MANAGED HONEYBEES AS POLLINATORS AND VECTORS OF BIO-CONTROL AGENT (*TRICHODERMA HARZIANUM)* AGAINST GREY MOLD DISEASE FOR INCREASED STRAWBERRY YIELD AND QUALITY IN KENYA

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Managed honeybees as Pollinators and Vectors of Bio-Control Agent (*Trichoderma harzianum)* Against Grey Mold Disease for Increased Strawberry Yield and Quality in Kenya

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DECLARATION

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

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this work to all those who believe in the pursuit of science, technology, and innovation

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPINDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BCA	Bio –control Agent
BVT	Bio vectoring Technology
CAVS	College of Agriculture & Veterinary Sciences
CFU	Colony forming units
IPPM	Integrated pest pollinator management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KU	Kenyatta University
NMK	National Museums of Kenya
OP	Open cage
RBD	Randomized Block Design
UoN	University of Nairobi

ABSTRACT

Pollination by bees and other animals significantly increase both crop yields and quality. Bees also support the transfer of bio-control agents for suppression of crop pests and diseases through bio- vectoring technology that has not been applied in Africa. Two farms were set up to test the ability of honeybees to disseminate *Trichoderma harzianum* to control *Botrytis cinerea,* on strawberries. At on-station farm, three treatments (bee-vectoring inoculum, spraying and control) with 4 replicates each were set up; while on-farm, normal farmer practices were employed. A nuclear beehive fitted with a two- way dispenser was loaded with two grams of *T. harzianum* inoculum. Fifteen bees and flowers from each treatment were picked and cultured in the laboratory. Fruits and flowers infected with *B. cinerea* were recorded. Healthy fruits were counted, weighed and both the equatorial and polar diameter determined. Each bee carried $22.4\pm4.9\times10^2$ colony -forming units of *T. harzianum.* Flowers from the sprayed treatment had significantly higher Colony-Forming Unit's ($P<0.05$) than the bee- vectored treatment. Grey mold disease levels on fruits were significantly lower ($P>0.05$) in sprayed, bee-vectored and control treatment than in farmer's practice treatment. Fruits from spray treatment weighed significantly higher than those from control treatment ($P< 0.05$). The number of seeds, equatorial and polar diameter per berry were significantly higher, $P<0.05$) in farmer's practice treatment. Honeybees proved effective in vectoring *T. harzianum* but, sufficient Colony-forming units had to be delivered for effective control of grey disease. The use T. *harzianum* and open pollination by feral bees proved effective in improving the quality and yield of strawberry plants.

Key words: *Trichoderma harzianum* grey mold, strawberry, biocontrol agent, honeybees, bio-vectoring technology.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Availability and access to quality food is paramount to all living organisms. During the Covid -19 pandemic a global increase of 9.9 percent from 8.4 percent in the number of persons affected by hunger was realised, hence need for bold actions to address major drivers of food insecurity (FAO 2020). In this regard agriculture sector plays an importance role in improving the availability of food and achieving food security. (Smutka *et al.,* 2009). In Kenya agriculture is the backbone of country's economy. It accounts for over 26 per cent of Gross Domestic Product (GDP), 20 per cent employment,75 per cent labour force and over 50 percent revenue generation from export (Kenya Economic survey 2017). It is, therefore, an important economic sector in terms of food production, creation of employment, production of raw material for industries, foreign exchange earnings and raising rural income levels to alleviate poverty.

Strawberry farming is one of the most profitable ventures in Kenya. There is ready market and a high demand especially in urban areas (Mwangi *et al.,* 2016). However, most of the Kenyan horticultural crops including strawberry are rarely grown due to unreliable sources of pre- harvest information (Wainaina, 2013). Therefore, there has been a short supply of quality strawberry fruits in the Kenyan markets. The low supply of the quality strawberry fruits in the market has been driven partially due to limited production of strawberry fruits from the firms since most farmers are predominantly small-scale growers. Other drivers associated to limited supply includes: - High cost of seedlings, poor crop management practices, refrigeration cost, inadequate knowledge on the huge market opportunities (Mwangi *et al.,* 2016) and inadequate access to knowledge and information in areas of early warning systems such as the presence of pest and diseases and new innovative agricultural technologies (Mayaka *et al.,* 2013).

However, edaphic factors and climatic factors have favoured the production of strawberry in almost all eco-regions in kenya. Strawberry is known to adapt well to highly varying climatic conditions (Pramanick *et al.,* 2012). They also do well in acid soils ranging between 6-6.2 pH, and soils with good drainage capacity but, irrigation is necessary after planting, during flowering and the entire growing period.

Recent studies by Menzler-Hokkanen (2017) reported that the conventional growers lose between 25-35% of their strawberry yield to grey mold disease. Grey mold disease caused by a fungi *Botrytis cinerea* is one of the most economically important biotic threat to strawberry plants. Conventional growers have intensely used fungicides to control grey mold disease in every growing season (Menzler-Hokkanen 2017). Most of the fungicides used have resulted to development of resistance by the pathogen (Fernández-Ortuño *et al.,*2014). In addition, the fungicides used have known to reduce the market value of the strawberry due to the toxic residues in the fruit (Lopez *et al.,* 2012). A recent report has further revealed that the viability of the strawberry reduces following the application of fungicides during flowering stage and this reduces the harvestable yield. (Kovach *et al.,* 2000).

To manage grey mold disease in strawberry plants, farmers have resulted to intensive use of synthetic chemicals and cultural methods, but the efforts have proved futile due to increased resistance to insecticides and hence most farmers have neglected and abandoned strawberry farming. Advancement in innovation and technologies have led to introduction of a new strategy of integrated pest pollinator management (IPPM) that ensure crop produced are of high quality and yield. Many countries in Europe have reviewed their agricultural innovations in recent years in response to concerns about lack of adoption of innovation and the need to increase performance to respond to emerging and pressing challenges (Plan, 2016). Therefore, there is need to work with farmers to validate and adapt technologies in an integrated way for successful acceptance and adaption. Some of the notable new technologies that are transforming agriculture from a labour intensive industry to capital intensive include apivectoring technology (Mommaerts & Smagghe, 2011). This technology involves the use of insects as vectors

for bio-control agents. The apivectoring technology is a dual -win scenario where it significantly improves the crop quality and yield through crop protection and pollination in organic cropping systems (Menzler-Hokkanen 2017).

A successful outcome of apivectoring technology has been realized mostly in some developed countries such as Brazil, Serbia and Colombia. The technology is especially useful in large variety of pollination-dependent crops. Managed bees, honeybees and bumble bees have been used to vector inoculum of fungi, bacteria, and viruses from the hive to flowers (Kevan *et al.,* 2008). The technology has been evaluated for the dissemination of *Trichoderma harzianum* T39 and *T. harzianum* 1295-22 against *B. cinerea* under field conditions using honeybees and bumble bees (Shafir *et al.,* 2006; Kovach *et al.,* 2000). Other bio control agents evaluated against various pest and diseases using honey include:- *Bacillus subtilis* to blueberry flowers against mummy berry disease (Dedej *et al*.,2004) *Metarhizium anisopliae* against pollen beetle(*Meligethes aeneus)* and cabbage seed weevils(*Ceutorhynchus assimilis)* (Carreck *et al.,* 2007), *Trichoderma spp* against sunflower head rot (*Sclerotinia sclerotiorum)* (Escande *et al.,* 2002), However, despite this innovative approach, many African countries including Kenya have not embraced it as part of integrated pollinator pest management.

The success of the technology, therefore, depends on the ability of the target vector to efficiently disseminate the biological control agent to the target crop. In the present study, Effectiveness of African honeybees as vector of *T. harzianum* to strawberry crop under field conditions in Nairobi County was evaluated, *T. harzianum* against grey mold disease and the effect of simultaneous pollination and disease control using African honeybee on strawberry quality and yields was also determined.

1.2 Statement of the problem

Most of East African studies toward promotion of agricultural production, have concentrated on plant breeding and use of organic and inorganic agrochemicals.

However, the use of agrochemicals has been faced by issues of resistance development by pests, environmental health issues, banns in European markets due to toxic residues on produce, pollinator decline, and low-quality yield in agricultural production. For instance, synthetic pesticides do not often provide an effective control of grey mold disease in strawberry plants. The increased occurrence of Benz- imidazole and dicarboximide is a resistant strains of *Botrytis cinerea* the causative agent of grey mold disease in strawberry plants. (Schnabel & Weber, 2017). Therefore, there has been decrease in production of strawberries of good quality and quantities and loss of biodiversity. Most of the synthetic pesticides used are of wide spectrum leading to elimination of both natural enemies and beneficial insects such as lady birds and bees. Lady birds have been used extensively to suppress herbivorous pest of economic importance Rondoni *et al., 2021* while bees forage on flowering plants and offer pollination service to the target plant.

Recent studies from several countries in the world such as Canada, Mexico, USA have revealed the use of managed bees as effective pollinator and vectors of bio control agents in controlling pest and diseases in most crops both in green houses and open field (Kovach *et al.,* 2000). However, in Kenya the small-scale strawberry growers have continuously used synthetic pesticides to control strawberry pests such as thrips and fungal diseases such as grey mold hence reducing the market value of the produce as well as reducing the biodiversity mostly of the beneficial and natural enemies. Unfortunately, farmers have not been sensitized on the correct regime of application, for instance, spraying the crops during a calm day to avoid chemical drifting to un-intended sites. More so, use of honeybees for pollination and dispensation of biocontrol agent using the apivectoring technology has not been exploited in any crop. Therefore, there is a knowledge gap in apivectoring technology in many cash crops in East Africa including strawberries which small scale farmers have continuously grown in their home garden for their own consumption as well as for local markets.

1.3 Justification and significance of the study

Strawberries are the most popular perennial small fruits grown in home gardens. There is a high demand for the strawberry leaves, which are an important component of bouquets due to their beautiful shape and sweet scent. Strawberries' gardening is a golden opportunity for the youths in Kenya to improve their livelihood through self-employment. Strawberries are easy to grow, and they can fit within a small space with no specialised equipment's needed. Strawberry thrives well in a wide range of temperatures ranging from mild to hot mainly in temperate and tropic regions of the world. Strawberries have shallow roots, so it is easy to grow them in pots, both indoors and out. They can be placed on a balcony, patio, or indoors in front of a sunny window. They are early maturity and a long bearing period. They have market and demand both local and international.

