Biological Control of *Myzus persicae* and *Aphis gossypii* on Potato Crop (*Solanum tuberosum* L) in Kenya

Joseph Maina Machangi

A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in Zoology (Agricultural Entomology) in the Jomo Kenyatta University of Agriculture and Technology

2013

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature ..... Date 29.8.2013

Joseph Maina Machangi

This thesis has been submitted with our approval as University supervisors

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DEDICATION

I dedicate this thesis to my wife, Anne Muthoni Maina, to our children, daughter Elizabeth Wanjeri Maina and Son, Anthony Irungu Maina for their moral and material support throughout my studies.

To my entire family and friends for their prayers and encouragement in different ways.

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LIST OF ACRONYMS AND ABBREVIATIONS

AEZs	Agro-ecological zones
ANOVA	Analysis of variance
APU	Arthropod Pathology Unit
CIP	International Potato Centre
CPC	Crop Protection Compendium
CRD	Completely randomized design
EPPO	European Plant Protection Organisation
g/l	Grams per litre
GHWF	The green-house whitefly
GPS	Geographical Positioning System
icipe	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
Ksh	Kenya Shillings
LC50	Lethal Concentration to 50% Mortality
LH	Lower highland
LM	Lower Midland
m.a.s.l	Metres above sea level
NPRC	National Potato Research Centre
^{o}C	Degrees Centigrade
PCR	Polymerase Chain Reaction

PLRV	Potato leaf roll virus
PVS	Potato virus S
PVX	Potato virus X
PVY	Potato virus Y
RCBD	Randomized complete block design
RH	Relative humidity
SAS	Statistical analysis system
SDA	Sabouraud (4%) Dextrose Agar
SPSS	Statistical Package for Social Scientists
t/ha	Tonnes per hectare
UH	Upper Highland
UK	United Kingdom
UM	Upper Midland
USA	United States of America

ABSTRACT

Potato (*Solanum tuberosum* L) is the second most important food crop in Kenya after maize. However the main problem facing potato production in Kenya is low yields due to diseases and insect pests. Among the insects, aphids are considered the most important because they are vectors of potato viruses. Thus, their control is crucial, especially in seed potato production. Aphids have developed resistance to a range of synthetic chemical insecticides. Search for alternative control measures is therefore necessary. This study was therefore done to investigate the possible use of biological control agents for the control of *M. persicae* and *A. gossypii* aphid species on potatoes in Kenya.

The study was done in four main stages. The first stage was a field survey in potato farms to collect aphids and all associated insects in the potato farms in four major potato growing counties in Kenya. This was followed by the identification of these insects in the laboratory which were classified to aphid pest species and the associated predators and parasitoids. Pathogens associated with two main aphid species *Myzus persicae* Sulzer and *Aphis gossypii* Glover, were isolated from cadavars of these aphids and also identified in the laboratory. The next main stage was to evaluate the most prevalent natural enemies in each category (i.e predators, parasitoids and the fungal pathogens) on their effectiveness in causing mortality against the two target aphid species, *M. persicae* and *A. gossypii*. This evaluation was in three stages first in the laboratory, then in the

greenhouse and finally in the open field. The study in the laboratory involved screening the major natural enemies to find if they were effective in close contact with the aphid in Petri dishes. Two predators, one parasitoid and one fungal pathogen isolate were then evaluated on aphids on the potato crop in the green house in cages. The best performing predator and the parasitoid and pathogen evaluated in the green house were then evaluated in the open field on potato crop under natural conditions. An evaluation of the common pesticides used to control aphids on the potato crop locally was also done at all stages from the laboratory, green house and in the open field to compare the performance of the natural enemies with that of the currently used chemicals for the control of aphids on the potato crop. A control treatment where no aphid control measure was applied was also included in the experiment at each level. The final experiment was an evaluation of the compatibility of the commonly used pesticides in the control of aphids on the potato crop with the above natural enemies to find out if they can be used together in an integrated pest management of the two aphid species.

The results of the field survey showed the presence of four aphid species, sixteen predator species, three parasitoid species and four fungal entomopathogenic species. The most prevalent natural enemies in each category that were evaluated for their effectiveness in the control of *M. persicae* and *A. gossypii* were *Hippodamnia convergens* and *Harmonia axyridis* for predators, *Aphidius colomani* for parasitoids and *Metarhizium anisopliae* icipe 62 isolate for the fungal entomopathogens. All these proved to be very effective in the control of the two aphid species at all stages evaluated

from the laboratory, the green house and the open field. It is therefore recommended that these natural enemies be mass produced and evaluated further under on-farm field trials with the aim of commercialising them for use in the control of the two aphid species on potato farms in Kenya. Evaluation of the compatibility of the current common pesticides used for the control of aphids on potato farms showed that they negatively affected all these natural enemies. Hence where the above potential bio control agents are to be used, none of these chemical pesticides should be used.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Potato (*Solanum tuberosum* L) is the second most important food crop in Kenya after maize (CIP, 1996; NPRC, 2011). The potato crop in Kenya earns farmers a total of 5.5 billion Kenya Shillings annually (NPRC, 2011). The main varieties grown in Kenya are Tigoni, Asante, Nyayo, Kerr's pink, Ngure, Arika, Desiree, Roslin Tana, Dutch Robjin (all National Potato Research Centre – NPRC- varieties), Komesha, Meru Mugaruro, Black blue and Tana Kimendu (local varieties at the farms). New varieties by NPRC recently released include Kenya Karibu, Kenya Sifa, Kenya Mavuno and Kenya Faulu released in 2007 while the latest varieties released in 2010 are Kenya mpya, Kenya sherehekea and Purple gold (NPRC, 2011).

Four species of aphids, *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* Kaltenbach, have been reported in potato fields in Kenya (Nderitu and Mueke, 1986; Machangi *et al.*, 2003) of which, *M. persicae* and *A. gossypii* are the most common and have been reported to transmit potato viruses (Kennedy *et al.*, 1962; Ebert and Cartwright, 1997; Machangi, 2003; CPC, 2006). Four potato viruses, Potato virus S (PVS), Potato leaf roll virus (PLRV), Potato virus Y (PVY) and Potato virus X (PVX) have been reported in the potato fields in Kenya of which Potato virus Y and Potato leaf roll virus both of which are transmitted by the two

aphids, were among the most prevalent. A significant positive correlation between population of the two aphid species *M. persicae* and *A. gossypii* and the virus incidence of the two viruses PLRV and PVY has been established (Machangi, 2003).

Resistance of aphids to chemicals insecticides has been a problem associated with more than 40 Chemical insecticides used to control these aphids world-wide (Gao *et al.*, 1992; Hockland *et al.,* 1992; Herron and Rophail, 1994, CPC, 2006). In a few cases, chemical treatments have resulted in more damage than would have occurred without the treatment (CPC, 2006, Machangi, 2013). They may also result in pest resurgence due to a reduction in natural enemies among other factors. For example, cotton fields treated with sulprophos had elevated numbers of *A. gossypii* (Kerns and Gaylor, 1993) while Potato plants treated with Lambda cyhalothrin and Dimethoate in the field had higher numbers of *M. persicae* and *A. gossypii* than untreated plants (Machangi, 2013). Cotton plants treated with disulfoton, phorate, dimethoate and lindane had bigger aphids, (Sithanantham *et al.,* 1973) while Okra treated with phorate had more aphids than the untreated Okra (Regupathy and Jayaraj, 1994).

1.1.1 Biological Control Agents

Natural enemies used in biological control of insect pests basically fall in two groups (Loomans, 2003). These are; (i) The Macrobials: - Which include Predators (Riudavets, 1995; Sabelis and van Rijn, 1997) and Parasitoids (Loomans *et al.*, 1997) and (ii) The

Microbials: - Which include the entomopathogens mainly the entomopathogenic fungi (Butt and Brownbridge, 1997) and the entomopathogenic nematodes (Loomans *et al.*, 1997).

Biological control of insect pests on crops has been used successful in other parts of the world. For instance, studies carried in Iran showed that the cost of a biological control programme in cotton was three times cheaper than the cost of chemical treatments (Heydari *et al.*, 1997). Records are also available of successful biological control being practised in the field. Successful control of *A. gossypii* using biological control has been achieved in Egypt by releases of predators *Chrysoperla carnea* at a ratio of 1:5 (predator: aphid) and *Coccinella undecimpunctata* at a ratio of 1:50 in okra crop (Zaki *et al.,* 1999). Many attempts have also been made with biological control in Kenya particularly by *icipe* though not on aphids. These include biocontrol by predators (Vasconcelos *et al.*, 2008; Huseynov *et al.*, 2008; El Banhawy and Knapp 2009; Jackson *et al.*, 2010; Omondi *et al.*, 2011; Jackson and Nelson, 2012), parasitoids (Billah *et al.*, 2008; Bruce *et al.*, 2009; Gitau *et al.*, 2010; Amanuel, *et al,* 2011; Obonyo *et al.,* 2011 ; Musundire *et al.,* 2012) and fungal pathogens (Wekesa *et al.*, 2008; Bugeme *et al.*, 2008 ; Mburu *et al.*, 2009; Nchu *et al.*, 2010; Migiro *et al.*, 2011; Nana *et al.*, 2012; Niassy *et al.*, 2012; Maniania *et al.*, 2012).

1.2 LITERATURE REVIEW

1.2.1.1 Aphids

1.2.1.2 *Aphis gossypii*

Aphis gossypii adults range from 1-1.5 mm in body length. The minimum diameter is just over 0.34 mm (Bethke and Paine, 1991). *Aphis gossypii* can range in colour from yellow to very dark (almost black) green. The smaller yellow form occurs during warmer conditions. The green form is larger and occurs during cooler temperatures and uncrowded conditions. *Aphis gossypii* reproduction is mostly asexual with either alate or apterous females. In warmer environments, *A. gossypii* exhibits an anholocyclic life cycle (i.e. a simplified life cycle without a sexual phase), while in cooler areas it exhibits either a heteroecious (host-alternating) or autoecious (same host) holocyclic life cycle (i.e sexual reproduction during part of its life cycle) (Slosser *et al.*, 1989; Zhang and Zhong, 1990). *Aphis gossypii* takes 5.2 days to reach maturity on cotton at 28°C. The optimal temperature for reproduction is 20-25°C when the aphid can produce an average of 2.8 nymphs per day (Isely, 1946; Akey and Butler, 1989).

The host range of *A. gossypii* includes over 92 plant families among them food and fibre crops, ornamentals and flowers. Affected plant stages include flowering stage, fruiting stage and vegetative growing stage. Affected plant parts include growing points, inflorescence, leaves, stems and whole plant. *Aphis gossypii* settles on older mature leaves. It moves to younger tissues only when population pressure forces it to; thus aphid

populations are always greatest on the older leaves (Banerjee and Raychaudhuri, 1985; Ebert and Cartwright, 1997; Machangi *et al.*, 2003). However, *A. gossypii* will attack most parts of the plant if population density is high enough. It is extremely polyphagous and very damaging to many economically important crops, including cotton, citrus, coffee, melon, okra, peppers, potato, squash and sesame. It has a worldwide distribution and it is particularly abundant in the tropics.

The most important impact *A. gossypii* has on world agriculture is through its ability to transmit plant viruses (Kennedy *et al.*, 1962; Ebert and Cartwright, 1997). It transmits over 30 plant viruses, among them Potato leaf roll virus (PLRV) and Potato virus Y (PVY) (CPC, 2006). There is little quantitative information on exact crop losses due to these aphids (CPC, 2006). However, losses caused by the viruses transmitted such as the PLRV and the PVY in potatoes have been reported to result in up to 90% yield loss (Salazar, 1996). Despite the lack of quantitative data on exact yield reductions caused by *A. gossypii*, there are reports on the control action threshold. In Sudan on cotton, this level was 30% infestation of the plants during the first two months of the season (Stam *et al.*, 1994).

1.2.1.3 *Myzus persicae*

The species *M. persicae* was first described by Sulzer in 1776 as *Aphis persicae*. Its numerous synonyms are listed by Börner (1952) and Remaudière and Remaudiere (1997). Adults are oval-bodied, 1.2-2.1 mm in body length, of very variable colour;

whitish green, pale yellow-green, grey green, mid-green, dark green, pink or red. The tobacco form (nicotianae) varies even more and can also be bright yellow, or almost black. Apart from genetically determined colour variation, any one genotype will be more deeply pigmented green or magenta in cold conditions. Immature stages are quite shiny, but adults are less so. Winged morphs have a black central dorsal patch on the abdomen. Immatures of the winged females are often pink or red, and immature males are yellowish. *Myzus persicae* is heteroecious holocyclic (host alternating, with sexual reproduction during part of life cycle) between Prunus (usually peach) and warmer season host plants, but anholocyclic on secondary (warm season) hosts in many parts of the world where peach is absent, and where a mild climate permits active stages to survive throughout the cold season. It is usually anholocyclic in tropics and sub-tropics with some exceptions. For example, Ghosh and Verma (1990) reported apterous oviparous females of *M. persicae* for the first time on *Prunus persica* in India.

Myzus persicae feeds mostly on older senescing leaves, often along the leaf veins. Emden van *et al.,* (1969) described how a range of host-plant variables affected aphid development and fecundity. Plant nutrition is a factor in the induction of winged forms, along with temperature, but there is also a strong genetic component. In laboratory experiments, low temperature promoted, while high temperature tended to suppress, the development of winged forms. *Myzus persicae* is relatively cold resistant. Howling *et al.*, (1994) described mortality of aphids at various cold temperatures and their results suggested that an acclimatized population of *M. persicae* would persist without significant mortality after a period of 7-10 days with -5°C frosts each night. Between six

and eight generations developed on sugar-beet plants during the growing season in the Czech Republic, where as 10-25 generations a year were possible on potatoes in southwestern USA. Wingless parthenogenetic females produce 30-80 progeny each. Higher growth rates have been observed on virus-infested plants. Winged females alight fairly indiscriminately on warm season hosts, as expected for a polyphagous species, although they have a landing preference on yellow and yellow-green surfaces (CPC, 2006).

Myzus persicae is highly polyphagous. It is found in over 90 host plant species which are in over 40 different families including Brassicaceae, Solanaceae, Poaceae, Leguminosae, Cyperaceae, Convolvulaceae, Chenopodiaceae, Compositae, Cucurbitaceae and Umbelliferae (CPC, 2006). The hosts include many economically important plants. Affected plant stages are flowering stage, post-harvest, seedling stage and vegetative growing stage. Affected plant parts are growing points, inflorescence, leaves, stems and whole plant. Its habitat is in the open vegetation which includes crops and herbaceous plants in open situations and peach orchards. *Myzus persicae* is probably of Asian origin, like its primary host plant (*Prunus persica*), but its distribution now occurs everywhere in the world except where there are extremes of temperature or humidity (CPC, 2006).

The *M. persicae* aphid species is the most important aphid virus vector. It has been shown to transmit well over 100 plant virus diseases, in about 30 different families, including many major crops. *Myzus persicae* is a major pest everywhere potatoes are grown. It is the most important vector of potato leafroll virus (PLRV), which causes leaf

roll and tuber rot necrosis. Seed potatoes have low tolerance for PLRV and low aphid populations can be very damaging (CPC, 2006). Direct feeding damage can be of economic importance in some crops, an effect enhanced by toxic effects of aphid saliva (Emden van *et al.*, 1969). Honeydew production is less than for many other aphids because dense colonies are not formed, but it may be economically important in greenhouse plants due to growth of black sooty mould. On many crop plants such as potato, brassicas and sugarbeet, *M. persicae* only occurs at low densities, particularly on older leaves, but can still transmit viruses of these crops at low densities. It is therefore difficult to detect on the crop before the damage is done. Control of this aphid species is, therefore, very important even at very low populations.

1.2.2 Aphid Natural Enemies

Natural enemies of aphids can be divided into three groups: parasitoids (e.g parasitic wasps), predators (e.g. ladybeetles, lacewings and hoverflies) and pathogens (mainly parasitic fungi) (Steenis *et al.*, 1995). Few aphid natural enemies are host-specific; rather they are attracted to aphids in particular habitats. Thus, the important natural enemies attacking particular aphid pests on crops tend to vary according to the crop and the circumstances in which it is grown and the climate (CPC, 2006).

1.2.2.1 Parasitoids

Parasitoids develop in or on a host and cause it to die. Parasitoids lay eggs on or in a pest host, which the resulting larvae consume and ultimately kill. Most parasitoids are far

smaller than their host, and therefore mass rearing and release of parasitoids can be relatively convenient. Parasitoids are found only in the insect order Hymenoptera and the Tachinid family of flies (Diptera). Parasitoids usually exhibit high host specificity and many are entirely monophagous (IPM, 2007). Many aphid parasitoids are members of species complexes, morphologically very similar but with different host preferences and geographical distributions. Most aphid parasitoids belong to the hymenopterous families Aphelinidae and Braconidae (subfamily Aphidiinae). The most common genera are *Aphidius, Lysiphlebus* and *Trioxys* (Ferrari and Burgio, 1994). More than 30 species of parasitoids have recently been used for the control of *M. persicae* and/or *A. gossypii* of which the most commonly used was *Aphidius colemani* Viereck (Hymenoptera: Braconidae) followed by *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) (CPC, 2006).

A particular feature of aphid-parasitoid systems is the existence of a delay between parasitisation (sting) and the death of the host (i.e. mummification). This biological trait is generally not considered important for population stability, except if the delay is very long, and hence it is ignored in most population dynamics studies. However, many crops have relatively short durations, and these time delays may have important consequences and cannot be ignored in a dynamics model (Rochat, 1997). Different studies have been done with parasitoids for the control of the two aphid species *M. persicae* and *A. gossypii* as highlighted below.

***Aphis gossypii* Parasitoids:** The most common parasitoids of *A. gossypii* are insects in the Hymenoptera. Additional parasitoids include members of the Diptera and some mites. While these parasitoids are able to parasitize all life stages of *A. gossypii*, they prefer later instars (Ebert and Cartwright, 1997). More than 35 species of *A. gossypii* parasitoids have been reported which include *Allothrombium* sp. (about three), *Aphelinus* sp. (about six species); *Aphidius* sp. (about five species); *Ephedrus* sp. and *Lipolexis* sp. (three each); *Lysiphlebia* sp. and *Trioxys* sp. (six sp. each) and a few other minor species(CPC, 2006).

Changes in parasitism based on age structure of *A. gossypii* populations feeding on cotton were reported for the parasites *Trioxys* spp. and *Aphelinus* spp; these parasites rarely parasitized first- and second-instar aphids. Thus, the percentage of parasitism increased as the proportion of older aphids increased (Luo and Gan, 1986). This has survival value for both the parasite and *A. gossypii* because aphids which are parasitized as older nymphs or as adults have a chance to reproduce. Aphids parasitized by *A. colemani* had a fecundity of 0.5-1.3 nymphs/female when parasitized in the fourth-instar, and 10.5-13.3 when parasitized as adults (Steenis van and El-Khawass, 1995). Aphids which survived an attack had lower fecundity but equal longevity relative to aphids that were not attacked (Steenis van and El-Khawass, 1995).

***Myzus persicae* parasitoids:** *Myzus persicae* is attacked by over 45 species of primary parasitoids (CPC, 2006). Almost all of them are recorded to attack nymphs and adults of *M. persicae* but a few have been reported as attacking only nymphs of the aphid.

Although most also attack a range of other aphid species, some are host-specific, such as *Trioxys similis* n. sp. (Hymenoptera: Braconidae, Aphidiinae). *Trioxys angelicae* Haliday (Hymenoptera: Aphidiidae) is a parasitoid on Prunus only. *Myzus persicae* is the preferred host for *Aphidius matricariae* Haliday (Hymenoptera: Aphidiidae) and *Aphelinus semiflavus* Howard (Hymenoptera: Aphelinidae) in the USA. *Aphidius colemani* is also an important natural enemy in many parts of the World (CPC, 2006). The second and third aphid nymphal instars are usually preferred by ovipositing parasites (Hagvar and Hosfgang,1991), with older nymphs usually avoided as they result in small parasite adults emerging which leave few offspring, though *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) prefers third and fourth instars (Emden van *et al.*, 1969).

The parasitoid, *A. matricariae,* has been widely used as a biological control agent against *M. persicae* in greenhouses on aubergine in France, chrysanthemums in the UK, sweet pepper in the Netherlands and Russia, and tomatoes in Canada. The releases were often made in combination with the predatory aphid midge, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) (Granges and Leger, 1995). *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) has been successfully mass-reared and released in greenhouse control of *M. persicae* and other aphids in China (Xin, 1986). *Ephedrus cerasicola* Stary (Hymenoptera: Aphidiidae), *A. colemani* and *Aphelinus abdominalis* Dalman (Hymenoptera: Aphelinidae) have also been used in biological control against *M. persicae* (Hagvar and Hofsvang, 1991). Parasites often use aphid honeydew as a host-finding cue (Hagvar and Hofsvang, 1991).

There are no available records of the use of parasitoids for the control of the two aphid species in Kenya and East Africa.

1.2.2.2 Predators

Predators require feeding on a number of prey organisms during their lifetime and they are active organisms which seek their food. Normally they are superior in size and mobility compared with their prey. Predators can be monophagous or polyphagous. Major groups of predators include the insects in Orders Hemiptera, Neuroptera, Diptera, Coleoptera, and Hymenoptera, the Arachnida, and vertebrates such as snakes, birds, and fish (IPM, 2007). Predators used for the control of *M. persicae* and/or *A. gossypii* include more than 50 predator species of which the most commonly used is *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) followed by *A. aphidimyza*, *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae).

The 'problem' with many predators is that they may only lay their eggs in the presence of sufficient aphids to ensure the survival of the larvae to maturity, and by then the damage to the crop may have been done. Non-specific predators with good searching ability that are active early in the year, such as Carabidae and Staphylinidae, are important when aphid numbers are low (CPC, 2006). The use of coccinellids in the biological control of aphids is restricted to the release of larvae because adults tend to fly away. Non-flying adults may stay longer in one place and so they and their progeny could give longer term protection to plants (Ferran *et al.,* 1998). In Kenya, Seventy species of insects were

found on the potato crop of which, 52 of them were pests and 17 were predators of those pests (Nderitu, 1991). A literature search on use of natural enemies of aphids in East Africa also revealed only use of predators (Le Pelly, 1959).

***Aphis gossypii* predators**: The predators of *A. gossypii* include a number of species in the Coccinellidae, Syrphidae, Chrysopidae, Hemerobiidae, and many small spiders. Although reports suggest that the predators will attack all life stages, there is no record of predators attacking the egg (Ebert and Cartwright, 1997). Over 90 species of predators have been recorded attacking *A. gosspii* (CPC, 2006). All of these predators are reported only attacking nymphs and adult stages. They include *Scymnus* sp., *Harmonia* sp., *Chrysopa* sp., *Orius* sp., *Coccinella* sp., *Cheilomenes* sp.; *Chrysoperla* sp., *Cycloneda* sp., *Deraeocoris* sp., *Mallada* sp., *Oenopia* sp.; *Hippodamia* sp., *Ischiodon* sp., *Propylea* sp., *Sphaerophoria* sp. and many other single species the most important and common of which is *Aphidoletes aphidimyza* (CPC, 2006). The effectiveness of predators is highly variable, depending on the availability of alternative prey, host plant and environmental factors. Syrphid flies have shown potential in controlling aphid populations under greenhouse conditions (Babayan and Hovhannisian, 1984; Chambers, 1986; Adashkevich and Karelin, 1988). However, colonization by the syrphid was decreased on older plants, and older larvae would not transfer from young plants to more mature plants (Adashkevich and Karelin, 1988). The suggested cause for the latter effect was leaf pubescence (i.e hairiness or surface hair cover).

The occurrence of chrysopids on cotton in relation to *Helicoverpa armigera* Hübner (Lep., Noctuidae) and *A. gossypii* was studied in Tanzania between 1988 and 1991(Kabissa *et al.*, 1995). Only *Mallada desjardinsi* Navas (Neuroptera: Chrysopidae), and *Chrysoperla congrua* Walker (Neuroptera: Chrysopidae) occurred on cotton when both *H. armigera* and *A. gossypii* were present. Because of its longer larval period, and higher consumption of *A. gossypii*, *M. desjardinsi* was found to be better suited for use against *A. gossypii* than *C. congrua* (Kabissa *et al.*, 1995, 1996). Successful biological control of *A. gossypii* by the predatory syrphid *Syrphus corollae* Fabricius (Diptera, Syrphidae) [*Eupeodes corollae* Fabr.] has been reported in Sardinia for a period of over 20 years (Luciano, 1996). The predatory activity of larvae of *Paragus borbonicus* Macquart (Diptera: Syrphidae) was observed in the laboratory and in the field in Cameroon. This species preferred *A. gossypii* to *Rhopalosiphum maidis* Fitch (Ekukole, 1996).

***Myzus persicae* predators:** Over 85 predators have been reported attacking *M. persicae* (CPC, 2006). All of them were recorded attacking nymphs and adults. Adults and larvae of Coccinellids are important predators worldwide, particularly *Adonia* spp., *Coccinella* spp., *Hippodamia* spp. and *Scymnus* spp. *Coccinella septempunctata* and *Chilomenese sexmaculata* Fabricius (Coleoptera: Coccinellidae) are the most abundant predators in potatoes and other crops in India (Raj, 1989; Gupta and Yadava, 1989). Important syrphid larvae predators worldwide include *Episyrphus balteatus* De Geer (Diptera: Syrphidae), *Ischiodon scutellaris* Fabricius (Diptera: Syrphidae), *Metasyrphus corollae*

Fabricius (Diptera: Syrphidae) and *Scaeva pyrastri* Linnaeus (Diptera: Syrphidae). The aphid-eating gall midge, *A. Aphidimyza,* has been widely used as one of the biological control agents for the control of several species of aphids (Jeoung *et al.*, 2003). It has been released in greenhouses and plastic tunnels in China and in greenhouses in Canada.

1.2.2.3 Pathogens

Among the pathogens (bacteria, fungi, viruses, protozoa), fungi are the only pathogens that have been reported to attack aphids (CPC, 2006). They include *Neozygites fresenii* Nowakowski (Entomophthorales: Neozygitaceae), *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Zygomycetes: Entomophthorales) and *Lecanicillium lecanii* Zimmermann (Hypocreales: Incertae sedia). Mycosis among aphids is widely reported (Hatting and Wraight, 2007). Natural infection of aphids is most commonly caused by the Zygomycetes (Order: Entomophthorales) especially *Entomophthora, Pandora, Zoophthora, Conidiobolus* and *Neozygites* (Humber, 2005). On the other hand, entomopathogenic fungi (EPF) belonging to order Hypocreales such as *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Isaria* (= *Paecilomyces*) *fumosorosea, I. farinosus,* and *Lecanicillium lecanii* (Humber, 2005), have been receiving increasing attention as commercial biocontrol agents of insects. A number of formulations based on these fungi have been registered in many countries (Kabaluk and Gazdik, 2005). These EPF species occur widely in a variety of habitats from temperate to tropical climates and are ubiquitous in soils across the entire planet where conditions allow survival (Jaronski, 2007).

Although very few entomopathogenic hyphomycetous fungi seem to be specific to aphids, the genera *Lecanicillium, Beauveria, Metarhizium* and *Isaria* (species formally assigned to the genus *Paecilomyces*) are considered the most important (Humber, 2005). Development of entomopathogenic fungi as mycoinsecticides against aphids has been restricted to species of hyphomycetous fungi (Copping, 2001; Hatting and Wraight, 2007). A common objective in the early stages of many biopesticide development projects is the screening of pathogen isolates to identify those most promising for further development (Marcus and Eaves, 2000).

1.3 PROBLEM STATEMENT

The problem facing potato production in Kenya is low yields due to diseases such as early blight (caused by *Alternaria solani*), late blight (caused by *Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum* (= *Pseudomonas solanacearum*) and insect pests mainly aphids, white flies and potato tuber moth. The average yield under farmers' conditions where pests and diseases are prevalent is 10 t/ ha while it is 30 to 40 t/ha when pests and diseases are controlled under research conditions (Kinyae *et al.*, 1994). Among the insects, aphids are considered the most important because they are vectors of potato viruses; thus, their control is crucial, especially in potato seed production. Current control method is by use of chemical pesticides which has drawbacks due to aphids developing resistance to the chemical pesticides. Resistance of aphids to chemicals insecticides has been a problem associated with more than 40 Chemical insecticides used to control these aphids world-wide (Gao *et al.*, 1992; Hockland *et al.,* 1992; Herron and Rophail, 1994, CPC, 2006).

1.4 JUSTIFICATION FOR THE STUDY

The common control measure currently used against the aphid pests in Kenya is the use of synthetic chemical pesticides mainly Duduthrin (Lambda cyhalothrin 17.5 g/l – a non systemic synthetic pyrethroid) and Dimethoate (Dimethoate 40% ww emulsifiable concentrate – a systemic organophosphate). Although effective, this method is both expensive to farmers and also lead to environmental pollution, in addition to the aphids developing resistance to chemical pesticides. Aphids have already developed resistance to a range of synthetic chemical insecticides Worldwide. Control of these aphids by insecticides has, therefore, had only limited or transient success. With these negative attributes to chemical pesticide use, search for alternative control measures is, therefore, necessary. Biological control is both environmentally friendly and cheaper to the farmer if an appropriate natural enemy is identified. This study, was therefore, done to investigate the use of biological control agents for *M. persicae* and *A. gossypii* aphid species control on potatoes in Kenya.

1.5 HYPOTHESES

1 Predators, parasitoids and pathogens of *M. persicae* and *A. gossypii* are not present on potato fields in Kenya.

2 There are no effective predators, parasitoids and pathogens in Kenya for biological control of *M. persicae* and *A. gossypii*.

3 Pesticides (fungicides and insecticides) used in potato production have no negative effects on indigenous biological control agents of *M. persicae* and *A. gossypii*.

1.6 OBJECTIVES

1.6.1 General objective

To identify and evaluate biological control agents of the two major aphid species, *M. persicae* and *A. gossypii,* on potato crop in Kenya.

1.6.2 Specific Objectives

1. To identify predators, parasitoids and pathogens associated with *M. persicae* and *A. gossypii* in major potato growing areas of Kenya.
2. To evaluate the effectiveness of the most prevalent predators, parasitoids and pathogens for the control of *M. persicae* and *A. gossypii*.
3. To evaluate the effect of the main synthetic pesticides (fungicides and insecticides) used in potato production in Kenya on indigenous biological control agents on the crop.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Survey of Aphids and their natural enemies in the field

2.1.1 Study area

In 2008 and 2009, a field survey to determine the abundance and species composition of aphids and their primary and secondary parasitoids, predators and pathogens in potato crop was conducted in four major potato growing areas in Kenya (Fig.2.1), in the counties of Kiambu (Latitude S01°12.906' to S00°59.370', Longitude E036°45.055' to E036°37.188' and Altitude 1795m to 2417.5m a.s.l), Nyandarua (S00°52.178' to S00°02.311', E036°39.036' to E036°15.223' and Elevation of 2393m to 2770.5m a.s.l), Meru (S00°02.979' to N00°08.006', E037°35.569' to E037°17.059' and 1931m to 2490m a.s.l) and Nakuru (S00°20.670' to S00°16.434', E035°46.865' to E035°39.875' and 2496m to 2771m a.s.l). The areas surveyed in Kiambu County were Kiambu west district (Limuru, Lari and Kikuyu divisions) and Kiambu east district (Githunguri, Gatamaiyo and Kiambaa divisions). In Nyandarua County, the survey was done in Nyandarua south district (South Kinangop, Nyakio, Njabini, North Kinangop and Kipipiri divisions) and in Nyandarua North district (Ol-Kalou and Ol-Joro-Orok divisions) while in Meru County the areas surveyed were Meru Central district (Kibirichia and Abothuguchi west divisions), Imenti South district (Nkueni division) and Imenti North district (Timau

division). In Nakuru County, the survey was done in Molo district (Molo, Kamara, Mona, Keringet and Elburgon divisions). These are the major potato growing areas in Kenya with an average annual rainfall of between 1200mm to 2200mm and mean temperatures of between 10°C (mean minimum) and 25°C (mean maximum). Soils in these areas are of moderate to high fertility, well drained, moderately deep to very deep, dark redish brown to dark brown clay loam with humic top soil (humic andosols). These areas also represent the different Agro-ecological zones (AEZs) supporting potato growing in Kenya. These are Lower Midland (LM), Upper Midland (UM), Lower highland (LH) and Upper Highland (UH).

