EFFECT OF HERBIVORE-INDUCED PLANT VOLATILES OF *BRACHIARIA BRIZANTHA* ON NEIGHBOURING MAIZE FOR *CHILO PARTELLUS* MANAGEMENT IN SUBA DISTRICT, HOMA-BAY COUNTY, KENYA

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DECLARATION

Student declaration

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

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Supervisors' declaration

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DEDICATION

To my late father Magara Otieno and beloved mother Teresa Orenge, brothers and sisters for their patience, love and inspiration while doing this great work.

ACKNOWLEDGEMENT

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ACRONYMS AND ABBREVIATIONS

ANOVA   Analysis of variance

BIOASSAY   Biological assay

CIMMYT   International Maize and Wheat Improvement Centre (Centro Mejoramiento de Maiz y Trigo-in Spanish)

D.A.P   Diammonium phosphate

DCM   Dichloromethane

EAD   Electroantennodetector

GC   Gas Chromatography

GC-MS   Linked Mass Spectrometer to Gas Chromatography

HIPVs   Herbivore Induced Plant Volatiles

icipe   International Centre of Insect Physiology and Ecology

IPM   Integrated pest management

IRMA   Insect Resistant Maize for Africa

ITOC   *icipe* Thomas Odhiambo Campus

Ltd   Limited

MS   Mass Spectrometer

SEMIOCHEMICALS Chemicals mediating intraspecific (same species) and interspecific

(different species) interactions among organisms

SSA   Sub Saharan Africa

WH505   Western Hybrid 505

PH4   Pwani Hybrid 4

R   Is stasitical software used to analyse experimental data.

ABSTRACT

The Lepidopteran stemborer *Chilo partellus* is a key constraint to cereal production in most resource-poor farming systems as it causes damage to the crop, accruing up to 80%. When plants are damaged by herbivorous insects, they produce herbivore-induced plant volatiles (HIPVs) which can act as repellants for these insect pests and as attractants for organisms antagonistic to these pests (parasitic wasps and predators). Plants can also produce signals that warn other plants of impending attack. Thus, HIPVs potentially have a practical role in crop protection by directly deterring further colonization in already infested and neighbouring plants and indirectly by attracting natural enemies of the pests, particularly parasitoids. The study sought to evaluate the effect of herbivore-induced plant volatiles on maize plants exposed to neighbouring *Brachiaria brizantha* (A.Rich) Stapf (Poaceae) grass oviposited by the spotted stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). This was done using six maize varieties; four local varieties and two hybrids. Treatments comprised of maize seedlings exposed to *B. brizantha* with *C. partellus* eggs versus non-exposed ones and or maize seedlings exposed to *B. brizantha* without *C. partellus* eggs. Responses of stemborers and larval parasitoids to HIPVs collected from maize plants exposed to *B. brizantha* with and without stemborer eggs was determined by olfactometer and oviposition bioassays respectively. Qualitative and quantitative analyses of these HIPVs was determined through gas chromatography (GC) and GC-Mass spectrometry (GC-MS) to identify electrophysiologically active compounds. The results of this study demonstrated that exposing farmers' local maize varieties to *Brachiaria brizantha* with *Chilo partellus* eggs induced the plants to emit volatile blends that are attractive to the parasitoid *Cotessia sesamiae* Cameron (Hymenoptera: Braconidae) parasitoid. Also the same volatile blends were repellent to *Chilo partellus*. On the other hand the hybrids exposed to *Brachiaria brizantha* with *Chilo partellus* eggs were not induced. The biologically active chemical compounds responsible for the behaviour of *Cotessia sesamiae* and *Chilo partellus* obtained from local maize exposed to *B. brizantha* with eggs were (E)-4, 8-Dimethyl-1, 3, 7-nonatriene, Decanal, (E)-Caryophyllene, Linalool, linalool (plus Nananal), E-β-fernesene, Methyl salicylate and (3E, 7E)-4, 8, 12-trimethyl-1, 3, 7, 11-tri-decatetraene. Hybrids did not produce any biologically active chemical compounds. Attraction of larval parasitoids implies that natural enemies can be recruited to attack the newly hatched stemborer larvae before they cause damage to the crop. In addition, this grass can be used as a trap crop to ward off *Chilo partellus* from the maize crop in the field. The results reported in this study are of great significance for the management of *Chilo partellus* stemborers as they provide insights into possible exploitation of the signalling of defence responses in the smart maize varieties using an equally smart plant. In addition, it also would open up opportunities for a more efficient exploitation of natural enemies as they would be recruited earliest before larvae of the pest hatch from eggs to cause damage to maize plants. It is recommended that resource poor farmers adopt the findings of this research to increase food on the table and cash in the pocket.

CHAPTER ONE

INTRODUCTION

1.1 Background

The Lepidopteran stemborer *Chilo partellus* (Swinhoe) (Crambidae) is a major constraint to efficient production of maize (*Zea mays* L.) (Poaceae), the most important staple food crop in Africa (Khan *et al*., 2010). It is the most destructive insect pest of the crop in sub-Saharan Africa (Seshu, 1998; Khan *et al*., 2007; Tamiru *et al*., 2011). Yield losses of up to 80% in some places have been recorded on maize depending on cultivar, developmental stage of the plant at infestation, infestation rate, prevailing environmental conditions and system of planting adopted, among other factors (Kfir *et al*., 2002; Tamiru *et al*., 2007; Khan *et al*., 2010). In addition to maize, stemborers have been discovered in sorghum and wild grasses that act as reservoirs during non-cropping period (Khan *et al*., 1997a; 2000).

Small-holder farmers are resource constrained and are unable to afford expensive synthetic pesticides (recommended by the Ministry of Agriculture) for crop protection. More so, relying on chemical control may result to undesirable effects such as pesticide resistance, upsurge of secondary pests, environmental pollution and poisoning of the farmers (Chadwick and Marsh, 1993; Obonyo *et al.*, 2008; Bruce *et al*., 2010). Semiochemicals involve chemicals mediating intraspecific communication among organisms such as pheromones and interspecific communication among organisms such as allomones, kairomones and synomones (Metcalf and Metcalf, 1992; Cook *et al*., 2007). This has prompted the need to develop alternative stemborer pest management strategies which are

affordable and ecologically friendly. These include exploiting semiochemicals produced by plants (van den Berg and Nur, 1998; Khan *et al.,* 2010). Plants typically respond to insect attack by producing what are generally referred to as herbivore-induced plant volatiles (HIPVs), a group of volatile organic compounds that could protect the plant either directly, by inhibiting further colonization by the pest, or indirectly, by attracting the pests' natural enemies (Turlings and Tumlison, 1992; Dicke and van Loon, 2000; Khan *et al*., 2008). These HIPVs could also signal neighbouring plants to produce volatile organic compounds, with similar consequences. Semiochemicals emitted by plants have been exploited to effectively manage insect pests by manipulating the behaviours of both pests and their natural enemies. For example, in the push-pull strategy, where semiochemicals from companion plant to maize has been employed to control stemborers and in the Lima beans project to control aphids (Khan *et al*., 2001; Heil and Kost, 2006).

Studies have shown that like other plants in the Poaceae family, the Signal grass *Brachiaria brizantha* (A. Rich) Stapf emits green-leaf volatiles which serve as cues that attract female *C. partellus* moths to the plant to deposit eggs (Bruce *et al*., 2010). Following egg deposition by *C. partellus*, this plant emits HIPVs that have dual functions: attracting stemborer natural enemies, notably *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), an indigenous parasitoid that attacks the destructive larval stage of stemborers and inhibiting further egg laying by other *C. partellus* moths (Bruce *et al*., 2010).

Previous studies have shown that in some host plant species HIPVs emitted as a result of pest attack can signal neighboring undamaged host plants, hence tailoring their defense

against attack from the herbivores (Turling *et al.*, 1995; Kessler and Torto, 2001; Dicke and Torto, 2010; Khan *et al.*, 2010). The current study is aimed at evaluating whether maize crop can be induced to defend itself against stemborer damage by herbivore induced plant volatiles from neighbouring *B. brizantha* grass following oviposition by *C. partellus*.

1.2 Statement of the problem

Maize is an important food and cash crop for millions of rural people in SSA. However, its production by small scale farmers in the region is seriously constrained by stemborer infestation and damage, caused by the feeding of the stemborer larvae. The young larvae destroy the crop by feeding on leaf whorls while the older larvae tunnel into the stem of the crop. Damage occurs as a series of small holes in lines in younger leaves and/or patches of transparent leaf epidermis (window panes) in older leaves. Holes in stem caused by larvae tunnelling into the stem can result in broken stems or drying and eventual death of the growing point of the maize (deadheart) (Kfir *et al.*, 2002).

Infested plants have poor growth and reduced yield and are more susceptible to secondary infections and wind damage. Estimates of yield losses due to stemborer are in the neighbourhood of up to 80% of the total yields (Seshu and Walker, 1990; van Rensburg and van den Berg, 1992; Kfir *et al.*, 2002), which is partly responsible for the high rates of food insecurity and poverty in the region.Various methods have been tried in a bid to control these pests, including biological control. Here, the focus has been to find an exotic natural enemy that will successfully fit into the community of invasive *C. partellus*.

Hampered by a lack of economic and convenient tools, however, advances in biological control have been largely overshadowed by the rush to exploit insecticides and the ready availability and comparative simplicity of cultural methods. But that is changing; the use of insecticides recommended to farmers by the Ministry of Agriculture for controlling the stemborers is difficult or unwise because of cryptic nature of the stemborer in the maize stem, making it difficult for an insecticide to reach and kill the stemborer. Also, the cost of synthetic pesticides has proved to be unaffordable to the resource poor farmers. Moreover, relying on chemical control may result to undesirable effects such as effects on non-target organisms, resistance development, up-surge of secondary pests, environmental pollution and poisoning of the farmers (Chadwick and Marsh, 1993; van den Berg *et al*., 1998; Bruce, 2010; Karuku, 2012). This has called for alternative methods to manage the stemborer. One such strategy is the combined use of plant semiochemicals and biological control for stemborers.

1.3 Justification of the study

Stemborers cause serious damage to cereal crops, leading to reduced yields (Seshu, 1998; Khan *et al*., 2010; Tamiru *et al*., 2011), hence the need for effective of methods control. Although several methods have been used to control the stemborers, they have drawbacks. For instance, chemical control recommended by the government to resource poor farmers is difficult since these pesticides are expensive, often toxic and relatively ineffective to these insects, because they burrow inside the plants (Chadwick and Marsh, 1993; Tamiru *et al*., 2011). Natural enemies have also been tried, but are less abundant to manage the pest.

Cultural methods too are faced by shortage of labour, finance and crop residues to be used are scarce.

Therefore, plant signalling has been adopted as an alternative strategy of controlling the stemborers. This strategy is environmentally friendly, technologically and economically feasible. In addtion, the strategy is conducive since it does not use chemical toxins but plant semiochemicals which are produced by the plants and are highly suitable to resource poor SSA farmers. Furthermore, the strategy can easily be applicable in the polycropping system of farming practiced by these farmers.

Recent studies by icipe and Rothamstead research scientists have shown that the Signal grass, *B. brizantha,* naturally defends itself against attack by the spotted stemborer *C. partellus* by suppressing emission of one of the key green leaf volatiles, (Z)-3-hexanyl acetate (Z3HA) following egg deposition by moths of this pest (Hilker and Meiners 2006; Bruce *et al*., 2010). This suppressed emission of Z3HA alters ratios of the individual compounds in the volatile blend, which then becomes attractive to *C. sesamia* but less preferable for *C. partellus* moths, resulting in reduced further colonization of the plants. Recent studies have reported the role of HIPVs in signalling defense responses in neighbouring plants, which then condition their responses against damaging pests.

The aim of the current research was to determine whether HIPVs emitted by *B. brizantha* following egg deposition by gravid *C. partellus* could be used to induce neighbouring

maize crop to emit HIPVs to protect itself from damage by *C. partellus*, hence contributing to new and useful knowledge in insect control.

1.4 Research Questions

i. What are the effects of HIPVs emitted by Signal grass *B. brizantha* with *C. partellus* eggs on neighbouring maize plants?

ii. What are the behavioural responses of the stemborer larval parasitoid, *C. sesamiae,* towards the volatile cues from maize plants exposed to Signal grass *B. brizantha* with *C. partellus* eggs?

iii. What are the effects of volatile cues from maize varieties exposed to Signal grass *B. brizantha* with eggs on host finding behavioural of the gravid *C. partellus* moths?

iv. What biologically active components of the volatiles are responsible for observed behavioural patterns in the parasitic wasp, *C. sesamiae* and gravid stemborer moth *C. partellus*?

1.5 Hypotheses

i. Herbivore-induced plant volatiles emitted by Signal grass *B. brizantha* have no effect on neighbouring maize.

ii. Herbivore-induced plant volatiles emitted onto by Signal grass *B. brizantha* following oviposition by *C. partellus* have no behavioural effects on *C. sesamiae.*

iii.   Herbivore-induced plant volatiles emitted by Signal grass *B. brizantha* following oviposition by *C. partellus* have no significant effect on the host finding behaviour of gravid female *C. partellus* moths.

iv.   There are no biologically active compounds in the volatiles emitted by different maize varieties exposed to Signal grass *B. brizantha* grass following oviposition by gravid *C. partellus* moths.

1.6 Objectives

1.6.1 General objective

To evaluate the effect of herbivore-induced plant volatiles of Signal grass *B. brizantha* on neighbouring maize plants for *C. partellus* management.

1.6.2 Specific objectives

i.   To evaluate the effects of Signal grass *B. brizantha* with *C. partellus* eggs on neighbouring maize.

ii.   To evaluate the behavioural responses of gravid female, *C. sesamiae* to volatiles from various maize varieties exposed to Signal grass *B. brizantha* following oviposition by gravid female spotted stemborer, *C. partellus*.

iii.   To assess the effects of volatile cues from maize varieties exposed to Signal grass *B. brizantha* with eggs on host finding by gravid female, *C. partellus*.

iv. To characterize biologically active compounds of the volatiles responsible for any observed behavioural patterns in the gravid female *C. sesamiae* and gravid female *C. partellus*.

1.7 Significance of the study

Plant to plant communication which results due to chemical ecology of HIPVs is an important area of study in multitrophic interactions as it influences egg deposition by female stemborers and the parasitoids. Research findings from the current study would enable exploitation of plant to plant communication through HIPVs in the management of maize stemborer pests. The electrophysiologically active compounds responsible for the repellency of gravid female stemborer moths and attraction of gravid female parasitoids can be used as signals for protection against *C. partellus* infestation. The study has practical applications in enhancing biological control programs against the spotted stemborer in western Kenya.

This study has adopted the push-pull strategy approach to managing C. partellus populations. Push-pull involves a combination of various behaviour-modifying stimuli to manipulate the prevalence and distribution of pest species and beneficial organisms for pest management. The study findings will maximize pest control efficacy, efficiency, sustainability, and output, while minimizing negative environmental effects. The biologically active coumpounds emitted by local maize exposed to *B. brizantha* with eggs could be a repellant, antifeedant or an oviposition deterrent natural agent to the *C. partellus*, and the a kairomone, aggregation, sex or oviposition pheromones or a selective

control agent to the natural enemies for the *C. partellus* (Picket *et al*., 1997; van den Berg, 2006; Cook *et al*., 2007). This approach has been successfully applied in managing stemborer pests of maize and sorghum in East Africa (Khan *et al*., 2010). Results of the current study indicate that *B. brizantha* can be employed as a trap plant at the perimeter of maize plants field to protect against *C. partellus.* However, *B. brizantha* would have to be established earlier before planting the maize, to boost or offer them more efficient protection from *C. partellus*. *Brachiaria brizantha* is a valuable livestock feed and its usage in cereal crop protection can facilitate livestock integration with an overal improvement in the livelihood of the smallholder farmers. This study also provides underpinning science for the use of the inducing plants in field crops pest control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Stemborers

There exists more than 18 species of *Lepidopteran* stemborers with varied degree of damage to maize and sorghum recorded in Africa (Polazsek, 1998; Muyekho *et al*., 2005). In SSA, two species are the most destructive to cereal crops and these are *Busseola fusca* and *C. partellus. Busseola fusca* (Fuller) is native to Africa and is found in mid to high altitudes (1230 m and above above sea level). *Chilo partellus* is an invasive stemborer from Asia and is common in low to mid altitudes of zero (0) to 1230 m above sea level) (Seshu, 1983; Polaszek, 1998; Infonet-Biovision, 2011).

2.1.1 Pest status and distribution of *Chilo partellus*

Chilo partellus is the most damaging stemborer pest of cereal crops, causing serious yield losses in maize and sorghum; the losses range between 20-80% depending on the crop related factors and its surrounding (Seshu and Walker, 1990; Kfir *et al*., 2002)

Chilo partellus is an exotic species accidentally introduced to Africa in early 1930s from Asia (Tams, 1932) and has since spread to nearly all countries of Southern, Eastern and Central Africa (Nye, 1960; Kfir, 1998). In Kenya, *C. partellus* is common at the coastal (lowland) (0m above sea level) and western (1200m above sea level) parts of the country where it is causing devastating damage to Graminaceae family crops (Seshu, 1983; Polaszek, 1998; Kfir *et al*., 2002; Obonyo *et al*., 2008; Karuku, 2012).