Use of honeybees in apivectoring technology has proved effective against strawberry grey mold disease using bio control fungus *Gliocladium catenulatum* disseminated by European honeybees and *Bombus bees* in European countries (Hokkanen *et al.*, 2015). However in this study african honeybees (*apis mellifera*)have been identified as appropriate vector for *T. harzianum* and pollinators for improved quality and yield in strawberry plants. Availability of beekeepers and honey bee colonies have made this technology viable while the use of *T. harzianum* as bio control agent against *B. cinerea* is a naturally occuring agent that supress the diseases by augumentation thus no environmental pollution(Reino *et al,*. 2008). Use of honeybees to supress grey mold disease is ecologically friendly and can enhance organic cerfitication of agricultural products hence fetching good prices in the market that will translate to improved standards of living. While combined pollination and crop protection by honeybees increases crop quality and yield leading to increased food security in the country (Kevan *et al.,* 2015). Apivectoring technology is less labour intensive because bees undertake the spread of the bio control agent as compared to sprayng method of disease control where human labour is hired raising the cost of prodution. Apivectoring technology is therefore a viable alternative method in increasing crop production and improving the

livelihood,. The technology is in line with the kenyan 'Big four' agenda on food security. This research project thus contributes heavily to Kenya's bargaining power in global agricultural market and ten percent annual economic growth rate envisaged under the pillar in the vision 2030 thus, improving the standards of living for all Kenyans through provision of quality food and nutritional security.

1.4 Research Questions

To meet the specific objectives, the following research questions were used to guide the study:

1. Are honeybees *(Apis mellifera*) effective in delivering the bio-control agent *Trichoderma harzianum* to strawberry flowers?
2. Is *T. harzianum* effective in controlling grey mold disease of strawberry?
3. What are the effects of simultaneous pollination service and grey mold disease control on quality and yield of strawberry?

1.5 Objectives

1.5.1 General Objective

To determine the effect of managed bees (*Apis mellifera)* as pollinators and vectors of bio control agent against grey mold disease for improved strawberry quality and yields.

1.5.2 Specific Objectives

1. To determine the effectiveness of managed bees (*A. mellifera*) in delivering bio-control agent *Trichoderma harzianum* to strawberry plant.
2. To determine the effectiveness of *T. harzianum* against grey mold disease of strawberry.
3. To assess the effect of simultaneous pollination and control of grey mold disease by managed bees on strawberry quality and yield.

1.6 Scope of the study

Strawberry plants are known to adopt well in diverse eco-regions and different farmers grow different strawberry varieties at different times of the year. To collect consistent data, the study was carried out within Nairobi County in an experimental plot and in a farmer's practice farm who previously grew strawberries.

1.7 Limitation and delimitation of the study

The study was faced by several challenges which include: - putting the hives in cages where honeybees were protected from foraging from other areas, however it was found that honeybees do not orient well in enclosures and therefore change of study site to an open environment free from caging and human interruption. There was also heterogeneity in strawberry farms, strawberries farmers had different varieties of strawberries that were planted at different times, this led to selection of farms within the same environment and planted strawberries of the same variety to ensure uniformity in data collection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This section gives a brief description of what is already known on strawberry ecology, farming and honeybees as vectors of bio- control agents. It also discusses the threat to pollinators, effect of pollination on strawberry quality and yield, and control of strawberry grey mold disease. The chapter identifies the gaps to be filled by this research study. It also shows how existing work contributed to the design of this study.

2.2 Strawberry ecology and farming

2.2.1 Classification of strawberry Varieties

Strawberry varieties are classified based on aroma type namely: Peachy, Pineapple- like, Fruity, and floral like variety (Sheng *et al.,* 2021). They are also classified based on the shape of the strawberry fruit (*Ishikawa et al., 2018*). Strawberry belongs the family Rosaceae under the genus *Fragaria* that have five species. *Fragaria virginiana* a native variety grown during colonial times. The species was hardy and with ability to withstand drought and cold temperature. Later *Fragaria chiloensis* a wild strawberry was cultivated for their large fruit, but it did not suite in wide range of climates. *Fragaria vesca* a woodland strawberry was grown for its flowers. During modern strawberry cultivation, *Fragaria x ananassa* which is a natural hybrid of a natural crossing of *F. virginiana* and *F. chiloensis was* developed and its cultivation spread globally. (Husaini *et al.,* 2016). Garden strawberry plant (*Fragaria x ananassa)* is a perennial and arises from a crown of meristematic tissue. The plant has five basic anatomical structures that includes, leaf, stolon, crown, roots, and fruits. Strawberry plant is distributed throughout the world and it is a popular fruit growing in the northern hemisphere in temperate and sub temperate environment (Khatik *et al.,* 2019). It is also known to grows well in tropical and subtropical regions (Mir *et al.,* 2019). Berries requires full sun for the

highest yield approximately six hours a day. The soil requirement differs according to the variety, for the Chandler variety, it requires soil that is rich in organic matter and well drained (Natsheh *et al.,* 2015).

2.2.2 Economic and nutritional importance of strawberry

Strawberry fruits are favored in the world because of their good flavor that comes as a result of well-balanced sugars and organic acids(Guimarães *et al.,* 2016). The fruit forms part of balanced diet in households since it contains vitamins and minerals necessary for a healthy life(Guimarães *et al.,* 2016). As a good source of vitamin C, Vitamin B1, B2, iron, calcium, potassium, copper and proteins ,strawberry fruits are considered very nutritious for human consumption (USDA, 2015).

2.2.3 Production of strawberry in the world

In ancient times strawberry farming was practiced by romans in large scale for medicinal purposes (Memon *et al.,*2014). Before the arrival of colonialist, native Americans Indians were still consuming strawberry. In1780 the first strawberry hybrid was developed in United State of America (USA) (Dubey *et al.,*2019), However in Africa strawberry was introduce back in 1656 in south Africa (Simirgiotis & Schmeda, 2010). Currently there are three main classes of strawberry varieties grown for commercial purposes, these includes, June bearing, Ever-bearing and Day-neutral (Petran e*t al.,* 2017) .

The selection for the strawberry variety is dependent on the time of fruiting, the hardiness of the fruit after ripening and the type of fruit. Strawberry farming has picked over time and the annual world production greatly increased in quantity in the last twenty years to over 2.5 million tones (FAO, 2014). While their uses have also advanced where in the current time strawberry are eaten either fresh or in such prepared foods as preserves, fruit juice, pies, ice creams, milkshakes, and chocolates. The demand and consumption of strawberries has increased due to the antioxidants effect in them .

The antioxidant like ellagic acids, anthocyanin's, ellagitannins, catechin and quercetin reduce the risk of atherosclerosis and protect the heart against bad cholesterol (Afrin *et al.,* 2016).

Studies showed that women who take three serving of strawberry have a lower risk of heart attach (Cassidy *et al.,* 2013). Consumption of strawberry has also been associated with prevention of oral and liver cancer, regulate blood pressure, boost immunity, improve brain function and protect the eyes from free radical scavenging activities (Giampier *et al.,* 2017). Strawberry has therefore gained popularity all over the world and considered as one of the best agricultural venture in many different ecosystems (Republic of South Africa, 2008).

2.2.4 Production of strawberry in Kenya

Advancement in technology especially in media sector has made information accessibility more efficient. However, strawberry production in kenya is dominated by small scale growers. The industry is faced by several challenges including farmers with little or no information about nutritional benefits of strawberry fruits, high cost of planting materials, fruit waste and loses due to the high rate of perishability, production of fruits of lower quality, lack of access to refrigerators and cool boxes and minimal or lack of production skills (Mwangi *et al.,* 2016). There is a ready market for strawberry especially in urban areas. However, strawberry fruits are produced in short supply and are expensive in the market. This is because there is limited production due to lack of proper crop management skill especially in disease control (Mwangi *et al.,* 2009).

2.3 Pollinators and flower visitors of strawberry.

Strawberry plant produces flowers which attract many types of insects including butterflies, syrphids flies' beetles, solitary bees, and honeybees (Abrol, *et al.,* 2019). however, only bees are of real consequences in transferring pollen effectively without injuring the flower parts. The various groups of bees include, honeybees (*Apis*

mellifera), and solitary bees such as, sweat bees (*Lasioglossum sp and Halictus sp*), leaf cutter bees (*Megachile_sp*), small carpenter bees (*Caratini sp*), Mining bees (*Hyleus sp*), and carpenter bees (*Xylocopa sp*). According to (Abrol, *et al.,* 2019), Honeybees are the most important pollinators of strawberry as they visit the strawberry flowers frequently and in numerous numbers throughout the day. Nevertheless, Abrol *et al.,* 2019 recorded that there was no significant difference in pollination rate when *Apis mellifera*, native bees and Syrphidae categories of strawberry flower visitors were analysed, thus diversification of pollinator sources would be encouraged for quality production.

Several studies have been carried with the aim of assessing pollination effectiveness with different strawberry floral visitors. According to (Hodgkiss *et al.,* 2018) aphidophagous syrphine hoverflies **(***Eupeodes latifasciatus)* has been found as effective pollinators of commercial strawberry and could also offer crop protection against aphids. Further studies by (Dimou *et al.,* 2008) showed that bumble bees (*Bombus terrestris*) have been widely used as pollinators of strawberry in greenhouse where fruits of quality shape and yield of marketable fruits was realized as compared to control treatment. In Brazil members of stingless bees have been found effective pollinators of strawberry flowers thus promoting a significant increase in strawberry quality and yield production Meléndez Ramírez *et al.,* 2018. However, there has been a global decline in bee abundance and diversity (Zattara *et al.,*2021).

2.4 Pollination requirement of strawberry

Strawberry breeding and selection for commercial cultivars has resulted in the hermaphrodite's strawberry flowers. The male and female flower organs are within the same flower and can self-fertilize and produce viable fruits. Unlike the earlier cultivars before breeding and selection technology in agriculture developed, strawberry flowers without stamens never produced fruits unless cross pollinated from a staminate flower (Abrol, *et al.,*2019).

Currently most strawberry growers do not use bees for pollination because strawberry are primarily self-pollinating and can be pollinated by wind and gravity (Kovach *et al.,* 2000).However, hermaphrodite's flowers may not be sufficiently self-fertilizing for maximum strawberry production, Therefore, additional pollination for all the pistils of a flower is necessary for maximum berry size and increased yield. According to Vaissiere *et al.,* (2010,) a perfectly shaped berries develops when all pistils receive sufficient pollination. If only few of its stigma is pollinated, an irregularly shaped berries develop and would be discarded in the market .Kovach *et al.,* 2000 also found 22 percent increase in size and 26-40 percent increase in weight of strawberries visited by bees as compared to non- bee visited treatment.