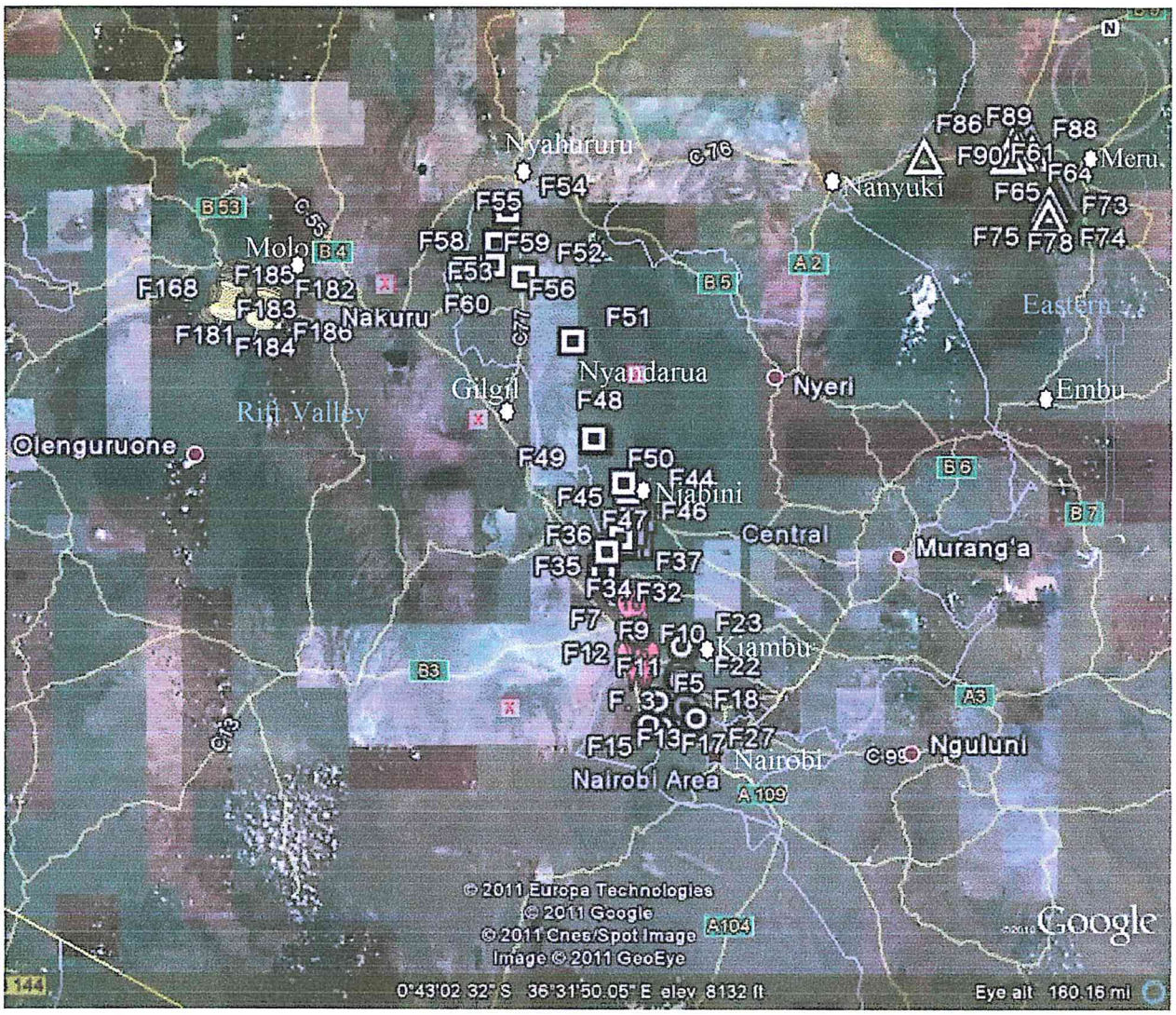

Fig. 2.1 Map of the major potato growing areas in Kenya surveyed – Kiambu, Nyandarua, Meru and Nakuru Counties. (Source: Google maps)

Key

- ● - Farms Surveyed in Kiambu County – F1 to F30
- ■ - Farms Surveyed in Nyandarua County – F31 to F60
- △ - Farms Surveyed in Meru County – F61 to F90
- ▯ - Farms Surveyed in Nakuru County – F162 to 191

Each of these areas has more than one potato growing AEZ and during the survey, sampling was done across the different AEZs represented in each area. The survey was done in both the long rains (April – July) and the short rains (November – January) seasons. Respondents/farmers were selected at random through stratified systematic sampling in each survey area with 30 per locality making a total of 120 farmers in the four survey areas.

2.1.2 Experimental Design and Description of Sampling

The survey design was the descriptive/monitoring survey with sampling. A total of 120 farms from throughout the four major potato growing areas in Kenya were sampled for aphids and their associated natural enemies. The survey was conducted in the months of January to February, 2008 for the short rains crop in Kiambu, Nyandarua and Meru Counties. A second survey was done in the month of June, 2008 for the long rains crop in all the four target areas, Kiambu, Nyandarua, Meru and Nakuru counties. Another survey was done in the month of January, 2009 in Nakuru County alone to cover the short rains crop in the area which had been missed in the first survey in January-February, 2008 due to post election violence in the area. So, at the end the field survey, each of the four target areas had been surveyed twice, i.e. once for the short rains season crop and once for the long rains season crop.

In each of the four areas, 30 potato farms were sampled with sample size chosen through expedience. The average size of the farms was 6.34 acres (2.57 ha). The farms in each

region were randomly selected through stratified systematic random sampling maintaining a distance of at least 500 metres between the farms sampled and only picking farms with potato crop at flowering stage. At each farm, the survey started with the collection of basic data about the farm using a survey questionnaire (Appendix 1 and Plate 2.1).

In each farm, 30 plants were then randomly selected through systematic random sampling in a quarter acre of the potato farm and sampled for insects. The sampling was done by beating the standing plant on a white cloth placed underneath the potato plant (Plate 2.2) so that insects that fall from the plant are collected on the cloth. The insects collected here were then transferred to a plastic container of ½ litre capacity (12 cm diameter top by 15 cm high) which was then closed with an aerated lid. In each container, a few potato leaves were enclosed as food for the insects being the host plant from which they had been collected. The container was then put in a cool box until the samples were later taken to a cold room at the International Centre of Insect Physiology and Ecology (*icipe*) at Duduville, Nairobi where they were preserved awaiting identification of the insect species collected. Apart from the 30 standing plants sampled by the beating method, another 10 plants were randomly selected in the ¼ acre potato field and from here, destructive sampling was done by cutting the whole potato stem with its branches and leaves at the ground and putting it in a plastic paper bag to sort out the insects on the plant later. These were also put in a cool box for extraction of insects on the plants which were actively searched later in the day, removed with a camel brush and put in the plastic container for that farm with the insects from the 30 plants collected

by the beating method. This was to capture insect species which are more stuck on the plant and might not have been captured by the beating of the plant. Insects collected from different farms were put in separate containers.

Farms sampled had potato plants at the flowering stage above 50% as this is the stage with highest number of insects (Machangi *et al*, 2003) and hence the highest chance of getting all types of insects found in the survey area. The plants sampled were also one to two month-old because it is the age at which the plant is not too young or too old but most actively growing and attracting maximum number of insects (Machangi, 2003). Samplings were done between 8am to 5pm during the day. All insect species at all stages were collected using a camel brush. They were then brought to the laboratory for identification. The insects collected were put in a cool box during transportation to the laboratory. Live predators and parasitoids of *M. persicae* and *A. gossypii* as well as the aphids were maintained live in rearing units at *icipe*. The live and dead aphids, predators, parasitoids and other insects of each species identified were counted for determination of the species diversity, abundance and distribution in these major potato growing regions.

Plate 2.1: Administering a questionnaire to a farmer before sampling the potato plants during the field survey

Plate 2.2: Sampling by beating a potato plant on a white cloth underneath to trap the insects from the plant

2.1.3 Specimen identification

2.1.3.1 Identification of insects

Identification was done using morphological features with a dissecting stereo microscope. After the completion of each survey, the insects collected were taken to the *icipe* laboratories where they were examined under a dissecting stereo microscope for

identification. Identification of specimens was based on taxonomic keys of Prinsloo (1997) and those of Clarke and Erik (1985). The insect samples (Aphids, predators, parasitoids and other insects) were also compared with specimens from the icipe's insect taxonomy and biosystematics unit laboratories. The different species identified were preserved separately in a drop of glycerol placed on a glass slide and clearly labelled. Their prevalence in the various areas was recorded.

2.1.3.2 Identification of fungal pathogens

Sampling for fungal pathogens was done by allowing the aphid cadavars to stay on a moistened filter paper placed in a sterile Petri dish so that any fungus in the aphids could grow. The fungi were then grown in Sabouraud (4%) Dextrose Agar (SDA) artificial media where the different isolates which grew were picked and sub cultured several times in the same media until pure isolates were obtained. Identification of fungal pathogens was done at the icipe's Arthropod Pathology Unit's (APU) Laboratories. Aphids with signs of external fungal growth were examined under a dissecting stereomicroscope. Fungal structures were mounted in lactophenol–aceto-orcein (LPAO) 1:1 or stained with 1% aceto-orcein plus glycerine for semi-permanent mounts, and examined with a phase contrast microscope. Measurements of fungal structures from fresh infected cadavars were made to enable specific identification. Fungal species were identified according to taxonomic keys and monographs of Keller (1987, 1991), Balazy (1993) and Humber (1989).

2.1.4 Data Analysis

Data was recorded for the species collected from the different areas covered in the four major potato growing areas. The insect species identified were classified to the different groups, viz. predators, parasitoids, aphid species and other insects. The fungi were also categorized to different species to identify aphid entomopathogenic species. The data collected was subjected to analysis of variance (ANOVA) using SPSS version 12.0.1.308 and the statistical analysis system (SAS) version 9.2 (SAS institute Inc., 2007) computer programmes.

2.2 Evaluation of predators, parasitoids and pathogens for the control of *Myzus persicae* and *Aphis gossypii*

Experiments were carried out in the laboratory, greenhouse as well as in the open field to evaluate the efficacy of predators, parasitoids and pathogens in the control of *M. persicae* and *A. gossypii*. Treatments of aphids with predators, parasitoids or pathogens in the laboratory and the green house were set up in Completely Randomized Design (CRD) with four or five replicates while they were set up in a Randomized Complete Block Design (RCBD) in the open field with four replicates. The laboratory and green house experiments were carried out at *icipe*, Duduville campus, Kasarani while the field experiments were carried out at the University of Nairobi's faculty of Agriculture, Kabete campus field station in collaboration with the CIP, Nairobi.

2.2.1 Source of Aphids

Adult *M. persicae* and *A. gossypii* aphids for the laboratory and green house experiments were obtained from a colony maintained in the green house (Plate 2.3) at ICIPE, Nairobi, Kenya positioned at 1°13'14.92''S and 36°53'44.37''E at an elevation of 5271 ft (1607 m) a.s.l. The initial stock culture was obtained in 2008 from infested potato plants in farmers' fields during a field survey in Kiambu, Nyandarua, Nakuru and Meru Counties in Kenya. The aphids were reared on Tigoni variety potato plants in ventilated cages, 100 x 50 x 50 cm (Plate. 2.4) placed on benches in the green house at *icipe* and maintained at ambient temperature (23-30 °C) and relative humidity 40-70% (r.h.) under a photo period of 12L: 12D. The two aphid species *M. persicae* and *A. gossypii* were reared in separate cages. Fresh young and clean potato plants were regularly supplied to the aphids.

Plate 2.3: One of the green houses used in evaluation experiments of the different aphid control agents

Plate 2.4: Cages used in the green house for the evaluation of the different aphid control agents

2.2.2 Source and Management of Potato Plants

Potato plants used in the experiments consisted of Tigoni variety grown from clean certified seed obtained from NPRC headquarters at Tigoni in Kiambu County. The seed potato was planted in pots (20 cm diameter and 18cm high) half filled with potting soil

(a 4:2:1:1 mixture of loam soil, manure, sand and ballast pebbles) also obtained from the research station already mixed in the above proportion then steam sterilised. The plants (one per pot) were grown in the pots put inside the ventilated cages described above and placed on greenhouse benches (Plate 2.5). The pots were watered once a week till the plants emerged and then twice a week thereafter until the plant matured or dried up due to aphid feeding. Plants were infested with aphids when 15-20 cm tall. Aphids were periodically moved to new potato plants as old ones declined in vigour due to aphid infestation. A constant supply of fresh young potato plants was therefore maintained for this purpose.

Plate 2.5: A pot used in planting potatoes for rearing aphids used in the evaluation experiments of the different aphid control agents

2.2.3 Evaluation of the predators, parasitoids and pathogens for the control of the aphids in the laboratory

The first experiments to evaluate the efficacy of predators, parasitoids and pathogens against *M. persicae* (Plate 2.6) and *A. gossypii* (Plate 2.7) were carried out in the laboratory.

Plate 2.6:- Green peach aphid (*Myzus persicae*)

Plate 2.7:- Cotton aphid (*Aphis gossypii*)

2.2.3.1 Evaluation of *Harmonia axyridis* and *Hippodamnia convergens* Predators for their efficacy in the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

2.2.3.1.1 Source of Predators

Adults of *H. axyridis* (Plate 2.8) and *H. convergens* (Plate 2.9) which were the two most commonly encountered predator species in the survey, were collected in the field from potatoes in the survey areas, in the month of June, 2009 before experiments took place. These ladybird beetles were reared at 25 ± 1 °C, 75 ± 5% RH and a photoperiod of 12L:12D in a screened plastic cage (30 × 30 × 30 cm) covered by clear glass and mesh. The predators were provided with enough food of mixed diet of different developmental stages of aphids *A. gossypii* and *M. persicae* reared on potatoes (2.2.1 and 2.2.2 above), and a solution of honey diluted in water (30%) applied to cotton. The mixed diet was provided to avoid food adaptation (Rana *et al.*, 2002) and to supply a more widened group of nutrients to the predator. Individuals (4th instar larvae and adults) produced in this rearing system were used for experiments.

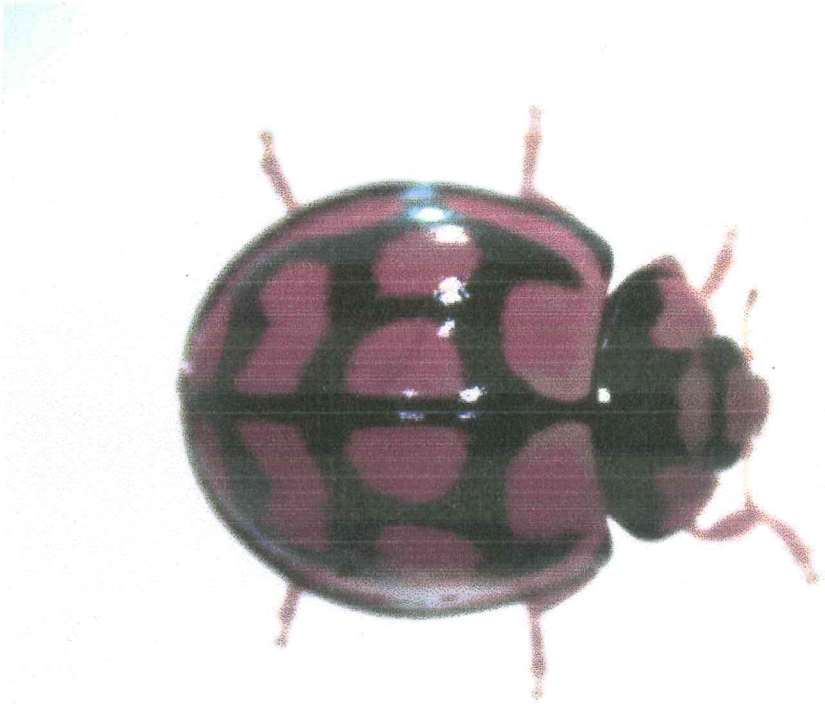

Plate 2.8:- Pumpkin lady beetle (*Harmonia axyridis*)

Plate 2.9: - Convergent lady beetle (*Hippodamnia convergens*)

2.2.3.1.2 Laboratory evaluation trials of the predators

Experiments were carried out in plastic Petri dishes (9 cm diameter, 1.5 cm height) with a mesh-covered hole in their lid (3 cm diameter). A potato leaf was placed on the bottom of each dish. Ten aphids (*M. persicae* or *A. gossypii*) adults were transferred from the rearing potatoes in the wood framed cages and placed gently on each leaf using a camel brush and left undisturbed for 1 h to settle. Each aphid species was placed in its own Petri dish.

When the predator nymphs had developed to the fourth instar, they were caged on potato plants at 25 °C, with a relative humidity of 75 ± 5% and a photoperiod of 12L:12D and were deprived of prey for 24 h before the experiments were initiated. Then, one starved fourth instar nymph of the predator, *H. axyridis* was introduced into each dish with aphids. After 24 h the predator was removed from the dish and the numbers of totally consumed or unconsumed (sucked) aphids in each dish were recorded. The same was done with the other predator, *H. convergens.* Aphids (*M. persicae* or *A. gossypii*) were offered at increasing densities in order to evaluate the predation rate. In total 10, 20 and 30 aphids per Petri dish were offered for *M. persicae* while 10, 20, 30, 50 and 100 aphids were used for *A. gossyppii* since its population in the field is usually much higher than that of *M. persicae*. This set up was replicated 5 times.

2.2.3.1.3 Voracity of the predators

The comprehension of the interactions between predator and prey (such as voracity rates) allows us to evaluate the predator's potential as a biological control agent (ElHag and Zaitoon, 1996). Each individual of the 4th instar, *H. axyridis* or *H. convergens* ladybird beetle was provided with one of the following prey densities: 10, 20, 30, 50 and 100 adult aphids of *M. persicae* or *A. gossypii* in a CRD set up. After 24 h the number of surviving prey was recorded in each treatment. All treatments were performed at 25 ± 1 °C, 75 ± 5% RH and a photoperiod of 12L:12D. In order to evaluate the ratio of natural mortality of prey, control treatments were performed with the above-mentioned prey densities, but in the absence of predators. Abiotic conditions were the same as previously mentioned. Five replicates for each treatment were carried out. Voracity (V_o) was determined according to the following equation (Soares *et al.*, 2003): $V_o=(A-a_{24})ra_{24}$ where V_o is the number of aphids eaten, A is the number of aphids available, a_{24} is the number of aphids alive after 24 h and ra_{24} is the ratio of aphids alive after 24 h in the control treatment.

2.2.3.1.4 Functional response of the predators

Functional response refers to the change in number of prey consumed by a predator per unit time in relation to prey density (McCaffrey and Horsburgh, 1986, b). Functional response studies are central to the successful use of natural enemies in an augmentative approach to suppress pest populations (Wiedenmann and Smith, 1997, Gitonga *et al.*, 2002). Voracity data is fitted to the modified Holling disk equation. In this experiment

Voracity data were fit to the ''random-predator" equation according to Rogers (1972), a modification of Holling's (1959) disk equation, that is regarded as more appropriate because it considers prey density to be affected by prey consumption (Hazzard and Ferro, 1991): $N_a/TP=\alpha N/(1+\alpha T_h N)$ where N_a is the number of prey attacked, T is the total time of prey exposure, P is the number of predators, N is the initial prey density, α is the attack rate (or searching efficiency) and T_h is the handling time (i.e., the time spent handling each prey attacked). In this experiment, $T = P = 1$, because prey were exposed to one predator for one day. The parameters α and T_h were estimated using the number of aphids consumed as dependent variable.

2.2.3.1.5 Statistical data analysis

One-factor ANOVA was used to compare the voracity of *H. axyridis* and *H. convergens* under different prey densities. When ANOVA showed significant differences ($P < 0.05$) among data sets, paired comparisons of each mean were made using Fisher's protected LSD tests (Zar, 1996). All analyses were performed using SPSS v. 12.0.1 for Windows (SPSS, Inc., 2004). Functional response model parameters were calculated and the curve was plotted for untransformed data, using the nonlinear regression module of SPSS v. 12.0.1 for Windows (SPSS, Inc., 2004). Significance of the regression model was evaluated by ANOVA and the variance explained by the model was expressed by the coefficient of determination.

2.2.3.2 Evaluation of *Aphidius colemani* Parasitoid in the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

2.2.3.2.1 Source of the *Aphidius colemani* parasitoid

Aphidius colemani parasitoid was used in this experiment as it was the only parasitoid that emerged from the aphid mummies of both *M. persicae* and *A. gossypii* collected from the field during the field survey. The parasitoids were collected from the Tigoni National Potato Research Centre (NPRC) potato crop from where a lot of mummified aphids were harvested, Marimba NPRC sub centre in Meru, and from another farm in Meru and in Nyahururu. These were brought to the laboratory at *icipe* later and put in Petri dishes from where the parasitoids emerged. The aphid mummies were first identified to see from which aphid species the parasitoids were developing. Mummies of *M. persicae* (Plate 2.10) were put together and those of *A. gossypii* (Plate 2.11) also put together in separate Petri dishes. They were each observed for emergence of the parasitoids after the parasitoid larvae inside the aphid (Plate 12) matured to an adult and emerged by cutting a circular hole at the rear back side of the aphid mummy. These were then observed and identified under a stereo dissecting microscope. For both aphid species, the parasitoids that emerged were all *A. colemani* (Plate 2.13 a, b) species. These were introduced to aphids in the caged potted plants onto which was released 20 mixed-sex parasitoids per cage once at the start of the trials at the green house where the parasitoids were reared on both *M. persicae* and *A. gossypii* aphids for use in the laboratory and green house evaluation experiments for aphid control.

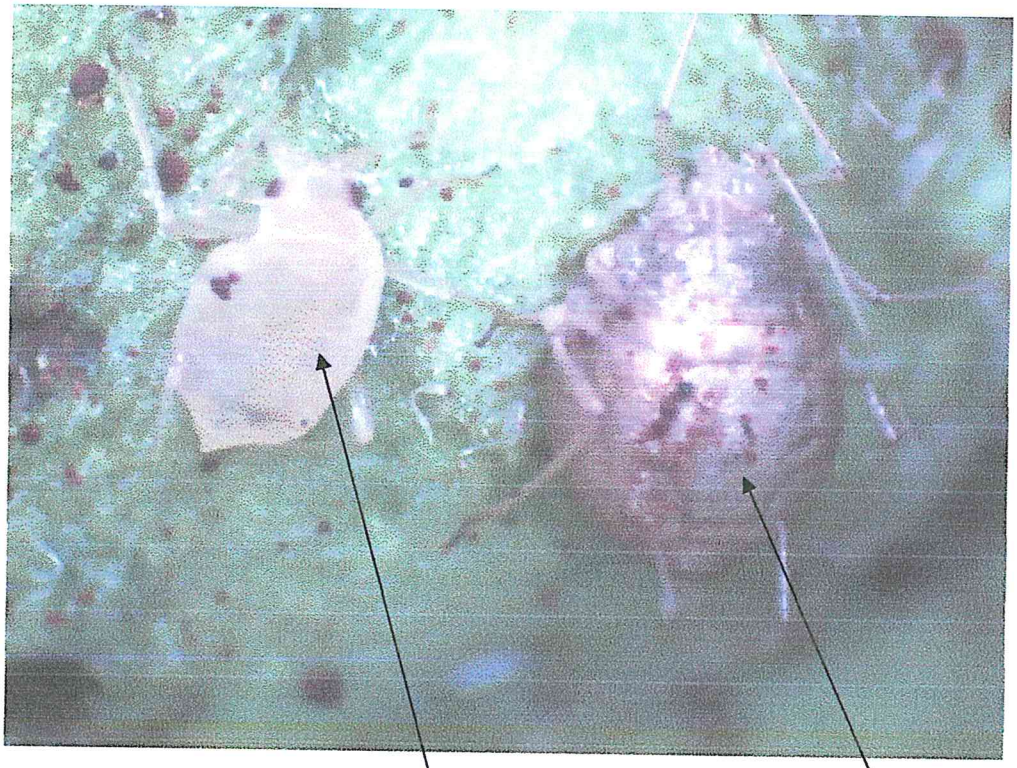

Plate 2.10: Normal size unparasitized *Myzus persicae* and silvery enlarged parasitized *Myzus persicae* mummy

Plate 2.11: Normal size unparasitized *Aphis gossypii* and 3 silvery enlarged parasitized *Aphis gossypii* mummies

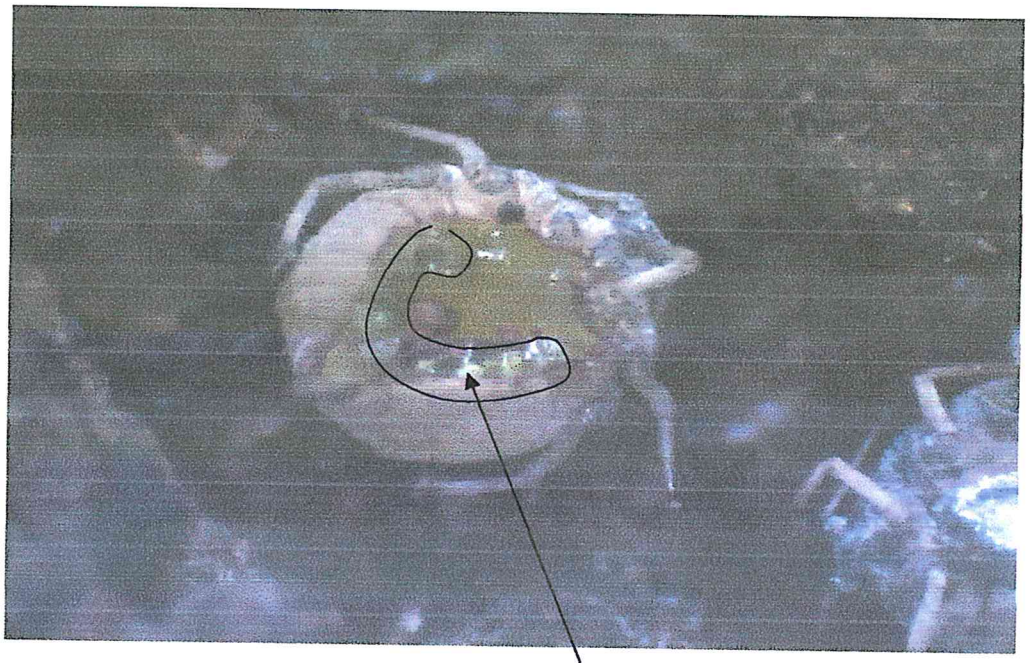

Plate 2.12: Lower side of a parasitized aphid mummy detached from the potato leaf to show the developing parasitoid larva inside

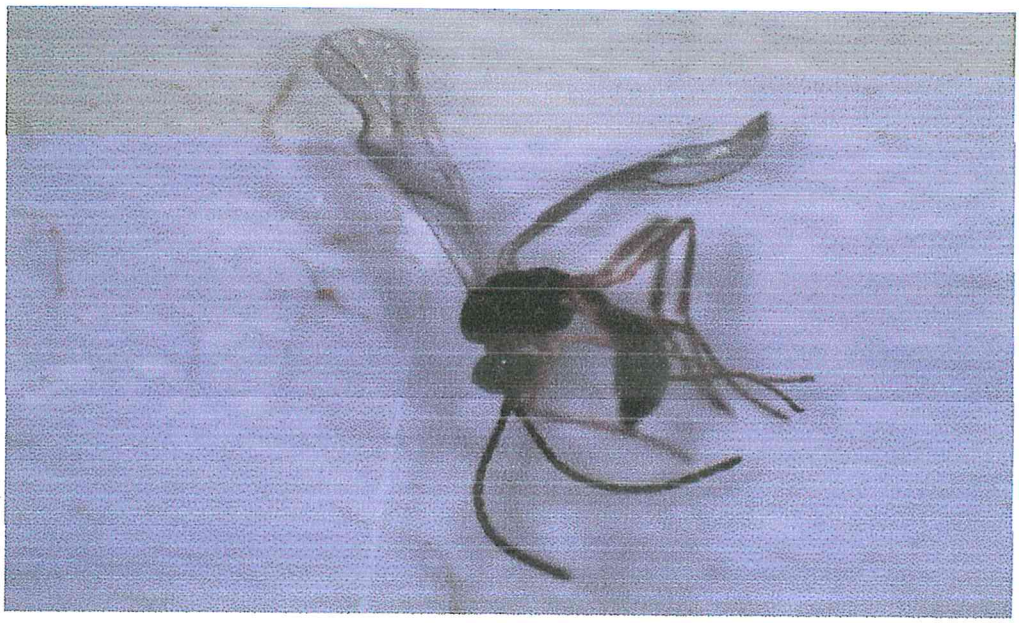

Plate 2.13a: Emerged adult female *Aphidius colemani* parasitoid

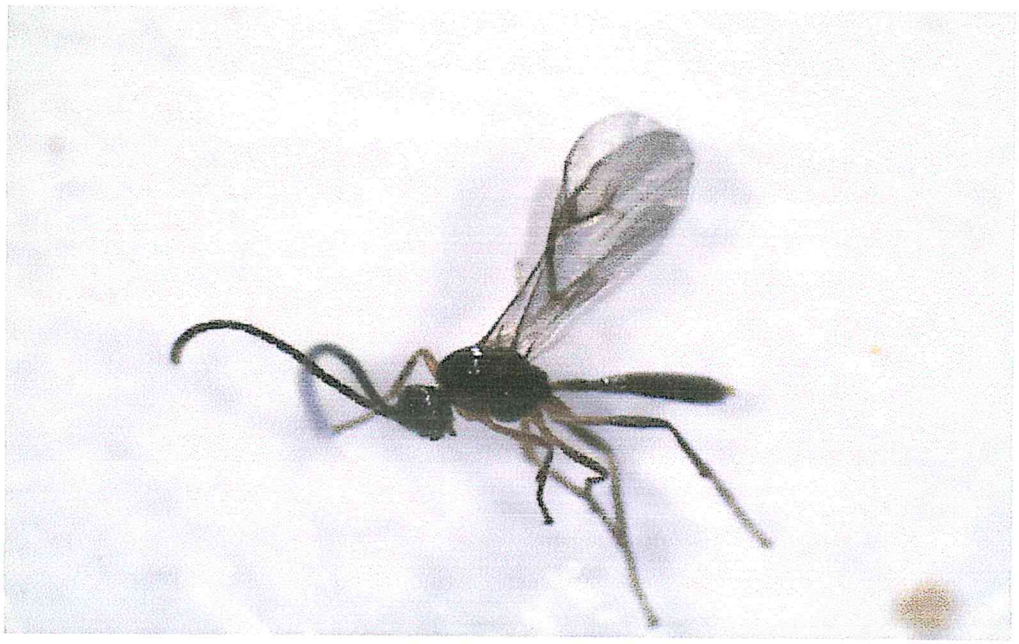

Plate 2.13b: Emerged male *Aphidius colemani*

2.2.3.2.2 Laboratory evaluation trials of the Parasitoids

Wasps reared from *M. persicae* and *A. gossypii* on potato plants in the green house were used for all tests. Adult *M. persicae* and *A. gossypii* were harvested from potato plants in the green house and transferred into a Petri dish (9 cm diameter). Ten apterous adult aphids of each species were used and put in separate Petri dishes and one adult female *A. colemani* was then introduced in each Petri dish. The aphids were exposed to one mated, 1–2 day old naïve adult female *A.colemani* for five days in the petri dish. Water and honey were provided for the wasps *ad libitum*. Daily checks were conducted on parasitoid survival and dead or missing parasitoids were replaced to maintain the presence of a parasitoid in each treatment till the end of the trial five day exposure period. The experiment was repeated in five Petri dishes for each species to make five replications. The aphids were observed daily for mortalities or any changes in their body form due to parasitisation by the parasitoid. After the 5 days, parasitized aphids (mummies) were collected and reared individually in vials until emergence of the adult parasitoid to determine the number of emerging wasps.

2.2.3.2.3 Statistical Data analysis

Analysis of variance (ANOVA) was used to analyse all data. Count data was log transformed and percentage data square-root transformed. Means were separated using the student-Newman-Keuls (SNK) test (SAS Software version 9.2 - SAS institute Inc., 2007).

2.2.3.3 Evaluation of entomopathogenic fungi in the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

2.2.3.3.1 Source of Aphids

Adult *M. persicae* and *A. gossypii* aphids were obtained from a colony maintained in the screenhouse at *icipe*, Nairobi, Kenya. The initial stock culture was obtained in 2008 from infested potato plants in farmers' fields during a field survey in Kiambu, Nyandarua, Molo and Meru counties in Kenya. The aphids were reared on potato plants (Tigoni variety) in ventilated cages (100 x 50 x 50 cm) placed in the screen house and maintained at ambient temperature (23-30 □C) and relative humidity 40-70% (r.h.) under a photo period of 12L: 12D. Fresh young and clean potato plants were regularly supplied to the aphids. Healthy 2 to 3 days old adult aphids were used in the experiments.

2.2.3.3.2 Source of Entomopathogenic Fungi

Fifteen (15) fungal isolates (5 *M. anisopliae,* 5 *B. bassiana*, 1 *Isaria* and 4 unidentified) were used in the present study (Table 2.1). With exception of the 4 unidentified isolates, all the isolates were obtained from the ICIPE's Athropod Germplasm Centre. Four isolates were isolated from aphid cadavers (Plate 2.14) collected during field surveys in 2008 and temporary identified as *Beauvera* sp. and *Lecanicillium* sp. The isolates were cultured on Sabouraud Dextrose Agar (SDA) medium (Oxoid, Basingstoke, Hampshire,

England) in 9-cm Petri dishes and incubated at 25 ± 2°C in complete darkness. Conidia were harvested from 2 to 3-week-old surface cultures by scrapping with a sterile spatula and suspending in 10 ml sterile distilled water containing 0.05% Triton X-100 (Fluka, Sigma-Aldrich, UK) and 3 mm glass beads in universal bottles. Conidial suspension was vortexed for 5 minutes to homogenize the suspension. Spore concentrations were determined using a haemocytometer (Hausser, Scientific Horsham, USA). Different spore concentrations were obtained through serial dilutions to obtain the desired concentrations. Viability of conidia was determined before each bioassay by spread-plating 0.1 ml of conidial suspension titrated at 3.0×10^6 conidia ml^{-1} on SDA plates. Sterile microscope cover slip was placed on each plate and plates were incubated at 25 ± 2°C and examined after 15-18 h. Percentage germination was determined from 100 spore counts at x 40 magnification. Each plate was replicated five times. Over 90% of conidia germinated in all tests.