2.1.2 Biology of the, *Chilo partellus*

The spotted stemborer *Chilo partellus* undergoes a complete metarmophosis. Each stage

takes different number of days depending on prevailing abiotic and biotic factor prevailing

(Seshu, 1983; Kfir *et al*., 2002). Here, growth and development involves the eggs hatching

into larvae. The larva then changes into pupa stage and then finally adult.
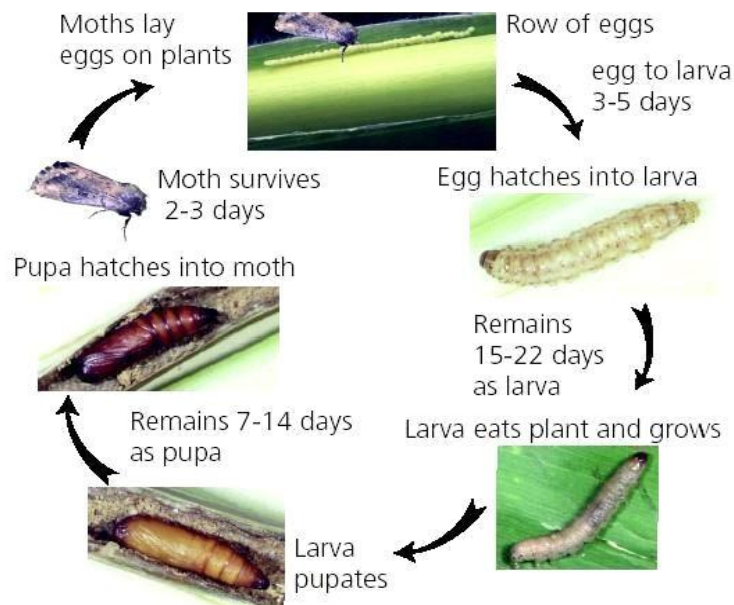
Figure 1: Life cycle of *Chilo partellus*

Chilo partellus lays 10-80 overlapping eggs per batch on both upper and lower leaf

surfaces, usually close to the midrib (Seshu, 1983; Polaszek, 1998; Kfir *et al*., 2002; CABI

International, 2006). These eggs hatch into larvae 3-5 days (Figure 1).Young larvae feed in

the leaf whorl while older larvae tunnel into stems, eating out extensive galleries, within

which they live, feed and grow for about 15-22 days (Overholt *et al*., 2001). When larvae are fully grown, they pupate and remain inside the maize stem for 7-14 days. Afterwards, adults emerge from pupae and come out of the stem through the exit windows. The moths usually mate soon after the female emerges and stay for 2-3 days before they oviposit on maize plants again and continue damaging the crop. During the dry season, *C. partellus* may enter a state of diapause for several months and will only pupate at the start of rains. Adults emerge from pupae in the late afternoon or early evening. They are active at night and rest on plants and plant debris during the day. They are rarely seen, during the day unless they are disturbed (Overholt *et al*., 2001).

The whole life cycle takes about 25-50 days (Figure 1), varying according to temperature and other factors. Five or more successive generations may develop under favourable conditions. In regions where there is sufficient water and an abundant of host plants, the spotted stemborer can reproduce and develop all year-round (Harris, 1990).

2.2 Methods of managing stemborer pests

There are many methods of managing *C. partellus*, and includenatural enemies, microbial agents, chemical control, cultural control, classical biological control, host plant resistance and use of semiochemicals. These methods can be used singly or integrated.

2.2.1 Natural enemies of *Chilo partellus*

This is a method of controlling the pest using other living organisms. It relies on predation, parasitism and herbivory or other natural mechanisms, but typically also involves human management role.

2.2.1.1 Predators

A Predator is an organism that hunts and kills other organisms for food to live. Therefore *C. partellus* has several predators such as the ant, *Dorylus helvolus* (L.) (Hymenoptera: Formicidae) and the mouse, *Mastomys natalensis* (Smith) (Rodentia: Muridae). Other predators of importance are *Cardiophorinae* larvae (Coleoptera: Elateridae), the doryline ant *Aenictus* sp. (Hymenoptera: Formicidae), larvae of *Astylus atromaculatus* Bl. (Coleoptera: Melyridae), tenebrionid larvae (Coleoptera: Tenebrionidae), larvae and adults of *Heteronychus arator* F. (Coleoptera: Scarabaeidae), carabid larvae (Coleoptera: Carabidae), staphylinids (Coleoptera: Styphylinidae) and Earwigs (Dermaptera: Labiduridae) (Dwumfour *et al*., 1991; Songa *et al*., 2001; Kfir *et al*., 2002; Khan *et al*., 2007).

2.2.1.2 Parasitoids

Parasitoids are natural enemies that oviposit on or in the various stages of *C. partellus*; their larvae thus feed on the various pest stages, resulting in death of the prey after the parasitoids have pupated inside coccons (Overholt *et al*., 1994a; Kfir, 1998; Obonyo *et al*., 2010). The egg parasitoids that have shown significant reduction in *C. partellus* populations, thereby contributing to control of the pest include *Trichogramma bournieri* (Pintureau and Babault) [Hymoneptera: Trichogrammatidae], *Telonomus busseolae*

(Hymenoptera: Scelionidae), *Telonomus isis* (Hymenoptera: Scelionidae) and *Chelonus curvimaculatus* (Hymenoptera: Braconidae). They also parasitize first and second instar of *C. partellus*. The larval parasitoids are *C. flavipes* (Hymenoptera: Braconidae) and *C. sesamiae* (Hymenoptera: Braconidae) that parasitize third to fifth instar of *C. partellus*. *Cotesia sesamiae* is one of the most important local larval parasitoids of stemborers in many countries of SSA (Bonhof *et al*., 1997), attacking third to fifth larval instars of both exotic and indigenous borer species (Mohyuddin, 1971).

Cotessia sesamiae is the major larval parasitoid in SSA (Polaszek and Walker, 1991; Overholt *et al*., 1994a), but it is ineffective in managing *C. partellus* populations (Overholt *et al*., 1994b). In Kenya, there are two biotypes of *C. sesamiae*; the coastal and the inland, all expressing different capacities to develop in *C. partellus* (Ngi-Song *et al*., 1995). The inland biotype successfully develops in *C. partellus*, while the coastal one does not (Ngi-Song *et al*., 1995). This variation in parasitism of *C. partellus* by *C. sesamiae* is due to physiological unsuitability and an encapsulation mechanism by which oviposited eggs are melanised in *C. partellus* (Ngi-Song *et al*., 1995, Mochiah *et al*., 2002). Encapsulation of parasitoid eggs reduces the efficiency of a given parasitoid species mostly in regions where the unsuitable host is the predominant pest species (Ngi-Song *et al*., 1995; Obonyo *et al*., 2008). During oviposition, most parasitic hymenoptera co-inject stings with the egg factors that are responsible for the suppression of the host's immune response and the venom from their accessory glands (Obonyo *et al*., 2008). They may also inject polydnaviruses that work in synergy to bring about host regulation and immune suppression (Richards and Parkinson, 2000). The presence of the virus is asymptomatic in the wasp but causes major

physiological disturbances in host larvae in which several viral genes are expressed. The most commonly observed pathologies in infected caterpillars are suppressed immunity and developmental arrest prior to metamorphosis. These two conditions are essential for the survival and growth of the wasp larvae inside its hosts (Beckage and Gelman, 2004).

Experiments with calyx fluid show that the substances co-injected by the inland strain of *C. sesamiae* during parasitism of *C. partellus* suppress the host immune system, while that from the coastal strain does not. Consequently, the two strains of *C. sesamiae* are termed as virulent and avirulent *C. sesamiae*, respectively (Mochiah *et al*., 2002). There is relatively less information on the biology of the local parasitoid *C. sesamiae* as opposed to that of its exotic counterpart *C. flavipes*. This is because it came into the lime light during the initiation of the classical biological control program at *icipe* in 1993. Thus, it was assumed that its biology and behavioural attributes resemble those of *C. flavipes*. The pupae are attacked by *Dentichasmiasis busseolae* (Hymonoptera: Ichneumonidae) (Seshu and Walker, 1990; Kfir *et al*., 2002; Khan *et al*., 2007).

2.2.1.2.1 Host finding behaviour of *Cotesia sesamiae*
In order to become established in a new host, a parasitoid must first locate and be able to discriminate between suitable and unsuitable hosts. Behavioural events leading to successful parasitism include host habitat location, host location, host acceptance and suitability (Godfray, 1994; Obonyo *et al*., 2010). During location of hosts, the parasitoids typically exploit long and short range stimuli emanating from the host habitat (Godfray, 1994), followed by stimuli directly associated with the host and its products (Vet and

Dicke, 1992; Godfray, 1994). However, stumili from the habitat do not convey sufficiently reliable information on the suitability of host species but are mere indicators of the presence of the herbivores (Ngi-Song and Overholt, 1997). As a result, *C. sesamiae* is often attracted to plants infested with unsuitable species of stemborers (Ngi-Song *et al*., 1996; Obonyo *et al*., 2008). Therefore, it is suggested that *C. sesamiae*, though not capable of recognizing a suitable host species from a distance, are able to distinguish suitable from non-suitable hosts on contact. There exists some information on the host searching and selection behaviour of *C. sesamiae* parasitizing larvae feeding inside plants (Gohole *et al*., 2005). Studies have shown that *C. sesamiae* use their antennae for host recognition, and both their antennae and tarsi for final acceptance of a host for oviposition. Also, the tactile and contact-chemoreception from the host play a major role in guiding *C. sesamiae* in making a decision to oviposit (Obonyo *et al*., 2010).

2.2.2 Microbial agents

Microbial agents are pathogens such as bacteria, fungi and viruses and enthomopathogenic nematodes usually formulated as biopesticides. Stem borer pathogens in SSA Africa have received very little attention. Examples of microbial agents of *C. partellus* are: a microsporidian, *Nosema partelli* Walters & Kfir (Microsporidia: Nosematidae), cytoplasmic polyhedrosis virus, granulosis virus, nuclear polyhedrosis virus, the entomopathogenic fungi *Beauveria bassiana* (Balsamo) (Fungi imperfecti) and *Entomophthora* sp. (Phycomycetes), the bacteria *Serratia marcescens* Bizio (gram-negative facultative anaerobic pathogen, non-sporeformer) and *Bacillus thuringiensis* Berliner (endospore-forming rods and cocci, an obligate pathogen). Microorganisms that have been

isolated from cadavers of *C. partellus* include cytoplasmic polyhedrosis virus, entomopox virus, the fungi *Beauveria bassiana, Entomophthora* sp. and *Aspergillus* sp. (Fungi imperfecti) the bacteria *Streptococcus* sp. (gram-nagative cocci), *Serratia maecescens* and *Bacillus thuringiensis* and the microsporidian *Nosema partelli* were isolated (Oloo, 1989; Obonyo *et al*., 2008).

2.2.3 Chemical control

There are several insecticides that have been discovered to be effective as dust, leaf whorl placement granules or applied as sprays. They include endosulfan, synthetic pyrethroids, carbaryl, delthamethrin and carbofuran (Ajayi, 1989; Minja, 1990; van Rensburg *et al*., 1992). The disadvantages of using chemical control are that they are expensive for small scale farmers due to requirement for regular application, may lead to pesticide resistance, upsurge of secondary pests, environmental pollution and other hazards (Chadwick and Marsh, 1993; van den Berg and Nur, 1998).

2.2.4 Cultural methods

Cultural methods are aimed at reducing population growth. The commonly used methods include: tillage, mulching, right spacing, manipulating planting time, crop rotation, fertilizer application and crop residue management (Seshu, 1998). The disadvantage of using cultural methods are that crop residues have many uses, there is shortage of labor and finance (Skovgard and Pats, 1996).

2.2.5 Host plant resistance

Use of resistant crop varieties is the most important and promising way to reduce damage and yield loss due to stemborers (van Resburg *et al.*, 1988). Several mechanisms are utilized by resistant maize varieties against the attack by *C. partellus.* These include non-preference for oviposition, reduced feeding and tunneling, tolerance to leaf damage, dead heart and stem tunneling and antibiosis (Polaszek, 1998). Nwanze (1997) and Singh *et al.* (1983) indicated that host plant resistance is generally the most farmer friendly pest control option. Efforts are also being made by International Maize and Wheat Improvement Center (CIMMYT) and Kenya Agricultural Research Institute (KARI) to develop insect resistant maize for Africa (IRMA, 2009).

2.2.6 Classical biological control

This refers to importation of specialist natural enemies from the home land of a pest of foreign origin. The objective is to establish populations of these natural enemies to attack the pest and reduce its number (Overholt, 1993; Kfir, 1998; Getu *et al.*, 2003; Khan *et al.*, 2008).

Natural enemies are usually not sufficiently abundant to keep stemborer populations at low levels (Oloo, 1989; Kfir, 1995). A combination of the different approaches of classical biological control, conservation of indigenous natural enemies, application of commercially produced micro-organisms and redistribution of locally important natural enemies may provide a suitable management strategy for the sustainable control *of C. partellus* in Africa (Bonhof *et al.*, 1997). Efforts have been made to introduce exotic

parasitoid, *C. flavipes*, for control of *C. partellus* in Africa (Overholt, 1993). Reports indicate that the parasitoid is currently established in Kenya, Tanzania, Uganda, Zambia and Ethiopia (Omwega *et al*., 1995; Overholt *et al*., 1997; Getu *et al*., 2003).

2.2.7 Semiochemicals

Semiochemicals, also known as behavior-modifying chemicals, are chemicals that convey a signal from one organism to another so as to modify the behavior of the recipient. They are emitted by one individual and cause a behavioral response in another (Law and Regnier, 1971; Khan *et al*., 2000; Bruce *et al*., 2005a; Cook *et al*., 2007). Since the control of stemborers using synthetic chemicals has proved to be expensive to resource poor farmers and environmentally unfriendly, researchers in SSA are working around the clock to come up with cheap and environmentally friendly strategies to manage the stemborers. One of these strategies is the use of semiochemicals from companion plants of maize (Khan *et al*., 2006b). Volatile semiochemicals are detected by olfaction while non volatile semiochemicals are detected by contact chemoreception. An attractant and repellent are two groups of semiochemicals referring to the behavior changing olfactory active compounds which do not need contact with the source. The words stimulant and deterrent denote groups of behavior changing chemicals that are active only at close range (Foster and Harris, 1997; Cook *et al*., 2007).

2.2.7.1 Types of semiochemicals

Semiochemicals involve chemicals mediating intraspecific (same species) and interspecific (different species) interactions among organisms. Intraspecific communication chemicals are known as pheromones and consist of alarm pheromones and spacing or epideictic pheromones that can be used as repellents, sex and aggregation pheromones. Sex and aggregation pheromones can be used as attractants (Metcalf and Metcalf, 1992; Cook *et al*., 2007). On the other hand, interspecific communication chemicals include allomones, kairomones and synomones. Allomones are allelochemicals that favor the emitter while kairomones benefit the receiving species (Bruce *et al*., 2005a; Cook *et al*., 2007). The former type may include herbivore repellents in the case of host plant volatiles while the latter could be plant chemicals that attract pests, or herbivore emitted chemicals that attract natural enemies (Metcalf and Metcalf, 1992; Khan *et al*., 2000; Bruce *et al*., 2005a; Cook *et al*., 2007). Synomones benefit both the emitter and receiver, as in the case of the chemicals mediating pollination (Meiners and Hilker, 2000; Cook *et al*., 2007).

2.2.7.2 Semiochemicals as stemborer management strategy

Plants, when infested and damaged by phytophagous insects, emit herbivore induced volatiles (Bruce *et al*., 2010). Attraction of insects to plants and other host organisms involves detection of specific semiochemicals or specific ratios of these semiochemicals (Pickett *et al*., 2006). Understanding the chemical ecology of insects and plants leads to development of novel techniques in pest management (Khan *et al*., 2010). There is a large array of compounds produced by at least 100,000 species of flowering plants (Metcalf and Metcalf, 1992). There is an intricate chemical interaction 'arms race' between plants,

herbivores and herbivore enemies, as they have been coevolving for almost 400 million years, resulting in inter-trophic 'conversations' (Hsiao, 1985; Metcalf and Metcalf, 1992; Khan *et al.,* 2008). Particular host plant chemicals can affect the behavior of insects in various ways, such as attractants and oviposition or feeding stimulants (kairomones), and as repellents and oviposition or feeding deterrents (allomones).

Attractants and repellents can be long range or short range. Long range attraction results when plants provide information in the form of chemical cues, which aid parasitoids and pests to locate their hosts at some distance. These cues are highly reliable; they have high detectability (Vet and Dicke, 1992) because they are generally produced in large amounts, and are very volatile. They tend to act mainly as long distance kairomones. Short range attractants are semiochemicals employed by the parasitoids and pests when they have successfully located the suitable habitat of their host. The parasitoid and pest must then find their host.

The parasitoid employs cues from host byproducts such as frass, faeces or silk while the pest employ plant features such as hairs, texture, trichomes and chemical components. These by products of the pest and plant features provide the most reliable source of information on the presence, identity, availability and suitability of the host to the foraging parasitoid and pest (Vet and Dicke, 1992; Foster and Harris, 1997; Khan *et al*., 2000; Cook *et al*., 2007). Although these cues are highly reliable, they are limited by their low detectability because they are generally produced in small amounts, and are not very volatile; they tend mainly to act as contact kairomones. Long range repellents result when

plants provide information in the form of chemical cues, which aid pests to locate and avoid their unsuitable hosts at some distance. Short range repellents are semiochemicals employed by the pests when they have already successfully located the suitable habitat of their host. The pest must then find their host (Vet and Dicke; 1992, Metcalf and Metcalf, 1992; Foster and Harris, 1997; Cook *et al*., 2007).

Plants attacked by herbivores 'cry' for help from herbivore enemies and also alert and prime neighboring plants for impeding attack. Parasitic and predatory arthropods recognize plant 'cry' as 'dinner bells' (Takabayashi *et al*., 1994, de Moraes *et al.,* 1998; Hilker and Meiners, 2002; Turlings and Ton, 2006; Heil, 2008) and move towards the plants to find the signaling food. These 'cry' or semiochemical compounds, once identified, could be of practical use in crop protection by virtue of repelling herbivores or call in herbivore enemies earlier and more effectively (Khan *et al.*, 2008; Bruce *et al*., 2010; Tamiru *et al*., 2011). In the past few decades, efforts have been made by scientists to devise methods for utilizing semiochemicals in pest management, such as the push pull strategy (Miller and Cowles, 1990; Cook *et al*., 2007).

