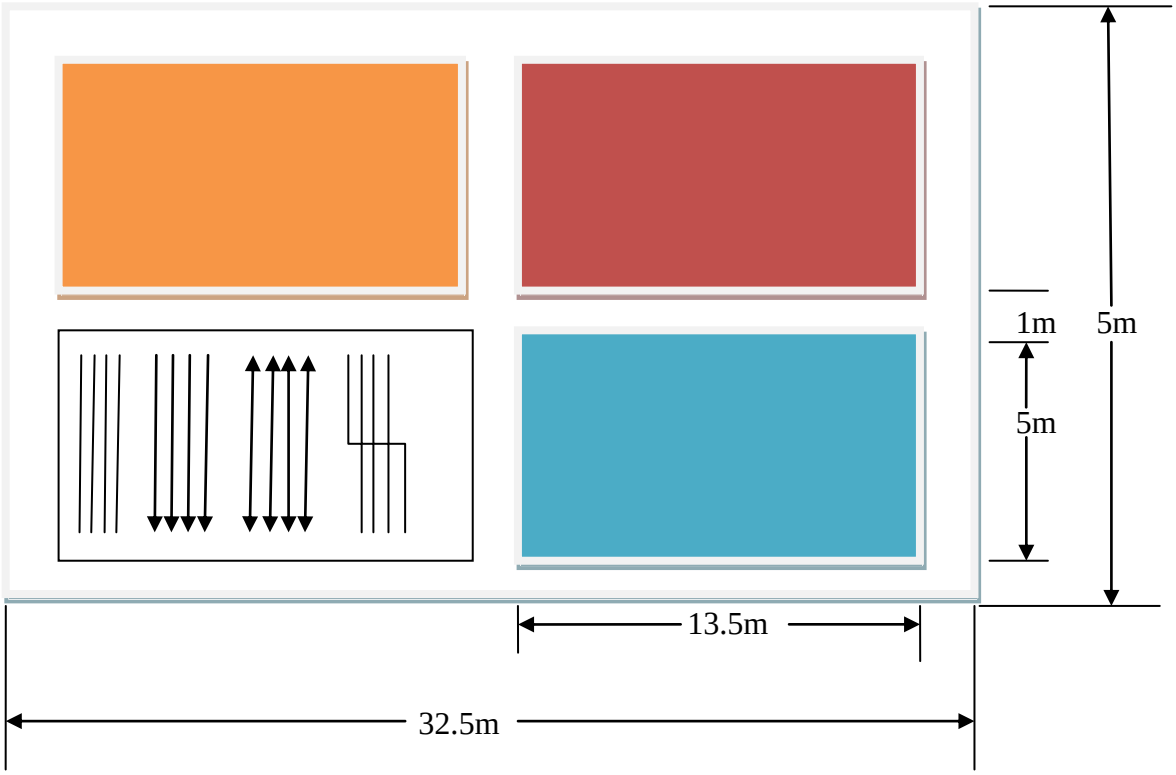
3.1 Study area

The study was conducted between March 2012 and February 2013 in CIMMYT experimental stations in Alupe and in Kibos. Alupe is located in Busia County Western Kenya. Its geographical coordinates are 0^o 29´ N, 34^o 07´E and it is 1189 meters above sea level. The region receives approximately 1148mm of rainfall annually with an annual mean temperature of 29^oC. (Atera, 2012). Kibos on the other hand, is situated in Kisumu County; its geographical coordinates are 0° 40' S 34° 49' E and it is 1135 meters above sea level. The region receives approximately 1287mm of rainfall annually with an annual mean annual temperature of 22.3 °C (Climate data, 2012; Maplandia, 2012). The two benchmark sites have different soils, Kibos having loamy sandy soils while Alupe has red clay soils (Apollo n.d).

3.2 Study design

Four cropping systems or plant combinations were tested to evaluate the effect of plant cover on the emergence of *S. hermonthica*. The cropping systems were: maize intercropped with *Desmodium uncinatum*, maize intercropped with groundnuts, maize rotated with soybeans and pure maize. Four maize varieties were also tested for their influence on the emergence of the weed. These were; IR Hybrid- seeds coated with imazapyr herbicide, bred from two parents (inbred), cannot be replanted and have a narrow genetic base. IR OPV – seeds coated with imazapyr herbicide, open pollinated variety bred from many parents, can be replanted; they have a wide genetic base. *Striga* resistant hybrid (STR HYB) – bred to resist *Striga*. WH 403 – a commercial maize variety susceptible to *Striga*. Each maize variety was planted in four replicates in each cropping system. This set up constituted a plot and each plot was in three replicates

(Figure 2). This design was laid down in Kibos and in Alupe. The whole farm was 97.5 meters long and 33 meters wide in each benchmark site. Each plot measured 32.5×11m with the subplots for each cropping system measuring 13.5×5m. A single row of IR OPV was planted around each maize variety as the guard road and two rows around each subplot. Between the maize varieties within the subplots a 1m space was maintained. The *S. hermonthica* seeds planted in the two benchmark sites were harvested from the previous planting season in Alupe while the maize seeds planted were purchased from Kenya Seed Company; seeds for IR Hybrid and IR OPV were coated with imazapyr prior to planting.



KEY:

- Maize intercropped with groundnuts.
- Maize intercropped with *Desmodium uncinatum*.
- Maize rotated with soybeans.
- Pure maize.

- IR- Hybrid.
– IR – OPV.
- WH 403.
- *Striga* resistant hybrid

Figure 2: Experimental layout of a plot

3.3 Planting of maize and *Striga hermonthica* in the experimental blocks

The land was prepared by digging using a jembe. Planting was done in April 2012 in the bench mark sites. Maize was planted with 1 teaspoon of diammonium phosphate (DAP) fertilizer per hole placed at the side of the hole. The insecticide-Regent 3G was also applied at planting by mixing it with the soil used to cover the hole. The spacing applied was 75x50 cm and for each hill 2 maize seeds were planted and later thinned to 1plant per hill. The planting density was 66,666 plants/ha. For each maize hole 2000 to 3000 *S. hermonthica* seeds were also inoculated at planting, since the *Striga* seeds had not been tested for viability 2000 to 3000 seeds were planted so as to rule out incidences of *Striga* not germinating. Planting of intercrops was also done at the same time with maize. Foliar spray called synergise^{R} was applied 4 and 6 weeks after planting. Top dressing using calcium ammonium nitrate (CAN) fertilizer was done 8 weeks after planting. Weeding was done 4 times. The first weeding was done 3 weeks after planting using a jembe. The second, third and fourth were done by hand at weeks 6, 8 and 10 after planting respectively so as not to interfere with *S. hermonthica* emergence. All weeds were removed except *S. hermonthica.* The system was rain fed.

3.4 Determination of *Striga hermonthica* weed density in Alupe and Kibos

Based on the developmental and morphological features as described by Ramaiah et al., (1983) and Parker and Riches (1993), the emerged *S. hermonthica* plants were identified and counted at weeks; 8,10,12 and 15 after planting.

3.5 Assessment of the soil physical, chemical and biological parameters in the experimental blocks

3.5.1 Collection of soil samples from the experimental blocks

The soil samples were collected 15 weeks after planting. Soil from each subplot and within each maize variety in each replicate plot was sampled, so as to get adequate data pertaining to the soil physical, biological and chemical parameters. The top soil was augured at a depth of 0-25 cm and 500g of soil collected. Soil samples were collected from three spots randomly selected for each maize variety and then mixed to form a composite soil sample representative of each maize variety. This was done for all replicates in the two benchmark sites.

3.5.2 Soil sample preparation and analysis

The soil samples were air-dried, crushed and screened through a 2 mm sieve. The samples were then sent to the Kenya Agricultural research institute (KARI) laboratory for analysis as described by IITA, (1982). The soil parameters that were tested included: macro and micronutrients, soil pH, soil texture, amounts of bacteria, actinomycetes and fungi in g /dry weight.

The soil pH was determined in a 1:1 (w/v) soil-water suspension with a pH meter. The organic carbon was analyzed by dichromate oxidation according to Walkley and Black, (1934). The total N in the soil was determined by the Kjeldhal digestion (Bremner and Mulvaney, 1982). Available nutrient elements; potassium, phosphorus, sodium, calcium, magnesium and manganese were extracted using Mehlich-3 extracting solution containing 0.02 M acetic acid, 0.25 M ammonium nitrate, 0.01 M ammonium fluoride, 0.01 M nitric acid and 0.001 M EDTA (Mehlich, 1984). Levels of elements such as potassium, sodium and calcium were determined by flame photometry while levels of elements such as phosphorus magnesium and manganese determined spectrophotometerically. The available trace elements were extracted with 0.1M

hydrochloric acid and the levels of iron, zinc and copper determined with atomic absorption spectrophotometer. Soil texture and coarse fragments was determined according to Eriksson and Holmgren, (1996) while the soil particle size distribution was determined using guidelines authored by FAO (2006). The colony forming units of bacteria, fungi and actinomycetes were estimated according to Stotzky et al., (1993).

3.6 Isolation and characterization of fungal species from diseased *Striga hermonthica* and assessing their virulence efficacy against the weed

3.6.1 Isolation of fungal species infecting *Striga hermonthica*

Diseased *S.hermonthica* plants showing necrosis, spots on the leaves and stem, wilting and other abnormalities were uprooted, placed in brown paper bags and transported to the laboratory. The diseased parts were cut into pieces of about 4-7mm, surface-sterilized with 1% sodium hypochlorite (NAOCL) for 1½ minutes and rinsed 3 times in sterile distilled water. The cut plant parts were then dried with sterilized filter papers and some placed on Peptone PCNB Agar (PPA) and others on potato dextrose agar (PDA) half strength (Rahjoo et al., 2008). For purification and subsequent morphological identification of the fungus which grew, the isolates were transferred on to potato-dextrose agar (PDA), Spezieller Nahrstoffarmer Agar (SNA) and Carnation Leaf Agar CLA (Kwasna and Bateman, 2007). All the cultures were incubated at 25ºC for two to four weeks. Cultural characters were assessed by eye and by microscopic examination. Colony morphology was recorded from cultures grown on PDA while the morphology of macroconidia, microconidia, conidiogenous cells and the chlamydospores was assessed from cultures grown on SNA, PDA and CLA media. Morphological identifications of the *Fusarium* isolates were made using the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006). The non *Fusarium* isolates were identified using Dugan and Dugan (2006) identification key.

3.6.2 Molecular characterization of the isolated fungi

3.6.2.1 DNA extraction

The fungal isolates were grown on PDA plates for 7 days. The mycelia were harvested and resuspended in nuclease free water. Total DNA was extracted from the resuspended mycelia of each isolate (50-100 mg wet weight) using a ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo research, South Africa) according to the manufacturer's instructions.

3.6.2.2 PCR Amplification

The PCR amplifications were performed using the DreamTaq Green PCR Master mix (Thermo Scientific). The amplification reactions were carried out in volumes of 50 µL containing; 25 µL DreamTaq Green PCR Master mix, 1 µM of each primer i.e the forward primer and the reverse primer, 1µg of the template DNA and 23 µL of nuclease free water. The PCR reaction was carried out in a thermal cycler as follows: 1) 1 cycle of initial denaturation at 95^0C for 3 minutes; 2) 35 cycles of the following: denaturation at 95ºC for 30 seconds, annealing at 57ºC for 30 seconds and extension at 72°C for 1 min; 3) final extension at 72°C for 10 min, followed by cooling at 4°C until recovery of the samples. Amplification products were visualized in1.2% agarose gels stained with ethidium bromide. The gel was photographed under UV light at 254 nm (Kwasna and Bateman, 2007). The primers used in the PCR were; TEF primers (TEF1 5'-ATGGGTAAGGARGACAAGAC-3' and TEF2 5'-GGARGTACCAGTSATCATGTT-3') for the *Fusarium* species and ITS primers (ITS F 5 '-AACTCCCAAACCCCTGTGAACATA-3' and ITS R 5 '-TTTAACGGCGTGGCCGC-3') for the non *Fusarium* species. The PCR products were then sequenced and edited using the geneious program. The obtained DNA sequences were blasted using the NCBI BLAST (National Center for Biotechnology Information - Basic Local Alignment Search Tool) to reveal their identities. To reveal the relatedness of the isolates

alignment of all the sequences was done using Clustalx 2.1 software and Phylogenetic analyses conducted using MEGA version 5.1 using the Neighbour Joining (NJ) method (Tamura et al., 2011). In the NJ analysis, distances were calculated using the Kimura 2-Parameter model and bootstrap tests performed with 1,000 replications (Tamura et al., 2004; Felsenstein, 1985)

3.6.3 Green house tests for virulence efficacy of the fungal isolates on *Striga hermonthica*

3.6.3.1 Inoculum preparation

Seven days old fungal hyphae of was scraped into Armstrong medium and incubated in an incubator shaker at 25^{o}C at 100rpm for 5 days to produce spores. The spores were filtered through two layers of sterile cheesecloth into a 50ml falcon tube and centrifuged at 3500rpm for 10minutes, the supernatant was discarded and the pellet (conidia) washed twice with de-ionized autoclaved water. The spores were suspended in 350ml sterile distilled water. With the aid of a haemocytometer the conidia concentration was adjusted to 2×10^{6} conidia per ml. Tween 20 surfactant (Polyoxyethylene 20-sorbitan monolaurate) was added to the conidial suspension before inoculation at the rate of 3 drops per liter (Booth, 1971).

3.6.3.2 Green house trials

Maize was grown in 5 liter plastic pots, in a greenhouse at the CIMMYT experimental center in Kibos. The pots were filled with 5 kg of soil mix comprised of manure, sand and clay in a ratio of 2:5:1. Five replicates were used for each fungal isolate being tested. Prior to sowing, the pots were infested by mixing 2000 to 3000 *S. hermonthica* seeds into the soil layer 5-10 cm below the surface. Prior to inoculation, all the non *S. hermonthica* weeds growing in the pots were uprooted. Excess *S. hermonthica* were also uprooted so that each pot had only 10 *S.hermonthica* plants. Eight weeks after planting, when the *S. hermonthica* plants were approximately 5-15 cm

tall, they were inoculated with the different fungal isolates. The inoculum was sprayed throughout the *S.hermonthica* plants. Following inoculation, the *S. hermonthica* plants were assessed for disease symptoms at a 5 day interval for 3weeks. Plants with observed lesions and abnormal color changes on the leaves and stems were designated as infected. After infection fungi were isolated from the infected *S hermonthica* onto PDA plates to confirm if the infection was as a result of the fungus inoculated. The infection and the mortality rates were used to determine the most virulent isolate.

3.7 Statistical analysis

ANOVA was used to analyze the data and find out whether there was significant difference in the number of *S. hermonthica* in Alupe and Kibos. ANOVA was also used to find out whether the levels of the soil parameters assessed significantly varied in the two benchmark sites. A test of within subject contrast- ANOVA was used to find out if the weed density and the soil parameters significantly varied within the cropping systems and within the maize varieties in each benchmark site. A post hoc ANOVA test-LSD (Least significance difference test) was carried out to elucidate the differences in weed density and soil parameters between the cropping systems and the maize varieties in each area. A t test was done to find out if the fungal isolates could significantly cause infection and mortality to *S. hermonthica* in the green house.

CHAPTER FOUR

4.0 RESULTS

4.1 Germination of maize and *Striga hermonthica* in the experimental blocks

Maize emerged 7 days after planting and by the 10th day 96% of maize had germinated. *Striga hermonthica*, on the other hand, emerged five weeks after planting. The weed density increased progressively with time then drastically fell after the weed had flowered at week 12 (Figure 3). The number of live weeds reduced from week 12.

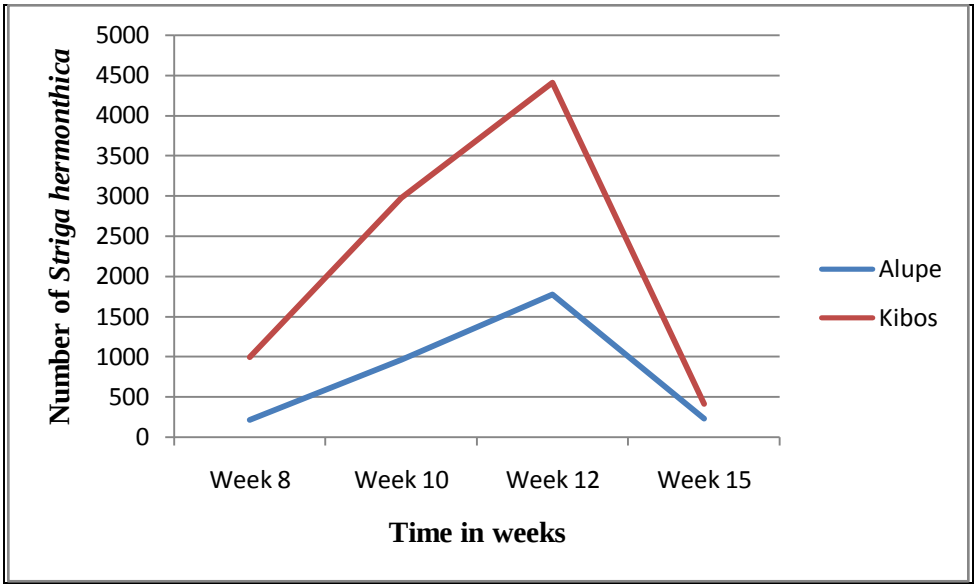


Figure 3: *Striga hermonthica* germination in Alupe and in Kibos

The weed density was higher in Kibos than Alupe and this difference was significant at $F_{1,96}$ =8.772; p=0.004. The interaction between the weed density and the cropping systems in the two regions was not significant at $F_{3,96}$ = 1.372; p=0.256 meaning that the cropping systems in the two regions had similar weed distributions. However, the interaction between the weed density and the maize varieties in the two regions was significant at $F_{3,96}$ = 3.243; p=0.025 meaning that the maize varieties had different weed distributions in the two regions (Table 2).

Table 2: Comparison of weed density in Alupe and Kibos: ANOVA

Dependent Variable: Weed density

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Values	Significance level
Corrected Model	292219.259[a]	31	9426.428	3.759	.000
Intercept	112256.842	1	112256.842	44.764	.000
Areas	21998.318	1	21998.318	8.772	.004
Cropping systems	28433.047	3	9477.682	3.779	.013
Maize varieties	147888.312	3	49296.104	19.658	.000
Areas * Cropping systems	10325.077	3	3441.692	1.372	.256
Areas * Maize varieties	24401.235	3	8133.745	3.243	.025
Cropping systems * Maize varieties	42073.730	9	4674.859	1.864	.067
Areas * Cropping systems * Maize varieties	17099.541	9	1899.949	.758	.655
Error	240742.636	96	2507.736		
Total	645218.737	128			
Corrected Total	532961.895	127			

*means 'AND' for example; areas*cropping systems means areas and cropping systems.

4.2 Influence of cropping systems and maize varieties on *Striga hermonthica* emergence in Kibos and Alupe

Weed density varied with cropping systems and this variation was significant at $F_{3,96} = 3.779$; p= 0.013 (Table 2). Maize rotated with soybeans had the highest number of weeds (394) followed by pure maize (209) then maize intercropped with groundnuts (156), maize intercropped with *Desmodium uncinatum* had the least number of weeds (67). The weed density also varied with maize variety planted and this variation was significant at $F_{3, 96} = 19.658$; p= 0.00 (Table 2). Subplots with the maize variety WH403 had the highest number of weeds (602) followed by subplots with *Striga* resistant hybrid (194) then subplots with IR Hybrid (19), subplots IR OPV had the least number of the weed (11) as shown in figure 4 below.