Table 2.1: Fungal Isolates tested against *M. persicae* and *A. gossypii* adults and their origin

Fungal species	Isolate	Source	Locality, Country	Year of Isolation
Isolates from ICIPE's Germplasm Centre				
Metarhizium anisopliae	ICIPE 62	Soil	Kinshasa, DRC	1990
	ICIPE 30	*Busseola fusca*	Kendubay, Kenya	1989
	ICIPE 69	Soil	Matete, DRC	1990
	ICIPE 18	Soil	Mbita, Kenya	1989
	ICIPE 84	*Ornitacris turbida cavroisi*	Kaffraine, Senegal	2003
Beauveria bassiana	ICIPE 273	Soil	Mbita, Kenya	2006
	ICIPE 622	Soil	Kericho, Kenya	2008
	ICIPE 279	Coleopteran larvae	Kericho, Kenya	2005
	ICIPE 620	Soil	Kericho, Kenya	2008
	ICIPE 664	Soil	Bungoma, Kenya	2008
Isaria (*Paecilomyces*	ICIPE 682	Soil	Diani, Kenya	2008

fumosoroseus)				
Isolates from the Field Survey				
Beauveria bassiana	Aphid B.b-1	*Aphis gossypii*	Kiambu, Kenya	2008
	Dd-Aphid B.b	*Myzus persicae*	Molo, Kenya	2008
	Wd-Aphid Bb	*Aphis gossypii* (winged)	Meru, Kenya	2008
Lecanicillium sp.	Wd-Aphid-V.1	*Aphis gossypii* (Winged)	Nyandarua, Kenya	2008

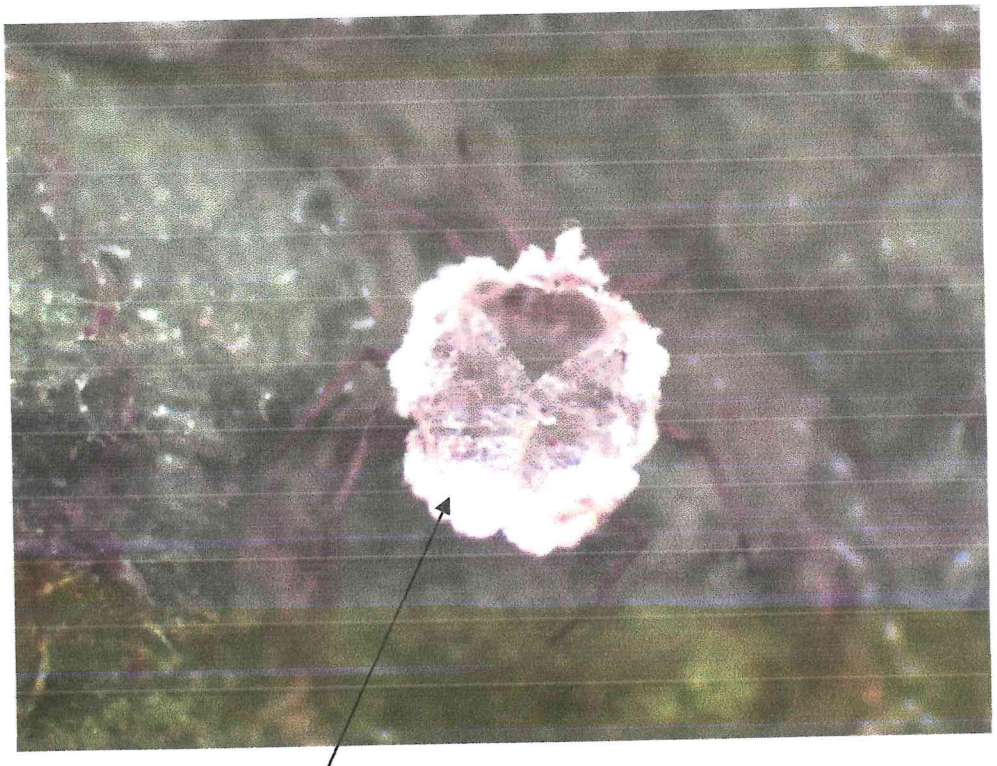

Plate 2.14: Fungal growth on a dead aphid collected from potatoes in the field

2.2.3.3.3 Treatments

Ten milliliters of standard concentration of $1x10^9$ conidia ml^{-1} in sterile distilled water containing 0.05% Triton X-100 were directly sprayed on aphids using Burgerjon's (1956) spray tower (INRA, Dijon, France) for the screening bioassays. Aphids in the control treatments were only sprayed with sterile distilled water containing 0.05% triton X-100. Treatments consisted of 30 aphids per replicate and the experiment was replicated five times. Experiments took place in plastic Petri dishes (9 cm diameter, 1.5 cm height). A potato leaf was placed on an abaxial surface on top of a layer of cotton wool lined with filter paper disks and moistened with water, which was placed on the bottom of each dish. Aphids (*M. persicae* or *A. gossypii*) adults were transferred from the rearing potatoes in the wood framed cages and placed gently on each leaf using a camel brush and left undisturbed for 1 h to settle. Each aphid species was placed in its own Petri dish. The aphids were maintained in an incubator at 26 $\pm$ 2°C and 70 -80% RH. Mortality was recorded daily for seven days. Dead aphids were transferred to Petri dishes lined with moist filter paper to allow the growth of the fungus on the surface of the cadavers. Mycosis was confirmed by microscopic examination of the hyphae and spores on the surface of the cadaver. Based on the results of mortality, lethal time 50% mortality (LT_{50}) values and optimal sporulation on the cadavers, two fungal isolates were selected for lethal concentration to 50% mortality (LC_{50}) bioassays. Four concentrations were used: 1.0×10^6, 1.0×10^7, 1.0×10^8 and 1.0×10^9 conidia ml^{-1}. All the experimental procedure remained the same as described earlier.

2.2.3.3.4 Statistical Data Analysis

Percent mortality data were corrected for natural mortality in controls using Abbott's formula (Abbott, 1925) and then arcsine transformed to normalize the variance (Sokal and Rohlf 1981) before being subjected to analysis of variance (ANOVA) using PROC GLM, at 95% level of significance. Student-Newman-Keuls (SNK) analysis was used to separate the means as a post-ANOVA procedure ($\alpha = 0.05$). Non transformed means are presented in the tables. The lethal time mortality and lethal concentration mortality were determined for each replicate using the probit analysis method for correlation data (Throne *et al.*, 1995) and compared among themselves using ANOVA (α=0.05)and means separated using Student-Newman-Keuls test. Probit analysis remains an attractive method for bioassay in invertebrate pathology because it was designed specifically to handle batch-treatment of test subjects (i.e. treating test subjects in a group, e.g., by spray application rather than administering doses on an individual basis) (Hatting and Wraight, 2007). These analyses were carried out using GENMOD procedure of SAS version 9.2 (SAS institute, 2007). The level of significance was set at 5% for all analyses.

2.2.3.4 Evaluation of Duduthrin (Lambda cyhalothrin) and Dimethoate chemical pesticides for the control of *A. gossypii* and *M. persicae* in the laboratory

In this experiment, the influence of the commonly used pesticides in aphid control in the potato crop in the field, Duduthrin (Lambda cyhalothrin) and Dimethoate, on mortality of *A. gossypii* and *M. persicae* was evaluated. Thirty individuals of each aphid species were treated. Prior to the insecticide treatments, adult aphids were held in separate Petri dishes (3 cm in depth and 5 cm in diameter) on a potato leaf. Lambda cyhalothrin or Dimethoate was applied as separate treatments per each species at the field rate recommended by the manufacturer for the control of aphids using a hand sprayer. Each Petri dish was sprayed with 10ml of the Duduthrin (Lambda cyhalothrin) preparation at the recommended rate of 65 ml per 20 litres water which translated to 0.325ml per 100ml water. The dosage used for Dimethoate was 0.2 ml per 100ml water derived from the recommended rate of 30 to 40 ml per 20 litres of water. Controls were sprayed only with distilled water. After spraying, aphids were allowed to dry for 1 h before their transference to separate untreated Petri dishes (3 cm in depth and 5 cm in diameter). All treatments were performed at 25 ± 1 °C, 75 ± 5% RH and a photoperiod of 12L:12D. Each treatment was replicated 5 times. Mortality data was recorded daily till all aphids had died (2-6 days). Data collected was analysed using the SAS 9.2 (SAS institute, 2007) statistical programme.

2.2.4 Evaluation of the aphid natural enemies in the Green house

After the evaluation in the laboratory, the two predators *H. axyridis* and *H. convergens*, the parasitoid *A.colemani* and one entomopathogenic fungi *Metarhizium anisopliae* ICIPE 62 which performed best in causing mortality to the two aphid species in the laboratory were selected for evaluation in the greenhouse.

2.2.4.1 Evaluation of Predators *Harmonia axyridis* and *Hippodamnia convergens* for the control of *Aphis gossypii* and *Myzus persicae* in the green house

Potato plants, variety Tigoni, were planted in 2 kg plastic pots, 20 cm diameter and 18cm high (Plate 2.5). The potato plants were planted on the First of July, 2009 and placed in netting cages (45cm x 45cm x 100cm) in the green house at *icipe*. The front side was fitted with a sliding door (Plate 2.4). One week after the crop emergence, 20 adult aphids were harvested from the rearing potato crop in the green house (Section 2.2.1 above) and introduced on each of the caged potted potato plants. The aphids were allowed to establish on the potato plants for one week before predators were introduced. Three potato plants in different cages were used for one replication per aphid species. The first plant was treated with the introduction *H. Axyridis*, the second plant treated with *H. convergens* and the third plant was the control with no aphid predator treatment introduced. One predator was introduced per caged plant while no predator was introduced in the control cage. The experimental design was completely randomised design (CRD) with 4 replications. The number of aphids on each plant was counted before and after the introduction of the predator using a hand lens. The population was

monitored weekly in both treated and controls until the crop matured or dried up and no aphids were found on the crop in the cage. Whole plant counts of aphids were made weekly on each of the inoculated plants. The data was recorded and later analysed using the SAS 9.2 (SAS institute, 2007) statistical programme.

2.2.4.2 Evaluation of the parasitoid *Aphidius colemani* for the control of *Aphis gossypii* and *Myzus persicae* in the green house

The parasitoid *A. colemani* was evaluated against both *M. persicae* and *A. gossypii*. Parasitoids reared from *M. persicae* and *A. gossypii* on potato plants were used for all tests. The experimental design was similar to that used when evaluating predators. Two one-week-old potted potato plants (15-20 cm high) in different cages (Plate 2.5) were used per aphid species per replication. One week after the crop emergence, the plants were infested with 20 aphids of *M. persicae* introduced on each of the two potted potato plants. Two other potted potato plants were each infested with 20 aphids of the second species, *A. gossyppii*. After letting the aphids establish on the plants for four days, 20 parasitized aphid mummies (6-8 days into parasitisation) were picked from potato plants in a screen house where the parasitoids were being reared and introduced on the first plant put in the cage with 20 *M. persicae*. The second caged plant with 20 *M. persicae* was left without any parasitoids introduced as the control. The same treatments were applied on the plants with the second aphid species (*A. gossyppii*). This was replicated four times making 16 caged plants (eight per aphid species). The aphid mummies were observed under a dissecting microscope to ensure that the parasitoid had not yet escaped from the mummy before being introduced on the aphids in the caged plants. Before the

parasitoids were introduced, the total number of aphids on each plant was counted with the aid of a hand lens and recorded. The population growth of the aphids was then monitored every two weeks by counting all the aphids on each plant to compare the populations where the parasitoid was present with those in the control where no parasitoid was introduced. The response (aphid numbers per plant on initially inoculated plants) was recorded each 2 weeks for 12 weeks on each plant.

The plants were also observed for any development of more mummies on the new aphids due to infestation by the *A. colemani* parasitoid. The parasitoids developed in the aphid mummy from oviposition to adult eclosion and then re-emerged and re-infested new aphids within the cage hence killing the aphid and new aphid mummies developed until all aphids were killed or the plant dried up at maturity. Only one *A. colemani* parasitoid emerged from each aphid mummy. Monitoring was done until the crop matured or dried up and no live aphids were found on the crop in the cage. Parasitization rate of the parasitoid species was recorded as percent aphids mummified. The data was recorded and later analysed using the SAS (9.2) statistical programme.

2.2.4.3 Evaluation of the fungal isolate *Metarhizium anisploae* icipe 62 for the control of *Aphis gossypii* and *Myzus persicae* in the green house

Of the 15 fungal isolates screened and evaluated in the laboratory, the isolate *Metarhizium anisploae* icipe 62 that caused the highest mortality of the two aphid species *M. persicae* and *A. gossypii* by mycosis was selected for evaluation in the control of these aphids on potato plants in the green house. The design was similar to that used

when evaluating predators and the parasitoids above. Hence four potted potato plants in cages were used per replication for the two aphid species. 20 aphids of one species were introduced on each of the potted potato plants, two plants with *M. persicae* and two with *A. gossypii*. The fungal isolate icipe 62 prepared as a spray formulation in triton water at a concentration of 1x 10^8 was then applied as a spray, 50 ml on one plant with *M. persicae* and on one with *A. gossyppii* aphid species. The other two plants one with 20 *M. persicae* and the other with 20 *A. gossyppii* were left as control treatments and were only sprayed with 50ml of triton water each without any fungal isolate. These treatments were repeated on four plants each to make four replicates. The aphid population growth on the different treatments was observed weekly to compare the growth in the control with those of the fungal isolate treatment. The data was recorded and later analysed using the SAS statistical computer package SAS 9.2 (2007)

2.2.4.4 Evaluation of chemical pesticides Lambda cyhalothrin and Dimethoate for the control of *M. persicae* and *A. gossypii* in the greenhouse

Duduthrin (Lambda cyhalothrin) and Dimethoate were evaluated for their efficacy in the control of the two aphid species *M. persicae* and *A. gossypii* on the potato crop in the green house. The design was similar to that used when evaluating predators, parasitoids and the entomopathogens above. Four potted potato plants in different cages (Plate 2.5) were used per replication for the two aphid species. Each plant was infested with 20 aphids of one species introduced one week after crop emergence, two plants with *M. persicae* and two with *A. gossypii*. After letting the aphids establish for one week, the

first treatments were applied as follows. One plant with *M. persicae* and another with *A. gossyppii* aphid species were sprayed with 100 ml of Duduthrin each prepared as a spray formulation at the recommended rate of 65ml per 20 litres of water which translated to 0.325 ml per 100ml of water. The other two plants one with *M. persicae* and the other with *A. gossypii* were left as control treatments and were only sprayed with 100ml of tap water each without any chemical pesticide. These treatments were repeated on four plants each to make four replicates. Since it is recommended to alternate a contact insecticide like Duduthrin with a systemic insecticide like Dimethoate in aphid control (NPRC, 2011) on the potato crop in the field, this was similarly done in this experiment in the green house. Hence, one week after this treatment with Duduthrin, a similar treatment was repeated on the same plants using the second chemical, Dimethoate at the recommended rate of 40 ml per 20 litres of water which translated to 0.2 ml per 100 ml of water per plant. The control plants were similarly sprayed with 100 ml of water in this repeat treatment. The aphid population growth on the different treatments was observed weekly to compare the growth in the control with those of the chemical pesticide treatment. Whole plant counts of aphids were made on each of the inoculated plants. The data was recorded and later analysed using the SAS 9.2 (SAS institute Inc., 2007) statistical computer programme.

2.2.5 Evaluation of aphid natural enemies in the open field

The predator *H. convergens*, parasitoid *A. colemani* and entomopathogen *M. anisploae* icipe 62 that were assessed as the best in the control of *M. persicae* and *A. gossypii* from the laboratory and the greenhouse evaluations were taken to the open field for assessment of their potential to control aphids in the natural open field situation as grown by farmers.

2.2.5.1 Study site

The studies were carried out at the field station of the University of Nairobi's faculty of Agriculture at Kabete (Plate 2.15). The Kabete field station experimental site was at latitude 1°14'52.74''S and longitude 36°44'23.35''E and an altitude of 6092ft (1857m) a.s.l. The site has 2 rainy seasons: March – June and October - December, with an average annual rainfall of 1046mm.

Plate 2.15: On-Station Field Experiment Site at the University of Nairobi's Upper Kabete Campus Field Station. (Source: Google Maps)

2.2.5.2 Fungus and mass production of the inoculum

The most virulent isolate in the laboratory bioassay, *M. anisopliae* icipe 62 was selected for field experiments to evaluate its efficacy in the control of natural aphid infestations on potatoes in the field. This strain of *M. anisopliae* was initially isolated from a soil sample from Kinshasa, Democratic Republic of Congo (DRC), in 1990 using the "*Gallerie* bait method" (Zimmermann 1986), and maintained at *icipe* germplasm centre as fungal isolote 'icipe 62'. For this field experiment, Conidia were mass produced on whole rice substrate in Milner bags (60 cm long by 35 cm wide). Rice was autoclaved for 1 h at 121°C and inoculated with a 3-day-old culture of blastospores (Jenkins *et al.*, 1998). The sterile rice was incubated for 21days at 20-26°C, 40-70%RH, and allowed to dry for 5 days at room temperature. Conidia were harvested by sifting the substrate through a sieve (295-μm mesh size). The conidia were stored in 100g sachets (plate 2.16) at 4-6°C in the refrigerator until used.

Plate 2.16: A sachet of the fungal pathogen

Viability of conidia was determined based on germ tube formation (Goettel and Inglis 1997) before each fungal application in the field. Conidial suspensions (0.1ml) titrated to 3x 10^6 conidia/ml were spread plated on Petri dishes (9 cm) containing SDA medium. Four replicate plates per isolate were used. A sterile microscope coverslip (2 by 2 cm) was placed on top of the agar in each plate. Plates were incubated in complete darkness at 25 ± 2°C and examined after 20 hours. Percentage germination of conidia was determined by counting the number of germinated conidia (a germ tube two times the

diameter of the propagule) from 100 spores counted randomly on the surface area covered by each coverslip under the light microscope (400x) (Goettel and Inglis 1997). In the viability tests, over 90% of conidia germinated after 24 hours on Sabouraud dextrose agar.

2.2.5.3 Source of Predators

Adults of the predator *H. convergens* were collected in the field from potatoes in the survey areas, in the month of November, 2009 and May, 2010 before experiments took place. Prior to their use in the experiment, these ladybird beetles were reared and maintained at 25 ± 1 °C, 75 ± 5% RH and a photoperiod of 12L:12D in screened plastic cages (30 x 30 x 30 cm) in the Laboratory at *icipe* as described in section 2.2.3.1.1 above.

2.2.5.4 Source of Parasitoids

The parasitoids were collected as mummified aphids from the NPRC - Tigoni potato crop, Marimba NPRC sub centre in Meru and from another farm in Meru and in Nyahururu. These were observed and identified under a stereo dissecting microscope and then introduced to aphids in the caged potted plants at the green house where the parasitoids were reared on both *M. persicae* and *A. gossypii* aphids as described in section 2.2.3.2.1 above.

2.2.5.5 Source and Formulation of Insecticides

Duduthrin (Lambda cyhalothrin) and Dimethoate chemical pesticides sprays are used for the control of aphids on potatoes in Kenya. The two were purchased from a pesticide shop and applied alternatively as recommended as standard check to compare with the three natural enemies being tested in the control of *M. persicae* and *A. gossypii*. Each was prepared as a spray formulation at the recommended rate of 65ml per 20 litres of water for Duduthrin and 40 ml per 20 litres of water for Dimethoate.

2.2.5.6 Field Plots Layout and Preparation

Field plots for the on-station field experiment were prepared and laid out as follows (fig. 2.2). The field was devided in 10 x 6 m plots. Each plot was separated by 1.5m and each block (replicate) by 3m. Seed Potato tubers of 'Tigoni' variety were planted spaced at 30cm within rows and 75 cm between rows. Each plot had six rows of potatoes with 33 plants in each row giving a population of 198 potato plants per plot. There were three guard rows of Maize around all sides of each plot. The different blocks (replicates) were separated by six rows of Maize. Di-ammonium phosphate (DAP) fertilizer was applied at planting at the rate of 200kg ha^{-1}. Plants in the plots were hand-weeded and irrigated as necessary using an overhead sprinkler. The crop was planted for two seasons: first season (November 2009 - February, 2010) short rains and second season (May - August, 2010) long rains

2.2.5.7 Treatments

Five treatments were applied and consisted of (1). *Hippodamia convergens* predator introduced weekly for four weeks (2). *Aphidius colemani* parasitoid released weekly for four weeks. (3). *Metarhizium anisploae* icipe 62 fungal entomopathogen applied weekly for eight weeks. (4). Duduthrin (Lambda cyhalothrin) and Dimethoate chemical pesticides applied weekly alternatively (as done on the farms) for eight weeks (5). Untreated control. The treatments were arranged in a randomised complete block design (RCBD) with four replicates (fig. 2.2). The potato plants were under natural aphid infestations and were not artificially infested with aphids. The prevailing natural insect population was monitored in each treatment until the crop matured and dried up. The fungus and the chemical insecticides (Duduthrin and Dimethoate) were applied with different knapsack sprayers (Plate 2.17).

In the first season (Short rains 2009), the treatments commenced in the fourth week after crop emergence to allow aphids establish on the crop before treatments start. But after the result showed that aphids had built up so much almost to the peak in those four weeks, the treatments in the second season (Long rains, 2010) commenced the first week immediately the crop emerged.

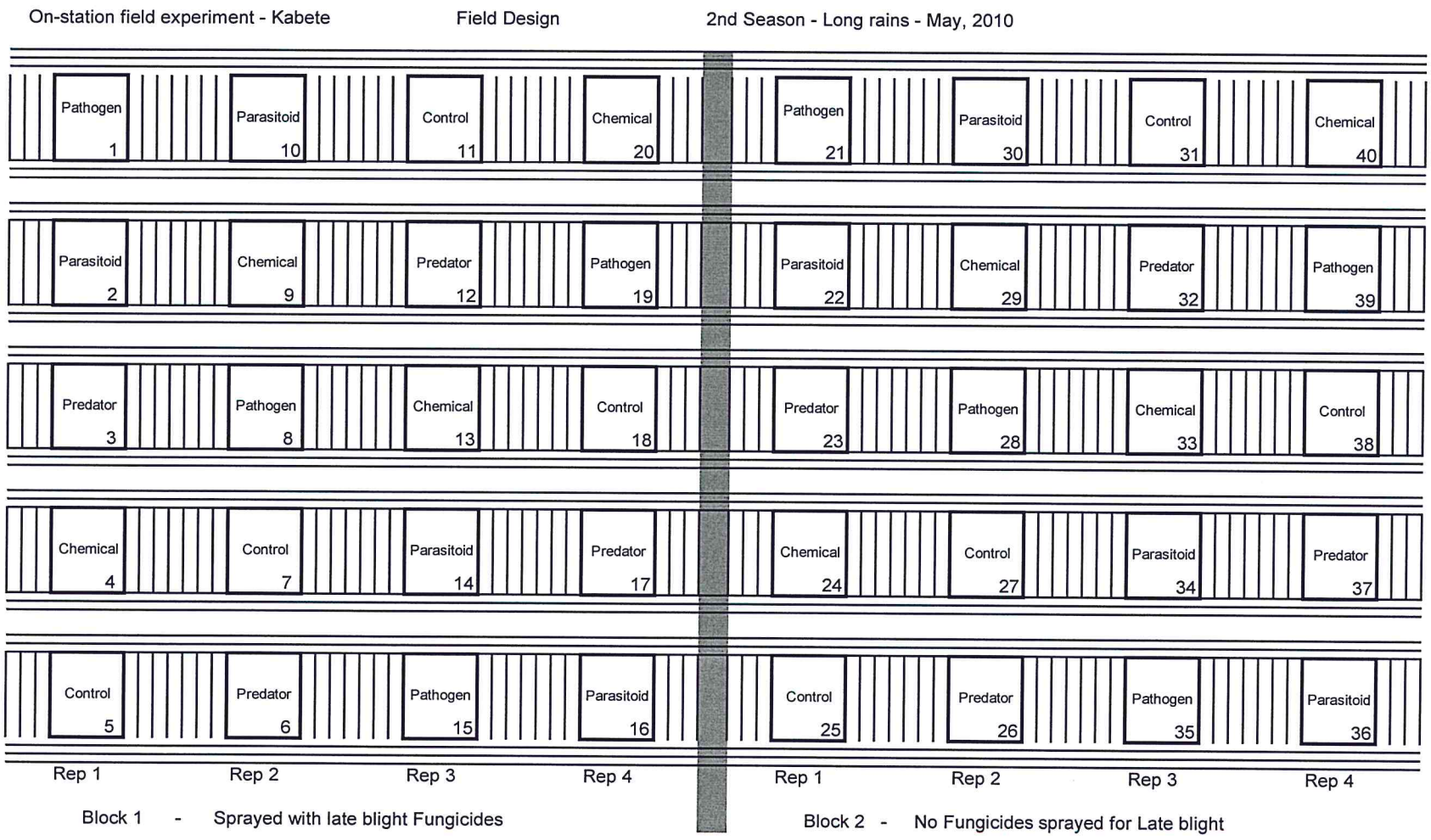

Figure 2.2: Field layout of the on-station experiment on the field evaluation of Biological control agents of aphids on the Potato crop

Plate 2.17: Spraying a fungal pathogen on one of the experimental potato plots.

For spray applications, conidia of *M. anisopliae* icipe 62 were suspended in water containing 0.05% triton water and 3% corn oil (Elianto) added to the inoculum as a protectant and bait to complete the formulation (Maniania, 1993). The fungus was applied at the rate of 1x 10^8 conidia ml-1 which had been determined from the laboratory evaluations (section 2.2.3.3 above) as the optimal concentration for the highest aphid mortality. Spore concentrations were quantified with a bright line haemocytometer.

Serial dilutions were prepared to obtain the desired concentration ($1x10^8$). Duduthrin (Lambda cyhalothrin) and Dimethoate insecticides were sprayed at the recommended rates of 65ml per 20 litres of water and 40 ml per 20 litres of water respectively. Untreated control plots were not sprayed. Spray applications were performed in the evenings between 17:00h and 18:00h to lessen the adverse effects of ultraviolet radiation (Moore and Prior, 1993). For the predator, 200 live beetles collected from the field and maintained in cages in the laboratory as in section 2.2.3.1.1 above were released in all the six rows of the plot well spread (about one per plant). For the parasitoid, the aphid mummies collected from the field and reared further in the green house at *icipe* were also released (1000 per plot) on all the six rows of the plots treated with the parasitoid and spread uniformly (about 5 per plant) across each row. Since the potato crop is usually susceptible to late blight and farmers almost always spray to control late blight, these treatments were all repeated in another block where late blight control pesticides (Ridomil and Dithane M 45 at the recommended rates) were also applied as opposed to the first treatments where no late blight pesticide was applied.

2.2.5.7.1 Evaluation of treatments

For determination of the efficacy of the treatments, 18 plants were picked at random from each plot distributed equally in the six potato rows, three plants per row, one from near each end of the row and one from the centre. From each plant, three compound leaves were plucked, one each from the top, middle and bottom part of the plant. Leaves

from each level of the plant were put in separate plastic paper bags (12 x 15cm). The samples were transported in a cool box to the *icipe* laboratory. The aphids on each leaf were counted under a dissecting microscope in the laboratory and each species identified and recorded.

2.2.5.8 Statistical analysis

The data was subjected to analysis of variance for a randomised complete block design and means were separated by Student-Newman-Keuls (SNK) test (P=0.05) using the ANOVA or GLM procedure of SAS (SAS institute Inc., 2007). Analysis of aphid counts was based on data transformed to $\log_{10}(x + 1)$. For fungicide treated and untreated plots, data from post-spray samples were pooled over the entire season after the initial spray applications and mean values of all (12) samplings are presented.

2.3 Evaluation of Compatibility of Biological Control Agents *A. colemani*, H. *convergens* and *M. anisploae* icipe 62 with Common Pesticides Used in Potato Production in Kenya

To assess the effect of the commonly used chemical pesticides on the predator, parasitoid and entomopathogen evaluated in this study for their efficacy in the control of the two aphid species on the potato crop, specific experiments were set up in the Laboratory where the Fungicides used for spraying against late blight in potatoes ie

Ridomil and Dithane M45 were assessed for any effect on the fungal entomopathogen *M, anisploae* icipe 62, while the chemical insecticides used against aphids on potatoes i.e Duduthrin (Lambda cyhalothrin) and Dimethoate were assessed for their effects on the two insect bio-control agents i.e the predator *H. convergens* and the parasitoid *A. colemani*.

2.3.1 Evaluation of the Compatibility of *A. colemani* Parasitoids and *H. convergens* Predators with insecticides Duduthrin (Lambda cyhalothrin) and Dimethoate

The goal of this experiment was to determine if two common pesticide products used to control aphids, formulations of Duduthrin (Lambda cyhalothrin) and Dimethoate, were compatible with *A. colemani* adults (via contact with freshly dried residues) and *H. convergens* adults (via direct sprays). If compatible, such materials might be used to control species not parasitized by *A. colemani* and/or predated by *H. convergens.*

Pesticide Rates. Duduthrin (Lambda cyhalothrin) was applied at the label-recommended rate for aphid suppression (65 ml per 20 litres water = 0.325ml per 100ml water). Dimethoate was used at the high end of the labelled range for aphids (0.2 ml per 100ml water derived from the label-recommended rate of 30 to 40 ml per 20 litres of water). Each Petri dish was sprayed with 10ml of the pesticide solutions applied with small, hand pumped, spray bottles. Water was applied as a control.

Source of Predatory Beetle and *Parasitoid Wasp.* All *H. convergens* and *A. colemani* used in these experiments were obtained from *icipe* in Nairobi, Kenya where they were reared (sourced originally from potatoes in the field) as described in sections 2.2.3.1 and 2.2.3.2 above. Parasitoids were collected as aphid mummies, and typically adult wasps were just beginning to emerge on the day of the experiment.

Exposure of Adult A. colemani to Pesticide Residues. Adult parasitoids were exposed to freshly dried residues of pesticide sprayed on cotton wool lined with a filter paper in a Petri dish (9 cm diameter) in groups of 10. Petri dishes were treated individually with 10ml of spray from a spray bottle containing either an insecticide solution or water, until cotton wool and filter paper were coated to run off. The Petri dishes were then inverted to allow excess liquid to drain out. After filter paper surfaces were dry, 10 adult wasps (unsexed) were aspirated into each Petri dish (= 1 replicate). For ventilation, a 10-mm diameter hole was cut in each Petri dish top lid and a fine-meshed polyester screening then secured over the top of the Petri dish by the remainder of the lid. Parasitoids were collected with aspirators from emergence containers and allowed to walk from the aspirator into the test Petri dishes. Dishes with wasps were held in the laboratory at ambient conditions 22 - 25° C, 50-75% RH, and 12:12 L:D. The Petri dishes were examined under a dissecting microscope and the number of dead wasps counted each hour up to 12 hours . Each treatment was replicated five times.

Exposure of H. convergens to Pesticide Sprays. Groups of 10 *H. convergens* were placed on blotting paper on plastic dishes lined with cotton wool with a fine paintbrush. Each group was sprayed directly with one of the test pesticide solutions as described above and allowed to dry. The cotton wool absorbed the spray chemical to assist in faster drying and avoid any death of the beetles by drowning. The blotting paper lining assisted in preventing the predator beetles from burrowing into the cotton wool and possibly die by drowning in the wet chemical in the cotton wool. Each replicate consisted of 10 *H. convergens* ladybeetles which were held in a clean Petri dish (90 mm) secured with fine mesh polyester fabric and a ventilated lid. The ladybeetles were held in the laboratory at 22-25° C, 50-75% RH, 12:12 L:D photoperiod and the number of dead insects were counted every hour for 8 hrs. Each treatment was replicated five times.

2.3.2 Evaluation of the Compatibility of fungal entomopathogen *Metarhizium anisopliae* icipe 62 with fungicides Ridomil and Dithane

To evaluate the effect of fungicides Ridomil and Dithane on the fungal entomopathogen *M. anisopliae* icipe 62, the experiment was set as follows. As previously done when evaluating the efficacy of pathogens in the control of the two aphid species *A. gossypii* and *M. persicae*, the same was done here but an additional set of treatment added where after spraying the pathogen on the aphids, the test fungicide was then prayed additionally on the pathogen to see if it would lead to a reduction in the percent mycosis on the aphids by the fungal entomopathogen. Hence three sterile petridishes were prepared per replicate per aphid species. As in earlier evaluations, a piece of cotton wool was placed

at the bottom spread uniformly and some distilled water sprinkled to make it moist to provide some humidity which is conducive for fungal entomopathogen activity. A sterile filter paper was then placed on top of the cotton wool in each plate to protect the aphids from burrowing in the cotton wool and hence dying from drowning.

Thirty aphids of one species, *M. persicae* were placed on each petri dish plate. As done in earlier pathogen evaluations the plates were taken to the Burgerjon sprayer tower and the first plate was prayed with 10 ml of triton water as the untreated control. The other two plates were sprayed with 10 ml of the test entomopathogen *M. anisploae* icipe 62 isolate at a concentration of 1x 10^9 as done in the screening experiment earlier for this pathogen. The plates were then removed and one of the plates treated with the entomopathogen was sprayed again with one of the fungicides Dithane M45 to see the effect of this fungicide on the efficacy of the pathogen in controlling this aphid species. The three plates were sealed with parafilm and left at room temperature. This was repeated using the second aphid species *A. gossypii* with the same fungicide Dithane M45. Hence six plates were used in total per replicate for the two aphid species. This was repeated to make five replicates hence a total of 30 plates for the first fungicide Dithane M 45. These were all put in the laboratory at room temperature and observed daily for aphid mortality till all aphids were dead in all the treatments as well as in the control. After all the aphids in each petri dish were dead, they were removed and placed on a sterile moist filter paper placed in another sterile petri dish and left for at least seven days in an incubator at 25°C. This was done for all the 30 petri dishes which were then

observed for mycosis on the aphids and recorded separately for each petri dish. After completion of this evaluation for the first fungicide Dithane M45, another experiment was set up to evaluate the second fungicide, Ridomil MZ where the above procedure was repeated but using Ridomil instead of DithaneM45.

2.3.3 Statistical analysis

The data was subjected to analysis of variance and means were separated by Student-Newman-Keuls (SNK) test (P=0.05) using the ANOVA or GLM procedure of SAS (SAS institute Inc., 2007). Analysis of aphid counts was based on data transformed to $\log_{10}(x + 1)$.

CHAPTER THREE

3.0 OCCURRENCE OF NATURAL ENEMIES OF *MYZUS PERSICAE* AND *APHIS GOSSYPII* IN MAJOR POTATO GROWING AREAS IN KENYA

3.1 Introduction

Under natural conditions, a range of organisms including predators, parasitoids and pathogens regulate insects and other arthropod populations (Nielsen *et al.*, 2007). Biological control is based on the use of natural enemies of pests, often referred to as Biological Control agents. These are predators, parasitoids and pathogens of invertebrate pests and herbivores attacking weed pests (Driesche van *et al.*, 2008). Almost all Biological Control methods come under one of three categories: natural, classical and augmentative.

Natural Biological Control is used to describe the effects of the indigenous natural enemies already present in natural or managed ecosystems. In a healthy ecosystem, these natural enemies act to keep the populations of many (or all) pests at acceptable levels, below the economic threshold at which a control intervention is justified. There are a variety of methods to increase the number, diversity and impact of these naturally occurring Biological Control agents, and this interventionist approach is often referred to as conservation Biological Control. Natural Biological Control is widely recognised as the foundation of integrated pest management (IPM), and in the interests of the public and the environment.

Classical Biological Control, also referred to as introduction Biological Control (Waage, 2007), is the introduction of one or more Biological Control agents, usually from a pest's area of origin, to control the pest in an area where it is introduced. Once introduced, the Biological Control agent will become established, reproduce and spread, so that no further intervention is needed for the Biological Control agent to have its effect on the target pest. Thus, the introduced Biological Control agent in a classical Biological Control programme becomes part of the natural Biological Control in the ecosystem, working in combination with it.

Augmentative Biological Control using invertebrates involves the production and release of Biological Control agents into specific crop situations, where they cause mortality of the target pest, but are not expected to persist from one cropping cycle to the next. A great proportion of augmentative Biological Control is applied to greenhouse crops, but field crops are also treated. The Biological Control agents used in augmentative Biological Control may be indigenous or exotic. Where they are exotic, they should under best practice be evaluated before use in a similar way to Biological Control agents for classical Biological Control, which is now common practice in several countries (Lenteren van *et al.*, 2006).