2.2.7.3 Push-pull as a biological control strategy for stemborers

Push-pull is a stimulo-deterrent diversionary strategy developed by exploiting semiochemicals to repel insect pests from the crop ('push') and attract them into trap crops ('pull') (Miller and Cowles, 1990; Hilker and Meiners, 2002; Cook *et al*., 2007). The most successful example of a 'push-pull' strategy was developed by *icipe* and Rothamsted Research (Khan *et al.*, 2001) for the control of cereal stemborers. The technology involves

intercropping maize or sorghum, with desmodium (*Desmodium uncinatum or D. intortum*) or Molasses grass, *Melinis minutiflora* (Beauv.) and planting Sudan grass (*Sorghum vulgare Sudanense*) or Napier grass, *Pennisetum purpureum* (Schumach) around the crop. Chemicals produced by the desmodium and molasses repel (push) stemborer pests and encourage them to lay eggs in the grass instead of in the maize (Khan *et al.*, 2000; Matthes *et al.*, 2003). Moreover, Napier grass does not allow stemborer larvae to develop on it (Khan *et al.,* 2006a; van den Berg, 2006) as it produces a gummy substance that traps newly hatched larvae (Khan *et al.*, 2001). Planting molasses grass between rows of maize not only causes a drastic decrease in stemborer infestation but also significantly increased parasitism of the stemborer larvae by *C. sesamiae* (Khan *et al.*, 1997a). The push pull technology has shown a high adoption rate by over 50,000 resource poor small scale farmers in the Western Kenya region. The number of farmers adopting the technology is increasing daily as a result of the natural delivery of semiochemicals from companion plants to maize because of its low cost and is environmentally friendly. This has resulted in a significant impact on food security, human health, poverty reduction and environmental sustainability through increased farm productivity of 3.5-4 tons per hectare up from 1 ton (Hassanali *et al.*, 2008; Khan *et al.*, 2011). Therefore, the resulting benefits of push-pull technology has prompted research on feasibility of using *B. brizantha* grass as a companion plant for maize plants, with a possibility of inducing defense mechanisms in these plants and also acting as a trap plant for managing *C. partellus* in maize.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Screen house and bioassay studies were carried out at the International Centre of Insect Physiology and Ecology (*icipe*) Thomas Odhiambo Campus (ITOC), Mbita point. The site is found in Western Kenya at latitude and longitude 0°25'S, 34°12'E. The altitude is approximately 1,200m above sea level) (Figure 2). This is an area where *C. partellus* are commonly found destroying cereal crops. Gas Chromatography and Gas Chromatography-Mass Spectrometry (GC-MS) analyses of volatiles was carried out at Rothamsted Research, United Kingdom.
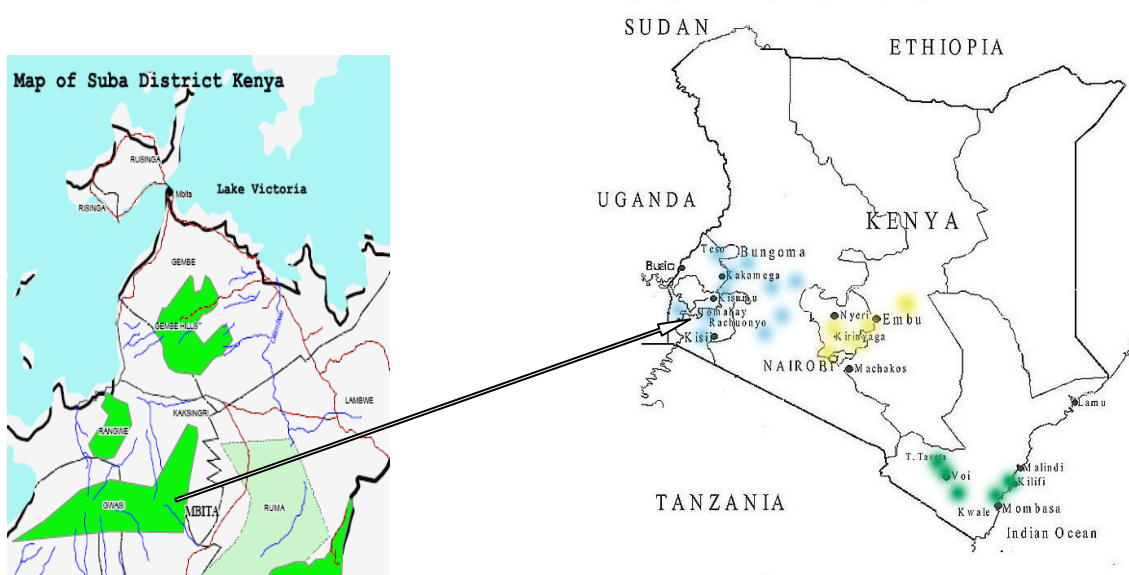
Figure 2: A map of Kenya showing *icipe* Mbita (Infonet-Biovision, 2011)

Note

Shaded area shows places where stemborers are prevalent
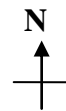
3.2 Materials

3.2.1. Plant material

Seeds of six different maize varieties, namely: Nyamula (Plate 1), Endere (Plate 2), Jowi (Plate 4) (smallholder farmers' own seed variety) were obtained from farmers in Western Kenya) while Cuba 91 (Plate 3) was obtained from CIMMYT. Hybrid 505 (Plate 5) was obtained from Western Seed Company and PH4 (Plate 6) was obtained from Kenya Seed Company. Signal grass *B. brizantha* (Plate 7) was collected from the field at ITOC. These plants were grown individually in plastic pots filled with soil. Planting holes were made using a dibbling stick and then 3.95 grams of Diammonium phosphate fertilizer added and mixed with some soil to prevent scorching of the seeds. The sowing of maize seeds and planting splits of grass was done at ITOC screen houses (25± 3°C, 65±5% RH, 12L: 12D photoperiod). During planting, 16 pots of maize for various varieties were planted at an interval of three days so that all the 12 plants needed for entrainment and oviposition bioassay were used when they were exactly 28 days old. This was aimed at preventing errors which could result due to plant age differences. These plants were used in the experiments when four weeks old, because this is the stage at which crops are colonized under natural conditions.

Plate 1: Nyamula seeds

Plate 2: Endere seeds

Plate 3: Cuba 91 seeds

Plate 4: Jowi seeds
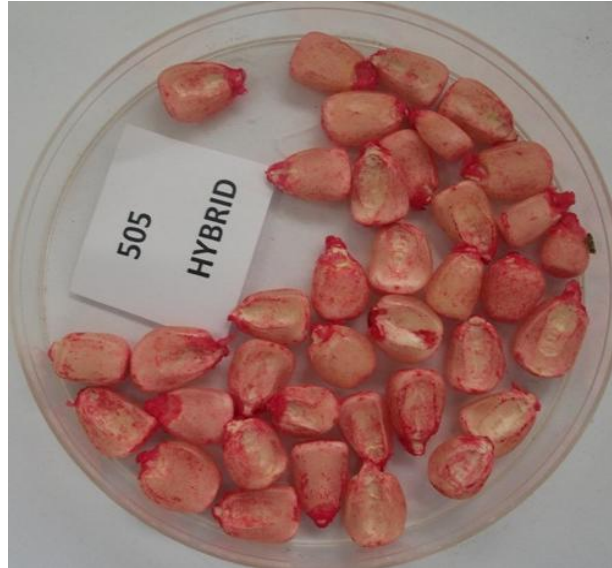
Plate 5: Western Hybrid 505 seeds

Plate 6: Pwani hybrid (PH4) seeds

Plate 7: Signal grass splits

3.2.2 Insect culture

Chilo partellus and the larval parasitoid, *C. sesamiae* were obtained from the mass rearing unit at ITOC (12L: 12D photoperiod, 24±2 °C, 70±5% RH). The *C. partellus* were reared using protocols described by Ochieng *et al.* (1985) on a semi-synthetic diet comprising of the sorghum, *S. bicolor*. *Cotesia sesamiae* were reared using the protocol described by Overholt *et al.* (1994a), on stemborer larvae. Adult *C. sesamiae* were provided with 20% honey solution soaked in cotton wool as diet. One-day-old, mated naive female *C. sesamiae* were used in all experiments.

3.3. Methods

Ten potted *B. brizantha* plants were removed from the screen house and each was placed into an oviposition cage (80 x 40 x 40 cm) (Plate 8) where twenty gravid stemborer moths were introduced per cage and kept overnight for oviposition.The four stands of each cage were dipped in lids containing clean water overnight to prevent predatory insects such as brown ants and spiders from preying on the stemborer moths and their eggs. The following day, the *B. brizantha* grass with eggs laid on them were removed from the cages and taken back into the screen house whereby they were arranged into two rows 1m apart. Each row comprised of five plants. One row of maize comprising of five potted maize plants was placed in between the two rows of signal grass for three days. The above set up was repeated for another five maize plants but with *B. brizantha* not exposed to gravid stemborer moths; the setup was left for three days. Another setup comprised of clean maize which had no prior exposure to any kind of grass. The experiment was replicated twelve times.

Plate 8: Introducing Signal grass into oviposition cage

3.3.1 Collection of volatiles from maize plants by air entrainment

Volatile compounds from test potted maize plants, placed in between *B. brizantha* with and without stemborer eggs for three days were collected by entrainment of intact plants using portable equipment developed at Rothamsted (Agelopoulos *et al*., 1999; Birkett *et al*., 2003). The above procedure was repeated on non-exposed maize plants. Care was taken not to damage the plants while setting up the entrainment to avoid possible release of volatiles in response to mechanical damage of the plant.

Headspace volatiles were collected from these plants after 3 days, starting at the last two hours of photophase on the third day, for 48 hours. At each entrainment, leaves of maize plants (averaging 45 grams) exposed to *B. brizantha* with eggs, without eggs and clean maize leaves (with no prior exposure to *B. brizantha*) were enclosed in polyethyleneterephthalate (PET) bags (volume 3.2 liters) heated to 150°C before use and fitted with a Swagelock inlet and outlet. Purified (charcoal filtered) air was pumped (600 ml/min) through the inlet port and volatiles were collected using an absorbent (0.05g of Porapak Q) in filter tubes inserted in the outlet port through which air was drawn at 450ml/min. Pumping rates were controlled using flow meters on entrainment kits to make sure more purified air was pumped in than drawn out, to avoid influx of unfiltered air from outside. After the entrainment, volatiles were eluted from the Porapak Q filters with 0.5ml Dichloromethane and used immediately in a bioassay or stored at -20°C for later use.

3.3.1.1 Gas chromatography (GC) analysis

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Gas chromatography is used to separate the different components of a mixture (the relative amounts of such components can also be determined).

The chemical components of vacuum distilled volatile compounds from various maize lines were eluted in Dichloromethane. Gas chromatography analysis was carried out by injecting l-2µl of sample onto a non polar (50m × 0.32mm I.D.HP1) separation column using Hewlett-Packard 6890 gas chromatography (GC) system (Agilent Technologies), equipped with temperature programmable on-column injectors (230° C) and flame ionization detectors (FIDs)). Hydrogen was the carrier gas. The GC was equipped with a HP-1 capillary column (50m × 0.32mm id., 0.25µ m film thickness). The oven temperature programme was maintained at 30°C for 0.5 minutes initially, then a rise to 150^0 C at 5minutes, a hold at 150° C (0.1 minute) and then another programmed rise to 250° C at 5° C/min. A final hold was maintained at 250° C for 45 minutes. The results were obtained with an enhanced integrator (HP Chemstation), and confirmed by peak enhancement on GC using authentic compounds (Birkett *et al.,* 2008). In GC analysis, quantification of volatile compounds was carried out by calculating and comparing peak area with known amounts of authentic standards. Gas chromatography was done to determine the effect of herbivore induced plant volatiles on various neighbouring maize varieties.

Oviposited *B. brizantha* grass

(a) Maize plants exposed to *B. brizantha* grass

    with eggs

(b) Maize plants exposed to *B. brizantha* grass without eggs

(c) Non-exposed maize plants (Not exposed to any kind of *B. brizantha* grass)

Plate 9: Experimental plant set-up in the screen house (a) Maize plants exposed to *B. brizantha* grass with eggs; (b) Maize exposed to *B. brizantha* without eggs; and (c) Non-exposed to *B. brizantha* maize plants.
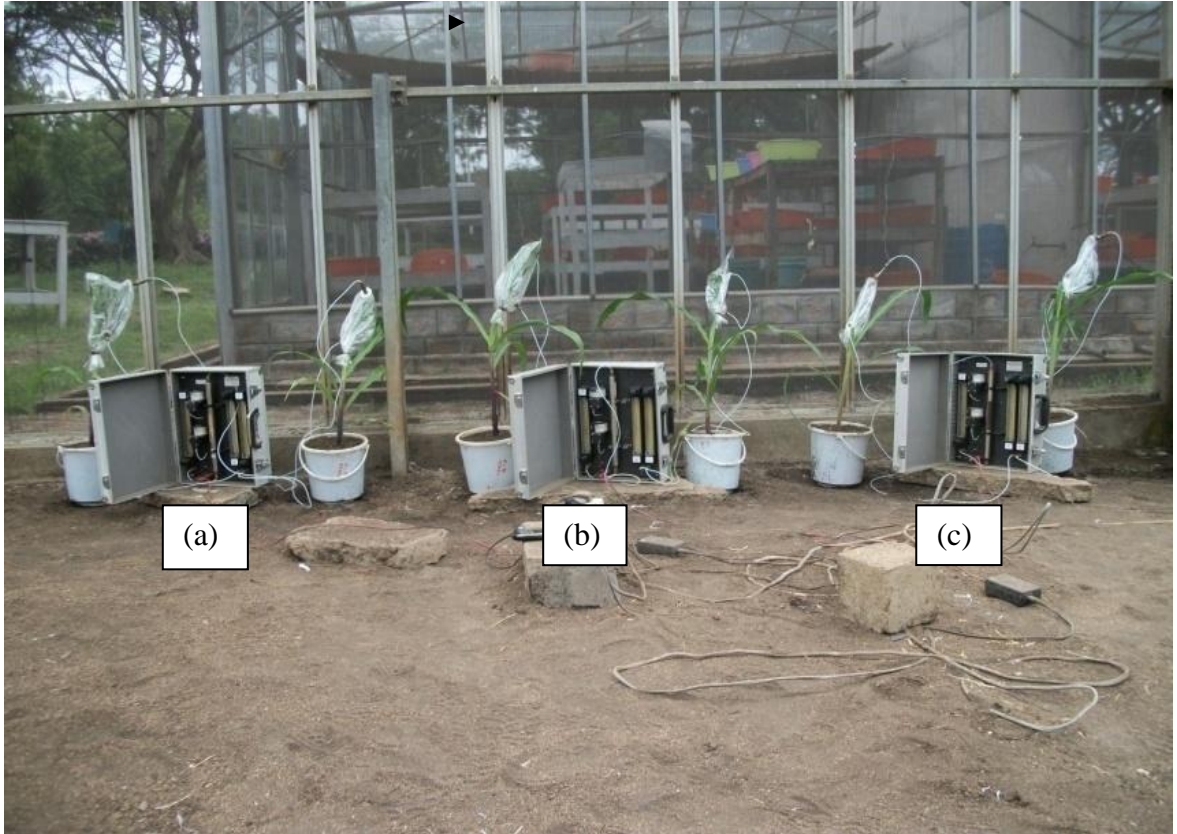
Plate 10: Collection of volatile chemicals from exposed and nonexposed maize
seedlings

Key

(a) Potted Maize seedlings exposed *B.brizantha* with eggs

(b) Potted Maize seedlings exposed *B.brizantha* without eggs

(c) Potted non-exposed Maize seedlings

3.3.2 Behavioural responses of gravid female parasitoid *C. sesamiae* to volatiles emitted by maize plants

The samples of volatiles collected in experiment one were tested for attractiveness to gravid female parasitoid, *C. sesamiae* in olfactometer bioassays using a Perspex 4-arm olfactometer lined at the base with filter paper (Whatman NO.1) (Whatman Ltd, Maidstone, England) and lit from above with diffuse uniform lighting (Petterson, 1970; Birkett *et al*., 2004). The olfactometer consisted of three layers of 6 mm thick transparent Perspex screwed together. A four pointed star shaped exposure chamber was milled into a circular layer measuring 12cm×12cm×1.2 cm, with a hole (3 mm diameter) drilled into the walls at each of the four cardinal points where the arms of the olfactometer are inserted (Plate 11). Another layer (10.2×10.2×0.6 cm) served as the floor and a third layer of the same size but with an apperture (4 mm diameter) in its centre, served as the cover.

The olfactometer side arms were inserted through the holes of the chamber walls. Air stream through the olfactometer was supplied by the air entrainment system (Plate 12) (KNF Neuberger, Germany) through Teflon tubing measuring 3.2 mm I.D. (Cabal Ltd, UK). The air from the pump was pushed through one flow meter (GPE Ltd, Leighton Buzzard, UK). This was to give one air flow which was directed into the olfactometer through a central hole in the cover plastic plate by the help of teflon tubing fixed into a pipette tip which was directed into the behavioral chamber. The air flow was drawn through the four arms towards the centre allowing mixing at 480ml per minute (a flow rate through each individual arm being) from the pump. Each side glass arm inlet was covered with a net to prevent *C. sesamiae* entry into the arm. The experiment was replicated twelve times.

The different odour sources (treatments) used to compare the responses of gravid *C. sesamiae* included: Nyamula, Endere, Cuba 91, Jowi, Hybrid 505 and Hybrid PH4. The olfactometer was coded into five regions (Plate 11). The arms were numbered 1-4 while the central hole on the upper plastic cover was given number 5. During the bioassay arm 3 held 10 µl aliquots of headspace sample from the above maize varieties exposed to *B. brizantha* with eggs and arm 1 held 10 µl aliquots of head space sample from non-exposed maize plants respectively; arms 2 and 4 were blank controls extracted with the 10 µl of dichloromethane (DCM) solvent. The same procedure was repeated for headspace samples from the experimental maize plants exposed to *B. brizantha* with eggs versus headspace samples from the experimental maize plants exposed to *B. brizantha* without eggs. Lastly, the above procedure was repeated for headspace samples from experimental maize seedlings exposed to *B. brizantha* without *C. partellus* eggs placed in arm 3 while volatiles from clean maize was placed in arm 1. The other two opposite arms were blank control, with DCM only. The olfactometer bioassay methodology was adopted from Tamiru *et al*. (2011).