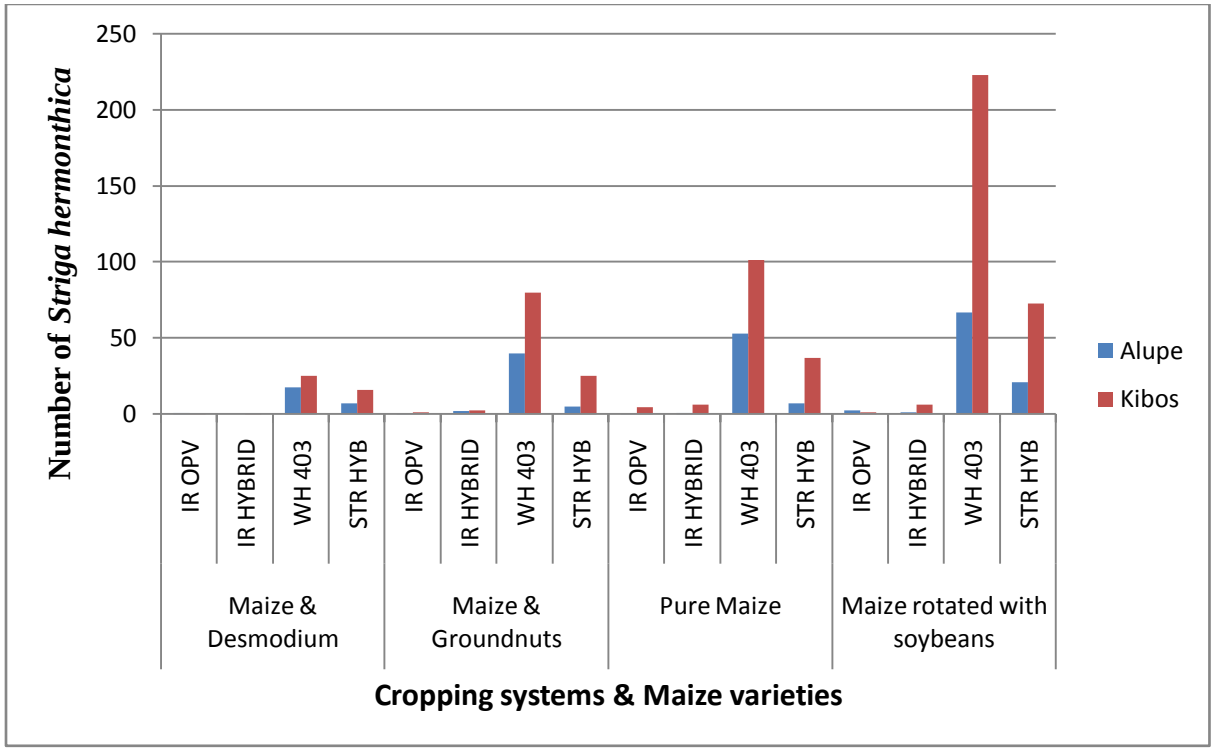


Figure 4: Influence of cropping systems and maize varieties on *Striga hermonthica* emergence in Alupe and Kibos

In Alupe, the weed density differed within the cropping systems and within the maize varieties planted and these differences were significant at $F_{3,48} = 3.940$; $p = 0.017$ and $F_{3,48} = 14.442$; $p = 0.000$ respectively (Table 3). However, the interaction between the weed density, cropping systems and maize varieties was not significant at $F_{9,48} = 1.148$; $p = 0.360$ as shown in table 3. Meaning that the weed distribution was similar amongst the replicate plots in Alupe.

Table 3: Effects of cropping systems and maize varieties on *Striga hermonthica* emergence in Alupe: Test of within subject contrast-ANOVA

Measure: Weed density

Source: Alupe	Weed density	Type III Sum of Squares	Degrees of freedom	Mean Square	F- Value	Significance level
Weed density	Linear	263.344	1	263.344	22.633	.000
Weed density * Cropping systems	Linear	137.531	3	45.844	3.940	.017
Weed density * Maize varieties	Linear	504.115	3	168.038	14.442	.000
Weed density * Cropping systems * Maize varieties	Linear	120.177	9	13.353	1.148	.360
Error(Weed density)	Linear	372.333	48	11.635		

* means ‘AND’ for example; areas*cropping systems means areas and cropping systems.

In Alupe, weed density in maize rotated with soybeans differed significantly with the weed densities in the other cropping systems (significance levels were 0.002, 0.006 and 0.009) (Table 4). However, no significant difference in weed density was observed amongst maize intercropped with *Desmodium uncinatum*, maize pure and maize intercropped with ground nuts (significance levels were 0.138, 0.085 and 0.081) as shown in table 4 below.

Table 4: Effects of cropping systems on *Striga hermonthica* emergence in Alupe: Post hoc ANOVA LSD

(I) Cropping System	(J) Cropping System	Mean Difference (I-J)	Standard Error	Significance level	95% Confidence Interval	
					Lower Bound	Upper Bound
Maize & Desmodium	Maize & Groundnuts	-3.0000	1.96938	.138	-7.0115	1.0115
	Pure Maize	-3.5000	1.96938	.085	-7.5115	.5115
	Maize rotated with Soy	-6.7500*	1.96938	.002	-10.7615	-2.7385
Maize & Groundnuts	Maize & Desmodium	3.0000	1.96938	.138	-1.0115	7.0115
	Pure Maize	-.5000	1.96938	.801	-4.5115	3.5115
	Maize rotated with Soy	-3.7500	1.96938	.006	-7.7615	.2615
Pure Maize	Maize & Desmodium	3.5000	1.96938	.085	-.5115	7.5115
	Maize & Groundnuts	.5000	1.96938	.801	-3.5115	4.5115
	Maize rotated with Soy	-3.2500	1.96938	.009	-7.2615	.7615
Maize rotated with Soy	Maize & Desmodium	6.7500*	1.96938	.002	2.7385	10.7615
	Maize & Groundnuts	3.7500	1.96938	.006	-.2615	7.7615
	Pure Maize	3.2500	1.96938	.009	-.7615	7.2615

In Alupe, the weed densities in the subplots with WH 403 were significantly higher than the weed densities in the subplots with IR OPV, IR Hybrid and STR Hybrid (the significance levels were less than 0.001 for all the maize varieties) (Table 5). However, the weed densities in the subplots with maize varieties; IR OPV, IR Hybrid and STR Hybrid were not significantly different from each other (significance levels were 0.995, 0.180 and 0.178) as shown in table 5 below.

Table 5: Effects of maize varieties on *Striga hermonthica* emergence in Alupe: Post hoc ANOVA LSD

(I) Maize varieties	(J) Maize varieties	Mean Difference (I-J)	Standard Error	Significance level	95% Confidence Interval	
					Lower Bound	Upper Bound
IR OPV	IR HYBRID	-.0581	9.23914	.995	-18.5392	18.4229
	WH 403	-49.7081*	9.23914	.000	-68.1892	-31.2271
	STR HYB	-12.5825	9.23914	.178	-31.0635	5.8985
IR HYBRID	IR OPV	.0581	9.23914	.995	-18.4229	18.5392
	WH 403	-49.6500*	9.23914	.000	-68.1310	-31.1690
	STR HYB	-12.5244	9.23914	.180	-31.0054	5.9567
WH 403	IR OPV	49.7081*	9.23914	.000	31.2271	68.1892
	IR HYBRID	49.6500*	9.23914	.000	31.1690	68.1310
	STR HYB	37.1256*	9.23914	.000	18.6446	55.6067
STR HYB	IR OPV	12.5825	9.23914	.178	-5.8985	31.0635
	IR HYBRID	12.5244	9.23914	.180	-5.9567	31.0054
	WH 403	-37.1256*	9.23914	.000	-55.6067	-18.6446

In Kibos, the weed density differed within the cropping systems and within the maize varieties planted, these differences were significant at $F_{3,48}= 2.79$; p= 0.49 and $F_{3,48} = 11.284$; p=0.000 respectively as shown in table 6. The interaction between the weed density, cropping systems and the maize varieties was however, not significant at $F_{9,48} = 1.418$; p= 0.207 (Table 6). Meaning that the weed distribution was similar amongst the replicate plots in Kibos.

Table 6: Effects of cropping systems and maize varieties on *Striga hermonthica* emergence in Kibos: Test of within subject contrast- ANOVA

Measure: Weed density

Source: Kibos	Weed density	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Weed density	Linear	55708.299	1	55708.299	25.873	.000
Weed density * Cropping systems	Linear	18019.622	3	6006.541	2.790	.049
Weed density * Maize varieties	Linear	72885.744	3	24295.248	11.284	.000
Weed density * Cropping systems * Maize varieties	Linear	27481.131	9	3053.459	1.418	.207
Error(Weeddensity)	Linear	103349.460	48	2153.114		

*means 'AND' for example; areas*cropping systems means areas and cropping systems.

In Kibos, weed density in maize rotated with soybeans differed significantly with the weed densities in the other cropping systems (significance levels were 0.048, 0.046 and 0.026) (Table 7). However, no significant difference in weed density was observed amongst maize intercropped with *Desmodium uncinatum*, maize pure and maize intercropped with ground nuts (significance levels were 0.225, 0.362 and 0.760) as shown in table 7 below.

Table 7: Effects of cropping systems on *Striga hermonthica* emergence in Kibos: Post hoc ANOVA LSD

(I) Cropping Systems	(J) Cropping Systems	Mean Difference (I-J)	Standard Error	Significance level	95% Confidence Interval	
					Lower Bound	Upper Bound
Maize & Desmodium	Maize & groundnuts	-35.7225	29.13448	.225	-94.0001	22.5551
	Pure maize	-26.7837	29.13448	.362	-85.0614	31.4939
	Maize rotated with soy	-66.4619*	29.13448	.026	-124.7395	-8.1842
Maize & groundnuts	Maize & Desmodium	35.7225	29.13448	.225	-22.5551	94.0001
	Pure maize	8.9387	29.13448	.760	-49.3389	67.2164
	Maize rotated with soy	-30.7394	29.13448	.046	-89.0170	27.5383
Pure maize	Maize & Desmodium	26.7837	29.13448	.362	-31.4939	85.0614
	Maize & groundnuts	-8.9387	29.13448	.760	-67.2164	49.3389
	Maize rotated with soy	-39.6781	29.13448	.048	-97.9558	18.5995
Maize rotated with soy	Maize & Desmodium	66.4619*	29.13448	.026	8.1842	124.7395
	Maize & groundnuts	30.7394	29.13448	.046	-27.5383	89.0170
	Pure maize	39.6781	29.13448	.048	-18.5995	97.9558

In Kibos, the weed density in the subplot with WH 403 was significantly higher compared to the weed densities in subplots with IR OPV, IR Hybrid and STR Hybrid (significance levels were 0.001 for STR Hybrid and less than 0.001 for IR OPV and IR Hybrid) (Table 8). The weed density in the subplot with STR Hybrid was also significantly different from the weed densities in the subplots with IR OPV and IR Hybrid (significance levels were 0.036 and 0.043 respectively) (Table 8). However, the weed densities in subplots with IR OPV and IR HYBRID were not significantly different from each other (significance level was 0.935) as shown in table 8 below.

Table 8: Effects of maize varieties on *Striga hermonthica* emergence in Kibos: Post hoc ANOVA LSD

(I) Maize Variety	(J) Maize Variety	Mean Difference (I-J)	Standard Error	Significance level	95% Confidence Interval	
					Lower Bound	Upper Bound
IR OPV	IR HYBRID	-.3333	4.08264	.935	-8.5613	7.8947
	WH 403	-22.6667*	4.08264	.000	-30.8947	-14.4387
	STR HYB	-8.8333*	4.08264	.036	-17.0613	-.6053
IR HYBRID	IR OPV	.3333	4.08264	.935	-7.8947	8.5613
	WH 403	-22.3333*	4.08264	.000	-30.5613	-14.1053
	STR HYB	-8.5000*	4.08264	.043	-16.7280	-.2720
WH 403	IR OPV	22.6667*	4.08264	.000	14.4387	30.8947
	IR HYBRID	22.3333*	4.08264	.000	14.1053	30.5613
	STR HYB	13.8333*	4.08264	.001	5.6053	22.0613
STR HYB	IR OPV	8.8333*	4.08264	.036	.6053	17.0613
	IR HYBRID	8.5000*	4.08264	.043	.2720	16.7280
	WH 403	-13.8333*	4.08264	.001	-22.0613	-5.6053

4.3 Variation of soil nutrients in Alupe and Kibos

The levels of nitrogen, organic carbon and copper were significantly higher in Alupe compared to Kibos, while the levels of iron and potassium had no significant difference in the two bench mark sites. On the other hand, the levels of phosphorus, magnesium, calcium, manganese, zinc and sodium were significantly higher in Kibos than in Alupe (Table 9).

Table 9: Table of variation of soil nutrients in Alupe and Kibos.

Nutrient being compared in Alupe and Kibos	Average level in Alupe	Average level in Kibos	Class of the levels as described by KARI	Statistical test and Significance level	Reference
Nitrogen	0.15%	0.08%	Low both in Alupe and Kibos	ANOVA 0.001 at p=0.005	Appendix 1
Organic carbon	1.36%	0.75%	Moderate in Alupe but low in Kibos	ANOVA <0.001 at p=0.005	Appendix 2
Phosphorus	174ppm	396ppm	High both in Alupe and Kibos	ANOVA <0.001 at p=0.005	Appendix 3
Magnesium	0.99me%	2.25me%	Low in Alupe but adequate in Kibos	ANOVA <0.001 at p=0.005	Appendix 4

Calcium	1.14me%	1.74me%	Low both in Kibos and Alupe	ANOVA <0.001 at p=0.005	Appendix 5
Manganese	0.38me%	0.62me%	Adequate both in Alupe and Kibos	ANOVA 0.005 at p=0.005	Appendix 6
Zinc	1.38ppm	1.71ppm	Low both in Alupe and Kibos	ANOVA 0.001 at p=0.005	Appendix 7
Iron	36.75ppm	34.70ppm	Adequate both in Alupe and in Kibos	ANOVA 0.533 at p=0.05	Appendix 8
Potassium	0.17me%	0.22me%	Low both in Alupe and Kibos	ANOVA 0.65 at p=0.05	Appendix 8
Copper	4.62ppm	2.68ppm	Adequate both in Alupe and in Kibos	ANOVA 0.010 at p=0.05	Appendix 9
Sodium	0.14me%	0.20me%	Adequate both in Alupe and Kibos	ANOVA 0.001 at p=0.005	Appendix 10

4.4 Microbial populations and soil pH in Alupe and Kibos

The soil pH was acidic in both areas however; it was significantly different in the two areas with Kibos having a higher pH than Alupe. The microbial populations of bacteria, fungi and actinomycetes on the other hand, were not significantly different in the two areas (Table 10).

Table 10: Table of soil pH and microbial populations in Alupe and Kibos

Factor being compared in Alupe and Kibos	**Average level in Alupe**	**Average level in Kibos**	**Class of the levels as described by KARI**	**Statistical test and Significance level**	**Reference**
Soil pH	4.79	5.45	Acidic both in Alupe and Kibos	ANOVA <0.001 at p=0.005	Appendix 11
Bacteria	1.19×10^{6} g dry wt	1.04×10^{6} g dry wt	Low both in Alupe and Kibos	ANOVA 0.517 at p=0.05	Appendix 12
Fungi	5.32×10^{5} g dry wt	6.54×10^{5} g dry wt	Adequate both in Alupe and Kibos	ANOVA 0.244 at p=0.05	Appendix 12
Actinomycetes	8.71×10^{5} g dry wt	9.84×10^{5} g dry wt	Low both in Alupe and Kibos	ANOVA 0.674 at p=0.05	Appendix 13

4.5 Soil texture in Alupe and Kibos

The soil texture was sandy in Kibos and clay in Alupe. Sand levels were significantly higher in Kibos than Alupe while clay and silt levels were significantly higher in Alupe than Kibos (Table 11).

Table 11: Table of soil texture in Alupe and Kibos

Soil component being compared in Alupe and Kibos	**Average level in Alupe**	**Average level in Kibos**	**Statistical test and significance level**	**Reference**
Sand	29.5%	54.1%	ANOVA <0.001 at p=0.005	Appendix 14
Silt	23.8%	15.4%	ANOVA <0.001 at p=0.005	Appendix 15
Clay	46.6%	29.9%	ANOVA<0.001 at p=0.005	Appendix 16

4.6 Virulence efficacy of the fungal isolates as biological control agents against the weed

4.6 Fungal species isolated from *Striga hermonthica*

Ten fungal species were recovered from diseased *S. hermonthica* collected from Alupe and Kibos (Table 12). *Fusarium sp* were isolated from *S. hermonthica* collected from Alupe as well as from Kibos. *Hypocrea stellata* was isolated from *S. hermonthica* collected from Alupe, while *Colletotrichum gloeosporioide*s and *Artroderma otae* were isolated from *S. hermonthica* collected from Kibos. *Fusarium oxysporum* was the most frequent fungal species isolated followed by *F. chlamydosporium* then *F. equiseti*. The least frequent species were *Colletotrichum gloeosporioide*s and *Hypocrea stellata*.

Table 12: Fungal species isolated from *Striga hermonthica*

NAME OF THE FUNGUS	ORIGIN (AREA)	ORIGIN (PART OF PLANT)	FREQUENCY	PERCENTAGE FREQUENCY
Fusarium equiseti	Kibos	Leaves	11/20	14.49%
	Alupe	Leaves	9/20	
Fusarium verticilloides	Kibos	Leaves	8/18	13.04%
	Alupe	Leaves	10/18	
Fusarium oxysporum	Kibos	Leaves	12/26	18.84%
	Alupe	Leaves	14/26	
Fusarium incarnatum	Kibos	Leaves	8/16	11.59%
	Alupe	Leaves	8/16	
Fusarium chlamydosporium	Kibos	Leaves	10/22	15.94%

	Alupe	Leaves	12/22	
Gibberella moniliformis	Kibos	Stem	8/17	12.31%
	Alupe	Stem	9/17	
Gibberella intricans	Kibos	Stem	9/15	10.87%
	Alupe	Stem	6/15	
Colletotrichum gloeosporioide	Kibos	Stem	1/1	0.72%
	Alupe		0/1	
Hypocrea stellata	Kibos	Roots	0/1	0.72%
	Alupe		1/1	
Arthroderma otae	Kibos	Roots	2/2	1.45%
	Alupe		0/2	

4.7.2 Morphological description of the fungal isolates

4.7.2.1 *Fusarium equiseti* (Corda) Saccardo

The fungus grew rapidly on PDA producing white aerial mycelium after 2 days. As the culture aged the mycelia turned brownish and formed annular zonations. The under surface of the culture was brick red. Conidia were formed when the fungus was grown on CLA. The macroconidia were septate - with most having 4-6 septa, thick walled and sickle shaped. Microconidia were absent. The condiophores were unbranched but the monophialides were branched (Figure 5).