Aphids are serious insect pests throughout the world and are one of the most important factors limiting horticultural crop production (Botto, 1999). The aphids *M. persicae* and *A. gossypii* (Hemiptera: Aphididae) are major pests of the Potato crop in Kenya (Nderitu,

1991, Machangi *et al.*, 2003). This study was, therefore, intended to investigate and determine possible indigenous biological control agents for the aphid pests *M. persicae* and *A. gossypii* on potatoes in Kenya.

3.2 Materials and Methods

The survey of aphids and their natural enemies was done as described in detail at section 2.1 (subsections 2.1.1 to 2.1.4).

3.3 RESULTS

3.3.1 Aphids and their Natuoral Enemies found in the Field Survey

3.3.1.1 Aphid species found in the \survey areas

Four aphid species were found in the survey areas, namely *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas and *Aulacorthum solani* Kaltenbach (Fig.3.1). *Aphis gossypii* species had the highest population overall in the four survey areas with a mean of 132 aphids per farm, followed by *M. euphorbiae* (97 aphids) and *M. persicae* (85). *Aulacorthum solani* population was the lowest. This species is usually found in stored potato and this may explain its low density.

The populations of Aphids were highest in Molo area with a mean of approximately 180 aphids per farm. This was followed by Kiambu area with a mean of 62 aphids per farm and Meru area with an average of 58 aphids per farm. Nyandarua area had the lowest population of aphids with a mean of just about 12 aphids per farm. The mean population of each aphid species in each of the four survey areas is shown in fig 3.1.

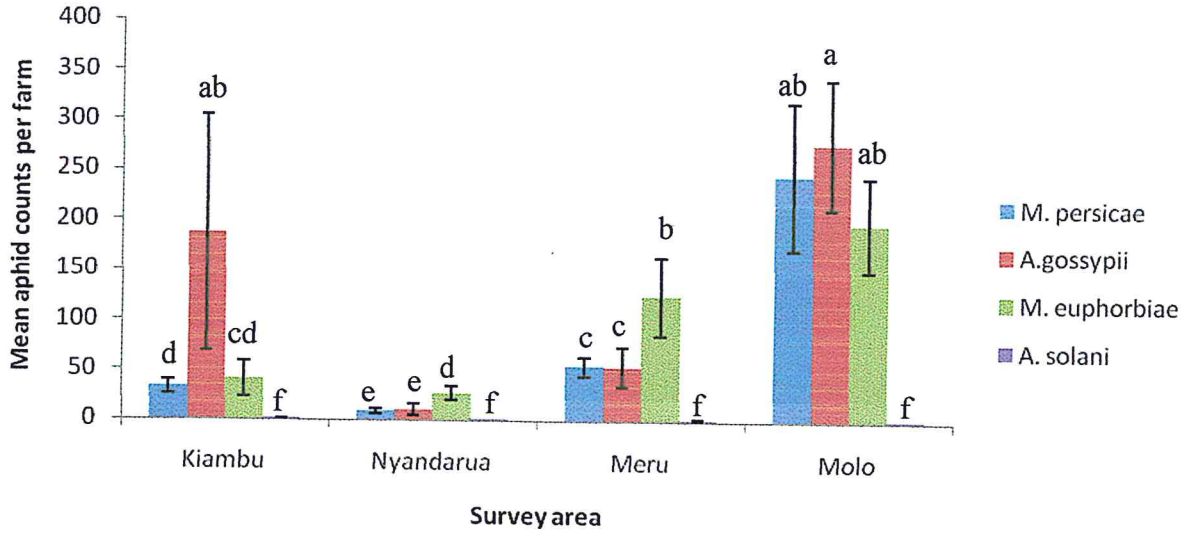

Figure 3.1: Population (Mean ± S.E) of each aphid species per farm in each of the four survey areas. Means with the same letter are not significantly different by Student-Newman-Keuls ($P = 0.05$) test.

3.3.1.2 Predators found associated with aphids in the survey area

Sixteen predator species were collected from the four survey areas (Table 3.1). The most prevalent predators were the ladybeetles *Hippodamnia convergens* with a mean of 7.17 per farm and *Harmonia axyridis* at 6.25 per farm, followed by the aphid eating gall midge *Aphidoletes aphidimyza* (5.23) and the minute pirate bugs, *Orius* spp. (4.83). The other predators present in low numbers included the dwarf spiders – *Erigone* spp., (white crab spider and brown crab spiders), 1.43, Rove beetles (*Paederus spp.*), 1.08, big eyed bug (*Geocoris* spp.) at 0.88, ladybird *Scymnus* spp. at 0.81, Lacewings (*Chrysoperla* spp.) 0.73 and Syrphid flies (Diptera: Syrphidae, *Syrphus* spp.) with a mean of 0.67 insects per farm. Other six species occurred at very low populations and included, Damsel bugs (*Nabis* spp.) 0.26, Tachnid fly (0.21), Assasin bugs (Hemiptera:Reduviidae, *Zelus* spp.) at 0.08, ladybug *Coccinela semptuncutata* (0.07), Lygus bugs (0.07), and Praying mantis (Mantodea: Mantidae) at 0.03. In general the predator populations were highest in Kiambu (2.71 per farm), followed by Nyandarua (2.14), Meru (2.03). The predators were lowest in Molo (0.58) which was significantly lower ($P<0.05$) than the other areas above. The abundance of each predator species in each of the four survey areas is as shown in Table 3.1.

Table 3.1: Mean Population (± S.E) of the different predators of aphids found in the four survey areas, Kiambu, Nyandarua, Meru and Nakuru counties

Predator	Population (Mean ± S.E)				
	Kiambu	Nyandarua	Meru	Molo	Grand mean
Harmonia axyridis	9.00 ± 2.85	6.00 ± 2.18	9.33 ± 0.23	0.67 ± 0.05	6.25 ± 2.01
Hippodamnia convergens	12.33 ± 0.37	7.00± 0.25	5.67 ± 0.20	3.67 ± 0.10	7.17 ± 1.85
Scymnus spp.	0.47 ± 0.37	0.73 ± 0.28	0.97 ± 0.33	1.07 ± 0.22	0.81 ± 0.13
Coccinela semptuncutata	0.00 ± 0.00	0.10 ± 0.06	0.13 ± 0.13	0.03 ± 0.03	0.07 ± 0.04
Orius spp.	5.07 ± 1.12	9.4 0± 2.69	4.13 ± 1.49	0.73 ± 0.20	4.83 ± 1.78
Geocoris spp.	0.36 ± 0.24	2.03 ± 0.51	1.07 ± 0.21	0.07 ± 0.05	0.88 ± 0.44
Aphidoletes aphidimyza	9.03 ± 3.37	3.50 ± 1.50	7.83 ± 6.94	0.57 ± 0.23	5.23 ± 1.96
Nabis spp.	0.10 ± 0.07	0.33 ± 0.18	0.23 ± 0.14	0.37 ± 0.14	0.26 ± 0.06
Erigone spp.	2.50± 0.50	2.60± 0.74	0.23 ± 0.11	0.40 ± 0.15	1.43 ± 0.65
Syrphus spp.	1.10 ± 0.30	0.73 ± 0.53	0.43 ± 0.22	0.40 ± 0.19	0.67 ± 0.16
Paederus spp.	2.53 ± 0.67	0.20 ± 0.11	1.50 ±1.04	0.07 ± 0.05	1.08 ± 0.58
Preying Mantis	0.00 ± 0.00	0.07 ± 0.05	0.07 ± 0.05	0.00 ± 0.00	0.03 ± 0.02
Chrysoperla spp.	0.00 ± 0.00	1.23 ± 0.84	0.80 ± 0.33	0.90 ± 0.18	0.73 ± 0.26
Lygus bugs	0.13 ± 0.13	0.10 ± 0.06	0.03 ± 0.03	0.00 ± 0.00	0.07 ± 0.03
Zelus spp.	0.23 ± 0.17	0.07 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.06
Tachinid parasites	0.33 ± 0.12	0.10± 0.06	0.03 ± 0.05	0.37 ± 0.18	0.21 ± 0.08
Mean	2.71 ± 0.99	2.14 ± 0.73	2.03 ± 0.76	0.58 ± 0.22	

3.3.1.3 Parasitoids found associated with aphids in the potato crop in the survey areas.

Three hymenopteran parasitoids were found in the survey areas. These were i) Braconids – Hymenoptera: Braconidae ii) Ichneumonids – Hymenoptera: Ichneumonidae and iii) Chalcids – Hymenoptera: Chalcidae. The most abundant of these were the Braconids (mean of 1.4 per farm) followed by chalcids at 0.57 then the ichneumonids (0.17) in all the survey areas as shown in fig. 3.2 below. These differences were all significantly different ($P<0.05$) from one another. Overall, the parasitoids were most abundant in Meru survey area (mean of 3.2 per farm) followed by Nyandarua (2.1) then Molo (1.4) and least in Kiambu area (0.6). These differences were also all significantly (<0.05) different from one another Fig. 3.2.

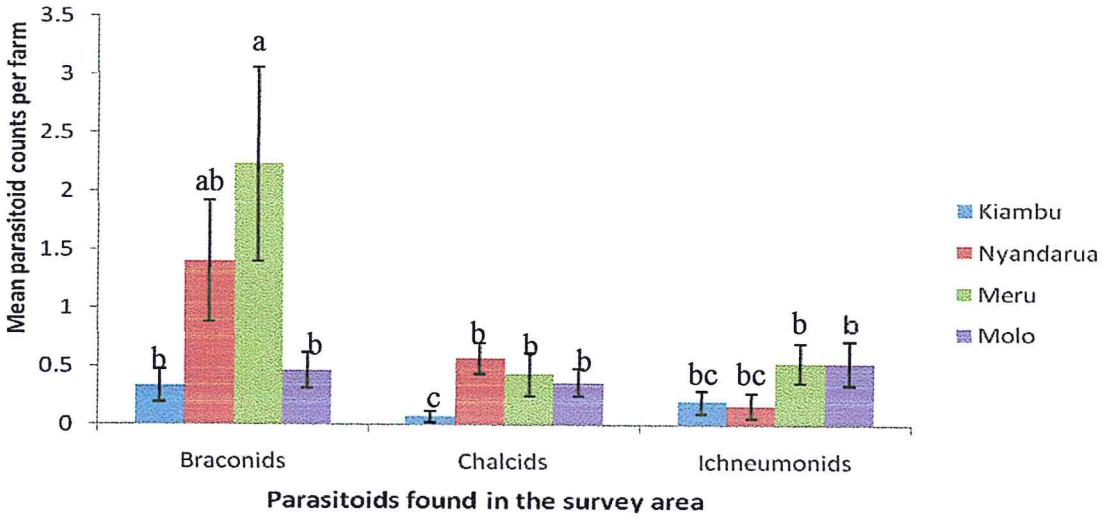

Figure 3.2: Mean Population (± S.E) per farm for the different Parasitoids found in the four survey areas. Means with the same letter are not significantly different by Student-Newman-Keuls ($P = 0.05$) test.

3.3.1.4 Entomopathogens associated with aphids on the potato crop in thesurvey areas

Four fungal entomopathogens were identified from aphids in the survey area. The most abundant species was *Beauveria bassiana* (Balsamo) Vuillemin (a mean of 13.75 aphids per farm were infested by this fungus species) followed by *Verticillium lecanii* (Zimm.) Viegas (6.75) then *Metarhizium anisopliae* (Metschnikoff) Sorokin (6.00). The fourth and least abundant species in the survey area was *Pandora neoaphidis* (Remaudiere and Hennebert) Humber (Zygomycetes: Entomophthorales) (1.75) (figure3.3a). Overall, the entomopathogens were highest in Nyandarua (mean of 12.5 per farm) followed by Molo (9.25), Kiambu (3.25) and Meru (3.25) survey areas (Figure 3.3b).

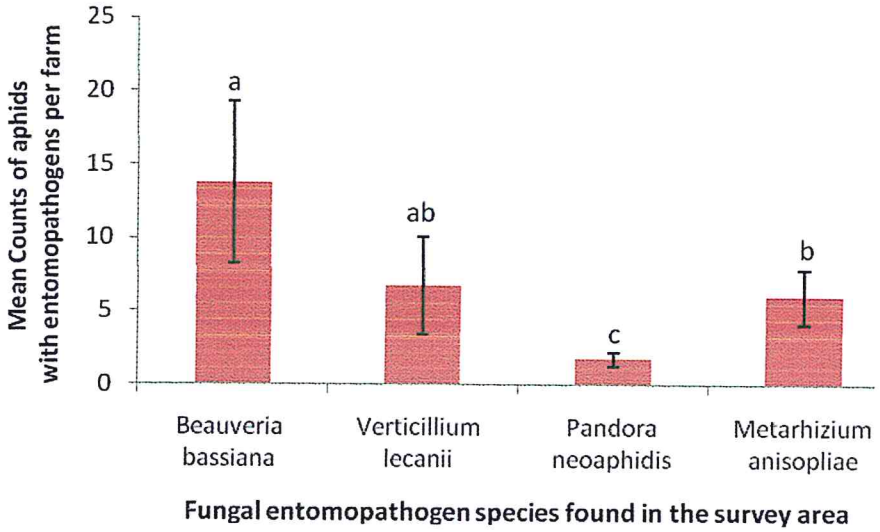

Figure 3.3: Mean population (± S.E) of aphids found infested with entomopathogens in the survey areas. Means with the same letter are not significantly different by Student-Newman-Keuls ($P = 0.05$) test.

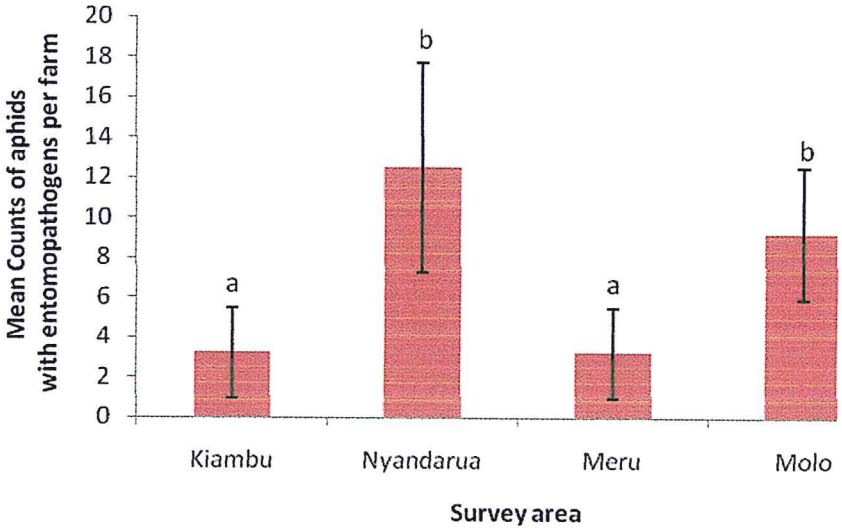

Figure 3.4: Mean population (± S.E) of aphids found with entomopathogens in each of the four survey areas. Means with the same letter are not significantly different by Student-Newman-Keuls ($P = 0.05$) test.

3.4 Discussion

The results present the survey of indigenous natural enemies of aphids on potatoes in the main potato growing are in Kenya. The results obtained show that there are many natural enemies of aphids in Kenya. Other studies done earlier in Kenya (Nderitu, 1991) and East Africa (Le Pelly, 1959) also agree with this. Insects and pathogens classified here as natural enemies are those that have earlier been reported to be effective in the control of

aphids in studies done in other countries (CPC, 2006). For instance, the most prevalent and abundant predators in the field surveys *H. axyridis, H. convergens*, *Orius* spp. and *A. aphidimyza* have been reported as very effective in the control of both *M. persicae* and *A. gossypii* aphid species on potatoes (CPC, 2006). The same is the case with the parasitoid *A. colemani* that has been reported as effective in the search and control of the two aphid species on potatoes both in the field and in the screen house (CPC, 2006; Hagvar and Hofsvang, 1991). As for the entomopathogens, the most abundant species *B. bassiana* followed by *V. lecanii* and *M. anisopliae* have all been reported as potential effective biological control agents of *M. persicae* and *A. gossypii* aphid species on potatoes (Kish *et al.,* 1994; CPC, 2006). It has also been reported that *Pandora neoaphidis* has a worldwide distribution and only recorded in aphid species but is considered a generalist amongst aphids, having been recorded from over 70 species of aphids on annual and perennial crops, weeds and wildflowers (Pell *et al.*, 2001). The prevalence of entomophthoralean fungi in insects and mite populations may at certain period of the year reach very high levels and can lead to complete suppression of insect populations (Pell *et al.,* 2001). Contrasting abiotic and biotic (e.g host range/virulence/voracity) requirements modulated by behavioural and host plant interactions are just some of the mechanisms that have been reported to allow predators, parasitoids and fungal pathogens to differentiate niches and co-exist rather than compete in ecosystems. In this way, they are likely to function in complementary or facilitatory way rather than interfere with each other (Pell, 2007). Overall, studies in caged aphid populations have demonstrated that a combination of predators, parasitoids and *P.*

neoaphidis has the greatest impact on aphid population suppression, although it can also lead to exclusion of some species in the short term (Baverstock, 2004). The Survey results have shown that aphids on potatoes in Kenya harbour a number of natural enemies, however, there is need to assess their effectiveness against the two target aphid species on potatoes with the aim of using them for the biological control of these aphids.

CHAPTER FOUR

4.0 EVALUATION OF PREDATORS, PARASITOIDS AND PATHOGENS FOR THE CONTROL OF *MYZUS PERSICAE* AND *APHIS GOSSYPII* ON POTATOES IN THE LABORATORY, GREEN HOUSE AND OPEN FIELD

4.1 Introduction

Aphids (Homoptera: Aphididae) are widely recognized as economically damaging pests of field and greenhouse crops (Brown and Czosnek, 2002; Van Emden and Harrington, 2006). Species of aphids are typically r-strategists characterized by high fecundity and short generation time (Hatting and Wraight, 2007). Resistance against certain chemical insecticides has, therefore, been reported for at least 24 species of aphids. These include major pests like cotton aphid, *Aphis gossypii* Glover, and green peach aphid *Myzus persicae* (Sulzer), (Whalon *et al.*, 2003). Such phenomena underscore the importance of seeking alternative control options concentrating on the use of natural antagonists such as pathogens, predators and parasitoids (Faria and Wraight, 2001; Gerling *et al.*, 2001; Naranjo, 2001; Gould *et al.*, 2008). Under natural conditions, a range of organisms including predators, parasitoids and pathogens regulates insects and other arthropod populations.

Research on biological control of aphids involves a selection of potential candidates for the control of the aphids and then, the development of an efficient method for the introduction of the natural enemies in the laboratory, glasshouse or the field. Due to the large reproductive capacity of aphids, the continuous presence of natural enemies is very important. This continuous presence might be realized by an open rearing system of the natural enemies (Steenis van, 1992). Because of the good correspondence between laboratory and glasshouse experiments, it is suggested that bad performance of an aphid natural enemy species in a simple laboratory trial might be sufficient evidence to disregard this species for further tests in the greenhouse and the open field (Steenis van, 1995). For instance, experiments were performed in the laboratory and small glasshouses to evaluate the performance of three parasitoids *A. colemani, E. cerasicola* and *L. testaceipes* in the control of *A. gossypii* on cucumbers (*Cucumis sativus* L. cv. 'Aramon'). As in the laboratory test, *A. colemani* performed best in the greenhouse; significantly more colonies were found and parasitization rates in the colonies were higher by *A. colemani* than by *E. cerasicola* and *L. testaceipes.* Among parasitoids, *A. colemani* has been reported in several experiments to perform better than other parasitoids in the control of *A. gossypii and M. persicae*. *Aphidius colemani* seems, therefore, a promising candidate for biological control of the two aphid species (Steenis van, 2009)

Among the pathogens, fungi uniquely posses the ability to penetrate the host cuticle, thus they do not require to be ingested by the host in order to initiate infection (Nielsen *et al.*,

2007). There exists a high potential of utilizing entomophthoralean fungi for biological control of insects. However, *in vitro* production of most entomophthoralean fungi is difficult and, as a result, they are often not suitable for inundative application strategy. Unlike fungi in the Hypocreales, they have proven difficult to mass produce and impossible to apply by spraying (Pell *et al.*, 2001, Freimoser *et al.*, 2001). Entomopathogenic Hyphomycetes fungi (EPF), Particularly the Ascomycetes *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus, P. farinosus,* and *Lecanicillium lecanii,* have been receiving increasing attention as commercial biocontrol agents of insects. A number of formulations based on these fungi have been registered in many countries (Kabaluk and Gazdik, 2005.). Entomopathogenic Hyphomycetes are well suited for the inundative strategy based on ease of production and formulation. The host ranges of the entomopathogenic Hyphomycetes isolates are not restricted to their original insect host species (Goettel *et al.*, 2005) allowing enlargement of the market of each mycoinsecticide based on single fungal isolate formulation (Vidal and Fargues, 2007). Mycoinsecticide products have all been developed from entomopathogenic fungi in the Ascomycota, order Hypocreales and are used following the conventional chemical paradigm i.e for inundative application, often as stand-alone sprays that have an immediate suppressive effect on pest populations but require repeated applications. Mitosporic entomopathogenic fungi have a worldwide distribution, a good safety record towards non-target organisms (Hokkanen and Lynch, 1995) and can be mass produced using low-input technology (Jackson *et al.*, 2000).

Most, if not all, coccinellids rely on non-prey foods as part of their life history. Under field conditions, even the most stereotypical entomophagous coccinellids consume sugar, pollen, fungus, fruit and vegetation, often as an integral part of their diets. Non-prey foods support survival of the coccinellid predators in the absence of prey, improve reproductive capacity, and increase survival through quiescent periods. The importance of non-prey foods to coccinellids provide opportunities for pest managers to manipulate the abundance of coccinellids as biological control agents and increases biological control of key pests by coccinellid predators (Wade *et al.*, 2008; Evans, 2009; Lundgren, 2009).

In this study, experiments were done in the laboratory, the greenhouse and in the open field to evaluate the potential of most prevalent predators, parasitoids and pathogens from the field survey for the control of *Myzus persicae* and *Aphis gossypii* as described in detail in 2.2 above.

4.2 Materials and Methods

The experiments for the evaluation of predators, parasitoids and pathogens for the control of *M. persicae* and *A. gossypii* in the laboratory, green house and the open field were done as outlined in detail at section 2.2 (2.2.1 to 2.2.5).

4.3 Results

4.3.1 Evaluation of predators, parasitoids and pathogens against *Myzus persicae* and *Aphis gossypii* in the Laboratory

4.3.1.1 Evaluation of *Harmonia axyridis* and *Hippodamnia convergens* predators for the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

4.3.1.1.1 Voracity of the predators

Both *H. axyridis* and *H. convergens* predator species exhibited very high predation rates for both *M. persicae* and *A. gossypii* aphid species as shown in figures 4.1 to 4.4. For both species, the predation rate increased with increase in the prey density. At higher prey densities, both predators consumed approximately one aphid every minute for the first 10 to 30 minutes. However, at low aphid prey density, the consumption rate was much lower. There was no significant difference ($P>0.05$) between the aphid predation rate by the two predator species ($P>0.05$). However, for both predator species, the aphid consumption rate was significantly different ($P<0.05$) at the different prey densities tested (10, 20, 30, 50 and 100) for both aphid species offered (fig. 4.5 – 4.8).

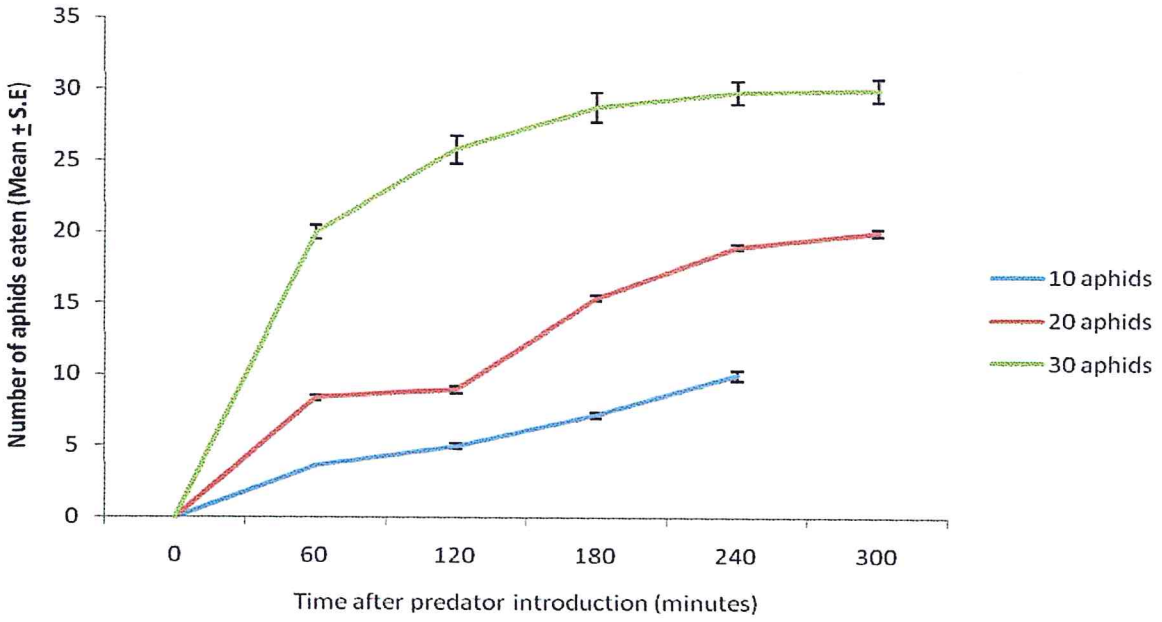

Figure 4.1: Voracity of the predator *Harmonia axyridis* preying on *Myzus persicae* at different prey densities in the laboratory

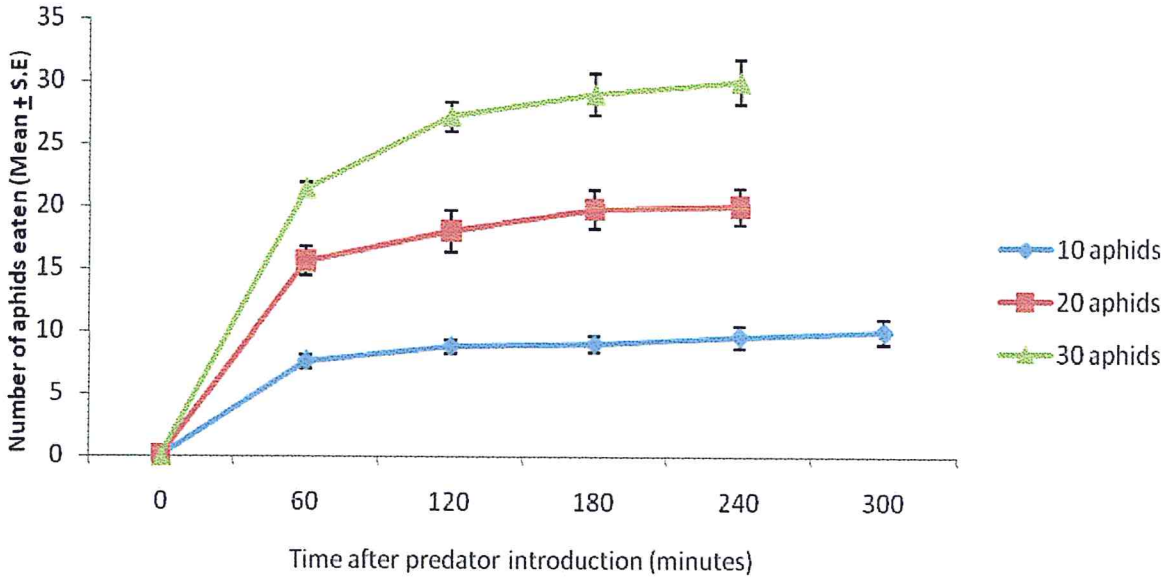

Figure 4.2: Voracity of the predator *Hippodamia convergens* preying on *Myzus persicae* at different prey densities in the laboratory

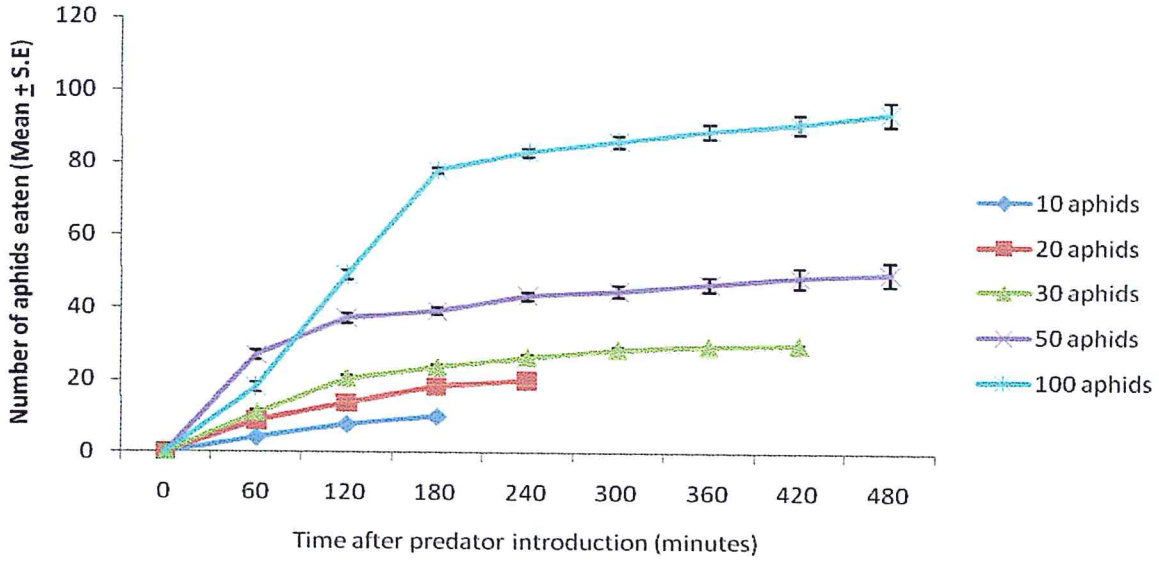

Figure 4.3: Voracity of the predator *Harmonia axyridis* preying on *Aphis gossypii* at different prey densities in the laboratory

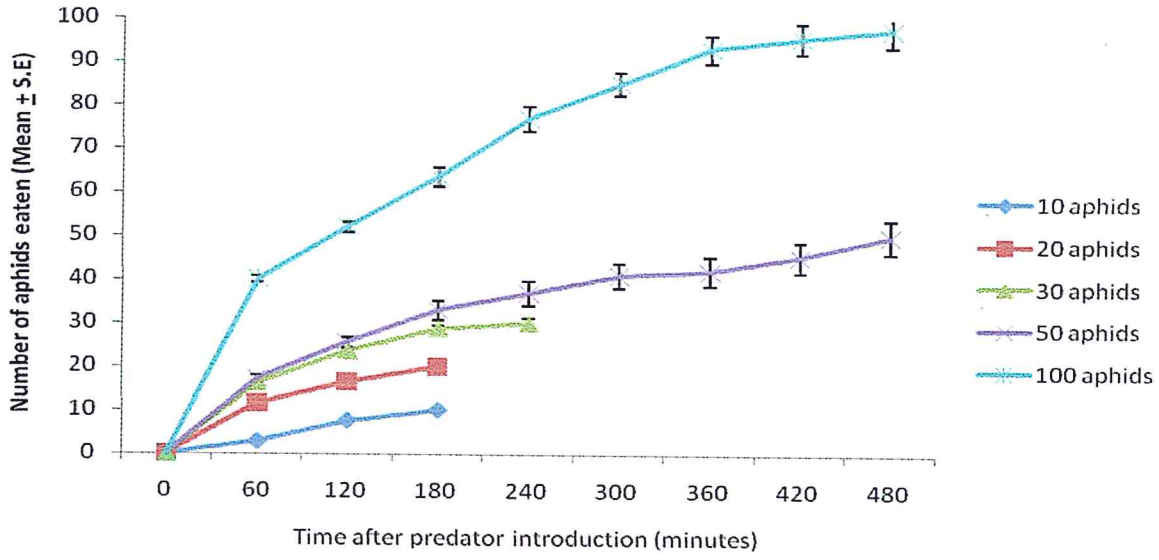

Figure 4.4: Voracity of the predator *Hippodamia convergens* preying on *Aphis gossypii* at different prey densities in the laboratory

Voracity (V_o) was determined according to a modification of the equation by Soares *et al.*, (2003a): $V_o=(A-a_{24})ra_{24}$ where V_o is the number of aphids eaten, A is the number of aphids available, a_{24} is the number of aphids alive after 24 h and ra_{24} is the ratio of aphids alive after 24 h in the control treatment. Since all aphids were eaten before 24 h, the voracity was calculated for the times observed 1 h – 8 h thus replacing a_{24} with a_i where i represents the i^{th} hour when the observation was made on the number of aphids alive after i hours. Since all aphids were still alive in the controls within the 8 hours maximum period it took for the predators to eat all aphids at all prey densities, the ratio of aphids alive after i hours (ra_{24} modified to ra_i) in the control treatment was equal to 1 in all cases hence V_o was equal to the number of aphids eaten in the treatment where the predator was present.

4.3.1.1.2 Functional response of the predators

Voracity data were fit to the ''random-predator" equation according to Rogers, (1972), a modification of Holling's (1959) disk equation. $N_a/TP=\alpha N/(1+\alpha T_h N)$ where N_a is the number of prey attacked, T is the total time of prey exposure, P is the number of predators, N is the initial prey density, α is the attack rate (or searching efficiency) and T_h is the handling time (i.e., the time spent handling each prey attacked). In this experiment, the unit time was in hours instead of days.