Test solutions were applied using a micropipette to a piece of filter paper (4×25mm) and solvent allowed to evaporate for 30 seconds. The filter paper strips were then placed in an inlet port at the end of the olfactometer arms. One *C. sesamiae* gravid female was transferred individually from a small glass cage into the central chamber of the olfactometer using a custom made piece of glass tubing (made from a Pasteur pipette heated over a Bunsen burner to remove the narrower end). Each parasitoid was observed visually for 12 minutes as it made choices among the different olfactometer arms. Each of

the olfactometer arms was considered a separate compartment when recording the *C. sesamiae* response to test volatiles. The parasitoid was considered to have entered a given arm when its whole head crossed the zone boundary. A computer program for collecting and analyzing behavioural data with the four arm olfactometer called "Olfa" software developed by Francesco Nazzi (33100 Udine, Italy) was used to get the data (plate 12). The data recorded included the time spent by the *C. sesamiae* in the different zones of the olfactometer and the number of entries into each olfactometer arm. The olfactometer was rotated at 90° every three minutes to avoid directional effects (Petterson, 1970; Bruce *et al*., 2010). Each experiment was replicated 12 times. Olfactometers were changed between replicates, while the aliquots (odours samples) were replaced after each replicate. The assignment of treatments to the olfactometer arms were the same throughout the experiment.
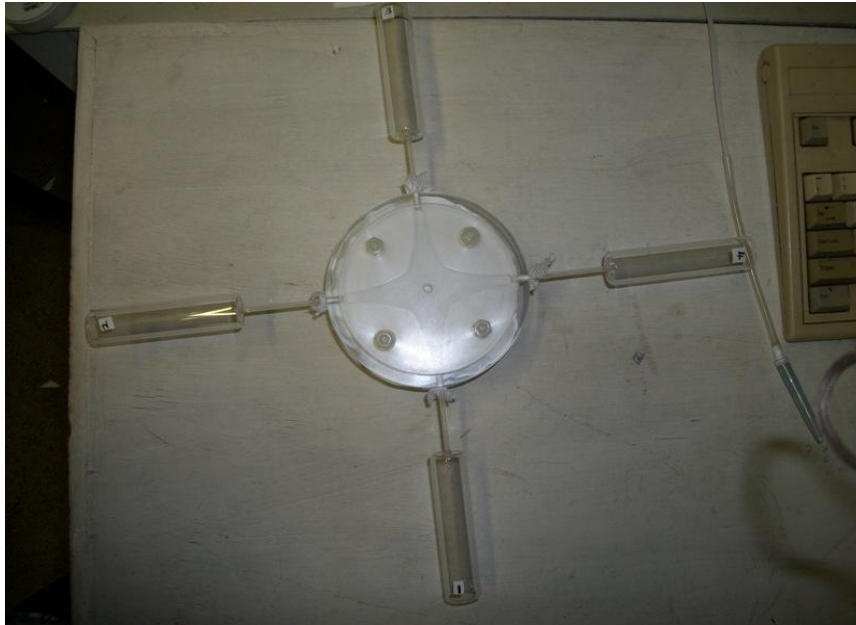
Plate 11: Olfactometer

Plate 12: Olfactometer bioassay set up

3.3.3: The effect of volatile cues from maize varieties exposed to *B. brizantha* with eggs on host finding by gravid female, *C. partellus*.

The responses of gravid female stemborer moths, *C. partellus* to oviposition-induced plant volatiles from selected maize lines was carried out in an ovipostion cage measuring 80 x 40 x 40 cm (Tamiru *et al*., 2012). Two 4 week old potted maize plants, one non-exposed (no prior exposure to HIPVs of *B. brizantha*) and one exposed to HIPVs of *B. brizantha* with *C. partellus* eggs were removed from the screen house and taken to an open oviposition bioassay field at *icipe* Thomas Odhiambo Campus where they were placed side by side without touching each other, on a clean oviposition table measuring 1.2m x 1.2m x 0.3m (Plate 13). This was to prevent any communication through contact. A pad of cotton wool moistened with clean water in a petri dish (10 cm diameter) was placed on a marked point on the table at the centre in between the two experimental plants at a distance of 20

cm from each plant. This was to provide water for the moths. Seven pairs (male and female) of *C. partellus* moths in a Petri dish (20 cm diameter) were introduced at a marked circular point on the table.

Large cylindrical oviposition cages (Diameter=100 cm and height=100cm) made of fine mesh netting were carefully introduced to cover the two maize seedlings, to prevent the gravid *C. partellus* from escaping. Care was taken to prevent the breaking of the leaves, which could lead to the test plants producing mechanical-damage induced volatiles. The cage was raised up slightly at the point where the Petri dish containing the moths was, so as to remove the lid of the Petri dish to release the moths. Then the cage was dropped back immediately to prevent the moths from escaping and fastened to the table to prevent it from being blown away by the wind and the moths from escaping. The experiment was carried out during the last two hours of photophase when there was more emission of volatiles by the test plants (Chamberlain *et al*., 2006). The gravid female moths were allowed to oviposit overnight.

Plate 13: Cage oviposition bioassay set up

At 7.00 a.m. the following day, plants were removed and the egg batches cut from both plants and placed into Petri dishes labelled with the variety, treatment (exposed or non exposed), the date of harvesting the eggs and the replication (1, 2 and 3). The egg batches were then taken to the laboratory where they were kept in a lockable drawer for 3-5 days, depending on prevailing weather conditions. The purpose of this was to allow the heads of the larvae to turn black for easy counting (Plate 14). The numbers of eggs on each plant were counted under a light microscope at Mg ×6.5 and recorded. The above set up was repeated for the maize seedlings exposed to *B. brizantha* without *C. partellus* eggs versus clean maize seedlings. The experiment was replicated three times whereby the replicate tables were placed at a distance of 4 m from each other and 2m from the edge to prevent edge effect.

Plate 14: *Chilo partellus* eggs with black heads

3.3.4 Analysis of volatiles by Coupled gas chromatography-mass spectrometry (GC-MS)

Data outputs from GC-MS linked electrophysiological study were analyzed with the software package Synthech from the Netherlands to define biologically active compounds that caused the observed behaviour in *Cotessia sesamiae* wasps and *Chilo partellus* moths (Wadhams, 1990; Birkett *et al*., 2004).

The GC-MS of active compounds of various maize lines exposed to *B. brizantha* with eggs extract samples eluted in Dichloromethane fractions were analyzed using a silica capillary GC column (50 m x 0.32 mm id. HP 1), fitted with an on-column injector, which was directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments, Manchester, UK). Ionization was done by electron impact at (70 eV, source temperature 250°C) with Helium as the carrier gas. The oven temperature was maintained at 30° C for 5 minutes and then programmed at 5^0 C/minute to 250° C. Tentative GC-MS identifications were confirmed by peak enhancement with an authentic sample on two GC columns of different polarities (Ngi-Song *et al*., 2000). The data generated included peaks of various active physiological compounds.

3.4 Statistical analyses

Statistical analyses are methods used to summarise the data collected into values and figures so as to make sense to readers. The method used to analyse the data depends on number of treatments considered during the experiment.

Data on behavioural response of gravid female *C. sesamiae* to volatiles emitted by various varieties of maize plants was analysed by one way-analysis of variance in a generalized linear model (GLM) followed by separation of significant means using Tukey's Studentized Range Test. The time spent in each arm and the number of entries into each olfactometer arm by *C. sesamiae* were the parameters selected for assessment of the difference between plant volatiles and control. Analysis was performed using R software (R, 2005), with confidence interval level set at 95%. The time spent in each arm was treated as compositional data (Aitchison and Egozcue, 2005) and were thus converted into proportions. All data were checked for normality before analysis and log ratio transformation was done for the non-conforming data.

Data on behavioural response of gravid female *C. partellus* to volatiles emitted by various varieties of maize plants was analysed using paired t-test. The number of eggs and egg batches per maize variety was treated as compositional data (Aitchison and Egozcue, 2005) and were thus converted into proportions. Data on chemical compounds of vacuum distilled volatiles from various maize lines eluted in dichloromethane was done by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Data outputs from GC-linked electrophysiological study were analyzed with a software package Synthech, from the Netherlands to define biologically active compounds (Wadhams, 1990; Birkett *et al*., 2004). Tentative GC-MS identifications were confirmed by peak enhancement with an authentic sample on two GC columns of different polarities (Ngi-Song *et al*., 2000). The data generated included peaks of various active physiological compounds.

CHAPTER FOUR

RESULTS

4.1 The effects of Signal grass *B. brizantha* with *C. partellus* eggs on neighbouring maize

Volatile analysis by gas chromatography bioassays using collected volatiles from exposed maize to Signal grass *B. brizantha* with *C. partellus* eggs, exposed maize to Signal grass *B. brizantha* with *C. partellus* eggs and non-exposed ones revealed that Signal grass *B. brizantha* with *C. partellus* eggs induces small holders-farmer's own varieties. Comparison of GC profiles of volatiles emitted from maize seedlings exposed to *B. brizantha* with *C. partellus* eggs revealed that the smallholder farmers' own varieties (Nyamula, Endere, Cuba 91, and Jowi) emitted more HIPVs than the non-exposed ones and those exposed to *B. brizantha* without *C. partellus* eggs (Figures 3). The degree of inducement varied with the maize variety. On the other hand comparison of GC profiles of volatiles emitted from hybrid (WH505 and PH4) maize seedlings exposed to *B. brizantha* with *C. partellus* eggs showed that they were not induced by HIPVs from this grass. A similar result was noted with the same hybrids exposed to *B. brizantha* without *C. partellus* eggs and the non-exposed hybrid seedlings (Figures 4).

(a) Nyamula

(b) Endere

(c) Cuba 91

(d) Jowi
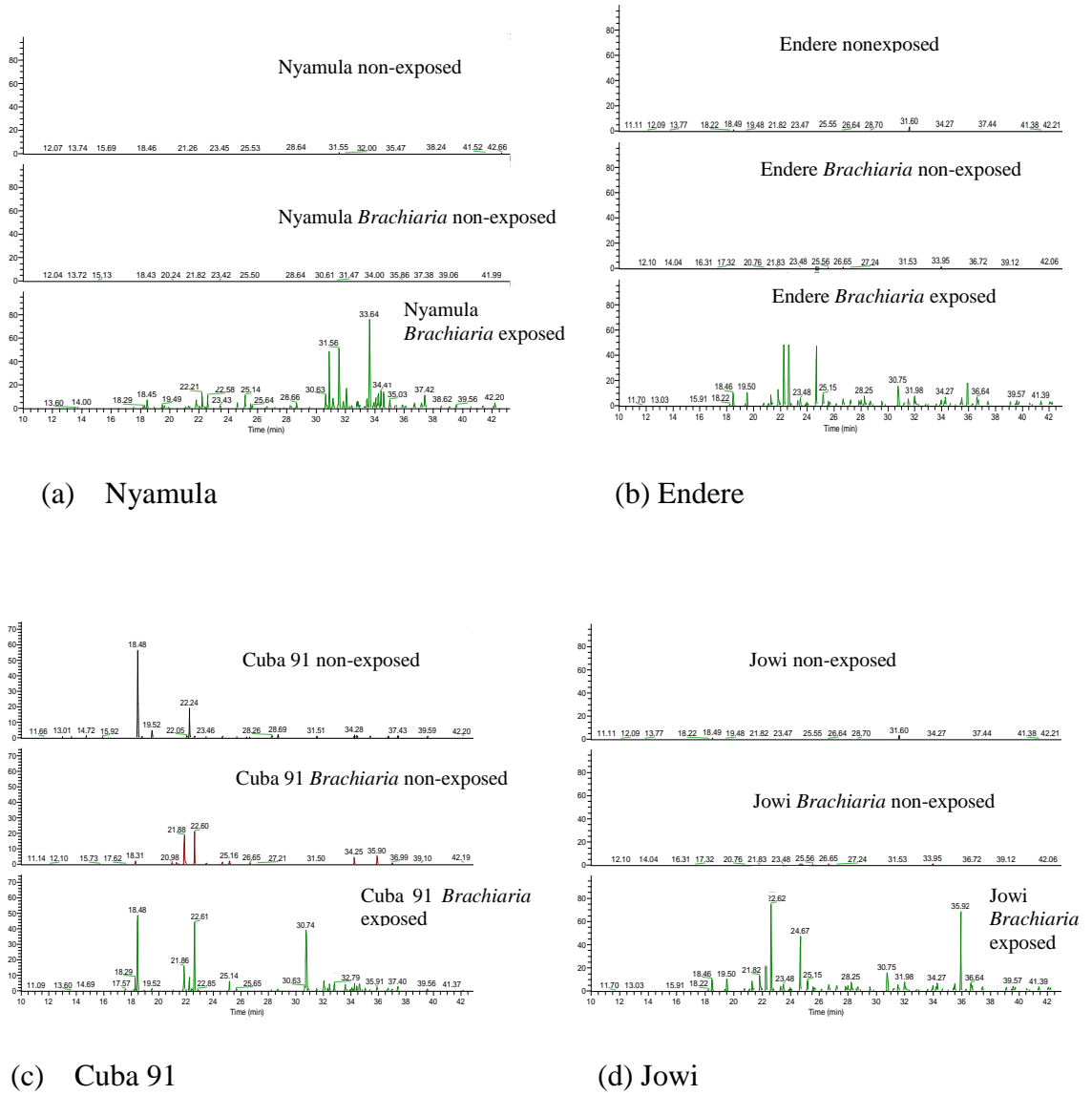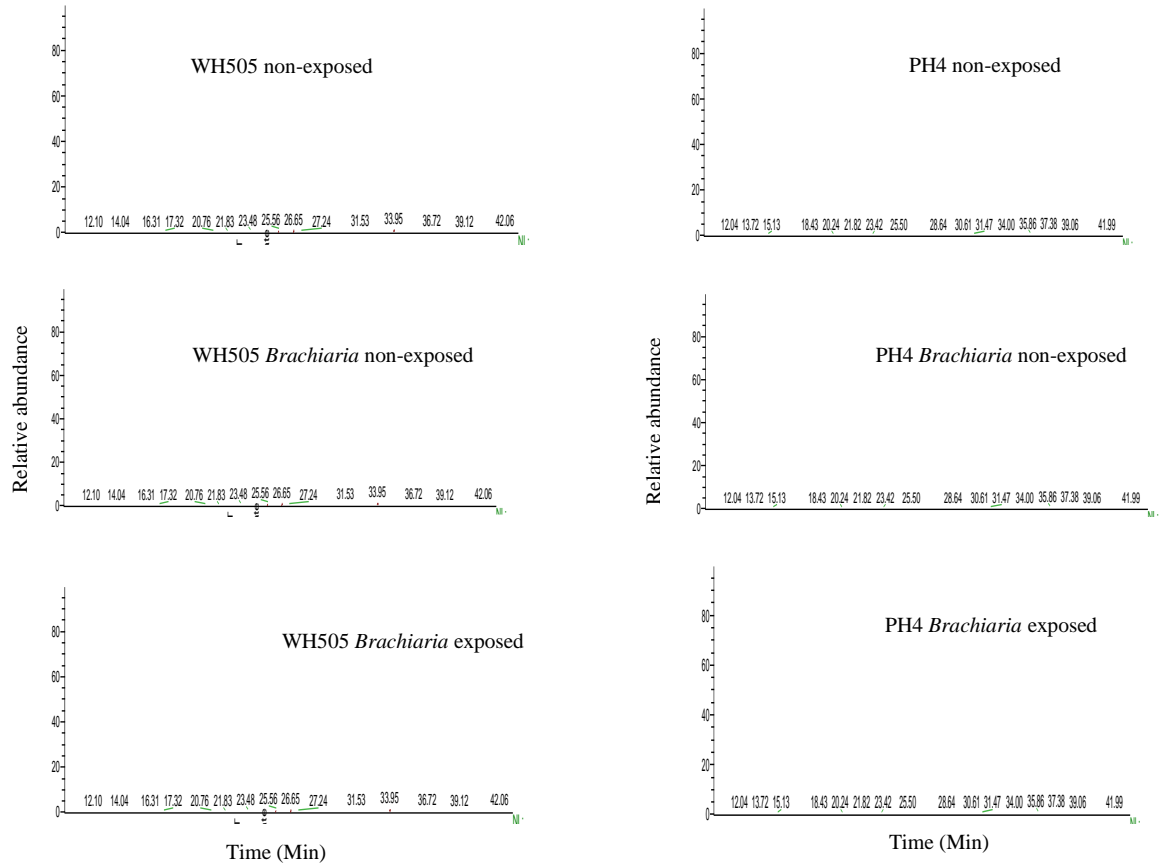
Figure 3: GC analysis of volatiles collected from small holders-farmer's own varieties. Peaks indicate the maize variety was induced

(a)   WH5O5   (b) PH4

Figure 4: GC analysis of volatiles collected from hybrid (WH505 and PH4) maize varieties