Abrol, *et al.,* 2019 carried out an experiment in a strawberry field to investigate insect's pollinators visiting chandler strawberry blossoms and their impact on fruit production. They found that the strawberry that were in open pollination plots had a higher per cent of fruit set as compared to enclosed plots, the fruits from open pollinated plots were in good quality and large while the malformed fruits were only 11.2 percent unlike the enclosed plot which had 17.44 percent. They concluded that self-fertilizing alone of strawberry was not sufficient for maximum fruit set since they set few fruits, thus diversifying pollinator sources is paramount for quality strawberry production

2.5 Managed bees as pollinators and their threats to pollination service.

Members of the bee family *Apidae* have been managed globally by farmers for commercial crop pollination. Klein *et al.,* 2007, found that over 35 percent of global food production come because of cross pollination dependent on or enhanced by animals. Honeybees (*Apis mellifera*) are the key group of animal pollinators utilized in agriculture for improved crop production (Kevan, 1999). In the United States *A. mellifera* are managed through beekeeping and translocated in hives from one region to the other for the purpose of pollinating a wide variety of crops that are dependent on bees (Melin *et al.,*2018). In addition, other feral bees such as members of the bee family *Halictidae- Lasioglossum sp, Megachilidae- Osmia sp,* are used effectively in

pollination of fruits such as almond (Koh *et al.,* 2018,) However, anthropogenic activities such as land fragmentation, habitat loss, introduction of alien species, pesticides application have adverse effect on pollinator diversity (Potts *et al.*,2010). Nevertheless, the global stock of managed honeybees' colonies has gradually increased (Aizen &Harder,2009), thus making apivectoring technology using honeybees applicable.

2.6 Apivectoring technology using managed honeybees

Apivectoring technology is an innovative technology where bees have been used as vectors for bio-control agents (Hokkanen, Menzler-Hokkanen, & Lahdenpera, 2015). This approach incorporates different components such as pollinators, bio control agents and pest pathogen/ insect pest interactions. Introduction of apivectoring technology as a bio control strategy is primarily to reduce application of synthetic chemicals which have impacts on human health and environment in addition to pest/pathogen developing resistance (Kapongo *et al.*,2008).Three types of bees have been used as vectors(honeybees, Bombus spp and bumble bees) with different bio control agent in various crops (Peng *et al.*,1992). Kovach *et al.*,(2000) used *Bombus impatiens* and honeybees to deliver T. *harzianum* against grey mold in strawberry.

Honeybees have also been used effectively as vectors of *clonostachys rosea,* a potential antagonist of *Botrytis cinerea* that causes grey mold in strawberry (Kapongo *et al.*,2008). Honeybees use their head, thorax, legs, and abdomen to carry the inoculum from the hive dispenser to the strawberry plants (Peng *et al.,* 1992).Kovach *et al.*,2000 found that most of the *T. harzianum* propagules found on the honeybees exiting treated hives was detected more on the bee l at 58 percent on legs as compared to head (5%) , Thorax(23%) and abdomen (14%).

Successful results on effectiveness of bees in delivering bio control agent to plants were also found on apple and pear for management of fire blossom blight (Roselló *et al.,* 2013), crimson clover flowers for management of *Helicoperva zea* ,and sunflowers

plant for management of banded sunflower moth (Jyoti & Brewer, 1999). Apivectoring technology is organically certified, and economically viable (Kevan *et al.,* 1990). The technology has been in practice in Canada and other counties such as Brazil but, not yet applied in Kenya at the time of this study.

2.7 Bio control agent

The bio control agent (BCA) also referred to as biological control agent. It refers to deliberate introduction of living organism with aim of antagonising the effect of one or more pathogen causing disease to the plant rather than the disease resistance host plant ((Pal & Gardener, 2006). BCA are also considered environmentally friendly because they are naturally occurring in the environment. Various beneficial fungi such as *Clonostachys rosea Trichoderma harzianum* and *Gliocladium catenulatum*, were found effective in controlling *B. cinerea on strawberry plants* (Hokkanen *et al.*, 2015; Karise *et al.*,2016)

2.7.1 Use of bio control agent in Agriculture

The use of bio control agent in agriculture has gained popularity in countries such as Mexico, USA, Canada, as a new sustainable agriculture practise due to its nature as a natural occurring remedy with no environmental risk to human and the rest of the biodiversity (Peng *et al.*, 1992). Various groups of microorganisms have been exploited as BCA, and these include, bacteria, and fungi. According to Fravel, 2005 fourteen bacterial and twelve fungi have been registered with the Environmental Protection Agency (EPA) of the USA for the control of plant diseases.

However, the success of the bio control agent against plant diseases depend on: the isolation of the microorganism from their natural ecosystems, and the screening process to obtain a pure strain of the intended BCA(Chaube *et al.,* 2003). The isolates obtained are then screened for their action against the target pathogen in the laboratory and in the experimental green houses before implementing in the open field (Junaid *et al.,* 2013).

2.7.2 Mode of action of bio control agents

Different BCA exhibit either direct or indirect mode of action against the pathogen. other BCA exhibit indirect antagonism mode of action whereby they compete with the pathogen for essential micronutrients and space from the soil to get established to the environment. The BCA are capable of efficiently up taking and utilizing the micronutrients than the pathogen. The pathogens are excluded by depletion of food base and by physical occupation of site (Singh *et al.,* 2018).

According to (Pal & Gardener, 2006), hyper parasitism is the most direct physical contact mode of action, which involves tropical growth of BCA towards the target organism, the BCA coils the pathogen, and finally enzymatically disbands the pathogen cell wall rendering it harmless .Hyper parasitism is the major mode of action exhibited by *Trichoderma spp.*

2.8 Management of grey mold disease in strawberry

Strawberry plants are susceptible to several pathogens including fungi, bacteria, and virus (Petrasch *et al.,* 2019). Fungi are the most economically impactful pathogen of strawberry where they cause extensive loss to field and stored strawberries. Amongst the fungal pathogen, *Botrytis cinerea* causing grey disease is considered the primary pathogen of harvested strawberry. The fungi are necrotrophic in nature and are known to cause an extensive and expensive losses to crops in both the field and greenhouses before and after harvest (Willianson *et al.,* 2007). The pathogen affects the strawberry when the conidium germinates, penetrate, and invade the tissue during humid and wet weather conditions. The invaded tissue becomes soft and finally rot lowering the production yield. The infested crops become a primary source of infection while the spores of the pathogen get dispersed by wind to other plant host (Carisse, 2016).

2.8.1 Cultural methods of grey mold disease control

Conidia of *B. cinerea* is found everywhere in the environment and therefore can be dispersed easily by wind, water, insects' human, and agricultural tools during cultural operations. Sanitation therefore is the first strategy to limit the amount of initial pathogen inoculum and reduce the spread of grey mold disease during strawberry crop production. *Botrytis cinerea,* generally, infects the aging tissue. The removal of dead and infected plant materials from the farm, will therefore reduce the conidia and mycelium of *B. cinerea.* Sterilizing agricultural tools such as panga, jembe will also limit the spread of conidia of *B. cinerea.* Further treatment of cut flowers by dipping in hot water is a phytosanitary method of limiting the spread of conidia. (Bika *et al.*,2021). Planting less susceptible cultivars, creating space between the beds and seedlings has said to offers a limited effect on grey mold disease control (Legard *et al.,* 2002). Therefore, sanitation alone is inadequate in managing grey mold disease when inoculum loads are high.

2.8.2 Chemical method of grey mold disease control

Control of grey mold disease is based predominantly on routine synthetic fungicides application as the most used agrochemical. The fungicides are known to have a negative impact on living organisms and environment (Tomas-Grau *et al.,* 2020). The repeated application of fungicides in greenhouses also reduces the market value of the berry due to the accumulation of toxic residues on the fruits (Wang *et al.,* 2021). Kovach *et al.,* 2000) also recorded that application of fungicides at a flowering stage may reduce the viability and hinder fruit formation leading to low or no harvestable yield. Use of fungicides to control grey mold have been very expensive since it requires four to five times of application from bloom through harvest to supress the disease (Kovach *et al.,* 2000). This has prompted the strawberry growers to search for affordable and safe method of controlling *B. cinerea*.

2.8.3 Biological method of grey mold disease control

Biological control using naturally occurring bio control agents is being studies as an alternative way of controlling grey mold disease. Antagonist biocontrol agent (*Clonostachys. rosea*) is potentially used to control the fungi (*B. cinerea*) in strawberry plants. However, application of the *C. rosea* through spraying on flowers have resulted in unsatisfactory control of the fungi since not enough spores reach the target. The spore reaching time is also transversal making the spraying method unsatisfactory Cota *et al.*, 2008).

2.8.4 Integrated pest pollinator management

Introduction of integrated pest pollinator management technologies (IPPM) have been evaluated for effective control of crop diseases. Among the strategies, apivectoring technology has been evaluated for the control of pest and diseases among several crops. Use of honey bee and bombus bees have been tested in delivering *Trichoderma harzianum* against grey mold disease in strawberry. Kovach *et al.*,(2000) found that each honeybee used as a vector of *T. harzianum* against grey mold disease carried about 1×10^5 of colony forming units of *T. harzianum* hence providing a better *B. cinerea* control than that applied by spraying method. Bees are timely when they visit the flowers to collect nectar and pollen. They also apply satisfactory colonies of bio control agent on time to the crop. Apivectoring technology has proved effective in controlling crop diseases and offer pollination service. However, no studies have been done in Africa using African bees to deliver bio control agent to crops. This research, therefore, aims at using African honeybees in delivering *T. harzianum* in controlling grey mold disease in strawberry plants.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

This section describes the study area and the criteria for the study site selection. It provides descriptive procedure of how the study plots were prepared for the study. The section explains how the research plots were designed, and data collection by different techniques to achieve the objectives of the study. The section summarizes the systematic application of statistical and logical techniques that were used to describe, summarize, and compare the data that was obtained from the study.

3.2 Description of the Study area

The study was conducted in two experimental farms. These farms included the University of Nairobi (UoN) farm and a private farm in Loresho in Nairobi County. The UoN farm was located at the field station of Upper Kabete Campus, College of Agriculture & Veterinary Sciences (CAVS). The college of agriculture and veterinary science is located off Kapenguria road, 15 Kilometres to the Northwest of Nairobi at a geographical location E -1.25, S 36.742554, while Loresho farm was situated in Loresho area along Kaimoni road approximately five kilometres away from CAVs at a geographical location E -1.253, S 36. 751(Figure 3.1). In Loresho farm there were other cash crop grown in the farm such as apple, kales and peach grown for home consumption. While in CAVs the area consisted of various crops grown for demonstrations and for student's experiments. The study area in CAVs farm is at an altitude of 1840m while Loresho farm is at 1822m above sea level (ASL), the area receives an average rainfall of 950mm per annum with bi-annual type of rainfall that falls as long rains in the month of March to May and short rains that comes in the month of October to December.