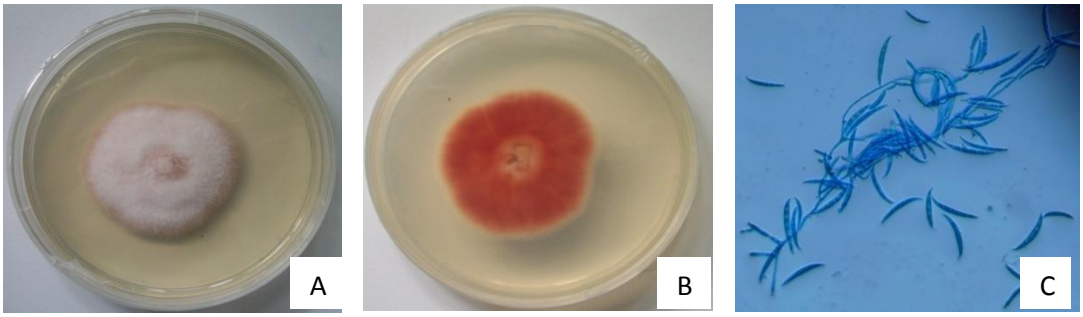


Figure 5: *Fusarium equiseti* growing on potato dextrose agar (A) White aerial mycelia (B) Brick red under surface (C) Sickle shaped septate macroconidia.

4.7.2.2 *Fusarium verticilloides* (Saccardo) Nireberg

The fungus grew on PDA producing whitish mycelia which became orange with age. The under surface of the culture was orange. Upon growth on CLA the fungus formed relatively long, slender, slightly straight, thin walled macroconidia with slightly curved apical cells. Microconodia were few, oval shaped and without a septum. Chalmydiospores were absent (Figure 6).

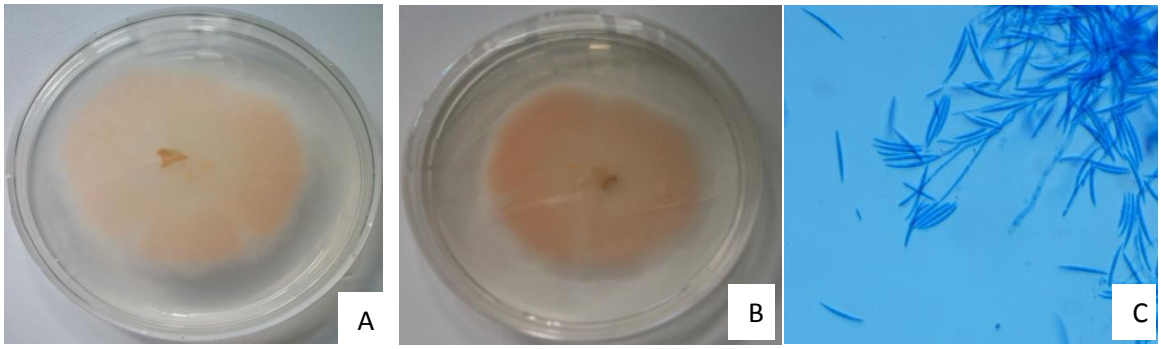


Figure 6: *Fusarium verticilloides* growing on potato dextrose agar (A) Whitish aerial mycelia (B) Orange undersurface (C) Long slender and slightly straight macroconidia.

4.7.2.3 *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen

Growth was rapid on PDA, with the fungus producing whitish aerial mycelium after 3days which became violet with age. The under surface of the culture was brick red. Macroconidia were formed when the fungus was grown on CLA. The macroconidia were slightly sickle shaped, thin walled with a foot shaped basal cell. Microconodia were generally few single celled and oval shaped (Figure 7).

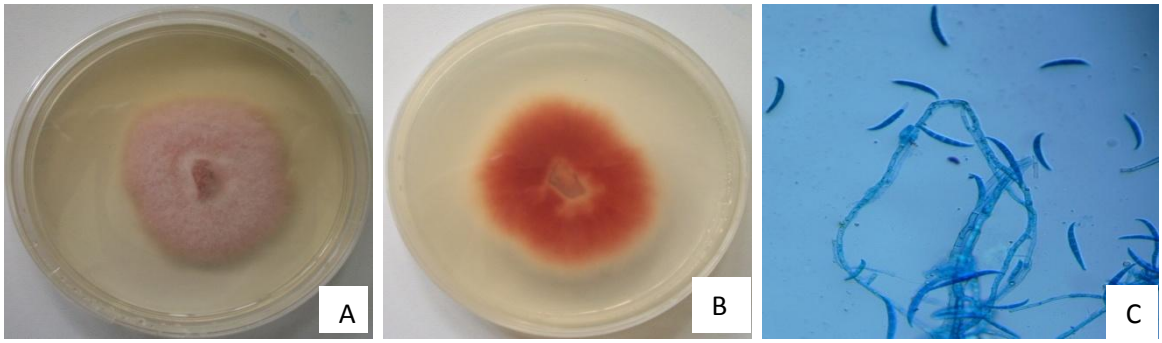


Figure 7: *Fusarium oxysporum* growing on potato dextrose agar (A) Violet aerial mycelia (B) Brick red undersurface (C) Thin walled, slightly sickled, foot shaped macrconidia.

4.7.2.4 *Fusarium incarnatum* (Roberge) Sacc.

Growth was rapid on PDA, with the fungus producing whitish aerial mycelia after 3 days. The culture appeared somehow powdery with age. The undersurface of the culture was orange. Upon growth on CLA the fungus produced macroconidia , which were somehow straight, spindle shaped, and tapering at both ends with around 3-4 septa. Chlamydospores were absent (Figure 8).

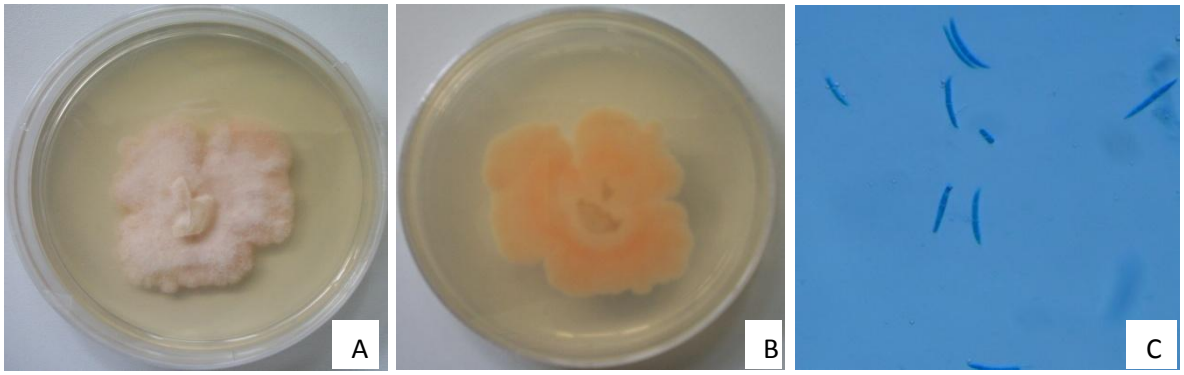


Figure 8: *Fusarium incarnatum* growing on potato dextrose agar (A) Whitish aerial mycelia (B) Orange undersurface (C) Spindle shaped, somehow straight macroconidia.

4.7.2.5 *Fusarium chlamydosporium* Wollenweber and Reinking

The fungus grew rapidly on PDA producing violet aerial mycelium after 3 days. Annular zonations were formed with age. The under surface of the culture was generally dark brown. Upon growth of the fungus in CLA numerous spindle shaped microconidia having 1septa were formed. Macroconidia on the other hand were very few (Figure 9).

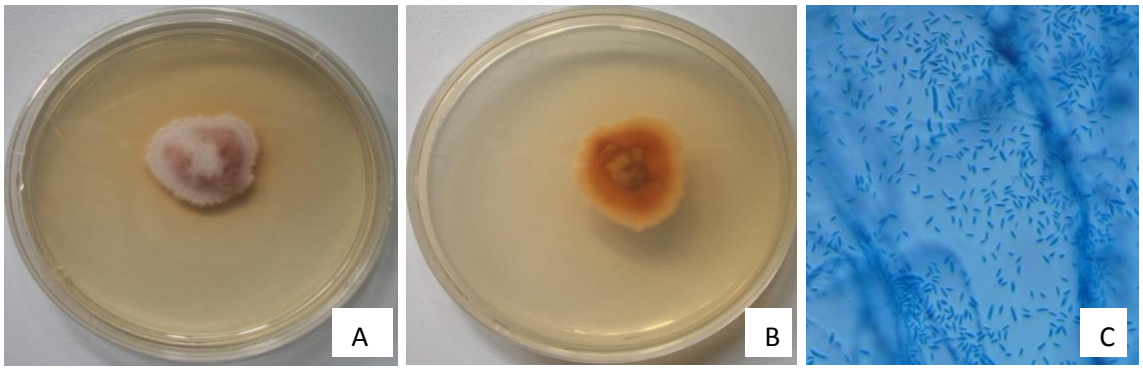


Figure 9: *Fusarium chlamydosporium* growing on potato dextrose agar (A) Violet aerial mycelium (B) Brownish undersurface (C) Spindle shaped microconidia of *Fusarium chlamydosporium*.

4.7.2.6 Gibberella moniliformis (Wineland)

The fungus grew rapidly on PDA producing purplish aerial mycelia after 3days. The undersurface of the culture was orange in color. Annular zonations were formed with age. Macroconidia were formed when the fungus was grown on CLA. The macroconidia were slender and slightly straight. Microconodia and chalmydiospores were absent. The conidiophores were branched (Figure 10) (Isolate name as in the NCBI Blast).

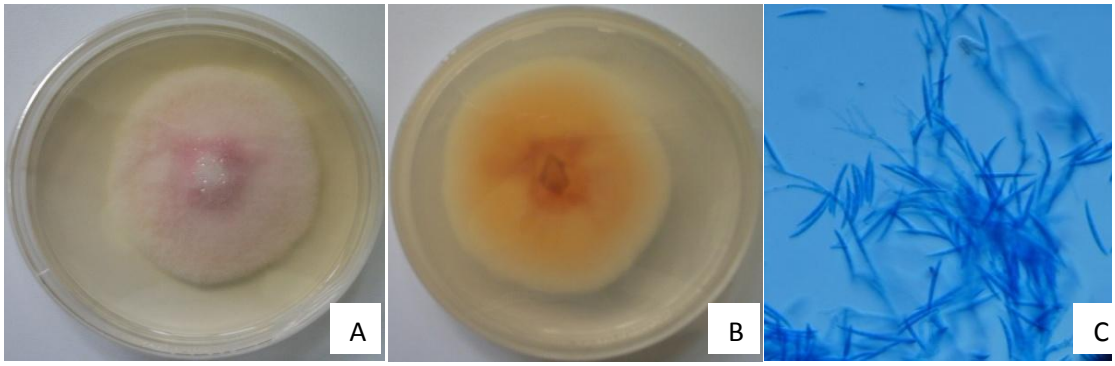


Figure 10: *Gibberella moniliformis* growing on potato dextrose agar (A) Purplish mycelia (B) Orange undersurface (C) Slender slightly straight macroconidia

4.7.2.7 *Gibberella intricans* (Wollenweber)

The fungus grew rapidly on PDA producing whitish aerial mycelium after 3 days which turned purplish as the culture aged. Annular zonations were also formed with age. The under surface of the culture was reddish in color. Macroconidia were formed when the fungus was grown on CLA. The macroconidia were septate, sickle shaped and thick walled (Figure 11) (Isolate name as in the NCBI Blast).

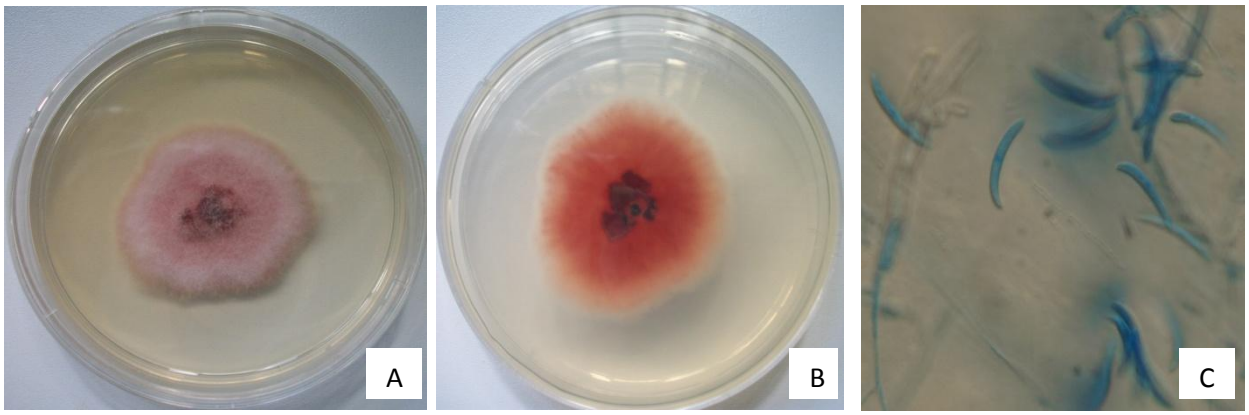


Figure 11: *Gibberella intricans* growing on potato dextrose agar (A) Purplish aerial mycelia (B) Reddish undersurface (C) Sickle shaped, thick walled septate macroconidia.

4.7.2.8 *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

The fungus grew rapidly on PDA to fill the entire plate after 4 days producing whitish aerial mycelia which turned grey with age. The undersurface of the culture was whitish. The fungus also developed cylindrical conidia which measured on average 14 µm long and 5 µm wide, confirming the findings of Cano, *et.al,* (2004) (Figure 12).

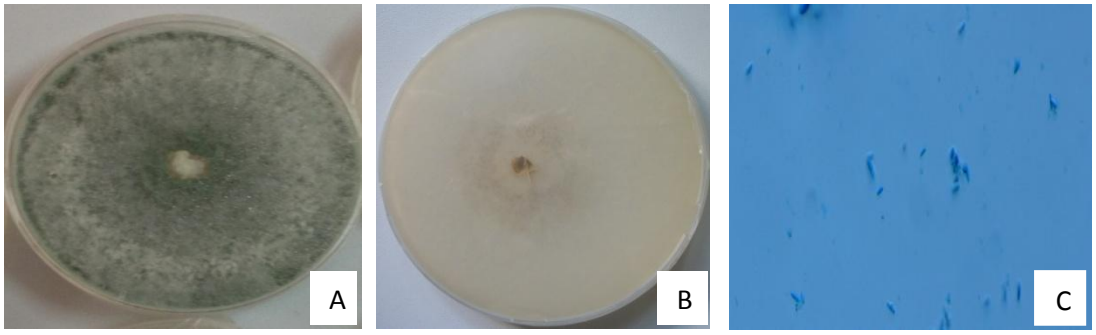


Figure 12: *Colletotrichum gloeosporioides* growing on potato dextrose agar (A) Greyish mycelia (B) Whitish undersurface (C) Cylindrical shaped conidia.

4.7.2.9 *Hypocrea stellata* B.S. Lu, Druzhin. & Samuels

The fungus grew rapidly on PDA producing whitish aerial mycelia. The fungus also produced conidia which were clustered confirming the findings of Chaverri and Samuels, 2003. Figure 13 below are plates of *Hypocrea stellata.*

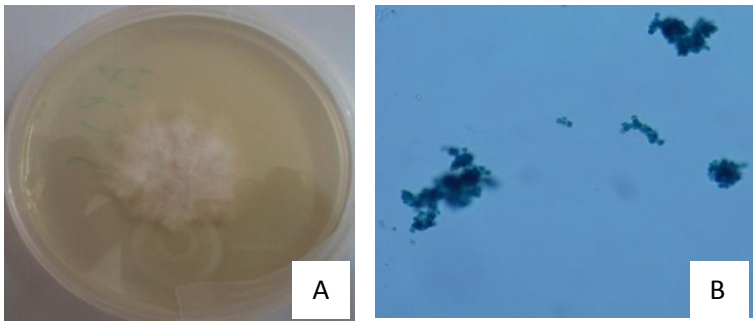


Figure 13: A, Whitish aerial mycelia of *Hypocrea stellata* growing on potato dextrose agar. B, Clustered conidia of *Hypocrea stellata.*

4.7.2.10 *Arthroderma otae* (A. Haseg. & Usui) McGinnis, Weitzman, A. A. Padhye & Ajello

The fungus grew on PDA, producing whitish aerial mycelia with a yellow pigmentation. The fungus also developed oval shaped conidia with distinct septa confirming the findings of Padhye and Carmichael, 1972. The conidia had on average 5 – 7 septa (Figure 14).

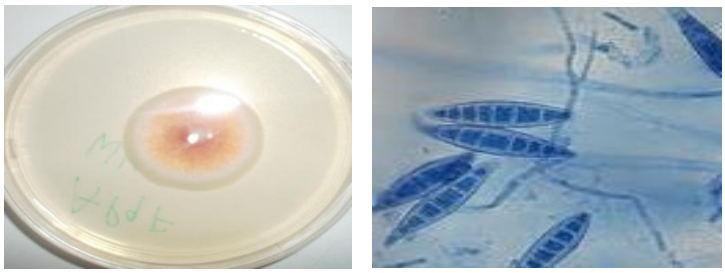
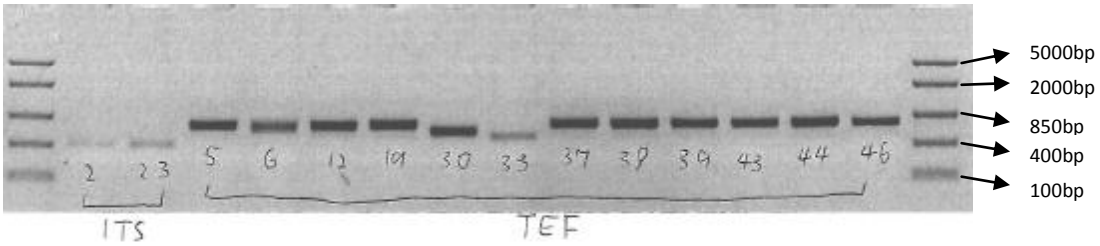


Figure 14: A, Whitish mycelia of *Arthroderma otae* growing on potato dextrose agar. B, Oval shaped septate conidia of *Arthroderma otae.*

4.8 Molecular characterization of the fungal isolates

4.8.1 PCR Amplification

Isolates morphologically identified as *Fusarium sp* and *Gibberella sp* were positively amplified using TEF1 (20 base pairs) and TEF2 (21 base pairs) primers. The isolates morphologically identified as *Hypocrea stellata* and *Colletotrichum gloeosporioides,* were amplified using the ITSF (24 base pairs) and ITSR (17 base pairs) primers as shown in figure 15 below.



KEY:

2- *Hypocrea stellata;* **23**- *Colletotrichum gloeosporioides;* **5**- *Gibberella monoliformis;* **6**- *Fusarium equiseti;* **12**- *Fusarium incarnatum;* **19**- *Fusarium oxysporum*; **30**- *Gibberella intricans*; **33**- *Arthroderma otae;* **37**- *Fusarium oxysporum*; **38**- *Fusarium oxysporum*; **39**- *Fusarium oxysporum*; **43**- *Fusarium chlamydosporum*; **44**- *Fusarium chlamydosporum* ; **46**- *Fusarium verticilloides*.

Figure 15: Micrograph showing amplified DNA bands.

4.8.2 Identification of the fungal isolates using their sequences

The DNA extracted and showing positive bands in figure 15 above were sequenced and their identities agreed with morphological identification (Table 13).