4.2 Behavioural responses of gravid female *C. sesamiae* to volatiles emitted by various varieties of maize plants

Olfactometer bioassays showed that female *C. sesamiae* wasps were significantly attracted to HIPVs from the smallholder farmers' own varieties Nyamula, Endere and Jowi and the landrace Cuba 91, exposed to Signal grass *B. brizantha* with *C. partellus* egg*s* (Nyamula $F_{2,33}$ =8.32, *P*=0.0012; Endere $F_{2,33}$ = 15.35, $P < 0.0001$; Cuba 91 $F_{2,33}$ = 12.68, $P < 0.0001$; Jowi $F_{2,33}$ = 17.21, $P < 0.0001$) compared to volatiles from the non-exposed plants and the blank controls (Figure 5). A similar behavioural response was seen in the volatiles from the plants exposed to *B. brizantha* with *C. partellus* eggs compared to those from the plants exposed to *B. brizantha* without *C. partellus* eggs and the blank controls (Nyamula $F_{2, 33}$ =23.53, *P*<0.0001; Endere $F_{2, 33}$ =10.55, *P*=0.0003; Cuba 91 $F_{2, 33}$ =12.68, *P*<0.0001; Jowi $F_{2, 33}$ =17.21, *P*<0.0001) (Figure 6). In contrast, olfactometer bioassays showed that female *C. sesamiae* were not significantly attracted to HIPVs from the plants exposed to *B. brizantha* without *C. partellus* eggs, just as volatiles from the non-exposed plants and the blank controls (Nyamula $F_{2, 33}$ =2.25, *P*=0.1214; Endere $F_{2, 33}$ =3.79, *P*=0.0331; Cuba 91 $F_{2, 33}$ =2.63, *P*=0.0868; Jowi $F_{2, 33}$ =2.8, *P*=0.0705) (Figure 7).
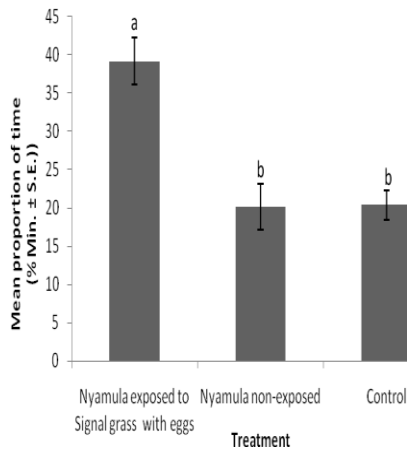
(a) Nyamula
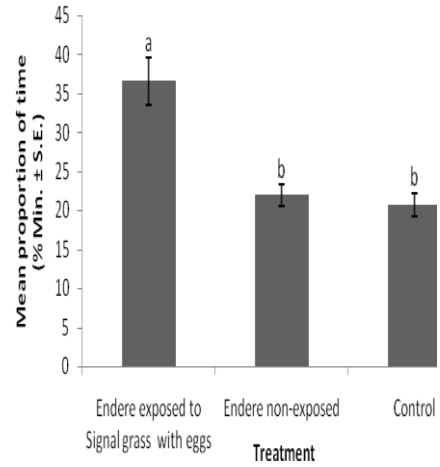
(b) Endere
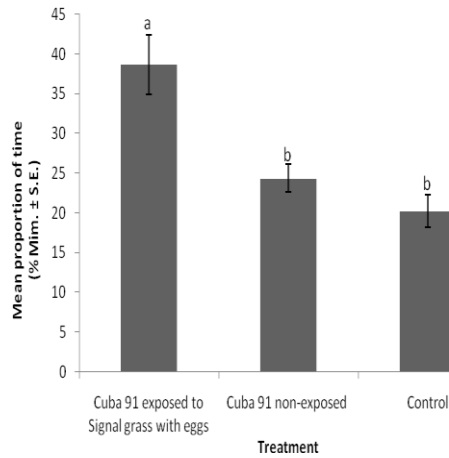
(c) Cuba 91

(d) Jowi

Figure 5: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B.brizantha* with *C. partellus* eggs versus volatiles from non-exposed and blank control in a four way olfactometer.

(a) Nyamula

(b) Endere

(c) Cuba 91

(d) Jowi

Figure 6: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B.brizantha* with *C. partellus* eggs versus volatiles from the maize seedlings exposed to Signal grass *B. brizantha* without eggs and blank control in a four way olfactometer.
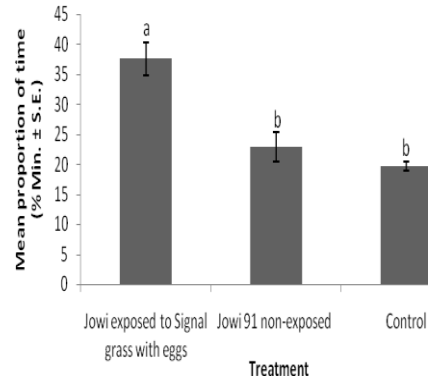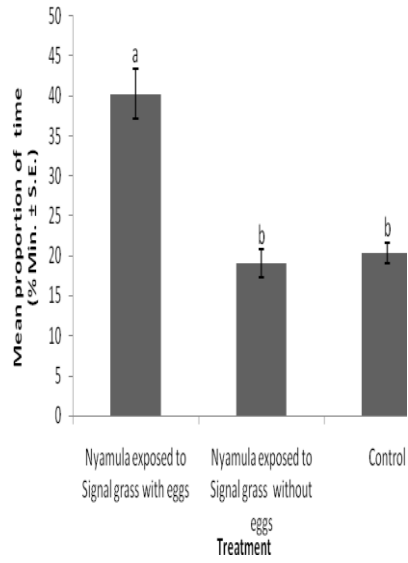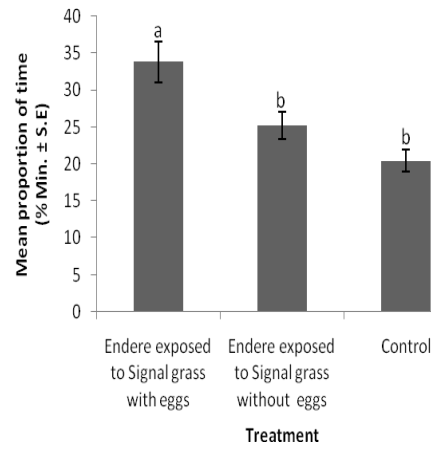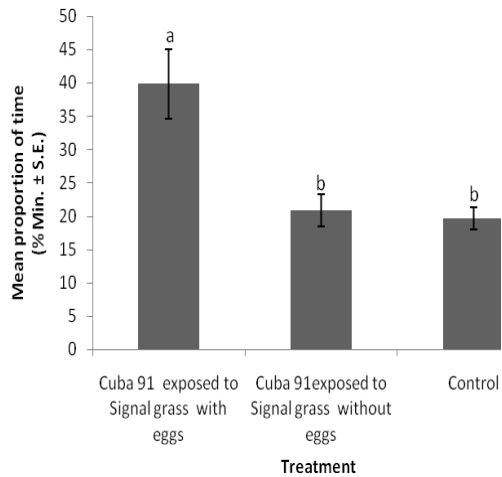
(a) Nyamula
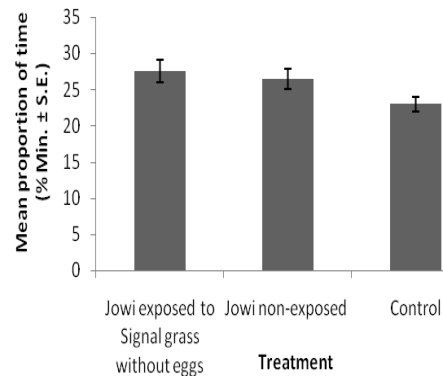
(b) Endere

(c) Cuba 91

(d) Jowi

Figure 7: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B.brizantha* without eggs versus volatiles from the non-exposed and blank control in a four way olfactometer.

Notably, inconsistent results were obtained with the volatiles collected from the two standard commercial hybrid varieties WH505 and PH4 following exposure to Signal grass *B. brizantha* with *C. partellus* eggs. These volatiles were on some occasions not more attractive to *C. sesamiae* compared to volatiles from nonexposed plants and plants exposed to *B. brizantha* without eggs and the blank control arms (WH505: $F_{2,\,33} = 4.63$, $P = 0.0168$; PH4 $F_{2,\,33} = 4.04$, $P = 0.0269$ and WH505 $F_{2,\,33} = 3.96$, $P = 0.0288$; PH4: $F_{2,\,33} = 3.08$, $P = 0.0593$ respectively) (Figures 8 and 9). Similarly, volatiles collected from these two hybrids following exposure to *B. brizantha* with no eggs were not more attractive (WH505: $F_{2,\,33} = 2.86$, $P = 0.0718$; PH4: $F_{2,\,33} = 2.59$, $P = 0.0900$) compared to volatiles from nonexposed plants and the blank control arms (Figure 10)

(a) WH505   (b) PH4
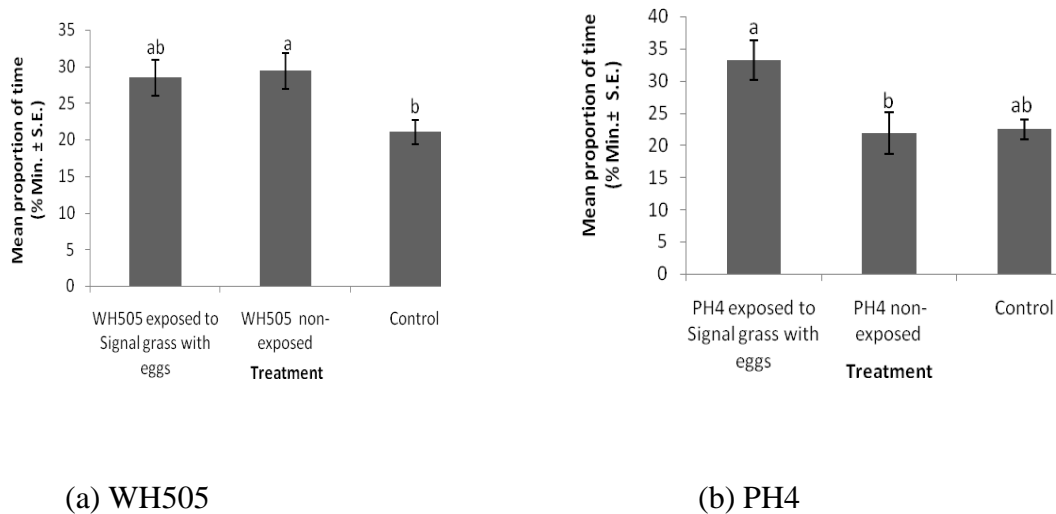
Figure 8: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B.brizantha* with *C. partellus* eggs versus volatiles from the non-exposed and blank control in a four way olfactometer.
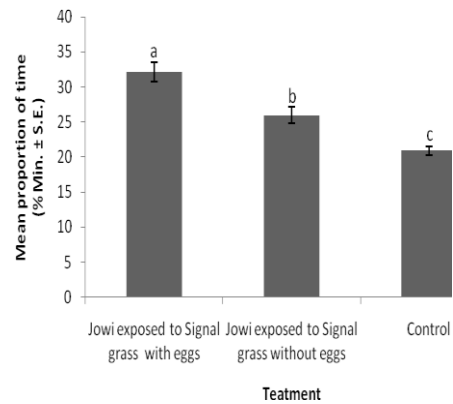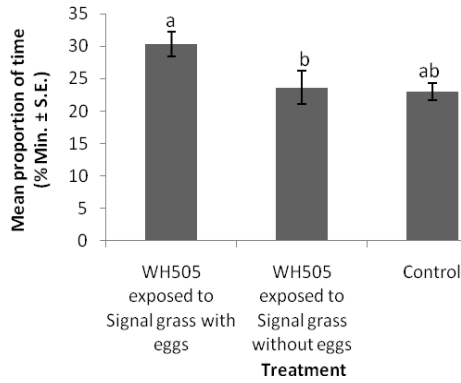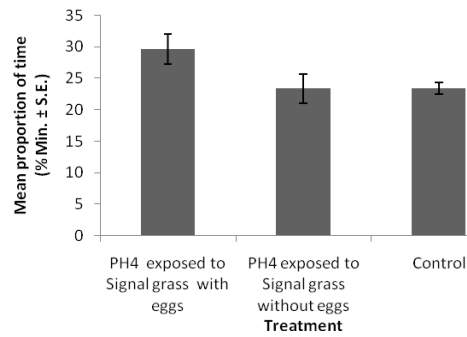
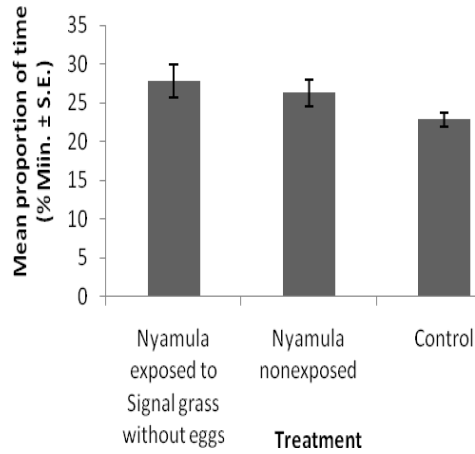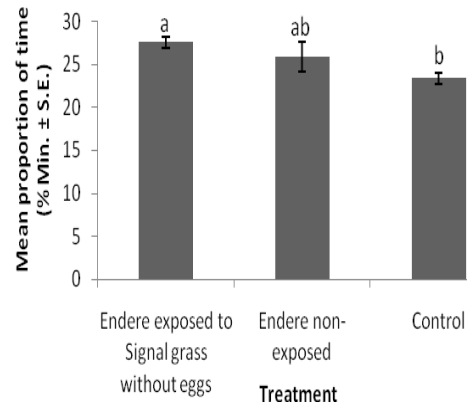(a) WH505   (b) PH4

Figure 9: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B.brizantha* with *C. partellus* eggs versus volatiles from maize seedlings exposed Signal grass *B. brizantha* without eggs and blank control in a four way olfactometer.
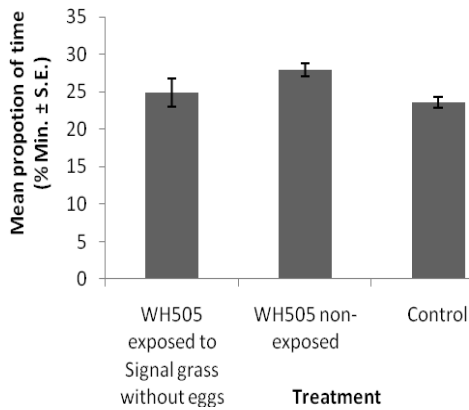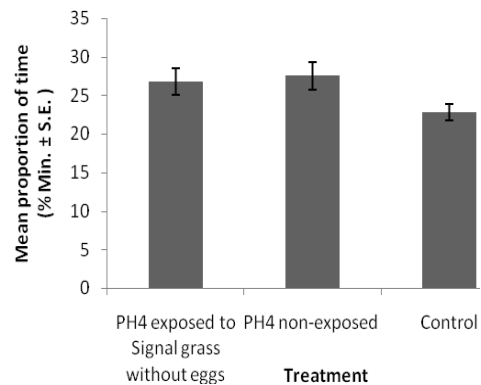
(a) WH505   (b) PH4

Figure 10: Mean proportion (%) of time spent in each arm by gravid *C. sesamiae* in response to volatiles from WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B.brizantha* without *C. partellus* eggs versus volatiles from the non-exposed maize plants and blank control in a four way olfactometer.

4.3 **The effects of volatile cues from maize varieties exposed to Signal grass *B. brizantha* with eggs on host finding by gravid female, *C. partellus*.**

Cage oviposition preference bioassay showed that compared to the smallholder farmers' own varieties, the landrace plants were significantly less preferred for oviposition following expossure to *B. brizantha* with *C. partellus* eggs (Nyamula, d.f. =34, t=- 3.98, p=0.0003; Endere, d.f. = 34, t = -2.55, p= 0.0153; Cuba 91, d.f. = 34, t= -3.16, p=0.0033; Jowi, d.f. =34, t= -3.42, p=0.0017) (Figure 11). Similarly, the numbers of egg batches deposited by gravid female *C. partellus* on the plants exposed to *B. brizantha* with *C. partellus* eggs were significantly lower than those oviposited to the non-exposed plants (Nyamula, d.f. =34, t= -2.92, p= 0.0061; Endere, d.f. =34, t = -3.04, p = 0.0046; Cuba 91, d.f. = 34, t= -2.45, p=0.0195; Jowi, d.f. = 34, t= -3.72, p=0.0007) (Figure 12). On the other hand, oviposition bioassays showed no significant difference in the mean number of eggs oviposited on the plants exposed to *B. brizantha* without *C. partellus* eggs and those oviposited on the non-exposed plants (Figure 13). These seedlings showed no significant difference in the number of eggs deposited on them by the female *C. partellus* (Nyamula, d.f. = 34; t= 1.11; p = 0.2733; Endere, d.f. =34; t=0.87; p= 0.3881; Cuba 91, d.f. =34; t= 0.34; p=0.7374 and Jowi, d.f. =34; t= -1.70; p=0.0975). A similar behavioural response was observed in the number of egg batches deposited by the gravid female *C. partellus* on the plants exposed to *B. brizantha* without *C. partellus* eggs (Nyamula, d.f. = 34, t= -0.02, p =0.9834; Endere, d.f. =34, t=0.15, p=0.8830; Cuba 91, d.f. =34, t= -0.31, p=0.7556; Jowi, d.f. =34, t= -1.03, p=0.3081) compared to non-exposed plants (Figure 14).
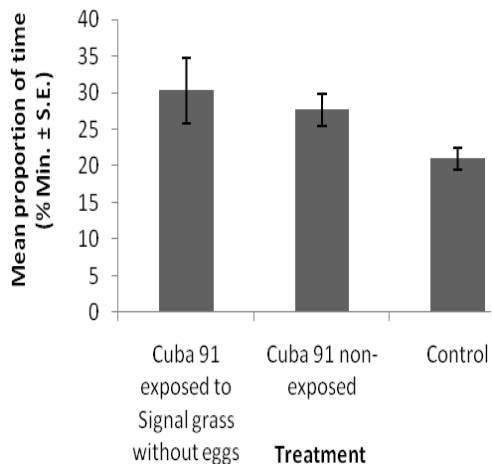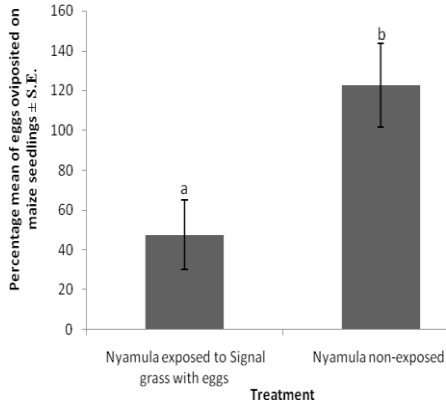
(a) Nyamula
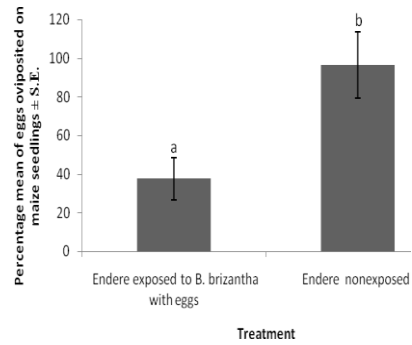
(b) Endere
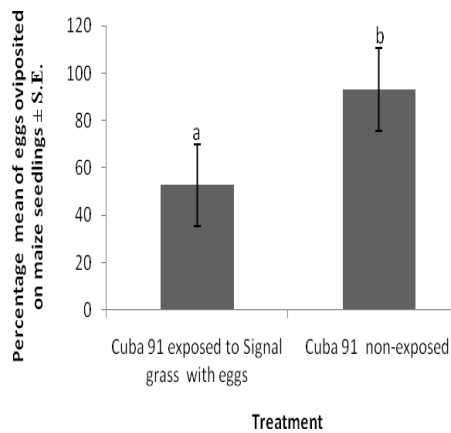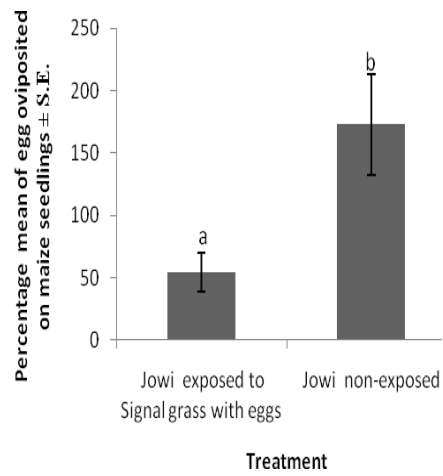
(c) Cuba 91