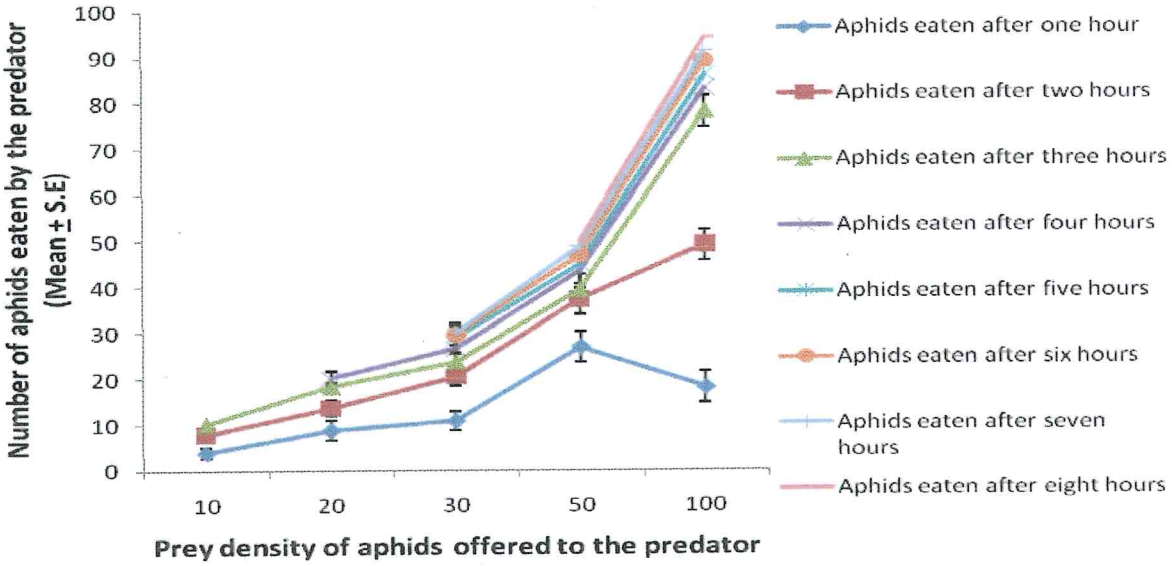

Figure 4.5: Functional response *of Harmonia axyridis* preying on *Aphis gossypii* in the Laboratory

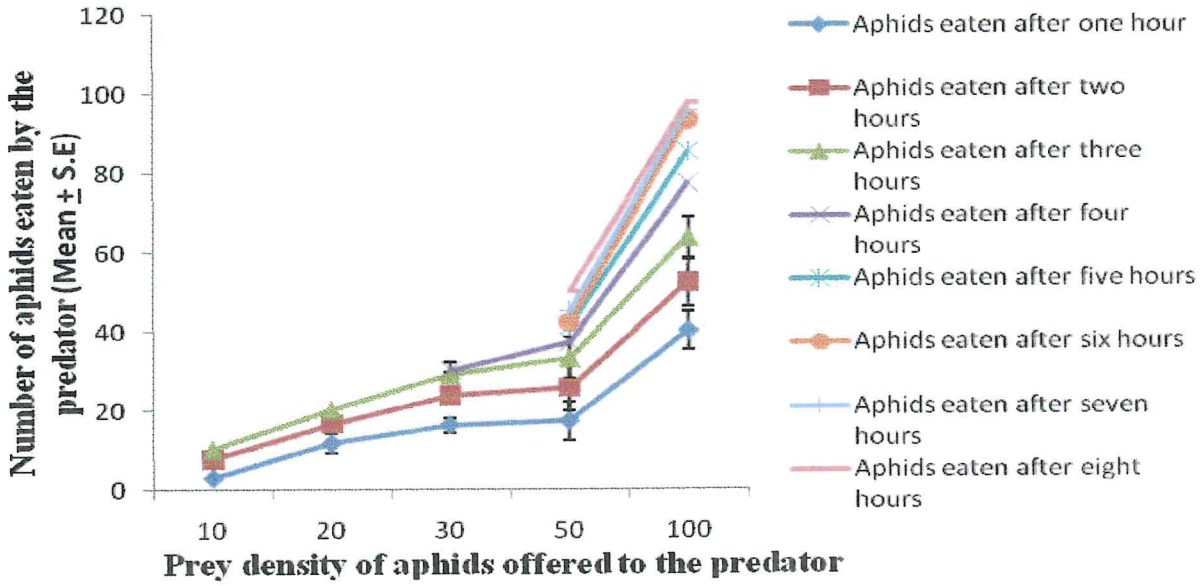

Figure 4.6: Functional response *of Hippodamia convergens* preying on *Aphis gossypii* in the Laboratory

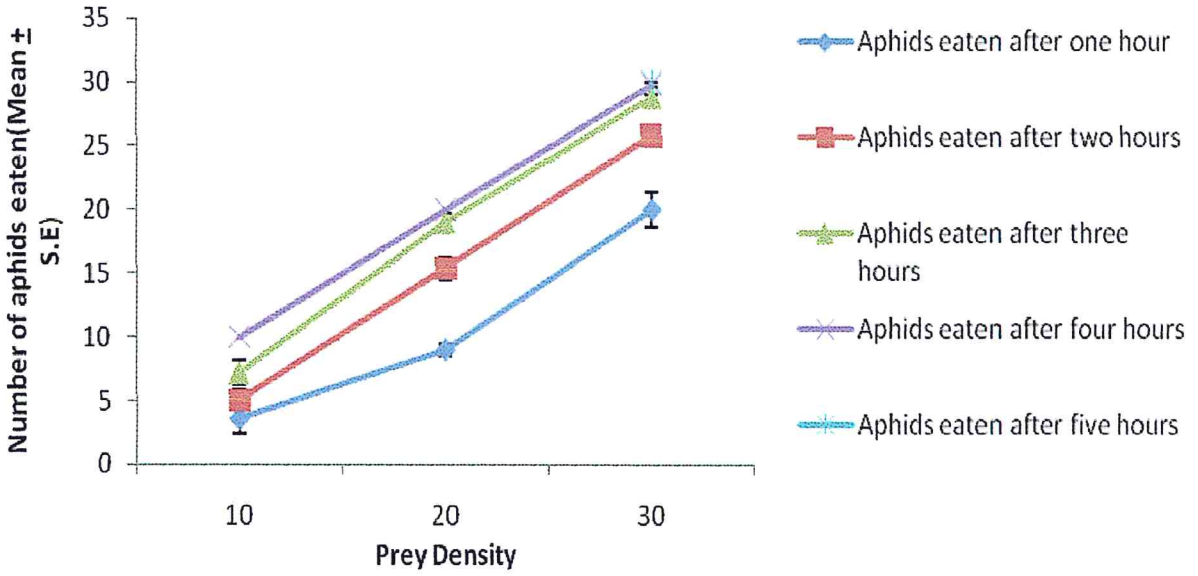

Figure 4.7: Functional response of *Harmonia axyridis* preying on *Myzus persicae* in the Laboratory

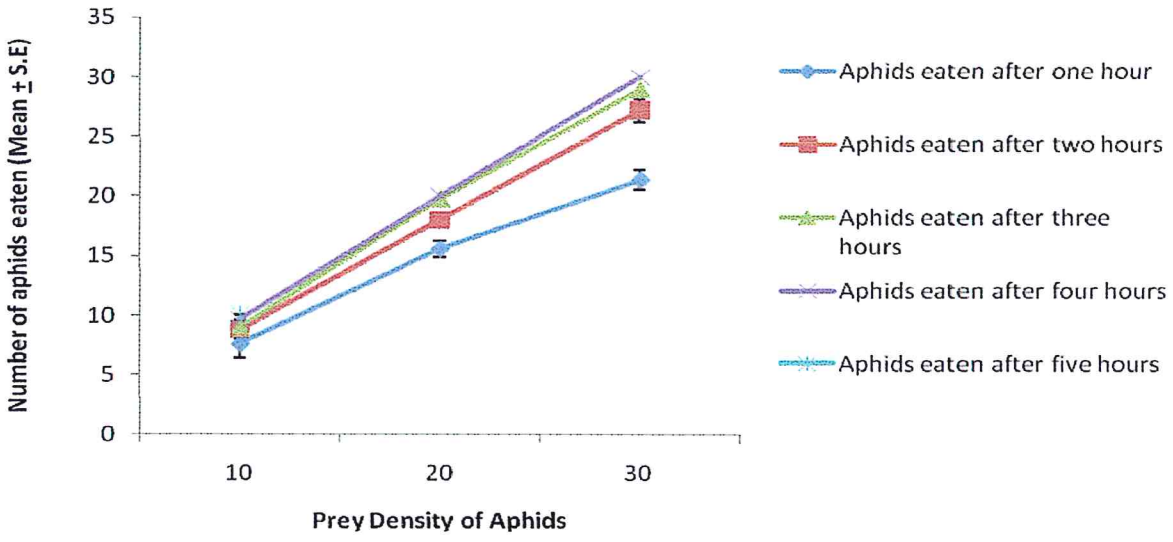

Figure 4.8: Functional response of *Hippodamia convergens* preying on *Myzus persicae* in the Laboratory

4.3.1.2 Evaluation of *Aphidius colemani* Parasitoid in the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

Figure 4.9 shows results of the laboratory evaluation of the effect of the parasitoid *A. colemani* on the two aphid species. From the results, it showed that parasitization of *M. persicae* species was slow at the beginning but increased at an increasing rate (hyperbolic curve) reaching to over 60% by the 5th day. The parasitization of *A. gossypii* was however more uniform from the beginning and increased at a constant rate for the entire 4 days observed forming a more or less straight line in the increase. The parasitization had reached just above 30% by the 4th day slightly more than that of *M. persicae* which was just about 30% on the 4th day. This difference was however not significant (P>0.05).

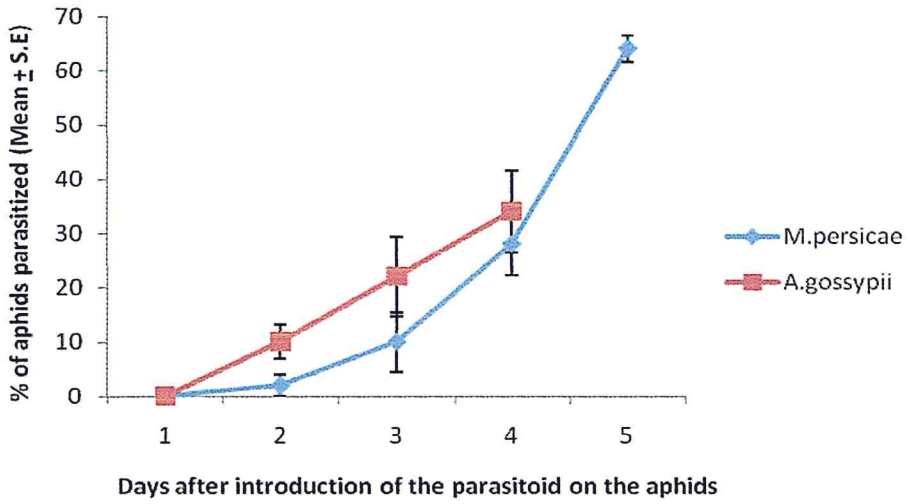

Figure 4.9: Parasitization of *Myzus persicae* and *Aphis gossypii* by *Aphidius colemani* in the laboratory

4.3.1.3 Evaluation of entomopathogenic fungi for the control of *Myzus persicae* and *Aphis gossypii* in the laboratory

The results of the evaluation of the effectiveness of the different entomopathogens in the control of the two aphid species *M. persicae* and *A. gossypii* in the laboratory are as shown in Table 4.1.

Table 4.1: Mean percent mycosis of the dead aphids treated with the different fungal isolates

	Isolate	**Aphid spp.**	**Treatment**	**Mycosis**			
	Icipe isolates			per species		Overall per Isolate	
	M. anisploae			Mean%	Rank	Mean %	Mean Rank
1	Icipe 62	*A. gossypii*	treated	79.56	1	80.91	1
			control	2.36			
		M. persicae	treated	82.26	1		
			control	2.5			
2	Icipe 30	*A. gossypii*	treated	52.14	8	40.44	11
			control	0.66			
		M. persicae	treated	28.74	11		
			control	0			

3	Icipe 69	*A. gossypii*	treated	72.58	4	69.91	3
			control	13.44			
		M. persicae	treated	67.24	4		
			control	1.34			
4	Icipe 18	*A. gossypii*	treated	62.86	6	59.55	6
			control	3.38			
		M. persicae	treated	56.24	6		
			control	2.36			
5	Icipe 84	*A. gossypii*	treated	64.9	5	68.04	4
			control	8.58			
		M. persicae	treated	71.18	3		
			control	3.36			
	B. bassiana						
6	Icipe 273	*A. gossypii*	treated	49.26	9	46.52	8
			control	2.36			
		M. persicae	treated	43.78	8		
			control	2.5			
7	Icipe 622	*A. gossypii*	treated	24.86	13	29.7	13
			control	1			
		M. persicae	treated	34.54	9		
			control	3.34			

8	Icipe 279	*A. gossypii*	treated	9.8	15	10.9	15
			control	0.52			
		M. persicae	treated	12	14		
			control	0.7			
9	Icipe 620	*A. gossypii*	treated	49	10	40.76	10
			control	0			
		M. persicae	treated	32.52	10		
			control	13.7			
10	Icipe 664	*A. gossypii*	treated	57.2	7	42.85	9
			control	0			
		M. persicae	treated	28.5	12		
			control	0			
	Field survey isolates						
	B. bassiana						
11	Aphid B.b-1	*A. gossypii*	treated	47.2	12	33.6	12
			control	0			
		M. persicae	treated	20	13		
			control	0			
12	Dd-Aphid B.b	*A. gossypii*	treated	48.95	11	52.635	7
			control	0			
		M. persicae	treated	56.32	5		

			control	0			
13	Wd-Aphid Bb	*A. gossypii*	treated	72.66	3	61.98	5
			control	0			
		M. persicae	treated	51.3	7		
			control	0			
	Verticillium						
14	Wd-Aphid -	*A. gossypii*	treated	20.32	14	14.996	14
	(Verticillium)		control	1.066			
	Bacilliomycetes	*M. persicae*	treated	9.672	15		
			control	4.362			
	Icipe isolate						
	Isaria (Paocilomyces)						
15	Icipe 682	*A. gossypii*	treated	77.912	2	76.863	2
			control	1.176			
		M. persicae	treated	75.814	2		
			control	3.028			

4.3.1.3.1 Pathogenicity of fungi to *Myzus persicae*

The ability of fungi to infect and kill the host adult varied considerably according to the fungal isolate and species used (Table 4.2). Mortalities caused by isolates of *M.*

anisopliae on *M. persicae* were all above 50% ranging from 50.87% to 81.81% apart from one isolate at 28.74%. In contrast, Mortalities caused by isolates of *B. bassiana* on *M. persicae* were all below 50% ranging from 11.37% to 42.35% apart from two isolates obtained from the field aphids which caused mortality of 51.3% and 56.32%. So generally, Isolates of *M. anisopliae* performed better than those of *B. bassiana* in their pathogenicity to *M. persicae*. The only isolate of *Paecilomyces fumosoroseus* (Wize) Brown and Smith tested was highly virulent causing mortality of 75.05% on *M. persicae* which was the second highest after one from an isolate of *M. anisopliae* (ICIPE 62). The other fungal species tested *Verticillium lecanii* (Zimm.) Viegas which had also only a single isolate obtained from the aphid cadavars from potatoes in the field had little pathogenic effect with a mortality of only 9.67% on *M. persicae*. LT_{50} values ranged from 1.6 to 2.9 days for *M. anisopliae* and 4.15 to 4.7 days for *B. bassiana* isolates (Table 4.2).

4.3.1.3.2 Pathogenicity of fungi to *Aphis gossypii*

Pathogenicity of the various fungal isolates on *A. gossypii* followed almost the same tread as for *M. persicae*. All of the fungal species tested were pathogenic to *A. gossypii* but mortality to the aphids differed for the different isolates of the fungi assayed (Table 4.3). The most pathogenic isolate on *A. gossypii* was *M. anisopliae* ICIPE 62 just as was the case for *M. persicae*. The mortality on *A. gossypii* was 79.07 % just slightly less than but not significantly different ($P>0.05$) from the 81.81% mortality on *M. persicae*. The

least virulent isolate was *B. bassiana* Icipe 279 which caused a mortality of only 9.33% on *A. gossypii*. This isolate also performed poorly on *M. persicae* causing a mortality of only 11.38% which was also the second least virulent on *M. persicae*. As in the case of *M. persicae*, Isolates of *M. anisopliae* were highly virulent on *A. gossypii* killing over 60% of the test aphids with all isolates causing mortality ranging from 61.56% to 79.08% apart from one that caused a mortality of 50.87%.

In contrast, all isolates of *B. bassiana* caused mortality of less than 50% ranging from 9.33% to 48.03% apart from one isolate obtained from aphids collected from the field which caused a mortality of 72.66% on *A. gossypii*. This is the same isolate from the field that performed best among the *B. bassiana* isolates on *M. persicae*. *Paecilomyces fumosoroseus* isolate ICIPE 682 again ranked as the second most virulent isolate causing mortality of 77.65% on *A. gossypii* which was very close to that of the best isolate *M. anisopliae* ICIPE 62 at 79.07% thus maintaining the same trend as for *M. persicae*. The isolate of *V. lecanii* that caused lowest mortality on *M. persicae* did not do any better with *A. gossypii* either and was ranked second last causing a mortality of only 19.46%. LT_{50} values ranged from 1.7 to 3.0 days for *M. anisopliae* isolates and 2.45 to 4.1 days for *B. bassiana*.

Table 4.2: Pathogenicity of entomogenous fungi to adults of *M. persicae*. Percent mortality and lethal time (LT_{50}) of different isolates at the concentration of 10^9 conidia ml^{-1} at 6 days after inoculation

Fungal species	Isolate	Percent mortality (Mean ± SE)	LT_{50} (Days) (Mean ± SE
ICIPE's Germplasm Centre Isolates			
M. anisopliae	ICIPE 62	81.81 ± 4.08a	1.6 ± 0.05f
	ICIPE 30	28.74 ± 7.17fg	-
	ICIPE 69	66.80 ± 4.14bc	2.45 ± 0.15d
	ICIPE 18	55.18 ± 5.75d	2.9 ± 0.13c
	ICIPE 84	70.31 ± 12.65abcd	2.15 ± 0.05[e]
B. bassiana	ICIPE 273	42.35 ± 6.90ef	-
	ICIPE 622	32.28 ± 11.35fgh	-
	ICIPE 279	11.38 ± 9.10ij	-
	ICIPE 620	21.80 ± 10.45gh	-
	ICIPE 664	28.50 ± 3.73g	-
P. fumosoroseus	ICIPE 682	75.06 ± 5.94ab	2.75 ± 0.12c
Field Survey Isolates			
B. bassiana	Aphid B.b-1	20.00 ± 4.11hi	-
	Dd-Aphid B.b	56.32 ± 12.30cde	4.15 ± 0.05b
	Wd-Aphid Bb	51.30 ± 7.62de	4.7 ± 0.1a
V. lecanii	Wd-Aphid	5.55 ± 4.25j	-

Means within columns followed by the same letter are not significantly different, ANOVA and Student-Newman-Keuls' test, α=0.05, n=30

Table 4.3: Pathogenicity of entomogenous fungi to adults of *A. gossypii*. Percent mortality and lethal time (LT_{50}) of different isolates at the concentration of 10^9 conidia ml^{-1} at 6 days after inoculation

Fungal species	Isolate	Percent mortality (Mean ± SE)	LT_{50} (Days) (Mean ± SE
ICIPE's Germplasm Centre Isolates			
M. anisopliae	ICIPE 62	79.08 ± 11.11ab	1.7 ± 0.1f
	ICIPE 30	50.87 ± 2.63d	3.0 ± 0.12b
	ICIPE 69	68.32 ± 10.21ab	2.5 ± 0.1de
	ICIPE 18	61.56 ± 7.87bc	2.7 ± 0.1cd
	ICIPE 84	61.60 ± 7.50bc	2.35 ± 0.05[e]
B. bassiana	ICIPE 273	48.03 ± 10.24cd	-
	ICIPE 622	24.58 ± 4.41e	-
	ICIPE 279	9.33 ± 6.10f	-
	ICIPE 620	49.00 ± 6.78d	-
	ICIPE 664	57.20 ± 0.25c	4.1± 0.02a
P. fumosoroseus	ICIPE 682	77.65 ± 7.07a	2.8 ± 0.1bc
Field Survey Isolates			
B. bassiana	Aphid B.b-1	47.20 ± 18.69cde	-
	Dd-Aphid B.b	48.95 ± 11.87cd	-
	Wd-Aphid Bb	72.66 ± 7.92ab	2.45 ± 0.05e
V. lecanii	Wd-Aphid	19.46 ± 4.10ef	-

Means within columns followed by the same letter are not significantly different, ANOVA and Student-Newman-Keuls' test, α=0.05, n=30

4.3.1.3.3 Dose – response mortality

Between the two most virulent isolates selected for dose response mortality bioassay i.e, *M. anisopliae,* ICIPE 62 and *P. fumosoroseus* ICIPE 682, the isolate with the lower LC_{50} was *M. anisopliae,* ICIPE 62 at 0.4 x 10^7 conidia ml^{-1} on *A. gossypii* and 0.6 x 10^7 conidia ml^{-1} on *M. persicae* which was much lower than that of *P. fumosoroseus*, ICIPE 682 at 0.2 x 10^9 on *M. persicae* (Table 4.4). Due to these good performances criteria including highest virulence to both target aphid species ranking the best in causing highest percent mortality, having the shortest LT_{50} and the low LC_{50}, *M. anisopliae,* ICIPE 62 was selected for further evaluation as a potential biological control agent of the two aphid species *M. persicae* and *A. gossypii* on potatoes in the greenhouse and in field trials. The results of these trials are presented at section 4.3.2.3 and 4.3.2.5.

Table 4.4: Lethal concentration (LC_{50}) values of the isolates of *M. anisopliae*, ICIPE 62 and *P. fumosoroseus,* ICIPE 682 against *M. persicae* and *A. gossypii* [a]

Fungal Isolate	Host spp.	LC_{50} (95% Fiducial Limits)	Slope (± SE)	x^2-test[b]
M. anisopliae, ICIPE 62	*M. persicae*	0.6 x 10^7 (0.4 – 0.9) x 10^7	0.58 ± 0.03	365.9
	A. gossypii	0.4 x 10^7 (0.3 – 0.5) x 10^7	0.40 ± 0.02	245.6
P. fumosoroseus, ICIPE 682	*M. persicae*	0.2 x 10^9 (0.1 – 0.3) x 10^9	00.60 ± 0.04	369.8
	A. gossypii	0.9 x 10^6 (0.6 – 1.1) x 10^6	0.89 ± 0.03	589.7

[a] Five replicates per treatment of 30 insects [b] d.f = 1

4.3.1.4 Evaluation of Duduthrin (Lambda cyhalothrin) and Dimethoate chemical pesticides for the control of *Aphis gossypii* and *Myzus persicae* in the laboratory

The results of the effect of treatments with Dimethoate (Fig. 4.10 and 4.11) and Duduthrin (Fig.4.12 and 4.13) on the two aphid species *M. persicae* and *A.gossypii* showed that both chemical pesticides, Dimethoate and Duduthrin were very effective in the control of both *M. persicae* and *A. gosssypii* aphid species. The chemicals killed all aphids within the first day after treatment apart from one case where one *A. gosssypii* aphid was still alive until the second day after the treatment with Dimethoate. In contrast, the aphids in the control treatment which were only sprayed with distilled water survived up to four days after the treatment with all aphids still alive on the first day after treatment apart from one case where five *A. gosssypii* aphids had died one day after treatment. This is in sharp contrast to the pesticide treated aphids where all aphids apart from one had died in the first day after treatment. There was a significant difference ($P<0.05$) between the number if aphids killed by the two pesticides and those dead in the control treatment right from the first day after the treatments. However, there was no significant difference ($P>0.05$) between the number of aphids killed by Dimethoate and those killed by Duduthrin and the two chemical pesticides were equally effective in the control of the two target aphid species.

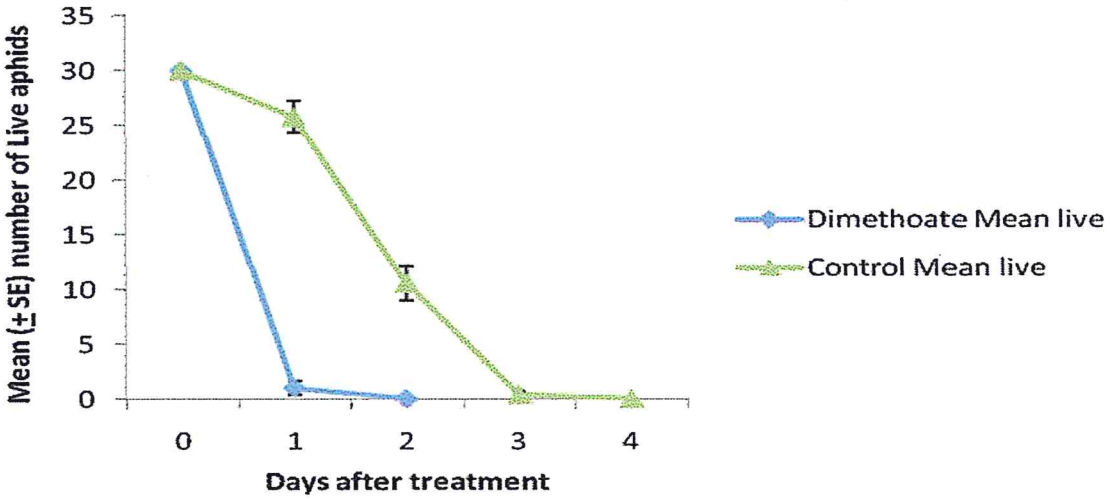

Figure 4.10: Mortality of *Aphis gossypii* in the Dimethoate and control treatments in the laboratory

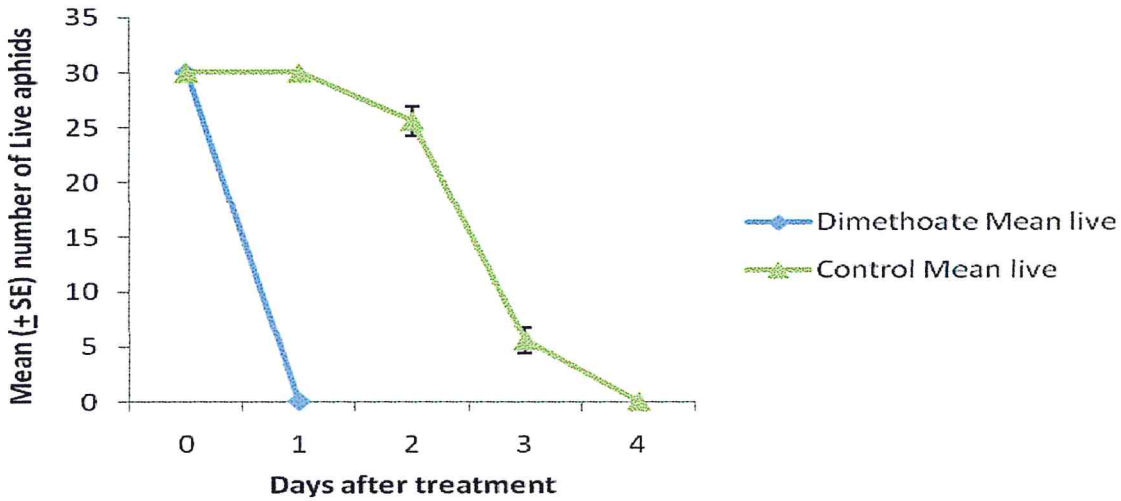

Figure 4.11: *Myzus persicae* mortality in the Dimethoate and control treatments in the laboratory

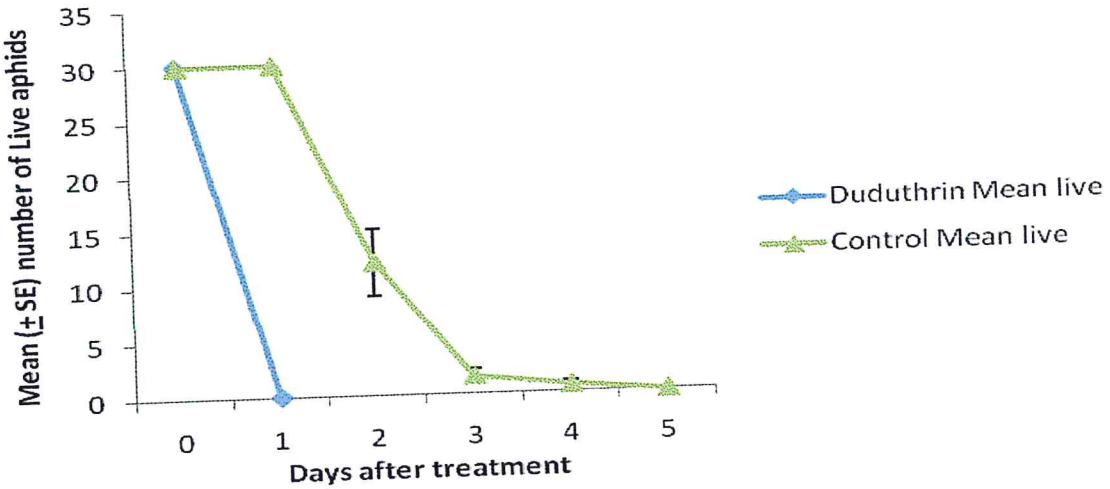

Figure 4.12: Mortality of *Aphis gossypii* in the Duduthrin and control treatments in the laboratory

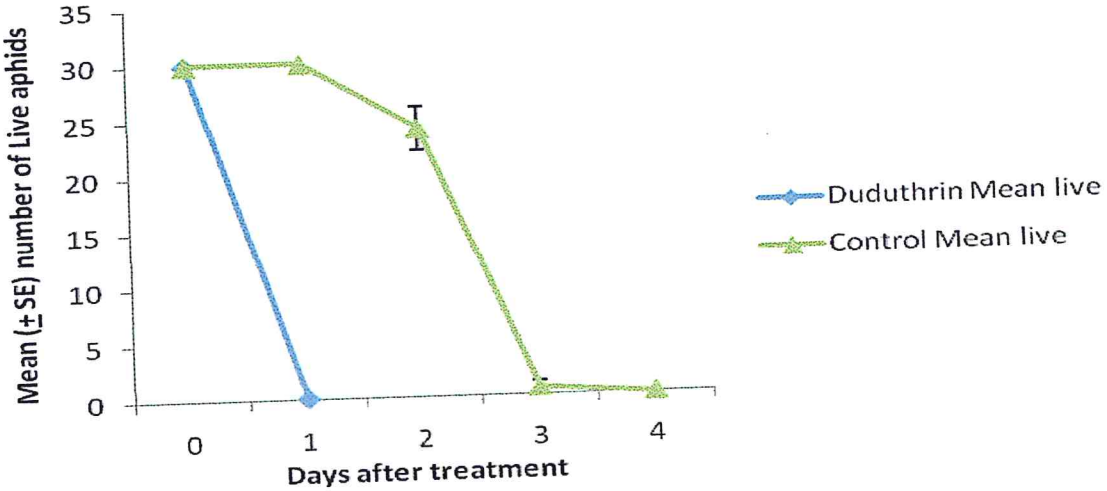

Figure 4.13: *Myzus persicae* mortality in the Duduthrin and control treatments in the laboratory

4.3.2 Evaluation of the aphid natural enemies in the Green house

4.3.2.1 Evaluation of Predator *Harmonia axyridis* and *Hippodamia convergens* for the control of *Aphis gossypii* and *Myzus persicae* in the green house

From the results (Fig. 4.14 – 4.15), the two predators *H. axyridis* and *H. convergens* were very effective in the control of both *M. persicae* and *A. gossypii* species on potato plants in the green house. There was a significant difference ($P<0.05$) between the aphid populations in the treatments and that of the control. However, the two species were almost equally effective in their control of both aphid species with no significant difference in the aphid populations of treatments of either species of the predator. Generally, the aphid populations of *A. gossypii* increased much faster than those of *M. persicae.*

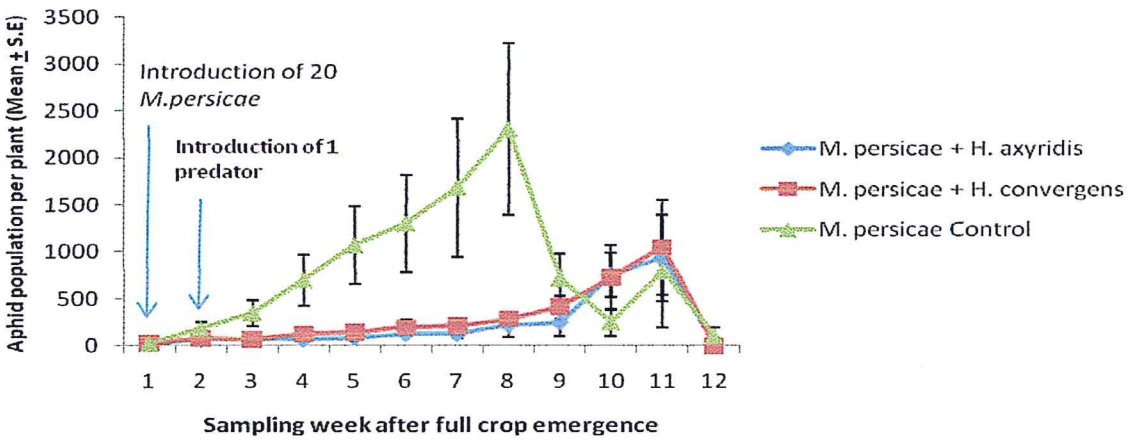

Figure 4.14: Evaluation of *Harmonia axyridis* and *Hippodamia convergens* in the control of *Myzus persicae* in the green house

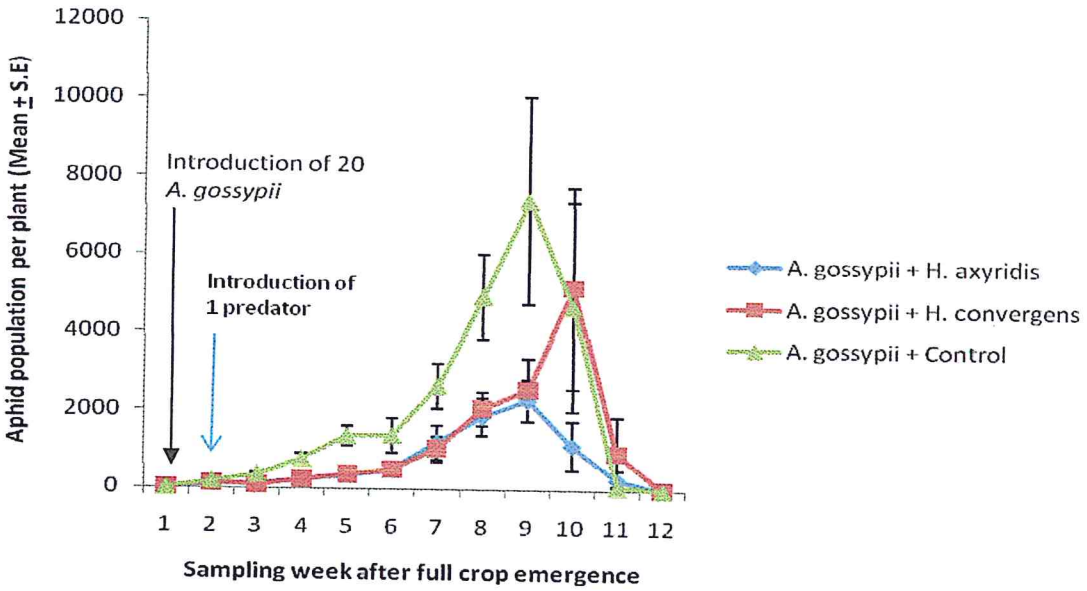

Figure 4.15: Evaluation of *Harmonia axyridis* and *Hippodamia convergens* in the control of *Aphis gossypii* in the green house

4.3.2.2 Evaluation of parasitoids in the Green house

The results of the evaluation of the parasitoid *A. colemani* in the control of *M. persicae* and *A. gossypii* on the potato crop in the green house are as shown below (Fig. 4.17 and 4.18).These results show that the parasitoid *A. colemani* is also very effective in the control of both *M. persicae* and *A. gossypii*. As shown in Figure 4.17, the population of *A. gossypii* reached to a high of over 13,000 aphids per plant in the control, but the population of the same species in the treatments by the parasitoid *A. colemani* reached a mean maximum of about 4000 aphids per plant. This was a reduction of the aphid population by about 70% to only about 30% the population in the control where no parasitoid was introduced for aphid control. This difference was significant ($P < 0.05$).