Table 13: Names of the fungal isolates as in the National Center for Biotechnology Information - Basic Local Alignment Search

Isolate lab no.	Species name as in the NCBI Blast	Corresponding strain in the NCBI Blast
5	*Gibberella monoliformis*	Voucher GFLC190
12	*Fusarium incarnatum*	Isolate lb2
30	*Gibberella intricans*	Strain LVPEI.H4599
6	*Fusarium equiseti*	Strain DBT – 102
19	*Fusarium oxysporum*	Strain NRRL 52937
43	*Fusarium chlamydosporum*	Strain JL-26
44	*Fusarium chlamydosporum*	Isolate AC638
38	*Fusarium oxysporum*	Isolate FSY0953
46	*Fusarium verticilloides*	Strain 25 ALH
2	*Hypocrea stellata*	Strain GJS 99-222
23	*Colletotrichum gloeosporioides*	Isolate s-7
33	*Arthroderma otae*	Strain CBS 113480
37	*Fusarium oxysporum*	f.sp melonis Strain ISPAaVe1070
39	*Fusarium oxysporum*	Isolate IBSD-GF13

4.8.3 Relatedness of the fungal isolates: phylogenetic analysis

Alignment of the sequences (appendix 17) grouped the *Fusarium* sp. isolates into 3 clades. *Fusarium sp* isolates demonstrated relatedness; however, they showed no relationship with *Arthroderma otae*. Close relationships were observed; amongst the different strains of *F.oxysporum* and between *F. verticilloides* and its sexual state. Relatedness was also observed amongst the different strains of *F. chlamydosporum* and between *F. equiseti* and its sexual state. A relationship was observed amongst the *Fusarium* sp isolated from the leaves of *S hermonthica*, however no relationship was observed amongst the *Gibberella* sp isolated from the stems (Figure 16).

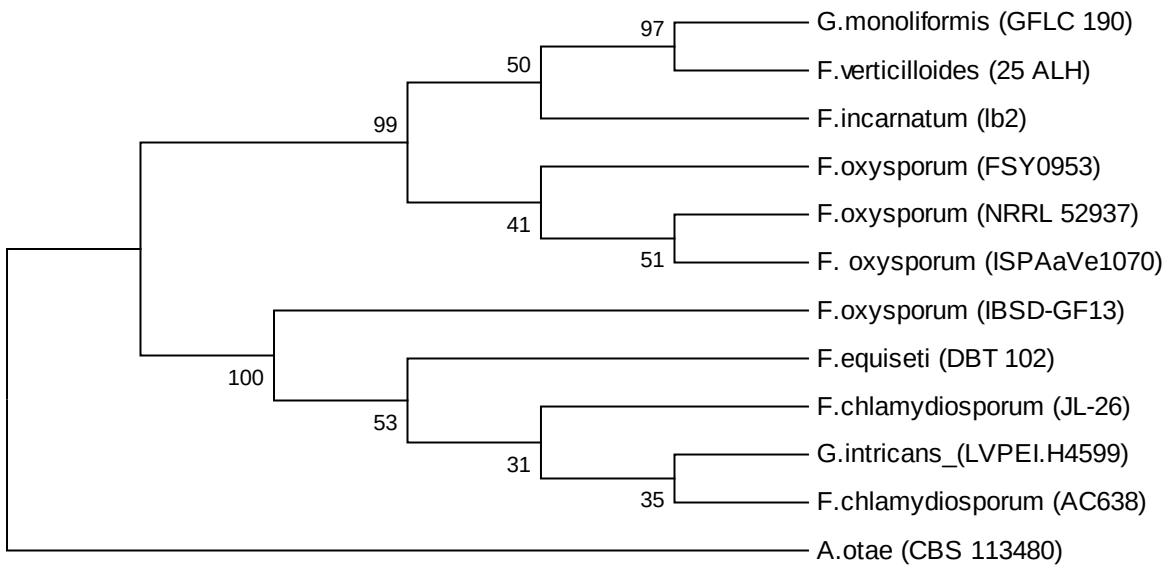


Figure 16: Relatedness of the isolated fungal species

4.9 Virulence efficacy of the fungal isolates on *Striga hermonthica* in the green house

All the nine fungal isolates tested for their efficacy against *S. hermonthica* caused infections on the weed producing different symptoms as shown in figures 17-23 below.

Gibberella monoliformis caused lesions on both the stem and leaves of the weed. The leaves turned dark maroon, followed by blackening of the stems and eventual drying and death of the weed (Figure 17).

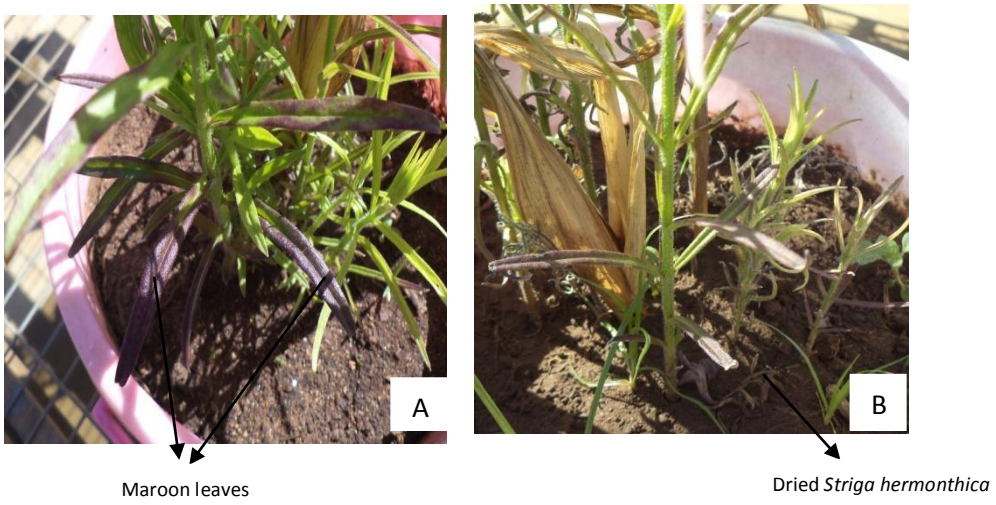


Figure 17: A, Leaves of *Striga hermonthica* turning maroon 7 days after infection by *Gibberella monoliformis*. B, Dried *Striga hermonthica* 15 days after infection by *Gibberella monoliformis*

Fusarium incarnatum caused lesions on the leaves. The leaves became twisted and developed an ashy burned appearance at the tip. The weed then dried and died (Figure 18).

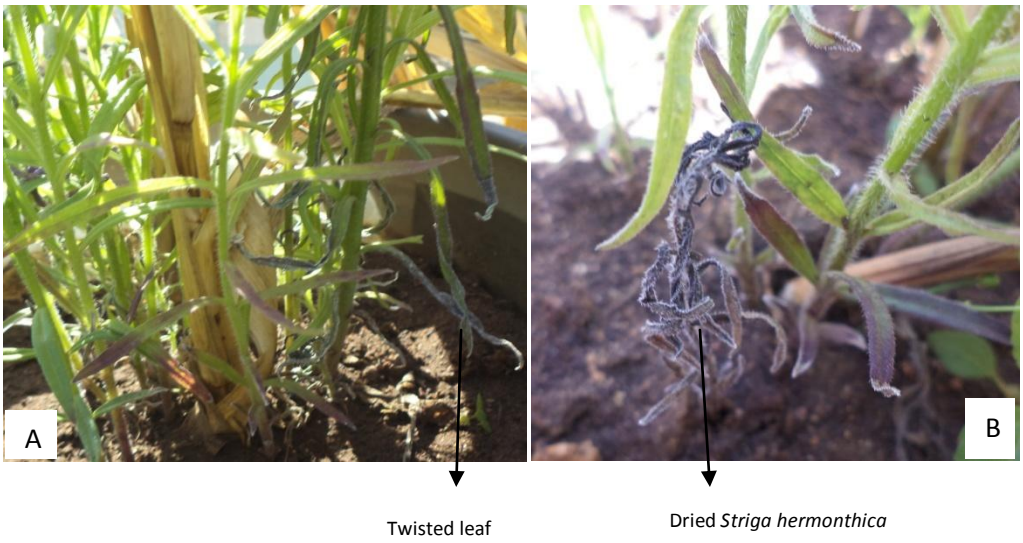


Figure 18: A, Leaves of *Striga hermonthica* becoming twisted 7 days after infection by *Fusarium incarnatum*. B, Dried *Striga hermonthica* 15 days after infection by *Fusarium incarnatum*.

Infection with *Gibberella intricans* caused the leaves to turn pale, then maroon and became twisted. The stems darkened from the ground up the plant this was followed by drying and eventual death of the weed (Figure 19).

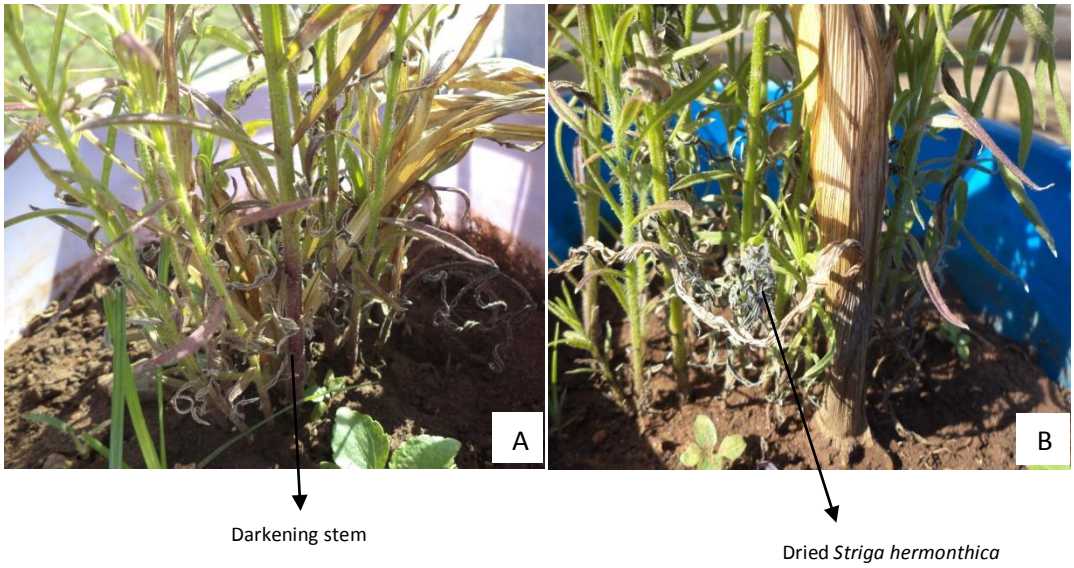


Figure 19: A, Darkening stem of *Striga hermonthica* 7 days after infection by *Gibberella intricans*. B, Dried *Striga hermonthica* 15 days after infection by *Gibberella intricans*.

Fusarium equiseti caused lesions on the leaves. The leaves became pale, rough textured and developed spots. Some leaves also curled and become twisted. The weed then dried and died (Figure 20).

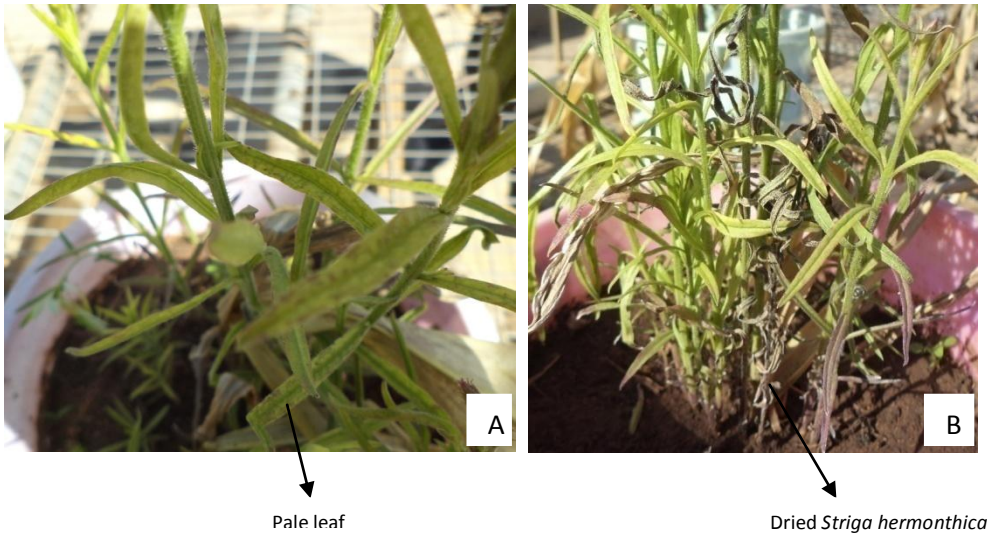


Figure 20: A, Pale leaves of *Striga hermonthica* 7 days after infection with *Fusarium equiseti*. B, Dried *Striga hermonthica* 15 days after infection with *Fusarium equiseti.*

Fusarium oxysporum caused lesions on the leaves and the stem. The leaves turned pale then maroon with curling and twisting. Some leaves developed spots which perforated them. The stem darkened from the ground up the plant, followed by drying then death of the weed (Figure 21).

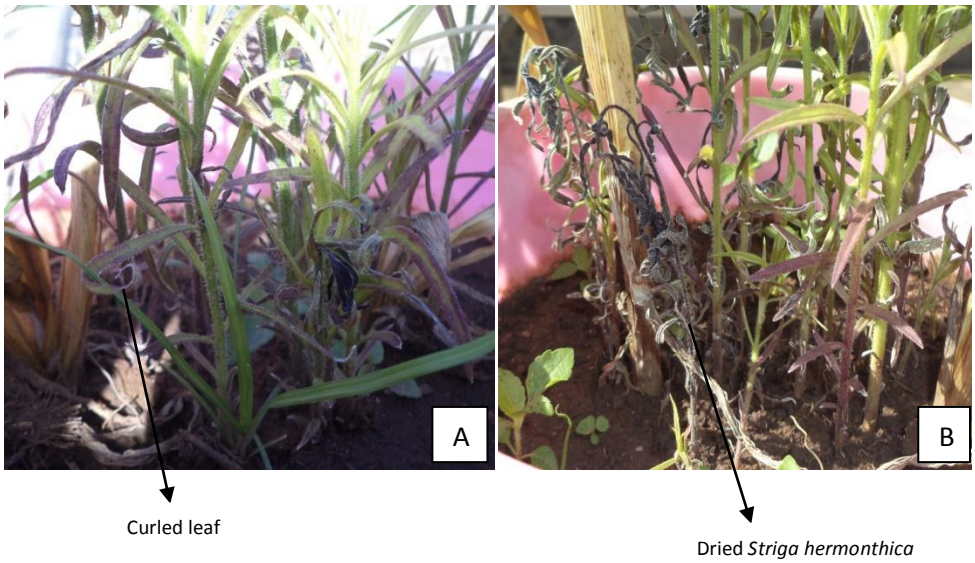


Figure 21: A, Curled maroon leaves of *Striga hermonthica* 7 days after infection with *Fusarium oxysporum*. B, Dried *Striga hermonthica* 15 days after infection with *Fusarium oxysporum*.

Fusarium chlamydosporum caused lesions on the leaves and on the stem. The leaves turned brownish, developed whitish spots, curled, became twisted and then dried. The weed dried from the tip down the stem and eventually died (Figure 22).

Figure 22: A, Leaves of *Striga hermonthica* with whitish spots 7 days after infection with *Fusarium chlamydosporum*. B, Dried *Striga hermonthica* 15 days after infection with *Fusarium chlamydosporum.*

Fusarium verticilloides caused lesions on the leaves. The leaves turned maroon, developed whitish spots and an ashy burned appearance at the tip and on the edges of the leaves. The plant then dried and died (Figure 23).

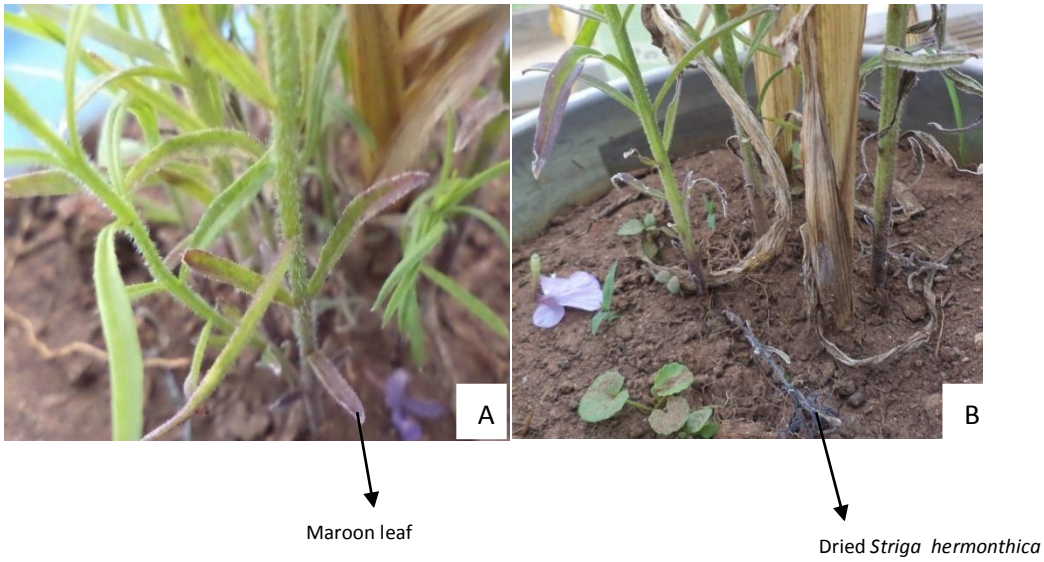


Figure 23: A, Maroon leaves of *Striga hermonthica* 7 days after infection with *Fusarium verticilloides*. B, Dried *Striga hermonthica* 15 days after infection with *Fusarium verticilloides*.

4.9.1 Infection and mortality rates of the fungal isolates on *Striga hermonthica*

The number of weeds with observed lesions on the leaves and stems increased progressively with time. *Fusarium oxysporum* had the highest number of weeds with observed lesions after 5 days followed by *F. verticilloides* then *F. chlamydosporum*. After 20 days, *F. incarnatum* had the highest number of plants with observed lesions while *F. oxysporum* had the least. For each fungal isolate, 50 *S. hermonthica* plants were inoculated. *Fusarium oxysporum* had the highest number of dead weeds followed by *F. equiseti* then *F. verticilloides* (Table 23).

Table 14: Infection of *Striga hermonthica* after inoculation with the fungal isolates

Isolate name	**No. of weeds with lesions after 5 days.**	**No. of weeds with lesions after 10 days.**	**No. of weeds with lesions after 15 days.**	**No. of weeds with lesions after 20 days.**	**No. of dead weeds after 22 days**
Gibberella monoliformis (Voucher GFLC 190)	20	28	36	44	20
Fusarium incarnatum (Isolate lb2)	24	32	39	46	21
Gibberella intricans (Strain LVPEI.H4599)	25	36	42	45	18
Fusarium equiseti (Strain DBT- 102)	26	33	39	43	23
Fusarium oxysporum (Strain NRRL 52937)	31	35	38	40	30

Fusarium chlamydosporum (Strain JL-26)	26	35	40	45	19
Fusarium chlamydosporum (Isolate AC638)	24	33	39	42	20
Fusarium oxysporum (Isolate FSY0953)	29	33	36	38	29
Fusarium verticilloides (Strain 25 ALH)	27	34	41	45	22
Control	0	1	2	2	0

The fungal isolates demonstrated high infection rates (significance level less than 0.001 at p=0.05 as shown in table 15) with *F. incarnatum* having the highest infection rate of 92%, followed by *Gibberella intricans*, *F. verticilloides* and *F. chlamydosporum* at 90% each. *Fusarium oxysporium* strains had the highest mortality rates (significance level less than 0.001 at p=0.05 as shown in table 16) of 60% and 58% followed by *F. equiseti* at 46%. *Gibberella intricans*, on the other hand, had the least mortality rate of 36% (Figure 24).