(d) Jowi

Figure 11: Mean number of eggs oviposited by *C. partellus* gravid female on Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B. brizantha* with *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
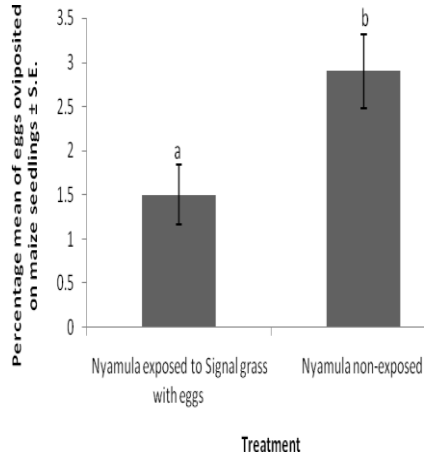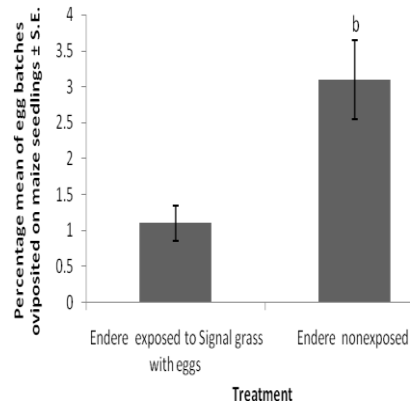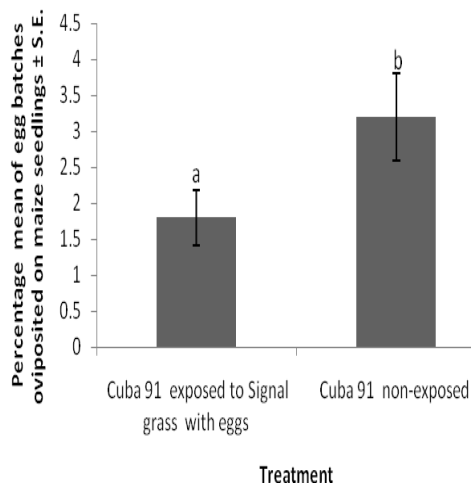
(a) Nyamula
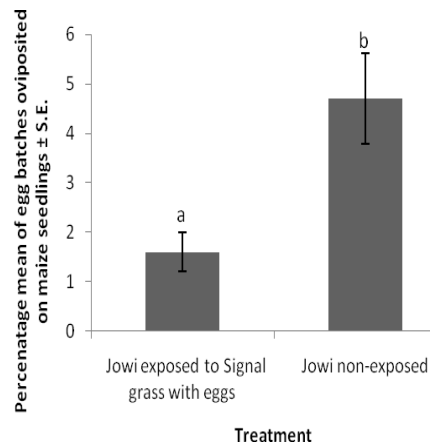
(b) Endere

(c) Cuba 91

(d) Jowi

Figure 12: Mean number of egg batches oviposited by *C. partellus* gravid female on Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B. brizantha* with *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
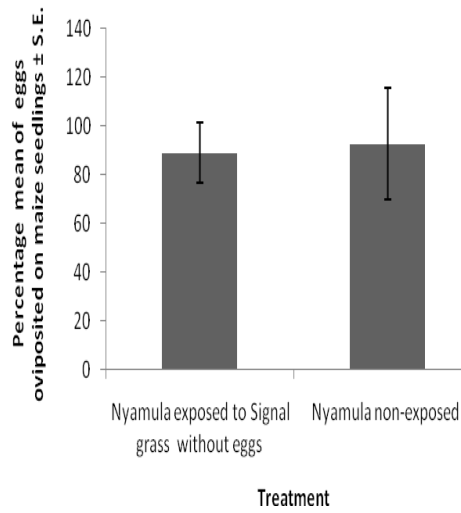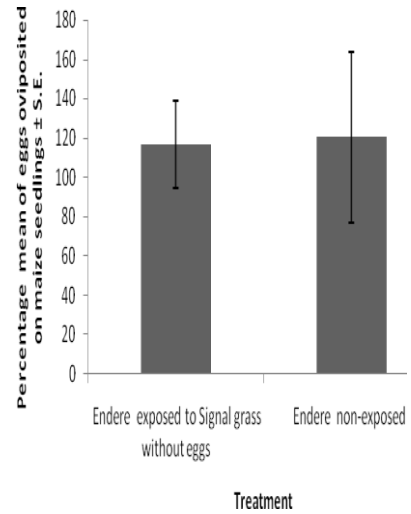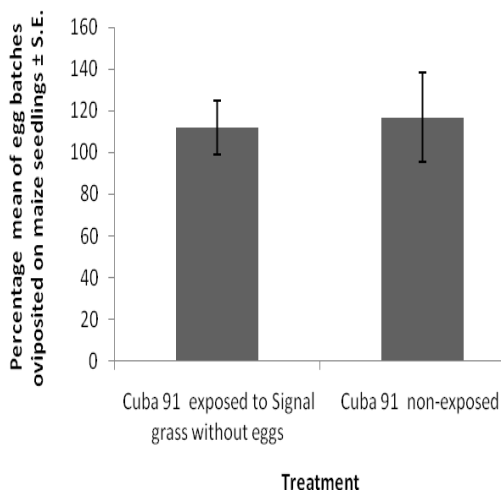
(a) Nyamula
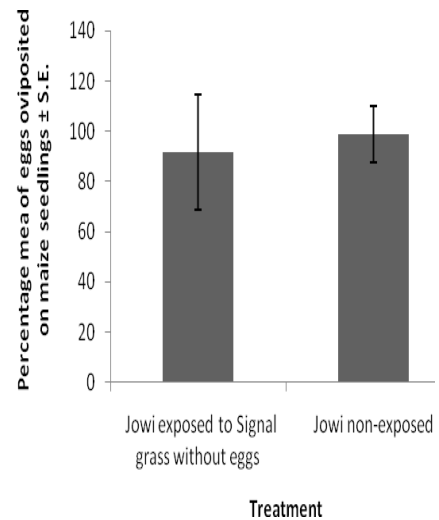
(b) Endere

(c) Cuba 91

(d) Jowi

Figure 13: Mean number of eggs oviposited by *C. partellus* gravid female on Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B. brizantha* without *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
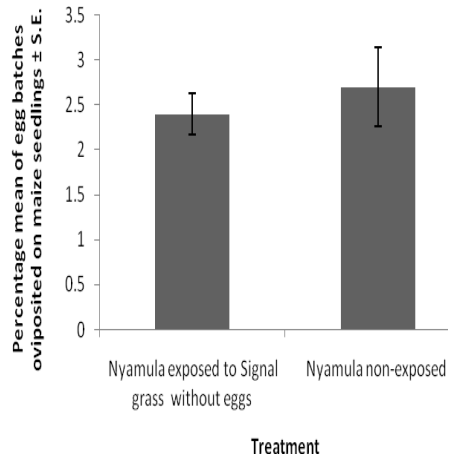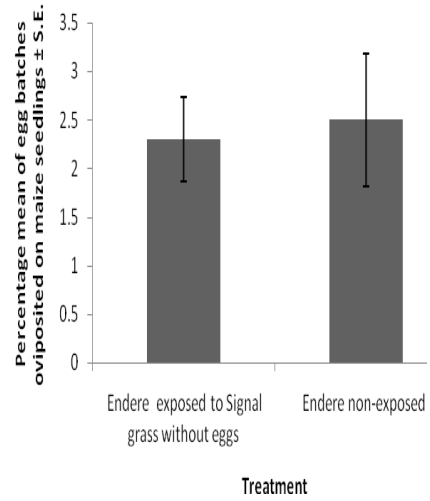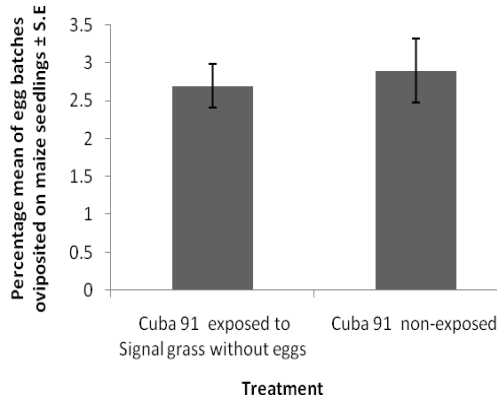
(a) Nyamula
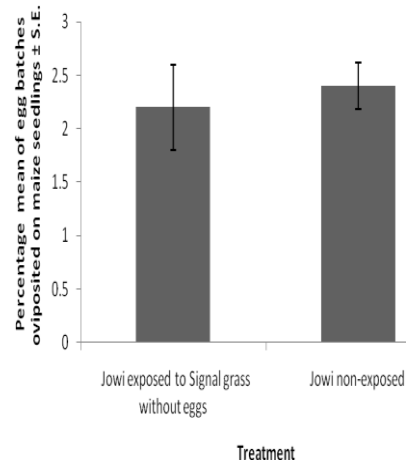
(b) Endere

(c) Cuba 91

(d) Jowi

Figure 14: Mean number of egg batches oviposited by *C. partellus* gravid female on Nyamula (a), Endere (b), Cuba 91 (c) and Jowi (d) maize seedlings exposed to Signal grass *B. brizantha* without *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.

A contrast was observed when two standard commercial hybrid varieties [Western Seed Company Ltd (WH505) and Kenya Seed Company Ltd (PH4)] were used. Cage oviposition preference bioassay revealed that for these varieties, plants exposed to *B. brizantha* with *C. partellus* eggs showed no significant difference in the number of eggs oviposited on the maize plants exposed to *B. brizantha* with eggs and the non-exposed plants (WH505, d.f. =34, t= -0.73, p=0.4694; PH4, d.f. =34, t= -1.03, p=0.3094) (Figure15). A similar behavioural response was observed in the number of egg batches oviposited by the gravid female *C. partellus* on WH505 and PH4 seedlings exposed to *B. brizantha* with *C. partellus* eggs relative to those oviposited on the non-exposed plants (WH505, d.f. = 34; t= -0.46; p=0.6496 and PH4, d.f. =34; t= -1.01; p=0.3199) (Figure 16). Moreover, there were no significant differences between the number of eggs oviposited on plants exposed to *B. brizantha* without *C. partellus* eggs and those oviposited on the non-exposed plants (WH505, d.f. =34, t= -0.28, p=0.7797; PH4, d.f. =34, t=0.77, p=0.4448) (Figure 17). A similar behavioural response was observed in the number of egg batches deposited by the gravid female *C. partellus* on WH505 and PH4 exposed to *B. brizantha* without *C. partellus* eggs and those oviposited on nonexposed plants (WH505 d.f.= 34, t= -0.20, p=0.8435; PH4 d.f. =34, t=0.82, p=0.4163) (Figure 18).

(a) WH505   (b) PH4
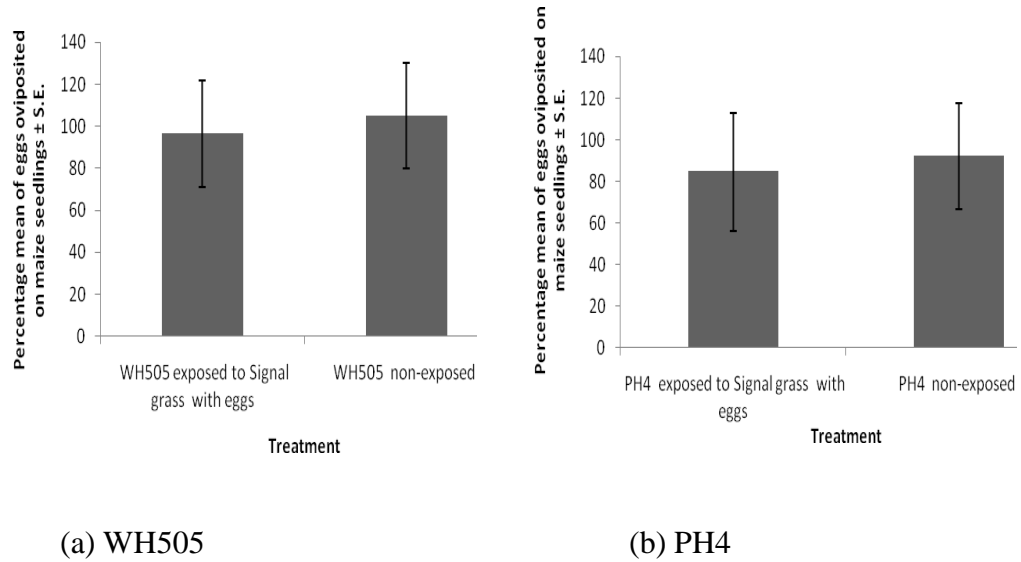
Figure 15: Mean number of eggs oviposited by *C. partellus* gravid female on WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B. brizantha* with *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
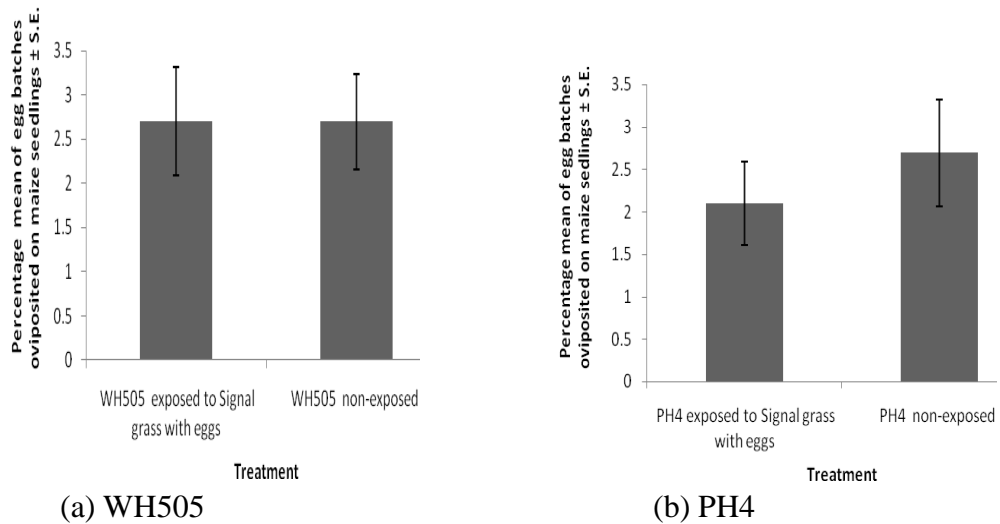
(a) WH505   (b) PH4

Figures 16: Mean number of egg batches oviposited by *C. partellus* gravid female on WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B. brizantha* with *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
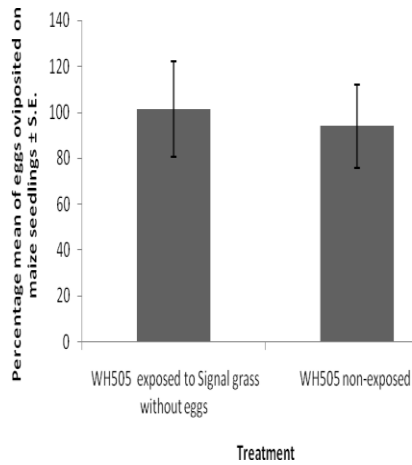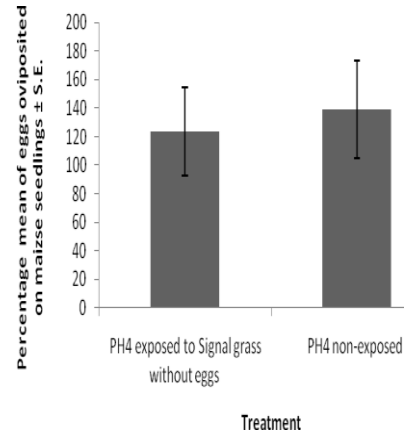
(a) WH505

(b) PH4

Figure 17: Mean number of eggs oviposited by *C. partellus* gravid female on WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B. brizantha* without *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.
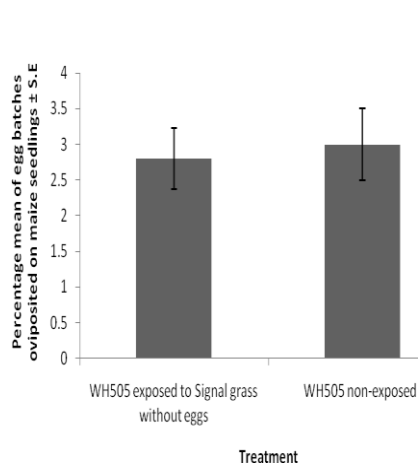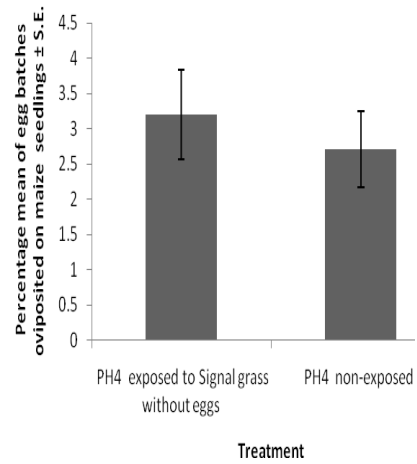
(a) WH505

(b) PH4

Figures 18: Mean number of egg batches oviposited by *C. partellus* gravid female on WH505 (a) and PH4 (b) maize seedlings exposed to Signal grass *B. brizantha* without *C. partellus* eggs and the non-exposed maize seedlings in an oviposition cage.