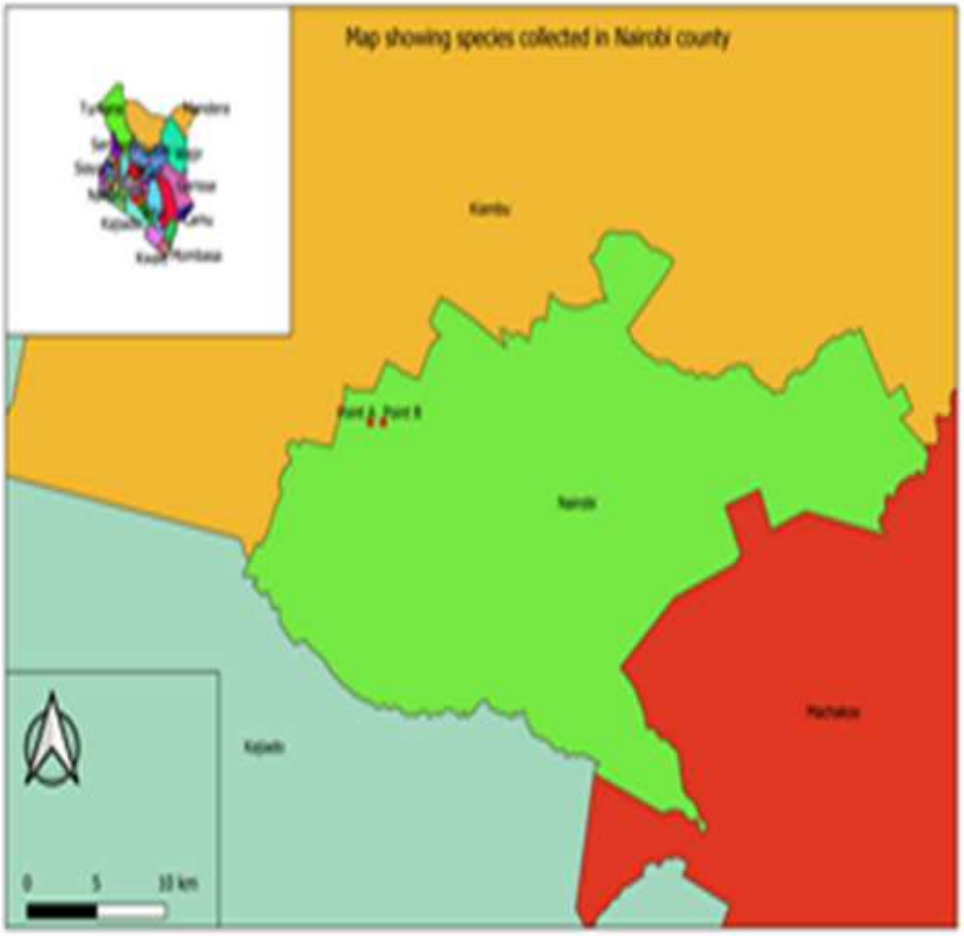

Figure 3.1: Map of Kenya showing the location of the study area.

3.3 Selection of the study area

Factors considered in selection of the study sites included: - area free from human settlement to avoid interfering with colony of bees installed at the study site, suitable edaphic factors (soil composition, organic matter) as well as climatic conditions (temperature, humidity) favouring growth of strawberries. Proximity of the sites to required resources such as accessibility to nearby flowering plants that provided bees with alternative resources when strawberry plants were not on bloom, ploughing services, labour, irrigation facilities and expertise in cage construction formed basis for selection of the study sites.

3.4 Study sites

A preliminary survey was carried in Nairobi County for the purpose of selecting the best strawberry experimental plots where the study would be carried out. Two farms were selected for the experiment. These included the UoN farm and a private farm at Loresho. The two farms were stratified into two main categories namely: Open pollination without introduction of managed bees and managed bee farm. In each category the best representative plots were selected depending on the location of the plot from the human settlements, the surrounding vegetation and access to water for irrigation. One farm was selected from Loresho area and the other farm in CAVS farm station at UoN. The sites were ranked, and names given according to management status of grey mold disease, status of managed bees and surrounding vegetation's. (Table 3.1.)

Table 3.1: Summary of the two study sites and their description

Site name	Definition of the site name/ site description
Loresho Farm -Open pollination without introduction of managed bee colonies.	• There were no colonies of managed bees installed at the site, strawberries depended on the feral bees that were within the surrounding. • Strawberries were not sprayed with any fungicides. • There was open pollination from all the pollinator diversity. • There was no limitation of the surrounding crops/vegetation. • The surrounding vegetation included farmer's choice of crop.
CAVs-Managed bees	• Colonies of managed bees were installed at the strawberries site. • Bio control agent (*T. harzianum*) was used against grey mold disease, delivered by managed bees and through spraying. • There were surrounding wild flowering plants from nearby farms. • Cages made of stiff net were used for plots that received bio control agent through spraying and for treatment that received neither bio control agent nor visited by managed bees.

3.5 Study design

3.5.1 Experimental design

The study focused on managed bees, bio control agent (*T. harzianum*), grey mold disease and quality yield of the strawberry. Two experimental farms (CAVS and Loresho) were purposively selected to: determine the effectiveness of managed bees as vectors of *T. harzianum* to strawberry flowers, effect of *T. harzianum* against grey mold disease and the impact of increased managed bee on strawberry quality and yield. The plot at CAVs which measured 50 m by 20m was divided into four sub plots on which three treatments were randomly distributed. The treatments included the following: Treatment 1(BVT)- Open plot that enhanced visits by managed honeybees vectoring *T. harzianum* to strawberry plants. The treatment contained one modified beehive colony of managed bees situated within the plot (Figure 3.2); Treatment 2(spray)- Caged plot where managed bees and other feral bees were protected from foraging on the strawberry plants. The treatment was caged using a stiff net. Strawberry plants received T. harzianum through spraying using a hand sprayer.: Treatment 3(Control)- The treatment was caged also to protect bees and other flower visitors from foraging on the strawberry plants. Stiff net was used to cage the plot. No *T. harzianum* was used in this treatment (Figure 3.3). Each treatment was replicated 4 times.

Loresho farm was classified as an open pollination plot under farmer's practice. The plot was left open to enhance normal farmer's practise in crop production. *T. harzianum* was not applied in the plot. The strawberry plants relied on the farmers' practise of manual weeding to remove the competing weed species and debris that could be potential hosts for the *B. cinerea.*

Figure 3.2: A modified beehive on site

Figure 3.3: Caged treatments at CAVS farm

3.5.2 Land preparation and strawberry planting

3.5.2.1 CAVs Farm and Loresho farm

A plot measuring 20 by 50 meters was used to set up the experiment at CAVs field station (Figure 3.4). The plot was cleared off the weeds that could form a potential host for pest and diseases affecting strawberries. Ploughing was then employed to overturn the soil and expose the soil nutrients that were previously leached. After two weeks, harrowing was done to break the hard pans of soil and improve the soil structure for proper spreading of the shallow roots of the strawberry plants. Sprinkler Irrigation was done continuously for two hours for two consecutive days till the soil was saturated.

The plot was then divided into four blocks of 10m by 20m. The blocks were two meters apart. A two-metre buffer was left. Seven strawberry beds of one (1) meter-wide and 10 meters long were established in each block. The beds were one meter apart. Each bed consisted of a single row on which chandler strawberry variety was planted. The spacing was maintained at 50 centimetres between plant to plant to allow for maximum growth, reduce competition for nutrients and reduce the high chances of pest and diseases infestation. In each bed, twenty certified strawberry seedlings obtained from Kenya

Forest research institute (KEFRI) were planted in a row. The strawberry seedlings were planted in such a way that the crown was left above the soil surface to encourage more sprouting and vegetative growth. Prior to planting, the strawberry seedlings were cleaned off the dead leaves and induced flower abortion to avoid withering and hasten the growth.

Each block with strawberry seedlings was further sub- divided into three experimental plots that were distributed randomly within the block resulting to a total of twelve experimental plots. Two experimental plots measuring 2.5 x7m in each block were caged. Each cage consisted of fifteen strawberry plants. While the third experimental plot was left undemarcated with most of strawberry plants from which fifteen strawberry seedling were randomly selected as the sample size and used for data collection.

Weeding was done regularly to remove all the weeds that absorbed considerable nitrogen and compete for space while care was taken not to interfere with the delicate and shallow strawberry roots. Irrigation was done for two hours daily during the first month of planting to compensate for lack of rainfall. Induced flower abortion was done on daily basis for the first one months after transplanting. This was to enhance vegetative growth and allow plant to obtain enough nutrient to support quality fruit. Top dressing of the strawberry plants was done prior to flowering stage using inorganic fertilizer Calcium Nitrate (CN). One teaspoonful was CN was mixed with soil at the crown surface of the strawberry plant. Top dressing was done to enhance sufficient formation of flowers and quality fruits (Hosseini, *et al.*, 2021).

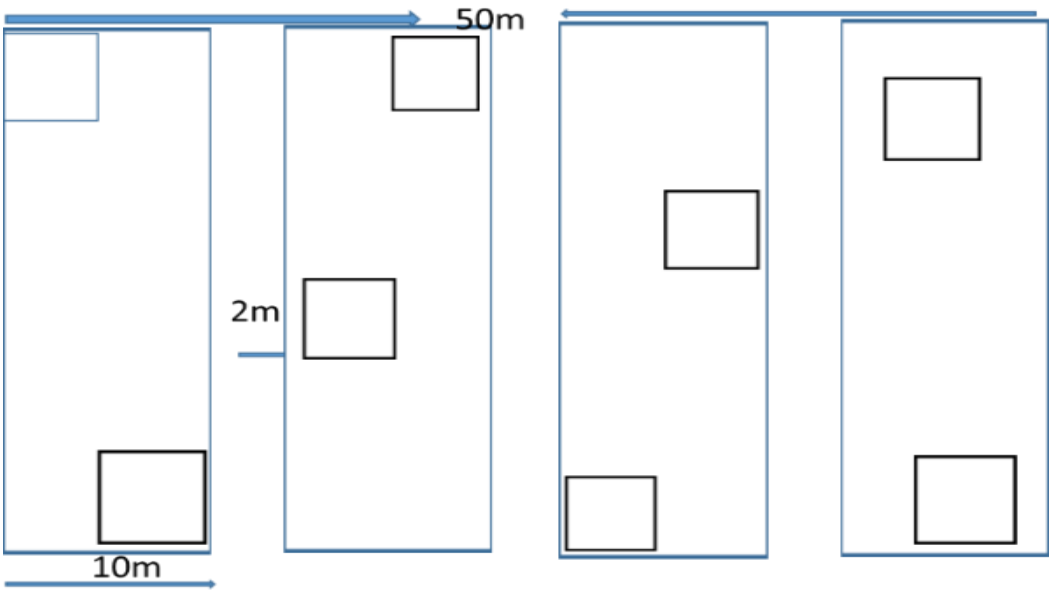

Figure 3.4: Cavs Plot demarcation

At the private plot in Loresho, the plot was identified in an area where there were other crops that were growing, some of these crops within the plots included peach, avocado, kales and pumpkins. Two experimental plots each measuring 7m by 10m were identified within the plot. The experimental plots were 10 meters apart. The plots were selected from the open areas where the canopy of the growing crops was not shading the plot. The plots were then subdivided into two subplots each measuring 7 by 2.5m resulting to four replicates. The subplots were 2meters apart (Figure 3.5).