Similarly, the aphid population of *M. persicae* reached a mean maximum of about 9000 aphids per plant in the control treatment while in the treatment with *A. colemani* parasitoid, the aphid populations of the same species only reach a maximum of less ths 2000 aphids per plant. This is a reduction of over 80% in the population of *M. persicae* to only less than 20% the population in the treatment where no parasitoid was added for for the control of the aphids. This difference was also significant at $P<0.05$.

Looking at the percentage of the aphids that were parasitized and mummified due to the the presence of the parasitoid larvae inside their bodies (Figure 4.18), it shows that this parasitoid was very effective. The percentage of aphids that had mummified after being parasitized by *A. colemani* increased steadly after the introduction of the parasitoid reaching 100% parasitism to all the aphids on the crop by the 5^{th} week after the release of the parasitoid to the aphids. This was the case for both species of aphids with *A. gossypii* showing a higher rate of parasitization than *M. persicae* but this difference was not significant ($P > 0.05$). The aphids in the control treatment were not parasitized apart from one plant which exhibited parasitization towards the end (Probably due to entry of a parasitoid to the cage late after escape from the cages with parasitoids). This parasitization also inceased very fast reaching 100% within three weeks.

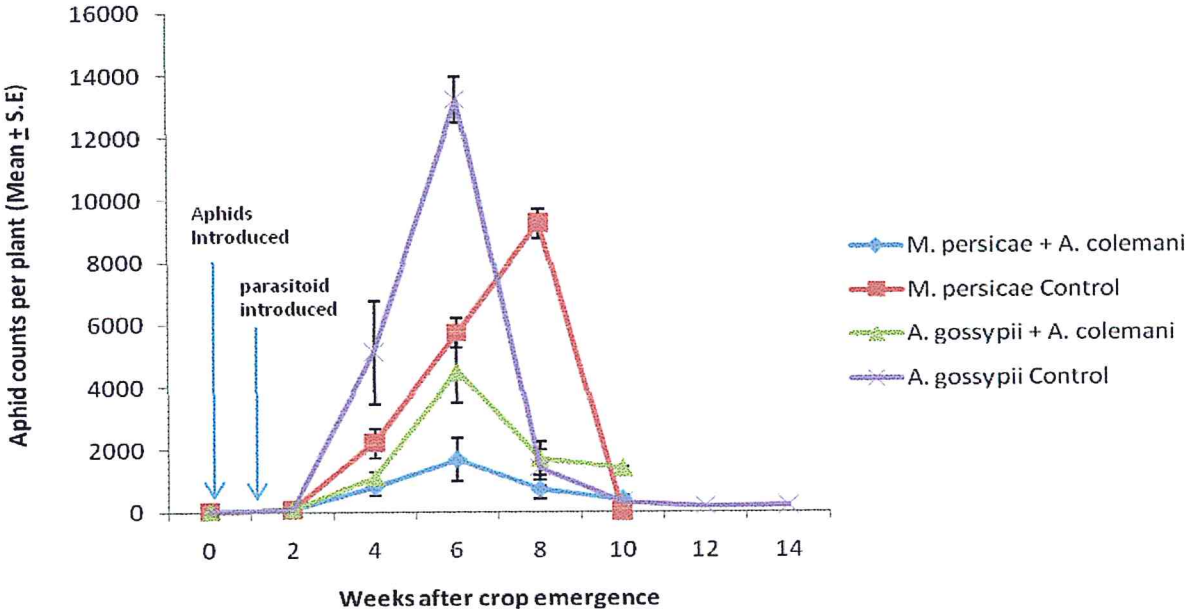

Figure 4.17: Mean (± S.E) aphid population growth per plant after the parasitoid and control treatments

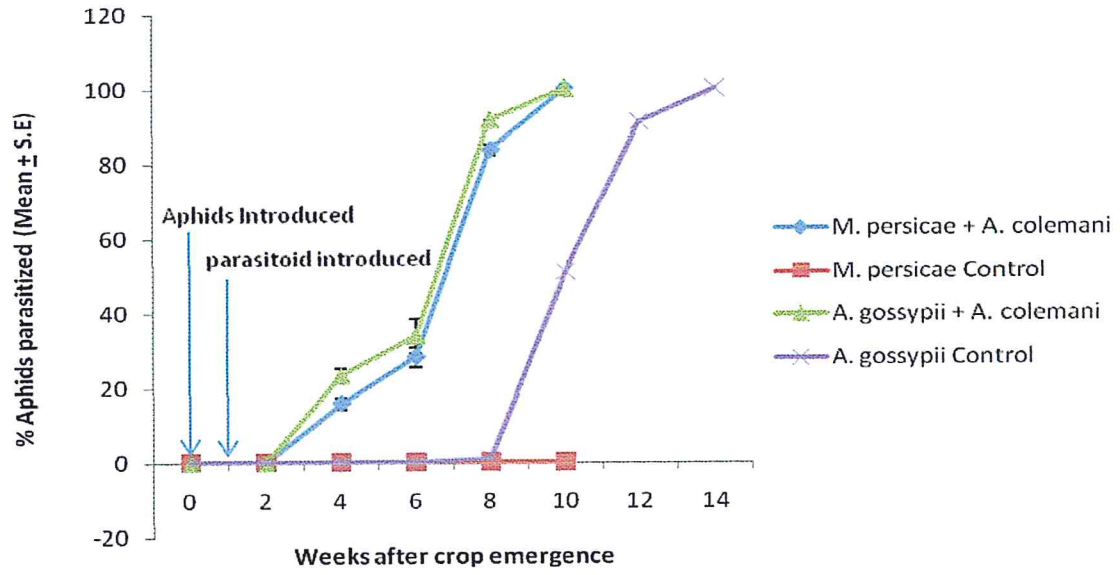

Figure 4.18: Percentage of aphids parasitized per plant after the parasitoid and control treatments

4.3.2.3 Evaluation of the fungal isolate *Metarhizium anisploae* icipe 62 for the control of *Aphis gossypii* and *Myzus persicae* in the green house

The results of the evaluation of entomopathogens for the control of *M. persicae* and *A. gossypii* in the green house are as shown (Fig.4.19) below. From the results, it is clear that the entomopathogen *M. anisploae* icipe 62 was effective in the control of both *M. persicae* and *A. gossypii*. Though it took longer (over 2 weeks) for the effect of the entomopathogen to be seen as opposed to the other biocontrol agents (predators and parasitoids) whose effect appeared in less than the 2 weeks (Section 4.3.2.1 and 4.3.2.2), the control was effective after that period. The difference in the population of aphids in the *M. anisploae* icipe 62 treated plants and that in the control plants (with no treatment with this pathogen) was significant ($P<0.05$). The plants not treated with the *M. anisploae* icipe 62 had much higher aphid population than those where the pathogen was applied for both *M. persicae* and *A. gossypii* aphid species. The mean population of *A. gossypii* rose to a maximum of over 11,500 aphids per plant and that of *M. persicae* to over 6,200 in the control treatments, but the maximum mean aphid population in the pathogen treated plants was only about 1500 aphids per plant for both *A. gossypii* and *M. persicae* species. This translates to over 85% control for *A. gossypii* as the maximum population in the *M. anisploae* icipe 62 treatment was only 12.5% that in the control treatment and 75% control for *M. persicae* as the maximum population in the pathogen treatment was 25 % that in the control treatment (Fig. 4.19). The overall average control of the two aphid species by this pathogen was therefore 81%. These results compared very closely to the results of the evaluation of this pathogen *M. anisploae* icipe 62 in the

laboratory where overall average mycosis for the two aphid species by this pathogen was 81% with mean mycosis for *M. persicae* at 82% and for *A. gossypii* at 80% (Table 4.2 and 4.3)

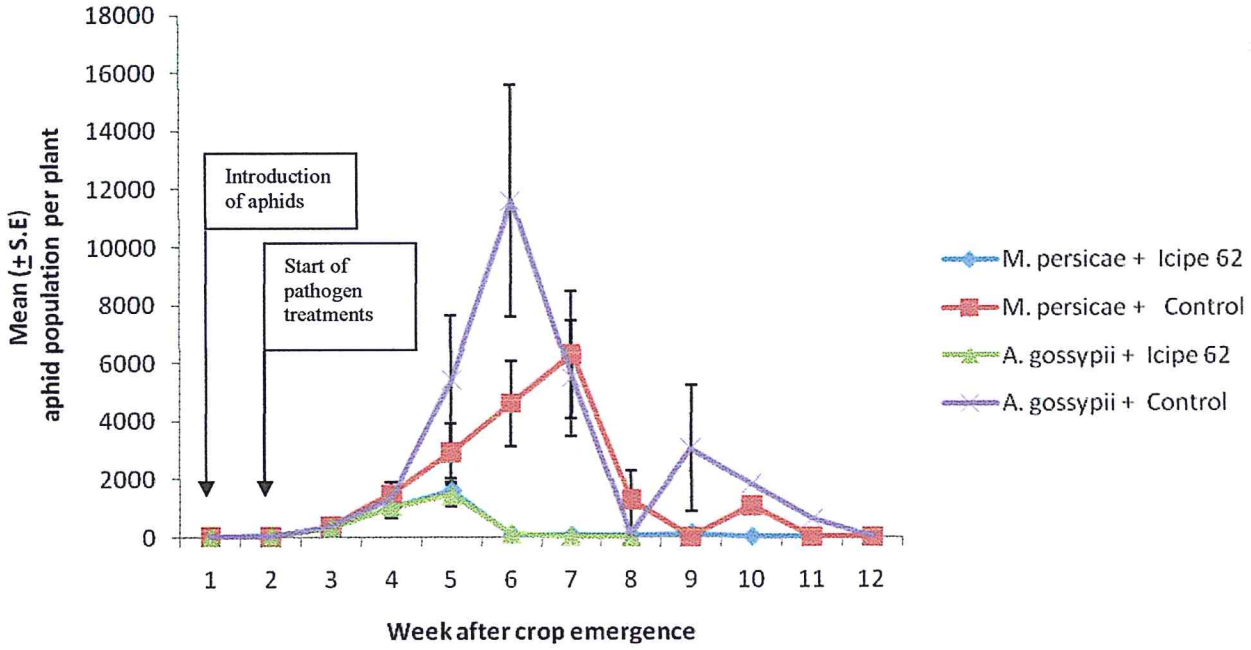

Figure 4.19: Evaluation of the entomopathogen *Metarhizium anisploae* icipe 62 in the control of *Myzus persicae* and *Aphis gossypii*

4.3.2.4 Evaluation of chemical pesticides Lambda cyhalothrin and Dimethoate for the control of *Myzus persicae* and *Aphis gossypii* in the greenhouse

Results of the evaluation of chemical pesticides in the control of *M. persicae* and *A. gossypii* in the green house are as shown in Figs 4.20 to 4.22. From these figures, it is clear that chemical pesticides were the most effective products in the control of aphids

providing upto 100% control. The chemicals were the only aphid control products that reduced the aphid population to zero after just one application in several treated plants. They were more effective in control of *M. persicae* (Fig. 4.20) where all aphids were killed after just one chemical application apart from one plant which had six aphids remaining. However, *A. gossypii* (Fig. 4.21) was more resistant and all plants still had some aphids remaining after one chemical spray with one plant having more than 800 out 2000 still remaining live after the first chemical spray. After two applications, all treated plants had all the aphids killed and the population was zero in all treatments for both *A. gossypii* and *M. persicae* aphid species. The population remained at zero for the next five weeks for *M. persicae* and only reappeared in one plant while no aphid appeared again in the other three plants till the crop matured at more than 14 weeks after the first chemical application. For *A. gossypii* the aphids reappeared after just one week in one of the plants and started buiding up very fast till the population reached over 4000 aphids in just five weeks killing the plant a week later. Aphid re-infestation also occurred in a second plant after being aphid free for five weeks after chemical application and in the third plant after six weeks and started building up both plants. Only one out of the four plants (four replications) remained with no aphid re-infestation for *A. gossypii* till the crop matured.

This shows that the chemical pesticides currently being used in aphid control on potatoes are very effective especially on *M. persicae* species if applied correctly. They are also effective on *A. gossypii* but this species exhibits a lot of resistance to the chemicals as

well as faster reinfestation and build up hence requires more frequent re-applications of the chemicals otherwise, the aphids rebuilds very fast killing the plant much earlier before the natural maturity period of the crop. As shown in fig. 4.22, the aphid population build up was much higher for *A. gossypii* than for *M. persicae* both reaching peak at fifth week after crop emergence for the control plants where no chemicals were sprayed. After introduction of 20 aphids per plant for both *A. gossypii* and *M. persicae* one week after crop emergence, the population of *A. gossypii* had by the fifth week reached a mean of about 9000 aphids which was double that of *M. persicae* which was then at a mean of about 4500 aphids per plant. This led to the death of plants with *A. gossypii* due to high aphid pressure causing all plants to die two weeks later by the seventh week after crop emergence as opposed to treated plants which continued growing upto over 14 weeks after crop emergence. The aphid pressure for this species thus killed the plants when they had barely gone through half of their natural life span. On the other hand, plants with *M. persicae* species survived upto the 12^{th} week after crop emergence before they were all killed by the aphids which was about a month later than for *A. gossypii* infested plants and about a month earlier than for plants treated with the chemical pesticides which had low or no aphids on them hence survived to the natural maturity period of the potato crop. The difference between the mean aphid population for the plants treated with the chemical pesticides and the control plants was significant (P=0.05) for both *A. gossypii* and *M. persicae* aphid species. The difference in the mean aphid populations of *A. gossypii* and *M. persicae* for the control plants not treated with the chemicals was also significant (P=0.01).

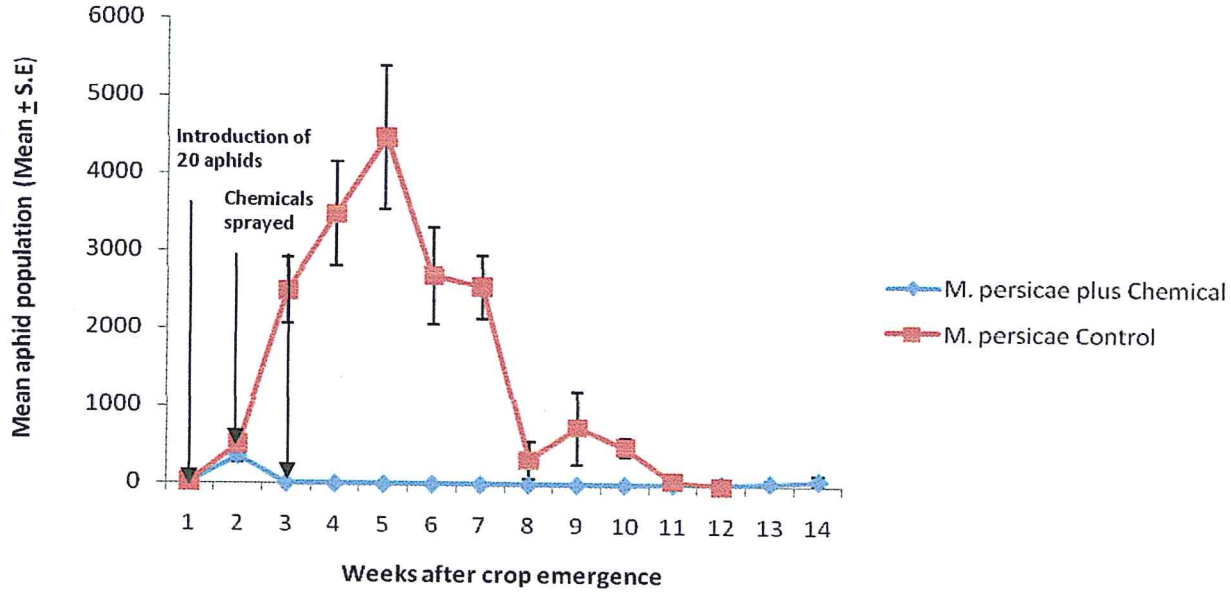

Figure 4.20: Evaluation of chemical pesticides for the control of *Myzus persicae* in the green house

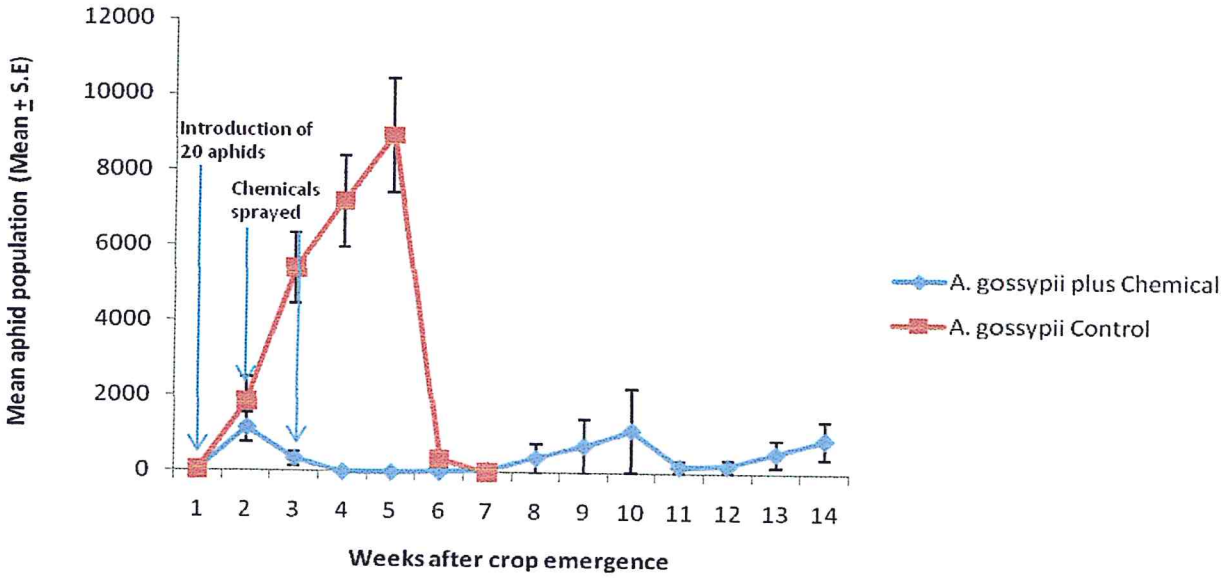

Figure 4.21: Evaluation of chemical pesticides for the control of *Aphis gossypii* in the green house

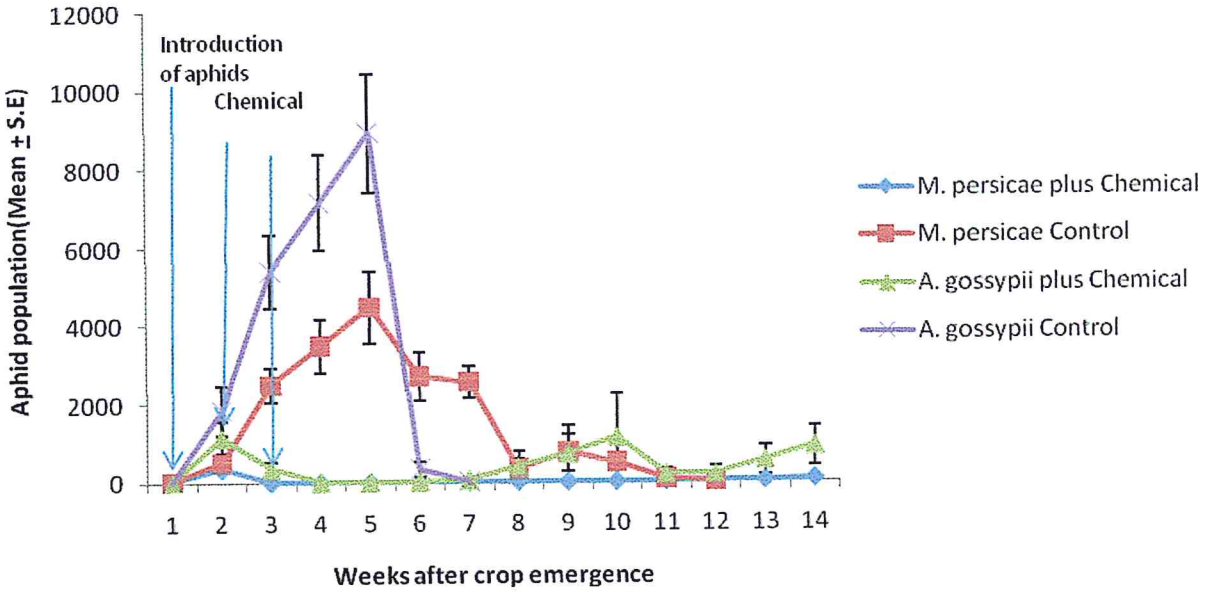

Figure 4.22 Evaluation of chemical pesticides for the control of *Myzus persicae and Aphis gossypii* in the green house

4.3.2.5 Evaluation of aphid natural enemies in the open field

Results of the treatments with the different control agents in the open field during the first season - short rains (figures 4.23 to 4.30) and the second season – long rains (fig. 4.31 to 4.40) clearly showed that in both seasons, all the three biocontrol agents, predators, parasitoids and pathogens were quite effective in the control of aphid populations on the potato crop for both *M. persicae* and *Aphis gossypii* species as compared to the untreated control.

4.3.2.5.1 First Season (Short rains, 2009) results

The first season's results showed that aphid populations had built up to almost their peak by the time the treatments started in the fourth week after crop emergence (Fig. 4.23 to 4.30). However, after the treatments commenced plots treated with biocontrol agents, the predator *H. convergens* (Fig. 4.23 and 4.24), the parasitoid *A. colemani* (Fig. 4.25 and 4.26) and the pathogen *M. anisploae* icipe 62 (Fig. 4.27 and 4.28) all had their aphid populations reduced within a week lower than the populations in the untreated control plots in both the block treated with late blight fungicides and the block not treated with the fungicides for both aphid species *A. gossypii* and *M. persicae*. The decline in aphid populations continued and was significantly lower ($P<0.05$) than the population in the control plots from the second week after the treatments commenced i.e sixth week after crop emergence to the eigth or ninth week after crop emergence. However in this season, the results showed that for the plots treated with the chemical insecticides Duduthrin - Lambda cyhalothrin- and Dimethoate (used on potatoes in the field for the control of aphids), aphid counts were actually highest here sometimes even higher than the counts in the untreated control plots (Fig. 4.29 and 4.30) in the block treated with the late blight control fungicides..

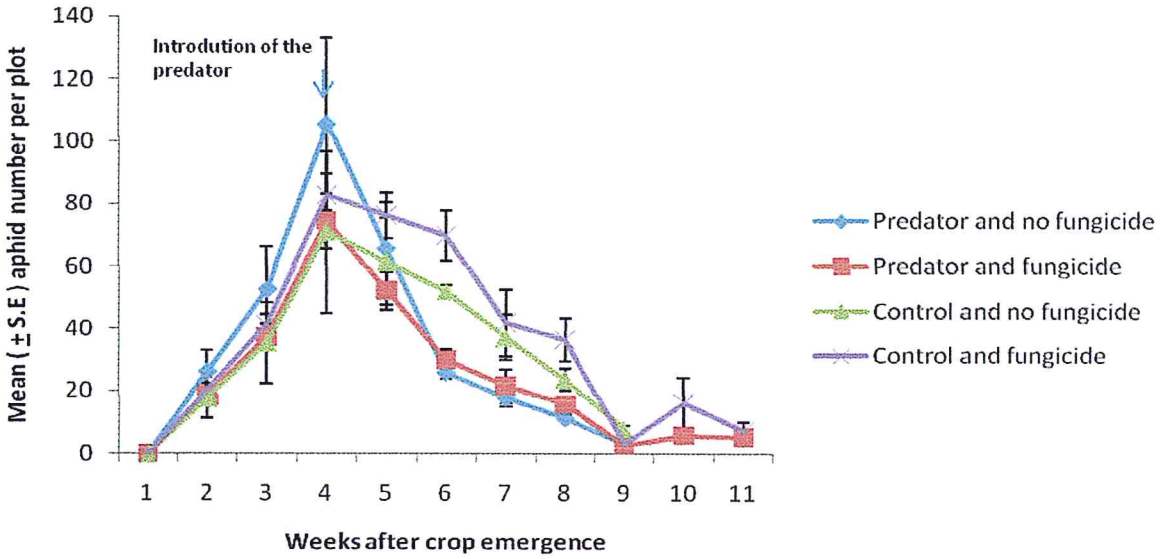

Figure 4.23: Evaluation of efficacy of the predator *Hippodamia convergens* in the control of *Aphis gossypii* on potatoes in the open field during the short rains, 2009

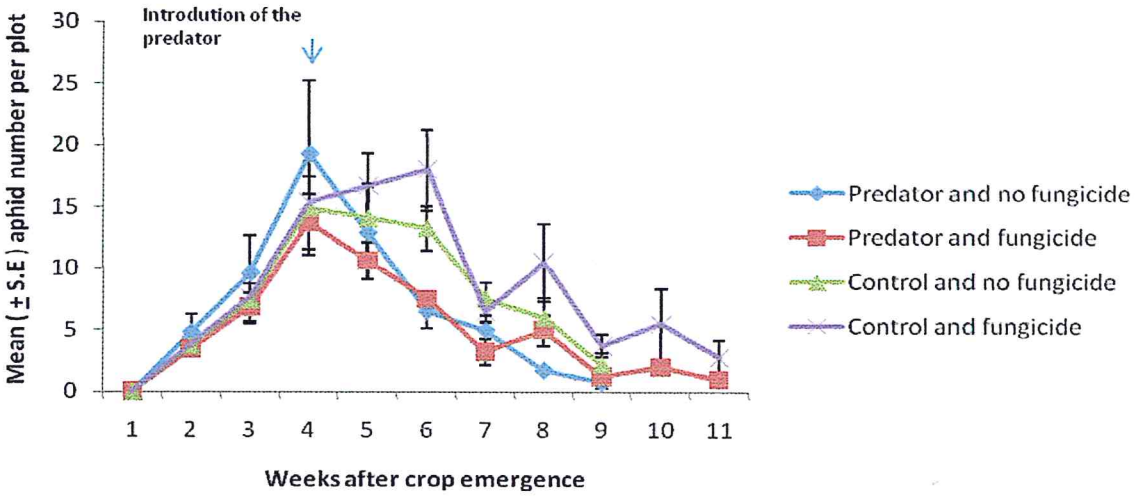

Figure 4.24: Evaluation of efficacy of the predator *Hippodamia convergens* in the control of *Myzus persicae* on potatoes in the open field during the short rains, 2009

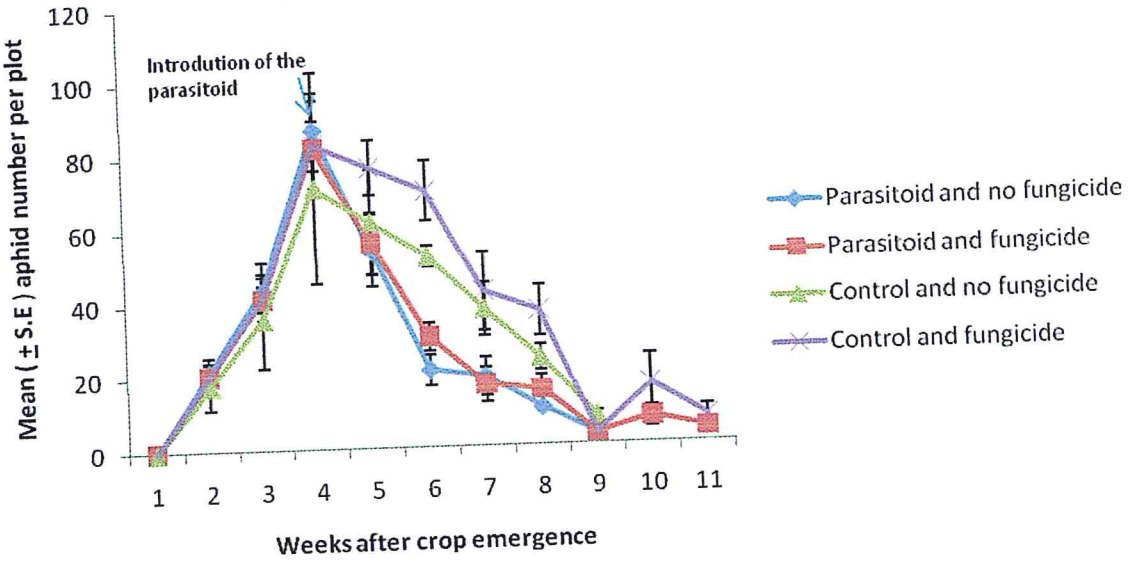

Figure 4.25: Evaluation of efficacy of the parasitoid *Aphidius colemani* in the control of *Aphis gossypii* on potatoes in the open field during the short rains, 2009

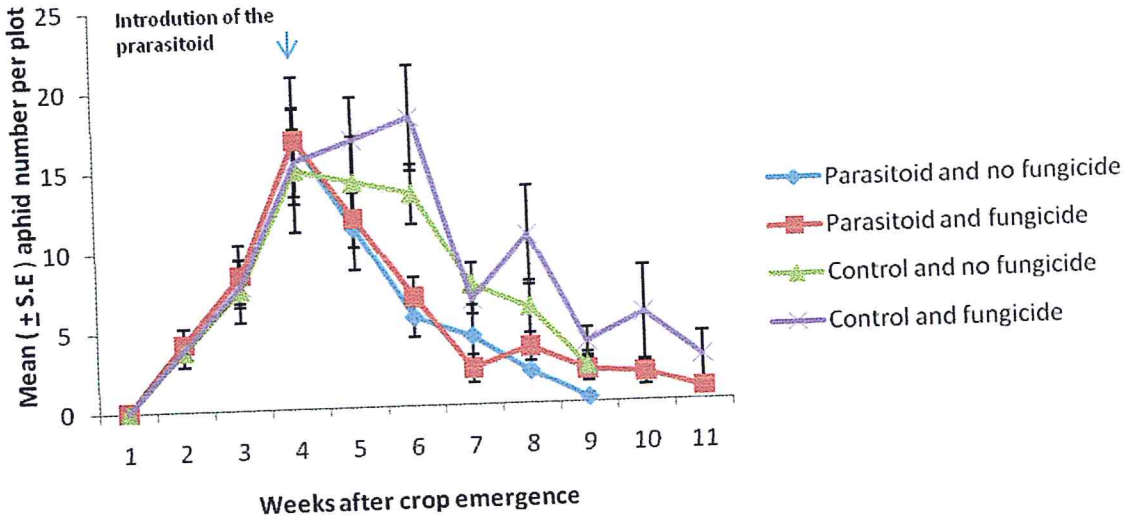

Figure 4.26: Evaluation of efficacy of the parasitoid *Aphidius colemani* in the control of *Myzus persicae* on potatoes in the open field during the short rains, 2009

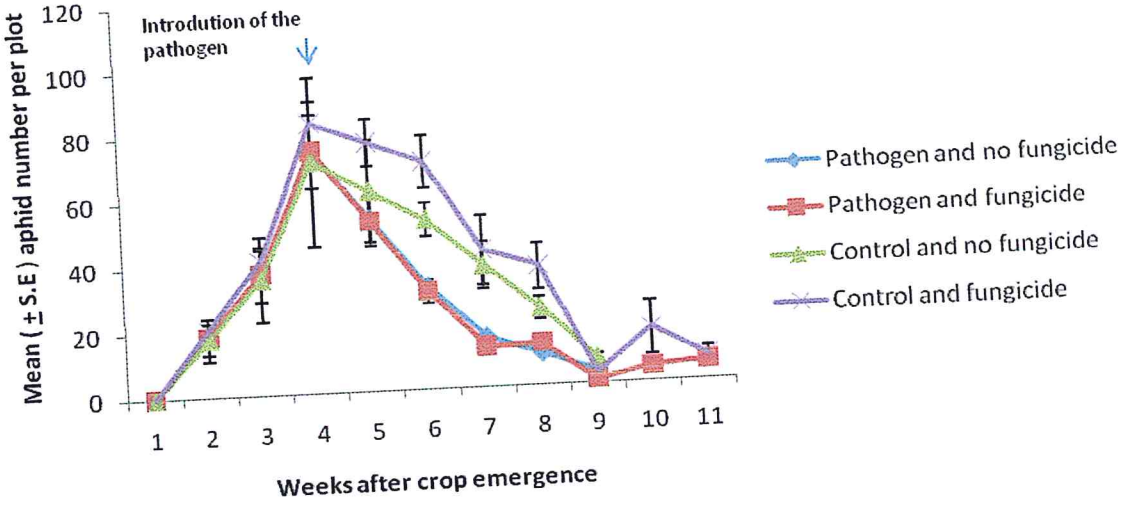

Figure 4.27: Evaluation of efficacy of the entomopathogen *Metarhizium anisploae* icipe 62 in the control of *A. gossypii* on potatoes in the open field during the short rains, 2009