**4.4 Biologically active compounds of the volatiles responsible for the observed behavioural pattern in the gravid female parasitoid, *C. sesamiae* and gravid female *C. partellus***

Plants release volatile organic compounds which act as chemical signals when attacked by herbivorous insects or pathogens to serve as attractants, repellents and aggregation stimulants of chemical ecology. The chemical identity of the volatile compounds varies with the plant species and with the herbivorous insect species. These volatiles attract both parasitic and predatory insects that are natural enemies of the herbivores. They may also induce defense responses in neighboring plants. Such chemicals, which function in communication between and among species, as well as those that serve as messengers between members of the same species, are called semiochemicals.

4.4.1 Chemical components of Gas chromatography-Mass spectrometry of volatiles from representative landrace maize lines exposed to *B. brizantha* with *C. partellus* eggs

Comparison of GC-MS profiles of volatiles emitted from maize seedlings exposed to *B. brizantha* with *C. partellus* eggs revealed that the smallholder farmers' own seed varieties (Nyamula, Cuba 91, and Jowi) emitted more HIPVs than the non-exposed ones or those exposed to *B. brizantha* without *C. partellus* eggs (Figures 19, 20 and 21).

The GC-MS analyses of the volatiles from three representative maize lines (Nyamula, Cuba 91 and Jowi) exposed to *B. brizantha* with *C. partellus* eggs revealed the presence of seven key compounds. The most abundant components from Nyamula were identified as: (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Decanal and (*E*)-Caryophyllene. The most abundant

components from Cuba 91 were identified as: Linalool, (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT) and (*E*)-β-fernesene, while the most abundant components from Jowi were identified as Linalool, Nananal, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Methyl Salicylate and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecattetraene (TMTT). The above compounds were induced in the maize exposed to *B. brizantha* with *C. partellus* eggs but were not insignificant in the maize exposed to *B. brizantha* without *C. partellus* eggs and the non-exposed ones as expressed in the landraces maize varieties. The variation in induction could be because of genetic variability amongst the various landraces maize varieties as reflected by the representative peaks (Figures 19, 20 and 21).WH505 and PH4 maize seedlings exposed to *B. brizantha* with and without *C. partellus* eggs and non-exposed maize plants did not emit any of the above chemical compounds and thus did not have their defense systems induced in any way.
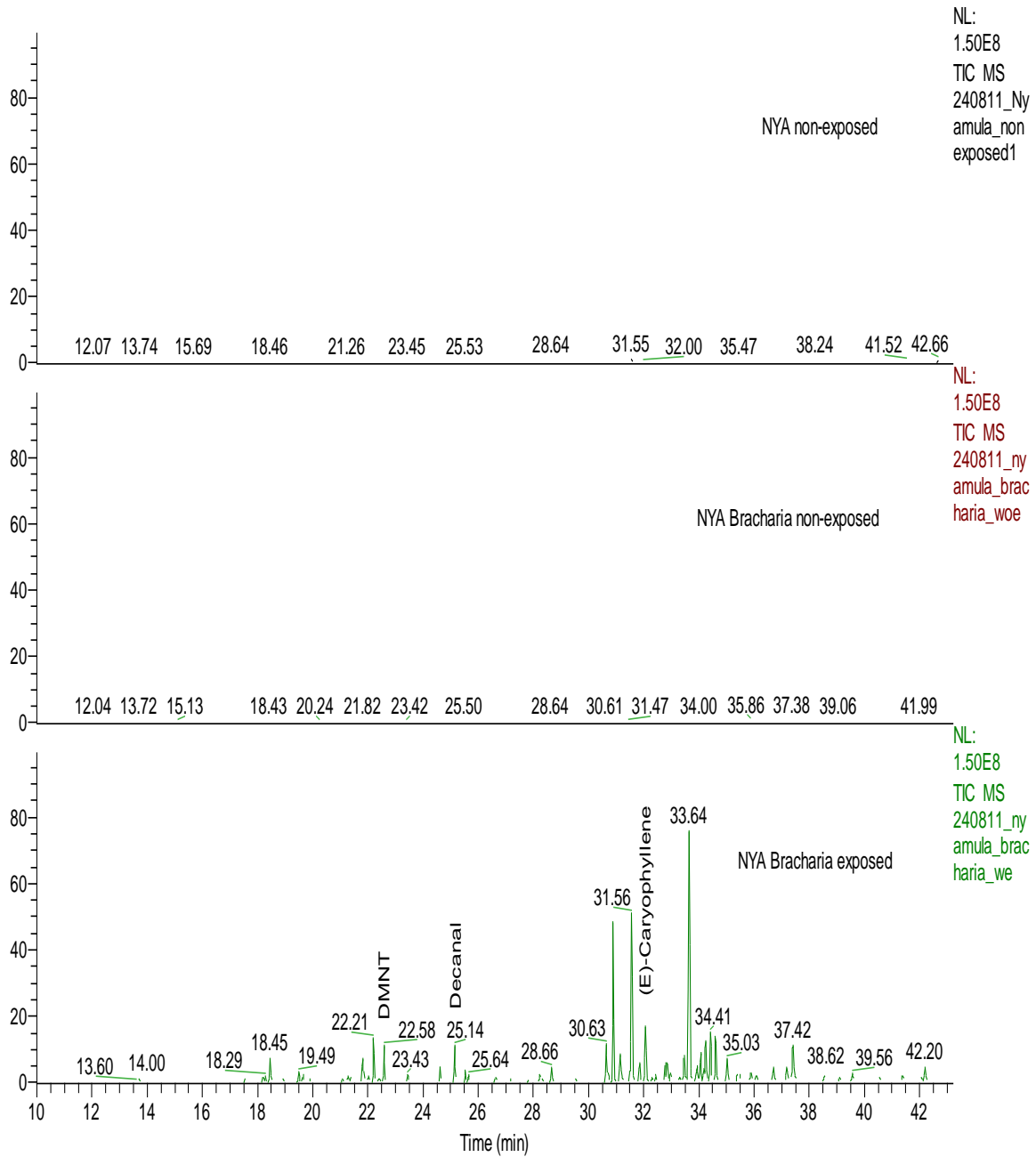
Figure 19: Total ion chromatogram (TIC) obtained by coupled GC-MS analysis of volatiles of Nyamula (NYA) maize attractive to *C. sesamiae*.
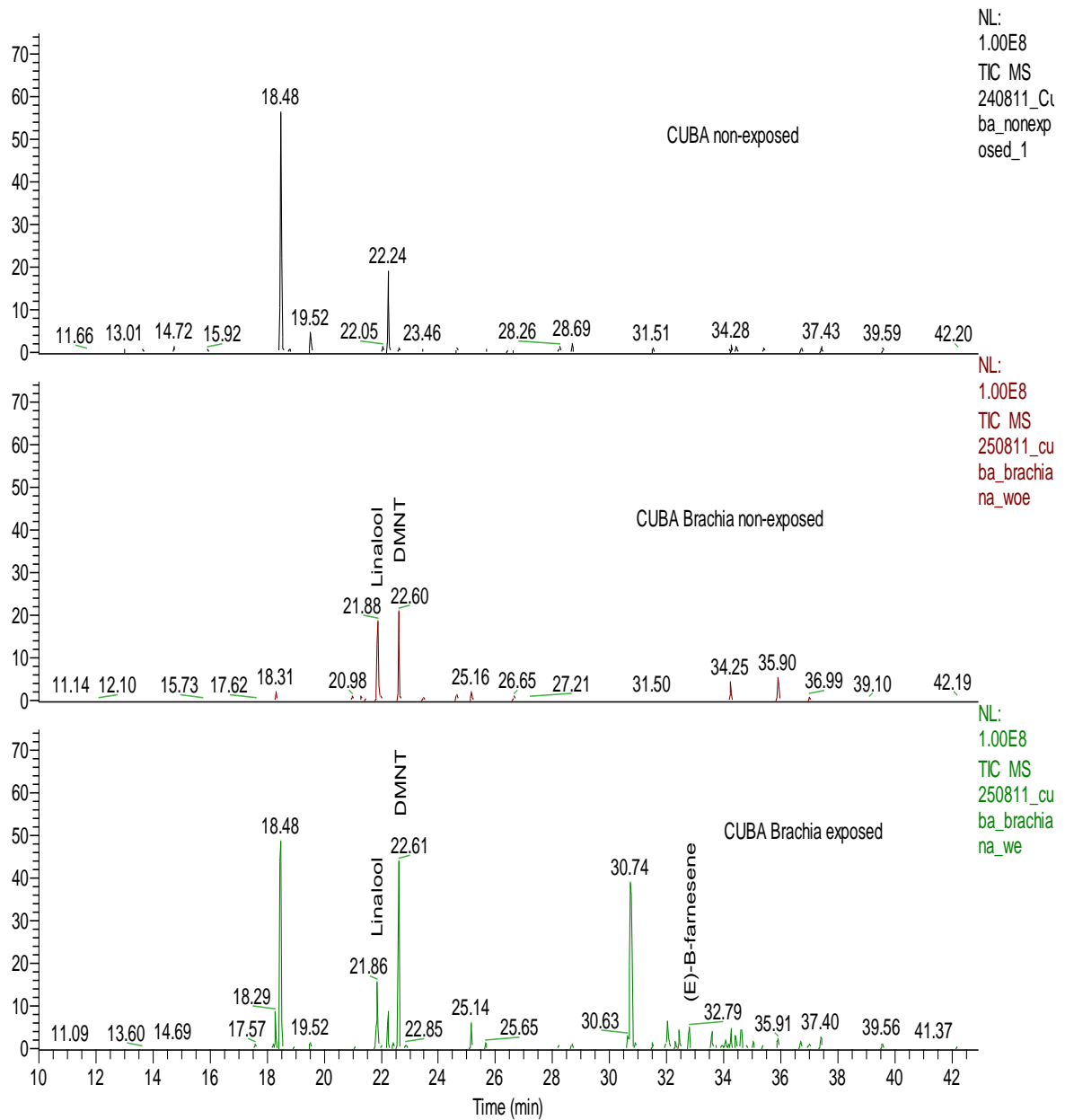
Figure 20: Total ion chromatogram (TIC) obtained by coupled GC-MS analysis of volatiles of Cuba 91 maize attractive to *C. sesamiae*.
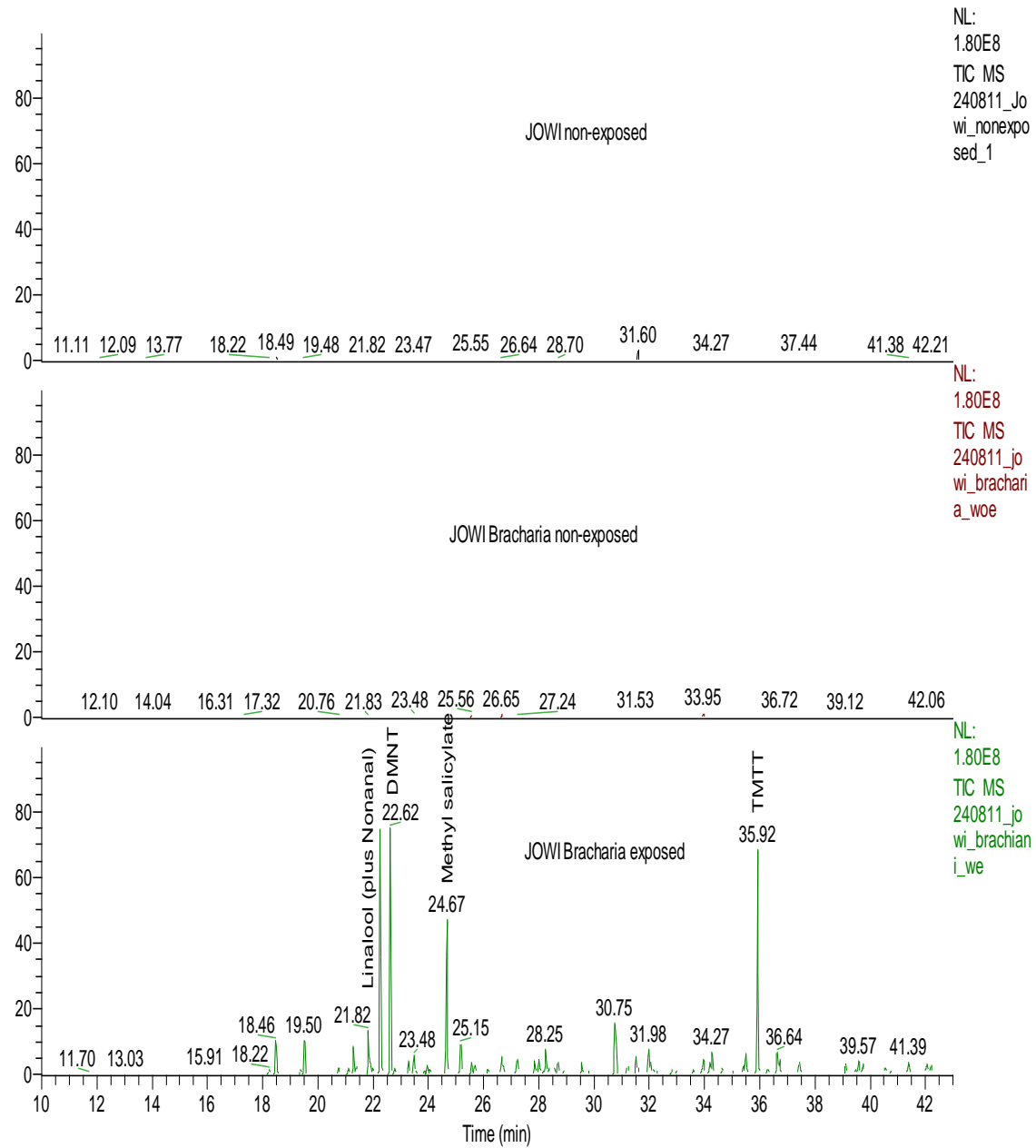
Figure 21: Total ion chromatogram (TIC) obtained by coupled GC-MS analysis of volatiles of Jowi maize attracive to *C. sesamiae*.

4.4.2 Chemical structures of Nyamula, Cuba 91 and Jowi volatiles eluted in DCM

GC-MS analysis of behaviourally active HIPVS of various maize lines eluted in DCM showed the presence of one major compound from each extract (Figures 18, 19 and 20). A major compound identified from Nyamula and other maize lines was (*E*)-4,8-Dimethyl-1, 3,7-nonatriene. The other compounds varied depending on the local maize variety. The stereochemistry of these compounds was determined by enantioselective gas chromatography (GC) using authentic samples (Figures 21, 22, 23, 24, 25, 26, 27 and 28).
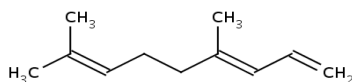
Figure 22 (*E*)-4,8-Dimethyl-1,3,7-nonatriene
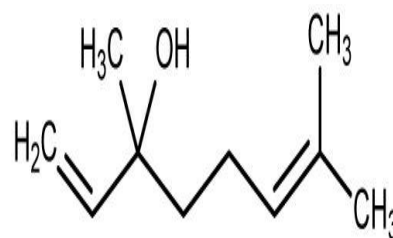
Figure 23 Linalool
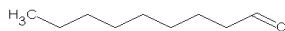
Figure 24 Nonanal
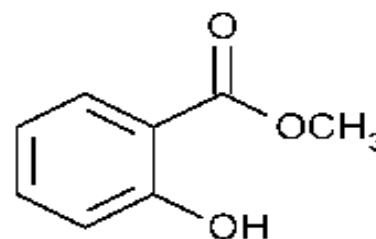
Figure 25 Methyl salicylate

Figure 26 Decanal
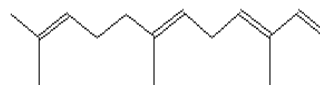
Figure 27 (*E*)-β-Farnesene
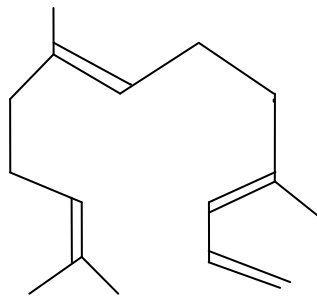
Figure 28 (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tri-decatetraene (TMTT)
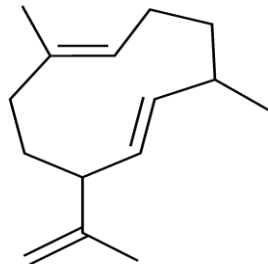
Figure 29 (*E*)-Caryophyllene

CHAPTER FIVE

DISCUSSION

The main aim of this study was the identification and use of semiochemicals for the control of the maize stemborer, *C. partellus* in Kenya. The use of semiochemicals has stimulated much interest because they may be used to improve current monitoring as well as direct means of controlling field crop insect pest species (Khan *et al*., 2010). Laboratory olfactometry and semi field oviposition preference experiments were designed to investigate the inducement properties of Signal grass, *B. brizantha* following oviposition by the *C. partellus* on field maize. Gas chromatography (GC) and coupled gas chromatography-mass spectrometry (GC-MS), were carried out to identify the chemical constituents that confer attractancy of the *C. sesamiae* to the exposed maize varieties to *B. brizantha* oviposited by *C. partellus* and repellency of *C. partellus*. The aim was to investigate the possibility of using the *B. brizantha* as a trap crop and as a source of inducing signals for maize plants in the field, to recruit *C. sesamiae*, a parasitoid of *C. partellus* in a novel, environmentally sound, small-scale crop protection strategy in Kenya.