Three strawberry beds of one (1) meter-wide and 2.5 meters long were established in each subplot. The beds were 1meters apart. Each bed consisted of a single row. In each row, five certified strawberry seedlings obtained from Kenya forest research institute (KEFRI)were planted resulting to fifteen strawberry seedling per subplot. Spacing of strawberry seedlings was 50 centimetres in each row. Prior to planting, the strawberry seedlings were cleaned off the dead leaves to avoid pest and diseases infestation. Induced flower abortion was also done to remove all the flowers that had sprouted before the plant had developed enough leaves to support quality and quantity fruits.

Weeding was done weekly removing all the competing weeds. However, no pesticides were used in the farm to control any diseases or pest while irrigation was done in absence of rainfall.

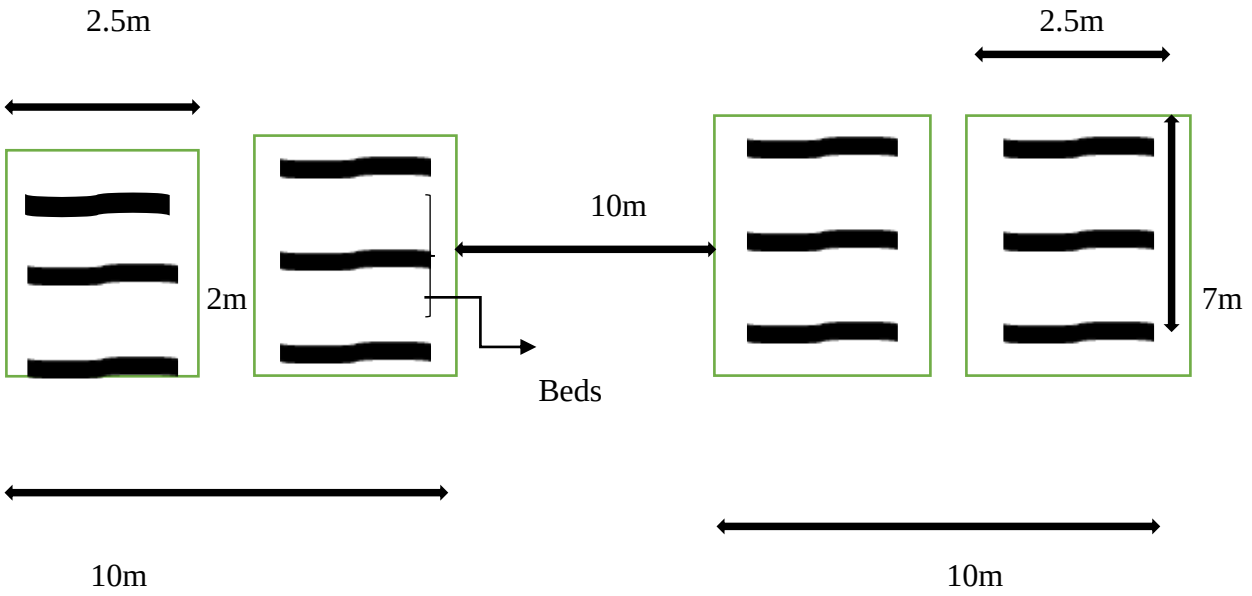

Figure 3.5: Experimental plot at Loresho

3.5.3 Sampling design

A simple random sampling method was applied where all the strawberry plants in the BVT plots were given a unique number. Using a table of random numbers, fifteen strawberry samples were selected and marked as the sample size from which data was collected. For the sprayed, caged, and in farmer's practises plots, the entire population of fifteen strawberry plants were picked for data collection ag.

3.6 Data collection techniques

3.6.1 Determining the effectiveness of honeybees in delivering T. *harzianum* to strawberry plants.

To assess the effectiveness of honeybees in delivering the bio control agent *(T. harzianum)* to the target plant, a healthy colony of honeybees in a two- way modified Langstroth hive was installed in the plot. The placement of the hive was done two weeks prior to data collection. The honeybees were given time to acclimatize to the new hive design and orient to the new environment before filling the dispenser with the formulated inoculum. The inoculum was formulated from 50-gram tin commercial product of *T. harzianum* which had 1.6×10^{9} colony forming units per gram to form 1.2×10^{9} of CFU. The concentration of the commercial *T. harzianum* was diluted using sterilized corn flour. Corn flour also acted as an inert carrier for the colonies.

The hive dispenser was then filled with two grams of formulated inoculum (1.2×10^{9} CFU/g) after every six days. Samples of fifteen honeybees per sampling were picked after refilling the dispenser with inoculum. The bees were picked using sterilized sweep net and sterilize forceps and put into a sterilized vial for further analysis in the microbiology laboratory at the university of Nairobi, chiromo campus. Bees were picked throughout morning to afternoon when exiting the hive after refilling the dispenser with the inoculum. Bee data was collected once per week for three consecutive weeks. In the first week, fifteen bee samples were picked immediately after refilling the dispenser with 2grams of *T. harzianum.* In the second week, bees were picked on the second day after refilling the dispenser and in the third week, bees were picked on the third day after refilling the dispenser with the inoculum. The *T. harzianum* was refilled into the disperser before 8.00 in the morning before bees started moving out of the hive to forage. Samples were taken to UoN, Chiromo campus microbiology laboratory where they were cultured on potato dextrose agar (PDA) through spreading method. The inoculated plates were then incubated at 25^{o}C for four to seven days after which the total number of colonies forming units (CFU) were calculated.

To determine whether the vectored spores of *T. harzianum* were delivered to the strawberry flowers, fifteen flowers from each treatment were excised using a sterilised pair of scissors and placed individually in a manila paper bag. The samples were then taken for further processing at UoN, Chiromo campus microbiology laboratory where the samples were cultured individually in PDA at room temperature of 25^{o}C in dark cabinets and after 4-7 days there was growth and the number of CFU were calculated. Figure 3.6.

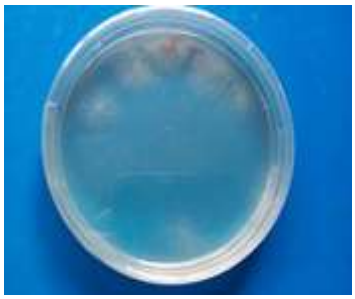

Figure 3.6: Colonies of T. harzianum growing in Potato dextrose agar after six day

3.6.1.1 Preparation of potato dextrose agar

Potato dextrose agar (PDA) was used as media to culture the wash solution that was obtained from the freshly collected honeybees and flowers. The media was prepared at a full strength of 39g PDA in 1000ml of distilled water (Bacteriological Analytical Manual,1998). The media bottle was used where the cap was loosened to create enough space for pressure to be built during autoclaving. Autoclaving was done at 121 pressure for 15 minutes (Figure 16). The content was allowed to lose the pressure then cool before transferring it to the fume chamber which was sterilized by cleaning it with cotton wool soaked in 70% alcohol. At hand touch temperature of PDA media, 1 gram of streptomycin powder was added to inhibit bacterial growth and the media swirled gently to avoid bubbles forming. The media bottle was flame sterilized on the bottle mouth to dispense the media into the labelled sterile petri plates which were arranged in the fume chamber. The media was left to cool and form a jell. After solidification of the

media, plates were inverted upside down to prevent the accumulation of condensed water that could contaminate the culture.

3.6.1.2 Processing of Honeybee and strawberry flowers Samples in the Laboratory.

The freshly collected samples of honeybees and strawberry flowers from the field were put each in a labelled 25ml glass bottle. Ten millilitre sterilised water was added into each glass containing the sample. The mixture was then shaken vigorously using rotary machine to dislodge all the adhering powder from the surface. The content was then passed through a laboratory test sieve of 200ml pores size to separate the liquid from the solid content into an empty sterilized 25ml glass bottle. The wash solution obtained was diluted in ten- fold was inoculated on Petri dishes half filled with Potato Dextrose Agar (PDA) media. The entire process was done in a fume chamber to protect contamination from the surrounding environment.

3.6.1.3 Inoculation of honeybees and strawberry wash solution on Petri dishes containing potato dextrose agar

The serial dilutions were made by taking 1ml of wash solution obtained from freshly collected honeybees and strawberry flowers. The 1 ml wash solution was placed into 9ml volume of distilled water to make 10 ml dilute solution. The dilute solution produced had 1ml of extract /10ml, producing a 10-fold dilution. The amount of wash solution in each ml of the diluted solution is 0.1ml. The process was repeated to make five successive dilutions depending on how *T. harzianum* spores per sample were widely spread for ease calculation. One ml of inoculum from each sample was drawn from three to four consecutive dilutions and spread over the Petri dish containing half -full the PDA media. The inoculated plates were sealed with a para-film to avoid any invasion of air contamination during incubation. Incubation was done at room temperature of 20- 25^{o}C in dark cabinets for 4- 7 days under close observation.

3.6.1.4 Colony forming units counting.

Growth of *T. harzianum* was observed f between day 4 to day 6. Using a colony counter machine, the number of growing colonies were calculated and recorded per plate per dilution. The total number of CFU per sample were calculated using the following formular,

$$\text{CFU/ml} = \frac{\text{number of colonies x total dilution factor}}{\text{Volume of culture plate in ml}}.$$

Where colonies were densely populated and difficult to separate them during counting, plates with a higher dilution were grown for sparsely distributed number of colonies. (Figure 3.7 a &b).