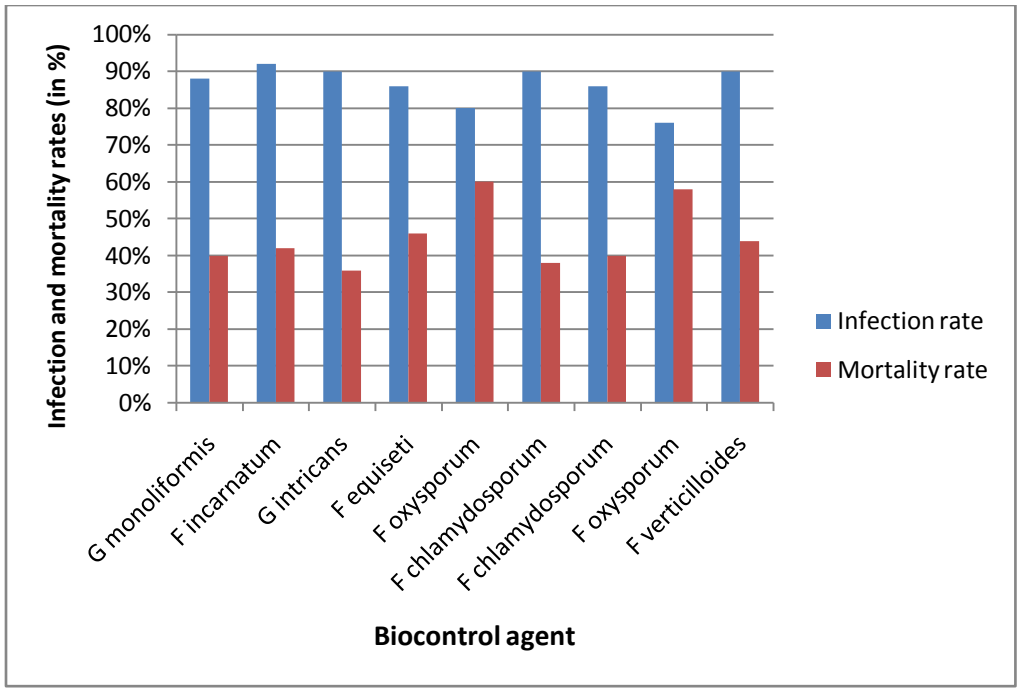


Figure 24: Infection and mortality rates of the fungal isolates on *Striga hermonthica*

Table 15: Infection of *Striga hermonthica* by the fungal isolates in the green house: T- test.

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Infection	7.716	8	.000	6.77778	4.7522	8.8034

Table 16: Mortality of *Striga hermonthica* by the fungal isolates in the green house: T- test.

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Mortality	19.336	8	.000	27.55556	24.2693	30.8418

CHAPTER FIVE

5.0 DISCUSSION

Cropping systems influenced weed density with maize rotated with soybeans having the highest number of the weed followed by pure maize then maize intercropped with groundnuts and finally maize intercropped with *Desmodium uncinatum* having the least number of the weed. This can be possibly due to the fact that soybeans are heavy feeders, therefore, when they are planted they utilize most of the nutrients in the soil hence making the next crops planted after them in the rotation cycle vulnerable due to lack of sufficient nutrients. Maize intercropped with *Desmodium uncinatum* and maize intercropped with groundnuts on the other hand, had the least numbers of *S. hermonthica* respectively. Since *Desmodium uncinatum* and groundnuts are nitrogen fixing plants. Possibly, they fixed nitrogen which complemented the planted maize and thus discouraged the growth of *S. hermonthica.* As a legume trap crops *Desmodium uncinatum* can release exudates which can induce suicidal germination of *S. hermonthica* without being parasitized itself. *Desmodium uncinatum* is also a cover crop which reduces the growth of weeds (Odhiambo et al., 2011).

The IR OPV and IR Hybrid maize varieties were the most effective in controlling the weed possibly due to the fact that the herbicide (imazapyr) coating on the seeds was *S. hermonthica* repellant. *Striga* resistance hybrid was the third best maize variety in *Striga* management followed by WH 403 which had the highest number of the weed.

The density of *Striga hermonthica* was significantly higher in Kibos than in Alupe suggesting agro-ecological differences. The two benchmark sites lie within the same altitude and experience similar climatic conditions (Climate data, 2012; Maplandia, 2012). However, the two regions

showed differences in the soil parameters assessed. High levels of nitrogen have been shown to discourage germination of *S. hermonthica* and this was the case in Alupe. Nitrogen is a critical nutrient in the production of a *Striga* germination stimulant known as strigolactones. With increasing nitrogen content, less strigalactones is usually produced by the crop plant hence less *Striga* germinates and vice versa (Cardosoa et al., 2011). Since the nitrogen content was significantly higher in Alupe than Kibos, this could be a possible reason why Alupe had less *S.hermonthica* emergence compared to Kibos.

The organic carbon levels were significantly higher in Alupe than in Kibos with the levels being moderate in Alupe but low in Kibos (table 9) a possible reason why Alupe had a lower *S.hermonthica* emergence than Kibos. Effects of organic carbon alone, on *S. hermonthica* are not well known, however, increasing amounts of organic carbon and nitrogen leads to the production of fewer capsules per *S.hermonthica* plant. This in turn leads to production of fewer seeds and consequently fewer *S. hermonthica* plants in the subsequent seasons (Kudra et al., 2012).

The effect of phosphorus alone on *Striga* is not well known. However, together with nitrogen they usually play a crucial role in determining the amount of strigolactone exuded by the host plant. With increasing levels of nitrogen and phosphorus strigolactone exudation by the host plants usually decreases. The decrease in strigolactone exudation results in lower *S. hermonthica* germination and infection (Jamil et al., 2012). Kibos had significantly higher amounts of phosphorus than Alupe but to the contrary it also had a higher weed density than Alupe. This could be due to the fact that Alupe had higher nitrogen levels than Kibos and the effects of nitrogen on strigolactone exudation could be more pronounced than the effects of phosphorus, or due to the fact that phosphorus levels in the two sites were higher than the recommended amounts (table 9), hence detrimental rather than beneficial to the plants.

Effects of adequate, inadequate or excess copper in *S. hermonthica* germination and emergence are not well understood (Ikie et al., 2006). Since the levels of copper were adequate in the two sites and significantly higher in Alupe than Kibos (table 9), probably increase in the amount of copper can discourage germination of *Striga*.

The effect of manganese on *Striga* emergence is not well understood. Kibos had significantly higher manganese levels than Alupe and to the contrary it also had a higher weed density suggesting that probably increase in the amount of manganese can encourage germination of *Striga*. It is not clear whether and how magnesium affects *S. hermonthica* emergence but in conjunction with other nutrients and factors probably magnesium can influence the emergence of the weed. The levels of magnesium were adequate in Kibos but low in Alupe (table 9).

Even though calcium may not have direct effects on *S. hermonthica* emergence, if it is applied as calcium ammonia nitrate fertilizer, it can significantly reduce emergence of the weed (Olakojo and Olaoye, 2007). The calcium levels were below the recommended amounts both in Kibos and Alupe with Kibos having significantly higher amounts than Alupe. The effects of zinc on *S. hermonthica* emergence are not well known. The levels of zinc were below the recommended amounts both in Alupe and Kibos with the levels in Kibos being significantly higher than the levels in Alupe (table 9).

High levels of potassium are known to have a negative effect on the emergence of *Striga* sp. In the absence of applied nitrogen, potassium applications can lead to more than a 4-fold increase in the incidence of *Striga asiatica* (Farina et al., 2006). The levels of potassium were below the recommended amounts in the two areas, however, they were not significantly different. The

effect of iron on *Striga* sp germination is not well understood. The level of iron was adequate and similar in the two areas (table 9).

Low pH values can increase *S.hermonthica* infection, how this happens is not fully understood but it can be attributed to the fact that low pH in the soil results to a reduction in available exchangeable bases and vice versa and it is known that exchangeable bases boosts plants immunity, hence the more the availability of exchangeable bases in the soil the more the plants can be tolerant to *S. hermonthica* and thus leading to its reduction and vice versa (Tarfa et al., 2006). The soil pH in Alupe was more acidic than Kibos. On average the pH of Alupe was 4.79 and that of Kibos was 5.45 (table 10). To the contrary Alupe had a lower weed density than Kibos this can be due to the effects of other factors being more pronounced than the effects of soil pH.

Microbial composition in the two sites was similar (table 10). The role of bacteria, fungi and actinomycetes on *S. hermonthica* emergence is not well understood. However, microbes are known to be degraders and they can possibly degrade the seeds of *S. hermonthica* in the soil.

Type of soil can influence the emergence and growth of *S. hermonthica,* sandy and well drained soils favors the growth of *S. hermonthica* while clay soils on the other hand, have poor drainage and excess moisture which discourages *S. hermonthica* germination by lowering the soil temperature and diluting the germination stimulant (Kayeke et al., 2007). The levels of clay and silt were high in Alupe than Kibos, while the levels of sand were high in Kibos than Alupe. This gave Alupe a clay soil texture while Kibos a sandy soil texture. This could be a possible explanation as to why Kibos had a higher *S. hermonthica* emergence than Alupe.

Striga thrives in nutrient deficient soils and the weed can be used as an indicator of soil infertility and once the weed has established itself, it is usually almost impossible to eliminate it without correcting the infertility status of the soil (Oswald, 2005). The high number of the weed in the two benchmark sites can be attributed to the fact that the soils were infertile with most nutrients being either above or below the recommended amounts. The nitrogen content was below the recommend amounts in both areas, the phosphorus levels were higher than the recommended amounts in both areas. In Alupe the organic carbon content was moderate but below the recommended amount in Kibos. The levels of calcium and zinc were below the recommended amounts however; other micronutrients like manganese, potassium and iron were in adequate amounts with magnesium being adequate in Kibos but low in Alupe.

Fusarium sp was the most abundant fungal species isolated from *S. hermonthica*. All the *Fusarium* sp were isolated from the leaves of *S. hermonthica* with *Gibberella intricans*, *Gibberella monoliformis* and *Colletotrichum gloeosporioide* being isolated from the stems and *Hypocrea stellata* and *Arthroderma otae* being isolated from the roots. Molecular characterization of the fungal isolates revealed that the amplified genomic DNA of the fungal isolates ranged between 100bp to 850bp, indicating their potential in genetic modification in producing more virulent strains for use as biocontrol agents. Phylogenetic analysis of the fungal isolates, revealed that the *Fusarium* sp were related to each other, close association was also observed amongst the species isolated from the leaves of the weed. However, no relatedness was observed in terms of virulence.

In the green house, all the fungal isolates tested showed high virulence efficacy on the weed revealing a wide variety of choice in developing a biological control agent for controlling the weed. All the fungal isolates demonstrated infection rates greater than 75% indicating that the

isolates tested could easily colonize the weed. However, it was only the two strains *Fusarium oxysporum* which had mortality rates greater than 50%, one having 60% and the other having 58% agreeing with the findings of Elzein et al., (2008). This demonstrated the suitability of *F. oxysporum* as a boicontrol agent against the weed. The less than 50% mortality rate exhibited by most of the isolates can be attributed to the time of inoculation of the weed with the fungal isolates. The fungal isolates were inoculated when the *S.hermonthica* were 8 weeks old, probably a higher mortality rate could have been achieved with early inoculation, because perhaps young *S. hermonthica* are more susceptible. The use of *Fusarium* sp as bioherbicides against *S. hermonthica* sp usually reduces the growth vigor and consequently the biomass of the weed. Once the *S. hermonthica* plant has been infected there is usually a great reduction in the number of flowering and fruiting plants. Thus, the application of *Fusarium* spores on *S. hermonthica* in the field could limit the increase of the soil seed bank (Yonli et al., 2010).

CHAPTER SIX

6.1 CONCLUSION

In conclusion, appropriate cropping systems and maize varieties can be used to alleviate the *Striga* menace. Maize intercropped with *Desmodium uncinatum* was the best cropping system against *S.hermonthica* since it had the least number of the weed. For the maize varieties, IR hybrid and IR OPV were the best since they had the least number of the weed.

Soil fertility and good soil texture should be maintained in effective management of *S. hermonthica*. Nitrogen is the most crucial nutrient that has direct effects to the emergence of *S. hermonthica*. Increasing the levels of nitrogen in the soil can lead to a decrease in the emergence of *S. hermonthica.* Soil texture can directly influence the emergence of *S. hermonthica.* The emergence of *S. hermonthica* increases with an increase in sand quantity in the soil and vice versa.

There is a wide variety of choice all in developing a biocontrol agent against *Striga,* all the fungal isolates tested as bicontrol agents against the weed could cause infection and consequently kill the weed. *F. oxysporum* was the most aggressive recording the highest mortality rates of more than 50%.

6.2 RECOMMENDATIONS

- Intercropping food crops with leguminous plants like groundnuts can help to ease the production constrains imposed by *S. hermonthica* to farmers.
- The use of improved maize varieties can be used as tool to circumvent the production constraints imposed by *S. hermonthica* in Kenya.
- The *S. hermonthica* menace in the Western Kenya Region, can be alleviated by adding organic amendments rich in nitrogen to the soil.
- Proper tillage and maintenance of a balanced soil structure is essential in the elimination of *S. hermonthica.*
- Further research should be done on the fungal isolates tested as mycoherbicides so as to elucidate their maximum potential and find out how best they can be used and exploited as biocontrol agents against the weed on a commercial scale.

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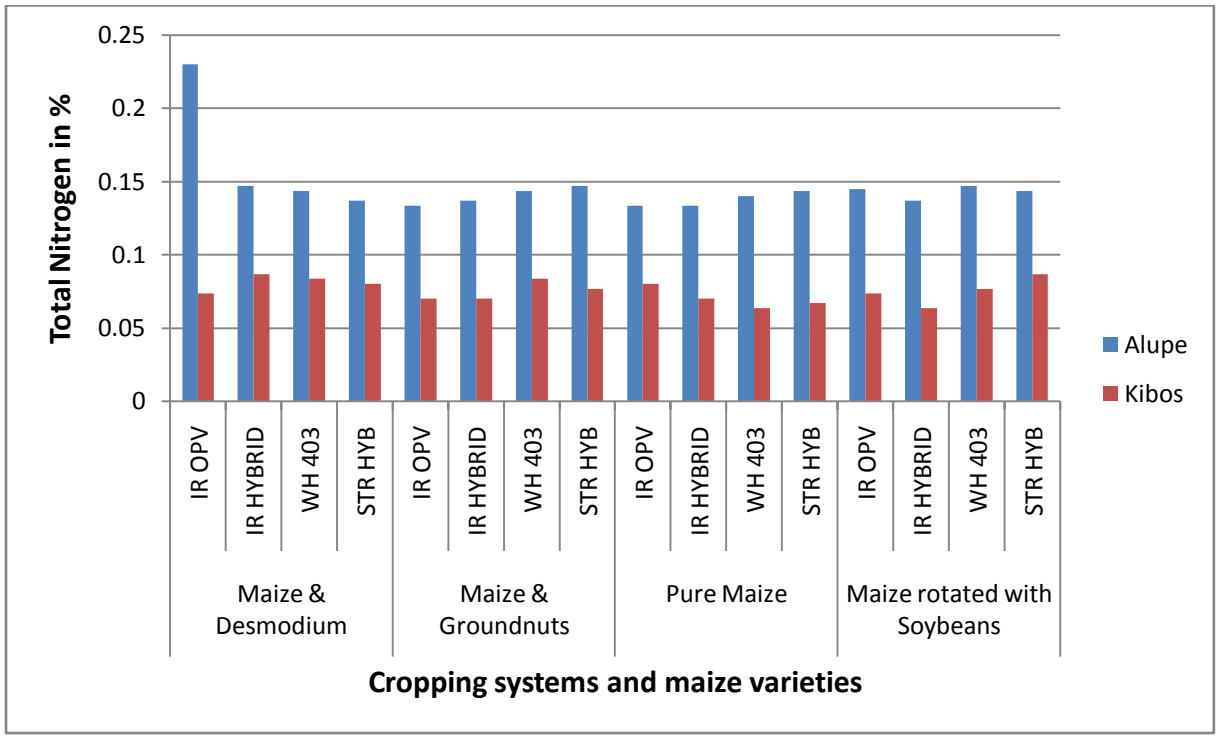
APPENDICES

Appendix 1

Comparison of nitrogen in Alupe and in Kibos: ANOVA

Dependent Variable: Total Nitrogen

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	.682[a]	31	.022	1.339	.161
Intercept	1.453	1	1.453	88.472	.000
Areas	.219	1	.219	13.364	.001
CSYTM	.045	3	.015	.906	.443
MaizeVar	.061	3	.020	1.244	.301
Areas * CSYTM	.042	3	.014	.844	.475
Areas * MaizeVar	.064	3	.021	1.297	.283
CSYTM * MaizeVar	.123	9	.014	.834	.588
Areas * CSYTM * MaizeVar	.128	9	.014	.864	.561
Error	1.051	64	.016		
Total	3.186	96			
Corrected Total	1.733	95			



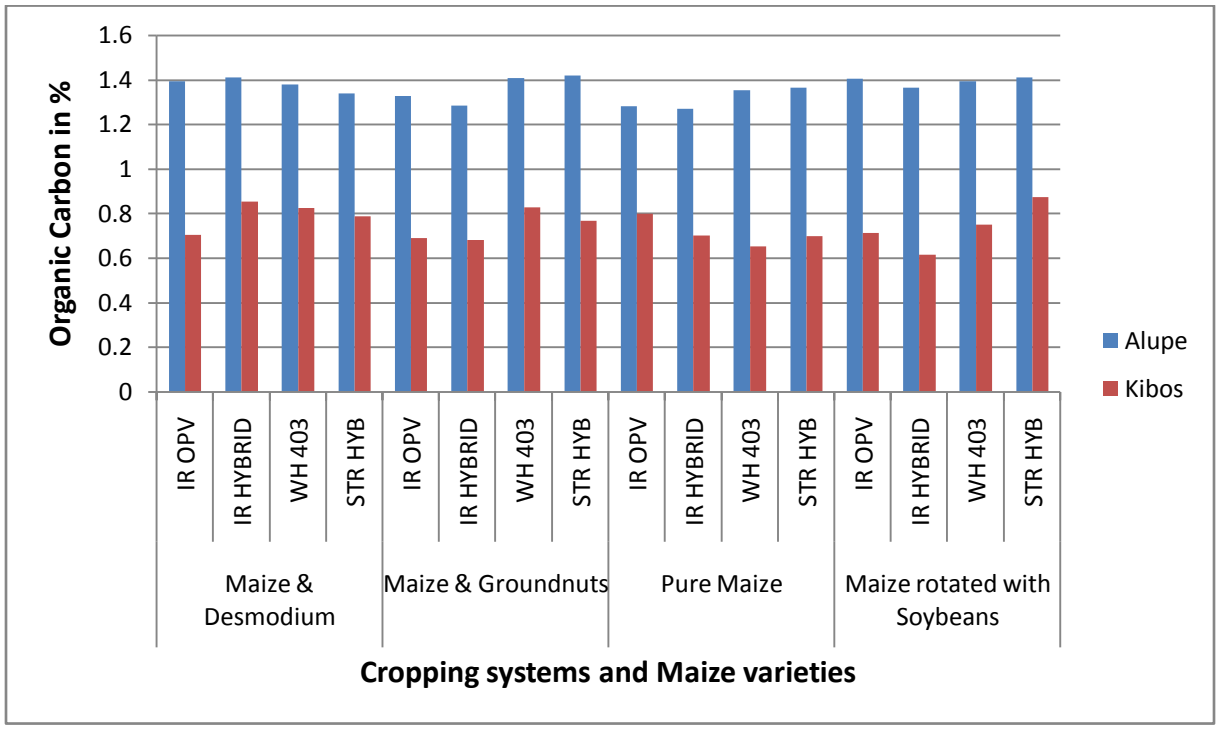
Nitrogen levels in Alupe and Kibos.