5.1 The effects of Signal grass *B. brizantha* with *C. partellus* eggs on neighbouring maize.

The current study has identified the potential of inducing defence responses in maize varieties by the *B. brizantha* with *C. partellus* oviposition. Signal grass, an African fodder grass, is a 'clever' plant in that once the stemborer moth has laid its eggs on it, there is supressed emission of attractive plant volatiles leading to reduced further egg laying on the plant as revealed by oviposition bioassays. At the same time, the grass releases volatile

organic compounds that recruit parasitic wasps that then attack the larvae as they emerge from the eggs, thereby preventing them from completing their life cycle (Colazza *et al*., 2004; Bruce *et al*. 2010; Tamiru *et al*., 2011). Such chemical compounds which play a dual role include: (*E*) - Caryophyllene, (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT), Decanal, Linalool, (*E*)-β-farnesene, TMTT, methyl salicylate and Nananal (Bruce *et al*. 2010). The current study has shown that this grass is more attractive for egg laying by *C. partellus* due to the listed chemical components. The grass induced farmers' own maize varieties to produce HIPVs similar to those which were emitted by *B. brizantha* plants laid on by gravid *C. partellus*. This makes the grass to qualify as an ideal trap plant candidate for use in the push-pull strategy for stemborer management by smallholder farmers in western Kenya and indeed in similar agro-ecologies where the stemborer pests are a menace. This approach should be introduced or activated in maize plants (Bruce, 2010 ;Tamiru *et al*., 2012).

5.2 Behavioural responses of gravid *Cotessia sesamiae* to the induced maize plant volatiles

Olfactometer bioassays conducted with gravid female *C. sesamiae* and volatiles from various maize varieties following exposure to *B. brizantha* with *C. partellus* eggs showed clear orientation choices by *C. sesamiae. Cotessia sesamiae* responded with positive and consistent anemotaxis to air plumes/currents passed over 10µl of Nyamula, Endere, Cuba 91 and Jowi maize varieties extracts. The attractivity of *C. sesamiae* towards local maize volatiles was due to attractive semiochemicals from the *B. brizantha* with *C. partellus* eggs which were induced into these maize plants. Nyamula exposed to *B. brizantha* with eggs

emitted (*E*) - Caryophyllene, (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT) and Decanal, key compounds known to repel *C. partellus* pest away from the maize. Cuba 91 exposed to *B. brizantha* with eggs emitted (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Linalool and (*E*)-β-farnesene. Exposed Jowi to *brizantha* with eggs emitted DMNT, TMTT, methyl salicylate and Linalool (plus Nananal), compounds known to have similar effects as for Nyamula. This implies that the defense systems of the local maize varieties were induced by the *B. brizantha* with *C. partellus* eggs. Then this local maize attracted the parasitic wasps that preyed on the stemborer even before any pest damage occurred. This was not observed in the controls with the solvent and volatiles of the non-exposed maize plants. This conforms to the findings by Bruce *et al*. (2010) and Tamiru *et al*. (2011) who demonstrated that oviposition by *C. partellus* induces maize landraces to release volatiles that attract egg and larval parasitoids. *Cotesia sesamiae* did not show a positive and consistent anemotaxis to air plumes/currents passed over 10µl of the hybrid maize varieries (WH505 and PH4) tested. The fact that the induction of the plants' defense systems was only observable in the local maize varieties and not consistently in the hybrids implies that the innate defense systems in the latter plants might have been compromised by the breeding processes that yield these hybrids (Rasmann *et al.*, 2005; Kollner *et al.*, 2008; Tamiru *et al*., 2011). Local farmers' maize varieties have undergone far less improvement processes and the results of the current study imply that their defence systems are still intact and functioning.

Insect host location frequently involves detection of volatile signals in the environment by olfactory receptors located in minute or microscopic hair-like structures (setae) in the

antennae (Bruce *et al.,* 2005b; Couty *et al*., 2006). This implies that the insect species are able to detect a suitable host while walking or in flight, and also that host selection can depend on a lack of repellency. During detection, the insect recognizes the active components in the signal by chemoreceptors according to their structures or loosely their 'size and shapes' or spatial arrangement (enantiomeric composition) (Torto, 2005). These chemoreceptors then relay a message through the antennal nerve to the central nervous system (brain) that decodes the messages via cellular and metabolic activity. This may in turn activate sets of muscles whose reaction may manifest a behavioural response (van den Berg *et al.*, 2008).

Under natural conditions, this mechanism may take place when the first *C. sesamiae*, preferably a male, arrives at a food source and releases aggregation pheromones. In combination with food odours, the pheromones contribute to the aggregation of small colonies of conspecific during the initial stage of *C. sesamiae* infestation (Muyekho *et al*., 2005). This is in agreement with findings by Tamiru *et al*. (2011) who reputed that *C. sesamiae* was attracted to the 'smart' maize plants infested by *C. partellus* eggs.

Overall, the results of this study indicated that exposing farmers' local maize varieties to *B. brizantha* with *C. partellus* eggs induced the plants to emit volatile blends that were attractive to *C. sesamiae.* The attraction of larval parasitoids meant that natural enemies could be recruited to attack the newly hatched stemborer larvae before they cause damage to the crop. These results reported here are of significance for the management of *C. partellus* as they provide insights into possible exploitation of signalling of defence responses in smart maize varieties using an equaly "smart" trap plant. It would also open

up opportunities for a more efficient exploitation of natural enemies as they would be recruited by the earliest stagte of pest attack before larvae hatch from the eggs and damaging the plants. This may thus lead to identification of further candidates for use as 'inducer' plants in pre-harvest crop protection especially at traditional African small-scale farmer level.

5.3 The effects of volatile cues from maize varieties exposed to Signal grass *B. brizantha* with eggs on host finding by gravid female, *C. partellus*.

Results showed that gravid female *C. partellus* displayed clear orientation choices between the maize plants exposed to *B. brizantha* with *C. partellus* eggs and the non-exposed plants. Nyamula, Endere, Cuba 91 and Jowi exposed to *B. brizantha* with *C. partellus* eggs were found to be less attractive to *C. partellus* for oviposition relative to the non-exposed plants and those which were exposed to *B. brizantha* without *C. partellus* eggs. The less attractivity of *C. partellus* towards local maize by gravid *C. partellus* was due to repelling semiochemicals from the *B. brizantha* with *C. partellus* eggs which were induced into these maize plants. Nyamula exposed to *B. brizantha* with eggs emitted (*E*) - Caryophyllene, (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT) and Decanal, key compounds known to repel *C. partellus* pest away from the maize. Cuba 91 exposed to *B. brizantha* with eggs emitted (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Linalool and (*E*)-β-farnesene. Jowi seedlings exposed *to brizantha* with eggs emitted DMNT, TMTT, methyl salicylate and Linalool (plus Nananal), compounds that were repellent to gravid *C. partellus* . On the other hand non-exposed maize and the maize exposed to *B. brizantha* without *C. partellus* eggs were highly attractive to gravid *C. partellus* since they could not emit the above semiochemicals

respectively. These observations were not seen in experiments with the commercial hybrids. These findings obtained conform to the findings of Tamiru *et al*. (2012) who showed that when landrace maize are laid on by gravid stemborer, they produce DMNT and ocimene which act as repellents to stemborers preventing them from further laying eggs on them and also primes neighbouring maize plants. The results of this study suggest that the local farmers' varieties exposed to *B. brizantha* with *C. partellus* eggs were induced to emit volatile blends that were less attractive to gravid *C. partellus*, a feature that was not observed in the hybrids. This seems to follow from the section above and imply that the breeding process, that often involves selection of traits such as faster growth and higher yields, could have compromised the inducible defense trait in these elite hybrids (Rasmann *et al.*, 2005; Kollner *et al.*, 2008; Tamiru *et al*., 2011; Tamiru *et al*., 2012). The unattractiveness of volatiles from the induced maize plants could be used to mask or alter odours emitted by the field maize crop leading to reduced 'visibility' in olfactoric terms of the plants for attack by gravid *C. partellus* moths.

The presence of phagostimmulatory compounds have been considered crucial in the infestation by the stemborers and *C. sesamiae* (Khan *et al*., 2011). The high attractiveness of the maize exposed to oviposited *B. brizantha* to gravid *C. sesamiae* in the present study provides the understanding of the importance of HIPVs cues in host habitat location by the *C. sesamiae* parasitoids. van den Berg *et al.* (2008) showed that three antennaly active compound of sorghum, *Sorghum bicolor* (L. Moench) panicles namely; benzyl alcohol, linalool and 2-phenlethanol specific ratios play a great role in the host attraction by the pollen beetle, *Astylus atromaculatus* Blanchard. Similarly, decanal and nonanal collected

from *Z. mays* and *S. bicolar* by air entrainment have been reported to cause a repellent response in the stemborer, *B. fusca*, a major pest of cereal crops in Africa (Birkett *et al*., 2006). Furthermore, (*E*)-4, 8-dimethyl-1,3,7-nonatriene produced by corn has been reported to be an attractant of the *C. sesamiae* and a repellent of the egg laying *C. partellus* (Torto, 2005; Hooper *et al*., 2010). Webster *et al.* (2008) also reported that the winged *Aphis fiba* Scop, was attracted to volatiles from the air entrainment sample of the faba bean, *Vicia faba* in olfactometry behavioural and electrophysiological responses, and identified decanal and methyl salicylate among the 16 electrophysiologically active compounds.

Plant signalling is not limited to communication between *B. brizantha* and maize plants only. Couty *et al*. (2006) reported that the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae), the major pest of cruciferous crops such as cabbages, uses volatile plant chemical cues to locate and promote landing on their hosts even in a complex mixed-crop environment in large scale. Khan *et al.* (2011) studied the odour-mediated effects of Molasses grass, *Mellinis minutiflora*, on maize plants and host searching behaviour of the *C. partellus* and its parasitoid, *C. sesamiae* in oviposition bioassays and in 4-way olfactometry bioassays respectively. They reported that *M. minutiflora* was able to induce maize plants and volatiles from the exposed maize plants were significantly attractive to *C. sesamiae* and significantly repellant to *C. partellus*. Therefore it may be possible to mask host plant odours with non-host plants in order to disrupt host finding by the insect pest species, hence protecting the desired field crop.

5.4 Biologically active compounds from the induced local maize varieties responsible for observed behavioural patterns in the gravid female parasitoids *Cotessia sesamiae* and the gravid female stemborers *Chilo partellus*

The present study demonstrated that Nyamula, maize variety emitted a different blend of volatile organic compounds following exposure to *B. brizantha* with *C. partellus* eggs. There was emission of (*E*) - Caryophyllene, DMNT and Decanal, key compounds known to attract *C. sesamiae* and repel *C. partellus* in the push-pull companion cropping system (Khan *et al*., 1997a; Goninguene *et al*., 2001; Degen *et al*., 2004). Also, there was emission of volatile organic compounds by Cuba 91, a landrace variety from Latin America, following exposure to *B. brizantha* with *C. partellus* eggs. These included DMNT, Linalool and (*E*)-β-farnesene, key compounds also known to attract *C. sesamiae* and also repel *C. partellus* in the push-pull system. Furthermore, the study also revealed that Jowi, an African local maize variety, emitted a different blend of volatile organic compounds when exposed to *B. brizantha* with *C. partellus* eggs. The key compounds in the volatiles blend of Jowi comprised DMNT, TMTT, Methyl salicylate and Linalool (plus Nananal), compounds known to have similar effects as above.

The behavioural responses observed in *Cotessia sesamia and Chilo partellus* could be attributed to these chemical compounds. The attraction of larval parasitoids was particularly very interesting as it means that natural enemies can attack the newly hatched *C. partellus* larvae, preventing them from causing damage to the maize crop. Again repulsion of gravid female means the maize crop will be protected by warding off these females from further laying their eggs on the crop. Previous reports have indicated possible

loss of direct defenses (Sotelo, 1997) and below-ground indirect defences (Rasmann *et al.*, 2005; Kollner *et al.*, 2008) during breeding and domestication processes. This is the first demonstration of an above-ground indirect defence characteristic elicited by insect eggs that is present in local maize varieties but absent in commercial hybrid maize varieties.

The results reported in the current study are of significance for the management of *C. partellus*. When *B. brizantha* is oviposited on by *C. partellus*, it emits volatiles not only from the damaged tissues but in the whole plant system, including undamaged parts (Bruce *et al.*, 2010). Among the chemicals produced by herbivore-infested *B. brizantha* that have been reputed to induce maize to tailor its own volatile emission include (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT), Decanal, (*E*)-Caryophyllene, (*E*) -β-farnesene, Linalool, Nananal, Methyl Salicylate and (3*E*,7*E*)-4,8,12-trimethy-1,3,7,11-tri decattetraene (TMTT) (Ngi-Song *et al.*, 2000; Mathes *et al.*, 2003; Bruce *et al.*, 2010). These chemical compounds are long range attractant semiochemicals that provide information in the form of chemical cues, which aid the larval parasitoid *C. sesamiae* to its host, *C. partellus* as reputed by Ngi-Song *et al.* (1996). In addition, these same chemical compounds act as long range repellent semiochemicals that repel *C. partellus* away from the masked maize plant, leading to reduced crop damage as reputed by Tamiru *et al.* (2012).

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

i. The current study has identified the potential of inducing defence responses in the smallholder farmers' own maize varieties and Latin America landrace by use of *B. brizantha* with *C. partellus* oviposition.

ii. The results of the current study demostrated the attractiveness of *C. sesamiae* to volatiles from the maize varieties exposed to *B. brizantha* with *C. partellus* eggs.

iii. Similarly, unattractiveness of *C. partellus* to the local maize varieties and the landrace Cuba 91 exposed to *B. brizantha* with oviposition was demonstrated in the oviposition choice tests with *C. partellus* gravid female moths.

iv. The identified electrophysiological inducing compounds from *B. brizantha* to various maize varieties were (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene (DMNT), Decanal, (*E*)-Caryophyllene, (*E*)-β-farnesene, Linalool, Nananal, Methyl Salicylate and (3*E*, 7*E*)-4, 8, 12-trimethy-1, 3, 7,11-tri decattetraene (TMTT). The findings confirm the crucial role of plant volatiles in mediating responses of *C. partellus* to oviposition substrates.

Signal grass is "a clever" plant in that once the stemborer moth has laid its eggs on it, the grass stops producing attractive plant volatiles, leading to no more eggs laid. At the same

time, the grass releases the chemical compounds that recruit parasitoids which attack the larvae as they emerge from the eggs and thereby preventing the larvae from completing their life cycle. This makes this grass to qualify as an ideal HIPVs agent and trap plant candidate to be used in push-pull strategy of stemborer management by small scale farmers in Kenya and other countries. The findings for the current study would contribute to the development of management tactics that rely on the exploitation of plant to plant communication through semiochemicals to manipulate oviposition behaviour of the maize stemborers. Therefore, the use of stimulo-deterrent diversionally strategy that takes advantage of naturally-occurring semiochemicals in small scale maize farms in Kenya appears to be feasible in the near future.

6.2 Recommendations

6.2.1 Recommendations for Policy makers

i.   Policy makers should be sensitised to come up with policy to ensure the availabity of *B. brizantha* seeds at affordable price to farmers.

ii.   There should be capacity buiding by the ministry of agriculture and sensitisation of all stake holders on the benefits of using *B. brizantha* in Push-Pull technology.

iii.   The Government and other stake holders should have budgetary allocation for research, development and dissemination of the Push-Pull technology using HIPVs of *B. brizantha*.

iv.   Farmers need to be adviced by agricultural extension officers on the importance of using *B. brizantha* in management of the stemborer *C. partellus*.

6.2.2 Recommendations for research institutions

i. Researchers should engage on more research to identify other potential plants which can be employed to induce maize and other agricultural crops to release semiochemicals repellent to insect pests and attractive to natural enemies of the pests.

ii. Plant breeders should come up with maize varieties which naturally emit HIPVs to ward off pests and recruit parasitoids to control the pest.

iii. The research institutions should come up with demonstration fields for farmers to educate them on how to grow *B. brizantha* at the border of maize plots and how the technology works.

6.2.3 Recommendations for Farmers

Resource poor farmers should use the findings of this research in their small scale farms to manage stemborers. This would be through sensitization by the Agricultural extension officers and visiting *icipe* research station to see for theselves.

6.3 Suggestions for futher research

The following areas are proposed for further research

i. Further research should be done using egg parasitoids to confirm whether the above result obtained using larval parasitoids can be achieved again to ensure maximum employment of natural enemies for maize protection.

ii.   There is a need to evaluate the effectiveness of the results obtained in the above study under natural field conditions.

iii.   There is a need to evaluate the possible impact of the obtained result of the study on smallholder farmers' livelihoods.

iv.   There is need to identify the key compounds in the volatile blend emitted by *B. brizantha* following oviposition that is responsible for inducing of defence in the local maize varieties and the landrace Cuba 91.

v.   Further research is needed to identify other potential plants which can be employed to induce maize and other field crops to release semiochemicals repellent to insect pests and/but attractive to natural enemies of these pests.

vi.   There is also need to identify the biologically active compounds released by these other potential plants, to mediate behavioural responses in the target insects. This would open up opportunities for preparation of synthetic formulations of volatile blends for use in management of *C. partellus* thus expanding appeal of this approach among farmers belonging to different socio-economic strata.

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