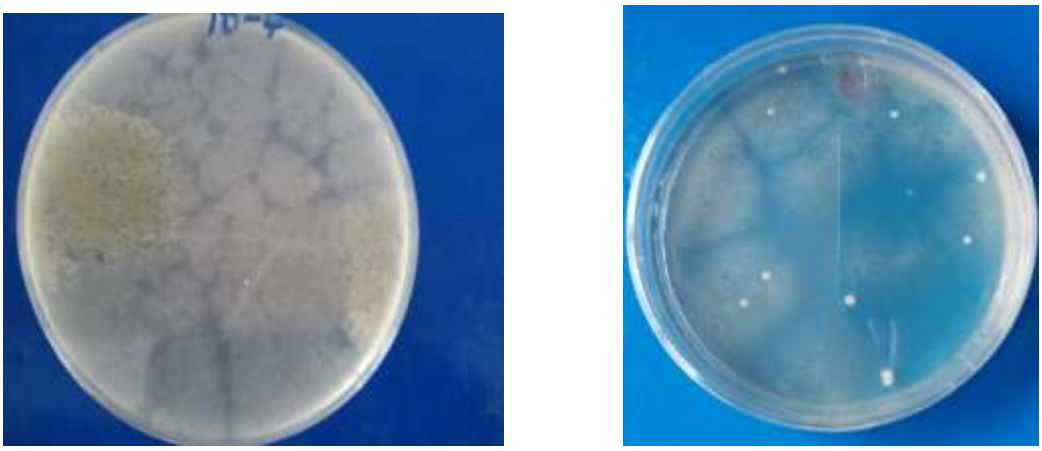

Figure 3.7: (a) Densely populated CFU of *T. harzianum* and (b) Sparsely populated CFU of *T. harzianum*

3.6.2 Determining the effect of *T. harzianum* against grey mold disease

Total number of flowers and ripe berries per plant were recorded from each plot on weekly basis for three months. The data also included the total number of strawberry fruits and flowers that had signs of grey mold disease (Figure 3.8 a& b). The data

collected was used to calculate the percent disease incidence in strawberry plant using the formular described by Waller *et al.,* 2002.

$$Disease\ incidence\ \% = \frac{Number\ of\ structures(\ fruits\ or\ flowers)with\ B.cinerea}{Total\ number\ of\ structures(\ fruits\ or\ flowers)} x100$$

The data was treated independently for each treatment since different treatment showed different results for *B. cinerea* in both flowers and fruits.

Figure 3.8: (a) Grey mold disease on a strawberry fruit and (b) Grey mold disease on a strawberry flower

3.6.3 Determining the effect of pollination by managed bees on strawberry quality and yield.

The indicators of strawberry quality and quantity in the study was determined using the following fruit parameters; The total number of seeds per berry, the equatorial diameter, polar diameter, and the weight of the berry (Colak *et al.*,2017). The diameter of the berry was measured in units of centimetres(cm) while the weight was measured in units of grams(g). Harvesting of the ripe fruits was done one month after planting. Harvesting was done once in every seven days for a period of three months. Ripe berries characterised by red colour from different treatment were picked and packed in different manila bags. In each harvesting time, the total berries were counted per harvest per plot

during each harvest. The harvested berries were taken to the Center for bee biology and pollination ecology at National museums of Kenya (NMK) for further analysis. Each berry per treatment was weighed using analytical sensitive weighing balance, equatorial and polar diameter determined using a Vernier calliper (Figure 3.9), number of seed counted, and all the data obtained was recorded in an excel data sheets for further statistical analysis.

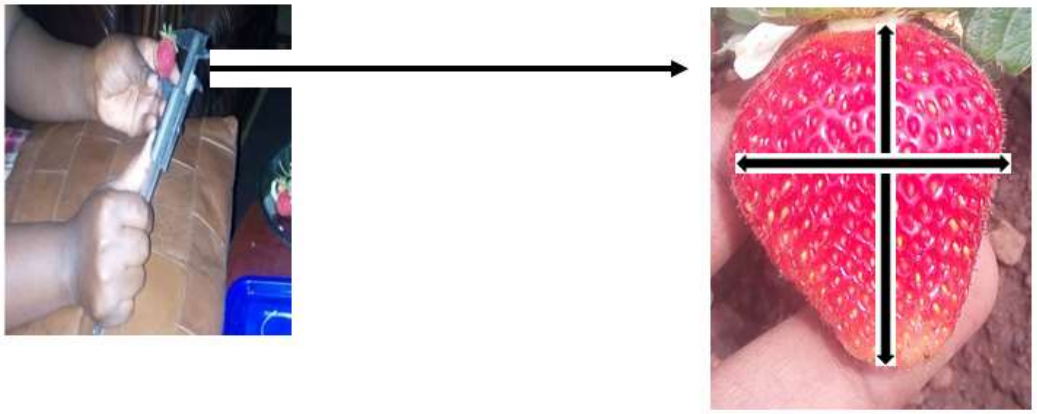

Figure 3.9: Determining the size of the strawberry fruit using a vernier calliper

3.7 Data Analysis, Techniques, and presentation

Statistical techniques were used to describe, summarize, and compare data. Data were analysed using STATISTICA program (Stat Soft.Inc.(2007) version 8.0. The data were first tested for normality using Shapiro Wik; s W- test and histogram. Where data did not show a normal distribution, it was subjected to a normal distribution curve to check for outliers and then transformed at $Log_{10}(x+1)$. Statistical test was set at a significant level of 0.05. The data were analysed using a one-way analysis of variance (ANOVA) to show whether there was any significant difference among means of all the treatments. Where data showed a significant difference in means, the results were further analysed using Tukey's test to show the level of significance difference and where the significance difference occurred among means of different treatments. The results

obtained from analysis were presented in graphs, charts, and tables for visual interpretation.

CHAPTER FOUR

RESULTS, ANALYSIS, AND INTERPRETATION.

4.1 Introduction

This section presents major findings of the study organized into tables, figures, charts, and graphs. It covers findings of each objective: the effectiveness of managed bees as vectors of *T. harzianum* to strawberry flowers, the effectiveness of *T. harzianum* against *B. cinerea* in strawberry, and the combined effect of managed bees as both vector and pollinator of strawberry on quality and yield production.

4.2 Effectiveness of managed bees in delivering the T. harzianum to strawberry flowers

Results indicated that on average a honeybee carried 22.4 ±4.85x $(10)^2$ colony forming units of *T. harzianum* when leaving the hive. Honeybees collected on the first day after refilling the dispenser with the inoculum recorded a significantly higher mean of colony forming units (25.8x 10^2) compared to honeybees sampled on the third day (18.2x10^2) after refilling the dispenser ($F=2,12$, $P<0.05$) (Figure 4.1).

There was a significant difference in mean number of colonies forming units of *T. harzianum* per strawberry flower among treatments ($F=3,140$, $P<0.05$). Spray treatment recorded significantly high mean of CFU per flower (20.56 ± 0.48 x$(10)^2$) while Caged and Farmers practice treatment significantly recorded the lowest mean of CFU per flower (0.3610^2) respectively. (Figure 4.2). Results also revealed that the mean of *T. harzianum* $\pm 0.11 \; and \; 0.33 \pm 0.08)$ CFU delivered among the three days were significantly different. The significance difference was found between day one and day three of the BVT treatment and spray treatment ($F=11,132$, $P<0.05$). Spray treatment in day one recorded significantly the highest mean of *T. harzianum* CFU per flower.

(22±0.79x 10^2) while BVT in day three recorded the lowest mean of CFU per flower (9.42$\pm 0.47x10^2$) P<0.05). Figure 4.3).

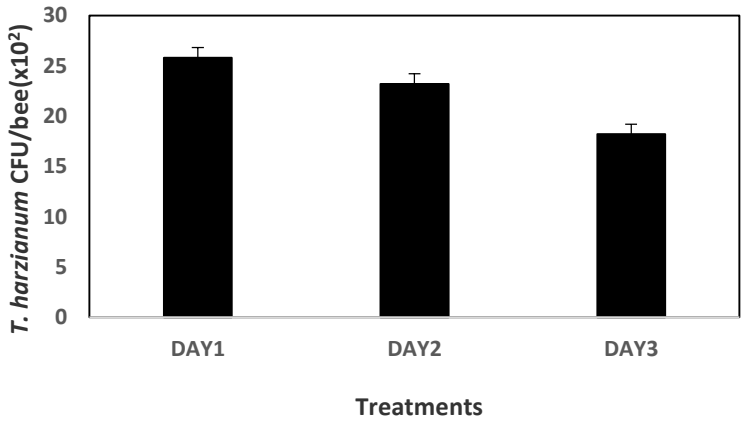

Figure 4.1: Population of *T. harzianum* in honeybee sampled at different days after refilling the beehive dispenser with inoculum of the biocontrol agent.

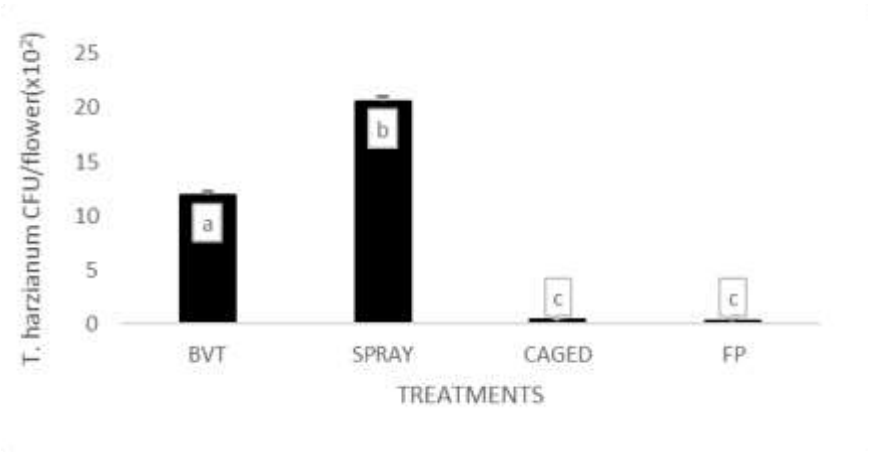

Figure 4.2: Population of *T. harzianum* on strawberry flowers sampled from different treatments.