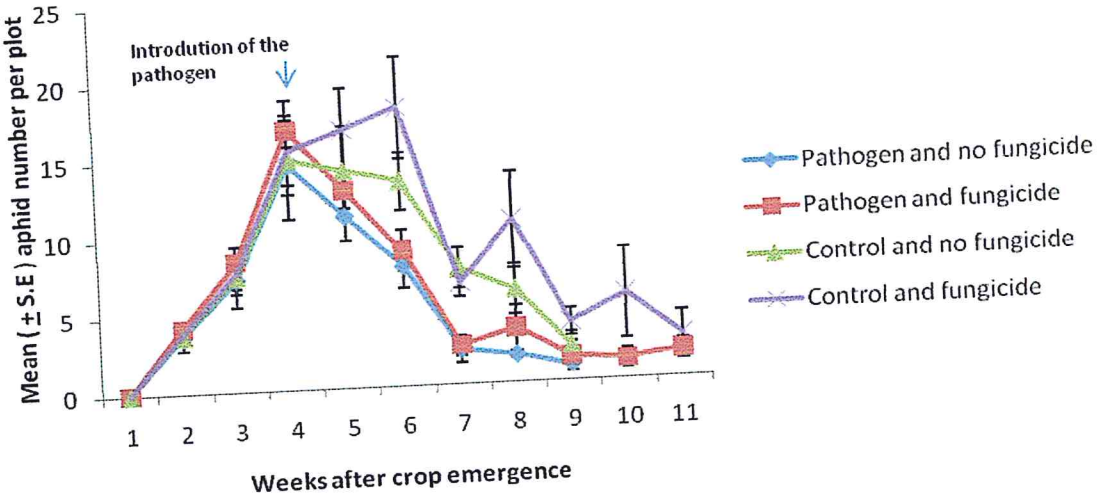

Figure 4.28: Evaluation of efficacy of the pathogen *Metarhizium anisploae* icipe 62 in the control of *Myzus persicae* on potatoes in the open field during the short rains, 2009

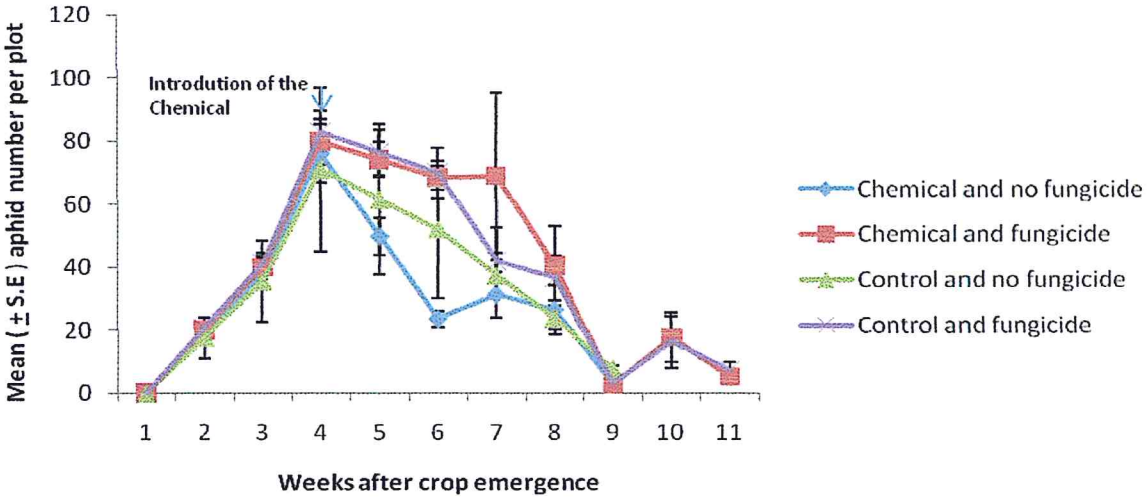

Fig. 4.29: Evaluation of efficacy of the Chemical insecticides Duduthrin and Dimethoate in the control of *Aphis gossypii* on potatoes in the open field during the short rains, 2009

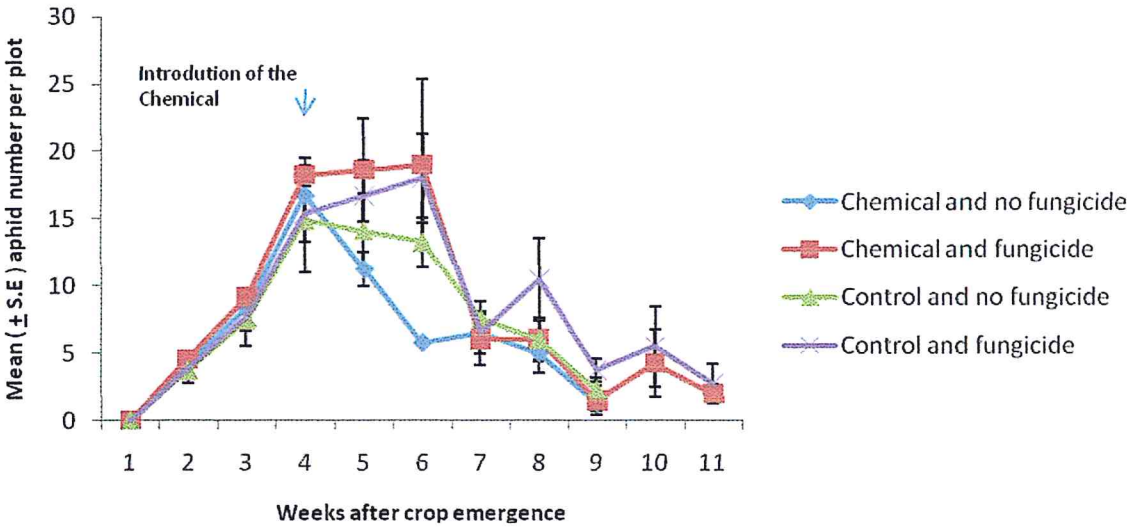

Figure 4.30: Evaluation of efficacy of the Chemical insecticides Duduthrin and Dimethoate in the control of *Myzus persicae* on potatoes in the open field during the short rains, 2009

4.3.2.5.2 Second Season (Long rains) results

In the second season (long rains, 2010) all aphid control treatments including the chemicals were effective as the aphid populations in the treatments were all lower than the populations in the control (Fig. 4.31 to 4.34). For *A. gossypii,* the differences in aphid populations between the control and the treatments were highest and significantly different ($P<0.05$) from the third to seventh week after which the aphid population collapsed to almost zero from the eighth week for all the treatments as well as for the control. For *M. persicae*, the population build up was slow initially and though the control plots had higher populations than the treatments, this was minimal and not significantly different ($P>0.05$) until the eighth week when their population shot up after the population of *A. gossypii* had collapsed. The differences in aphid populations between the control and the treatments for *M. persicae* were highest and significantly different ($P<0.05$) from the seventh to the tenth week after which the population also decreased to almost zero from the 11^{th} week after crop emergence for all the treatments as well as for the control.

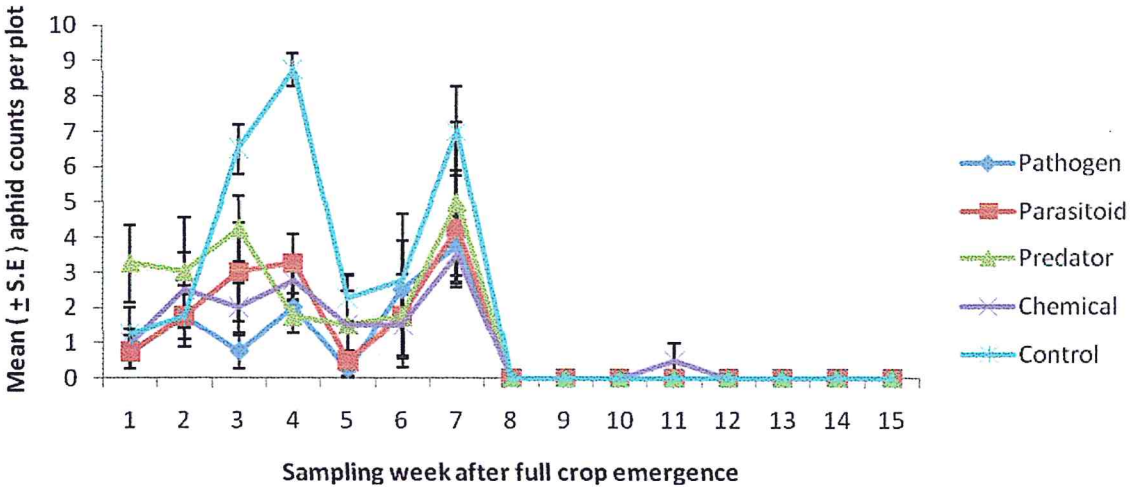

Fig. 4.31: *Aphis gossypii* counts (Mean ± S.E) for the different aphid control treatments on potatoes at the fungicide sprayed block in the open field during the long rains, 2010

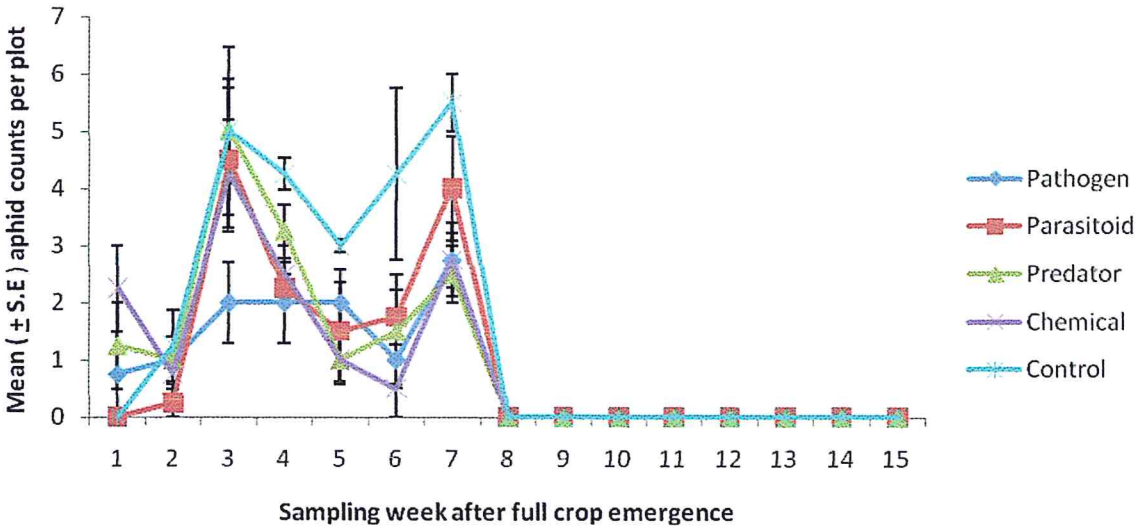

Figure 4.32: *Aphis gossypii* counts (Mean ± S.E) for the different aphid control treatments on potatoes at the block not sprayed with fungicides in the open field in the long rains, 2010

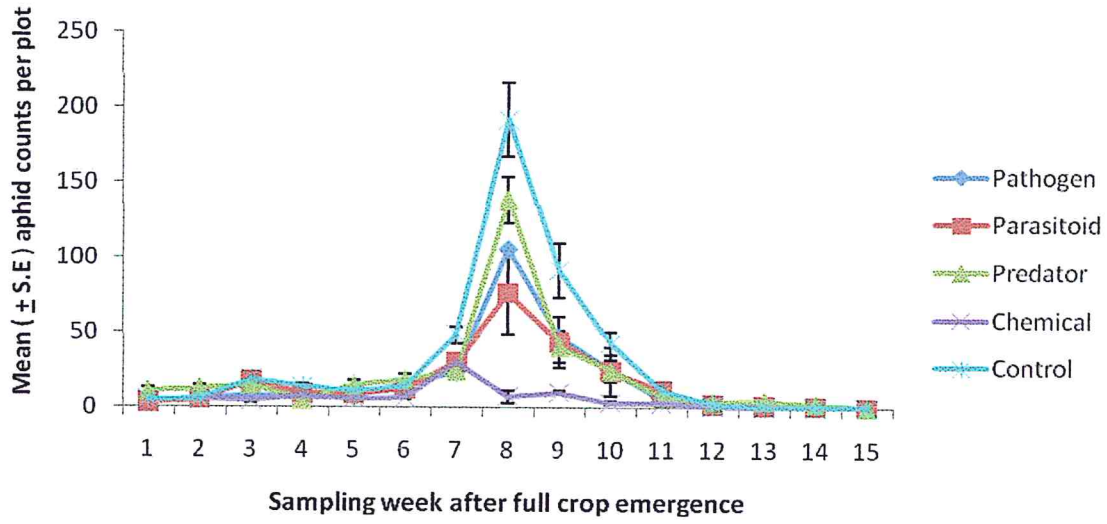

Fig. 4.33: *Myzus persicae* counts (Mean ± S.E) for the different aphid control treatments on potatoes at the fungicide sprayed block in the open field during the long rains, 2010

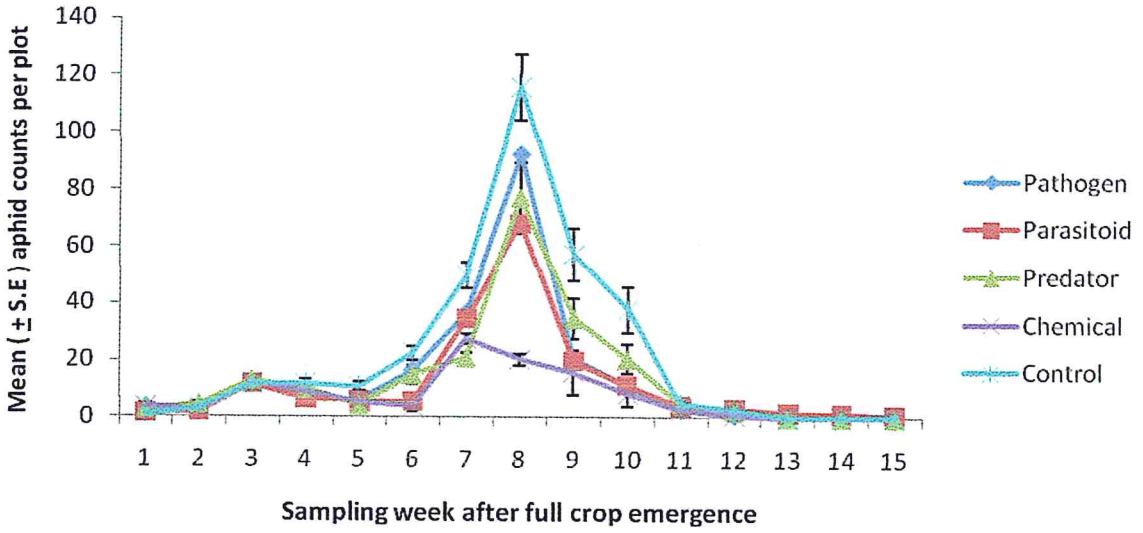

Figure 4.34: *Myzus persicae* counts (Mean ± S.E) for the different aphid control treatments on potatoes at the block not sprayed with fungicides in the open field in the long rains, 2010

4.3.2.5.3 Seasonal means for the different treatments

Comparing the overall performance of the different treatments for aphid control for the two species *M. persicae* and *A. gossypii*, (Fig. 4.35 to Fig. 4.40), the pathogen (*M. anisopliae* icipe 62 isolate) performed best in the control of *A. gossypii* with the mean aphid counts in plots treated with the pathogen being significantly lower ($P < 0.05$) than the aphid counts in all other treatments. This was followed by the parasitoid (*A. colemani*) and chemical (Dimethoate and Duduthrin) treatments whose mean aphid counts over the second season though not significantly different ($P>0.05$) from one another, were significantly lower than aphid counts in plots treated with the predator (*H. convergens*) and the control plots ($P < 0.05$). Predators ranked fourth in their effectiveness in the control of *A. gossypii* but were also effective compared to the control since the mean aphid counts in plots treated with the predator were also significantly lower ($P<0.05$) than aphid counts in the control plots. This ranking of the different aphid treatments was the same in the blocks sprayed with late blight fungicides as well as in the block not sprayed with the fungicides (Fig. 4.35 and 4.36).

For *M. persicae* species, the aphid treatment that performed best was the chemical spray whose mean aphid counts were significantly lower ($P< 0.05$) than the aphid counts in all the other plots. This was followed by the parasitoid treatment then the pathogen and lastly the predator. All these treatments' mean aphid counts were significantly different from one another and also significantly lower than the mean aphid counts in the control

($P < 0.05$). This was the case in both the fungicide treated block and the block not treated with fungicides apart from the predator and parasitoid treatments which were not significantly different from one another in the block not sprayed with the fungicide (Fig. 4.37 and 4.38).

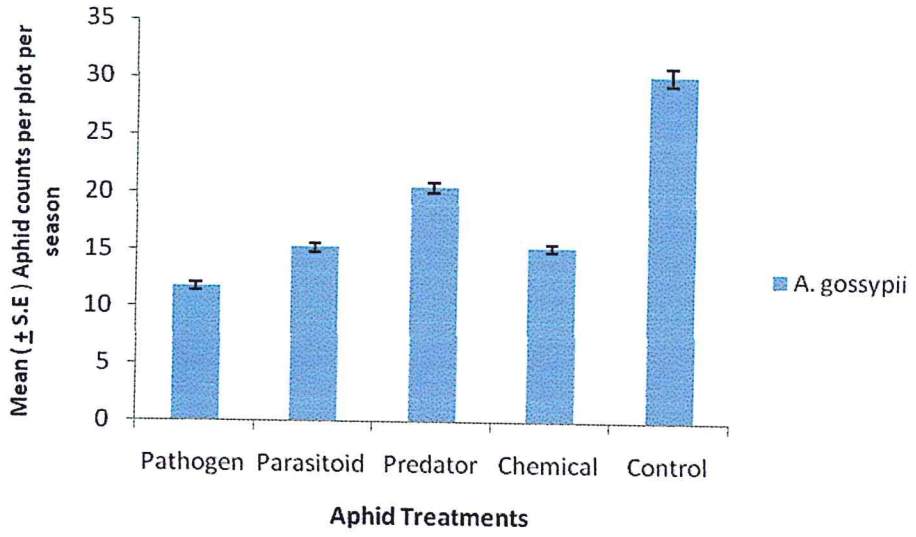

Figure 4.35: *Aphis gossypii* seasonal mean (± S.E) counts per plot for the different treatments in the fungicide sprayed block during the second season (Long rains, 2010)

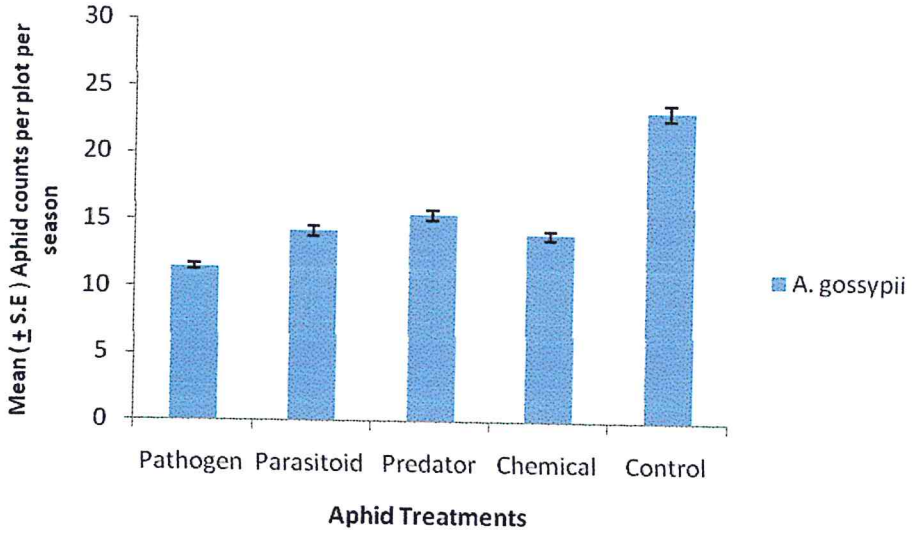

Figure 4.36: *Aphis gossypii* seasonal mean counts (± S.E) per plot for the different treatments in the block not sprayed with fungicides during the second season (Long rains, 2010)

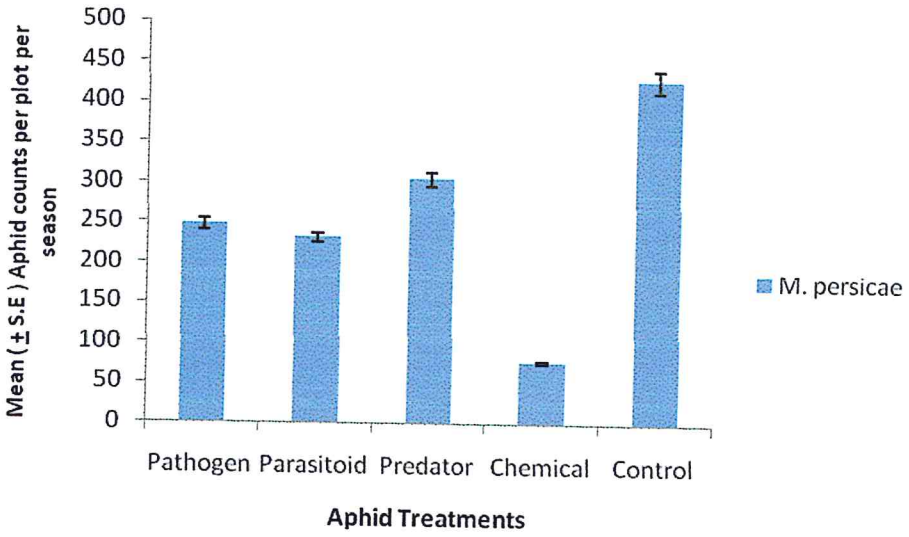

Figure 4.37: *Myzus persicae* seasonal mean counts (± S.E) per plot for the different treatments in the fungicide sprayed block during the second season (Long rains, 2010)

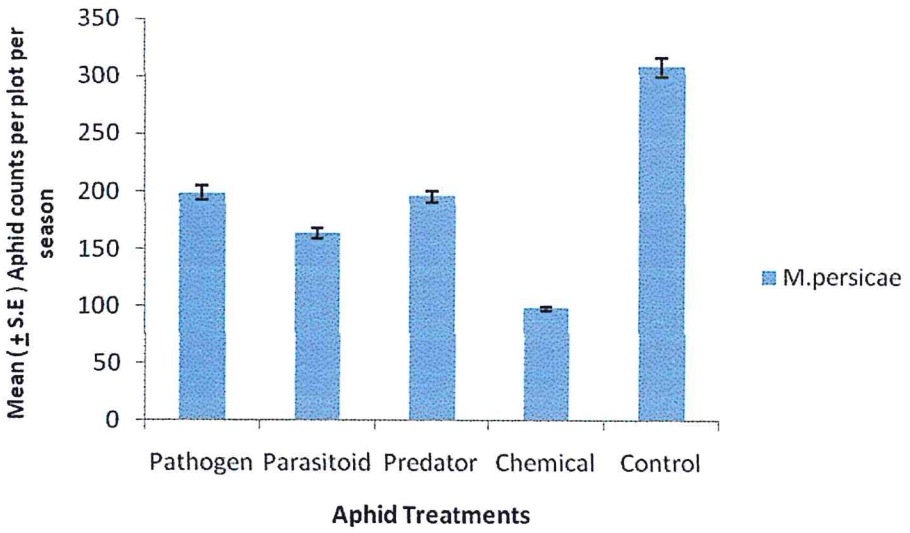

Figure 4.38: *Myzus persicae* seasonal mean counts (± S.E) per plot for the different treatments in the block not sprayed with fungicides during the second season (Long rains, 2010)

4.3.2.5.4 Comparison of the treatments in the blocks sprayed and not sprayed with fungicides

For both seasons, the results of the blocks where the potatoes were sprayed with late blight control fungicides, Dithane M45 and Ridomil as recommended in the field showed that the fungicide treated plots had slightly higher aphid populations than the plots where the late blight fungicides were not applied for the three biocontrol agents predators, parasitoids and pathogens (Fig. 4.39 and 4.40). The effect was however more pronounced in the plots treated with the chemical pesticides for control of aphids in the first season but not in the second season. The plots where the fungicide and the chemical

insecticides were both applied had much higher aphid populations than the plots where only the chemical pesticide was applied but no fungicide. Infact, plots with both fungicide and chemical insecticide treatments had the highest aphid populations even higher than the control plots in the first season (short rains December 2009 – February 2010). It is however worth to note that the fungicide treated plots remained green for over two weeks more after the crop in the block not sprayed with the late blight fungicides had dried up.

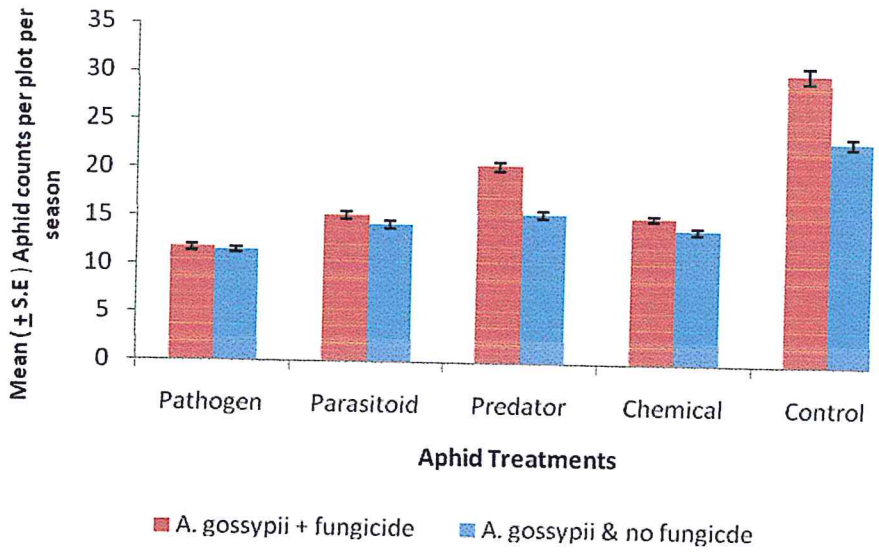

Figure 4.39: *Aphis gossypii* seasonal mean counts (± S.E) per plot for the different treatments in the blocks sprayed and not sprayed with fungicides during the second season (Long rains, 2010)

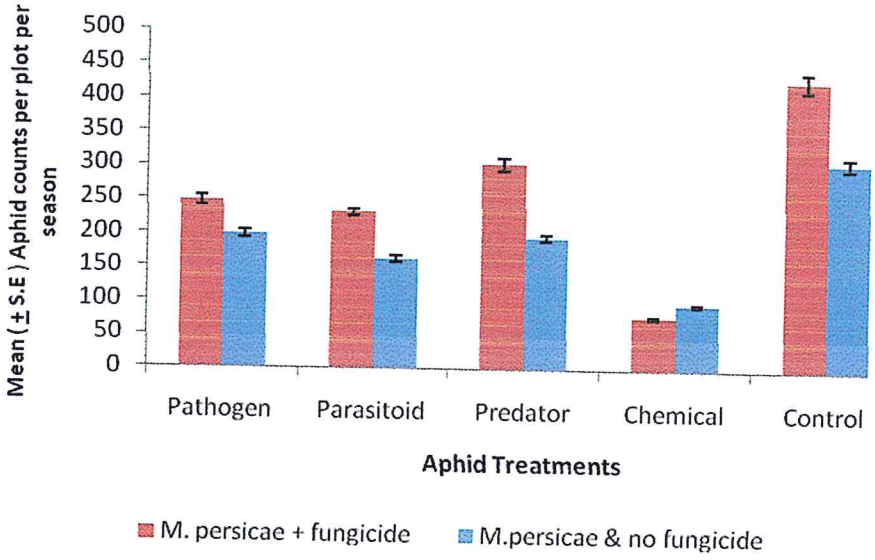

Figure 4.40: *Myzus persicae* seasonal mean counts (± S.E) per plot for the different treatments in the blocks sprayed and not sprayed with fungicides during the second season (Long rains, 2010)

4.3.2.6 Discussion

From the results above, it was clear that in both seasons, all the three biocontrol agents, predators, parasitoids and pathogens were quite effective in the control of aphid population on the potato crop for both *M. persicae* and *A. gossypii* species as compared to the untreated control. The first season's results for the plots treated with the chemical pesticides used on potatoes in the field for the control of aphids were unepected. Whereas this was where it was expected to have the lowest or no populations of aphids after the treatments, it turned out that aphid populations were actually highest here sometimes even higher than the populations in the untreated control plots. This shows that these chemicals were completely ineffective for the aphid control and they could

have even affected other natural enemies of aphids leading to aphid populations building even higher than the untreated control. If this happened in the field, it could be a tragedy to farmers who will have spent extra resources to treat their crop with the chemical pesticides only to end up with even higher aphid populations than when no aphid control measure was applied. This finding agrees with some earlier research findings. For example, cotton fields treated with sulprophos had elevated numbers of *A. gossypii* than untreated fields (Kerns and Gaylor, 1993). Cotton plants treated with disulfoton, phorate, dimethoate and lindane had bigger aphids than untreated plants, (Sithanantham *et al.*, 1973) Okra treated with phorate had more aphids which were also physically bigger than those from untreated okra (Regupathy and Jayaraj, 1994).

However, in the second season (long rains, 2010) all treatments for control of the aphids including the chemicals were effective as the aphid populations in the treatments were all lower than the populations in the control.

For both seasons, the results of the blocks where the potatoes were sprayed with late blight control fungicides, Dithane M45 and Ridomil as recommended in the field showed that the fungicide treated plots had slightly higher aphid populations than the plots where the late blight fungicides were not applied for the three bio-control agents, predators, parasitoids and pathogens. The effect was however more pronounced in the plots treated with the chemical pesticides for control of aphids in the first season but not in the second season. The plots where the fungicide and the chemical insecticides were both applied had much higher aphid populations than the plots where only the chemical

pesticide was applied without fungicide. In fact, plots with both fungicide and chemical insecticide treatments had the highest aphid populations even higher than the control plots in the first season (short rains, 2009), which shows that the fungicides reduces the effect of the chemical pesticides more than the natural enemies in the control of aphids on the potato crop.

It is however worth to note that the fungicide treated plots remained green for over two weeks after the crop in the block not sprayed with the late blight fungicides had dried up due to the protection the fungicides offer to the potato crop against infection by late blight disease. This shows that the fungicides are important in potato growing and hence should be used with aphid control measures that are more compatible with these fungicides. Hence the bio-control agents used here are more compatible with fungicides for aphid control than the conventional chemical pesticides currently in use. These results show that the late blight fungicide use is much more compatible with the bio-control agents for the control of the aphids than it is with the chemical insecticides.

4.3.2.6.1 Predators

Both predator species were highly effective in the predation of the two aphid species as shown in section 4.3.1.1 and 4.3.2.1 with no significant difference between the aphid predation rate by the two predator species $P>0.05$). For both species, the predation rate increased with increase in the prey density. However, at low aphid prey density, the consumption rate was much lower as the predators seemed not able to trace their prey as

fast. This showed that these predators prey on the two aphid species more by coming into contact with them rather than by actively searching for them. They would be therefore more useful in aphid control where aphid populations are high to rapidly reduce their populations. However, at low aphid populations, other aphid control measures would be required to prevent the aphid populations from increase because the predators will turn to other sources of food when aphid populations are low as studies have reported that coccinellid predators have several sources of food (IPM, 2007). It has been reported that Ladybirds (Coleoptera: Coccinellidae) are major predators of aphids and both the adults and the larvae are predatious. However, adults can also feed on pollen, nectar or fungi though feeding on aphid prey is required for egg production (Sundby 1968). The predatory lacewing *Dichochrysa prasina* Burmeister (Neuroptera: Chrysopidae) has also been reported to favour nymphs of *M. persicae* as the most preferred prey for enhancing development and adult longevity and fecundity (Pappas *et al.*, 2007). However, the prey is recognised by direct contact and prey search is random and in periods of prey absence, cannibalism occurs (Canard and Duelli, 1984).

In some studies, it has been reported that most, if not all, coccinellids rely on non-prey foods as part of their life history (IPM, 2007). Under field conditions, even the most stereotypical entomophagous coccinellids consume sugar, pollen, fungus, fruit and vegetation, often as an integral part of their diets. Non-prey foods support survival in the absence of prey, improve reproductive capacity, and increase survival through quiescent periods (Wade *et al.*, 2008). The importance of non-prey foods to coccinellids provides

opportunities for pest managers to manipulate the abundance of coccinellids as biological control agents and increases biological control of key pests by coccinellid predators (Wade *et al.*, 2008; Evans, 2009; Lundgren, 2009).

In this study, the population of *H. convergens* was very high in the field as compared to that of *H. axyridis*. For this reason, this species was chosen for use in the on-station field evaluation of predators for the control of the two aphid species.

4.3.2.6.2 Parasitoids

The parasitoid used in this experiment proved very efficient in the search and pasitization of both *M. persicae* and *A. gossypii* in the green house as well as in the open field. Unlike the chemical pesticides which required proper coverage of the foliage for them to be effective against the aphids and also unlike the predators which consumed aphids only when the aphid was within a short distance from the predator, the parasitoid *A. colemani* was able to actually search for the aphids from a distance upto over five meters (width of the green house) in the green house and even more in the open field whether the aphids were on the upper or lower side of the potato leaves. This shows that this parasitoid *A. colemani* is very effective in the control of the two aphid species *M. persicae* and *A. gossypii* especially when it is introduced early when the crop is young and the aphid populations just begginning to establish due to its searching efficiency..

These results agree with some earlier research findings where experiments were performed in small glasshouses for the control of aphids on cucumbers (*Cucumis sativus* L. cv. 'Aramon') with three parasitoids, *A. colemani*, *E. cerasicola* and *L. testaceipes*. As in the laboratory test *A. colemani* performed best; significantly more colonies were found and parasitization rates in the colonies were higher by *A. colemani* than by *E. cerasicola* and *L. testaceipes*. *Aphidius colemani* Viereck parasitized 72 to 80 percent of the aphids (steenis van, 2009).