Appendix 2

Comparison of organic carbon content in Alupe and Kibos: ANOVA

Dependent Variable: Organic Carbon

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	9.497[a]	31	.306	5.415	.000
Intercept	106.640	1	106.640	1.885E3	.000
Areas	9.127	1	9.127	161.314	.000
CSYTM	.066	3	.022	.386	.763
MaizeVar	.059	3	.020	.345	.793
Areas * CSYTM	.014	3	.005	.084	.968
Areas * MaizeVar	.002	3	.001	.010	.999
CSYTM * MaizeVar	.126	9	.014	.247	.986
Areas * CSYTM * MaizeVar	.105	9	.012	.206	.993
Error	3.621	64	.057		
Total	119.758	96			
Corrected Total	13.118	95			



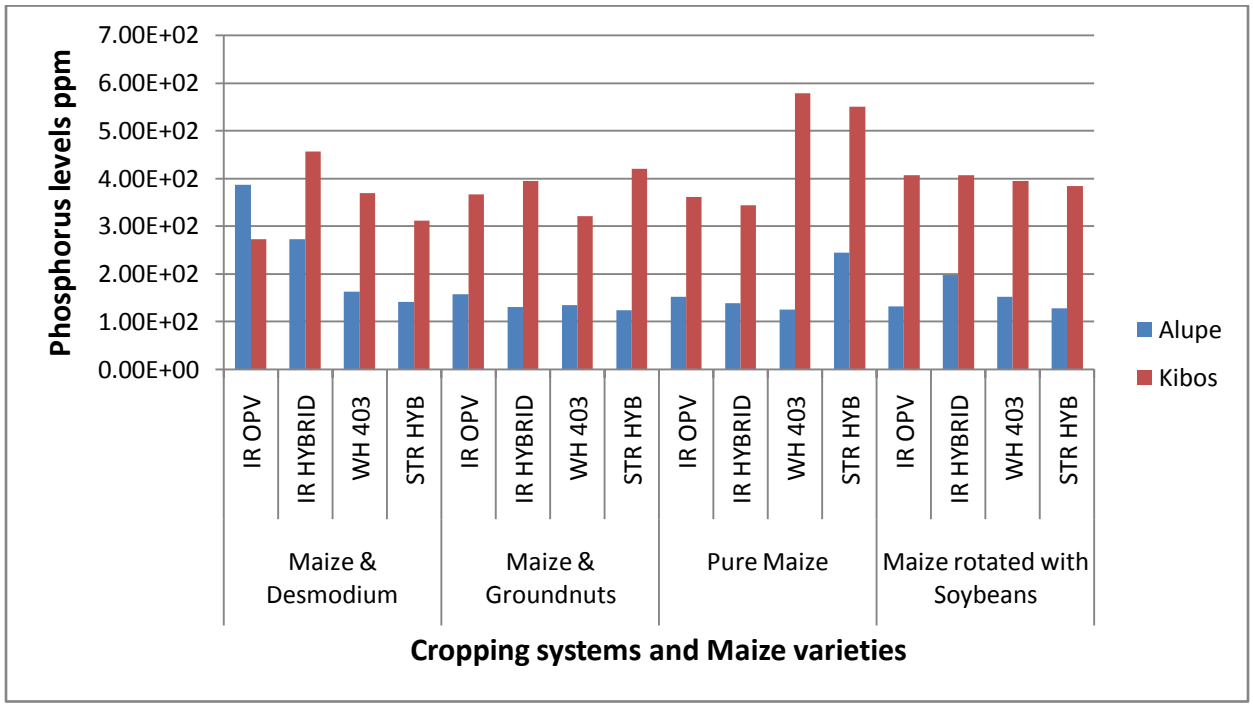
Organic carbon content in Alupe and Kibos

Appendix 3

Comparison of phosphorus in Alupe and in Kibos: Anova

Dependent Variable: Phosphorous

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.696E6[a]	31	54724.360	2.769	.000
Intercept	7813568.167	1	7813568.167	395.311	.000
Areas	1182816.000	1	1182816.000	59.842	.000
CSYTM	42973.833	3	14324.611	.725	.541
MaizeVar	2880.417	3	960.139	.049	.986
Areas * CSYTM	108124.333	3	36041.444	1.823	.152
Areas * MaizeVar	58229.083	3	19409.694	.982	.407
CSYTM * MaizeVar	181800.250	9	20200.028	1.022	.432
Areas * CSYTM * MaizeVar	119631.250	9	13292.361	.672	.731
Error	1264998.667	64	19765.604		
Total	1.078E7	96			
Corrected Total	2961453.833	95			



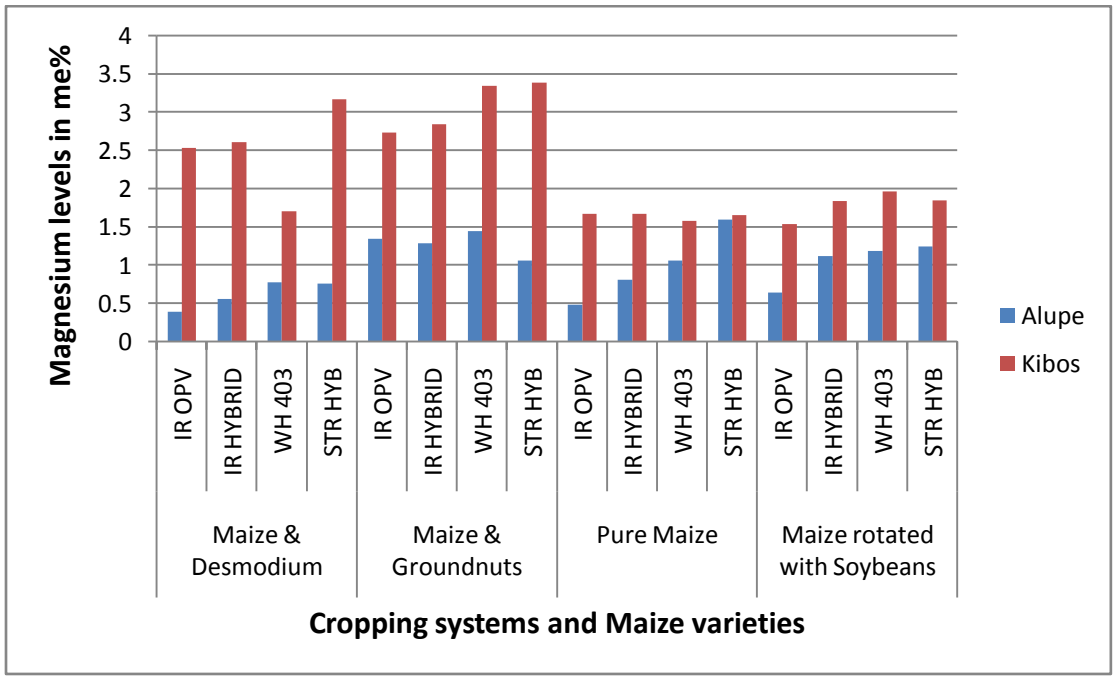
Phosphorus levels in Alupe and Kibos

Appendix 4

Comparison of Magnesium in Alupe and Kibos: Anova

Dependent Variable: Magnesium

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F- Value	Significance level
Corrected Model	64.843[a]	31	2.092	2.528	.001
Intercept	251.845	1	251.845	304.430	.000
Areas	38.621	1	38.621	46.685	.000
CSYTM	10.732	3	3.577	4.324	.008
MaizeVar	2.144	3	.715	.864	.464
Areas * CSYTM	7.738	3	2.579	3.118	.032
Areas * MaizeVar	.488	3	.163	.197	.898
CSYTM * MaizeVar	1.838	9	.204	.247	.986
Areas * CSYTM * MaizeVar	3.282	9	.365	.441	.908
Error	52.945	64	.827		
Total	369.633	96			
Corrected Total	117.788	95			



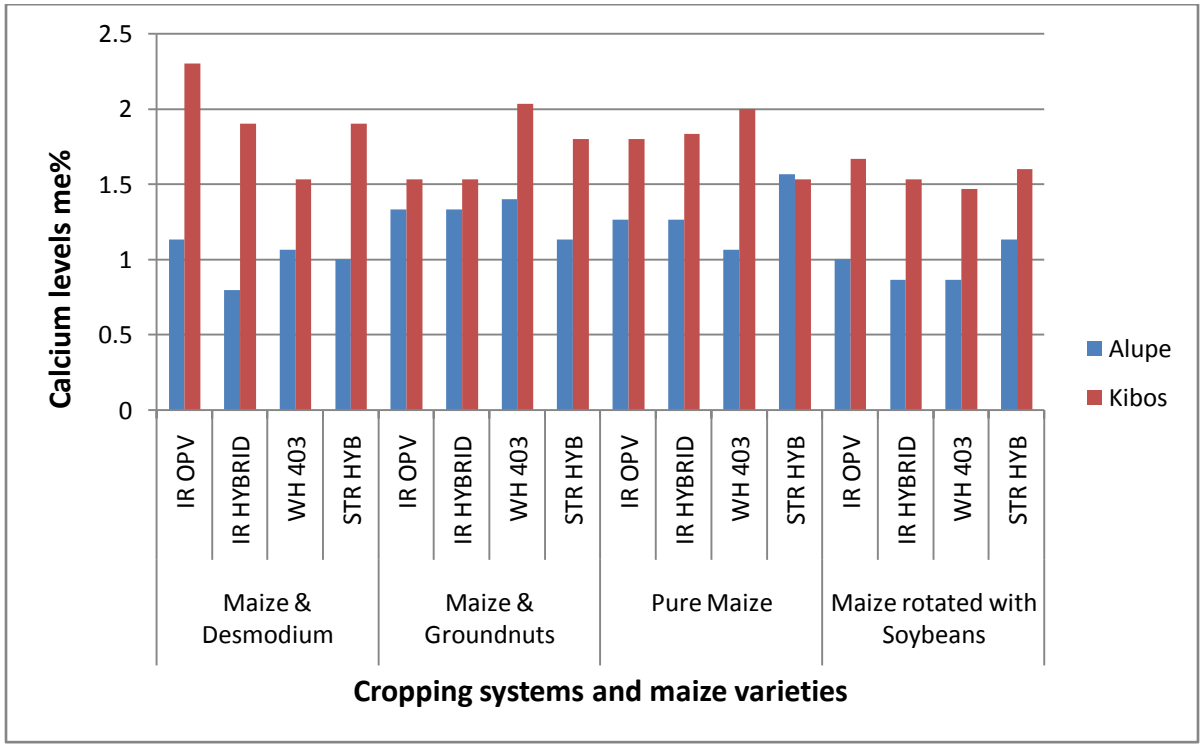
Magnesium levels in Alupe and Kibos

Appendix 5

Comparison of Calcium in Alupe and Kibos: Anova

Dependent Variable: Calcium

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	13.436[a]	31	.433	1.811	.023
Intercept	200.104	1	200.104	835.943	.000
Areas	8.882	1	8.882	37.104	.000
CSYTM	1.099	3	.366	1.530	.215
MaizeVar	.185	3	.062	.258	.855
Areas * CSYTM	.813	3	.271	1.131	.343
Areas * MaizeVar	.096	3	.032	.133	.940
CSYTM * MaizeVar	.949	9	.105	.440	.908
Areas * CSYTM * MaizeVar	1.413	9	.157	.656	.745
Error	15.320	64	.239		
Total	228.860	96			
Corrected Total	28.756	95			



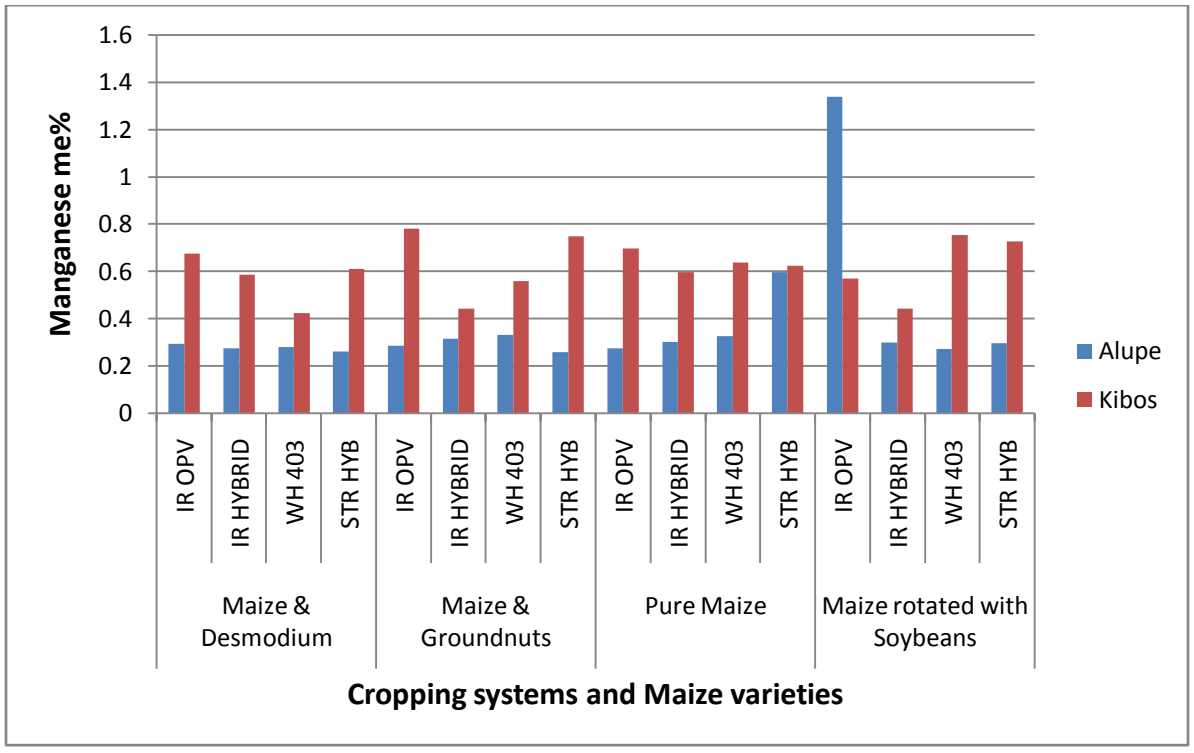
Calcium in Alupe and in Kibos

Appendix 6

Comparison of Manganese in Alupe and Kibos: Anova

Dependent Variable: Manganese

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	5.195[a]	31	.168	.995	.492
Intercept	23.691	1	23.691	140.631	.000
Areas	1.394	1	1.394	8.277	.005
CSYTM	.342	3	.114	.676	.570
MaizeVar	.586	3	.195	1.159	.332
Areas * CSYTM	.245	3	.082	.485	.694
Areas * MaizeVar	.129	3	.043	.255	.858
CSYTM * MaizeVar	.792	9	.088	.522	.853
Areas * CSYTM * MaizeVar	1.708	9	.190	1.127	.358
Error	10.782	64	.168		
Total	39.668	96			
Corrected Total	15.977	95			



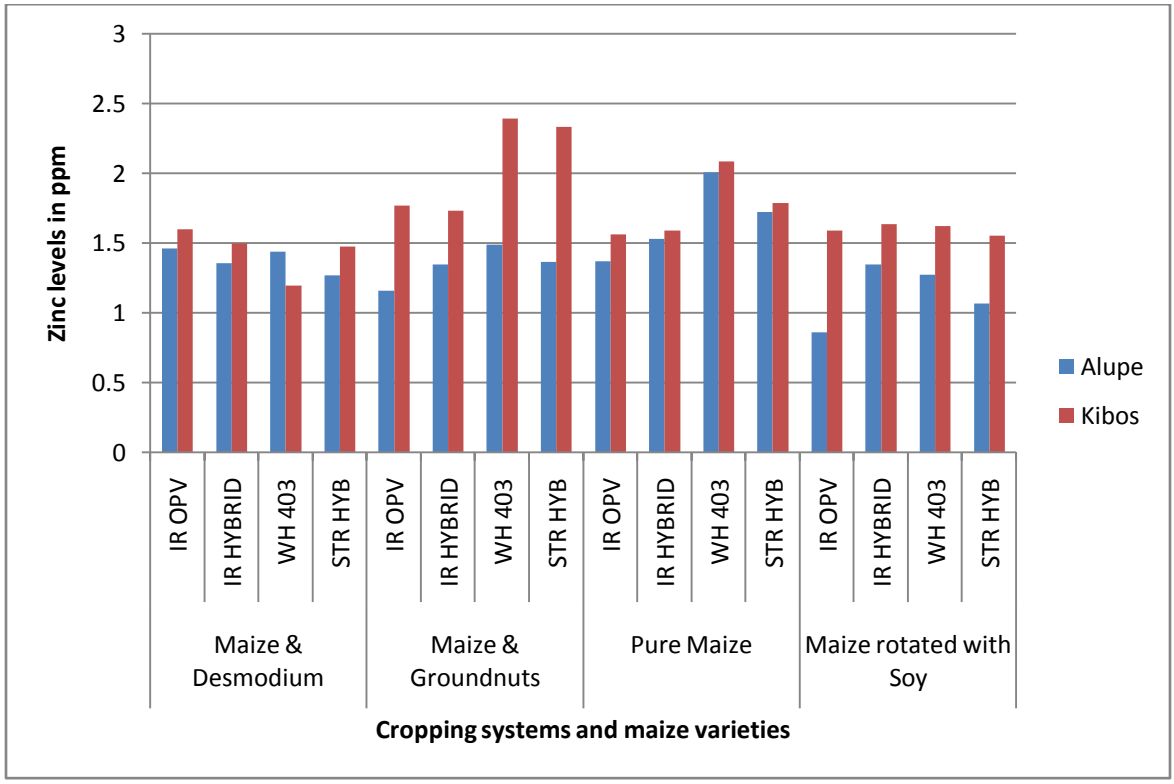
Manganese in Alupe and Kibos

Appendix 7

Comparison of Zinc in Alupe and Kibos: Anova

Dependent Variable: Zinc

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	10.085[a]	31	.325	1.413	.122
Intercept	229.402	1	229.402	996.302	.000
Areas	2.687	1	2.687	11.668	.001
CSYTM	2.360	3	.787	3.416	.023
MaizeVar	.907	3	.302	1.314	.278
Areas * CSYTM	1.767	3	.589	2.558	.063
Areas * MaizeVar	.200	3	.067	.289	.833
CSYTM * MaizeVar	1.662	9	.185	.802	.616
Areas * CSYTM * MaizeVar	.503	9	.056	.243	.987
Error	14.736	64	.230		
Total	254.223	96			
Corrected Total	24.821	95			