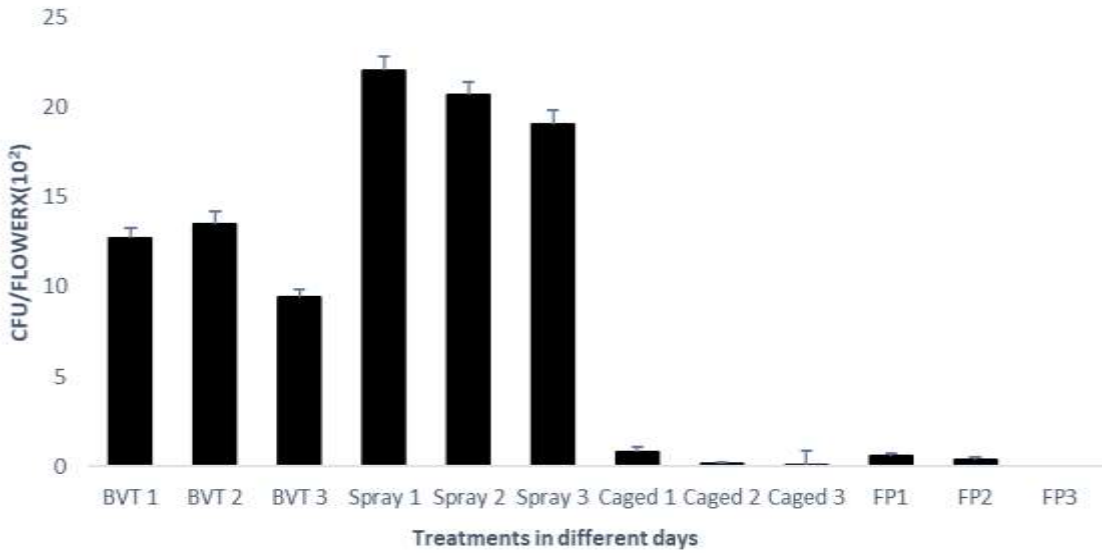

Figure 4.3: Population of *T. harzianum* on strawberry flowers sampled at different days from different treatments.

4.3 Effectiveness of *T. harzianum* against grey mold (*Botrytis cinerea*) on strawberry plant.

A preliminary survey done to assess the presence of *B. cinerea* in the soil showed the presence of the fungi in both the CAVS and Loresho farm (0.58±0.2 and 0.33±0.1) $x10^2$ per gram of soil respectively ($F=1.11$ $P > 0.05$) There was a significant difference in percentage disease incidence on strawberry flowers among treatments ($F=3,12$, $p<0.05$). Bio vectoring technology and caged treatment recorded significantly high mean of flower disease incidence (54% and 53%) than the farmers practice treatment (34%) ($p=0.03$) (Figure4.4). In strawberry fruits there was a significance difference in disease incidence level between farmer's practice treatment and spray treatment. The farmers practice treatment recorded the highest percent disease incidence (63%) while the spray treatment recorded the lowest disease incidence (26%) $p=0.01$. caged treatment recorded

(37% diseases incidence while BVT recorded 38% diseases incidence) and BVT treatment recorded 37 p>0.05 (Figure 4.5).

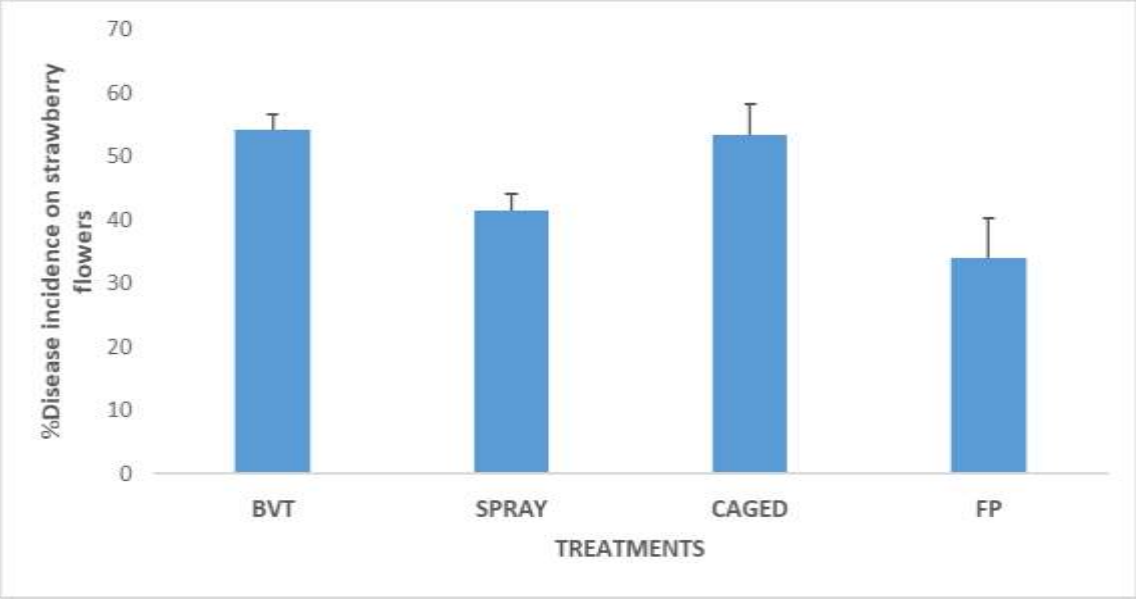

Figure 4.4: Percent disease incidence of strawberry flowers from different treatments

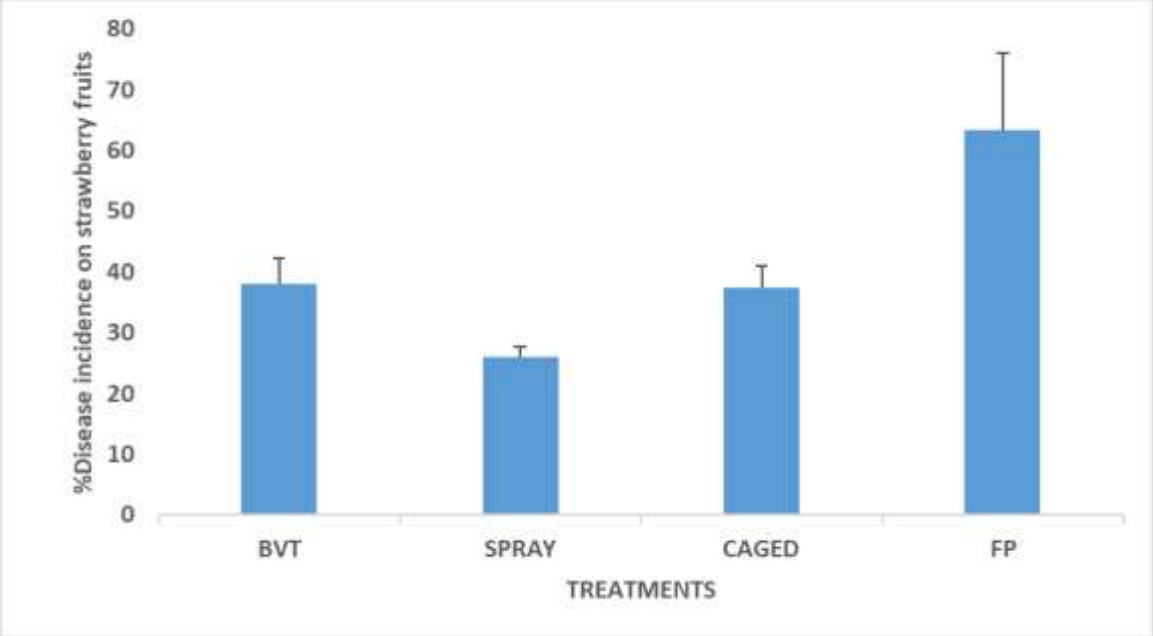

Figure 4.5: Percent disease incidence in strawberry fruits from different treatments.

4.4 The effect of pollination by managed honeybees on strawberry quality and yield

Data on total harvest, weight, polar diameter, equatorial diameter, and the number of seeds in strawberry fruits were used in this study to assess the simultaneous effect of inoculated managed honeybees on strawberry quality and yield**.** It was found that the mean of the total number of strawberry fruits harvested from spray treatment was higher as compared to those harvested from BVT, caged and farmers practice treatment ($p>0.05$) Spray treatment showed a significant difference in mean weight of strawberry fruits (4.9g) as compared to the mean weight of fruits in caged treatment (4.1g) ($F=3,2122$, $P<0.05$). Farmers practice treatment produced fruits with significantly high polar diameter (2.5cm) ($F=3,2122$, $P<0.05$) as compared to caged treatment(1.6cm) which showed the lowest polar diameter size. Bio vectoring and spray treatment showed a polar diameter of 2.0 cm ($p>0.05$. Similarly, equatorial diameter of the fruit was

significantly higher in FP plot(1.9cm) ($P<0.05$). A significant difference was recorded in the mean seeds of the strawberry where farmers practice recorded significantly higher mean seeds (242) as compared to caged treatment that recorded significantly lowers mean seeds (125) (F- 3. 2122, $P<0.05$ (Table 4.1).

The abundance of bees and other flower vising insects were recorded from farmer's practices treatment and BVT treatment before and after installation of managed beehive. Honeybees, solitary bees, dipteran, lepidopteran, coleopteran, orthopteran, Aranea and wasps were identified from the study site (Table 4.2).

Table 4.1: Means of parameters that were used as indicators of strawberry quality and yield

	Mean ± SE					
Treatment	**No. of flowers**	**No. of fruits**	**Weight(g)**	**PD (cm)**	**ED (cm)**	**No. of Seeds**
BVT	186.25 ± 21.07a	69.75± 2.78a	4.85±0.18ab	1.96±0.08b	1.55±0.04b	160±4.8b
Spray	204.5±28.6a	85.5±2.50a	4.89±0.20a	2.02±0.08ab	1.44±0.05bc	135±5.1c
Caged	157.25± 11.50ab	57.5 ± 3.38a	4.13 ± 0.20b	1.64±0.09c	1.31±.0.05c	125±5.8c
FP	88.75± 21.99b	54.25±20.14a	4.48±.42ab	2.48±0.18a	1.93±0.10a	241±11.1a

Means with same letters within a column are not significantly different(P<0.05)

Table 4.2: Total number of bees and other visiting insects sampled at CAVs and Loresho Plot

Insect visitors	**Before BVT**	**After BVT**	**FP**
Diptera	912	112	69
Honeybees	4	107	23
Solitary bees	48	0	30
Coleoptera	0	1	30
Orthoptera	1	1	5
Aranea	2	3	0
Lepidoptera	3	10	0
Wasp	10	26	0

CHAPTER FIVE

DISCUSSION

5.1 Introduction

This chapter present the discussion of the study findings. The results are interpreted using the existing knowledge on strawberry pollination and use of bio control agent (*T. harzianum*) vectored by managed honeybees. In some cases, the findings are justified using the actual data collected and prevailing factors of the study site.