It was found that, the parasitoid has a fecundity of 302 eggs per female at 20°C and 388 eggs per female at 25°C, a developmental period of 12.7 days at 20°C and 10.0 days at 25°C, and an immature mortality of 14.1 % at 20°C and 27.8 % at 25°C. The intrinsic rate of increase of the parasitoid is both at 20 and at 25°C comparable to the intrinsic rate of increase of the cotton aphid. *Aphidius colemani* seems, therefore, a promising candidate for biological control of cotton aphid. (Steenis van, 2009).

Because of the good correspondence between laboratory and glasshouse experiments, it is suggested that bad performance of an aphid parasitoid species in a simple laboratory trial might be sufficient evidence to disregard this species for further tests (Steenis van, 1995).

Aphids, successfully parasitized by *Aphidius colemani* Viereck (Hymenoptera: Braconidae) only reproduced when they were parasitized after the third instar. Fecundity was 0.1 to 0.9 and 10.5 to 13.3 nymphs/♀ for aphids parasitized in the fourth instar or as

adults, respectively. Reproduction of aphids that were stung but survived the attack was lower than for aphids not stung. Average longevity of these aphids was equal to the longevity of aphids not stung by *A. colemani* (Steenis van and El-Khawass, 1995).

4.3.2.6.3 Pathogens

All the fungal isolates tested were pathogenic to adult *M. persicae* and *A.gossypii*. However, there was a variation in the virulence for the different fungal isolates. Such variations have previously been reported with different host species including leaf miners (Migiro *et al.*, 2010), termites, (Mburu *et al.*, 2009), sweet potato weevils, (Ondiaka *et al.*, 2008), tomato spider mites, (Wekesa, *et al*, 2006) and Stem borers, (Maniania, 1992) among other hosts. This emphasizes the need for strain selection (Soper and Ward, 1981). *Metarhizium anisopliae* and *B. bassiana* are ubiquitous pathogens recorded on many hosts (Veen, 1968) and can be, therefore, tested against insects that are not associated with them in nature and developed as biopesticides (Ondiaka *et al.*, 2008). In this study, all *M. anisopliae* isolates performed very well in their virulence against the two target aphid species and included the most virulent isolate ICIPE 62 against both *M. persicae* and *A.gossypii*, yet none of the tested isolates originated from the host. These results agree with earlier findings e.g by Migiro, *et al.*, (2010) where in their study, none of the tested isolates originated from the host or closely related species but some isolates of both *M. anisopliae* and *B. bassiana* were highly virulent to the target insect, the pea leaf miner, *Liriomyza huidobrensis*

(Blanchard) (Diptera: Agromyzidae). This shows that isolates of diverse origin can be equally pathogenic to other host insects.

On the other hand, it is generally admitted that strains isolated from a particular host remain highly virulent to that host (Ferron *et al.*, 1972; Fargues, 1976; Maniania, 1992). In the current study, this was also true for *B. bassiana* isolates. Strains isolated from aphids collected from potatoes in the field generally performed better than the isolates obtained from the icipe germplasm centre which had originally been isolated from other hosts. This underlines the importance of careful selection of fungal isolates for use as microbial agents.

4.3.2.6.4 Pesticides

From the results above, it is clear that both chemical pesticides used, Dimethoate and Duduthrin were very effective in the control of both *M. persicae* and *A. gosssypii* aphid species in the laboratory and in the green house, however, in the open field the effectiveness varied. The chemical pesticides were not effective in the first season but were effective in the second season. This could be attributed partly to the effectiveness of foliar coverage by the chemical pesticide during spraying whereby all aphids on both sides of leaves came to direct contact with the chemical pesticide by spraying both the upper and lower side of the leaves in the second season,. Aphids reside on the lower side of the potato leaves. Whereas in the first season the spray application of the pesticides

was done typically on the top side of the plants as is the practice by farmers at the farm level, the application was done differently in the second season after results in the first season showed that the pesticide treatment was not effective.

In the second season deliberate effort was made to spray both the upper side as well as the lower side of the potato leaves so as to ensure direct contact of the pesticide to the aphids on the lower side of the potato leaves. This better coverage of the foliage gave much better results for the pesticide treatment which was now as effective as the treatments with the biological control agents all of which had significantly lower aphid populations than the control treatments. The results show that, if a farmer has to use chemical insecticides, it has to be ensured that spray coverage is done both on the upper and lower side of the potato leaves otherwise, the treatments will be a waste of funds as spraying only in the upper side leaves most of the aphids on the lower side of the leaves live which then multiply creating even more resistant aphid populations due to the suboptimal level of the chemical pesticides exposed to these aphids (Shi *et al.*, 2011).

CHAPTER FIVE

5.0 EVALUATION OF COMPATIBILITY OF BIOLOGICAL CONTROL AGENTS *APHIDIUS COLEMANI*, *HIPPODAMIA CONVERGENS* AND *METARHIZIUM ANISOPLIAE* ICIPE 62 WITH COMMON PESTICIDES USED IN POTATO PRODUCTION IN KENYA

5.1 Introduction

Aphids are one of the main agricultural pest groups (Aeschlimann *et al.*, 1993). Considerable research has been done on their natural enemies, and it has been clearly established that aphid pathogens, predators and parasites can limit the incidence of aphids and, in some cases, reduce insecticide use by varying degrees. Unfortunately, aphid natural enemies are directly exposed to pesticides, and the use of non-selective products can greatly affect their performance, resulting in rapid aphid outbreaks Vickerman and Sunderland (1977), Horn, (1983), Borgemeister and Poehling, 1989 . To derive the maximum benefit from the activity of aphid natural enemies and limit insecticide application, the use of selective products is recommended in the context of organic farming and the development of integrated pest management (IPM). However, various laboratory studies indicate that most insecticides are toxic to several species of beneficial arthropods (Ledieu *et al.*, 1989, Castagnoli *et al.*, 2005, Medina *et al.*, 2007, Duso *et al.*, 2008, Kraiss and Cullen, 2008). Although the effects of these

products in the field are probably limited over time owing to their instability (Crosby, 1995), the question arises as to the impact of such toxic products on beneficial arthropods. There is a paradox between on the one hand trying to maximise the activity of pests' natural enemies and on the other hand affecting their populations by non-selective products.

Integrated pest management (IPM) has been described as a process involving compatible use of multiple tactics for simultaneous control of all classes of pests while considering economics and the environment (Prokopy, 2003). This description suggests that pest management tactics should be used in an integrated sense, and as such, should not interfere with each other. Integration and integration failure can be vertical (within one class of pests) or horizontal (among classes of pests) (Ehler, 2006). Applications of insecticides have been shown to disrupt the efficacy of predatory natural enemies (Hattingh and Tate, 1995; Grafton-Cardwell and Gu, 2003), exhibiting vertical integration failure, and fungicide applications have been observed to reduce local abundance of predators and parasitoids of arthropod pests (Martinson *et al.*, 2001; Michaud, 2001), exemplifying horizontal integration failure. These concepts can be applied to disease management as well. Indeed, it has been demonstrated that fungicide applications can be detrimental to disease biocontrol programs involving fungal antagonists (Fravel *et al.*, 2005; Tobin et *al.*, 2008). Discussions of IPM disruption, however, have yet to address instances of vertical integration failure with respect to mycophagous arthropods. Biological control of plant pests may offer solutions to

resistance and other pesticide-related problems such as residues in food crops, worker health and safety, and negative effects to non-target organisms.

To assess the effect of the commonly used chemical pesticides on the predators, parasitoid and entomopathogen evaluated in this study for their efficacy in the control of the two aphid species on the potato crop, specific experiments were set up in the Laboratory. The fungicides used for spraying against late blight in potatoes ie Ridomil MZ and Dithane M45 were assessed for any effect on the fungal entomopathogen *M. anisopliae* icipe 62, while the chemical insecticides used against aphids on potatoes i.e Duduthrin and Dimethoate were assessed for their effect on the two insect bio-control agents i.e the predators *H. convergens* and *H. axyridis* and the parasitoid *A. colemani*.

5.2 Materials and Methods

Experiments for the evaluation of compatibility of *aphidius colemani, hippodamia convergens* and *metarhizium anisopliae* icipe 62 with common pesticides used in potato production were done as described at section 2.3 (2.3.1 to 2.3.3).

5.3 Results

5.3.1 The compatibility of fungal entomopathogen *Metarhizium anisopliae* icipe 62 with fungicides Ridomil and Dithane

Results of this evaluation are as shown in figures 5.1 to 5.4. The results show that, the two fungicides, Ridomil and Dithane, used locally for the control of the fungal pathogen *Pytophthora infestans* that causes late blight in potatoes also affect other fungi on the potatoes significantly. In all assessments, the test entomopathogen *M. anisploae* icipe 62 caused significantly higher mycosis ($P<0.05$) on the two aphid species *A. gossypii* and *M. persicae* when applied alone on the aphids than in the treatment where either Ridomil or Dithane were sprayed additionally after spraying the aphid species with the test entomopathogen *M. anisploae* icipe 62. The mycosis in the treatments where the test entomopathogen *M. anisploae* icipe 62 was applied alone was also significantly higher ($P<0.05$) than in the control treatment where the aphids were only sprayed with triton water. The results also show that the two fungicides also affected other fungi on the aphids. This is clearly evident because mycosis in the treatments where the two fungicides were applied was significantly lower ($P<0.05$) than the mycosis in the control treatments. This shows that apart from these fungicides killing the introduced fungal entomopathogen icipe 62, they also killed other fungi that were already on the aphids used in the experiment hence leading to almost total eradication of any fungi and thus very low (almost zero) percent mycosis in all treatments where fungicides were used (Fig.5.1 – 5.4).

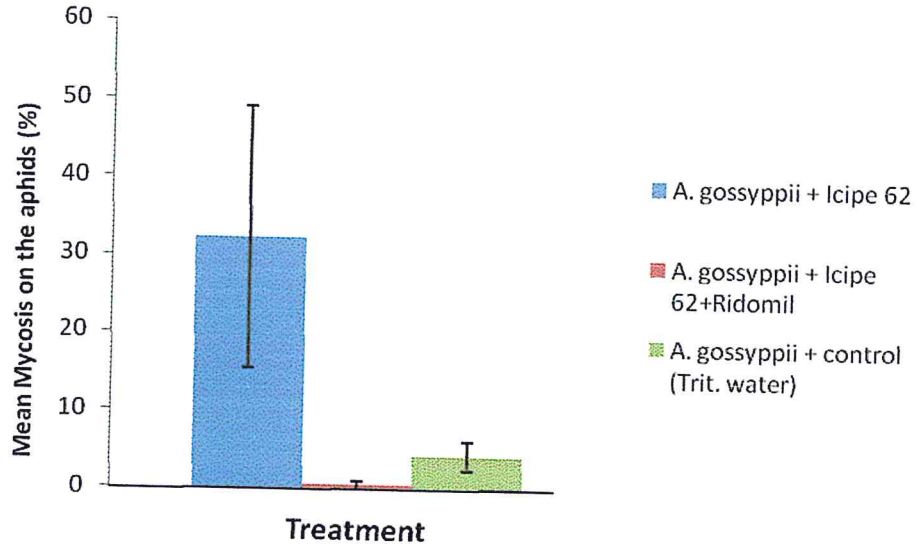

Figure 5.1: Effect of Ridomil fungicide on the entomopathogenic fungi *Metarhizium anisploae* icipe 62 sprayed on *Aphis gossypii* in the laboratory.

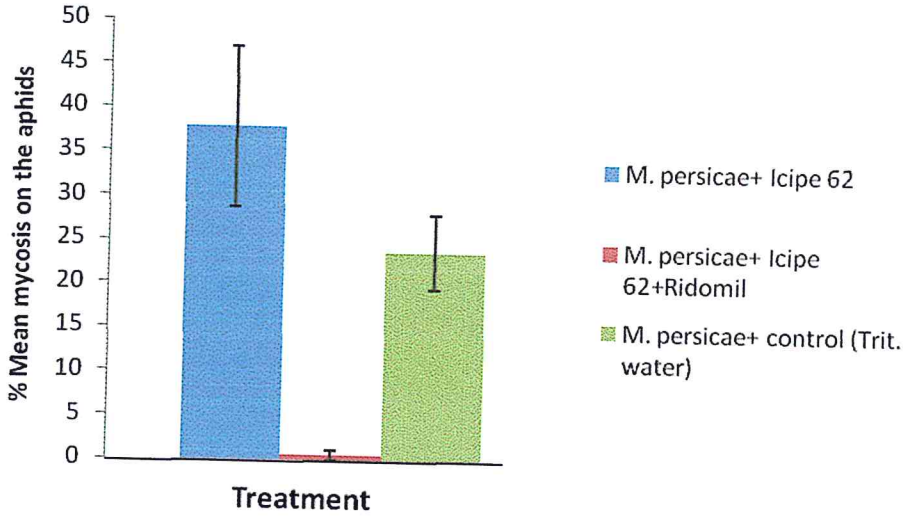

Figure 5.2: Effect of Ridomil fungicide on the entomopathogenic fungi *Metarhizium anisploae* icipe 62 sprayed on *Myzus persicae* in the laboratory.

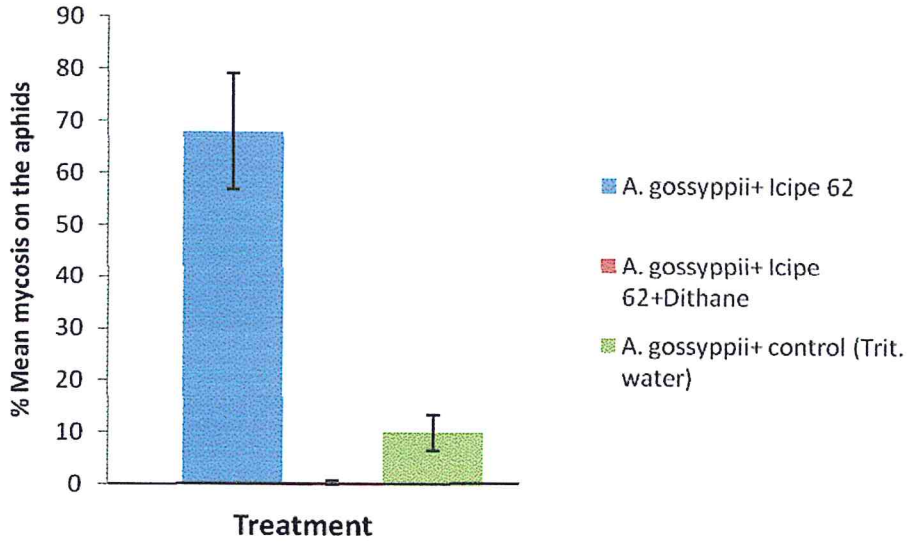

Figure 5.3: Effect of Dithane fungicide on the entomopathogenic fungi *Metarhizium anisploae* icipe 62 sprayed on *Aphis gossypii* in the laboratory.

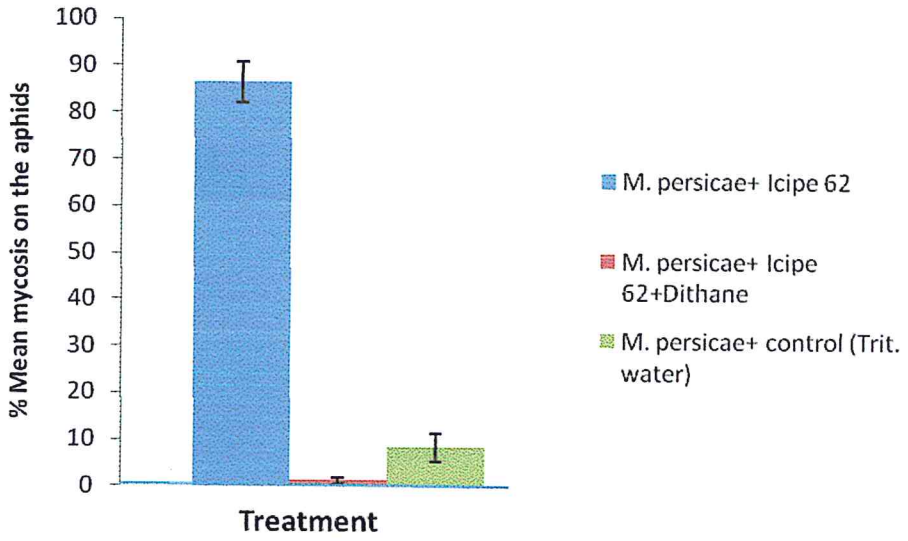

Figure 5.4: Effect of Dithane fungicide on the entomopathogenic fungi *Metarhizium anisploae* icipe 62 sprayed on *Myzus persicae* in the laboratory.

5.3.2 The compatibility of *Aphidius colemani* parasitoids and *Hippodamia convergens* predators with insecticides Duduthrin (Lambda cyhalothrin) and Dimethoate

Fig.5.5 and 5.6 show that the two insecticides Duduthrin(Lambda cyhalothrin) and Dimethoate used commonly in aphid control on the potato crop locally also highly affect and kill the predators *H. convergens* and *H. convergens* and also the parasitoid *A. colemani* under study here for bio-control of aphids on potatoes. The two insecticides killed the predators above in less than 1 hour (60 minutes) in all the five trials done (fig.5.5). This was significantly ($P<0.05$) less time than that taken by the control where no chemical pesticide was applied but only sprayed with distilled water.

The same was the case for the parasitoid *A. colemani* which also died within one hour (fig. 5.6) after application of the insecticide. The control plates with no insecticide had their predators as well as the parasitoids still alive even up to five days after the experiment was set up.

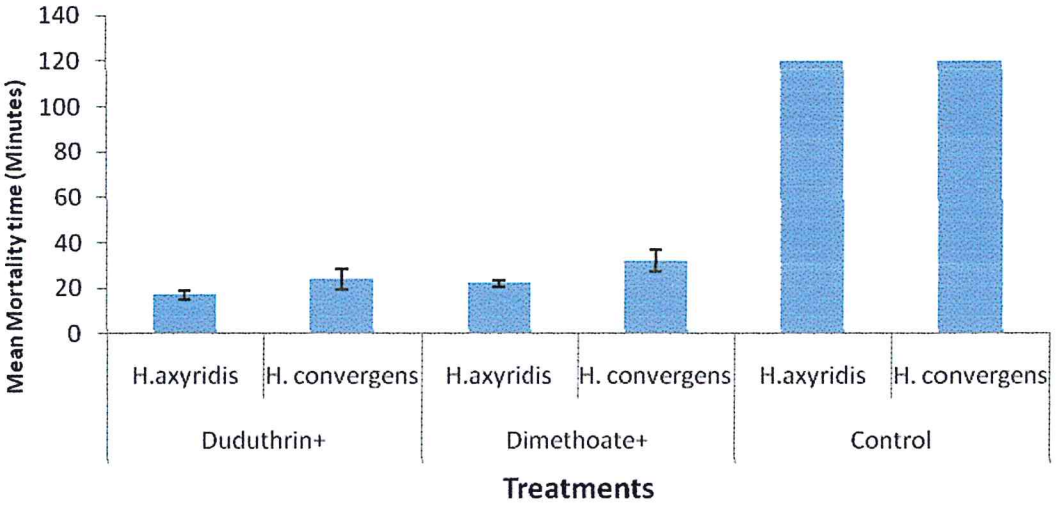

Figure 5.5: Effect of Duduthrin (Lambda cyhalothrin) insecticide on *Harmonia axyridis* and *Hippodamia convergens* predator species in the laboratory.

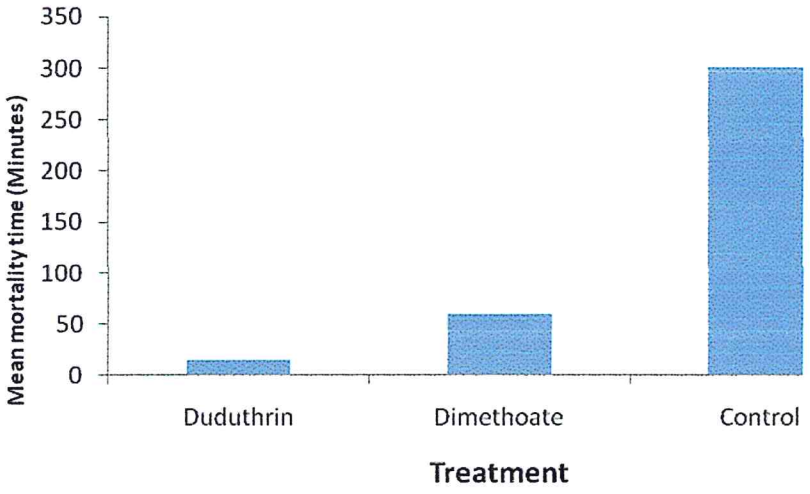

Figure 5.6: Effect of Duduthrin (Lambda cyhalothrin) and Dimethoate insecticides on *A. colemani* parasitoid in the laboratory.

5.4 Discussion

These results show that current fungicides used locally in potato farms are not compatible with use of fungal entomopathogen *M. anisploae* icipe 62 for the control of aphids in potatoes because they end up killing these fungi while killing the fungus that causes late blight in potatoes. Hence, the fungal entomopathogen *M. anisploae* icipe 62 should only be used for the control of aphids in potatoes when the weather is not favourable for late blight attacks on potatoes hence no need to apply the late blight control fungicides. When there is need to apply the fungicides, then other compatible aphid control measures should be used but not the fungal entomopathogens. The results also showed that apart from these fungicides killing the introduced fungal entomopathogen icipe 62, they also killed other fungi that were already on the aphids used in the experiment hence leading to almost total eradication of any fungi and thus very low (almost zero) percent mycosis in all treatments where fungicides were used. This suggests that use of fungal entomopathogens for control of aphids on potatoes might not be compatible with fungicide use as the fungicides will kill most of the fungi on the potato crop.

These results agree with previous research findings where it has been found that, fungicides applied to potato can enhance green peach aphid (*M. persicae*) outbreaks by interference with entomopathogenic fungi (Lagnaoui and Radcliffe, 1998). Minnesota potato growers reported high green peach aphid numbers in both 1995 and 1996, years

of intensive fungicide spraying. Consequently, there was a marked increase in the incidence of Potato Leaf Roll Virus (PLRV) in seed lots entered for testing. In the laboratory, fungicides were shown to have direct effects on these entomopathogens *Conidiobolus obscures, P. neoaphidis* and *Entomophthora planchoniana* Cornu from field-collected aphids (Lagnaoui and Radcliffe, 1998).

Results of the current study also showed that the two insecticides Duduthrin (Lambda cyhalothrin) and Dimethoate are not compatible with the biological control agents predators *H. convergens* and *H. convergens* and also the parasitoid *A. colemani* since they kill them almost immediately. Hence, use of these insecticides should be stopped when the above bio-control agents are being used for aphid control on the potato crop.

This also agrees with some previous studies where the toxicities of pyrethrins were assessed in the laboratory on the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez), the ladybird *Adalia bipunctata* (L.), the rove beetle *Aleochara bilineata* (Gyll.) and the carabid beetle *Bembidion lampros* (Herbst.). The methods selected were residual contact toxicity tests on inert and natural substrates. The pyrethrin products led to 100% mortality in the adult parasitic wasps and ladybird larvae on glass plates and plants (Jansen *et al.*, 2008)

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General Discussion

The results of this study have shown that there are effective indigenous biological control agents of the aphids *M. persicae* and *A. gossypii* in Kenya. From the field survey, different kinds of Predators, Parasitoids and Fungal pathogens were found in all the major potato growing areas surveyed. Predators were the most commonly found natural enemies whereby, of the 23 natural enemies of aphids found, 16 species were predators, four fungal pathogens and three parasitoid species. An almost equal number (22) of other insect pest species were also found in the potato crop of which four of them were aphid species. This agrees with an earlier study by Nderitu (1991) in Kenya where seventy species of insects were found on the potato crop of which, 52 of them were pests and 17 were predators of those pests. A literature search on natural enemies of aphids in East Africa also revealed presence of only predators (Le Pelly, 1959).

The most prevalent predator species found in this study were the ladybird beetles *H. convergens* and *H. axyridis* followed by the aphid eating gall midge *Aphidoletes*

aphidimyza and minute pirate bugs (*Orius spp.)*. This partly agrees with previous studies where these predators have been among the most commonly used predators on the two aphid species. The aphid-eating gall midge, *A. aphidimyza* has been wildly used as one of the biological control agents for the control of several kinds of aphids (Jeoung *et al.*, 2003). Orius species (*Orius insidiosus* Say, *Orius tristicolor* White) (Heteroptera: Anthocoridae) in particular, have for a long time been known as generalist predators preying on a wide range of prey species such as spider mites, Lepidoptera eggs, aphids and whiteflies (McCaffrey and Horsburg, 1986). Coccinellids, adults and larvae, have also been reported as important predators worldwide, particularly *Adonia* spp., *Coccinella* spp., *Hippodamia* spp. and *Scymnus* spp (CPC, 2006). However, there were slight differences between the current study and some previous ones in that, in some previous studies, the most common ladybird beetles reported on the aphids *M. persicae* and *A. gossypii* have been the Coccinellids seven spotted ladybird beetle *Coccinella septempunctata* and *Chilomenese sexmaculata* Fabricius (Coleoptera: Coccinellidae) which were the most abundant predators in potatoes, and other crops, in India (Gupta and Yadava, 1989; Raj, 1989). The two most prevalent predators in this study, *H. convergens* and *H. axyridis* were however very effective in their consumption of the target aphid species *M. persicae* and *A. gossypii* at all stages tested in the laboratory, green house and in the open field suggesting their potential for use as biological control agent of these aphids.

As for the parasitoids, out of the three species collected from the field, the most prevalent species was *Aphidius colemani* and this was the only species that emerged from aphid mummies when reared in the green house. This species was extremely effective in parasitizing both target aphid species *M. persicae* and *A. gossypii* especially in the green house where 100% parasitization was achieved within six weeks of introduction of the parasitoid to the aphids on potted plants in the screen house. Unlike the predators, this parasitoid *A. colemani* was able to search for the aphids at all points of the plant achieving total parasitization and death of all the aphids. This agrees with previous studies regarding use of parasitoids for control of *M. persicae* and/or *A. gossypii* where it has been found that more than 30 species of parasitoids have been used of which the most commonly used was *Aphidius colemani* Viereck (Hymenoptera: Braconidae) followed by *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) (CPC, 2006).

Experiments on the use of fungal entomopathogens in the control of *M. persicae* and *A. gossypii* showed that there are some very effective fungal isolates that cause high mortalities on both aphid species. Isolates from the fungal species *Metarhizium anisopliae* were particularly effective in the control of these aphid species causing high mortalities compared to isolates from the other fungal species evaluated especially those from *B. bassiana*. However, it was also observed that isolates from the two target aphid species were also more pathogenic to these species than those isolated from other hosts as demonstrated in the case of *B. bassiana* isolates. Two other fungal species

Paecilomyces fumosoroseus and *Lecanicillium lecanii* where one isolate from each species were also evaluated in this study showed mixed results with the isolate from *P. fumosoroseus* which had been isolated from soil performing very well close to the best isolate of *M. anisopliae* while the isolate from *L. lecanii* which had been isolated from an aphid cadaver performed very poorly in the same category with the worst isolates of *B. Bassiana.* This shows the need for wide screening of fungal isolates from both the target host and other hosts to arrive at the best isolate for use as biological control agents against these aphids. Entomopathogenic Hyphomycetes fungi (EPF), Particularly the Ascomycetes *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus, P. farinosus,* and *Lecanicillium lecanii,* have been receiving increasing attention as commercial biocontrol agents of insects. A number of formulations based on these fungi have been registered in many countries (Kabaluk and Gazdik, 2005.).

Evaluation of the compatibility of pesticides used locally in potato fields against the above potential bio-control agents revealed that the current common pesticides in use reduce the effectiveness of these agents, the predators, parasitoids and the fungal entomopathogens in the control of the aphid species. This shows that, use of any of these agents should not be combined with use of any of the current pesticides on use in the potato fields. This agrees with some previous studies for instance, in the laboratory, where fungicides were shown to have direct effects on the fungal entomopathogens (Lagnaoui and Radcliffe, 1998). It has also been found that, fungicides applied to potato

can enhance green peach aphid (*M. persicae*) outbreaks by interference with entomopathogenic fungi (Lagnaoui and Radcliffe, 1998).

6.2 Conclusion

From the results of this study, it has been found that there are many indigenous natural enemies of the aphids *M. persicae* and *A. gossypii* in the potato fields in Kenya. The natural enemies that were most prevalent in the field have also proven to be effective biological control agents of the two aphid species after their evaluation in the laboratory, the green house and in the open field. These are, the predators *Harmonia axyridis* and *Hippodamia convergens*, the parasitoid *Aphidius colemani* and the fungal entomopathogen *Metarhizium anisopliae* icipe 62 isolate.

6.3 Recommendations

It is, therefore, recommended that;

1. Further on-station field trials be conducted on the four natural enemies, the predators *Harmonia axyridis* and *Hippodamia convergens*, the parasitoid *Aphidius colemani* and the fungal entomopathogen *Metarhizium anisopliae* icipe 62 isolate which have been found to be very effective potential biological control agents of the two target aphid species *M. persicae* and *A. gossypii* on the potato crop in Kenya. These should then be mass produced so that they can be used on farmers' fields in on-farm trials and later commercialised.

2. It is also recommended that more natural enemies which ranked very close to the above as potential biological control agents of the two aphid species be evaluated right from the laboratory to the green house and the open field and if found effective be tested further in the field to add on the potential biological control agents of these aphid species in the potato crop in Kenya. These natural enemies are, the aphid eating gall midge *Aphidoletes aphidimyza* and minute pirate bugs (*Orius spp.)* which were also very prevalent in potato fields locally and have been reported as very effective biological control agents of these aphid species in

studies done in other countries (Jeoung *et al.*, 2003, McCaffrey and Horsburg, 1986).

3. The fungal pathogen *Paecilomyces fumosoroseus* (Isaria – icipe 682 isolate) should also be evaluated further in the green house and the open field as it proved also very effective against the two aphid species in the laboratory ranking a close second to the *Metarhizium anisopliae* icipe 62 isolate which was evaluated in the laboratory and field in this study. More isolations of fungi from cadavars of the two aphid species *M. persicae* and *A. gossypii* from the potato crop should be done and evaluated for their pathogenicity on these aphid species since it was shown that isolates obtained from the target host have better potential of being more effective biological control agents against the same host than isolates obtained from other hosts.

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APPENDICES

Appendix 1: Field Survey Questionnaire

Potato Farms Field Survey Questionnaire

Serial Number (S. No.)..... Date.....

Name of the Farmer.....

Province..... District.....

Division.....Location.....

Village.....Agro-Ecological Zone (AEZ).....

GPS Co-ordinates: Lat/Longitudes.....Altitude (m).....

Farm size (ha).....Area under potatoes (ha).....

Main potato varieties grown (ranked by area allocated on the farm).....

.....

Age of the potato crop (weeks after planting).....Date planted.....

Types of fertilizers used.....

Are chemical pesticides used on the potatoes.....If yes, which ones;-

Name(s) of fungicides used.....

Name(s) of insecticides used.....

Is manual weeding practiced..... If yes, no. of weedings per season.....

Is chemical weeding practiced.....If yes, which herbicides.....

Date of last weeding.....Period since last weeding (days).....

Other crop protection practices on the farm……..……

Other main enterprises on the farm……..……

Type of vegetation or crops next to the potato farm……..……

Is crop rotation practiced…… If yes, with which crops……..……

Last crop before the potatoes on the current potato farm……..……

Last time crop rotation done; Year…….Month(s)……how many seasons ago?……

Other cultural practices on the farm e.g Irrigation …… …… …

……

Appendix 2: Some natural enemies of aphids in East Africa (Le Pelley, 1959)

	Predator	Host Insect	Country
1.	**Coleoptera:Coccinellidae**		
	i) *Adalea intermidia* Crotch	Aphid on wheat	Kenya
	ii) *Alasia striata* F.	*Aphis gossypii*	Tanzania
		Aphid on wheat	Kenya
	iii) *Brumus suturalis* F.	Aphid on cotton	Tanzania
	iv) *Cheilomenes lunata* F.	*Aphis gossypii*	Tanzania / Kenya
		Aphis sp.	Tanzania
	v) *Cheilomenes posticalis* Fairm	*Aphis gossypii*	Tanzania
	vi) *Cheilomenes quadrilineta* Muls.	*Aphis gossypii*	Tanzania
		Aphis sp.	"
	vii) *Cheilomenes vicini* Muls.	*Aphis gossypii*	Uganda
	viii) *Chilocorus angolensis* Crotch	*Aphis gossypii*	Uganda
	ix) *Exochomus ventaralis* Gerst.	*Aphis* sp.	Tanzania
	x) *Platynaspis capicola* Crotch	*Aphis gossypii*	"
	xi) *Platynaspis kollari* Muls.	"	"
	xii) *Platynaspis maginate* Sic.	Aphids on citrus	Kenya
		Aphids on Tea	Uganda
	xiii) *Platynaspis rufipenis* Gerst	Aphis sp. on citrus	Tanzania

		Aphids on Tea	Uganda
	xiv) *Platynaspis solaamensis* Weise	Aphis sp. on citrus	Tanzania
		Aphids on Tea	Uganda
	xv) *Platynaspis sexguttata* Sic.	Aphis sp. on Coffee	Uganda
	xvi) *Scymnus morosus* Weise	Aphid on Penisetum sp.	"
	xvii) *Scymnus scapuliferus* Muls.	*Aphis gossypii*	"
	xviii) *Scymnus trepidulus* Weise	"	Tanzania
2.	**Diptera:Syrphidae**		
	i) *Melanostoma annulipes var. automenes* Wlk.	Aphid on cabbage	Kenya
	ii) *Paragus borbonicus Macq.*	*Aphis gossypii*	Kenya / Uganda
	iii) *Syrphus adligatus* Wied.	*Aphis gossypii*	" "
	iv) *Syrphus trisectus* Loew.	Aphid on cabbage	Kenya
	v) *Xanthogramma scutellare aegyptica* Wied.	*A. gossypii*	Kenya
3.	**Hemiptera:Miridae**		
	Deraeocolis sp.	*A. gossypii*	Uganda
4.	**Neuroptera:Chrisopidae**		
	Chryopa congrua Wlk. (*Chrysopa bequaerti*)	*A. gossypii*	Tanzania