Zinc levels in Kibos and Alupe

Appendix 8

Comparison of Iron in Kibos and Alupe: Anova

Dependent Variable: Iron

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	5603.337[a]	31	180.753	.700	.861
Intercept	122513.172	1	122513.172	474.152	.000
Areas	101.414	1	101.414	.392	.533
CSYTM	21.333	3	7.111	.028	.994
MaizeVar	38.903	3	12.968	.050	.985
Areas * CSYTM	3949.072	3	1316.357	5.095	.003
Areas * MaizeVar	152.402	3	50.801	.197	.898
CSYTM * MaizeVar	379.462	9	42.162	.163	.997
Areas * CSYTM * MaizeVar	960.750	9	106.750	.413	.924
Error	16536.568	64	258.384		
Total	144653.077	96			
Corrected Total	22139.905	95			

Comparison of Potassium in Kibos and Alupe: Anova

Dependent Variable: Potassium

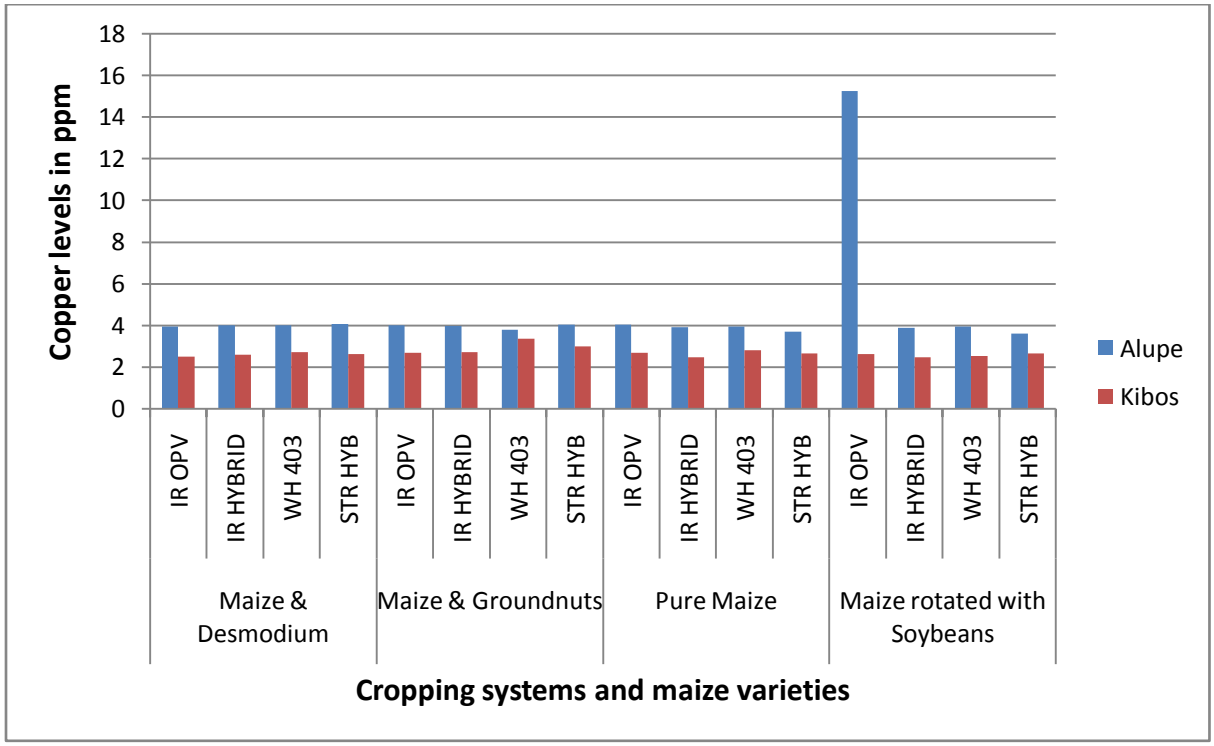
Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	.567[a]	31	.018	.890	.631
Intercept	3.753	1	3.753	182.483	.000
Areas	.073	1	.073	3.531	.065
CSYTM	.084	3	.028	1.368	.260
MaizeVar	.033	3	.011	.536	.659
Areas * CSYTM	.032	3	.011	.526	.666
Areas * MaizeVar	.036	3	.012	.584	.628
CSYTM * MaizeVar	.100	9	.011	.543	.838
Areas * CSYTM * MaizeVar	.208	9	.023	1.126	.358
Error	1.316	64	.021		
Total	5.636	96			
Corrected Total	1.883	95			

Appendix 9

Comparison of Copper in Alupe and Kibos: Anova

Dependent Variable:Copper

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	454.987[a]	31	14.677	1.160	.303
Intercept	1276.479	1	1276.479	100.871	.000
Areas	90.482	1	90.482	7.150	.010
CSYTM	29.789	3	9.930	.785	.507
MaizeVar	35.872	3	11.957	.945	.424
Areas * CSYTM	38.081	3	12.694	1.003	.397
Areas * MaizeVar	40.552	3	13.517	1.068	.369
CSYTM * MaizeVar	113.993	9	12.666	1.001	.449
Areas * CSYTM * MaizeVar	106.219	9	11.802	.933	.503
Error	809.890	64	12.655		
Total	2541.357	96			
Corrected Total	1264.877	95			



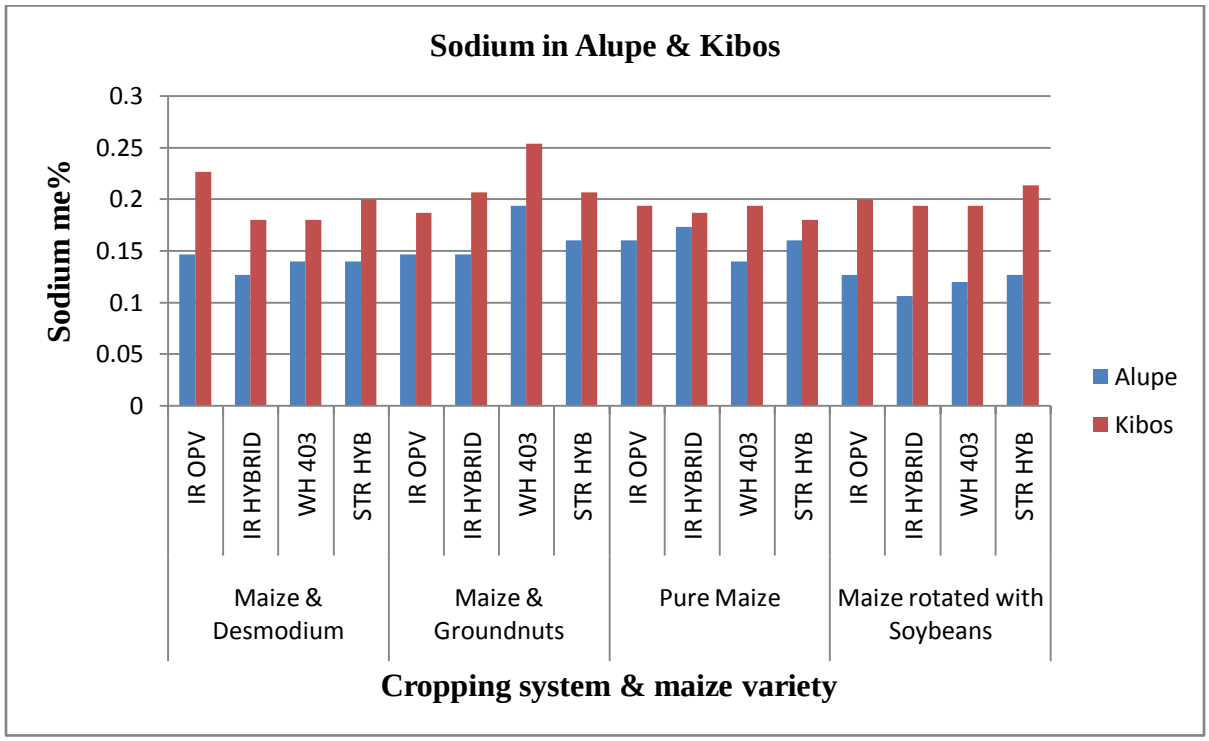
Copper in Alupe and Kibos

Appendix 10

Comparison of Sodium in Alupe and Kibos: ANOVA

Dependent Variable: Sodium

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F- Value	Significance level
Corrected Model	.110[a]	31	.004	2.194	.004
Intercept	2.843	1	2.843	1.754E3	.000
Areas	.073	1	.073	44.792	.000
CSYTM	.010	3	.003	2.005	.122
MaizeVar	.002	3	.001	.367	.777
Areas * CSYTM	.008	3	.003	1.570	.205
Areas * MaizeVar	6.667E-5	3	2.222E-5	.014	.998
CSYTM * MaizeVar	.015	9	.002	1.036	.422
Areas * CSYTM * MaizeVar	.003	9	.000	.226	.990
Error	.104	64	.002		
Total	3.057	96			
Corrected Total	.214	95			



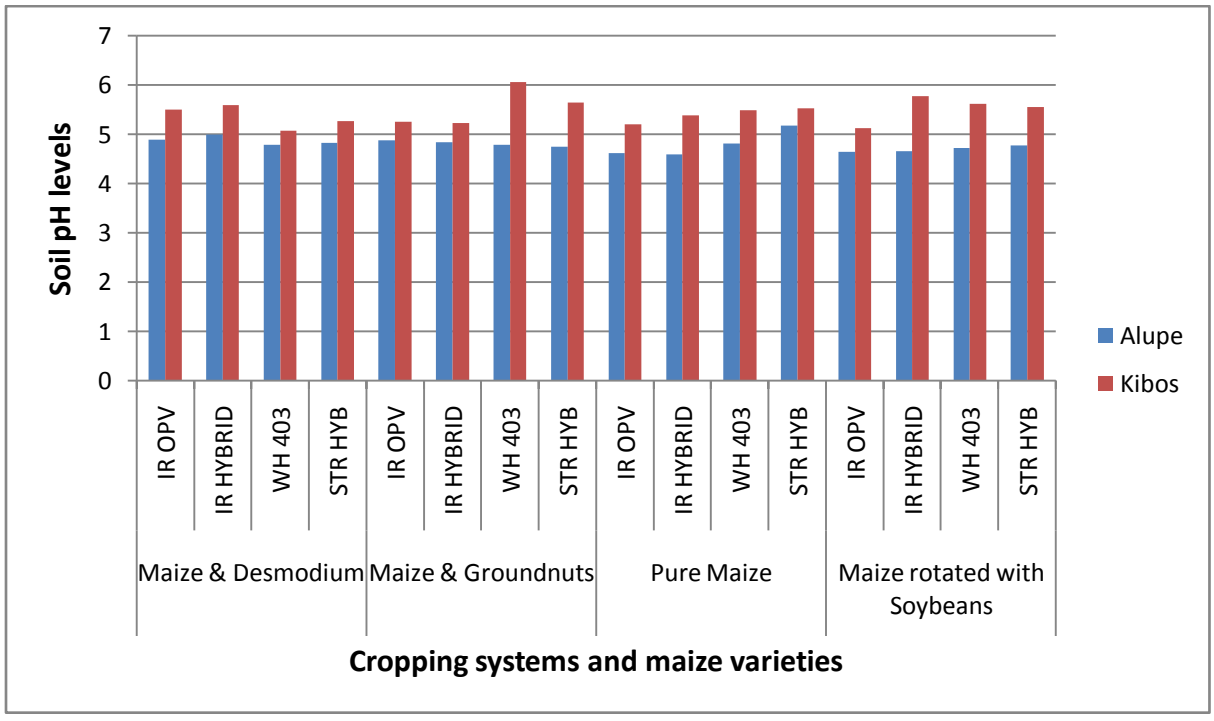
Sodium levels in Alupe and Kibos

Appendix 11

Comparison of soil pH in Alupe and Kibos: Anova

Dependent Variable: Soil PH

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	14.480[a]	31	.467	1.761	.028
Intercept	2514.842	1	2514.842	9.482E3	.000
Areas	10.435	1	10.435	39.342	.000
CSYTM	.093	3	.031	.117	.950
MaizeVar	.452	3	.151	.568	.638
Areas * CSYTM	.384	3	.128	.483	.695
Areas * MaizeVar	.255	3	.085	.321	.810
CSYTM * MaizeVar	1.695	9	.188	.710	.697
Areas * CSYTM * MaizeVar	1.166	9	.130	.488	.877
Error	16.975	64	.265		
Total	2546.296	96			
Corrected Total	31.455	95			



Soil pH levels in Alupe and in Kibos.

Appendix 12

Comparison of Bacterial population in Kibos and Alupe: Anova

Dependent Variable: Bacteria

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	3.386E13[a]	31	1.092E12	.855	.678
Intercept	1.196E14	1	1.196E14	93.565	.000
Areas	5.437E11	1	5.437E11	.425	.517
CSYTM	6.459E12	3	2.153E12	1.685	.179
MaizeVar	3.321E12	3	1.107E12	.866	.463
Areas * CSYTM	5.116E11	3	1.705E11	.133	.940
Areas * MaizeVar	3.590E12	3	1.197E12	.936	.428
CSYTM * MaizeVar	1.268E13	9	1.409E12	1.102	.374
Areas * CSYTM * MaizeVar	6.762E12	9	7.514E11	.588	.802
Error	8.179E13	64	1.278E12		
Total	2.352E14	96			
Corrected Total	1.157E14	95			

Comparison of Fungal population in Kibos and Alupe: Anova

Dependent Variable: Fungi

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	1.080E13[a]	31	3.484E11	1.352	.154
Intercept	3.373E13	1	3.373E13	130.924	.000
Areas	3.565E11	1	3.565E11	1.384	.244
CSYTM	1.421E12	3	4.736E11	1.838	.149
MaizeVar	1.225E12	3	4.082E11	1.584	.202
Areas * CSYTM	2.345E11	3	7.817E10	.303	.823
Areas * MaizeVar	1.774E10	3	5.912E9	.023	.995
CSYTM * MaizeVar	1.318E12	9	1.464E11	.568	.818
Areas * CSYTM * MaizeVar	6.227E12	9	6.919E11	2.685	.010
Error	1.649E13	64	2.576E11		
Total	6.102E13	96			
Corrected Total	2.729E13	95			

Appendix 13

Comparison of actinomycetes population in Kibos and Alupe: Anova

Dependent Variable :Actinomycetes

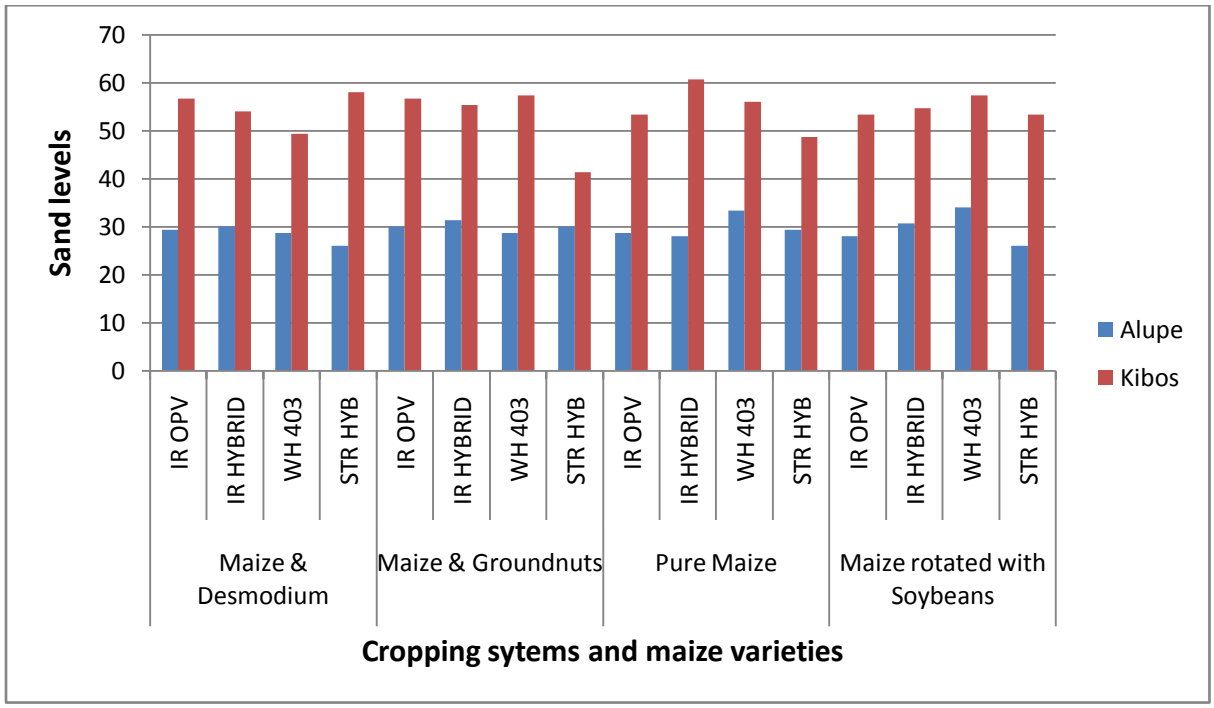
Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	3.580E13[a]	31	1.155E12	.682	.877
Intercept	8.264E13	1	8.264E13	48.843	.000
Areas	3.022E11	1	3.022E11	.179	.674
CSYTM	8.009E12	3	2.670E12	1.578	.203
MaizeVar	3.905E12	3	1.302E12	.769	.515
Areas * CSYTM	3.760E12	3	1.253E12	.741	.532
Areas * MaizeVar	3.860E12	3	1.287E12	.760	.520
CSYTM * MaizeVar	1.071E13	9	1.190E12	.703	.704
Areas * CSYTM * MaizeVar	5.249E12	9	5.833E11	.345	.956
Error	1.083E14	64	1.692E12		
Total	2.267E14	96			
Corrected Total	1.441E14	95			

Appendix 14

Comparison of sand levels in Alupe and Kibos: ANOVA

Dependent Variable: Sand

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F- Value	Significance level
Corrected Model	15712.625[a]	31	506.859	2.255	.003
Intercept	167835.375	1	167835.375	746.765	.000
Areas	14553.375	1	14553.375	64.754	.000
CSYTM	15.458	3	5.153	.023	.995
MaizeVar	257.125	3	85.708	.381	.767
Areas * CSYTM	35.458	3	11.819	.053	.984
Areas * MaizeVar	56.458	3	18.819	.084	.969
CSYTM * MaizeVar	314.042	9	34.894	.155	.997
Areas * CSYTM * MaizeVar	480.708	9	53.412	.238	.988
Error	14384.000	64	224.750		
Total	197932.000	96			
Corrected Total	30096.625	95			