5.2 Effectiveness of managed bees in delivering the *T. harzianum* to strawberry flowers.

From the results, it was evident that honeybees leaving the hive with two-way dispenser were inoculated with *T. harzianum* spores. The findings agree with work done by Bilu *et al.,* 2004 on use of Triwaks; a dispenser type that evidently dispensed a high level of *Trichoderma spp* consistently throughout the day. Flowers collected from all the treatments showed the presence of *T. harzianum* although at different concentration. The results were an indication that honeybees were able to carry and disseminate the bio control agent (*T. harzianum)* to the strawberry crop. The results agree with those of Kovach *et al.,* 2000 who did a study in Geneva with *Trichoderma harzianum* and observed that honeybees can successfully deliver *T. harzianum* to strawberry plant for biological control of *Botrytis* fruit rot.

The study also revealed that honeybees carried more of bio control agent (*T. harzianum*) in day one compared to day three after refilling the dispenser. The results concur with findings of Freeman *et al.,* 2004, where application of bio control agent after every two days resulted in better control of *B. cinerea* than less frequent application of every seven to ten days.

The spores of *T. harzianum* were recorded more on flower samples derived from spray and BVT plots as compared to those derived from caged and farmers practice plots. This finding concurs with work done by Shafir *et al.,* 2006 where on average 22000 CFU of *T. harzianum* per flower were delivered by honeybees to strawberry plants through a Triwaks dispenser. However, despite the spray treatment showing the highest number of spores landing on the flower, the spraying practice is not economically viable for large scale or commercial farming because the practice is labor intensive, and a lot of water is used during spraying resulting to high cost of production. Shafir *et al.,*2006 also found similar challenge with application of bio control agent whereby frequent use of a sprayer may cause mechanical damage to the fruit and foliage whereas use of managed honeybees to deliver the bio control agent requires no manpower and the bees double up as pollinators of the target crop. The flower samples obtained from BVT plot were believed to have lost some spores as a results of honey bee moving from one flower to the next and this also corresponds to a study carried by Shafir *et al.,* (2006) who concluded that there was a possibility of the bio control agent being lost as the bee fly from one flower to the next, hence the amount delivered to the next flower becomes less. Additionally, bio control agent could have been lost during the process of catching the bees using the sweepnet , drift by the air and some of the *T. harzianum* could have been washed away during irrigation.

Traces of *T. harzianum* spores were found on flowers in caged and farmers practise plots, although at a lower percentage than flowers in spray and BVT plots. This observation provides evidence that the fungi (*T. harzianum)* are naturally occurring in the soil at a very small concentration that require to be augmented for crop protection. It could also be assumed that the *T. harzianum* spores were drifted through the stiff net from the spray and BVT plots to the non-T. *harzianum* loaded plots (Caged and Farmers practise). These findings were supported by work done by Shafir *et al.,* 2006 who explained that some of those bio control agents in non-target plots could be naturally occurring. Kovach *et al.,* 2000 also indicated that a drift effect of bio control agent occurred whereby non-target areas were found to have the inoculum. Elad *et al,* 1993,

also found a significant population of *T. harzianum* in untreated control plants when the bio control agent was sprayed in the treated plots of cucumber plants.

5.3 Effectiveness of *T. harzianum* against *B. cinerea*

It was evident that use of *T. harzianum* on strawberry plants influenced strawberry flower production that led to high production of strawberry fruits. It is also revealed that *T. harzianum* used in the strawberry plots either through use of managed honeybees or spraying had a positive effect on reducing grey mold disease incidence on strawberry fruit. It was evident that when bio control agent *(T. harzianum)* was applied on strawberry plant the disease suppression was more expressed on strawberry fruits than on flowers. These results were attributed to the short life span of the strawberry flowers. The strawberry flowers took between one to three days to fruit formation, while the strawberry fruits took between five to seven days before ripening and ready for harvesting. Additionally, high grey mold disease incidence on flowers in BVT plot could be because of effect of sprinkler irrigation that washed away most of the delivered *T. harzianum* inoculum. Introduction of managed honeybees at the study site could also contributed to increased disease incidence as referenced by Dedej *et al*.,2004, who found that the incidence of mummy berry disease in blueberry plant increased because of increased honeybee density, but when the honeybees disseminated the bio control *Bacillus subtilis,* the diseases reduced.

The study proved the effectiveness of *T. harzianum* against grey mold disease in strawberry. This result agrees with the results by Shafir et al.,2006 who found that at low to medium grey mold disease levels, there was best control in inoculated honeybee - visited strawberry plots. Elad, (2000) also found suppression of *Botrytis cinerea* on greenhouse crops when *T. harzianum* was applied. While Soliman et al.,2015 recorded that when *T. harzianum* was applied on cucumber in green house, there was a significant reduction in grey mold incidence.

The concept of using bio control agent *T. harzianum* therefore, proved to be effective in suppressing *B. cinerea* that cause grey mold to strawberry plant. It was evident that the spray treatment recorded the lowest percent of disease incidence and though caged treatment did not receive any bio control agent, the disease incidence was reduced on fruits as compared to flowers. This showed the protective effect of the stiff net that was used in caging the plot which prevented entry of strong winds that would have drifted the pathogen into the cage. The stiff net also hindered direct sunlight, water droplets and contaminated insects from reaching the strawberries.

5.4 Effect of pollination by managed honeybees on strawberry quality and yield

It was apparent in this study that pollination by insects and wind on strawberry plant played a significant role in influencing the quality and yield of strawberry crop. Results showed a significant difference in number of seeds, weight, total harvest, and the diameter of strawberry fruit among treatments. The number of managed honeybees, solitary bees and wind pollination is thought to have influenced the quality and quantity of the strawberry fruits. Albano *et al.,* 2009, showed a relationship between the number of fertilized ovules and berry size of strawberry when they were pollinated by both honeybees and native bees. Therefore, it may be assumed in this study that the numerous numbers of pollinating solitary bees contributed to improved quality and quantity of strawberry yield in the farmers practice plot.

Comparison in results between BVT plot and farmers practice plot revealed a strong contribution of multiple pollinators for strawberry pollination. In contrast, managed honeybees alone were found effective in strawberry pollination according to Colak *et al.,* (2017) where a significant difference in weight of strawberry was realized for honeybee pollinated strawberry plots as compared to treatment without honeybee pollination. However, the current study found that reliance on several pollinators (managed honeybees and feral bees) results to improved quality and quantity in strawberry. This result supports work done by Albano *et al.,*2009 on diversification of pollination sources, avoiding the dependence on a single specific group of pollinators.

The consistent higher fruit weight, polar diameter and number of seed in spray plot compared to caged plot is likely to results from effect of *T. harzianum* given that enclosure of the plot using stiff net was applied in both treatments and the effect of stiff net was also across the both treatments. Kovasch *et al.,* (2000) showed that stiff net frequently used by fruits and vegetable growers, protect plants from cold and hence accelerate plant growth. However, despite the influence of pollinators on strawberry yield, it was confirmed from this study that the commercial strawberry cultivar has a degree of self-pollination and can be wind pollinated. According to Albano *et al.,* (2009) flowers of all the commercial strawberry are hermaphrodites and self-fertile. It was noted that flowers of both open BVT and farmers practice, and stiff- netted plots (caged and spray) were similarly agitated under windy conditions, indicating that the stiff net was not a major barrier to wind pollination. Abrol *et al.,* (2019) showed a significant increase in pollination of strawberry yield among honeybees' pollination, wind pollination and native bees' pollination.

It was noted that the numerous numbers of managed bees in BVT plot could have been influenced by introduction of honeybee's colony within or close to the plot. Hansted *et al.,* 2015 showed a rise in fruit set and yield when bees were kept close or within an orchard. However, relatively higher quality strawberry yield was recorded in farmer's practise plot that had recorded a numerous number of solitary bees. Roubik (2009) showed that increased managed bees in an area silently compete with solitary bees interfering with their foraging activity.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The study revealed that honeybees could effectively vector bio control agent (*T. harzianum*) from a hive with a two –way dispenser to the strawberry plants.
2. The bio control agent *(T. harzianum*) disseminated either by managed honeybees or through spraying application could effectively be used to suppress grey mold disease (*B. cinerea*) on ripe strawberry fruits. However, more colonies of *T. harzianum* is required to be disseminated by managed honeybees for effective control of grey mold disease on strawberry plants.
3. The study concluded that managed honeybees could be used simultaneously as pollinators and vectors of *T. harziunum* in strawberry plants. However, diversification of pollinator host plants that attracts feral bees could lead to strawberries of good quality and yield.

6.2 Recommendations

i. There is need for furthers studies on efficient number of *T. harzianum* colonies forming units (CFU) delivered by managed honeybees to effectively suppress grey mold disease.

ii. There is need to focus on use of managed honeybees in farms as pollinators and vectors of bio control agent (T. *harzianum*) for improved strawberry quality and yield.

iii. There is need to sensitize farmers on contribution of managed honeybees in agriculture and adoption of apivectoring technology as a management tool within a structured program of pest and disease control.

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APPENDICES

Appendix I: Checklist of strawberry flower visiting insects in the study site

Insect visitors	**Before BVT**	**After BVT**	**FP**
Diptera	912	112	69
Apis mellifera	4	107	23
Ceratina sp.	3	0	7
Lasioglossum sp.	32	0	7
Patellapis sp	9	0	7
Seladonia sp	1	0	9
Megachile sp	1	0	0
Macrogalea candida	2	0	0
Coleoptera	0	1	30
Orthoptera	1	1	5
Aranea	2	3	0
Lepidoptera	3	10	0
Wasp	10	26	0

Appendix II: Grey mold disease on strawberry fruits

Appendix III: Managed and some solitary bees sampled from strawberry plots at CAVs and Loresho plots.

African honeybee ***(****Apis mellifera****)***

Lipotriches sp.

Leaf cutter bee *(Megachile sp.)*

Small carpenter bee *(Ceratina sp.)*

Appendix 1V: Certificate of participation in BVT Workshop in University of Belgrade

CERTIFICATE OF PARTICIPATION

СЕРТИФИКАТ О УЧЕШЋУ

THIS ACKNOWLEDGES

Jane Macharia

First International Advanced Course on Using Managed Pollinators for Dissemination of Biological Control Agents for Suppression of Insect, Fungal & Other Pests of Crops

Први међународни напредни курс о коришћењу узгајаних опрашивача за ширење агенаса биолошке контроле за сузбијање инсеката, гљивица и других штеточина гајених биљака

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SIGNED, Dr. Peter Kevan & Dr. Ljubiša Stanisavljević May 10th, 2019