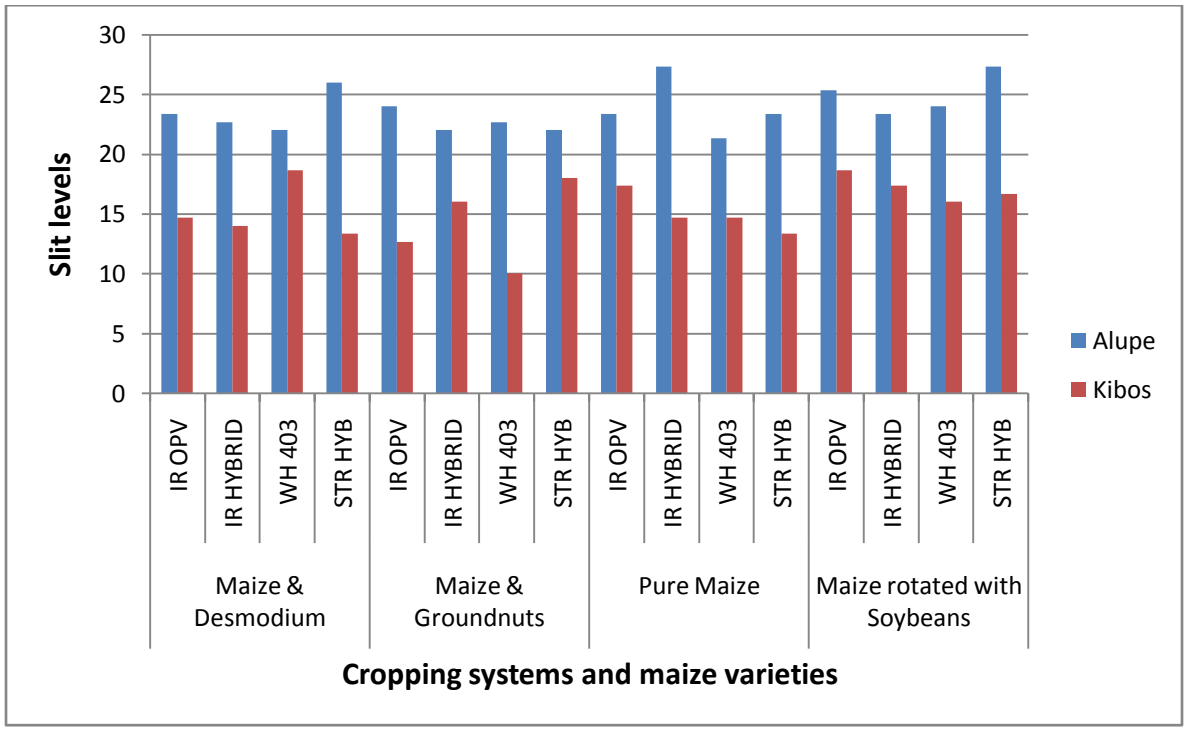
Sand levels in Alupe and in Kibos

Appendix 15

Comparison of silt levels in Alupe and Kibos: Anova

Dependent Variable: Silt

Source: Alupe and Kibos	Type III Sum of Squares	Degrees of freedom	Mean Square	F-Value	Significance level
Corrected Model	2097.625[a]	31	67.665	3.024	.000
Intercept	36738.375	1	36738.375	1.642E3	.000
Areas	1683.375	1	1683.375	75.235	.000
CSYTM	88.792	3	29.597	1.323	.275
MaizeVar	27.125	3	9.042	.404	.751
Areas * CSYTM	3.125	3	1.042	.047	.987
Areas * MaizeVar	8.792	3	2.931	.131	.941
CSYTM * MaizeVar	89.042	9	9.894	.442	.907
Areas * CSYTM * MaizeVar	197.375	9	21.931	.980	.465
Error	1432.000	64	22.375		
Total	40268.000	96			
Corrected Total	3529.625	95			



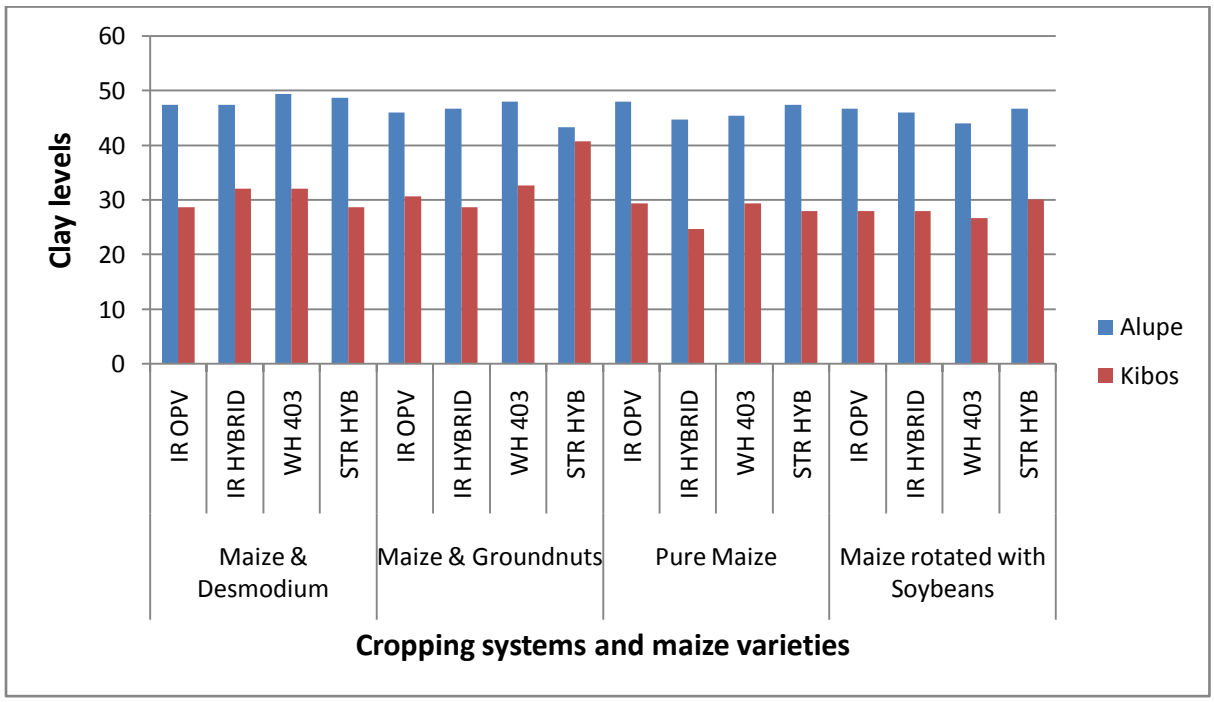
Silt levels in Alupe and Kibos

Appendix 16

Comparison of clay levels in Alupe and Kibos: Anova

Dependent Variable: Clay

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7382.958[a]	31	238.160	1.642	.047
Intercept	140301.042	1	140301.042	967.593	.000
Areas	6700.042	1	6700.042	46.207	.000
CSYTM	136.792	3	45.597	.314	.815
MaizeVar	45.458	3	15.153	.105	.957
Areas * CSYTM	122.458	3	40.819	.282	.839
Areas * MaizeVar	40.458	3	13.486	.093	.964
CSYTM * MaizeVar	129.375	9	14.375	.099	1.000
Areas * CSYTM * MaizeVar	208.375	9	23.153	.160	.997
Error	9280.000	64	145.000		
Total	156964.000	96			
Corrected Total	16662.958	95			



Clay levels in Alupe and Kibos.

Appendix 17

Sequences of the isolated fungal species

Lab no.2 Name: *Hypocrea stellata* (Strain GJS 99-222)

GGAAGCCGCCGAACTCGGCAAGGGTTCCTTCAAGTACGCGTGGGTTCTTGACAAGCT
CAAGGCCGAGCGTGAGCGTGGAATCACCATCGACATTGCCCTCTGGAAGTTCGAGA
CTCCCAAATACTATGTCACCGTCATTGGTATGTTTTCATTCCTTCGTATTCGATACTG
TAGAGATACACCAGTGCCAACAACACCTGACAGATGCTCCCGGTCACCGTGATTTCA
TCAAGAACATGATCACTGGTACTTCCC

Lab no. 23. Name: *Colletotrichum gloeosporioides* (Isolate s-7)

GGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGTACCATCGAGAAGTTCGAG
AAGGAGGCCGCCGAATTGTATTTCACCCTCACCTCATCACATCGTCGTTTATTAACA
AATCTATAGGGGCAAGGGCTCCTTCAAGTATGCCTGGGTCGGTTCCCCGTTTTCTCG
CGATCCTGAAATTGTCTTGCTGACTCTCTACAGGTTCTTGACAAGCTCAAGGCCGAG
CGTGAGCGTGGTATCACCATCGACATTGCTCTCTGGAAGTTCGAGACTCCCAGATAC
TACGTCACCGTCATTGGTAAG

Lab no. 5. Name: *Gibberella monoliformis* (voucher GFLC190)

TCATCATCGGGCCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTACCCTTGACG
ATGAGCTTATCGGCCATCGTAAACCCGGCCAAGACCTGGCGGGGGATTTCTCAAAG
AAAACATACTGATATCGCTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTAT
CGACAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTTCCTTCTATCGCGC
GTTCTTTGCCCATCGATTCCCCCCTACGACTCGAAACGTACCCGCTACCCCGCTCGA
GCCCAAAAATTTTGCGATACGACCGTAATTTTTTCTGGTGGGGCATTTACCCCGCCA
CTCGAGCGGCGCGTTTCTGCCCTCTCCCATTCCACAACCTCACTGAGCTCATCGTCAC
GTGTCAAGCAGTCACTAACCATCCGACAATAGGAAGCCGCTGAGCTCGGTAAGGGT
TCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATC
ACCATCGATATCGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTG
GTATGTTGTCGCTCTTACTCCGTTCTATATCTCCTATTACTAACACATCACATAGACG
CTCCCGGTCACCGTGATTTCATCAAGAACATGATGATGGGTACCCTCCC

Lab no. 6. Name: *Fusarium equiseti* (Strain DBT – 102)

ATAACATACCATAGAACCCCCCACATTGGGAACGCGGGTTACCGCAGTCCCAACAC
CAACTAACTTGAGGGTTGAAATGACCTCGAACAGGCATGCCCGCCAAAATACTGGC
GGGCGCAATGTGCGTTCAAAAATTCAATGATTCACTGAATTCTGCAATTCACATTAC
TTATCCATTTTGCTGCTTCTTCATCATGCCAAAACCAAAAAATCCTTGTTGAAATTTT
GATTTTTTGTTTGTTTTACTCAAAATCCAAAAAAAATTGGGTCCTCGGGGGGCCGTC
CTTTTTACGGGGGGGGTGATCCCCAGGCAACGATGTCMCGGGGTTGGATTA

Lab no.12. Name: *Fusarium incarnatum* (Isolate lb2)

TACAATCGGCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTACCCACGATGACC
TGCCTATCACAGTCATCAACCCCGCCATATGTGGCGGGGTAATTTCAACCTGAATAT
TTGTTGACAAGATTGTATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACA
AGCGAACCATCGAGAAGTTCGAGAAGGTTGGTTTCCATTTCCCTCGATCGCACGACA
TCTACCCATCGATCCATCAGTCGAATCAGTCTTACGACGATTGAATATGCGCCTGTT
ACCCCGCTCGAGTACAAAATTTTGCGGTTCAACCGTAATTTTTTGGTGGGGTTTCAA
CCCCGCTACTCGAGTGACAGGCGTTTGCCCTTCCCACAATGCGCATCACGTGTCAAT
CAGTCACTAACCACCCGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAA
GTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGA
TATCGCCCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTG
TCAACACTTACACTCATCATCTTCTCATGCTAACGTGTGCTTCAGACGCTCCCGGTCA
CCGTGATTTCATCAAGAACATGATATGGGGTCCCCAAA

Lab no.19. Name**:** *Fusarium oxysporum* (Strain NRRL 52937);

TCATATCGGCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTCTCCTCGACAATG
AGCATATCTGCCATCGTCAATCCCGACCAAGACCTGGCGGGGTATTTCTCAAAGTCA
ACATACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGA
CAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGCGTC
CTTTGCCCATCGATTTCCCCTACGACTCGAAACGTGCCCGCTACCCCGCTCGAGACC
AAAAATTTTGCAATATGACCGTAATTTTTTTGGTGGGGCACTTACCCCGCCACTTGA
GCGAAGGGAGCGTTTGCCCTCTTACCATTCTCACAACCTCAATGAGTGCGTCGTCAC
GTGTGAAGCAGTCACTAACCATTCAATAATAGGAAGCCGCTGAGCTCGGTAAGGGT
TCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATC
ACCATCGATATTGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTG
GTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCGTACTAACATATCACTCAGACG
CTCCCGGTCACCGTGATTTCATCAAGAACATGATATGGTACTCCA

Lab no.30. Name**:** *Gibberella intricans* (Strain LVPEI.H4599)

GATTACTACGCTATGGAAGCTCGACTGACCGCCAATCATTTGGGGAACGCGGGTTAC
CGCAGTCCCAACACCAAGCTGAGCTTGAGGGTTGAAATGACCTCGAACAGGCATGC
CCGCCARAATACTGGCGGGCGCAATGTGCGTTCAAAATTCAATGATTCACTGAATTC
TGCAATTCACATTACTTATCGCATTTTGTGCTTCTTCATCATGCCAAACCAAAATCCG
TTGTTGAAATTTTGATTTATTTGTTTGTTTTACTCAAATTCCACTAAAAACAAGTTTA
GGGTCCTCGGGGGGCCGTCCCGTTTTACAGGGCGCGGGCTGATCCCCAGGCAACGT
ATAGGTATGTTCACGGGGTTTGGAGTTA

Lab no.33. Name**:** *Arthroderma otae* (Strain CBS 113480)

TCGGTCGTCGCCTGCTACCGTTGCGCCACTGAACGTACGCAAGCGTAGCACCAGCAG
TGATGATGATCTTCACACTCCGGTACAGCGTCTGAATGTGCGTACTTCGAACGAGCG
ATTGGACAAAAAGCGCAGCATCGAACCGATTGCCATCTTGGAACCGATTGAAGAGG
ACTCGGTCCTCCAGAGCCCCACTGCCGTTCGCAAGAAGAGAGCTGGTTGTTTGGAGA
AAGACGCCAGAGCCTGACTTAGCATCGGCCAGACCTGAGGCCACTTCAGCAGCCTT
GGATCTTGGTGACCCGTCTGCGAAGCGTTCGTCGTATGTCTTGAGCAAGTTACAGAA
GCCGCTGCCACAAGATCCTCCCGTATCTGCTTTGAGCAGCGAGTTCCCAATCCGGAA
GAAGAGGTTCGGTGGAGGCAAGACGGGCTTCTCAAAGTGGTTGAGCATAAAGAGCG
CTGACAAACATGATGACTGGGTACCTCCCA

Lab no.37. Name**:** *Fusarium oxysporum* (f.sp melonis Strain ISPAaVe1070)

TATCATATCGGGCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTCTCCTCGACA
ATGAGCATATCTGCCATCGTCAATCCCGACCAAGACCTGGCGGGGTATTTCTCAAAG
TCAACATACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTAT
CGACAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGC
GTCCTTTGCCCATCGATTTCCCCTACGACTCGAAACGTGCCCGCTACCCCGCTCGAG
ACCAAAAATTTTGCAATATGACCGTAATTTTTTTGGTGGGGCACTTACCCCGCCACTT
GAGCGAAGGGAGCGTTTGCCCTCTTACCATTCTCACAACCTCAATGAGTGCGTCGTC
ACGTGTGAAGCAGTCACTAACCATTCAATAATAGGAAGCCGCTGAGCTCGGTAAGG
GTTCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTA
TCACCATCGATATTGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCAT
TGGTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCGTACTAACATATCACTCAGA
CGCTCCCGGTCACCGTGATTTCATCAAGAACATGATGATGGTTACCCTC

Lab no.38. Name**:** *Fusarium oxysporum* (Isolate FSY0953)

TCATATCGGCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTCTCCTCGACAATG
AGCATATCGTATCGTCAATCCCGACCAAGACCTGGCGGGGTATTTCTCAAAGTCAAC
ATACTGACATCGTTTCACAGACTGGTCACTTGATCTACCAGTGCGGTGGTATCGACA
AGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGCGTCCT
TTGCCCATCGATTTCCCCTACGACTCGAAACGTGCCCGCTACCCCGCTCGAGACCAA
AAATTTTGCAATATGACCGTAATTTTTTTGGTGGGGCACTTACCCCGCCACTTGAGC
GACGGGAGCGTTTGCCCTCTTACCATTCTCACAACCTCAATGAGTGCGTCGTCACGT
GTCAAGCAGTCACTAACCATTCAACAATAGGAAGCCGCTGAGCTCGGTAAGGGTTC
CTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCAC
CATCGATATTGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGT
ATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCGTACTAACATATCACTCAGACGCT
CCCGGTCACCGTGATTTCATCAAGAACATGATSAGGGGCCC

Lab no.39. Name**:** *Fusarium oxysporum* (isolate IBSD-GF13)

TACGCTATGGAAGCTCGACGTGACCGCCAATCAATTTGAGGAACGCGAATTAACGC
GAGTCCCAACACCAAGCTGTGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCC
CGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTC
TGCAATTCACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGA
GATCCGTTGTTGAAAGTTTTGATTTATTTATGGTTTTACTCAGAAGTTACATATAGAA
ACAGAGTTTAGGGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGGGCTGATCC
GCCGAGGCAACAAGTGGT ATGTTCACAGGGGTTTGG

Lab no.43. Name: *Fusarium chlamydosporum* (Strain JL-26)

TATGGAAGCTCGACGTGACGGCCAATCGATTTGGGGAACGCGGGTTACCGCGAGTC
CCAACACCAAGCTGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCA
GAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAA
TTCACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC
GTTGTTGAAAGTTTTGATTTATTTGTTTGTTTTACTCAGAAGTTCCACTAAAAACAGA
ATTTAGGGGTCCTCGGGCGGGCCGTCCCTTTTTACGGGGCGCGGGCTGATCCGCCGA
GGCAACGTATAGGTATGTTCACAGGGGTTGGGA

Lab no.44. Name: *Fusarium chlamydosporum* (Isolate AC638);

GGGTTGATTACTACCTATGGAAGCTCGACGTGACCGCCAATCGATTTGGGGAACGCG
GGTTACCGCAGTCCCAACACCAAGCTGAGCTTGAGGGTTGAAATGACCTCGAACAG
GCAGCCCGCCAAATACGGCGGGCGCAATGTGCGTTCAAAATTCATGATTCACTGAAT
TCTGCAATTCACATTACTTATCGCATTTTGCTGCGTTCTTCATCATGCCAAACCAAAA
TCCGTTGTTGAAAGTTTTGATTTATTTGTTTGTTTTACTCAAATTCCACTAAAAACAA
TTTAGGGTCCTCGGGCGGGCCGTCCCGTTTTACGGGGCGCGGGCTGATCCCCAGGCA
ACGTATAGGTATGTTCACGGGGTTGTGGATA

Lab no.46. Name: *Fusarium verticilloides* (Strain 25 ALH)

CACGTTCGGCACGTCGACTCTGGCAGTCGACCACTGTGAGTACTACCCTTGACGATG
AGCTTATCGGCCATCGTAAACCCGGCCAAGACCTGGCGGGGGATTTCTCAAAGAAA
ACATACTGATATCGCTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGA
CAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTTCCTTCTATCGCGCGTT
CTTTGCCCATCGATTCCCCCCTACGACTCGAAACGTACCCGCTACCCCGCTCGAGCC
CAAAAATTTTGCGATACGACCGTAATTTTTTCTGGTGGGGCATTTACCCCGCTACTCG
AGCGGCGCGTTTCTGCCCTCTCCCATTCCACAACCTCACTGAGCTCATCGTCACGTGT
CAAGCAGTCACTAACCATCCGACAATAGGAAGCCGCTGAGCTCGGTAAGGGTTCCT
TCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCA
TCGATATCGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTAT
GTTGTCGCTCTTACTCCGTTCTATATCTCCTATTACTAACACATCACATAGACGCTCC
CGGTCACCGTGATTTCATCAAGAACATGATATGGGTCTCCC